Host status of plants associated to coffee shady agroecosystems to Meloidogyne paranaensis

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Research Article

Keywords: Root-knot nematodes, alternative hosts, coffee corky-root disease

Posted Date: July 27th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-3098216/v1

Version of Record: A version of this preprint was published at Journal of Plant Diseases and Protection on February 27th, 2024. See the published version at https://doi.org/10.1007/s41348-024-00882-5.

Abstract

The root-knot nematode *Meloidogyne paranaensis* is one of the main problems for coffee production in Latin American countries. In Mexico, this nematode is found damaging shaded coffee plantations, with a wide variety of associated vegetation. The plant species present in these agroecosystems could serve as nematode alternative hosts, helping to maintain and disperse the population of *M. paranaensis* even when control measures are carried out in coffee trees. The aim of this work was to evaluate the ability of *M. paranaensis* to reproduce in 13 plant species commonly associated with shade-grown coffee plantations. The plants were inoculated with eggs and J2 of *M. paranaensis*, 10 months later the population density, the multiplication rate, and the host susceptibility index were calculated. *Meloidogyne paranaiensis* reproduced in 11 of the evaluated plants at different levels. *Citrus aurantium, Citrus reticulata, Inga jinicuil, Inga vera* and *Musa* AA, were highly susceptible compared to *Coffea arabica* and *Coffea canephora.* On the other hand, *Macadamia integrifolia* and *Psidium guajaba* are considered resistant to moderately resistant with a reproduction rate less than one and a susceptibility index less than 10 with respect to *C. arabica* and less than 25 with respect to *C. canephora*. *Persea schiedeana* and *Syzygium jambos* did not allow the *M. paranaensis* reproduction, so they are considered highly resistant. The results of this study provide important information for the *M. paranesnsis* management in infested shade-grown coffee plantations. It is necessary to evaluate other woody and herbaceous plant species to improve control measures for this nematode.

Introduction

The root-knot nematode *Meloidogyne paranaensis* is one of the main phytosanitary problems in coffee (*Coffea* spp.) production in Latin America. (Carneiro et al. 1996; Villain et al. 2013). This nematode is associated with the development of the coffee corky-root disease, which begins with the typical thickening and galls on the roots. However, as the infection progresses, more severe symptoms are seen, such as deep cracked cortical tissues with a cork-like appearance, along with the presence of fungi and bacteria inside the root tissues (López-Lima et al. 2020; Lamelas et al. 2020). The disease leads to necrosis and atrophy of the root system, causing the plant death in a period of 2 to 4 years, significantly affecting coffee production (Bertrand et al. 2000). *Meloidogyne paranaensis* has been detected in Brazil, Guatemala, Hawaii, and Mexico, where coffee production is affected due to the increased distribution of this nematode (Carneiro et al. 2004; Villain et al. 2013; López-Lima et al. 2020).

In Mexico, coffee corky-root has been recorded since the 1960s, however the disease was distributed only in small areas of the Veracruz and Chiapas states. In 2015, *M. paranaensis* was identified in heavily affected plants with corky-root in several shade-grown coffee plantations in the main producing areas of the Veracruz and Puebla states (López-Lima et al. 2015; Alcasio-Rangel et al. 2017). Currently, *M. paranaensis* is a threat to coffee production in Mexico, it is estimated that up to 35% of replanted *Coffea arabica* plants are lost every year in many municipalities of Veracruz, forcing producers to frequently replace affected plants (Téliz-Ortíz et al. 1993; INIFAP 2005; López-Lima 2021). In Mexico, most of the coffee plantations are found in different agroforestry schemes, with a variety of both native and

introduced vegetation, which functions as shade for coffee plants and provides additional income to farmers (Licona-Vargas et al. 2006; Hernández-Martínez et al. 2009). This type of biodiverse agroecosystems also functions as a refuge for many species of plants and animals, in addition to contributing to the recharge of groundwater tables and carbon sequestration (Ruelas-Monjardín et al. 2014). Among the main plant species associated with shade coffee plantations are *Persea americana*, *Persea schiedeana*, *Inga vera*, *Inga jinicuil*, *Quercus* spp. and some introduced species such as *Musa* spp. *Macadamia* spp., and *Citrus* spp. (Moguel and Toledo 1999; López-Gómez et al. 2008; Castro-Luna and Galindo-González 2012; Ramos-Reyes et al., 2019). Recently, *M. paranaensis* was found parasitizing roots of *Musa* spp. in infested coffee plantations, this is important for the nematode management, since the plants associated with shaded-coffee agroecosystems could contribute to the dispersion and maintenance of *M. paranaensis* populations and to the infection of replanted coffee trees, even when prophylactic measures are taken. In addition to *C. arabica*, *C. canephora* and *Musa* spp., the nematode *M. paranaensis* has been recorded parasitizing 36 plants, mainly herbaceous such as *Brachiaria plantaginea, Galinsoga cilliata, Impatiens balsamina, Ilex paraguariensis* and *Raphanus raphanistrum* (Carneiro et al. 1996; Santiago et al. 2000; Roese and Oliviera 2004; Campos and Villain 2005; Machado et al. 2014; Da Silva et al. 2015; Mendoca et al. 2017). However, the hosts of *M. paranaensis* in plants associated with coffee agroecosystems, which is where this nematode is currently most widely distributed, are not known. Knowing the status of *M. paranaensis* hosts in coffee agroecosystems will provide important information for decision-making when implementing coffee corky-root disease management strategies. Therefore, the objective of this work is to determine the host status of *M. paranaensis* in 13 plant species commonly associated with shaded-coffee agroecosystems.

Materials and Methods

Meloidogyne paranaensis population

The eggs and J2 used for the plants inoculation were obtained from infested coffee roots collected in the La Lagunilla town, municipality of Cosautlán de Carvajal, Veracruz, Mexico. Population was previously identified with specific SCAR markers (Randig et al. 2002; López-Lima et al. 2015) and reproduced in tomato plants (*Solanum lycopersicum* cv Rio Grande).

Plant species

Thirteen plant species commonly associated with coffee plantations were selected, either as shade or for use: *Citrus aurantium, Citrus reticulata, Enterolobium cyclocarpum, Eriobotrya japonica, Inga jinicuil*, *Inga vera, Macadamia integrifolia, Musa* AA, *Persea americana, Persea schiedeana, Psidium guajaba, Quercus xalapensis* and *Syzygium jambos*. The plants were obtained at the Tierra Vital nursery (Coatepec, Veracruz). Likewise, *C. arabica* CV Costa Rica 95 and common *C. canephora* plants were obtained from Paso Grande nursery (La Estanzuela, Veracruz) and were included as susceptible controls, the age of the plants was 12 months on average. All the plants were taken out from their original pots to remove the remains substrate with running water and verify the health of the roots. Subsequently, they were

transplanted into 25x40 cm polypropylene bags with a sterile substrate composed by soil, peat moss and perlite in 1.5 1 and 0.5 proportions. The plants were distributed in a completely random design in a shade house in the experimental area of the Faculty of Agricultural Sciences of the Universidad Veracruzana, *campus* Xalapa and were allowed to acclimatize for 45 days. To stimulate the roots and foliage development, 100 mL of rooting fertilizer were applied to the base of the stem at a concentration of 2 gr L^{-1} (Rotex®, Cosmocell) and 20 mL of foliar fertilizer (Bayfolan® Bayer) at a concentration of 4 mL L^{-1} to each plant three times with 15-day intervals. Likewise, to control the insects and phytopathogenic fungi populations, the insecticide imidacloprid (Dinastia® Agroquimica Tridente 0.5 mL L^{-1}), the fungicides methyl thiophanate (Cerfutrin 70® Agroquimica Tridente 3 g L^{-1}) and cyproconazole (Alto100® Syngenta 0.5 mL L^{-1}) were applied once. The plants were watered every 72 hours.

Meloidogyne paranaensis inoculation

Nematodes were extracted from the tomato roots by crushing in sodium hypochlorite, sieving, and centrifuging to obtain eggs and second stage juveniles (J2) (Carneiro et al. 2004). Subsequently, the number of viable eggs was quantified according to their morphology (Calderón-Urrea et al. 2016) and live J2 juveniles according to their mobility in a Sedgwick-Rafter counting chamber (Wildlife Supply Company, Model 1801-A10) under a light microscope (Nikon, Alphaphot YS2), at 100X. Once the number of viable specimens in the sample was determined, the suspension was adjusted with sterile water to 200 eggs and J2 mL^{-1}. Prior to inoculation, the plants were watered, and two holes were made in the substrate near to the stem base to expose the roots. Subsequently, 50 mL of the *M. paranaensis* eggs and J2 suspension were added to each plant divided into the two holes with the help of a 25 mL syringe (10,000 eggs and J2 per plant). After inoculation, the plants remained in the shade house for 10 months, with irrigation every 72 hours and foliar fertilization every 30 days (20 mL of Bayfolan® at 4 mL L^{-1}).

Evaluation of galling and reproduction of M. paranaensis

After the experimental period, the plants were removed from the pots, the roots were washed with running water and dried with absorbent paper to obtain the fresh weight and observe the galls formation or corky symptoms and assign a number on the galling scale (Coyne and Rose 2014). Afterwards, the roots were cut into fragments of 1–2 cm and 50 gr were taken to extract eggs and J2 by the crushing, sieving and centrifuging technique (Carneiro et al. 2004). The roots that weighed less than 50 gr were processed whole. A 10 mL suspension of eggs and J2 was obtained from which three one mL aliquots were taken and counted in a Sedgwick-Rafter chamber under a 100X light microscope. The average of the nematode count and the total root weight was used to calculate the population density, the final population, and the reproduction rate in each plant species.

Host susceptibility index and resistance level

The host susceptibility index (HSI) expressed as a percentage was calculated taking as reference the population density in the roots of the main hosts *C. arabica* and *C. canephora* and comparing it with the nematode population density in the other evaluated plant species. From this parameter, the plants were

classified by their resistance level (RL) according to the criteria used by Shigueoka et al. (2016), where: 0 to 1% = highly resistant (HR); 1.01 to 10% = resistant (R); 10.01 to 25% = moderately resistant (MR); 25.01 to 50% = moderately susceptible (MS); 50.01 to 75% = susceptible (S); 75.01 to 100% = highly susceptible (HS). Resistance indicates the ability of the plant to suppress the nematode reproduction and susceptibility is determined by the normal nematode reproduction in the main host.

Data analysis

Data on population density and final population were analyzed for normality by the Shapiro-Wilk test and homogeneity of variances by Levene's test at 5%. The data were subjected to a Kruskal-Wallis analysis of variance and the test of multiple comparisons of means at 1% probability in the Statistica 12 program for Windows.

Results

The plants presented different symptomatology on the roots (Table 1). Numerous light and elongated thickenings were observed in the roots of *C. aurantium* and *C. reticulata*, but without evidence of necrosis. *Coffea arabica*, *C. canephora, I. jinicuil* (Fig. 1a), *I. vera* (Fig. 1b) and *M. integrifolia* (Fig. 1c) showed light to medium galling and developed characteristic symptoms of corky-root disease as hyperplasia in the cortical tissue with cracks. *Musa* AA showed a medium to high galling level, with elongated galls and beginnings of necrosis (Fig. 1d). When making cuts in the galls, numerous females and egg masses embedded in the tissue were observed (Fig. 1e-h). The highest population density of *M. paranaensis* was recorded in *I. vera* plants, followed by *Musa* AA, *C. aurantium* and the main host *C. arabica*. The highest final population (Table 1) and reproduction rate (Table 2) was found in *Musa* AA followed by *I. vera*, *C. aurantium*, *C. reticulata* and *C. arabica*. Taking as reference the population density values of *C. arabica* to calculate the HSI and RL, *C. aurantium, C. reticulata, I. jinicuil, I. vera* and *Musa* AA were highly susceptible, since allowed the nematode reproduction above 80%. On the other hand, *C. canephora* and *E. cyclocarpum* were moderately resistant, while *E. japonica*, *M. integrifolia*, *P. americana*, *P. guajaba* and *Q. xalapensis* were classified as resistant (Table 2). Likewise, when the values of the population density in *C. canephora* were taken to calculate the HSI and the RL, *C. arabica*, *C. aurantium*, *C. reticulata*, *E. cyclocarpum*, *I. jinucuil*, *I. vera* and *Musa* AA resulted highly susceptible, while *M. integrofolia* and *P. guajaba* were classified as moderately resistant. *Meloidogyne paranaensis* could not reproduce in *P. schiedeana* and *S. jambos*, so were classified as highly resistant (Table 2).

Table 1
Galling index, population density and final population of Meloidogyne paranaensis in the roots of the evaluated plants.

Specie	Corky-root symptoms	Galling index	Population density (eggs and J2 gr root^{-1})	Final population
Citrus aurantium	No	2	656.5 def	60631 de
Citrus reticulata	No	2	315.7 def	41660 cde
Enterolobium cyclocarpum	No	1.1	76.7 cdef	5553 bcde
Eriobotrya japonica	No	1.1	14.4 abcd	1096 abc
Inga jinicuil	Yes	2	334.8 abcde	18655 abcd
Inga vera	Yes	2.5	3804.3 f	570548 e
Macadamia integrifolia	Yes	2.1	12.6 abc	1652 ab
Musa AA	No	3.5	1923.4 f	1521423 e
Persea americana	No	1	21.2 abcd	2756 abcd
Persea schiedeana	No	1	0 a	0 a
Psidium guajaba	No	1	9.1 ab	1525 abc
Quercus xalapensis	No	1	20.1 abcde	955 abc
Syzygium jambos	No	1	0 a	0 a
Coffea canephora	Yes	2	55.156 bcde	2514 bcd
Coffea arabica	Yes	2.5	390.586 ef	31413 de

Different letters in the columns indicate significant differences in the population density and final population of *M. paranaensis* between the plant species after the Kruskal-Wallis test and multiple comparisons of means at 0.01%.

Table 2 Reproduction rate, host susceptibility index and resistance/susceptibility level against *Meloidogyne paranaensis* in the evaluated plants.

Specie	Reproduction rate	*Coffea arabica*		*Coffea canephora*	
		HSI %	RL	HSI %	RL
Citrus aurantium	6.0	100	HS	100	HS
Citrus reticulata	4.1	80.5	HS	100	HS
Enterolobium cyclocarpum	0.5	19.6	MR	86.0	HS
Eriobotrya japonica	0.1	3.7	R	26.2	MS
Inga jinicuil	1.8	85.7	HS	100	HS
Inga vera	57.0	100	HS	100	HS
Macadamia integrifolia	0.2	3.2	R	22.9	MR
Musa AA	152.1	100	HS	100	HS
Persea americana	0.2	5.4	R	38.6	MS
Persea schiedeana	0	0	HR	0	HR
Psidium guajaba	0.15	2.3	R	16.5	MR
Quercus xalapensis	0.09	5.1	R	36.4	MS
Syzygium jambos	0	0	HR	0	HR
Coffea canephora	0.3	14.1	MR	100	HS
Coffea arabica	3.1	100	HS	100	HS

HSI= Host susceptibility index; RL= Resistant level; HR= highly resistant; R= resistant; MR = moderately
resistant; MS= moderately susceptible; S= susceptible; HS= highly susceptible.

Discussion

Coffee agroecosystems in Mexico are main socioeconomic and environmental importance, conserving them is a priority for many people and private and public organizations. However, coffee corky-root disease represents a threat to the continuity of these plantations. The focus of this study was to determine the host status of some plant species commonly associated with shade coffee plantations in Veracruz, the observations indicate the potential of *M. paranaensis* to reproduce adequately in at least 5 species evaluated with different degrees of susceptibility. In general, *Meloidogyne* species are polyphagous, which makes their management difficult because they can remain in other plants in the absence of the main host; however, in the case of *M. paranaensis*, knowledge about the host range is still limited.

Meloidogyne paranaensis was able to reproduce in the two evaluated citrus species. Another species, such as *Meloidogyne indica*, causes significant damage to citrus in India, causing the dieback of the plants in one year (Kumar and Arthurs 2021). Likewise, *Meloidogyne javanica, Meloidogyne incognita* and *Meloidogyne arenaria* affect citrus species in Asian and African countries (Bakr et al. 2011; Onkendi et al. 2014). In this work, despite the high reproduction rate in *Citrus* spp., no major damage to the roots or aerial symptoms were observed; however, we do not recommend the use of this plants in *M. paranaensis* infested fields since they allow reproduction even more than the main host.

Our results indicate that *M. paranaensis* was able to reproduce in *E. cyclocarpon*. This plant is very common in coffee plantations due to its wide crown, which leaves extensive shaded areas. Although other species of this genus are highly susceptible to *Meloidogyne* spp., *E. cyclocarpon* has no recorded significant problems with nematodes (Falkowski et al. 2019).

Erythrobotria japonica limited the reproduction of *M. paranaensis*, according to our bibliographical review, there are no records of significant affectations by *Meloidogyne* or other nematode genera on this plant. In contrast, whole plant ethanolic extracts have been reported to cause *Meloidogyne* J2 mortality *in vitro* (Sultana et al. 2014). It is possible that the plant has defense mechanisms against nematodes, so it is necessary to investigate its possible nematicidal effect in the field.

Inga species are the shade trees most used by coffee growers in Veracruz, due to their rapid growth, shade level, and nitrogen fixation in the soil (Ávila-Bello et al. 2023). According to our bibliographic review, there are no records of nematodes associated with *Inga* species; however, our results indicate that the two species evaluated are very susceptible to *M. paranaensis*, so we do not recommend their use in infested sites.

Macadamia integrifolia limited the reproduction of *M. paranaensis* compared to the main hosts, however, some root areas development galling and initial symptoms similar to those of corky-root disease. In another study, seven macadamia varieties were evaluated against *M. paranaensis* and none of them reproduced adequately or developed galls (Costa et al. 2020). However, these authors used a lower population density and shorter interaction period (120 days) than in this work. It is possible that the nematode requires more time for the development of galling symptoms as occurs in common *C. canephora* (Sera et al. 2006), but it can cause damage to macadamia plants in the medium or long term. Pathogenicity studies should be carried out with different infestation levels and interaction periods to determine the macadamia susceptibility, since this is one of the most widespread crops associated with coffee plantations and could be at risk from this nematode.

The susceptibility of banana plants was confirmed. This crop was highly susceptible due to its non-woody root and rapid development. It is known that *M. paranaensis* affects banana plants in coffee plantations (Villain et al. 2013; Lopez-Lima et al. 2015). *Musa* spp. are very susceptible to nematodes, mainly of the genera *Radopholus*, *Pratylenchus* and *Meloidogyne* (Seenivasan and Senthilnathan 2018; Peraza-Padilla et al. 2020). However, it is necessary to carry out pathogenicity tests with *M. paranaensis* to know if it can represent a risk for banana plantations.

The *Persea* spp. plants did not show damage symptoms, although *P. americana* allowed reproduction at low levels. Low populations of *Meloidogyne hapla* and *Meloidogyne trifoliophila* are known to be associated with *Persea* sp. in New Zealand orchards, but no major damage reported (Knight 2001). Likewise, another study indicates that *Meloidogyne incognita* race 2 cannot reproduce or cause galling in potted avocado seedlings. *Persea* spp. seems to be more susceptible to genera such as *Pratylenchus*, *Radopholus* and *Helicotylenchus* (El-Borai and Duncan 2005). According to these results, we think that *P. ameriacana and P. schiedeana* are an option for planting in *M. paranaensis* infested sites.

Psidium guajaba, is severely affected by *M. enterolobii*, which together with *Fusarium* spp. cause the guava decline and similar symptoms to those of coffee corky-root disease (Khan et al. 2022). However, our results indicate that it is not susceptible to *M. paranaensis*. This plant is commonly associated with coffee plantations in Mexico, as part of the shade vegetation. This work indicates that its use is viable in infested coffee plantations.

There are records of *Meloidogyne* species parasitizing *Quercus* spp. (Brito et al. 2015; Sohrabi et al. 2015; Brito et al. 2016). However, under the conditions of this work it seems that *Q. xalapensis* is not very susceptible to *M. paranaensis*. As a native species, its maintenance in coffee agroecosystems is environmentally important.

Syzygium jambos is suitable for use in infested coffee plantations since not allow the reproduction of *M. paranaensis*, also there are no records of nematode involvement in this plant. In addition, the *S. jambos* extracts of leaves and bark have antimicrobial activity, however it is necessary to investigate its nematicidal activity (Djipa et al. 2000; Mohanty and Cock 2010).

Knowing the *M. paranaensis* hosts within the plants present in the shade-grown coffee agroecosystems is important since it influences the permanence and increase of nematode populations. Management strategies should consider possible alternative hosts for *M. paranaensis*, even when using tolerant rootstocks, since avoiding exposure to high population densities is crucial to maintaining the productivity and longevity of new plantings. The results of this study provide important information for the management of *M. paranesnsis* in infested shade-grown coffee plantations. It is necessary to evaluate the pathogenicity of *M. paranaensis* in plants that were susceptible, as well as in other woody and herbaceous plant species present in coffee agroecosystems.

Declarations

Competing Interests: The authors declare that they have no conflict of interest.

Acknowledgments

The first author thanks the National Council of Humanities, Sciences and Technologies (CONAHCYT) for the scholarship granted to carry out her postgraduate studies (PhD in Agricultural Sciences) at the Universidad Veracruzana (UV).

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Figures

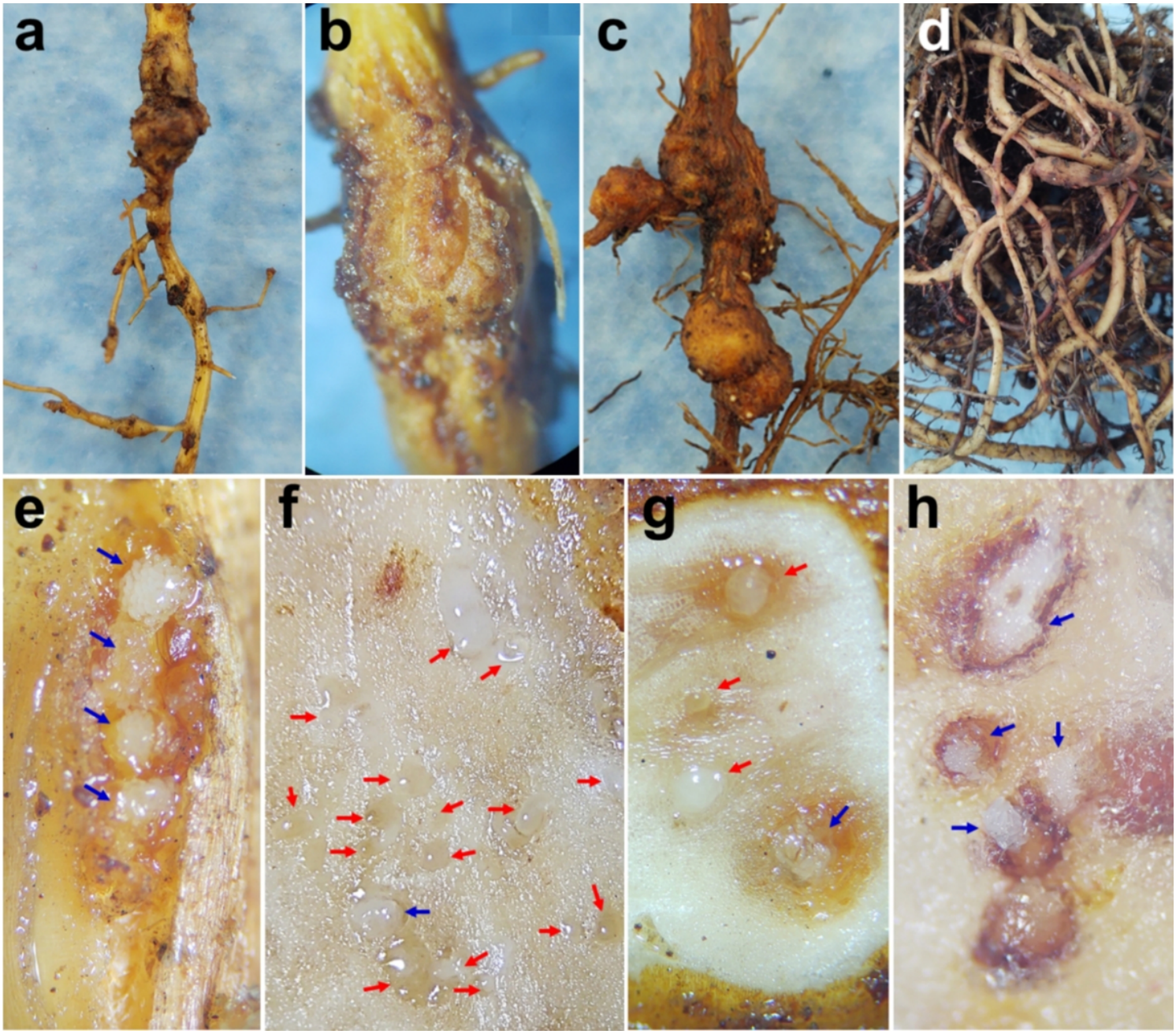

Figure 1

Galling roots of *Inga jinicuil* (a), *Inga vera* (b), *Macadamia integrifolia* (c) and *Musa* AA (d). *Meloidogyne paranaensis* egg masses and females embedded in the root tissue of *Inga jinicuil* (e), *Inga vera* (f), *Macadamia integrifolia* (g) and *Musa*AA (h). Red arrows indicate the presence of females and blue arrows indicate the presence of egg masses