Parasitic Nematodes Associated with Tree Tomato (Solanum betaceum Cav.) in the Ecuadorian Highlands

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ABSTRACT.- A nematological survey was conducted in four tree tomato plantations (Solanum betaceum Cav.), located in the Ecuadorian highlands. The purpose was to study the occurrence of parasitic nematodes associated with this crop. A total of 64 soil and 34 root samples were processed and analyzed in duplicate, in which nematodes from at least 12 genera in different population arrangements were found. Differences in soil conductivity measurements were significant, whereas pH was not. The most frequent genus was Meloidogyne spp., with a mean average of 363.5 nematodes per 100 g of soil sample, and 290.4 for 10 g of roots among the 64 and 34 soil and root samples collected at all four locations. Pratylenchus spp. Followed, with a mean average of 146.3 and 97.4 per 100 g of soil and for 10 g of root samples, respectively. The occasional appearance of the nematode genus Hoplolaimus in 33 of the 64 soil samples (52%) is a significant finding, since it is the first report of this genus associated with tree tomato crops in Ecuador. Around half of the total nematode population found in soil as well as in root samples (with a mean average of 530 and 518, respectively) were from the saprophytic genera.

KEYWORDS: Ecuador, phytonematodes, population, Solanum betaceum, tree tomato.

INTRODUCTION

Tree tomato, also known as “tamarillo”, “tomatillo” or “tomate de árbol” is a solanaceous plant, originally from the Latin American Andean region. It is also found widely throughout tropical and subtropical areas in New Zealand, Haiti, Mexico, Malaysia and Uganda (Moreno et al. 2007). It is a small, half-woody tree reaching a height of 2 m. It has a perennial vegetative life cycle, and fruit production is continuous throughout the entire year. Production begins 1-2 years after planting. Maximum yield is reached at 4-5 years, but minor production can occur up to 10 years (Amaya and Julca 2006).
Tree tomatoes grow in both warm and cold areas between 1000-3000 m above sea level. The optimal habitat temperature is 16-19°C; high humidity is not a factor. Soil requirements include pH values between 5.6 and 7.7; mostly sandy soil, and the presence of organic matter (Cadena 2001).

The fruit contains high levels of vitamins A, B, C and E; it is also a good source of calcium, iron and phosphorus, as well as antioxidants found in carotenes, pectins, polyphenols, proteins and fibers (PAVUC 2008).

In Ecuador, tree tomato production is located mainly on small farms in the Andean highlands, especially in the provinces of Imbabura, Tungurahua, Chimborazo, Azuay and Pichincha (Cadena 2001). The crop constitutes an important economic activity for indigenous low-income farmers. Its production has been directed towards satisfying the needs of local markets; however, since 2005, part of the yield has been redirected to international markets such as the United States, Canada, Spain and other European countries (CICO 2006).

There are six commercially relevant varieties of tree tomatoes: Yellow or “Incan Gold” which is preferred by the industry for its taste, size and durability in shipment and transportation; Purple or “Purple Red”, which is the market’s second choice; Black or “Heights Tomato”; Pointy Tree Tomato; Round Tree Tomato, and Giant Yellow. All of these varieties are reported to be attacked by plant soil and root parasitic nematodes to an unequal degree (Cadena 2001).

In crop production it is estimated that nearly 20% of the harvest worldwide is lost annually due to nematodes and related diseases. This value is greatly underestimated, however, since infections are often confused with nutritional or water deficiencies or other plant diseases; 40% might be a closer approximation (Ríos 2006). The identification of nematodes in the tree tomato is essential for the diagnosis of their impact on production, so as to evaluate crop losses due to these parasites and establish adequate pest management programs (Solano et al. 2014; Khan et al. 2011), in order to improve the quality and quantity of the harvest. There is little information available on nematodes associated with tree tomatoes in other similar regions (Ramirez et al. 2015; Prohens and Nuez 2005).

The purpose of this study was to determine the incidence of root and soil nematode infection associated with tree tomato farms located in the Pichincha province of Ecuador, including pH and soil conductivity measured during the time of the study. Existing methods for nematode analysis were adapted to our facilities in order to record and process the data collected.

**MATERIALS AND METHODS**

This study was conducted in four yellow tree tomato plantations in areas close to 2200 m altitude, near the towns of Puembo, Yaruquí and Checa: Daniela Verónica, 1 ha (Location 1: 78°21’15.78”W; 0°10’30.06”S); Los Guabos, 1.5 ha (Location 2: 78°19’30.78”W; 0°9’23.01”S); Santa María, 2 ha (Location 3: 78°19’30.78”W; 0°9’23.01”S); Santa Rita, 0.7 ha (Location 4: 78°18’52.83”W; 0°7’42.09”S) (Figure 1).
All of these plantations were between 15-27 months old. The study was conducted from April to July of 2008. Sixty-four soil samples were collected, taking one sample weekly from each of the four locations; thirty-four root samples were also collected. Soil pH and conductivity were measured weekly for 16 weeks at all four locations, by mixing a 10 g soil sample in 100 ml of distilled Milli Q water, using a Metler Toledo® SevenEasy pH-meter and conductivity that was calibrated prior to each measurement. The 10 g sample was taken out of a pool of mixed 1 kg sample collected every week from five random 20 cm deep soil samples (200 g each) from each of the four locations.

Each soil sample was obtained using small steel shovel a distance of 20 cm from each plant’s step and at a depth of 20 cm. The sampling surface was previously cleaned of organic and inorganic debris. Soil samples were taken from the around the secondary and tertiary roots. Approximately 200 g of soil were collected from each of five randomly selected plants. These soil samples were mixed together to obtain a sample of approximately 1 kg for each five plant group selected, which collectively represented one sample out of the 64 soil samples studied.

Root samples were collected from randomly selected and subsequently labeled plants, at the same distance and depth as described above, using a knife disinfected with alcohol to cut exposed secondary and tertiary roots. Once the root samples were taken, the soil was quickly replaced to cover the exposed roots. Approximately 5 g of tertiary-only roots were collected from each of the five different randomly selected plants. These were then mixed together in order to obtain a 25 g root sample, representing one out of the 34 samples analyzed: eight samples each from locations 2 and 4, and nine each for locations 1 and 3 (farmers allowed only these numbers of samples to be taken).

All soil and root samples were labeled accordingly, transported to our laboratory in individual, hermetically sealed plastic bags, stored at room temperature (± 20°C) in a dark, dry environment, and then processed and analyzed the day following collection using a modification of the Cobb (1918) sieving and decanting technique, which has been standardized in accordance with our laboratory facilities. The original methodology has been described by Townshead (1962) and Thorne (1961). Samples were collected on a single basis either from a given place or from an individual plant sample.

One hundred grams of soil were processed from each thoroughly mixed 1 kg soil sample. These 100 g of soil were stirred vigorously into 1 L of tap water for two minutes and were allowed to settle for 30 seconds. Leaving the sediment for a second wash treatment, the liquid phase was completely filtered through a 150 mesh (106 μm) sieve over a 350 mesh (45 μm) sieve that was inclined to 45º. While the liquid phase passed through the sieves, approximately 1 g of small soil particles, including the nematodes, remained on the 350 mesh sieve; this last sieve was turned over and was then washed with portions of tap water over wet filter paper (Whatman No.1) so as to retain the remaining soil particles while allowing the nematodes to pass through and be collected in a small plastic container.

Filtration lasted for two days and small volumes of water were added continuously to avoid filter paper desiccation while assuring maximum nematode retention. In order to maximize the number of nematodes collectable from each sample, an extra 1 L of tap water was added to the first sediment which was stirred, decanted and filtered again as previously described; the volume of these last two filtrates, containing the nematodes, was adjusted to 100 ml with tap water.

Each 25 g root sample was washed with running tap water and gently blotted with a paper towel. Ten grams of roots were randomly selected from each sample and cut in smaller pieces (± 1 cm length). The roots were gently macerated and mixed homogeneously for 15 seconds in 100 ml of tap water using a blender at maximum speed. This mix was decanted and washed through the 150 mesh sieve over the 350 mesh sieve; both filters were inclined at 45º. Water was allowed to pass entirely through the sieves while the small pieces of roots, debris and nematodes remained on the sieves. Both sieves were turned over, then washed with tap water over soaked filter paper (Whatman No.1) in order to retain the final debris, allowing the nematodes to pass through and be collected into a small plastic container.

Filtration lasted two days and small volumes of water were added continuously to avoid filter paper desiccation while assuring maximum nematode recovery. The volume of this final filtrate containing the nematodes was adjusted to 100 ml with tap water; each of the 64 soil and the 34 root samples was processed and analyzed twice as two subsamples, and the results were reported as the mean of the two counts in order to minimize errors in nematode counting and identification.
A 10 ml sample of each filtrate was placed in a small Petri dish to be studied under a microscope. Nematode identification and counting were done using only the live specimens found in each sample. Due to degradation processes that could alter the results, dead nematodes were not included in the counting or identification. The quantity of nematodes found in each 10 ml sample was multiplied by 10, and reported as the number of nematodes found in the 100 g soil sample or in the 10 g of processed roots. Nematodes were identified with an inverted microscope (ZEISS® Telaval 3) at 5x. Identification of genera was possible with the aid of several taxonomic keys found in Tiwari et al. (2001), Nickle (1991), Eisenback et al. (1981), Tarjan (1973) and Golden (1971). Photos were taken with a Canon Powershot® A540 camera with a 4x zoom positioned over the microscope optic lens at 5x optics and a 4x zoom.

The SPSS® statistical program was used to organize and analyze data collected. Original data were transformed into Log x+1 to avoid statistical errors. The statistical model used to analyze these data was an ANCOVA for soil samples (in order to compare nematode quantities with pH and conductivity measures) and a RBD ANOVA for root samples (since neither pH nor conductivity measurements were carried out on roots). The frequency of each genera in each location was calculated as the number of samples containing a nematode specie divided by the number of samples collected, multiplied by 100 (Barker 1985).

RESULTS

Soil samples from location 1 showed an average, acid, pH of 6.7; alkaline pH 7.4 in location 2; pH 7.2 in location 3 and neutral pH 7 in the soils from location 4 (Figure 2).

ANCOVA in DBCA analysis showed that the soil pH measurements were not significant with respect to the quantity of total soil nematodes found at each of the four locations (p=0.675; H₀ = 0.93). Soil conductivity was found to be signifi-

Figure 2.- Weekly soil pH (A) and conductivity (B) measurements of each sample collected in all four studied locations
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significant as a parameter that could affect the total number of nematodes found at each location (p=0.081; H1=0.05). The mean average soil conductivity among all samples was 1.14 mSiem/cm. In location 1, the mean conductivity among the samples collected was 1.75 mSiem/cm; a low 0.54 mSiem/cm in location 2; 0.88 mSiem/cm in location 3; and 1.36 mSiem/cm in location 4.

Nematodes were found in the soil and in roots of tree tomatoes at the four locations. The nematodes found represented at least 12 genera in 10 families of the Tylenchida order; the remaining 2 (Aphelenchoïdes and Aphelenchus) belonged to one of two families in the Aphelenchida order; others that could not be identified were grouped as “other genera”. Means and frequencies of these nematodes are reported (Table 1 and 2) and were calculated without any Log x+1 transformation.

Species diversity was similar at all four locations and within soil and roots. The smallest nematode was *Meloidogyne* spp. (250 μm) found with other larger genera such as *Pratylenchus* spp., *Aphelenchus* spp., *Paratylenchus* spp., *Radopholus* spp., *Rotylenchus* spp., *Heterodera* spp. and *Tylenchus* spp. were found in small numbers, around 20 each in all four locations. Others, such as *Criconemella* spp., were detected in even smaller numbers (x=7,6).

The occasional appearance of *Hoplolaimus* spp. was observed in 33 of the 64 soil samples analyzed (x=7,1). About 50% of the total nematodes counted were saprophytes (700-1000 μm long) from several unidentified genera. Other less damaging nematodes, such as *Rotylenchoïdes* spp., *Dorilaymus* spp. and *Radopholoides* spp., reported as other genera, were also found, but their total mean number was x=26,1. Numbers of nematodes found in soil samples from the four different locations (Figure 5) were similar at locations 1 and 2, with larger numbers in location 3, and at even greater quantities in location 4, where each of the two largest peaks represented maximum numbers of *Meloidogyne* spp. and saprophytes.

With the exception of *Hoplolaimus* spp., all genera encountered in soil samples were also found in roots. *Meloidogyne* spp., *Pratylenchus* spp. and *Helicotylenchus* spp. were also the most frequent nematodes associated with the root system (x=290,4; x=97,4 and x=56,8 respectively).

### Table 1. Frequency and mean density of tomato tree nematodes found in 100 g soil samples: 16 samples taken from each of the four locations. Results were calculated from the original data without any log x+1 transformation.

|      | 1 Daniela Verónica | 2 Los Guabos | 3 Santa María | 4 Santa Rita |
|------|---------------------|--------------|--------------|-------------|
| **Genera** | **Frequency** | **Mean** | **Frequency** | **Mean** | **Frequency** | **Mean** | **Frequency** | **Mean** |
| **Aphelenchoïdes** | 75 | 8 | 89 | 16 | 100 | 18 | 100 | 33 |
| **Aphelenchus** | 88 | 6 | 89 | 16 | 89 | 12 | 75 | 8 |
| **Criconemella** | 38 | 4 | 78 | 11 | 44 | 3 | 38 | 6 |
| **Helicotylenchus** | 100 | 52 | 100 | 72 | 100 | 52 | 100 | 61 |
| **Heterodera** | 75 | 8 | 89 | 15 | 89 | 22 | 88 | 26 |
| **Hoplolaimus** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Meloidogyne** | 100 | 175 | 100 | 212 | 100 | 356 | 100 | 479 |
| **Pratylenchus** | 100 | 21 | 100 | 22 | 89 | 25 | 100 | 29 |
| **Radopholus** | 100 | 28 | 100 | 37 | 100 | 21 | 75 | 16 |
| **Rotylenchus** | 88 | 19 | 100 | 23 | 100 | 26 | 100 | 24 |
| **Tylenchus** | 75 | 10 | 100 | 23 | 89 | 22 | 100 | 31 |
| **Otros géneros** | 88 | 21 | 100 | 18 | 100 | 24 | 100 | 32 |
| **Saprophytes** | 100 | 167 | 100 | 241 | 100 | 496 | 100 | 1170 |

Frequency is the result of dividing the number of the samples with a given nematode for the total number of samples analyzed in a given location, times 100.
The absence of *Hoplolaimus* spp. in all root samples, together with a decrease in numbers of *Radopholus* spp. and *Rotylenchus* spp., was also evident. Other less damaging phytoparasitic nematodes, such as *Rotylenchoïdes* spp. and *Radopholoides* spp., reported as one group, were also found in smaller numbers (*x* = 23.7). The number of saprophytes (45.8% of the total number of nematodes observed) was greater than that of any of the phytoparasites encountered.

The number of nematodes found in root samples was also compared between the four different locations (Figure 6) Roots from plants at locations 1, 2, and 3, showed similar numbers of parasites. Location 4 had higher numbers of *Meloidogyne* spp., and saprophytes were even higher. The original data from soil and root samples were transformed to Log x+1 due to the presence of some data with zero values. Results showed highly significant differences within and among genera and samples (*p* = 0.00 in both categories). Highly significant differences were also found in the number of nematodes reported for each location (*p* = 0.00). These differences were a result of the larger number of saprophytes and *Meloidogyne* spp reported at location 4 in both root and soil samples, and also, to a lesser degree, to the amount of other parasitic nematodes found in each distinct location.

In accordance with similarities found between the averages of each genus, it was possible to create frequent genera that could be separated into ei-
DISCUSSION

Parasitic nematode infections in the roots of tree tomatoes and in surrounding soil samples at four different locations were studied and found to host similar genera. The population density of each genera in both the soil and the roots was statistically different. The processed soil sample weighed 10 times that of a root sample, and the results clearly indicated the importance of nematode proximity to roots in accordance to nourishment, invasion and survival needs. Furthermore, De Waele et al. (2006) reports that the host suitability for a given plant based on nematodes per root system and nematodes per root unit can differ.

The results also showed a large root and soil population of *Meloidogyne* spp., *Helicotylenchus* spp. and *Pratylenchus* spp. with a clear predominance of *Meloidogyne* parasites in soil and root samples at all four locations; other studies (Ramirez et al. 2015) also found similar results in similar crops. Our results indicated that this nematode has adapted to crops such as tree tomatoes and is capable of existing in close relationship within root nodula, or freely in the soil. This latter habitat is an important means of rapid and continuous dissemination of second-stage parasites.
thin and long free-living juvenile parasites in crops, while eggs and nodules are secluded spaces for parasite development and reproduction in plant tissues, including dissemination over the time (Soria 2009).

The pathological damage that *Meloidogyne* spp. can cause (Baicheva et al. 2002) is in association with the common root gall. The nodule may interrupt the flow of nutrients within the plant, and the root damage caused by the digestive enzymes will contribute to the development of plant diseases associated with invasive bacteria, fungi or viruses (Haseeb et al. 2005). Therefore, in association with tree tomato crops, *Meloidogyne* spp., constitutes another suitable model for the study of nematode pest management and applied phytopathology research.

The low numbers of the other phytonematodes (*Aphelenchoides* spp., *Aphelenchus* spp., *Paratylenchus* spp., *Radopholus* spp., *Rotylenchus* spp., and *Tylenchus* spp.) or others with even smaller populations (*Criconemella* spp., *Hoplolaimus* spp. or those grouped as “other genera”) found in this study may indicate their ability to coexist as internal and external plant parasites. The number of nematodes are not necessarily to be taken as an absolute indication of high or low infestations, perhaps due to differences in isolation techniques according to size and motility nematode habits. It may also indicate the different life cycles for each genera that may take place in the soil or in root tissues, which should be taken under consideration when implementing control strategies. These are good reasons to choose an appropriate standardized isolation technique (McSorley and Parrado 1981), and an extended sampling to study at least two generations of nematodes (Barker 1985).

*Hoplolaimus* spp. is a very large nematode usually reported in turf or other kinds of grasses; damage to the crop may appear as yellowing patches across the field, sometimes confused with being caused by nutritional deficiency or drought (Tiwari et al. 2001; Mateille 1994; Rhoades 1986). Small tertiary roots are most affected by this parasite, since their growth and function are notably diminished (Nickle 1991). The presence of *Hoplolaimus* spp. represents nematological diversity in the tree tomato. While its numbers were small, and it was found only in soil surrounding the roots, its presence suggests the possibility of established relationships with tree tomato plantations. The low numbers found in the soil need to be confirmed. *Hoplolaimus* spp. is the largest and probably the most massive of the phytoparasitic nematodes found, and it may very well be that it was decanted together with soil particles or possibly trapped on the last sieve or on the filter paper prior to analysis; it thus may be more abundant than we observed. In contrast, *Meloidogyne* spp. was the most numerous and frequently found parasite in this study, probably because the collection technique may favor small parasites (McSorley and Parrado 1981).

**Figure 6.** Comparison of the mean number of nematodes found in root samples (10 g) from tree tomato (*Solanum betaceum* Cav.) in 4 different locations.
The occurrence of a large saprophytic nematode population associated with soil and roots constitutes a reference for soil quality. Usually, a relatively high number of saprophytes suggest that there is enough organic matter in the soil for the plant to grow adequately (Nickle 1991) and for free living nonpathogenic saprophytes to closely coexist with tertiary roots. Ecological competition with phytoparasitic nematodes, not for food, but for space, is also of great importance for the saprophyte balance in soil; particularly in external root tissues where nematodes were found to contribute to the processing of organic molecules that specially modified soil niche conductivity. While the genera found are the same in all four locations, note should also be made of the heterogeneity of nematodes in relation to the significant differences in numbers and frequencies found among genera and locations, in spite of the similarities in pH measurements and the differences in soil conductivity. Particular note should be made of location 1, where conductivity was significantly higher than in other locations, but parasites were less numerous than those in location 4, where conductivity was around 0.3 mSiem/cm lower than in location 1. Increased conductivity as a result of sodium or related salts added as fertilizers or released by saprophytic organic debris processing lowered the nematode population. This is perhaps due to unfriendly environment for metabolic exchange, which includes altered parasite cellular osmotic pressure. These factors may also help to explain the natural grouping in repetitive frequencies for each genus in each of the four farms.

These small plantations were located close to one another at approximately the same altitude, and were exposed to similar weather conditions; however, frequencies encountered are directly proportional to differences in organic matter and conductivity, the presence of certain fungi or bacteria acting as bio-controllers (Safiuddin et al. 2015), and neighboring nematode contamination.

The number of nematodes found in soil is different than those reported for roots. This result might be explained both by differences in the quantity of sample processed as well as by the association of certain nematodes with roots in preference to the soil. It could likewise indicate the comparative number of nematodes that actually penetrate the root tissue during part of their life cycle (*Meloidogyne* spp.), in relation to the number of nematodes that remain outside, which may feed on the exterior byproducts of roots while moving to other resources in order to minimize ecological competition.

![Graph](image)

**Figure 7.** Results of the Tukey test with significant levels of 0.05 on the means of all genera found in the 64 soil samples collected and analyzed on the 4 locations of tree tomato plantations. Data was grouped as 4 significantly different subsets.
In view of these results, it is possible that new and improved soil, crops, and pest management techniques are needed in order to increase yields and crop quality as well as to ensure successful and competitive large scale production of tree tomatoes in Ecuador and in other regions.

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