EPIDEMIOLOGY OF *MELOIDOGYNE EXIGUA* IN AN UPLAND COFFEE PLANTATION IN BRAZIL

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Summary. A 24-month study was conducted in a commercial non-irrigated arabica coffee plantation to assess the influence of air temperature, rainfall and soil moisture content on the epidemiology of *Meloidogyne exigua*. The second stage juvenile (J2) populations in soil and roots showed clear seasonal fluctuations, with an increase in their levels during the dry months (April through September) and a decrease in the rainy months (October through March). The number of galls/5 g of roots induced by the nematode did not show consistent fluctuations through the seasons, although a path analysis indicated that this variable was negatively correlated to mean air temperature and rainfall, but not correlated to soil moisture content. The results of this study suggest that, in the environmental conditions of south-east Brazil, sampling coffee plantations during the dry months may provide a more accurate assessment of J2 populations of *M. exigua* than sampling during the rainy season.

Key words: Coffee root-knot nematode, *Coffea arabica*, epidemiology, population dynamics.

Coffee (*Coffea* sp.) is one of the most important commodities worldwide. Its vigorous trading strengthens the economy and the social stability in many countries, promoting directly and indirectly related rural and urban economic activities (http://www.ico.org/, visited on April 14th, 2007).

Plant-parasitic nematodes are amongst the most important pests of coffee. In Brazil, coffee plantations have been decimated by root-knot nematodes (*Meloidogyne* spp.) in several areas since the late 19th century (Jobert, 1878, cited by Santos, 2000). In Central America, several nematode species are responsible for major yield losses, while in Vietnam, India and Indonesia *Pratylenchus* spp. and *Radopholus* spp. are becoming a concern as the production system shifts from low input to intensive cultivation (Villain *et al*., 1999; Dhanam and Sreedharan, 2008; Wiryadiputra and Loang, 2008).

Meloidogyne exigua Goeldi is the most widespread root knot nematode species in the Americas (Campos and Villain, 2005), and can cause large yield losses in Arabica coffee (*C. arabica* L.) plantations under conducive edaphic and climatic conditions associated with inappropriate management practices (Arruda and Reis, 1962; Gonçalves, 1997; Barbosa *et al.*, 2004).

A few studies have dealt with ecological aspects affecting the biological activity of *M. exigua*. Lima and Ferraz (1985a) observed a slower embryogenesis in eggs kept at 15°C, in comparison to 20 and 25°C, and a 50% egg death rate at 30°C. Second-stage juvenile (J2) eclosion *in vitro* was higher at 25°C, and similar at 15, 20 and 30°C (Santos and Ferraz, 1977). In a growth chamber, the number of root galls induced by *M. exigua* correlated positively to the air temperature, which was kept constant at 16, 20, 24 or 28°C. Nematode egg production was greater at 20 and 24°C than at 16 and 28°C (Tronconi *et al.*, 1986). These studies suggest an adaptation of those *M. exigua* populations to a montane, tropical temperature regime.

In a non-irrigated plantation, Huang *et al.* (1984) found a marked fluctuation of *M. exigua* populations during the rainy and dry seasons. In nearby areas, Almeida *et al.* (1987) obtained results that contradicted those of Huang *et al.* (1984), and Maximiniano *et al*. (2001) found no statistical correlation between the number of J2 in the soil and mean air temperature and rainfall.

These apparent contradictory results indicate the need for epidemiological studies to be conducted under the climatic and edaphic conditions that characterize the geographical areas affected by *M. exigua* infestations across the Americas. Also, such studies should cover longer sampling periods, and assess different population variables.

This article reports the results of a 24-month study conducted in a upland coffee plantation in south-east Brazil to determine the influence of air temperatures, rainfall and soil moisture content on the epidemiology of *M. exigua*.

MATERIALS AND METHODS

The study was conducted in a commercial, non-irrigated, six-year-old Arabica coffee plantation (cultivar 'Catuaí') in the municipality of Varre-Sai, State of Rio de Janeiro, Brazil. This area is characterized by a montane, tropical climate (Aw according to Köppen´s Cli-

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mate Classification System), with an altitude around 700 m asl. The historical (1931-1990) minimum, mean, and maximum average temperatures are 15-18, 21-24, and 27-30°C, respectively (http://www.inmet.gov.br/, visited on July 20th, 2007). The historical monthly rainfall ranges from 0 to 80 mm from April through September, 80 to 160 mm in October, February and March, and 160 to 240 mm in November through January. The plantation was established in a red-yellow latosol (oxisol in the USDA system), with 30, 17, and 53% of sand, silt and clay, respectively, and 4.8% of organic matter. The field had a declivity of 30%, and no infestation by other *Meloidogyne* species besides *M. exigua*.

In a 1.5 hectare plantation, an experimental plot composed of five contiguous planting rows (1.8 m apart) of 34 plants each (1 m between plants), totaling 170 plants, was selected. The plants were randomly choosen and tagged so that 10 plants (two per planting row) were sampled every three weeks, from October/2002 through October/2004 (32 sampling dates). During this period, every plant was re-sampled once, 12 months after being first sampled.

Soil cores were collected with an 8 cm diam. and 16 cm long bucket auger (approximately 800 cm^3 of soil) at distances of 20 and 40 cm (under the plant canopy) from the trunk, at depths of 0-25 and 25-50 cm, and at 80 cm from the trunk (at the edge of the canopy), at a depth of 0-25 cm. At this distance from the trunk no samples were collected at 25-50 cm depth because only few roots were present. A total of 50 samples were collected on each sampling date. The samples were placed in plastic bags and processed in the laboratory.

Newly-grown feeder roots present in each sample were removed from soil and weighed (old, thick, brownish root segments that are not infected by *M. exigua* J2 were discarded). Galls induced by *M.* exigua on these feeder roots were counted, and expressed as number of galls/5 g of roots.

The roots from each sample were then homogenized in a commercial blender (Arno®, model LE) for 10 seconds and sieved through a 60-mesh sieve nested onto a 500-mesh sieve by spraying water on the upper sieve. Nematode stages on the 500-mesh sieve were collected in a beaker. To prevent hatching of the nematode eggs, the resulting suspension was stored at 4°C until it was entirely (not by aliquots) examined using a dissecting microscope. The root population of vermiform J2 was expressed as number of J2/5 g of roots.

Finally, the soil of every sample was thoroughly mixed and a sub-sample of 100 cm^3 was processed by centrifugation according to Jenkins (1964), with sieving as described above. The resulting suspension was kept at 4 °C until it was entirely examined and the number of J2 counted. Soil nematode population was expressed as number of J2/100 cm^3 of soil. All sampling, processing and counts were carried out by the same operator (the second author).

The levels of root galling and J2 population were correlated to air temperature, rainfall and soil moisture content, recorded at each sampling date. The moisture content in each sample was determined by drying the soil at 105ºC for 72 h. Mean air temperature and rainfall were recorded in a meteorological station located 15 km from the experimental site.

The original data of nematode populations, not log-transformed, were used in regression analysis over time. A path analysis was conducted according to Singh and Chaudary (1979) (cited by Vencovsky and Barriga, 1992) to measure the effect of the different environmental variables on the number of galls/5 g of roots.

RESULTS AND DISCUSSION

The fluctuation of mean air temperature, rainfall and soil moisture content recorded during this study are shown in Fig. 1. The mean air temperature was always above 15°C, around which seems to be the minimum threshold required for development of *M. exigua* populations from south-east Brazil, as suggested by previous studies (Santos and Ferraz, 1977; Lima and Ferraz, 1985a; Tronconi *et al.*, 1986). Although the rainfall and soil moisture content fluctuated during the study, no plant wilting or leaf falling occurred due to water stress.

During the rainy months (October through March) the soil J2 density was low at all sampling distances from the trunk and depths (Fig. 2; for this and the following graphics, the polynomial equations and R^2 are shown in Table I). This suggests that, after eclosion, the J2 readily infects the newly-grown feeder roots. Almeida *et al.* (1987) also found considerably less J2 in the soil during this season. Huang *et al.* (1984) found the opposite trend (more J2 in the soil during the rainy months),

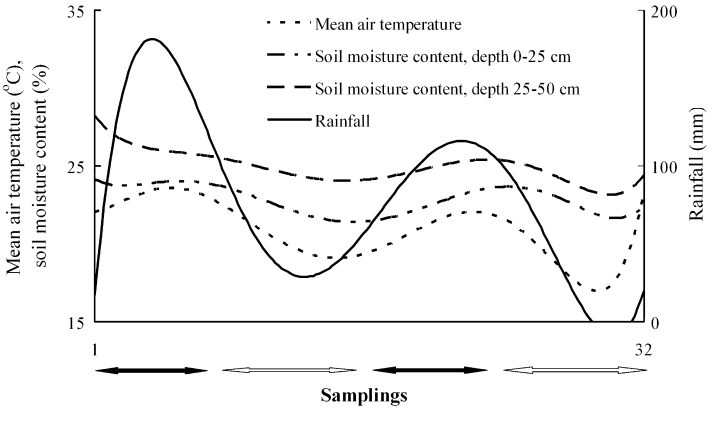

Fig. 1. Mean air temperatures, rainfall and soil moisture content recorded during a two year period (October 2002-October 2004) in a coffee plantation infested by *Meloidogyne exigua* in southeast Brazil. Rainfall amounts are cumulative for three-week periods. Mean air temperatures are averages of daily records for each period. Soil moisture contents were assessed every three weeks. Rainy and dry seasons are marked by black and white arrows, respectively.

Table I. Descriptive statistical data for the regression analysis shown in Figures 2 through 5 involving epidemiological variables of *Meloidogyne exigua* recorded during a two year period (October 2002-October 2004) in a coffee plantation in southeast Brazil.

Trunk distance/depth/year	Polynomial equations	R^2
	Figure 2	
20/0-25 cm	$y = -5E\text{-}05x^6 + 0.004x^5 - 0.1274x^4 + 1.7903x^3 - 10.156x^2 + 13.678x + 76.205$	0.30
/25-50 cm	$y = -2E\text{-}05x^6 + 0.0015x^5 - 0.029x^4 - 0.1324x^3 + 8.8004x^2 - 69.729x + 190.98$	0.24
40/0-25 cm	$y = -7E\text{-}05x^6 + 0.0066x^5 - 0.2107x^4 + 2.9454x^3 - 16.115x^2 + 16.493x + 81.875$	0.24
/25-50 cm	$y = -3E\text{-}05x^6 + 0.0023x^5 - 0.0646x^4 + 0.6194x^3 + 0.6692x^2 - 30.396x + 109.62$	0.36
80/25-50 cm	$y = -1E\text{-}05x^6 + 0.0011x^5 - 0.0287x^4 + 0.1987x^3 + 2.0934x^2 - 28.578x + 97.585$	0.48
	Figure 3	
40/0-25 cm	$y = -7E\text{-}05x^6 + 0.0043x^5 - 0.0314x^4 - 2.7665x^3 + 63.631x^2 - 423.05x + 870.75$	0.36
	Figure 4	
20/25-50 cm	$y = -5E\text{-}06x^6 - 0.0005x^5 + 0.0662x^4 - 2.1899x^3 + 29.427x^2 - 154.17x + 399.36$	0.70
	Figure 5	
	J2	
40/0-25 cm/year 2002-2003	$y = -0.008x^6 + 0.3647x^5 - 6.1833x^4 + 48.086x^3 - 168.67x^2 + 212.15x + 17.567$	0.65
/25-50 cm/year 2002-2003	$y = 0.0045x^6 - 0.6692x^5 + 40.742x^4 - 1308x^3 + 23357x^2 - 219965x + 853624$	0.64
	Root galls	
40/0-25 cm/year 2002-2003	$y = 0.007x^6 - 0.3851x^5 + 8.1884x^4 - 83.733x^3 + 417.14x^2 - 890.37x + 735.88$	0.65
/25-50 cm/year 2003-2004	$y = 0.007x^6 - 1.055x^5 + 65.791x^4 - 2165.2x^3 + 39646x^2 - 382789x + 2E+06$	0.65

but the authors did not prevent possible egg hatching during laboratory processing. Furthermore, the short duration of their sampling precluded confirmation of this trend. In the present study, the fast production of root flushes by the coffee trees during the rainy months probably explains the low density of J2/5g of roots observed at 40 cm from the trunk, at a depth of 0-25 cm (Fig. 3), a pattern observed similarly at all other sampling locations (not shown).

In contrast, during the dry months (April through September), densities of J2 in the soil increased (Fig. 2), confirming findings by Almeida *et al.* (1987). Eclosed J2 possibly found few feeder roots available to infect, thus remaining in the soil. Nonetheless, results by Huang *et al.* (1984), Maximiniano *et al.* (2001) and Dimmy Barbosa (Universidade Estadual do Norte Fluminense, Brazil, personal communication) suggest that severe, long droughts eventually reduce hatch of eggs of *M. exigua* in coffee fields, hence reducing the J2 density in the soil. During the dry months, the increase in the J2 density in the roots (Fig. 3) is possibly due to the reduced root system, in comparison to the rainy season.

The number of galls/5 g of roots did not fluctuate seasonally, with the exception of the sampling at 20 cm

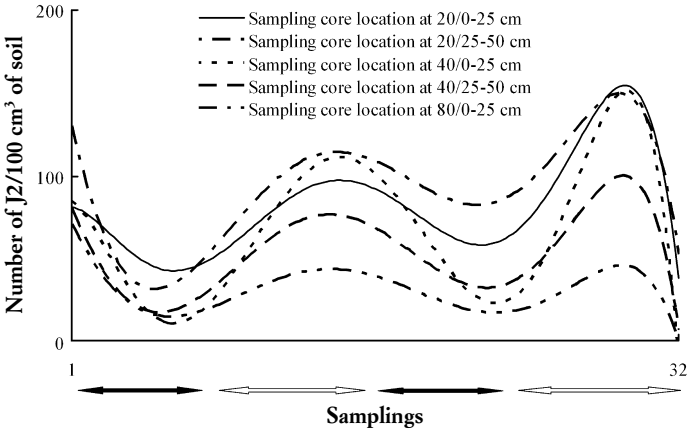

Fig. 2. Soil population fluctuation of second-stage juveniles (J2) of *M. exigua* during a two year period (October 2002-October 2004) in a coffee plantation in southeast Brazil. Samples were collected every three weeks, at different distances from the plant trunk, at two depths. Nematode counts (not transformed) are averages of 10 replicates (plants). Rainy and dry seasons are marked by black and white arrows, respectively.

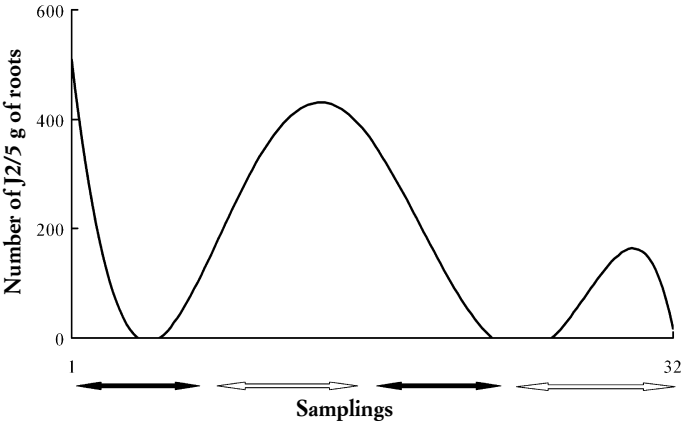

Fig. 3. Root population fluctuation of second-stage juveniles (J2) of *M. exigua* during a two year period (October 2002-October 2004) in a coffee plantation in southeast Brazil. Samples were collected every three weeks, at 40 cm distance from the trunk and at a depth of 0-25 cm. Nematode counts (not transformed) are averages of 10 replicates (plants). Rainy and dry seasons are marked by black and white arrows, respectively.

from the trunk, at depth of 25-50 cm (Fig. 4). This is probably a result of the mutual interaction between the components of this variable, since the increased number of root galls induced during the rainy months results in an increase in feeder root weight, ensuring a constant pattern to this epidemiological variable. The path analysis conducted to measure the effect of the environmental variables on the number of galls/5 g of roots revealed that mean air temperature and rainfall had direct, negative effects on root galling (Table II).

When the number of galls/5 g of roots is plotted for the years 2002-2003 and 2003-2004 separately, intraseasonal fluctuations are evident at 40 cm from the trunk and at the depth of 0-25 cm (Fig. 5), as well as at all other sampling depths and distances from the trunk (not shown). The same pattern was observed for J2 in soil (Fig. 5). The eight- to nine-week cycles seen in soil and root nematode populations and root galling resemble generations undergone by *M. exigua*. The generation time observed in this study is longer than the 32-42 days observed by Lima and Ferraz (1985b) at temperatures ranging from 25 to 30 °C. For *M. incognita* parasitizing coffee, Jaehn (1991) calculated the generation time to vary between four and ten weeks, depending on the mean air temperature and the nematode race involved.

The results of the present work do not support previous reports of an increase of *M. exigua* population levels in late October or November, when the air temperature rises, rainfall increases and new root flushes are produced by coffee trees in south-east Brazil. Although more root galls are seen in the top layer of the soil during the rainy months as a consequence of the new root flushes, the number of root galls and J2 per unit root actually declines during this period, as does the density of J2 per volume of soil.

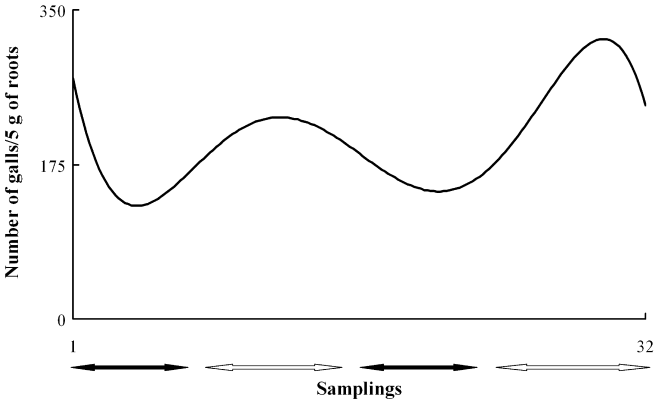

Fig. 4. Variation of *M. exigua*-induced root galls during a two year period (October 2002-October 2004) in a coffee plantation in southeast Brazil. Samples were collected every three weeks, at 20 cm distance from the trunk and at a depth of 25-50 cm. Gall counts (not transformed) are averages of 10 replicates (plants). Rainy and dry seasons are marked by black and white arrows, respectively.

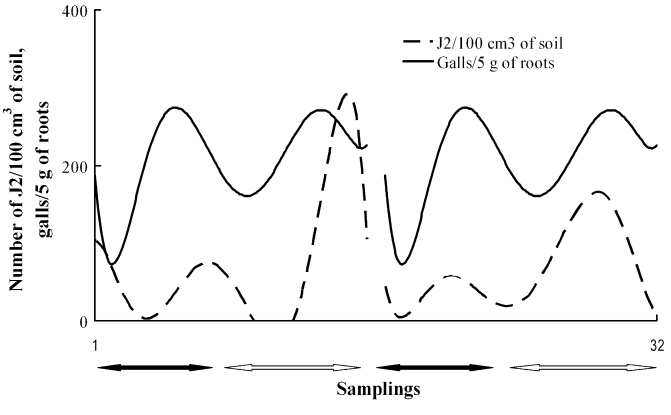

Fig. 5. Variation of *M. exigua*-induced root galls and soil population of second-stage juveniles (J2) during a two year period (October 2002-October 2004) in a coffee plantation in southeast Brazil. Samples were collected every three weeks, at 40 cm distance from the trunk and at a depth of 0-25 cm. Gall and nematode counts (not transformed) were plotted separately for the years 2002-2003 and 2003-2004 and are averages of 10 replicates (plants). Rainy and dry seasons are marked by black and white arrows, respectively.

Table II. Path analysis performed to assess the direct and indirect effects of environmental variables on the number of galls/5 g of roots induced by *Meloidogyne exigua* during a two year period (October 2002-October 2004) in a coffee plantation in southeast Brazil.

Rainfall	
Direct effect	-0.3225
Indirect effect through mean air temperature	-0.1862
Indirect effect through soil moisture content, depth 0-25 cm	-0.0142
Indirect effect through soil moisture content, depth 25-50 cm	0.0624
Total	-0.4607
Mean air temperature	
Direct effect	-0.4233
Indirect effect through rainfall	-0.1419
Indirect effect through soil moisture content, depth 0-25 cm	-0.0145
Indirect effect through soil moisture content, depth 25-50 cm	0.0677
Total	-0.5121
Soil moisture content, depth 0-25 cm	
Direct effect	-0.0268
Indirect effect through rainfall	-0.1713
Indirect effect through mean air temperature	-0.2293
Indirect effect through soil moisture content, depth 25-50 cm	0.0970
Total	-0.3306
Soil moisture content, depth 25-50 cm	
Direct effect	0.1202
Indirect effect through rainfall	-0.1674
Indirect effect through mean air temperature	-0.2384
Indirect effect through soil moisture content, depth 0-25 cm	-0.0216
Total	-0.3074
Coefficient of determination	0.3373
Effect of the residue	0.8140

In addition to the environmental variables assessed in the present work, host-derived cues could also play a role in the population dynamics of *M. exigua*, such as the amount and composition of root exudates, acting on the J2 in the soil, and hormonal and nutritional composition of the roots, acting on J2 and parasitizing females. Exceedingly tightly controlled experiments would be needed to investigate such interactions.

In conclusion, this work shows that experimental results are needed on the epidemiology of *M. exigua* in different climatic regions, as a platform for improving nematode management. For example, in Brazil water-soluble nematicides are applied during the rainy months. The results of the present work warrant a comparison of the effect of nematicide applications in the rainy *versus* dry months, since more J2 are present in the soil during the latter, providing the necessary soil moisture for nematicide solubilization is monitored and provided by irrigation, if necessary. Also, the use of biological agents aimed at controlling J2 migrating through the soil could be more efficient if applied during the winter, providing the soil temperature is favourable to the activity of the biocontrol organisms.

LITERATURE CITED

Almeida V.F., Campos V.P. and Lima R.D., 1987. Flutuação populacional de *Meloidogyne exigua* na rizosfera do cafeeiro. *Nematologia Brasileira, 11*: 159-175.

Arruda H.V. and Reis A.J., 1962. Redução nas duas primeiras colheitas de café, devida ao parasitismo de nematóide. *O Biológico*, *28*: 349.

Barbosa D.H.S.G., Vieira H.D., Souza R.M., Viana A.P. and Silva C.P., 2004. Field estimates of coffee yield losses and damage threshold by *Meloidogyne exigua*. *Nematologia Brasileira*, *28*: 49-54.

Campos V.P. and Villain L., 2005. Nematode parasites of coffee and cocoa. Pp. 529-579. *In*: Plant Parasitic Nematodes in Subtropical and Tropical Agriculture - Second edition (Luc M., Sikora R.A. and Bridge J., eds). CABI Publishing, Wallingford, UK.

Dhanam M. and Sreedharan K., 2008. India. *In*: Plant Parastic nematodes of Coffee (Souza R.M., ed.). Springer, Dordrecht, The Netherlands, in press.

Gonçalves W., 1997. Resistência do cafeeiro a *Meloidogyne* spp. *Fitopatologia Brasileira*, *22*: 230 (Abstract).

Huang S.P., Souza P.E. and Campos V.P., 1984. Seasonal variation of a *Meloidogyne exigua* population in a coffee plantation. *Journal of Nematology*, *16*: 115-117.

Jaehn A., 1991. Estimativa do número de gerações de três raças de *Meloidogyne incognita* em cafeeiro para o Estado de São Paulo. *Nematologia Brasileira*, *15*: 143-151.

Jenkins W.R., 1964. A rapid centrifugal-flotation technique for separating nematodes from soil. *Plant Disease Reporter*, *48*: 692.

Lima R.D. and Ferraz S., 1985a. Biologia de *Meloidogyne exigua*. I. Desenvolvimento embriogênico e efeito da temperatura na embriogênese. *Revista Ceres*, *32*: 339-347.

Lima R.D. and Ferraz S., 1985b. Biologia de *Meloidogyne exigua*. II. Desenvolvimento pós-embriogênico em cafeeiro `Mundo Novo´. *Revista Ceres*, *32*: 349-361.

Maximiniano C., Campos V.P., Souza R.M. and Almeida A.R., 2001. Flutuação populacional de *Meloidogyne exigua* em cafezal naturalmente infestado por *Pasteuria penetrans*. *Nematologia Brasileira, 25*: 63-69.

Santos J.M., 2000. Fatos e feitos relevantes na História da Nematologia no Brasil e principais desafios para o início do novo século. Pp. 9-13. *In*: Anais do XXII Congresso Brasileiro de Nematologia, February 20-25, 2000, Uberlândia, Brasil.

Santos J.M. and Ferraz S., 1977. Efeito de exsudatos radiculares e temperatura sobre a eclosão de larvas de *Meloidogyne exigua* Goeldi, 1887. Pp. 137-138. *In*: Resumos do V Congresso Brasileiro de Pesquisas Cafeeiras, Guarapari, Brasil.

Tronconi N.M., Ferraz S., Santos J.M. and Regazzi A.J., 1986. Influência da temperatura na patogenicidade e reprodução de *Meloidogyne exigua* em mudas de cafeeiro. *Nematologia Brasileira*, *10*: 69-83.

Vencovsky R. and Barriga P., 1992. *Genética Biométrica no Fitomelhoramento*. Sociedade Brasileira de Genética, Ribeirão Preto, Brasil, 496 pp.

Villain L., Anzueto F., Hernández A. and Sarah J.L., 1999. Los nematodos parásitos del cafeto. Pp. 163-171. *In*: Desafios de la Caficultura en Centroamerica (Bertrand B. and Rapidel B., eds). IICA, Promecafe, and Cirad, San José, Costa Rica.

Wiryadiputra S. and Loang T.K., 2008. Indonesia and Vietnam. *In*: Plant Parastic Nematodes of Coffee (Souza R.M., ed.). Springer, Dordrecht, The Netherlands, in press.

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