RESEARCH/INVESTIGACIÓN

MIGRATION AND REPRODUCTION OF *MELOIDOGYNE INCOGNITA* IN TWO SOIL TEXTURES

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ABSTRACT

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The purpose of this study was to evaluate the migration of second-stage juveniles (J2) of *Meloidogyne incognita* at different distances from the root system of tomato cultivated by direct sowing or transplanted into either clay or sand, and to determine the effect of soil type on nematode infectivity and reproduction. Cylinders of 10 and 20 cm diameter, enclosed in a 0.36-mm diameter mesh and filled with clay soil or sand were placed in the center of 1-m^2 wooden boxes. Second-stage juveniles were placed inside the cylinder and tomato seedlings cv. 'Kada' were sown or transplanted externally surrounding the cylinder. The control consisted of two tomato plants cultivated in the center of the box without cylinder with clay or sand and infested with J2. The motility and neutral lipid content from J2 used before and those sampled 20 days after inoculation from the cylinders were estimated. Infectivity and reproduction of *M. incognita* were evaluated 30 days after inoculation. More J2 were recovered from the cylinders containing sand than clay soil, but the number of J2 was not affected by the type of planting (sowed or transplanted). The motility and the neutral lipid content of J2 extracted from the 20-cm cylinders filled with sand containing transplanted seedlings decreased drastically when compared to the control. Regarding the content of neutral lipids from J2 recovered from the cylinders, there was a significant interaction between soil texture and type of planting for neutral lipid content, and between the type of planting and the distance from inoculum deposition. Independent of the type of planting, lower nematode infectivity and reproduction were observed in J2 in sand than in clay soil. An interaction also was seen between soil texture and the type of planting for infectivity and reproduction. This is the first report of using a three-dimensional environment to monitor the distance *M. incognita* J2 migrate to a host root system, as influenced by soil texture, mediated by lipid loss and the type of planting on infectivity and reproduction of *M. incognita*.

Key words: Infectivity, neutral lipids, root-knot nematodes, *Solanum lycopersicum*.

RESUMO

Rocha, F. S., V. P. Campos, M. F. G. Fernandes, e M. F. S. Muniz. 2016. Migração e reprodução de *Meloidogyne incognita* sob duas texturas de solo. Nematropica 46:162-171.

O propósito deste estudo foi avaliar a migração de juvenis do segundo estádio (J2) de *M. incognita* colocados a diferentes distâncias do sistema radicular de tomateiros cultivados por semeadura direta ou transplantados em solo argiloso ou areia e seu efeito sobre a infectividade e reprodução. Para isto, no centro de caixas de madeira de 1m^2 foram colocados cilindros de 10 e 20 cm de diâmetro, revestidos nas laterais com tela de 0,36 mm de diâmetro de poro, contendo solo argiloso ou areia. Em seguida, os J2 foram colocados no interior dos cilindros e externamente ao seu redor foram semeadas ou transplantadas mudas de tomateiros cv. Kada. No controle duas plantas de tomate foram cultivadas no centro das caixas sem cilindro e inoculadas com J2. Estimaram-se a mobilidade e o teor de lipídios neutros dos J2 usados na infestação do solo e daqueles extraídos do interior dos cilindros aos 20 dias após infestação. Aos 30 dias após infestação, avaliaram-se a infectividade e a reprodução dos J2. Observou-se uma maior quantidade de J2 recuperados dos cilindros contendo areia do que em solo, mas não diferiram significativamente entre o tipo de plantio (semeadura direta ou transplantio). No entanto, a motilidade e teor de lípidos neutros dos J2 extraídos dos cilindros de 20 cm com areia contendo mudas transplantadas diminuíram drasticamente em comparação

com o controle. Em relação ao teor de lipídios neutros dos J2 recuperados dos cilindros houve interação entre o tipo solo e o tipo plantio, o tipo de plantio e a distância do inóculo. Independentemente do tipo de plantio, menor infectividade e reprodução foram observadas nos J2 colocados na areia em relação àqueles colocados em solo argiloso. Para a infectividade e a reprodução houve interação apenas entre o tipo de solo e o tipo de plantio. Este é o primeiro relato da utilização de um ambiente tridimensional para monitorar a migração de J2 de *M. incognita* em relação com a distância do sistema radicular da planta hospedeira, textura do solo mediada pela perda das reservas lipídicas e o tipo de plantio sobre a infectividade e reprodução de *M. incognita*.

Palavras chave: Infectividade, lipídios neutros, nematóide das galhas, *Solanum lycopersicum.*

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the most important vegetable crops grown in the world and consumed as fresh and(or) processed fruit. Root-knot nematodes, *Meloidogyne incognita*, *M. javanica*, and *M. hapla*, are the most common plant-parasitic nematodes in tomato-growing regions. Of these species, *M. incognita* is the most important root-knot nematode species causing economic losses in warm and sandy soils (Overman, 1991; Silva, 1991; Charchar *et al.*, 2003).

The infective stage of *Meloidogyne* spp. is the second-stage juvenile (J2), which penetrates the root near the root cap (Campos *et al*., 2011). The penetration process depends upon the directional movement of the J2 in response to attractive exudates released by the host plant and the lipid reserves of the J2 (Bird, 1959; Christophers *et al*., 1997; Zhao *et al.*, 2000; Campos *et al*., 2006). The distance from the stimulus source can change the orientation of the nematode toward the root due to the loss of stimulus, increasing the residence time in the soil and decreasing the body lipid content and the ability of the nematode to survive (Bergeson, 1959; Campos *et al*., 2006; Freire *et al*., 2007; Das *et al*., 2011). Other factors such as soil texture and structure, temperature, soil moisture, water flow, photoperiod, oxygen concentration, carbon dioxide concentration, pH, and concentration of the attractant and repellant exudates influence the migration and survival of the nematodes (Van Gundy and Stolzy, 1963; Wallace, 1968; Robbins and Barker, 1974; Prot and Van Gundy, 1981a, 1981b; Castro *et al.*, 1989; Castro *et al*., 1991; Robinson, 1995; Zhao *et al*., 2000; Melakeberhan *et al*., 2004; Freire *et al*., 2007; Fujimoto *et al*., 2009). For each nematode species there is an optimal size of soil particle for their movement in the soil (Wallace, 1958a, b).

On average *Pratylenchus penetrans*, *Heterodera schachtii*, *M. hapla*, and *M. incognita* species move between 2 and 20 cm toward a host root (Viglierchio, 1961; Lavallee and Rohde, 1962; Prot and Van Gundy, 1981a; Rocha *et al.*, 2008), but some populations of *M. javanica* and *M. incognita* have been reported to migrate up to 75 cm (Prot, 1976; Prot, 1978). The water-soluble chemical exuded from the plant roots are mainly for short-range chemotaxis, while the volatile substances, CO_2, and temperature are stimuli for longer range chemotaxis (Prot, 1980).

The distribution and diffusion of soluble attractive substances in water is more uniform in a homogeneous environment than in heterogeneous substrates. The ideal location for the behavioral study of nematode migration should allow natural movement of the nematode in a three-dimensional environment (soil), which has not been appropriately studied. Despite the migration studies reported in the literature, few studies emphasize the distance between the nematode and the root system of the host plant relative to successful infection of the host. The objectives of this study were to evaluate the migration of *M. incognita* J2 at different distances from the roots of direct sowed or transplanted tomato plants, in different soil textures, and the relationship of these factors to the lipid reserves and reproduction of the nematodes under greenhouse conditions.

MATERIALS AND METHODS

Meloidogyne incognita J2 used in this study were obtained from a greenhouse population maintained on tomato cv. Kada. Eggs were obtained by sectioning 1-cm long pieces of infected roots and grinding in 0.5% NaOCl for 40 sec in a blender (Hussey and Barker, 1973). Kaolin was used to remove root debris from the egg suspension (Coolen and D'Herde, 1972). The egg suspension was placed in a hatching chamber at 28°C, and only 24-hr-old J2 were used in the experiments.

The migration of *M. incognita* J2 was studied in a root-restricting chamber inserted into a planter box using the experimental apparatus shown in Figure 1A. The planter box was a 1-m^2 wooden box with 15 cm high borders. Ten and 20 cm diameter cylinders of the same height were positioned in the center of the box (Fig. 1B). Cylinders were made with an open wood ring at the top and a closed circular wood bottom. The side wall was covered and stapled with a 0.36-mm pore size screen to prevent root growth inside cylinder, but allowing the J2 to migrate through. Second-stage juveniles placed in the center of each cylinder would need to move either 5 or 10 cm and pass through a root-preventing mesh to reach tomato roots surrounding the cylinders.

Two migration experiments were conducted in these systems in a greenhouse. For experiment one, soil from a coffee plantation classified as Red Latosol (Oxisol) with clay texture was sieved through a 12-mm pore diameter metal sieve to remove large debris. This soil was mixed with plaster sand (coarse washed river sand) 1:1 (volume:volume) to improve soil physical characteristics for greenhouse experiments. Analyses of the clay and plaster sand composition showed the following physico-chemical characteristics: 65% clay, 18% silt, 17% sand, pH = 6.2 in water, 0.6% organic matter. The composite clay soil was autoclaved for 1 hr at 121°C to eradicate any plant-parasitic nematodes and then added to the boxes and inside the cylinders. Tomato plants were established outside the cylinders in the planter boxes by either direct sowing or transplanting. For direct sowing, several tomato seeds of cv. Kada were planted in 6 and 8 holes surrounding the 10- and 20-cm diam. cylinders, respectively, containing 50 cm^3 of Plantmax® substrate (Eucatex, Brazil). Seedlings were thinned to one per hole after emergence and maintained for 35 d in the greenhouse. For transplanting, 35-d-old tomato seedlings of cv. Kada grown in trays containing Plantmax® in the greenhouse were transplanted to the holes as previously described.

The J2 obtained from the hatching chambers were counted under an inverted microscope (Leitz, Oberkochen, Germany) at 100× magnification and the suspension was adjusted to 1,700 J2 per ml. Each cylinder was inoculated with approximately 17,000 J2 in 10 ml of suspension into a 5 cm deep hole made in the soil with a glass rod at the center of the cylinder. After infestation of the soil, the top of the cylinder was covered with aluminum foil to prevent loss of soil moisture. Two plants cultivated in the center of the boxes that were filled with clay soil without cylinders and inoculated with 17,000 J2 were used as controls. In control one, J2 were inoculated near the roots of transplanted tomato seedlings, whereas in control two, J2 were inoculated in the zone of developing roots of direct-sowed tomato seedlings. Plants were watered daily as needed by spraying and fertilized with basic solution as described in Hoagland and Arnon (1950). Soil temperature was measured with Davis k6470 sensors, installed next to the cylinders (~ 8 cm deep) and connected to a data logger (Davis Instruments, Hayward, CA, USA) placed in two boxes filled with soil. Temperature was automatically recorded hourly. The experiment was set up in a completely randomized design, with four replicates per treatment, in a factorial arrangement with three inoculation distances (0, 5 and 10 cm) and two types of planting (direct sowed and transplanted). The experiment was repeated once.

Aliquots of 10 ml from the suspension used in the soil infestation were collected to determine the motility and neutral lipid content of *M. incognita* J2. The J2 were stained according to Christophers *et al.* (1997) with modifications as described by Rocha *et al.* (2010). After lipid staining, 20 randomly selected J2 were mounted in glycerin on microscope slides. Each slide-mounted J2 was photographed, and the stained body area corresponding to the lipid

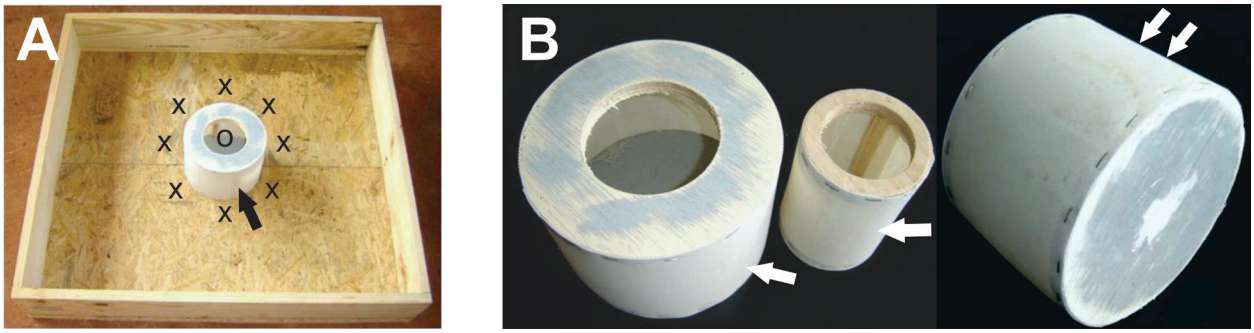

Fig. 1. Schematic view of the experimental box and cylinders used in the experiment. A) Wooden box with cylinder at the center (O: local infestation of *Meloidogyne incognita* second –stage juveniles (J2); X: sowing or transplanting location of the tomato seedlings; ⟶ : Arrows indicate screen); B: Cylinders 10 and 20 cm in diameter.

reserves as well as the full area of the J2 body were estimated using the Image-Pro Plus software for Windows version 4.1 (Media Cybernetics, Bethesda, MD). The observations and documentation were processed using a photomicroscope (Olympus AX70TRF, Olympus Optical, Tokyo, Japan) attached to a Canon Power Shot S40® digital camera (Canon Inc., Tokyo, Japan). A total of 120 J2 were analyzed. The motility of J2 was evaluated under an inverted microscope. Second-stage juveniles were considered immotile when they did not move or when they remained straight or curved after settling in water for 12 h.

Twenty days after infestation, the cylinders from the boxes of each treatment were carefully removed avoiding soil disturbance around the tomato plants. The same composite soil (clay and sand composite) was added in the place of the cylinder. The composite soil from each cylinder was removed with a spatula and placed in a plastic bag. In the laboratory, the soil was mixed in a plastic bag and *M. incognita* J2 were extracted from 100 cm^3 subsamples by centrifugal flotation technique (Jenkins, 1964). Four subsamples were processed representing a total of 400 g of soil used for J2 extraction per treatment. Second-stage juveniles were examined and counted under inverted light microscope where motility percentage and neutral lipid reserves in each soil sample were estimated as described previously.

Thirty days after inoculation, tomato plants were cut at soil level and the root systems were removed from soil and washed in water to remove adhering soil. Each root system was weighed, and egg masses were stained according to the method of Rocha *et al.* (2005) using artificial food dye. The number of egg masses and galls per root system were counted, and eggs were collected from each root system by the Hussey and Barker (1973) procedure. The number of egg masses, galls, and eggs per gram of fresh root were then determined. A reproduction factor (R_f) was calculated using the formula $R_f = P_f/P_i$ (Seinhorst, 1967), where P_i was the initial population of J2 of *M. incognita* and P_f was the final nematode population density (eggs).

The second experiment was similar to the first one, but the clay soil (soil prepared with mixture of field soil and sand) was replaced with sand. The sand used was the same steam-pasteurized plaster sand used in experiment one, sieved before the experiment as described previously. The average temperature in the clay soil (experiment 1) was 22.4°C, and in the sandy soil (experiment 2) the temperature was 20.8°C. In clay soil, the average daytime and nighttime temperature during the study were 24.4°C and 20.4°C respectively, while in the sandy soil they were 21.5°C and 19.2°C.

Statistical analyses

Statistical analyses were performed using the statistical software R, version 3.2.4 (R Development Core Team, 2016). A value of $P < 0.05$ was considered statistically significant. General linear models (GLM) were constructed using the error distribution that best fit the nature of the data. To analyze the influence of soil type, type of planting and distance, and their interactions (explanatory variables) on the percentage of neutral lipids and motility of J2, the binomial error distribution and overdispersion corrected with quasibinomial distribution were used. For interactions of the above explanatory variables with the number of J2 inside cylinders, the number of galls, egg masses, and eggs per gram of root were analyzed by the Poisson error distribution and overdispersion corrected with negative binomial distribution with the MASS package, 20 days after infestation. The Gaussian error distribution was used to verify the influence of explanatory variables on reproduction factor. The means of qualitative variables with three levels or more were compared by contrast analysis with the car package.

RESULTS

Soil temperature during the experiments was suitable for *M. incognita* movement and reproduction. There was no interaction between soil texture and the method of planting, or distance of inoculum from the host and the soil texture for the number of J2 extracted from the cylinders at 20 days after inoculation. The total number of J2 recovered from the cylinders containing sand was higher than from the clay soil, but did not differ between majority of treatments (Table 1), except for the clay soil, where fewer J2 were observed in transplanted tomato plants inside the 10 cm diameter cylinder.

There was an interaction between the type of planting and soil texture, and the distance and soil texture on nematode motility. Motility of J2 was always lower in the sand than clay soil regardless of planting type (directly sown or transplanting) and distance from the roots in the J2 were placed (5 or 10 cm). However, the motility fluctuated with the type of planting (sowed or transplanted), and was lower when inserted 10 cm away from the roots in sand. The opposite was the case when the juveniles were placed in clay (Fig. 2). Second-stage juvenile motility recovered from the 10- and 20-cm diameter cylinders with sand was 72 and 85% lower than with the control.

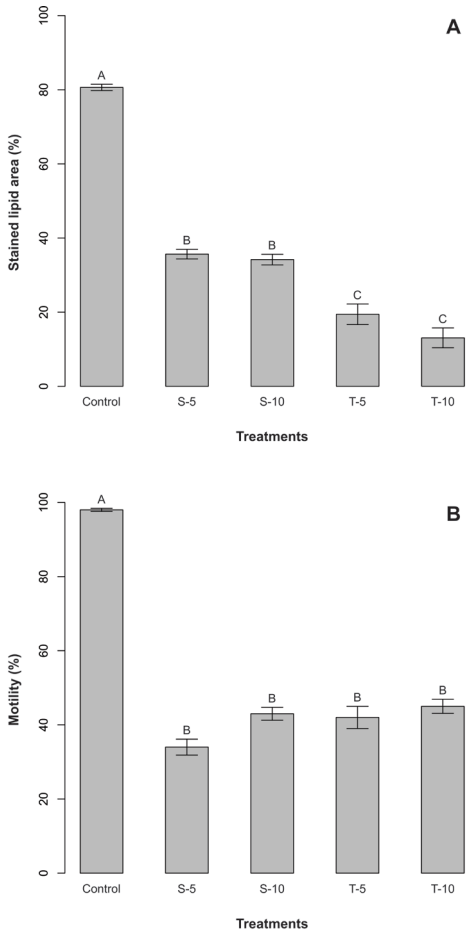

Fig. 2. Percentage of neutral lipids (A) and (B) motility of *Meloidogyne incognita* second-stage juveniles (J2) 20 days after introduction into the center of cylinders containing clay. Control: J2 used in the soil infestation. S-5: J2 extracted from cylinder with radius of 5 cm and cultivated outside by sowing tomato seeds. S-10: J2 extracted from cylinder with radius of 10 cm and cultivated outside by sowing tomato seeds. T-5: J2 extracted from cylinder with radius of 5 cm and cultivated outside by transplanting seedlings. T-10: J2 extracted from cylinder with radius of 10 cm and cultivated outside by transplanting seedlings. Bars followed by the same letter are not significantly different according to contrast analysis at 5% probability. Error bars represent standard errors of the means. Values are means of four replicates.

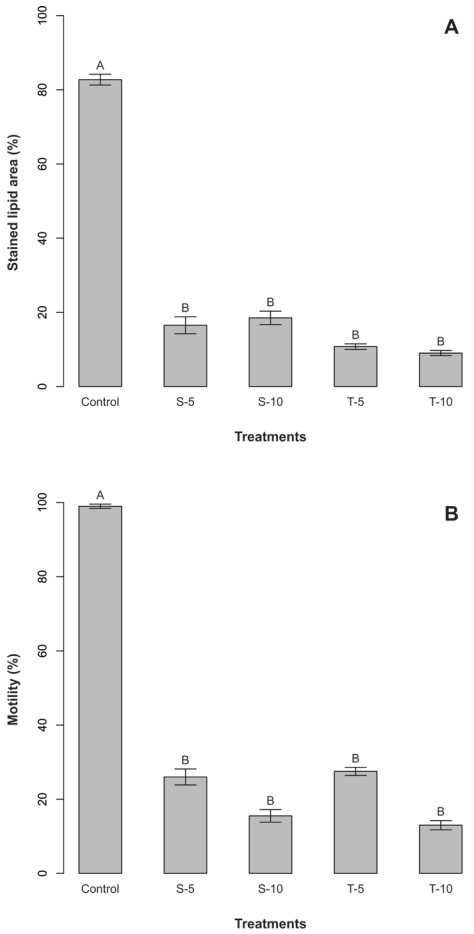

Fig. 3. Percentage of neutral lipids (A) and (B) motility of *Meloidogyne incognita* second-stage juveniles (J2) 20 days after introduction into the center of cylinders containing sand. Control: J2 used in the soil infestation. S-5: J2 extracted from cylinder with radius of 5 cm and cultivated outside by sowing tomato seeds. S-10: J2 extracted from cylinder with radius of 10 cm and cultivated outside by sowing tomato seeds. T-5: J2 extracted from cylinder with radius of 5 cm and cultivated outside by transplanting seedlings. T-10: J2 extracted from cylinder with radius of 10 cm and cultivated outside by transplanting seedlings. Bars followed by the same letter are not significantly different according to contrast analysis at 5% probability. Error bars represent standard errors of the means. Values are means of four replicates.

Regarding the content of neutral lipids of J2 recovered from the cylinders, there was interaction between soil texture and type of planting, and between the type of planting and distance of inoculum (Table 2). The lipid content of J2 recovered from cylinders from both type of planting (sowed or transplanted) did not change with the placement of the J2 (5 or 10 cm). However, lipid content was always lower for transplanted than sowed plants for any soil texture (Fig. 3). The neutral lipid content was lower in J2 recovered from cylinders containing sand and transplanted tomato seedlings, regardless of the distances tested. Second-stage juveniles in the cylinder with sand and transplanted seedlings lost 88% of their lipid content when compared to the control. Although there was no significant difference between the distances of 5 and 10 cm relative to the type of planting, we observed that transplanted seedlings also resulted in greater loss of J2 lipid reserves, with lipid content varying between 81 and

Table 1. Mean values and standard deviations of *Meloidogyne incognita* second-stage juveniles (J2) in 100 cm^3 of soil extracted from either 10- or 20-cm diameter (radius 5 or 10 cm) inoculation cylinders depending on soil texture and treatments, 20 days after infestation from tomato plants cultivated either by sowing or transplanted tomato.

	J2/100 cc soil	
Treatments	Clay	Sand
Sowing-5 cm	31 ± 14.2Ab[z]	269 ± 99.9aA
Sowing-10 cm	36 ± 9.1aB	548 ± 209.1aA
Transplanting-5 cm	13 ± 4.4bB	297 ± 258.6aA
Transplanting-10 cm	33 ± 22.9aB	456 ± 100.2aA

[z]Means followed by the same lower case letters in the column and capital letters in the rows do not differ significantly according to contrast analysis at 5% probability. Values are based on four replicates.

Table 2. A summary of statistical analysis on influence of type of planting, distance of inoculum, soil type, and their interactions on the percentage of motility, neutral lipid content, infectivity, reproduction, and reproduction factor (R_f) of *Meloidogyne incognita*.

Response variables	Explanatory variable	Df	Deviance	Resid. Dev	F	P-value
Motility	Planting type	2	1,423.15	238.68	872.54	$< 2.20 \times 10^{-16}$
	Distance	1	4.29	234.39	5.26	0.03
	Soil texture	1	155.97	78.42	191.25	4.78×10^{-15}
	Planting type:Soil texture	2	10.58	67.84	6.49	0.004
	Distance:Soil texture	1	40.00	27.84	49.05	6.16×10^{-8}
Neutral lipid	Planting type	2	1,171.81	112.41	669.68	$< 2.20 \times 10^{-16}$
	Distance	1	1.71	110.70	1.96	0.17
	Soil texture	1	53.48	57.22	61.13	6.43×10^{-9}
	Planting type: Distance	1	4.77	52.45	5.45	0.03
	Planting type: Soil texture	2	23.93	28.52	13.68	5.09×10^{-5}
Galls	Planting type	2	958.66	576.73	182.18	$< 2.20 \times 10^{-16}$
	Distance	2	1.82	574.91	0.35	0.44
	Soil texture	1	378.19	196.72	143.74	1.73×10^{-14}
	Planting type: Soil texture	2	88.40	108.32	16.80	2.39×10^{-5}
Egg masses	Planting type	2	620.86	550.99	94.48	7.67×10^{-15}
	Distance	2	1.86	549.12	0.28	0.59
	Soil texture	1	334.37	214.75	101.76	2.89×10^{-12}
	Planting type: Soil texture	2	81.08	133.67	12.34	0.0003
Eggs	Planting type	2	107,909.00	51,676.00	189.46	$< 2.20 \times 10^{-16}$
	Distance	2	134.00	51,542.00	0.24	0.65
	Soil texture	1	32,568.00	18,974.00	114.36	5.34×10^{-13}
	Planting type: Soil texture	2	8,046.00	10,927.00	14.13	0.0001
R_f	Planting type	2	1,328.91	849.71	79.02	1.23×10^{-13}
	Distance	2	2.11	847.60	0.13	0.98
	Soil texture	1	253.38	594.22	30.13	3.25×10^{-6}
	Planting type: Soil texture	2	257.87	336.35	15.33	5.06×10^{-5}

Table 3. Mean values and standard deviations of infectivity, reproduction, and reproduction factor (R_f) of *Meloidogyne incognita* second-stage juveniles (J2) depending on type of planting and soil texture 30 days after infestation.

Soil type	Planting type	Galls	Egg masses	Eggs	R_f
Clay	Control 1[x]	86.3 ± 39.6a[z]	58.5 ± 15.2 a	7,862 ± 3,575.9a	17.9 ± 9.0a
	Control 2[y]	83.0 ± 8.7a	54.0 ± 35.1a	7,398 ± 2,039.5a	16.8 ± 5.1a
	Sowing	14.5 ± 4.4d	13.1 ± 8.9b	1,429 ± 970.2c	1.8 ± 1.3c
	Transplanting	21.6 ± 3.2c	8.7 ± 3.2b	663 ± 198.6d	0.9 ± 0.3c
Sand	Control 1	34.7 ± 16.1b	18.0 ± 3.2b	2,845 ± 50.0b	6.4 ± 0.5b
	Control 2	34.5 ± 6.5 b	16.7 ± 1.7b	2,690 ± 0.0b	6.1 ± 0.5b
	Sowing	0.9 ± 0.9e	0.0 ± 0.0c	9.7 ± 5.1e	0.01 ± 0.01c
	Transplanting	1.2 ± 0.7e	0.0 ± 0.0c	9.1 ± 4.7e	0.01 ± 0.01c

[x]Control 1: J2 inoculated near the base of transplanted tomato seedlings.
[y]Control 2: J2 inoculated near the base of directly sowed tomato seedlings.
[z]Means followed by the same letter, in the same column, are not significantly different according to contrast analysis at 5% probability. Values are based on four replicates.

86.5%.

For infectivity (galls, egg masses) and reproduction (eggs, R_f) of *M. incognita*, there was an interaction between soil texture and type of planting. The distance of the inoculum did not influence *M. incognita* infectivity or reproduction. Fewer J2 migrated through sand to infect tomato roots whether by transplanted seedlings or seeded directly (Table 3). Migration of J2 decreased reproduction when placed on cylinders regardless of soil texture and type of planting, as expressed by the number of eggs per gram of root and the reproduction factor, compared to the controls. Greater reproductive reduction occurred when J2 were placed on sand, followed by those placed in clay with transplanted seedlings (Table 3).

DISCUSSION

Environmental factors such as temperature and soil moisture are important factors affecting the *M. incognita* migration process and reproduction. In our study, the average daytime and nighttime soil temperatures (24.4°C; 21.5°C and 20.4°C; 19.2°C, respectively) in both experiments were favorable to migration of *M. incognita* J2, as indicated by Prot and Van Gundy (1981b) who reported that the optimum temperature for *M. incognita* migration was between 18 and 22°C.

Soil texture affects nematode motility and host recognition (Table 1). Van Gundy *et al.* (1967) reported that the activity of *M. javanica* J2 increased in the soil when a susceptible host was present. In these experiments, a susceptible tomato cv. Kada (Rocha *et al*., 2010; Rocha *et al*., 2015) was used to evaluate root-knot nematode infectivity and reproduction. However, the two distances between the root system and J2 tested are hypothesized to have influenced in the distribution and diffusion of root exudate substances, which prevented the recognition of a concentration gradient in the soil by J2, which thereby caused significant disorientation, reducing motility and increasing consumption of neutral lipid contents of J2 when compared to the freshly collected second-stage juveniles. A greater reduction in nematode motility and lipid content (86.5% losses) occurred in J2 extracted from the 20-cm cylinder with sand and transplanted tomato. Similarly, J2 of *M. exigua* and *M. javanica* lipid losses were estimated at 89 and 91%, respectively, after 12 days of incubation in water at 28ºC (Campos *et al*., 2006; Rocha *et al*., 2010). The greater loss of J2 lipid content from soils with transplanted tomato plants is thought to be associated with the lower physical distribution of roots surrounding cylinders when compared to the direct seeded plant, which reduced the quantity and/or quality of attractive substances exuded by the roots. Therefore, the interaction between soil texture and type of planting coupled with the lipid required to move to the root resulted in a drastic reduction in body lipid reserves, which impeded most J2 from reaching the host. In fact, more pronounced negative effect was observed in sandy soil interaction with transplanted seedlings on those J2 inoculated that penetrated the roots, which had greater lipid loss at 20 d and, consequently, lower infectivity and reproduction in relation to those that migrated and infected the plants in clay soil. The average reduction in the reproduction of those J2 (eggs) placed at sand cylinders with transplanted tomato plants around was 99.6%, compared with the control 1. Prot and Van Gundy (1981a) found that

in pure silica sand less than 1% of *M. incognita* J2 migrated 20 cm and were able to penetrate in tomato plant roots.

Tomato plants cv. Kada, both transplanted and seeded in the sandy soil, were considered poor hosts with a low R_f of 0.01 and 0.01, respectively, when analyzing the reaction of tomato to root-knot nematode (J2 inoculated) based on the criterion of Seinhorst (1967). In clay soil, only transplanted tomatoes into the boxes behaved as poor hosts (R_f = 0.9), while the sown and control treatments were good hosts (R_f > 1), which indicates that both soil types and planting types inhibited the ability of J2 to infect tomato plants. However, the sandy soil texture demonstrated an additional effect on J2 migration. Consequently, infectivity and reproduction of *M. incognita* J2 were lower in the sandy soil. Rocha *et al.* (2008) studied the timing effect of soybean seeding and the storage of *M. incognita* J2 placed at different distances from the root system and observed that the storage period of 5 d in sand before planting soybeans caused drastic reduction in the body lipid content (23%), migration (81.8%), and infectivity (94%).

Since the study was conducted in a three-dimensional environment, the data suggests that plant exudates that served as attractant cues incurred greater leaching in the sandy soil, which negatively affected attraction and migration of J2 towards tomato plants positioned outside of the cylinder. Consequently, there was greater movement of disoriented J2 causing greater loss of lipid reserves than in clay soil. Prot and Van Gundy (1981a) studied the migration of *M. incognita* J2 introduced at distances of 20 cm from the roots and observed no J2 migration in silica sand, but when a mixture of 5 or 10% clay was combined with silica sand, 34 and 36% of J2 were able to migrate 20 cm and penetrate roots, respectively. This suggests that clay particles play a significant role in the attraction and migration of J2 by adsorbing and providing for capillary spread of root exudates or other substances, enabling a concentration gradient that supports nematodes in locating and migrating towards roots. Moreover, the ability of J2 migration depends on the diameter and distribution of pores in the soil (Wallace, 1958b; Otobe *et al*., 2004). Since sandy soils have greater pore spaces than clay soils, this would account for a greater movement of J2 and leaching of exudates in the cylinders with sand. It is also conceivable that screen pore clogging by soil particles may have interfered with J2 movement through the screen, thereby limiting infection of the tomato plant. Fujimoto *et al.* (2009) studied the movement of J2 of *M. incognita* using the Marriot reservoir connected to a column filled with different textures of soil maintained at a constant water flow flux of 36 cm h^{-1} and demonstrated that the number of J2 passing through the column (outflow point) was much lower in the Andisol than in the sand column. Thus, clay soil may prevent the spread of more than 60% of J2 because they are incapable of passing through soil pores less than 20 µm (Wallace, 1958b; Fujimoto *et al.*, 2009). This suggests that pore size limitations explain the 64% reduction in reproduction of *M. incognita* J2 (eggs) in the control boxes with sand when compared to the clay soil. Thus, under field conditions the further spread of nematodes in sandy soil can be explained by the higher movement of J2 associated with soil disturbance.

In our studies, we have demonstrated the negative effect of distance from the root system in combination with differences in soil texture and planting type on migration of *M. incognita* J2, as well as the dynamic behavior and reproduction of J2 on tomato.

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