



Characterization of Plant-Parasitic-Nematode Communities Associated with three Species used in Hedges in Western Niger and their Susceptibility to *Meloidogyne Javanica*

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Abstract

A study was undertaken in three important vegetable producing areas to determine the plant-parasitic nematodes associated with hedge trees and a pot experiment was conducted to assess hedge trees (*Acacia senegal*, *Bauhinia rufescens* and *Prosopis juliflora*) behavior to the root-knot-nematode, *Meloidogyne javanica*. Soil and roots analysis revealed the presence of 10 genera of plant-parasitic nematodes, including *M. javanica*. The behavior test seems to indicate that all three plants were susceptible to *Meloidogyne javanica* with root gall index ranging from 1 to 2.8, and nematode multiplication rate ranging from 2.78 to 5.70.

Keywords: Nematodes, Hedges, *Meloidogyne*, Vegetable, Niger

Introduction

Chronic drought of the decades 70 and 80, combined with high population pressure in some Western countries of Africa resulted in deforestation and overexploitation of natural resources. Subsequently, fallowing decreased, soil fertility lost, and agricultural lands were lost due to erosion and degradation.

To mitigate effects of the phenomenon, reforestation of the affected areas and setting up defense and land reclamation strategies were

necessary measures to be taken (Duponnois *et al.*, 1997a). Consequently, several range management techniques were tested including usage of hedges composed of one or more forest trees.

Legumes are the most commonly used species because they are able to grow on poor soils due to the capacity of their root system to fix atmospheric nitrogen (Duponnois *et al.*, 1999). In Niger, most commonly used trees are *Acacia senegal*, *Ziziphus mauritiana*, *Bauhinia rufescens*, *Prosopis juliflora* and *Euphorbia balsamifera* (Balde, 2013). The latter species is more used in the eastern part of the country. These species are present in single or multi-species communities.

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Hedges play several functions at once: (i) decrease the rate of runoff, soil erosion main factor, (ii) serve often as park of woods, firewood, and timber; (iii) serve as food for humans and livestock and game refuge; (iv) promote the recovery of the forest environment (Dibloni et al., 2000). Hedges are also used for the delineation of farms and prevent the damage caused by stray animals on crops (Yossi et al., 2006).

Despite all their usefulness on farmers' fields, trees hedges have shortcoming to be live relay hosts not only to pests such as insects, mites and rodents (Garba, 2012) of cultivated plants in their immediate vicinity, but also to disease agents such as plant-parasitic nematodes, mainly root-knot-nematodes *Meloidogyne*.

These nematodes are limiting factor in vegetable crop production in the Sahel (Haougui and Kollo, 2006). In Niger, tomato producers can experience losses exceeding 57% as a result of nematode infestation (Haougui et al., 2003). Nematodes extend their attack to trees such *Acacia*. However, only limited studies have been conducted so far as to establish these trees susceptibility to nematodes.

Well known work in this field was carried out on *Acacia* spp by team of IRD researchers based in Dakar (Senegal) since these species constitute the bulk of plants used in hedges (Duponnois et al., 1995; Duponnois et al., 1997a; Duponnois et al., 1997b; Cadet and Sanogo, 2007). Before this current research, no such study was undertaken in Niger.

Objectives of this work were to characterize the parasitic nematodes associated with forest trees used in the hedges, and to test their susceptibility to *Meloidogyne javanica*, the widespread root-knot nematode in Niger.

Materials and Methods

Faunistic Study

Site Characteristics

Three representative sites of areas using hedges to fence farms were chosen. Ganguel (13 ° 50'N, 2 ° 40'E), Karey Gorou (13 ° 52'N, 2 ° 10 E) and Yowaré (13 ° 47'N, 2 ° 10 ') all three were along road side to Burkina Faso. Soil type

at Ganguel and Karey Gorou was sandy loam, and at Yowaré, it was sandy clay loam. Trees on the farms were mainly native fruit trees like *Mangifera indica*, *Psidium guava*, *Carica papaya*, *Citrus* and exotic trees like *Eucalyptus* spp. and *Azadirachta indica*. In addition to the above common trees, others specific to sites were present. Ganguel had some edible shrubs (*Moringa oleifera*) and inedible ones (*Ricinus communis*), at Karey Gorou (*Leptadenia hastata*) and at Yowaré *Waltheria indica*. Herbaceous layer was dominated by *Cassia occidentalis*, *Acanthospermum hispidum*, *Sida cordifolia*, *Cenchrus biflorus*, *Panicum pedicellatum*, *Comelina bengalensis* and *Mitracarpus* spp. Ganguel and Yowaré had mono-specific hedges made up with *Bauhinia rufescens* and *Acacia Senegal* respectively. At Karey-Gorou, hedges were multispecies with *B. rufescens*, *A. senegal* and *Prosopis juliflora*.

Sampling and Nematode Extraction

Sampling was conducted in mid August 2012 and for each tree species found in a hedge, fifteen samples were collected each at 5 m intervals on straight lines not including first three border plants as to avoid border effect. Each sample (2 kg of soil) was composed of three sub-samples taken at 5-30 cm depth using a trowel and placed in a plastic bag and sent to the Nematology Laboratory of the Agrhyment Regional Centre of Niamey.

Nematodes were extracted from 250 cm³ of soil taken from each sample using method described by Seinhorst (1962). Importance of each nematode genus or species established using Fortuner and Merny (1973) Diagram of Frequency / Abundance. Frequency (F) is percentage of samples containing given genus or species. This parameter was estimated on the basis of the following formula:

$$F = \frac{e}{n} \times 100$$

e = total number of samples containing given genus/species of nematode

n = total number of samples at given site

Abundance (A) of nematode genus/specie is the average density per samples in which nematode

was found. It was calculated using the following formula:

$$A = \frac{\sum X_i}{e}$$

X_i = number of nematodes per liter of soil

e = number of samples in which the given nematode was present.

Calculated A values were then log transformed. A genus/specie was considered abundant if present minimally at about 300 individuals per liter of soil and it were considered frequent when present in at least 30% of the samples.

Study of the reaction of forest trees to *Meloidogyne javanica*

Plant Material

Tree species used in the test were: *Acacia senegal* (obtained from Baleyara and harvested in January 2012), *Bauhinia rufescens* (collected in Loga and harvested in January 2012), and *Prosopis juliflora* (collected in Niamey and harvested in October 2012). They were obtained from the Regional Forestry Services of Niamey. Tomato (*Lycopersicon esculentum*) cv Roma VF purchased on local market was used as susceptibility control to *Meloidogyne* spp.

Animal Material

Animals were second stage juveniles (J2) of root-knot nematodes *Meloidogyne javanica*. Nematodes were reared on eggplants grown in greenhouse at ARC of Niamey. Nematodes extraction from roots of eggplants was performed using Seinhorst (1950) method. However, only 24 hours old juveniles were used.

Nematodes Inoculation and data Analysis

A paired-treatment experiment was conducted in a screen house at The Agrhyment regional Centre

of Niamey Niger. Pots with 1.5 Kg autoclaved soil were seeded with the three trees species and tomato. One half of pots containing 24 old seedlings were inoculated with 500 juveniles (J2) of *M. javanica* and the other half remained as control.

Ten weeks after inoculation, plants were carefully uprooted and roots washed under running tap water. Nematode damage to plant was estimated using the scale of root gall index spread over six classes: 0 = no galls, 1 = 1-5 galls, 2 = 6-20 galls, 3 = more than 20 galls, 4 = galls coalescing throughout the root, 5 = root system completely decomposed (Duponis *et al.*, 1999).

Nematode reproduction efficiency or reproduction factor (RF) was calculated using the following formula: $RF = P_f/P_i$, where P_f is final nematode number and P_i , the initial nematode population (500 J2 inoculated to the plants). Nematological data were processed with GenStat 9 software using ANOVA and completed by the Student Newman Keurl test. Dry weight of shoot and root biomass were measured on inoculated and inoculated plants and the comparison done using the "t-test" at the probability of 0.05.

Results

Faunistic study

Plant-parasitic nematodes extracted from rhizosphere of all selected tree sites (Ganguel, Karey Gorou, Yoware) showed for the three plant species (*A. senegal*, *B. rufescens*, and *P. juliflora*) the presence of 10 genera. Two (2) of them were endoparasites (*Meloidogyne* and *Pratylenchus*), one (1) was semi-endoparasite (*Rotylenchulus*) and others were ectoparasites (TableI).

Table I: Nematodes Parasites Found on Plants in Different Sites

Nematode genera	Sites				
	Ganguel	Karey-Gorou			Yowaré
	<i>B.rufescens</i>	<i>B.rufescens</i>	<i>P.juliflora</i>	<i>A.senegal</i>	<i>A.senegal</i>
<i>Melodoigyne</i>	-	+	+	-	+
<i>Tylenchorhynchus</i>	-	+	+	-	+
<i>Hoplolaimus</i>	+	-	+	-	+
<i>Helicotylenchus</i>	+	+	-	+	+
<i>Rotylenchulus</i>	-	-	-	-	+

<i>Pratylenchus</i>	-	-	-	-	+
<i>Criconemella</i>	-	-	+	-	-
<i>Filenchus</i>	-	-	+	-	-
<i>Longidorus</i>	-	-	-	-	+
<i>Xiphinema</i>	+	-	+	-	+

(+) presence of nematode
 (-) absence of nematode

Acacia Senegal

A. senegal rhizosphere showed 8 genera of plant-parasitic nematodes of which 5 are common but not abundant (Figure 1). These were: *Hoplolaimus*, *Longidorus*, *Pratylenchus*, *Xiphinema* and *Helicotylenchus*. *Tylenchorhynchus* and *Rotylenchulus* were frequent and rare while *Meloidogyne* was abundant but infrequent.

The majority of parasitic nematode community found on *A. Senegal* was ectoparasites (Figure 2). They represented 71% of the total and endoparasites such as *Meloidogyne* and *Pratylenchus* representing 21% of the community, and the remaining (8%) was representative of the semi-endoparasites, *Rotylenchulus* (Figure 2).

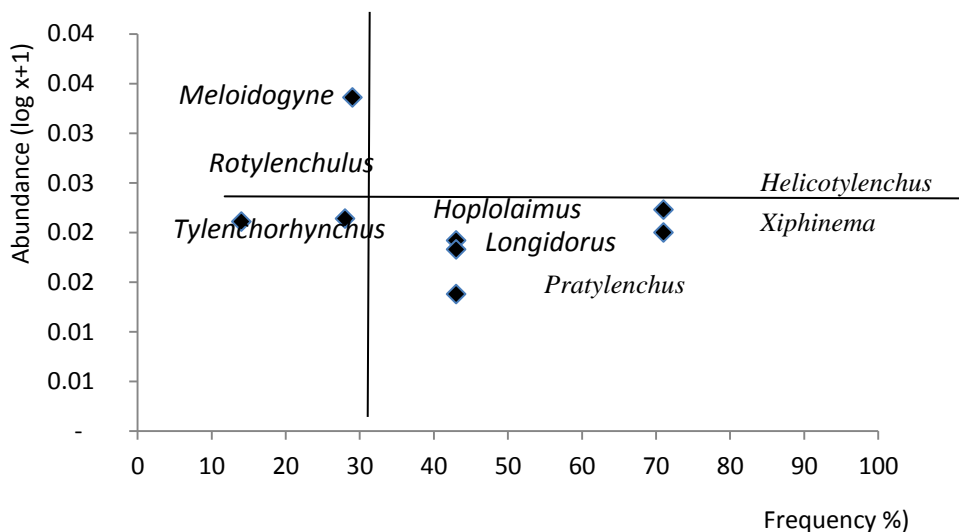


Figure 1: Importance of Plant-parasitic-Nematode Populations on Acacia Senegal

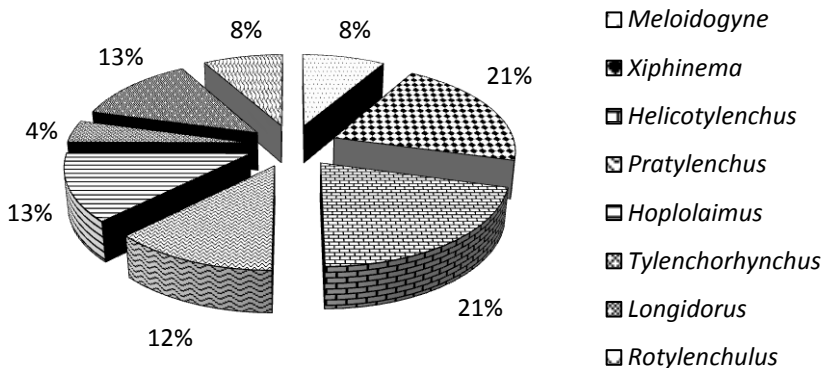


Figure 2: Plant-parasitic-nematodes Community Structure on A. Senegal

Bauhinia Rufescens

Five (5) different genera of phytonematodes were found in rhizosphere of *Bauhinia rufescens* and out of these, only *Xiphinema* was common but not abundant. The three (3) others genera made up of *Helicotylenchus*, *Hoplolaimus* and *Tylenchorhynchus* were scarce, while *Meloidogyne* was abundant but it was not frequent (Figure 3).

Ectoparasites represented 80% of the nematode community on *B. rufescens* with greater presence of the genus *Xiphinema* which solely made up 50% of the ectoparasite community and represented about 40% of the whole phytonematode community found in *B. rufescens* rhizosphere (Figure 4). The genus *Meloidogyne* accounted for 20% of densities (Figure 4).

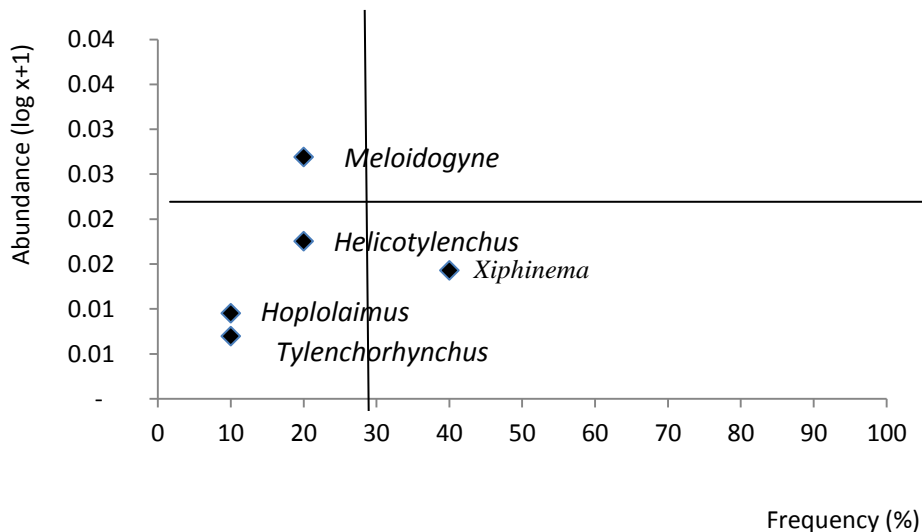


Figure 3: Importance of Plant-parasitic Nematodes on *Bauhinia Rufescens*

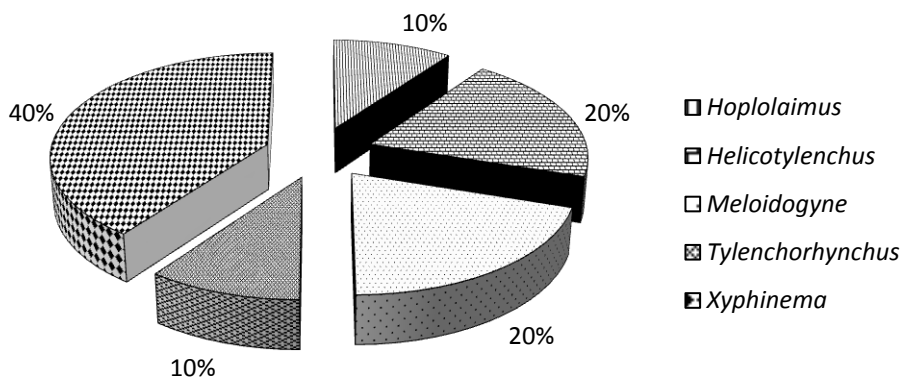


Figure 4: Plant-parasitic Nematode Community on *Bauhinia Rufescens*

Prosopis Juliflora

Six (6) different genera were found in *Prosopis juliflora* rhizosphere including three common but less abundant ones that were *Meloidogyne*,

Xiphinema and *Tylenchorhynchus*, and three genera were sporadic and comprised *Hoplolaimus*, *Filenchus* and *Criconemella* (Figure 5).

Figure 6 gives the relative frequency of different nematodes genera encountered associated with *P. juliflora*. While genus *Meloidogyne* represented 33% of the community of parasitic

nematodes, ectoparasites accounted for 67% with greatest presence of *Xiphinema*, 33% of ectoparasites.

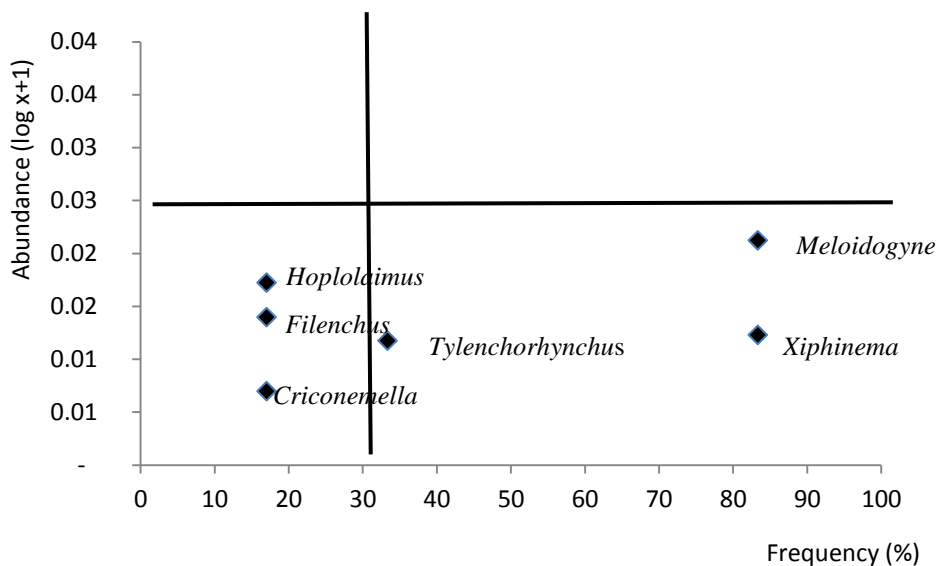


Figure 5: Importance of Plant-parasitic-Nematodes on *Prosopis Juliflora*

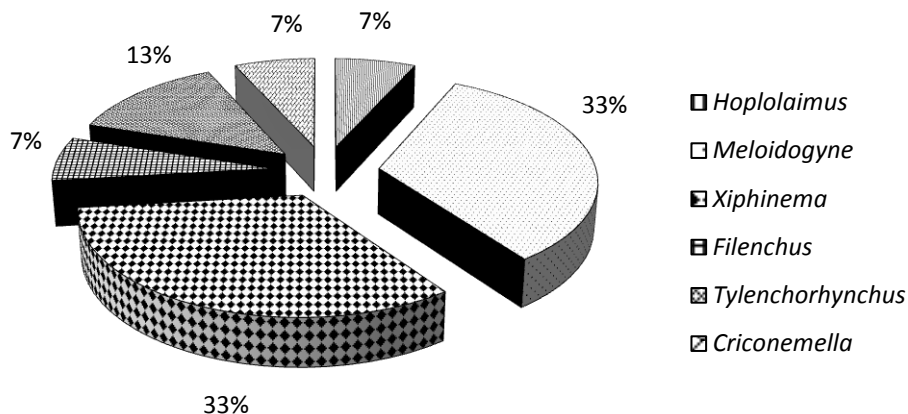


Figure 6: Plant-parasitic Nematode Community on *Prosopis Juliflora*

Response of Forest Species to *M. javanica* in Greenhouse

✓ **Nematological Parameters**
Root Gall Index

Gall index results are reported in Table II and indexes ranged from 1.2 on *A. senegal* to 2.8 on *L. esculentum* with mean index of 2 for the test. Statistical analysis revealed three (3) distinct groups of sensitivity and *A. Senegal* seemed less

susceptible to *Meloidogyne* compare to *L. esculentum* the control plants while *B. rufescens* and *P. juliflora* were as susceptible as the control

plant (tomato). Statistical analysis showed that the final populations on *B. rufescens* and *P. juliflora* are not significantly different from the control one. All plants had relatively a good multiplication rate of the nematode with RM ranging from 2.78 on *A. senegal* to 6.09 on tomato.

Final Populations

Table III shows final population of *Meloidogyne javanica* on the three tree species and on control

Table 2: Root Gall Index and *Meloidogyne* Final Population, 10 Weeks after Inoculation

Plants	Root gall index	Final population (Pf)	Pf/Pi*
Tomato	2,8 a	3045a	6,09
<i>Bauhinia rufescens</i>	2,4 ab	2851a	5,70
<i>Prosopis juliflora</i>	1,8 bc	2187ab	4,37
<i>Acacia senegal</i>	1,2 c	1390b	2,78

*Pi = initial population (500 juveniles inoculated to seedlings)

Final populations were subjected to ANOVA after log transformation (x +1). Numbers followed by the same letter are not significantly different (p ≤ 0.05).

✓ **Growth Parameters**

Shoot Weight

Dry weight of aerial part was recorded in Table III. In general, while inoculation of *Meloidogyne* on *P. juliflora* resulted in reduced dry weight of

aerial parts, on the other two tree species and on tomato, difference between the two parameters was not significantly different. Reduction rate varied from 4.12% in tomato to 52.38% in *P. juliflora* (Figure 7).

Table 3: Weight of Above Ground Part of Plants 10 Weeks after Inoculation with *M. Javanica*

Plants	Shoot weight of inoculated plants	Shoot weight of uninoculated plants
<i>A.senegal</i>	0,44 b	0,50 a
<i>B.rufescens</i>	0,13 c	0,14 a
<i>P.juliflora</i>	0,11 c	0,21 b
Tomato	0,93 a	0,97 a

The numbers in the same row followed by the same letter are not significantly different (p ≤ 0.05)

Roots weights

Weights of dry roots were reported in Table IV. In general, on all plant species used in the trial including tomato, inoculation with *Meloidogyne*

reduced dry root weight. The rate of reduction varied from 40% on *A. senegal* to 91% on *P. juliflora* (Figure 7).

Table 4: Dry Roots Biomass 10 Weeks after Inoculation with *m. Javanica*

Plants	Shoot weight of inoculated plants	Shoot weight of uninoculated plants
<i>A.senegal</i>	0,06a	0,05a
<i>B.rufescens</i>	0,03b	0,08a
<i>P.juliflora</i>	0,02b	0,22a
Tomate	0,03b	0,16a

The numbers in the same row followed by the same letter are not significantly different (p ≤ 0.05)

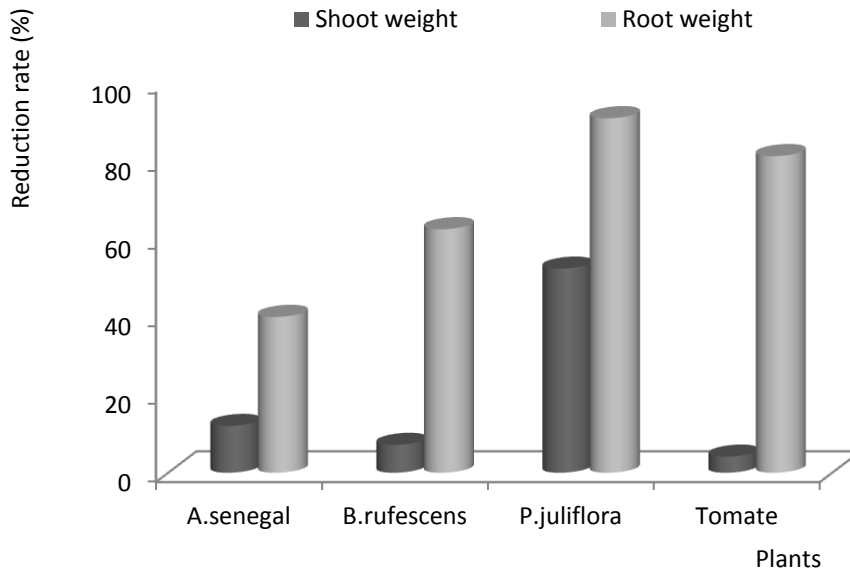


Figure 7: Weights Reduction Rates In Dry Roots And Above Ground Part Of Plants

Discussions

Faunistic study inherent to phytonematodes species associated with hedges was undertaken for the first time in Niger. It enabled identification of 10 genera of plant-parasitic nematodes in three (3) surveyed plants (*Acacia Senegal*, *Bauhinia rufescens* and *Prosopis juliflora*).

In Senegal, Cadet and Sanogo (2007) found 7 genera associated with *Acacia* spp used in hedges. In Niger, several studies with the same objectives showed up to 10 genera on vegetable crops (Haougui, 1999; Zakari, 2008; Djibey, 2012; Nourh, 2012).

Presence of different plant-parasitic-nematodes in rhizosphere of the three (3) trees demonstrated potential for this nematode to act as reservoirs of parasitic phytonematodes. Worrysome was that at maturity these plants did not exhibit symptoms that might alert of presence of harmful nematodes. This observation confirmed previous report by Cadet and Sanago (2007) who considered *A. senegal* and *P. juliflora* as infestation source of vegetable crops in Senegal. Use of above described species of trees in hedges could be threat to vegetable crops growers. It has been

reported that *P. juliflora* promoted proliferation of root-knot-nematodes *Meloidogyne* that swiftly devastated vegetable crops (Prot, 1986).

Our results showed that this nematode, the most destructive on vegetable crops was present on all three (3) tree species used as hedges in Niger. This is in accordance with the fact that root-knot nematodes can attack more than 200 plant species worldwide and thus colonize many habitats (Nourh, 2012).

Widespread distribution of *Meloidogyne* spp. on hedges might also be due to exchange of plant material from a site to another. Indeed, in Niamey’s region, seedlings of vegetable and tree crops are subject to intense trading. Hence, very important passive dispersal of the nematodes was observed on these sites. In Eastern Niger at Aguié, researchers found that several pepper growers bought seedlings produced in nurseries from neighborhood country of Nigeria and observations of roots of several lots of seedlings for sale revealed presence of root galls induced by *Meloidogyne* (Haougui and Bizo, 2009). *Meloidogyne* was less abundant but present in up to 30% of samples on *A. senegal* and *B. rufescens*, and on *P. juliflora*, it was rather common but in less than 300 individuals per liter of soil. This result confirmed earlier report by Oumar (2010) who worked in the same geographical region during the rainy season, but

on plants from Solanaceae family. Dry season surveys undertaken in the same region showed that *Meloidogyne* was both frequent and abundant on many vegetable crops (Haougui 1999; Zakari, 2008; Haougui and Bizo, 2009; Nourh, 2012). Low frequency and abundance of root-knot-nematodes found in rainy season could be explained by the fact that all eggs laid at end of dry season were still in diapause, thus only few were able to hatch at that period (Prot, 1980). Furthermore, some juveniles might also migrate to depth with the humidity front. Such juveniles escaped our sampling of upper 5-30 cm layers of soil.

Relative abundance of *Helicotylenchus* was characteristic of long-term fallow or mature fallow where biological diversity tended to be high (Villenave and Cadet, 1998) and parasitic and non-parasitic nematodes density very high (Serigne *et al.*, 2003).

Susceptibility tests to *Meloidogyne* of trees used in hedges showed that all trees in trial were vulnerable to root-knot nematodes. Using 500 juveniles per pot produced gall indexes of 1.2 to 2.4 on the three trees. On control tomato plants cv Roma VF, the same initial inoculum generated gall index of 2.8 (Table 2). Moreover, significant reduction in root biomasses of infected plants were observed (Table 3). Several authors have already reported these observations on *Acacia* spp. (Duponnois *et al.*, 1997a. Duponnois *et al.*, 1997b; Duponnois *et al.*, 1999), and also on *Ziziphus mauritiana*, *Bauhinia rufescens* and *Acacia nilotica* (Cadet and Sanogo, 2007).

Susceptibility of plants used in hedges to *Meloidogyne* combined with the fact that they are healthy carriers thus displays no visible symptoms of attack make them great reservoir for vegetable crops contamination and reinfestation source. They can be compared to papaya (*Carica papaya*) or Moringa tree (*Moringa oleifera*) which are well documented sources for proliferation and the spread of root-knot-nematodes belonging to the genus *Meloidogyne* on vegetable production sites (Prot, 1984; Haougui, 1999). Cadet and Sanogo (2007) reported that the presence of Hedges purportedly increase nematode population densities relative to neighboring fields without

hedges (Cadet and Sanogo, 2007). Hedges effect can radiate from 2 to 8 m around, and as deep as 40 cm depth.

Conclusion

Safe agricultural practice recommend that prior to using plant species in a hedge, they may be thoroughly assessed relative to parasitic phytonematodes, especially of the genus *Meloidogyne*. This will minimize risk of spreading these important threats to cultivated plants. As cautionary measures, usage of *A. Senegal*, *B. rufescens* and *P. juliflora* must be completely avoided in hedges around susceptible vegetable crops.

Acknowledgements

The authors express their gratefulness to all the vegetable producers on the three sites used for their collaboration, and also to personal of Nematology Laboratory of the Regional Center of Agrhymet for their assistance during this study. They also express their gratitude to Dr Abdourhamane Alou who helped in manuscript edition.

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