

MAGALHÃES, CC; CARVALHO, ADF; BISCAIA, D; MELO, RAC; SOUZA, LR; ALVES, CS; SILVA, GO; PINHEIRO, JB. 2022. Adjustment of inoculum levels for the evaluation of carrot genotypes resistance to *Meloidogyne incognita* Horticultura Brasileira 40: 321-325. DOI: <http://dx.doi.org/10.1590/s0102-0536-20220311>

Adjustment of inoculum levels for the evaluation of carrot genotypes resistance to *Meloidogyne incognita*

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ABSTRACT

One of the main phytopathogens that cause enormous economic losses in several agricultural species, including carrots, are the root-knot nematodes belonging to the genus *Meloidogyne*. Thus, in order to identify the best population level for inoculum of the root-knot nematode (*Meloidogyne incognita*) for maximum expression of symptoms of this nematode attack on carrots, an experiment was carried out to evaluate the carrot cultivar Brasília (CBR) and the population Pop-750 (CRO). The experiment was carried out in a greenhouse at Embrapa Vegetable, Brasília-DF, in 5 L pots, with six replications, in a 4x2 factorial scheme in a completely randomized design, in the dosages of 0, 1, 5 and 10 thousand eggs and occasional juveniles of 2nd stage (E+J2R). 'Rutgers' susceptible tomato cultivar was used as control to verify inoculum efficiency. Inoculation was carried out 30 days after sowing and evaluation 60 days after inoculation. Gall index (GI), egg mass index (EMI), number of eggs plus occasional second stage juveniles per gram of root (E+J2R) and reproduction factor (RF) were performed. There were differences between genotypes and between inoculum levels for all variables evaluated. For CRO, inoculum levels of *M. incognita* from 1,000 E+J2 the plants already manifested symptoms and changes in all evaluated variables, with ideal levels around 5 to 7 thousand E+J2R, above 7 thousand E+J2R nematode multiplication to express symptoms decreased. For CBR the response variables E+J2 at root and RF inoculum levels close to 5 thousand E+J2R also present the best results, but when the characterization is based on the evaluation of GI and EMI, suitable inoculum levels would be close to 9 and 12 thousand E+J2R.

RESUMO

Ajuste de níveis de inóculo para avaliação da resistência de genótipos de cenoura a *Meloidogyne incognita*

Um dos principais fitopatógenos que causam prejuízos econômicos em diversas espécies agrícolas, inclusive cenoura, são os nematoides-das-galhas, pertencentes ao gênero *Meloidogyne*. Assim, com o objetivo de identificar o melhor nível populacional de inóculo de *Meloidogyne incognita* para a máxima expressão de sintomas do ataque de nematoides-das-galhas em cenoura, foi realizado um experimento que avaliou os genótipos de cenoura cv. Brasília (CBR) e a população Pop-750 (CRO). O experimento foi conduzido em casa de vegetação da Embrapa Hortaliças, Brasília-DF em vasos de 5 L, com seis repetições, em esquema fatorial 4x2 em delineamento inteiramente casualizado, nas dosagens de 0, 1, 5 e 10 mil ovos e eventuais juvenis de segundo estágio (O+J2R). O tomateiro suscetível 'Rutgers' foi utilizado como testemunha para verificar eficiência do inóculo. A inoculação foi realizada 30 dias após a semeadura e, a avaliação, 60 dias após a inoculação. Foi realizada a avaliação dos índices de galhas (IG) e de massas de ovos (IMO), do número de ovos mais eventuais juvenis de segundo estágio por grama de raiz (O+J2R) e do fator de reprodução (FR). Houve diferenças entre os genótipos e entre os níveis de inóculo para todas as variáveis avaliadas. Para CRO, os níveis de inóculo de *M. incognita* a partir de mil O+J2 as plantas já manifestaram sintomas e alterações para todas as variáveis avaliadas, com os níveis ideais em torno de 5 a 7 mil O+J2R, sendo que acima de 7 mil O+J2R a multiplicação do nematode para expressar sintomas diminuiu. Para a CBR, as variáveis índices O+J2R na raiz e FR, os níveis de inóculo próximos a 5 mil O+J2R também apresentam os melhores resultados. Quando a caracterização for baseada na avaliação da formação de galha (IG) e massa de ovos (IMO), os níveis de inóculo adequados seriam entre 9 e 12 mil O+J2R.

Keywords: *Daucus carota*, resistance, root-knot nematodes, dose.

Palavras-chave: *Daucus carota*, resistência, nematoides-das-galhas, dose.

Received on April 19, 2022; accepted on August 24, 2022

Carrot (*Daucus carota*) is the most important species of the Apiaceae family due to its social and economic significance. Worldwide more than 39 million tons are produced in an area of 1.1 million hectares, with an average yield of 35 t/ha. China is the largest producing country, achieving a value of 18 million tons (FAO, 2020). In Brazil,

carrot is among the most popular and consumed vegetables, with an annual volume of 700 thousand tons grown in an estimated area of 20,000 hectares. This carrot growing area is represented primarily by the Alto Paranaíba region in Minas Gerais State, which encompasses half of the country production volume, followed by Marilândia do Sul (Paraná state), Caxias do Sul (Rio Grande do Sul state), Cristalina (Goiás state), and Irecê (Bahia state) (IBGE, 2017; Cunha *et al.*, 2021).

Several diseases can occur during the carrot growing stages, but root-knot nematodes (RKN) are of particular interest, given that these plant-parasitic nematodes cause significant losses not only in the afore mentioned regions but as well as in all global carrot production areas (Viljoen *et al.*, 2019). The amplitude of these damages goes from discarding of products classified as unsuitable for marketing, up to areas suffering from recurring infestation, as a result of lack of sound agricultural practices, or its complete abandonment (Pinheiro, 2017).

Meloidogyne incognita, *M. javanica*, and *M. hapla* are the major RKN species responsible for carrot losses in Brazil (Cunha *et al.*, 2021). Their attack is characterized by cell hyperplasia and hypertrophy of the taproots, compromising its classification (Charchar *et al.*, 2000), though in certain circumstances the weight loss is not evident, resulting mostly in forking (Huang *et al.*, 1986). RKN can also induce galling, plant stunting, and root fasciation (Cunha *et al.*, 2021). The use of chemical control is not recommended given the fact that its efficiency is uncertain and also expensive (Pinheiro, 2017). Additionally, in Brazil, there are few nematicides registered for carrots, with most of them being highly toxic and pollutant, making this practice inappropriate (Silva *et al.*, 2011). Crop rotation with legumes, brassicas, or grasses previous to carrot cultivation is being used as a control measure (Charchar & Aragão, 2003). However, this practice is not effective in all situations, besides making the production system more complex.

Thus, improving genetic resistance is considered the best method to overcome RKN. Sources of resistance in carrots have been identified since 1982 by Embrapa Vegetables, and as a result the cultivars Brasília and Tropical were released in the '80s (Pinheiro, 2017). Boiteux *et al.* (2000) identified the MJ-1 gene located in chromosome 8 in two carrot genotypes derived from 'Brasília' (891091 and 971252). This gene expresses a complete resistance to *M. javanica* and a partial resistance to *M. incognita*. Other Quantitative Trait locus (QTLs) were identified in chromosomes 1, 2, 8, and 9, that explained 27.3% of the genetic effects, all considered of additive nature (Parsons *et al.*, 2015). More recently, another gene was identified in the genotype PI 652188, called Mj-2, and also located in chromosome 8. This gene confers a high level of resistance to *M. javanica* and *M. incognita* compared to the 'Imperator 58' used as susceptible control (Ali *et al.*, 2014).

Even though resistance sources to RKN are well known, difficulties in the evaluation of progenies in field conditions are patent (Silva *et al.*, 2011). These difficulties comprise the visual identification of individual characteristics of resistant plants, in addition to the effects of environmental conditions, resulting in values of heritability close to zero, making the selection process compromised.

Studies in the literature report inoculation doses ranging from 1,000 (Viljoen *et al.*, 2019) to 50,000 (Simon *et al.*, 2000) eggs and eventual second-instar juveniles in experiments evaluating carrots. However, this amount varies according to the purpose of the work. It should be noted that low doses may not cause symptoms and high doses may lead to competition between individuals, also with negative effects on the appearance of symptoms.

This study aimed at the adjustment of inoculum levels for the expression of plant reaction to *M. incognita* by analyzing the resistance and susceptibility of carrot genotypes from breeding populations derived from cultivar Brasília (CBR), and from population Pop-750 (CRO).

MATERIAL AND METHODS

The experiment was held from May 21 to October 29, 2019, at Embrapa Vegetables, Brasília-DF, Brazil (15°55'54"S; 48°08'40"W, 1.014 m altitude) in a glass-glazed greenhouse. It consisted of a completely randomized design with a factorial scheme (4x2) with six replications. Four inoculum levels (0; 1,000; 5,000 and 10,000) containing eggs and eventual second-stage juveniles (E+J2R) of *M. incognita* represented the first factor and two breeding populations, Pop-750 of purple skin color identified as (CRO), and from cv. Brasília of orange skin color identified as (CBR) represented the second factor. The tomato cv. Rutgers, susceptible to RKN, was used as control to verify the inoculum viability. Sowing was made in 5 L pots filled with a sterilized substrate mixture of subsurface soil (a clayey red Oxisol commonly encountered in the Cerrado Biome region), washed sand, manure, and carbonized rice hulls, in a proportion of 1:1:1:1. It was fertilized with 300 g of 4-30-16 NPK formulation, and 300 g of calcined ground dolomitic limestone per 300 kg of this mixture. All other cultural practices to maintain the plants (irrigation, top dressing fertilizers, and others) were done according to Coyne & Ross (2014).

Thirty days after sowing (30 DAS) thinning was performed, leaving two plants per pot, to proceed the inoculation. The inoculum was maintained in tomato plants and was extracted according to Boneti & Ferraz (1981) methodology. Afterward, the suspension was calibrated to contain the afore said levels, being diluted in 5 mL water, and applied around the plant's stalk. Sixty days after the inoculation (60 DAI), the root system was washed thoroughly, preserving the galls and eggs. All infected roots were stained with phloxine B for J2 and egg masses, respectively.

Egg mass index (EMI) was obtained according to Taylor & Sasser (1978) using a scoring scale from 0 to 5, wherein: 0 = roots without egg masses; 1 = presence of 1 to 2 egg masses; 2 = presence of 3 to 10 egg masses; 3

= presence of 11 to 30 egg masses; 4 = presence of 31 to 100 egg masses and 5 = presence of more than 100 galls or egg masses. Gall index (GI) was quantified according to Taylor & Sasser (1978) likewise, using the aforementioned scoring scale for the number of galls. The reproduction factor (RF) was obtained by dividing the initial and final nematode population ($RF = Pf/Pi$), considering the initial population (Pi) the one inoculated and the final population (Pf) present (Oostenbrink, 1966). Data were subjected to analysis of variance (ANOVA), and transformation of $\sqrt{x + 0.5}$ was used to approximate the residuals to a normal distribution to enable homoscedasticity. The inoculum levels were analyzed by regression. All computations were performed using SAS/GLM (Statistical Analysis System, Cary, NC, USA).

RESULTS AND DISCUSSION

The significance of the inoculum levels was expressed by regression curves (Figures 1 to 4). Concerning the GI (Figure 1), crescent levels resulted in a quadratic fit for CRO and CRB, with a coefficient of determination (R^2) presenting values of 0.93 and 0.70, respectively, which show the usefulness of both models. As for CRO, the level of 6,948 resulted in a higher E+J2R value. Thus, values superior to this would decrease GI number. At the same time, for CBR the maximum value of 11,902 E+J2R is fundamentally close to the higher inoculum level evaluated. These results are consistent with those reported previously for carrot genotypes/cultivars. Silva *et al.* (2011) described that it was possible to distinguish resistant genotypes from susceptible ones using an inoculum level of 6,000 E+J2R, with progenies from 'Brasília' regarded as tolerant when compared to the control 'Kuronan'. Yet, the authors stated that improvements in this methodology were still necessary for the selection of superior progenies, aiming at precise results.

GI is a primary character for the evaluation of resistance in carrot progenies, substantiated by several studies (Boiteux *et al.*, 2004). A high correlation between egg production and

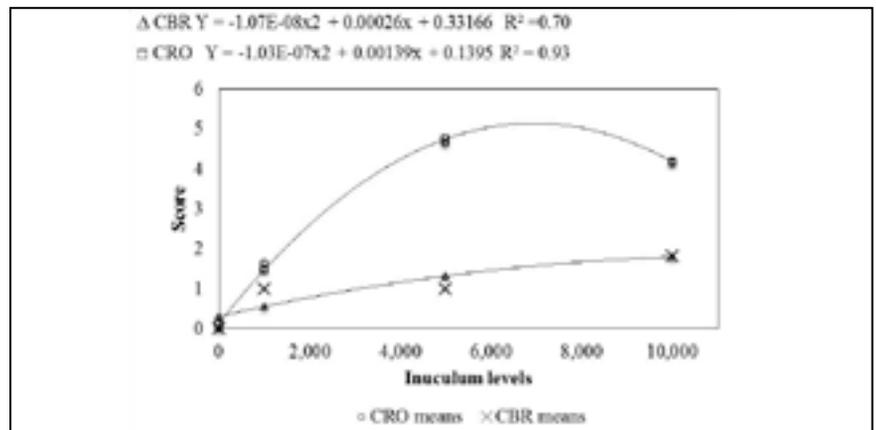


Figure 1. Regression fitting for the variable gall index scores observed in carrot genotypes under different *Meloidogyne incognita* inoculum levels. Brasília, Embrapa Hortaliças, 2019.

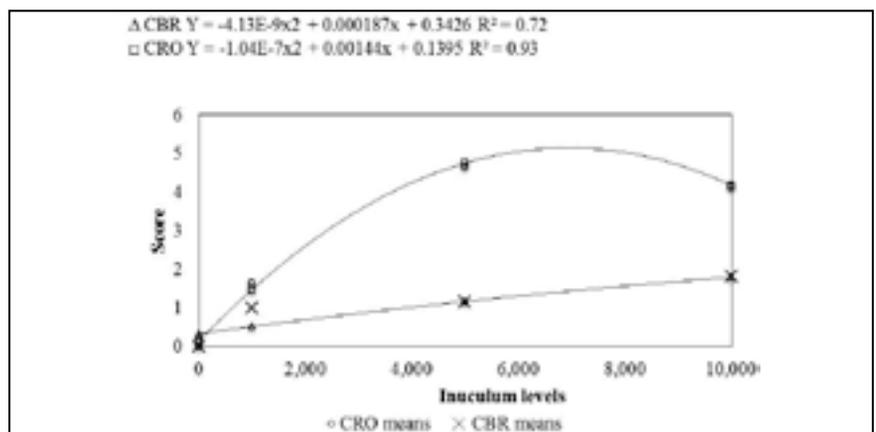


Figure 2. Regression fitting for the variable egg mass index scores observed in carrot genotypes under different *Meloidogyne incognita* inoculum levels. Brasília, Embrapa Hortaliças, 2019.

gall formation allows the identification of resistant carrot genotypes (lines or populations) based on this characteristic. Seo *et al.* (2014) evaluated 170 carrot lines about their resistance to *M. incognita* race 1, inoculating 1,000 E+J2R, evaluating the GI 7 weeks after inoculation, observing values ranging from 0.20 to 4.20.

For the EMI (Figure 2), similar results to GI were observed, hence, CRO displayed a larger quantity with a peak of around 7,000 E+J2R. After this inoculum level, symptoms began to become less evident. For CBR, there was no maximum expression at the evaluated inoculum levels. The estimate to obtain the maximum expression of EMI in Brasília would be 11,472 E+J2. Khan *et al.* (2018) evaluated 13 carrot cultivars utilizing an inoculum level of 3,000 E+J2R of *M. incognita*. These authors identified, with varying

degrees of resistance, that all main roots showed symptoms. Thus, they emphasize that the management of this RKN in production fields is a challenge and requires a combination of genetic and cultural methods.

Regarding the E+J2R per g of roots (Figure 3), in all inoculum levels evaluated, CRO presented higher values compared to CBR. Yet an equivalent fitting was observed, where increasing the inoculum would result in E+J2R values being elevated in different proportions for both genotypes. An inoculum level of 6,329 E+J2 would result in an approximate population of 25 E+J2R per gram of roots, considering the higher quantity of this RKN present in the roots.

The reduction of E+J2R values from certain inoculum levels can be explained mostly by competition, as a result of higher levels that interfere in

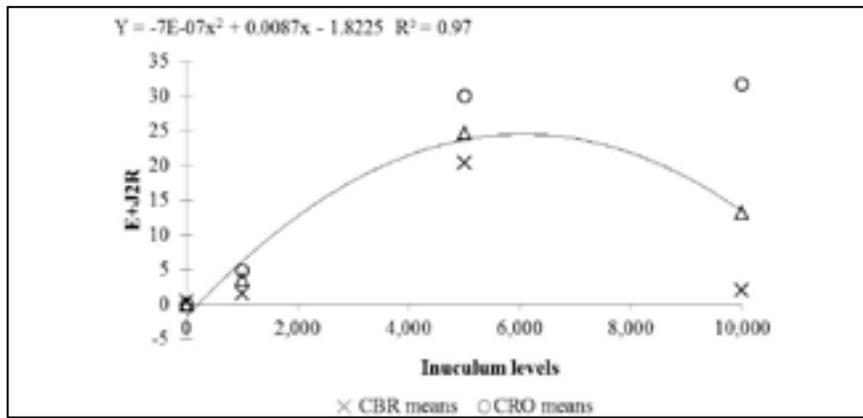


Figure 3. Regression fitting for the variable E+J2R/g of roots scores observed in carrot genotypes under different *Meloidogyne incognita* inoculum levels. Brasília, Embrapa Hortaliças, 2019.

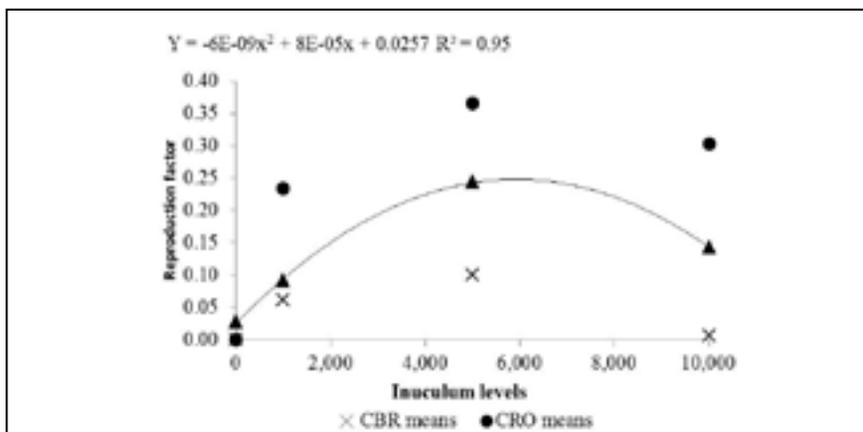


Figure 4. Regression fitting for the variable reproduction factor (Rf) scores observed in carrot genotypes under different *Meloidogyne incognita* inoculum levels. Brasília, Embrapa Hortaliças, 2019.

the infection and multiplication of RKN. Jaiteh *et al.* (2012) described this effect in a tomato trial with inoculum levels of 100 to 2,000 E+J2 of *Meloidogyne* spp., which was also noticed by Kayani *et al.* (2017) with cucumber genotypes inoculated with levels ranging from 500 to 8,000 E+J2 of *Meloidogyne incognita*.

For comparison purposes, the susceptible tomato 'Rutgers' used to verify the efficiency of the inoculum, presented a mean value of 103 E+J2R per gram of roots. Carrot is classified as one of the most susceptible vegetable species, with high E+J2R values, like the obtained in tomato, okra, eggplant, and green peas, but lesser compared to cauliflower, radish, and cabbage (Anwar & McKenry, 2010). The lower number of E+J2R per gram of roots of the present work may be associated with

the high and intermediate resistance of CBR and CRO, respectively, pointing to the resistance of the genotypes that form their derivate population against *M. incognita*.

The RF values (Figure 4) observed for both CBR and CRO, were inferior to 1. This denotes a lesser quantity of nematodes per root compared to the inoculated treatments, therefore, proving a populational reduction of *M. incognita* of these genotypes, mainly CBR, that behaved as more tolerant. An inoculum level of 5,959 E+J2 would result in a Rf value of 0.30. These low Rf values obtained from genotypes derived from 'Brasília' are commonly observed when compared to other parental lines.

Pop-750 is derived from a crossing between 'BRS Planalto' (also derived from 'Brasília') and 'Cosmic', being selected for six successive cycles

for resistance to leaf blight diseases and against premature bolting, but not directly selected to RKN. A high susceptibility level of 'Cosmic' was identified by Pinheiro (2017) where severe symptoms in the taproots were described. Contrarily, the resistance of genotypes derived from 'Brasília' to RKN is largely discussed in other works (Singh *et al.*, 2019; Simon *et al.*, 2000). This cultivar is a standard parent for RKN resistance in breeding programs in Brazil and other countries. As an example, populations 891091 and 971252 possess a complete resistance level to *M. javanica* obtained in the USA (Boiteux *et al.*, 2004). So, when GI and EMI are evaluated, a higher inoculum level is necessary to differentiate tolerant progenies, which can be obtained by utilizing 'Brasília' as a source of resistance.

In the evaluation of populations with different degrees of resistance to nematodes, the inoculum level must be adequate for each response variable evaluated and for each genotype studied. In the inoculation process for carrot populations like CRO, with a lower tolerance level to RKN, the most adequate levels of inoculum ranged from 5,000 to 7,000 eggs and eventual second stage juveniles of *M. incognita*, to obtain the maximum manifestation of nematological characters. Levels higher than 7,000 would decrease multiplication efficiency, due to competition for feeding sites. Regarding carrot genotypes with resistance degrees comparable to 'Brasília', which is known to have resistance to *M. javanica* and tolerance to *M. incognita* (Boiteux *et al.*, 2004), for the quantification of the number of E+J2R per gram of roots and RF, inoculum levels close to 5,000 are sufficient to maximize the expressions of nematode variables. Inoculation levels higher than that will present the same competition for feeding sites. However, when the characterization is based on the evaluation of gall index (GI) and egg mass index (EMI), the appropriate inoculum level would range between 9,000 to 12,000 E+J2 to maximize symptoms. This necessity for higher levels of inoculum for this RKN species multiplication is partially explained by

the tolerance mechanism of cultivar Brasília.

ACKNOWLEDGEMENTS

To Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) by the first author's scientific initiation scholarship.

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