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Plant Parasitic Nematodes Associated with Okra in the Forest Savanna Transition and Semi-Deciduous Forest Agro-Ecologies of Ghana

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Abstract
Okra is an important vegetable crop in Ghana. Plant-parasitic nematodes infect okra, reducing the quality and quantity of fresh immature and dry fruits. This study was carried out to determine diversity, density and distribution of plant parasitic nematodes attacking okra in the Forest savanna transition and Semi-deciduous forest agro-ecologies of Ghana. In each agro-ecology, soils from okra rhizosphere were sampled from 15 farms for plant-parasitic nematodes assessment. Nematodes were extracted using the modified Baermann nematodes extraction method. Six nematode genera were identified; *Meloidogyne, Pratylenchus, Xiphinema, Helicotylenchus, Rotylenchulus* and *Scutellonema. Meloidogyne* occurred with 64.9% relative abundance and 100% frequency. *Rotylenchulus, Pratylenchus,* and *Helicotylencus* nematodes also occurred with 100% frequency. *Xiphinema* were the least abundant (0.05%) and the least frequent (15%). The findings have shown that a large number of nematodes exist on okra plants and therefore there is the need for educational campaigns to be stepped up by Agricultural Extension Services of the Ministry of Food and Agriculture on plant parasitic nematode infections in okra production.

Keywords: Dagger nematodes; Root knot nematodes; Root lesion nematodes; Spiral nematodes; Yam nematodes.

Nématodes Parasites des Plantes Associés au Gombo dans la Transition de la Savane Forestière et Agro-écologies Forestières Semi-décidues du Ghana

Résumé
Le gombo est une culture maraîchère importante au Ghana. Les nématodes parasites des plantes infectent le gombo, réduisant la qualité et la quantité de fruits frais immatures et secs. Cette étude a été réalisée pour déterminer la diversité, la densité et la répartition des nématodes parasites des plantes attaquant le gombo dans la transition de la savane forestière et les agro-écologies forestières semi-caduques du Ghana. Dans chaque agroécologie, les sols de la rhizosphère du gombo ont été échantillonnés dans 15 fermes pour l'évaluation des nématodes parasites des plantes.Les nématodes ont été extraits à l'aide de la méthode d'extraction modifiée des nématodes de Baermann. Six genres de nématodes ont été identifiés; Meloidogyne, Pratylenchus, Xiphinema, Helicotylenchus, Rotylenchulus et Scutellonema. La mélancogyne s'est produite avec une abondance relative de 64,9 % et une fréquence de 100 %. Les nématodes Rotylenchulus, Pratylenchus et Helicotylencus se sont également produits avec une fréquence de 100%. Les

Xiphinema étaient les moins abondants (0,05%) et les moins fréquents (15%). Les résultats ont montré qu'il existe un grand nombre de nématodes sur les plantes de gombo et qu'il est donc nécessaire que les services de vulgarisation agricole du Ministère de l'alimentation et de l'agriculture intensifient les campagnes d'éducation sur les infections par les nématodes parasites des plantes dans la production de gombo.

Mots clés: Nématodes du poignard; Nématodes à nœuds racinaires; Nématodes de la lésion racinaire; Nématodes en spirale; Nématodes de l'igname.

Introduction

Okra is a common fruit vegetable belonging to the genus *Abelmoschus*. It is widely distributed in the tropics (Petropoulos *et al*., 2018). It is estimated that more than 700 million metric tons (MT) of okra is produced globally with Ghana contributing over 60,000 MT (FAOSTAT, 2012). According to Babatunde *et al*. (2007), okra ranks high among other vegetable crops in Sub-Saharan Africa in production and utilization. It is of high nutritional and economic importance (Eke *et al*., 2008: FAO, 2013). The young immature fruits are eaten as vegetable and can be consumed when fried or boiled. In Ghana, okra is usually boiled in water resulting in slimy soups and sauces which are relished. The leaves may be used as good cattle feed whilst the mucilage is useful in the medical and confectionery industries. It is successfully grown across the Forest and Savanna agro-ecological zones in Ghana.

Economically important plant parasitic nematodes (PPN) found infecting and causing yield reduction of okra include *Meloidogyne* species and *Pratylenchus coffeae* (Bharadwaj and Sharma, 2007: Danso and Kwoseh, 2016). *Meloidogyne* species particularly, produce conspicuous galls on okra roots. This makes the roots succumb to other microbial infections leading to root rots. The damaged roots result in reduced water and nutrient uptake. Leaves of heavily infected okra plants become yellow, resulting in a lower photosynthate production; impacting negatively on yield (Sikora and Fernandez, 2005; Bharadwaj and Sharma, 2007; Coyne *et al*., 2014). Plant-parasitic nematodes identification is important for management strategies, uncovering emerging nematode threats, predicting crop host range, and plant quarantine requirements (Adam *et al*., 2007). Information on PPN diversity, density and distribution on okra is scanty in Ghana. This study generated basic data on PPN genera, density and distribution on the crop to drive the design and execution of management strategies.

Materials and Method

Soil samples collection

Okra plant rhizosphere soil sampling was done in okra farms in the Forest savanna transition and Semi-deciduous forest agro-ecologies of Ghana. The two agro-ecological zones studied experience a bimodal rainfall pattern. Locations of the districts involved in each zone has been presented in Table 1.

In each agro-ecology, 15 okra farms were sampled. A systematic soil sampling technique was employed in a zigzag manner. The sampling was done with a soil augur up to 30 cm soil depth. For an acre okra farm, 20 core sub-samples were taken to make a composite sample (1 kg). Each sample was kept in polythene bag and properly labelled. The samples were kept in insulated cool boxes (ice chests) to prevent excessive heating and desiccation and transported to the laboratory. Types of intercrops found in the okra farms were recorded for each agro-ecology.

Table 1. Locations of sampling districts in two agro-ecological zones

Agro-Ecological Zone	District	GPS Location
Forest savanna transition	Kintampo North	8°11.157′N 01°34.194′W
	Atebubu Amantin	7°46.675′N 01°02.711′W
	Wenchi	7°47.813′N 02°06.599′W
Semi-deciduous forest	Ejura Sekyedumase	7°28.003′N 01°17.919′W
	Offinso North	7°24.628′N 01°56.808′W
	Atwima Nwabiagya South	6°41.345′N 01°47.116′W

Extraction and identification of plant-parasitic nematodes

Seven days after sampling, plant-parasitic nematodes were extracted from the soil samples. The extraction method used was according to Whitehead and Hemming (1965), and Hooper (1993). Each soil sample was sieved to get rid of foreign materials. One hundred ml soil sample was spread evenly on a two-ply tissue paper and placed in a plastic sieve. The plastic sieve and its content were placed on a plastic plate. About 100 ml distilled water was poured by the side of the plastic sieve into each plate. The set-up was left undisturbed on a laboratory bench for 48 h to allow the nematodes to settle in the water in the tray. The nematode-water suspension was poured into 100 ml beaker and allowed to stand for 24 h, after which the supernatant was removed by decanting and reduced to 20 ml. The concentrated nematodes were heat-killed in warm water (60°C; 3 min) and fixed in TAF (7.6 ml of 37% formaldehyde, 2 ml of Tri-ethylamine and 90.4 ml of distilled water). Nematodes' identification was done based on morphological characteristics under binocular stereoscopic and compound microscopes with the aid of established standards.

Soil property analysis

Collected soil samples were analyzed for texture, pH, electrical conductivity (EC), and organic matter (Rowell, 1994). Soil pH was measured with a pH-meter on a 1:1 soil/water suspension (Page, 1982). Soil electrical conductivity was measured with a conductometer on a 1:2.5 soil/water suspension (Yadav *et al*., 1979). Soil particle size distribution was determined with a densimeter using Calgon as a dispersing agent and classes referred to the International Soil Science Society (ISSS) standards as demonstrated by the BTSM (1991). Soil organic matter carbon was determined following a modified Walkley-Black wet oxidation method [(% C X 1.724; (C = carbon content, 1.724 is the conventional Van Bemellen factor)] (Nelson and Sommers, 1982).

Data collection and analysis

The okra cropping systems found on each farm visited was observed and recorded. The number of different genera of plant-parasitic nematodes observed was recorded. This was used to calculate the following;

(I) population density =
Total nematodes per 100 ml rhizosphere soil sample

(ii) frequency of distribution =

$$\frac{\text{farms in which a particular nematode was found}}{\text{total number of farms surveyed}} \times 100$$

Fig. 1: Okra rhizosphere soil sampling locations in Ghana

(iii) relative abundance =

$$\frac{\text{population of a particular nematode}}{\text{total nematode population}} \times 100$$

Nematode count data was log transformed [(log (x + 1)] to improve homogeneity of variance before statistical analysis using ANOVA with GENSTAT 12.1, and back-transformed for presentation. Mean differences were separated using LSD at 5%.

Results

Cropping systems

Four intercrop patterns were found; okra-rice, okra-plantain-tomatoes, okra-maize-plantain, and okra-pepper (Table 2).

Genera of nematodes found in the study area

Six nematodes' genera namely *Xiphinema, Helicotylenchus, Meloidogyne, Pratylenchus, Rotylenchulus and Scutellonema* were identified across the study area (Table 3). All the six nematode genera identified were found in the Semi-deciduous forest agro-ecology whilst five of them except *Xiphinema* were found in the Forest savanna transition agro-ecology (Table 3). The nematodes identified belong to the order Tylenchida with one *viz Xiphinema* belonging to the order Dorylaimida. Four of the nematodes species namely *Meloidogyne* (fig. 2B), *Helicotylenchus* (Fig. 2A), *Pratylenchus* (Fig. 3F) and *Rotylenchulus* (Fig. 2C) were observed to be widely distributed across the study area. *Xiphinema* (3E) were the least distributed. *Meloidogyne* spp. were the most abundant nematodes identified (64.9% relative abundance) (Table 4). *Rotylenchulus, Pratylenchus,* and *Helicotylencus* recorded a relative abundance of 17.6%, 6.7%, and 4.7%, respectively. Four of the nematodes (*Meloidogyne Rotylenchulus, Pratylenchus,* and *Helicotylencus*) recorded a frequency of 100% being extracted and identified in all samples collected whilst *Xiphinema* spp. was the least frequent (15%) and least abundant (0.05%) (Table 4)

Frequency and relative abundance of nematodes

Population of nematodes

In the Semi-deciduous forest agro-ecology, *Meloidogyne* population density was significantly higher (1,975 juveniles per 100 ml soil). *Xiphinema* recorded significantly, the least (4 juveniles per 100 ml soil). In the Forest-savanna transition, *Helicotylenchus* and *Rotylenchulus* populations did not differ significantly (Table 4).

Characteristics of soils at the study area

Most of the soils were slightly acidic, recording pH levels ranging between 6.4 and 6.2. (Table 5). Sandy loam texture was common. Soil electrical conductivity (EC)

Table 2. Okra intercropping systems in two agro-ecologies of Ghana

Agro-ecology	Okra-rice	Okra-plantain-tomatoes	Okra-maize-plantain	Okra-pepper
Forest Savanna Transition	2	3	3	3
Semi-deciduous Forest	4	3	2	3

The figures represent number of okra-intercropped farms ranged between 5.2 and 32.5 µS/cm. Soil organic matter content ranged from 0.8% to 3.9%.

Discussion

Table 3. Pest nematodes retrieved from 54 okra farms in two agro-ecologies of Ghana

	Agro-ecology	
Nematode genus	Forest Savanna Transition	Moist Semi-deciduous Forest
Xiphinema	0	+
Helicotylenchus	+	+
Meloidogyne	+	+
Pratylenchus	+	+
Rotylenchulus	+	+
Scutellonema	+	+

+ (Present) and 0 (absent)

Meloidogyne, *Pratylenchus, Rotylenchulus* and *Helicotylenchus* nematodes were found to be widely distributed. This was not surprising as these plant-parasitic nematodes have been reported as having wide host range and extensively distributed in tropical soils (Rich *et al*., 2009). *Xiphinema* occurred only in the Ejura Sekyedumase District of the Semi-deciduous forest zone. Their low prevalence could be linked to the crop (okra) under study which has been found not to support populations of the pest. Cordero *et al*. (2012) found *Xiphinema* mostly on permanent, ornamental and landscape crops. On the other hand, the relatively high density and wide distribution of *Meloidogyne* may be due to the high susceptibility of okra to the pest and favourable environmental conditions. According to Crow and Dunn (2005), the short life cycle (20-30 days) and high reproductive rate of *Meloidogyne* species under favourable environmental

Fig. 2A *Helicotylencus multicintus*; 2B *Meloidogyne* species; 2C *Rotylenchulus reniformis*

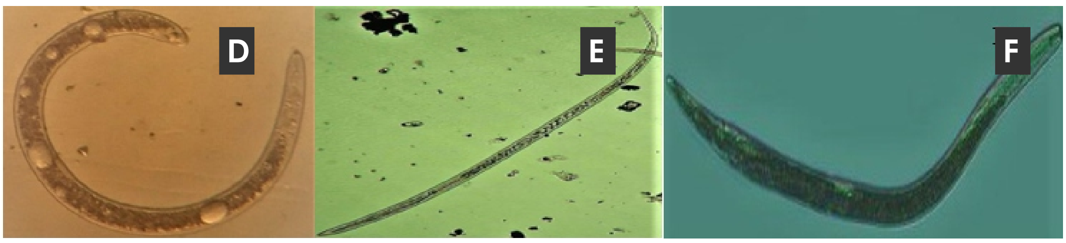

Fig. 3D *Scutellonema bradys;* 3E *Xiphenema Americanum;* 3F *Pratylenchus coffeae*

Table 4. Frequency and relative abundance of pest nematodes in okra farms in two agro-ecologies of Ghana

Nematode genus	Population / 100 ml soil	Frequency[1] (%)	Relative abundance (%)[2]
Xiphinema	15	15	0.05
Helicotylenchus	1,521	100	4.70
Meloidogyne	21,183	100	64.90
Pratylenchus	2,201	100	6.70
Rotylenchulus	5,729	100	17.60
Scutellonema	1,967	70	6.03

[1]Ratio of okra farms in which a particular nematode was found to the total farms sampled x 100
[2]Ratio of a particular nematode population to the total nematode population x 100

Table 4: Pest nematode population densities from okra farms in two agro-ecologies of Ghana

Nematode genus	Plant parasitic nematode populations per 100 ml soil	
	Moist Semi-deciduous Forest	Forest Savanna Transition
Xiphinema	4 e	0 e
Helicotylenchus	222 d	287 d
Meloidogyne	1,975 a	5,082 a
Pratylenchus	353 c	379 c
Rotylenchulus	1,554 b	276 d
Scutellonema	162 d	497 b

Means followed by different letters are significantly different at 5% probability level; the figures are means of 15 replications

conditions may result in a rapid population build-up. Osei *et al*. (2004) found *Meloidogyne* and *Pratylenchus* as the most abundant nematode genera recovered from yam farms in the Forest savanna transition zone of Ghana. Similarly, *Meloidogyne* was the most abundant plant-parasitic nematode genera recovered from tomato farms in the Semi-deciduous forest, Forest savanna transition, and Guinea savanna zones of Ghana (Osei *et al*., 2012). Therefore, the high population of the pest in this study is supported by these earlier reports.

Soil pH, electrical conductivity, organic matter and texture may influence plant-parasitic nematode populations and distribution (Monfort *et al.*, 2007). According to Nisa (2021), these soil factors influenced nematode population dynamics directly and indirectly. Soils in this study were generally slightly acidic. Such soils are suitable for crop growth and plant-parasitic nematodes multiplication and establishment (Abu-Garbieh *et al*., 2005; Seifi *et al*., 2010). Most of the soils encountered in the study area were of sandy loam texture. This reflects the most common type of soil used for growing okra and other food crops. Soil texture influenced plant-parasitic nematode populations and distribution horizontally and vertically (Le Saux and Quénéhervé, 2002). According to Robinson (2005), sandy soils harboured larger plant-parasitic nematode populations than clayey soils. This is because sandy soils

Table 5. Some physical and chemical characteristics of okra rhizosphere soils

Agro-Ecology / District	pH	EC* (µs/cm)	% Organic matter	% Sand	% Silt	% Clay	Textural Class
Forest Savanna Transition	6.4	7.5	1.5	80.1	8.6	11.4	sandy loam
Semi-Deciduous Forest	6.2	19.7	2.4	80.1	8.6	11.4	sandy loam

The values are means of 15 replicates

were more porous and aerated. Taylor *et al*. (1982) and Koenning *et al*. (1996) observed that few *Meloidogyne* populations were recovered from soils with higher clay and silt content, because small soil particles reduced soil pore spaces and hindered free nematodes movement.

Conclusion

This work showed spatial distribution of plant-parasitic nematodes in okra rhizosphere soils. Educational awareness campaigns must be stepped up by Agricultural Extension Services and other stakeholders on the damage potentials of plant-parasitic nematodes in okra production. It is important to develop sustainable integrated management strategies by Crop Protectionists for adoption by okra farmers to combat the menace. Those plant parasitic nematodes confined to specific locations must be contained and not allowed to spread to contaminate other farmlands. Management campaigns must be considered against the plant parasitic nematodes that were found to be widely distributed and abundant to minimize their damage effects in okra.

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