Relationships Among *Pratylenchus jaehni* and *P. coffeae* Populations from Brazil

Silvia R.S. Wilcken[1]*, Edson S. Mori[1], Maurício Bacci[2], Luiz Carlos C.B. Ferraz[3], Claudio Marcelo G. Oliveira[4] & Mário M. Inomoto[3]

[1]Departamento de Produção Vegetal, Faculdade de Ciências Agrárias, Universidade Estadual Paulista (FCA - UNESP), 18603-070 Botucatu (SP) Brasil.
[2]Departamento de Bioquímica e Microbiologia e Centro de Estudos de Insetos Sociais, Instituto de Biociências, UNESP, 13506-900 Rio Claro (SP) Brasil.
[3]Setor de Zoologia, Escola Superior de Agricultura Luiz de Queiroz, Universidade de São Paulo (ESALQ / USP), 13418-900 Piracicaba (SP) Brasil.
[4]Instituto Biológico, C. Postal 70, 13001-970 Campinas (SP) Brasil.
*Corresponding author: srenata@fca.unesp.br

Received for publication on December 10, 2007. Accepted on April 22, 2008
Edited by Guilherme Asmus

Summary - Wilcken, S.R.S., E.S. Mori, M. Bacci, L.C.C.B. Ferraz, C.M.G. Oliveira & M.M. Inomoto. 2008. Relationships among *Pratylenchus jaehni* and *P. coffeae* populations from Brazil.

The relationships among *Pratylenchus jaehni* (C_1) and six amphimitic *Pratylenchus* populations from Brazil (three from banana, PcB_1, PcB_2, and PcB_3; one from *Aglaonema* sp., M_2; one from coffee, K_5; and one from citrus, C_2) were studied through morphological and molecular analysis, and pathogenicity test. The analysis of morphometric characteristics of PcB_1, M_2, C_2 and K_5, compared with *P. jaehni* and *P. coffeae* (K_6) data obtained from literature, delineated three groups: *P. coffeae* (K_6), PcB_1 and M_2; *P. jaehni* (C_1) and C_2; and K_5. RAPD analysis of PcB_1, PcB_2, PcB_3, M_2, C_2, K_5 and *P. jaehni* (C_1) demonstrated that these populations form three distinct genetic groups: PcB_1, PcB_2, PcB_3 and M_2; *P. jaehni* and C_2; and K_5. In pathogenicity test, K_5 reproduced well on coffee and caused extensive root necrosis, but C_2 did not. Population C_2 reproduced well on Rangpur lime, which was previously rated as poor host to K_5. Therefore, the results demonstrated that C_2 is *P. jaehni*, K_5 is an undescribed species of *Pratylenchus*, and PcB_1, PcB_2 and PcB_3 are *P. coffeae*. The taxonomic status of M_2 remains inconclusive.

Key words: *Musa* sp., *Aglaonema* sp., *Coffea arabica*, *Citrus limonia*, lesion nematode, molecular analysis, taxonomy.

Resumo – Wilcken, S.R.S., E.S. Mori, M. Bacci, L.C.C.B. Ferraz, C.M.G. Oliveira & M.M. Inomoto. 2008. Relações entre populações de *Pratylenchus jaehni* e *P. coffeae* do Brazil.

As relações entre *Pratylenchus jaehni* (C_1) e seis populações anfimíticas de *Pratylenchus* do Brasil [três de bananeira (PcB_1, PcB_2 e PcB_3); uma de *Aglaonema* sp. (M_2); uma de cafeeiro (K_5); e uma de citros (C_2)], foram estudadas por meio de análises de características morfológicas e moleculares e teste de patogenicidade. A análise das características morfométricas de PcB_1, M_2, K_5 e C_2, comparadas com as de *P. jaehni* e *P. coffeae* (K_6) obtidas da literatura, delineou três grupos: *P. coffeae* (K_6), PcB_1 e M_2; *P. jaehni* (C_1) e C_2; K_5. Análise molecular (RAPD) de PcB_1, PcB_2, PcB_3, M_2, C_2, K_5 e *P. jaehni* (C_1) demonstrou que essas populações formam três grupos genéticos distintos: PcB_1, PcB_2, PcB_3 e M_2; *P. jaehni* (C_1) e C_2; e K_5. No teste de patogenicidade, a população K_5 reproduziu-se e causou necroses extensas nas raízes de cafeeiro, mas C_2 não. A população C_2 reproduziu-se em limoeiro cravo, que, entretanto, apresentou-se mau hospedeiro de K_5. Portanto, os resultados demonstraram que C_2 é *P.*

jaehni, K_5 é uma espécie ainda não descrita de *Pratylenchus*, e PcB_1, PcB_2 e PcB_3 são *P. coffeae*. A posição taxonomica de M_2 permanece inconclusiva.

Palavras-chaves: *Musa* sp., *Aglaonema* sp., *Coffea arabica*, *Citrus limonia*, nematóide das lesões, análise molecular, taxonomia.

Introduction

The taxonomic status of the coffee lesion nematode [*Pratylenchus coffeae* (Zimmerman) Filipjev & S. Stekhoven] is under debate, because the populations of this species exhibit high morphological and molecular variabilitity (Duncan *et al*., 1999). One population (C_1 from Duncan *et al*., 1999), collected in a citrus orchard in Itápolis (SP) Brazil, was recently elevated to species level as *P. jaehni* Inserra, Duncan, Troccoli, Dunn, Santos, Kaplan & Vovlas (Inserra *et al*., 2001). Duncan *et al.* (1999) have studied six other putative *P. coffeae* populations from Brazil, and two of them, C_2 (from a citrus orchard in Conchal, SP) and K_5 (from a coffee plantation in Marília, SP) were considered morphometrically similar to *P. jaehni* regarding relative distance of vulva from anterior end (V%), body length / greatest body width ratio (a), and stylet length (St). Both C_2 and K_5 populations mated successfully with *P. jaehni* (Inserra *et al*., 2001), so they may be co-specific to *P. jaehni*. According to Campos (2002), three other *Pratylenchus* populations from citrus (a, b and c) colleted in São Paulo state, together with K_5, were morphologically similar to *P. jaehni*. However, K_5 populations did not reproduce on Rangpur lime (*Citrus limonia* Osbeck), perhaps belonging to a race that is different from C_1 and C_2 (Silva & Inomoto, 2002).

Considering that *P. coffeae* is a major pest of coffee and citrus in the Americas and Asia, and some populations like K_5 and C_2 are sympatric, an accurate characterization is required for the management of this nematode. Thus, the aim of this research was to study the relatioships among *P. jaehni* and putative *P. coffeae* populations from Brazil, using morphologic, morphometric and molecular features and pathogenicity test.

Material and Methods

Inocula preparations. The population of *P. jaehni* (C_1) was collected from citrus at the same area of the type locality [Sítio das Antas, Itápolis (SP)]. Another *Pratylenchus* population from citrus (C_2) was collected from an orchard at Conchal (SP). Three populations of *P. coffeae* were obtained from banana roots from Jaíba (MG), Janaúba (MG) and Itariri (SP), and were named PcB_1, PcB_2, and PcB_3. Two other *Pratylenchus* populations that were studied by Duncan et al. (1999) and Silva & Inomoto (2002) were included in this research, K_5 (from arabica coffee roots from Marília, SP) and M_2 (from the ornamental *Aglaonema* sp. roots from Rio de Janeiro, RJ).

All seven populations were reared on alfalfa callus (Riedel *et al*., 1973). Motile stages of the populations were obtained from tissue cultures using a Baermann modified method (Hooper, 1986).

Morphometric analysis. Female specimens of C_2, PcB_1, K_5 and M_2 were heat-killed at 60 °C, mounted in glass slides with 4 % formaldehyde medium, and examined using light microscopy. Eleven morphometric characters were measured: body length (L), body width, stylet length, stylet knob height, tail length, distance from excretory pore to head end, distance of vulva from anterior end *x* 100 / body length (V%), body length / greatest body width (a), body length / distance from anterior end to junction of esophagus and intestine (b), body length / tail length (c), and overlap of esophageal gland. The data were analyzed using a principal component analysis (PCA) (Community Analysis Package, PISCES Conservation Ltd, Lymington UK). For comparison, morphometry of fixed specimens of the type population of *P. jaehni* (C_1) and one population of *P. coffeae* from Java (K_6), both data from the literature (Inserra *et al*., 2001), were included in the analysis.

Molecular analysis. DNA was extracted by smashing a single specimen individually with 5 µ*l* of lysis buffer (1X PCR buffer containing 50 µg / m*l* proteinase K) (Sambrook *et al*., 2001) and kept frozen at – 20 °C. After one week, the tubes were incubated at 60 °C for 1 h and then 95 °C for 15 minutes. DNA

extracts from 50 individuals from a given population were pooled and 5 µl of that pool were added to 20 µl PCR mix (0.9 units of Taq DNA polymerase, 2.5 µl of 10X PCR buffer, 5.75 mM of MgCl, 0.143 mM of DNTPs, 11.3 µl of water and 1 µl of a randomic primer). PCR was performed in a MJ Research Thermocycler using a pre-heated block (94 °C) and 50 cycles (94 °C, 1 minute; 55 °C, 1 minute; 72 °C, 1.5 minute). Seventy-seven primers were tested and only seven (OPAJ06, OPAJ09, OPAJ11, OPAD08, OPQ18, OPAK04 and OPAK09) showed polymorphic bands. The electrophoresis run was done in 3 % agarose gel for 3 h at 80 V. The gels were stained with 0.25 % ethidium bromide and photographed. For consistency, the experiments were repeated at least twice. The absence or presence of polymorphic bands were arranged in a matrix that was analyzed through the maximum parsimony method as implemented by the software PAUP* 4.0b4a (Swofford, 2000) utilizing branch-and-bound search (Felsenstein, 1985).

Pathogenicity test. One greenhouse experiment was carried out in order to evaluate the pathogenicity of K_5 and C_2 on arabica coffee (*Coffea arabica* L. 'Catuaí'). In the present work, pathogenicity is defined as the capacity of the nematode to cause disease on the plant, reproductive fitness as the ability of the nematode to reproduce on the plant, and virulence as the amount of damage caused by the nematode on the plant (Shaner *et al.*, 1992). Coffee seeds were sowed in a box containing autoclaved sand, and the seedlings were transplanted just after emergence to 500 cm^3-plastic pots. Seedlings with two pairs of true leaves were inoculated with the initial densities (Pi) of 2,000 specimens of K_5 or 2,000 and 8,000 specimens of C_2 / plant, by pouring an aqueous suspension containing the nematodes into two holes made on the soil near the plants. This experiment was set up in a completely randomized design, with five individual pot replications. Infectiveness of C_2 inoculum was assessed inoculating plants of Rangpur lime with 2,000 specimens of C_2. The plants were fertilized twice with NH_4SO_4 and, three months after inoculation, were transplanted to 1,800 cm^3-clay pots. Pathogenicity of K_5 and C_2 on 'Catuaí' was evaluated 257 days after inoculation, by measuring plant height, shoot dry weight (dried at 70 °C for 1 week) and extracting nematodes from soil (Jenkins, 1964) and roots (Coolen & D'Herde, 1972). After counting final nematode densities (Pf), nematode reproduction factor (RF = Pf / Pi) was calculated.

Differences among treatment means for growth data were determined by analysis of variance using the SANEST software [Ciagri and Departamento de Matemática e Estatística, ESALQ / USP, Piracicaba (SP)], and the comparisons of means were compared by Tukey's honestly significant difference test.

Results

All populations were formed by amphimitic and two lip annuli specimens. There are a wide range of the standard morphometric values among the specimens of each population (K_5, M_2, PcB_1 and C_2), resulting in an overlap of the values among the populations (Table 1). However, the PCA analysis of these morphometric characters and the data of the type population of *P. jaehni* (C_1) and the *P. coffeae* from Java (K_6) delineated one group comprised by the populations PcB_1 and M_2, closely associated with K_6. Another group was formed by C_2 and the type population of *P. jaehni*. The remaining population from coffee (K_5) was separated from both groups (Figure 1).

The RAPD method generated 95 polymorphic bands that were arranged in a matrix that was submitted to the maximum parsimony method. The analysis resulted in two most parsimonious trees (see consensus tree in Figure 2), in which the populations were separated into three distinct genetic groups. Group 1 was formed by populations from banana (PcB_1, PcB_2 and PcB_3), which were closely related to each other, as well as to *Pratylenchus* population from *Aglaonema* sp. (M_2). Group 2 was formed by *P. jaehni* (C_1) and C_2. The population from coffee (K_5) belonged to a genetically distinct group.

In the pathogenicity test, K_5 (Pi = 2,000) caused extensive necrosis in roots of arabica coffee plant. Conversely, both Pi (2,000 and 8,000) of C_2 did not cause any signal of necrosis in coffee roots. Plant height and shoot dry weight of coffee were also depressed by K_5 (Table 2). The population density of K_5 increased to very high levels on arabica coffee plant (Pf / Pi = 19.87), but C_2 densities decreased until to

Table 1 - Morphometrics (mean, standard deviation and range) of Brazilian *Pratylenchus* females from coffee K_5 (Marília SP), *Aglaonema* sp. M_2 (Rio de Janeiro RJ), banana PcB_1 (Jaíba MG), and citrus C_2 (Conchal SP). Measurements in µm.

Character	n	Populations			
		K_5	M_2	PcB_1	C_2
Body length	22	484 ± 31.6 (435-652)	583 ± 55.3 (450-702)	603 ± 30.6 (551-660)	480 ± 26.8 (425-522)
Body width	22	17.8 ± 1.7 (14.5-20.0)	20.6 ± 3.1 (14.0-25.4)	21.3 ± 2.1 (17.2-27.9)	18.4 ± 1.6 (16.4-23.2)
Stylet length (St)	20	15.0 ± 0.6 (14.2-16.4)	17.0 ± 0.6 (16.0-17.8)	16.3 ± 0.6 (15.2-17.5)	14.5 ± 0.6 (13.4-15.9)
Stylet knob height	10	2.9 ± 0.2 (1.8-2.5)	2.5 ± 0.3 (2.2-2.8)	2.6 ± 0.1 (2.5-2.9)	2.0 ± 0.2 (1.8-2.5)
Tail length	10	23.3 ± 2.9 (15.6-26.5)	30.0 ± 3.8 (24.0-35.3)	27.4 ± 2.8 (21.2-30.6)	27.1 ± 3.2 (23.2-34.8)
Excretory pore to head end	19	85.8 ± 7.2 (71.3-108.4)	90.0 ± 7.2 (77.8-103.3)	93.1 ± 3.0 (88.8-100.7)	80.1 ± 4.0 (70.3-86.2)
V%	22	79.9 ± 1.1 (77.2-82.2)	80.0 ± 1.4 (76.9-82.3)	79.0 ± 1.1 (76.8-80.5)	77.7 ± 0.9 (76.2-79.9)
Ratio (a)	22	27.3 ± 2.3 (23.6-32.9)	28.7 ± 3.2 (22.7-36.0)	28.5 ± 2.0 (23.3-33.2)	26.2 ± 2.1 (21.2-27.8)
Ratio (b)	17	5.0 ± 0.4 (4.5-5.8)	5.4 ± 0.4 (4.6-6.1)	5.8 ± 0.5 (5.0-6.6)	5.4 ± 0.5 (4.6-6.6)
Ratio (c)	10	20.5 ± 3.1 (18.6-29.3)	21.1 ± 4.1 (16.8-30.6)	22.1 ± 2.6 (20.2-29.8)	17.6 ± 1.4 (13.8-18.4)
Pharyngeal overlap	17	32.3 ± 5.9 (24.7-45.1)	37.9 ± 7.6 (25.8-54.5)	43.8 ± 9.3 (29.8-60.4)	22.8 ± 5.2 (12.7-30.4)

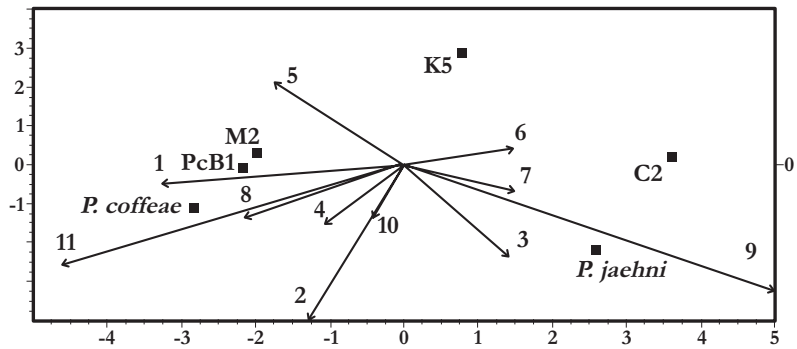

Figure 1 - Principal component analyses (PCA) of lesion nematode populations from banana (PcB_1), *Aglaonema* sp. (M_2), coffee (K_5) and citrus (C_2), compared with *P. coffeae* (K_6) and *P. jaehni* (C_1), using body length (1), body width (2), stylet length (3), stylet knob height (4), tail length (5), excretory pore to head end (6), parameters V%, a, b and c, (7), (8), (9) and (10) respectively, and pharyngeal overlap (11).

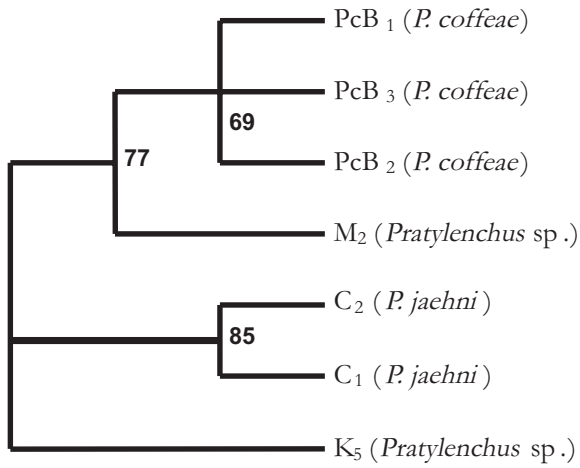

Figure 2 - Observed phylogenetic consensus tree showing relationships among *P. jaehni* (C_1) and six amphimitic lesion nematode populations from banana (PcB_1, PcB_2 and PcB_3), *Aglaonema* sp. (M_2), coffee (K_5) and citrus (C_2) based on analysis of 27 informative characters generated from RAPD markers and submitted to the maximum parsimony method. Length = 108, CI = 0.8241, RI = 0.5128. Numbers above branches indicate bootstrap support from 1,000 replicates with 100 random addition heuristic searches per replicate. Bootstrap values below 50 are not shown.

Table 2 - Pathogenity of *Pratylenchus* populations (K_5 and C_2) on arabica coffee plants (*Coffea arabica* 'Catuaí').

Treatment	Plant height (cm)	Root fresh weight (g)	Shoot dry weight (g)	Pf / Pi
Check	48.3 a	73.9 a	41.4 a	0
C_2 2,000	49.2 a	72.0 a	33.5 a	0
C_2 8,000	42.4 ab	69.6 a	32.3 ab	0
K_5 2,000	32.1 b	12.2 b	19.8 b	19.87

Data are means of five replicates. Means within a column followed by a common letter are not different according to Tukey's test ($P < 0.05$).

undetectable levels (Pf = 0). The consistent increase of C_2 on Rangpur lime (Pf / Pi = 16.08, mean of four replicates) demonstrated that the inoculum used was infective.

Discussion

Comparing the present morphometric data with those reported by Duncan *et al.* (1999), Inserra *et al.* (2001) and Campos (2002), there appears to be great similarity among *P. coffeae*, *P. jaehni* and K_5, and a wide variability among the populations of *P. coffeae* and *P. jaehni*. Campos (2002) used the values of stylet length, stylet knob height and V% to conclude that K_5 is *P. jaehni*. However, in the same report, cluster analysis of eight morphometric characteristics of five populations of *Pratylenchus* from citrus (a, b, c, *P. jaehni* and C_2), one from coffee (K_5), one from yam (*P. coffeae*) and one from banana (*P. coffeae*) suggests another interpretation of those data. According to cluster analysis, *P. coffeae* populations (from yam and banana) were separated from the six other populations, and among them, the five citrus populations were closely related to each other but separated from K_5. Thus, the identification *P. coffeae*, *P. jaehni* and K_5 based on a low number of morphometrics characters is not reliable. However, these nematodes can be separated using a multivariate analysis, as demonstrated in the present study.

The results of the pathogenicity test are in agreement to a previous report, in which K_5 reproduced on arabica coffee, but C_2, PcB_2 and PcB_3 did not (Wilcken *et al.*, 2003). In addition, K_5 and M_2 did not reproduce on Rangpur lime (Silva & Inomoto, 2002), and a recent work demonstrated that K_5 reproduces and is very virulent on arabica coffee, compared to M_2, which is probably non-pathogenic to arabica coffee (Inomoto *et al.*, 2007). Furthermore, arabica coffee and Rangpur lime are useful plants to differentiate K_5, C_2 and M_2, as the former reproduce on arabica coffee but not on Rangpur lime. C_2 population reproduces on Rangpur lime but not on arabica coffee, and M_2 do not reproduces on either of those plants. Thus, pathogenicity tests, PCA and RAPD analyses are in agreement concerning identity of C_2 and K_5, indicating that the former is *P. jaehni* and that K_5 is a different species from *P. jaehni* or *P. coffeae*. However, the identity of M_2 is less obvious, considering the results of previous pathogenicity and reproductive fitness tests.

In conclusion, based on the combined multivariate and molecular analyses in the present study, K_5 is likely a different species from *P. coffeae* and *P. jaehni*, probably constituting an undescribed species. From the populations of *Pratylenchus* tested herein, M_2 and the banana populations are the most closely related each other based in RAPD. Based in PCA, M_2 and the banana populations grouped with *P. coffeae* from Java (K_6), but they exhibited low reproductive fitness on coffee, probably constituting species complex. It is clear that a thorough and combined morphological and molecular investigation is required to unravel the complexities of the *P. coffeae* populations and closely related species. Future studies, such as those taken to clarify taxonomic questions surrounding *Xiphidorus* species (Oliveira *et al.*, 2004) and *Xiphinema krugi* populations (Oliveira *et al.*, 2006) using rDNA sequences or mtDNA prospecting (Blouin *et al.*, 1998; Blouin, 2002) may provide more conclusive evidence.

Acknowledgement

The first author thanks Fundação de Apoio à Pesquisa do Estado de São Paulo (FAPESP) for the financial support (protocol nº. 1999 / 0906-2).

Literature Cited

BLOUIN, M.S. 2002. Molecular prospecting for cryptic species of nematodes: Mitochondrial DNA versus internal transcribed spacer. International Journal for Parasitology 32 (5): 527–531.

BLOUIN, M.S., C.A. YOWELL, C.H. COURTNEY & J.B. DAME. 1998. Substitution bias, rapid saturation, and use of mtDNA for nematode systematics. Molecular Biology and Evolution 15 (12): 1719–1727.

CAMPOS, A.S. 2002. Distribuição de *Tylenchulus semipenetrans* e *Pratylenchus jaehni* em citros, no estado de São Paulo, e estudo morfométrico comparativo de populações anfimíticas de *Pratylenchus* spp. (Dissertação de Mestrado). Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista, Jaboticabal, 65 p.

COOLEN, W.A. & J.C. D'HERDE. 1972. A Method for Quantitative Extraction of Nematodes from Plant Tissue. State Nematology and Entomology Research Station, Ghent (Belgium), 71 p.

DUNCAN, L.W., R.N. INSERRA, W.K. THOMAS, D. DUNN, I. MUSTIKA, L.M. FRISSE, M.L. MENDES, K.MORRIS & D.T. KAPLAN. 1999. Genetic and morphological relationships among isolates of *Pratylenchus coffeae* and closely related species. Nematropica 29 (1): 61-80.

FELSENSTEIN, J. 1985. Confidence limits on phylogenies: an approach using bootstrap. Evolution 39: 783-791.

HOOPER, D.J. 1986. Extraction of free-living stages from soil. In: SOUTHEY, J.F. (ed). Laboratory Methods for Work with Plant and Soil Nematodes. Her Majesty's Stationery Office, London UK, p. 5-30.

INOMOTO, M.M., R.K. KUBO, R.A., SILVA, C.M.G. OLIVEIRA, M.D. TOMAZINI & P. MAZZAFERA, 2007. Pathogenicity of two *Pratylenchus coffeae* populations from Brazil on coffee plants. Nematology 9 (6): 853-858.

INSERRA, R.N., L.W. DUNCAN, A. TROCCOLI, D. DUNN, J.M. SANTOS, D. KAPLAN & N. VOVLAS. 2001. *Pratylenchus jaehni* sp. n. from citrus in Brazil and its relationship with *P. coffeae* and *P. loosi* (Nematoda: Pratylenchidae). Nematology 3 (7): 653-665.

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

OLIVEIRA, C.M.G., L.C.C.B. FERRAZ, A.R. MONTEIRO, B. FENTON, G. MALLOCH & R. NEILSON. 2004. Molecular and morphometric analyses of *Xiphidorus* species (Nematoda: Longidoridae). Nematology 6: 715–727.

OLIVEIRA, C.M.G., L.C.C.B., FERRAZ & R. NEILSON. 2006. *Xiphinema krugi*, species complex or complex of cryptic species? Journal of Nematology, 38: 418-428.

RIEDEL, R.M., J.G. FOSTER & W.S. MAI. 1973. A simplified medium for monoxenic culture of *Pratylenchus penetrans* e *Ditylenchus dipsaci*. Journal of Nematology 5: 71-72.

SAMBROOK, J., D.W. RUSSEL & J. SAMBROOK. 2001. Molecular Cloning: a Laboratory Manual. Cold Spring Harbor Laboratory Press, New York (USA).

SHANER, G., E.L. STROMBERG, G.H. LACY, K.R. BARKER & T.P. PIRONE. 1992. Nomenclature and concepts of pathogenicity and virulence. Annual Review of Phytopathology, 30: 47-66.

SILVA, R.A. & M.M. INOMOTO. 2002. Host-range characterization of two *Pratylenchus coffeae* isolates from Brazil. Journal of Nematology 34: 135-139.

SWOFFORD, D.L. 2000. Phylogenetic analysis using parsimony. Version 4.0b4a. Illinois Natural History Survey, Champaign.

WILCKEN, S.R.S., C.A.R. DEMANT & M.M. INOMOTO. 2003. Estudo da variabilidade de *Pratylenchus coffeae* e *P. jaehni* pela reação de genótipos de cafeeiro. Nematologia Brasileira 27: 265 (Resumo).