

Effect of Plant-Parasitic Nematodes Associated with Tea in Nigeria

Samuel B. Orisajo

Plant Pathology Section, Crop Protection Division,
Cocoa Research Institute of Nigeria, P.M.B. 5244, Ibadan, Nigeria

Abstract: Several species of plant parasitic nematodes have been encountered in soils of different tea-growing areas of the world. Roots and soil were sampled for the presence of plant-parasitic nematodes from tea plantations in Kusuku, the only major tea growing location in Nigeria. Six genera of plant-parasitic nematodes were recovered from the rhizosphere soil. *Meloidogyne* spp. *Pratylenchus coffeae* and *Helicotylenchus coffeae* were predominant with prominence values of 380, 212 and 88 respectively and occurred in 84, 63 and 58% from all soil samples of 250 cm³ soil respectively. This was followed in descending order by *Rotylenchulus reniformis*, *Radopholus* spp. and *Xiphenema* spp. with frequency of occurrence 43, 39 and 29 respectively. *Meloidogyne* spp. was the only nematode species recovered from the sampled tea roots with 21% frequency of occurrence. Root-knot nematodes, *Meloidogyne incognita*, reduced the leaf area of selected tea clones seedlings significantly ($P < 0.05$) compared to the control 4 weeks after inoculation. The nematode reproduced successfully on all the tea clones but China clones exhibited some level of tolerance to the nematode. There is the need of concert effort for controlling this nematode in tea, most especially with the recent move in Nigeria to cultivate tea in the lowland.

Key words: Plant-parasitic nematodes • Tea clones • *Meloidogyne incognita*

INTRODUCTION

Tea is an evergreen shrub from the genus *Camellia* that includes 82 species [1]. Of all the *Camellia* spp. tea is the most important both commercially and taxonomically and is cultivated to produce a stimulant brew. Tea, *Camellia sinensis* L. belongs to the plant family Theaceae. Cultivated tea varieties are natural hybrids of the original tea species *C. sinensis* L. O. Kuntze, *C. assamica* (Masters) and *C. assamica* subsp. *lasiocalyx* (Planchon ex Watt) [2]. The species *sinensis*, is known as 'China tea', originated from Sichuan province, south-western China, is suitable for growing in marginal areas of the subtropics and is more drought-tolerant and can survive short frost periods. Similarly, the species *assamica* is also known as 'Assam tea', originated from the forests of Assam, north-eastern India, is a tropical variety sensitive to dry and cold weather conditions [3].

The tea crop has been introduced to other parts of the world with diverse climatic conditions from its native habitat ranging from a Mediterranean-type climate to hot

humid tropical and subtropical regions from as far north as Georgia (42° N) and as far south as Argentina (27° S) and between sea level and 2,460 m altitude [4]. The major tea producing countries are China, India, Sri Lanka, Kenya, Indonesia, Turkey, Iran, Georgia, Japan, Vietnam, Bangladesh, Argentina, Malawi, Uganda and Tanzania. Tea is grown in Nigeria with commercial cultivation on the Mambilla plateau along Gembu, Ardo-Gori, Kusuku, Karaka, Maizat-mari and Nguroje axis [5, 6].

Diversity of forest environment provides a stable micro-climate and continuous supply of food for many pests and diseases. Tea, being a monocrop, loses the diversity and becomes prone to pests and disease infestation, especially after long periods of cultivation. Several species of plant parasitic nematodes have been encountered in tea soils of different tea-growing areas in the world. However, no positive evidence of pathogenicity has been established with respect to the majority of these nematodes. The species that are either known or suspected to be pathogenic to tea includes *Pratylenchus* spp. *Radopholus similis*, *Meloidogyne* spp.

Hemicriconemoides kanayaensis, *Rotylenchulus reniformis*, *Helicotylenchus* spp. *Paratylenchus curvatus*, *Hoplolaimus* sp. *Rotylenchus* sp. and *Xiphinema* sp. [7]. There is limited information on the plant-parasitic nematodes associated with tea in Nigeria. Therefore, a survey of the tea plantations in Nigeria and determination of the susceptibility of tea seedlings to plant-parasitic nematodes become imperative, hence the essence of the present study.

MATERIALS AND METHODS

Nematode Survey: Nematological surveys of tea plots were carried out in Kusuku (6° 50'N; 11° 07'E), Taraba State of Nigeria, located at about 11 km off Ngoroje-Gembu Road on the Mambilla Plateau. Kusuku, the only major tea growing location in Nigeria, is situated on an elevation of about 1,460 meters above sea level with maximum temperature between 21.4 °C to 30 °C, while the minimum temperature ranges between 1.9°C to 16 °C. The total annual rainfall is between 1800 mm and 2000 mm. Soil and root samples were collected from the rhizosphere region about 50-70 cm from the base of the plants and at a depth of 20 cm.

Processing of Soil and Root Samples: Aliquots of 250cm³ sub-sample soil from 500cm³ each composite sample were assayed for nematodes by sieving and decanting [8]. After decanting, the sediment was assayed for nematodes using the Whitehead and Hemming [9] tray modification of Baermann [10] technique as described by Coyne [11]. The root samples were washed, pooled, chopped into approximately 1-cm-pieces and thoroughly mixed. A 5g sub-sample was put in 100ml water in a kitchen blender. The root was macerated 3 times for 10 seconds, separated by 5 seconds intervals and the nematodes were extracted from the homogenate using sieve method [12]. The nematode suspension was diluted with water in a graduated cylinder to 10ml.

Prior to counting, solution containing nematodes were agitated thoroughly and nematode populations were determined in 1 ml distilled water suspension in a counting dish [13] under a stereomicroscope and expressed per 250cm³ soil or 5g roots. A mean of 3 counts was taken in each case. Nematodes were transferred with an eye lash picker to a slide with a drop of water, covered (with a cover slip) and examined under a compound microscope with a 40, 60 and 100X objective for identification using taxonomic keys [14] and counted. The identification and counting was repeated three times and mean population of nematodes/sample calculated.

Pathogenicity Tests: Tea clones seedlings collected from the location were allowed to stabilize in the greenhouse for two weeks and thereafter subjected to pathogenicity tests. The pots with the tea seedlings were inoculated with 5,000 *Meloidogne incognita* eggs obtained from the pure culture on the roots of *Celosea argentea* using Hussey and Barker [15] sodium hypochlorite (NaOCl) method. Uninoculated units served as control. Normal watering of seedlings as obtains in tea nurseries was carried out. Fortnightly, growth parameters such as stem girth, leaf area and numbers of leaves were recorded. The experiment was terminated 24 weeks after inoculation. To assess infection the roots were carefully freed of soil, washed under a gentle stream of tap water, mopped and galls counted using a hand lens at 3-5 X magnification. Root galling was assessed using the 0-5 gall index [16]. Nematode eggs were collected from each root system using sodium hypochlorite method (NaOCl) of Hussey and Barker [15] and counted. Aliquots of 250cm³ soil samples from each pot were assayed for juveniles of *M. incognita* using the modified Baermann technique [11].

Data Analysis: Prior to statistical analyses, data were checked for normality and homogeneity of variances and transformed where necessary. A log transformation [$\log_{10}(x + 1)$] was applied to the data on nematodes (densities per 250cm³ soil, densities per gram root and gall index). The determination of disease incidence was based on the nematode population per root system and it was expressed by the number of egg masses and gall index. The numbers of egg masses were counted and gall index (GI) and egg mass index (EMI) were determined on the following scale: 0 = 0; 1 = 1 - 2; 2 = 3 - 10; 3 = 11 - 30; 4 = 31 - 100 and 5 = greater than 100 galls or egg masses per root system [17, 16]. Reproduction factor of the nematode (Rf) = Final nematode population (pf) × Initial nematode population (pi)⁻¹. To estimate the susceptibility and resistance of the tea clones surveyed, the prominence values (PV = nematode population density × (frequency of occurrence)^{1/2} × 10⁻¹) were calculated for each species All data collected were subjected to analysis of variance and significant differences between means were evaluated using Least Significant Difference Method at P<0.05. All analyses were performed using GENSTAT (version 7.1, VSN International Ltd. Lawes Agricultural Trust, Hempstead, UK).

RESULTS

Plant-parasitic nematodes recovered from the tea plantations were *Meloidogyne* spp. *Pratylenchus coffeae*,

Table 1: Prominence value (PV), frequency of occurrence and mean population density of predominant plant-parasitic nematodes recovered from soils and roots of tea on the Mambilla plateau in Nigeria.

Nematode species	PV	Frequency of occurrence (%)	Mean population density	PV	Frequency of occurrence (%)	Mean population density
	-----Soil*-----			-----Root#-----		
<i>Meloidogyne</i> spp.	380	84	415	32	21	69
<i>Pratylenchus coffeae</i>	212	63	267	-	-	-
<i>Helicotylenchus coffeae</i>	88	58	115	-	-	-
<i>Rotylenchulus reniformis</i>	45	43	69	-	-	-
<i>Radopholus</i> spp.	27	39	43	-	-	-
<i>Xiphenema</i> spp.	10	29	18	-	-	-

* nematodes recovered from 250 cm³soil samples from the tea rhizosphere

nematodes recovered from 5g tea roots

Table 2: Effect of root-knot nematode, *Meloidogyne incognita*, on some growth parameters of tea seedlings at 24 weeks after inoculation.

Tea clones	No of leaves* (count)	Leaf area (cm ²)	Stem girth (cm)	GI/EMI (count)	Reproduction factor (Rf) (pf/pi) #
236	15a	185.2e	1.21b	4/4	12.1
Control	15a	197.1c	1.26a	-	-
143	16a	186.3e	1.28a	2/2	7.4
Control	16a	196.4c	1.27a	-	-
318	15a	192.1d	1.22b	3/3	9.2
Control	15a	199.7c	1.28a	-	-
China	16a	206.4b	1.30a	1/1	2.6
Control	16a	209.5a	1.29a	-	-

*Means followed by the same letter in the same column are not significantly different (P<0.05). pf = Final nematode population, pi = Initial nematode population. Where GI = Gall index, EMI = Egg mass index.

Helicotylenchus coffeae, *Rotylenchulus reniformis*, *Radopholus* spp. and *Xiphenema* spp. (Table 1). *Meloidogyne* spp. *Pratylenchus coffeae* and *Helicotylenchus coffeae* were predominant with prominence values of 380, 212 and 88, respectively and occurred in 84, 63 and 58% of all soil samples per 250 cm³ soil, respectively. This was followed in descending order by *Rotylenchulus reniformis*, *Radopholus* spp. and *Xiphenema* spp. with frequency of occurrence of 43, 39 and 29, respectively. *Meloidogyne* spp. was the only nematode species recovered from the sampled tea roots with 21% frequency of occurrence (Table 1). Root-knot nematodes, *Meloidogyne incognita*, reduced the leaf area of selected tea clones seedlings significantly (P<0.05) compared to the controls 24 weeks after inoculation (Table 2). There were no significant differences in the number of leaves of various tea clones between the inoculated and the control. Stem girth of tea clones 236 and 318 was reduced due to nematode inoculation, while there were no significant differences between the inoculated and the control of 143 and China clones (Table 2). The nematode reproduced successfully on all the tea clones, but China clones exhibited some level of

tolerance to the nematode (Table 2). Tiny galls typical of root-knot nematodes were observed on the roots of the inoculated seedlings.

DISCUSSION

There have been several instances of slow decline of tea, ultimately leading to death of affected tea bushes, especially following pruning in the high elevation tea areas and in a few of the mid-elevation tea areas of Sri Lanka caused by plant-parasitic nematodes [18]. Root knot nematodes, *Meloidogyne javanica*, *M. incognita*, *M. brevicaua* are widely distributed nematode species in tea plants, especially in India where tea has been grown for a long period of time [19]. The first two species affect tea seedlings in the nursery and the third one affects mature tea. The infested seedlings are not always killed outright, but patches are found to be stunted, wilted, chlorotic or generally unhealthy. Sometimes root tips are devitalized and their growth is arrested. Such damage impairs root-spread and the ability of the plants for uptake of water and nutrients is reduced. The roots of tea when infested with *M. incognita* tend to form very small galls

that look like beads on a string leading to contamination of tea plant [20]. Generally the infestation is very severe between the months of June and August in north-east India [21]. Similar observations were observed on the tea clones investigated in the present study in Nigeria. There is the need for concerted effort to control this nematode on tea, most especially with the recent move to cultivate tea in the lowland.

REFERENCES

1. Banerjee, B., 1992. Botanical classification of tea. In: Willson, K.C. Clifford, M.N. (Eds), Tea: Cultivation to consumption. Chapman and Hall, London, pp: 25-51.
2. Mondal, T.K., A. Bhattacharya, M. Laxmikumaran and P.S. Ahuja, 2004. Recent advances of tea (*Camellia sinensis*) biotechnology. *Plant Cell, Tissue and Organ Culture*, 76: 195- 254.
3. Van der Vossen, H.A.M. and M. Wessel, 2000. Plant resources of South-East Asia (PROSEA) No. 16 Stimulants. Backhuys Publishers, Leiden, The Netherlands, pp: 55-63.
4. Carr, M.K.V., 1972. The climatic requirements of tea: A review. *Experimental Agriculture*, 8: 1-14.
5. Awaro, E.N., 1983. Bulletin for the commissioning ceremony of the Mambilla Tea project. Nigerian Beverages Production Company Ltd, Yola. pp: 1 - 23.
6. Shaib, B., 1985. Speech, Federal Minister of Agriculture, Water Resources and Rural Development. The Mambilla Tea Project Commissioning Ceremony, 26th March 1985. Government Printers. Yola, pp: 1-3.
7. Gnanapragasam, N.C. and K.M. Mohotti, 2005. Nematode Parasites of Tea. In: Plant parasitic nematodes in subtropical and tropical agriculture. Luc, M. Sikora, A. & Bridge, J. (Eds.). CABI Publishing, Wallingford, UK. pp: 581-609.
8. Cobb, N.A., 1918. Estimating the mean population of the soil. *Agricultural Technical Circular Bureau of Plant Industries, United States Department of Agriculture*, 1, 48.
9. Whitehead, A.G. and J.R. Hemming, 1965. A comparison of some quantitative methods of extracting small vermiform nematodes from soil. *Annals of Applied Biology*, 55: 25-38.
10. Baermann, G., 1917. Eine einfache Methode zur Auffindung von Ankylostomum (Nematodes) Larven in Erdproben. *Geneesk. Tijdschr. Ned. India*, 57: 131-137.
11. Coyne, D.L., J.M. Nicol and B. Claudius-Cole, 2007. Practical plant nematology: a field and laboratory guide. SP-IPM Secretariat, International Institute of Tropical Agriculture (IITA), Cotonou, Benin. pp: 84.
12. Speijer, P.R. and D. De Waele, 1997. Screening of Musa germplasm for resistance and tolerance to nematodes. INIBAP Technical Guidelines 1. Montpellier, France, International Network for the Improvement of Banana and Plantain, pp: 48.
13. Doncaster, C.C., 1962. A counting dish for nematodes. *Nematologica*, 7: 33-34.
14. Hunt, D.J., M. Luc and R. H. Manzanilla-López, 2005. Identification, Morphology and Biology of Plant Parasitic Nematodes. In: Plant parasitic nematodes in subtropical and tropical agriculture. Luc, M. Sikora, A. & Bridge, J. (Eds.). CABI Publishing, Wallingford, UK. pp: 11-52.
15. Hussey, R.S. and K.R. Barker, 1973. A Comparison of methods of collecting inocula for *Meloidogyne* spp. including a new technique. *Plant Disease Reporter*, 57: 1025-1028.
16. Sasser, J.C., C.C. Carter and K.M. Hartman, 1984. Standardization of host suitability studies and reporting of resistance to root-knot nematode. USAID (United States Agency for International Development), Raleigh, North Carolina, U.S.A. pp: 7.
17. Taylor, A.L. and J.N. Sasser, 1978. Biology, Identification and control of root knot nematodes (*Meloidogyne* spp.). Coop. Pub. Deptt. Plant Pathol. North Carolina State University and United States Agency for International Development, Graphics, Raleigh, pp: 111.
18. Gnanapragasam, N.C., 2002. Slow decline observed in nematode tolerant tea cultivar. *International Journal of Nematology*, 12: 232-233.
19. Glover, P.M., 1961. Nematodes in N.E. India. Two and a Bud, 8: 6-7.
20. Bidarigh, S. and E. Azarpour, 2011. The study effect of cytokinin hormone types on length shoot in vitro culture of tea (*Camellia sinensis* L.). *World Applied Sciences Journal* 13(7): 1726-1729
21. UPASI, 1987. Handbook of tea culture. United Planters Association of Southern India, Tea Research Institute, Valparai, Coimbatore, India