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Fungal endophytes in bananas cv Manzano affected by *Fusarium*

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Endophytes microorganisms have the potential to control vascular diseases caused by *Fusarium* spp. which does not have an effective chemical control. In this study, endophytes populations present in Manzano -apple bananas- affected by *Fusarium oxysporum* f. sp. *cubense* race 1 were studied. Endophytes were isolated in two commercial farms in Urabá-Colombia, taking leaf, pseudostem, corm and root tissues from healthy and diseased plants. Two disinfestation methods were used: conventional (2% hypochlorite + 70% Ethanol) and chlorine gas (6.25% sodium hypochlorite + 37% hydrochloric acid). 143 isolates with 11 genera were obtained from healthy plants with the following frequencies: *Fusarium* sp. (18.67%), *Nigrospora* sp. (8%), mycelia sterilia (48%), among others. Also, eight genera were found in diseased plants, *Fusarium* sp. (23.53%), *Colletotrichum* sp. (17.76%), mycelia sterilia (47.06%). All endophytic fungi are ascomycetes, except for *Pythium* sp., oomycete that was isolated only from diseased plants. *Pythium* sp. which, was isolated from healthy plants, constitutes the first reports in musaceas. According to the Simpson and Shannon-Wiener diversity indices, a higher diversity of fungi was found in healthy plants (0.282 and 1.729) than in infected ones (0.294 and 1.532); it depends on disinfection method as demonstrated here, suggesting that tissue cleaning and disinfection methodologies modulate the microbial populations obtained. This work contrasted endophytic fungi in symptomatic plants attacked by Foc R1 with healthy plants and also the genus of endophytic fungi described in this study have already been reported in previous research in *Musa,* except for the oomycete *Pythium*

Key words: Biological control, diversity, Foc, microbiota, vascular disease.

INTRODUCTION

Banana is next to rice, wheat, and maize as a food crop. Though banana is a major crop around the world in international trade, more than 85% of bananas are grown for local consumption in tropical and subtropical regions

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(Perrier et al*.,* 2011). This crop is affected by numerous diseases, but *Fusarium* wilt, formerly known as Panama disease, caused by the fungus *Fusarium oxysporum* f. sp*. cubense* (Foc), is the most destructive disease of the Musaceae family (Ploetz, 2015). It has four races, and Race 1 (R1) destroyed more than 80.000 hectares of the Gros Michel clone in Latin America in 1890 and mid-1950s, which forced the banana industry to replace this cultivar with bananas of the Cavendish subgroup resistant to R1. However, the problem of Foc R1 persists in smallholding production systems, where susceptible varieties such as Gros Michel (AAA), Manzano (Apple) (AAB), Prata (AAB) and Bluggoe-type cooking bananas (ABB) are still cultivated (Viera and Pérez-Vicente 2009; Ordoñez et al., 2015). Currently, a Foc population variant known as Race 4 Tropical (Foc R4T) is present in Asia, Oceania and Africa, but not in America. In the areas where it is present, the disease has caused extensive damage, including serious economic and productive consequences. A marginal damage cost of $2.3 billion by Foc R1 has been estimated, but there is no consolidated official data concerning RT4. However, it has been estimated that around 100.000 ha are infested globally, being the Philippines (15.500 ha), China (40.000 ha) and Jordan (80% of the production area in banana Valery) the countries with the highest loses (Ordoñez et al*.,* 2016). It is expected that damage by R4T may affect 1.6 million hectares of musaceas crops in 2040, whose production today is 36 million tons, worth 10 billion dollars (Scheerer et al., 2016).

Market conditions must change in the short and medium term due to conventional management measures are inefficient to counteract the problem (Ploez, 2015). Therefore, it is necessary to study, design and adapt control measures. One of the proposed alternatives to control is the use of endophytes, a group of microorganisms that colonize the internal tissues of plants without causing immediate negative effects. These microorganisms are linked with various beneficial interactions with their host, providing protection against a wide variety of biotic and abiotic stress factors (Porras-Alfaro and Bayman 2011; Aly et al., 2011). Microbiological composition of plants depends on multiple factors such as the host, phytosanitary status, physiological age, availability of nutrients and environmental conditions (Stone et al., 2004; Zimmerman, 2012). Studies in banana have shown the presence of endophytic microorganisms (Photita et al., 2001; Rossmann et al., 2012), and their activity spectrum can include plant growth promoters (Ting et al., 2008; Marcano et al., 2016) and Foc antagonists (Cao et al., 2005; Lian et al., 2008; Nuñez et al., 2013), among others. Endophytic fungi have been associated with plants for over 400 million years; they are ubiquitous and occur within all known plants, including a broad range of host orders, families, genera and species. Endophytic fungi mainly consist of members of the Ascomycota or their mitosporic fungi, as well as some taxa of the Basidiomycota, Zygomycota and Oomycota phyla (Sun and Guo, 2012). The aim of this study is to observe fungal endophyte populations in banana cv. Manzano, in healthy plants and plants infected with Foc R1 as an approximation to the dynamics of these organisms in pathogenesis processes, using two disinfection methods on tissues.

MATERIALS AND METHODS

Plant material

Healthy (H) banana plants cv. Manzano in a state of development of floral differentiation and phenological state 5090 of the BBCH scale (Meier, 1997) were collected in productive area in Urabá (Colombia) in the farm 'Villa Carmen' (18 years without the presence of Foc R1) (lat. 7°51'12'' N, long. 76°41'19'' W). Also, symptomatic (S) bananas plants cv. Manzano with similar developmental stages were collected in the farm 'La Isla Bonita' (12 years with the presence of Foc R1) (Lat. 7°48'08'' N, long. 76°41'25'' W). These plants had initial stages of Foc R1 symptoms consisting of chlorosis in the first three leaves. Four plants were selected in each location; roots, pseudostems, corms, 300 g sections from leave number three, and segments of each plant were collected and then immediately taken to the laboratory of Cenibanano on Carepa (Colombia).

Isolation and preservation of endophytic fungi

Under laboratory conditions, tissues were washed with tap water and cut into pieces of 0.5 cm^3. Then, samples were subjected to two sterilization methods: i) Conventional disinfection with liquid chlorine (LCD), in which each sample was immersed in 2% liquid NaOCl and then in 70% ethanol for 1 min, then it was rinsed twice with sterile distilled water for 1 min. ii) Gaseous chlorine disinfection (GCD), in which each sample was placed on filter paper and inside a hermetically sealed container. Then it was suspended for 30 min in a Cl gaseous atmosphere produced by the reaction of a mixture containing 100 mL of 6.25% NaOCl and 5 ml of 37% HCl (Marshall et al., 1999).

Tissues were placed on Petri dishes containing potato dextrose agar (PDA) + Streptomycin 150 ppm and incubated at 25°C during 8-10 days. For the incubation period, samples were observed every day, and any newly emerged fungal spot was immediately picked out using autoclaved toothpicks and transferred to another fresh PDA plate. The resulting fungal isolates were conserved in 20% glycerol in 2 ml cryovials, and stored at -20°C.

Endophytic fungi morphotypes

Fungi strains were identified based on macroscopic and microscopic features according to Barnett and Barry (1998), Hanlin (1990) and Seifert et al. (2011). Morphotypes of nonsporulating strains were determined based on macroscopic characteristics.

Diversity analyses of endophytic fungi

Using morphotypes as the unit, the number of isolates (N) was counted and the isolation frequency (IF) was calculated for each endophytic microorganism in different tissues. The species diversity was evaluated by the Shannon–Wiener index (H′) and Simpson's

dominant index (λ). The indices were estimated using the EstimateS program (Version 9.1.0) (Colwell, 2013).

RESULTS AND DISCUSSION

Isolation of endophytic fungi

A total of 143 isolates of endophytic fungi were recovered (Tables 1 and 2), 45.46% from leaf, 20.28% from root, 18.18% from pseudostem and 16.08% from corm. Fourteen morphotypes of endophytic fungi, one oomycete (Chromista) and 68 sterile mycelia strains were categorized (Tables 1 and 2). It was found that 14 genera of endophytic fungi are Ascomycota (Fungi), and were identified in three classes: Sordariomycetes, Dothideomycetes, Eurotiomycetes distributed in eight orders: Hypocreales (35.7%), Sordariales and Eurotiales (14.3% each), and Pleosporales, Trichosphaeriales, Diaporthales, Capnodiales, Glomerellales (7.1% each). Additionally, 47.5% of the isolates showed sterile mycelium belongs to Ascomycota or Basidiomycota based on mycelia septation.

Prevalence of sterile mycelial fungi (Ascomycota/ Basidiomycota) in this study is the highest detected in *Musa*, with 68 strains (Figure 1). Previous reports in this genus of plants conducted by Photita et al. (2001) found 14 sterile mycelial fungi from 61 collected, and Brown et al. (1998) reported 24 strains from 100. Sun and Guo (2012) summarized that 54% of total isolates obtained in recent research on endophyte fungi did not sporulate in cultures, which suggest a considerable diversity of these fungi with sterile mycelia in plant tissues. It is consistent with finding here, in both groups of plants, healthy and diseased plants, nonsporulating strains were common (Table 1) (48 and 47.06% respectively), followed by *Fusarium, Nigrospora, Phomopsis* and *Cladosporium*, all common and reported endophytes in *Musa* (Cao et al., 2002; Photita et al., 2001; Sun and Guo, 2012; Ting et al., 2008; Zakaria and Rahman, 2011). On healthy tissues are present too *Verticillium, Curvularia, Purpureocillium, Trichoderma, Stachybotrys, Aspergillus* and *Sordaria*. On plants affected by Foc R1 *Colletotrichum, Chaetomium, Penicillium* and *Pythium* were isolated exclusively.

Common fungal morphotypes of healthy and diseased plants can be part of the usual microbiome, and within these, *Fusarium* sp. was the most frequently found morphotype in healthy (18.67%) and diseased plants (23.53%), being an endophyte widely isolated in different tissues in several banana studies (Brown et al., 1998; Photita et al., 2001; Ting et al., 2008; Zakaria and Rahman, 2011). *Nigrospora, Phomopsis* and *Cladosporium* are common to both groups of plants, and could present affinity with some tissues, such as *Phomopsis* sp. which was isolated only from foliar tissue in healthy and diseased plants under the two disinfection methods used. Similar results have been reported by Gazis and Chaverry (2010), who found that *Phomopsis* aff. *theicola* was located in leaves, while diverse genera of endophytic fungi were located in leaves and in the sapwood of wild rubber trees. Fungi belonging to the genus *Cladosporium* sp. also showed a type of affinity with roots and leaves (Photita et al., 2011; Cao et al., 2002); five isolates were found in symptomatic plants, but an individual was isolated from healthy plants in the corm, the tissue adjacent to the root. These results are in accordance with Hamayun et al. (2009) who reported the presence of *Cladosporium sphaerospermum* in soybean roots and its activity as a plant growth promoter. However, *Fusarium, Colletotrichum, Verticillium, Penicillium, Nigrospora* and *Curvularia* are reported as causal agents of crown rot in banana in different production areas (Perez et al., 2001; Kamel et al., 2016). For this reason, some endophytic fungi can be considered latent pathogens (Stone et al., 2004) under conditions such as plant senescence and biotic and abiotic stresses, but we did not probe if those endophytic isolates had pathogenic characteristics.

Verticillium, Sordaria Stachybotrys Curvularia, Aspergillus Purpureocillium, Trichoderma were obtained from healthy tissues (Table 1). Some of them were involved in biological control with active compounds against *Fusarium* on *Musa* (Brown et al., 1998; Cao et al., 2004; Nuñez et al., 2013), or in other pathosystemas such as *Stachybotrys elegans* is a fungus native to the soil, mycoparasitic properties have been widely described (Chamoun and Jabaji, 2011). *Purpureocillium* spp. is a fungus widely analyzed in the biological control of pathogens, as specified by Munawar et al. (2015), who demonstrated its biocontrol capacity in the tomato wilt complex. Others like *Sordaria* sp. have been reported as an endophyte fungus in beet (Abdelwehab et al., 2014) and conifers (Hoffman et al., 2008) but not reported in banana, so this is the first report in these plants.

On symptomatic plants, eight genera of endophytic fungi were collected (Table 1), also fungi of mycelia sterilia. Identified morphotypes included *Fusarium* (23.5%) followed by *Colletotrichum* (11.76%) and *Cladosporium* (7.53%). *Colletotrichum* sp., *Penicillium* sp., *Chaetomium* sp. and *Pythium* sp. were isolated exclusively from symptomatic plants; these genera of fungi are involved in the decomposition of tissues, a typical symptom of decay or wilting also caused by *Fusarium*, but when deep disinfection is performed, those are only on pseudostem. Rodrigues (1994) mentions that the affinity with tissues of endophytes suggests that these microorganisms have the capacity to use specific substrates or habitat, in addition to making a differential use of substrate to reduce competition among endosymbionts and prevent excessive population of endophytes in the host plant (Gamboa and Bayman, 2001; Venkatachalam et al., 2015). Presence of *Chaetomium* sp. on speudostem on infected plants suggests tissue decomposition since it is a cosmopolitan fungus and cellulose degrader (Lee and

Table 1. Morphotypes of fungal endophytes in bananas cv. apple.

Kingdom	Phylum	Class	Order	Genus/ Morphotype	Healthy			Symptomatic			Total strains
					Organ	Strains	Frequency (%)	Organ	Strains	Frequency (%)	
Fungi	Ascomycete/Basidiomycete	indeterminated	Indeterminated	Mycelia sterilia	r,c,s,l	36	48				36
Fungi	Ascomycete/Basidiomycete	indeterminated	Indeterminated	Mycelia sterilia				r.c.s.l	32	47.06	32
Fungi	Ascomycete	Sordariomycetes	Hypocreales	*Fusarium*	r,c,s,l	14	18.67	c.s.l	16	23.53	30
Fungi	Ascomycete	Sordariomycetes	Trichosphaeriales	*Nigrospora*	c,s,l	6	8	r.c	2	2.94	8
Fungi	Ascomycete	Sordariomycetes	Diaporthales	*Phomopsis*	*l*	2	2.67	*l*	1	1.47	3
Fungi	Ascomycete	Dothideomycetes	Capnodiales	*Cladosporium*	*c*	1	1.33	*r*	5	7.35	6
Fungi	Ascomycete	Sordariomycetes	Hypocreales	*Verticillium*	*r,c,l*	5	6.67		0	0	5
Fungi	Ascomycete	Dothideomycetes	Pleosporales	*Curvularia*	*r,l*	3	4		0	0	3
Fungi	Ascomycete	Sordariomycetes	Hypocreales	*Purpureocillium*	*c,l*	3	4		0	0	3
Fungi	Ascomycete	Sordariomycetes	Hypocreales	*Trichoderma*	*c,s*	2	2.67		0	0	2
Fungi	Ascomycete	Sordariomycetes	Hypocreales	*Stachybotrys*	*c*	1	1.33		0	0	1
Fungi	Ascomycete	Eurotiomycetes	Eurotiales	*Aspergillus*	*l*	1	1.33		0	0	1
Fungi	Ascomycete	Sordariomycetes	Sordariales	*Sordaria*	*l*	1	1.33		0	0	1
Fungi	Ascomycete	Sordariomycetes	Glomerellales	*Colletotrichum*		0	0	*r.s.l*	8	11.76	8
Fungi	Ascomycete	Sordariomycetes	Sordariales	*Chaetomium*		0	0	*s*	2	2.94	2
Fungi	Ascomycete	Eurotiomycetes	Eurotiales	*Penicillium*		0	0	*r*	1	1.47	1
Chromista	oomycota	Peronosporide	Peronosporales	*Pythium*		0	0	*r*	1	1.47	1
				Total		75	100		68	100	143

r: root, c: corm, s: pseudostem; L: leaf.

Table 2. Amount of endophytic morphotypes obtained by means of two disinfection methods of tissues in bananas cv. apple.

Plants	Disinfection method	Tissue				Total
		Root	Corm	pseudostem	leaf	
Healthies	L	11	11	3	19	44
	G	5	5	6	15	31
Symptomatics	L	6	4	11	19	40
	G	7	3	6	12	28
Total		29 (20.28%)	23 (16.08%)	26 (18.18%)	65 (45.46%)	143 (100%)

L: liquid chlorine disinfection
G. Gas chlorine disinfection.

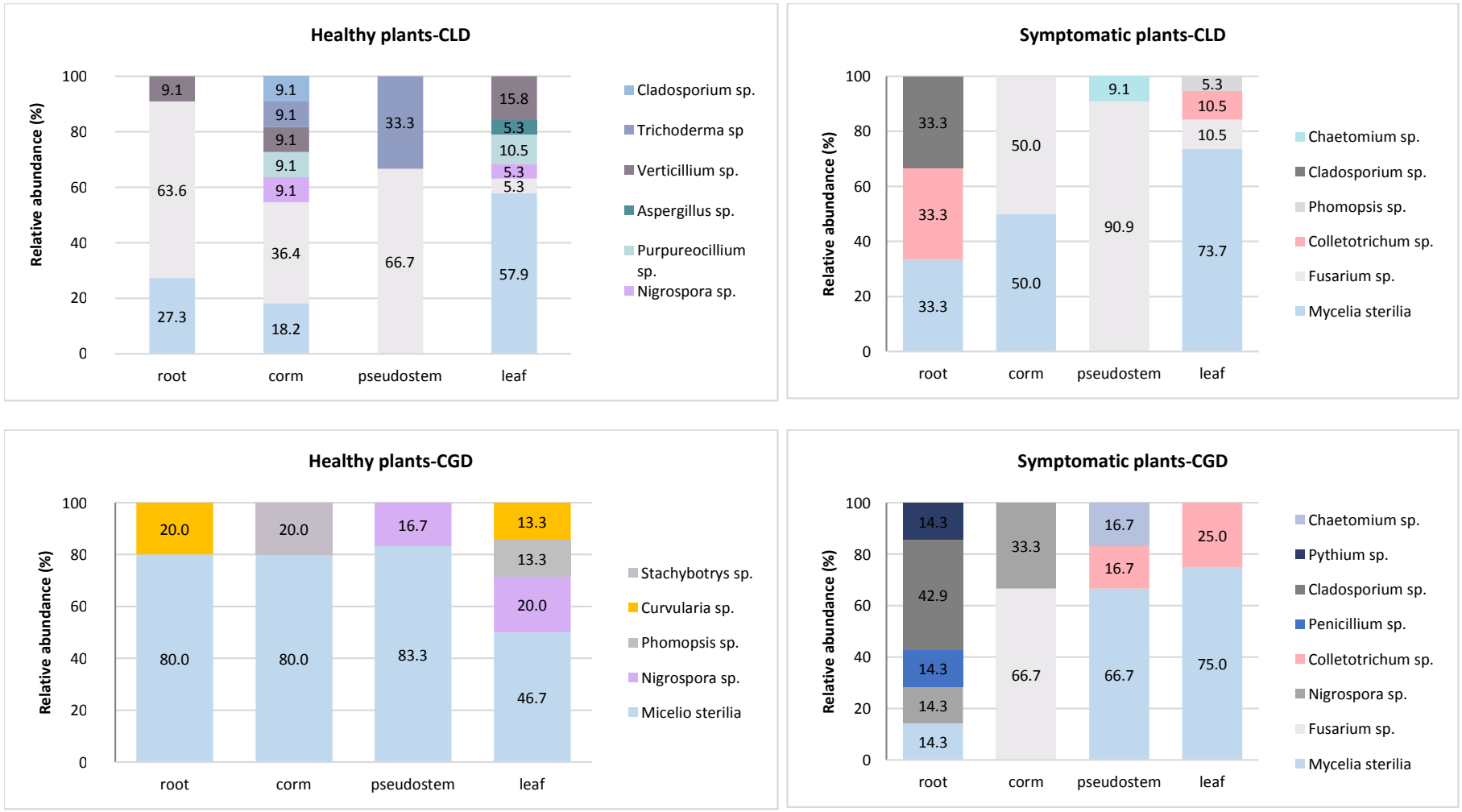

Figure 1. Richness, dominance and heterogeneity of endophytic fungi in healthy and inoculated plants of banana with F. oxysporum race 1 (CLD: Chlorine Liquid Disinfection. GCD: Chlorine Gas Disinfection).

Hanlin, 1999). Huang et al. (2015) determined that after soil disinfection of a crop affected with Foc, the presence of *Chaetomium* sp. was highlighted within the microbial community of the soil, while the population of the pathogen (Foc) decreased. It can be inferred that both genera can be common in the soil of the banana farms in the study area, and the condition of diseased plants can favor the endophytic condition of this fungus.

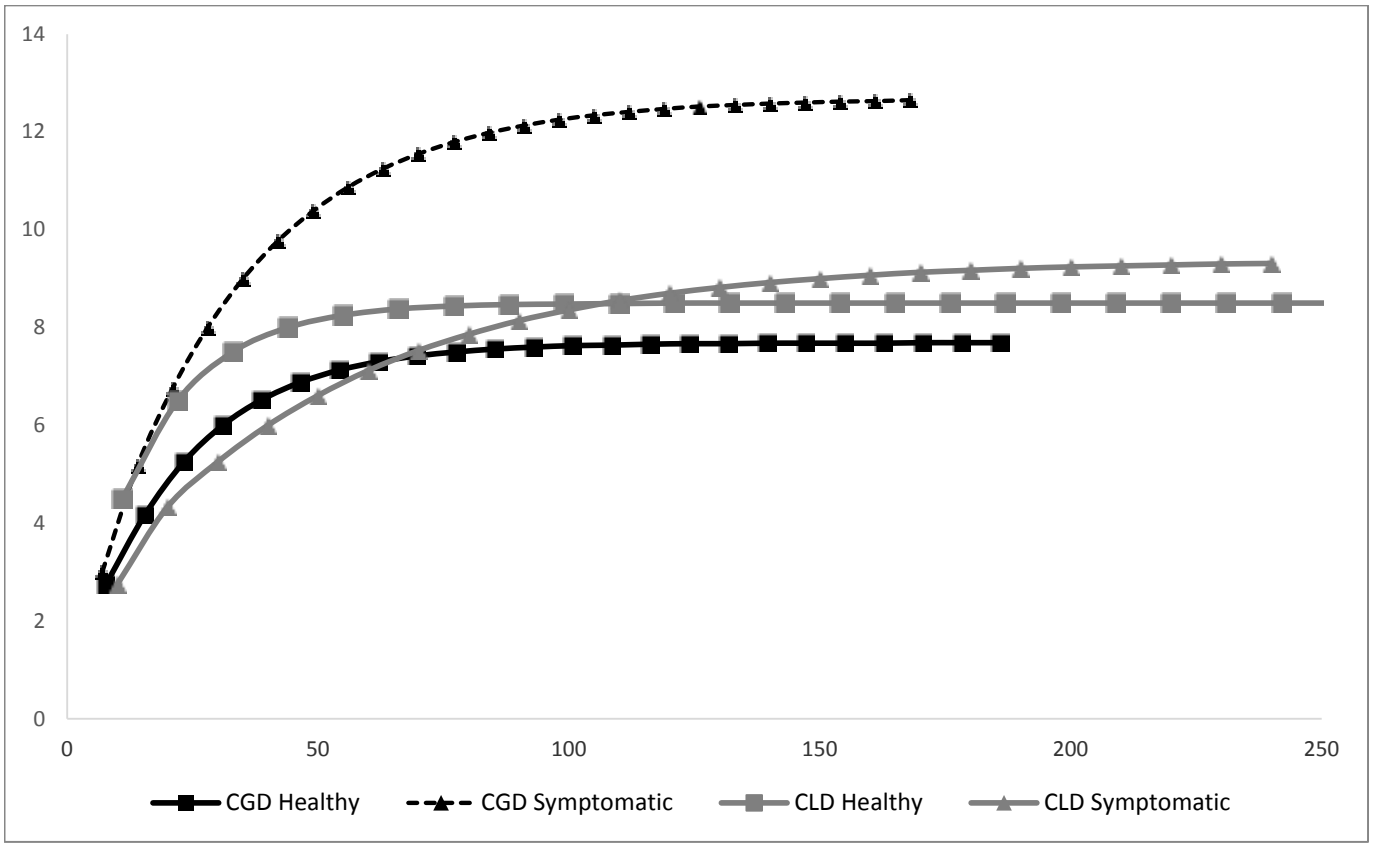

Figure 2. Rarefaction curves of endophytic fungal species for healthy and symptomatic plants under gas(CGD) and liquid (LCD) chlorine disinfection.

Pythium sp. (Oomycota: Chromista) constitutes the first report of an Oomycete in banana as endophyte on roots, obtained by means of GCD.

Previously, Oomycota had been sampled as a phylloplane inhabitant in *Musa* AAB (Urdaneta et al., 2002), but existence of *Pythium* on root tissues in banana can be explained as an opportunistic inhabitant of root rot. This genus is its direct causal agent of root rot in several crops (Manjunath et al., 2010; Gichuru et al., 2016; Charkowski, 2016). In *in vitro* banana plants, *Pythium debaryanum* has been associated with damping-off symptoms (Herrera et al., 1995). However, *Pythium* was recently reported as a microorganism that confers cross-resistance to diseases, which suggests several ecological functions in plant tissues (Yacoub et al., 2016).

Disinfection methods

In our results leaf and root tissue showed a high proportion of endophytes (Table 2, Figure 1); leaf contains the greatest diversity of endophytic fungi, but, disinfection method seems to modulate proportion of strains obtained. Leaf contains high diversity and more when in a healthy tissue, but root is as well a tissue that carries a high diversity on disease process, and again is dependent on disinfection method (Figures 1 and 2). It leads to analyze not only diversity, but also function of population inside a tissue. As was mentioned, on leaf some genuses obtained are involved in biological control activity; in contrast with some from roots from diseased plants, this community seems to be active in decomposition tissues. Context of biological stage of plants is important to analyze diversity of endophytes. It is similar with the results of Pocasangre et al. (2000).

Cultivation-dependent techniques used in this study included surface sterilization of plant tissue to eliminate superficial microorganisms (Hallmann et al., 2006). Theoretically, the sterilizing agent should kill any microbe on the plant surface without affecting the host tissue and the endophytic microorganisms. For the purposes of this study, two disinfection methods were used: the first one consisted of using gaseous chlorine (GCD), which is more efficient to remove contaminants from surfaces (Marshall et al., 1999), and the second one used conventional disinfection with liquid chlorine (LCD). The use of gaseous chlorine resulted in 30% less isolatesfrom healthy and symptomatic plants compared with the usual

Table 3. Ecological index of endophytic fungi in healthy and inoculated plants of banana with *F. oxysporum* race 1.

Comparison		Strains (n)	Richness (S)	Simpson (λ)	Shannon-Wiener (*H'*)
Plant	Healthy	75	12	0.282	1.729
	symptomatic	68	9	0.294	1.532
Gas chlorine disinfection	Healthy	31	6	0.448	1.170
	symptomatic	28	8	0.296	1.590
Liquid chlorine disinfection	Healthy	44	8	0.256	1.610
	symptomatic	40	6	0.280	1.290

disinfestation method. It is required in tissues like roots, corm and pseudostems of banana plants which have porous surfaces that can create air chambers that prevent this substance from deep cleaning them (Tables 1 and 2). As an example, *Fusarium* morphotypes were obtained from leaves in healthy plants, but when gas chlorine was used, they were not isolated from tissues. The same was observed with *Verticillium* morphotypes, which were present in leaves with superficial disinfection with liquid chlorine. In contrast, *Stachybotrys* and *Sordaria* were present on corms and leaves with deep disinfection, respectively and *Pythium* on roots in symptomatic plants when tissue was cleaned with gaseous chlorine. These findings indicate that the disinfection method leads to diversity of microorganisms that can be found by molecular or microbiological methods.

Diversity

Richness (S), dominance (λ) and heterogeneity (H´) of fungal endophyte morphotypes were calculated in healthy and diseased banana plants. Richness refers to the number of groups of genetically or functionally related individuals, in this case fungi morphotypes. In general, S of endophytic fungal morphotypes in bananas cv. Manzano was higher in healthy plants (12 morphotypes) compared with diseased plants with Foc (nine morphotypes) (Table 3). Our data suggested a high diversity of the endophytic fungal community of healthy plants in contrast to diseased plants (Table 3). Regarding S comparisons between microorganisms obtained by GCD, infected plants have more diversity or richness suggesting again that deep disinfection of tissues allows one to analyze some microorganisms as weak competitors in axenic media, but present on tissues. As discussed above, in LCD disinfestation, surfaces of healthy and diseased plants kept competitive or opportunistic strains that can survive on air chambers of tissues; those strains are more competitive in a Petri dish than any other obtained by GCD.

The Simpson diversity index (λ) is the estimated probability that two individuals randomly selected from the same habitat will be of the same species, which is a measure of dominance. This index in healthy plants was 0.282, while in diseased plants it was 0.294 (the λ index oscillates between 0 and 1, 1 being a population with 1 species). When healthy and infected plants were compared, they did not exhibit any differences, but when GCD values were observed, healthy plants tended to be less diverse.

On the other hand, the Shannon-Wiener index (H') indicates how heterogeneous or uniform the representation of the species in abundance is, considering all species, assuming that all of them are represented in a sample and that they are randomly sampled. Values range from 0 to 5. Typical values are generally between 1.5 and 3.5 in most ecological studies where 1.5 represents the lowest diversity and 3.5 the highest. This index was 1.729 in healthy plants, being slightly higher than in diseased plants, which showed a value of 1.532. The same tendencies were observed with LCD. In contrast, tissues of infected plants disinfected with GCD are more heterogeneous in terms of fungal endophyte morphotypes (Table 3).

Rarefaction curves allow comparing the number of genera between healthy and diseased plants, when finding different numbers of isolates. The results confirm that the method of disinfection is important because it allows us to understand the richness of the species in terms of their abundance in deep tissues, so that they can be considered endophytes and not superficial invaders. In the case of the evaluations between healthy and diseased plants subjected to GCD, results showed that infected plants contained more diversity of organisms, specifically in roots, but that these organisms are essentially tissue decomposers. In healthy plants, this endophytic population had other functions such as protection, and that is why although populations count numerically, it is also important to analyze their ecological significance within the tissues of the plant. This also necessarily implies that the methods of organism identification with sterile mycelium and the counting of non-cultivable ones are also two necessary and pertinent methodological components for this type of analysis

(Figure 2).

Cao et al. (2004) reported a higher number of Actinomycete morphotypes in diseased banana plants with Foc R4T in comparison to healthy plants. This fact suggests that Actinomycetes diversity increases in the presence of the pathogen. However, 50% of isolates from healthy plants showed antagonism with Foc, while only 27% of the isolates from diseased plants showed antagonistic activity. Lian et al. (2008) stated that the presence of pathogens (Foc) in banana plants triggers a cascade of reactions that leads to the synthesis of stress metabolites such as H_2O_2, phytoalexins, abscisic acid, jasmonic and salicylic acid, which can generate changes in endophytic populations. In lemon plants, a greater diversity of endophytic fungi has been found in healthy leaves compared to yellow leaves (with nutritional deficiency). This difference suggests that the yellowing of leaves can facilitate the incidence of certain endophytic fungi such as *Colletotrichum gloeosporioides* and impose growth inhibition on the other endophytes (Douanla-Meli et al., 2013), which is a similar condition to that presented in Manzano banana plants affected by Foc. Even more information is needed to understand the endophyte-host relationship, since the effects attributed to the endophytes present in healthy plants can change when host plants are grown under less favorable conditions, or even in conditions of stress triggered by the presence of pathogens or an unfavorable environment (Hardoim et al., 2015).

Conclusions

In general, healthy plants have greater diversity compared with plants infected with *Fusarium*. The attack of *Fusarium* initially concentrated in roots and, therefore, decomposer organisms was found there. Although these organisms are indicators of diversity, they are part of the necrotrophic degradation of the tissue, and for this reason, the amount of endophytic organisms found in a tissue must be analyzed in depth in the context of microbial ecology. It is necessary then, to begin to study not only the abundance but also the functionality of the microorganisms found in terms of pathogenic, symbiotic and beneficial interactions. In this work, it was demonstrated that the tissues analyzed leaf is the carrier of greater diversity of endophytic fungi, but the root from diseased plants also contains a considerable number of microorganisms associated to a decomposition of tissues. In contrast, many of the genera found in the leaf have biocontrol activity reported in literature.

As well, the type of disinfection leads to the findings of endophytes in terms of diversity. Methodology of disinfection used on tissues to obtain endophytes is crucial because it determines if surface organisms are extracted from tissues. Chlorine gas disinfection prevents air chambers of tissues on which superficial micro-organisms can be hosted, and then obtained as endophytic. In the case of *Musa* where there is prevalence of aerenchyma and irregular surfaces in some cases, the disinfection method is crucial to obtain reliable results that allow us to understand the context of the presence of a species in an endophytic community.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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