

# Resistance evaluation of a F<sub>1</sub> hybrid of *Solanum quitoense* Lam. to *Neoleucinodes elegantalis* (Guenée) and *Meloidogyne incognita* (Kofoid & White)

## Evaluación de la resistencia de un híbrido F<sub>1</sub> de *Solanum quitoense* Lam. a *Neoleucinodes elegantalis* (Guenée) y *Meloidogyne incognita*

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## Abstract

The aim of this study was to cross lulo plants of cv. La Selva to obtain a hybrid with tolerance to the fruit borer *Neoleucinodes elegantalis* Guenée (Lepidoptera: Crambidae) and to the nematode *Meloidogyne incognita* (Kofoid & White) (Nematoda: Heteroderidae), to obtain better-adapted plants to the conditions of the Colombian coffee-growing region and with bigger and non-dehiscent fruits. La Selva cultivar is a hybrid developed from the interspecific backcross of *Solanum quitoense* Lam. × *Solanum hirtum* Vahl with plants of lulo cv. Castilla Larga Vida. The experiment was conducted in the municipality of Dosquebradas, Risaralda, Colombia, located at 1,465 m a.s.l. F<sub>1</sub> plants were obtained from reciprocal crossings. When inoculated with the nematode *M. incognita*, plants showed susceptibility

in their seedling stage; however, when we carried out the evaluation six months after transplantation under field conditions, nematode infestation was less than 1%, which likely indicates the tolerance of these materials to the nematode. When assessing the resistance of the hybrid to the attack of *N. elegantalis*, we found that the evaluated materials were resistant to this insect. The resulting hybrids showed good agronomic characteristics, such as a good morphological structure and vigor, high productivity, good solar exposure adaptation, large fruits (5.6 cm average diameter) similar to those of cv. Castilla, with yellow peel, green pulp and non-dehiscent fruits with pleasant aroma and flavor. As an undesirable characteristic plants had thorns on leaves and stems.

**Keywords:** diversity, fruit borer, hybrids, plant nematodes, plant breeding, *Solanum quitoense*

## Resumen

En este estudio se cruzaron plantas de lulo del cultivar La Selva, híbrido del retrocruzamiento interespecífico de *Solanum quitoense* Lam. × *Solanum hirtum* Vahl (Solanaceae), con plantas del cultivar Castilla Larga Vida, con el fin de obtener un material de frutos grandes, no dehiscente, adaptado a la zona cafetera colombiana y con resistencia al pasador del fruto *Neoleucinodes elegantalis* Guenée (Lepidoptera: Crambidae) y al nematodo *Meloidogyne incognita* (Kofoid & White) (Nematoda: Heteroderidae). El experimento se realizó en el municipio de Dosquebradas, Risaralda (Colombia), a 1.465 m s. n. m. Al ser inoculadas con el nematodo *M. incognita*, las plantas F<sub>1</sub>, obtenidas de los cruzamientos recíprocos, mostraron susceptibilidad en la etapa de almácigo.

Sin embargo, al realizar la evaluación seis meses después del trasplante en condiciones de campo, se encontró menos del 1% de infestación del nematodo, lo que hace presumir la tolerancia de los híbridos a este organismo. De igual forma, se evidenció la resistencia de los materiales al ataque de *N. elegantalis*. Los híbridos resultantes presentaron buenas características agronómicas, como vigor y estructura buenos, alta productividad, buena adaptación a condiciones de libre exposición solar, frutos grandes (similares a los del lulo cv. Castilla), corteza amarilla y pulpa verde, sin rajamiento, y de aroma y sabor agradables. Las plantas presentaron una característica indeseable, que consistió en espinas en tallos y hojas.

**Palabras clave:** diversidad, fitomejoramiento, híbridos, nematodos de plantas, pasador del fruto, *Solanum quitoense*

## Introduction

Lulo, *Solanum quitoense* Lam. (Solanaceae) Castilla cultivar, is one of the Andean fruits with a huge commercial potential, given its wide acceptance in national markets, and because it is also considered a promissory product for international markets. However, the demand has been reduced due to the current production system used, characterized by a high consumption of pesticides, which causes the detection of traces of active ingredients not allowed in international markets (Muñoz, 2011).

During 2014, 33,522 t of lulo were produced in Colombia, with an average yield of 9.6 t/ha per year. Antioquia is the main producer department with 10,477 t, corresponding to 31.25% of the total production, followed by the departments of Huila, Cundinamarca and Santander.

In the period from January to July 2014, the main destinations of this fruit were France, contributing 49% of the demand, with a value of US\$38,990.50, followed by Panama (14%), Spain (11%) and Aruba (4%) (National Administrative Department of Statistics [DANE], 2015).

Some authors have recognized two botanical varieties: *Solanum quitoense* var. *septentrionale*, which has thorns, and *Solanum quitoense* var. *quitoense*, which is thornless (Fory et al., 2010; Morton, 1987; Schultes, & Cuatrecasas, 1953; Whalen, Costich, & Heiser, 1981), but there is still controversy regarding this aspect.

Lulo cv. La Selva is the product of two backcrosses in search of a spineless population of the hybrid obtained by Professor C. Heiser (Indiana University, USA) of *Solanum quitoense* × *Solanum hirtum* (locally known as "lulo de perro") (Bernal, Lobo, & Londoño, 1998), and is characterized for not having thorns neither in its leaves nor in the stem. It is a precocious cultivar, which begins its production between six and seven months after planting.

Its production can reach up to 20 t/ha and it is destined for the agribusiness because it has a very short

postharvest life, since its pulp oxidizes quickly and has high dehiscence; this causes the fruit to crack even before reaching its full maturity. However, it has tolerance to nematodes and to *N. elegantalis* (Medina, Lobo, Martínez, & Riaños, 2004).

Lulo cv. Castilla and cv. Castilla Larga Vida have thorns in stems and leaves, they start producing between seven and nine months after transplantation and reach a production of 8 to 10 t/ha, destined mostly for the fresh market. Among the pests that affect severely the crop are *Neoleucinodes elegantalis* (Guenée) (Lepidoptera: Crambidae) which is the main pest, and the nematode *Meloidogyne incognita* (Kofoid & White) (Nematoda: Secernentea: Tylenchida: Heteroderidae) (Bernal & Díaz, 2006).

*Neoleucinodes elegantalis* is a moth that in its larval state drills and bores lulo fruits, feeding on these until it reaches adulthood. Larvae enters the fruit from the third instar and stays there until the fifth instar, and then drops to the ground where it pupates. However, when leaving, the larvae leaves an exit hole that allows the entry of other insects and microorganisms, damaging completely the fruit (Instituto Colombiano Agropecuario [ICA], 2011, Marcano, 1991, Obando, 2011, Parra, Barona, & Vallejo, 1994).

Due to their particular habits in which the larvae live inside the fruit, chemical control is ineffective (Díaz-Montilla, 2013). Moreover, it is considered a quarantine pest in Chile, the US, and Peru, which limits fruit exports (Obando, 2011).

Regarding the nematode *M. incognita*, according to Tamayo (2001), it is a pest that attacks lulo in all producing areas of Colombia. The attack starts in the nursery, producing galls and nodes in roots, which prevents the absorption of water and nutrients; this weakens plants that show symptoms such as smaller leaves, chlorosis of lower leaves and temporary withering on sunny days. Moreover, it can reduce production by more than 50% (Franco et al., 2002).

Control based on agrochemicals is the most used by farmers but has huge disadvantages. As products

used are extremely toxic and residual, this pollutes the environment and, when applied repeatedly, they cause resistance from the organism to the product, aggravating further the problem (Andrés, 2002).

Therefore, the best control method for these two crop-limiting pests is the use of resistant cultivars and varieties. Lobo and Medina (2000) have reported that lulo cv. La Selva is resistant to nematodes and to *N. elegantalis*. The definition of varietal resistance to pests is variable, but, in practical agricultural terms, we can say that a resistant cultivar is one that yields more than a susceptible one when facing the invasion of a pest (Maxwell & Jennings, 1980). Therefore, the resistance of plants is relative, and is based on the comparison with those that lack that quality, that is, the susceptible ones. According to Panda and Khush (1995), varietal resistance has the following fundamental characteristics: a) is heritable and controlled by one or more genes; b) is relative and can only be measured when compared to a susceptible cultivar of the same species; c) is qualitatively or quantitatively measurable; and d) is variable and can be modified by biotic and abiotic factors.

According to the aforementioned, the aim of this study was to evaluate the resistance or varietal tolerance of the F<sub>1</sub> materials obtained from the reciprocal crossing between lulo cv. Castilla, natural selection Larga Vida and cv. La Selva to the nematode *M. incognita* and to the moth *N. elegantalis*.

## Materials and methods

### Collection of parental material

Plant material of lulo cv. La Selva was collected in the town of Caucayá, municipality of Belén de Umbría, Risaralda (Colombia), with the following geographic coordinates: 05°13' N and 75°48' W, at an altitude of 1,320 m a.s.l., with an average temperature of 26 °C and an annual rainfall of 2,150 mm.

Cuttings of basal suckers from selected plants were obtained due to their excellent production and

good phytosanitary condition. Cuttings were planted in plastic bags with a substrate composed of three parts of soil plus one of organic matter. Once planted, a drench application with *Trichoderma* spp. was made to prevent the attack of the damping-off complex (*Fusarium oxysporum*, *Phytophthora infestans*, *Sclerotinia sclerotiorum*) (ICA, 2011).

For the case of natural selection of lulo cv. Castilla Larga Vida, fruits were harvested from the best plants of a commercial cultivation that were isolated from any other cultivar in the town of Andica de Belén de Umbría, Risaralda. The location has an altitude of 1,370 m a.s.l., an average temperature of 25 °C, an average annual precipitation of 2,030 mm, and have the following geographic coordinates: 05°14' N and 75°52' W.

Seeds were extracted from fruits weighing more than 150 g and a diameter greater than 8 centimeters, mixed as a mass selection and planted in a germinator. Twenty-eight days after planting, seedlings that emerged were registered, achieving a germination of 70.3%. When seedlings emitted the third pair of leaves, they were transplanted to plastic bags, where they remained for four months until they had three true leaves.

### Parent plant management

Seedlings from both parents were planted in a plot of Universidad Nacional Abierta y a Distancia (UNAD) in the municipality of Dosquebradas, Risaralda, located at an altitude of 1,460 m a.s.l., with an average temperature of 21 °C, an average annual rainfall of 2,200 mm, and an average relative humidity of 85%.

Plants were sown in holes of 30 × 30 × 30 cm in a square arrangement with distances of 2 m between plants and 3 m between rows. We added 1.5 kg of composted organic matter to each hole as well as 150 g of commercial mycorrhizae to favor a better phosphorus absorption.

All the traditional cultural management practices such as weed control, soil fertilization with NPK

and secondary elements, as well as foliar fertilization with minor elements were carried out. Shape pruning was also done to eliminate shoots or suckers from the stem that were below 30 cm in height.

### Crossings

Four months after transplantation flowering occurred. At that time, flowers with long pistils

were selected in both materials (lulo cv. La Selva and lulo cv. Castilla Larga Vida), and reciprocal crosses were made, that is, the two materials were used as parent, i.e. as mother and as father plants. Flowers were emasculated removing the stamens, and the chosen flowers were pollinated as females. In figure 1, we can see the fruits resulting of these crosses during their development process.



**Figure 1.** Fruits in development obtained from the crosses of lulo cv. Castilla Larga Vida × cv. La Selva.

From these crosses, fruits were obtained from which the F<sub>1</sub> seed was extracted (figure 2). The seeds of all the F<sub>1</sub> fruits of each reciprocal cross were kept separate as a mass selection. Three hundred and forty-four seeds of each of these crosses were sown and the percentage of germination was 73%. One hundred F<sub>1</sub> plants were selected from each crossing, which were transferred to plastic bags, and then a part of these was taken to the field to perform the morphoagronomic character evaluation.

The F<sub>1</sub> plants of the crosses of the lulo cv. Castilla × cv. La Selva, cv. La Selva × cv. Castilla, and cv. La Selva × cv. Larga Vida were sown in a plot located in the installations of UNAD, in the municipality of Dosquebradas, Risaralda. The study plots were located at an altitude of 1,460 m a.s.l. with an average

temperature of 21 °C, an annual rainfall of 2,200 mm and a relative humidity of 80%. The region corresponds to a very humid premontane forest (bmh-PM) according to the Holdridge life zone classification.

Planting was carried out in a completely random design with 25 plants per treatment, for 100 plants in total. Every seven days the following quantitative morphoagronomic descriptors were obtained: plant height, stem diameter, fruit volume, number of leaves per week, number of buttons per cushion, number of cushions, number of fruits per bunch, number of bunches, and leaf area. From each of the treatments 10 rooted plants were plucked six months after sowing to evaluate level of root infestation by nematodes.



Figure 2. F<sub>1</sub> lulo fruits. a. cv. Castilla Larga Vida × cv. La Selva; b. cv. La Selva × cv. Castilla Larga Vida.

## Resistance evaluation to nematodes

### Nematode isolation

Pure cultures of *M. incognita* were obtained from infected roots of lulo cv. Castilla and from the soil surrounding these, using the Baermann funnel method.

### Experiment set up for evaluation of resistance to *Meloidogyne incognita*

To evaluate the resistance of F<sub>1</sub> plants to nematodes, seedlings with four or five well-formed leaves were planted in plastic jars with a capacity of 2 kg, with a substrate composed of three parts of soil and one part of organic matter without disinfecting.

A completely random design was used. The experimental unit corresponds to 10 plants, and the sampling unit was a complete plant by treatment and by repetition. Treatments correspond to the interaction of crosses by inoculation with or without nematodes, as follows.

T1: F<sub>1</sub> plants of cv. Castilla Larga Vida × cv. La Selva, without nematode inoculation

T2: F<sub>1</sub> plants of cv. Castilla Larga Vida × cv. La Selva, inoculated with nematodes

T3: Plants of cv. Castilla Larga Vida, inoculated with nematodes (control)

T4: F<sub>1</sub> plants of cv. La Selva × cv. Castilla Larga Vida, inoculated with nematodes

T5: F<sub>1</sub> plants of cv. La Selva × cv. Castilla Larga Vida, without nematode inoculation

For plant inoculation, four holes were made in the soil inside each plot and around the neck of the root, where 50 cm<sup>3</sup> of distilled water containing approximately 10,000 nematodes, between adults and eggs, were inoculated. These plants were kept inside a greenhouse for 60 days, at an average temperature of 23 °C and 85 % relative humidity.

At the end of this period, plants were extracted from their pots, and the presence or absence of nodes in the root system was evaluated, according to a scale of 0 to 10, where 0 corresponds to a healthy plant without root nodes, and 10 corresponds to plants with a root system completely covered in nodes. Plants were considered resistant when the mean root-node index was less than 2 and susceptible when that value was higher. Response variables used were level of infestation, root length and fresh root weight.

## Nematode resistance evaluation

### Multiplication of *Neoleucinodes elegantalis*

Adults of *N. elegantalis* were obtained from lulo fruits (from an undetermined cultivar) collected from plants in the area, which had entry holes but

no exit holes. They were taken to the laboratory where four or five fruits were placed inside transparent plastic jars with wet sand and pieces of cloth paper in the bottom (figure 3). Once the larvae left the fruits, they started the pupation process on paper fabrics and adults emerged later. Adults obtained were sexed by wing color and by their genital opening.



**Figure 3.** Multiplication process of *Neoleucinodes elegantalis* in the laboratory using infested lulo fruits inside plastic jars.

### Experiment set up for the evaluation of tolerance to *Neoleucinodes elegantalis*

To evaluate the tolerance of the F<sub>1</sub> generations to *N. elegantalis*, entomological sleeves (40 × 15 cm) were used, selecting a branch of the middle third of the plant, with fruits in the first formation phase, in which two male and two female *N. elegantalis* moths were introduced.

Daily observations were made in order to record oviposition. For the evaluation of tolerance to *N. elegantalis*, a completely randomized design of the progenies of the reciprocal crosses of lulo cv. Castilla Larga Vida × cv. La Selva, and as control, plants from cv. Castilla Larga Vida were used. An experimental unit corresponded to a plant with its respective entomological sleeve, and a sampling unit with 10 plants per treatment.

Four quantitative descriptors were used: percentage of affected fruits, average number of exit holes per

fruit, percentage of fruits with exit holes, and average number of larvae per fruit. Data obtained from the experiments in relation to the evaluation of resistance to nematodes and tolerance to *N. elegantalis* were subjected to an analysis of variance (Anova) and mean comparison tests (Duncan) using the statistical program SPSS V15.0.

## Results

### Resistance evaluation of the F<sub>1</sub> materials to the nematode *Meloidogyne incognita*

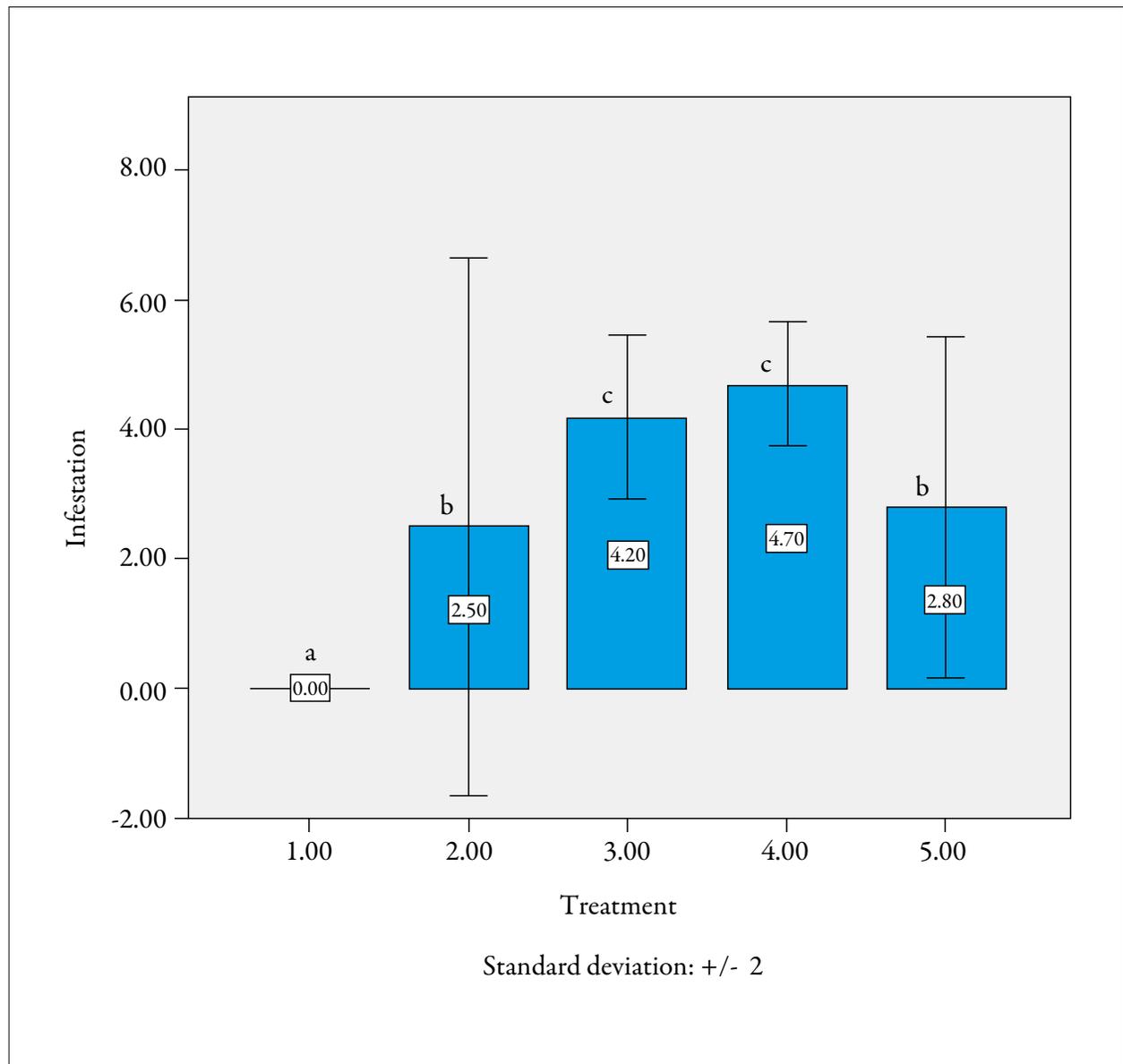
An analysis of variance for the variables level of infestation, root length and fresh weight of the root in seedlings 60 days after inoculation were carried out in function of the resistance of F<sub>1</sub> plants of the reciprocal crosses of lulo cv. La Selva × cv. Castilla Larga Vida to the nematode *M. incognita*; results showed highly significant differences between treatments.

### Infestation level of *Meloidogyne incognita* in seedlings

When analyzing the level of infestation of the root by the nematodes in seedlings 60 days after inoculation, treatment 1 (F<sub>1</sub> plants cv. Castilla Larga Vida × cv. La Selva, without nematode inoculation) showed a level of nematode infestation of zero (0). Moreover, treatments 2 (F<sub>1</sub> plants cv. Castilla Larga Vida × cv. La Selva, inoculated with nematodes) and 5 (F<sub>1</sub> plants cv. La Selva × cv. Castilla Larga

Vida, without nematode inoculation) showed a low percentage (2.5%).

On the other hand, treatments 3 (cv. Castilla plants, inoculated with nematodes [control]) and 4 (F<sub>1</sub> plants cv. La Selva × cv. Castilla Larga Vida, inoculated with nematodes), were the most susceptible, with a level of infestation of 4.2% and 4.7%, respectively. Furthermore, 50% of the plants of treatment 2 showed levels of infestation between 0 and 2 and can be suggested as resistant lines (figure 4).



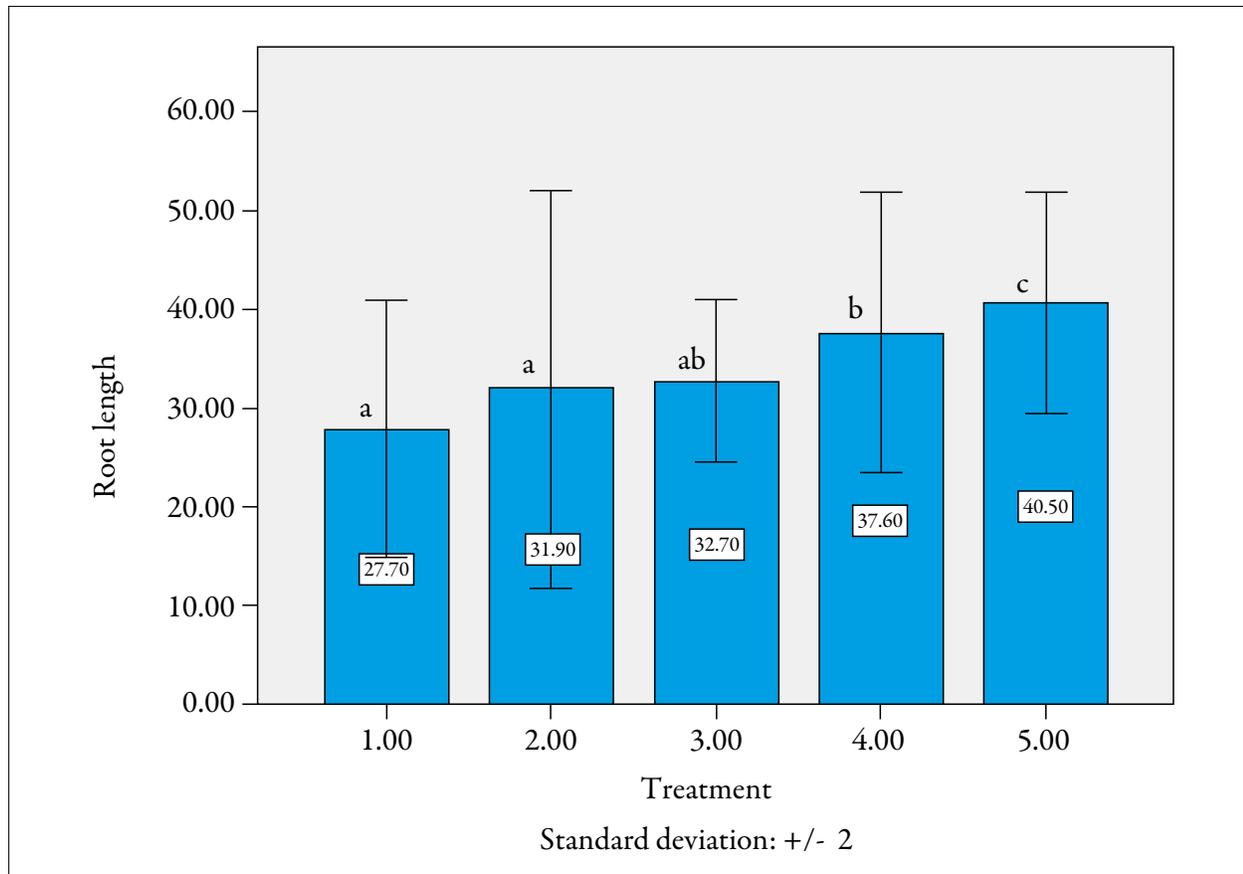
**Figure 4.** Evaluation of the level of infestation of the nematode *Meloidogyne incognita* in roots of seedlings of F<sub>1</sub> lulo plants. Same letters on the bars mean that they do not differ statistically ( $p > 0.05$ ).

Source: Prepared by the authors

## Root length

There were significant differences among treatments. Roots of treatment 5 (F<sub>1</sub> plants cv. La Selva × cv. Castilla Larga Vida, without nematode inoculation) were the longest, with an average length of 40.5 cm, followed by those of this same material, but inoculated with nematodes (figure 5).

Although in this case we can say that nematodes affected root lengthening, this did not occur in the F<sub>1</sub> plants cv. Castilla Larga Vida × cv. La Selva material. This might be due to an oligogenic effect provided by the parent cv. Castilla Larga Vida when it is used as a male (figure 5).



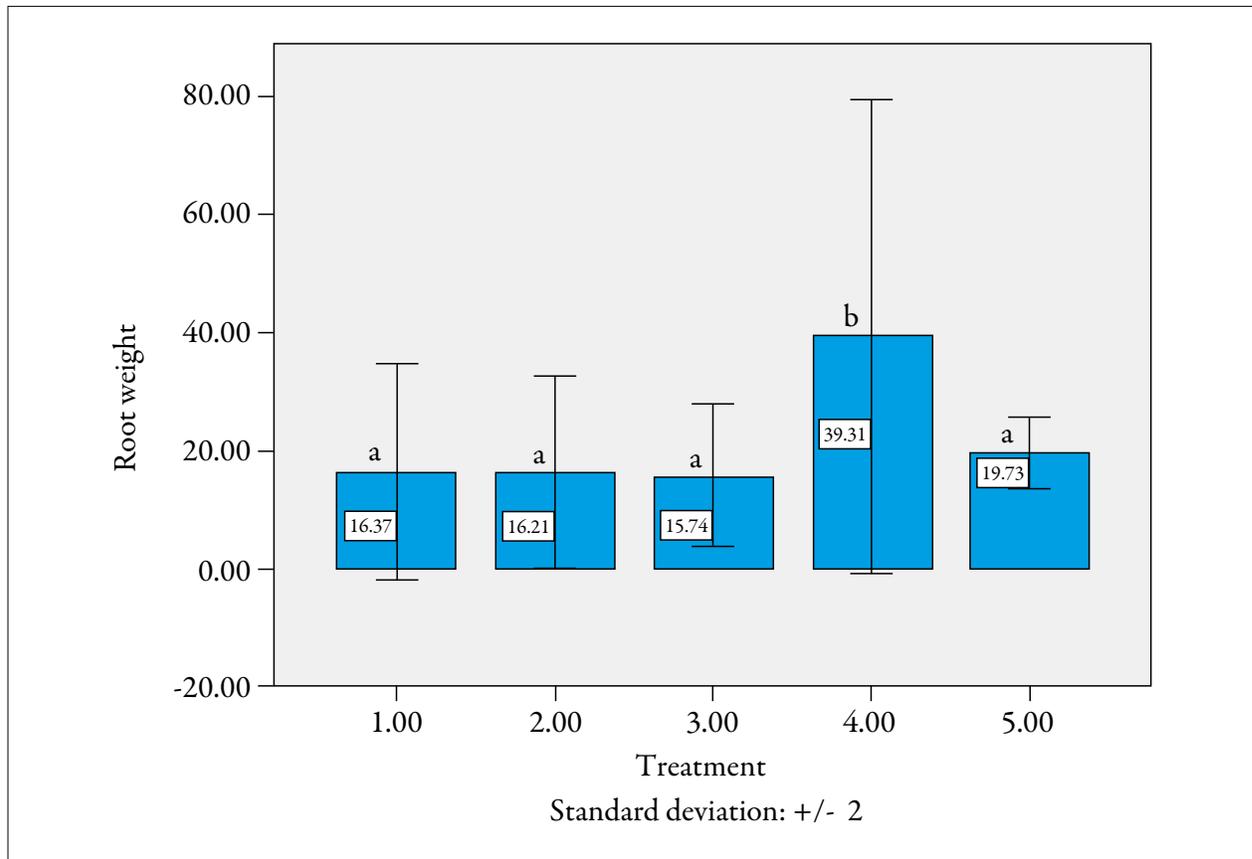
**Figure 5.** Root elongation affection of F<sub>1</sub> lulo plants by infestation of the nematode *Meloidogyne incognita* in seedlings. Same letters on the bars mean that they do not differ statistically ( $p > 0.05$ ).

Source: Prepared by the authors

## Fresh weight of roots

Fresh weight of roots alone did not prove to be a good susceptibility or tolerance indicator to the nematode *M. incognita*, although in treatment 4 (hibrid plants: cv. La Selva × cv. Castilla Larga Vida, inoculated with nematodes) there was a highly significant difference of 39.3 g compared to all other treatments (figure 6).

A higher root weight found in this treatment may correspond to the hyperplastic effect of an infection by *M. incognita*, which increased root volume compared to those that were not infected, as reported in the literature (Hafez & Sundararaj, 2000; Hashmi, Huettel, Hammerschlag, & Krusberg, 1993, Perrotta, Magnano, & Bassi, 1980).



**Figure 6.** Root fresh weight in the seedling phase of F<sub>1</sub> lulo plants evaluated for resistance to the nematode *Meloidogyne incognita*. Same letters on the bars mean that they do not differ statistically ( $p > 0.05$ ).

Source: Prepared by the authors

In addition, figure 7 shows the development of plants 60 days after the treatment was applied. In the first place, there is a lower plant vigor obtained from the crosses when the parent cv. Castilla Larga Vida was used as the mother; meanwhile the progenies of the crossing that used cv. La Selva as a mother showed a more vigorous development.

Control plants of cv. Castilla Larga Vida inoculated with *M. incognita*, in addition to showing a poor development, showed yellowing, i.e. symptom of the affection by the nematode. Apparently, the F<sub>1</sub> materials inoculated with *M. incognita* showed no symptoms of such affection when plant infestation was evaluated.

From the previous results and subsequent from the comparison of treatments T1 and T5, we can conclude that there is no cytoplasmic effect on the

resistance or tolerance to the nematode; however, this can be a result of an oligogenic effect, since a greater resistance is observed when the lulo cv. La Selva is used as a male parent.

Nonetheless, as stated by Dahms (1972), it is necessary to bear in mind that this type of experiment, in which high inoculum pressures are exerted through the artificial inoculation of treatments, may mask the results, so it may be possible that we cannot detect if these F<sub>1</sub> materials are really resistant.

Nevertheless, it should be considered that, because they are normally cross-pollinated plants, and since cv. La Selva is the result of a backcrossing, both parents are heterozygous, and segregation in the progeny obtained from crosses may be present. This causes that each plant shows differential levels of resistance to these crop pests.



Figure 7. Lulo plants of five treatments cultivated in plastic containers 60 days after inoculation.

When performing a correlation analysis between variables, we observed a non-significant inverse relationship between levels of nematode infestation and root weight, and a direct, non-significant relationship between that level of infestation and root

length. On the other hand, the correlation between root fresh weight and root length shows a direct and highly significant relationship, as is expected according to the biological logic in plant development. However, this is a fact that does not account for the tolerance or susceptibility to nematodes (table 1).

**Table 1.** Pearson's correlation analysis among response variables used in the evaluation of the susceptibility of lulo materials to the nematode *Meloidogyne incognita*

Response variables/ Variation source	Treatment	Infestation level of nematodes	Root length	Root fresh weight
Treatment	1	0.819*	0.282	-0.035
Infestation level of nematodes		1	0.012	-0.224
Root length			1	0.547*
Root fresh weight				1

\* Significance at 1 %.

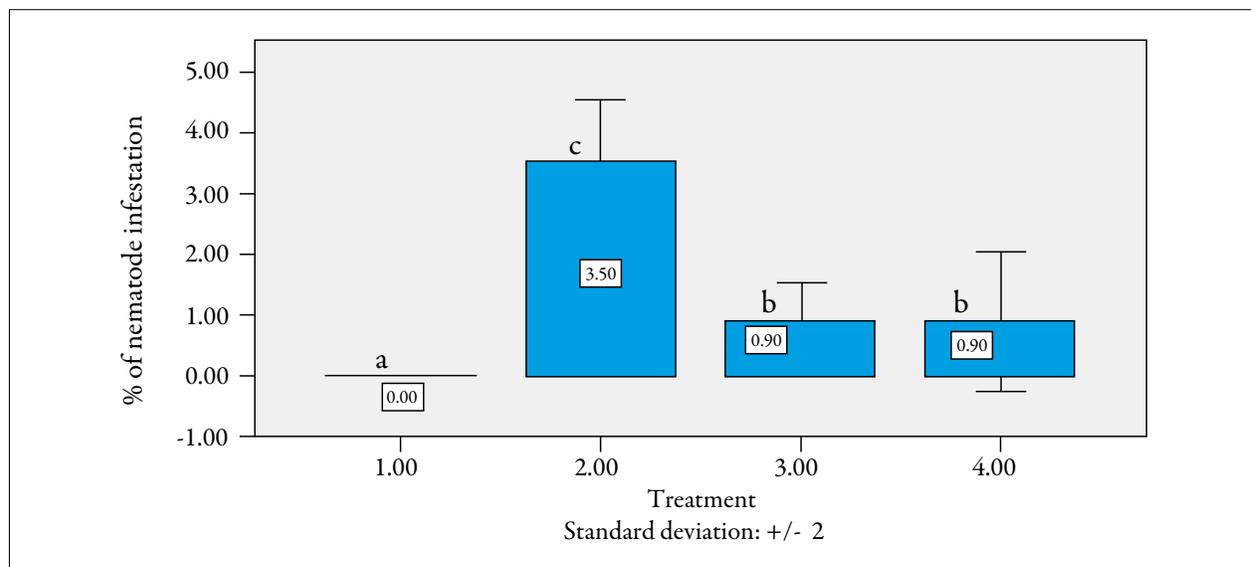
Source: Prepared by the authors

### Infestation level of *Meloidogyne incognita* under normal field conditions

The F<sub>1</sub> plants were evaluated under normal field conditions during the fruiting phase (six months after field transplantation). Because it was a destructive test, and since other phenological variables of the plants continued to be evaluated, only ten plants of each reciprocal crossing and of the evaluated cultivars were randomly removed; results show highly

significant statistical differences between the materials related to the infestation of *M. incognita*.

Based on the comparisons between the means of the evaluated materials regarding root infestation with *M. incognita*, the degree of infestation of both reciprocal crosses was less than 1%, which indicates a high level of resistance of these materials to nematodes, e.g. cv. La Selva (0). On the other hand, cv. Castilla Larga Vida was highly susceptible (3.5 %) (figure 8).

**Figure 8.** Evaluation of the level of infestation of *Meloidogyne incognita* in the roots of two cultivars and two F<sub>1</sub> materials of lulo under field conditions. Same letters on the bars mean that the values do not differ statistically ( $p > 0.05$ ).

Source: Prepared by the authors

### Evaluation of the resistance to *Neoleucinodes elegantalis*

The F<sub>1</sub> materials of both reciprocal crosses of lulo cv. La Selva × cv. Castilla Larga Vida did not show *N. elegantalis* larvae inside the fruits, nor exit holes in these. On the contrary, plants of the cv. Castilla Larga Vida used as a control were largely affected (60%) and showed, on average, two exit holes and two larvae per fruit (table 2).

The high percentage of fruits affected and the high average of larvae that attacked each fruit in the control treatment indicated that, using the entomological sleeves, the level of infestation was adequate to evaluate

the resistance of the F<sub>1</sub> materials obtained with the reciprocal crosses of cv. La Selva × cv. Castilla Larga Vida to the nematode *M. incognita*.

The F<sub>1</sub> materials showed resistance to the moth *N. elegantalis*, probably due to an antixenotic effect in the adult insect, based mainly on physical and chemical factors caused by the presence of trichomes throughout the plant and especially in the fruits, which secrete terpenoids, alkaloids, phenolic substances and others. These can be olfactory or gustatory repellents for the insect (Aragão, Dantas, & Gandolfi, 2000; Cardona, & Mesa, 2011; Simmons, & Gurr, 2005). However, it is necessary to continue with the observations of the lulo crop under commercial conditions.

**Table 2.** Average values of the evaluated descriptors to establish the resistance of the F<sub>1</sub> plants of the crosses of lulo cv. La Selva × cv. Castilla Larga Vida to the moth *Neoleucinodes elegantalis*

Descriptor	Descriptors		
	Percentage of fruits affected by <i>N. elegantalis</i>	Average number of exit holes per fruit	Average number of larvae per fruit
F <sub>1</sub> La Selva × Castilla Larga Vida	0 <sup>a</sup>	0 <sup>a</sup>	0 <sup>a</sup>
F <sub>1</sub> Castilla Larga Vida × La Selva	0 <sup>a</sup>	0 <sup>a</sup>	0 <sup>a</sup>
Castilla Larga Vida (control)	60 <sup>b</sup>	3 <sup>b</sup>	2 <sup>b</sup>

Averages with different letters in the same column are significantly different.

Source: Prepared by the authors

### Morphoagronomic behavior of hybrids

Hybrid plants of the reciprocal crosses proved to be vigorous, with an average height of 145 cm and a stem diameter of 4.5 cm, a good structure, large leaves, a length of 40.6 cm, a width of 41.5 cm, a petiole of 14 cm, and adapted to full solar exposure.

First flowering began 60 days after transplantation, with an average of four inflorescences per plant, which formed ca. 13 fruits, with an average diameter

of 5.6 cm, similar to the lulo cv. Castilla Larga Vida. Fruit maturation and harvest occurred 155 days after the beginning of flowering.

Fruit epidermis was yellow and the pulp green, without dehiscence (cracking), and with pleasant aroma and flavor. However, plants showed abundant and pronounced thorns in stems, branches and leaves (figure 9), a condition that is classified as unfavorable by producers, since it hinders cultivation work.



**Figure 9.** F<sub>1</sub> plants, four and a half months after transplantation to free sun exposure locations. a. Crossing of lulo cv. La Selva × cv. Castilla Larga Vida: stems and leaves with very strong thorns are observed in large quantity and with basal ramifications; b. Crossing of lulo cv. Castilla Larga Vida × cv. La Selva: shows thorns in stem and leaves.

## Conclusions

When inoculated with a high level of infestation of the nematode *M. incognita*, the F<sub>1</sub> plants obtained from the reciprocal crosses between the parents of lulo cv. Castilla Larga Vida and cv. La Selva turned out to be susceptible. However, when evaluating these same materials under field conditions, where the presence of the nematode was detected in previous samples, plants showed a root-node index lower than 1%, which indicates that the materials are highly tolerant to the nematode, and that there is a possible segregation of this resistance character.

In seedlings, fresh weight of the root in inoculated plants was affected by the infestation of *M. incognita*, causing a reduction of its weight, which affects normal plant development. Although root growth was significantly affected in plants subject to a high inoculum pressure of *M. incognita*, superior

vigor and higher tolerance were demonstrated in F<sub>1</sub> plants, especially when the cv. Castilla Larga Vida was used as the mother parent.

Apparently, the F<sub>1</sub> plants of the reciprocal crosses of cv. La Selva × cv. Castilla Larga Vida showed resistance to the moth *N. elegantalis* due to an antixenotic effect.

In subsequent trials, it is necessary to analyze separately fresh weight of roots and nodules of *M. incognita*, in order to establish real differences in weight attributable to the node formation caused by nematodes.

Moreover, it is necessary to continue with the evaluations of both the tolerance to nematodes and fruit quality (Brix degrees, total solids, ascorbic acid, among others), under commercial growing conditions, aiming at obtaining materials without

thorns, but keeping the outstanding agronomic characteristics found in plants in this study, and those that have a high consumer acceptance.

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## Disclaimers

All co-authors made significant contributions to the document and agree with its publication. Moreover, all authors state that there are no conflicts of interest in this study.

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