EFFECT OF 1,3 DICHLOROPROPENE AND ROOTSTOCKS ALONE AND IN COMBINATION ON *TYLENCHULUS SEMIPENETRANS* AND CITRUS TREE GROWTH IN A REPLANT MANAGEMENT PROGRAM

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ABSTRACT

F. J. Sorribas, S. Verdejo-Lucas, M. Galeano, J. Pastor, and C. Ornat 2003. Effect of 1, 3 dichloropropene and rootstocks alone and in combination on *Tylenchulus semipenetrans* and citrus tree growth in a replant management program. Nematropica 33:147-156.

The effects of 1,3 dichloropropene and rootstocks alone and in combination on *Tylenchulus semipenetrans* population densities and tree growth were evaluated during three years in a nematode-infested citrus orchard. The experiment was a 2 × 2 factorial with six replications. One factor was fumigation and the second, citrus nematode resistant and susceptible rootstocks. Soil population densities of the citrus nematode remained below detectable levels in fumigated plots planted with the resistant rootstock Cleopatra mandarin × *Poncirus trifoliata* 03.01.5 throughout the study. The nematode was not recovered from fumigated plots with Carrizo citrange until after the third year, when it appeared sporadically in some of the plots. The nematode was not recovered from citrus roots of either rootstock in fumigated plots. In non-fumigated plots, the resistant rootstock suppressed nematode reproduction. The number of juveniles per 250 cm^3 soil, females per gram of root, and eggs per gram of root were 8, 6, and 0.3%, respectively, compared to Carrizo citrange by the third year. The trunk diameter of Orogrande mandarin scion was greater on the resistant than on the susceptible rootstock in non-fumigated plots, but there was no difference in fumigated plots. Fumigation with 1,3 D combined with the resistant rootstock 03.01.5 retarded nematode reinfestation for at least three years. Also, 1,3 D delayed reinfestation of the plots with the susceptible rootstock. The resistant rootstock suppressed nematode reproduction, and the growth rate of the nematode on this rootstock was slower than on the susceptible one.

Key words: Citrus nematode, fumigation, integrated pest management, nematicide, nematode management, resistance, citrus rootstocks, susceptibility.

RESUMEN

F. J. Sorribas, S. Verdejo-Lucas, M. Galeano, J. Pastor, and C. Ornat 2003. Efecto de 1,3 dicloropropano y patrones individualmente y en combinacion sobre *Tylenchulus semipenetrans* y el crecimiento de citricos en un programa de manejo de replantacion. Nematropica 33:147-156.

Se evaluó el efecto de la fumigación con 1,3 dicloropropeno sola o en combinación con un patrón resistente o susceptible a *Tylenchulus semipenetrans* sobre los niveles poblacionales del nematodo y el crecimiento de los árboles durante tres años en una parcela infestada por el nematodo. El diseño experimental fue factorial 2 × 2 con seis repeticiones por tratamiento. Los factores fueron la fumigación y el segundo, patrón resistente y susceptible al nematodo de los cítricos. Las densidades de población del nematodo en suelo en las parcelas fumigadas y plantadas con el patrón resistente permanecieron por debajo de los niveles de detección durante los tres años del estudio. El nematodo no se detectó en las parcelas fumigadas y plantadas con citrange Carrizo hasta el tercer año cuando aparecieron esporádicamente en alguna de las repeticiones. El nematodo no se detectó en las raíces de ninguno de los dos patrones en las parcelas fumigadas. En las parcelas no fumigadas, el patrón resistente inhibía la reproducción del nematodo. El número de juveniles por 250 cm^3 de suelo, hembras y huevos por gramo de raíz fue 8, 6 y 0,3%, respectivamente, en comparación con citrange Carrizo, tres años después de plantar. El diámetro del tronco del cultivar Orogrande fue mayor sobre el patrón resistente que sobre el susceptible en las parcelas no fumigadas pero no se encontraron diferencias en las parcelas fumigadas. La combinación fumigación con 1,3 D y patrón resistente 03.01.5 retrasaba la reinfestación del suelo por el nematodo al menos durante tres años. La desinfestación con 1,3 D también retrasaba la reinfestación de las parcelas con el patrón susceptible. El patrón resistente suprimía la reproducción del nematodo y su tasa de crecimiento era más lenta que en el patrón susceptible.

Palabras clave: fumigación, manejo integrado de plagas, manejo de nematodos, nematodo de los cítricos, nematicida, patrones de cítricos, resistencia, susceptibilidad.

INTRODUCTION

Replanted citrus trees in orchards that have been previously planted to citrus often show growth retardation that may be due to several factors, including the citrus nematode (*Tylenchulus semipenetrans* Cobb) (Tsao *et al.*, 1989). This nematode causes the disease known as slow decline, named for the slow rate of nematode population growth in newly replanted orchards and the slow development of symptoms in the host (Reynolds and O'Bannon, 1958; O'Bannon and Tarjan, 1973, Duncan and Cohn, 1990). High nematode population densities eventually will be associated with severe decline symptoms on infected trees, although low population densities have also been associated with advanced stages of decline due to severe root damage (Reynolds and O'Bannon, 1963a).

Sour orange (*Citrus aurantium* L.) was formerly the most widespread citrus rootstock in Spanish orchards, with more than 95% of the trees grafted on this rootstock. However, the severity of citrus tristeza virus (CTV) on this rootstock forced the replacement of sour orange with other citrus trees tolerant to the virus (Cambra, 1994). Sour orange still remains in 60% of the old orchards that are reported in progressive decline. At present, the CTV-tolerant Carrizo citrange [*Citroncirus webberi* Ingram & Moore × *Poncirus trifoliata* (L.) Raf] is the rootstock most widely used to establish new citrus orchards in soils not previously planted to citrus. This rootstock also is commonly used to replace sour orange trees killed by CTV in old orchards. More than 80% of the citrus trees in Spanish nurseries are grafted on this rootstock. However, Carrizo citrange does not grow well in calcareous soils, the predominant soil type of Spanish citrus (Forner and Pina, 1992).

Resistance to a Mediterranean biotype of *T. semipenetrans* has been identified in new hybrid citrus rootstocks from the breeding program of the Instituto Valenciano de Investigaciones Agrarias (Verdejo-Lucas *et al.*, 1997, and 2000). Some rootstocks have agronomic characteristics that are promising for commercial use. The selection of Cleopatra mandarin (*C. reshni* Hort) × *P. trifoliata* Rubidoux 03.01.5 is resistant to CTV and is more tolerant to soil salinity and lime-induced chlorosis than Carrizo citrange (Forner *et al.*, 2003). This rootstock retained its resistance to *T. semipenetrans* when exposed to increasing inoculum densities of the nematode (Galeano *et al.*, 2003), but its resistance was somehow reduced under a continuous inoculum source (Verdejo-Lucas *et al.*, 2003).

The objectives of this study were to evaluate *i)* the effect of 1,3 dichloropropene and rootstocks alone or in combination on reproduction of the citrus nematode in citrus orchard that had been replanted, and ii) the influence of the nematode infection on early growth of Orogrande mandarin (*C. reticulata* Blanco) scion grafted onto the resistant Cleopatra mandarin × *P. trifoliata* 03.01.5 and onto the susceptible Carrizo citrange.

MATERIALS AND METHODS

Plant material

Seeds of the resistant selection of Cleopatra mandarin × *P. trifoliata* 03.01.5 and of the susceptible Carrizo citrange were germinated in seedbeds on 19 December 1996. Seedlings were transplanted into 3-liter black-plastic bags containing a steam sterilized potting mixture on 10 April 1997. Seedlings were grafted with Orogrande mandarin on 2 July 1997, maintained on a greenhouse bench for 12 months, and then transplanted into the orchard on 1 July 1998.

Orchard site

The orchard, located in Alcanar, Tarragona, Spain, was previously planted to Clementine mandarin (*C. reticulata* Blanco) on sour orange and infested with residual populations of *T. semipenetrans* that remained in the soil after the removal of the citrus trees in February 1998. After uprooting the old tree trunks, soil was subsolated at about 40 cm depth to remove big root pieces and stones. Then, soil was prepared for planting by passing a cultivator cross way three times at periodical intervals. The soil was a sandy loam (58% sand, 15.5% clay and 26.5% silt), pH (water) 8.2, electric conductivity 0.21 dS/m, 2.4% organic matter, 6% active lime, 52% CO_3Ca, and contained 14 ppm N, 68 ppm P, 368 ppm K, and 252 ppm Mg. Experimental plots were prepared for planting on 3-m wide and 40-cm raised beds. Beds were divided into main plots 24-m long. On 27 May 1998, half of the plots were fumigated with 1,3 dichloropropene (1,3 D) by injecting the nematicide at a depth of 30 cm in 1.5 m wide strips at a rate of 600 liter /ha. Chisel spacing was 30 cm, and the tractor was passed twice to obtain a 3-m wide fumigated band. The experiment was a 2 × 2 factorial with six replications. One factor was fumigation with 1,3 D, and the second, citrus nematode-resistant and susceptible rootstocks. Treatments were randomly assigned to plots within a replication. Individual plots, in fumigated and non-fumigated soil, were planted with three trees of 03.01.5 and another three of Carrizo citrange in a row. Trees were spaced 4 m apart within the row; there was 6 m between rows, and two guard trees of Orogrande mandarin onto 03.01.5 were planted between contiguous plots. The orchard was managed according to standard practices in the area. Supplemental irrigation was provided from March to October through a drip irrigation system, and fertilizer was delivered with the irrigation water.

Nematode population densities

Tylenchulus semipenetrans population densities were determined before removing the old trees by collecting composite soil and root samples to a depth of 20 cm at the drip line on 21 January 1998. Roots were separated from soil by sieving. Screened soil was mixed thoroughly, and nematodes extracted from 500 cm^3 soil subsample using Baermann trays. Nematodes migrating to the water were collected 1 week later, concentrated on a 25 µm-pore screen, and counted. The number of nematodes was expressed per 250 cm^3 of soil. Roots were washed free of soil, cut into 1 cm sections and macerated in a 0.5% NaClO solution in a food blender at approximately 1000 rpm for two successive 15 s intervals (McSorley *et al.*, 1984). The resulting suspension was passed through a 74 µm sieve to remove root debris. Nematodes were concentrated on a 20 µm-pore screen. The population densities of *T. semipenetrans* were 120 ± 100 females (mean ± standard deviation), 380 ± 310 eggs per gram of root, and 2220 ± 1340 juveniles per 250 cm^3 soil. To determine preplanting population densities (post-fumigation), soil samples were collected to a depth of 20 cm in each plot on 17 June 1998. Individual samples consisted of three soil cores (one core per tree of each rootstock) taken to a depth of 20 cm from the planting spot. Soil was mixed and nematodes were extracted as described previously. Post-planting densities were determined by collecting composite soil and root samples in May-June and in November each year for three consecutive years. Individual samples consisted of three soil cores per plot (one core per tree of each rootstock) taken with a soil auger at a depth of 20 cm midway between the trunk and the drip line. Samples were processed and nematodes extracted from soil and roots as described above.

Plant growth

Tree trunk diameters (cm) were measured 10 cm above the grafting union with a digital caliper at transplanting and in May each year for three consecutive years. In the third year, tree canopy volumes were determined according to the formula $V = 0.52/h/d^2$ (Turrells, 1946) where h is total height of the tree minus height from the first branch to the ground, and d is the average diameter of the canopy measured at right angles (north-south and east-west). Yield was not assessed.

Statistical analyses

Numbers of nematodes in soil and roots were transformed log (x+1) and subjected to analysis of variance. To compare plant growth, a repeated measure analyses was performed, considering trunk diameter at planting as covariate. Tukey-Kramer adjustment for multiple comparisons was used when the F test for main effects and interactions were significant ($F < 0.05$). Analyses were made using the GLM procedure of SAS version 8 (SAS Institute Inc., Cary, NC).

RESULTS AND DISCUSSION

Main effect of rootstocks on nematode population densities and plant growth in fumigated plots

Citrus nematodes remained below detectable levels in soil of fumigated plots planted with 03.01.5 through the study, and they were recovered sporadically from those with Carrizo citrange with average numbers of 23 ± 27 juveniles per 250 cm^3 soil by the third year. The fumigation effect prevented any detectable nematode root infection. Consequently, no citrus nematodes were recovered from roots of either rootstock in fumigated plots for the duration of the study. The Orogrande scion on the resistant and susceptible rootstocks showed similar trunk diameters in fumigated plots. The diameters of 03.01.05 and Carrizo citrange at planting were 5.7 ± 1.3 and 6.4 ± 0.9, and they increased to 29.2 ± 6.57 and 27.4 ± 9.7 respectively by the third year (Fig. 4).

Effect of rootstocks on nematode population levels and plant growth in non-fumigated plots

Site preparation reduced *T. semipenetrans* from 2220 ± 1340 juveniles per 250 cm^3 soil to preplanting densities of 225 ± 130 juveniles per 250 cm^3 soil in non-fumigated plots. In these plots, *T. semipenetrans* was not detected until 12 months after planting in soil samples from plots with either rootstock and was recovered thereafter in all sampling dates. Nematodes reached levels of 2400 ± 2245 juveniles per 250 cm^3 by the third year. Nematode distribution in non-fumigated plots with 03.01.5 was erratic, and the average soil densities were 198 ± 288 juveniles per 250 cm^3 soil at the end of the study. Soil nematode population densities in plots with either rootstock were not statistically different for the first five sampling dates, but they were greater ($P < 0.05$) in plots with Carrizo citrange than in those with 03.01.5 by the third year (Fig. 1). Nevertheless, the nematode population increased more slowly on the resistant than on the susceptible rootstock. Roots of 03.01.5 supported 44 ± 73 females and 20 ± 20 eggs per gram of roots, whereas those of Carrizo citrange, 730 ± 1110 females and 6040 ± 10990 eggs per gram of root in non-fumigated plots by the third year. The amount of fibrous roots recovered from these young citrus trees was small, which contributed to the variability of the results. The number of females per gram of root and eggs per gram of root on 03.01.5 in non-fumigated plots were lower ($P < 0.05$) than those on Carrizo citrange in November 2000 and May 2001, but they did not differ in the previous samplings (Figs. 2, and 3, respectively). Other genera of plant-parasitic nematodes present in the orchard were *Helicotylenchus*, *Heterodera*, *Paratylenchus*,

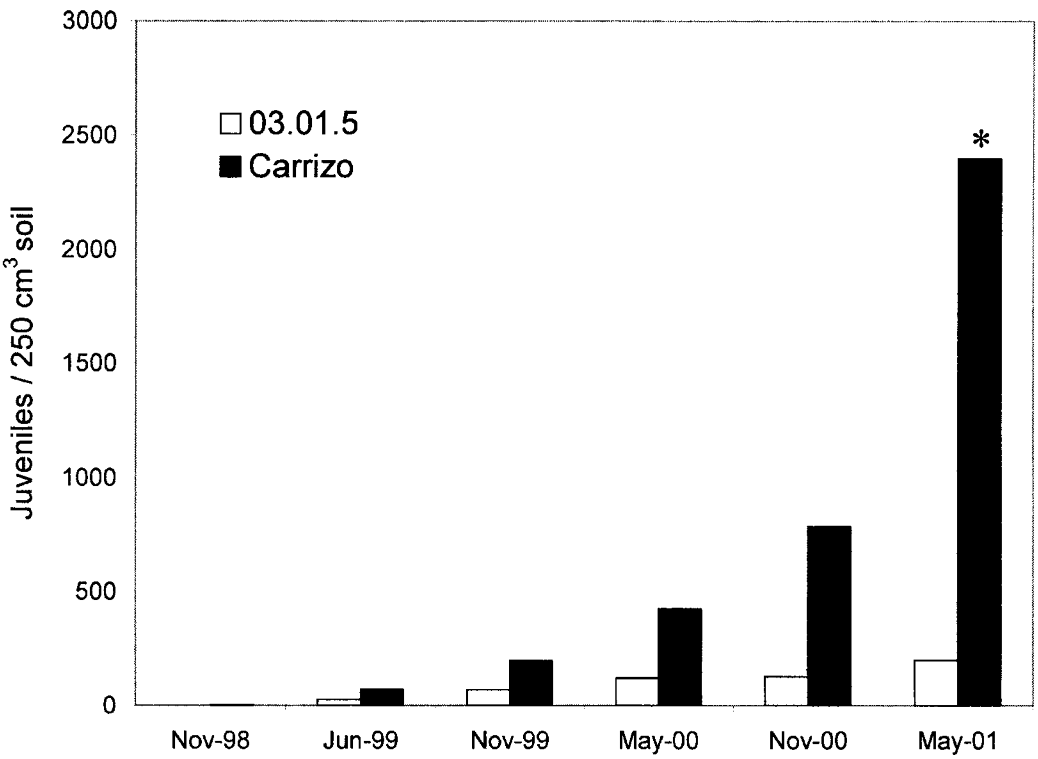

Fig. 1. Number of juveniles per 250 cm^3 soil of *Tylenchulus semipenetrans* in non-fumigated plots planted with the resistant rootstock Cleopatra mandarin × *Poncirus trifoliata* 03.01.5 and the susceptible Carrizo citrange in an orchard replanted in July 1998. Values are mean of six replicated plots, three trees of each rootstock per plot. * significant differences ($P < 0.05$) between rootstocks at a given sampling time.

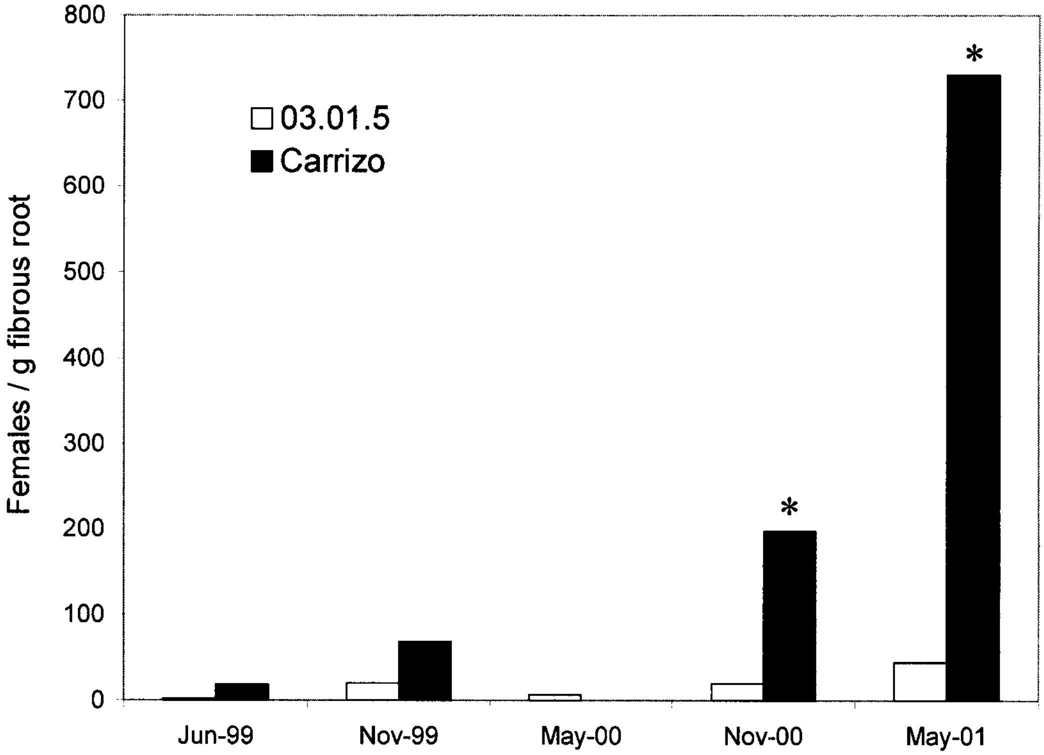

Fig. 2. Number of females per gram fibrous root of *Tylenchulus semipenetrans* on the resistant rootstock Cleopatra mandarin × *Poncirus trifoliata* 03.01.5 and on the susceptible Carrizo citrange in non-fumigated plots established in an orchard replanted in July 1998. Values are mean of six replicated plots, three trees of each rootstock per plot. * significant differences ($P < 0.05$) between rootstocks at a given sampling time.

Tylenchorhynchus, and *Zygotylenchus* species. These nematodes were detected only occasionally and in very low numbers. The trunk diameters of Orogrande scion on 03.01.5 and Carrizo citrange at planting were 5.9 ± 1.7 and 6.4 ± 1.1, and changed to 29.7 ± 6.2, and 24.4 ± 4.9, respectively, by the third year. The resistant rootstocks in non-fumigated plots had a greater ($P < 0.05$) trunk diameter than the susceptible rootstock in the second and third year (Fig. 4). Also, trees on 03.01.5 had greater ($P<0.05$) canopy volume than those on Carrizo citrange by the third year (data not shown).

Trees were replanted four months after removing the old trees. The land was not left fallow for one year, although doing so is the common practice in the area. This agronomic practice was adopted to find out whether fumigation, the use of resistant rootstock or the combination of both could reduce the fallowing period. Fumigation with 1,3 D combined with the resistant rootstock 03.01.5 retarded nematode reinfestation for at least three years. Also 1,3 D, delayed reinfestation of the plots with the susceptible rootstock; the nematode was only detected sporadically after three years. In non-fumigated plots, however, numbers of *T. semipenetrans* on Carrizo citrange roots were greater than those existing in the old orchard three years after planting the new trees. The resistant

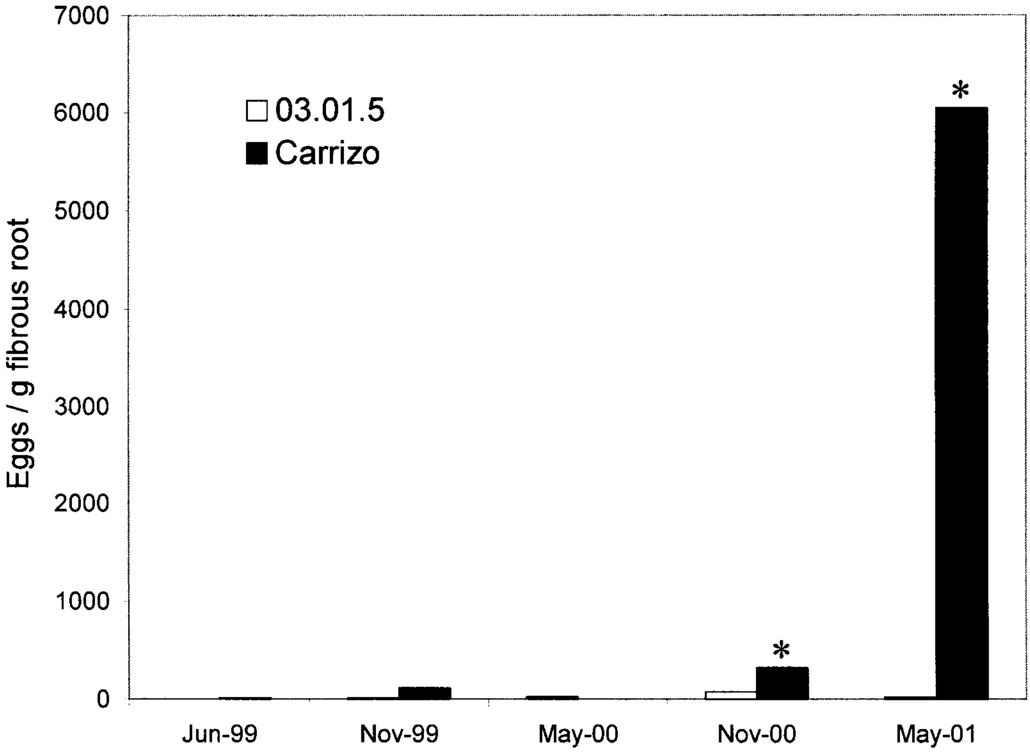

Fig. 3. Number of eggs per gram fibrous root of *Tylenchulus semipenetrans* on the resistant rootstock Cleopatra mandarin × *Poncirus trifoliata* 03.01.5 and on the susceptible Carrizo citrange in non-fumigated plots established in an orchard replanted in July 1998. Values are mean of six replicated plots, three trees of each rootstock per plot. * significant differences ($P < 0.05$) between rootstocks at a given sampling time.

rootstock suppressed nematode reproduction, and population density increase was slower than on the susceptible rootstock. Thus, the number of juveniles, females, and eggs were 8, 6, and 0.3% of those on Carrizo citrange three years after exposure of the rootstocks to moderate densities of the nematode.

These results confirm others on the reduced growth potential of the nematode on 03.01.5 (Galeano *et al.*, 2002). The reduced growth rate may be explained by the longer generation times of citrus nematode on resistant rootstocks (Cohn, 1965). Fumigation with 1,3 D maintained nematode densities below detectable levels for longer time than did the resistant rootstock alone, although this rootstock reduced the rate of population growth, and affected scion growth similarly in fumigated and non-fumigated plots. The 03.01.5 will be useful for low impact environmental production systems such as integrated production or organic farming.

Site preparation resulted a good tactic for nematode management in this orchard since it reduced drastically the citrus nematode population, probably due to successive cultivator passing that mixed the upper soil layers and exposed nematodes in soil and within small root fragments to the desiccation effect of sun and wind.

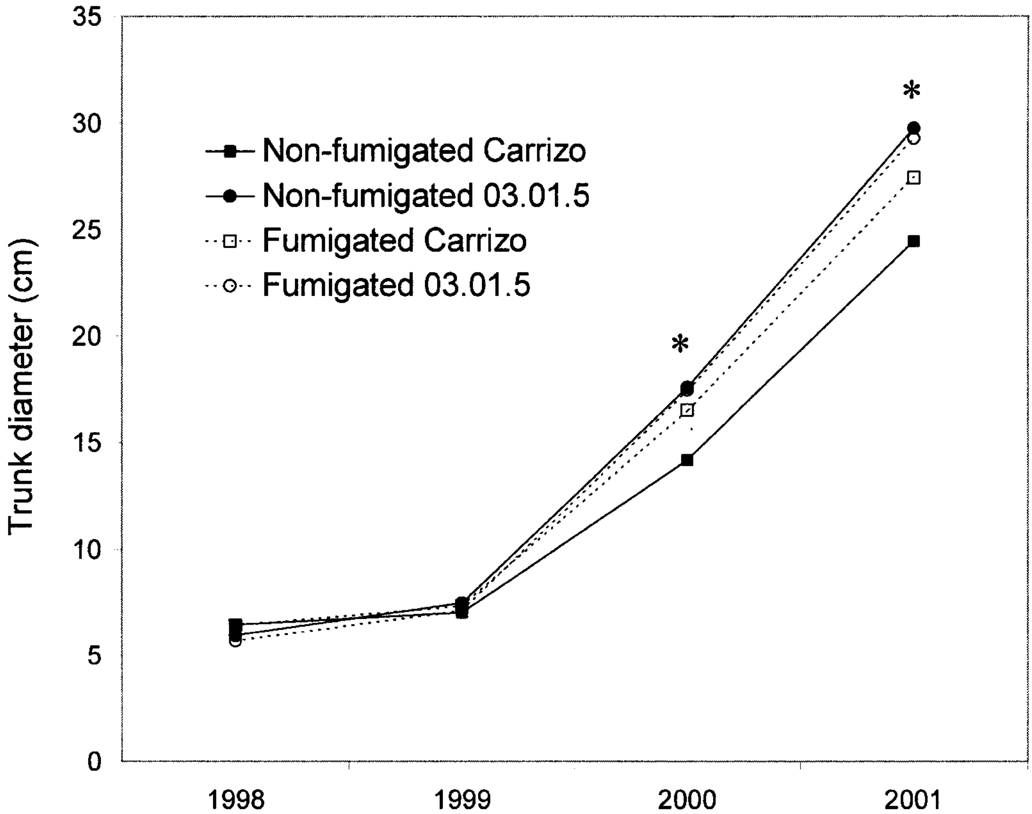

Fig. 4. Trunk diameter (cm, measured 10 cm above the grafting union.) of Orogrande mandarin grafted onto the resistant rootstock Cleopatra mandarin × *Poncirus trifoliata* 03.01.5 and the susceptible Carrizo citrange in fumigated and non-fumigated plots established in an orchard replanted in July 1998. Values are mean of 18 trees (six replicated plots, three trees of each rootstock per plot). * significant differences ($P < 0.05$) between rootstocks only in non-fumigated plots.

Nevertheless, the residual *T. semipenetrans* life stages left in the orchard after removing the old infected trees re-emerged and were detected in all non-fumigated plots one year after replanting. These nematodes were able to infect and reproduce on the new citrus roots and they did so more efficiently on the susceptible than on the resistant rootstock. Citrus nematode numbers probably will continue to increase in this orchard in future years before reaching a plateau that usually occurs when trees reach maturity (Cohn *et al.,* 1965). Mature orchards in Spain often have higher nematodes numbers than those detected in this young orchard, probably due to more feeder root available for nematode feeding (Navas *et al.*, 1992; Sorribas *et al.*, 2000). Population build-up would occur more rapidly once trees have developed sufficient canopies to shade the soil and so provide an environment more favorable for nematode development (Reynolds and O'Bannon, 1963b; le Roux *et al.*, 1998).

Since the study was done at one site, the results presented here are not conclusive. However our findings corroborate those of others on differential reproduction of the citrus nematode on the resistant rootstock in greenhouse and microplots (Verdejo-Lucas *et al*, 1997, 2000, Galeano *et al.*, 2003). New citrus rootstocks adapted to edaphic conditions and resistant to major plant pathogens prevalent in the production areas are needed to conduct crops according to good agricultural practices, and 03.01.5 appears to be a good candidate for such purpose. Monitoring nematode densities on this young citrus orchard is in progress to determine the effect of the 1,3 D treatment and rootstock on yield and the rate of nematode population growth as trees reach maturity.

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