African Journal of Agricultural Research Vol. 5(14), pp. 1889-1896, 18 July, 2010
Available online at http://www.academicjournals.org/AJAR
ISSN 1991-637X ©2010 Academic Journals

Full Length Research Paper

Occurrence and distribution of soil nematodes in cotton (*Gossypium hirsutum* L.) production areas of Kenya

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Accepted 24 June, 2010

A baseline survey was conducted to determine the occurrence and distribution of soil nematodes associated with cotton in major growing areas in Kenya. Such baseline data on soil nematode abundance, diversity and ecosystem function in cotton ecosystems are valuable in providing a basis for comparison with organisms from transgenic cotton fields. Transgenic cotton plants expressing Cry1Ac and Cry2Ab proteins, from the soil bacterium *Bacillus thuringiensis* (Bt), provide effective control of lepidopteran pests. However, the potential effects of these proteins on soil nematofauna are unknown in Kenya. Soil samples were collected from nine locations of western (Odiado, Angorom and Ochundo locations), coast (Baharini, Mpeketoni and Witu locations) and central (Kajiji, Tebere and Nyangati locations) Province. Nematodes were extracted and recovered from soil samples using the Whitehead and Hemming tray method and identified under a light microscope according to their morphological characters. They were classified according to their feeding habits. Twenty seven genera of plant parasites, bacteriovores, fungivores, predators and omnivores were identified. Bacterial, fungal feeding and parasitic nematodes were the most abundant trophic groups across all Provinces. There were significant differences in the numbers of bacteriovores ($P \leq 0.01$) and plant parasites ($P \leq 0.05$) between the Provinces but no difference was observed in the numbers of fungal feeding nematodes. There was a significant difference in genus richness within locations in western and coast Provinces ($P \leq 0.001$). The combined maturity index ($\sum$MI) did not vary significantly within the locations. The Shannon index (H') showed variations within locations in western ($P < 0.001$) and coast Province ($P \leq 0.01$). Soil texture, P and K were correlated with abundance of some nematode genera. The bacteria feeders, *Acrobeles* and *Rhabditis* showed positive correlations to K ($r = 0.592$, $P < 0.05$ and $r = 0.128$, $P \leq 0.05$) and P ($r = 0.406$, $P \leq 0.05$, and $r = 0.252$, $P < 0.05$) while *Aphelenchus* was positively correlated to P ($r = 0.375$, $P \leq 0.05$). The plant parasitic genera *Meloidogyne* and *Pratylenchus* showed significant negative correlation to N ($r = -0.513$, $P \leq 0.05$ and $r = -0.226$, $P \leq 0.05$). It is clear from this baseline data that plant parasitic and free living nematodes are widespread in cotton fields and any potential effects of Bt cotton on these nematodes may affect the nematode community structure and their ecosystem functions.

Key words: Cotton, soil nematodes, survey.

INTRODUCTION

Soil species and their interactions can influence different ecosystem processes. Nematodes are abundant, diverse in all soils and participate in many functions at different levels of the soil food web (Yeates, 1979). Bacterial and fungal feeding nematodes release a large percent of nitrogen when feeding on their prey groups and are thus

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responsible for much of the plant available nitrogen in majority of soils (Bongers and Bongers, 1998). Cotton fields contain high nematode diversity that is influenced by cultural practices including tillage, use of pesticides and fertilizers (Koenning and Barker, 2004). In Kenya, cotton is mainly grown in the semi-arid regions of Eastern, Central, Nyanza, Coast, Western and Rift valley Provinces. It is an important fibre crop that provides a source of income to farmers and fibre to the textile industries. The seeds provide an important source of oil and feed cake for humans and livestock (Lutrell et al., 1994). For decades now, cotton production in Kenya has been characterized by low yields. Pest management in cotton accounts for about 57% of the total production cost (Ikiara and Ndirangu, 2003). Considering the environmental and human health concerns associated with high pesticide usage on cotton, there has been a strong need for more integrated approaches to pest management that minimize pesticide requirement. Transgenic crops expressing insecticidal protein genes from *Bacillus thurigensis* (Bt) represent one new approach for integrated pest management (IPM) of cotton (Fitt, 2003; Wilson et al., 2004).

Concerns about the impacts of genetically modified crops on soil biota have been raised, in part because of the chemical and biological properties of soil (McGregor and Turner, 2000). Soil materials have large sorptive capacities for biological molecules, including insecticidal bacterial proteins and DNA. Laboratory studies (Saxena and Stotzky, 2001; Saxena and Stotzky 2002; Tapp and Stotzky, 1998) have shown that insecticidal Cry proteins from Bt are readily adsorbed and bound to clay minerals and humic acids, and they persist in soil. Consequently, the issue of the impact of Bt proteins released to soil from roots and biomass of Bt crops on soil nematodes is an important one. Transgenic plants produce and release relatively large amounts of a variety of novel proteins, including the active toxins. Microbial processes have been shown to be particularly responsive to protein substrates (Wheatley et al., 2001). Changes in the root exudates of a transgenic plant may significantly alter the rhizosphere community associated with it (Brusetti et al., 2004). Bt proteins are present as active toxins in most of the cells of a Bt transformed plant and so are present in all plant residues, and may be released into the soil through various routes depending on the crop and environment (Mendonca et al., 2006). A baseline study to document species diversity and abundance for a particular ecosystem is crucial before cultivation of transgenic plants. Lack of baseline information on soil fauna, found in an agro-ecosystem, to compare with microorganisms in ecosystems containing transgenic crops have been cited as a major problem in evaluating the impact of transgenic crops on soil microbial diversity (Dale et al., 2002). Therefore, documentation of nematodes found in cotton ecosystems in different agro-ecological zones was carried out to provide a basis for comparison with nematodes from cotton fields cultivated with Bt cotton.

MATERIALS AND METHODS

Soils were sampled from cotton growing fields in nine locations of Western (Odiado, Angorom and Ochundo locations), Coast (Baharini, Mpeketoni and Witu locations) and Central (Kajiji, Tebere and Nyangati locations) Provinces of Kenya. Two composite samples (5 cm diameter, 30 cm deep) were independently collected per field. Two linear transects were randomly and diagonally located along rows of crops and composite samples were collected along these transects (Neher, 1999). The samples were thoroughly mixed and 200 ml of each soil samples was used for nematode extraction using Whitehead and Hemming tray technique. Nematode numbers were counted and identification to genus level was done under a compound microscope at a magnification of ×400 - ×1000.

Nematode genera were assigned to trophic groups (bacterial and fungal feeders, plant parasites, omnivores and predators) as described by Yeates et al. (1993). Taxonomic groups were also assigned to colonizer-persistor (cp) values according to Bongers (1990). Physical and chemical properties of soil were measured. Total nitrogen (N) was measured through Kjedahl method. Total organic carbon (C) was estimated through calometric method. Phosphorous (P) was analyzed by colomitery, potassium (K) and sodium (Na) by flame emission spectrophotometry, and calcium (Ca) and magnesium (Mg) by atomic absorption spectro-photometry (Gallaher et al., 1975). Cation exchange capacity (Jackson, 1958), pH, electrical conductivity (Smith and Doran, 1996) and soil texture (Gee and Bauder, 1985) were also analyzed.

The following nematode parameters were computed: Genus richness index (d=S-1) log N, where S = number of genera and N = total number of nematodes, abundance and Shannon Wiener's diversity index ($H'=-\sum p_i \log_2 p_i$).Maturity index for all free living nematodes except taxa with cp value of 1 (MINO), combined maturity index for free living and plant parasitic nematodes ($\sum MI$) and ratio of fungivorous to bacterivorous nematodes was also computed. Nematode data based on relative abundance of genera was subjected to analysis of variance. Relationships between soil chemical properties and nematode genera/indices were derived using correlation analysis. All statistical computations were performed using XLSTAT version 7.5 (Addin software, New York).

RESULTS

Twenty seven genera were identified in nine locations of Western (Odiado, Angorom and Ochundo locations), Coast (Baharini, Mpeketoni and Witu locations) and Central (Kajiji, Tebere and Nyangati locations) Province. The plant parasitic genera identified were *Meloidogyne, Pratylenchus, Trichodorous, Helicotylenchus, Rotylenchus, Hoplolaimus, Xiphinema, Tylenchus, Filenchus, Longidorous, Tylenchorhynchus* and *Scutollenema*, the bacteriovores consisted of *Acrobeles, Rhabditis, Cervidellus, Eucephalobus, Cephalobus, Heterocephalobus, Plectus, Wilsonema* and *Tylocephalus.* Two fungal genera (*Aphelenchus and Aphelenchoides)* were recorded. Nematodes from higher trophic groups in the genera *Labronema, Chromadora* and *Prodorylaimus* were also identified (Table 1). Only one genus (*Mononchus*) of predatory nematodes was observed.

Bacterial, fungal feeding and parasitic nematodes were the most abundant trophic groups across all Provinces. There were significant differences in the numbers of bacteriovores ($P \leq 0.01$) and plant parasites ($P \leq 0.05$)

Table 1. Nematode genera, cp values and abundance in Central, Coast and Western Province.

Nematode genera	cp value	Central Province	Coast Province	Western Province	ANOVA		
					Province	Genera	Province×Genera
Bacterial feeders							
Acrobeles	2	190.125	97.500	29.083			
Cephalobus	2	360.750	224.250	.000			
Cervidellus	2	124.313	372.750	.000			
Eucephalobus	2	124.313	.000	.000			
Heterocephalobus	2	131.625	338.813	.000			
Plectus	2	.000	4.875	.000			
Rhabditis	1	34.125	24.375	50.000			
Tylocephalus	2	19.500	87.292	.000			
Wilsonema	2	29.250	.000	.000			
Mean number of bacterial feeders		**112.660**	**127.76**	**8.79**	****1**	**	**ns**
Fungal feeders							
Aphelenchoides	2	58.500	282.750	4.167			
Aphelenchus	2	168.188	173.063	31.250			
Mean number of fungal feeders		**113.344**	**227.906**	**17.708**	**ns**	**ns**	**ns**
Plant parasites							
Filenchus	2	7.313	.000	.000			
Helicotylenchus	3	319.000	309.667	87.500			
Hoplolaimus	2	33.000	180.000	104.167			
Longidorous	5	17.063	.000	.000			
Meloidogyne	3	112.917	191.208	68.750			
Pratylenchus	3	97.667	75.042	91.667			
Rotylenchus	3	18.667	346.000	91.667			
Scutellonema	3	225.000	320.000	56.250			
Trichodorous	4	71.667	139.333	39.583			
Tylenchorhynchus	2	12.188	.000	.000			
Tylenchus	2	80.667	67.667	116.667			
Xiphinema	5	50.333	10.000	97.917			
Mean number of plant parasites		**87.123**	**136.576**	**62.847**	*	***	**ns**
Omnivores							
Chromadora	3	48.750	.000	20.833			
Labronema	4	104.813	143.813	.000			
Prodorylaimus	5	7.312	58.500	.000			
Mean number of omnivores		**53.625**	**67.438**	**6.944**	**ns**	**ns**	**ns**

1*, **, indicate significance at $P < 0.05$ and $P < 0.01$, respectively; ns = not significant.
[a]Colonizer-persister scale (cp1-5) describing life history strategy of nematodes.

between the Provinces but no difference was observed in the numbers of fungal feeding nematodes. In western Province the common genera were *Acrobeles, Rhabditis, Aphelenchoides, Aphelenchus, Meloidogyne, Pratylenchus, Trichodorous, Helicotylenchus, Rotylenchus, Hoplolaimus, Xiphinema, Tylenchus, Scutellonema* and *Mononchus*. Similar genera were found in coast and central Province except *Mononchus*. In addition, the bacteria feeding genera, *Cervidellus, Eucephalobus, Cephalobus, Heterocephalobus Plectus* and *Tylocephalus* were also identified in the two Provinces. The genus *Wilsonema* was only found in central Province. Omnivorous nematodes from the genera *Chromadora* were identified in western and central Province while *Labronema* and *Prodorylaimus* were present in coast and central Provinces (Table 1).

There was a significant difference in genus richness within locations in western and coast Provinces ($P \leq 0.001$). The Shannon index (H') showed variations within locations in western Province ($P \leq 0.001$) and coast Province ($P \leq 0.01$). The combined maturity index ($\sum$MI) did not show significant variation within locations.

Table 2. Nematode richness, abundance and diversity indices in western, central and coast Province.

	Western Province			Central Province			Coast Province		
	Odiado	**Angorom**	**Ochundo**	**Tebere**	**Nyangati**	**Kajiji**	**Witu**	**Mpeketoni**	**Baharini**
Genus richness	4.925±0.095[b]	5.38±0.067[a]	5.2±0.051[a]	5.228±0.142	5.637±0.142	4.976±0.201	5.05±0.177[a]	4.128±0.177[b]	4.119±0.102[b]
Abundance	172±27.823	262.5±19.674	221.43±14.872	739.875±56.963	769.109±56.963	652.594±80.559	934±54.037[a]	807±54.037[ab]	680.50±31.198[b]
MINO	0.395±0.111	0.5975±0.078	0.396±0.059	1.263±0.146	1.343±0.146	1.310±0.207	1.405±0.123	1.125±0..123	0.93±0.071
Combined MI	3.225±0.231	3.238±0.163	2.964±0.124	2.480±0.084	2.508±0.084	2.620±0.119	2.695±0.0128	2.415±0.0128	2.723±0.074
F/B+F	0.415±0.084	0.35±0.060	0.336±0.045	0.273±0.043[a]	0.165±0.043[a]	0	0.225±0.124	0.383±0.124	0.242±0.072
Shannon index	2.34±0.018[c]	2.498±0.012[a]	2.431±0.009[b]	2.525±0.037	2.428±0.037	2.385±0.052	2.64±0.065[a]	2.365±0.065[b]	2.325±0.038[b]

NB: Means followed by different letters along the row for each Province are significantly different ($P \leq 0.05$).

Nematode abundance, richness and H' varied significantly within coast Province (Table 2.). All Provinces had high ∑MI which did not significantly vary across locations. Nyangati had the highest genus richness (5.64) while the lowest was in Baharini (4.12). MINO varied across locations in the 3 Provinces with Witu having the highest value. The lowest value of F/B+F was in Kajiji and the highest H' was found in Witu (Figure 1). There were differences in soil properties across the Provinces (Table 3). Soil texture, Phosphorous (P) and Potassium (K) were corre-lated with abundance of some nematode genera. *Acrobeles* showed strong positive correlations to K ($r = 0.592$, $P \leq 0.05$) while *Rhabditis* exhibited weak positive correlation ($r = 0.128$, $P \leq 0.05$). The two genera showed weak positive correlation to P (for *Acrobeles* and $r = 0.406$, $P \leq 0.05$ and $r = 0.252$, $P \leq 0.05$ for *Rhabditis*). There was a weak positive correlation between and P ($r = 0.375$, $P \leq 0.05$). The plant parasitic genera *Meloidogyne* and *Pratylenchus* showed significant negative correlation to nitrogen ($r = -0.513$, $P \leq 0.05$ and $r = -0.226$, $P \leq 0.05$). A strong positive correlation was also found between ∑MI and silt content ($r = 0.666$, $P \leq 0.05$).

DISCUSSION

Nematodes in an agro-ecosystem are potential bio-indicators due to their diversity and their relationships to soil processes (Yeates and Bongers, 1999). The nematode community structure can be used to assess changes in soil since these organisms respond rapidly to new resources (Bongers and Bongers, 1998). Cotton fields in different regions had different abundances of nematode functional groups. Common genera of parasitic nematodes associated with cotton were identified across the Provinces, which is in agreement with the findings of Lawrence and Mclean (1995). The root knot nematode genus, (*Meloidogyne*) that causes serious yield losses in cotton, was found in all the regions surveyed indicating the need for nematode management strategies in cotton. Bacteria and fungal feeding nematodes indirectly affect primary production due to their role as key intermediaries in the decomposition process and nutrient cycling (Ruess and Ferris, 2004). Bacteriovores were among the dominant trophic groups present in all the Provinces. The genera were mainly in the cp 2 category which is associated with dry conditions in the soil environment (Griffiths et al., 1995). Large numbers of fungal feeders were also identified and this could be an indication of decomposition of substrates with high C : N ratios by fungi. The high abundance of fungal and bacterial feeding nematodes could also be attributed to colonization of cotton roots by large numbers of bacteria and fungi (Hallman et al., 1999; Feng et al., 2003; Koening and Barker, 2004). Predators and omnivores which are at a higher trophic level were not abundant in the surveyed areas. Freckman and Ettema (1993) associated low numbers of these groups of nematodes to disturbances in agro-ecosystems such as use of fertilizers and cultivation.

The number of genera in a soil habitat is a reflection of its biodiversity (Ou et al., 2005). Variation in nematode diversity shown by values of H' is an indication of environmental disturbances (Yeates and Bongers, 1999). Genus richness, MINO and H' values in cotton fields were comparable to values in other agricultural systems in Kenya (Kimenju et al., 2009). Nematode genus richness varies across soil ecosystems and an abundant food source at the rhizosphere accommodates great richness (Macchia et al., 2003). The

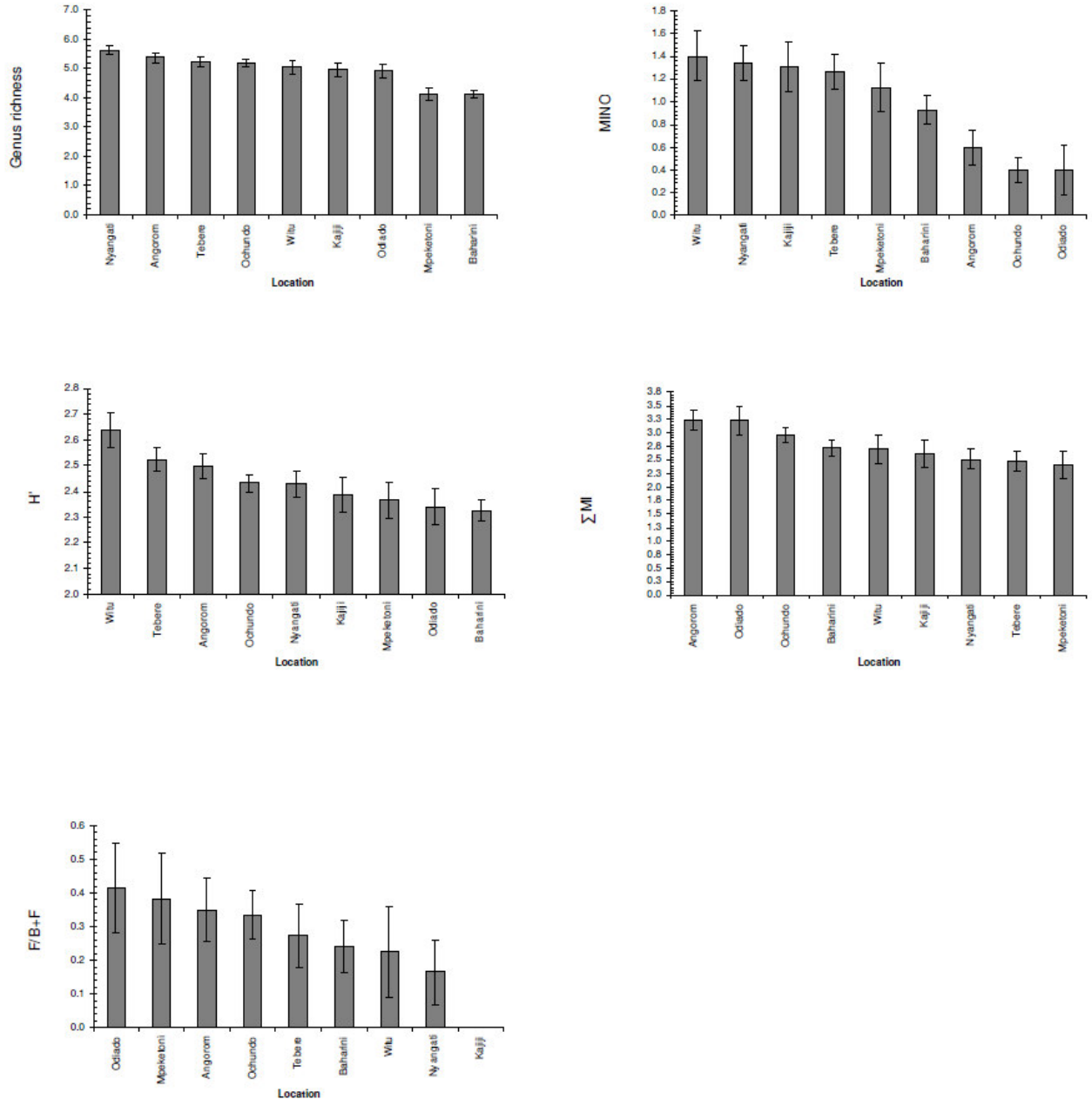

Figure 1. Nematode richness and indices in cotton fields from different locations of western, central and coast Province.
NB: MINO-Maturity index with no ones; H'- Shannon Wiener's diversity index; ∑MI-Combined maturity index; F/B+F-Fungivores:Bacteriovores ratio.

ecological measure MI is important in assessing the status of soil food webs in terrestrial ecosystems (Yeates et al., 1999; Neher et al., 2005). The soil food web condition can also be assessed using ∑MI. High values of ∑MI in this study were realized due to the inclusion of plant feeding nematodes in the calculation (Zuckerman

Table 3. Physical and chemical properties of soil samples collected from different locations in western, central and coast Province.

Soil properties	Western Province			Central Province			Coast Province		
	Odiado	Angorom	Ochundo	Tebere	Nyangati	Kajiji	Baharini	Witu	Mpeketoni
pH	6.7	5	5	5.83	5.54	5.09	6.18	6.88	7.05
Total nitrogen (%)	0.16	0.12	0.09	0.09	0.11	0.11	0.05	0.19	0.09
Phosphorous (ppm)	130	30	24	179	19	24	31	204	32.5
Electrical conductivity (*mS/cm)	0.2	0.1	0.1	0.08	0.08	0.06	0.06	0.13	0.12
Carbon (%)	1.3	0.8	0.7	0.9	0.7	0.7	0.3	1.1	0.4
Sand (%)	20	20	56	8	2	4	90	70	86
Silt (%)	28	58	24	20	24	16	0	6	2
Clay (%)	52	22	20	72	74	80	10	30	12
Texture class	**Clay**	**Clay**	**Sandy clay**	**Clay**	**Clay**	**Clay**	**Sandy**	**Sandy clay loam**	**Loam sandy**
Calcium (**me%)	7.8	4.1	1.5	7.5	5.2	2.4	3.1	14.2	5.6
***CEC (me %)	20	23.7	19.3	19.2	14	6.8	3.5	12.4	3.1
Magnesium (me%)	2.5	1.3	0.5	2.3	1.4	0.8	0.1	1.1	0.5
Potasium (me%)	1.8	0.2	0.3	2.7	1.3	0.9	0.4	1.2	0.6
Sodium (me%)	0.8	0.6	0.6	0.2	0.1	0.2	0.4	0.5	0.5

*mS/cm-millisiemen/centimeter; **me-milliequivalents. ***CEC- Cation exchange capacity.

and Coleman, 2007). However, there was no variation in ∑MI across the Provinces. Neher (1999) also reported a lack of variation in the ∑MI across conventionally and organically managed soils. The highest F/B+F ratio was in Odiado (Western Province) and lowest in Kajiji. Fungivore: bacteriovore ratios provided information on detrital pathways in soils (Zuckerman and Coleman, 2007) and can also be used as an index reflecting the microbial community structure (Yeates et al., 1993). The F/B+F ratios in this study were within the range reported for agricultural ecosystems. Agro-ecosytems have an inter-mediate fungivore to bacteriovore ratios (0.38) compared to grasslands (0.19) and forests (0.74) (Wasilewska, 1979). The low F/B+F ratio in Kajiji may be an indication of a bacteria-based food web which is characterized by higher decomposition rates than fungi-based food webs (Porazinska and Coleman, 1995). Crop species, use of fertilizers and herbicides may also influence the F/B+F ratio (Neher, 1999).

Soil physical characteristics, different nutrients and elements influence the occurrence, distribution and population dynamics of nematodes (Norton et al., 1971; Mcsorley, 1998; Wang et al., 2004). Significant positive correlations were found between K and the bacteriovores, *Acrobeles* and *Rhabditis* while the fungal feeder *Aphelenchus* was positively correlated to P. Similar results were obtained by Ingham et al. (1985) and Wang et al. (2004) and they attributed the correlations to high levels of nutrient mineralization that occur where large numbers of free living nematodes are present (Ferris et al., 1998). Negative correlations between plant parasitic genera and soil nutrients may be due to the fact that plant roots may have taken up nutrients from the soil (Wang et al., 2004). Correlations between nematode genera and soil physical characteristics in Kenya have been documented (Kandji et al., 2001; Chirchir et al., 2008). Soils with sand, silt or loam texture have different nematode composition and maturity indices (Yeates et al., 1997). Although some soil physico-chemical properties are correlated with nematode community structure and indices, there are other factors such as season and type of crop that may influence soil quality and ultimately the nematode composition (Norton et al., 1971; Mendoza et al., 2008).

Parasitic nematodes that cause damage to cotton (Gazaway and Mclean, 2003) and microbial grazers that regulate rates of decomposition (Yeates and Coleman, 1982) and nutrient mineralization were identified in the cotton fields surveyed. Crops affect the quality of resources that enter the soil through supply of organic substrates from sloughing off plant cells, root exudates and aging root epidermis (Nguyen, 2003). These substrates consequently influence the community composition (Wardle et al., 2003). Genetically modified plants offer various benefits over conventional crops; however their products may have potential adverse effects on soil organisms (Rui et al., 2005). Bt toxins engineered into plants could persist, accumulate and retain their insecticidal activity due to their binding to humic acids and clay particles (Tapp and Stotzky, 1998). This could be

hazardous to non target organisms such as nematodes. Transgenic plants have been reported to have no toxic effects to nematodes (Saxena and Stotzky, 2001) but there are Bt crystal proteins that have been shown to contain nematicidal properties (Wei et al., 2003). Manachini and Lozzia (2002) reported a decrease in bacteriophagous nematodes and an increase in mycophagous nematodes in Bt maize field trials in Italy. There are no reports on the effects of Bt cotton on soil nematodes in Kenya and it is therefore important to evaluate the potential risks of its release to the environment. Post release monitoring should also be done once Bt cotton is commercially cultivated in order to detect and prevent adverse effects on soil nematodes. Despite these concerns, Bt crops such as cotton have the potential to significantly increase yield and reduce pesticide use if the technology is applied in an environmentally acceptable and sustainable manner.

ACKNOWLEDGEMENTS

This is publication No. 18 of the BiosafeTrain project funded by the Danish International Development Agency (DANIDA). The authors would like to acknowledge the Kenya Agricultural Research Institute, National Agricultural Research Laboratories (KARI-NARL) for providing laboratory space and equipments. We also thank Maurice Okomo and Mary Ndunguli for technical support and Elias Thuranira for assistance in data analysis.

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