Nematicidal plants for root-knot nematode (Meloidogyne spp.) management in vegetable cropping systems

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Abstract

Root-knot nematodes (RKN), *Meloidogyne* species, are a top global threat associated with economic crop yield losses. They are difficult to detect and control, especially given the recent restrictions on environmentally harmful chemicals. Thus, there is a need for alternative solutions for sustainable RKN management, such as nematicidal plants (non-hosts or poor hosts). Despite the advanced literature, the information for nematicidal plant species, cultivars, and specific RKN species is incomplete or inconsistent. We evaluated the host suitability of 28 nematicidal plant candidates in controlled climate chambers using a susceptible tomato and pepper as controls. The assessment was based on gall and egg mass counts after one RKN cycle. All screened candidates were less infected with *M. incognita*, *M. arenaria*, and *M. enterolobii* than tomatoes, suggesting all the candidates are either non/ poor hosts, except *Allium fistulosum*. Only *Tagetes patula* and *T. erecta* were consistently non-hosts to the three RKN species. Other candidates exhibited RKN species-specificity and varied in their poor host or non-host status depending on the variety. Selected nematicidal plants were further assessed for RKN juvenile penetration and had significantly lower *M. incognita* penetration than tomato. However, *Crotalaria juncea* had significantly higher *M. incognita* penetration than tomato. This suggests that the tested plants inhibit root penetration of most *M. incognita* juveniles at the rhizosphere level while *C. juncea* attracts the nematodes and restricts reproduction. There is potential for most of the nematicidal plants to be used in cropping systems for sustainable integrated RKN management.

Introduction

Root-knot nematodes (RKN), *Meloidogyne* species, are the globally top-ranking plant parasitic nematodes (Jones et al. 2013). Several control techniques have been deployed to regulate RKN including the now-restricted chemical nematicides, soil solarisation, resistant crop cultivars, and biological control (Wang 2007; Djian-Caporalino et al. 2005; Moens et al. 2009; Smith 2015; Sorribas et al. 2020). For instance, a few RKN-resistant genes have been identified, for example, the *Mi-1* and *Me(s)* genes in tomato and pepper (Djian-Caporalino et al. 2011; Regmi and Desaeger 2020) but they have limited durability, starting to be overcome, and ineffective when temperatures are over 30°C for *Mi-1*. Moreover, these genes do not provide resistance against *M. enterolobii* (Castagnone-Sereno 2012; Brito et al. 2007). RKN are also exceedingly difficult to control because they are highly polyphagous and can remain hidden in the soil or plants. There is a need for agroecological alternatives to chemical nematicides as part of a combined package of RKN multi-control strategies.

Particular attention could be paid to nematicidal plants for their traits related to RKN control. They have sometimes been effective in crop rotation, intercropping, and cover cropping agronomic practices to reduce plant parasitic nematodes in vegetable production systems (Djian-Caporalino et al. 2005; Ploeg 2002). However, it is crucial to study their host suitability to the RKN and mechanisms of action, which are still often misunderstood. Plants can be categorised as good, poor, or non-hosts (Djian-Caporalino et al. 2005). Susceptible hosts (good hosts) are attacked, permitting elevated accumulation and normal development of RKN. Poor host plants disrupt the prolific development and multiplication of RKN. Non-

host plants are not attacked by RKN as they can inhibit egg hatching, root penetration, or subsequent RKN development and multiplication. Nematicidal compounds can be phytoanticipins (constitutive) implying they exist regardless of whether pests or diseases are present or phytoalexins (induced)- only accumulate upon detection of pests or diseases. They could also be both phytoanticipins and phytoalexins as elucidated in the review on defence metabolites involved in plant parasitic nematode control (Desmedt et al. 2020). The review further explains the importance of penetration tests which can reveal key information about the stage when plant resistance to nematodes occurs ultimately influencing the choice and how to use a nematicidal plant.

Several plants have been reported for their host suitability and potential use in nematode management. Despite the advances in the availability of this information, it is still incomplete (studies do not cater for all the nematicidal plant cultivars and nematode species), inconsistent, and mired with contrasting performance among nematicidal plant species/ cultivars. An example is a study on 18 plant species and cultivars where three cultivars of *C. juncea*, three cultivars of sorghum sudangrass, and *Avena strigosa* all interchangeably exhibited varied suitability for RKN, from non-host to poor host and susceptible to *M. incognita*, *M. arenaria*, and *M. javanica* except for *T. erecta* which was consistently non-host to three *Meloidogyne* species (Marquez et al. 2022).

Marigolds, particularly *T. patula*, *T. erecta*, and *T. minuta*, are largely renowned for efficiently controlling a broad range of RKN species through mechanisms such as non-host or poor host, presence of bioactive compounds like thiophenes and flavonoids, toxic compounds released from soil incorporated biomass, trapping, and fostering nematode antagonistic flora/ fauna (Wang et al. 2001; Wang et al. 2018; Djian-Caporalino et al. 2005; Krueger et al. 2007; Wang 2007; Bhattacharyya 2017; Karakas and Bolukbasi 2019). However, the response of *Tagetes* to RKN varies depending on plant species or variety and nematode species (Krueger et al. 2007).

Sunn hemp, especially *Crotalaria juncea*, has also been studied for nematode management. It employs modes of action such as being a non/ poor host, production allelochemicals like monocrotaline a pyrrolizidine alkaloid, trapping, and promoting antagonistic flora and fauna (Wang et al. 2002; Colegate et al. 2012; Rech et al. 2022). However, responses of *C. juncea* to *Meloidogyne* spp. have shown inconsistencies (McSorley 1999; Bui and Desaeger 2021; Khanal and Harshman 2022).

Sorghum's response to *Meloidogyne* spp. have also been shown to vary from non-hosts, poor hosts to even good hosts (Bui and Desaeger 2021; Khanal and Harshman 2022; Djian-Caporalino et al. 2019). The differential resistance between sorghum genotypes is not due to dhurrin content as often thought, given both low and high dhurrin cultivars showed similar poor host statuses (Djian-Caporalino et al. 2019). Instead, inherited resistance factors in the sudangrass genome (Harris-Shultz et al. 2015) and hypersensitive-like reactions, similar to those in Mi-1.2 resistant tomatoes (Paulson and Webster 1972), likely contribute to the lack of nematode reproduction. Additionally, toxic root exudates, including sorgoleone, may repel nematode juveniles (Czarnota et al. 2003).

Brassicas like *Raphanus sativus* (radish) and *Diplotaxis tenuifolia* (perennial wall rocket) contain bioactive compounds such as glucosinolates (Bell and Wagstaff 2014; Teklu et al. 2014; Ngala et al. 2015), but significant variability was observed among cultivars in their poor host status to different *Meloidogyne* spp. (Edwards and Ploeg 2014; Daneel et al. 2018; Waisen et al. 2019).

The host suitability of several other plants to *Meloidogyne* species still needs to be explored. Millets (*Pennisetum glaucum*) are known to be susceptible to *M. ethiopica*e (Lima et al. 2009) yet non-hosts for *M. enterolobii* (Khanal and Harshman 2022). *Fagopyrum esculentum* (buckwheat) is known as a poor host to *M. javanica* (Melo et al. 2023; Sipes and Arakaki 1997) and *M. enterolobii* (Khanal and Harshman 2022). *Avena strigosa* is a poor host for *M. ethiopica*e (Lima et al. 2009). *Allium fistulosum* (Welsh onion) reduced the incidence of *M. incognita* in cucumber (Li et al. 2018). *A. cepa* is renowned for its sulfur amino-acid precursors, which, upon cellular degradation, break down into dimethyl disulfide and dipropyl disulfide which may affect nematodes. According to a study (Carneiro and Carneiro 1982), *Phacelia tanacetifolia* (phacelia) which is well-known in insect pest management, suppresses the development of *M. incognita*. It is also difficult to find information on the nematode host status of *Valeriana locusta*, commonly known as corn salad, which is grown as a crop during winter in France and used by farmers as a poor host crop to manage RKN.

This study aims to provide a framework for testing plants for RKN host suitability and to determine their potential as nematicidal plants. Because a large pool of nematicidal plants is useful to breeders as suitable and confirmed sources of resistance, and to farmers as a rich portfolio to help with their preferences when using these plants based on their agronomic easiness, resources, labour intensity, production season, and local climate, we screened 27 nematicidal plant candidates to determine their host suitability to the RKN: *M. incognita*, *M. arenaria*, *and M. enterolobii*. *M. incognita* is regarded as the most damaging crop pathogen worldwide (Sasser 1977; Trudgill and Blok 2001). A previous survey (Djian-Caporalino 2012) reported that RKN had already been posing a growing problem to French vegetable production by then, with *M. incognita* often appearing in a mix with *M. arenaria* in the farmers' fields in France. On the other hand, *M. enterolobii* is a recently emerging pathogen in worldwide vegetable systems (Philbrick et al. 2020).

RKNs induce infection and ravage their host plant by feeding on the roots causing root galls, stunting, wilting, or chlorosis through limiting plant nutrient and water uptake resulting in yield and quality loss (Djian-Caporalino 2012; Wang 2007). The RKN life cycle consists of the exophyte and endophyte phases (Ibrahim et al. 2019), usually lasting six weeks depending on the host, temperature, moisture, and soil type. The exophyte begins from the egg which hatches into second-stage juveniles (J2), and the endophytic stage where the development of the feeding site at the central cylinder of the root occurs allowing the establishment, development, and reproduction of the nematode (Abad et al. 2009). Plant-nematode interactions start in the soil, then the root interface and inside the roots. Plant response could lead to non-hosts (no EM and often no gall), poor hosts (galls, few EMs), or susceptible (several galls and egg masses). Our set-up consists of counting galls and egg masses to prove the ability of nematodes to penetrate and reproduce inside the plant roots. In addition, we set a bioassay to evaluate *M. incognita's*

root penetration on selected nematicidal plant species to determine the role that root penetration plays in the mechanism and host suitability of these plants. Our study also sheds more light on some of the inconsistencies of the reports on the host suitability of nematicidal plants and their varietal effects on RKN exposure. This will aid in identifying nematicidal plants that pose a low risk of exacerbating nematode populations when grown.

Finally, among the 27 screened plant species, we selected both summer and winter plants. Having a range of summer and winter nematicidal plants is crucial for RKN management, as the winter nematicidal plants or crops can be grown in rotation with summer crops. Potential summer nematicidal plants include marigolds, sunn hemp, buckwheat, millet, and forage sorghums. Winter nematicidal plants, grown mostly as crops, include radish, fennel, rocket, onions, oats, and phacelia. Some are already being used by the farmers in the South of France.

Materials and Methods

Plant species

We evaluated 15 summer and 12 winter nematicidal plant cultivars or accessions belonging to 16 species and nine plant families (Table 1.) These nematicidal plant candidates were chosen from the bibliography and in consultation with different stakeholders in France. Tomato cv. Saint Pierre and pepper cvs. Calibello and Lipari usually susceptible to RKN were used as controls.

Table 1

List of the 15 summer and 12 winter nematicidal plant cultivars or accessions screened against three root-knot nematodes (*M. incognita, M. arenaria*, and *M. enterolobii*) with susceptible tomato and pepper as controls. Names of cultivars are provided when known. S: Summer Plants; W: Winter Plants.

Botanical name	S/W	English / French common names	Cultivars	Family	Providers
Solanum lycopersicum L.	S	Tomato / Tomate	St. Pierre	Solanaceae	Syngenta
Capsicum annuum L.	S	Pepper / Poivron	Calibello, Lipari	Solanaceae	Sakata/ Clause
Allium cepa L.	W	Onion / Oignon	Rebouillon	Amaryllidaceae	Gautier
Allium fistulosum L.	W	Welsh onion / Ciboule	Totem	Amaryllidaceae	Baumaux
Avena strigosa Schreb.	W	Oats / Avoine rude	Max, Otex	Poaceae	Agrosemens
Crotalaria juncea L.	S	Sunn hemp / Crotalaire	Crescent Sunn, unknown	Fabaceae	unknown/ LIDEA
Diplotaxis tenuifolia (L.) de Candolle	W	Perennial wall rocket / Roquette	Tiara, Soria	Brassicaceae	Semences de France /Gautier
Fagopyrum esculentum Moench.	S	Buckwheat / Sarrasin	unknown	Polygonaceae	GRAB
Foeniculum vulgare Mill.	W	Fennel / Fenouil	Rondo, Solaris	Apiaceae	Bejo
Pennisetum glaucum (L.) Morrone	S	Millet / Millet perlé	ADR 300, Nutrient C	Poaceae	LIDEA/ GRAB
Phacelia tanacetifolia Benth.	W	Lacy Phacelia / Phacelie	unknown	Boraginaceae	GRAB
Raphanus sativus L. var. *oleiformis* Pers.	W	Radish / Radis	Doublet	Brassicaceae	Semences de France
Sorghum bicolor (L.) Moench	S	Sorghum / Sorgho	Jumbo	Poaceae	Alta Seeds by Advanta
Sorghum bicolor (L.) Moench x	S	Sorghum / Sorgho	Jumbo Star, Piper, Sudal	Poaceae	LIDEA

Botanical name	S/W	English / French common names	Cultivars	Family	Providers
S. sudanense					
Tagetes erecta L.	S	African marigold / Rose d'Inde	CrackerJack	Asteraceae	LIDEA
Tagetes patula L.	S	French marigold / Œillet d'Inde	Nana, Princess of Orange, Proud Mary, unknown	Asteraceae	Girerd et fils/ Lammers Seed Options B.V/ LIDEA
Tagetes minuta L.	S	Mexican marigold / *Tagetes* des decombres	unknown	Asteraceae	Germinance
Valerianella locusta (L.) Betcke	W	Corn salad / Mâche	Gala, Trophy	Caprifoliaceae	Clause

Root-knot nematode species

M. incognita Morelos, *M. arenaria* Marmande, and *M. enterolobii* Jan Mayric populations were reared and multiplied on susceptible tomato cultivar St. Pierre at the INRAE Sophia Antipolis' experimental unit in France. As mentioned before, *M. incognita* and *M. arenaria* are found alone or in a mix on vegetable farms in southern France. *M. enterolobii* is a recently emerging pest in vegetable systems, being detected in Europe. After two propagation cycles (four months after inoculating two-month-old plants), cleaned roots with galls and egg masses (EMs) were blended in chlorine solution at 0.5% to release the eggs from the surrounding mucilaginous mass and let them pass through a series of sieves to remove root debris and the chlorine solution. Lastly, the eggs were suspended in fresh water and counted under a microscope.

Host suitability experiments

Four independent trials were designed in 2020–2023 and randomly arranged in a complete block design, with each treatment replicated six or twelve times (n = 6 or 12). All the host suitability assays on *M. incognita* and *M. arenaria* were conducted in climate-controlled rooms. *M. enterolobii* was tested in a special greenhouse compartment because of its quarantine status. All plant species were grown in 7x7x7cm small pots filled with sandy-loam autoclaved soil.

In the first trial, summer plant species were grown at 24°C, 12h light and relative humidity (RH) of 60–70%. Winter plant species were grown in a different climate chamber at 16°C, 11h light and RH 60–70% throughout the trial. There was an exception for radishes and oats that were kept together with the summer plants because they germinated and grew well at 24°C. After a month, plants were inoculated with 2000 eggs of *M. incognita* or *M. arenaria*. The trial was terminated six weeks after inoculation for

plants at 24°C and 10 weeks for plants at 16°C as they needed more time to complete their nematode cycle. Plant roots were cleaned and stored in a freezer until counting for EMs and for the galls- a root gall index (GI) on a scale of 0 to 3 was used (0 = no gall, 1 = less than 20 galls, 2 = between 20 and 50 galls, and 3 = more than 50 galls).

An improvement to the previous protocol was implemented in the following trials. The winter plant species were only kept at 16°C until RKN inoculations, then transferred to the other plant species at 24°C to homogenise the environmental conditions for the RKN cycle. Plants for the *M. enterolobii* screening were maintained in a greenhouse. Five weeks post-inoculation allowing one nematode cycle, the number of EMs and galls were counted on the eosin-stained roots.

In all our experiments, non-host plants were categorised based solely on the total absence of EMs, regardless of the presence of galls. Plants with more than 10 EMs were considered susceptible. Galls indicate just the reaction of some plants to RKN penetration: some plants can show galls but cannot multiply RKN (no EM). Conversely, some plants may have EMs without visible galls.

Meloidogyne incognita penetration assays

A selected number of nematicidal plants: *C. juncea*, *F. vulgare*, *Sorghum bicolar* x *S. sudanense* cv. Jumbo Star, *T. erecta*, *T. patula* (cv. Princess of Orange, Proud Mary and an unknown variety) and *T. minuta* were further tested for the penetration of *M. incognita* second-stage juveniles (J2). The plants were grown in 7x7x7cm pots with autoclaved sand-loam soil in the climate chamber at 24°C, with each treatment replicated 10 times (n = 10). At one month, 600 *M. incognita* J2 were inoculated in the first assay, and 2000 J2 were inoculated in the second one. Eight days later, the plant roots were cleaned and stained with fuchsin acid (Byrd et al. 1983) to count the juveniles that penetrated the roots using a microscope and compare them with the controls.

Data analysis

The statistics normality distribution for model residuals was checked for with the Shapiro-Wilk test, including the residuals. Since egg mass counts and gall counts/ indices followed non-standard statistical distributions, with over 50% being zeros, we modelled these variables accordingly. To analyse the variables in the first experiment, a Zero-inflated Poisson (ZIP) was used for egg masses using the '**zeroinfl**' and logit link function from the '**pscl**' package in R. We used the Conjugate Gradient method for optimisation. Then to analyse the gall index (ordinal outcomes) an Ordered Logistic Regression was used using the '**polr**' function and '**MASS**' package. The choice of different models for the other experiments was necessary to provide reliable results. A Bias Reduction in Generalized Linear Models (BRGLM) Poisson family was then used for modelling the number of galls and egg masses for all the data from other experiments using the '**brglm2**' package in R. The '**brglmFit**' a fitting method for GLM using bias reduction methods to the maximum likelihood estimates was applied. A negative binomial generalised mixed model (NBGLM) was used to model the number of juveniles counted in the penetration test using the '**glm.nb**' function from the '**MASS**' package in R. We used 'emmeans' for multi-

means comparisons adjusted using Tukey's HSD (honestly significance difference test). The R software (version 4.3.2) was used for all the statistical analyses.

Results

1. Host response to M. incognita, M. arenaria, and *M. enterolobii*

First trial (Fig. 1) - tomato plants are confirmed as good hosts whilst all the nematicidal plant candidates were either non-hosts or poor hosts of *M. incognita* and *M. arenaria*, and nematode infection differed significantly among plant and nematode species. The following were non-hosts (no EMs and often no galls) of *M. incognita*: *D. tenuifolia* cv. Soria and *F. vulgare* (cv. Rondo and Solaris), whereas *A. strigosa* and *P. tanacetifolia* were non-hosts of *M. arenaria* (Fig. 1a and b). Also, *F. vulgare* cv. Rondo (*M. incognita*: gall index = 0), *A. strigosa* cv. Max and *P. tanacetifolia* (*M. arenaria*: gall index = 0) were statistically different from all other nematicidal plants for both the *Meloidogyne* species (gall index = 1–2) but all significantly lower than tomato (gall index = 3) ($p < 0.05$; Fig. 1c and d).

All the other tested plants were poor hosts (few EMs and galls) of *M. incognita* and *M. arenaria*. All the plant species identified as non-hosts for *M. arenaria* are poor hosts for *M. incognita* (the opposite is also true).

Second trial (Fig. 2) - all the nematicidal plants, except *A. fistulosum*, were significantly less infected with the RKN than tomato and pepper controls ($p < 0.05$).

All the *Tagetes* species, *C. juncea* cv. Crescent Sunn, and *F. esculentum* were non-hosts of *M. incognita*, based on the absence of EM and even so, had no visible galls (Fig. 2a). Despite a few galls, *P. glaucum* cv. Nutrient C, *Raphanus sativus* cv. Doublet, and *P. tanacetifolia* were also non-hosts for *M. incognita*, as they had no EMs. Similarly, all *Tagetes* species and *Sorghum* hybrid cv. Piper were non-hosts to *M. arenaria*, showing no EMs and no galls (Fig. 3).

A. cepa cv. Rebouillon, *A. strigosa* cv. Max, *C. juncea*, *D. tenuifolia* cv. Soria, *D. tenuifolia* cv. Tiara, *F. vulgare* cv. Rondo, *F. vulgare* cv. Solaris, *P. glaucum* cv. ADR300, *Sorghum* hybrid (cv. Piper and Jumbo Star), *V. locusta* cv. Gala, and *V. locusta* cv. Trophy were all poor hosts of *M. incognita*, as they allowed some reproduction. Considering the reaction of the plants (number of galls) *D. tenuifolia* cv. Tiara and *F. vulgare* cv. Solaris were significantly different from the rest but not, *C. juncea*, *Sorghum* hybrid cv. Sudal, and *P. tanacetifolia* ($p < 0.05$). However, all of them had significantly fewer galls than the tomato and the two peppers ($p < 0.05$). For *M. arenaria*, *F. esculentum*, *Sorghum* hybrid (cv. Jumbo Star and cv. Sudal), and *P. glaucum* (cv. ADR300 and cv. Nutrient C) were poor hosts with < 10 EMs and visible galls and significantly different from tomato ($p < 0.05$). The two pepper cultivars were also poor hosts to *M. arenaria* with very few EMs and galls and were statistically the same with all the nematicidal plants. Finally, only *A. fistulosum* was susceptible to *M. incognita* and *M. arenaria* (EMs greater than 10 and a similar number of galls). The number of egg masses for all the non-host and poor host nematicidal

plants was statistically similar and significantly different to *A. fistulosum*, which was susceptible, $p < 0.05$.

Third and fourth trials (Fig. 3 & 4)- tomato was significantly infested with *M. incognita*, *M. arenaria*, and *M. enterolobii* unlike all the other plant species evaluated, including pepper (Fig. 3 & 4, $p < 0.05$). All the *Tagetes* species except *T. minuta* were non-hosts with no EMs or galls for the three *Meloidogyne* species. *T. minuta* was a non-host (no EMs and no galls) for *M. incognita* and *M. enterolobii* whilst a poor host to *M. arenaria* with a few occasional EMs and galls. Sorghum cv. Jumbo was a non-host to *M. enterolobii* (Fig. 4). Regardless there was no statistical difference among all the non-host and poor host nematicidal plants ($p < 0.05$). Pepper cv. Calibello was a non-host of *M. arenaria* and showed varied susceptibility to *M. enterolobii* but was ultimately a poor host. We had no results for *M. incognita* as all the pepper plants died.

2. Penetration test of M. incognita juveniles into the roots

The number of *M. incognita* second-stage juveniles (J2) inside the roots significantly varied with plant genotype ($p < 0.05$). In our first experiment (Fig. 5a), *M. incognita* J2 were significantly less in *F. vulgare* and all the *Tagetes* species than in tomato and pepper ($p < 0.05$). *C. juncea* had the most J2 counts inside its roots compared to all the plant species, including the controls. However, *M. incognita* J2 counted inside pepper were significantly more than in tomato ($p < 0.05$). A similar trend is observed in our second experiment (Fig. 6b), where the tomato has significantly more J2 inside the roots than the rest of the nematicidal plants despite a thrice increase in inoculation density ($p < 0.05$). There were no statistical differences among *Sorghum* hybrid cv. Jumbo, *T. minuta*, and *T. patula cv*. Proud Mary at $p < 0.05$.

Discussion

Our study suggests that all the screened nematicidal plant candidates, except for *Allium fistulosum*, are either non or poor hosts to the RKN (*M. incognita*, *M. arenaria*, and *M. enterolobii*). Non-host status in this study was based on the absence of EMs, regardless of the galls, which are not reliable indicators of the plant's susceptibility. Egg masses are the most important indicator of the host status of plants because they reflect the quantity of nematodes that make it to reproductive maturity (Hajihassani et al. 2020; Marquez et al. 2022). Nematicidal plants were shown to be less infected with RKN than tomato and sometimes pepper, showing potential for RKN suppression. Only *Tagetes patula* and *T. erecta* were consistently non-hosts of *M. incognita*, *M. arenaria*, and *M. enterolobii*, indicating potential in control areas where these nematodes could exist in an association. Other nematicidal plants exhibited varietal- and RKN species-dependant effects in their poor-host or non-host plant status. Although we observed some inconsistencies, there were barely any significant differences between non-hosts and poor hosts, with a few exceptions. Some selected nematicidal plants were further assessed for the penetration of *M. incognita* juveniles. Root penetration tests provide knowledge of whether the plants impede nematode

penetration. *T. patula*, *T. erecta*, and *F. vulgare* had significantly lower *M. incognita* penetrations than tomato and pepper. However, *C. juncea* had significantly higher *M. incognita* penetration.

Generic non-host plants - *Tagetes erecta* and all the *T. patula* cultivars in our study were consistently non-hosts to *M. incognita*, *M. arenaria*, and *M. enterolobii* with no EMs or galls and substantially low penetration by nematodes. Similarly, previous works have reported *T. erecta* and *T. patula* as non-host to *M. incognita, M. arenaria*, and *M. javanica* (Buena et al. 2008; Marquez et al. 2022). Our study further fills gaps in the existing literature by demonstrating that both *T. erecta* and *T. patula* are non-hosts for the recently emerging vegetable pathogen, *M. enterolobii*. Furthermore, the non-host status appears to be consistent across different varieties: indeed, all four *T. patula* cultivars (Nana, Princess of Orange, Proud Mary, and the unknown) were non-hosts to the three *Meloidogyne* species.

Tagetes species are known to separately or simultaneously employ multi-modes of action against plant parasitic nematodes, including producing allelopathic compounds, trapping nematodes or creating a conducive environment for nematode antagonistic flora or fauna (Hooks et al. 2010; Wang et al. 2001). Their nematicidal effect has been mainly attributed to the thiophenes compounds, particularly *α*-terthienyl (Marotti et al. 2010; Szarka et al. 2006; Arroo et al. 1997; Tang et al. 1987) that can also be found in the rhizosphere and to a lesser extent other compounds like flavonoids. Alpha-terthienyl is an allelochemical that induces oxidative stress, infiltrating the nematode hypodermis and inducing its nematicidal effects (Hamaguchi et al. 2019). Hence, the non-host and low nematode penetration we observed in the *Tagetes* species could be due to defence by thiophenes as phytoanticipins (Kagan 1991; Hamaguchi et al. 2019) in the rhizosphere by inhibiting egg hatching and repelling or killing the juveniles in the soil before root penetration. Additionally, upon their attempt to penetrate roots, juveniles could be killed by both phytoanticipins and induced phytoalexins. Whether the roots of *Tagetes* have a physical barrier effect to inhibit nematode penetration remains unclear. Those nematodes that manage to penetrate could be killed inside the roots by toxic compounds or starved off, leading to a failure in the establishment of feeding sites, given that we observed no galls or any other morphological changes on the roots of our *Tagetes*. It is plausible that the nematicidal compounds in *Tagetes* could be both constitutive and induced but the degree to which certain assumed typical thiophene components of *Tagetes* might be outcomes of phytoalexins remains uncertain. However, a nematicidal compound can be both a phytoanticipin and phytoalexin (Desmedt et al. 2020). *Tagetes minuta* was also a non-host with no egg mass or gall for *M. incognita* and *M enterolobii* and could follow the same mode of action pathways of *T. erecta* and *T. patula*, as explained above. However, we observed occasional egg masses of *M. arenaria* on *T. minuta*, which were inconsistent with observations from the other *Meloidogyne* species.

Between non-host and poor host plants - Several nematicidal plant candidates had varied responses among the three trials- they acted as non-hosts in one trial and robust poor hosts in another, also depending on the nematode species. *A. strigosa*, *P. tanacetifolia*, and Sorghum cv. Piper were non-hosts to *M. arenaria* whilst *D. tenuifolia* cv. Soria, *F. esculentum*, *F. vulgare* cv. Rondo and Solaris, *P.* glaucum cv. Nutri C, *P. tanacetifolia*, and *R. sativus* cv. Doublet were non-hosts to *M. incognita.* Sorghum cv. Jumbo

was a non-host to *M. enterolobii*. In the rest of the cases, they were poor hosts. These differences that we observed for *A. strigosa*, three cultivars of sorghum sudangrass, and three *C. juncea* cultivars, according to the RKN species, are similar to what was obtained for other nematicidal plant species (Marquez et al. 2022). This observation points out the importance of knowing the RKN species in the field before advising the use of a given nematicidal plant cultivar.

For sorghums, we observed plants that were non-hosts or poor hosts depending on cultivars and nematode species. However, there were no statistical differences, indicating they could all be effective in the control of *M. incognita*, *M. arenaria*, and *M. enterolobii*. Our experiments confirm previous results that reported sorghums as either non-host or poor hosts to *M. ethiopicae*, *M. incognita*, *M. arenaria*, *M. javanica*, and *M. enterolobii* and that their mode of action to control RKN may depend on their genotypes (Lima et al. 2009; Bui and Desaeger 2021; Khanal and Harshman 2022; Djian-Caporalino et al. 2019; Curto et al. 2012). The epidermal cells of sorghum, sudangrass and their hybrids contain dhurrin, a cyanogenic glucoside (De Nicola et al. 2011). When root tissues are damaged, dhurrin is hydrolysed by dhurrinase in mesophyll cells, producing hydrogen cyanide (toxic to nematodes), p-hydroxybenzaldehyde, and glucose. Nevertheless, the differential level of resistance between genotypes could not be accounted for by dhurrin content, because cultivars with low levels of dhurrin and cultivars with high levels of dhurrin were both very poor hosts, with no significant difference in EM numbers on their roots (Djian-Caporalino et al. 2019). The authors hypothesised inherited resistance factors that may be present in the sudangrass genome as already mapped (Harris-Shultz et al. 2015) accounting for the lack of RKN reproduction on cv. Piper. Several hypersensitive-like reaction (HR) sites also indicate a response to infection similar to that in *Mi-1.2* resistant tomato plants (Paulson and Webster 1972). Their roots may also repel juveniles, due to the toxic root exudates, as reported for the hybrid *Sorghum bicolor x S. sudanense* 'SX-15' and 'SX-17' (Czarnota et al. 2003). Sorgoleone, the phenolic compound identified as a predominant constituent in exudates, could be potentially responsible for this suppressive effect.

Like previous studies, we report *Raphanus sativus* with inconsistencies as it was a poor host to *M. incognita* in the first experiment yet a non-host in the second one. Further, we observed both the presence and absence of reproduction for *M. arenaria*. Similarly, four cultivars of *R. sativus* had varied host statuses ranging from susceptible to poor host for *Pratylenchus penetrans*, a migratory semi-endoparasitic nematode (Neupane and Yan 2023) whilst *R. sativus* was a poor host to *M. incognita*, *M. ethiopicae*, and *M. javanica* (Lima et al. 2009; Daneel et al. 2018; Waisen et al. 2019). Also, although *R. sativus* was a poor host of *M. incognita*, *M. hapla*, and *M. javanica*, significant differences were still observed (Edwards and Ploeg 2014). It follows that the application of *R. sativus* to plant parasitic nematodes in the field is mired with mixed results, sometimes working, sometimes not (Ngala et al. 2015; Daneel et al. 2018; Waisen et al. 2019). However, there is a consensus that *R. sativus* is capable of highly producing glucosinolates, which are toxic to nematodes, and being used as a cover crop for nematode control (Ngala et al. 2015; Waisen et al. 2019). Perhaps the inconsistencies that we observed in our study could be due to other factors that influence the production of these bio-toxic compounds, which are known to be actively produced through the plant growth phase. Regarding *Diplotaxis tenuifolia*, there is a lack of information available on its host suitability which we have shown to be a poor host to

M. incognita and *M. arenaria*. However, we attribute our results, like most Brassicaceae, to glucosinolates, identified in *D. tenuifolia* (Bennett et al. 2007; Ntalli and Caboni 2012 ; Bell and Wagstaff 2014). Interestingly, we identified a varietal effect for *D. tenuifolia* (cv. Soria and Tiara), that might be linked to differences in the quality/quantity of glucosinolates, highlighting the importance of testing several varieties.

The host suitability results for *Foeniculum vulgare*, *Phacelia tanacetifolia*, and *Fagopyrum esculentum* were convincing despite the inconsistencies of being non-hosts and poor hosts. *F. esculentum*, whose parasitism by RKN has received little investigation, was a non-host to *M. incognita* whilst a poor host to *M. arenaria* in our assays. Similarly, *F. esculentum* is a known poor host of *M. javanica* (Melo et al. 2023; Sipes and Arakaki 1997). *P. tanacetifolia* did not support the reproduction of *M. incognita* in the second experiment, although it acted as a poor host in the first one and a non-host for *M. arenaria*. The mechanisms and compounds involved in *F. esculentum* and *P. tanacetifolia*'s nematicidal effects on RKN are less known if not documented and need to be studied. Both cultivars of *F. vulgare* were non-hosts to *M. incognita* in the first experiment but varied in the second one as both were poor hosts, though the majority of the replicates in our experiment were non-hosts. It is not clear what mechanisms could be responsible for this. However, our penetration test showed that *F. vulgare* significantly reduced *M. incognita* J2 penetration than tomato. Like *Tagetes*, perhaps *F. vulgare*'s mechanism of action is mainly at the rhizosphere level by being a hatching inhibitor, repellent/ toxic to the juveniles, or killing the J2 upon attempt to penetrate the root, with further activity to stifle the establishment of those that would have successfully penetrated inside. Five terpene compounds—D-limonene, estragole, anethole, gamma-terpenes, and beta-myrcene—were identified in fennel rhizosphere soil and root exudates (Yang et al. 2022). These compounds were found to inhibit *Phytophthora capsica* infection. D-limonene, in particular, attracted zoospores through positive chemotaxis. The combined effect of all five terpenes showed a strong synergistic action, significantly disrupting the infection process by causing zoospore rupture (Yang et al. 2022).

Both Alliums (*A. fistulosum* and *A. cepa*) host responses differed in our experiment despite both being known to produce nematicidal compounds. *A. cepa* was a poor host for *M. incognita* and we had no results for *M. arenaria* as the plants died from other causes during the experiment. *A. cepa* is renowned for its sulfur amino-acid precursors, which, upon cellular degradation, break down into dimethyl disulfide and dipropyl disulfide which could act on nematodes (Haroutunian 2015). On the opposite, *A. fistulosum* has to be considered carefully as it was a host for *M incognita* and *M. arenaria* though with significantly low *M. arenaria* infestation that tomato despite being shown to be an *M. incognita* egg hatching inhibitor mostly likely due to the root exudate compound 4-hydroxybenzeneethanol (Li et al. 2018).

Varietal effects were observed for *P. glaucum* (cv. ADR 300 and Nutrient C). Cv. Nutrient C was a non-host for *M. incognita* while cv. ADR 300 was a poor host. Both cultivars were poor hosts for *M. arenaria*. No varietal effect on *V. locusta* (cv. Gala and Trophy) was observed for *M. incognita* and *M. arenaria*.

The specific pattern of a trap plant - *Crotalaria juncea* cv. Crescent Sunn had mixed results in our experiments as it was a non-host or poor host for *M. incognita*. This cultivar also differed from the other *C. juncea*, cultivar unknown, which was consistently a poor host for *M. incognita* in both experiments. Further, the significantly higher *M. incognita* penetration for *C. juncea* cv. unknown than tomato in our work concurs with some previous works (Curto et al. 2015; Marla et al. 2008). Marla et al. (2008) observed all developmental stages of *M. incognita* within the roots of *C. juncea*. Additionally, a few mature females produced egg masses, but the eggs did not hatch after a week of incubation. However, these results on the number of hatched juveniles are considered insufficient, as egg hatching was only monitored for one week. However, our results contradict other previous reports, where *C. juncea* P1207657 and *C. juncea* cv. Tropic Sun were resistant to penetration by *M. javanica*, unlike a susceptible tomato (Araya and Caswell-Chen 1994) and when *C. juncea* root exudates had nematicidal effects on *M. incognita* (Danahap and Wonang 2016). This indicates varied modes of actions and varietal effects for the *Crotalaria* genus. *Crotalaria* species have pyrrolizidine alkaloids like monocrotaline that are antagonistic to the RKN (Moens et al. 2009; Colegate et al. 2012; Rech et al. 2022). However, some *Crotalaria* spp. have different responses to RKN with some non-hosts or poor hosts (trap plants) to certain nematode species. Moreover, most of the research has focused on the use of the *Crotalaria* genus as a biofumigant green manure to benefit the most from its aerial parts. Our study suggests that *C. juncea* cv. unknown could act as a good dead-end trap plant as it had significant penetrations yet low nematode reproduction.

Conclusion and perspectives

With this work, we have filled some gaps regarding the host suitability of some nematicidal plant candidates for certain nematode species and brought some insights into inconsistencies found in the literature. We show that several nematicidal plants also work against *M. enterolobii* which can infect crops resistant to the other *Meloidogyne* species. Information on the varietal effect is also crucial as we have shown that in some plant species, one variety could be more effective in the control of one nematode species than the other. Further research should be conducted to fill the remaining gaps on nematicidal plants and fully understand their mechanisms of action. Broad-spectrum host quality studies are useful to nematicidal plant growers and breeders seeking sources of nematode resistance to propose nematicidal service plants. The use of the nematicidal plants for RKN management would depend on their host suitability, their mode of action, agronomical considerations, the strategy to implement, and the crop to protect. Non-host plants like *Tagetes* can be used before the crop or in crop rotation to clean and kill the nematodes in the soil. They can also be used as companion plants if they are toxic to nematodes and don't have disservices on the growth of the crop; but not when the dominant mode of action is repulsive as nematodes can be repelled to the crop. Implementation strategy has been demonstrated to be key. Intercropping *Tagetes* species or growing them before the crop has been shown to suppress nematodes in tomato, cucumber, and pepper (Ploeg 1999, 2002; Taha 2020; Tesleem et al. 2014). Poor host plants can also be used the same way as non-host plants: sorghum, for instance, could be effectively used as a rotation crop or green manure where their biomass is incorporated into the soil

as has been successfully shown in the South of France where sorghum is the most widely used green manure in vegetable production (Djian-Caporalino et al. 2019). When they have a high trapping effect, like *C. juncea* which had high J2 penetration with very low multiplication, nematicidal plants could be used as companion plants to attract nematodes away from the crop. The possibility of having nematicidal plants also grown as crops such as fennel, corn salads, radish, and rockets included in our crop rotation sequence to suppress RKN, brings more economic value from their yield. Finally, having the choice between summer or winter nematicidal plants provides a solution regardless of the main economic growing season practised. Subject to validation by field evaluations, our results pave the for the incorporation of nematicidal plants, whether in summer or winter, in vegetable cropping systems for sustainable management of nematodes, but with considerations on how and when to use them.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

All authors approve the submission

Competing interests

We declare no competing interest

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Author Contribution

CN and CDC conceived and designed the research. CN and CC conducted the experiments. CN, AVL, and FM performed the statistical analysis. CN wrote the first draft and together with CDC and AVL wrote the final manuscript. All authors reviewed the submitted manuscript.

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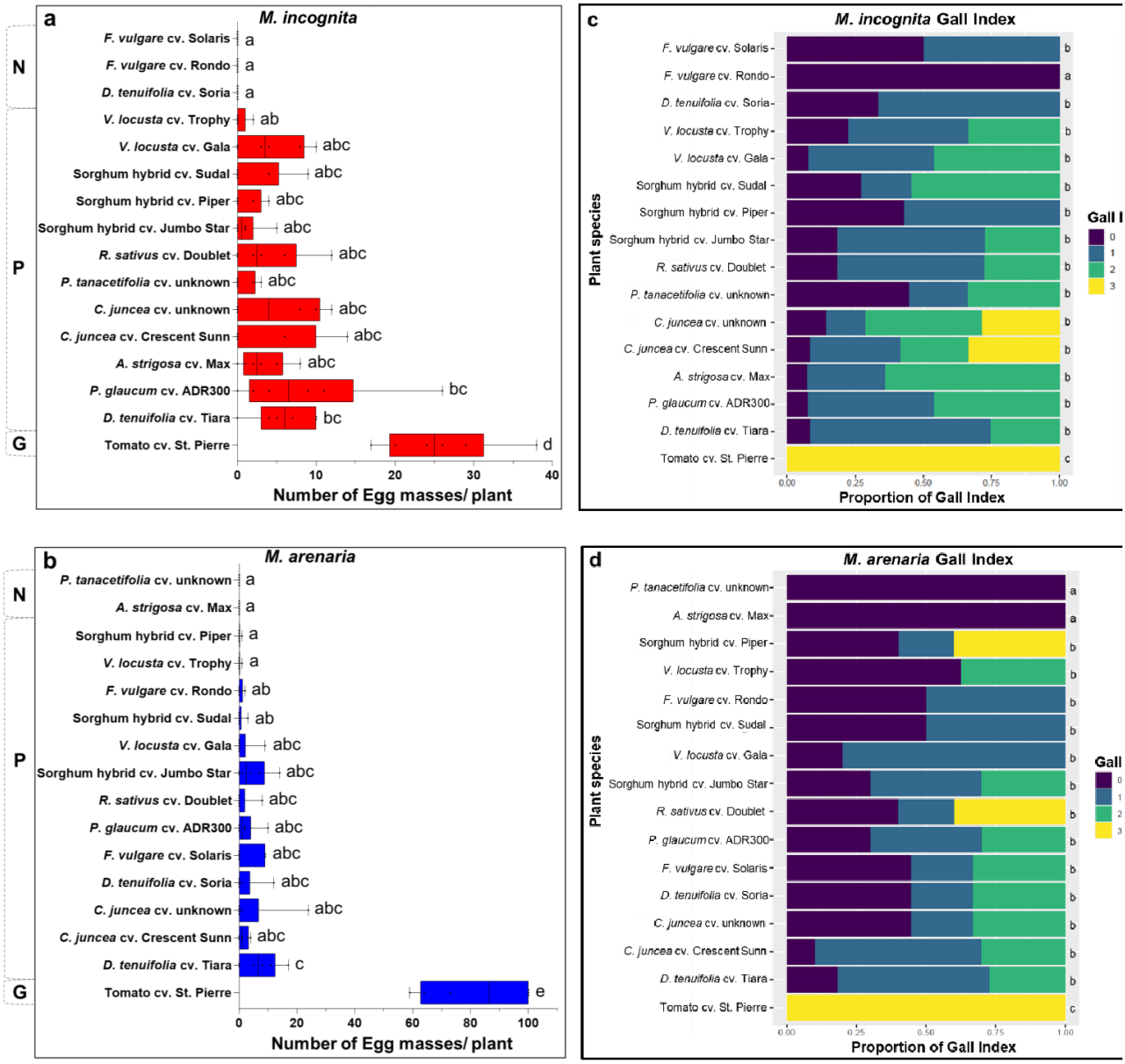
Figures

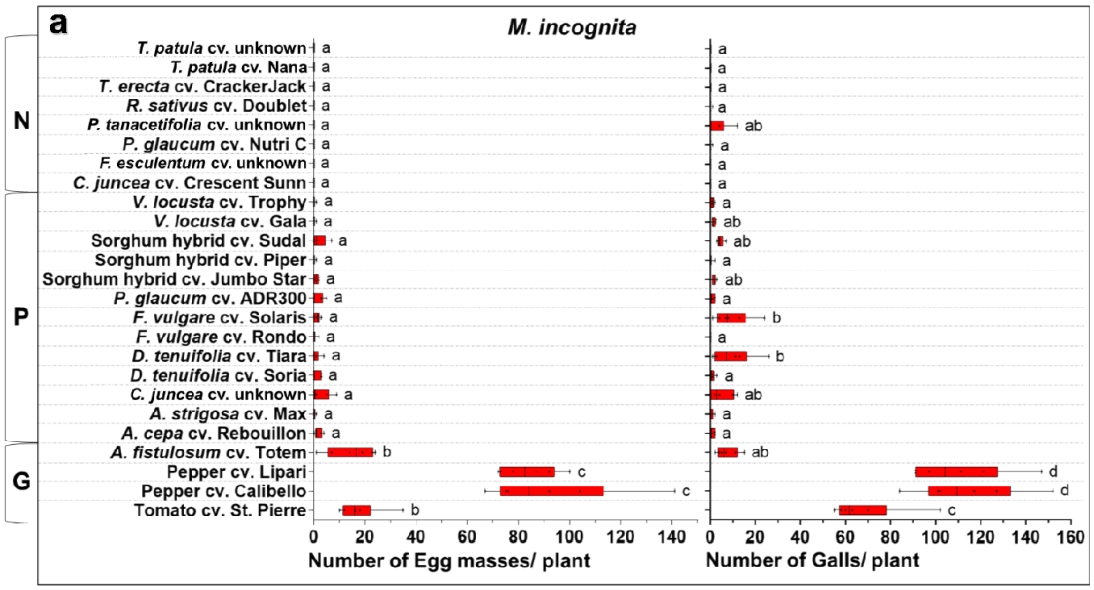


Explanatory variables	Number of egg masses for *M. incognita* & *M. arenaria*		
	df	LRT	P-value
NULL			
Plant species	31	1467.2136	0.0000

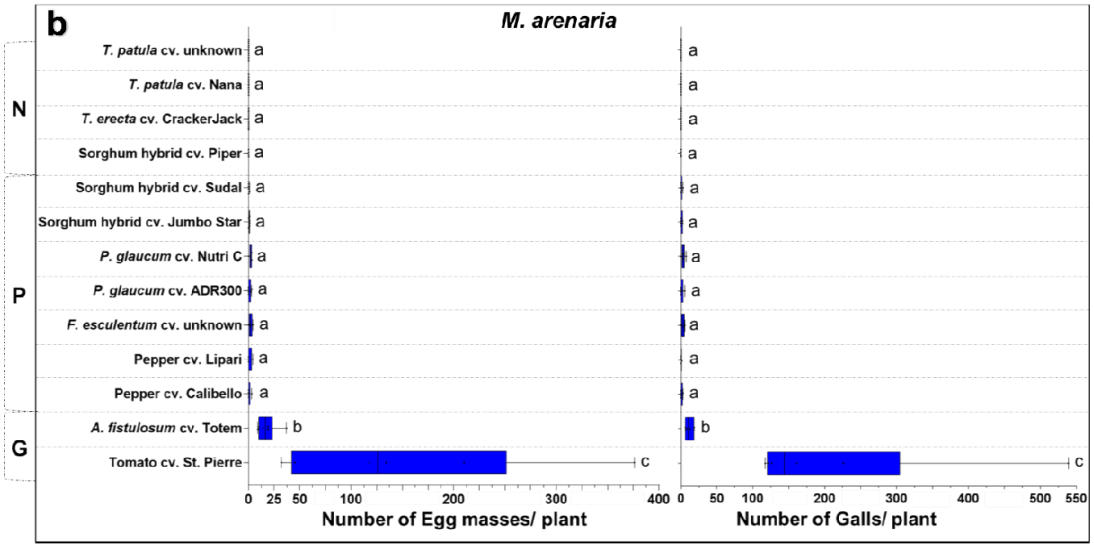
Figure 1

Mean number of egg masses and gall index per plant species after inoculation with 2000 eggs of *M. incognita* and *M. arenaria*(first experiment): means (n= 6) ± standard error. **a** & **b**: Number of egg masses- the Zero-Inflated Poisson Regression (ZIP) and Tukey's HSD post hoc test used to compute the significant differences; $p < 0.05$. **c** & **d**: Proportion-based gall index for *M. incognita* and *M. arenaria*, respectively with Ordered Logistic and Tukey's HSD post hoc test used to compute the significance; p <

0.05. Gall index: 0= no gall, 1= less than 20 galls, 2= between 20 and 50 galls, and 3= more than 50 galls. N= non-host, P= poor host, and G= good host/ susceptible.



Explanatory variables	Number egg masses					Number of galls				
	df	Deviance	Residual df	Residual Dev	Pr(>Chi)	df	Deviance	Residual df	Residual Dev	Pr(>Chi)
NULL			164	5304.8				164	7159.2	
Plant species	24	5063.7	140	241.1	< 2.2e-16 ***	24	6842	140	317.2	< 2.2e-16 ***

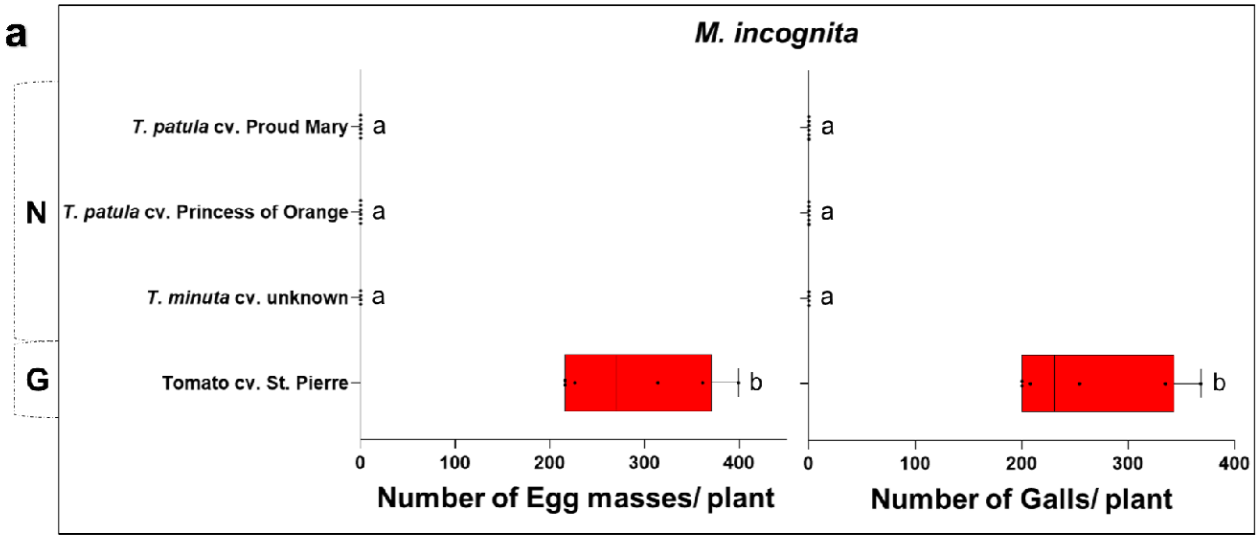


Explanatory variables	Number egg masses					Number of galls				
	df	Deviance	Residual df	Residual Dev	Pr(>Chi)	df	Deviance	Residual df	Residual Dev	Pr(>Chi)
NULL			86	5134.4				86	6988.4	
Plant species	12	4547.7	74	586.6	< 2.2e-16 ***	12	6405.9	74	582.5	< 2.2e-16 ***

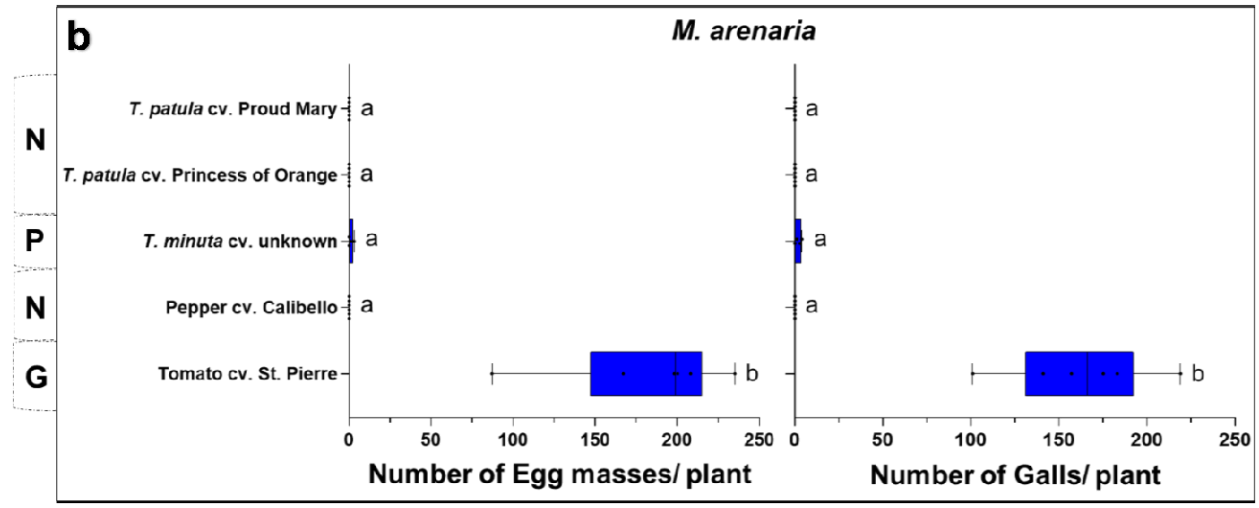
Figure 2

Mean number of egg masses (left) and galls (right) per plant species after inoculation with 2000 eggs of *M. incognita* **(a)** and *M. arenaria* **(b)**: means (n= 6 and 12 for *Tagetes* spp.) ± standard error. Different

letters indicating significant differences were computed by the Bias reduction GLM followed by Tukey's HSD post hoc test; $p < 0.05$. N= non-host, P= poor host, and G= good host/ susceptible.



Explanatory variables	Number egg masses					Number of galls				
	df	Deviance	Residual df	Residual Dev	Pr(>Chi)	df	Deviance	Residual df	Residual Dev	Pr(>Chi)
NULL			21	4612.1				21	4167.7	
Plant species	3	4497.7	18	114.4	< 2.2e-16 ***	3	4063.8	18	103.9	< 2.2e-16 ***

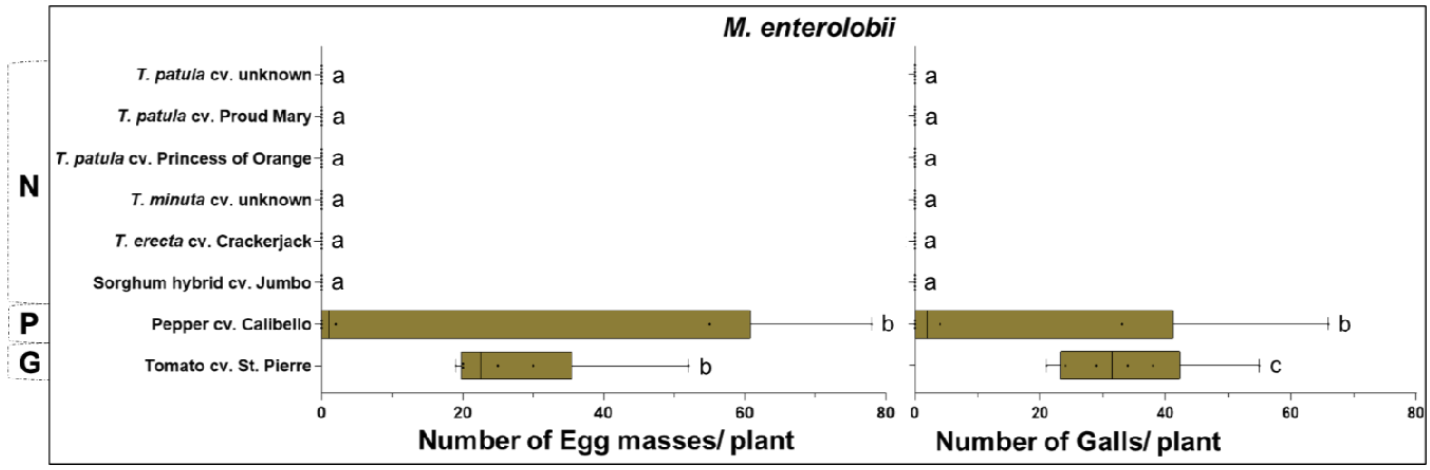


Explanatory variables	Number egg masses					Number of galls				
	df	Deviance	Residual df	Residual Dev	Pr(>Chi)	df	Deviance	Residual df	Residual Dev	Pr(>Chi)
NULL			27	3426.4				27	3002.97	
Plant species	4	3333.2	23	93.2	< 2.2e-16 ***	4	2942.1	23	60.84	< 2.2e-16 ***

Figure 3

Mean number of egg masses and galls for **a)** *M. incognita* and **b)** *M. arenaria* per plant species after inoculation with 2000 eggs of *M. incognita* or *M. arenaria* (third experiment): means (n= 6) ± standard

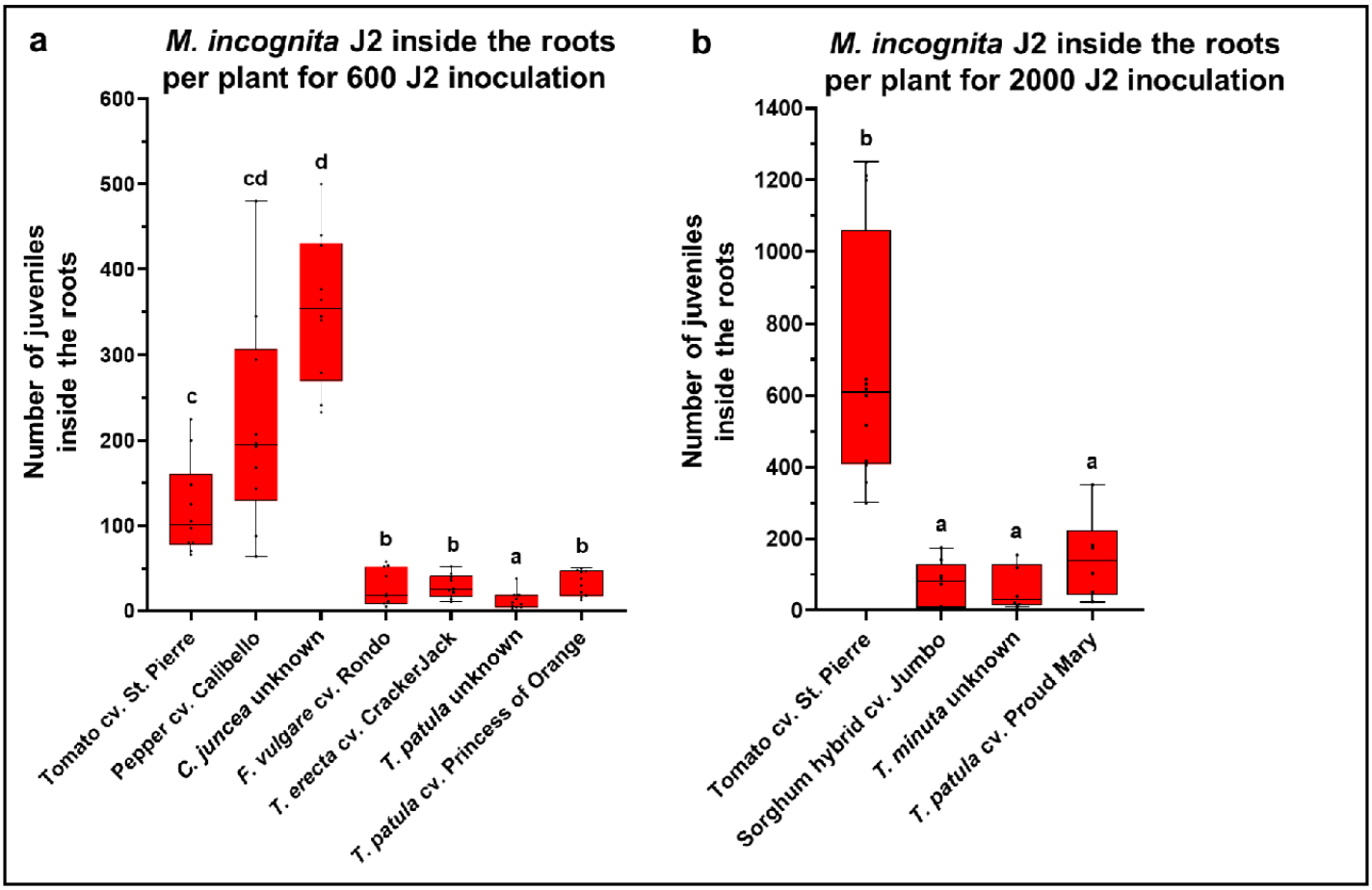
error. Different letters indicate significant differences computed by Bias reduction GLM followed by Tukey's HSD post hoc test; $p < 0.05$. N= non-host, P= poor host, and G= good host/ susceptible.



Explanatory variables	Number egg masses					Number of galls				
	df	Deviance	Residual df	Residual Dev	Pr(>Chi)	df	Deviance	Residual df	Residual Dev	Pr(>Chi)
NULL			47	1145.50				47	1105.41	
Plant species	7	831.75	40	313.76	< 2.2e-16 ***	7	869.03	40	236.38	< 2.2e-16 ***

Figure 4

Mean number of egg masses (left) and galls (right) per plant species after inoculation with 2000 eggs of *M. enterolobii* (fourth experiment): means (n= 6) ± standard error. Different letters indicate significant differences computed from Bias reduction GLM followed by Tukey's HSD post hoc test; $p < 0.05$. N= non-host, P= poor host, and G= good host/ susceptible.



Explanatory variables	Number J2 inside roots/ 600 J2 inoculation					Number of J2 inside roots/ 2000 J2 inoculation				
	df	Deviance	Residual df	Residual Dev	Pr(>Chi)	df	Deviance	Residual df	Residual Dev	Pr(>Chi)
NULL			69	400.56				31	86.915	
Plant species	6	321.91	63	78.66	< 2.2e-16 ***	3	51.502	28	35.412	3.823e-11 ***

Figure 5

Mean number of *M. incognita* J2 inside the roots of the different plant species tested 8 DAI from two independent experiments. **a) Inoculated with 600 J2** (n= 10 ± standard error). **b) Inoculated with 2000 J2** (n= 6-12 ± standard error). Different letters indicate significant differences at P < 0.05 (Negative Binomial GLM followed by Tukey's HSD post hoc test).