

RESEARCH NOTE/NOTA DE INVESTIGACIÓN

INFLUENCE OF TWO *NACOBBUS ABERRANS* ISOLATES FROM ARGENTINA ON THE GROWTH OF THREE TOMATO CULTIVARS

V. A. Cabrera*¹, N. Dottori¹, and M. E. Doucet²

¹Laboratorio de Morfología Vegetal, IMBIV, Universidad Nacional de Córdoba, Vélez Sársfield 299, CP 5000 Córdoba, Argentina; ²Laboratorio de Nematología, IDEA, Universidad Nacional de Córdoba, Rondeau 798, CP 5000 Córdoba, Argentina; *Corresponding author: verocabrera8@hotmail.com

ABSTRACT

Cabrera V. A., N. Dottori, and M. E. Doucet. 2019. Influence of two *Nacobbus aberrans* isolates from Argentina on the growth of three tomato cultivars. *Nematropica* 49:42-48.

Nacobbus aberrans attacks weeds and cultivated plants, causing drastic crop yield losses. The tomatoes ‘Superman’ and ‘Mykonos’ are marketed as nematode resistant, but with no specifications of the nematode species. Moreover, no studies have recorded the response of these cultivars to infection by *N. aberrans*. We analyzed the influence of two Argentine isolates of *N. aberrans* (Lules and Río Cuarto) on the growth of these cultivars and Platense, a known susceptible used as a positive control. Plant growth variables, number of galls and egg masses, and gall size were measured. Mykonos infected with the Lules isolate exhibited a decrease in stem diameter. In Superman, all infected plants showed reduced shoot dry weight. Platense did not show differences ($P>0.05$) in growth variables between treated and control plants, however, this cultivar showed the largest galls. The Lules isolate was more aggressive than the Río Cuarto isolate and produced a greater number of galls and egg masses on Mykonos and Platense than on Superman. These findings indicate that Superman and Mykonos were efficient hosts of *N. aberrans* and cannot be considered resistant to either of the two nematode isolates.

Key words: infection, phytoparasitic nematode, response, tomato

RESUMEN

Cabrera V. A., N. Dottori N., y M. E. Doucet. 2019. Influencia de dos aislados de *Nacobbus aberrans* de Argentina en el crecimiento de tres cultivares de tomate. *Nematropica* 49:42-48.

Nacobbus aberrans ataca tanto malezas como plantas cultivadas, causando drásticas pérdidas en el rendimiento de los cultivos. ‘Superman’ y ‘Mykonos’ son cultivares de tomate redondo comercializados como resistentes a nematodos, aunque no se especifica a qué especie/s, ni hay estudios acerca de su respuesta ante la infección con *N. aberrans*. En este trabajo analizamos la influencia de dos aislados argentinos del nematodo (Lules y Río Cuarto) en el crecimiento de Superman y Mykonos; se utilizó como control positivo al cultivar Platense que es susceptible al parásito. Se midieron variables de crecimiento,

cantidad de agallas y masas de huevos por sistema radical y tamaño de las agallas. En el cultivar Mykonos disminuyó el diámetro del tallo cuando se infectó con el aislado Lules; en Superman, las plantas infectadas evidenciaron menor peso seco aéreo que las sanas. No se encontraron diferencias significativas para las variables de crecimiento en el cultivar Platense, aunque fue el que presentó las agallas de mayor tamaño. El aislado proveniente de Lules fue más agresivo que el de Río Cuarto, generando mayor cantidad de agallas y masas de huevos en Mykonos y Platense que en Superman. Los cultivares evaluados fueron hospedadores eficientes del parásito, por lo que no pueden considerarse resistentes a los aislados utilizados.

Palabras claves: infección, nematodo fitoparásito, respuesta, tomate

The false root-knot nematode *Nacobbus aberrans* (Thorne, 1935) Thorne & Allen, 1944 is a sedentary endoparasite that feeds on roots of a wide host range, including both cultivated plants and weeds (Manzanilla-López *et al.*, 2002). The establishment and development of the female and its feeding site (syncytium) alter the anatomy of roots, affecting their normal functioning and reducing crop yield (Doucet and Lax, 2005; Curtis, 2008). This nematode is considered one of the 10 plant-parasitic nematode species of greatest scientific and economic importance (Jones *et al.*, 2013). It causes severe losses to basic food plants in Latin America; more than 80% in potato (*Solanum tuberosum* L.), between 50% and 90% in tomato (*S. lycopersicum* L.), and 35% in common bean (*Phaseolus vulgaris* L.) (Manzanilla-López *et al.*, 2008). Accordingly, its regulation as a quarantine pest has been recommended (EPPO, 2015). Physiological races were distinguished among the populations of *N. aberrans* according to their host preference by Inserra *et al.* (1985) and subsequently by other authors (Castiblanco *et al.*, 1999; Manzanilla-López *et al.*, 2002; Lax *et al.*, 2011). With evidence of morphometric, molecular, and physiological variations among populations from different geographical regions, it is assumed that the genus includes a higher number of species, therefore, the taxonomy is currently under revision (Manzanilla-López *et al.*, 2002; Manzanilla-López, 2010).

In Argentina, several plants serve as hosts for this nematode, with the most important ones being pepper (*Capsicum annuum* L.), sugar beet (*Beta vulgaris* L.), potato, eggplant (*S. melongena* L.) and tomato, all of which can be severely damaged

under both field and greenhouse conditions (Doucet and Lax, 2005). Tomato is one of the most important horticultural crops worldwide, due to the by-products obtained and the income generated (Costa and Heuvelink, 2005). Some important horticultural tomato production centers in Argentina are in La Plata (Buenos Aires), Lules (Tucumán), and Río Cuarto (Córdoba). These three regions are affected by the presence of *N. aberrans* (Doucet and Lax, 2005).

Developing tomato cultivars resistant to *N. aberrans* would be of benefit and significantly contribute to horticultural production in countries where *N. aberrans* is present (Veremis *et al.*, 1997; Manzanilla *et al.*, 2002). ‘Superman’ and ‘Mykonos’ are round tomato cultivars of indeterminate and determinate growth, respectively, that breeders employ as germplasm for high yield and productivity. These cultivars also have desirable fruit traits like large size (220-250 g), firm texture, attractive color, and desirable flavor. These cultivars are commercialized as nematode resistant, but with no specification of the nematode species. These cultivars have been evaluated, both under greenhouse and field conditions, by examining plant vigor and fruit quality (Ávila, 2009), however, no studies have analyzed growth variables in plants infected with *N. aberrans*. Considering the severe damage this parasite inflicts on agricultural production and on tomato production in particular, a thorough analysis of relationship between tomato cultivars and *N. aberrans* populations is warranted. Therefore, the aim of this work was to compare the effect of two Argentine isolates of the *N. aberrans* on growth of the commercial tomatoes Superman

and Mykonos.

Nematode inocula of two isolates were produced using *S. lycopersicum* cultivar Platense, which is susceptible to *N. aberrans* (Tordable *et al.*, 2010). Seeds of Platense were sown in germination trays containing 2:1 autoclaved soil and vermiculite, held at 25°C with a 12-hr photoperiod and manually watered every other day. Seedlings at the 4-leaf stage were transplanted into disposable 180-ml plastic pots. Five months later, egg masses from each nematode isolate were removed and placed in 250- μ m mesh sieves fastened to a Petri dish lid. The bottom of the sieve was partially immersed in water contained at the base of the Petri dish, and was maintained at 25°C \pm 2°C (Lax *et al.*, 2011). First-stage juveniles (J1) that were inside the eggs molted to J2 (infective stage), hatched, and were collected from the water. Using a Pasteur glass pipette, 3,600 J2 were collected from each isolate, transferred to a 250-ml graded tube, and the volume adjusted to obtain a concentration of 100 J2/ml. *N. aberrans* were from two Argentine localities: a greenhouse of Río Cuarto, Córdoba province and field plots in Lules, Tucumán province. According to the physiological race scheme, both isolates belong to the “sugar beet group”, whose individuals do not parasitize potato but do infest sugar beet and tomato (Inserra *et al.*, 1985; Lax *et al.*, 2011).

An experiment was conducted at the greenhouse of the Centro de Zoología Aplicada (Facultad de Ciencias Exactas, Físicas y Naturales, UNC, Argentina) to determine susceptibility of the tomato cultivars Superman, Mykonos (marketed as resistant to nematodes), and Platense (nematode susceptible). Seedlings were obtained from certified seeds using the procedures described above. Four-leaf stage seedlings of each cultivar were transplanted individually to disposable 180-ml plastic pots containing autoclaved soil and vermiculite (2:1). The root system of each seedling was left uncovered to allow direct application of the inoculum on the root. The inoculum for each seedling consisted of 1 ml of nematode suspension containing 100 J2. For this, the inoculum was homogenized by bubbling with air, 1 ml was taken with a plastic Pasteur pipette and applied directly on the roots of each plant to ensure J2-host contact. Then, the roots were covered with the soil/vermiculite mixture.

Each tomato cultivar was tested against each nematode isolate resulting in six treatments: Platense inoculated with the Lules isolate (Pla-L), Platense inoculated with Río Cuarto isolate (Pla-RC), Mykonos inoculated with Lules isolate (Myk-L), Mykonos inoculated with Río Cuarto isolate (Myk-RC), Superman inoculated with Lules isolate (Sup-L), and Superman inoculated with Río Cuarto isolate (Sup-RC). The negative control consisted of tomato plants that were not inoculated with *N. aberrans* (Pla-C, Myk-C, and Sup-C). Plants were maintained in the greenhouse of the Centro de Zoología Aplicada for 90 days at a mean temperature of 25°C to allow completion of the entire nematode life cycle. The experimental design consisted of a randomized complete block with two factors (cultivars and nematodes) at three levels each (3x3 = three cultivars and two nematode isolates plus the uninoculated) with 12 replications per treatment.

After 90 days, the stem height from the base to the apex (centimeters), stem diameter at the base (centimeters), total number of leaves, and shoot dry weight (grams) were measured and recorded. For shoot dry weight, the shoots of each plant were placed in a paper envelope, dried at 65°C for 24 hr and weighed. Each root system was carefully washed, and the numbers of galls and egg masses present counted. The size of each gall was also recorded by measuring the greatest diameter.

An Analysis of Variance (ANOVA) was performed using InfoStat (Di Rienzo *et al.*, 2016). Assumptions of independence and normality of residuals, variance homogeneity, and additivity of the block-treatment effects were previously confirmed for all variables. When the assumptions were not met, data were $\log_{10}(x + 2)$ transformed. A multiple comparison of means was performed using the LSD Fisher test at a significance level of 0.05.

As with other plant-parasitic nematodes, *N. aberrans* does not produce specific unique symptoms in infected plants. Patches of plants showing chlorosis and poor development are observed occasionally in field crops. The inoculated plants did not show severe signs of wilting or chlorosis compared to those in the control plants in the greenhouse. Since Platense and Superman are of indeterminate growth and Mykonos is of determinate growth, comparisons of

of growth variables were made within each cultivar instead of among cultivars. Stem diameter of Mykonos was reduced by the Lules isolate compared to the control ($F = 8.18$; $P = 0.0014$). No differences in stem height ($F = 1$; $P = 0.3782$), total number of leaves ($F = 0.6$; $P = 0.5535$) or shoot dry weight ($F = 0.06$; $P = 0.9423$) were observed in this cultivar (Table 1). The presence of thin and weak shoots in Myk-L is a symptom of nutrient (potassium and/or nitrogen) deficiency, which might be attributed to an alteration in mineral transport caused by the disorganization of the vascular system caused by the nematode (Taiz and Zeiger, 2006; Tordable *et al.*, 2010). Indeed, other plant-parasitic nematodes have been found to reduce the transport of P, K, N, and sugars (Carrancio *et al.*, 2014).

The presence of the nematode was detrimental to the cultivar Superman, since it produced a decrease on shoot dry weight, reducing the biomass production (Taiz and Zeiger, 2006). Both nematode isolates reduced the shoot dry weight of Superman ($F = 7.20$; $P = 0.0026$). Sup-L and Sup-C had the lowest mean values in stem diameter ($F = 9.30$; $P = 0.0007$). No significant differences were found in stem height ($F = 0.41$; $P = 0.6665$) or total number of leaves ($F = 2.10$; $P = 0.1394$) (Table 1).

Platense tolerated infection by both nematode

isolates showing no differences in stem diameter ($F = 0.43$; $P = 0.6557$), stem height ($F = 2.28$; $P = 0.1190$), total number of leaves ($F = 0.83$; $P = 0.4438$), or shoot dry weight ($F = 1.29$; $P = 0.2891$) (Table 1). Continuous irrigation applied in this experiment might have mitigated damage from the nematode, since the symptoms produced by the presence of the parasite are more severe in water-stressed soils (Chew Madinaveitia and Jiménez Díaz, 2002). However, Platense had the largest galls at 2.93 mm ($F = 3.35$; $P = 0.0418$). The large size of galls observed in Platense is important because a significant proportion of eggs can remain inside the root tissue, protected from fungal attack or other antagonist organisms from the rhizosphere (Flores-Camacho *et al.*, 2007).

None of the cultivars evaluated showed a decrease in number of leaves or stem height due to the presence of nematodes. Gaviria (1999) attributed this effect to the initial physiological response of plants to the nematode parasitism, i.e., greater water uptake and an increase in length.

Both nematode isolates developed and reproduced in the roots of the three tomato cultivars. The tomato presents a gene Mi that confers resistance to three species of *Meloidogyne*, a genus of phytoparasitic nematodes, inhibiting nematode reproduction considerably (Ornat *et al.*, 2001). This gene, however, does not confer

Table 1. Response of three tomato cultivars to two *Nacobbus aberrans* isolates 90 days after inoculation with 100 second-stage juveniles (J2).

Tomato cultivar	Nematode isolate	Stem diameter (cm) ^z	Stem height (cm) ^z	Number of leaves	Shoot dry weight (g) ^z
Mykonos	Uninoculated	1.49 b	27.43	9	0.83
	Lules	1.23 a	25.28	10	0.78
	Río Cuarto	1.45 b	24.26	10	0.81
Superman	Uninoculated	1.54 x	24.63	10	1.35 y
	Lules	1.50 x	24.22	9	0.96 x
	Río Cuarto	1.72 y	22.83	9	1.10 x
Platense	Uninoculated	1.53	25.96	11	1.05
	Lules	1.47	26.23	10	0.93
	Río Cuarto	1.51	29.98	12	0.80

^zValues are means of 12 replicates. Different letters with in a column by tomato cultivar indicate significant differences according to the LSD Fisher test ($P < 0.05$). The absence of a letter indicated no significant difference among values within a tomato cultivar.

resistance to *N. aberrans*, as could be demonstrated in this work. The Lules isolate induced a greater number of galls ($F = 7.04$; $P = 0.0017$) and egg masses ($F = 7.37$; $P = 0.0013$) than the Río Cuarto isolate on both Platense and Mykonos, while in the cultivar Superman the values were lower (Fig. 1). The effect caused by *N. aberrans* in the histology of roots of these cultivars has been analyzed, and although all the cultivars were susceptible to the nematode when comparing the anatomical alterations caused, the cultivar Superman was the least affected (Cabrera *et al.*, 2017). Although both the Lules and Río Cuarto nematode isolates belong to the same sugar beet physiological race, Lules was more aggressive than Río Cuarto, producing more galls and egg masses on plants. These findings confirm the

results of other experiments conducted by Lax *et al.* (2006) and Flores-Camacho *et al.* (2007), who observed differences in aggressiveness and virulence among isolates of *N. aberrans* from different origins. Thus, when evaluating the response of cultivars to parasitism by *N. aberrans*, it is necessary to conduct experiments with more than one population, since isolates of different geographical origin can have different degrees of aggressiveness on a host (Lax *et al.*, 2006).

The susceptibility of the tomato cultivar Mykonos to Lules nematode isolate observed in our experiment should discourage tomato growers from using this cultivars in the Tucumán area where this isolate is present. However, field studies in plots infested with this nematode isolate are necessary to observe the response of the two

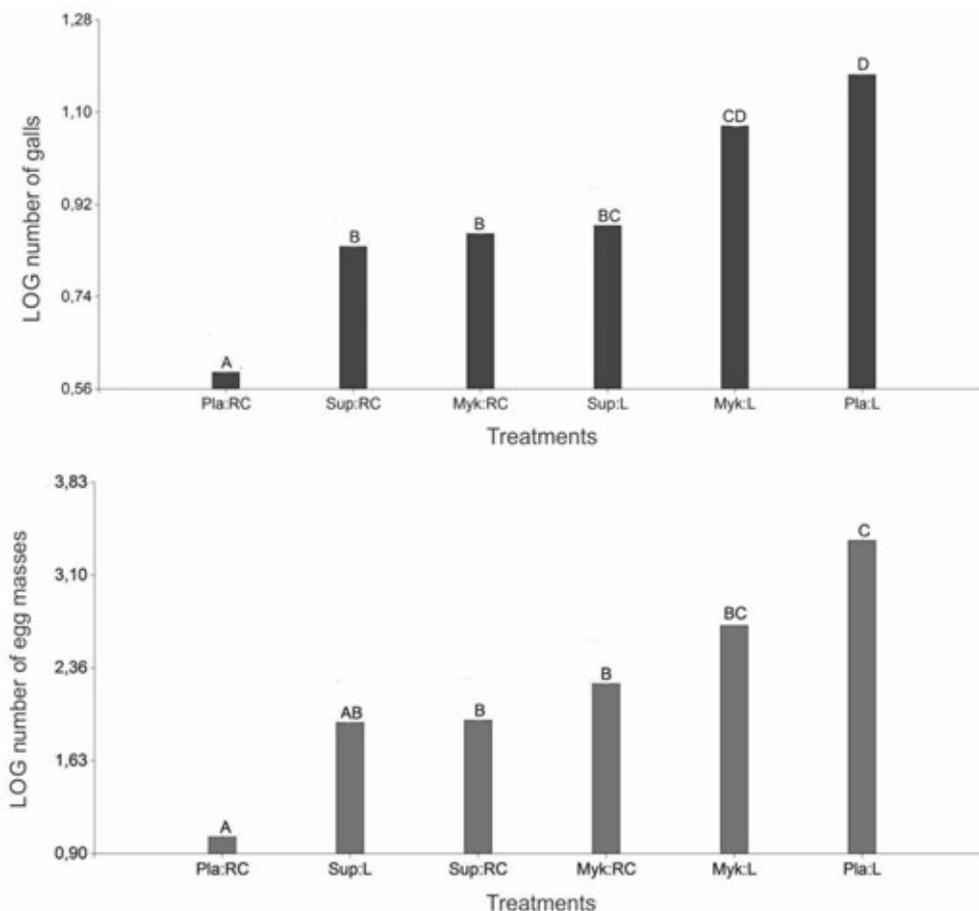


Figure 1. Number of galls and egg masses per root system in tomato cultivars infected with two isolates of the nematode *N. aberrans*. Data were $\log_{10}(x + 2)$ transformed. Different letters indicate significant differences according to the LSD Fisher test ($P < 0.05$). Treatments: Pla=tomato cultivar Platense, Myk=tomato cultivar Mykonos, Sup=tomato cultivar Superman, L= inoculated with nematode isolate Lules, RC=inoculated with nematode isolate Río Cuarto.

tomato cultivars under field conditions. These cultivars, which are marketed as nematode-resistant, cannot be considered resistant to *N. aberrans*.

Given the importance of the green belts in Río Cuarto and Lules for tomato production, further studies evaluating the response of other tomato cultivars to these nematode isolates are necessary to provide relevant information for selecting the most suitable cultivar for the specific conditions of each region.

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