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Diversity and population densities of plant-parasitic nematodes in commercial and smallholder pineapple production in Kenya

Agnes W. KIRIGA [1], Danny COYNE [2], Janet G. ATANDI [2], Nikolai V. BEEK [3,†] and Solveig HAUKELAND [1,4,*]

[1] *International Centre of Insect Physiology and Ecology (icipe), Department of Plant Health, P.O. Box 30772-00100, Nairobi, Kenya*
[2] *International Institute of Tropical Agriculture, IITA East Africa, icipe campus, P.O. Box 30772-00100, Nairobi, Kenya*
[3] *Kenya Biologics Limited, P.O. Box 4560-01002, Thika, Kenya*
[4] *Norwegian Institute of Bioeconomy Research, P.O. Box 115, 143 As, Norway*

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Summary – Plant-parasitic nematodes (PPN) cause significant yield reduction in commercial pineapple (*Ananas comosus*) worldwide. In Kenya, few nematode studies have been conducted, although the main commercial pineapple producer has sole dispensation to use Telone II (1,3-Dichloropropene) indicating the magnitude of the nematode problem. This study was conducted with the aim to investigate the population densities and diversity of nematodes in two commercial plantations with two contrasting management practices. We additionally assessed the influence of crop age and compared this with nearby smallholder pineapple production systems. Soil and root samples were collected from fields of different ages in each commercial plantation and from 29 smallholder fields. A total of 18 genera were associated with pineapple, with a relatively greater diversity found in smallholder than commercial farms. The most prevalent genus was *Meloidogyne* spp. (*M. javanica*) followed by *Helicotylenchus* spp., *Tylenchus* spp. and *Aphelenchoides* spp. PPN densities were higher in relatively older fields of 24 and 36 months than from fallow and 3-month-old fields. Regression analysis additionally demonstrated the rise of PPN densities with age of pineapple fields, especially *Meloidogyne* spp., while free-living nematode densities declined. This study provides an indication of the high level of PPN infection in pineapple in Kenya, which would constitute an important factor contributing to low yields. The study confirms an obvious need for pineapple producers to control PPN to improve crop yields.

Keywords – *Ananas comosus*, *Meloidogyne* spp., plant-parasitic nematodes, yield loss.

Pineapple (*Ananas comosus*) is the third most economically important fruit crop worldwide, after banana and mango (FAO, 2020). It is primarily produced in tropical regions, in over 82 countries on approximately 1.1 million ha and with an annual production of over 27.9 million metric tons in 2018 (FAOSTAT, 2018). The largest producers are Costa Rica, the Philippines, Brazil and Thailand, which together amount to nearly 50% of total world production (UNCTAD, 2016). In Africa, Nigeria leads as a pineapple producer, but Kenya is also important, and one of the main contributors to the 19.8% pineapple global production that Africa exports (Kormelinck & Janssen, 2012; FAOSTAT, 2018). In Kenya, pineapple is mostly cultivated on commercial plantations centred around two locations in central Kenya. Commercial production accounts for *ca* 90% of production, while the remaining 10% is produced by medium- and smallholder growers (Ndungu, 2014), primarily for income generation but also for home consumption (Koech *et al*., 2013).

Pineapple is cultivated principally for its fruit, which is consumed fresh, canned or juiced. The 'Smooth Cayenne' is by far the most popular cultivar, which predominates production throughout the world (Sipes & Chinnasri, 2018). In commercial plantations, pineapple is monocultured as a perennial crop, vegetatively propagated using crowns (the fruit top), suckers (formed at the base of the trunk or obtained from plantlets that develop between the leaves of the 'mother' plant) or slips (formed underneath the fruits) (Rohrbach & Apt, 1986; Kormelinck & Janssen, 2012). The type of the vegetative material deter-

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* Corresponding author, e-mail: shaukeland@icipe.org

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mines the initial development of the root system and the duration of the first crop cycle, which varies between 12 and 24 months, depending on cultivar and climate (Coppens d'Eeckenbrugge *et al*., 2011). Following harvest of the plant crop, crowns and slips are replanted, or suckers may be left on the plant, providing new growth axes and a ratoon crop. The use of suckers theoretically provides a faster successional harvest, as the plant is already established. However, fruit size is often reduced and less uniform, so production is usually limited to one or two ratoon cycles, after which the root system tends to have deteriorated, resulting in non-economical production (Rohrbach & Apt, 1986; Coppens d'Eeckenbrugge *et al*., 2011). The crop is therefore terminated and incorporated back into the soil to decompose (Rohrbach & Apt, 1986). As the plant is a xerophyte it survives well throughout the year, including during periods of drought (Sipes & Chinnasri, 2018). It requires a well-distributed annual rainfall of at least 1000 mm, ideal temperatures (20-30°C) and deep sandy loam soils with high organic matter for optimum production (Kormelinck & Janssen, 2012; Koech *et al*., 2013).

The pineapple plant attracts a wide variety of pests and diseases, with plant-parasitic nematodes (PPN) (Gianessi, *et al*., 2002) and mealybug wilt of pineapple the main biotic threats worldwide (Lacerda *et al*., 2009; Ferreira *et al*., 2015). Although over 100 species of PPN have been reported from pineapple root systems, just a few are found to be economically important: *Meloidogyne javanica*, *M. incognita*, *Rotylenchulus reniformis* and *Pratylenchus brachyurus* (Sipes & Schmitt, 2000; Sipes & Chinnasri, 2018). Infection by the root-knot nematodes (RKN) *M. javanica* and *M. incognita*, which are sedentary endoparasites, results in swelling and distortion of the roots. The lesion nematode, *P. brachyurus*, is a migratory root endoparasite that causes necrotic lesions through intra- and intercellular migration of the nematodes in the root cortex. The reniform nematode, *R. reniformis*, is a sedentary semi-endoparasitic nematode that feeds in the root cortex and causes mechanical breakdown of the cortical cells, thus providing suitable entry sites for secondary infection by pathogenic fungi (Jones *et al*., 2013). Nematode infection of pineapple roots results in reduced root function; they then deteriorate and eventually die, reducing yield and fruit quality. Interactions between PPN and other pathogens, or between nematode species in a mixed community are common and can result in extensive damage or disease complexes (Ferreira *et al*., 2014). In Hawaii, yield reductions of pineapple production were associated with PPN parasitism (Sipes & Schmitt, 2000). In Queensland, Australia, *M. javanica* was reported to be the most damaging PPN (Stirling, 1993), and in South Africa, *M. javanica* and *P. brachyurus* were reported as the most important species, causing considerable losses (Rabie, 2017). *Meloidogyne javanica* and *R. reniformis* are the most frequently occurring and most damaging PPN in the main pineapple production areas of Brazil (Costa *et al*. 1998; Ferreira *et al*., 2015). Some reports have demonstrated that *R. reniformis* can reduce yield by 60% in the plant crop and around 40% in the second (ratoon crop) harvest (Ferreira *et al*., 2015).

In Kenya, there is scarce information on the occurrence and distribution of PPN associated with pineapple or of the yield losses they incur. Nematode species, such as the RKN, are particularly challenging to manage due to their polyphagous nature, ability to reproduce rapidly and undergo multiple generations within a short time (Trudgill & Blok, 2001). The availability of susceptible pineapple plants and continuous cultivation in the same field year after year often aggravates the nematode problem. Under commercial production systems soil fumigants (*e.g.*, 1,3-Dichloropropene; Telone II™, Corteva Agriscience) are used and relied upon to manage problematic nematodes, such as RKN (Stirling & Pattison, 2008; Daramola & Afolami, 2014). However, the application of such chemicals has negative impacts on the environment and human health, which has led to greater interest in alternative environmentally sensitive strategies (Stirling & Pattison, 2008). Nonetheless, even though there is only limited information in Kenya, efforts and resources are committed to nematode management, especially in commercial pineapple production where the level of awareness is greater and resources more available than in smallholder systems.

In light of the sparse knowledge on nematodes affecting pineapple production in Kenya this study was conducted to determine and compare the diversity and population densities, in relation to plant age, of PPN associated with pineapple under large scale commercial production and in smallholder farms.

Materials and methods

Description of sampling sites and production practices

Sampling was conducted at two commercial pineapple sites located in the counties of Kiambu (01°03′S lat-

itude and 37°05′E longitude) and Murang'a (0°58′S latitude and 37°16′E longitude) and from 29 smallholder pineapple farms also in Kiambu county (0°90′S latitude and 36°81′E longitude). The area is a major producer of pineapples by smallholders. All sites have a similar climate but differing management and cropping systems. The commercial farm in Kiambu relies heavily on the use of the fumigant Telone II for nematode control, while the one in Murang'a relies more on biologically-based nematode management. Kiambu receives an average rainfall of 1200 mm with mean annual temperatures of 19.8°C, while Murang'a receives mean annual rainfall of between 1400-1600 mm with mean annual temperatures between 14-18°C. The commercial farm in Kiambu has over 18 000 ha under pineapple production, where sandy loam soils are well-drained with a high organic matter content (Ndungu, 2014; Delmonte, www.Delmonte.co.ke). The use of Telone II is highly restricted for sole use in the Kiambu site, approved by the Pest Control Products Board (PCPB) in Kenya, which is applied under plastic tarping in the fallow fields prior to planting. At planting the crowns are treated with fungicides to control phytophthora and insecticides for control of mealybugs. Following the first harvest, Oxamyl (Vydate®) (DuPont de Nemours South Africa) is drip-irrigated to control PPN. Di-Ammonium Phosphate (DAP) is applied in the nursery and NPK in the field. Following the second harvest, fields are fallowed for 5-6 months.

The commercial farm in Murang'a has over 100 ha under pineapple production (Ndungu, 2014). The farm relies on Phytoprotect® (Sineria Holland), an unrefined crude sesame oil for PPN management, which is applied during the 6-month fallowing period following the second harvest. Both farms uproot the plant material and incorporate it back to the soil after the second harvest of the crop, for it to decompose and provide organic matter.

The smallholder farmers use DAP (diammonium phosphate) during planting and a later top dressing of NPK. During land preparation most farmers apply organic manure. Manual weeding is also conducted in the early stages of the crop. At 2 months after planting, most farmers spray their crop using an organophosphate pesticide (Dursban® (Chlorpyrifos), Corteva Agriscience) and a fungicide, mostly Mancozeb-based. To our knowledge these farmers use no management strategies to control plant-parasitic nematodes. Farmers leave the suckers on the plant after the first crop cycle, which usually takes around 24 months, to provide new growth axes for further production cycles. The cropping cycle varies but may extend for multiple years, with some up to 7 years.

Sample collection, nematode extraction and identification

In the two commercial farms, three (replicate) fields for each crop age (0, 3, 5, 8, 11, 24 and 36 months) were sampled and processed separately. In each field, a composite soil sample (*ca* 1 kg) comprising 25 subsamples (cores) was removed randomly along a zigzag pattern using a hand shovel to a depth of *ca* 25 cm, after scraping aside the top 5 cm of soil (Coyne *et al*., 2018a). Roots were collected at the same time (but not for the fallow and 3-month-old fields) and placed in the same bag with the soil. The samples were placed in polythene bags, labelled and stored in a cool box before processing in the laboratory. For the smallholder fields, composite samples of soil and roots were collected as described above, and the crop age recorded.

In the laboratory, nematodes were extracted from 100 ml soil sub-samples for each field after mixing thoroughly. Roots were rinsed free of soil debris, dabbed dry, chopped finely, mixed and 5 g sub-samples removed. Nematodes were extracted using a modified Baermann technique and collected after 48 h, the extraction was reduced to 10 ml and all nematodes (both PPN and non-PPN) counted under a Leica 12.5 stereomicroscope; nematodes were identified to genus level after killing in hot water (60°C) and fixing with formaldehyde (Coyne *et al*., 2018a). Twenty females were obtained from each field (*ca* 1 ha), where ten were used for morphological characterisation using perineal pattern and the other ten for molecular identification. *Meloidogyne* species were identified to species level using the perineal patterns of mature live egg-laying females obtained by cutting the vulva region, according to Jepson (1987). The results were confirmed using PCR species-specific SCAR primer set Fjav/Rjav (*M. javanica*) (Zijlstra *et al*., 2000). Species-specific primers for other root-knot nematodes (*M. enterolobii*, *M. incognita* and *M. hapla*) were also used.

Statistical treatment of data

Nematode data were subjected to two-way analysis of variance (ANOVA) using R software version 3.2.3 (R Core Team, 2015) to investigate the effect and interactions between the two commercial sites and crop age. General Linear Model procedures were used to analyse and dis-

tinguish occurrence and distribution of PPN across sites and crop age. Significantly different means at $P \leqslant 0.05$ were separated using Tukey-HSD. Linear and quadratic regression of nematode densities against pineapple field age were also conducted.

Percentage frequency of occurrence was calculated as: $((n/N) \times 100)$, where n = the total number of times a nematode genus occurred in samples and N = sample size. Percentage nematode proportion was calculated as: ((In/TN) × 100), where In = individual nematode density, TN = total density of nematodes present in the sample.

Results

Nematode characterisation and frequency of occurrence

A total of 72 and 57 samples were collected from commercial fields and smallholder farms, respectively. Across all samples 19 genera of PPN were identified, with 14 taxa recovered from roots and 17 from soil. Eight genera were present in the two commercial plantation sites, whereas all 19 genera were present in the smallholder farms (Table 1). The majority of PPN occurred infrequently. The most commonly occurring taxa were *Meloidogyne* spp., *Helicotylenchus* spp., *Tylenchus* spp. and *Aphelenchoides* spp. *Meloidogyne* spp. were present at most sites in both the commercial and smallholder farms. *Xiphinema* spp. were present in 72% of smallholder fields but were not detected in the commercial plantations. *Rotylenchulus* and *Pratylenchus* were present at low frequencies across all sites. Free-living nematodes were also recovered from all samples, including bacterial feeders, fungal feeders, omnivores and predators.

Morphological and molecular identification of *Meloidogyne* species

The results from the morphological perineal pattern observations identified *M. javanica* as the sole root-knot nematode species present. The pattern had a low dorsal arch and double lateral lines, which are the main characteristic features of *M. javanica* (Fig. 1).

Molecular identification using the specific SCAR primers Fjav/Rjav (*M. javanica*) consistently confirmed the results and the products were readily amplified from DNA of individual females (Fig. 2). The MI-F/MI-R

Table 1. Frequency of occurrence of nematodes associated with pineapple in two commercial farms and 29 smallholder farms in Kenya.

Genus	Frequency of occurrence (%)					
	Commercial plantation Kiambu		Commercial plantation Murang'a		Smallholder farms	
	Soil (n = 21)	Roots (n = 15)	Soil (n = 21)	Roots (n = 15)	Soil (n = 29)	Roots (n = 28)
Meloidogyne	95	80	100	100	97	86
Pratylenchus	29	20	0	40	45	4
Rotylenchulus	19	0	0	0	10	0
Xiphinema	0	0	0	0	72	7
Helicotylenchus	100	73	86	50	90	61
Tylenchorhynchus	0	0	0	0	7	7
Trichodorus	0	0	0	0	41	4
Hoplolaimus	0	7	0	0	0	4
Rotylenchus	0	0	0	0	7	0
Scutellonema	0	0	0	0	0	4
Criconema	0	0	0	0	3	0
Hemicycliophora	0	0	0	0	3	0
Aphelenchoides	100	100	100	100	100	82
Globodera	0	0	0	0	17	14
Tylenchus	100	100	100	100	100	82
Filenchus	14	20	100	80	100	57
Diptherophora	0	0	0	0	55	4
Not identified (plant-parasitic)	0	0	0	0	24	0
Free-living nematodes	100	100	100	100	100	100

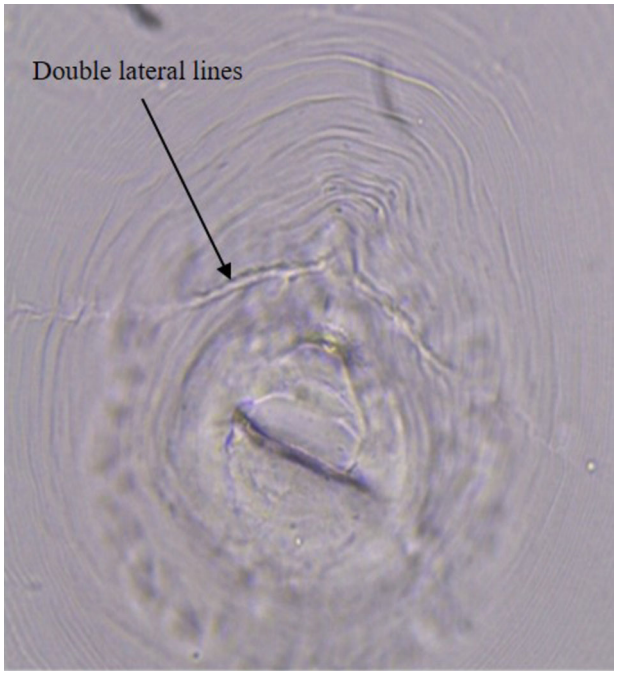

Fig. 1. Perineal pattern from a female specimen of *Meloidogyne javanica*.

M. incognita-specific SCAR primers, JMV *M. hapla* primers and MK7-F/Mk7-R *M. enterolobii* primers gave no amplification products from single females (Fig. 3).

Nematode occurrence under different management systems

Across all sites, *Meloidogyne* spp., *Helicotylenchus* spp., *Tylenchus* spp. and *Aphelenchoides* spp. occurred at higher densities in soil compared to other taxa (Table 2). The percentage proportion of *Meloidogyne* spp. was similar at both commercial sites (Kiambu 7.4%; Murang'a 7.2%). Recorded in low percentage proportions were *Filenchus* spp., *Pratylenchus* spp. and *Rotylenchus* spp. (Kiambu) and *Filenchus* spp. and *Tylenchus* spp. (Murang'a) (Figs 4A; 5A). The density of free-living nematodes was higher in Kiambu (2633 $\pm$ 233 (mean $\pm$ s.d.) nematodes (100 ml soil)$^{-1}$) compared to Murang'a farm (1683 $\pm$ 84).

In roots, *Meloidogyne* spp. (225 $\pm$ 73 (mean $\pm$ s.d.) nematodes (5 g roots)$^{-1}$ Kiambu; 158 $\pm$ 45 Murang'a) and *Tylenchus* spp. (170 $\pm$ 52 Kiambu; 88 $\pm$ 16 Murang'a) were recovered in high densities at both sites followed by *Aphelenchoides* spp. (95 $\pm$ 26 Kiambu; 106 $\pm$ 22 Murang'a). A number of genera were barely detectable with just a few individuals recovered. The density of free-living nematodes was higher in Murang'a (400 $\pm$ 82) than Kiambu (183 $\pm$ 20) (Table 2). Recorded in low proportions were *Filenchus* spp., *Pratylenchus* spp. and *Hoplolaimus* spp. in Kiambu and *Pratylenchus* spp. in Murang'a (Figs 4B; 5B).

In smallholder fields *Meloidogyne* spp. was present in higher densities in both soil and root samples than other taxa with percentage proportions of 11.4 and 22.9%, respectively (Fig. 6A, B), being recorded. This was followed by *Filenchus* spp. and *Tylenchus* spp. respectively for the soil samples. *Xiphenema* spp. occurred in a relatively high proportion of fields (72%; mean density of 22 $\pm$ 4.1 (100 ml soil)$^{-1}$) (Tables 1, 2). *Scutellonema* spp. and *Hoplolaimus* spp. were not encountered in the soil samples but occurred in low mean densities in root samples (Table 2).

Nematode population densities under different crop ages

In soil, *Meloidogyne* spp. was quite ubiquitous across the different aged fields (Table 3) with densities generally increasing with age from 3 to 36 months in Kiambu and significantly higher ($P \leqslant 0.05$) at 36 months compared to other ages. In Murang'a, the population density increased with age up to 11 months and then declined. Kiambu had a significantly higher *Meloidogyne* spp. density at 36 months compared to Murang'a. *Rotylenchus* spp. and *Pratylenchus* spp. occurred in low densities. *Tylenchus* spp. had higher ($P \leqslant 0.05$) densities in all fields in Kiambu than Murang'a, with the highest density occurring at 24 months. *Helicotylenchus* spp. were present in most fields across the different ages in both sites, except in fields at 24 months in Murang'a. Compared to Murang'a, higher ($P \leqslant 0.05$) population densities of *Helicotylenchus* spp. were found in the older fields (8, 11, 24 and 36 months) in Kiambu, whilst the densities were significantly higher ($P \leqslant 0.05$) in younger fields (0, 3 and 5 months) in Murang'a. *Aphelenchoides* spp. was common in fields of all ages at both sites. *Filenchus* spp. occurred in all Murang'a fields but was recovered only from 36-month-old fields in Kiambu (Table 3).

In the sampled roots, *Meloidogyne* spp. were recovered in all fields across the different ages at both sites, except at 8 months in Kiambu, with densities increasing with age from 5 to 36 months in Kiambu and the highest occurring at 36 months (Table 4). Compared to Kiambu, higher ($P \leqslant 0.05$) population densities of *Meloidogyne* spp. were found at 5, 8 and 24 months in Murang'a, while population densities were higher in Kiambu at 36 months.

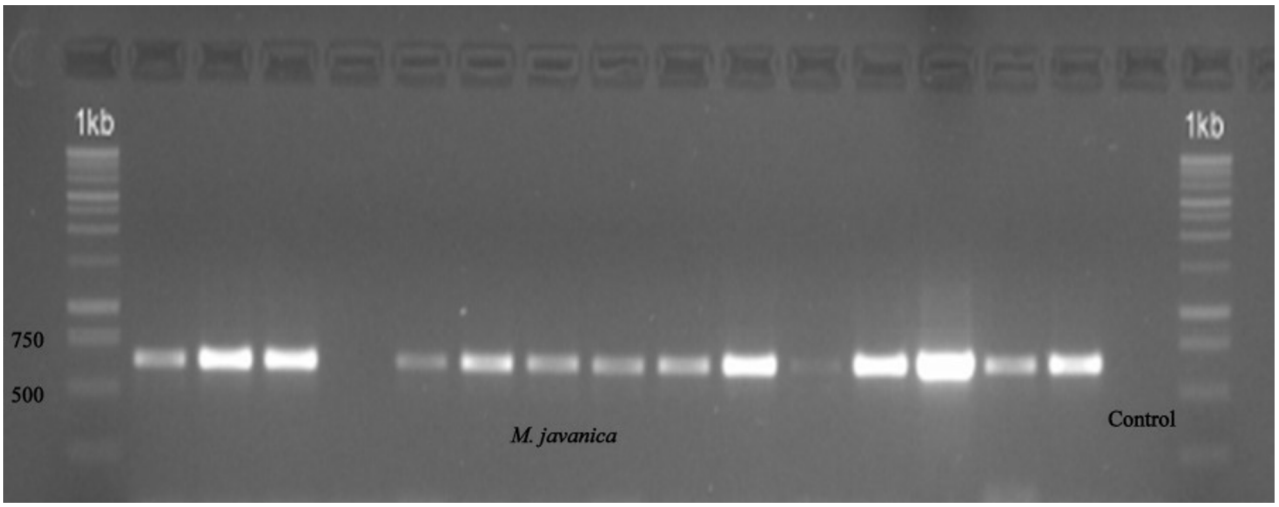

Fig. 2. PCR products (720 bp) obtained from amplification of DNA from single females of *Meloidogyne* spp. from pineapple farms using Fjav/Rjav *Meloidogyne javanica*-specific primers.

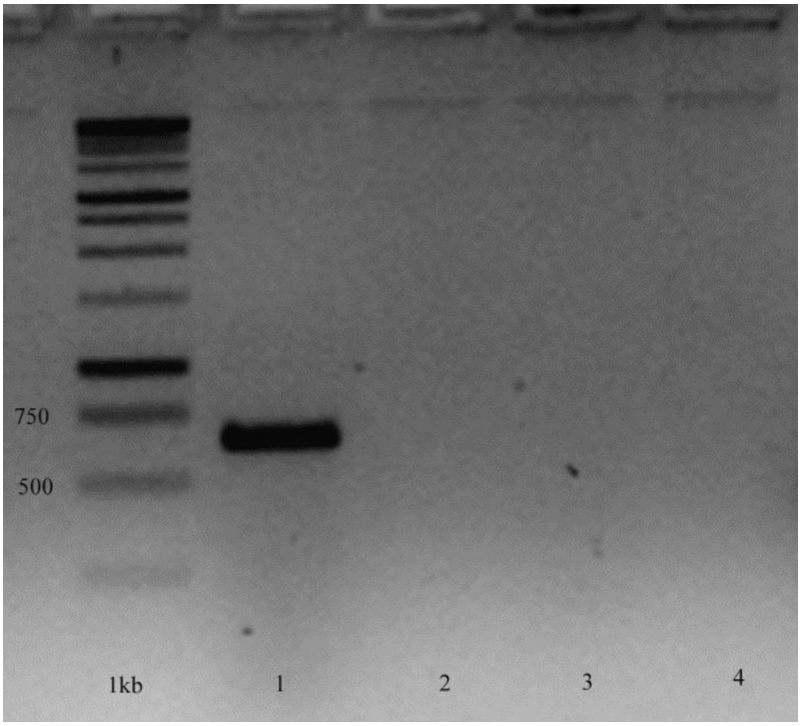

Fig. 3. DNA amplification using species-specific primers for: 1: *Meloidogyne javanica*; 2: *M. incognita*; 3: *M. enterolobii*; 4: *M. hapla*.

The densities at 11 months had no significant difference between the two sites. *Pratylenchus* spp. was found only at 11 and 24 months in Murang'a and at 36 months in Kiambu. *Helicotylenchus* spp. were present in all crop ages in Kiambu, except in fields at 11 months with the highest density occurring at 36 months, but were only found at 11 and 24 months in Murang'a. *Filenchus* spp. occurred in all crop ages in Murang'a, except in fields at 11 months, with higher densities ($P \leqslant 0.05$) found in older (24 and 36 months) as opposed to younger (5 and

Table 2. Mean nematode population density ± S.E. in soil and roots[1] associated with pineapple in two commercial plantations and smallholder farms in Kenya.

Genus	Kiambu		Murang'a		Smallholder farms	
	Soil	Roots	Soil	Roots	Soil	Roots
Meloidogyne	196 ± 67	225 ± 73	121 ± 18	158 ± 45	121 ± 22.5	42 ± 8.0
Pratylenchus	14 ± 5	3 ± 2	0 ± 0	26 ± 10	18 ± 3.3	11 ± 2.2
Helicotylenchus	407 ± 74	77 ± 33	161 ± 24	26 ± 11	57 ± 10.6	14 ± 2.6
Tylenchorhynchus	0 ± 0	0 ± 0	0 ± 0	0 ± 0	3 ± 0.5	1 ± 0.3
Trichodorus	0 ± 0	0 ± 0	0 ± 0	0 ± 0	10 ± 1.9	1 ± 0.2
Rotylenchus	7 ± 4	0 ± 0	0 ± 0	0 ± 0	1 ± 0.1	0 ± 0
Hoplolaimus	0 ± 0	1 ± 1	0 ± 0	0 ± 0	0 ± 0	1 ± 0.1
Rotylenchulus	0 ± 0	0 ± 0	0 ± 0	0 ± 0	1 ± 0.2	0 ± 0
Scutellonema	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	1 ± 0.1
Criconema	0 ± 0	0 ± 0	0 ± 0	0 ± 0	1 ± 0.1	0 ± 0
Hemicycliophora	0 ± 0	0 ± 0	0 ± 0	0 ± 0	1 ± 0.1	0 ± 0
Globodera	0 ± 0	0 ± 0	0 ± 0	0 ± 0	3 ± 0.5	2 ± 0.5
Aphelenchoides	208 ± 18	95 ± 26	163 ±	106 ± 22	62 ± 11.6	16 ± 3.1
Xiphinema	0 ± 0	0 ± 0	0 ± 0	0 ± 0	22 ± 4.1	1 ± 0.1
Tylenchus	290 ± 41	170 ± 52	94 ± 16	88 ± 16	65 ± 12	29 ± 5.6
Filenchus	14 ± 8	7 ± 4	80 ± 7	150 ± 34	97 ± 18.0	18 ± 3.4
Diptherophora	0 ± 0	0 ± 0	0 ± 0	0 ± 0	23 ± 4.2	0 ± 0
Unidentified (plant-parasitic)	0 ± 0	0 ± 0	0 ± 0	0 ± 0	9 ± 1.7	0 ± 0
Total free-living nematodes	1499 ± 138	183 ± 20	1063 ± 62	400 ± 82	570 ± 105.9	46 ± 8.8
Total plant-parasitic nematodes	1135 ± 149	579 ± 121	620 ± 36	554 ± 103	520 ± 96.6	140 ± 26.5
Total nematodes	2633 ± 233	762 ± 139	1683 ± 84	954 ± 181	1067 ± 198.1	185 ± 34.9

[1](100 ml soil)$^{-1}$ or (5 g roots)$^{-1}$.

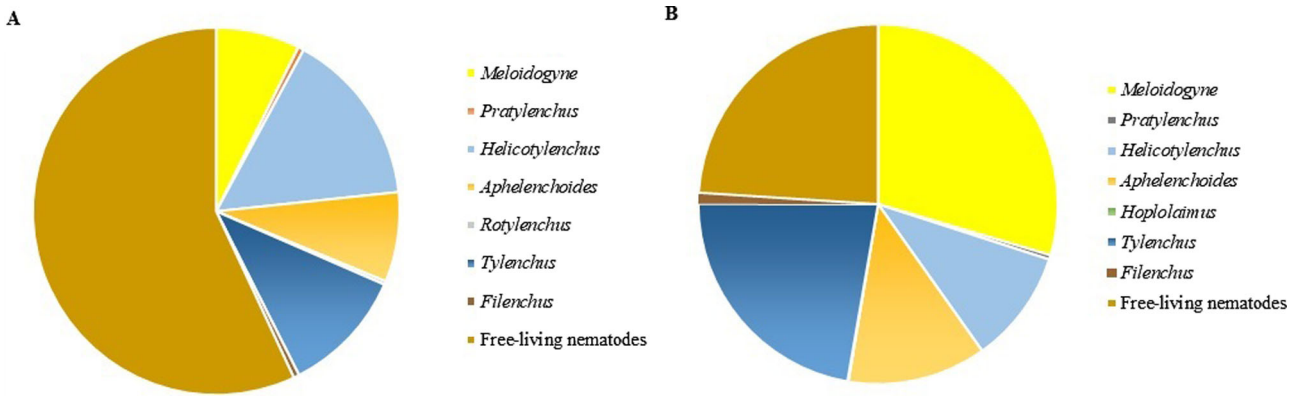

Fig. 4. Percentage nematode proportions in Kiambu commercial pineapple farm in Kenya. A: Soil; B: Roots.

8 months) fields and were only detected at 36 months in Kiambu. *Tylenchus* spp. occurred in all fields across the ages at both sites, with significantly ($P \leqslant 0.05$) higher densities at 24 months in Kiambu compared to other fields. *Aphelenchoides* spp. were recovered from all fields at both sites. Free-living nematodes were found in all fields at the two commercial sites with higher densities occurring in Kiambu than Murang'a (Table 4).

In Kiambu commercial fields, the population density of *Meloidogyne* spp. in roots was positively correlated with

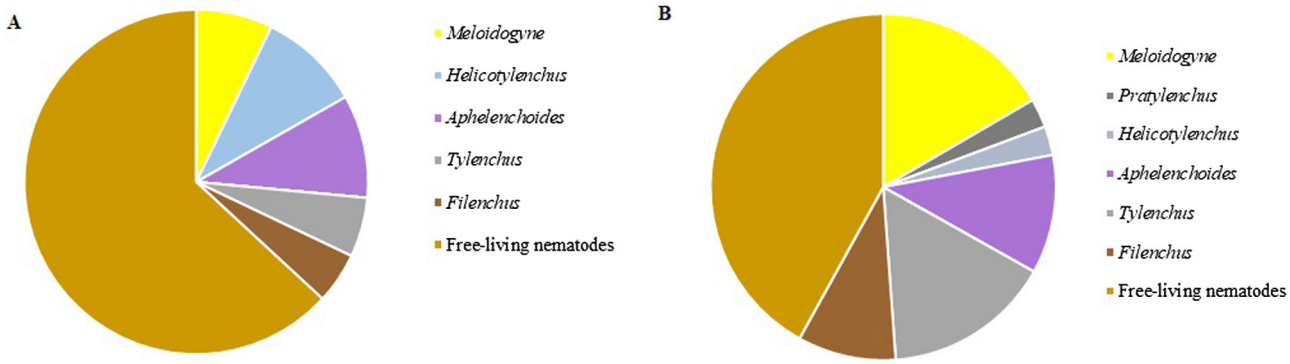

Fig. 5. Percentage nematode proportions in Murang'a commercial pineapple farm in Kenya. A: Soil; B: Roots.

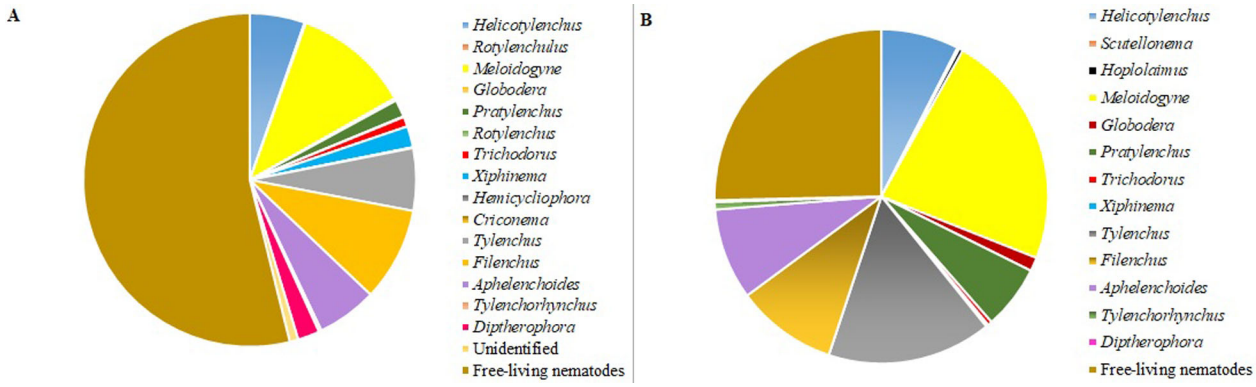

Fig. 6. Percentage nematode proportions in Kenyan smallholder farms. A: Soil; B: Roots.

years of production ($R^2 = 0.97$; $P \leqslant 0.001$) (Fig. 7A) as were total PPN ($R^2 = 0.86$; $P \leqslant 0.001$) (Fig. 7B), whilst no correlation was found for free-living nematodes ($R^2 = 0.004$; $P = 0.776$).

For the Murang'a commercial farm, no correlation with crop age was found for the population density of *Meloidogyne* spp. in roots ($R^2 = 0.15$; $P = 0.16$), whereas for the total PPN density there was a positive correlation ($R^2 = 0.60$; $P \leqslant 0.001$) (Fig. 8A); by contrast, there was a negative correlation with crop age observed for free-living nematodes ($R^2 = 0.35$; $P \leqslant 0.005$) (Fig. 8B).

In the smallholder farms samples, the population density of *Pratylenchus* spp. in roots increased with crop age ($R^2 = 0.36$; $P \leqslant 0.05$) Fig. 9A. For *Meloidogyne* spp., the density appeared to decline in the first 1-3 years, but thereafter increased with crop age (Fig. 9B). Total PPN in sampled roots increased significantly with crop age ($R^2 = 0.76$; $P \leqslant 0.05$) (Fig. 9C), whereas a negative correlation was observed with crop age for free-living nematodes in soil ($R^2 = 0.64$; $P \leqslant 0.02$) (Fig. 9D).

Discussion

Our study represents the first known documentation of the diversity and population densities of nematodes on pineapple in Kenya and it appears that *M. javanica* is the principal nematode pest species present. Nineteen genera were recovered across sites, most of which were represented by few individuals or low mean densities, and probably of no or limited threat to pineapple. Many have previously been reported from pineapple fields elsewhere with just a few regarded as real threats to the crop (Stirling, 1993; Nath *et al*., 1997; Daramola & Afolami, 2014; Rabie, 2017). *Meloidogyne javanica* would appear to be the primary nematode pest in Kenya, as it is in other pineapple-growing areas (Babatola, 1985; Stirling, 1993; Stirling & Kopittke, 2000; Rabie, 2017). In our study it was the only species of *Meloidogyne* identified, although we cannot rule out the presence of other *Meloidogyne* species on pineapple in Kenya, as a relatively small proportion of samples were used for species characterisation. Globally, however, *M. javanica* is the most

Table 3. Mean nematode population densities in soil[1] across different ages (months) of a pineapple crop in two commercial plantations in Kenya.

Age	*Meloidogyne*		*Pratylenchus*		*Helicotylenchus*		*Rotylenchus*		*Filenchus*		*Tylenchus*		*Aphelenchoides*		Free-living nematodes	
	Kiambu	Murang'a	Kiambu	Murang'a	Kiambu	Murang'a	Kiambu	Murang'a	Kiambu	Murang'a	Kiambu	Murang'a	Kiambu	Murang'a	Kiambu	Murang'a
0	87bA	30dB	43aA	0aB	290cA	330aA	47aA	0aB	0bB	50cdA	123eA	70abB	103 dB	280aA	2226 ± 50	900 ± 15
3	17cdB	100bcA	0bA	0aA	20eB	180bA	0bA	0aA	0bB	30dA	250bcA	10cB	313aA	70cB	1087 ± 37	1150 ± 21
5	53bcB	110bcA	0bA	0aA	83dB	200abA	0bA	0aA	0bB	90abA	250bcA	110abB	130cdA	130bA	847 ± 19	1280 ± 31
8	7dB	150bA	0bA	0aA	417bcA	30cB	0bA	0aA	0bB	90abA	340bA	120aB	280aA	120bB	1503 ± 44	1090 ± 49
11	130bB	290aA	0bA	0aA	273cA	200abB	3bA	0aA	0bB	130aA	147deA	90abB	293aA	90bcB	1430 ± 50	1410 ± 15
24	167bA	100bcA	0bA	0aA	773abA	0dB	0bA	0aA	0bB	70bcA	703aA	240aB	187bB	380aA	2550 ± 53	1120 ± 15
36	910aA	70cB	53aA	0aB	990aA	190bB	0bA	0aA	97aA	100abA	213cdA	20bcB	150bcA	70cB	847 ± 29	490 ± 10

Means within columns shows significance differences of nematode genera among the ages of the crop and are followed by small letters; means within rows shows significance differences of each nematode genus between the two farms and are followed by capital letters. Means followed by the same letter(s) are not significantly different ($P \leqslant 0.05$).
[1](100 ml soil)$^{-1}$.

Table 4. Mean nematode population densities in roots[1] across different ages (months) of a pineapple crop in two commercial farms in Kenya.

Age	*Meloidogyne*		*Pratylenchus*		*Helicotylenchus*		*Hoplolaimus*		*Filenchus*		*Tylenchus*		*Aphelenchoides*		Free-living nematodes	
	Kiambu	Murang'a	Kiambu	Murang'a	Kiambu	Murang'a	Kiambu	Murang'a	Kiambu	Murang'a	Kiambu	Murang'a	Kiambu	Murang'a	Kiambu	Murang'a
5	13 dB	60cA	0bA	0cA	7dA	0cA	0aA	0aA	0bB	20cA	103bA	50cB	30bA	20eA	140 ± 6	290 ± 21
8	0eB	80bcA	0bA	0cA	17bcA	0cB	0aA	0aA	0bB	150bA	53cB	100bA	27bB	130bA	70 ± 15	270 ± 6
11	40cA	60cA	0bB	30bA	0eB	20bA	0aA	0aA	0bA	0dA	107bA	40cB	267aA	30 dB	187 ± 9	40 ± 6
24	393aB	490aA	0bB	100aA	43bB	110aA	3aA	0aA	0bB	300aA	553aA	50cB	133aA	100cB	253 ± 19	960 ± 10
36	680aA	100bB	17aA	0cB	320aA	0cB	0aA	0aA	37aB	280abA	33cB	200aA	20bB	250aA	263 ± 13	440 ± 12

Means within columns shows significance differences of nematode genera among the ages of the crop and are followed by small letters; means within rows shows significance differences of each nematode genus between the two farms and are followed by capital letters. Means followed by the same letter(s) are not significantly different ($P \leqslant 0.05$).
[1](5 g root)$^{-1}$.

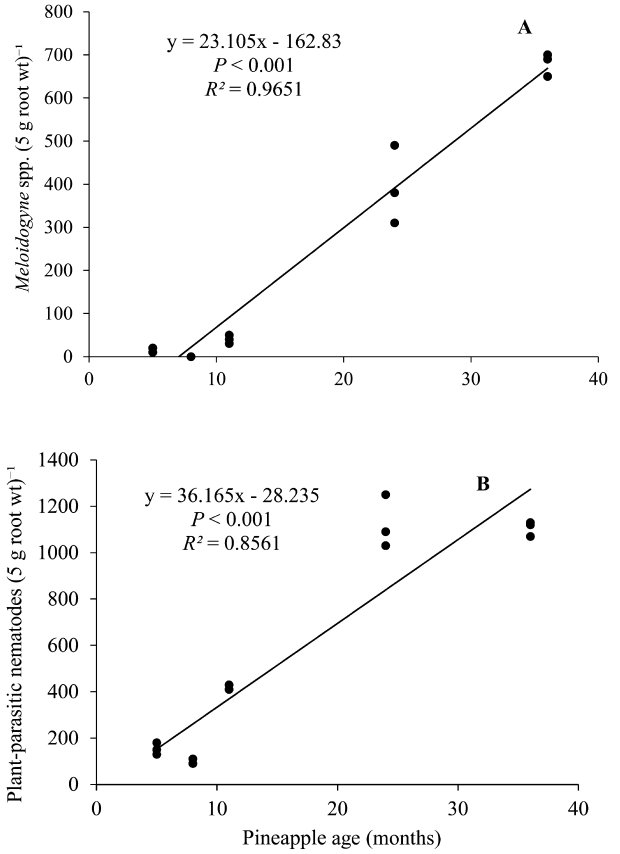

Fig. 7. Effect of pineapple age on the population of *Meloidogyne* spp. (A) and plant-parasitic nematodes (B) in Kiambu commercial pineapple farm in Kenya.

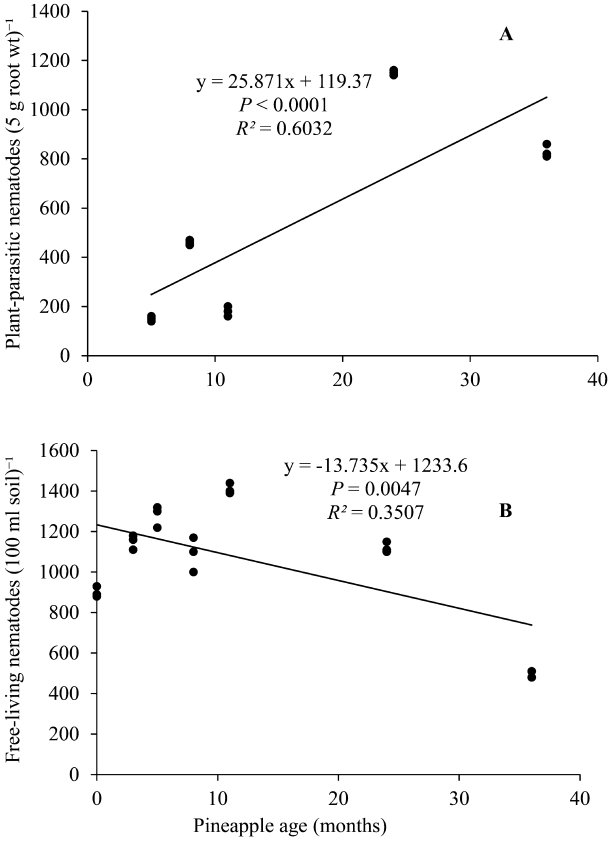

Fig. 8. Effect of pineapple age on the population of plant-parasitic (A) and free-living (B) nematodes in Murang'a commercial pineapple farm in Kenya.

prominent RKN species occurring on pineapple, hence our study is probably a good reflection of the RKN species incidence on the crop in Kenya. The widespread distribution of *M. javanica*, in both commercial and smallholder farms demonstrate its pest status and likely impact on production. This is pertinent in view of the management measures undertaken in the commercial production systems, especially in Kiambu, where the use of Telone II is routine. *Meloidogyne* spp. occurred in all fields, across the various crop ages and in both commercial and smallholder pineapple productions. Although population build-up of *Meloidogyne* spp. is slower on pineapple compared with other crops (Sipes & Chinnasri, 2018), their impact can be substantial on yields (Rohrbach & Apt, 1986; Stirling & Nikulin, 1993; Stirling & Kopittke 2000), with a single second-stage juvenile (J2) of *M. javanica* in a sample interpreted as a potential problem (Stirling & Kopittke, 2000; Rabie, 2017). Reports indicate that early infection with *M. javanica* reduces the development of pineapple plants (Costa & Matos, 2000; Stirling & Pattison, 2008; Sipes & Chinnasri, 2018). With densities of less than 10 J2 (200 ml soil)$^{-1}$ at 12 months, Stirling & Kopittke (2000) reported yield losses of 10%, while in ratoon crops yield losses of 30-60% can be experienced when *M. javanica* is left uncontrolled (Stirling & Nikulin, 1993). When relating nematode density with crop age, *M. javanica* densities generally increased with age from 3 to 36 months at the Kiambu site, while in Murang'a densities increased up to 11 months before declining up to 36 months. However, in general, and across all sites, *M. javanica* percentage proportion and densities increased with age of crop. Differences in nematode management probably play a role in nematode population trends between the two commercial sites. The use of fumigants at Kiambu during fallow periods will initially knock down the PPN population and negatively impact beneficial organisms and nematode pathogens and predators, thereby creating a favourable environment for the surviving PPN to resume

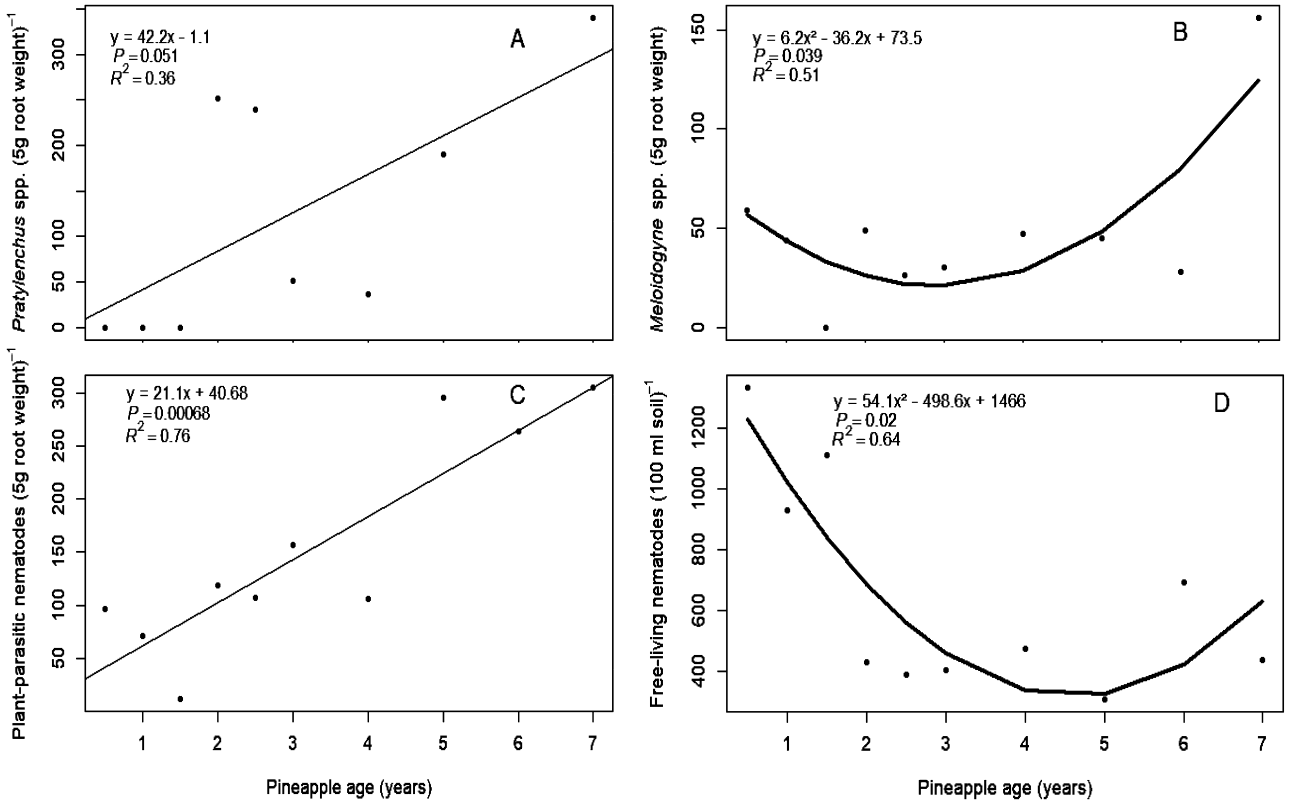

Fig. 9. Effect of pineapple age on the population of *Pratylenchus* spp. (A), *Meloidogyne* spp. (B), plant-parasitic (C) and free-living (D) nematodes in Kenyan smallholder pineapple farms.

reproduction at higher rates (Stirling, 2011; Sipes & Chinnasri, 2018). At both commercial sites and in smallholder systems, total PPN densities were highly positively correlated with age of pineapple fields, in contrast to free-living nematodes, which were negatively correlated with age in smallholder systems and the Murang'a site. In pineapple production, fumigation has been shown adequately to protect the plant crop but not the ratoon crops (Sipes & Chinnasri, 2018). In South Africa, average yield increases of 34% have been recorded following the use of nematicides, and on sandy soils especially, pre-plant nematode control is deemed essential for economic production (Rabie, 2017). Higher PPN densities in Kiambu compared with Murang'a could further be explained by pineapple cultivation over a longer period of approximately 60 years at Kiambu (www.Delmonte.co.ke). At both sites fallow periods for 6 months are employed after two croppings, which is recommended as a minimum for managing PPN below threshold levels (Rohrbach & Apt, 1986). The higher number of genera in smallholder farms could reflect the lack of nematode management, providing a basis for greater biodiversity, although the extended smallholder monoculture period does appear to be detrimental to free-living nematodes densities, while PPN densities proliferate.

Regarding other PPN species, the spiral nematodes (*Helicotylenchus* spp.) frequently occurred and in large densities, as has been reported previously (Nath *et al*., 1997; Costa *et al*., 1998; Daramola & Afolami, 2014; Sipes & Chinnasri, 2018). These nematodes tend to have wide host ranges and occur commonly, but a pineapple monocropping history over an extended period probably facilitates these nematodes, perhaps to a greater extent than other taxa. The impact of these nematodes on pineapple, as well as numerous other crops, remains speculative, however, with limited information demonstrating damage. *Helicotylenchus* spp. were implicated to be economically important on pineapple (Babatola, 1985; Ko & Schmitt, 1993) and reported significantly to reduce fruit weight and delay maturity (Ferreira *et al*., 2014). As ectoparasites,

Helicotylenchus spp. would normally not be recovered from roots, although some species (*e.g.*, *H. multicinctus*) are ecto-endoparasites feeding and living up to eight cells deep in the roots such as banana (Orion *et al*., 1999), which could explain their presence in our root extractions.

The occurrence and abundance of *Tylenchus* spp. and *Aphelenchoides* spp. in Kenya also confirms previous reports (Daramola *et al*., 2013), although their economic importance is yet to be established. Similarly, *Xiphinema* spp. have previously been reported from pineapple fields (Nath *et al*., 1997; Daramola *et al*., 2013) and were relatively common in smallholder pineapple farms. The impact of these nematodes on pineapple is unknown but at high densities could well pose a threat to production (Sipes & Chinnasri, 2018).

The predominant occurrence of *Meloidogyne* spp. on pineapple in Kenya supports observations on the rising problem of RKN as a key threat to crop production across a wide range of crops and across the tropics (Jones *et al*., 2013; Karssen *et al*., 2013; Coyne *et al*., 2018b). As demonstrated in our study, intensive pineapple monocropping leads to a concomitant rise in *Meloidogyne* spp. densities, leading to higher levels of damage, and a consequent need for their management. The use of Telone II and Phytoprotect provides protection for relatively short periods, but at a cost. In the case of Telone II, soil health, as reflected by free-living nematodes, is also affected, while Phytoprotect appears to be less detrimental. The lack of management in smallholder systems likely takes a heavy toll on pineapple yields, with PPN densities rising and soil health depleting, in relation to the length of continuous pineapple cropping. Consequently, there is a need to create greater awareness of these pests and their damaging potential to pineapple producers. The indistinct aboveground symptoms further complicate the issue around PPN, as they are difficult to differentiate from other constraints. The commercial producers are obviously aware of these pests, and of the economic damage PPN inflict, given the precautions taken and expense invested in nematode management. Smallholder farmers, however, appear completely unaware. In any case, the implications of nematode management can be costly both financially and environmentally and establishing alternative options to fumigation with Telone II will be advantageous to all. Furthermore, accurate diagnosis is crucial to develop and employ appropriate and effective management strategies. While it is clear that *M. javanica* is prevalent and resulting in undoubted loss to pineapple production, the damage caused by a number of other PPN to pineapple in Kenya is not clear and requires further investigation.

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