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 Brasilia (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.

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

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

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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 Paranáiba 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 fasciculation (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 Brasilia 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 ‘Brasilia’ (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 Brasilia (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, 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. Brasilia 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 (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 Brasilia 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 a 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...
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 ‘Brasilia’ are commonly observed when compared to other parental lines.

Pop-750 is derived from a crossing between ‘BRS Planalto’ (also derived from ‘Brasilia’) 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 ‘Brasilia’ 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 ‘Brasilia’ 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 ‘Brasilia’, 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. Levels higher than 5,000 are adequate to differentiate tolerant progenies, which can be obtained by utilizing ‘Brasilia’ as a source of resistance.
the tolerance mechanism of cultivar Brasília.

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