Phytopathologia Mediterranea
The international journal of the
Mediterranean Phytopathological Union

Firenze University Press
www.fupress.com/pm

Research Papers

Reactions of *Citrullus amarus* and *Cucumis metuliferus* to *Meloidogyne chitwoodi*, *Meloidogyne enterolobii* and *Meloidogyne luci*

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Summary. *Meloidogyne chitwoodi*, *M. enterolobii,* and *M. luci* are present in some EU countries, with restricted distributions, and plant resistance can be used to manage these nematodes. Two pot experiments were conducted under controlled conditions for 56 d to assess the host suitability of two potential rootstocks, *Cucumis metuliferus* BGV11135 and *Citrullus amarus* BGV5167, to one isolate of each nematode. The susceptible cucumber (*Cucumis sativus*) 'Dasher II', watermelon (*Citrullus lanatus*) 'Sugar Baby' and tomato (*Solanum lycopersicum*) 'Coração-de-Boi' were included for comparisons. A histopathological study using confocal-laser microscopy was also conducted 15 d after nematode inoculations. In the pot test, the rootstocks showed lower numbers of galls, egg masses, and eggs per plant than their susceptible ones. Reproduction indices of the rootstocks varied from immune to moderately resistant, depending on the isolate-rootstock combination. In the histopathological study, *M. enterolobii* and *M. luci* induced similar numbers of giant cells (GC) per feeding site in all germplasms. However, GC volumes and numbers of nuclei in rootstocks were lower than in the susceptible germplasms. GCs induced by *M. chitwoodi* were only detected in susceptible cucumber. These results emphasize the potential of *C. metuliferus* and *C. amarus* as effective, eco-friendly strategies for managing root-knot nematodes, and show the complex these host-pathogen interactions.

Keywords. Histopathology, plant resistance, root-knot nematodes, rootstocks.

OPEN ACCESS

Citation: A.M. Fullana, C. Maleita, D. Santos, I. Abrantes, F.J. Sorribas, A. Giné (2024) Reactions of *Citrullus amarus* and *Cucumis metuliferus* to *Meloidogyne chitwoodi*, *Meloidogyne enterolobii* and *Meloidogyne luci*. *Phytopathologia Mediterranea* 63(1): 79-90. doi: 10.36253/phyto-15108

Accepted: March 20, 2024

Published: April 29, 2024

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

Editor: Thomas A. Evans, University of Delaware, Newark, DE, United States.

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INTRODUCTION

Plant-parasitic nematodes (PPN) have significant economic impacts on agriculture (Jones *et al.*, 2013), leading to diminished crop yields qual-

ity (Elling, 2013). *Meloidogyne* spp., commonly known as root-knot nematodes (RKN), are obligate sedentary endoparasites of roots of many plant species, and are responsible for approx. half of crop yield losses attributed to PPN (Bent *et al*., 2008). In a compatible host, the RKN trigger formation of multinucleated giant cells (GC), from which the nematodes obtain the nutrients for development. RKN induce formation of host root galls, disrupting the uptake of water and nutrients and causing nonspecific symptoms in aerial plant parts, including stunting, nutrient deficiency, epinasty, and plant death, at high nematode population densities in soil. Disease severity depends on soil nematode population density at sowing or transplanting, and on host species and cultivar, cropping season, soil texture and presence of potential nematode antagonists (Sorribas *et al.,* 2020). Conversely, when compatibility between the host plant and the nematode is suboptimal, GCs often have inhibited growth, characterized by presence of multiple vacuoles, sparse nuclei, or cytoplasmic collapse. Another distinctive feature frequently observed is the absence of fluorescence in histopathological images, due to the probable accumulation of phenolic compounds surrounding the GCs, indicating hypersensitive responses to nematode infections (Phan *et al*., 2018; Expósito *et al*., 2020; Fullana *et al*., 2023). This defensive response results in suppression of nematode infection and reproduction, and, in some cases, increases in proportions of males in the populations (Ye *et al*., 2017).

Of the approx. 100 RKN species described to date, *Meloidogyne arenaria*, *M. incognita, M. javanica* (tropical species), and *M. hapla* (temperate species), are responsible for most yield crop losses attributed to *Meloidogyne* spp. (Jones *et al.,* 2013). However, other RKN species, such as *M. chitwoodi, M. enterolobii* and *M. luci,* are gaining importance, because of their high pathogenicity in several economically important crops despite their limited global distributions (Castagnone-Sereno, 2012; Elling, 2013; Maleita *et al*., 2022). *Meloidogyne chitwoodi* and *M. enterolobii* have been added to the EPPO A2 list of pests recommended for regulation as quarantine pests (EPPO, 2023a), and *M. luci* has been added to the EPPO Pest Alert List (EPPO, 2017). In Europe, populations of *M. chitwoodi* have been reported in Belgium, France, Germany, the Netherlands and Portugal in 2016 (EPPO, 2016). Currently, however, 17 other countries, including Spain, have been included (EPPO 2023b). The distribution of *M. enterolobii* is more limited than that of other *Meloidogyne* species, having been reported in Belgium, France, Italy, the Netherlands, Portugal and Switzerland (EPPO, 2023c). *Meloidogyne luci* is present in Greece, Italy, Portugal, Serbia, Slovenia and Turkey (EPPO, 2023d). Despite these restricted distributions, legislative measures have been implemented to eradicate these nematodes, and prevent their introduction into regions where they are absent. This emphasizes the need for increased surveillance and control measures against these emerging nematode species.

RKN control has traditionally relied on fumigant and non-fumigant nematicides. However, use of most of these have been prohibited or restricted, due to harmful environmental, human, and/or animal effects. In response, the European Union has adopted new policies that promote the use of integrated nematode management strategies, which prioritize environmentally friendly and safe approaches reflected in Directive 2009/128/CE and the European Green Deal. Plant resistance plays a key role in the available control strategies, because it suppresses nematode infection and/or reproduction (Roberts, 2002). Resistance is cost-effective, prevents nematode reproduction and crop yield losses (Sorribas *et al*., 2005), and its effect is extended to following susceptible crops (Ornat *et al*., 1997; Hanna, 2000). Commercially available resistant vegetable cultivars or rootstocks for tropical RKN species are limited to the *Solanaceae* and *Cucurbitaceae* including tomato, pepper, eggplant and watermelon. However, some of the minor and temperate RKN species can reproduce on these plants, or their reproductive capacity is unknown.

Meloidogyne chitwoodi, M. enterolobii and *M. hapla* can reproduce on tomato carrying the *Mi1.2* resistance gene and pepper germplasm carrying the *N* resistance gene (Brown *et al*., 1997; Koutsovoulos *et al.,* 2020). In addition, virulent isolates of *M. luci* able to overcome resistance conferred by the tomato *Mi1.2* gene have been reported (Aydinli *et al*., 2019). In cucurbits, the experimental melon rootstocks *Cucumis metuliferus* BGV11135 display resistance to *M. arenaria*, *M. incognita* and *M. javanica* (Expósito *et al*., 2018, and 2019), as well as *Citrullus amarus*, a commercial watermelon rootstock (García-Mendívil *et al.,* 2019; Waldo *et al.,* 2023). Additionally, three accessions of *C. metuliferus* 'Kino' exhibit resistance to *M. enterolobii*, *M. incognita* race 1, and *M. javanica* (Pinheiro *et al.,* 2019). Waldo *et al.* (2023) also evaluated 108 different accessions of *C. amarus*, and some of these were resistant to *M. enterolobii*. Nevertheless, there is currently no available knowledge about the host suitability of *C. metuliferus* and *C. amarus* for the emerging RKN species *M. chitwoodi* and *M. luci.*

Histopathological studies conducted with laser scanning confocal microscopy have shown that GCs in resistant germplasms are less voluminous and have fewer

nuclei than those in susceptible germplasm (Expósito *et al.,* 2020; Fullana *et al.,* 2023). The aim of the present study was to determine host suitability of *C. metuliferus* BGV11135 and *C. amarus* BGV5167 accessions for isolates of *M. chitwoodi, M. enterolobii,* and *M. luci.* Histopathological studies of each plant germplasm-RKN isolate combination were also carried out.

MATERIALS AND METHODS

Nematode inocula

Inocula consisted of second-stage juveniles (J2) of *M. chitwoodi* (PtCh), *M. enterolobii* (PtEn), and *M. luci* (PtL1) isolates selected from the RKN NEMATOlab collection (CFE, University of Coimbra) (Maleita *et al.*, 2021). The isolates were maintained on the susceptible tomato (*Solanum lycopersicum*) cultivar 'Coração-de-Boi' (Vilmorim-Mikado Ibérica, Alicante, Spain; Maleita *et al.*, 2022), in a growth chamber maintained at 24 ± 2°C and 16 h light 8 h dark daily cycle. One week before nematode inoculations, nematode egg masses were hand-picked and placed in Baermann funnels to allow J2 emergence. After 24 h, the emerged J2 were discarded, and the remaining J2 were collected daily and kept at 4°C until the beginning of the experiment, for a maximum of 5 d. Biochemical electrophoretic analyses of non-specific esterase enzymes were carried out to confirm the *Meloidogyne* species (Pais *et al.*, 1986).

Plant material

Seeds of the *C. metuliferus* BGV11135 and *C. amarus* BGV5167 (COMAV-UPV, Valencia, Spain) were used in this study. The cucumber (*Cucumis sativus*) 'Dasher II' (Seminis Seeds) and the watermelon (*Citrullus lanatus*) 'Sugar Baby' (Batlle Seeds) were used as cultivars susceptible to tropical RKN species for comparisons (Giné *et al.*, 2014; López- Gómez *et al.*, 2014). The susceptible tomato (*S. lycopersicum*) 'Coração-de-Boi' was included as a control, to assess the viability of the nematode inocula. Seeds were germinated in Petri dishes with sterile filter paper soaked with sterile distilled water at 24 ± 1°C for 3 d in the dark. After germination, seedlings were transplanted (one per pot) into 50 cm^3 pots containing a sterile mixture (1:1:2) of sandy loam soil, sand and a germination substrate (Siro Germinação bio®). This substrate contains 2 $kg{\cdot}m^{-3}$ of NPK 9-2-2. The seedlings were kept in a growth chamber for 3 weeks at 24 ± 2°C and a 16 h light 8 h dark daily cycle.

Host suitability

Plants were transplanted into 200 cm^3 capacity pots containing the soil mixture described above, and were each inoculated with 200 J2. The nematode inoculum was distributed in each pot into two 2 cm holes, located 1 cm away from the plant stem and 2 cm deep in the soil. Each plant germplasm-RKN isolate combination was repeated 10 times, and the experiment was conducted twice.

The plants were maintained in controlled climate chamber at 25 ± 2°C and 60% relative humidity with a 16 h light 8 h dark daily cycle for 56 d. The plants were watered at 2 d intervals, and were fertilized once each week with NUTREA 12-4-6 (Genyen, Crop Solutions), a liquid fertilizer containing 5% N, 8% P and 10% K. At the end of the experiment, plant roots were carefully washed free of soil with tap water, and were then immersed in a phloxine B (0.0015%) solution for 15 min to stain and visualize the nematode egg masses (Holbrook *et al.*, 1983). The number of root galls and egg masses per plant were counted to estimate nematode penetration (galls) and infectivity (egg masses). Nematode eggs were extracted from each whole root system by blending maceration in a 1% NaOCl solution, using the procedure outlined by Hussey and Barker (1973), eggs were counted to estimate the final nematode population densities (Pf). Nematode fertility was calculated as the number of eggs per egg mass per plant, and reproduction index (RI), as the percentage of reproduction of a given *Meloidogyne* isolate in the resistant germplasm relative to that in the susceptible germplasm [RI = (Pf in resistant germplasm/Pf in susceptible germplasm) × 100]. Levels of resistance were estimated according to the RI values, as immune (RI = 0), highly resistant (RI < 1%), resistant (1% ≤ RI < 10%), moderately resistant (10% ≤ RI < 25%), slightly resistant (25% ≤ RI < 50%), or susceptible (RI ≥ 50%), based on the scale of Hadisoeganda and Sasser (1982).

Histopathology

Fifteen plants of each plant germplasm (described above) were transplanted into 200 cm^3 capacity pots containing sterilized sand, and were maintained under the conditions described above. After 7 d, each susceptible plant germplasm-RKN isolate combination was inoculated with 200 J2, and each expected resistant plant germplasm-RKN isolate combination was inoculated with 600 J2, using the procedure described above. Each plant germplasm-RKN isolate combination was repeated five times. Fifteen days after nematode inoculation, five root systems

of each RKN isolate-plant combination were washed free of subtrate, and were then fixed and rinsed following the procedure of Expósito *et al.* (2020). Images were acquired using a laser scanning confocal microscope (LSM 710 Axio Observer Z1 microscope with QUASAR detection unit; ZEN Black software) using a Plan-Neofluar 10×/0.3 objective, and Argon/2 (488 nm) and HeNe633 (633 nm) lasers, all of which are components from Carl Zeiss. Volumes were acquired with Z-stacks with a step size of 10 µm. The volumes and numbers of nuclei per GC, the numbers of GCs, and the volumes and numbers of nuclei per feeding site were determined using ImageJ and the TrakEM2 ImageJ plugin (ImageJ, version 1.50). This study was conducted once.

Data analyses

Statistical analyses were carried out using GraphPad Prism 7.00 (GraphPad Software). The normality of the data distributions and homogeneity of variances were determined with non-transformed or $\log_{10}$ (x+1) transformed data for parametric or non-parametric analyses. The nonparametric Mann-Whitney test was used to compare penetration (number of galls per plant), infectivity (number of egg masses per plant), reproduction (number of eggs per plant), and fecundity (number of eggs per egg mass) between the experimental repetitions. When significant differences ($P \leq 0.05$) were observed, the values for each replicate were presented separately. Additionally, each parameter was compared between susceptible and the expected resistant germplasm of the same plant genus, or between paired comparisons of tomato plants and each of the susceptible cucurbit germplasms, by Student's t-test ($P \leq 0.05$) when the data exhibited a normal distribution or Mann-Whitney test ($P \leq 0.05$) if it did not. In addition, nonparametric Kruskal-Wallis analyses and Dunn's test ($P \leq 0.05$) were used to compare each parameter between RKN isolate by plant germplasm combinations.

The numbers of nuclei per feeding site and GCs per feeding site, the volume of each GC, and the number of nuclei per GC from the histopathological study were compared ($P \leq 0.05$), between expected resistant and susceptible germplasms per plant genus, as well as the paired comparisons between tomato plants and each of the susceptible cucurbit germplasms. Data were compared using Student's t-test if the data fitted normal distributions; otherwise, the nonparametric Mann-Whitney test was used. In addition, nonparametric Kruskal-Wallis analysis and Dunn's test ($P \leq 0.05$) were used to compare each parameter among the RKN isolate by plant germplasm combinations.

RESULTS

Host suitability

Although general trends were observed, statistically significant differences ($P < 0.05$) were found between the experiments, results for each experiment are presented separately (Table 1). Second-stage juveniles of all RKN isolates penetrated the roots of each plant germplasm, leading to the formation of galls (Table 1). Among the susceptible germplasms, *M. chitwoodi* produced fewer ($P < 0.05$) galls on the cucurbit than on the tomato plants, while no differences ($P > 0.05$) were found between *M. enterolobii* and *M. luci*. Among the resistant germplasms, all the RKN isolates induced fewer ($P < 0.05$) galls than the susceptibles (Table 1). For nematode reproduction, all the RKN isolates developed until the adult female stage producing eggs, in all germplasms, except for *M. chitwoodi* in *C. metuliferus* (Table 1). Fewer ($P < 0.05$) egg masses per plant were produced in the resistant germplasms than in the susceptible germplasms of the same plant genus, except for *M. chitwoodi* in *Citrullus* spp. (Table 1). Concerning the levels of resistance of *C. amarus* to the RKN isolates, performed as resistant to *M. luci* (RI = 4.3 and 4.3%) in both experiments, and resistant or moderately resistant to *M. enterolobii* (RI = 6.7 and 12.2%) and *M. chitwoodi* (RI = 5.3 and 19.1%), depending on the experiment. Meanwhile, *C. metuliferus* was immune to *M. chitwoodi* (RI = 0), highly resistant to resistant to *M. enterolobii* (RI = 0.3 and 3.8%), and resistant to *M. luci* (RI = 1.6 and 1.8%).

Regarding the RKN isolates, *M. chitwoodi* produced fewer ($P < 0.05$) egg masses and eggs per plant on tomato plants than the other RKN isolates. *Meloidogyne luci* reproduced means of 5.5 and 11.3 more times in tomato than *M. chitwoodi* in experiment 1, and 2.6 and 1.7 more times than *M. enterolobii* in experiment 2 (Table 1). In *C. sativus*, *M. chitwoodi* produced fewer ($P < 0.05$) egg masses and eggs per plant than *M. enterolobii* and *M. luci*, which were not different for the numbers of eggs per egg mass in the second experiment. For *C. metuliferus*, *M. chitwoodi* induced fewer ($P < 0.05$) root galls than the other RKN isolates, but no reproduction was detected. In *Citrullus* spp., *M. enterolobii* produced more ($P < 0.05$) egg masses (4.0 to 112.9 times more in *C. lanatus*; 5.3 to 42.0 times more in *C. amarus*) and eggs per plant (4.3 to 515.0 times more in *C. lanatus*; 6.8 to 680.0 times more in *C. amarus*) than the other RKN isolates (Table 1).

Histopathology

Fifteen d after nematode inoculations, only the *M. enterolobii* and *M. luci* isolates were able to infect the

Table 1. Number of galls, nematode egg masses and eggs per plant, and number of eggs per egg mass of *Meloidogyne chitwoodi, M. enterolobii* or *M. luci*, in susceptible plants of *Solanum lycopersicum* 'Coração-de-Boi', *Cucumis sativus* 'Dasher II', and *Citrullus lanatus* 'Sugar Baby', or *Cucumis metuliferus* BGV11135 or *Citrullus amarus* BGV5167 rootstocks 56 d after inoculations with 200 second-stage juveniles per pot, in a climatic chamber in the two experiments [a].

	Meloidogyne species	Plant species	Galls	Egg masses per plant	Eggs per plant (10^2)	Egg per egg mass	Reproduction index (%)[b]	Resistance level [c]
First experiment	*M. chitwoodi*	*S. lycopersicum*	>100 A	22 ± 4.0 C	74 ± 6.7 C	486 ± 111 A		
		C. sativus	59 ± 6 B * †	1.8 ± 0.6 B †	0.9 ± 0.2 B †	23 ± 5 B †		
		C. metuliferus	7 ± 1 C	0 ± 0	0 ± 0	nc	0	I
		C. lanatus	28 ± 9 B * †	0.9 ± 0.4 B †	2.0 ± 0.4 C * †	120 ± 10 A †		
		C. amarus	8 ± 1 C	0.1 ± 0.1 B	0.01 ± 0.01 B	nc	5.3	R
	M. enterolobii	*S. lycopersicum*	>100 A	42 ± 2.3 B	144 ± 9.5 B	346 ± 22 A		
		C. sativus	>100 A *	34 ± 2.3 A * †	44 ± 3.7 A * †	136 ± 16 A †		
		C. metuliferus	25 ± 2 B	0.8 ± 0.3 A	1.7 ± 1.4 A	179 ± 136 A	3.8	R
		C. lanatus	>100 A *	36 ± 3.0 A *	102 ± 9.0 A * †	295 ± 28 A		
		C. amarus	50 ± 5 B	4.2 ± 1.7 A	6.8 ± 2.5 A	191 ± 56 A	6.7	R
	M. luci	*S. lycopersicum*	>100 A	96 ± 4.5 A	382 ± 24.1 A	431 ± 25 A		
		C. sativus	>100 A *	34 ± 2.3 A * †	36 ± 2.2 A * †	112 ± 10 A †		
		C. metuliferus	53 ± 3 A	0.3 ± 0.2 A	0.7 ± 0.5 A	218 ± 43 A	1.8	R
		C. lanatus	>100 A *	9 ± 2.0 B * †	24 ± 5.6 B * †	242 ± 50 A †		
		C. amarus	79 ± 5 A	0.8 ± 0.3 B	1.0 ± 0.6 AB	157 ± 106 A	4.3	R
Second experiment	*M. chitwoodi*	*S. lycopersicum*	>100 A	27 ± 3.0 B	57 ± 7.3 C	221 ± 30 C		
		C. sativus	37 ± 2 B * †	5.6 ± 1.8 B †	5.6 ± 0.3 B †	90 ± 25 C †		
		C. metuliferus	20 ± 1 B	0 ± 0	0 ± 0	nc	0	I
		C. lanatus	33 ± 3 B * †	0.7 ± 0.4 B †	0.8 ± 0.5 C †	114 ± 39 B †		
		C. amarus	13 ± 2 C	0.2 ± 0.2 B	0.2 ± 0.2 B	nc	19.1	MR
	M. enterolobii	*S. lycopersicum*	>100 A	105 ± 9.0 A	380 ± 33.5 B	393 ± 55 B		
		C. sativus	>100 A *	34 ± 5.0 A * †	224 ± 39.3 A * †	801 ± 172 A *		
		C. metuliferus	39 ± 5 A	0.3 ± 0.2 A	0.6 ± 0.4 A	200 ± 16 B	0.3	HR
		C. lanatus	>100 A *	79 ± A 7.0 * †	412 ± 26.9 A *	548 ± 50 A †		
		C. amarus	66 ± 6 A	8.3 ± 1.5 A	50 ± 10.4 A	603 ± 70 A	12.2	MR
	M. luci	*S. lycopersicum*	>100 A	121 ± 6.0 A	647 ± 22.5 A	544 ± 26 A		
		C. sativus	>100 A *	36 ± 4.0 A * †	160 ± 23.0 A * †	442 ± 42 B		
		C. metuliferus	25 ± 2 A	0.4 ± 0.2 A	2.6 ± 2.1 A	510 ± 162 A	1.6	R
		C. lanatus	>100 A *	7 ± 2.0 B * †	27 ± 7.3 B * †	371 ± 65 AB †		
		C. amarus	50 ± 5 B	0.6 ± 0.2 B	1.1 ± 0.5 B	222 ± 89 B	4.3	R

[a] Data are means ± standard errors of ten replicates. Data in each column followed by different letters are significantly different ($P < 0.05$) between root-knot nematode (RKN) isolates for a given plant germplasm, according to Dunn's test. Data for each column and each RKN isolate followed by * indicate significant differences ($P < 0.05$) between germplasms of the same genus, and by † indicate differences ($P < 0.05$) between *Solanum lycopersicum* and *Cucumis sativus* or *Citrullus lanatus*, as shown by Student's t tests or Mann-Whitney tests. nc = Not calculated. [b] Reproduction index: percentage of the eggs produced in the resistant germplasm compared with those produced in the susceptible germplasm. [C] Resistance level: I = immune (RI = 0), HR = highly resistant (RI < 1%), R = resistant (1% ≤ RI ≤ 10%), MR = moderately resistant (10% < RI ≤ 25%), SR = slightly resistant (25% < RI ≤ 50%) or S = susceptible (RI > 50%), as categorized by Hadisoeganda and Sasser (1982).

roots of all the assessed plant germplasms (Table 2; Figures 1 to 4). *Meloidogyne chitwoodi* only infected tomato and cucumber roots (Table 2; Figures 1 and 4). Despite *M. chitwoodi* J2 being observed inside the roots of *C. metuliferus* and *C. lanatus,* no GCs were induced (Figure 4, b and c); therefore, comparisons were only valid between tomato and cucumber. The number and volume of GCs per feeding site and the number of nuclei per GC and per feeding site did not differ ($P > 0.05$) between the tomato and cucumber plants (Table 2).

Meloidogyne enterolobii induced a similar ($P > 0.05$) number of GCs in *C. metuliferus* and cucumber. However, the volumes of the GCs in *C. metuliferus* were six times less ($P < 0.05$) than in cucumber, resulting in a 9.5-fold reduction ($P < 0.05$) in the total volume of GCs per feeding site. The number of nuclei per GC and per

Table 2. Number of giant cells per nematode feeding site (GC·fs^{-1}), number of nuclei per giant cells (N·GC^{-1}), number of nuclei per feeding site (N·fs^{-1}), giant cell volume (GCV) and giant cell volume per feeding site (GCV·fs^{-1}), in *Solanum lycopersicum* 'Coração-de-Boi', *Cucumis sativus* 'Dasher II' and *Citrullus lanatus* 'Sugar Baby' plants, and *Cucumis metuliferus* BGV11135 and *Citrullus amarus* BGV5167 rootstocks, 15 d after nematode inoculations with 200 or 600 second-stage juveniles per pot [a], in susceptible or rootstocks respectively.

Meloidogyne species	Plant species	GC·fs^{-1}	N·GC^{-1}	N·fs^{-1}	GCV (μm^3 10^{-5})	GCV·fs^{-1} (μm^3 10^{-5})
M. chitwoodi	*S. lycopersicum*	5 ± 1.0 A	14 ± 3.2 B	44 ± 8.8 B	8 ± 1.1 B	26 ± 3.0 B
	C. sativus	4 ± 0.2 A	9 ± 1.7 B	29 ± 6.4 C	5 ± 0.9 C	22 ± 3.4 B
	C. metuliferus	na	na	na	na	na
	C. lanatus	na	na	na	na	na
	C. amarus	na	na	na	na	na
M. enterolobii	*S. lycopersicum*	5 ± 0.8 A	26 ± 3.1 A	131 ± 6.7 A	14 ± 1.9 A B	70 ± 7.8 A
	C. sativus	9 ± 0.9 A	20 ± 1.7 A *	181 ± 8.3 A *	12 ± 1.8 B *	114 ± 23.1 A *
	C. metuliferus	5 ± 0.8 A	7 ± 1.5 A	33 ± 5.6 B	2 ± 0.6 A	12 ± 2.0 A
	C. lanatus	5 ± 0.7 A	17 ± 2.8 A *	79 ± 10.7 A * †	40 ± 13.8 A * †	170 ± 32.8 A * †
	C. amarus	6 ± 1.7 A	5 ± 0.4 A	28 ± 3.2 A	3 ± 0.4 A	20 ± 3.0 A
M. luci	*S. lycopersicum*	4 ± 0.4 A	30 ± 5.2 A	138 ± 31.2 A	19 ± 4.7 A	87 ± 24.1 A B
	C. sativus	6 ± 0.4 A	16 ± 2.1 A †	89 ± 14.7 B	33 ± 5.5 A * †	181 ± 29.6 A * †
	C. metuliferus	9 ± 0.8 A	9± 1.4 A	59 ± 7.3 A	3 ± 0.8 A	15 ± 2.6 A
	C. lanatus	5 ± 0.5 A	22 ± 2.9 A *	112 ± 8.7 A *	12 ± 1.9 B *	65 ± 9.8 B *
	C. amarus	5 ± 0.7 A	7 ± 0.3 A	35 ± 3.3 A	5 ± 0.2 A	22 ± 1.8 A

[a] Data are means ± standard errors for five replicates. Data in the same column followed by different letters are significantly different ($P < 0.05$) between root-knot nematode (RKN) isolates by a given plant germplasm, according to Dunn's test. Data in each column and for each RKN isolate followed by * are significantly different ($P < 0.05$) between germplasms of the same genus. † indicates differences ($P < 0.05$) between *Solanum lycopersicum* and *Cucumis sativus* or *Citrullus lanatus*, according to Student's t or Mann-Whitney tests. na = No available data because no infection was observed.

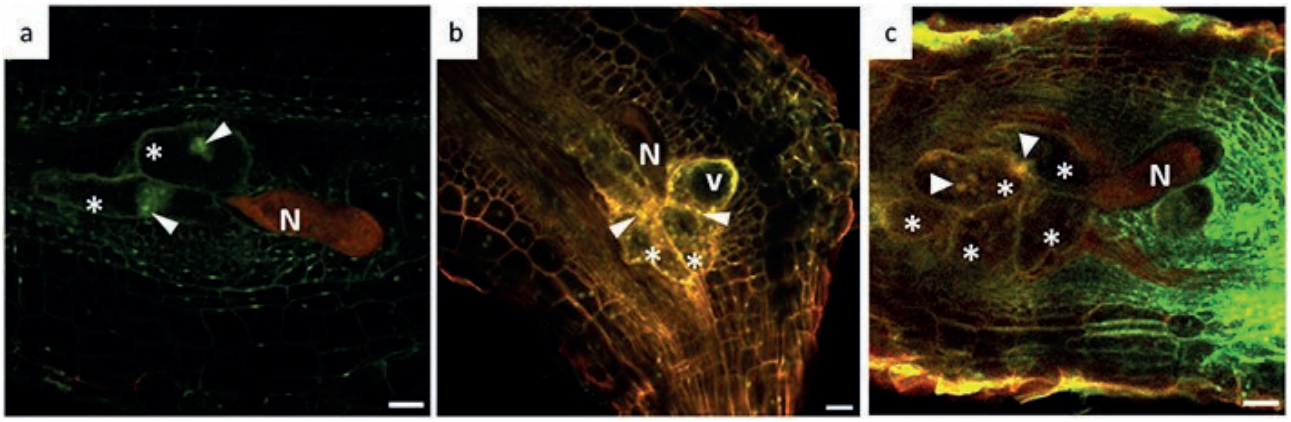

Figure 1. Laser scanning confocal microscope images of the infection sites of *Meloidogyne chitwoodi* (a), *Meloidogyne enterolobii* (b) and *Meloidogyne luci* (c), 15 dafter inoculation, in *Solanum lycopersicum* 'Coração-de-Boi'. Nematode (N); vacuoles (v); giant cells (asterisks); and some nuclei (white arrowheads) are indicated. Scale bars = 50 μm.

feeding site were 2.9 and 5.5 times greater ($P < 0.05$) in cucumber than in *C. metuliferus* (Table 2). Similar results were observed in watermelon. Although the nematodes induced similar ($P > 0.05$) numbers of GCs per feeding site in both *Citrullus* spp., the volumes per GC were 13.3 greater in *C. lanatus* and 8.5 times greater ($P < 0.05$) than in *C. amarus*. The numbers of nuclei per GC were 3.4 greater, and per feeding site were 2.8 greater ($P < 0.05$).

Meloidogyne luci induced a similar ($P > 0.05$) numbers of GCs in *C. metuliferus* and cucumber, but the GC volumes in *C. metuliferus* were 11 times less ($P < 0.05$) than in cucumber, resulting in a 12.1-fold reduction (*P*

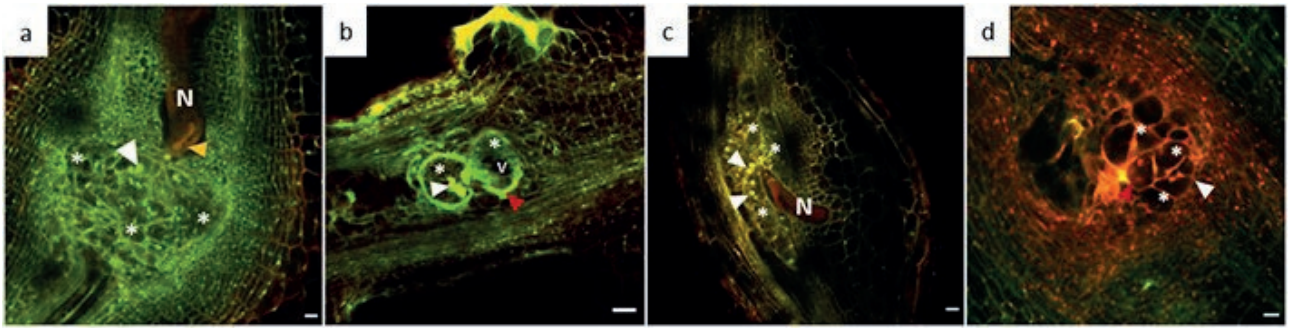

Figure 2. Laser scanning confocal microscope images of infection sites of *Meloidogyne enterolobii*, 15 d after inoculation, in *Cucumis sativus* 'Dacher II' (a), *Cucumis metuliferus* BGV11135 (b), *Citrullus lanatus* 'Sugar Baby' (c) or *Citrullus amarus* BGV5167 (d). Nematodes (N); vacuoles (v); giant cells (asterisks); some nuclei (white arrowheads); necrosed areas (red arrowheads); and a nematode oesophageal median bulb (yellow arrowhead) are indicated. Scale bars = 50 µm.

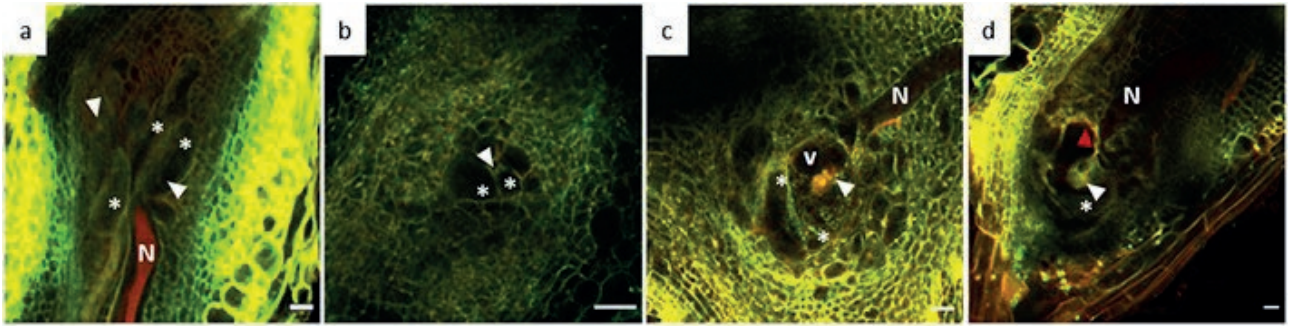

Figure 3. Laser scanning confocal microscope images of infection sites of *Meloidogyne luci* 15 d after inoculation in *Cucumis sativus* 'Dacher II' (a), *Cucumis metuliferus* BGV11135 (b), *Citrullus lanatus* 'Sugar Baby' (c), or *Citrullus amarus* BGV5167 (d). Nematodes (N); giant cells (asterisks); some nuclei (white arrowheads); and necrosed area (red arrowhead) are indicated. Scale bars = 50 µm.

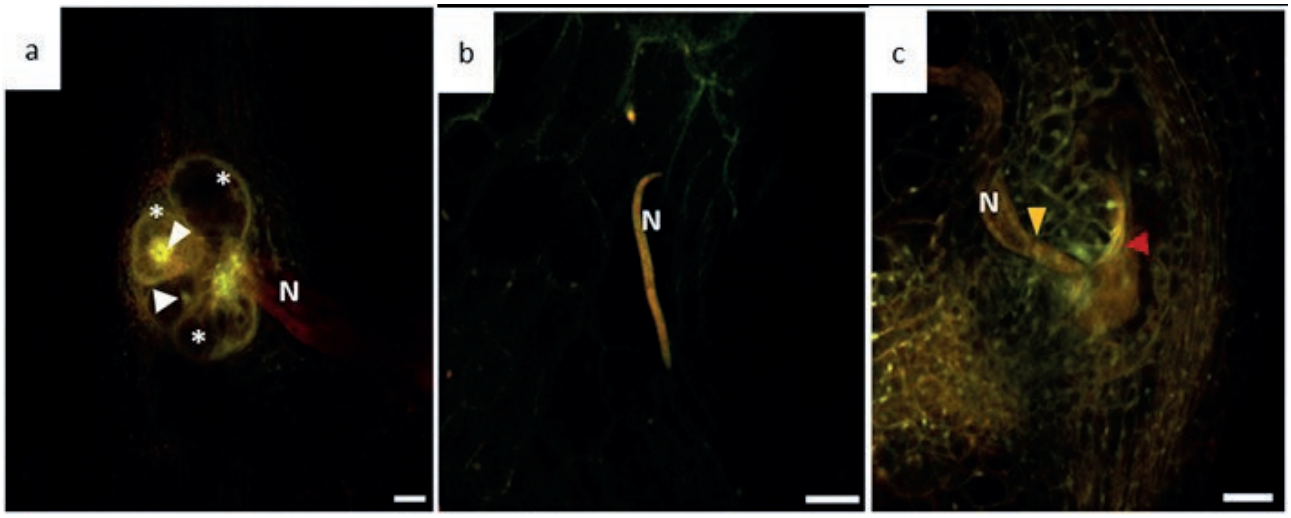

Figure 4. Laser scanning confocal microscope images of *Meloidogyne chitwoodi* infection sites, 15 d after inoculation in the cucumbers *Cucumis sativus* 'Dacher II' (a), *Cucumis metuliferus* BGV11135 (b), watermelon *Citrullus lanatus* 'Sugar Baby' (c), or *Citrullus amarus* BGV5167 (d). Nematodes (N); giant cells (asterisks); some nuclei (white arrowheads); necrosed area (red arrowhead), and an oesophageal median bulb (yellow arrowhead) are indicated. Scale bars = 50 µm.

< 0.05) in total volume of GC per feeding site. However, the numbers of nuclei per GC and per feeding site did not differ ($P > 0.05$) (Table 2). In both *Citrullus* species, *M. luci* induced similar numbers ($P > 0.05$) of GCs, but GC volumes and numbers per feeding site in *C. amarus* were 2.5 and 3 times less ($P < 0.05$) than in in *C. lanatus*. In addition, 3.1 times fewer nuclei per GC ($P < 0.05$) and 3.2 times fewer feeding sites were observed in *C. amarus* than in *C. lanatus*.

The majority of GCs induced by *M. enterolobii* and *M. luci* in *C. metuliferus* and *C. amarus* were almost empty, with few or no nuclei and with some necrotic areas compared to those in the respective susceptible plant germplasm (Figure 2 b and d, Figure 3 b and d).

Of the different RKN isolates, *M. enterolobii* induced formation of GCs that were 3.3 more voluminous ($P < 0.05$) than *M. luci* in *C. lanatus,* which resulted in a total mean GC volume per feeding site that was 2.6 times greater ($P < 0.05$). Nevertheless, no differences ($P > 0.05$) were observed in *C. amarus*. The numbers of nuclei per GC and per feeding site induced by *M. enterolobii* and *M. luci* in both *Citrullus* spp. did not differ ($P > 0.05$), but the numbers of nuclei per feeding site differed ($P < 0.05$) in *Cucumis* spp. (Table 2). Specifically, the number of nuclei per feeding site induced by *M. enterolobii* was 2 times greater in *C. sativus* and 0.56 times greater in *C. metuliferus,* compared to those induced by *M. luci* (Table 2). *Meloidogyne enterolobii* induced the formation of 1.8 times more GC volume ($P < 0.05$) in *S. lycopersicum* than *M. chitwoodi,* resulting in 2.7 more GC volume per feeding site ($P < 0.05$).

DISCUSSION

The main objective of this study was to determine host suitability of *C. metuliferus* BGV11135 and *C. amarus* BGV5167 for the nematodes *M. chitwoodi*, *M. enterolobii* and *M. luci*, to provide insights into the potential use of these rootstocks for melon and watermelon crops, and to provide this information to assist management of RKN species. Previous studies have reported resistance of some *C. metuliferus* accessions to *M. incognita, M. arenaria, M. hapla, M. javanica* and *M. enterolobii* (Walters *et al*., 2006; Ye *et al.,* 2017; Pinheiro *et al.,* 2019), and that of *C. amarus* to *M. arenaria, M. enterolobii, M. incognita* and *M. javanica* (García-Mendívil *et al.,* 2019; Waldo *et al.,* 2023). The present paper is the first report on levels of resistance of *C. metuliferus* and *C. amarus* to *M. chitwoodi* and *M. luci*. In addition, cucumber may be included as a potential plant host of *M. chitwoodi*, because this nematode reproduced in this plant species, as in watermelon which is listed as a plant host (EPPO, 2023b).

The results of the present study have shown that the levels of resistance of *C. metuliferus* ranged from immune (RI = 0) to *M. chitwoodi*, highly resistant (RI < 1%) to resistant (1% ≤ RI < 10%) to *M. enterolobii*, and resistant to *M. luci*. *Citrullus amarus* ranged from resistant to moderately resistant (10% ≤ RI ≤ 25%) to *M. chitwoodi* and *M. enterolobii*, and resistant to *M. luci*.

Several resistance mechanisms of *C. metuliferus* against RKN have been proposed, affecting root penetration, feeding site formation, nematode development, and sex differentiation (Fassuliotis, 1970; Walters *et al.,* 2006). Xie *et al.* (2022) reported the emission of 18 volatiles by the roots of the CM3 accession of *C. metuliferus*, which had repellent effects on *M. incognita*. In the present study, substantial reductions of J2s root penetration of all the RKN isolates were observed, compared to that in cucumber, and only a low proportion of J2 achieved the adult female stage laying eggs (0% for *M. chitwoodi,* 2% for *M. enterolobii* and 1.1% for *M. luci*; averaged over two experiments). Some studies comparing the transcriptome of *C. metuliferus* and cucumber plants inoculated with *M. incognita* have proposed putative resistance mechanisms (Ling *et al.,* 2017; Ye *et al.,* 2017; Li *et al.,* 2021). Ling *et al.* (2017) attributed resistance to differential expression in two host gene clusters related to cytoskeletons and RNA processing. Ye *et al.* (2017) attributed resistance to induction of phenylalanine ammonia-lyase and peroxidase activities after infection together with the expression of genes related to biosynthesis of phenylpropanoids and plant hormone signalling. Li *et al.* (2021) attributed resistance to upregulation of genes related to the Ca^{2+} signalling pathway at early stages of *M. incognita* infection, as well as the salicylic acid and jasmonate signalling pathways. In all these cases, nematode penetration and root infection were reduced, and nematode development was delayed. According to the present study results, the resistance mechanisms of *C. metuliferus* were highly effective against *M. chitwoodi*, because less J2 were able to penetrate, compared to *M. enterolobii* and *M. luci,* and no J2 reached the adult female stage. The histopathological analysis showed that *C. metuliferus* was not infected at 15 d after *M. chitwoodi* inoculation, and those that infected cucumber plants produced less voluminous GCs with a low numbers of nuclei per GC and per feeding site than did the other studied RKN species. For *M. enterolobii* and *M. luci,* reductions in nematode infection and reproduction were detected in *C. metuliferus* in comparison with cucumber, but J2, which were able to infect, to develop until the female stage and reproduce,

produced a similar number of eggs per egg mass than in cucumber (except for *M. enterolobii* in the second experiment). However, a reduction in female fertility of *M. incognita* on *C. metuliferus* has been reported previously (Ye *et al.*, 2017; Expósito *et al.,* 2020). This result is important, because it could be an indicator of adaptation of a given percentage of individuals that could reproduce and increase populations after repeated cultivation. The present histopathological study showed some differences from previous studies regarding the *C. metuliferus-M. incognita* relationship (Ye *et al.*, 2017; Expósito *et al.,* 2020), in which fewer nuclei per cell and per feeding site were reported.

Resistance of *C. amarus* to tropical *Meloidogyne* spp. has been attributed to its high root fibrosity in comparison with that of other cucurbits (Thies and Levi, 2007; Thies *et al.*, 2015; García-Mendivil *et al.,* 2019). Waldo *et al.* (2023) suggested that resistance to *M. enterolobii* is modulated by 11 single-nucleotide polymorphisms. Those in the locus QTL 3.1 influence root galling and egg mass formation, while those in QTL 4.1, 4.2, and 8.1 are associated with nematode egg production. In the present study, compared with those of watermelon, J2 root penetration of all the RKN isolates was reduced, and only a low proportion of J2 achieved the adult female stage laying eggs: 1.4% for *M. chitwoodi,* 10.5% for *M. enterolobii*, and 1.1% for *M. luci* (averaged for the two experiments). Watermelon is considered a poor host for the tropical *Meloidogyne* spp. due to their reduced reproduction rates (López-Gómez *et al.*, 2014), but is a main host for *M. enterolobii* (EPPO, 2023b). This was observed in the present study, achieving levels of reproduction close to those in tomato. However, *M. enterolobii* reproduction in *C. amarus* reached 9.45% of that observed on watermelons, defining the *C. amarus* rootstock as an effective tool for managing this RKN.

Histopathological analyses revealed that neither *C. lanatus* nor *C. amarus* were infected by *M. chitwoodi* 15 d after inoculations. Reductions in the numbers of nuclei and GC volumes were observed in the combinations of remaining RKN-isolates in *C. amarus* compared with watermelon, which may affect nematode development and reproduction.

The results from the present study will provide valuable information for farmers to facilitate decision-making for implementing integrated RKN control strategies, including scenarios with a co-occurrence of RKN species and/or virulent nematode populations to specific host resistant genes. Resistance of these plant species to tropical RKN species in pot and field experiments (Ye *et al.,* 2017; García-Mendívil *et al.,* 2019), and the effectiveness for managing virulent RKN populations to the *Mi1.2* resistance gene in tomato (Expósito *et al.,* 2018; Fullana *et al.,* 2023) have been demonstrated. In addition, several accessions of *C. metuliferus* and *C. amarus* are resistant to other pathogens and diseases, such as *Fusarium oxysporum*, gummy stem blight, powdery mildew, and potyvirus (Gusmini *et al.*, 2005; Guner *et al.*, 2008; Tetteh *et al.*, 2010; Keinath *et al.*, 2019). These characteristics enhance agronomic value of these plant germoplasm. The strategic use of these rootstocks in rotations with other resistant plant germplasms can alleviate the impacts of RKN on crop yield and contribute to reducing reliance on pesticides, as has been previously reported (Expósito *et al.*, 2018; Fullana *et al.*, 2023).

ACKNOWLEDGMENTS

The UPC authors acknowledge funding from the R+D+i project AGL2017-89785-R, financed by MCIN/AEI/10.13039/501100011033 and by the FEDER and FSE (PRE2018-084265, Aïda Magdalena Fullana). In UC, this research was supported by FEDER funds through the Portugal 2020 (PT 2020), COMPETE 2020 and by the Portuguese Foundation for Science and Technology (FCT), under contracts UIDB/00102/2020, UIDP/00102/2020 (CERES), and UIDB/04004/2020 (DOI identifier 10.54499/UIDB/04004/2020), UIDP/04004/2020 (DOI identifier 10.54499/UIDP/04004/2020) (CFE); and by 'Instituto do Ambiente, Tecnologia e Vida'. Duarte Santos is funded by FCT through the grant SFRH/BD/146196/2019. The authors are also grateful to the Institut de Conservació i Millora de l'Agrodiversitat Valenciana from Universitat Politècnica de Valencia (COMAV-UPV) for providing seeds of *Cucumis metuliferus* BGV11135 and *Citrullus amarus* BGV5167. Dr Teresa Rodrigues, iLAB – Microscopy and Bioimaging Lab, a facility of the Faculty of Medicine of University of Coimbra and a member of the national infrastructure PPBI-Portuguese Platform of BioImaging (POCI-01-0145-FEDER-022122) and supported by FSE CENTRO-04-3559-FSE-000142, gave technical support with the laser scanning confocal microscopy. The NEMATO-lab members are also acknowledged for their technical laboratory assistance.

LITERATURE CITED

Aydinli G., Kurtar E.S., Mennan S., 2019. Screening of *Cucurbita maxima* and *Cucurbita moschata* genotypes for resistance against *Meloidogyne arenaria*, *M. incognita*, *M. javanica*, and *M. luci*. *Journal of Nema-*

tology 51: 2019–2057. https://doi.org/10.21307/jofnem-2019-057

Bent E., Loffredo A., McKenry M.V., Becker J.O., Borneman J., 2008. Detection and investigation of soil biological activity against *Meloidogyne incognita*. *Journal of Nematology* 40(2): 109–118.

Brown C.R., Mojtahedi H., Santo G.S., Williamson V.M., 1997. Effect of the *Mi* gene in tomato on reproductive factors of *Meloidogyne chitwoodi* and *M. hapla*. *Journal of Nematology* 29(3): 416-419.

Castagnone-Sereno P., 2012. *Meloidogyne enterolobii* (= *M. mayaguensis*): profile of an emerging, highly pathogenic, root-knot nematode species. *Nematology* 14(2): 133–138. https://doi.org/10.1163/156854111X601650

Elling A.A., 2013. Major emerging problems with minor *Meloidogyne species*. *Phytopathology* 103: 1092–1102. https://doi.org/10.1094/PHYTO-01-13-0019-RVW

EPPO, 2016. PM 7/41 (3) *Meloidogyne chitwoodi* and *Meloidogyne fallax*. *Bulletin OEPP/EPPO* 46(2): 171–189.

EPPO, 2017. EPPO Alert List: Addition of *Meloidogyne luci* together with *M. ethiopica*. EPPO Reporting Service, 2017/2018. Available at: https://gd.eppo.int/reporting/article-6186. Accessed August 05, 2023

EPPO, 2023a. EPPO Alert List 2013-10. Available at: https://www.eppo.int/ACTIVITIES/plant_quarantine/alert_list. Accessed November 03, 2023

EPPO, 2023b. *Meloidogyne chitwoodi.* EPPO global database. Available at: https://gd.eppo.int/taxon/MELGCH Accessed November 03, 2023

EPPO, 2023c. *Meloidogyne enterolobii*. EPPO global database. Available at: https://gd.eppo.int/taxon/MELGMY Accessed August 05, 2023

EPPO, 2023d. *Meloidogyne luci*. EPPO global database. Available at: https://gd.eppo.int/taxon/MELGLC Accessed August 05, 2023

Expósito A., Munera M., Giné A., López-Gómez M., Cáceres A., … Sorribas F.J., 2018. *Cucumis metuliferus* is resistant to root-knot nematode *Mi1.2* gene (a)virulent isolates and a promising melon rootstock. *Plant Pathology* 67: 1161–1167. https://doi.org/10.1111/ppa.12815

Expósito A., García S., Giné A., Escudero N., Sorribas F.J., 2019. *Cucumis metuliferus* reduces *Meloidogyne incognita* virulence against the *Mi1.2* resistance gene in a tomato-melon rotation sequence. *Pest Management Science* 75: 1902–1910. https://doi.org/10.1002/ps.5297

Expósito A., Pujolà M., Achaerandio I., Giné A., Escudero N., … Sorribas F.J., 2020. Tomato and melon *Meloidogyne* resistant rootstocks improve crop yield but melon fruit quality is influenced by the cropping season. *Frontiers Plant Science* 11: 560024-14. https://doi.org/10.3389/fpls.2020.560024

Fassuliotis G., 1970. Resistance of *Cucumis* spp. to the root-knot nematode, *Meloidogyne incognita acrita*. *Journal of Nematology* 2: 174–178.

Fullana A.M., Expósito A., Escudero N., Cunquero M., Loza-Alvarez P., … Sorribas F.J., 2023. Crop rotation with *Meloidogyne*-resistant germplasm is useful to manage and revert the (a)virulent populations of Mi1.2 gene and reduce yield losses. *Frontiers Plant Science* 14: 1133095-13. https://doi.org/10.3389/fpls.2023.1133095

García-Mendívil H.A., Munera M., Giné A., Escudero N., Picó M.B., … Sorribas F.J., 2019. Response of two *Citrullus amarus* accessions to isolates of three species of *Meloidogyne* and their graft compatibility with watermelon. *Crop Protection* 119: 208–213. https://doi.org/10.1016/j.cropro.2019.02.005

Giné A., López-Gómez M., Vela M.D., Ornat C., Talavera M., … Sorribas F.J., 2014. Thermal requirements and population dynamics of root-knot nematodes on cucumber and yield losses under protected cultivation. *Plant Pathology* 63(6): 1446–1453. https://doi.org/10.1111/ppa.12217

Guner N., Wehner T.C., Pitrat M., 2008. Overview of potyvirus resistance in watermelon. In: *Proceedings of the IXth EUCARPIA Meeting on Genetics and Breeding of* Cucurbitaceae. (M Pitrat ed.), Institut National de la Recherche Agronomique, Avignon, France, 445–451.

Gusmini G., Song R., Wehner T.C., 2005. New sources of resistance to gummy stem blight in watermelon. *Crop Science* 45(2): 582–588. https://doi.org/10.2135/cropsci2005.0582

Hadisoeganda W.W., Sasser J.N., 1982. Resistance of tomato, bean, southern pea, and garden pea cultivars to root-knot nematodes based on host suitability. *Plant Disease* 66: 145–150.

Hanna H.Y., 2000. Double-cropping muskmelons with nematode-resistant tomatoes increases yield, but mulch color has no effect. *HortScience* 35: 1213–1214. https://doi.org/10.21273/HORTSCI.35.7.1213

Holbrook C.C., Knauf D.A., Dickson D.W., 1983. A technique for screening peanut for resistance to *Meloidogyne incognita*. *Plant Disease* 57: 957–958.

Hussey R.S., Barker K.R., 1973. A comparison of methods of collecting inocula of *Meloidogyne* spp., including a new technique. *Plant Disease Report* 57: 1025–1038.

Jones J.T., Haegeman A.J., Danchin E.G., Gaur H.S., Helder J.K., … Perry R.N., 2013. Top 10 plant-parasitic nematodes in molecular plant pathology.

Molecular Plant Pathology 14: 946–961. https://doi.org/10.1111/mpp.12057

Keinath A.P., Wechter W.P., Rutter W.B., Agudelo P.A., 2019. Cucurbit rootstocks resistant to *Fusarium oxysporum f.* sp. niveum remain resistant when coinfected by *Meloidogyne incognita* in the field. *Plant Disease* 103: 1383–1390. https://doi.org/10.1094/PDIS-10-18-1869-RE

Koutsovoulos G.D., Poullet M., Elashry A., Kozlowski D.K., Sallet E., … Danchin E.G., 2020. Genome assembly and annotation of *Meloidogyne enterolobii*, an emerging parthenogenetic root-knot nematode. *Scientific Data* 7(1): 324. https://doi.org/10.1038/s41597-020-00666-0

Li X., Sun Y., Yang Y., Yang X., Xue W., … Chen S., 2021. Transcriptomic and histological analysis of the response of susceptible and resistant cucumber to *Meloidogyne incognita* infection revealing complex resistance via multiple signalling pathways. *Frontiers Plant Science* 12: 675429-675441. https://doi.org/10.3389/fpls.2021.675429

Ling J., Mao Z., Zhai M., Zeng F., Yang Y., Xie B., 2017. Transcriptome profiling of *Cucumis metuliferus* infected by *Meloidogyne incognita* provides new insights into putative defense regulatory network in Cucurbitaceae. *Scientific Reports* 7: 3544–3559. https://doi.org/10.1038/s41598-017-03563-6

López-Gómez M., Giné A., Vela M.D., Ornat C., Sorribas F.J., … Verdejo-Lucas S., 2014. Damage functions and thermal requirements of *Meloidogyne javanica* and *Meloidogyne incognita* on watermelon. *Annals of Applied Biology* 165(3): 466–473. https://doi.org/10.1111/aab.12154

Maleita C., Cardoso J.M., Rusinque L., Esteves I., Abrantes I., 2021. Species-specific molecular detection of the root knot nematode *Meloidogyne luci*. *Biology* 10*:* 775–788. https://doi.org/10.3390/biology10080775

Maleita C., Correia A., Abrantes I., Esteves I., 2022. Susceptibility of crop plants to the root-knot nematode *Meloidogyne luci*, a threat to agricultural productivity. *Phytopathologia Mediterranea* 61: 169–179. doi: 10.36253/phyto-13369

Ornat C., Verdejo-Lucas S., Sorribas F.J., 1997. Effect of the previous crop on population densities of *Meloidogyne javanica* and yield of cucumber. *Nematropica* 27: 85–90.

Pais C.S., Abrantes I., Fernandes M.F.M., Santos M.S.N.A., 1986. Técnica de electroforese aplicada ao estudo das enzimas dos nemátodes-das-galhas-radiculares, *Meloidogyne* spp. *Ciência Biológica Ecology and Systematics* 6: 19–34.

Phan N.T., Waele D., Lorieux M., Xiong L., Bellafiore S., 2018. A hypersensitivity-like response to *Meloidogyne graminicola* in rice (*Oryza sativa*). *Phytopathology* 108(4): 521–528. https://doi.org/10.1094/PHYTO-07-17-0235-R

Pinheiro J.B., Silva G.O.D., Oliveira V.R., Amaro G.B., Morais A.A.D., 2019. Prospection of genetic resistance resources to root-knot nematodes in cucurbit genotypes. *Horticultura Brasiliera* 37: 343–347. https://doi.org/10.1590/S0102-053620190314

Roberts P.A., 2002. Concepts and consequences of resistance. In: *Plant Resistance to Parasitic Nematodes* (J.L. Starr, R. Cook, J. Bridge, ed.), Wallingford, UK: CAB International, 25–41.

Sorribas F.J., Ornat C., Verdejo-Lucas S., Galeano M., Valero J., 2005. Effectiveness and profitability of the Mi-resistant tomatoes to control root-knot nematodes. *European Journal of Plant Pathology* 111: 29–38. https://doi.org/10.1007/s10658-004-1982-x

Sorribas F.J., Djian-Caporalino C., Mateille T., 2020. Nematodes. In: *Integrated pest and disease management in greenhouse crops* (M.L. Gullino, R. Albajes, P.C. Nicot, ed.), Springer Cham, Switzerland, 147–174.

Tetteh A.Y., Wehner T.C., Davis A.R., 2010. Identifying resistance to powdery mildew race 2W in the USDA-ARS watermelon germplasm collection. *Crop Science* 50(3): 933-939. https://doi.org/10.2135/cropsci2009.03.0135

Thies J.A., Levi A., 2007. Characterization of watermelon (*Citrullus lanatus* var. citroides) germplasm for resistance to root-knot nematodes. *HortScience* 42(7): 1530–1533. https://doi.org/10.21273/HORTSCI.42.7.1530

Thies J.A., Ariss J.J., Hassell R.L., Buckner S., Levi A., 2015. Accessions of *Citrullus lanatus* var. citroides are valuable rootstocks for grafted watermelon in fields infested with root-knot nematodes. *HortScience* 50: 4–8. https://doi.org/10.21273/HORTSCI.50.1.4

Waldo B.D., Branham S.E., Levi A., Wechter W.P., Rutter W.B., 2023. Distinct Genomic Loci Underlie Quantitative Resistance to *Meloidogyne enterolobii* Galling and Reproduction in *Citrullus amarus*. *Plant Disease* 107(7): 2126–2132. https://doi.org/10.1094/PDIS-09-22-2228-RE

Walters S.A., Wehner T.C., Daykin M.E., Barker K.R., 2006. Penetration rates of root-knot nematodes into *Cucumis sativus* and *C. metuliferus* roots and subsequent histological changes. *Nematropica* 36: 231–242.

Xie X., Ling J., Mao Z., Li Y., Zhao J., … Xie B., 2022. Negative regulation of root-knot nematode parasitic behavior by root-derived volatiles of wild relatives of

Cucumis metuliferus CM3. *Horticulture Research* 9: uhac051. https://doi.org/10.1093/hr/uhac051

Ye D.Y., Qi Y.H., Cao S.F., Wei B.Q., Zhang H.S., 2017. Histopathology combined with transcriptome analyses reveals the mechanism of resistance to *Meloidogyne incognita* in *Cucumis metuliferus*. *Journal of Plant Physiology* 212: 115–124. https://doi.org/10.1016/j.jplph.2017.02.002