RESEARCH NOTE/NOTA DE INVESTIGACIÓN

REACTION OF GREEN LEAF LETTUCE GENOTYPES TO THREE SPECIES OF ROOT-KNOT NEMATODES ACCORDING TO TWO EVALUATION METHODS

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ABSTRACT

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This experiment employed two evaluation methods to determine the reaction of green-leaf lettuce genotypes to *Meloidogyne enterolobii*, *M. javanica*, and *M. incognita*. Two hundred second-stage juveniles (J2) of each nematode species were separately inoculated on eight green leaf lettuce inbred lines developed by UNESP/FCAV. The cultivar 'Vanda' was also evaluated, as well as tomato cultivar 'Santa Cruz Kada' to aid in detecting a pattern of susceptibility. The host-plant reactions to the nematodes were determined by the root gall index (GI), egg mass index (EMI) and reproduction factor (RF). Treatments were arranged in a completely randomized design, in factorial 3 x 9 with seven replications. The results changed according to the method. The inbred line L_1 was moderately resistant to all the nematodes based on GI. However, all the genotypes were considered susceptible to root-knot nematodes considering RF reaction.

Key words: gall index, *Lactuca sativa* L., *Meloidogyne* spp., reproduction factor, resistance

RESUMO

Diniz, G. M. M., W. S. Candido, H. O. Rabelo, M. V. Marín, L. T. Braz, e P. L. M. Soares. 2018. Reação de alface-crespa ao parasitismo de três espécies de nematoide de galha utilizando dois métodos de análise. Nematropica 48:1-4.

Este experimento empregou dois métodos de avaliação para determinar a reação de genótipos de alface crespa para *Meloidogyne enterolobii*, *M. javanica* e *M. incognita*. Duzentos J2 de cada espécie de nematoides foram inoculadas separadamente em oito linhagens de alface crespas desenvolvidas pela UNESP / FCAV. A cultivar 'Vanda' também foi avaliada, bem como a cultivar de tomate 'Santa Cruz Kada' para auxiliar na detecção de um padrão de susceptibilidade. As reações da planta hospedeira aos nematoides foram determinadas pelo índice de galhas da raiz (IG), índice de massa de ovo (IMO) e fator de reprodução (FR). Os tratamentos foram organizados em delineamento inteiramente casualizado, em fatorial de 3 x 9 e sete repetições. Os resultados mudaram de acordo com o método. A linhagem L_1 foi moderadamente resistente a todos os nematoides, com base em IG. No entanto, todos os genótipos foram considerados suscetíveis a nematoides de nó de raiz considerando a reação de FR.

Palavras-chave: *Lactuca sativa* L., fator de reprodução, índice de galhas, resistência

Considered the most consumed leafy-type vegetable species in Brazil, lettuce (*Lactuca sativa* L.) is cultivated year round in all regions. Six types of lettuce (green leaf, butterhead, iceberg, mimosa, romaine, and red leaf) are preferred by Brazilian customers. In Brazil, the most cultivated lettuce types, in order of importance, are green-leaf, iceberg, butterhead, and romaine lettuce (Sala and Costa, 2012). One problem that has worried farmers is the greater occurrence of parasitism by nematodes as result of the intensification and expansion of lettuce fields. Nematodes have been increasing in population densities due to consecutive cultivation of susceptible lettuce cultivars (Carneiro *et al*., 2008).

The most economically important nematodes on lettuce are *Meloidogyne incognita* and *M. javanica*. Reports of *M. enterolobii* in lettuce fields are more frequent. Lettuce roots attacked by root-knot nematodes are deformed and uptake less water and nutrients from soil. The lettuce plants become smaller, have chlorotic leaves, smaller leaf area, and consequently are rejected by consumers (Pajovic, 2007).

Several studies have identified host-plant resistance to root-knot nematodes in lettuce (Carvalho Filho *et al*., 2008; Silva *et al*. 2008). Resistance is available to *M. incognita*, *M. javanica* and *M. hapla* (Oliveira *et al*., 2015; Rosa *et al*., 2015). The cultivar 'Simpson', for example, is considered resistant with an RF=0.3, and the cultivars 'Grandes Lagos 659' (green leaf) and 'Mônica' (iceberg) highly resistant to *M. enterolobii* (Bitencourt and Silva, 2010). In another study, 'Elisa', 'Luisa', 'Mirella', 'Vera' and 'Salinas 88' were rated as moderately resistant, whereas 'Júlia,' 'Hortência,' 'Verônica,' 'Grand Rapids,' and 'Babá de Verão' were highly resistant to *M. enterolobii* based on infection indices (De Melo *et al*., 2011). However, none of the current sources of resistance have been effective against *M. enterolobii* as measured by nematode reproduction (Kaur *et al*., 2007; Rosa *et al*., 2015). The present work evaluated the reaction of inbred lines of green-leaf lettuce to *M. enterolobii*, *M. javanica*, and *M. incognita*.

The experiments were conducted in a greenhouse at the Department of Vegetable Crops and Aromatic-Medicinal Plants of São Paulo State University "Júlio de Mesquita Filho", Faculty of Agriculture and Veterinary Sciences (UNESP-FCAV), Campus of Jaboticabal-SP, Brazil. The site is located at 21º14'05'' S, 48º17'09'' W and 614 m altitude.

Nematode inoculum was obtained from greenhouse cultures. Nematode eggs were extracted from infected roots using the NaOCl method (Hussey and Barker, 1973). *Meloidogyne enterolobii*, originally obtained from roots of guava 'Paluma' in Taquaritinga-SP, was grown on okra. *Meloidogyne incognita* and *M. javanica* were originally obtained from cotton (*Gossypium hirsutum* L.) and okra (*Abelmoschus esculentus* L.) collected in Barreiras-BA and Piatu-SP, respectively. *Meloidogyne incognita* was cultured on cotton and *M. javanica* on okra plants. Eggs and second-stage juveniles (J2) were counted in a 1-ml Peters counting slide with a light microscope. Thereafter, the suspension was transferred to a Baermann funnel (Southey, 1986) and placed in an incubation chamber at 30ºC for 3 days to collect hatched J2. The J2 obtained after the first hour were discarded. J2 were collected at 24, 48, and 72 hr, and the average number of J2 was measured using a 1-ml Peters counting slide.

Eight green-leaf lettuce inbred lines (L_1, L_2, L_3, L_4, L_5, L_6, L_7, L_8) developed by the breeding program for horticultural crops of UNESP/FCAV were assessed. In addition, the green-leaf lettuce cultivar 'Vanda' was evaluated. The tomato cultivar 'Santa Cruz kada' was used as a comparative susceptible host. Treatments were arranged in a completely randomized design in factorial 3 x 9, with seven replications, and one plant per plot. Seedlings were produced in 128-cell polystyrene trays contained in a greenhouse equipped with sprinkler irrigation. Seedlings were transplanted 25 d after sowing into 2-L plastic pots filled with a mixture of autoclaved soil, sand, and cattle manure in 1:1:1 proportions. Pots were inoculated with 5-ml suspensions containing 40 J2 ml^{-1}, giving an initial population (Pi) of 200 J2/pot. The J2 were injected at four equidistant positions surrounding the plants to a depth of 3 cm. The plants were irrigated daily and fertilized according to recommendations for lettuce (Trani *et al*., 1996). Phytosanitary treatments were used, but no pesticide with nematicidal properties was applied.

Plants were harvested 60 d after inoculation. Plants were removed from the pots, roots gently rinsed to remove any adhering soil, and the shoots discarded. Roots were immersed in Phloxine B (0.5 $g.m^{-1}$) for 15 minutes to stain egg masses and galls. Root evaluation was performed at 10x magnification. Egg mass (EMI) and gall indices (GI) were assigned to each plant (Charcar *et al*., 2003). For EMI, the scale was: 1) roots without egg mass; 2) roots with 1 to 5 egg masses; 3) roots with 6 to 15 egg masses; 4) roots with 16 to 30 egg masses; and 5) roots with more than 30 egg masses. For GI, the scale was: 1) roots without any galls; 2) roots with up to 10 small galls; 3) roots with 11 to 50 small galls; 4) roots with more than 50 small galls and up to 10 large galls; and 5) roots with more than 50 small galls and more than 10 large coalesced galls. Galls greater than 3-mm diameter were considered large. The reactions of lettuce genotypes to root-knot nematode infection were based on the average score of the seven replications as highly resistant (HR) – EMI and GI from 1.0 to 1.8; resistant (R) – EMI and GI from 1.9 to 2.6; moderately resistant (MR) – EMI and GI between 2.7 and 3.4; susceptible (S) – EMI and GI from 3.5 to 4.2; and highly susceptible (HS) – EMI and GI greater than 4.2 (Charchar *et al*., 2003). After EMI and GI ratings were made, roots were processed to extract eggs and J2 (Hussey and Barker, 1973). The final population (Pf) of each pot was obtained with aid of a 1-mL Peters counting slide and light microscope. The final population (Pf) was used to calculated the Reproduction Factor (RF) where RF=Pf/Pi. Plants with RF<1 were considered as resistant, and those plants with RF≥1 were susceptible (Oostenbrink, 1966). The data were transformed (log x) and subjected to analysis of variance. Mean reaction scores were compared using the Scott-Knott Effect Size Difference. All statistical analyses were obtained using AgroEstat (Barbosa and Maldonado, 2010).

The analysis of variance indicated differences (P<0.01) among lettuce genotypes and nematodes that may be indicative of resistance (Table 1). The coefficient of variation (CV) was low, despite the interaction between nematodes and plants. High CVs have been associated with research involving nematode host-plant resistance evaluation (Carvalho Filho *et al*., 2011). The inoculation of *M. javanica*, *M. incognita*, and *M. enterolobii* was efficient, evidenced by abundant nematode multiplication on roots of tomato Santa Cruz Kada. Santa Cruz Kada showed high levels of susceptibility in all traits evaluated (Table 1).

Table 1. Average gall index (GI), egg mass index (EMI) and reaction of green-leaf lettuce genotypes inoculated with second-stage juveniles of *Meloidogyne enterolobii* (Me), *M. javanica* (Mj), and *M. incognita* (Mi).

	GI			EMI			Reaction (GI/EMI)[x]		
Genotype	Me	Mj	Mi	Me	Mj	Mi	Me	Mj	Mi
L_6	3.85 Bb[y]	4.00 Aa	5.00 Aa	3.14 Bb	4.71 Aa	5.00 Aa	S/MR	S/HS	HS/HS
L_8	3.57 Ac	3.57 Ab	3.28 Ac	2.42 Bc	4.42 Aa	2.57 Bc	S/MR	S/HS	MR/R
L_2	3.14 Ac	3.57 Ab	3.00 Ac	2.28 Bc	4.85 Aa	2.85 Bc	MR/R	S/HS	MR/MR
L1	2.85 Ac	3.42 Ab	3.14 Ac	2.14 Bc	5.00 Aa	2.71 Bc	MR/R	MR/HS	MR/MR
L_5	4.00 Ab	3.42 Ab	4.14 Ab	2.85 Bb	5.00 Aa	3.42 Bb	S/MR	MR/S	S/S
L_4	2.71 Bc	3.57 Ab	4.14 Ab	2.14 Cc	4.57 Aa	3.42 Bb	MR/R	S/HS	S/S
L_3	2.85 Bc	4.14 Aa	4.71 Aa	2.14 Cc	4.85 Aa	3.71 Bb	MR/R	S/HS	S/S
L_7	3.42 Ac	4.14 Aa	4.28 Ab	2.57 Bc	4.85 Aa	3.00 Bc	MR/R	S/HS	S/MR
'Vanda'	4.28 Ab	4.42 Aa	5.00 Aa	3.00 Bb	5.00 Aa	3.57 Bb	S/MR	HS/HS	HS/S
Tomato[z]	5.00	5.00	5.00	5.00	5.00	5.00			
Mean		3.89			3.70				
CV%		19.40			15.96				
F test		2.17**			5.83**				

[x]Resistance reactions: HR – highly resistant; R – resistant; MR – moderately resistant; S – susceptible; HS – highly susceptible.

[y]Means followed by the same lowercase/uppercase letters in columns and lowercase/uppercase letters in lines do not differ significantly by Scott-Knott test (P<0.05).

[z]Referential of susceptibility to the nematodes species. Data were transformed using log x.

The lettuce genotypes differed in their reactions to the different nematode species. Some genotypes were susceptible and some resistant (Tables 1 and 2). The inbred lines L_1 and L_5 were moderately susceptible to *M. javanica* by GI. For EMI, all genotypes were susceptible or highly susceptible (Table 1). L_1 and L_2 were moderately resistant and resistant to *M. enterolobii* and *M. incognita* by GI and EMI, respectively. Using RF, all lettuce genotypes were susceptible to all three nematode species (Oostenbrink, 1966). However, the lettuce genotypes had different number of eggs, J2, and RF to each nematode species evaluated (Table 2). Although all inbred lines were susceptible, lower RFs were observed for *M. enterolobii*. L_1, L_4, and L_3 had relatively low EMIs (2.14) but supported reproduction of all nematode species, RFs of 3.0, 6.0, and 6.0, respectively. L_1 is moderately resistant to *M. enterolobii*, *M. javanica*, and *M. incognita* based on GI. All the lettuce genotypes are susceptible to *M. enterolobii*, *M. javanica*, and *M. incognita* based on RF. Both GI and RF distinguished lettuce genotypes tolerant to *M. enterolobii* and susceptible to *M. javanica* and *M. incognita*. In general, there was resistance in the inbred lines to *M. enterolobii* (Table 1). Despite susceptibility indicated by RF, RF and GI exhibit similar behavior across the inbred lines.

Our evaluation suggests some inbred line genotypes have resistance, although other researchers might consider the genotypes susceptible (Ferreira, 2010). Ferreira *et al*. (2011) indicate that resistance screening for root-knot nematodes may use EMI and GI. Carvalho Filho *et al.* (2012) also report that the assessment of lettuce plants using traits like EMI and GI may be used to

Table 2. Average of number of eggs and second-stage juveniles (NEJ), reproduction factor (RF), and reaction of green-leaf lettuce genotypes inoculated with 200 second-stage juveniles of *Meloidogyne enterolobii* (Me), *M. javanica* (Mj), or *M. incognita* (Mi).

	NEJ[w]			EMI[w]			Reaction[x]		
Genotype	Me	Mj	Mi	Me	Mj	Mi	Me	Mj	Mi
L_6	2,185 Cc[y]	8,742 Bc	16,185 Ab	14.00 Cc	43.71 Bc	80.92 Ab	S	S	S
L_8	1,300 Bd	6,428 Ad	6,871 Ac	3.00 Bd	32.14 Ad	34.35 Ac	S	S	S
L_2	1,157 Bd	7,714 Ac	6,885 Ac	3.00 Bd	38.57 Ac	34.42 Ac	S	S	S
L_1	1,335 Bd	13,942 Ab	4,971 Bd	4.00 Bd	69.71 Ab	24.85 Bd	S	S	S
L_5	3,228 Cb	11,757 Ab	6,771 Bc	9.00 Bb	58.78 Ab	33.85 Bc	S	S	S
L_4	1,300 Bd	9,657 Ac	7,914 Ac	6.00 Bd	48.28 Ac	39.57 Ac	S	S	S
L_3	1,585 Bd	8,742 Ac	10,087 Ac	6.00 Bd	43.71 Ac	50.43 Ac	S	S	S
L_7	1,300 Bd	7,542 Ac	8,257 Ac	3.00 Bd	37.71 Ac	41.28 Ac	S	S	S
'Vanda'	2,200 Bc	15,257 Ab	13,828 Ab	11.00 Bc	76.28 Ab	69.14 Ab	S	S	S
Tomato[z]	6,857	50,714	30,685	30.00	253.57	153.42	S	S	S
Mean		9,180			45.90				
CV%		3.95			10.22				
F test		16.65**			16.65**				

[w]Data were transformed using log x.

[x]Reaction: RF<1 – resistant (R); R≥1 – susceptible (S), according to Oostenbrink (1966).

[y]Means followed by the same lowercase/uppercase letters in columns and lowercase/uppercase letters in lines do not differ significantly by Scott-Knott test (P<0.05).

[z]Referential of susceptibility to the nematodes species.

select genotypes with resistance to root-knot nematode. The use of traits that are in consonance with RF may bring better evaluations by highlighting correlation symptoms, i.e. galling and measures of nematode population (Silva, 2001). EMI and GI evaluation is advantageous because it is non-destructive, which allows individual and unique genotypes to be evaluated for resistance and then replanted for other objectives such as for crossings, self-pollination, or even to advance generations in breeding programs. Evaluations using GI allow the assessment of other traits, earning one selection cycle by the possibility of producing seeds from the same plants.

LITERATURE CITED

Barbosa, J.C., and W. Maldonado, Jr. 2010. AgroEstat. Sistema para análises estatísticas de ensaios agronômicos. Versao 1.0. Universidade Estadual Paulista "Júlio de Mesquita Filho" - Faculdade de Ciências Agrárias e Veterinárias, Jaboticabal, Brasil.

Bitencourt, N. V., and G. S. Silva. 2010. Reprodução de *Meloidogyne enterolobii* em olerícolas. Nematologia Brasileira 34:181-183.

Carneiro, R. M. D. G., M. R. Almeida, I. Martins, J. F. Souza, A. Q. Pires, and M. S. Tigano. 2008. Ocorrência de *Meloidogyne* spp. e fungos nematófagos em hortaliças no Distrito Federal, Brasil. Nematologia Brasileira 32:135-141.

Carvalho Filho, J. L. S., L. A. Gomes, and R. R. Costa-Carvalho. 2012. Incidência de galhas de *Meloidogyne incognita* raça 1 em progênies de F2: 3 (‘Salinas 88’x ‘Colorado’) de alface. Scientia Plena 8:2.

Carvalho Filho, J. L. S., L. A. A. Gomes, R. R. Silva, S. Ferreira, R. R. C. Carvalho, and W. R. Maluf. 2011. Parâmetros populacionais e correlação entre características da resistência a nematoides de galhas em alface. Revista Brasileira de Ciências Agrárias 6:46-51.

Carvalho Filho, J. L. S., L. A. A. Gomes, J. N. Westerich, W. R. Maluf, V. P. Campos, and S. Ferreira. 2008. Inheritance of resistance of ‘Salinas 88’ lettuce to the root-knot nematode *Meloidogyne incognita* (Kofoid & White) Chitwood. Revista Brasileira de Agrociência 14:279-289.

Charchar, J. M., L. B. Giordano, and L. S. Boiteux. 2003. Metodologia para seleção de hortaliças com resistência a nematoides: Famílias Convolvulaceae e Solanaceae/*Meloidogyne* spp. Brasília: Embrapa Hortaliças, Embrapa Hortaliças. Comunicado Técnico 21:4.

De Melo, O. D., W. R. Maluf, R. J. Sousa Gonçalves, Á. C. G. Neto, L. A. A. Gomes, and R. de Castro Carvalho. 2011. Triagem de genótipos de hortaliças para resistência a *Meloidogyne enterolobii*. Pesquisa Agropecuária Brasileira 46:829-835.

Ferreira S., L. A. A. Gomes, W. R. Maluf, V. P. Campos, J. L. S. de Carvalho Filho, and D. C. Santos. 2010. Resistance of dry bean and snap bean cultivars to root-knot nematodes HortScience 45:320-322.

Ferreira, S., V. L. F. Vieira, L. A. A. Gomes, W. R. Maluf, and J. L. S. D. Carvalho Filho. 2011. Identificação de linhagens avançadas de alface quanto à resistência a *Meloidogyne javanica*. Ciência e Agrotecnologia 35:270-277.

Hussey, R. S., and K. R. Barker. 1973. A comparison of methods collecting inocula of *Meloidogyne* spp. including a new technique. Plant Disease Reporter 57:1025-1028.

Kaur, R., J. A. Brito, and J. R. Rich. 2007. Host suitability of selected weed species to five *Meloidogyne* species. Nematropica 37:107-120.

Oliveira, G. H. F., S. E. R. E. A. Santana, R. C. N. Fonseca, R. L. E. de Lima, L. A. A. Gomes, and J. E. L. S. de Carvalho Filho. 2015. *Meloidogyne incognita* resistant strains of leaf lettuce. African Journal of Agricultural Research 10:4660-4667.

Oostenbrink, M. 1966. Major characteristics of the relation between nematodes and plants. Landbouw, Mededelingen 66:1-46.

Pajovic, I. 2007. The incidence of root-knot nematodes *Meloidogyne arenaria*, *M. incognita*, and *M. javanica* on vegetables and weeds in Monteegro. Plant Disease 91:1514.

Rosa, J. M. O., J. N. Westerich, and S. R. S. Wilcken. 2015. Reprodução de *Meloidogyne enterolobii* em olerícolas e plantas utilizadas na adubação verde. Revista Ciência Agronômica 46:826-835.

Sala, F. C., and C. P. Costa. 2012. Retrospectiva e tendência da alfacicultura brasileira. Horticultura Brasileira 30:187-194.

Silva, J. F. V. Resistência genética de soja a nematóides do gênero *Meloidogyne*. 2001. Pp. 95-127 *in* Ferraz, L. C. C. B., G. L. Asmus, R. G. Carneiro, P. Mazaffera, and J. F. V. Silva, (eds.) Relações parasito-hospedeiro nas meloidogynoses da soja. Londrina: Embrapa/CNPSo.

Silva, R. R., L. A. A. Gomes, A. B. Monteiro, W. R. Maluf, J. L. S. de Carvalho Filho, and J. A. Massaroto. 2008. Linhagens de alface-crespa para o verão resistentes ao *Meloidogyne javanica* e ao vírus mosaico-da-alface. Pesquisa Agropecuária Brasileira 43:1349-1356.

Southey, J. F. 1986. Principles of sampling for nematodes: Laboratory methods for work with plant and soil nematodes. London: Ministry of Agriculture, Fisheries and Food.

Trani, P. E., F. A. Passos, and J. A. Azevedo Filho. 1996. Bol. Tec. Inst. Agron. Campinas, n.100, p. 168-9.

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