DESCRIPTION OF PLANT PARASITIC NEMATODES ASSOCIATED WITH COFFEE IN ETHIOPIA

T. Mekete1,2*, R.A. Sikora2, S. Kiewnick3 and J. Hallmann1

1Federal Biological Research Center for Agriculture and Forestry, Institute for Nematology and Vertebrate Research, Toppbeideweg 88, D-48161, Münster, Germany
2Institute for Crop Science and Resource Conservation (INRES), Nematology in Soil Ecosystems, University of Bonn, Naßallee 9, D-53115 Bonn, Germany
3Agroscope Changins-Wädenswil, Plant Protection and Ecotoxicology, P.O.Box 1085, CH-8820 Wädenswil, Switzerland

Summary. A nematode survey was conducted in the major coffee (Coffea arabica) agroecologies of Ethiopia during 2004 and 2006. Soil samples were collected from 132 different coffee plantations and examined in the laboratory. Plant parasitic nematodes of the orders Tylenchida and Dorylaimida were examined taxonomically. Four species of Helicotylenchus (H. dihystera, H. multicinctus, H. californicus and H. gerti), one of Scutellonema (S. paralabiatum), one of Rotylenchus (R. unisexus), one of Tylenchorhynchus (T. agri), one of Quinisulcius (Q. capitatus), Xiphinema insigne, X. basilgoodeyi and two non-identified populations of the Xiphinema americanum group were found. Detailed descriptions, including morphometrics, illustrations and key features are given for each species. Scutellonema paralabiatum, Tylenchorhynchus agri, Q. capitatus, Xiphinema insigne, X. basilgoodeyi and X. americanum-group are first records for Ethiopia.

Key words: Coffea arabica, Helicotylenchus spp., Quinisulcius sp., Rotylenchus sp., Scutellonema sp., Tylenchorhynchus sp., Xiphinema spp.

Coffee (Coffea arabica L.) is an important cash crop worldwide. In Ethiopia, the place of its origin, the agriculture-based economy is highly dependent on coffee production and export, contributing 35 percent of the country’s foreign currency earnings (Woods, 2003). Due to its important economic role, all possible measures should be taken to ensure high yield and quality. Worldwide, plant-parasitic nematodes are among the main constraints reducing coffee yield. This is particularly true for root-knot nematodes, Meloidogyne spp. The most important root-knot nematode species in terms of their damage and distribution are M. exigua Goeldi, M. incognita (Kofoid et White) Chitw., M. coffeicola Lordello et Zamith and M. paranaensis Carneiro, Carneiro, Abrantes, Santos et Almeida. Other species with local importance include M. africana Whitehead, M. decalineata Whitehead, M. megadora Whitehead, M. arabidicida López et Salazar, M. javanica (Treub) Chitw., M. arenaria (Neal) Chitw. and M. hapla Chitw. (Campos et al., 1990; Campos and Villain, 2005).

In Ethiopia, very little information is available on plant-parasitic nematodes. Since 1975, only few plant-parasitic nematodes from various crops have been reported. Among them are Meloidogyne incognita, M. javanica, M. ethiopica Whitehead and Pratylenchus goodeyi Sher et Allen (O’Bannon, 1975; Godfrey et al., 1988; Abebe and Geraert, 1995; Swart et al., 2000; Wondirad and Tesfamariam, 2002; Bogale et al., 2004; Van Den Berg et al., 2004; Marais et al., 2005). As there is almost no information on nematodes in coffee, the objective of this study was to characterize the spectrum of plant-parasitic nematodes associated with coffee in Ethiopia. Therefore, a survey was conducted during the wet season in August 2004 and dry season in April 2006 to describe nematodes of the orders Tylenchida and Dorylaimida in soil samples taken from the rhizosphere of coffee.

MATERIALS AND METHODS

Soil samples were taken from 132 field sites in different coffee growing agro-ecologies of Ethiopia. Each 1-1.5 kg sample consisted of four to ten cores taken from the top 25-30 cm of soil around coffee trees with a spade. The samples were kept in plastic bags in an ice box during transportation and taken to the laboratory for nematode extraction.

Nematodes were extracted from 200 g soil sub-samples by combining the Cobb’s sieving, decanting method with a modified Baermann’s funnel method (Hooper, 1985a). Then they were killed in hot water at 65 °C and fixed in a formal acetic acid fixative (FA 4:1) solution of 10 ml formalin, 1 ml glacial acetic acid, 2 ml glycerol and distilled water up to 100 ml (Hooper, 1985b). The nematode specimens were dehydrated through the rapid glycerin method and mounted in pure glycerin on glass slides supported by glass rod of a diameter slightly larger than that of the nematodes (Hooper, 1985b).

Morphometric analyses of the nematode specimens were made using light microscopy and image analyzing software Leica IM 500 Version 1.20 release 19. The
species were identified using various keys and descriptions by various authors.

RESULTS AND DISCUSSION

The species identified are reported hereafter, grouped by genus, and their main characteristics are described.

**Helicotylenchus Steiner, 1945**

The genus *Helicotylenchus*, as reviewed by Firoza and Maqbool (1994), is classified to species level by a combination of 16 diagnostic characters: habitus, body length, a, c, c', V%, position of dorsal ossephageal gland opening, stylet length, shape of stylet knobs, head shape and annulation, tail shape and annulation, phasmid distance from anus expressed in terms of number of cuticular annules, presence and absence of males, and female posterior genital branch. In our studies, four species were identified from different agroecologies of coffee, namely: *H. dibystera*, *H. multicinctus*, *H. californicus* and *H. gerti*.

**Helicotylenchus dibystera** (Cobb, 1893) Sher, 1961

Females assume a spiral body shape; lip region is hemispherical with 4-5 annules. Cephalic region well developed extending 3-4 annules posteriorly; stylet well developed, 23-29 µm long; stylet knobs rounded posteriorly and hooked anteriorly. Dorsal ossephageal gland opening (DGO) 10-17 µm posterior to the base of the stylet. Reproductive system is didelphic with opposed branches; intestine does not extend beyond rectum. Tail dorsally convex-conoid with broadly pointed hyaline terminus (Fig. 1A); phasmsids located 5-8 annules anterior to anus.

**Measurements** (Female, n = 42). L = 0.6-0.8 mm (0.7 ± 0.1), a = 20.8-32.3 (26.9 ± 2.3), c = 37.7-72.2 (50.3 ± 6.8), c' = 0.8-1.4 (1.0 ± 0.1), O = 38.6-60.1 µm (50.1 ± 5.7), V% = 60.3-65.8, stylet length = 22.5-29.1 µm (26.7 ± 1.8).

The morphological and morphometric characters are in line with descriptions by Siddiqi (1972), Van Den Berg and Heyns (1975), Van Den Berg and Kirby (1979) and Firoza and Maqbool (1994).

Specimens of this species were collected from coffee plantations in all field sites of four different sampling locations at Shebe, Agaro, Mechara and Metu. *Helicotylenchus dibystera* is especially known as a parasite of several tropical plants such as rice, sugarcane, maize and banana (Coyne and Plowright, 2002). It has been reported as a minor pest of coffee in India (Giribabu and Saha, 2003). Detailed studies on host ranges and damage to crops were recorded by Sher (1966), Siddiqi (1972) and O’Bannon and Inserra (1989).

**Helicotylenchus multicinctus** (Cobb, 1893) Golden, 1956.

Female body C-shaped; hemispherical lip region, slightly offset with 3-5 annules and sclerotized frame-work. Reproductive system is didelphic with opposed branches; spermatheca rounded and slightly offset; intestine does not extend beyond rectum. Tapering tail with a hemispherical annulated terminus (Fig. 1B); no mucro or any ventral projection; pore-like phasmid, 1-6 annules anterior to anus.

**Measurements** (Female, n = 13). L = 0.6-0.8 mm (0.7 ± 0.1), a = 21.4-34.4 (31.2 ± 3.2), c = 33.4-57.2 (47.6 ± 6.5), c' = 0.9-1.6 (1.3 ± 0.2), O = 0.6-0.8 µm (0.6 ± 0.1), stylet length = 23-28 µm (26.2 ± 1.5).

These specimens correspond well with various descriptions by Sher (1966) and Siddiqi (1973). Damage to crops by this species alone and in association with other species is reported in Costa Rica, Panama and India, causing considerable yield reduction in banana and as a minor pest on coffee (Siddiqi, 1973). This species was collected from five sampling locations at Abella-Morroco, Limu, Apollo, Jimma and Hagere Selam.

**Helicotylenchus californicus Sher, 1966**

Body curved ventrally, lip region rounded, not set off, with 4-5 distinct annules. Well developed stylet. Epitypyna not seen. Spermatheca distinct and filled with sperms. Intestine does not extend beyond rectum. Tail region with ventral projection (Fig. 1C and D). Phasmid located 4-10 annules anterior to anus.

**Measurements** (Female, n = 12). L = 0.6-0.8 mm (0.7 ± 0.18), a = 24.0-32.3 (27.7 ± 2.1), c = 46.3-53.8 (49.2 ± 2.5), c' = 0.8-1.3 (1.1 ± 0.1), V% = 58-63 (61 ± 2.2), DGO = 11.5-16.8 µm (14.5 ± 1.9), O = 43.4-59.9 µm (53.7 ± 4.5), stylet length = 25.4-29 µm (27.1 ± 1.3).

These specimens agree with descriptions by Sher (1966) and Van Den Berg and Heyns (1975). Specimens of this species were collected from the rhizosphere of coffee in four sampling locations at Areka, Jimma-Seka, Bonga and Agaro. To our knowledge there is no report of damage caused by this species to coffee.

**Helicotylenchus gerti Marais, Mekete et Tiedt, 2005**

Body spiral; lip region hemispherical, not set off, with 3-4 annules; well developed stylet. Epitypyna folded into vagina (n = 4); reproductive system didelphic with opposed branches. Intestine does not extend beyond rectum. Non-annulated, digitate ventral tail projection (Fig. 1E); phasmid located in middle of lateral field.

**Measurements** (Female, n = 7). L = 622.3-649.3 µm (637.4 ± 9.9), a = 28.2-31.4 (29.7 ± 1.2), c = 40.1-57.2 (47.7 ± 6.7), c' = 1.1-1.3 (1.2 ± 0.1), DGO = 10.4-13.8 µm (12.2 ± 1.4), stylet = 23-25 µm long.

These specimens are similar to those described by Marais et al. (2005), in the only previous report for Ethiopia. So far, there is no evidence that *H. gerti* causes damage to coffee or any other crops. Specimens of this species were collected from four sampling locations at Chelenko, Chena, Aletawondo and Aposto.

**Scutellonema Andrassy, 1958**

Identification of the genus *Scutellonema* was done
following the key developed by Sher (1963). *Scutellonema paralabiatum* is the only species of this group found associated with coffee in Ethiopia.

**Scutellonema paralabiatum Siddiqi et Sharma, 1994**

Body ventrally curved; maximum body length 0.83 mm; cephalic region not offset, slightly tapering to a flat disc (Fig. 2A), with 4-6 annules; framework strongly sclerotized, with outer margins extending into body; stylet with rounded knobs; spermatheca not developed; intestine does not extend beyond rectum, tail rounded to hemispherical (Fig. 2D); lateral field not areolated in scutellum (Fig. 2E).

*Measurements* (Female, n = 29). $L = 0.68-0.83$ mm ($0.75 \pm 0.03$), $a = 26.4-31.4$ (28.5 ± 1.19), $b = 4.29-20.98$ ($5.81 \pm 3.26$), $c = 62.83-103.84$ (86.85 ± 12.79), $c' = \ldots$

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**Fig. 1.** Photomicrographs of *Helicotylenchus* species associated with coffee from Ethiopia. A, rounded tail with terminal projection of *H. dihystera*; B, hemispherical annulated tail terminus of *H. multicinctus*; C and D, irregular tail projections of *H. californicus*; E, tail with non-annulated ventral projection of *H. gerti*; F, dorsal gland opening to show distance from stylet end.
0.39-0.65 (0.49 ± 0.06), O = 5-27 µm (10.38 ± 7.43), stylet = 21-27 µm long, DGO 4-7 µm behind stylet knobs, scutellum diameter = 3-5 µm (3.68 ± 0.36); tail length = 6-12 µm (8.66 ± 1.37).

This species was originally described from pigeon pea in Kenya and found also in sweet potato in Uganda (Siddiqi and Sharma, 1994). The epiptygma projected outwards from the vulva in only a few specimens; otherwise, in most of the specimens, the epiptygma is single and not projected (Fig. 2B). This is the first report of this species from Ethiopia. Specimens of this species were found associated with coffee plants at Agaro, Bonga, Wushwush, Mizan, Areka-Hossaina, Areka, Boditi-Shone, Alewendo and Abea-Morrocho.

**Rotylenchus Filipjev, 1936**

The identification of species of the genus *Rotylenchus* was done following the tabular key developed by Castillo and Vovlas (2005). The most important differential and diagnostic characters used are: lip annulation, lip region shape, lateral field areolation, body longitudinal striations, tail shape, separation of lip region, stylet length and vulval position. Only one species was found.

**Rotylenchus unisexus Sher, 1965**

Specimens of this species have 4-5 lip annules, lateral field areolation only in the oesophasus region. Lip region hemispherical, contiguous with body contour. Labial framework well developed (Fig. 3A). Vulva 50-70% of body length. Tail 15-23 µm long with hemispherical shape (Fig. 3C).

Measurements (Female, n = 12). L = 0.84-1.02 mm (0.85 ± 0.23), a = 28.44-34.15 (31.23 ± 2.04), b = 5.99-10.18 (7.81 ± 1.40), b' = 4.6-9.92 (8.25 ± 1.33), c = 38.66-61.16 (47.36 ± 6.85), stylet length = 24.6-29.1 µm (26.7 ± 1.02), DGO = 4.7 µm, O = 0.16-0.25 µm (0.19 ± 0.03).

*Rotylenchus unisexus* was originally described from Rhodes grass in Kenya (Sher, 1965). Our specimens fit the original and various descriptions of the species very well. They were collected from ten sampled locations at Mechara, Mesela, Jimma-Seka, Tmenja-Yaje, Mizan, Tepi, Metu, Hossaina, Areka, Aletawendo.

**Tylenchorhynchus Cobb, 1913**

The genus *Tylenchorhynchus* was established by Nathan A. Cobb in 1913 when he described *T. cylindricus* found in soil from reclaimed coastal swamplands in southern California. The original genus has now been subdivided into several others. One of the most important characters used in distinguishing genera previously considered as *Tylenchorhynchus* is the number of lines in the lateral field, ranging from three to six. The species currently considered to be of the genus *Tylenchorhynchus* are those having four lines in the lateral field. Tarjan (1973) gave a valuable key and a table of diagnostic data of species. Only one species, *T. agri*, was found during our survey.

**Tylenchorhynchus agri Ferris, 1963**

Lateral field with four lines. Excretory pore opens at a level of anterior end of basal oesophageal bulb (Fig. 4B). Hemizonid 1-2 annules anterior to excretory pore and clavate tail shape (Fig. 4E).

Measurements (Female, n = 7). L = 0.56-0.74 mm (0.63), a = 28.5–33.4 (30.5), c = 12.9-16.2 (14.7), V% = 52.9-56.9 (55.4), stylet = 14-21 µm long.

*Tylenchorhynchus agri* was originally described from corn fields of the University of Illinois, USA, in 1963 (Ferris, 1963). Specimens from Ethiopian coffee fit the original descriptions of the species well. This is the first report of this species from Ethiopia. Specimens of this species were found associated with coffee plants from Bedessa, Mechara, Hosaina, Shebe, Aposto, Bonga, Wushwush and Teppi.

**Quinisulcius Siddiqi, 1971**

The genus *Quinisulcius* was established by Siddiqi, 1971. One of the most important characters used in dis-
Distinguishing this genus is the presence of five lateral lines (Siddiqi, 2000). In our survey we found only the species \textit{Q. capitatus} (Allen, 1955) Siddiqi 1971.

\textbf{Quinisulcius capitatus (Allen, 1955) Siddiqi 1971}

Lateral field with five lines, ends near the tail terminus; stylet slender with well developed knobs; oval-shaped median oesophageal bulb; tail conoid bluntly pointed with a characteristic enlarged tip with annules (Fig. 4F).

\textit{Measurements} (Female, \(n = 10\)). \(L = 0.63-0.79\) mm \((0.74)\), \(a = 30.9-38.6\) \((34.0)\), \(c = 15.3-17.6\) \((16.4)\), \(V\% = 54.7-63.6\) \((57.5)\), stylet = 15-18 \(\mu\)m long, DGO = 3-4 \(\mu\)m behind the styled base.

\textit{Quinisulcius capitatus} was first described as \textit{Tylenchorhynchus capitatus} by Allen in 1955, later as \textit{T. acti} by Hopper in 1959 and finally transferred to the genus \textit{Quinisulcius} by Siddiqi in 1971 (Siddiqi, 2000). Specimens from Ethiopian coffee fit the original descriptions of the species well. They were found in three sampling locations at Mechara, Wendo Genet and Dire Dawa. This is the first report of this species from Ethiopia.

\textbf{Xiphinema Cobb, 1913}

The genus \textit{Xiphinema} Cobb (1913) as revised by Loof and Luc (1990) has a total of 213 nominal species, including 172 valid species, 25 synonymised species, thirteen species inquirendae and three transferred to other genera. Species belonging to this genus can cause damage to many economically important crops by direct feeding on their roots and some by transmitting plant viruses.

The specimens were identified using the polytomous keys of Loof and Luc (1990) and Lamberti \textit{et al.} (2004). The morphology and morphometric descriptions were compared with previous descriptions of the same species from different parts of the world (Coomans, 1965; Loof and Maas, 1972; Luc and Southey, 1980).

Several populations of \textit{Xiphinema} belonging probably to four different species were found. Two of them are \textit{Xiphinema insigne} and \textit{X. basilgoodeyi}, whose morphological and morphometric characters are discussed and compared with reports in the literature. These two species are reported for the first time from Ethiopia.

In our survey we have also found two species of the \textit{X. americanum}-group. \textit{Xiphinema americanum} is considered to be a complex of more than 51 species, many of them difficult to identify. Species of this group are characterized by a rather small body length of less than 2.2 mm, vulva position between 50 and 56\%, short tail, and bacteria-like inclusions in the genital tract (Lamberti \textit{et al.}, 2000).

\textbf{Xiphinema insigne Loos, 1949}

Body habitus an open C when heat-relaxed; body average length of 2.51 mm; lip region separated from body by weak depression; odontostyle robust; odontophore flanged; guide ring typical of the genus; cuticle smooth, two layers; vulva situated at about 34\% of the body length; genital tracts amphidelphic and reflexed; well developed anterior genital tract but small in size; Z-organ absent. Tail long, conoid, ventrally arcuate and tail tip sharply curved with two lateral pores (Fig. 5A). Juveniles have body habitus and tails as in adult female (Fig. 5G). Male not found.

\textit{Measurements} (Female, \(n = 15\)). \(L = 2.34-2.70\) mm
(2.48), a = 55.79 (50.53-62.50), c = 16.98 (16.26-18.39),
c’ = 7.03 (6.23-7.52), V% = 34.37 (32.45-38.31), odontostyle = 111.18 µm (n = 7) (108.5-114.0), odontophore = 60.3 µm (n = 7) (56 -65), total stylet length = 171.48 µm (167-176).

The Ethiopian *X. insigne* population is not different from populations described from different parts of the world (Loof and Maas, 1972; Luc and Southey, 1980; Loof and Luc, 1990). This species has a worldwide distribution and was first reported from soil around the roots of soursop, coconut and grasses from Kurengala, Ceylon. *Xiphinema insigne* is a highly variable species with different forms. These forms are regarded primarily as an expression of geographical variation. Tarjan and Luc (1963) reported great variation in tail length of *X. insigne*. The Ethiopian *X. insigne* population has a long tail compared with other descriptions. The only population with comparable tail length to the Ethiopian population is one from Malawi (Luc and Southey, 1980). This is the first report of this species from Ethiopia. It was found in 13 sampling locations at Mechara, Agaro, Jimma-Seka, Bonga, Wushwush, Chena, Temenja Yaje, Mizan, Tepi, Gore, Metu, Areka and Aleta Wondo.

*Xiphinema basilgoodeyi* Coomans, 1965

Cuticle with two layers, lip region continuous with the body; oesophago-intestinal junction consists of a small conically rounded structure. Female gonads didelphic and reflexed. Tail ventrally curved with small peg 9.3 µm (8.5-10) long (Fig. 5B). Juvenile body habitus

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**Fig. 4.** Photomicrographs of *Tylenchorhynchus* and *Quinisulcius* spp. associated with coffee from Ethiopia. A, slender stylet with well developed knobs inclined posteriorly and DGO opening of *T. agri*; B, oval-shaped median bulb; C, lateral fields with four lines of *T. agri*; D, lateral field with five lines of *Q. capitatus*; E, clavate tail shape of *T. agri*; F, conoid tail of *Q. capitatus*.
and tails similar to adult female. Male not found.

Measurements (Female, n = 9). L = 3.01 mm, a = 48.15 (40.69-53.52), b = 13.81 (8.03-16.57), V% = 46.7 (42.1-52.6), odontostyle = 128.33 µm (125-132), odontophore = 79.22 µm (76-83), stylet length = 207.96 µm (216-191), thickness of body cuticle = 2.9 µm (2.3-3.7).

All morphometric measurements coincide with the original descriptions with the exception of the high b ratio. Even though there are some reports of the presence of *X. basilgoodeyi* in coffee plantations, we could not find any morphometric description of the species other than the original description by Coomans (1965). This species was first reported from soil around the roots of coffee from Congo. We found this species in only one sample site, at Gore Masha, a semi-forest coffee plantation type. This is the first report of this species from Ethiopia.

*Xiphinema americanum 'a'*

Body ventrally more curved in posterior than in anterior part; lip region continuous, cylindrical. Odon-
tostyle, odontophore and guide apparatus typical for the *X. americanum*-group. Female reproductive system amphidelphic, both branches equally developed. Ovaries filled with bacteria. Tail conoid, terminus bluntly rounded (Fig. 5C). Specimens of this species were collected from the rhizosphere of coffee in just one location at Bedele and are the first report from Ethiopia.

Measurements (Female, n = 5). L = 2.22 mm, odontostyle = 97-100 µm; odontophore = 72-75.2 µm long, V% = 52.2-56, tail length = 21.7-23 µm. Second stage juveniles (n = 2): L = 364 µm, total spear length = 24 µm.

*Xiphinema americanum 'b'*

This species can be distinguished from the other species of the *X. americanum* group by its short odontostyle and conoid tail shape (Fig. 5D).

Measurements (Female, n = 3). L = 1.6-1.8 mm, odontostyle = 76-89 µm, odontophore = 42-51 µm long, V% = 49.8-54, tail length = 20-21 µm. Juveniles and males not found.

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**Fig. 5.** Photomicrographs of *Xiphinema* spp. associated with coffee from Ethiopia. A, ventrally arcuate long conoid tail of *X. insignis*; B, ventrally curved tail of *X. basilgoodeyi* with small peg; C, dorsally convex tail with bluntly rounded terminus of *X. americanum 'a'*; D, tail shape of *X. americanum 'b'*; E-G, Body habitus of *Xiphinema* species. Scale bars: A-D = 25 µm; E-G = 200 µm.
Specimens of this species were collected from two sampling locations at Apostle and Wendogenet and are the first report for Ethiopia.

For both populations of the *X. americanum*-group, the species identity requires confirmation and detailed descriptions. In our survey, only a small number of specimens was recovered, which made the identification of the species difficult.

CONCLUSIONS

Many genera and species of plant-parasitic nematodes have been found associated with coffee in many countries, including very damaging nematodes causing great losses to the coffee farmers and the local economy of developing countries (Campos et al., 1990; Campos and Villain, 2005). The most important and widely distributed nematodes in coffee plantations are species of *Meloidogyne* and *Pratylenchus* (Bertrand et al., 1995; Campos and Villain, 2005). Although information about their damage potentials is lacking, species of the genera *Helicotylenchus*, *Hoplolaimus*, *Criconemella*, *Longidorus*, *Trichodorus*, *Paratrichodorus*, *Scutellonema*, *Xiphinema*, *Rotylenchulus*, *Tylenchorhynchus* and *Ogma* have been reported associated with coffee (Campos and Villain, 2005).

In neighbouring eastern African countries, plant-parasitic nematodes are a major problem on coffee. A unique group of root-knot nematode species, known as the African coffee root-knot nematodes, also occur in Africa, causing severe root damage to coffee in Tanzania and Kenya. The species identified from the areas sampled are *Meloidogyne africana*, *M. decalineata*, *M. kikuyensis* and *M. megadora* (Swai, 1981; Bridge, 1984; Campos and Villain, 2005). In Tanzania, sixteen genera of plant-parasitic nematodes were recorded from coffee, but only three of plant-parasitic nematodes were recorded from coffee, *Meloidogyne* spp., namely *M. decalineata*, *M. africana* and an unidentified species, were considered important. A survey in Tanzania revealed a minimum of 20% loss attributable to nematode infection in coffee plantations in the northern coffee districts (Bridge, 1984).

However, during this survey, we did not recover the most economically important endoparasitic and semi-endoparasitic nematodes. Whether the ectoparasitic nematodes that we did find have the potential to damage coffee in Ethiopia needs to be ascertained.

LITERATURE CITED


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