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Full Length Article

Effect of different Maize Cropping Systems on Plant-Parasitic Nematode Populations in Côte d'Ivoire

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Abstract

The food system in northern Côte d'Ivoire is based on cereals, mainly maize. However, maize, like all crops, faces numerous biotic and abiotic constraints. Among the biotic constraints, viruses and fungal diseases are the most widely studied. However, very few studies have been carried out on plant-parasitic nematodes of maize. In view of this situation, the present study was initiated to make an inventory of maize cropping systems and to assess their impact on the regulation of nematode populations in the Tchologo region. The aim was to identify the most sustainable cropping systems among prevailing systems in the region. To do this, a survey of growers was carried out in 2021 and 2022 during the months of September and October in the departments of Ferkessédougou, Kong and Ouangolodougou in northern Côte d'Ivoire. This survey was followed by root sampling of maize plants associated with the different crops to identify the various species of nematode pests. The results showed that the maize-legume cropping system is the predominant type in the region. The nematofauna consists mainly of the genera *Helicotylenchus*, *Radopholus*, *Pratylenchus*, *Hoplolaimus*, *Tylenchus*, *Longidorus*, *Rotylenchulus* and *Meloidogyne*. The most frequent and abundant nematodes are the genera *Pratylenchus*, *Radopholus* and *Meloidogyne*, with densities of 56944.44; 32933.33 and 22569.44 individuals/100 g of roots respectively. Our knowledge of the various maize-based cropping systems in rural areas and the population levels observed suggest that these nematodes may be responsible for significant losses.

Keywords: Maize; Plant-parasitic nematodes; Cropping system; Legumes; Côte d'Ivoire

Introduction

Cereals are among the most widely cultivated plants in the world, thanks to their ability to adapt to climatic conditions and their ease of starch storage. What's more, their versatility in culinary preparation makes them popular with consumers and farmers on every continent (Cormick 2020). For example, maize (*Zea mays* L.), with annual world production estimated in 2021 at over 1.22 million tonnes on some 206 million hectares, or an average yield of 5.88 t/ha, is the most widely produced cereal worldwide, ahead of wheat and rice (FAOSTAT 2023). This cereal is the most widely consumed in the world and the most widely sold on many markets, with far greater outlets than other cereals such as wheat and rice (Yara 2022).

However, in Côte d'Ivoire, maize is the second most cultivated cereal after rice, with annual national production estimated at around 1.33 million tonnes in 2021 on a cultivated area of 592272 ha (Yara 2022). Maize is present in all rural areas of Côte d'Ivoire and plays a major role as subsistence, commercial and socio-cultural crop (Kouakou *et al.* 2010; Yara 2022). It is produced for human consumption as fresh cobs in coastal areas and as semolina in the north of the country, where it is a staple food. It is also used in animal feed and in modern livestock farming (Kouakou *et al.* 2010). N'Guessan *et al*. (2019) report that in cotton-growing areas, cotton cultivation has long supported agricultural development by being the main crop

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in rotations of varied annual crops such as maize. Furthermore, as part of the project "Revitalising production, market access and agricultural advice for the food and commercial sectors in northern Côte d'Ivoire", observations revealed that maize is produced as pure crop on more than 80% of plots, with a preference for pure crop rotation systems over association systems. In fact, when producers choose associations, they give priority to legumes, which facilitate soil cover, thus reducing the presence of weeds, and compete little with maize for the availability of nitrogen in the soil. Maize is also sometimes combined with rice or vegetable crops on small plots, particularly in peri-urban areas where land pressure is high (Chigata 2014).

However, like all crops, maize productivity depends on an extremely diverse set of factors, which can be referred to as the "production situation". A production situation is represented by all the biotic, abiotic, social and economic factors that affect agricultural productivity (Savary 1992). Speaking of biotic constraints, they concern the abundance of weeds (particularly striga) and diseases such as stripe, helminthosporiosis, curvulariosis and rust (Traoré 2016). Furthermore, nematodes are capable of causing considerable losses during crop development and aggravate plant damage under conditions of water stress. This is the case of the genera *Helicotylenchus*, *Pratylenchus*, *Hoplolaimus*, *Rotylenchulus* and *Tylenchorhynchus*, which can be considered the most widespread and likely to cause significant yield losses in maize crops (Sawadogo *et al.* 2020).

This study focuses on the relationships between the components of biodiversity in cultivated fields and the ecosystem services provided by African multispecies cropping systems. Of course, among the ecosystem services expected, pest control is one of the most important in terms of the socio-economic and environmental benefits provided by the cropping system. With this background, the general aim of present study was to draw up an inventory of maize cropping systems and assess their impact on the potential for regulating plant-parasitic nematode populations in each maize cropping system in the Tchologo region, with a view to identifying the most sustainable ones.

Materials and Methods

Presentation of the study area

This study was carried out by the National Center for Agricultural Research (CNRA) in Ferkessédougou, in the Tchologo region (Fig. 1). The Tchologo region, located in the north of Côte d'Ivoire and covering an area of 1.728 km², is an administrative district and a territorial collectivity whose capital is the town of Ferkessédougou (Yeo *et al.* 2023). It is characterized by a monotonous relief, with varying altitudes, between 300 and 400 m, topped in places by a few isolated elevations that can exceed 500 m in altitude. There are also lateritic plateaus sometimes reaching 3 m in height. These plateaus have very gentle, even slopes. The forest galleries along with the watercourses are made up of Sudano-Guinean vegetation, with a predominance of wooded and grassy savannah (Brou 2005). The geological formations found in the study area consist of a sequence of bands of schistose, magmatic and plutonic rocks. These are essentially granites, granodiorites, undifferentiated schists and sericite schists (Yace 2002), from which several soil types are derived, such as, Ferralsols, Cambisols, Fluvisols and Luvisols (Koné *et al.* 2009).

This region is influenced by a dry tropical climate with two main seasons, including a dry season from November to April. The dry season is accompanied by the harmatan between November and February, followed by heat peaks between March and May. Another season is considered as a rainy season from June to October, with peaks in August and September, and an annual rainfall of around 1.200 mm (Brou 2005). Thermal amplitudes in the Ferkessédougou area from 2021 to 2022 are values of 4.9°C with month shifts (warmest and coolest months). In terms of precipitation, the period from 2021 to 2022 had an amplitude of 285.20 mm. However, it was noted in both cases that August was the wettest month and January was the month having the lowest precipitation.

Plant material

The plant material consisted of maize root samples from different systems combining or not legumes such as soybean (*Glycine max* L.), groundnut (*Arachis hypogea* L.) and an improved CNRA soybean variety called NKN1.

Inventory of maize-based cropping systems

A survey was carried out in the different maize production zones of the Tchologo region in 2021 and 2022 during the months of September and October to identify the different maize-based cropping systems. Information on the types of cropping associations practiced was collected from different areas surveyed. Crops present on the same plot were then inventoried.

Sampling in cornfields

Maize and legume root samples were collected from the various types of plots visited in the Tchologo region. Samples were collected randomly from farmers' plots as well as from experimental plots between the plot rows, at the rhizosphere level of previously marked plants at soil depths of 2 and 10 cm. Five elementary samples were taken per crop and on each type of plot in order to make up composite batches of root samples. These batches were placed in polyethylene plastic bags and labelled according to the growing system, indicating the date and location of sampling.

Overall, six maize cropping system modalities were sampled as indicated and coded below:

- Maize grown as a pure crop or as a monocrop on fallow

land less than 2 years old (T0):
- Corn in pure cultivation on previously cultivated plots (T1),
- Maize in association with groundnuts (T2),
- Corn combined with soybeans inoculated with *rhizobium* (T3),
- Corn combined with soybeans not inoculated with *rhizobium* (T4),
- Corn in combination with NKN1, an improved soybean variety (T5).

The plastic-packed samples were kept cool in a refrigerated cooler and transported to the nematology laboratory of the Pedagogical and Research Unit on Plant Physiology and Pathology, Laboratory of Biotechnology, Agriculture and Valorization of Biological Resources, University Félix Houphouët-Boigny for the various nematological analyses.

Extraction of nematodes from roots by double centrifugation

Nematodes were recovered from maize roots using centrifugal floatation method using magnesium sulfate as floatation solution (Coolen and D'Herde 1972). The principle consists in recovering nematodes according to their physical characteristics, notably their size and density in relation to water (1 kg/L) and a magnesium sulfate solution (1.15 to 1.2 kg/L). Each root fraction was ground in water using a blender. The crushed material was rinsed with a jet of water over a battery of sieves (500, 80, 40 and 32 μm). The sieve was centrifuged at 2,500 rpm in the presence of 3 g kaolinite, and the resulting pellet was centrifuged again after addition of 50 mL magnesium sulfate solution $MgSO_4$. The supernatant was sieved (5 μm). The contents of the sieve were collected by spraying water from a pissette into a glass vial to obtain the nematode suspension.

Nematode identification and counting

The nematode suspension was transferred to a graduated cylinder defining a volume known as the suspension volume. Next, 2 mL of the suspension was shaken and spread on the counting plate for observation, identification, and enumeration of the nematodes under light microscope. Three observations were made for each sample then the number of individuals was reduced to 100 g of root samples for analysis.

Identification was carried out using microscopic objectives (X4 and X10) and morphometric characters reported by Luc *et al*. (2005) and Mekete *et al*. (2012). Focusing on the description of the genus, the identification made it possible to determine the populations of each genus based on the number of individuals identified in relation to the nematode population in each sample.

Nematological data interpretation

Calculation of the number of individuals in 100 g of samples of roots: The density of nematodes was calculated in 100 g of sample (X) using following formula (Yeo *et al*. 2023):

$$X = [(N \times V) / M] \times 100$$

Where **N**: denotes the number of nematodes per mL of suspension, **V**: suspension volume (mL) and **M**: root mass used for extraction (g).

Calculation of the frequency and abundance of the main nematode species: The importance of each nematode genus and species was determined using the Frequency/Abundance Diagram of Fortuner and Merny (1973). Frequency is the percentage of samples in which the genus was found. The Abundance of a nematode genus is the average of the densities of the samples in which the genus was found.

They are determined by the following formulas:

$$\text{Frequency} = (ni/N) * 100$$

$$\text{Abundance} = \log \sum Xi/ni$$

ni = number of samples where the genus is present
Xi = number of individuals of the genus in question per kg of soil or per gram of dry roots
N = total number of samples

Statistical data analysis

STATISTICA software version 7.1 was used to analyze the data collected. A one-factor analysis of variance (ANOVA) was also performed to assess the effect of different maize cropping systems on nematode population density. Normality was checked using the Shapiro-Wilk test. Means were then separated using the Newman-Keuls test at the 5% threshold.

Results

Maize cropping systems in the Tchologo region

Surveys carried out in the various departments of the Tchologo region revealed that maize has been grown by all the producers surveyed (100%). It has been grown in association with other crops (food, cereals and cash crops), as a pure crop or as a monoculture. Hut cultivation was also observed in the Tchologo region. The composition of cropping associations varied widely from one department to another. In general, maize was grown in association with ground nuts, beans, rice, soya and vegetable crops (tomatoes, eggplants and okra *etc*.). However, monoculture or pure maize cultivation was rarely observed.

Nematodes observed in plant roots on different types of plots

Nematological analysis was used to determine the densities and taxonomic composition of plant-parasitic nematodes in the different cropping systems surveyed.

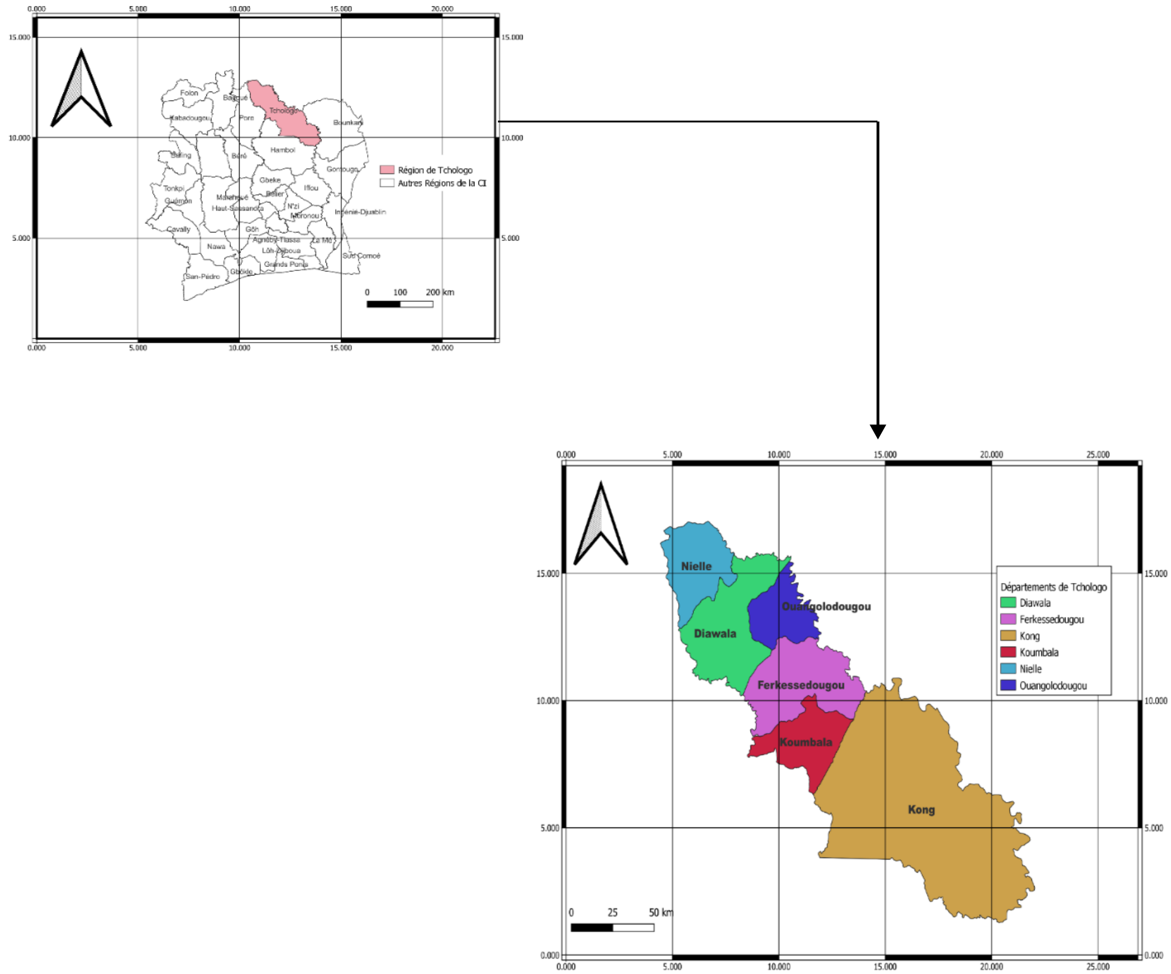

Fig. 1: Map of the Tchologo region

Eight genera of plant-parasitic nematodes belonging to five families and two orders were inventoried, including, *Pratylenchus*, *Radopholus*, *Tylenchus*, *Helicotylenchus*, *Hoplolaimus*, *Meloidogyne*, *Longidorus* and *Hirschmaniella*. A diversity of genera is observed with the order Tylenchida. Among these nematodes, four were endoparasitic and four were ectoparasitic in nature (Table 1).

Population density of plant-parasitic nematodes in maize roots from different cropping systems

The results in Table 2 show the population density of plant-parasitic nematodes present in the roots of maize plants in different cropping systems in the Tchologo region. Variability in nematode density from one cropping system to another and from one nematode genus to another was revealed. In general, nematode population densities were very low, ranging from 0 to 2922.58 individuals/100 g root area in plots of pure maize (T0), irrespective of nematode genus.

The highest densities of plant-parasitic nematodes were observed on plots T1 (maize in pure cultivation on previously cultivated plots) and T4 (maize-soya not inoculated). Thus, for the *Pratylenchus* genus, the number of individuals per 100 g of roots was 29821.43 and 34367.98 for treatments T4 and T1 respectively. For the *Radopholus* genus, treatments T1 and T4 showed values of 15652.19 and 16428.57 individuals/100g of roots respectively. As for the *Tylenchus* genus, the density of plant-parasitic nematodes was 3611.42 and 6785.71 individuals for treatments T1 and T4 respectively. For the *Helicotylenchus* genus, treatment T4 showed a respective value of 535.71 individuals, while T1 revealed a plant-parasitic nematode density equal to 2357.42 individuals/100 g of roots. For the *Hoplolaimus* genus, the number of nematodes obtained in 100 g of roots was respectively 1215.28 and 4107.14 individuals for treatments T1 and T4. The number of nematodes obtained in 100 g of roots in treatments T4 and T1 for the *Meloidogyne* genus was 11785.71 and 13816.83 individuals respectively. With the genus *Longidorus*, the respective value of 1415.53 individuals indicates the density of plant-parasitic

Table 1: Taxa and trophic types of plant-parasitic nematodes identified on the plots

Orders	Families	Genres	Trophic types
Dorylaimida	Longidoridae	*Longidorus*	Ectoparasites
Tylenchida	Tylenchidae	*Tylenchus*	
	Hoplolaimidae	*Helicotylenchus*	Semi-endoparasites
		Hoplolaimus	
	Meloidogynidae	*Meloidogyne*	Endoparasites
	Pratylenchidae	*Pratylenchus*	
		Radopholus	
		Hirschmanniella	

Table 2: Nematode density in 100 grams of maize roots in pure culture and associated with legumes

Genres	Number of individuals/100g of roots						Average	Coefficient of variation (%)	Probability (*P*)
	Treatments								
	T0	T1	T2	T3	T4	T5			
Pratylenchus	2922,58 ± 464,96 b	34367,98 ± 5752,45 a	19333,33 ± 3320,81 ab	21671,05 ± 641,22 ab	29821,43 ± 778,38 a	12857,14 ± 824,79 ab	19578,17 ± 2713,64	86,56	< 0. 001
Radopholus	1953,48 ± 503,16 b	15652,19 ± 3350,90 a	8000,00 ± 00,00 ab	9596,49 ± 1924,09 ab	16428,57 ± 472,46 a	8571,43 ± 00,00 ab	9431,98 ± 1403,39	92,92	0. 001
Tylenchus	200,32 ± 110,05 c	3611,42 ± 534,53 b	2000,00 ± 577,35 bc	530,70 ± 241,23 c	6785,71 ± 178,57 a	3809,52 ± 629,94 b	2223,35 ± 372,10	104,52	< 0. 001
Helicotylenchus	335,75 ± 117,62 b	2357, 42 ± 195,20 a	1000,00 ± 00,00 b	2184,21 ± 629,49 a	535,71 ± 00,00 b	00,00 ± 00,00 c	1282,83 ± 192,80	93,86	< 0. 001
Hoplolaimus	97,66 ± 51,00 b	1215,28 ± 664,19 ab	2000,00 ± 00,00 ab	1039,47 ± 205,95 ab	4107,14 ± 472,46 a	1428,57 ± 00,00 ab	1143,57 ±263,93	144,13	0. 003
Meloidogyne	795,23 ± 213,32 b	13816,83 ± 2317,49 a	4833,33 ± 1301, 71 ab	5096,49 ± 326,41 ab	11785,71 ± 309,30 a	9761,90 ± 1037,83 a	7309,40 ± 1125, 90	96,19	< 0. 001
Longidorus	229,17 ± 119,68 c	1415,53 ± 221,46 ab	500,00 ± 00,00 b	750,00 ± 315,41 b	3928,57 ± 643,85 a	1428,57 ± 412,39 ab	1072,00 ± 186,69	108,76	< 0. 001
Hirschmanniella	00,00 ± 00,00 c	418,31 ± 133,49 b	500,00 ± 00,00 b	00,00 ± 00,00 c	00,00 ± 00,00 c	714,29 ± 00,00 a	222,12 ± 56,96	160,15	< 0. 001

On the same line, values with the same letter do not differ significantly according to the Newman-Keuls test at the 5% threshold

- **T0:** Maize grown as a pure crop or as a monocrop on fallow land;
- **T1:** Maize grown as a pure crop on previously cultivated plots;
- **T2:** Maize in association with groundnuts;
- **T3:** Corn combined with soybeans inoculated with *rhizobium*;
- **T4:** Corn combined with soybeans not inoculated with *rhizobium*;
- **T5:** Corn in association with NKN1, an improved soybean variety

nematodes in 100 g of roots for treatment T1. For treatment T4, the number of individuals was equal to 3928.57 in 100 g of roots. Finally, for the genus *Hirschmanniella*, the density of nematodes was zero for treatment T4 but 418.31 individuals in 100 g of roots for treatment T1.

Moreover, the lowest plant-parasitic nematode densities in the case of cropping combinations were observed on the T3 plots (inoculated corn-soybean).

Evolution of phyto-parasitic nematode populations in relation to legumes

In the roots of legumes associated with maize, a low density of nematode populations was observed in the samples. These total densities of plant-parasitic nematodes varied to a greater or lesser extent from one legume to another (Fig. 2), with an extreme value of 11799 individuals/100 g of roots in the case of inoculated soybeans. For non-inoculated soybeans, the total number of individuals of the various plant-parasitic nematode genera was equal to 10.000/100 g of roots. The total nematode density for groundnuts was 5572 individuals per 100 g of roots. Finally, the improved legume NKN1 awakened a total density of plant-parasitic nematodes per 100 g of root equal to 1000 individuals, the lowest value.

Distribution of populations according to species frequency in maize and legume roots under different cropping systems

The frequency diagram shows that the nematodes *Pratylenchus*, *Radopholus*, *Tylenchus*, *Hoplolaimus*, *Meloidogyne* and *Longidorus* were the most frequently (100%) present in all maize root samples taken from the different plot types (Fig. 3). As for legumes, four nematode genera were identified namely *Helicotylenchus*, *Pratylenchus*, *Radopholus* and *Tylenchulus* (Fig. 4). The genera *Pratylenchus*, *Radopholus*, *Tylenchus* and *Helicotylenchus* with a frequency of 100%, were the plant-parasitic nematodes found in both maize and the legumes associated with it.

Population distribution according to species abundance in maize and legume roots under different cropping systems

Variability in relative abundance was observed among the different genera of nematodes extracted from maize roots (Fig. 5A). The most abundant genus was *Pratylenchus* with prevalence of 46.3%, followed by *Radopholus* and *Meloidogyne* with prevalence of 22.30 and 17.30%, respectively. The remaining ones had the least abundancy with low prevalence.

Like maize, the relative abundance of plant-parasitic nematodes extracted from legume root samples varies from one genus to another (Fig. 5B). However, *Pratylenchus* was the most abundant genus with prevalence of 35.8%. followed by *Radopholus* and *Meloidogyne* with prevalence of 29.4 and 13.6%, respectively. Other genera were having abundance ranging from 0.9 to 8% (Fig. 5B).

The plant-parasitic nematodes *Pratylenchus* and *Radopholus* are the most frequent and abundant in both maize and legume roots.

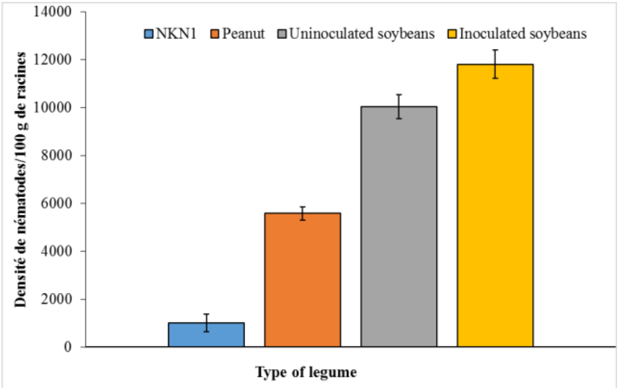

Fig. 2: Plant-parasitic nematode population density in 100 g of roots as a function of legume type

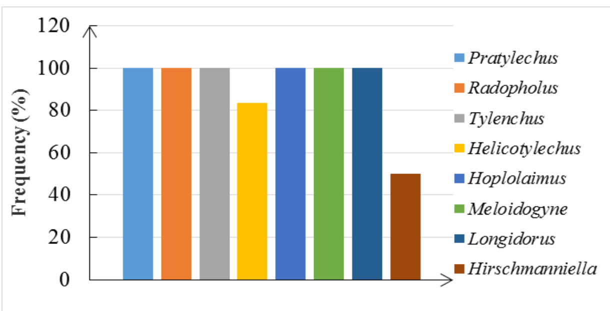

Fig. 3: Frequency of nematode genera: case of maize

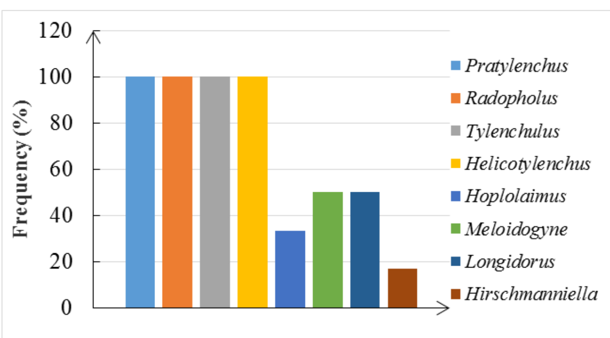

Fig. 4: Frequency of nematode genera: case of legumes

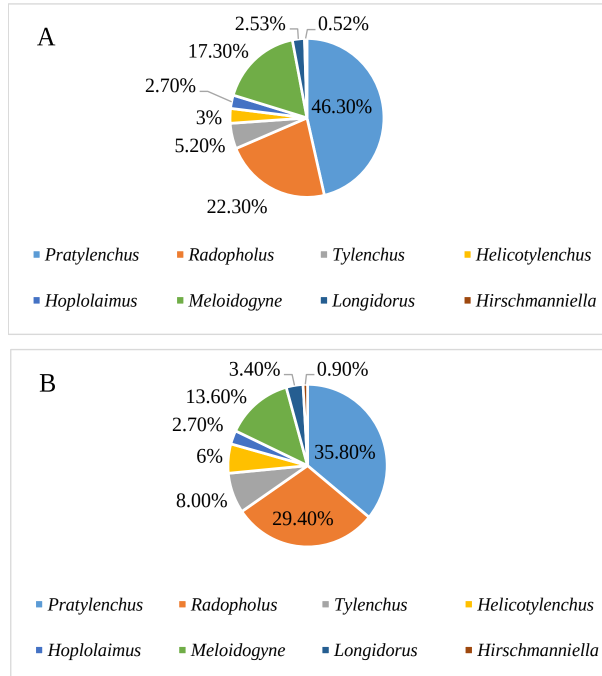

Fig. 5: Relative abundance of nematode genera in maize (A) and legumes (B)

Discussion

Like other cereal crops, corn is a plant that attracts a number of pests. Among these, plant parasitic nematodes are considered important, as they contribute to a direct or indirect drop in crop yields by disrupting the functioning of their root systems. The inventory of parasitic nematodes of maize is very useful, as it enables us to update data on plant-parasitic nematode communities of maize in Côte d'Ivoire (Kouadio 2022) and more specifically in the Tchologo region. The results of this study showed a very low density, with eight genera of nematodes identified on plots of pure maize cultivation on fallow land (T0). This low nematode density could be explained by the short duration of the fallow. Soils subjected to this cropping technique have not had sufficient time to recover naturally. As a result, they are less fertile. Our results are in line with those of Cadet *et al.* (2000), who showed that the number of plant-parasitic nematode species tends to increase with the length of fallow. In addition, several scientific works such as those by Berner (2013) prove that fertile soils harbor a rich diversity of living organisms, all of which participate in important processes. Among these, nematodes are revealed as bio-indicators of soil fertility. In their quest for dead organic matter, these organisms turn over the surface layers of the soil, creating galleries. These galleries ensure good soil aeration and improve water absorption and storage.

However, this study also showed that in plots where maize was not combined with legumes, and where other crops were grown (T1), nematode density was higher than in plots of pure crop on fallow land. This high nematode density is thought to be due to the presence of crop residues on these soils. This litter is a source of food for soil micro-organisms, in this case nematodes which decompose the organic matter. In a way, these plots are restituted with nutrients. Thus, enriching the soil with plant debris may induce significant mineralogical transformations that will affect the availability of certain soil elements (Kettering *et al.* 2000). In addition to improving the soil's organic and mineral qualities, crop residues will help to maintain good soil moisture levels. All of which is conducive to the reproduction of these nematodes.

In addition, other results from this study show that in plots (T2, T3, T4 and T5) where maize was combined with various legumes, plant-parasitic nematode density was generally higher in maize roots than in legume roots. Such results would suggest that the presence of legumes favored the presence of plant-parasitic nematodes. Indicating the level of enrichment of the environment, these nematodes therefore had the opportunity to multiply in these rich environments. This result can be explained by the quality of legume litter and rhizospheres, which are rich in nitrogen and easily degradable elements (DuPont *et al.* 2009; Schneider and Huyghe 2015).

As for the high density of nematodes in maize root samples, these results corroborate those of Luc *et al*. (2005), who reported that over 60 nematode species have been found associated with maize in different parts of the world. *Pratylenchus* is the genus most commonly found in subtropical and tropical zones. Other nematodes associated with maize include *Meloidogyne*, *Helicotylenchus*, *Longidorus* and others. Still in the case of the maize-legume association, our results showed significant differences between inoculated and non-inoculated maize-soybean associations for plant parasitic nematode densities in maize root samples. In fact, nematode densities were higher in the uninoculated maize-soybean association than in the inoculated maize-soybean association. This could be explained by the effect of inoculating soybeans with *Rhizobium bacteria*. These bacteria, present in the soil, undoubtedly considerably reduced the nematode population in plots where maize was associated with inoculated soybeans. According to Melnic *et al.* (2013), *Rhizobium bacteria* exert a lethal action on nematodes in proportions of 95 to 100%. Indeed, after 7 to 10 days of contact, they exert a destructive influence on the internal organs (intestines, ovaries and oviducts *etc*.) of parasitic nematodes, which lose their shape, become completely deformed and eventually die. Inoculation with *Rhizobium* can therefore be considered an effective method of regulating plant-parasitic nematode species.

Frequency diagrams show that the genera *Pratylenchus*, *Radopholus* and *Tylenchus* are the most frequent in both maize and legume roots. Relative abundance plots show that Pratylenchus, *Radopholus* and *Meloidogyne* are the most abundant genera on both sides. Our results corroborate those of Gnonhouri and Adiko (2005), whose study showed that *Meloidogyne incognita*, *Pratylenchus brachyurus* and *Radopholus similis* are the plant-parasitic nematodes most commonly found on plants in Côte d'Ivoire, with rates of 30; 21 and 19% respectively.

Conclusion

A total of eight plant-parasitic nematode genera were identified from prevailing agrosystems. Among them, the two genera were most dependent on maize, including *Pratylenchus* and *Radopholus*. It was also observed that a short fallow period has a regulating impact on nematode populations. However, the opposite phenomenon was observed on previously cultivated soils (T1) and in the case of cropping associations. However, the inoculated maize-soybean cropping association hindered the proliferation of these pests in this cropping system. In short, certain parameters such as previous cropping, humidity and cropping techniques such as fallow and intercropping would therefore have an impact on nematode population dynamics in Côte d'Ivoire.

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Author Contributions

G Yeo: Proposed the idea of research, conducted the study, collected data, wrote the protocol, performed the statistical analysis and wrote the first draft of the manuscript; OJA Anoma and S Sanogo: collaborated in writing the research protocol and writing the research and results and preparing the research for publication; S TUO and EYG KOUADIO: Performed literature review, edited and finalized the manuscript; F SORHO and D KONÉ: Conceived the idea, developed research methodology and read and approved the final manuscript.

Conflicts of Interest

The authors declare that they have no competing interests.

Data Availability

The data included in this paper can be obtained from the corresponding author upon reasonable request.

Ethics Approval

Not applicable

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