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PLANT-PARASITIC NEMATODES ASSOCIATED WITH COFFEE IN NIGERIA

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ABSTRACT
Plant-parasitic nematodes have a substantial economic impact on coffee in most coffee-producing countries but have hitherto received minimum research attention in sub-Saharan Africa. Roots and soil were sampled in 2010 and 2011 from coffee plantations in seven localities in six States, representative of the coffee producing areas of Nigeria, for the presence of plant-parasitic nematodes on *Coffea arabica* and *C. canephora*. Fourteen genera of plant-parasitic nematodes were recovered from the rhizosphere soil. The endoparasitic nematodes, *Meloidogyne* spp., *Pratylenchus coffeae* and *Rotylenchulus reniformis* were predominant and occurred in 82, 51 and 5% of all soil samples per 250 cm^3 soil, respectively. *Rotylenchulus reniformis* was recovered only from the highland Kusuku soil with 43% frequency of occurence. *Meloidogyne* spp. and *Pratylenchus coffeae* were the only plant-parasitic nematodes recovered from coffee roots during the survey. *Meloidogyne* spp. was present in root samples from all the 7 localities, while *Pratylenchus coffeae* occurred in root samples from 5 localities, but was present in soil samples from the 7 localities. Root-knot nematodes, *Meloidogyne incognita*, suppressed the growth of coffee seedlings significantly ($P<0.05$) 24 weeks after inoculation in the nursery. The nematode reproduced successfully on both *C. arabica* and *C. canephora* with reproduction factors of 15.1, and 16.4, respectively. There is therefore the need to develop strategies to control plant-parasitic nematodes on coffee in Nigeria.

KEY WORDS: plant-parasitic nematodes, coffee, *Meloidogyne incognita*

INTRODUCTION
Coffee is an important crop and extensively cultivated in about 60 tropical and subtropical countries. The main regions of coffee production over the world are South America (45%), Asia and Oceania (26. %), Africa (13 %), Mexico and Central America (16%) (ICO, 2010). Twenty-five countries produce over one million 60 kg bags/year (Waller *et al*., 2007); the top coffee producing countries being Brazil, Colombia, Indonesia, Vietnam, Mexico, Ethiopia, India, Guatemala, Cote D'Ivoire and Uganda (FAOSTAT, 2010). The production of arabica coffee is predominant in America, except for Brazil which also produces robusta coffee. In Africa, Vietnam and Indonesia, robusta coffee is predominant (Ha & Shively, 2008; Vieiria, 2008). Coffee is a major source for foreign exchange, and contributes significantly to the economy of developing countries (Waller *et al*., 2007). But, coffee production in Africa has largely stagnated over the past two decades. While the continent had attained a production level of 19.5 million bags of coffee in 1997, production in 2008 was only 17.5 million bags (ICO, 2009). In the same vein, there has been a downward trend in coffee production in Nigeria from 53,000 bags of coffee in 1995 to 34,000 in 2010 (ICO, 2011). This reduction in production has been attributed to a number of factors including poor management practices, soil infertility, inconsistent government policies, poor pricing and losses due to damage by pests and diseases (Musoli *et a*l., 2001, Ibiremo *et al*., 2011). Pest populations, nematodes for example, may build up to damaging levels in perennial crops, and coffee is no exception (Waller *et al*., 2007).

Plant-parasitic nematodes have a substantial economic impact on coffee in most coffee-producing countries. They are regarded as the major limiting factor in coffee production and worldwide coffee losses have been estimated to approximately 15% (Campos & Villain, 2005). Several plant-parasitic nematodes species are associated with coffee cultivation. The major species affecting coffee are *Meloidogyne* spp. and *Pratylenchus* spp. (Villain *et al*., 2000; Barbosa *et al*., 2004). Many other genera, however, have also been found associated with coffee trees worldwide (Campos and Villain, 2005). In Nigeria, there is a paucity of information regarding plant-parasitic nematode species associated with coffee. Hence, a survey of the major coffee-growing region of the nation becomes imperative. This will provide a good picture of the distribution of nematodes in different locations where coffee is grown and assist in identifying coffee cultivars resistant to these plant-parasitic nematodes. Therefore, an extensive survey of the coffee production areas of the country was conducted to obtain information on potentially harmful plant-parasitic nematodes associated with coffee species, both in soil and in roots.

MATERIALS AND METHODS
Nematode survey
Nematological surveys were carried out in seven localities in six States with coffee farms, experimental fields or germplasm plots, representative of the coffee producing areas of Nigeria in 2010 and 2011. These include: Kusuku (Taraba State) where *Coffea arabica* (Arabica coffee) is

grown, while Ajassor, Okundi (Cross-River State), Uhonmora (Edo State), Kabba (Kogi State), Owena (Ondo State) and Ibadan (Oyo State) grow *Coffea canephora* (Robusta coffee). A total of 320 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. The altitude of the sampled areas were 1500, 122, 121, 140, 440, 178 and 122m above sea level for Kusuku, Ajassor, Okundi, Uhonmora, Kabba, Owena and Ibadan, respectively.

Processing of soil and root samples

Aliquots of 250cm^3 sub-sample soil from 500cm^3 each composite sample were assayed for nematodes by sieving and decanting (Cobb, 1918). After decanting, the sediment was assayed for nematodes using the Whitehead and Hemming (1965) tray modification of Baermann (1917) technique as described by Coyne *et al*. 2007. 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 resulting homogenate using sieve method (Speijer and De Waele, 1997). 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 (Doncaster, 1962) under a stereomicroscope and expressed per 250cm^3 soil or 5 g 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 (Hunt *et al*., 2005) and counted. The identification and counting was repeated three times and mean population of nematodes/sample calculated.

Pathogenicity tests

Coffea arabica and C. canephora seedlings collected from the locations were allowed to stabilize in the sreenhouse for two weeks and thereafter subjected to pathogenicity tests. The pots with the coffee seedlings were inoculated with 5,000 *Meloidogne incognita* eggs obtained from the pure culture on the roots of *Celosea argentea* using Hussey and Barker (1973) sodium hypochlorite (NaOCl) method. Uninoculated units served as control. Normal watering of seedlings as obtains in coffee nurseries was carried out. Fortnightly, growth parameters such as plant height, 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 (Sasser *et al*., 1984). Nematode eggs were collected from each root system using sodium hypochlorite method (NaOCl) of Hussey and Barker (1973) and counted. Aliquots of 250cm^3 soil samples from each pot were assayed for juveniles of *M. incognita* using the modified Baermann technique (Coyne *et al*., 2007).

Data analysis

Prior to statistical analyses, data were checked for normality and homogeneity of variances, and transformed where necessary. A log transformation [log10(x + 1)] was applied to the data on nematodes (densities per 250cm^3 soil, densities per gram root and gall index).

The data were analyzed according to the prevalence of nematode population based on four factors; frequency, density, prominence value and disease incidence. The frequency of the nematode species was determined from the relationship between the numbers of samples in which the nematode species was observed divided by the total number of sample taken from that area or plants, multiplied by 100 to express as a percentage (Sawadogo *et al*., 2009). The population density of the nematode species was expressed as the population of nematode species in fixed volume of soil or root. 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 (Taylor and Sasser, 1978, Sasser *et al*., 1984). Reproduction factor of the nematode (Rf) = final nematode population (pf) × initial nematode population (pi) $^{-1}$. 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 AND DISCUSSIONS

Plant-parasitic nematodes were recovered from 82 out of the 320 collected root samples and from all 320 soil samples. Fourteen genera of plant-parasitic nematodes were recovered from the rhizosphere soil. The endoparasitic nematodes, *Meloidogyne* spp., *Pratylenchus coffeae* and *Rotylenchulus reniformis* were predominant and occurred in 82, 51 and 5% of all soil samples per 250 cm^3 soil, respectively (Tables 1 & 2). *Rotylenchulus reniformis* was recovered only from the highland Kusuku soil with 43% frequency of occurrence (Table 2). Ectoparasitic species identified from soil samples were mainly represented by *Helicotylenchus coffeae*, *Xiphinema* spp. and *Radopholus* spp. Other ectoparasites found were *Criconemoides xenoplax*, *Scutellonema brachyurus* and *Trichodorus* spp. Most of the detected species are among the most commonly observed and destructive nematodes on coffee (Campos and Villain, 2005). Although ecto- and semi-endoparasitic nematodes are reported associated with coffee by many surveys, they are considered of minor importance to coffee (Souza, 2008). However, *R. reniformis* was reported to cause damage to coffee in India (Anon., 1966), the Philippines (Valdez, 1968), in Brazil (Lordello, 1980), in New Guinea, Fiji and Western Samoa (Bridge, 1988). In our survey, *R. reniformis* was widespread and frequently encountered only from highland Kusuku soils and not from the roots.

TABLE 1. Distribution of plant parasitic nematodes associated with coffee in Nigeria

Nematode species	Locations						
	Kusuku	Ajassor	Okundi	Uhonmora	Kabba	Owena	Ibadan
Meloidogyne spp.	+++	+++	+++	+++	+++	+++	+++
Helicotylenchus coffeae	+++	+++	+++	+++	+++	+++	+++
Xiphenema spp.	+++	++	++	++	++	+++	+++
Criconemoides xenoplax	o	+	+	+	++	+	+
Pratylenchus coffeae	+++	+++	+++	++	+++	++	+++
Scutellonema brachyurus	o	++	++	++	+	++	++
Hemicycliophora spp.	o	++	++	+	+	++	++
Radopholus spp.	+++	+++	+++	+++	+++	+++	+++
Rotylenchulus reniformis	+++	o	o	o	o	o	o
Trichodorus spp.	o	++	++	++	+	++	++

o = not recorded; + = present in survey; ++ = common; +++ = widespread.

Meloidogyne spp. and *Pratylenchus coffeae* were the only plant-parasitic nematodes recovered from coffee roots during the survey. *Meloidogyne* spp. was present in root samples from all the 7 localities, while *Pratylenchus coffeae* occurred in root samples from 5 localities, but was present in soil samples from the 7 localities (Table 2). This is in agreement with the earlier submissions that *Meloidogyne* (root-knot nematodes) and *Pratylenchus* (root lesion nematodes) are the predominant genera and are widely distributed in coffee plantations, causing great economic losses to both farmers and industry (Villain, 1991; Bertrand *et al*., 1995; Campos and Villain, 2005). Damage by these nematodes have been reported throughout the world including Latin America (Villain *et al*., 2002; Campos and Villain, 2005), Vietnam (Trinh *et al*., 2009) and Central America (Salas and Echandi, 1961; Schieber and Soza, 1960; Fernández, 1968), where nematodes of these two genera are often found parasitizing the coffee trees simultaneously (Villain *et al*., 2002). Both genera were found to attack coffee roots simultaneously, *Meloidogyne* being the most common (Herrera, 2011). *Meloidogyne* species were recovered from coffee roots in all the locations covered in the survey. Predominance of *Meloidogyne* species over *P. coffea* has also been reported from coffee plantations in Guatemala (Hervé *et al*., 2005). Root-knot nematodes, *Meloidogyne incognita*, suppressed the growth of coffee seedlings significantly ($P<0.05$) 24 weeks after inoculation in the nursery. The nematode reproduced successfully on both *C. arabica* and *C. canephora*

TABLE 2. Frequency of occurrence of plant-parasitic nematodes recovered from soils and roots of coffee in selected coffee localities in Nigeria.

Nematode species	Frequency of occurrence (%) at various locations						
	Kusuku	Ajassor	Okundi	Uhonmora	Kabba	Owena	Ibadan
Meloidogyne spp.	82* (21) #	86 (20)	84 (18)	76 (4)	72 (25)	87 (29)	89 (30)
Helicotylenchus coffeae	45 (-)	41 (-)	59 (-)	49 (-)	63 (-)	51 (-)	53 (-)
Xiphenema spp.	46 (-)	20 (-)	23 (-)	18 (-)	26 (-)	37 (-)	34 (-)
Criconemoides xenoplax	- (-)	2 (-)	1(-)	1 (-)	12 (-)	1 (-)	1 (-)
Pratylenchus coffeae	58 (36)	37 (12)	34 (8)	17 (-)	41 (5)	10 (-)	31 (3)
Scutellonema brachyurus	- (-)	14 (-)	18 (-)	9 (-)	1 (-)	12 (-)	7 (-)
Hemicycliophora spp	- (-)	8 (-)	9 (-)	2 (-)	1 (-)	10 (-)	13 (-)
Radopholus spp.	34 (-)	44 (-)	32 (-)	38 (-)	49 (-)	41 (-)	32 (-)
Rotylenchulus reniformis	43 (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)
Trichodorus spp	- (-)	12 (-)	10 (-)	8 (-)	1 (-)	7 (-)	11 (-)

with reproduction factors of 15.1, and 16.4, respectively (Table 3). Galls typical of root-knot nematodes were observed on the roots of the inoculated seedlings. Root-knot nematode (*Meloidogyne* spp.) represents the major threat in all major coffee-growing (*Coffea arabica* L.) areas throughout the world. *M. exigua and M. incognita* are regarded as the most damaging species on coffee in Latin America (Carneiro and Cofcewicz, 2008). Once root-knot nematodes are established in a coffee plantation, they build up over time and certain conditions such as type and content of organic matter, can increase the nematode population (Waller *et al*., 2007). Coffee varieties cultivated in Nigeria are susceptible to root-knot nematodes, as it was reported in Central American countries (Bertrand *et al*., 2000). Chemical control, although the most reliable means to control root-knot nematodes, is expensive, with a minimal effectiveness when the symptoms of nematodes are already observed. These chemicals are also toxic to humans and environment (Villain *et al*, 2008). There is, therefore, the need to develop new strategies to control plant-parasitic nematodes on coffee through integrated management practices and tolerant coffee varieties (Bertrand *et al*., 2000).

TABLE 3. Effects of root-knot nematode, *Meloidogyne incognita*, on the growth of coffee seedlings.

Coffee seedlings	Plant height* (cm)	No of leaves (count)	Stem girth (cm)	GI/EMI (count)	Reproduction factor (Rf) (pf/pi) #
C. arabica	14.20b	15.13a	0.38b	4/4	15.1
C. canephora	12.67c	15.33a	0.36b	4/4	16.4
Un-inoculated	19.34a	15.14a	0.51a		

* 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.

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