Journal of Natural Sciences Research www.iiste.org
ISSN 2224-3186 (Paper) ISSN 2225-0921 (Online)
Vol.4, No.17, 2014

Cultivar Differences in the Level of Protection against Plant Parasitic Nematodes Conferred by Mycorrhizal Fungi Association on Plantain

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The study was funded by the International Foundation for Science through research grant number C/3859-1 awarded the first author.

Abstract

The experiment was set up to investigate the relative mycorrhizal dependence of a Falsehorn and a French plantain cultivars with a view to ascertaining the effect on plant parasitic nematodes infection. The experiment was laid out in a completely randomized design, all suckers were pared prior to planting and treatment assigned as either inoculated with the mycorrhizal inoculant or not. At 182 days after planting (DAP), all plants were uprooted and assessed for root and rhizome damage. Plant parasitic nematodes were extracted from both root and soil samples taken from the hole of each plant after uprooting. The results of the study suggests a negative impact of mycorrhizal fungi on the built-up of plant parasitic nematode population on plantain with mycorrhizal inoculation causing a considerable reduction of 37.1% in plant parasitic nematode population density recovered from the roots of False horn plantain compared to just 7.71% reduction recorded for the French plantain genotype. On the contrary, mycorrhizal fungi inoculation caused more reduction (40%) in the plant parasitic nematode population densities per/1 litre of soil sample from the rhizosphere of French plantain than that of the Falsehorn plantain genotype. Mycorrhizal inoculation affected the species composition within location and cultivar response differed. This implied that the benefit of mycorrhizal fungi in managing plant parasitic nematodes on plantain is cultivar dependent as the French plantain genotype may better benefit from mychorrhizal fungi association than the Falsehorn in terms of protection against plant parasitic nematodes.

Keywords: *Glomus* spp, mycorrhizal fungi, plant parasitic nematodes, plantain, genotypes, root health

1. Introduction

Decline in plantain yield had been attributed to a complex of factors including declining soil fertility, diseases, pests and weeds (INIBAP, 1986; Swennen *et al.,* 1998; Schill *et al.,* 1996). Moreover, problems associated with root damage predominate (Blake, 1996). Foremost in this instance is plant parasitic nematodes which constitute the most serious biotic constraint capable of destroying the whole root system, weakening the anchorage and reducing yield (Gowen and Quénéhervé, 1990). Rotimi *et al.* (2004) recorded from their studies in southeastern Nigeria, a range of 46-54% yield reduction due to species mixture of plant parasitic nematodes depending on the genotype planted, soil management and fertility status. The authors further noted a 33% absolute yield loss due to toppling of plant bearing immature bunches.

Musa spp genotypes differ in susceptibility and sensitivity to plant parasitic nematodes (Price, 1994; Fogain, 1996; Stoffelen, 2000). While some cultivars for instance Agbagba, suppressed nematode colonization and reacted to parasitism by better lateral root production (Rotimi *et al*., 2005), some have their root system prone to attack and damage by these parasites for example, the 'French' plantain. Moreover, it has been established that there are differences in the behaviour of the nematode population densities encountered in the root system at

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different growth stages of the plant (Quénéhervé, 1989). For instance, *Radopholus similis,* which is a primary root invader, has its levels of infestation decreased as the root system aged or decayed (Blake, 1961; Loos, 1962). Infections by nematodes species like *Helicotylenchus multicinctus, Hoplolaimus pararobustus* and *Pratylenchus coffeae* may accelerate root decay, thereby restricting the availability of healthy tissue to other endoparasites such as *R. similis*

Nematode induced lesions create a food base for weak, unspecialized fungal parasites, enabling them to invade the stele and to increase the amount of root necrosis (Pinochet and Stover, 1980). These fungi acting as secondary parasites can increase root breakage and consequently toppling for instance, *Fusarium oxysporium*, the causal organism for Fusarium wilt is of economic importance in this regard (Stover and Simmonds, 1987). Some endotrophic mycorrhizal fungi enhance nutrient uptake by plant roots and are known to have suppressive effect on some soil inhabiting pathogens including nematodes (Umesh *et al.*, 1988; Pinochet *et al.,* 1996; Jaizme-Vega *et al.,* 1997). The association of endo-mycorrhiza at the root zone also has a significant impact on other organisms' mainly via physical interaction, that is, occupying the entire root zone thus smothering out other organisms (Brundrett, 2002).

Like many plants, bananas are dependent on some vesicular arbuscular mycorrhizal (VAM) fungi which improve greatly their nutrition, especially under poor fertility conditions (Strullu, 1991.; Declerck et *al.,* 1995). Furthermore, mycorrhiza may play a role in the control of root pathogens, including nematodes (Umesh *et al.*, 1988; Pinochet *et al.,* 1996; Jaizme-Vega *et al.,* 1997). The way mycorrhizal fungi interact with root pathogens is not known, but they are supposed to increase the plant tolerance by improving nutrition, and they also may interact physically (site occupation) and/or have a suppressive effect on nematode reproduction due to alteration of root / shoot ratio as a result of enhanced root biomass (Hurt *et al.*, 2001). The study therefore, aimed at investigating the contribution of mycorrhizal fungi to comparative root health responses in plantain.

2. Materials and Methods

2.1 Site Description, Planting Materials and Treatment application

The experiment was carried out at the Crop Section of the Teaching and Research Farm of the Federal University of Technology, Akure. Suckers of plantain (*Musa* spp AAB-subgroup) cultivars Agbagba, a Falsehorn and Obino l'Ewai, a French were acquired from the commercial farm of Federal College of Agriculture, Akure. The cultivars were the ones commonly cultivated by farmers in the ecological zone. Also, bags of mycorrhizal innoculant with sand as carrier, bagged by the Ondo-State Accelerated Poverty Alleviation Authority (APAA) for the use of local farmers, were acquired for the experiment.

Treatment comprised of two factors, each with two levels: the first factor being plantain cultivars (Falsehorn (H), and 'French' (F) and the second factor mycorrhizal fungi (with mycorrhiza (M), and without Mycorrhizal (MN). There were a total of four (4) treatment combinations. All suckers were cleared by removing the roots and paring (i.e. peeling the rhizomes). Treatments were arranged in a completely randomized design of four rows, with five plants per row. Each treatment was randomized five times. All the four treatments were randomized through the balloting process. The experimental field was 15m x 15m. A total of 20 plants were spaced at 3m between rows and 2m within rows. Eighteen border plants were planted around the field area.

Pared suckers were planted directly into 30cm x 30cm x 30cm planting holes in the field. A 1.25kg of mycorrhizal inoculant infected soil containing spores of *Glomus* species (at no documented density) was poured around the base of each of the suckers receiving mycorrhizal treatment at 2 weeks after planting (WAP). Prior to planting, the field had been ploughed and harrowed. Slashing was done to keep weeds low before the field was ploughed. After planting, the field was again slashed at 4 and 12 weeks after planting (WAP).

2.2 Data Collection

Random soil samples were collected from the field prior to planting for nematode extraction to identify species of plant parasitic nematodes present in the soil and their levels. At 182 days after planting (DAP) all plants were carefully uprooted and the root and rhizome damage parameters namely root necrosis, feeder root health, dead roots and rhizome lesions were assessed according to the method described by Speijer and De Waele (1997). Nematodes were extracted from both root (5g) and 1 litre soil samples taken from the base of each plant. All roots on each plant were carefully removed and 25% of each was set aside for further assessment. Mean root length and diameter at point of severance from the rhizome were measured. The roots were later dried in the oven until a constant weight was attained after which the dry weight was estimated for 25% roots of each plant.

2.3 Data Analysis

The nematode population densities were log (x+1) transformed (Gomez and Gomez, 1984), damage parameters in percentages and scores were arcsine (x/100) and (x+0.5) transformed, respectively, while count data were square root transformed prior to using the generals linear model in SPSS. Where statistical differences were observed, means were separated using the Duncan Multiple Range Test at 5% significance level.

3. Results

3.1 Effects of plantain genotype and mycorrhization on plant parasitic nematode population recovered from roots and rhizosphere of plantain

At termination of the experiment, 6 months after planting, *Radopholus similis, Pratylenchus coffeae, Meloidogyne spp, Helicotylenchus multicinctus* and *H. dihystera* were extracted at varying densities from the roots and rhizosphere samples collected (Table 1). The densities were also different under interaction of plantain genotypes and mycorrhization (P≤0.05).

However, of all the plant parasitic nematode species extracted, only the *R. similis* extracted from the rhizosphere of non-mycorrhized French genotype (MNF) had significantly higher densities (P≤0.05) than from the mycorrhized Falsehorn (MH), even though it was not significantly higher than the non-mycorrhized Falsehorn (MNH) and mycorrhized French (MF). Other plant parasitic nematode population densities did not show significant difference in all the four treatment conditions.

Meanwhile, the Falsehorn cultivar seemed to support higher diversity of parasitic species, especially the non-mycorrhized treatments, which supported all the five species in the roots and rhizosphere although not at a significant level (P≤0.05) in most cases. The *H. multicinctus* extracted from the root of MNH plants was significantly different (P≤0.05) from that of MNF, but was not when compared with MH and MF respectively. *Helicotylenchus dihystera* was only detected in the root and rhizosphere of MNH while *R. similis* was detected only in the root and rhizosphere of MNH and MNF. *H. multicinctus* and *H. dihystera* were not found in both the root and rhizosphere of MNF while *H. multicinctus* was only found in the root of MF but absent in the rhizosphere.

The composition of plant parasitic nematode community in the roots differed when compared with that of the rhizosphere and were not consistent in percentages under the four treatments. The highest density recovered was that of *H. multicintus* being 450 nematodes in 100g fresh root of non-mycorrhized False Horn plantain, cvr. Agbagba. Of all the plant parasitic nematode species recovered, either from roots or rhizosphere, the contribution of *H. dihystera* was generally low in the plant parasitic nematode community as it was only recovered from the roots and rhizosphere of MNH only at 120 nematodes per 100g fresh roots and 60 per 1 litre of soil.

Table 1. Influence of genotypes and mycorrhization on plant parasitic nematode population under plantain

Hm = *Helicotylenchus multicinctus*; Hd = H. *dihystera*; Melo = *Meloidogyne* spp; Rs = *Radopholus similis*; Pc = *Pratylenchus coffeae*

Treatments	**Population Density/ 100g of Fresh Root**					**Population Density/1 Litre Soil**				
	Hm	**Hd**	**Melo**	**Rs**	**Pc**	**Hm**	**Hd**	**Melo**	**Rs**	**Pc**
Falsehorn										
Mycorrhized	120a	0a	180a	120a	240a	120a	0a	120a	0a	0a
Not Mycorrhized	450a	150a	150a	75a	225a	120a	60a	60a	150a	300a
French										
Mycorrhized	150a	0a	150a	150a	300a	0a	0a	300a	0a	300a
Not Mycorrhized	200a	0a	200a	200a	100a	300a	100a	200a	200a	200a

3.2 Effect of mycorrhization on root and rhizome health indices of plantain six months after planting

The root necrosis index, feeder root health, dead root, root length and root diameter were not significantly different under all the four treatment conditions (Table 2). Albeit this, the mycorrhized plants were still observed to have better performances in all these parameters.

Table 2. Effect of mycorrhization on root and corm damage parameters of plantain six months after planting

RNI = root necrosis index; FRH = feeder root health; DE = dead root; RtDWt = dry weight of 25% of plant roots; RtLt = mean length of 25% of plant roots; RtDia = mean diameter of 25% of plant roots; SL = small lesion on the root bases on rhizome; LL = large lesion on root bases on rhizome

Treatments	**RNI (%)**	**FRH**	**DE (%)**	**RtDWt (g)25%**	**RtLt (cm)**	**RtDia (cm)**	**SL (%)**	**LL (%)**	**EYE**
Falsehorn									
Mycorrhized	12.2a	2.00a	22.01a	1.71a	16.74a	0.52a	11.11ab	6.72ab	1.80a
Not Mycorrhized	17.0a	2.56a	37.18a	1.16b	14.56a	0.45a	17.48a	7.65a	1.35ab
French									
Mycorrhized	12.0a	2.00a	27.39a	0.79bc	18.67a	0.42a	9.88b	4.47ab	1.15b
Not Mycorrhized	14.33a	2.27a	29.28a	0.26c	11.40a	0.38a	18.56a	3.56b	0.65c

4. Discussion

Although the nematode population densities observed in this study under different treatment conditions were not significantly different from each other, the recorded means revealed a negative impact of mycorrhizal fungi on the build-up of plant parasitic nematode populations. This further underscores the importance of mycorrhizal fungi inoculation as an effective protection against plant parasitic nematodes (Azcon-Aguilar *et al.,* 2002); although the mechanism may be more than direct. Previous works have shown that the effective protection against plant parasitic nematodes is probably a consequence of several mechanisms (Elsen *et al*., 2003), some of which may have either direct or indirect impacts on nematodes. Azcon-Aguilar *et al.* (2002) proposed improved nutrient status, competition for nutrients and penetration sites, anatomical changes in the roots, microbial changes in the rhizosphere and activation of plant defense mechanisms as possible anti-nematode mechanisms that could be cast on plant by mycorrhizal inoculation. Moreover, since both plant parasitic nematodes and mycorrhizal fungi colonize the root tissues, the possibility of competition for space is also suspected. Also, mycorrhizal fungi symbiosis exerts a major impact on the root system (Azcon-Aguilar *et al*., 2002) often by promoting increased branching. Hence, a denser, more branched roots observed in mycorrhizal plants in this study will be less favorable for nematodes because they prefer primary roots (Stoffelen, 2000).

Moreover, mycorrhizal inoculation caused a considerable more reduction (37.1%) in plant parasitic nematode population density recovered from the roots of False horn plants compared to just 7.71% reduction recorded for the French plants. On the contrary, inoculation caused more reduction (40%) in the plant parasitic nematode population density/1`litre of soil sample from the rhizosphere of French plants than that of the False horn. This is an indication that in the absence of mycorrhizal fungi, False horn plants will support larger ppn population in their roots than French plants. Also, the findings revealed that inoculation was effective at causing a reduction in the populations of most of the important species detected in this study.

Populations of *Radopholus similis, Helicotylenchus multicinctus* and *Helicotylenchus dihystera* considerably decreased by inoculation while that of *Pratylenchus coffeae* was increased in all locations albeit the rhizosphere of False horn where it was reduced. In the same vein, inoculation affected the species composition within locations. For instance, *R. similis* and *H. dihystera* were absent in the mycorrhizosphere of False horn while *R. similis, H. dihystera* and *H. multicinctus* were all absent in the mycorrhizosphere of French. But in these locations, all the plant parasitic nematode species detected in this study were present in the not-mycorrhized rhizosphere of both cultivars. Meanwhile, all these species were present in the roots of the two cultivars except *H. dihystera* that was only detected in the roots of the not-mycorrhized False horn plants but not detected in the

roots of mycorrhized ones. It was also completely at a not detectable level in the roots of French cultivar, either mycorrhized or not.

All these findings are indicative of the fact that inoculation expectedly changed the microflora of the rhizosphere of the plants and each species of plant parasitic nematodes detected in this study reacted differently and in varying degree to this change. This is supporting the earlier findings that suggested that different species, being biologically different from each other, will react differently under different cultural management that will affect the microflora composition in their habitat. The findings also showed that mycorrhizal inoculation reduced diversity of species in all locations as the not-mycorrhized rhizosphere supported wider diversity of species. More so, *P. coffeae* has the highest percentage contribution to the plant parasitic nematode population found in the roots of mycorrhized plants while *H. multicinctus* was found to contribute more in the not-mycorrhized plants' roots especially that of False horn cultivar. This further underscores the importance of these species, most especially *P. coffeae,* at causing economic damage to plantain plantations especially in the agro-ecozones of this study. In all locations, *Meloidogyne* spp contributed more to the ppn population densities of the mycorhizosphere and mycorrhized roots than their not-mycorrhized counterparts. In this case, the rise in population of *Meloidogyne* spp may be attributed to the fact that it is sedentary in nature and would not have had much of their activities so adversely affected by symbiotic relationship of the mycorrhizal fungi with the roots.

The root necrosis index (RNI) and percentage dead roots could be associated with nematode population, irrespective of genotypes, and could thus better able to indicate nematode damage on plantain. This is in order with the earlier findings of Rotimi *et al.* (2004) that pointed to these two parameters as good indicators of nematode damage on plantain cv. Agbagba. Meanwhile, the low nematode population recorded in this study could be attributed to edaphic and climatic factors impact on these organisms, as soil moisture level was extremely low during the period of the study.

The positive effect of mycorrhizal inoculation on the management of plant parasitic nematodes problem was confirmed for the two plantain genotypes tested in this study. Mycorrhizal association gave better protection against plant parasitic nematodes to the rhizome of the French plantain as reflected in the intensity of small lesions on the rhizome. Hence, the response of plantain to mycorrhizal fungi association could be said to be genotype dependent and what this translates to in terms of yield differences would need to be explored. Moreover, inoculation caused a substantial decrease in nematode population density in the mycorrhizosphere of the inoculated plants. This is an indication that inter-specific competition between mycorrhizal fungi and nematodes could be exploited in the control of nematodes in plantain production.

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