Original Paper   http://ajol.info/index.php/ijbcs   http://indexmedicus.afro.who.int

Impact of arbuscular mycorrhizal fungus (*Rhizophagus irregularis*) on disease symptoms caused by the ascomycete fungus (*Mycosphaerella fijiensis* M.) in Black Sigatoka-resistant banana plantain

Corinne Coretta OYE ANDA[1*], Auguste NDOUTOUME NDONG[1],
Pamphile NGUEMA NDOUTOUMOU[1, 2] and Pierre Michel LOUBANA[3]

[1]*Centre National de la Recherche Scientifique et Technologique. Institut de Recherches Agronomiques et Forestières. Département d'Agronomie Générale. Laboratoire de Protection des végétaux. B.P. 2246 Libreville, Gabon.*
[2]*Université des Sciences et Techniques de Masuku. Unité de Recherche en Agrobiologie. Institut National Supérieur d'Agronomie et de Biotechnologies. Laboratoire de biodiversité. B.P. 99 Poto-Poto Franceville, Gabon.*
[3]*Centre Africain de Recherches sur Bananiers et Plantains (CARBAP), BP 832 Douala, Cameroun.*
[*]*Corresponding author ; E-mail : accorretta@yahoo.com; Tel.: +24166367759.*

ABSTRACT

Banana (*Musa spp.*) is grown throughout the tropical and subtropical areas. The Black Sigatoka, however, represents a major threat to world production. This study evaluates the effect of the arbuscular mycorrhizal fungus (AMF) *Rhizophagus irregularis* MUCL 41833 on four plantain cultivars with different resistance against *Mycosphaerella fijiensis*. The mycorrhized banana plantlets of four cultivars were grown under greenhouse and the effect of AMF on micropropagated banana plantlets was evaluated. After 35 days, the height and the diameter of the pseudostem, then the leaf area were measured. The significant effect of the cultivar on the height of the pseudostem and the leaf area and significant effect of *M. fijiensis* on the diameter of the pseudostem, the height of the pseudostem and the leaf area were obtained. Plants infected with *M. fijiensis* show more symptoms of the disease than healthy plants. Symptoms were delayed in bananas inoculated with AMF compared to bananas not inoculated with AMF. The disease progressed more rapidly in cultivars Bâtard and C292 than in cultivars CRBP39 and F568. These results suggest that AMF may decrease symptoms of Sigatoka, at the early stage of infection with *M. fijiensis*. AMF may be a promising tool for the pre-adaptation of micropropagated banana plantlets.
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Keywords: Plantain, *Rhizophagus irregularis*, arbuscular mycorrhiza fungi, *Mycosphaerella fijiensis*, disease control method.

INTRODUCTION

Black sigatoka (leaf-spot disease), caused by the fungal pathogen *Mycosphaerella fijiensis* Morelet, is the most devastating illness of banana and plantain worldwide (Jones, 2000; Churchill, 2011). The disease was detected for the first time in the Fiji Islands in 1963 and is nowadays widespread in banana crops worldwide (Ganry, 2010). The disease affects the leaves of banana crops by reducing

their functional surfaces necessary for the photosynthesis activity, the production of carbohydrate and banana fruits, as well as the pulp color and early ripening of fruits (Chillet et al., 2014).

Industrial banana producers control the leaf-spot disease using essentially repeated applications of systemic and protectant fungicides. The use of resistant cultivars is another cultural practice for controlling the leaf-spot disease caused by *M. fijiensis,* and this may be the most suitable option for small farmers, who usually are unable to afford chemicals due to limited finance (Almekinders et al., 2019). This cultural control practice has the advantage of decreasing the potential risks to human health and the environment (Churchill, 2011). For instance, in Cameroon, the tetraploid hybrid CRBP39 (Banana Plantain) is partially resistant to black sigatoka (Noupadja et al., 2007). Through the genetic improvement program conducted at the Centre Africain de Recherches sur Bananiers et Plantains (CARBAP) and its distribution via the International Network for the Improvement of Banana and Plantain (INIBAP), this hybrid of high productivity (47t/ha/year) has been evaluated in several countries in Central and West Africa (Tomekpé et al., 2011). In parallel to the application of fungicides and the utilization of resistant cultivars, a number of cultivation practices have been used to decrease the sources of inoculum and to circumvent the conditions which are favorable to the development of the pathogen. Among these practices are the elimination of infected leaves, proper watering, drainage and control of weeds (Tripathi et al., 2017).

Biological control is another option, often considered as an alternative to the control of several pests and diseases (Nega, 2014). It is based on the management of biological resources such as endophytic or rhizospheric microorganisms some of which are able to inhibit the activity of plant pathogens and thereby delay or suppress the appearance of disease symptoms. Therefore, microorganisms represent promising alternatives to minimize the use of chemicals by replacing them or

reducing their rate of application to control plant pathogens (Hajek & Eilenberg, 2018).

AMF has been reported to reduce damage caused by pathogens in numerous crops (Plouznikoff et al., 2016). In banana, their beneficial effects have been shown in the control of the nematodes such as *Radopholus similis* (Anene & Declerck, 2016) and *Pratylenchus species* (Quénéhervé et al., 2008) as well as the soil fungi *Cylindrocladium spathiphylli* (Declerck et al., 2002) and *Fusarium oxysporum* (Bawa, 2016).

Although several studies have demonstrated the effects of AMF on root pathogens, only a few were related to leaf pathogens and the results were less conclusive (Whipps, 2004). Increased resistance was observed in tomato against *Alternaria solani* (Fritz et al., 2006) and *Botrytis cinerea* (Jung et al., 2009); in cucumber against *Colletotrichum orbiculare* (Lee et al., 2005) and in potato against *Phytophthora infestans* (Gallou et al., 2011). Recently, Oye Anda et al., (2015) demonstrated under *in vitro* culture conditions that pre-mycorrhized banana (cv. Grande naine) plantlets had reduced leaf-spot disease symptoms caused by *M. fijiensis* compared to non-mycorrhized plants. The severity index measured in the mycorrhizal plantlets shows a decreased symptom by more than 80% and 60%, respectively 21 and 35 days after the infection by the pathogen. These results suggested that AMF may decrease, at least at the early stage of infection, leaf-spot symptoms caused by *M. fijiensis* and therefore, may represent an attractive option to consider in the context of an integrated control of this disease (Oye Anda et al., 2015). However, it is unknown whether the impact of AMF on the disease caused by *M. fijiensis* is also expressed in plantain plant, and if the control is more efficient in highly sensitive varieties relative to partially to highly resistant varieties, or vice versa. In the present study, the objective was to investigate the effect of AMF (*Rhizophagus irregularis* MUCL 41833) on four plantain cultivars that have contrasting levels of resistance against *M. fijiensis*.

MATERIALS AND METHODS
Biological material

- ***Musa spp.* cultivars**

  Four Musa (plantains) cultivars were used: Bâtard (AAB genome, sensitive to Black Sigatoka) and three plantain hybrids with different degree of resistance to the disease: F568 (AAA, resistant), CRBP39 (AAAB, partially resistant) and C292 (AAA, sensitive). These hybrids were obtained by crossing triploid female (Musa cv, AAB) with diploid male plantains.

  The plant material was obtained from fragments of stems bits, a technique developed by Kwa (2001). This technique allowed the activation of latent buds and the production of large quantities of healthy planting materials.

- **Arbuscular mycorrhizal fungus**

  A strain of *Rhizophagus irregularis* (Blaszk., Wubet, Renker & Buscot) as ['irregulare'] MUCL 41833 was supplied by the Glomeromycota *in vitro* collection (GINCO) on a root organ culture (ROC) of carrot (*Daucus carrot* L.) clone DC2 (Cranenbrouck et al. 2005). Spores were extracted from the ROC and associated to leek (*Allium porrum* L.) in pots on sterilized (121 °C for 1 h) volcanic lava (DCM, Grobbendonk, Belgium). The pots were placed in the greenhouse at 20/15 °C day/night under natural light. After six months of culture, roots were extracted from the substrate, cut into 0.5 cm fragments and mixed in sterilized (121 °C for 15 min.) Terragreen (Oil Dri, UK). The number of infective propagules (i.e. colonized root fragments, spores) in this substrate was estimated to 120 g^{-1} via the most probable number method.

- ***Mycosphaerella fijiensis***

  The strain of *M. fijiensis* was isolated from infected banana leaf tissues sampled at the CARBAP experimental station in Njombe, Cameroon. A piece of infected leaf was incubated in a Petri plate (90 mm diameter) on 3% agar medium and subsequently maintained for two weeks under continuous light at 22 °C to produce conidia. Conidia were then transferred on potato dextrose agar (39 g L^{-1}). After 2 weeks, the strain was transferred on V8 medium in Petri plates (90 mm diameter.) for another 10 days under continuous light at 22 °C.

Inoculum was prepared by grinding fungal mycelium in 10 ml of sterilized (121 °C for 15 min) water with a mortar and pestle. The grinded material was then sieved through sterile cheesecloth (one-layer pore size of approximately 150 μm) to separate the mycelium from conidia. The conidia were thereafter collected and suspended in distilled water. The concentration of conidia was determined with a hematological count cell (cell of malassez) and further adjusted to obtain a suspension of 10^4 conidia ml^{-1} (Abadie et al., 2008). Gelatine (1%) was added to the final conidia suspension for a better adhesion of this suspension on the banana leaves.

Experimental design

The experiment was conducted under greenhouse conditions (28/24 °C day/night, 70 - 90% relative humidity, under natural light intensity) at the CARBAP experimental station. Plants were grown in 1 L plastic pots containing a mixture of pasteurized (100 °C for 24 hours) sand and coffee husk (v:v 3:2) and were randomly positioned.

Three-month old banana plants of each of the four plantain cultivars were selected and separated in two homogenous groups based on a visual observation of plant size and leaf surface. Half of the plants were inoculated with 10 g (i.e. 1200 propagules) of the AMF inoculum while the other half was AMF-free. The plants were inoculated by introducing the

inoculum into the planting hole in close contact with the roots.

After eight weeks of growth, both groups of plants of each cultivar were again separated in two homogenous groups. One group was inoculated with *M. fijiensis* (+ *M. fijiensis*) and the other group remained free from the pathogen (- *M. fijiensis*). Before the inoculation, the leaf at stage 2 of cigar development of each plant was covered with a polyethylene bag for 48 hours to prevent natural contamination. The leaf was then gently rubbed with cotton soaked in distilled water to remove the waxy layer of the limb before the inoculation. The inoculation was performed by spreading 1 ml (i.e. 10^4 conidia) of the conidia suspension of the fungus on the underside of the leaf (Kablan et al., 2012). The non-inoculated plants (- *M. fijiensis*) received water. The plants were thereafter placed under shading and moisture was maintained at saturation during 72 h to activate the pathogen infection. Plants were subsequently placed under the same conditions as described above.

This study considered sixteen treatments using four cultivars of plantain (Bâtard, F568, CRBP39, C292), with or without AMF (+AMF or -AMF), and with or without *M. fijiensis* (+*M. fijiensis* or -*M. fijiensis*). The study also considered six replicates per treatment.

Data assessment

The plants were grown for 35 days following the inoculation with the pathogen. During this period, the inoculated leaves were observed twice a week for lesion development. The different symptom stages of the disease were monitored according to their appearance time. Symptom stages were recorded as described again by Oye Anda et al. (2015):

- stage 1, leaf-spot symptoms begin to show up with yellowish specks < 1 mm diameter on the abaxial surface of the leaf;
- stage 2, the initial specks elongate and enlarge to form reddish brown streaks up to 2 mm long. Streaks are visible on both sides of the leaf;

- stage 3, red-brown streaks reach 20-30 mm long and colour start to change from red to dark brown;
- stage 4, the streaks broaden and develop into elliptical spots, dark brown on the abaxial surface and black on the adaxial surface of the leaf;
- stage 5, the central area of the dark spots becomes totally black and necrotic, lesions are slightly depressed, and the black spots are surrounded by a bright yellow halo;
- stage 6, the center of the leaf-spots dried out, fade and become whitish to grey. The spots are surrounded by a dark brown to black border and are further depressed.

At the end of the experiment (i.e. 35 days after inoculation with *M. fijiensis*), the plants were harvested. The pseudostem height and diameter and the area of the last unwrapped leaf were assessed. The pseudostem height was measured from the base of the rhizome to the crossing point of the two last unfolded leaves. The pseudostem diameter was measured at 1 cm from the base of the rhizome. The length (l) and width (w) of the leaves were measured and the leaf area calculated as follows: Leaf Area = α.l.w, where α= 0.7 (Rufyikiri et al., 2000).

Root colonization by the AMF was estimated at harvest. The roots were cleaned from substrate and immersed in 10% KOH overnight at ambient temperature. They were rinsed several times with deionized water and incubated in 3% H_2O_2 for 30 min (Anene & Declerck, 2016). The roots were subsequently stained for 45 min in blue ink diluted (1:50 v: v) in 1% HCl. Root colonization was estimated according to McGonigle et al. (1990) to determine the proportion of root colonization (%RC), of arbuscules (%A), and of vesicles/spores (%V). For each sample, 150 root intersections were observed.

Statistical analysis

The data was analysed with the software Statistica (version, editor). Data for %RC, %A and %V was *arcsin√(x/100)*-transformed prior to analysis. Data was also analysed using the two-way and three-way ANOVA, and the means were separated using Tukey's Honest

Significant Difference (HSD) to identify significant differences (P ≤ 0.05) between treatments. Data on disease variables obtained from the four cultivars was analyzed using Kruskal Wallis rank sum test and means were separated using the Student-Newman-Keuls test (α = 0.05). Finally, the Tukey and Kramer (Nemenyi) test was used for the post-hoc analysis to determine the level of resistance of cultivars colonized by AMF.

RESULTS
Plant growth parameters
Plants were harvested 35 days after transfer into the pots (i.e. eight weeks after pre mycorhization and 35 days after infection by *M. fijiensis*). The pseudostem height and diameter and the leaf surface area are presented in Table 1. A significant effect (p<0.05) of the cultivar was noticed on pseudostem height and leaf surface area. There was no significant effect of AMF on the pseudostem height while the effect of AMF on the pseudostem diameter and leaf surface area was significant regardless the cultivar and whether *M. fijiensis* was present or absent. Similarly, there was a significant effect of *M. fijiensis* on pseudostem diameter and height and the leaf surface area. The plants infected by the pathogen had a significantly larger pseudostem height and diameter and the leaf surface area regardless the cultivar and whether AMF was present or absent. No significant interaction was noticed between cultivar and AMF, cultivar and *M. fijiensis,* AMF and *M. fijiensis* or between cultivar, AMF and *M. fijiensis* for any of the growth parameters.

Root colonization by AMF
Banana root colonization was evaluated at the end of experiment. The plants were harvested and the colonization evaluated in the presence or absence of *M. fijiensis* (Table 2). Hyphae, arbuscules and spores/vesicles were observed in the roots of all the cultivars infected or not infected by *M. fiji*ensis. A significant effect of the cultivar was observed on %RC and %A while no difference was noticed on %V. A significant effect of the *M. fijiensis* was noticed on the %RC and %A

while no difference was noticed on %V. The plants infected by *M. fijiensis* had higher %RC and %A relative to the plants not infected by *M. fijiensis.* No significant interaction was observed between the cultivar and *M. fijiensis* on %RC, %A and %V.

The post-hoc analysis revealed a significant difference between the hybrids F568 and C292 (p <0.05); F568 and Bâtard (p <0.01); and CRBP39 and Bâtard (p <0.08) infected with *M. fijiensis.* In addition, comparisons between all cultivars were significantly different except between Bâtard and C292 (p <0.8) and between CRBP39 and F568 (p <0.12) infected with *M. fijiensis.*

Disease development
Whatever the cultivar and regardless whether AMF was present or absent, the plants infected by the pathogen showed leaf-spot disease symptoms. There was a significant difference between the cultivars (p < 0.05) as well as whether AMF was present or absent (p < 0.003); however the appearance of disease symptoms depended on the cultivar. The appearance of symptoms was delayed in the cultivars inoculated with AMF relative to cultivars not inoculated with AMF (p < 0.003).

The disease incidence was more severe in sensitive cultivars (Bâtard, C292) (p < 0.89) relative to resistant cultivars (CRBP39, F568) (p < 0.62). Disease also progressed more rapidly in sensitive cultivars. Disease incidence varied between stage 1 and stage 4, but never exceeded stage 1 for the particular case of resistant cultivars.

The post-hoc analysis revealed a significant difference between hybrids C292 and CRBP39 (p < 0.05) and between hybrids C292 and F568 (p < 0.001). There was a significant difference between Bâtard and F568 (p < 0.001) and between Bâtard and CRBP39 (p < 0.001). By the end of the experiment, differences at the stage levels between resistant and sensitive cultivars were highly significant (p < 0.001).

Table 1: Growth parameters of pre-mycorrhized (+AMF) or non-mycorrhized (-AMF) banana plantains infected (+ *M. fijiensis*) or not (- *M. fijiensis)* by the pathogen. Four Plantain cultivars were considered from resistant (AAA, F568), to partially resistant (AAAB, CRBP39) or sensitive (AAA, C292 and AAB, Bâtart) to Black Sigatoka.

Cultivar	AMF Treatment	*M. fijiensis* Treatment	Pseudostem height (cm)	Pseudostem diameter (cm)	Leaf surface area (cm²)
F568	+ AMF	+ *M. fijiensis*	11.6 ± 1.0	1.5 ± 0.1	206 ± 23
		- *M. fijiensis*	14.9 ± 1.6	1.7 ± 0.1	289 ± 52
	- AMF	+ *M. fijiensis*	8.5 ± 0.7	0.9 ± 0.1	145 ± 32
		- *M. fijiensis*	13.8 ± 0.4	1.5 ± 0.1	260 ± 24
CRBP39	+ AMF	+ *M. fijiensis*	18.8 ± 1.2	1.4 ± 0.1	272 ± 15
		- *M. fijiensis*	24.1 ± 1.8	1.8 ± 0.1	309 ± 31
	- AMF	+ *M. fijiensis*	18.6 ± 1.5	1.3 ± 0.1	239 ± 47
		- *M. fijiensis*	23.1 ± 1.7	1.8 ± 0.1	331 ± 32
C292	+ AMF	+ *M. fijiensis*	15.9 ± 1.5	1.3 ± 0.1	249 ± 31
		- *M. fijiensis*	18.3 ± 2.1	1.8 ± 0.1	329 ± 39
	- AMF	+ *M. fijiensis*	13.6 ± 2.9	1.3 ± 0.2	207 ± 44
		- *M. fijiensis*	19.1 ± 2.1	1.7 ± 0.1	294 ± 35
Bâtard	+ AMF	+ *M. fijiensis*	22.4 ± 1.2	1.6 ± 0.1	315 ± 50
		- *M. fijiensis*	23.8 ± 0.9	1.7 ± 0.1	320 ± 22
	- AMF	+ *M. fijiensis*	20.0 ± 0.8	1.3 ± 0.1	233 ± 13
		- *M. fijiensis*	22.9 ± 0.9	1.5 ± 0.1	270 ± 37
Effect (p=)					
	Cultivar		< .0001***	0.1176	0.0473*
	AMF		0.1085	0.0039**	0.0288**
	M. fijiensis		< .0001***	< .0001***	0.0002***
	Cultivar x AMF		0.8892	0.2267	0.6772
	Cultivar x *M. fijiensis*		0.6323	0.5198	0.4302
	AMF x *M. fijiensis*		0.3618	0.2416	0.4302
	Cultivar x AMF x *M. fijiensis*		0.8328	0.7344	0.9710

Data are mean values ± standard error of 6 replicates. Main effects and interaction between the factors: "Cultivar", "AMF" and "*M. fijiensis*" are presented. Tests that revealed significant differences at values below 0.05, 0.01 and 0.001 α level are followed by *, **, ***, respectively.

Table 2: Root colonization of pre-mycorrhized banana plantains infected (+ *M. fijiensis*) or not (- *M. fijiensis)* by the pathogen. Four Plantain cultivars were considered from resistant (AAA, F568), to partially resistant (AAAB, CRBP39) or sensitive (AAA, C292 and AAB, Bâtart) to Black Sigatoka.

Cultivar	*M.fijiensis* Treatment	Root colonization (%)	Arbuscules (%)	Spores/Vesicles (%)
F568	+ *M.fijiensis*	35.4 ± 3.1	33.1 ± 2.6	2.3 ± 1.4
	- *M.fijiensis*	22.4 ± 2.6	21.5 ± 2.4	1.7 ± 1.1
CRBP39	+ *M.fijiensis*	31.8 ± 1.5	29.7 ± 2.3	1.1 ± 0.4
	- *M.fijiensis*	26.7 ± 1.2	24.0 ± 0.5	2.3 ± 0.9
C292	+ *M.fijiensis*	26.6 ± 3.5	26.2 ± 3.6	0.3 ± 0.2
	- *M.fijiensis*	14.8 ± 1.9	14.3 ± 1.7	0.4 ± 0.3
Bâtard	+ *M.fijiensis*	24.1 ± 2.9	22.9 ± 3.2	1.2 ± 0.5
	- *M.fijiensis*	14.4 ± 0.8	14.1 ± 0.6	0.3 ± 0.3
Effect (p=)				
	Cultivar	< .0001***	0.0004***	0.0523
	M.fijiensis	< .0001***	< .0001***	0.6654
	Cultivar x *M.fijiensis*	0.3363	0.4723	0.4128

Data are mean values ± standard error of 6 replicates. Main effects and interaction between the factors: "Cultivar"" and "*M. fijiensis*" are presented. Tests that revealed significant differences at values below 0.05, 0.01 and 0.001 α level are followed by *, **, ***, respectively.

DISCUSSION

We showed for the first time the effect of AMF (*R. irregularis*) on the disease symptoms caused by the ascomycete fungus (*M. fijiensis*) in black sigatoka-resistant banana plantain grown in greenhouse conditions. This study showed that the mycorhization of hybrids and banana plantain plants is possible. The AMF was able to colonize the root system of plantain plants and to accomplish his complete cycle of life in the *vivoplants*. The plantain plants were associated with the AMF in a poor substrate, sand + coffee husk. The mycorhization of plantain plants was made at the 8th week after the contact with the mycelium. We found no significant difference between the vegetative parameters (such as the diameter of the pseudostem of cultivars), between the plants with mycorrhiza and plants without mycorrhiza whether infected or not infected by the disease, as observed by Ambang et al. (2008) in their study of the effects of mycorrhizae on peanuts. This may be because the chosen substrate did not contain all the useful ingredients for the plant development, or maybe the volume of pots chosen (1 L) affected plant growth.

We found a significant difference in terms of the total root colonization of the AMF, with strong presence of arbuscular and vesicles in the plants with mycorrhizae that were infected by the disease as the p value indicated (p < 0.003). The high percentage of arbuscular in the plants with mycorrhizae relative to plants

without mycorrhizae can be explained by the induction of the mechanisms of defence in the presence of AMF in the roots.

Concerning the disease of black leaf streak disease, the development of the disease has been observed on the leaves, in the treatment with mycorrhizae and the treatment without mycorrhizae. The stages of the development of the disease (from stage 1 to and stage 4), according to Oye Anda et al. (2015), were observed on the leaves of hybrids plantains plants. Plantain plants have been treated with mycorrhizae during 8 weeks before the application of *M. fijiensis*. The dynamic of appearance of symptoms is similar in each cultivar. Symptoms appeared much earlier when cultivars were not inoculated with AMF. However, the development of the black leaf-spot disease was delayed in each cultivar treated with AMF. This was probably because the presence of *M. fijiensis* influenced the development of the AMF allowing the plantain plants to develop a systemic resistance to the disease. This was evidenced by the delay in the development of the disease in plantain plants with mycorrhizae treated with *M. fijiensis*. These results corroborate the studies on the systemic resistance of bananas to nematodes in pots culture conditions (Anene & Declerck, 2016). The AMF associate with plant roots help improve the use of water and nutrients, especially those with low mobility in the soil, and increase plant tolerance to various biotic and abiotic stresses (Bada & Fagbola, 2014).

The results after the analysis showed that the infection was less developed in plants with mycorrhizae than in plants without mycorrhizae, which could be explained by the morphologic transformation of the roots systems, the induction of the mechanisms of defence and the indirect competition between AMF and *M. fijiensis*. AMF induces the elicitation of plant defence genes in root colonization (Gallou et al., 2010). During mycorrhizal root colonization, transient defence mechanisms are activated (Oye Anda et al., 2015). The mechanisms that guide the reduction of the disease observed in our experiments remain speculative. Manga et al. (2017) showed the ability for *Acacia seyal* to form symbiotic associations with *G. aggregatum* despite the application of salt stress. In mycorrhized plants, it was noted an improvement in salt stress tolerance resulting in significantly higher dry biomass and N, P, K content than in non-mycorrhized plants. Dalpé (2005) showed that the interaction due to AMF during the biologic fight needs 5 mechanisms: *i)* plant growth stimulation through an increased nutritional contribution and, consequently, improved plant health; *ii)* the morphological transformation of the root system; *iii)* the induction of defence mechanisms in the plant; *iv)* a direct competition with AMF like with nutrient availability and infection sites; and *v)* the modification of the soil microflora and increase in organic matter. Haro et al. (2015) reported that the *G. aggregatum* arbuscular fungus could significantly enhance the growth of the flowering-fruiting KVX cowpea variety.

The results obtained during this experiment showed that the mycorrhization of plantain plants was beneficial in reducing the disease, which was confirmed in the study carried out by Oye Anda et al. (2015) *in vitro* culture on the cultivar "Grande naine".

Black sigatoka disease increased over time. The highest level of the disease symptoms occurred on the leaves of Bâtard (stage 4) and C292 (stage 3) after day 10 and day 15, respectively, while these symptoms did not occur on the leaves of CRBP39 and F568 until after day 25 (stage 1 and stage 2). This can be explained by the fact that there is a correlation between the occurrence of leaf-spots and the resistance of each cultivar. The frequency at which the stage of disease increases in CRBP39 and F568 (resistant varieties) is lower than in Batard and C292 (sensitive varieties). These results corroborate the study by Irish et al. (2013) who reported that the FHIA hybrids were consistently more resistant, and developed less disease than "Grande naine".

Moreover, the minimum duration of the progression of symptoms from stage 2 to stage 4 varied from 10 to 12 days for sensitive varieties. Consequently, the time of disease progression was higher in resistant varieties than in susceptible varieties. Consequently, infestation levels were higher in highly susceptible varieties relative to resistant varieties.

COMPETING INTERESTS

The authors declare that they have no competing interests

AUTHORS' CONTRIBUTIONS

The main writing was done by CCOA; the statistical and agronomic analyzes were carried out by CCOA and PML, while the revision of writing was done by PNN and ANN.

ACKNOWLEDGEMENTS

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