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Upward migration of second-stage juveniles of *Meloidogyne floridensis* and *M. incognita* under different plant stimuli

Diego Arruda Huggins de Sá Leitão · Elvira Maria Régis Pedrosa · Donald Ward Dickson · Janete Andrade Brito · Ana Karina dos Santos Oliveira · Mario Monteiro Rolim

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Abstract The vertical migration of second-stage juveniles (J2) of *Meloidogyne floridensis* and *M. incognita* was investigated in segmented 14-cm long PVC soil columns having their top ring in contact with soil of a potted plant. Both bottom and top rings were screened appropriately to allow only upward nematode movement and preventing root penetration from the potted plant into the soil in the column. Plants used for nematode's stimuli were the nematode attractive tomato (*Solanum lycopersicum* L.) 'Cobra' and the nematode repellent French marigold (*Tagetes patula* L.) var. Petite. Host-free columns were used as control. Columns with and without plants were placed in growth chambers at 20 °C and inoculated with each root-knot nematode species by injecting 1000 J2 through a hole in the basal ring of each column. Columns were dismantled at 3, 6 and 9 days after injection and J2 were extracted from soil of each ring and the planted pot; additionally, roots in the top pot were stained to observe J2 penetration. No preferential upward migration towards either plant stimuli was observed, but *M. floridensis* was more migrant than *M. incognita*, with 4.4% and 1.6% of active J2, respectively, reaching an upward distance of more than 13 cm regardless of plant stimuli. This study supports random migration of root-knot nematode J2, even without stimulus from a host plant.

Keywords Attraction assay · Behavior · Mobility · Root-knot nematode · Soil column · *Tagetes patula*

D. A. H. de Sá Leitão · D. W. Dickson
Entomology and Nematology Department, University of Florida, Gainesville, FL 32611, USA

E. M. R. Pedrosa (✉) · A. K. dos Santos Oliveira · M. M. Rolim
Agricultural Engineering Department, Federal Rural University of Pernambuco, Recife, PE 52171-900, Brazil
e-mail: elvira.pedrosa@ufrpe.br

J. A. Brito
Florida Department of Agriculture and Consumer Services, Division of Plant Industry, Gainesville, FL 32608, USA

Introduction

Plant-parasitic nematodes are major biotic stress factors of economically important agricultural crops (Sobkowiak et al., 2018). Their parasitism can cause yield losses that result in hundreds of billions of US dollars in losses (Kohli et al., 2018); about half of which may be attributed to *Meloidogyne* spp. (Fujimoto et al., 2010). The southern root-knot nematode, *M. incognita,* is the main soilborne pathogen of Solanaceae (Barbary et al., 2015) and it was recently ranked the most invasive root pathogen worldwide (Bebber et al., 2014). The peach root-knot nematode, *M. floridensis*, is a major pathogen of peach and is known to occur only in the United States, infecting peach rootstocks in Florida, South Carolina and California (Handoo et al., 2004; Reighard et al., 2019; Westphal et al., 2019). Additionally, this nematode species has been reported parasitizing field grown tomato (Church, 2005; Marquez et al., 2020), cucumber, eggplant, tomato, snap bean, and

squash (Brito et al., 2015; Smith et al., 2015). This species is able to overcome RMia and Mij genes in peach rootstocks used for the production of peach and almonds, *Mi-1*-carrying tomato cultivars, N gene in the pepper 'Charleston Belle', corn 'Mp-710' and tobacco 'N 95' (Claverie et al., 2004; Lu et al., 1999; Stanley et al., 2009; Vau, 2017).

The second-stage juveniles (J2) of *Meloidogyne* spp. move freely through soil pore spaces to find or locate host roots. This behavioral response may be altered by soil conditions (Fujimoto et al., 2009, Sharpe et al., 1969, Wallace, 1958a, Wallace, 1958b, Wallace, 1958c, Wallace, 1959), including the gradient of root exudates concentrations dissolved in the soil solution. Root exudates play a crucial role in J2 migration, as they are the main source of chemical cues to guide nematodes to the root tips to start the infection process (Dutta et al., 2012). Such exudates range from polysaccharides and proteins to small lipophilic molecules (Haichar et al., 2014; Rasmann et al., 2012). These molecules may act as repellents, attractants, or be neutral to nematode motility (Hiltpold et al., 2015).

Nematodes have co-evolved with plant species to recognize and respond to host stimuli (Dutta et al., 2012), thus chemotaxis is reported to be a leading factor for nematodes' host-location abilities (Reynolds et al., 2011). These authors observed that *M. incognita* and *M. graminicola* migrated towards preferred hosts (tomato and rice, respectively) and no significant attraction behavior was observed towards mustard (nonhost for both species). The hypothesis that plant-parasitic nematodes locate and freely move through soil to infect hosts (Steiner, 1925) has led to several studies about chemotactic responses. Chemotaxis have been evaluated mainly as two-dimensional and horizontal migration patterns by J2 on agar-filled plates (Castro et al., 1990; Dusenbery, 1983; Hewlett et al., 1997; Huettel & Jaffe, 1987). However, a three-dimensional soil system has been evaluated by Spence et al. (2008). The three-dimensional system includes using columns filled with sand or soil as the migration medium. The test nematode is placed at one end of the column and a bait plant placed at the opposite end, then J2 can migrate over different distances and periods of time. This system has been used by Prot (1976, 1978) and Prot and van Gundy (1981a) to evaluate the host-finding ability of *M. javanica* and *M. incognita*, and by Pinkerton et al. (1987) to assess preferential migration of *M. chitwoodi* in columns with or without tomato plants. However, there is no information regarding the ability of *M. floridensis* to move in vertical columns baited with host and nonhost plant species.

This study aimed to evaluate the vertical migratory ability of *M. floridensis* and *M. incognita* J2 in columns baited with different plant stimuli: tomato (*Solanum lycopersicum* L. 'Cobra'– attractive stimulus), French marigold (*Tagetes patula* L. var. Petite – repellent stimulus), and control treatment with no bait plant. Our objectives were to determine whether: i) both root-knot nematode species would migrate greater distances when exposed to plant stimuli; ii) J2 of both nematode species exhibit different migration patterns under tomato and French marigold; and iii) French marigold is less attractive than tomato to both *Meloidogyne* species. This research focused on understanding the migration behavior of *M. floridensis*.

Materials and methods

Nematode culturing

Isolates of *M. floridensis* and *M. incognita* were maintained and reared on tomato 'Cobra' in 25-cm-diameter clay pots filled with sandy soil in greenhouses at the University of Florida, Gainesville, FL, USA. Five thousand eggs of each root-knot nematode species were inoculated separately per plant.

Sixty-day old plants were selected for egg extraction using 0.52% NaOCl (Hussey & Barker, 1973). The egg suspension was poured into a modified Baermann funnel at 27 °C in 2-ply facial tissue paper (Rodríguez-Kábana & Pope, 1981). The J2 that hatched during the first 24 h were discarded. For the migration assays, we collected freshly hatched J2 daily for 3 days by pouring the suspension onto a 25-μm openings sieve and stored them at 4 °C until the beginning of the experiment.

Tomato and French marigold seedlings

Seeds of tomato 'Cobra' and French marigold var. Petite were sown, one seed per seedling tray cell, into plastic trays (Park Seed Co., Hodges, SC, USA). We used vermiculite as substrate to fill the trays cells and kept them under greenhouse conditions (mean daytime temperature of 28 °C). For the migration and penetration studies, 4-week-old tomato and French marigold seedlings were used. Roots were rinsed with tap water to remove vermiculite debris and transplanted into

Styrofoam cups, which were attached with a tape to the top of the columns 24 h before nematode injection.

Experimental columns

The J2 migration was assayed in polyvinyl chloride (PVC) columns (Fig. 1) (Pinkerton et al., 1987). We assembled each column using a water-resistant tape (3 M, St. Paul, MN, USA) to connect together three 4-cm long PVC rings placed on top of a 2-cm long injection ring and placed each column inside an empty support clay pot. A 1-cm-diameter hole was drilled into the center of the injection ring to allow for injecting J2 into the columns. The total length of each column was 14 cm with 4.4-cm internal diameter and 213-cm^3 internal volume. We filled the columns with heat-pasteurized (88 °C for 1 h) Candler sand (Hyperthermic, uncoated Lamellic Quartzipsamments) (Table 1) originally collected from a peanut field in Levy County, FL, USA. Soil inside the columns was kept at 1.2 kg dm^{-3} soil bulk density and 10% water content by weight, to mimic field conditions. The base of the injection ring was screened with Tegape nylon mesh (Tegape, Inc., Paraná, Brazil) with 15-μm openings to contain the J2 in the system. A parafilm sheet was placed on the top end of each column to prevent premature evaporative water loss until the beginning of the migration assays.

We attached one bottomless 500-cm^3 Styrofoam cups containing 300 g of the same soil at 10% water content to the top end of each column. One seedling of either tomato (attractive stimulus) or French marigold (repellent stimulus) was transplanted into the cups; untreated control consisted of seedling-free cups (no stimulus). Cups with plants had the bottom screened with a 35-μm Tegape nylon mesh, which was adhering to the soil of the top ring of each column to prevent root growth into the columns while allowing J2 to migrate through the column (Prot, 1976). Fully assembled columns (three per stimulus treatment for a total of 72 columns) were transferred to growth chambers at 20 °C, and a photoperiod regime of 16 h light/8 h dark.

Freshly-hatched J2 were counted under a stereomicroscope Labophot (Nikon, Tokyo, Japan) at 100× magnification and the suspensions were adjusted to at least 500 ± 50 J2 ml^{-1}. About 1000 ± 100 mobile J2 (≈2 ml of solution) were injected into each column through the hole in the injection ring (1 cm above the column base) after 24 h of acclimatization of columns inside growth chambers; the holes were sealed with waterproof tape following injection. No water was added to the columns at that moment to prevent premature nematode percolation. Columns were weighed daily, and enough water was added to replace that lost by evapotranspiration and maintaining the soil moisture at 10% throughout the experiment (Pudasaini et al., 2007). Water to moisturize the soil was kept at same temperature in plastic bottles inside the growth chambers, to prevent temperature fluctuation during and after watering. No fertilizers were applied to columns.

The columns for each treatment were dismantled 3, 6 and 9 days after J2 injection (DAI). Soil (80 g) was collected from each ring and planted Styrofoam cup separately at each harvesting time. We used a metal spatula to separate each ring and washed it thoroughly after each use. Nematodes from the soil of each column and cup were extracted by a centrifugal-flotation technique (Jenkins, 1964). Nematodes were counted to determine the total number of recovered and active J2 in each ring; we considered active those J2 that were moving regardless of the intensity. Concomitantly, tomato and French marigold root systems were washed free of debris and stained with acid fuchsin (Byrd Jr. et al., 1983). The J2 that penetrated the roots were counted under a stereomicroscope SteREO v. 20 (Carl Zeiss, Göttingen, Germany). Plant fresh shoot and root weights were recorded to verify the influence of the plant growth on nematode migration. Plant and nematode data are expressed as mean of four replications. The data obtained from the three treatments were also cumulated for their statistical analysis.

Statistical analysis

We used a completely randomized block design with four replications, totaling 72 soil columns and 360 experimental units. Nematode data were subjected to transformation via $\sqrt{x} + 0.5$-before statistical analysis to normalize the data. Data on the effect of plant stimuli (three stimuli – tomato, French marigold and no stimulus), distance migrated (five rings: <1, 1–5, 5–9, 9–13 and > 13 cm), and time (three times – 3, 6 and 9 DAI) on *M. floridensis* and *M. incognita* upward migration were subjected to analysis via a repeated measure Multivariate Analysis of Variance (MANOVA). When significant effects were observed, a chi-square (χ^2) test was performed to compare the distribution of J2 along the

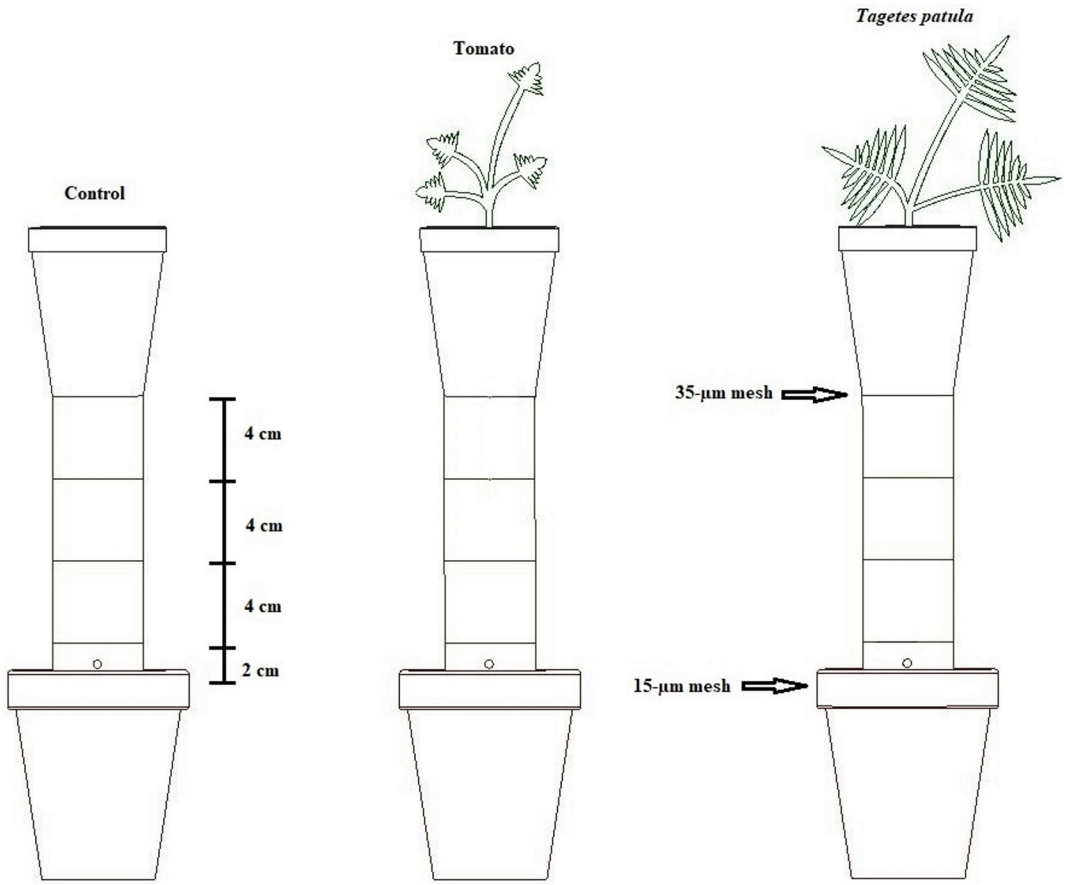

Fig. 1 Illustration of PVC soil column used in the experiment. Column contains three 4.4-cm-diameter × 4-cm long rings taped together to one 2-cm long ring (injection ring) each filled with sandy soil. Four-week-old tomato or French marigold (*Tagetes patula*) seedlings, or no seedling (control), were placed on the top of the columns to serve as migration stimuli. Freshly hatched juveniles were injected 1 cm above the base of the columns through an injection point

columns. Tukey's multiple comparison test was used for analysis of the number J2 detected per gram of roots. Pearson's correlation coefficients between plant variables and nematodes inside roots were also determined. All statistical analyses were performed in R Programming software R 3.5.1 (RStudio, Boston, MA, USA).

Results

No nematodes were found trapped in the bottom 15-µm or top 35-µm nylon mesh nor on the walls of the PVC rings, upon termination of each nematode extraction from soil. The distribution of recovered J2 along the

Table 1 Physical and chemical attributes of Candler soil used in nematode migration assays

Physical attributes	Unit	Depth (m) 0.00–0.40	Chemical attributes	Unit	Depth (m) 0.00–0.40
Sand	%	96	pH	(1:2)	5.6
Silt	%	2	Extractable P	mg Kg^{-1}	>135
Clay	%	2	Extractable K	mg Kg^{-1}	13
Texture		Sandy	Extractable Mg	mg Kg^{-1}	0
Bulk density	kg dm^{-3}	1.2	Extractable Ca	mg Kg^{-1}	164
Water content	%	10	Organic Matter	%	0.27

columns was influenced by plant stimuli ($P < 0.05$, Table 2). However, the presence of tomato or French marigold roots did not influence the rate of migration of active J2 as compared to the controls ($P \geq 0.05$, Table 2). There were significant differences between nematode species and distance migrated as well as time and distance migrated for J2 of *M. incognita* and *M. floridensis* ($P \leq 0.01$, Table 2).

Fewer J2 were recovered from the soil of control columns when compared to columns with plant stimuli ($P < 0.05$, Table 3). Although we found a greater number of J2 of *M. incognita* in the soil, the number of *M. floridensis* J2 inside the roots of both tomato and French marigold was three-fold greater than *M. incognita* ($P < 0.05$, Table 3).

Differences on the distribution of both recovered and active J2 of *M. floridensis* and *M. incognita* were observed over time and along the rings of the columns (Table 4). As there was no interaction between nematode species and plant stimulus, data for control, tomato and French marigold columns were combined (Fig. 2). A steady migration pattern was observed for *M. floridensis*, whereas most of *M. incognita* J2 remained in the injection ring (<1 cm). More than 1300 (40%) J2 of *M. floridensis* were recovered in the injection ring of tomato, French marigold and control columns, which was around 2-fold less than that observed for *M. incognita* (Fig. 2A). As the distance from the injection point increased, there was a decrease in the number of recovered J2 of both nematode species; however, 150 (5%) J2 of *M. floridensis* were found at distances greater than 13 cm (Fig. 2A). Although many J2 did not move away from the injection ring (<1 cm), 79 (1.6%) and 123 (4.4%) active J2 of *M. incognita* and *M. floridensis*, respectively, were able to migrate more than 13 cm towards the upper ring of the columns (Fig. 2B). The migration pattern of *M. incognita* was as follows: 14% of active J2 migrated up to 5 cm, 8% migrated up to 9 cm, 2% migrated up to 13 cm, while 33%, 21% and 10% of active J2 of *M. floridensis* migrated up to 5, 9 and 13 cm, respectively (Fig. 2B).

No significant interaction between nematode species with nematode travel distance and time was observed, thus data of *M. floridensis* and *M. incognita* were combined (Fig. 3). The highest percentages of J2 of both nematode species were found in the injection ring (<1 cm) at all harvesting times (Fig. 3). Nonetheless, there was a gradual decrease in the number of J2 recovered over time at <1 cm (Fig. 3). Although few J2 (< 0.5%) were able to reach the top of the column at 3 DAI, the vast majority remained in the injection ring (<1 cm) throughout the experiment, with an average of 3839 (84%), 2426 (64%) and 1522 (54%) J2 at 3, 6 and 9 DAI, respectively (Fig. 3A). An increase in the number of recovered J2 was observed on the upper rings over time, with almost 151 (6%) at the top of the columns at 9 DAI. At 3 DAI almost 80% of active J2 remained in the injection ring (<1 cm) and very few were observed at distances greater than 9 cm. At 6 DAI, 989 (34%) active J2 migrated 1 to 9 cm and 76 (2.7%) reached the Styrofoam cup (> 13 cm). At 9 DAI, 1093 (56%) active J2 were distributed throughout the length of the column, with approximately 76 (4%) at the Styrofoam cup (Fig. 3B).

The penetration of J2 did not differ between *M. incognita* and *M. floridensis* ($P \geq 0.05$) but the penetration was higher for tomato than French marigold ($P < 0.01$). Time alone influenced nematode penetration ($P < 0.0001$), and its interaction with stimulus ($P < 0.01$) was also significant (Table 5).

No J2 were found in roots of either plant species at 3 DAI regardless of the root-knot nematode species (Fig. 4), though 11 (0.4%) active J2 had already migrated up to >13 cm (Fig. 3A). Overall, the greatest average number of J2 inside roots was observed at 9 DAI under tomato, with an average of 2.2 J2 per g of fresh roots. The average number of J2 inside tomato roots at 6 DAI was very similar (0.56 J2 per g of fresh roots) as those in French marigold at 9 DAI (0.58 J2 per g of fresh roots) (Fig. 4).

Discussion

In the present study, less than 1% of *M. floridensis* and *M. incognita* J2 were able to migrate more than 13 cm vertically regardless of plant stimuli during a period of 9 DAI at 20 °C, which is an optimal temperature for maximum migration of *M. incognita* (Prot & van Gundy, 1981b). Similar recovery rates were observed for *M. chitwoodi* in 45-cm long columns (Pinkerton et al., 1987) and similar migration patterns were reported for *P. penetrans* in columns with different lengths (Pudasaini et al., 2007). Conflicting data were reported, where up to 50% of *M. javanica* J2 and 30% of *M. incognita* were recovered from tomato roots after migrating 50 cm and 20 cm from point of injection after 9 DAI, respectively (Prot, 1976; Prot & van Gundy,

Table 2 Repeated measure MANOVA summary of the effects of plant stimuli, distance migrated (rings) and time on recovered and active second-stage juveniles (J2) of *Meloidogyne incognita* and *M. floridensis* vertical migration along PVC columns filled with sandy soil at 20 °C

		Recovered J2				Active J2			
Source	df	SS	MS	F	*p* value	SS	MS	F	p value
Block	3	56.70	18.90	5.86	0.0007	64.90	21.60	7.61	<0.0001
Nematode (Nema)	1	116.90	116.90	36.23	<0.0001	43.20	43.20	15.18	0.0001
Stimulus (Stim)	2	22.60	11.30	3.50	0.0317	15.60	7.80	2.75	0.0660
Ring	4	2899.60	724.90	224.76	<0.0001	1632.70	408.20	143.49	<0.0001
Nema:Stim	2	3.80	1.90	0.60	0.5490	3.70	1.90	0.66	0.5185
Nema:Ring	4	895.60	223.90	69.43	<0.0001	524.30	131.10	46.08	<0.0001
Stim:Ring	8	11.20	1.40	0.45	0.8927	10.20	1.30	0.45	0.8903
Nema:Stim:Ring	8	18.40	2.30	0.71	0.6826	12.10	1.50	0.53	0.8308
Time	2	16.40	8.20	2.53	0.0818	20.70	10.40	3.64	0.0276
Time:Nema	2	0.80	0.40	0.14	0.8739	1.10	0.50	0.19	0.8283
Time:Stim	4	10.00	2.50	0.78	0.5400	12.90	3.20	1.13	0.3421
Time:Ring	8	330.40	41.30	12.80	<0.0001	177.40	22.20	7.80	<0.0001
Time:Nema:Stim	4	11.20	2.80	0.87	0.4855	6.80	1.70	0.60	0.6636
Time:Nema:Ring	8	42.20	5.27	1.64	0.1147	37.90	4.70	1.67	0.1064
Time:Stim:Ring	16	60.80	3.80	1.17	0.2897	49.20	3.10	1.08	0.3736
Time:Nema:Stim:Ring	16	43.20	2.70	0.83	0.6458	39.40	2.50	0.87	0.6091

df degree of freedom, *SS* Sum of squares, *MS* Mean square

1981a). Differences in soil texture and column dimension might explain such discrepancies.

Since nematode migration is influenced by soil physical attributes (soil pore size and connectedness, water content and percentage of silt and clay), coarser soils might hinder J2 movement towards plant roots. Nematode migration is dependent on pore size and J2 body diameter relation, as well as the thickness of water films adhered to soil particles (Wallace, 1968). In our experiment, we used a very sandy soil (96% sand), which does not hold as much water. Furthermore, the low percentage of clay particles in the Candler sand

Table 3 Recovery rates of cumulated second-stage juveniles (J2) of *Meloidogyne floridensis* and *M. incognita* along segmented PVC columns (14-cm long) under different plant stimuli

	Recovered J2	Active J2	J2 inside roots
	Nematode species[a]		
Meloidogyne floridensis	73.03b[b]	61.22b	3.42a
Meloidogyne incognita	175.07a	112.83a	1.00a
	Stimulus[c]		
Control (plant-free)	111.33b	78.47a	–
Tomato (*Solanum lycopersicum*)	128.87a	87.62a	3.50a
French marigold (*Tagetes patula*)	131.96a	94.99a	0.92b

[a] Average number of recovered or active J2 extracted from soil, or J2 per fresh root weight, refers to all three harvesting times. Data of control, tomato and French marigold were combined for nematode species effect

[b] Means followed by different letters within columns are statistically significant according to Tukey's test ($P \leq 0.05$)

[c] Average number of recovered or active J2 extracted from soil, or J2 per g of fresh roots, refers to all three harvesting times. Data of nematode species were combined for stimulus effect

Table 4 Contingency tables, χ^2 analysis for recovery rates of cumulated second-stage juveniles (J2) of *Meloidogyne floridensis* and *M. incognita* over time along rings of PVC columns (14-cm long) filled with sandy soil at 20 °C with and without plant stimuli

	Mean of recovered J2 per ring					χ^2 (p value)	Mean of active J2 per ring					χ^2 (p value)
	<1 cm	1–5 cm	5–9 cm	9–13 cm	>13 cm		<1 cm	1–5 cm	5–9 cm	9–13 cm	>13 cm	
Nematode species												
Meloidogyne incognita	717.11	85.22	49.47	13.81	9.72		419.78	77.17	46.42	12.03	8.78	
Meloidogyne floridensis	125.78	102.86	66.97	30.61	16.72	1918.1 (<0.01)	97.78	100.59	65.86	29.72	13.67	1378.0 (<0.01)
Time												
3 DAI[a]	639.83	81.58	39.58	1.42	1.83		362.17	72.92	38.08	1.42	1.83	
6 DAI	404.25	94.75	77.38	38.88	12.67		252.25	89.09	74.21	38.21	12.67	
9 DAI	253.58	105.79	57.71	26.33	25.17	1019.6 (<0.01)	161.92	102.38	56.13	23.67	12.67	679.4 (<0.01)

[a] DAI: Days after J2 injection

likely affects root-knot nematode J2 migratory ability. It is reported that clay particles hold root exudates and guide nematode towards roots to aid in penetration (Prot & van Gundy, 1981a).

The distance between the injection point and the host root is also important because nematodes might get disoriented, thereby consuming all their energy reserves before reaching the roots and become inactive or die (Pudasaini et al., 2007; Rocha et al., 2016). Another important factor governing nematode migration studies is the columns' internal diameter. Prot (1976) used columns with 1.2 cm internal diameter, but in our experiment, the columns' internal diameter was 4.4 cm. Smaller diameters will probably restrict nematode horizontal dispersal and impose vertical migration (Spence et al., 2008); therefore, it would be expected that a greater number of nematodes would be at the top of the experimental device if we used columns with smaller internal diameters.

The migration rate of *M. floridensis* and *M. incognita* along the columns with plant stimuli did not significantly differ from the control treatments. However, the downward percolation of irrigation water might have enhanced the juveniles' upward migration. Some studies highlight that entomopathogenic and plant-parasitic nematodes migrate towards and aggregate in wetter soil layers (Prot, 1978; Salame & Glazer, 2015; Wallace, 1960). No preferential migration of *M. chitwoodi* was observed in columns with and without tomato plants (Pinkerton et al., 1987); however, a greater number of

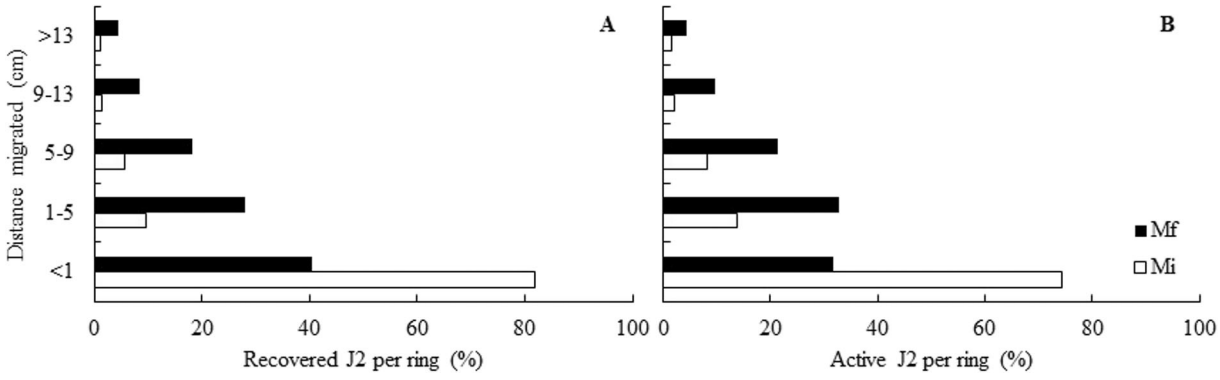

Fig. 2 Distribution of recovered (**A**) and active (**B**) second-stage juveniles (J2) of *Meloidogyne floridensis* (Mf) and *M. incognita* (Mi) at 20 °C along sandy soil columns, containing 4-cm long × 4.4-cm internal diameter rings. Bars represent the percentage of J2 extracted from control, tomato and French marigold columns for each nematode species ($n = 9$). Chi-square test results for recovered and active J2 are χ^2 (4) = 1918.1 ($P \leq 0.01$) and χ^2 (4) = 1378.0 ($P \leq 0.01$), respectively

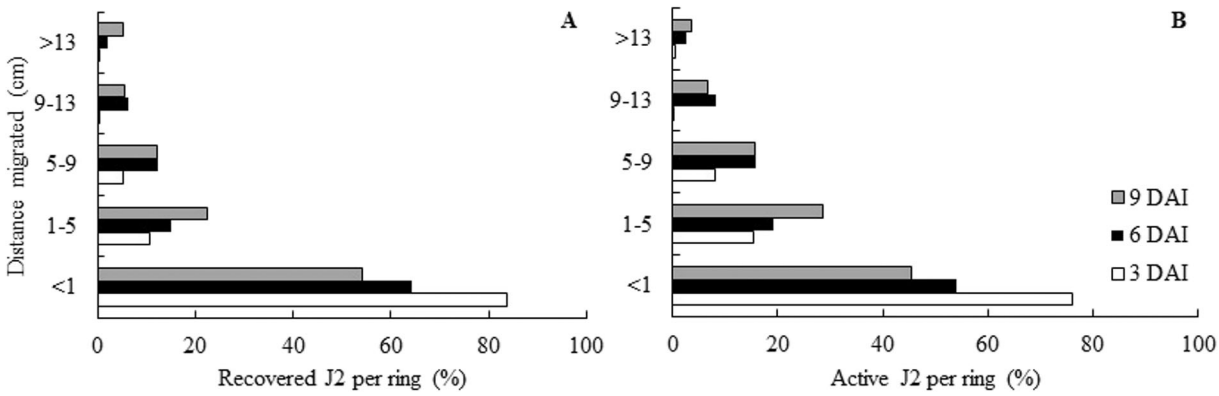

Fig. 3 Distribution of recovered (**A**) and active (**B**) second-stage juveniles (J2) of *Meloidogyne floridensis* and *M. incognita* at 20 °C along sandy soil columns, containing 4-cm long × 4.4-cm internal diameter rings. Bars represent the percentage of juveniles recovered at 3, 6 and 9 days after injection (DAI) ($n = 6$). Chi-square test results for recovered and active J2 are χ^2 (8) = 1019.6 ($P \leq 0.01$) and χ^2 (8) = 679.4 ($P \leq 0.01$), respectively

J2 reached the top of the control columns at 9 DAI, probably due to a moisture gradient created by top irrigation water.

Tagetes spp. have been reported as a nonhost for *M. floridensis* (Brito et al., 2015), where they showed a very low reproductive factor (Kokalis-Burelle & Nyczepir, 2004), and highly suppressive for *M. incognita* (Buena et al., 2008; Murga-Gutiérrez et al., 2012). Nevertheless, J2 may still migrate towards its roots, as we observed in our study. In fact, only a few J2 of both nematode species were able to penetrate French marigold roots after migrating over 13 cm; however, they did not show further development. There was no difference in the migration of *M. chitwoodi* in soil columns with tomato or *T. patula* extracts (Nježić et al., 2014).

In this study, the number of J2 recovered decreased as a function of distance migrated: at distances greater than 13 cm (Styrofoam cups), an average of 27 and 88 active J2 of *M. incognita* and *M. floridensis*, respectively, were found in the soil regardless of plant stimuli, whereas 831 and 141 active J2 of *M. incognita* and *M. floridensis*, respectively, were found in the injection ring (<1 cm) at 9 DAI. Horizontal and vertical migration studies with *Meloidogyne* and *Pratylenchus* spp. point out that most of the injected nematodes remain close to the injection ring in the short term (Francilino et al., 2017; Fujimoto et al., 2010; Nježić et al., 2014; Prot & van Gundy, 1981a; Pudasaini et al., 2007). Nevertheless, by the end of such studies J2 were almost evenly distributed within the columns. Our findings suggest that an even distribution of J2 of both nematode species would be expected if we had allowed the nematodes to migrate longer than 9 days.

Nematode host-recognition and migration are directly affected by allelochemicals released by plant roots; thus, root growth is crucial for migratory behavior of J2 of root-knot nematodes. When roots stop growing, the attractiveness to nematodes ceases, as it is proportional to the growth rate of the host root system (Prot, 1980). However, some species of root-knot nematodes do not need stimulus to migrate, as observed for *M. chitwoodi* (Pinkerton et al., 1987) and *M. arenaria* (Santos, 1973). Therefore, further studies about the migration of *Meloidogyne* spp. under different conditions and stimuli are important to understand their behavior within the soil.

Table 5 Repeated measure Multivariate Analysis of Variance (MANOVA) summary of penetration of second-stage juveniles (J2) of *Meloidogyne floridensis* and *M. incognita* into roots placed at 13 cm distance from the injection point at 20 °C over time

		J2 inside roots			
Source	df	SS	MS	F	p value
Block	3	0.21	0.07	1.32	0.2849
Nematode (Nema)	1	0.02	0.02	0.34	0.5639
Stimulus (Stim)	1	1.00	1.00	18.45	0.0001
Nema:Stim	1	0.14	0.14	2.51	0.1225
Time	2	2.99	1.50	27.67	<0.0001
Time:Nema	2	0.02	0.01	0.15	0.8656
Time:Stim	2	0.76	0.38	7.03	0.0029
Time:Nema:Stim	2	0.20	0.10	1.86	0.1719

df degree of freedom, *SS* Sum of squares, *MS* Mean square

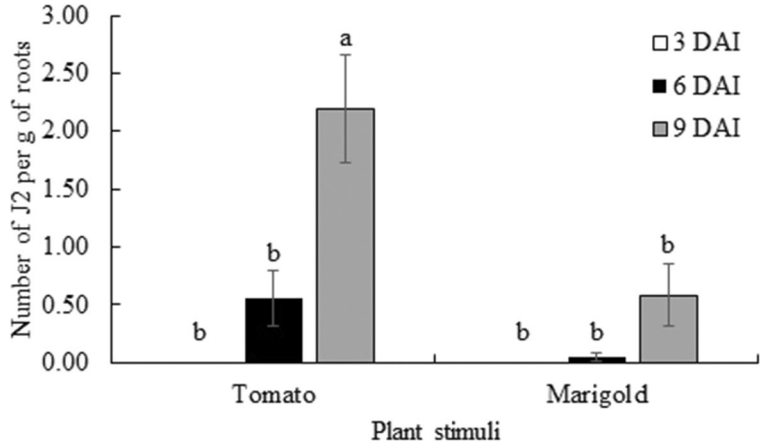

Fig. 4 Number of second-stage juveniles (J2) of *Meloidogyne floridensis* and *M. incognita* parasitizing tomato or French marigold (*Tagetes patula*) roots at 3, 6 and 9 days after injection (DAI). Bars followed by the same letter do not differ according to Tukey's multiple comparison test ($P \geq 0.05$)

In summary, both *M. floridensis* and *M. incognita* were able to migrate more than 13 cm and penetrate the roots of tomato and French marigold. Their upward migration occurred regardless of plant stimuli due to their random movement within the soil, but *M. floridensis* was more affected by plant stimuli than *M. incognita*. Although French marigold lured J2 of *M. floridensis* and *M. incognita* to their rhizosphere, few J2 penetrated their roots.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Research involving human participants and/or animal The research did not involve human participant or animals.

Informed consent N/A

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