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Parasitism of the root knot nematode *Meloidogyne incognita* (Kofoid and White) chitwood in five wild Solanaceae species

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Parasitismo del nematodo agallador de la raíz *(Meloidogyne incognita)* en cinco especies de solanáceas silvestres

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ABSTRACT

Keywords:
Solanaceae
Resistance
Susceptibility
Tolerance
Nematode

There is a high incidence of the nematode *Meloidogyne incognita* in several economically important species of Solanaceae. This nematode causes damage to the roots, leading even to the death of the plant, causing economic losses for the producer. This research was carried out in greenhouse to assess the response of five species of wild Solanaceae (*Solanum auriculatum*, *S.hirtum, S.hispidum*, *S.arboreum* and *Nicotiana glauca*) to the infestation of *M. incognita* was evaluated. A randomized block design with three replicates was used. The initial inoculum was obtained from infested roots of tree tomato (*S. betaceum* Cav.), which was propagated in kidney tomato plants (*Lycopersicum esculentum* Mill.) hibrid 'Sheila' which was used to inoculate the wild Solanaceae plus two susceptible controls (*S. betaceum* and *S. quitoense*) were inoculated with a dose of 2500 larvae. According to the reproduction factor of the nematode, *S. arboreum*, *S. hirtum* and *N. glauca* showed resistance because obtained values less than 1. In addition, *S. hirtum* (14.88) and *N. glauca* (22.67) showed the lowest number of root knots. In terms of foliage yield (dry weight), a response of tolerance was observed in all species except for the controls. It can be concluded that *S. hirtum* (compatible with *S. quitoense* - naranjilla) and *N. glauca* (compatible with *S. betaceum* - tree tomato) might be used as rootstocks of Solanaceae fruit crops of commercial importance in Ecuador, contributing to the integrated fruit production system.

RESUMEN

Palabras clave:
Solanaceae
Resistencia
Susceptibilidad
Tolerancia
Nematodo

Existe una alta incidencia del nematodo *Meloidogyne incognita* en varias especies solanáceas de importancia económica. Este nematodo causa daño a las raíces, llevando incluso a la muerte de la planta, causando pérdidas económicas para el productor. Esta investigación se llevó a cabo en invernadero para evaluar la respuesta de cinco especies de solanáceas silvestres (*Solanum auriculatum, S. hirtum, S. hispidum*, *S. arboreum* y *Nicotiana glauca*) a la infestación de *M. incognita*. Se utilizó un diseño de bloques al azar con tres repeticiones. Se obtuvo el inóculo inicial de raíces infestadas de tomate de árbol (*S. betaceum* Cav.), el cual se propagó en tomate riñón (*Lycopersicum esculentum* Mill.) híbrido 'Sheila' y se inoculó las solanáceas silvestres más dos controles susceptibles (*S. betaceum* y *S. quitoense*) con una dosis de 2500 larvas. De acuerdo al factor de reproducción del nematodo, *S. arboreum, S. hirtum* y *N. glauca* presentaron resistencia porque obtuvieron valores menor a 1. Además, *S. hirtum* (14,88) y *N. glauca* (22,67) presentaron el menor número de agallas en las raíces. En términos de rendimiento de follaje (peso seco), se observó una respuesta de tolerancia en todas las especies excepto en las plantas control. Se puede concluir que *S. hirtum* (compatible con *S. quitoense* - naranjilla) y *N. glauca* (compatible con tomate de árbol -*S. betaceum*) podrían ser utilizados como portainjertos de Solanáceas de importancia comercial en Ecuador.

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Nematodes of the genus *Meloidogyne* are considered worldwide as a major pest because they are the most numerous multicellular organisms on earth (González *et al*., 2010). Most of them are free-living and may be plant parasites and may become economically important in agricultural production, causing losses of more than 80% in areas of high infestation (Dong and Zhang, 2006; Guerena, 2006; Pakeerathan *et al*., 2009). *Meloidogyne* is a sedentary endoparasite that lives within the tissue and has a wide geographical distribution and forms root knots. There are more than 90 species of *Meloidogyne* identified, however *M. arenaria*, *M. javanica*, *M. hapla* and *M. incognita* are the most important because they cause major economic damage (De Jin *et al*., 2005; Rodríguez *et al*., 2009). Plants affected by nematodes showed symptomatology such as leaf yellowing, less growth and wilting and limited root development because of the formation of root knots, affecting the absorption of nutrients and decreasing yield (Daramola *et al*., 2015). Nematodes can interact with other diseases (i.e. *Fusarium* sp.) or breakdown genetic resistance to other enemies (Begum *et al*., 2012).

The control of nematodes has been generally done by the use of nematicides and soil fumigants. However, these products are no longer effective when populations of nematodes are high, in adittion they can also generate resistance and affect human health and the environment, and also reduce the biodiversity of the ecosystem (Akhtar and Malik, 2000; Pakeerathan *et al*., 2009). Currently, there is growing pressure to limit the use of agrochemicals (De Jin *et al*., 2005; Pakeerathan *et al*., 2009) and to develop environmentally friendly alternatives such as crop rotation, resistant cultivars, heat treatment, biological control and the application of organic amendments for good agronomic management (Castro *et al*., 2011). In this context, the use of rootstocks resistant to control this pest is a proposed option because had shown good results in agricultural crops (Marin *et al*., 2017).

The Solanaceae family contains approximately 96 genera and 2300 species including domesticated species and they have a cosmopolitan distribution, situated in tropical, subtropical and temperate climates at altitudes from 0 to 4000 m (Cuevas *et al*., 2008). The use of wild Solanaceae as rootstocks has been reported to prevent soil pest attack (Viteri *et al*., 2010). Different Solanaceae species have been reported showing resistance to *M. incognita* such as *Solanum torvum*, *S. sisymbriifolium*, *S. erianthum* and *Datura stramonium* (Rahman *et al*., 2002; Gonzáles *et al*., 2010).

The objective of this research was assessed the response of wild Solanaceae to nematode infestation caused by *M. incognita*, with the purpose of their subsequent use like rootstocks of Solanaceae fruit crops such as naranjilla (*S. quitoense*) and tree tomato (*S. betaceum*).

MATERIALS AND METHODS

Experimental location

The present research was carried out in the greenhouse of the Tumbaco Experimental Farm belonging to the Fruit Program (INIAP) and the nematological analysis in the Agrocalidad laboratories, located in the Quito Canton, Tumbaco Parish, Pichincha Province, at an altitude of 2348 m, latitude 0° 13' 0" S and longitude 78° 24'0" W. The average temperature in the greenhose varied from 12 °C to 32 °C and relative humidity was between 60 and 80%.

Inoculum of *M. Incognita*

The inoculum of *M. incognita* was obtained from the extract of infected roots of tree tomato plants. This procedure of extraction from roots used the technique of Stemerding (EPPO, 2016).

Identification of M. incognita

Morphometric identification was performed in J2 larvae obtained from infected tomato tree roots, using the taxonomic keys of Commonwealth Institute of Helminthology (Franklin, 1980). The specific primers MI-F GTGAGGATTCAGCTCCCCAG and MI-R ACGAGGAACATACTTCTCCGTCC (Meng *et al*., 2004) were used for the species confirmation.

Inoculum multiplication

Kidney tomato seedlings (*S. lycopersicum*) hybrid 'Sheila', were used for the inoculum multiplication. These seedlings were inoculated by placing 20 ml of the extract obtained from infected roots of tree tomato in four holes (4 cm deep) around the seedling. Once the roots of the tomato seedlings were galled, oval egg masses were obtained.

Inoculum preparation and inoculation of wild Solanaceae seedlings.

Using an optical stereoscope, it was found eggs and larvae of *M. incognita* embedded in the oval masses. These masses were separated from the plant tissue and placed in petri dishes covered with a plastic mesh and filter paper containing distilled water. They remained for 72 hours at a temperature of 28 °C, during which nematode eggs hatched and nematode juveniles J2 emerged. A 5 mL aliquot of the suspension was taken to account for the existing larvae and adjusted to the inoculation dose (2500 larvae per container of 1 kg capacity). An amount of 20 mL of the inoculum solution was placed in four holes (4 cm deep) around each Solanaceae plant. Sterilized water was placed instead of the inoculum in non-inoculated plants (controls).

Solanaceas species and variables analyzed

Treatments were constituted by different species of wild Solanaceae: *S. auriculatum* Aiton, *S. hirtum* Vahl, *S. hispidum* Pers., *S. arboreum* Dunal and *N. glauca* Graham; including 2 susceptible species such as *S. quitoense* Lam. and *S. betaceum* Cav. (Table 1). All Solanaceas were planted in pots (1.1 L) containing Andean black soil plus pomex in a proportion of 2:1.

Table 1. Solanaceae species assessed against the parasitism of *M. incognita*.

Solanacea	Province	Canton	Latitude	Longitude	Altitude (m)
S. auriculatum	Azuay	Paute	02° 47' 40"	78° 45' 59"	2201
S. hirtum	Morona Santiago	Palora	01° 42' 50"	77° 58' 24"	952
S. hispidum	Pichincha	Los Bancos	00° 04' 41"	78° 45' 11"	1498
S. arboreum	Morona Santiago	Palora	01° 50' 39"	78° 04' 35"	887
N. glauca	Tungurahua	Ambato	01° 14' 31"	78° 36' 06"	2379
S. quitoense	Napo	Archidona	00° 40' 30"	77° 45' 40"	1100
S. betaceum	Tungurahua	Pelileo	01° 19' 57"	78° 27' 49"	2405

The recorded variables in this study were the following:
Nematode final population: This was evaluated at 90 days after inoculation, at which time the plants showed nodulation in the roots. The roots of the different Solanaceae were processed by the Stemerding technique (EPPO, 2016) and then the final population of each treatment was counted.

Dry foliage weight (g): The fresh foliage of each seedling was placed in paper sleeves and dried in an oven at 60 °C for 72 hours, then weighed using a digital scale.

Number of root knots: This variable was recorded at 90 days after inoculation by counting individually the number of knots in the roots of each seedling evaluated.

Statistical analysis

Data were analyzed using the statistical package R version 3.3.2. The 95% confidence intervals were estimated to determine the rate of increase of the nematode population based on the Poisson model. When the reproduction factor (maximum value of the confidential interval/initial population) was less than 1 was considered resistant or superior than 1 was susceptible (Oostenbrink, 1966). With regard to host tolerance to nematode attack, a mean contrast (T test) was carried out between the yield (foliage dry weight) of the inoculated and non-inoculated plants for each Solanaceae, with the significance set at 5%. For number of root knots, the mean was estimated using the confidence interval at 95% and comparison of the means for Solanaceae was done through ANOVA, transforming the data by log transformation. The significance ranks were obtained by Duncan test at 5%.

RESULTS AND DISCUSSION

Identification of the nematode *M. incognita*

Individual larvae is obtained the following measurements: body length of 432.8 µm, body weight of 16.3 µm, stylet length of 10.2 µm and tail length of 42.1 µm; these measurements agree with those set by Eisenback *et al.* (1983). In adult females, the stylet cone was curved dorsally, and the anterior portion of the cone was cylindrical while the posterior was conical. In addition, specific primers MI-F and MI-R were used to amplify a band of 999 bp which confirmed the *M. incognita* species. Root knot nematodes (*Meloidogyne* spp.) are the most important nematode pests in the world, both because of their wide distribution and the high number of families and plant species that are affected (Gelpud, 2011), being the case of the commercial fruit crops in Ecuador such as tree tomato and naranjilla.

Evaluation of wild Solanaceae species inoculated with *M. incognita*

In Ecuador, fruit crops belonging to the Solanaceae family, such as tree tomato and naranjilla, have a high incidence of this nematode which causes damage to the plant and with the passage of the time can lead to death (Viteri *et al*., 2010). The estimated confidence interval for the nematode final population variable (Table 2) indicated that there was a resistance response by three species of wild Solanaceae (*N. glauca*, *S. hirtum* and *S. arboreum*) because their maximum value estimated in the confidence interval (1335, 1866 and 2484 larvae respectively) was inferior to the nematode initial population (2500 larvae) and the reproduction factor was less than 1. On the other hand, the control species (*S. quitoense* and *S. betaceum*) showed susceptibility because this value exceeded the initial population.

Table 2. Resistance or susceptibility response based on the nematode final population obtained in Solanaceae inoculated with *M. incognita*.

Solanaceae	Average final population	Confidence interval	Reproduction factor	Reaction
Solanum auriculatum	1881.1	1293.2 – 2736.3	1.09	Susceptible
Solanum hirtum	1283.2	882.1 – 1866.6	0.75	Resistance
Solanum hispidum	3574.9	2457.6 – 5200.2	2.08	Susceptible
Solanum arboreum	1711.6	1176.7 – 2484.8	0.99	Resistance
Nicotiana glauca	1024.8	786.2 – 1335.7	0.53	Resistance
Solanum quitoense	2636.1	1814.3 – 3838.9	1.53	Susceptible
Solanum betaceum	2823.7	1941.2 – 4107.4	1.64	Susceptible

The high inter and intra-specific variability in the *Meloidogyne* populations favors their adaptability and gives greater selective advantages over their hosts, being several Solanaceae species host of this nematode (González *et al*., 2010) how was observed in this study. Host-plant resistance to plant-parasitic nematodes is defined as the suppressive effect of the plant on the nematode's ability to reproduce (Cook and Evans, 1987). This definition of resistance in terms of reproduction of the nematode has been adopted by plant nematologists (Cook, 1974). The best sources of resistance to nematodes have been found in wild species that have no commercial value (Gonzáles *et al*., 2010). According to our results, *N. glauca*, *S. hirtum* and *S. arboreum* showed resistance (reproduction factor < 1), thus they can be considered as rootstocks that can be used in commercial Solanaceae such as tree tomato and naranjilla; however, *S. arboreum* showed a value very close to the initial population consequently the other two Solanaceae species are the best options. Piedra *et al*. (2005) demonstrated that a nematode resistance mechanism is considered good when it restricts or prevents the reproduction of nematodes how was observed in three Solanaceae species in this research. Unfortunately, the search for this type of plants requires years of research, an aspect that has limited the availability of resistant varieties (Anwar and McKenry, 2002).

Jacquet *et al*. (2005) and Devran (2004) have investigated the nematode resistance mechanism of the genus *Meloidogyne* spp. in tomato, concluding that the high amount of phenolic compounds synthesized in the plant and transferred to the roots confer resistance to nematode infestation. In addition, nematode-resistant plants have a higher level of phenylalanine ammonia, a key enzyme of phenolic metabolism known to be related to the resistance of plants to diseases (Sorribas *et al*., 2005). However, the sensitivity of a host depends not only on its genotype but on how many nematodes affect it. At high population densities the plants suffer a considerable reduction of their growth, but the sensitivity of the plant is also subject to the influence of the environment (Starr and Mercer, 2009).

On the other hand, Revelo and Sandoval (2003) demonstrated that the effect of a low initial population of *M. incognita* is easily supported by a Solanaceae species (*S. quitoense*) without decreasing its yield.

For the variable dry weight of the foliage (Table 3), a tolerance response to the nematode was found in all Solanaceae species except in the susceptible controls in which the p-values showed statistical significance at 5% (*S. quitoense*) and 1% (*S. betaceum*), indicating difference between the inoculated and non-inoculated plants (0.97 vs 1.23 and 1.03 vs 1.53, respectively).

Table 3. Response for the variable dry weight of foliage observed in Solanaceae inoculated with *M. incognita*.

Solanaceae	Inoculated plants (g)	Non-inoculated plants (g)	P-value	Response
Solanum auriculatum	1.05	0.80	0.066	Tolerant
Solanum hirtum	1.14	1.02	0.395	Tolerant
Solanum hispidum	1.66	1.29	0.165	Tolerant
Solanum arboreum	1.06	1.00	0.055	Tolerant
Nicotiana glauca	0.92	1.03	0.052	Tolerant
Solanum quitoense	0.97	1.23	0.048*	Non-Tolerant
Solanum betaceum	1.03	1.53	0.001**	Non-Tolerant

* Statistical significance, ** High statistical significance.

The response to Solanaceae sensitivity was based on the Cook (1974) criteria where inoculated plants that show a lower dry weight (yield) than non-inoculated plants and also show statistic differences were considered non-tolerant. The same author mentions that tolerance is a genetic character independent of the resistance but can be present in resistant or susceptible plants; occurring in *S. auriculatum* and *S. hispidun* in our study. On the other hand, further research is need to visualize the plant tolerance response in terms of fruit production in field conditions.

Duncan test at 5% showed two ranges of significance for the variable number of root knots, where the species *S. hirtum* (14.88), *N. glauca* (22.67) and *S. auriculatum* (25.11) were located in the first range of significance with the lowest root knot values (Table 4).

Table 4. Number of root knots counted in the different Solanaceae inoculated with the nematode *M. incognita*.

Solanaceae	Average number of root knots	Confidence interval
Solanum auriculatum	25.11 b	22.54 – 27.67
Solanum hirtum	14.88 a	12.67 – 17.11
Solanum hispidum	64.88 c	54.27 – 75.51
Solanum arboreum	81.22 d	69.75 – 92.70
Nicotiana glauca	22.67 b	19.59 – 25.74
Solanum quitoense	62.53 c	53.81 – 70.84
Solanum betaceum	87.88 d	84.17 – 91.60

All assessed Solanaceae species showed root knots, characteristic symptom of crops affected by nematodes of the genus *Meloidogyne* (González *et al.* , 2010). In Table 4, it can be seen a variation in the number of root knots in the different species, which can be related to the study of Gonzales *et al*. (2010), who inoculated Solanaceas species with a dose of 2500 larvae, finding species that did not showed galling such as *S. torvum*, *S, erianthum* and *Datura stramonium* and others that showed root knots as *S. mammosum* and *L. esculentum*. Oka *et al.* (2003) and Halbrendt (1996) mentioned that the decrease in root knot formation depends on the *Meloidogyne* species and also the plant species involved, the later can be seen in our study because there was a different number of root knots according to the species evaluated. According to Starr and Mercer (2009), the formation of root knots is not essential for the development of the nematode, therefore susceptible crops can show low levels of root knots and high nematode reproduction or vice versa; this can be confirmed with *S. auriculatum* which presented a low number of root knots (25.11) but its reproduction factor was greater than 1, showing susceptibility. Navarro *et al*. (2009) mentioned that the gall index should not be used as the only element to determine the resistance of a genotype or cultivar against *M. incognita*, even though it is considered an important element due to the damage caused by this pest. According to Gómez (2012), genetic resistance is one of the fundamental pillars of integrated pest management, thus grafted plants can be used to confer resistance to diseases and nematodes. According to our results, *S. hirtum* and *S. glauca* might be used as rootstocks because showed the best traits.

CONCLUSIONS

According to the reproduction factor, *S. arboreum, S. hirtum* and *N. glauca* showed resistance to the nematode *M. incognita*; however just the last two species showed a low number of root knots, for this reason they might be used as rootstocks for commercial Solanaceae fruit crops such as naranjilla and tree tomato, constituting an alternative to be involved in an integrated fruit production system.

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