

Plant Parasitic nematodes associated with sugarcane cultivation *Saccharum officinarum* L. in Ferkessédougou department (Tchologo region), Côte d'Ivoire.

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Abstract An inventory of the sugarcane parasitic nematode population in Ferkessédougou was carried out during the 2020-2021 and 2021-2022 cropping seasons. The aim of this research was to study the prevalence and abundance of the main parasitic nematodes associated with sugarcane. To this end, samples of sugarcane roots were taken from 10 agronomic trials. Nematodes were extracted from the roots of 15 cultivated varieties, at depths ranging from 02 to 10 cm. The results show twelve (12) genera of parasitic nematodes. The existence of a diversity of plant-parasitic nematodes of sugarcane was confirmed. *Radopholus*, *Pratylenchus*, *Meloidogyne*, *Hoplolaimus*, *Hirschmanniella*, *Caloosia*, *Xiphinema* and *Tylenchulus* were frequent and abundant, as they were observed in over 50% of root samples at population levels above 20 nematodes/g root. Varieties M1400/86, FR96070 and M2238/89 recorded the most plant-parasitic nematodes in the roots, with total densities of 52161.48, 28214.29 and 24528.10 nematodes/100g roots respectively. These 8 groups of nematodes can be considered the most damaging to sugarcane cultivation in the Ferkessédougou region, where they are important.

Keywords: Sugarcane parasitic nematodes, diversity, *Pratylenchus*, *Meloidogyne*, Côte d'Ivoire

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1. Introduction

Sugar cane (*Saccharum officinarum* L.) is a semi-perennial crop that is generally grown for at least 5 years and can remain in place for 20 years or more. It could also be considered an annual crop that is not replanted, and which grows back on its own [1]. This characteristic induces a differentiation in the technical itinerary between the first year in which the cane is planted (planted cane), and the following years in which it is the regrowth (offshoot or regrowth cane). These differences will be important in the choice of levers used to control pests and diseases [1]. In Côte d'Ivoire, numerous diseases and pests have been identified in all sugarcane plantations. These diseases and pests attack all stages of the crop and cause considerable yield reductions [2]. Among these pests, plant-parasitic nematodes cause extensive damage to be cutting roots, resulting in reduced tillering; while those

caused to stalk roots affect stalk elongation, both in virgin cane and in regrowth [3]. Plant-parasitic nematodes, whether ecto or endoparasitic, feed on the root system of cane plants and carry out all or part of their life cycle in the soil.

In response to these biological aggressors, on sugar plantations in Côte d'Ivoire, a control approach based on prevention has been adopted, involving thermotherapy treatments of cuttings destined for planting in primary and secondary nurseries that will be used for commercial plantations; and a control approach that relies mainly on chemical nematicides in planted cane [2]. An integrated control method, respectful of the environment and centered on varietal selection, also makes it possible to reasonably limit their action.

The growing presence of nematodes is alarming, and requires their numbers to be brought under control, or risk a very considerable drop in sugarcane yield in future years. To better control nematode damage in sugarcane

plantations, it is important to keep this population under control.

The aim of this study is to characterize the parasitic nematode communities associated with sugarcane in the Ferkessédougou region, to determine their importance and subsequently develop effective control methods, improve yields and sustainably increase sugar production.

2. Materials and Methods

2.1. Site Characteristics

The study was conducted under conditions of natural infestation in the Tchologo region, an area of high sugar production in Côte d'Ivoire.

The trial was carried out on the experimental site of the Centre National de Recherche Agronomique (CNRA) research station in Ferkessédougou, northern Côte d'Ivoire (9°35'54" N, 5°13' 30" W). The soil has an ochre-colored sandy-clay texture that is dominant throughout the perimeter of the station. It is marked by lateritic induration at medium depth (50-70 cm). The soil is predominantly low in organic matter (1.5% on average), with a strongly or weakly acidic pH (6 to 6.5) and low cation exchange capacity (8 meq/100 g) [4].

2.2. Plant Material

The plant material consists of sugarcane roots from various cane plantations in the Tchologo region under conditions of natural infestations in the field. The roots used come from varieties which are interspecific hybrids of *Saccharum officinarum* L. and *Saccharum spontaneum* L. [5,6].

2.3. Experimental Design

Sugarcane root samples were taken from various production plots in Ferkessédougou. Five plots consisting of 5 to 8 lines measuring 4 to 5 m were evaluated. The plots were planted with the following varieties: FR98122; VMC95119; SP711406; M2593/92; CP89-2377; FR97104; R570; FR97039; FR00259; VMC9588; FR98072; VMC9537; FR96070; M2238/89 and M1400/86. All plots were fertilized with compound fertilizers at a rate of 350 kg/ha.

2.4. Sampling and Preparation of Sugarcane Roots

Cane root samples were taken at a depth of 2 to 10 cm and within a radius of 1 to 30 cm, from five randomly selected clumps per elementary plot. Composite samples were also taken for each variety and treatment. The composite samples were placed in labelled plastic bags and transported to the laboratory under refrigerated conditions for nematode extraction. In the laboratory, the roots of each cane variety were rinsed twice in succession in tubs containing tap water, to remove sand and other debris, before being cut into small pieces of around 1 cm. A 50 g fraction of each sample was then taken for nematode extraction.

2.5. Nematode Extraction

Nematodes were extracted using the double centrifugation-flotting method developed by [7] and modified by [8]. 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 (MgSO₄). The supernatant was sieved (5 µm). The contents of the sieve were collected by spraying water from a pisette into a glass vial to obtain the nematode suspension.

2.6. Identification and Enumeration of Nematodes

The nematode suspension was transferred to a graduated cylinder and its volume determined. After homogenization using air blown into the suspension through the channel of a pipette, a volume of 2 mL was taken and spread on a grid-counting plate mounted under an "AMSCOPE" optical microscope. The latter was connected to a camera and a computer. Identification and enumeration of major plant-parasitic nematode populations were carried out at G x40 and G x100 magnifications based on their discriminating morphometric characters according to the determination keys of [9,10,11]. Three readings were taken per nematode suspension. The number of nematodes per taxon was related to the volume of the initial suspension and then to 100 g of roots, according to the following formula used by [12].

$$X = \frac{N \times V}{M} \times 100$$

X = number of nematodes per 100 g of roots; **N** = number of nematodes/ml of suspension

V = volume of suspension (ml) and **M** = mass of root fraction used for extraction (g)

2.7. Statistical Analysis and Data Processing

The data obtained were processed using XLSTAT version 2016 software. An analysis of variance (ANOVA) was performed on the parameters evaluated according to the treatments studied. Means were classified using the Student-Newman-Keuls test at the 5% threshold when significant differences were observed.

The importance of each nematode genus and species was determined using the Frequency/Abundance Diagram of [13]. This parameter is in decimal logarithm. These authors consider a genus to be abundant and frequent when it is present in at least 30% of samples, with at least 20 individuals per gram of roots. Below these values, it is considered infrequent and not very abundant. On the other

hand, a nematode genus may be considered frequent and scarce, or scarce and abundant.

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:

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

$$\text{Abundance} = \log \sum X_i/n_i$$

n_i = number of samples where the genus is present

X_i = number of individuals of the genus in question per kg of soil or per gram of dry roots

N = total number of samples

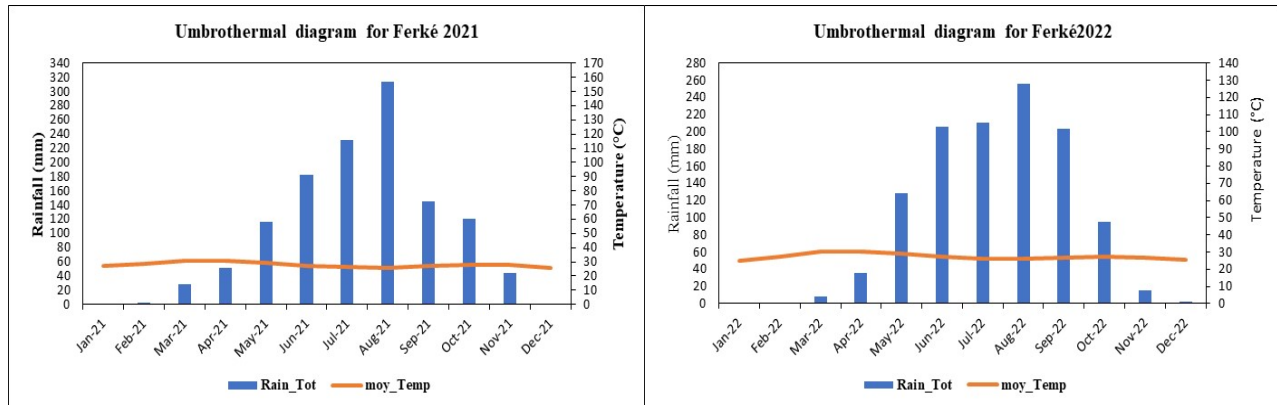


Figure 1. Umbrothermal diagram for Ferkessédougou from 2021 and 2022

3. Results

3.1. Climatic Conditions During the Test

The experimental site at the Ferkessédougou research station, due to its geographical location (northern Côte d'Ivoire), has benefited from a large part of the harmattan's humid saturated air. This site has been subjected to a dry tropical climate. Rainfall and temperatures over the test period indicate four seasons, with two short rainy seasons from February to April and October to November. These two short seasons are interspersed with a long rainy season from May to September, peaking in August. The short dry season runs from December to January. During the test year, monthly rainfall varied between 0 and 285.20 mm, giving an annual rainfall of 980.19 mm (Figure 1). On the other hand, the average monthly temperatures recorded, represented by the umbrothermal diagram in Figure 1, ranged from 24 to 30°C.

3.2. Nematode Taxa Observed in the Roots of Sugarcane Varieties

Six main species of plant-parasitic nematodes were frequently observed under the microscope when counting populations extracted from roots. These were *Radopholus similis*, *Pratylenchus* spp, *Helicotylenchus* spp, *Meloidogyne* spp, *Rotylenchulus reniformis* and *Hoplolaimus pararobustus*. In addition to these taxa, a few other plant-parasitic nematodes (*Hirschmanniella*, *Xiphinema*, *Caloosia*, *Longidorus*, *Trichodorus*, *Tylenchulus*) were also encountered. The results show that the Rhabditida order is the most common in sugarcane cultivation in the Ferkessédougou area. Identification revealed 12 genera of plant-parasitic nematodes, including 4 endoparasites, 4 ectoparasites and 4 semi-endoparasites belonging to 7 families and 4 orders (Table 1).

Table 1. Composition of plant-parasitic nematodes identified in sugarcane roots

ORDER	FAMILY	GENRE	TROPHIC TYPE
RHABDITIDA	Pratylenchidae	<i>Radopholus</i>	endoparasites
		<i>Pratylenchus</i>	
		<i>Hirschmanniella</i>	
DORYLAIMIDA	Meloidogynidae	<i>Meloidogyne</i>	ectoparasites
	Griconematidae	<i>Caloosia</i>	
	Longidoridae	<i>Xiphinema</i> <i>Longidorus</i>	
TRIPLONCHIDA	Trichodoridae	<i>Trichodorus</i>	semi-endoparasites
	Tylenchulidae	<i>Tylenchulus</i>	
TYLENCHIDA	Hoplolaimidae	<i>Rotylenchulus</i>	semi-endoparasites
		<i>Helicotylenchus</i>	
		<i>Hoplolaimus</i>	

3.3. Population Dynamics of Major Nematodes in Sugarcane Roots

Analysis of variance revealed a significant effect ($p < 0.000$) of root nematode infestation on all the samples collected. The main plant-parasitic nematodes associated with sugarcane were *Radopholus similis*, *Pratylenchus*, *Caloosia*, *Hirschmanniella* and *Meloidogyne*. The most abundant nematode based on total number of nematodes per species was *Radopholus similis* with a highest average population density of 3923.11 nematodes/100g root followed by *Pratylenchus* and *Caloosia* which had average population densities of 1944.37 and 1127.84 nematodes/100g root respectively. The taxa *Longidorus*, *Trichodorus*, *Rotylenchulus* and *Hoplolaimus* recorded the lowest population densities, with 223.54, 206.32, 203.73 and 111.05 nematodes/100g roots respectively (Figure 2).

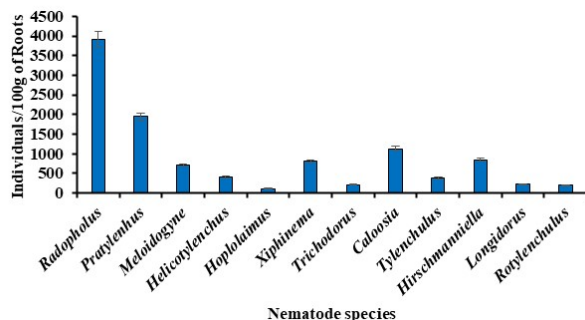


Figure 2. Population density of plant-parasitic nematode taxa in sugarcane roots

Statistical analysis showed a significant effect on the total number of nematodes in the roots of sugarcane varieties. The variety with the highest number of plant-parasitic nematodes was M1400/86, with a total density of 52161.48 nematodes/100g roots, followed by FR96070 and M2238/89, with a total density of 28214.29 and 24528.10 nematodes/100g roots respectively. The other varieties (VMC95119, VMC9537, FR98072, VMC9588, R570, CP89-2377, SP711406, FR97039, FR98122, FR00259, M2593/92) showed total plant-parasitic nematode densities well below 1000 individuals/100g roots (Figure 3).

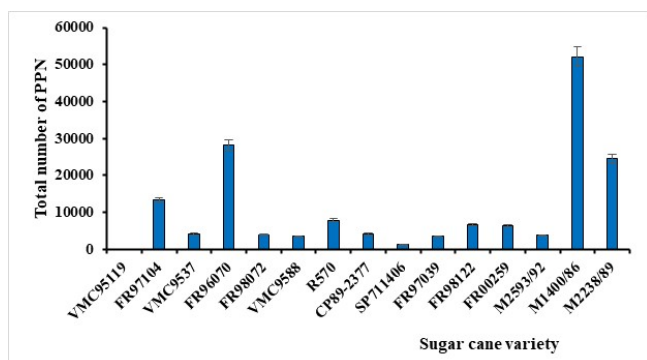


Figure 3. Total population density of plant-parasitic nematodes observed in the roots of sugarcane varieties

3.4. Distribution and Overall Structure of Nematodes in Sugarcane Roots

Application of the frequency-abundance diagram, based on calculation of the abundance and frequency of the taxa identified, reveals that plant-parasitic nematodes fall into two groups (Figure 4).

Nematodes in the first group (nematodes above and to the left) are abundant and infrequent. They were found in less than 30% of sugarcane varieties. Three genera of nematodes (*Helicotylenchus*, *Longidorus*, *Rotylenchulus* and *Trichodorus*) were extracted from roots and were abundant (more than 20 individuals/g of roots) but infrequent, as they were found in 26.66 and 20% of samples respectively (Figure 4).

Nematodes in the second group (nematodes above and to the right) are frequent and abundant. They are represented by *Radopholus*, *Pratylenchus*, *Meloidogyne*, *Hoplolaimus*, *Hirschmanniella*, *Caloosia*, *Xiphinema* and *Tylenchulus*. The genera *Radopholus*, *Meloidogyne* and *Pratylenchus* were the most abundant and frequent

endoparasites, present in 96%, 97% and 95% of samples respectively. Frequent and abundant nematodes were also represented by *Hoplolaimus*, *Hirschmanniella*, *Caloosia*, *Xiphinema* and *Tylenchulus*, present in 53, 33 and 60% of samples respectively (Figure 4).

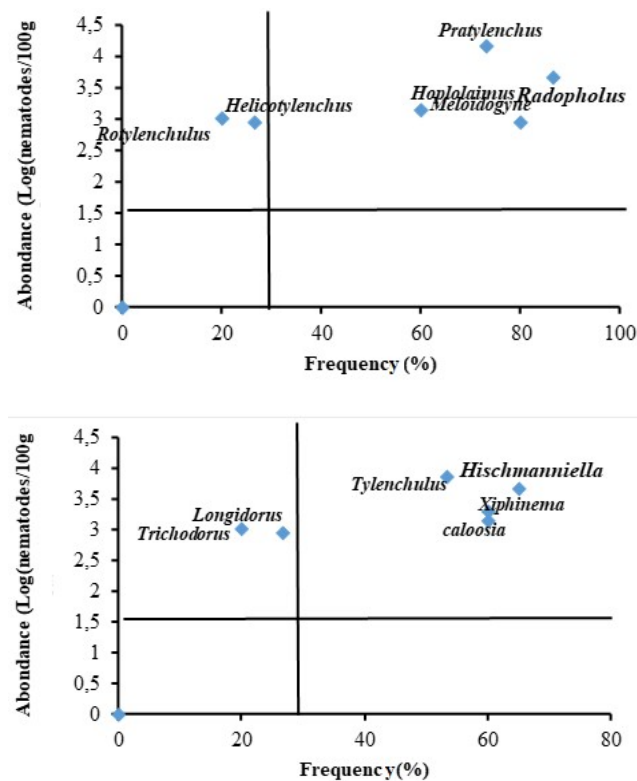


Figure 4. Frequency and abundance of sugarcane parasitic nematode genera and species observed in roots using the [13] method.

4. Discussion Diversity of Parasitic Nematode Communities in Sugarcane

A high diversity of parasitic nematode communities was observed in sugarcane cultivation plots. In fact, the nematode inventory yielded 12 genera from the samples taken, which can be explained by the fact that no nematicide treatments were applied during the cultivation of the sugarcane varieties. In addition, the soil texture and structure of the region is generally subject to high variability, and the water table would also influence the diversity and densities of parasitic nematode populations during the sugarcane cycle. In view of these results, biological diversity was relatively high compared with the research work of [2], which obtained 10 genera of plant-parasitic nematodes in the three sugar complexes of Côte d'Ivoire. Similarly, the order most frequently encountered is Rhabditida. Most of the nematodes identified in this study can be considered a major threat to sugar production and should be regarded as serious pests [14,15].

Prevalence and importance of sugarcane nematode parasites

Our results corroborate those of several research studies on sugarcane parasitic nematode communities. Indeed, several studies have noted the predominance of nematodes belonging to the *Radopholus*, *Meloidogyne* and

Pratylenchus genera, which are known to be the most frequent and damaging to sugarcane.

According to [16], *Pratylenchus* and *Meloidogyne* are the most pathogenic nematodes in sugarcane cultivation. *Pratylenchus* was even designated by these authors as the most abundant nematode on sugarcane plots in Côte d'Ivoire, which corroborates with our results, which also designate *Pratylenchus* as the most abundant genus. The root lesion nematode, *Pratylenchus*, is a migratory endoparasite of roots that feeds on cortical tissues. Their infections can lead to necrotic brown lesions and the formation of tunnels inside rootlets. This can interfere with the movement of water and nutrients within plant tissues, as well as increasing the leakage of harvested and stored food due to lesions [17]. The presence of these two very frequent and abundant endoparasitic genera, *Radopholus* and *Pratylenchus*, should raise the alarm and lead to monitoring of their populations.

Other nematode genera identified in this study are *Tylenchulus*, *Rotylenchulus*, *Longidorus*, *Hoplolaimus*, *Caloosia*, *Helicotylenchus* and *Xiphinema*. They are ectoparasites of root epidermal tissues and have not been documented as dangerous pests of sugarcane. Their feeding causes a reduction in root hair size and damage to epidermal tissues, which reduces the root's ability to absorb water and nutrients from the soil and results in poor foliage growth [18]. The presence of these ectoparasitic nematode genera has also often been observed in commercial vegetable crops [19, 20].

In terms of abundance and frequency, the genera *Radopholus* and *Pratylenchus* were the two found to be both abundant and frequent, in contrast to the work of [2], which found the genera *Xiphinema* and *Helicotylenchus* to be the most frequent and abundant. *Xiphinema* and *Longidorus* species, in addition to the direct damage caused to roots by their feeding, are known to transmit viral diseases such as tomato ring spot nepovirus (Tom RSV), tobacco ringspot nepovirus (Tob RSV) which infect tomato, tobacco and soybean and have a major economic impact on cucurbits [21]. As virus vectors, they can be harmful to sugarcane at very low population levels.

The presence of the genera *Pratylenchus*, *Meloidogyne*, *Xiphinema* and *Helicotylenchus* is not surprising, as they have also been present in the results of other authors on sugarcane cultivation [2,16,22]. Concerning the classification of sugarcane varieties according to nematode susceptibility, it was revealed that the M1400/86 variety is the most sensitive to plant-parasitic nematodes, to the *Radopholus* and *Pratylenchus* genera. On the other hand, VMC95119 was the most tolerant variety to all the plant-parasitic nematode genera encountered; this variety therefore requires particular attention with a view to its use in sugarcane cultivation. Variation in the frequency and density of each nematode species studied seems to be influenced by variety. It is reported that plant-parasitic nematodes in cultivated soil can be affected by the planting of cover crops, the use of alternate cropping sequences, soil types and fallow duration [23,24].

5. Conclusion

Nematodes belonging to the genera *Radopholus*, *Pratylenchus*, *Meloidogyne*, *Hoplolaimus*, *Hirschmanniella*, *Caloosia*, *Xiphinema* and *Tylenchulus* appear to be the most frequent and damaging to sugarcane cultivation in the Ferkessédougou region. These groups of nematodes are found in production plots and can cause significant damage if conditions are favorable for their multiplication. This information on the presence of nematodes in cane crops will be useful to growers in planning and administering plant-parasitic nematode management strategies to reduce nematode populations below their threshold levels. This study also suggests that the scale of the nematode problem needs to be taken seriously and should be the subject of research into control methods.

Conflicts of Interest

This study does not present any conflicts of interest.

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