

Response of lettuce cultivars to *Meloidogyne javanica* and *Meloidogyne incognita* race 1 and 2¹

Reação de cultivares de alface a *Meloidogyne incognita* raças 1 e 2 e *Meloidogyne javanica*

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ABSTRACT - The aim of this study was to evaluate resistance to *Meloidogyne javanica* and *Meloidogyne incognita* race 1 and 2 in the American group of lettuce cultivars. Two days after transplanting, the plants were inoculated with 5,000 eggs and potential second-stage juveniles of the nematode under test per pot. The Rutgers tomato was used as the standard for the viability of *Meloidogyne javanica* and *Meloidogyne incognita* race 1 and 2. The variables to be evaluated were gall index, egg mass index and nematode reproduction factor, which were evaluated 60 days after inoculation. The results showed that the Ithaca, RS-1397, Raider Plus, Challenge, L-104, IP-11, Salinas 88, Calona, Desert Queen, Classic and Vanguard 75 cultivars were resistant to *Meloidogyne incognita* race 1, with a reproduction factor which ranged from 0.19 to 0.88, and that the Desert Queen, L-104, Salinas 88, Vanguard 75, Robinson, RS-1397, Challenge, Raider Plus, Classic, Calona, Ithaca, Lady, IP-11 and Winterset cultivars were resistant to *Meloidogyne incognita* race 2, with a reproduction factor of between 0.23 and 0.93. All the cultivars under evaluation were resistant to *Meloidogyne javanica*, with a reproduction factor of less than 1.0.

Key words: *Lactuca sativa* L.. Root-knot nematodes. Reproduction factor.

RESUMO - O objetivo deste trabalho foi avaliar a resistência de *Meloidogyne incognita* raças 1 e 2 e *Meloidogyne javanica* em cultivares de alface do grupo americano. As plantas foram inoculadas com 5.000 ovos e eventuais juvenis de segundo estágio do nematoide em teste por vaso, dois dias após o transplante das cultivares. O tomateiro 'Rutgers' foi utilizado como padrão de viabilidade de *Meloidogyne incognita* raças 1 e 2 e *Meloidogyne javanica*. As variáveis avaliadas foram: o índice de galhas, o índice de massa de ovos e o fator de reprodução do nematoide, avaliadas 60 dias após a inoculação. Os resultados obtidos mostraram a resistência das cultivares Ithaca, RS-1397, Raider Plus, Challenge, L-104, IP-11, Salinas 88, Calona, Desert Queen, Classic e Vanguard 75 a *Meloidogyne incognita* raça 1, com fator de reprodução variando de 0,19 a 0,88; e das cultivares Desert Queen, L-104, Salinas 88, Vanguard 75, Robinson, RS-1397, Challenge, Raider Plus, Classic, Calona, Ithaca, Lady, IP-11 e Winterset, a *Meloidogyne incognita* raça 2, com fator de reprodução variando de 0,23 a 0,93. Todas as cultivares avaliadas foram resistentes a *Meloidogyne javanica*, apresentando fator de reprodução inferior a 1,0.

Palavras-chave: *Lactuca sativa* L.. Nematode das galhas. Fator de reprodução.

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INTRODUCTION

Lettuce (*Lactuca sativa* L.) is the most important leafy vegetable in the world, and is mainly consumed *in natura* in the form of salads (SALA; COSTA, 2012). In Brazil, crop production is largely located in the green-belt areas of medium and large cities (CAMARGO FILHO, CAMARGO, 2008). In some distribution centres, species of lettuce represent almost 50% of all the leafy vegetables that are marketed, and among these, American lettuce corresponds to almost 40% of the total (MORETTI; MATTOS, 2006).

Consecutive cultivation of this vegetable has increased the population of parasitic nematodes, leading to considerable economic damage. The species *M. incognita* (Kofoid and White) Chitwood, 1949 and *M. javanica* (Treib) Chitwood, 1949 stand out among the nematodes that are importance to the lettuce crop (WILCKEN; GARCIA; SILVA, 2005). Lettuce plants, when attacked by nematodes show intense debility caused by the dense formation of galls in the root system, which result in restrictions on the absorption and transport of water and nutrients from the soil; the plants become chlorotic, of small size, with little leaf volume and of no value for *in natura* consumption (CHARCHAR; MOITA, 2005; ORNAT; SORRIBAS, 2008).

The control of phytonematodes in infested areas of lettuce crops has proved to be problematic, and crop rotation, a method often recommended in the control of plant-parasitic nematodes, is difficult to employ due to the intense cultivation in these areas (WILCKEN; GARCIA; SILVA, 2005). The use of nematocides is also not recommended, as due to its short-cycle, the lettuce may contain residue of the applied product (FERREIRA *et al.*, 2011). The ideal method of controlling phytoparasitic nematodes in lettuce is therefore the use of resistant cultivars (FERREIRA *et al.*, 2011; MOENS; PERRY; STARR, 2009; WILCKEN; GARCIA; SILVA, 2005).

Identifying sources of resistance among commercial cultivars, and the development of resistant cultivars adapted to the various conditions of Brazil, have been the focus of research, especially since the early 90's (FIORINI *et al.*, 2005). Although there are a fair number of studies on the response of crisp and smooth lettuce to different species of *Meloidogyne*, studies that include the American group of lettuce cultivars are scarce (CHARCHAR; MOITA, 1996; FERREIRA *et al.*, 2011; ROSA; WESTERICH; WILCKEN, 2013).

Wilcken, Garcia and Silva (2005), using the nematode RF as a variable, verified the resistance of 13 cultivars of American lettuce to *Meloidogyne incognita* race 2, out of 23 being tested. Of those that

stood out, 'Salinas 88' proved to be promising. This cultivar was later used in crossings with the Colorado and Verônica cultivars in order to incorporate this and other characteristics of interest in the resultant strains, which were evaluated using the gall index as variable (CARVALHO FILHO *et al.*, 2012; SILVA *et al.*, 2008).

In the search for further information concerning the response of American-type lettuce cultivars to the root-knot nematode, the aim of this study was to evaluate the resistance of twenty-two lettuce cultivars to *M. javanica* and *M. incognita* race 1 and 2.

MATERIAL AND METHODS

The experiments were carried out in the greenhouse of the Department of Plant Protection at the School of Agronomic Sciences of the São Paulo State University, Botucatu Campus (FCA/UNESP). Separate experiments were conducted for each nematode species, each following the same methodology.

The lettuce cultivars used in this study were Lucy Brown, Robinson, Calona, Classic, Vanguard 75, Winterset, Salinas 88, Bnondaga, Ithaca, Raider Plus, Desert Queen, Sonoma, IP-11, Sundevil, Challenge, Summer Time, L-104, L-109, Tainá, Lady and RS-1397, all provided by Professor Noberto da Silva of the Department of Production and Plant Breeding, School of Agronomic Sciences, Botucatu Campus - FCA/UNESP.

To germinate, the seeds were transferred to polystyrene trays of 128 cells containing a sterilised substrate. At 25 days, they were transplanted into 2 L pots containing a substrate composed of previously autoclaved soil, sand and organic matter (1:2:1).

The *M. incognita* race 1 inoculum used in this study was obtained from root-tissue samples of lettuce plants collected in 2011 in the District of Pouso Alegre, in the State of Minas Gerais. The population of *M. incognita* race 2 was obtained from the roots of coffee plants originating in the District of Oswaldo Cruz, in São Paulo, while the population of *M. javanica* was obtained from roots of the 'Magali' tomato, from the District of Santa Rosa in Rio Grande do Sul. The species were identified by the perineal pattern of the females and by electrophoretic isoenzyme patterns, as per Oliveira *et al.* (2012), and were maintained in the Rutgers tomato in the greenhouse of the Department of Plant Protection. To identify the physiological races of *M. incognita*, a reaction test was carried out on different hosts.

Pure populations of *M. javanica* and *M. incognita* race 1 and 2 were multiplied in Rutgers tomato plants in 1 L pots containing autoclaved substrate. The plants were

then kept in a greenhouse for 60 days, when the highly infected roots of the plants were processed according to Hussey and Baker (1973) as modified by Bonetti and Ferraz (1981) to extract the eggs.

The number of eggs and potential newly hatched juveniles in the suspension was determined using a Peters slide under a light microscope. The lettuce plants, previously transplanted into 2 L pots containing sterilised substrate, were then individually inoculated with 5,000 eggs and potential second-stage juveniles (Pi) of the nematode population under test, two days after transplanting. Inoculation involved placing 2 mL of the inoculum suspension in two 3-cm deep holes in the rhizosphere of each plant. The Rutgers tomato was used as the standard for inoculum viability.

The experimental design was completely randomised, with five replications per treatment, and each plot comprising one plant.

The evaluations were made 60 days after inoculation. The root systems of the cultivars were washed individually under running water, weighed after removing the excess water with paper towels, and stained with Floxine B to facilitate counting of the egg masses (TAYLOR; SASSER, 1978).

The gall index (GI) and egg mass index (EMI) were obtained using a rating scale, and classified as: level 0 (no galls or egg masses), level 1 (1 to 2 galls or egg masses), level 2 (3 to 10 galls or egg masses), level 3 (11 to 30 galls or egg masses), level 4 (31 to 100 galls or egg masses) and level 5 (more than 100 galls or egg masses per root) (TAYLOR; SASSER, 1978). After counting the number of galls and egg masses, the root systems were processed following the method of Coolen and D'Herde (1972), using a 0.5% sodium hypochlorite solution in place of water to triturate the roots in a blender. The final number of eggs and newly hatched juveniles in the suspension was determined with the aid of a Peters slide under a light microscope. This number was used to obtain the reproduction factor (final nematode population (Pf) / initial population (number of eggs used in the nematode inoculations) (Pi)), as per Oostenbrink (1966), i.e. equal to or greater than 1.0, susceptible (S) and less than 1.0, resistant (R).

RESULTS AND DISCUSSION

According to the results (Table 1), differences can be seen between treatments in the reproduction factor (RF) of *M. incognita* race 1. These results indicate the existence of genetic variability among the tested materials, and are an important indication for

breeding programs aiming at lettuce resistance to root-knot nematodes. GI values ranged from 3.0 ('Sonoma') to 5.0 ('Lucy Brown'), and the EMI ranged from 1.6 ('Ithaca') to 4.6 ('Tainá'). The 'Rutgers' tomato, used as the control, had a reproductive factor of 5.18, confirming the viability of the inoculum.

The Ithaca, RS-1397, Raider Plus, Challenge, L-104, IP-11, Salinas 88, Calona, Desert Queen, Classic and Vanguard 75 cultivars had an RR <1.0 and therefore behaved as resistant to *M. incognita* race 1, with an RF ranging from 0.19 to 0.88. Within this group, the lettuces with an RF closest to zero were 'Ithaca' (RF=0.19), 'RS-1397' (RF=0.3), 'Raider Plus' (RF=0.41), 'Challenge' (RF=0.5), 'L-104' (RF=0.62) and 'IP-11' (RF=0.64); these did not differ statistically from one other.

The lettuces, 'Lady', 'Robinson', 'Sonoma', 'Winterset', 'Lucy Brown', 'Raider', 'Bnondaga', 'Summer Time', 'Sundevil', 'Tainá' and 'L-109' behaved as susceptible to *M. incognita* race 1, with an RF that ranged between 1.1 and 5.6. Among these materials, it is can be seen that 'Lady' (RF=1.1), 'Robinson' (RF=1.12), 'Sonoma' (RF=1.25) and 'Winterset' (RF=1.35), despite favouring nematode multiplication, were not statistically different from the resistant cultivars, Salinas 88 (RF=0.75), Calona (RF=0.77), Desert Queen (RF=0.82), Classic (RF=0.83) and Vanguard 75 (RF=0.88). The 'L-109' (RF=5.6) lettuce had the highest nematode reproduction factor of all the lettuces under study, followed by 'Tainá' (RF=5.1), 'Sundevil' (RF=4.53) and 'Summer Time' (RF=4.5).

The results of the experiment with *M. incognita* race 2 (Table 2) show that there were significant differences in reproduction factor (RF) for the cultivars under study. Values for GI ranged from 1.6 ('Raider') to 4.8 ('Bnondaga' and 'Raider Plus'), with the EMI ranging from 1.0 ('Vanguard 75') to 4.8 ('Bnondaga'). The Rutgers tomato had an RF of 17.41, confirming the viability of the *M. incognita* race 2 inoculum.

The Desert Queen, L-104, Salinas 88, Vanguard 75, Robinson, RS-1397, Challenge, Raider Plus, Classic, Calona, Ithaca, Lady, IP-11 and Winterset cultivars were resistant to nematode multiplication, with an RF <1.0, although there were significant differences between the cultivars.

Of the materials under evaluation, the 'Sonoma', 'Bnondaga', 'Lucy Brown', 'Tainá', 'Raider', 'L-109', 'Summer Time' and 'Sundevil' lettuces were susceptible to *M. incognita* race 2, with an RF >1.0. These materials afforded an initial increase in the nematode population, with an RF ranging from 1.39 to 4.64. The Sonoma (RF=1.39) and Bnondaga (RF=1.42) cultivars had the

Table 1 - Mean values for gall index (GI), egg mass index (EMI) and reproduction factor (RF) of *Meloidogyne incognita* race 1 in twenty-two American-group lettuce cultivars

Cultivar	GI	EMI	RF ¹	Response ²
Ithaca	3.6	1.6	0.19 a	R
RS-1397	3.4	1.8	0.30 a	R
Raider Plus	3.2	1.8	0.41 a	R
Challenge	3.6	2.8	0.50 a	R
L-104	4.6	4.2	0.62 a	R
IP-11	3.2	2.0	0.64 a	R
Salinas 88	3.6	2.6	0.75 b	R
Calona	4.6	3.4	0.77 b	R
Desert Queen	3.6	2.4	0.82 b	R
Classic	4.8	3.0	0.83 b	R
Vanguard 75	3.6	2.6	0.88 b	R
Lady	3.6	3.0	1.10 b	S
Robinson	4.0	3.0	1.12 b	S
Sonoma	3.0	2.4	1.25 b	S
Winterset	3.8	2.4	1.35 b	S
Lucy Brown	5.0	3.8	3.01 c	S
Raider	3.6	3.4	3.19 c	S
Bnondaga	4.8	4.2	3.48 c	S
Summer Time	4.6	4.4	4.50 d	S
Sundevil	4.4	4.0	4.53 d	S
Tainá	4.6	4.6	5.10 d	S
L-109	4.4	4.2	5.60 d	S
CV (%)			15.80	

¹Mean values followed by the same letter do not differ by Scott-Knott test at 5% probability. Analysis carried out with transformed data ($\sqrt{x + 0.5}$).

²R=resistant (RF <1.0) and S=susceptible (RF >1.0), as per Oostenbrink (1966)

Table 2 - Mean values for gall index (GI), egg mass index (EMI) and reproduction factor (RF) of *Meloidogyne javanica* in twenty-two American-group lettuce cultivars

Cultivar	GI	EMI	RF ¹	Response ²
Desert Queen	2.8	2.2	0.23 a	R
L-104	3.0	1.8	0.23 a	R
Salinas 88	1.8	1.4	0.24 a	R
Vanguard 75	2.0	1.0	0.27 a	R
Robinson	3.2	2.2	0.28 a	R
RS-1397	3.4	2.4	0.28 a	R
Challenge	2.4	1.4	0.33 a	R
Raider Plus	4.8	4.2	0.41 a	R
Classic	3.6	2.4	0.43 a	R
Calona	3.0	2.4	0.52 a	R
Ithaca	3.4	3.0	0.70 b	R
Lady	3.4	2.4	0.81 b	R

Continuation Table 2

IP-11	2.8	1.6	0.88 b	R
Winterset	2.6	1.6	0.93 b	R
Sonoma	2.8	1.4	1.39 c	S
Bnondaga	4.8	4.8	1.42 c	S
Lucy Brown	4.2	3.2	2.10 d	S
Tainá	2.4	2.0	2.37 d	S
Raider	1.6	1.4	2.78 e	S
L-109	3.2	2.2	4.0 f	S
Summer Time	2.8	2.0	4.32 f	S
Sundevil	3.6	2.4	4.64 f	S
CV (%)			9.08	

¹R=resistant (RF <1.0) and S=susceptible (RF >1.0), as per Oostenbrink (1966)

lowest values for RF, differing statistically from the other plants under test, while 'L-109' (RF=4.0), 'Summer Time' (RF=4.32) and 'Sundevil' (RF=4.64) had the highest mean values for RF, and were also statistically different from the other evaluated plants.

The results of Table 3 show that each of the lettuce cultivars under test had an RF <1.0 and were therefore considered resistant to *M. javanica*. The Rutgers tomato had an RF of 7.37, confirming the viability of the *M. javanica* inoculum.

The resistance of the 'Classic' lettuce was checked for a mixed population of *M. incognita* race 1 and *M. javanica* (CHARCHAR; MOITA, 1996). Their result agrees with those obtained here; although in the present study pure populations of *M. incognita* race 1 and *M. javanica* were used. The resistance of the

'Challenge', 'Salinas 88', 'Classic' and 'Vanguard 75' lettuces had already been reported for *M. incognita* race 2 (WILCKEN; GARCIA; SILVA, 2005). These cultivars also proved to be resistant to *M. incognita* race 1 and 2 in the present study. The resistance response of the Tainá and Lucy Brown cultivars to *M. javanica* had already been noted in the field and in the greenhouse (DIAS-ARIEIRA, 2012; LÉDO; SOUSA; SILVA, 2000). The susceptibility response seen in the Lucy Brown, Sonoma, Sundevil, Summer Time and Raider cultivars corroborate the results obtained by Wilcken, Garcia and Silva (2005).

The GI and EMI of *M. incognita* race 1 and 2 attributed to the lettuce cultivars under evaluation showed a relationship with the nematode RF, except for the 'L-104' lettuce to *M. incognita* race 1 and

Table 3 - Mean values for gall index (GI), egg mass index (EMI) and reproduction factor (RF) of *Meloidogyne javanica* in twenty-two American-group lettuce cultivars

Cultivar	GI	EMI	RF	Response ¹
Bnondaga	0	0	0	R
Raider Plus	0	0	0	R
Desert Queen	0	0	0	R
IP-11	0	0	0	R
Challenge	0	0	0	R
Ithaca	0.8	0.4	0.01	R
Lucy Brown	2.0	1.0	0.02	R
Calona	1.6	1.0	0.02	R
Classic	1.2	1.0	0.02	R
Vanguard 75	1.0	1.0	0.02	R

Continuation Table 3

Winterset	1.6	1.2	0.02	R
Salinas 88	1.6	1.0	0.02	R
Sonoma	1.0	1.0	0.02	R
Sundevil	1.4	1.0	0.02	R
Summer Time	1.0	1.0	0.02	R
L-109	2.0	1.2	0.02	R
Tainá	1.2	1.0	0.02	R
Raider	1.6	1.0	0.02	R
Lady	1.4	1.0	0.02	R
RS-1397	1.6	1.4	0.03	R
Robinson	2.0	1.0	0.05	R
L-104	2.4	1.6	0.06	R
CV (%)	0.75			

IR=resistant (RF <1.0) and S=susceptible (RF >1.0), as per Oostenbrink (1966)

'Raider Plus' to *M. incognita* race 2, which had a high GI and EMI and a low RF; this may be justified by the probable low number of eggs in each gelatinous mass. In the experiment with *M. javanica*, the GI and EMI were low, as was the nematode RF in all the evaluated cultivars, which indicates a greater resistance by these materials to this species, since the low GI values demonstrate the difficulty of the nematodes to establish parasitism in the roots of these lettuce cultivars.

CONCLUSIONS

1. The American-group lettuce cultivars Ithaca, RS-1397, Raider Plus, Challenge, L-104, IP-11, Salinas 88, Calona, Desert Queen, Classic and Vanguard 75 were resistant to *M. incognita* race 1 and 2;
2. The Robinson, Lady and Winterset cultivars were resistant to *M. incognita* race 2;
3. The Sonoma, Lucy Brown, Raider, Bnondaga, Summer Time, Sundevil, Tainá and L-109 cultivars were susceptible to *M. incognita* race 1 and 2;
4. The Lady, Robinson and Winterset cultivars were susceptible to *M. incognita* race 1;
5. All the cultivars were resistant to *M. javanica*.

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