EFFECT OF GRAFTING AND NEMATICIDE TREATMENTS ON DAMAGE BY ROOT-LESION NEMATODES (*PRATYLENCHUS* SPP.) TO *COFFEA ARABICA* L. IN GUATEMALA

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ABSTRACT

Villain, L., A. Molina, S. Sierra, B. Decazy, and J. L. Sarah. 2000. Effect of grafting and nematicide treatments on damage by the root-lesion nematode *Pratylenchus* spp. on *Coffea arabica* L. in Guatemala. Nematropica 30:87-100.

Root-lesion nematodes, *Pratylenchus* spp., are among the pests with the greatest negative impact on the economy of coffee production in Guatemala. A field experiment was undertaken in southwest Guatemala to assess damage due to a root-lesion nematode and to compare two methods of management: grafting onto *Coffea canephora* Pierre, 1897, and nematicide treatments (terbufos). The experiment was carried out for five years, and included the first three harvests. Root population densities of nematodes, coffee berry yield losses, and plant mortality rates were highest on ungrafted *C. arabica*, confirming its high degree of vulnerability to this root-lesion nematode population. Grafting onto *C. canephora* provided efficient control of populations of root-lesion nematodes and resulted in significantly greater yields compared to ungrafted plants. Nematicide treatments suppressed populations of this root-lesion nematodes only until the second year after planting. This was sufficient to significantly reduce rates of plant mortality in ungrafted plants. However, nematicide treatments did not result in significant increase of yield regardless of the rootstocks used. This work also provided evidence that growing grafted coffee trees under shade may be a beneficial tactic for managing these root-lesion nematodes.

Key words: chemical control, *Coffea* spp., grafting, Guatemala, pest management, *Pratylenchus*, root-lesion nematode, shaded perennials, terbufos.

RESUMEN

Villain, L., A. Molina, S. Sierra, B. Decazy y J. L. Sarah. 2000. Efecto del injerto y de los tratamientos nematicidas sobre los daños ocasionados por el nematodo lesionador *Pratylenchus* sp. sobre *Coffea arabica* L. en Guatemala. Nematrópica 30:87-100.

El nematodo lesionador *Pratylenchus* spp. es una de las plagas de mayor impacto sobre la economia del cultivo de café en Guatemala. Se desarrolló un experimento en el suroeste de este país para evaluar los daños causados por un nematodo lesionador así como para evaluar dos métodos diferentes de control: el injerto sobre *Coffea canephora* Pierre, 1897, y los tratamientos nematicidas (terbufos). El experimento tuvo una duración de cinco años, incluyendo las tres primeras cosechas formales. Las densidades poblacionales de nematodos en las raíces, las pérdidas de cosechas y las tasas de mortalidad de plantas fueron mas altas para el *C. arabica* sin injerto demostrando su alto grado de vulnerabilidad a esta población de nematodo lesionador. Se comprobó que el injerto sobre *C. canephora* es muy eficiente para controlar las poblaciones de nematodo lesionador, lo que resultó en niveles productivos significativamente más altos. Los tratamientos nematicidas bajaron los niveles poblacionales del nematodo año de cultivo solamente. Este efecto fue suficiente para bajar significativamente las tasas de mortalidad de plantas de los cafetos sin injerto. Sin embargo, los tratamientos nematicidas no permitieron ningún aumento significativo de la producción de plantas, con o sin injerto. Este trabajo trajo evidencias de que cultivar cafetos injertados bajo sombra puede ser una práctica benéfica para el manejo de estos nematodos lesionadores. Palabras claves: Coffea spp., control químico, cultivos perennes bajo sombra, Guatemala, injerto, manejo de plagas, nematodo lesionador, Pratylenchus, terbufos.

INTRODUCTION

The root-lesion nematode Pratylenchus coffeae (Zimmermann, 1898) is considered to be responsible for substantial damage to plantations of Coffea arabica L. throughout the world (Campos et al., 1990). In Central America, and particularly in Guatemala, P. coffeae has long been reported as the only damaging root-lesion species in coffee plantations (Chitwood and Berger, 1960; Schieber and Sosa, 1960; Schieber, 1966). Recent studies indicated that different species, morphologically closely related to P. coffeae could be involved in a pathogenic complex in coffee roots in Guatemala: P. gutierrezi Golden et al., 1992 (Inserra et al., 1998; Duncan et al., 1999); P. loosi Loof, 1960 (Anzueto and Sarah, 1992; Anzueto, 1993; Mohotti, 1998) and unidentified Pratylenchus spp. (Villain et al., 1998; Duncan et al., 1999; Villain, 2000). Results of routine analysis performed by the Guatemalan Association of Coffee (ANACAFE) over the last ten years indicated that rootlesion nematodes have been abundant in many of the C. arabica producing regions of Guatemala (Villain et al., 1999).

A method of hypocotyledonary grafting of *C. arabica* onto *C. canephora* Pierre, 1897 was perfected by Reyna (1966 and 1968) to control nematodes especially, *Pratylenchus* spp. Although this cultural practice is now widely implemented, some farmers still prefer to use nematicides since to them the effectiveness of grafting against nematodes has not yet been proven. Some other farmers who do use the grafting technique also apply nematicides without evidence of whether or not these applications are necessary. Therefore, the present study was aimed at evaluating the effectiveness of the hypocotyledonary grafting technique and nematicide treatments, either combined or used independently, for controlling *Pratylenchus* spp. (identification in process) in Guatemala. In this country, C. arabica is mainly cultivated under a shaded traditional cropping system. In addition to its environmental and socioeconomic importance as a source of fire-wood, shade trees work as an important climatic regulator in many coffee producing regions because of the presence of a marked dry season (Willson, 1985; Wrigley, 1988; Beer et al., 1998). Under these climatic conditions, shade trees have an important effect on the physiology of C. arabica trees, native to the cool shady rain forest of the Ethiopian highlands (Wrigley, 1988). Shade must then be considered for integrated management of nematodes because of its possible influence on the degree of tolerance and/or resistance of coffee trees to nematodes.

MATERIALS AND METHODS

Location: The experiment was conducted on a farm located on the southern slope of the Atitlan Volcano in the Sierra Madre mountains at an altitude of 900 m (Suchitepequez county). Average annual rainfall is 2 900 mm, 90% of which occurs from April to October. There is a long cool dry season from November to March, but there is also a short hot dry season in July-August, locally called canicule. The average annual temperature is 23°C with an absolute minimum and maximum of 12°C and 38°C. The soils are andisols resulting from volcanic wind ash deposits (Anonymous, 1989).

Experimental design: The experimental plot was located in a field heavily infested by root-lesion nematodes, which had previously been planted with C. arabica (cv. Bourbon) for approximately 20 years. In the present experiment, the selected C. arabica cultivar was Caturra. Hypocotyledonary grafting was done with approximately sixweek-old plants onto C. canephora. Both grafted and non-grafted C. arabica seedlings were kept in the nursery for 10 months in individual 2 dm³ polybags where all plantlets received three preventive applications of 0.1 g of terbufos at three-month intervals. The young plants were then planted in the field at a density of 5 000 per ha (2 m between rows and 1 m between plants in each row). Shade was provided by adult trees of Inga sp., spaced 8 m × 16 m apart. These shade trees were pruned every year in March-April at the end of the dry season to avoid excessive shading which exacerbates growth of detrimental fungal pathogens during the rainy season.

Half of the grafted and non-grafted plants did not receive any nematicide treatment. The other half were treated with terbufos 10G at a rate of 1 g per plant during the first 3 years and 2 g per plant the following years. The first application occurred in July 1991, 15 days after planting. The second application was in November at the end of the rainy season. In the following years, the first application changed to early May, at the beginning of the rainy season, and the second application continued in November just after harvest. Nematicide granules were scattered by hand over the entire undercanopy area of each coffee plant.

The factorial experimental design included four treatments (grafted/ ungrafted × treated/untreated) replicated four times, resulting in 16 plots arranged in a randomized complete block design. Each of the experimental plots included 84 plants arranged in seven rows of twelve plants. Observations were made on the 50 median plants (five rows of ten plants).

Measurements: To evaluate nematode population densities, two root samples were taken every year, one in February, during the dry season, and the second in July, during the rainy season. These dates corresponded to maximum population densities under local environmental conditions (Villain, 2000). Sampling began 11 months after planting (April 1992) when the root system was considered to be sufficiently developed to withstand the process. In each plot, root samples were taken from eight plants regularly spaced throughout each experimental plot (plant number 1, 9, 17, etc.). Plant number one was arbitrarily chosen as the first plant (East to West) on the first row (North to South). Every new sampling was carried out on the next eight plants of the plot (plant number 2,10, 18, etc.). Root samples were removed from a soil volume of 20×20 cm × 15 cm depth, taken in the row at 20 cm from the trunk. The eight soil samples for a given plot were pooled to obtain a single sample per plot.

The roots were then carefully washed with tap water. Only non- or slightly lignified secondary roots with a diameter of less than 3 mm were considered for nematode extraction. They were cut into 2 to 3 cm pieces. A 25 g aliquot was macerated in a blender (Oster, 3 speeds) for 15 seconds at intermediate and 15 seconds at full speed. The suspension was then passed through a column of sieves (850 µm, 150 µm, 45 µm, 38 µm and 25 µm) and the residues collected in the last three sieves were processed by centrifugal-flotation (Coolen and d'Herdes, 1972). Nematodes were finally collected in 100 ml of water and counted in 3 aliquots of 1 ml on a counting slide (Sedgewick rafter cell S50, Graticules Limited).

The annual yield of coffee berries was measured in every plot from 1993 to 1995, and expressed as kg of coffee berries per hectare. All plants were examined at each harvest, from 1992 to 1995. Unproductive and defoliated plants were recorded as dead and counted to determine the mortality rate in each plot from planting until the year of observation.

For each plant, a shade index was assigned by visually estimating the zenithal projection of the shade tree canopy on the plant in question. The scale was: 1 = 25%; 2 = 25-50%; 3 = 50-75%; 4 = >75% shade. A mean shade index was then calculated for each experimental plot from the estimated grades attributed to the 50 plants.

Statistical analysis: Nematode numbers per gram of roots were transformed to log (x + 1) for statistical analysis. The log transformed population data of 1992 were averaged to obtain the early population density. The log transformed population data from 1992 to 1995 were averaged to obtain the average population density for the duration of the experiment. Two-way ANOVA's of log-transformed nematode population, plant mortality rate and yield data were calculated. Means were separated using the Newman and Keuls' test. All statistical analyses were performed with STATITCF software from the Institut Technique des Céréales et Fourrages.

RESULTS

Nematode populations: There was no significant grafting onto *C. canephora* x nematicide treatment interaction for root-lesion nematode population densities in any year. In the first sampling year (1992), root-lesion nematode population densities were lower (P= 0.01) in the plots that had been treated with nematicide than in the untreated plots, whether or not the plants were grafted (Table 1). In the following years, there was no significant effect of nematicide treatment on nematode populations.

Root-lesion nematode population densities were lower (P = 0.001, P = 0.02, and P = 0.004 in 1992, 1994, and 1995, respectively) in plots where plants were grafted than in plots of ungrafted plants. The only exception was 1993, when no significant difference was observed because of high data variability (CV = 45.1%) between replicates.

In plots of grafted plants, the average root-lesion nematode population density was negatively correlated ($r^2 = 0.57$; P = 0.03) with the shade index (Fig. 1), whereas shade did not influence nematode populations ($r^2 = 0.15$; P = 0.34) in plots of ungrafted plants.

Plant mortality: During the first year, the plant mortality rate was less than 5% in each plot and there was no significant effect of nematicide treatments or grafting (Fig. 2). In the following years, there was a significant grafting × nematicide treatment interaction for plant mortality rate (P =0.01, P = 0.04, and P = 0.04 in 1993, 1994, and 1995 respectively). Plant mortality rates increased much more rapidly for the ungrafted plants than for the grafted plants (Fig. 2). The nematicide treatment reduced the mortality of ungrafted plants (P = 0.002, P = 0.01, and P = 0.01 in 1993,1994, and 1995 respectively). The average mortality rate for untreated plants increased from 24% (range = 12-36%) in 1993 to 50% (25-76%) in 1995. In contrast, the average mortality rate for treated plants was only 4.5% (2-10%) in 1993, and reached 23% (14-29%) in 1995. The final mortality rate was positively correlated ($r^2 =$ 0.76; P = 0.005) with the early root-lesion nematode population densities observed in 1992 (Fig. 3). The shade index was negatively correlated with the plant mortality rates observed in 1993 ($r^2 = 0.65$; P = 0.002) and 1994 ($r^2 = 0.52$; P = 0.04), but not significantly in 1995 ($r^2 = 0.42$; P = 0.08).

	Root-lesion nematode population densities (per g of roots)											
	1992 Grafting			1993 Grafting			1994			1995		
							Grafting			Grafting		
Nematicide	Without	With	Average	Without	With	Average	Without	With	Average	Without	With	Average
Without	54	12	33 a ^z	37	19	28 a	28	22	25 a	132	23	78 a
With	19	6	13 b	38	19	29 a	29	8	18 a	135	29	82 a
Average	$36 A^z$	9 B	23	38 A	19 A	28	29 A	15 B	22	134 A	26 B	80

Table 1. Effects of grafting onto *C. canephora* and nematicide treatments (two applications per year with 1 or 2 g of terbufos per plant) on root population densities of root-lesion nematode in plots of *C. arabica*.

'Averages in columns (A,B) or in rows (a,b) with the same letter do not differ according to Newman and Keuls' test (P = 0.05). Data were transformed to log (x + 1) prior to the ANOVA.



Fig. 1. Relationship between the shade index and root-lesion nematode population densities (mean of $\log [x + 1]$ transformed nematode numbers per gram of roots from 1992 to 1995) in plots of grafted *C. arabica*, with or without nematicide treatments (1 or 2 g of terbufos per plant twice a year).

The mortality rate for plants that were grafted remained very low over the years with no effect of nematicide treatment. The average mortality rate for grafted plants reached only 6% (0-15%) by the end of the experiment. The plant mortality rate was not correlated with nematode population densities ($r^2 = 0.00$; P = 0.93) or with the shade index ($r^2 = 0.04$; P = 0.65).

Yield: There was no significant grafting \times nematicide treatment interaction for coffee berry yield in any year. Nematicide treatments had no significant effect on yield in any year, whether or not the plants were grafted (Fig. 4). However the effect of nem-

aticide treatments approached significance for the ungrafted plants during the first harvest in 1993 (P = 0.07). The average yield of grafted plants was approximately 15 000 kg/ha for the three harvests. This was 250% greater than the average yield of ungrafted plots over the same period (Fig. 4).

The yield of grafted plants was not correlated with root-lesion nematode population densities or with the shade index over the three harvests. However, the yield of ungrafted plants in 1995 was negatively correlated with the average nematode population density ($r^2 = 0.87$, P = 0.001). The most heavily infested plots of ungrafted plants



Fig. 2. Plant mortality rates observed at harvest time, one, two, three, and four years after planting (1991) in plots of grafted and ungrafted *C. arabica* parasitized by root-lesion nematodes, with or without nematicide treatment (1 to 2 g of terbufos per plant twice a year). Data of the same year with the same letter do not differ according to Newman and Keuls' test at P = 0.05. (N.S.: not significant; P > 0.05).

yielded twelve-fold less than the least infested plots (Fig. 5). The yield of ungrafted plants also approached significant correlation with the shade index during the first two harvests ($r^2 = 0.47$, P = 0.06; $r^2 = 0.43$, P = 0.08 and $r^2 = 0.17$, P = 0.31, in 1993, 1994 and 1995 respectively).

The three plots of ungrafted plants with the lowest nematode densities had a similar average nematode population density to that of the three most heavily infested plots of grafted plants (approximately 25 nematodes per gram of roots). However, the yield in 1995 in the three plots of grafted plants was higher (P = 0.02) than in the three plots of ungrafted

plants: 16 000 kg/ha vs 5 300 kg/ha. In the same plots, the average mortality rate was 6% for the grafted plants vs 32% for the ungrafted plants.

DISCUSSION

The high root-lesion nematode population densities on ungrafted *C. arabica* was associated with severe plant die-back and low yields attesting to the high damage potential of this root-lesion nematode on *C. arabica*. Although the mortality rate for ungrafted plants was low in the first year after planting, even in plots with the highest levels of nematode infestation, mortal-



Fig. 3. Relationship between root-lesion nematode population density in 1992 (mean of $\log [x + 1]$ transformed nematode numbers per gram of roots) and plant mortality observed in 1995 in plots of ungrafted *C. arabica*, with or without nematicide treatments (1 or 2 g of terbufos per plant twice a year).

ity rates increased rapidly thereafter. In the most heavily infested plot of ungrafted plants, the plant mortality rate reached 75% by the third harvest. In such conditions, the economic consequences may be considerable because of crop losses and the high costs of replanting for a perennial crop such as coffee. Because the plant mortality rate observed at the end of the experiment (1995) was strongly correlated with the early nematode densities (1992), it appears likely that the initial root-lesion nematode population densities, during the early plant growth, are a critical factor for later plant development. This is the first study to evaluate the pathogenicity of root-lesion nematodes on *C. arabica* in the field in Central America.

The fact that the root-lesion nematode exhibited less damage potential on grafted plants is consistent with the perception of the most farmers. The nematode population densities on grafted plants were consistently below those of ungrafted plants indicating partial resistance of *C. canephora* to root-lesion nematodes. In India, partial resistance to *P. coffeae* was observed in a non-cloned *C. canephora* cv. Robusta progeny (Anonymous, 1971). In Indonesia (East Java), Toruan-Mathius *et al.* (1995) reported the existence of two clones of *C. canephora* cv. Robusta with high levels of



Fig. 4. Coffee berry yields in plots of grafted and ungrafted *C. arabica* parasitized by root-lesion nematodes, with or without chemical treatment (1 or 2 g of terbufos per plant twice a year), two, three and four years after planting (1991). The thin bars represent the standard deviation of each data bar. Data bars with the same letter are not different according to Newman and Keuls' test at P = 0.05.

resistance to *P. coffeae*. In South India, Kumar (1982), observed slower penetration dynamics of *P. coffeae* in a line of *C. canephora* cv. Robusta when compared with *C. arabica*. Those observations support our results obtained under field conditions showing that grafting onto *C. canephora* provided efficient control of rootlesion nematodes.

Nematode densities did not influence coffee yields or the mortality rate of grafted plants, which remained low ($\approx 15\%$) in all plots. Thus, the range of nematode densities observed in the plots of grafted plants appear to have been below the tolerance limit of the plant (Seinhorst, 1965 and 1998), and the yields of grafted plants may have been very close to their yield capacity in the absence of nematodes under these conditions.

At the third harvest in 1995, plots of grafted plants yielded three times more on average than the most productive plots of ungrafted plants, despite the fact that the most heavily infested plots of grafted plants had nematode densities similar to those of the most productive plots of ungrafted plants. A difference in tolerance levels may be involved, but data on the yield capacity of ungrafted plants in the absence of nematodes were lacking under our experimental conditions. Therefore, it cannot be definitively concluded that grafted plants were more tolerant to root-lesion nematode attacks (Bos and Parlevliet, 1995), or that there is a beneficial effect of C. cane-



Fig. 5. Relationship between average root-lesion nematode population density (mean of $\log [x + 1]$ transformed nematode numbers per gram of roots from 1992 to 1995) and coffee berry yield at third harvest (1995) in plots of ungrafted *C. arabica*, with or without nematicide treatments (1 to 2 g of terbufos per plant twice a year).

phora on yield capacity. Both effects may occur, but the relative importance of each remains to be determined.

On both ungrafted and grafted plants, nematicide treatments only suppressed nematode populations in the first year of sampling (1992), before the coffee plants began to yield. Thereafter, nematicide treatment had no effect on nematode populations density, even when the dose was increased to 2 g per plant at the end of the third year after planting. However, the significant decrease in early nematode populations due to nematicide applications in 1992 was linked to a delay in the die-back process of ungrafted plants, and significant lowering of their mortality rate throughout the experiment. In spite of this significant effect on plant mortality rates, nematicide treatments did not greatly influence the yield of ungrafted plants. A non-significant tendency toward higher yield in treated ungrafted plants plots diminished over time.

The terbufos dose used in this study complied with recommendations by the national association of coffee growers (Anonymous, 1998). Under experimental conditions, this nematicide rate clearly appeared insufficient for protection of susceptible ungrafted plants. Alternatively, a higher dose or frequency of nematicide applications could have a negative environmental impact and would substantially increase costs to levels that would likely be unacceptable to farmers. For partially resistant grafted plants, nematicide treatments did not have any significant effect on either plant mortality rates or yield. However, the higher yield of treated plots is a consistent trend throughout the experiment for the grafted plants. This demonstrates the superiority of resistance rootstocks compared to use of nematicides for management of this nematode.

Shade had different effects on coffee trees and nematode populations, depending on whether plants were grafted or not. Shade seemed to improve the tolerance of ungrafted plants to root-lesion nematodes during the initial growth phase as it restrained the die-back process of plants up to the first harvest. This result tallies with the observations of Sturdy (1935) and Montoya et al. (1961), who showed that die-back of coffee trees increases with light intensity. Shade also limits water stress in coffee trees during dry periods, by reducing ambient temperatures and hygrometric losses (Willson, 1985; Wrigley, 1988; Beer et al., 1998). This effect of shade may have been particularly important for the ungrafted plants whose root system was seriously damaged by root-lesion nematodes. O'Bannon and Reynolds (1965) showed that susceptibility of crops to drought was increased when they were heavily infected by root-knot nematodes. However the initial beneficial effect of shade did not influence final plant mortality rates significantly.

Shade suppressed root-lesion nematode population densities on grafted plants. This result may relate to a trend (non-significant, data not shown) for grafted plants to yield less with increased shade, in accordance with the well-documented negative effect of shade on yield when fertility conditions are adequate (Browning and Fisher, 1976; Cannell, 1985; Kuguru *et al.*, 1977; Wrigley, 1988). Hence, shade may favor partial resistance to root-lesion nematodes through plant trophic status in relation with fruit yielding. Gnanapragasam (1982) and Melakeberhan et al. (1997) elegantly showed that some nutritional deficits suppressed the levels of partial resistance of Camellia sinensis to P. loosi and Prunus avium L. rootstocks to P. penetrans respectively. These hypotheses should be tested because shade is an important agronomic component for coffee crops in most of the northern Latin American countries such as Guatemala (Rice and Ward, 1996). Shade did not influence nematode population densities on ungrafted plants, probably because of high susceptibility to this root-lesion nematode.

Schieber (1966) and Reyna (1968) recommended the use of C. canephora rootstocks to control P. coffeae in C. arabica plantations in Guatemala. Similar recommendations were made by Palanichamy (1973) in Indonesia. The results of this study are the first demonstration of the effectiveness of grafting onto C. canephora to control a root-lesion nematode in Central America. Substantial genetic variability exists within C. canephora, which is an allogamous species (Leroy, 1993). Hence, there is probably high genetic diversity within the plants used as rootstock in Guatemala, since they come from seeds obtained by open pollination and collected from different C. canephora trees. Furthermore, Anzueto (1993) showed the large variability of resistance levels in C. canephora clones from CATIE, Costa Rica (the main source of C. canephora propagation in Central America) to a population of Meloidogyne sp. (Hernandez et al., 1996) from Guatemala. In Indonesia, Toruan-Mathius et al. (1995) also reported the existence of a wide range of resistance levels to P. coffeae among clones of C. canephora cv. Robusta, some of them being very susceptible. Hence, resistance to Pratylenchus spp. needs to be included in the

coffee rootstock breeding programs already under way, which are primarily aimed at resistance to *Meloidogyne* spp. (Bertrand *et al.*, 1999; Villain *et al.*, 1999). Germplasm breeding should provide more reliability to the already satisfactory results obtained using non-selected and heterogeneous rootstock populations to control root-lesion nematodes. The diversity of root-lesion nematodes attacking coffee trees (see bibliographical references in the introduction) must also be taken into account.

To conclude, C. arabica appeared highly susceptible and sensitive to the root-lesion nematode, and effective control of that nematode cannot be achieved by nematicide treatments alone. Agronomic practices such as cropping under shade did not lead to sufficient improvement in C. arabica tolerance to the nematode. The Caturra cultivar studied here is currently the most widely cultivated C. arabica cultivar in Central America (Bertrand et al., 1999), and these results can probably be generalized for most currently cultivated C. arabica cultivars because of their very narrow common genetic pool (Orozco et al., 1994; Lashermes et al., 1996; Charrier and Eskes, 1997; Anthony et al., 1999). The exception could be some lines of the Catimor cultivar group, which have some introgressed genes of C. canephora cv. Robusta. Resistance to root-lesion nematodes should also be investigated among Ethiopian wild lines of C. arabica. Until then, grafting onto C. canephora should be recommended as an effective way of controlling root-lesion nematodes in Guatemala.

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