FOLIAR ENDOPHYTIC FUNGI OF *THEOBROMA CACAO* STIMULATE MORE THAN INHIBIT *MONILIOPHTHORA* SPP. GROWTH AND BEHAVE MORE AS AN ENDOPHYTES THAN PATHOGENS.

Miriam Villavicencio[1], Laura Schuller[2], Fernando Espinosa[1], Carlos Noceda[1,4], Daynet Sosa del Castillo[1,4,] Simón Pérez-Martínez[4*]

[1]Escuela Superior Politécnica del Litoral, ESPOL, CIBE. Campus Gustavo Galindo Km 30.5 Vía perimetral, P.O. Box 09-01-5863, Guayaquil, Ecuador.

[2]Department of Environmental Sciences. Universität Koblenz-Landau, Germany.

[3]Biología Celular y Molecular de Plantas (BOCEMP)/Biotecnología Industrial y Bioproductos, Departamento de Ciencias de la Vida y de la Agricultura, Universidad de las Fuerzas Armadas-ESPE, Av. General Rumiñahui s/n. Sangolquí, P.O. Box 171-5-231B, Ecuador.

[4]Facultad de Ciencias e Ingeniería. Universidad Estatal de Milagro, UNEMI. Calzada Universitaria km. 1.5 vía Milagro-km26, Milagro. Guayas, CP 090112, Ecuador.

*Author for correspondence: sperezm2@unemi.edu.ec

ORCID: orcid.org/0000-0001-7349-7036

SUMMARY

The two most disastrous diseases of *Theobroma cacao* in Ecuador are caused by the pathogenic fungi *Moniliophthora roreri* (MR, Frosty pod) and *M. perniciosa* (MP, Witches' broom). Both diseases are prevalent in Ecuador since its detection in 1916 and 1921, respectively. One hundred and twenty-six isolates were isolated from healthy *T. cacao* leaves, originated from five nurseries in the Ecuadorian Coast. Isolates were screened *in vitro* for their stimulation/ inhibition of growth of MR & MP. The endophytic fungi influenced the growth of *Moniliophthora* spp. in different manners, 91/126 of the tested isolates stimulated somehow the colony growth of both pathogens, and only 30 and 5 isolates always inhibited the mycelial growth of MR & MP, respectively. The highest percentage of growth inhibitions against MR *was 71%, but* quite lower for MP (18%). Only two isolates caused symptoms of chlorosis and necrosis in leaves and fruits *Phomopsis spp. (Ascomycota)* and *Psathyrella sp. (Basidiomycota).* Twenty-four isolates from 11 taxa caused necrosis only fruits (*Ascomycota: Phomopsis spp., Phoma sp., Colletotrichum gloeosporioides s.l., Fusarium decemcellulare, F. equiseti, F. solani, Nectria pseudotrichia, Pestalotiopsis microspora, Didymosphaeria futilis, Xylaria venosula and Basidiomycota: Ceriporia lacerata*) and four isolates/ taxa infected only leaves (*Phomopsis spp., Pestalotiopsis microspora, Nigrospora sphaerica and one unidentifiable isolate of Xylariaceae sp.*). The non-pathogenic isolate, *Hypoxylon investiens*, inhibited MR in 71 %, but also stimulate the growth of MP in 3%. The second-best isolate (*Lasiodiplodia theobromae*) only inhibit MR and MP in 58 y 15 %, respectively; and, although the isolate was not pathogenic, the specie is risky to develop a biological control agent. There was no promising isolate against both MR & MP.

Key words: endophytes, Theobroma, latent pathogen,

INTRODUCTION

Theobroma cacao L. fruits allow to manufacture chocolate. Ecuador's cacao production has become a major export commodity, particularly for fine-flavored cacao recognized for its unique flavor known as ''Arriba". This species is native to the Amazon Basin (Motamayor et al. 2008) and Ecuador is the world's largest producer of fine aroma cacao from Nacional genetic group (Boza et al. 2014) providing up to 75% of the world's supply in 2015 (data inferred from http://www.fao.org/faostat/en/#data/QC and from https://www.icco.org/about-cocoa/fine-or-flavour-cocoa.html).

Two of its main diseases of *T. cacao* in the Americas also originated in this region, the frosty pod disease (FP, caused by *Moniliophthora roreri*) in Colombia (Phillips-Mora and Wilkinson 2007) and witsches' broom (*Moniliophthora perniciosa*, WB) in Surinam and Brazil (Evans 2016b). The FP and WB diseases limit cocoa yield in the producing countries in this region (Evans 2016b; Evans 2016a; Hardy 1961; Purdy and Schmidt 1996). Particularly in Ecuador both diseases are endemic (Suárez-Capello 1983; Evans 2016b; Evans 2016a) and since the first reports as phytosanitary problems, for the FP in 1917 (Ciferri and Parodi 1933) and for WB in 1926 (Fowler and López R. 1949), have not stopped being the biggest challenge for production. This historical observation was corroborated experimentally in multi-environment trials where the diseased pods (FP + WB) per plot reached values between 34-60% for 12 genotypes across five locations in Ecuador over four years (Ramlachan et al. 2009; Sánchez-Mora et al. 2015). Even from the previous results it was observed that up to 30% of losses are reached in the genotypes with greater tolerance to diseases. Numerous strategies for control of both diseases have been investigated and some are recommended. These, invariably, center on cultural control with various supplementary control options, such as biological control (Hoopen and Krauss 2016).

Biological control of Moniliophthora spp. (*M. roreri*, MR & *M. perniciosa*, MP) can be categorized into two main approaches: classical and inundative (Krauss et al. 2010). The classical approach is defined in situations where a pest has been introduced into new regions, and the intentional introduction of an exotic, usually co-evolved, biological control agent for permanent establishment and long-term pest control. Inundative biocontrol resorts to antagonist native to the area where the pathogen is to be controlled, this are the case of MR & MP in Ecuador. The arguments in favor of the inundative approach are: i) the adaptation of the endophytic biocontrol agent to local agroecological conditions, and ii) the use of endophytic BCAs would probably have a larger window of opportunity to control Moniliophthora spp. since both pathogens exhibit a biotrophic phase in which they germinate, after arriving on the cocoa pod surface, and subsequently penetrate the host tissue. The biotrophic phase is followed by a necrotrophic phase within the brooms and/or cocoa pods during which the cocoa pod is destroyed from the inside out (Hoopen and Krauss 2016). The use of endophytic fungi as biological control agent offers the possibility of first infecting cocoa seedlings and occupy the niche before the arrival of MR & MP. This tactic must be done necessarily by seedlings or seed inoculation since the fungi are horizontally transmitted to the cacao (A. E. Arnold and Herre 2003), not the case for other BCAs such as actinobacteria that are transmitted via cocoa seeds (Tchinda et al. 2016).

Endophytic fungi are omnipresent, highly diverse, and naturally occurring inside the tissue (e.g. leaves, leave stalks, stems, bark, fruits) of all plant species (Rodriguez et al. 2009; Zabalgogeazcoa 2008; Porras-Alfaro and Bayman 2011). They form various symbiotic relationships with their host plants. Some of these endophyte associations are parasitic in some

part of its life cycle and harm their host, but in most instances the symbiotic lifestyles are mutualistic and can be beneficial for both symbionts (Partida-Martínez and Heil 2011). Through the colonization by endophytic fungi host plants can become more adaptable to stressful environments like water shortage, nematodes and herbivores by producing toxins which are a disincentive to insects (Yuan, Zhang, and Lin 2010; Porras-Alfaro and Bayman 2011) and fungal pathogens (Mejía et al. 2008; E. Arnold 2005; Holmes et al. 2004). The assemblage of taxa that inhabit the leaves is accommodated in time and space as a succession of species. The leaves during their life cycle are always colonized by endophytic fungi, in the beginning there is a great diversity of specimens and over time the diversity decreases but the density reaches 100% (Herre et al. 2007). The composition of species described in *T. cacao* is dominated by the ascomycete, and a few taxa belonging to the basidiomycetes (Evans, Holmes, and Thomas 2003; Rubini et al. 2005; Hanada et al. 2010).

In *T. cacao*, foliar endophytic fungi (FEF) have shown their potential as biological control agents at the field level (Mejía et al. 2008; A. E. Arnold et al. 2003; Krauss and Soberanis 2001). The origin of FEF strains reported as promising agents against MR & MP have been from both, wild species of Theobroma and Herrania genera (Evans, Holmes, and Thomas 2003; A. E. Arnold et al. 2000; Holmes et al. 2006) and from cultivated clones of *T. cacao* (Rubini et al. 2005; Villamizar-Gallardo et al. 2017; Hanada et al. 2010).

The ability of many fungal species to exist as endophytes and as pathogens (hemibiotrophic life styles) adds extra complication to our understanding of the ecology of host-fungi-environment interaction. Isolation from living plant tissue does not necessarily imply that the species is a latent pathogen with a hemibiotrophic phase (Cannon et al. 2012; Rojas et al. 2010), and distinguishing between the two life strategies is problematic. This study describes the richness of FEF species in *T. cacao* agroecosystems over 50 years of planted with Nacional cocoa clones in the Ecuadorian Coast. In addition, in vitro interactions of FEF with Moniliophthora spp. in terms of inhibition/stimulation of the mycelial growth of both pathogens, as well as the ability of the FEF to infect leaves and fruits of T. cacao were also investigated. Finally, implications of the development of biological control agents from endophytes from the perspective of its dual life style (endophytic/ pathogen) are discussed.

MATERIALS AND METHODS

Source of endophytic and pathogenic fungi

Endophytes were collected in five different cacao farms located in two counties in the Coast of Ecuador during 2014 (Tab 1). Only healthy leaves from plantations more than 50 years old and referenced by the owner as cocoa "National" were sampled. The whole leaves were cut in the field, kept in a cooler (< 24 h) until they were used in the laboratory. The leaves were washed with plenty of running water, dried with non-sterile paper towel, and then worked in laminar flow chamber. 2x2 cm fragments of the foliar lamina (without midribs) were cutted, disinfected consecutively for 2 min in 70% ethanol and 0.5% sodium hypochlorite and washed with sterile distilled water for three times. Subsequently the fragments were dried with sterile paper towel. Fragments were planted on 2% malt extract agar (A. E. Arnold and Herre 2003). Eight fragments were seeded per Petri dish (Ø90mm) and incubated at 25 °C in the dark for 10d. Every two days all colonies with different morphology were isolated and purified on potato-dextrose-agar.

Table 1. Origin of foliar fungal endophytes isolates from cacao Nacional from the Coast of Ecuador in 2014.

Clima	Province	County	Farm (N°isolates)	GPS-S	GPS-WO	Altitude (m.a.s.l)
Tropical de Monzón	Guayas	Balao (52)	Balao1 (18)	2°30'29,5"	79°46'34,8"	20
			Balao2 (28)	2°51'51,8"	79°37'20,8"	146
		Naranjal (36)	Naranjal1 (12)	2°30'49,2"	79°26'11,2"	50
			Naranjal2 (25)	2°40'35,2"	79°38'21,2"	32
Templado Permanente Húmedo	Azuay	Molleturo (38)	Molleturo (32)	2°30'49,2"	79°26'11,2"	50
Total N° Isolates[1, 2]		126	115			

[1]The difference in the quantity of isolates come from the uncertainty of the origin of some isolates

[2] Supplementary material 1 at the end of the manuscript.

The isolate of *M. roreri* (CIBE-A12.1) was isolated from a sporulated cacao pod in the Amazon region (Maridueña-Zavala et al. 2016) and *M. perniciosa* (CIBE-MP22) was isolated from symptomatic cacao stems in the Babahoyos in the province of Los Ríos (S-1°40'25'', O-79°32'44'', GenBank accession number KX913249), both from a 2011 field collections (Maridueña-Zavala 2011). All the isolates are conserved on both sterile distilled water and at -20°C (plus 25% of glycerol) in the Colección de Cultivos Microbianos del CIBE (WDCM 1151). The entire collection yielded 126 endophytic isolates, all were identified by sequencing the internal transcribed spacer, screened for their vis-à-vis antagonistic activity against both *M. roreri* and *M. perniciosa* and; their pathogenic capacity to their host plant *T. cacao.*

Taxa identification

DNA extraction. Fungi cultures were grown in a shaking incubator at 27°C for 3–5 days in 250 ml conical flasks containing 50 ml of culture media (7.0 g malt extract, 1.0 g Bacto peptone, 0.5 g yeast extract, in 1 l dH20). Mycelia were harvested by filtration (Whatman filter paper), washed with sterile distilled water, freeze-dried and ground. Total genomic DNA was extracted from mycelia (Sosa et al. 2016). The obtained DNA pellet was kept in 50 µl TE buffer at –20°C. **Amplification and sequencing of fungal internal transcribed spacers (ITSs).** ITS amplifications for sequencing were carried out by polymerase chain reaction (PCR) in a Mastercycler Eppendorf thermal cycler using ITS1 and ITS4 universal primers (White et al. 1990). The amplified fragments include ITS1, 5.8S and ITS2 regions of rDNA. Each PCR was performed in a 40 µl reaction mixture containing 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 0.1% Triton X-100, 1,5 mM MgCl2, 0.2 mM dNTPs, 0,25 µM primers, 1U GoTaq® Flexi Polymerase, and 40 ng of DNA. Amplifications were made 2–3 times to assess the reproducibility of the method. The thermal cycling program was as follows: initial denaturation at 94°C for 2 min, followed by 35 amplification cycles (denaturation at 94°C for 40 s, primer annealing at 53°C for 40 s and extension at 72°C for 40 s), and final extension at 72°C for 10 min. PCR products were purified with AccuPrep®PCR Purification Kit (Bio-NEER). Direct amplicon sequencing was performed on an ABI 3100 automated sequencer

following manufacturer's instructions (Applied Biosystems, Inc.). **Phylogenetic analysis**. The ITS1-5.8S-ITS2 sequences were aligned using method MUSCLE (Edgar 2004) with manual adjustments for visual improvement where necessary, in program MEGA version 6.0 (Tamura et al. 2007). Putative taxonomic affinities of sequence types (phylotypes) from BLAST searches of ITS sequences were inferred. The alignments for each studied genus were prepared including sequences of all relevant ex-type strains, from culture collection and/or published indexed journals sequences obtained from GenBank to ensure a more accurate identification.

Screening for vis-à-vis antagonistic activity of Moniliophthora spp.-endophytes

The assay for the *in vitro* antagonistic activity of fungal endophytes against the pathogens *Moniliophthora* spp. was carried out on Potato Dextrose Agar (PDA) on petri dishes by the dual culture method. The dual culture assay was realized for 126 endophytic fungi against the isolates of *M. roreri* (MR) and *M. perniciosa* (MP). The mycelial plugs (2 mm) of pathogens and endophytes were placed on the same petri dish five centimeters away from each other. Because of the slow growth of the fungus MP in culture, the isolates of the pathogen were plated seven days earlier than the endophytes. After inoculation the dishes were incubated at 26°C for ten days. During the trial period the distance of fungal growth (mm) from the point of inoculation to the other inoculation point was measured every single day after endophytes were placed into assay plates. These measurements were converted as percent of growth inhibition (PGI) of MR&MP respect to the growth of the pathogens alone (control). PGI values under cero was considered as one isolate stimulate the other, and over, that was inhibited. After the colonies of E-P contacts each other, four types of interactions were rated: i) endophyte (FEF) lose the pathogen, when a colony of the FEF was overgrown by MR or MP; ii) FEF win the pathogen, the opposite; iii) antibiosis: growth-inhibition determined by the presence of an inhibition halo <1cm (no contact); and iv) neutral, meeting of the mycelia without any overgrowth thus the interaction was supposed to be neutral. The experiment was performed with two replications of each treatment for ten days. Petri dishes inoculated with only one fungus (endophytes or pathogens) served as control for fungal growth rates.

The data of the measured distances was expressed as percent growth inhibition (PGI) of the pathogens *Moniliophthora* spp. The PGI was calculated for day two to ten of the incubation time: PGI [%] =(R−R1/R) ∗100, R represents the radius of the pathogen growth on the measuring days on the control dishes, R1 represents the distance of pathogen growth from the point of inoculation to its colony margin on the dual cultures. The PGI was calculated for every endophytic isolate and their interaction with the two pathogens MR and MP in the dual culture assay. The values of PGI show, whether the endophytes could repress the growth of the pathogen and how strong the growth inhibition was. Positive and high values of PGI suggest a strong antagonism against the pathogen, negative values indicate a stimulatory effect on the pathogen growth by the endophytic fungi. The day of each E-P colonies contact each other was also measured.

Screening for pathogenicity of endophytes on detached leaves and fruits

The healthy leaves and ripe pods used in this experiment were freshly cut and harvested shortly before starting the screening tests. The source of leaves and fruits were plantations of "cacao Nacional". The leaves and pods were washed with tap water and surface disinfected

with 95% ethanol. On each leaf four inoculation sites, two on each side of the midrib, were labelled with a permanent marker and wounded with a needle (Fig. 4). The cultured agar plugs, used for the inoculation, were punched around the actively growing zone of the fungus with a sterile cork borer (5 mm Ø) and were placed immediately after wounding on the top of each wounded inoculation site, with the mycelia touching the wound. On the right side of the midrib the two wounds were inoculated with the same endophyte strain and the two inoculation sites on the left side of the midrib were inoculated with PDA plugs without fungi and served as control. The leaf inoculation was performed with two replications of each treatment. The cacao pods were inoculated with three endophyte strains and one control, each with 3 repeats. Before inoculation, a cork borer (5 mm Ø) was used to create three wounds of 0.5 cm depth for every endophyte strain (Fig. 4). The inoculation´s plugs were punched around the actively growing zone of the colonies with the sterile cork borer in a laminar flow cabinet. The cultured agar plugs were placed inside the punched holes, with the fungus facing the inside of the fruit, and the holes were closed again with the saved fruit cover and fixed with sticky tape. The inoculated leaves and pods were placed inside plastic bags containing wet paper towels (Tahi et al. 2007). The leaves were incubated for four days and the pods for eight days in darkness at 26°C air temperature. At the end of the incubation time the appearance of chlorosis and necrosis surrounding the inoculation sites was recorded.

Statistical analysis.

Diversity. From the identified taxa, diversity was determined (expressed as average species richness) by farms sampled by county (Tab. 1). The taxa found only once (singleton) and those that appeared two or more times were differentiated. A hierarchical cluster analysis (average clustering) of the five localities based on the Bray-Curtis similarity matrix was made. The frequency of taxa by farms were transformed (√√, CLUSTER-PRIMER v6) in order to downweight the contributions of quantitatively dominant species to the similarities calculated between samples (Clarke and Gorley 2006). The Bray-Curtis similarity coefficient considers the abundance of each species in relation to the total sample. *Dual culture assays*. The PGI data (2nd to 10th d) were grouped manually and arbitrarily according to the average PGI of each FEF isolate vs. MR & MP on each trial day. Against MR the response of the FEF isolates was divided into four categories: PGI -68 to -30, PGI -29 to 0, PGI 1-45 and PGI 46 to 72. On the other hand, the response to MP was divided in two: PGI -22 to 0 and PGI 0 to 18. The relationships between the quantitative (PGI & day of colony contact) and qualitative variables (outcome of colony interactions & pathogenicity) were analyzed using contingency tables with the Pearson Chi-square statistic ($p \leq 0.05$), after categorization of the quantitative variables (Infostat v2016). Additional details of the analyzes are indicated as the results are displayed.

RESULTS

Taxa identified

Of the five sites evaluated, a total of 126 isolates were isolated, among which at least 35 taxa were identified: 29 to the species level, five to genus and one to family. There was a predominance of the Ascomycota division with 29 and only six Basidiomycota taxa (Tab.3). At least 24 taxa were found only once (singleton) in the three locations (Fig. 2, Tab.3). Community composition was dominated in 64% by five genus: Fusarium (*F. decemcellulare*),

Colletotrichum (*C. gloeosporioides* s.l.), Xylaria (*Xylaria spp.*), Pestalotiopsis (*P. microspora*) and Nigrospora (*N. sphaerica*), although only some few species by genus (Tab.4). 54.3% of the 35 taxa identified up to species or genus were found only once at all sampling sites. Figure 2 summarize the diversity in the cultivable fungal community on leaf from five farms.

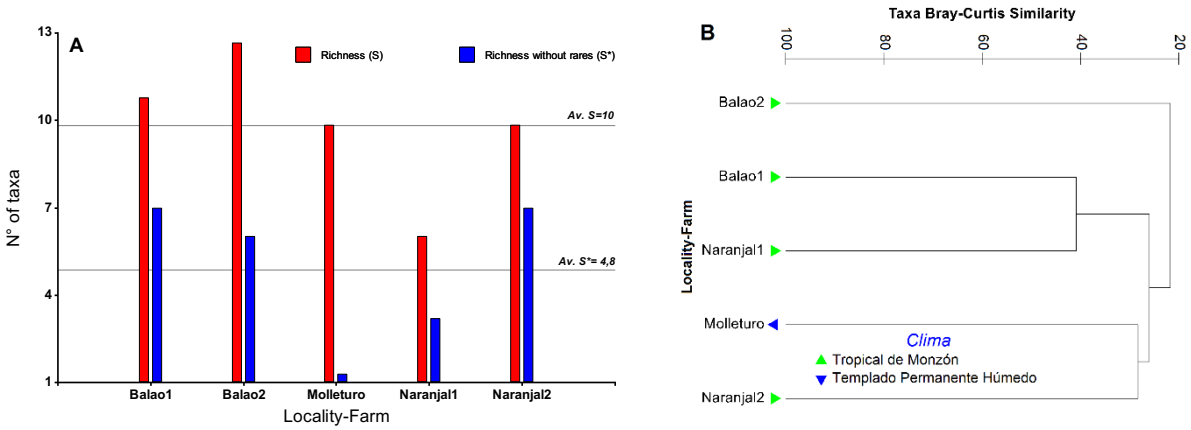

Figure 2. Summary of foliar endophytic fungal diversity identified by ITS from cacao Nacional plantations in the Coast of Ecuador in 2014. Twenty-nine taxa (118 isolates) belonging to Ascomycota and six taxa (8 isolates) to Basidiomycota. A) Richness of taxa by farms. Rare taxa (singleton) are included in red and those found 2 or more times in blue. The lines indicate the averages of richness for each case (DIVERS-PRIMER v6. B) Dendrogram based on the Bray-Curtis similarity index for the locality (hierarchical cluster, UPGMA). The original data of taxa frequencies were transformed √√ (CLUSTER-PRIMER v6).

The distribution of species richness was not homogeneous among farms (Fig. 2A), either when considering all species, or only singletons. The previous graph indicates that the diversity was lower in Molleturo and in Naranjal1. The cluster analysis (Fig. 2B) shows that the locality samples cannot be associated with any defined general pattern. The maximum similarity between the localities according to the composition of species was ca. 40% between Balao1 and Naranjal1 (Fig. 2B). This tendency is corroborated in the average% of dissimilarity between Balao, Molleturo and Naranjal with averages between 72 and 76% (SIMPER-PRIMER v6). As an approximation to the fungal diversity, the species accumulation curve was obtained. The resulting curve does not reach the *plateau* (data not shown) and therefore new species are expected to appear as the number of new isolates from these locations increases. Thus, the inventory of species obtained in these plantations is incomplete.

Colony interaction of foliar endophytic fungi vs. *Moniliophthora spp.* **on a vis-à-vis assays**

The FEF reactions against Moniliophthora spp. was differentiated according to the growth of both, the endophyte and the pathogen. Near half of the isolates inhibited the growth of MR with an average of the PGI > 0 (Fig.3A, Groups PGI 1-45 and PGI 46-72), although only seven isolates did it with a promising value (PGI > 46). On the other hand, 66 isolates stimulates MR, showing average values in a range from -67.38>PGI<-1.38 (data not shown). The group of isolates that most inhibited MR´s growth (PGI 46-72), and the one that least did (PGI -68 to -30), made contact on the eighth day of interaction (Fig. 3A). Group PGI 46-72

was composed of isolates that never showed a negative PGI value between the 2nd and 8th day (see the standard deviation Fig. 3A); but within the other three, several isolates showed alternatively positive and negative PGI through the confrontation period. The variation of the FEF´s response to M. perniciosa was less marked with only two groups, which on average inhibited and stimulated the growth of MP during the trial period (Fig. 3B). In this case, the group PGI -22 to 0 made contact on the ninth day of interaction.

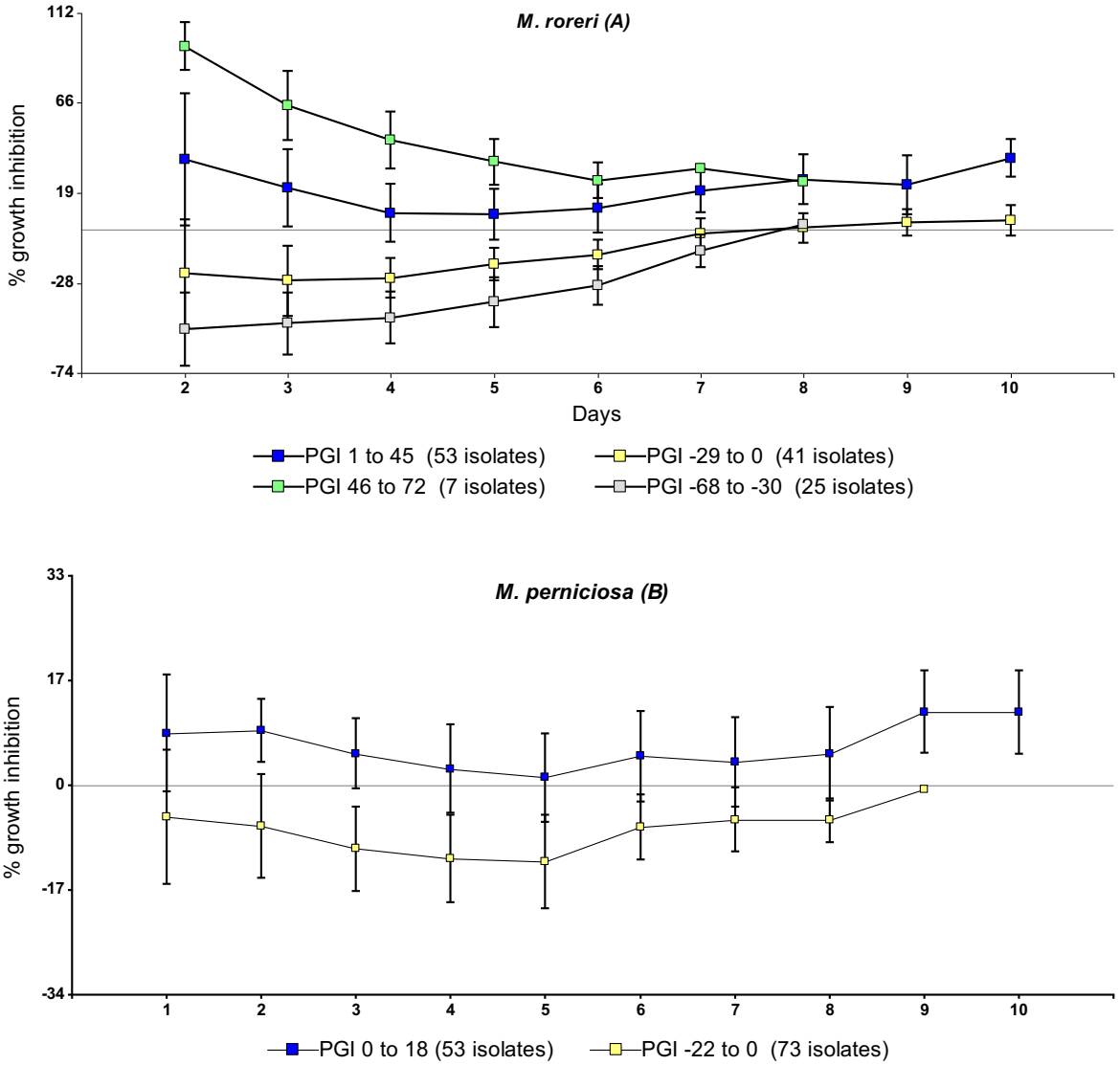

Figure 3. Dynamic of the growth stimulation/inhibition of *M. roreri* (A) and *M. perniciosa* (B) by foliar endophytic fungi groups on a 10 days dual culture assay (pathogen-endophyte). Groups were formed by the average PGI of each isolate. MR was seeded at the same day of endophytes and MP seven days before. Line indicate PGI=0, above it means inhibition of the growth of the pathogens respect to the pathogen alone (control without endophyte), under means stimulation of it. Bars indicate standard deviation.

By grouping the isolates not by the average of the PGI, but by the days in which they made contact, a relatively homogeneous distribution was obtained in the number of isolates by groups. Table 2 shows dynamics of the entire collections of FEF until the day each colony of the isolates contacted the pathogens. For example, on day 3 and day 8 of the confrontation with MR 30 isolates contacted at that times and never showed negative daily averages of PGI (Tab.2, groups D3 & D8), which means that they never stimulated the pathogen. In addition, the general PGI averages of D3 & D8 groups were low positive values of 12.5 and 10.79, respectively. However, most isolates stimulated (negative PGI) mycelial growth of MR at some point during the trial. In the response to MP only five isolates (Tab.2, group D10) showed daily positive averages of PGI, but a very low inhibitory capacity of the pathogen.

Table 2. Groups of endophytes and it´s percent of growth inhibition against *Moniliophthora spp*. by day of colony contact in a dual culture assay.

Contact day Groups	n	% of growth inhibition (PGI) at day 1 to 10										Average
		1	2	3	4	5	6	7	8	9	10	
						M. roreri**						
D3	16		12,49	12,51								12,5
D4	14		-2,39*	-11,31	-12,69							-8,79
D5	22		-10,6	-21,96	-22,21	-16,7						-17,87
D6	24		16,91	4,52	-5,78	-5,48	-3,05					1,42
D7	23		0	-13,76	-16,23	-10,25	-4,29	5,38				-6,52
D8	14		16,66	9,54	3,18	4,2	7,2	15,85	18,94			10,79
D9	6		0	-2,77	-14,8	-16,37	-10,82	0,3	4,48	7,53		-4,05
D10	7		-28,56	-23,81	-19,03	-12,24	-6,31	3,47	8,5	11,53	14,19	-5,81
											Total	-2,29
M. perniciosa**												
D3	10	1,64	-0,54	-3,61								-0,84
D4	13	1,14	1,46	-3,61	-6,19							-1,8
D5	43	3,77	1,63	-2,55	-5,31	-7,21						-1,93
D6	20	-4,92	-5,13	-8,31	-9,24	-9,31	-4,4					-6,89
D7	16	0	1,18	-2,11	-3,8	-4,46	-1,91	-1,07				-1,74
D8	16	-2,87	-3,21	-5,72	-7,34	-8,66	-4,24	-3,61	-3,08			-4,84
D9	3	-2,73	-0,9	-9,24	-7,24	-5,61	-0,56	1,04	2,41	5,52		-1,92
D10	5	4,26	7,03	5,54	3,48	2,57	8,47	8,75	8,99	10,46	11,66	7,12
											Total	-1,60

* Negative values indicate stimuli of pathogens *vis-a-vis*, positive inhibition respect to the pathogen alone (control without endophyte).
** *M. roreri* was seeded at the same day of endophytes and *M. perniciosa* seven days before.

On the sixth day of confrontation, at least 60% of the endophytes contacted the colonies of both pathogens. Only 10.3 and 6.3% of the isolates contacted at the end of the trial time (Tab.2, D9 & D10). The general averages of PGI for all endophytes against MR (-2.29) and MP (-1.60) were negative, indicating a trend towards the stimulation of these two pathogens (Tab.2). The values of PGI versus MP were, in general, lower than those of MR (Fig. 2).

Nectria pseudotrichia (Ec183), *Phomopsis sp*. (Ec073), *L. theobromae s.l.* (Ec027) and *Hypoxylon investiens* highlighted against MR (56.93>PGI<71.62). Against MP *L. theobromae* (Ec027), Fusarium equiseti (Ec025) and *Phomopsis sp*. (Ec013) were the most remarkable (15.38>PGI<18.44). Regarding the inhibition of the growth of MR & MP, the isolate Ec027 stood out, but with a remarkable difference in the capacity of inhibition of the growth of both pathogens. Conversely, the species that most stimulated the mycelial growth MR based on the general average of PGI were *Pestalotiopsis microspora* (2 isolates), and *F. decemcellulare*, *C. gloeosporioides* s.l., *Corynespora cassiicola*, *Bionectria ochroleuca* and *Eutypa sp*. with an isolate each (ranging from -55.70>PGI<-67.38). Against MP, *F. decemcellulare* (2 isolates), *Xylaria spp*. (2 isolates) and, one isolate each of *Pestalotiopsis microspora*, *C. gloeosporioides* s.l., *Nigrospora sphaerica* and *Corynespora cassiicola* (ranging from -18.66>PGI<-21.70) were highlighted. None of the most stimulating isolates against both pathogens matched, but the species do. Within *F. decemcellulare, C. gloeosporioides* s.l. and *Corynespora cassiicola* there were powerful growth stimulant isolates to MR & MP.

Once the E-P colonies made contact, the result of this interaction was recorded in terms of overgrowths of one colony over another. Between 67-80% of the interactions were neutral since they did not show any overgrowth (Tab.3). There were two strains of *Pestalotiopsis microspora* (Ec197 and Ec177) that did not contact MP, and were registered as antibiosis with an inhibition halo> 1 cm (Tab.3). At the contact zone, 11 strains of the FEF grew above MR, and 20 did so against MP.

Table 3. Frequency of colonies interaction outcomes of *Moniliophthora spp.*-foliar endophytic isolates of after colony contact in dual culture assay (rated at day 10).

Interaction	*M. roreri*		*M. perniciosa*	
	Absolute	Relative	Absolute	Relative
FEF lose Pathogen*	30	0,24	3	0,02
FEF win Pathogen*	11	0,09	20	0,16
Antibiosis (halo >1cm)**	-	-	2	0,02
Neutral***	85	0,67	101	0,80

*Overgrowth of one colony over the other, ** No contact, and *** No overgrowth

Pathogenic activity on detached fruits and leaf

Of the 126 isolates, 26 were pathogenic in fruits, inducing necrosis around the inoculation points and only six were considered pathogenic in leaves, inducing necrosis and / or chlorosis. Table 4 shows the detail of the specimens according to the induction of symptoms described elsewhere (Fig. 4).

Table 4. Outcome of inoculation of 35 taxa and 126 isolates of leaf endophytic fungi on detached leaflets and fruits of *T. cacao*.

Division	Taxa identified	Pathogenic on			Not Pathogenic	Total
		Leaf & Fruit	Fruit	Leaf		
Ascomycota	*Phomopsis spp.*	1	1	1	4	7
	Colletotrichum gloeosporioides s.l.		11		9	20
	Fusarium decemcellulare		3		8	11
	Nectria pseudotrichia		2		2	4
	Pestalotiopsis microspora		1	1	9	11
	Fusarium equiseti		1		7	8
	Fusarium solani		1		2	3
	Didymosphaeria futilis		1			1
	Phoma sp.		1			1
	Xylaria venosula		1			1
	Nigrospora sphaerica			1	5	6
	Xylariaceae spp.			1	3	4
	Xylaria spp.				11	11
	Lasiodiplodia theobromae s.l.				9	9
	Hypoxylon investiens				3	3
	Nigrospora oryzae				3	3
	Corynespora cassiicola				2	2
	Xylaria berteri				2	2
	Fusarium graminearum				1	1
	Bionectria ochroleuca				1	1
	Colletotrichum boninense				1	1
	Curvularia verruculosa				1	1
	Daldinia eschscholtsii				1	1
	Diaporthe phaseolorum				1	1
	Endomelanconiopsis endophytica				1	1
	Eutypa sp.				1	1
	Fusarium thapsinum				1	1
	Trichothecium roseum				1	1
	Xylaria feejeensis				1	1
Basidiomycota	*Psathyrella sp.*	1				1
	Ceriporia lacerata		1		2	3
	Phanerochaete chrysosporium				1	1
	Pseudolagarobasidium belizense				1	1
	Rigidoporus sp.				1	1
	Coriolopsis polyzona				1	1
Total isolates		2	24	4	96	126

* Outcome of inoculation: chlorosis and/or necrosis in leaflet, and only necrosis on fruit (See Fig. 4).

There was generally no coincidence in the capacity of an isolate to induce symptoms in leaves and fruits, only two were pathogenic in both organs (Ec013, *Phomopsis sp*. and Ec076, *Psathyrella sp*.). The pathogenic isolate Ec013 was the one that most inhibited the growth of MP. To measure of association between the organ inoculated (leaf-pod) and the outcome of inoculation (pathogenic-not pathogenic) we calculated the odd ratio from a 2x2 frequency table. Chi-squared test showed no pathogenic preference of FEF on fruit for our set of data (data not shown). However, it is striking that most of the FEFs were pathogenic in fruits, and

not in leaves, which suggests a tendency not to necrotize the organ where they live (or they were isolated).

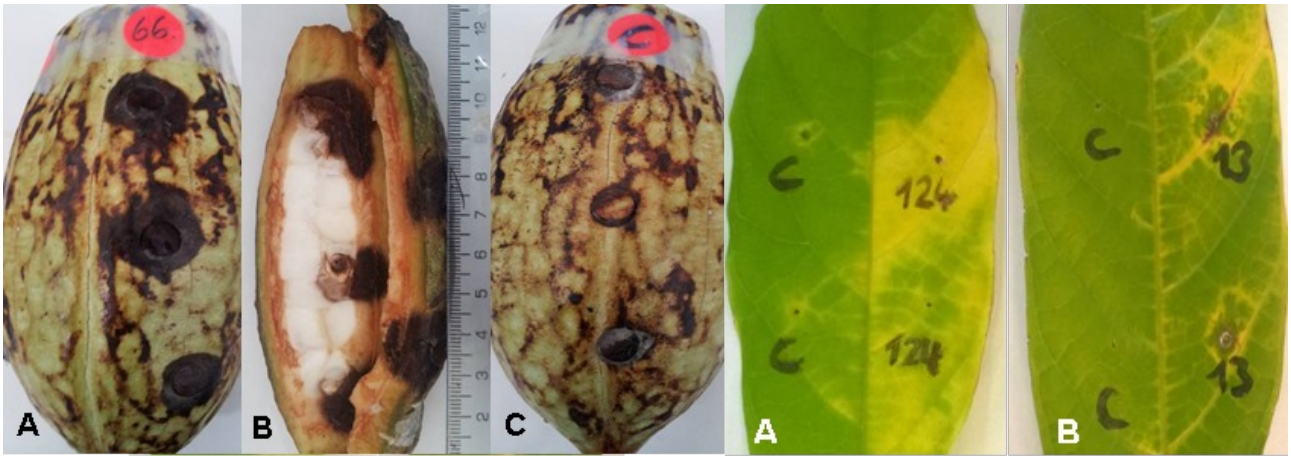

Figure 4. Symptoms of inoculations on detached fruits and leaf of *T. cacao* by foliar endophytic fungi of the same host. A-B, typical necrosis around the inoculation points of a isolated considered pathogenic on fruit. C, inoculation points with agar plug only (control) without necrosis. Chlorosis solely (D) and necrosis and chlorosis induced by pathogenic isolates (E) on right side of the leaf. Controls on black points located on the left side. Entrance of the endophyte were facilitated by wound (fruit) or a puncture (leaf) under de mycelial plug. Fruits showed only necrosis, and leaves both, chlorosis and/or necrosis.

Relationships among variables. The variables: PGI, day of contact, outcome of the colony interaction EP and pathogenicity (as a binary trait pathogenic/ non-pathogenic) of the 126 endophytic isolates against MR were all independent, according to the contingency analysis (Pearson Chi-square, $p \leq 0.05$). However, the variables day of contact and pathogenicity against MP were related (data not shown).

In the sense of selecting isolates with desirable characteristics for a BCA, it was observed that few isolates showed an average general PGI> 50 on both pathogens in the dual culture, that the contact time of the colonies was ≤ 6d, that it did not stimulate the pathogen in any time and that does not induce symptoms in leaves or fruits. Against MR four endophytes were able to satisfy most of the previous criteria (Tab.5); however, isolates Ec174 (*Hypoxylon investiens*) and Ec073 (*Phomopsis sp*.) stimulate MP growth (Average PGI -2.88 and -8.31, respectively). No promissory isolates against MP were found, since the maximum PGI within the strains was only 18. However, the two strains of *Pestalotiopsis microspora* that formed a halo of inhibition against MP and that could be considered promising (Rubini et al. 2005), stimulate this pathogen at the same time (Tab.3). It was not possible to elucidate if the formation of halo was because the slow growth of the colonies or the simultaneous excretion of some inhibitory / stimulating metabolite, considering that the pathogen grew faster. A strain that met all the criteria was not found.

Table 5. Summary of the characteristics of best endophytic isolates with antipathogen effects vis-à-vis with *M. roreri* (MR) and *M. perniciosa* (MP). All isolates were not pathogenic on fruit & leaflet.

Taxa	Isolate	Contact day		Stimulate the growth (PGI)*		Outcome of colonies interaction	
		MR	MP	MR	MP	MR	MP
Hypoxylon investiens	Ec174	6	6	No (71)	Yes (-3)	Neutral	Neutral
Lasiodiplodia theobromae	Ec027	3	4	No (58)	No (15)	Neutral	Neutral
Phomopsis sp.	Ec073	6	5	No (57)	Yes (-8)	Endophyte lose	Neutral
Nectria pseudotrichia	Ec183	5	4	No (56)	No (4)	Neutral	Neutral
Pestalotiopsis microspora	Ec177	6	6**	No (47)	Yes (-2)	Endophyte lose	Halo
Pestalotiopsis microspora	Ec197	7	3**	No (38)	Yes (-17)	Neutral	Halo

* PGI, Average % growth inhibition until contact. Negative PGI means stimuli, and positive vice versa.
**Indicates the day that each isolate stopped their growth, without colony contact.

DISCUSSION

Diversity of foliar endophytic fungi

All isolates purified from cacao leaves produce no spores on potato-dextrose-agar nor malt-extract-agar. ITS allowed to identify 35 taxa within 126 isolates (Tab. 4). ITS sequences are insiffient to differentiate among *C. gloeosporioides* (Weir BS, Johnston, and Damm 2012) or *L. theobromae* species complexs thus these species are treated as *sesu latu* (s.l.). The diversity of foliar endophytic fungi is high in cacao, characterized by the presence of a large number of singletons, and a smaller number of species that appear at least twice (A. E. Arnold, Maynard, and Gilbert 2001). In our case, 68% of the taxa were found only once. The Ascomycetes dominated the diversity of taxa, and to a lesser extent by Basidiomycota (6.3%). This has been reported for foliage (A. E. Arnold, Maynard, and Gilbert 2001) and cacao branches (Rubini et al. 2005).

In general, basidiomycetous endophytes are found more often in woody tissues than in foliage (A. E. Arnold 2007). The FEF community works on the cocoa leaf as an assemblage of species whose composition changes over time (≈ 8 wk) (Herre et al. 2007), but this endophytic mycobiota "wait" for a long time as endophyte and then complete their life cycle as saprotroph (Herre et al. 2007). A diversity of fungal spores infects young leaves of cacao that are transmitted horizontally (Herre et al., 2007). The forest canopy enhances the rate of initial endophyte colonization of seedling (A. E. Arnold and Herre 2003), but also from the leaf litter as primary source of inoculum of endophyte-free seedling (Herre et al. 2007). During rains, the shade trees and the foliar cacao canopy produces rivulets of water over the branches and trunk, while releasing rain droplets from the underside of the canopy that rebound at least 0.5m up the tree trunk (Fulton 1989). In the cacao leaves a succession of fungal communities occurs for about eight weeks (Herre et al., 2007), during this time high density is highlighted in terms of biomass of endophytic fungi colonizing the leaves and a high diversity in terms of the number of morphospecies present in them. This has been reported in other tropical species (A. E. Arnold, Maynard, and Gilbert 2001). Regardless of whether the diversity in the composition of FEF species is high, such as Panama (A. E. Arnold et al. 2003), or low in places where culture has been introduced more recently (Schmidt 2010), the pattern of tissue

colonization is similar in terms of that a few species predominate, and many rare species coexist with them.

Individual plants may harbor hundreds of species, and plant species across their native ranges may be inhabited by thousands of species (A. E. Arnold 2007). Novel taxa of endophytic fungi are frequently found in the case of cacao and some closely related species (Evans, Holmes, and Thomas 2003; Crozier et al. 2006; Sosa et al. 2016; Samuels et al. 2006). Generally, endophytic fungi from T. cacao are included on Class 3 of endophytes (Rodriguez et al. 2009). Class 3 endophytes are distinguished based on their occurrence primarily or exclusively in above-ground tissues; horizontal transmission; the formation of highly localized infections; the potential to confer benefits or costs on hosts that are not necessarily habitat-specific; and extremely high in planta biodiversity. In considering the ecology of Class 3 endophytes, Rodríguez et al (2009) pointed out it is important to think of plant–endophyte interactions in the context of ecological complexity: plants inhabited by highly diverse endophytes also host rhizosphere and phyllosphere fungi and bacteria and they are consumed by herbivores or parasite by pathogens. From an ecological perspective, the individual species does not seem to be determinant in the result of the plant-endophyte-pathogen interaction. The life-history strategies of the endophytic fungi vary depending on developmental stage of host and fungus, virulence of the fungus and the host defense response, but also on environmental factors which influence the phenotypic plasticity of both partners (Schulz and Boyle 2005).

The taxonomic (genus/ species) and functional (stimuli/ pathogenicity) diversity found in our work reflects a particular moment of the plant-endophyte-environment interaction (Schulz and Boyle 2005). The endophytes within the leaves of T. cacao type National harbor isolates with different pathogenicity (Tab.4), growth stimulation capacity (Fig. 3), and secondary metabolites producers (halos of *P. microspora*, Tab.5). These flexibilities were also within individual species. Within *P. microspora* were pathogenic and not pathogenic isolates, some isolates overgrew or stimulate the pathogen colonies, or produced halos. Within *F. decemcellulare* (no forming halos isolates) and *L. theobromae* (no pathogenic isolates) behave the same. Moreover, the same isolate could be pathogenic on fruit but causing no symptoms in leaf, and vice versa. These phenotypic plasticity is named in term of endophytic continuum (Schulz and Boyle 2005). Most plant species harbor a diverse assemblage of fungal endophytes, but it is notable that abiotic factors, the interaction between host genotype and environment, manipulation of host phenotype via crop breeding programs, and microbe–microbe interactions mediated by the production of secondary compounds can significantly impact endophyte communities (Saunders, Glenn, and Kohn 2010).

The most abundant genera obtained in this work (*Fusarium*, *Colletotrichum*, *Xylaria* and *Pestalotiopsis*) were cited in previous studies as endophytes on cacao leaves in Panama (A. E. Arnold et al. 2003) and from branch cortex and leaves in Bahia (Rubini et al. 2005; Hanada et al. 2010). However, more than 50% of fungi reported here had not yet been identified as cacao epi/endophytes or pathogens. Some have been identified as endophytes in other plants. It was somewhat surprising that *Trichoderma spp*. was not found on leaf, as it might be expected based on previous surveys (Hanada et al. 2010; Schmidt 2010; Villamizar-Gallardo et al. 2017; Rubini et al. 2005; Bailey, Strem, and Wood 2009).

Endophytes as stimulant of Moniliophthora spp.

Endophytes isolated from cocoa National leaves were screened for their ability to stimulate / inhibits the growth of *M. roreri* and *M. perniciosa*. Almost 90% of the isolates displayed some

stimuli in the vis-à-vis plate assay, both in their general average of PGI (Fig. 3) and in considering the individual average of each isolate between the 2nd to 10th day of confrontation (Tab.2). Since a high proportion of the tested endophytes stimulated somehow the growth of *Moniliophthora spp.*, it is supposed that the colonization by endophytic fungi can not only be beneficial for the host plants, but also for other (pathogenic) fungi. In the present study endophytic isolates of almost every taxonomic entity promoted the mycelial growth of the pathogens for a certain time. Facilitation can occur between fungal species, although it is rarely documented (Rodríguez 2011; Saunders, Glenn, and Kohn 2010). The water-soluble crude metabolites of two isolates of *Clonostachis spp*. and one of *Colletotrichum sp*. stimulated *M. perniciosa* by 5.47% compared to control (Rodríguez 2011). Metabolites seems to play role in some of our isolates. Of 17 of our isolates 76.5% showed a coincidence between the effects of crude extracts of their metabolites (inhibition / stimulation of mycelial growth) with the effects of vis-a-vis interaction with MR, and 58.9% in the case of MP (unpublished results). Sanders et al (2010) found that on synthetic medium, *F. verticillioides* was able to detoxify 2-benzoxazolinone (BOA), converts it to a less toxic product and increase the growth rate of commonly co-occurring maize endophyte species. However, in field-grown maize *F. verticillioides* interacted competitively with community members by preventing species with lower BOA tolerance from colonizing root tissue.

Fungal endophytes are known for develop several mechanism of inhibition of other fungal (pathogen) inhabiting within plant (Gao, Dai, and Liu 2010; Herre et al. 2007), including direct effect as antibiosis, competition for the substrate & mycoparasitism (Mejía et al. 2008; A. E. Arnold et al. 2003); indirect effects via defensive pathway (Mejía et al. 2014) and ecological effects (Gao, Dai, and Liu 2010; Mejía et al. 2008; A. E. Arnold et al. 2003). In relation to the host plant the metabolites produced by fungi are extremely diverse in structure and function. Of particular relevance are the compounds with proved hormonal role in plants such auxins, CKs and GAs, which are produced in significant amount and whose structure vary depending upon the fungal strain (Angel, Contreras-Cornejo, and Lopez 2015). Maybe, in some cases, the produced growth hormones or fungal secondary metabolites do not only enhance plant growth but also fungal growth.

Foliar endophytic fungi as T. cacao pathogen

In this study we have found isolates that have not been previously reported in cacao, or at least are not indexed in databases such as Fungal Databases, U.S. National Fungus Collections, ARS, USDA or Fungos Relatados em Plants no Brasil (EMBRAPA). The lack of records in these databases does not mean that it has not been reported, for example Psathyrella is not reported for cacao in those databases (Mendes and Urben 2017; Farr and Rossman 2017), however *Psathyrella coprinocepes* was recorded on *T. cacao* in Pichilingue, Ecuador (Hedger 1985). Special attention has been paid to locate the reports of pathogenicity of the taxa that induced symptoms of fruits and / or leaves in this work. Only two isolates showed the ability to induce symptoms in fruits and leaves, *Phomopsis sp*. and *Psathyrella sp*. (Tab.4), the first has been reported as a pathogen and the second as a saprophyte. At least two species of *Phomopsis*, *P. folliculicola* and *P. theobromae*, have been isolated and described from *T. cacao*. One of these species, the former, causes cacao pod rot, whereas the other, inhabits cacao leaves (Crous et al. 2015). Phomopsis sp. causes defoliation in *Theobroma grandiflorum* in Brazil (Mendes and Urben 2017) and grew from both diseased and healthy bark of cacao and were assumed to be common inhabitants of the outer layers of bark in Papua New Guinea (Keanae, Flentje, and Lamb 1972). A species of *Psathyrella*, *P. coprinocepes*, is

a lignicolous species of Basidiomycete isolated from freshly dead boles and branches of *T. cacao* (Hedger 1985). Both *Phomopsis* and *Psathyrella* known as stem pathogens or decomposers of dead plant tissue, showed in this work the ability to induce symptoms in leaves and fruits.

Colletotrichum gloeosporioides sensu lato is a well-known pathogen on multiple generated in multiple families of host plants. *T. cacao* harbor *C. gloeosporioides* s.l. as pathogen (Farr and Rossman 2017) and also as an endophyte (Rojas et al. 2010; Rubini et al. 2005). More than 50% of the isolates of this taxon were pathogenic in fruits, and none of the 20 were in leaves (Tab.3). *C. gloeosporioides s.l*. is a cryptic species within which 22 species have been defined (Weir BS, Johnston, and Damm 2012; Nam et al. 2013) at least three species have been associated with *T. cacao* (Rojas et al. 2010). *C. theobromicola* isolated from fruits and leaves with anthracnose; *C. tropicale* considered endophytic of *T. cacao* and other tropical species, and in turn as a pathogen in *Annona muricata* (Rojas et al. 2010) and *Manihot spp*. (Oliveira, Bragança, and Silva 2016); and *C. ignotum* species is referred to as endophyte in dicotyledonous plants, including *T. cacao* (Rojas et al. 2010). Later *C. ignotum* has been considered as a synonym of *C. fruticola* (Weir BS, Johnston, and Damm 2012; Nam et al. 2013).

Fusarium accounted for 22 isolates distributed in three species (Tab. 3) but dominated by *F. decemcellulare* (teleomorph *Albonectria rigidiuscula*). *F. decemcellulare* is a common fungi in cacao and recorded as a wound parasite of weakened trees (Farr and Rossman 2017), causes die-back and pod-spotting and also associated with cankers (Adu-acheampong 2009) and with gall disease on branches and trunks (Sosa et al. 2016). *F. equiseti* is rare on cacao, nevertheless is associated with fan galls on the field in Venezuela, and induce galls in artificial inoculation (Sosa et al. 2016). *F. solani* in cacao in Malaysia, Tanzania (Farr and Rossman 2017), Venezuela (Sosa et al. 2016) and in Ecuador (Zhang, Geiser, and Smart 2007) have been reported. Most of our Fusarium´s isolates were not pathogenic on fruit neither on leaf (Tab. 3).

Nectria pseudotrichia is a one of the common tropical fungi in the genus *Nectria* and is well known as a saprobe in tropical and warm temperate regions (Hirooka et al. 2012). *N. pseudotrichia* inhabit *T. cacao* in Ghana, Malaysia and Papua New Guinea (Farr and Rossman 2017; Hirooka et al. 2012) and was isolated from on woody substrate in Ecuador (Hirooka et al. 2012). The two of our isolates were pathogenic on fruits. *Pestalotiopsis microspora* was isolated as endophytic from cacao branches in Brazil (Rubini et al. 2005), but black spot, lesions and defoliation on fruit, leaves, twigs on several host families have been reported (Farr and Rossman 2017). In Ecuador was first reported having biodegradation of polyester polyurethane feature (Russell et al. 2011). Previously, *P. microspora* was reported as a producing taxol fungi, an anticancer drug (Strobel et al. 1996). Besides being a species that degrades the plastic and produces beneficial toxins, it can be pathogenic in cacao. Most isolates tested here were not pathogenic, but one in fruit and one in leaf (Tab. 3).

Single isolates of *Didymosphaeria futilis*, *Phoma sp.* and *Nigrospora sphaerica* were pathogenic too. *D. futilis* is recorded as cosmopolitan, inhabit on leaves and stems and can induce blight, canker and leaf spot on multiple species in multiple families, but not in cacao (Farr and Rossman 2017; Mendes and Urben 2017). Healthy fruits of *Citrus sinensis* harbor *D. futilis* in Ecuador (Moya-Maldonado 2016). *Phoma sp.* was isolated as endophyte in *T. cacao* y *T. grandiflorum* in Brazil (Hanada et al. 2010), and with *T. cacao* in Malaysia

Peninsula and Myanmar (Farr and Rossman 2017). *Nigrospora sphaerica* (synonym *N. oryzae* in Index Fungorum) was reported in Malaysia (Farr and Rossman 2017), and as human pathogen also (Fan et al. 2015).

Specimens from the Xylariaceae stand out, 17 of the 19 isolates were nonpathogenic, which coincides with the general reports for Xylaria spp. as endophytes of *T. cacao* in Ecuador, Mexico and Camerom (Crozier et al. 2006). As an exception, an isolate of Xylaria venosula and another of *Xylariaceae sp*. showed ability to induce symptoms in fruits and leaves, respectively. There are few reports of Xylaria as a pathogen in vegetative parts of cacao (Benjamin and Slot, n.d.) or in the seed (Mendes and Urben 2017). In addition, two other species have been registered associated with cacao in Ecuador *X. allantoidea* (JX476962) and *X. levis* (JX476951), as registered in the GenBank, although the origin of the strains is not specified. In general, specimens identified as Xylareaceae spp. there are 10 reports from Ecuador in GenBank, from both *T. cacao and T. gileri*. *X. venosula*, shown in this study as pathogenic in fruits is not reported on cacao (Farr and Rossman 2017).

Two basidiomycetes isolates were pathogenic on our study, an isolate of *Psathyrella sp*. in leaves and fruits and one out of three isolates of *Ceriporia lacerata* in fruit. *P. coprinocepes* were reported as pioneer colonizer of freshly dead bark of *T. cacao* (Hedger 1985) and P. aquatica growth on submerged wood in rivers (Gareth-Jones, Hyde, and Pang 2014). *C. lacerata* has been reported as an endophyte in several species (Farr and Rossman 2017); is known as a decay fungi, and grows on agar media in association with Vascular Streak Dieback (Ceratobasidium theobromae) -infected cocoa plants (McMahon and Purwantara 2016).

It has been pointed out that several diseases, often regarded as minor pests, affects production of the crop in various cocoa-producing regions and countries. These diseases may not be globally important, but they may have very great local impact on production wherever they are found (Akrofi et al. 2016). As we showed with the results, FEF could be a reservoir of such "latent" pathogens as environmental change throught time. On the other hand, the ability to induce symptoms observed within 13 of the 35 taxa analyzed in this study (Table 3) points to the importance of distinguishing not only between species, but between strains of the same species. Distinguishing between pathogenic and nonpathogenic isolates only takes place in pests of quarantine interest taxonomically (Weir BS, Johnston, and Damm 2012) , as in the case of *Colletotrichum kahawae* on the coffee berry disease (Silva et al. 2012). Isolated from endophytic species with pathogenic capacity, but not yet reported causing diseases, do not merit in practice be identified in greater detail.

Biological control agents with fungal endophytes

Based on the sampling effort made in our study, 4 out of 126 FEF isolates inhibited *M. roreri* between 56-71%, but none inhibited *M. perniciosa* at that levels (Tab 5). In a similar bioassay 2 out of 723 isolates of endophytic fungi from wild banana showed potential activity as a reduction of the radial mycelium growth of *Colletotrichum musae* (Nuangmek, McKenzie, and Lumyong 2008). Two of our "promising" isolates against MR stimulate, at the same time, the other cacao pathogen. Endophytes own a quite high risk potential to develop plant diseases, and it is difficult to assess this potential correctly because in distinguishing endophytes from pathogens many endophytes are closely related to pathogens (Porras-Alfaro and Bayman 2011). In many cases endophytes are described as fungi that inhabit plant tissue without

causing apparent symptoms (Schulz and Boyle 2005; Zabalgogeazcoa 2008), being inactive in early stages of plant growth and harming their host under changed environmental conditions or at post-harvest stages (Carroll 1988; Schulz and Boyle 2005). In the present study 79% of FEF did not cause symptoms in cacao leaves or pods. But it is possible that they are pathogenic to cacao under different conditions, or to other plant species within of the agroecosystem of *T. cacao*. Especially, since some of them are well known plant pathogens such as *Colletotrichum spp.* and *L. theobromae* (Purdy, Schmidt, and Gramacho 2017). Moreover, endophytes from one part of the plant, e.g. leaves, can cause diseases at other plant parts, e.g. fruits. In the present study it could be observed in some cases that some foliar endophytes did not cause any symptoms in cacao leaves but harmed the fruits (Tab. 3). Accordingly, endophytic fungi can be both beneficial and/or pathogenic to their host plant depending on the situation (Schulz 2005).

The endophyte ensamblage is a frame of time in a movie of plant-endophyte-pathogen coevolution. The fungi detected at any one moment in asymptomatic plant tissue and arbitrarily termed 'endophytes' include fungi with different life-history strategies. The life-history strategies of the endophytic fungi vary depending on developmental stage (momentary status) of host and fungus, virulence of the fungus and the host defense response, but also on environmental factors which influence the phenotypic plasticity of both partners (Schulz and Boyle 2005). The hypothesis that some endophytes are not pathogens, but evolved from pathogens, is described elsewhere (Porras-Alfaro and Bayman 2011). Inconsistency between results of greenhouse and in vitro experiments also complicates efforts to develop field applications of endophytes as BCA (Porras-Alfaro and Bayman 2011; Mejía et al. 2008).

CONCLUSIONS

Some of the tested foliar endophytes showed *in vitro* antagonism against *Moniliophthora spp*. with varying efficiencies. Four isolates of four species showed great potential as biological control candidates of *M. roreri* in terms of percent of growth inhibition between 56-71%. The growth inhibition of *M. perniciosa* by the foliar endophytes tested was quite lower and the strongest antagonists only reached average PGI values up to 18% . A combination of two or more endophytic strains with desirable characteristics should be considered in the formulation of a biocontrol product.

Moreover, a high proportion of the tested foliar endophytes stimulated the growth of *Moniliophthora spp*., isolates of all investigated taxonomic units promote the growth of the pathogens. Nearly ¼ of the isolates showed potential as pathogen on *T. cacao*; in addition, some inoculated foliar endophytes were not pathogenic to cacao leaves but caused symptoms of necrosis in cacao pods. Therefore, a high risk to develop endophytes as BCAs from leaves should be seriously considered.

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REFERENCES

Adu-acheampong, Richard. 2009. “Pathogen Diversity and Host Resistance in Dieback Disease of Cocoa Caused by Fusarium Decemcellulare and Lasiodiplodia Theobromae.” Imperial Colleage, London. http://eprints.imperial.ac.uk/bitstream/10044/1/4670/1/Adu-Acheampong-RK-2009-PhD-Thesis.pdf.

Akrofi, Andrews Y., Ismael Amoako-Atta, Kofi Acheampong, Michael K. Assuah, and Rachel L. Melnick. 2016. “Fruit and Canopy Pathogens of Unknown Potential Risk.” In *Cacao Diseases: A History of Old Enemies and New Encounters*, 361–82. doi:10.1007/978-3-319-24789-2.

Angel, Hexon, Lourdes Macias-Rodriguez Contreras-Cornejo, and Jose Lopez. 2015. “Fungal Biomolecules in Plant Growth Promotion.” In *Fungal Biomolecules: Sources, Applications and Recent Developments*, edited by Vijai Kumar-Gupta, Robert L. Mach, and S. Sreenivasaprasad, 1er ed., 345–62. Wiley-Blackwell.

Arnold, A.Elizabeth. 2007. “Understanding the Diversity of Foliar Endophytic Fungi: Progress, Challenges, and Frontiers.” *Fungal Biology Reviews* 21: 51–66.

Arnold, A.Elizabeth, and Edward Allen Herre. 2003. “Canopy Cover and Leaf Age Affect Colonization by Tropical Fungal Endophytes: Ecological Pattern and Process in Theobroma Cacao (Malvaceae).” *Mycologia* 95 (3). Mycological Society of America: 388–98. doi:10.2307/3761880.

Arnold, A.Elizabeth, Zuleyka Maynard, G S Gilbert, P D Coley, and T A Kursar. 2000. “Are Tropical Fungal Endophytes Hyperdiverse?” *Ecology Letters* 3 (4): 267–74. http://www.blackwell-synergy.com/doi/abs/10.1046/j.1461-0248.2000.00159.x.

Arnold, A.Elizabeth, Zuleyka Maynard, and Gregory S Gilbert. 2001. “Fungal Endophytes in Dicotyledonous Neotropical Trees : Patterns of Abundance and Diversity.” *Mycological Research* 105 (12): 1502–7.

Arnold, A.Elizabeth, Luis C Mejía, D Kyllo, Enith I. Rojas, Zuleyka Maynard, Nancy Robbins, and E Allen Herre. 2003. “Fungal Endophytes Limit Pathogen Damage in a Tropical Tree.” *Proc.Nat.Acad.Sci.USA* 100 (26): 15649–54. http://www.pnas.org/cgi/content/full/100/26/15649.

Arnold, Elizabeth. 2005. “Diversity and Ecology of Fungal Endophytes in Tropical Forests.” *Current Trends in Mycological Research*, 49–68.

Bailey, Bryan a., Mary D. Strem, and Delilah Wood. 2009. “Trichoderma Species Form Endophytic Associations within Theobroma Cacao Trichomes.” *Mycological Research* 113 (12). Elsevier Ltd: 1365–76. doi:10.1016/j.mycres.2009.09.004.

Benjamin, C.R., and Alice Slot. n.d. “Fungi of Haiti.” *Sydowia* 23 (50): 125–63.

Boza, Edward J, Juan Carlos Motamayor, Freddy Amores, Sergio Cedeño-Amador, Cecile L Tondo, Donald S Livingstone, Raymond J Schnell, and Osman A Gutiérrez. 2014. “Genetic Characterization of the Cacao Cultivar CCN 51: Its Impact and Significance on Global Cacao Improvement and Production.” *Journal of the American Society for Horticultural Science* 139 (2): 219–29. http://journal.ashspublications.org/content/139/2/219.abstract.

Cannon, P F, U Damm, P R Johnston, and Weir BS. 2012. “Colletotrichum - Current Status and Future Directions.” *Studies in Mycology* 73: 181–213. doi:10.3114/sim0014.

Carroll, George. 1988. “Fungal Endophytes in Stems and Leaves : From Latent Pathogen to Mutualistic Symbiont.” *Ecology* 69 (1): 2–9. http://www.jstor.org/stable/1943154.

Ciferri, R. E., and E. Parodi. 1933. “Descrizione Del Che Causa la ‘MONILIASI’ DEL CACAO.” *Phytopathologische Zeitschrift* 6: 539–42.

Clarke, KR, and RN Gorley. 2006. *PRIMERv6: User Manual/Tutorial*. Plymouth: PRlMER-E Ltd.

Crous, P W, M J Wingfield, J J Le Roux, D M Richardson, D Strasberg, R G Shivas, P Alvarado, et al. 2015. “Fungal Planet Description Sheets: 371–399.” *Persoonia : Molecular Phylogeny and Evolution of Fungi* 35 (December). Naturalis Biodiversity Center & Centraalbureau voor Schimmelcultures: 264–327. doi:10.3767/003158515X690269.

Crozier, Jayne, M Catherine Aime, Harry C Evans, and Keith A Holmes. 2006. “Molecular Characterization of Fungal Endophytic Morphospecies Isolated from Stems and Pods of Theobroma Cacao.” *Plant Pathology* 55: 783–91.

Edgar, R C. 2004. “MUSCLE: A Multiple Sequence Alignment Method with Reduced Time and Space Complexity.” *BMC Bioinformatics*.

Evans, Harry C. 2016a. “Frosty Pod Rot (Moniliophthora Roreri).” In *Cacao Diseases: A History of Old Enemies and New Encounters*, 63–96. Springer Berlin Heidelberg. doi:10.1007/978-3-319-24789-2.

———. 2016b. “Witches’ Broom Disease (Moniliophthora Perniciosa): History and Biology.” In *Cacao Diseases: A History of Old Enemies and New Encounters*, 137–77. doi:10.1007/978-3-319-24789-2.

Evans, Harry C, Keith A Holmes, and Sarah E. Thomas. 2003. “Endophytes and Mycoparasites Associated with an Indigenous Forest Tree, Theobroma Gileri, in Ecuador and a Preliminary Assess- Ment of Their Potential as Biocontrol Agents of Cocoa Diseases.” *Mycological Progress* 2 (2): 149–60.

Fan, Yi-Ming, Wen-Ming Huang, Wen Li, and Guo-Xue Zhang. 2015. “Onychomycosis Caused by Nigrospora Sphaerica in an Immunocompetent Man Nigrospora.” *Archive Dermatology* 145 (5): 611–12.

Farr, D F, and Amy Y Rossman. 2017. “Fungal Databases. Systematic Mycology and Microbiology Laboratory, ARS, USDA.” *Systematic Mycology and Microbiology Laboratory, ARS, USDA*. http://nt.ars-grin.gov/fungaldatabases/.

Fowler, Robert L, and Gustavo H. López R. 1949. “The Cacao Industry of Ecuador.” *Foreign Agriculture Report* 34. Washington D.C.: Office of Foreign Agricultural relations. U.S. Department of Agriculture: 1–48. https://ia700403.us.archive.org/29/items/cacaoindustryofe34fowl/cacaoindustryofe34fowl.pdf.

Fulton, Robert H. 1989. “The Cacao Disease Trilogy: Black Pod , Monilia Pod Rot , and Witches â€™ -Broom.” *Plant Disease* 73 (7): 601–3.

Gao, Fu-kang, Chuan-chao Dai, and Xiao-zhen Liu. 2010. “Mechanisms of Fungal

Endophytes in Plant Protection against Pathogens." *African Journal of Microbiology Research* 4 (13): 1346–51.

Gareth-Jones, E. B., Kevin D. Hyde, and Ka-Lai Pang. 2014. "Freshwater Basidiomycota." In *Freshwater Fungi and Fungal-Like Organisms.* Göttingen, Germany: Walter de Gruyter.

Hanada, Rogério Eiji, Alan William V Pomella, Heron Salazar Costa, José Luiz Bezerra, Leandro L. Loguercio, and José O. Pereira. 2010. "Endophytic Fungal Diversity in Theobroma Cacao (Cacao) and T. Grandiflorum (Cupuacu) Trees and Their Potential for Growth Promotion and Biocontrol of Black-Pod Disease." *Fungal Biology* 114 (11–12): 901–10. doi:10.1016/j.funbio.2010.08.006.

Hardy, Frederick. 1961. *Manual de Cacao*. Turrialba, Costa Rica: Instituto Interamericano de Ciencias Agrícolas. http://orton.catie.ac.cr/REPDOC/A8425E/A8425E.PDF.

Hedger, J. N. 1985. "Tropical Agarics: Resource Relations and Fruiting Periodicity." In *Developmental Biology of Higher Fungi. Symposium of the British Mycological Society*, edited by D Moore, LA Casselton, DA Wood, and JC Frankland, 41–86. Cambridge: Cambridge University Press.

Herre, EDWARD ALLEN, Luis C Mejía, Damond A. Kyllo, Enith I. Rojas, Zuleyka Maynard, Andre Butler, and Sunshine A. Van Baeel. 2007. "Ecological Implications of Anti-Pathogen Effects of Tropical Fungal Endophytes and Mycorrhizae." *Ecology* 88 (3): 550–58.

Hirooka, Y, A Y Rossman, G J Samuels, C Lechat, and P Chaverri. 2012. "A Monograph of Allantonectria, Nectria, and Pleonectria (Nectriaceae, Hypocreales, Ascomycota) and Their Pycnidial, Sporodochial, and Synnematous Anamorphs." *Studies in Mycology* 71 (1). CBS Fungal Biodiversity Centre: 1–210. doi:10.3114/sim0001.

Holmes, Keith A, Hans-Josef Schroers, Sarah E. Thomas, Harry C Evans, and Gary J. Samuels. 2004. "Taxonomy and Biocontrol Potential of a New Species of Trichoderma from the Amazon Basin of South America." *Mycological Progress* 3 (3): 199–210. doi:10.1007/s11557-006-0090-z.

Holmes, Keith A, Karina Solis, Mirjam Bekker, Rachel V Guerrero Chuez, Claudio Arroyo Robles, Sofia Peñaherrera, Sarah E. Thomas, et al. 2006. "Endophytic Biocontrol Agents; Most Effective Isolate, at the Right Time?" In *International Permanent Working Group for Cocoa Pests and Disease - INCOPED Newsletter*, 55–61.

Hoopen, G.M. ten, and Ulrike Krauss. 2016. "Biological Control of Cacao Diseases." In *Cacao Diseases: A History of Old Enemies and New Encounters*, 511–66. doi:10.1007/978-3-319-24789-2.

Keanae, PJ, NT Flentje, and KP Lamb. 1972. "Investigation of Vascular-Streak Dieback of Cocoa in Papua New Guinea." *Australian Journal of Biological Science* 25: 553–64. http://www.publish.csiro.au/bi/pdf/BI9720553.

Krauss, Ulrike, Eduardo Hidalgo, Roy Bateman, Valex Adonijah, Claudio Arroyo, Johnny García, Jayne Crozier, Neil a. Brown, G. Martijn ten Hoopen, and Keith A Holmes. 2010. "Improving the Formulation and Timing of Application of Endophytic Biocontrol and Chemical Agents against Frosty Pod Rot (Moniliophthora Roreri) in Cocoa (Theobroma Cacao)." *Biological Control* 54 (3). Elsevier Inc.: 230–40. doi:10.1016/j.biocontrol.2010.05.011.

Krauss, Ulrike, and W Soberanis. 2001. “Rehabilitation of Diseased Cacao Fields in Peru through Shade Regulation and Timing of Biocontrol Measures.” *Agroforestry Systems* 53: 179–84.

Maridueña-Zavala, María Gabriela. 2011. “Estudio de La Micobiota Patogénica de ‘cacao Criollo’ (Theobroma Cacao) En Cinco Provincias de La Costa Ecuatoriana Y Evaluación de La Efectividad in Vitro de Los Bioles Locales Para Su Control.” Ecuador: Escuela Superior Politécnica Del Litoral.

Maridueña-Zavala, María Gabriela, Mirian Elizabeth Villavicencio-Vásquez, Juan Manuel Cevallos-Cevallos, and Esther Lilia Peralta. 2016. “Molecular and Morphological Characterization of Moniliophthora Roreri Isolates from Cacao in Ecuador.” *Canadian Journal of Plant Pathology* 38 (4). Taylor & Francis: 460–69. doi:10.1080/07060661.2016.1261372.

McMahon, Peter, and Agus Purwantara. 2016. “Vascular Streak Dieback (Ceratobasidium Theobromae): History and Biology.” In *Cacao Diseases: A History of Old Enemies and New Encounters*, 307–55. doi:10.1007/978-3-319-24789-2.

Mejía, Luis C, EDWARD ALLEN Herre, Jed P. Sparks, Klaus Winter, Milton N. GarcÃ-a, Sunshine A. Van Bael, Joseph Stitt, et al. 2014. “Pervasive Effects of a Dominant Foliar Endophytic Fungus on Host Genetic and Phenotypic Expression in a Tropical Tree.” *Frontiers in Microbiology* 5 (479): 1–16. doi:10.3389/fmicb.2014.00479.

Mejía, Luis C, Enith I. Rojas, Zuleyka Maynard, Sunshine Van Bael, A.Elizabeth Arnold, Prakash Hebbar, Gary J. Samuels, Nancy Robbins, and Edward Allen Herre. 2008. “Endophytic Fungi as Biocontrol Agents of Theobroma Cacao Pathogens.” *Biological Control* 46 (1): 4–14. doi:10.1016/j.biocontrol.2008.01.012.

Mendes, M A S, and A F Urben. 2017. “Fungos Relatados Em Plantas No Brasil.” *Laboratorio de Quarentena Vegetal.Brasilia,DF*. Embrapa Recursos Geneticos e Biotecnologia. April 24. http://pragawall.cenargen.embrapa.br/aiqweb/michtml/fgbanco01.asp.

Motamayor, Juan Carlos, Philippe Lachenaud, Jay Wallace da Silva E Mota, Rey Loor, David N Kuhn, J Steven Brown, and Raymond J Schnell. 2008. “Geographic and Genetic Population Differentiation of the Amazonian Chocolate Tree (Theobroma Cacao L).” *PloS One* 3 (10): e3311. doi:10.1371/journal.pone.0003311.

Moya-Maldonado, Alexandra E. 2016. “Biodiversidad Fúngica Endófita Y Epífita de Citrus Sinensis, Naranjo Dulce, de Dos Localidades de La Región Litoral Del Ecuador.” Quito, Ecuador: Pontificia Universidad Católica Del Ecuador. http://repositorio.puce.edu.ec/bitstream/handle/22000/12506/TESIS ALEXANDRA MOYA.pdf?sequence=1.

Nam, Myeong Hyeon, Myung Soo Park, He Duck Lee, and Seung Hun Yu. 2013. “Taxonomic Re-Evaluation of Colletotrichum Gloeosporioides Isolated from Strawberry in Korea.” *The Plant Pathology Journal* 29 (3). Korean Society of Plant Pathology: 317–22. doi:10.5423/PPJ.NT.12.2012.0188.

Nuangmek, W., E.H.C. McKenzie, and S. Lumyong. 2008. “Endophytic Fungi from Wild Banana (Musa Acuminata Colla) Works Against Anthracnose Disease Caused by Colletotrichum Musae.” *Research Journal of Microiology* 3 (5): 368–74. doi:10.3923/jm.2008.368.374.

Oliveira, SAS, CAD Bragança, and LL Silva. 2016. “First Report of Colletotrichum Tropicale Causing Anthracnose on the Wild Cassava Species Manihot Dichotoma and M. Epruinosa in Brazil.” *Plant Disease* 100 (10). Scientific Societies: 2171. doi:10.1094/PDIS-10-15-1136-PDN.

Partida-Martínez, Laila P., and Martin Heil. 2011. “The Microbe-Free Plant: Fact or Artifact?” *Frontiers in Plant Science* 2 (December): 1–16. doi:10.3389/fpls.2011.00100.

Phillips-Mora, Wilbert, and M. J. Wilkinson. 2007. “Frosty Pod of Cacao: A Disease with a Limited Geographic Range but Unlimited Potential for Damage.” *Phythopathology* 97: 1644–47.

Porras-Alfaro, Andrea, and Paul Bayman. 2011. “Hidden Fungi, Emergent Properties: Endophytes and Microbiomes.” *Annu. Rev. Phytopathol* 49: 291–315. doi:10.1146/annurev-phyto-080508-081831.

Purdy, L H, and R a Schmidt. 1996. “Status of Cacao Witches’ Broom: Biology, Epidemiology, and Management.” *Annual Review of Phytopathology* 34 (1): 573–94. doi:10.1146/annurev.phyto.34.1.573.

Purdy, L H, R A Schmidt, and Karina Peres Gramacho. 2017. “Diseases of Cacao (Theobroma Cacao L.).” *APSnet Online*. http://www.apsnet.org/online/common/names/cacao.asp.

Ramlachan, N, J Agama, Freddy Amores, J Quiroz, D Vaca, C Zamora, J M Moore, J S Brown, R J Schnell, and Juan Carlos Motamayor. 2009. “Regional Selection of Hybrid Nacional Cocoa Genotypes in Coastal Ecuador.” *INGENIC Newsletter* 12: 25–35. http://ingenic.cas.psu.edu/documents/publications/News/Ramlachan12.pdf.

Rodríguez, Ginsberg. 2011. “Caracterización Morfológica de Hongos Endófitos Asociados a Cacao Nativo Y Su Capacidad Antagónica Para El Control de Moniliophthora Perniciosa.” Tarapoto - Perú, Perú: Universidad Nacional De San Martín. http://repositorio.unsm.edu.pe/bitstream/handle/11458/1191/ITEM%4011458-446.pdf?sequence=1&isAllowed=y.

Rodriguez, R J, J F White Jr, A.Elizabeth Arnold, and R S Redman. 2009. “Fungal Endophytes: Diversity and Functional Roles.” *New Phytologist* 182: 314–30. doi:10.1111/j.1469-8137.2009.02773.x.

Rojas, Enith I., SA Rehner, Gary J Samuels, Sunshine A Van Bael, Paul Cannon, Rui Chen, Junfeng Pang, Yaping Zhang, Yan-Quion Peng, and Tao Sha. 2010. “Colletotrichum Gloeosporioides S.l. Associated with Theobroma Cacao and Other Plants in Panamá: Multilocus Phylogenies Distinguish Host-Associated Pathogens from Asymptomatic Endophytes.” *Mycologia* 102 (6). doi:10.3852/09-244.

Rubini, M R, Rute T Silva-Ribeiro, A W V Pomella, Cristina S Maki, W L Araújo, Deise R dos Santos, and J L Azevedo. 2005. “Diversity of Endophytic Fungal Community of Cacao (Theobroma Cacao L.) and Biological Control of Crinipellis Perniciosa, Causal Agent of Witches’ Broom Disease.” *International Journal of Biological Sciences* 1: 24–33. http://www.biolsci.org/v01p0024.pdf.

Russell, Jonathan R, Jeffrey Huang, Pria Anand, Kaury Kucera, Amanda G Sandoval, Kathleen W Dantzler, DaShawn Hickman, et al. 2011. “Biodegradation of Polyester Polyurethane by Endophytic Fungi.” *Applied and Environmental Microbiology* 77 (17).

1752 N St., N.W., Washington, DC: American Society for Microbiology: 6076–84. doi:10.1128/AEM.00521-11.

Samuels, G J, Carmen Suárez-Capello, Karina Solis, Keith A Holmes, Sarah E. Thomas, A Ismaiel, and Harry C Evans. 2006. "Trichoderma Theobromicola and T. Paucisporum: Two New Species Isolated from Cacao in South America." *Mycological Research* 1 1 0: 381–92.

Sánchez-Mora, F D, S Mariela Medina-Jara, G T Díaz-Coronel, R A Ramos-Remache, J F Vera-Chang, V F Vásquez-Morán, F A Troya-Mera, F R Garcés-Fiallos, and R Onofre-Nodari. 2015. "Potencial Sanitario Y Productivo de 12 Clones de Cacao En Ecuador." *Revista Fitotecnia Mexicana* 38 (3): 265–74. http://www.scopus.com/inward/record.url?eid=2-s2.0-84941564486&partnerID=40&md5=347971fa65cc346f05f402c72a8f72a9.

Saunders, Megan, Anthony E. Glenn, and Linda M. Kohn. 2010. "Exploring the Evolutionary Ecology of Fungal Endophytes in Agricultural Systems: Using Functional Traits to Reveal Mechanisms in Community Processes." *Evolutionary Applications* 3 (5–6): 525–37. doi:10.1111/j.1752-4571.2010.00141.x.

Schmidt, Carsten. 2010. "Diversity and Distribution Patterns of Foliar Fungal Endophytes in Theobroma Cacao in Central Sulawesi and Interactions between Endophytes and Host Plant." Georg-August-Universität Göttingen.

Schulz, Barbara, and Christine Boyle. 2005. "The Endophytic Continuum." *Mycological Research* 109 (6): 661–86. doi:10.1017/S095375620500273X.

Silva, Diogo N, Pedro Talhinhas, Lei Cai, Luzolo Manuel, Elijah K Gichuru, Andreia Loureiro, Vítor Várzea, Octávio S Paulo, and Dora Ti Batista. 2012. "Host-Jump Drives Rapid and Recent Ecological Speciation of the Emergent Fungal Pathogen Colletotrichum Kahawae." *Molecular Ecology* 21: 2655–2670. doi:10.1111/j.1365-294X.2012.05557.x.

Sosa, Daynet, Dercy Parra, Carlos M. Noceda, and Simón Pérez-Martínez. 2016. "Co-Occurrence of Pathogenic and Not Pathogenic Fusarium Decemcellulare and Lasiodiplodia Theobromae Isolates within Cushion Galls Disease of Cacao (Theobroma Cacao L .)." *Journal of Plant Protection Research* 56 (2): 129–38. doi:10.1515/jppr-2016-0020.

Strobel, Gary, Xianshu Yang, Joe Sears, Robert Kramer, Rajinder S Sidhu, and W.M. Hess. 1996. "Taxol from Pestalotiopsis Microspora, an Endophytic Fungus of Taxus Wallachiana." *Microbiology* 142: 435–40. doi:10.1099/13500872-142-2-435.

Suárez-Capello, Carmen. 1983. "Problemática Del Cultivo Del Caccao En El Ecuador." *Comunicación Técnica*, no. 1: 10.

Tahi, G. M., B. I. Kébé, a. Sangare, C. Cilas, and a. B. Eskes. 2007. "Foliar Resistance of Cacao (Theobroma Cacao) to Phytophthora Palmivora as an Indicator of Pod Resistance in the Field: The Effect of Light Intensity and Time of Day of Leaf Collection." *Plant Pathology* 56 (2): 219–26. doi:10.1111/j.1365-3059.2006.01547.x.

Tamura, K, J Dudley, M Nei, and S Kumar. 2007. "MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) Software Version 4.0." *Molecular Biology and Evolution*.

Tchinda, R A, T Boudjeko, A M Simao-Beaunoir, S Lerat, E Tsala, E Monga, and C

Beaulieu. 2016. “Morphological, Physiological, and Taxonomic Characterization of Actinobacterial Isolates Living as Endophytes of Cacao Pods and Cacao Seeds.” *Microbes Environ* 31 (1): 56–62. doi:10.1264/jsme2.ME15146.

Villamizar-Gallardo, Raquel Amanda, Oscar Orlando Ortíz-Rodriguez, Jhon Wilmer Escobar, Raquel Amanda Villamizar-Gallardo, Oscar Orlando Ortíz-Rodriguez, and Jhon Wilmer Escobar. 2017. “Symbiotic and Endophytic Fungi as Biocontrols against Cocoa (Theobroma Cacao L.) Phytopathogens.” *Summa Phytopathologica* 43 (2): 87–93. doi:10.1590/0100-5405/2175.

Weir BS, P R Johnston, and U Damm. 2012. “The Colletotrichum Gloeosporioides Species Complex.” *Studies in Mycology* 73 (1). CBS Fungal Biodiversity Centre: 115–80. doi:10.3114/sim0011.

Yuan, Zhi lin, Chu long Zhang, and Fu cheng Lin. 2010. “Role of Diverse Non-Systemic Fungal Endophytes in Plant Performance and Response to Stress: Progress and Approaches.” *Journal of Plant Growth Regulation* 29 (1): 116–26. doi:10.1007/s00344-009-9112-9.

Zabalgogeazcoa, I. 2008. “Fungal Endophytes and Their Interaction with Plant Pathogens.” *Spanish Journal of Agricultural Research*.

Zhang, N, Dm Geiser, and Cd Smart. 2007. “Macroarray Detection of Solanaceous Plant Pathogens in the Fusarium Solani Species Complex.” *Plant Disease* 91 (12): 1612–20. doi:Doi 10.1094/Pdis-91-12-1612.

1 Supplementary material 1. Information from the isolates.

2

Strain	Province	Locality-Farm	S	WO	Division	Taxa	Average PGI-MR	Day of contact-MR	Colony interaction vs MR	Average PGI-MP	Day of contact-MP	Colony interaction vs MP	Patog_L	Pa
Ec002	Guayas	Balao2	2o51'51,8"	79o37'20,8"	Ascomycota	Nigrospora sphaerica	-29,60	4	Neutra MR	-20,05	6	Neutral MP	No Patog_L	Pa
Ec003	Guayas	Balao2	2o51'51,8"	79o37'20,8"	Ascomycota	Bionectria ochroleuca	-64,70	5	E win MR	3,27	8	E win MP	No Patog_L	Pa
Ec005	Guayas	Balao2	2o51'51,8"	79o37'20,8"	Ascomycota	Xylaria sp.	-23,12	6	E lose MR	-0,8	5	E lose MP	No Patog_L	Pa
Ec006	Guayas	Balao2	2o51'51,8"	79o37'20,8"	Ascomycota	Colletotrichum boninense	37,30	5	Neutra MR	2,21	7	Neutral MP	No Patog_L	Pa
Ec007	Guayas	Balao2	2o51'51,8"	79o37'20,8"	Ascomycota	Diaporthe phaseolorum	-3,06	6	Neutra MR	2,11	5	Neutral MP	No Patog_L	Pa
Ec008	Guayas	Balao2	2o51'51,8"	79o37'20,8"	Basidiomycota	Phanerochaete chrysosporium	8,35	3	Neutra MR	10,85	3	Neutral MP	No Patog_L	Pa
Ec012	Azuay	Molleturo	2o30'49,2"	79o26'11,2"	Ascomycota	Colletotrichum gloeosporioides	8,33	4	Neutra MR	-3,09	7	Neutral MP	No Patog_L	Pa
Ec013	Azuay	Molleturo	2o30'49,2"	79o26'11,2"	Ascomycota	Phomopsis sp.	3,70	4	Neutra MR	18,44	5	Neutral MP	Patog_L	Pa
Ec014	Azuay	Molleturo	2o30'49,2"	79o26'11,2"	Ascomycota	Phomopsis sp.	2,33	7	Neutra MR	0,97	8	Neutral MP	No Patog_L	Pa
Ec018	Azuay	Molleturo	2o30'49,2"	79o26'11,2"	Ascomycota	Nigrospora oryzae	-29,84	8	E lose MR	0,97	8	Neutral MP	No Patog_L	Pa
Ec019	Azuay	Molleturo	2o30'49,2"	79o26'11,2"	Ascomycota	Nigrospora sphaerica	16,36	6	Neutra MR	3,04	5	Neutral MP	No Patog_L	Pa
Ec020	Azuay	Molleturo	2o30'49,2"	79o26'11,2"	Ascomycota	Phomopsis sp.	-8,27	7	Neutra MR	0,8	6	Neutral MP	No Patog_L	Pa
Ec021	Azuay	Molleturo	2o30'49,2"	79o26'11,2"	Ascomycota	Corynespora cassiicola	-57,98	5	Neutra MR	6,85	6	Neutral MP	No Patog_L	Pa
Ec023	Azuay	Molleturo	2o30'49,2"	79o26'11,2"	Ascomycota	Fusarium equiseti	5,18	7	E lose MR	-15,03	6	Neutral MP	No Patog_L	Pa
Ec025	Azuay	Molleturo	2o30'49,2"	79o26'11,2"	Ascomycota	Fusarium equiseti	-39,10	7	Neutra MR	15,91	10	Neutral MP	No Patog_L	Pa

Ec026	Azuay	Molleturo	2o30'49,2"	79o26'11,2"	Ascomycota	Fusarium equiseti	-18,54	8	Neutra MR	9,72	5	Neutral MP	No Patog_L	Pa
Ec027	Azuay	Molleturo	2o30'49,2"	79o26'11,2"	Ascomycota	Lasiodiplodia theobromae	58,35	3	Neutra MR	15,38	4	Neutral MP	No Patog_L	Pa
Ec029	Azuay	Molleturo	2o30'49,2"	79o26'11,2"	Ascomycota	Nectria pseudotrichia	22,04	8	Neutra MR	3,83	5	Neutral MP	No Patog_L	Pa
Ec031	Azuay	Molleturo	2o30'49,2"	79o26'11,2"	Ascomycota	Nigrospora oryzae	28,38	5	Neutra MR	-12,15	4	Neutral MP	No Patog_L	Pa
Ec032	Azuay	Molleturo	2o30'49,2"	79o26'11,2"	Ascomycota	Fusarium decemcellulare	-3,07	8	E lose MR	10,21	8	Neutral MP	No Patog_L	Pa
Ec033	Azuay	Molleturo	2o30'49,2"	79o26'11,2"	Ascomycota	Fusarium equiseti	38,87	8	Neutra MR	4,35	5	Neutral MP	No Patog_L	Pa
Ec035	Azuay	Molleturo	2o30'49,2"	79o26'11,2"	Ascomycota	Lasiodiplodia theobromae	33,30	3	Neutra MR	4,98	4	Neutral MP	No Patog_L	Pa
Ec036	Azuay	Molleturo	2o30'49,2"	79o26'11,2"	Ascomycota	Phoma sp.	-33,30	3	E lose MR	-7,96	6	Neutral MP	No Patog_L	Pa
Ec037	Azuay	Molleturo	2o30'49,2"	79o26'11,2"	Ascomycota	Colletotrichum gloeosporioides	-15,08	5	Neutra MR	3,27	8	Neutral MP	No Patog_L	Pa
Ec038	Guayas	Balao2	2o51'51,8"	79o37'20,8"	Ascomycota	Pestalotiopsis microspora	37,11	8	Neutra MR	-5,59	4	Neutral MP	Patog_L	Pa
Ec039	Guayas	Balao2	2o51'51,8"	79o37'20,8"	Ascomycota	Xylaria venosula	-13,93	10	Neutra MR	-10,43	7	Neutral MP	No Patog_L	Pa
Ec040	Guayas	Balao2	2o51'51,8"	79o37'20,8"	Ascomycota	Xylaria berteri	-31,73	5	E win MR	-17,2	8	E win MP	No Patog_L	Pa
Ec043	Guayas	Balao2	2o51'51,8"	79o37'20,8"	Ascomycota	Xylaria sp.	25,90	4	Neutra MR	-18,83	5	E win MP	No Patog_L	Pa
Ec044	Guayas	Balao2	2o51'51,8"	79o37'20,8"	Ascomycota	Xylaria sp.	-15,90	7	E win MR	-11,05	6	E win MP	No Patog_L	Pa
Ec046	Guayas	Balao2	2o51'51,8"	79o37'20,8"	Ascomycota	Xylaria multiplex	-29,78	6	E win MR	1,73	5	E win MP	No Patog_L	Pa
Ec047	Guayas	Balao2	2o51'51,8"	79o37'20,8"	Ascomycota	Nigrospora sphaerica	34,04	10	Neutra MR	-11,13	6	Neutral MP	No Patog_L	Pa
Ec053	Guayas	Balao2	2o51'51,8"	79o37'20,8"	Ascomycota	Xylariaceae sp.	-31,73	5	E win MR	-10,17	8	E win MP	No Patog_L	Pa
Ec054	Guayas	Balao2	2o51'51,8"	79o37'20,8"	Ascomycota	Pestalotiopsis microspora	27,50	6	E lose MR	-1,52	5	E win MP	No Patog_L	Pa

Ec055	Guayas	Naranjal2	2o40'35,2"	79o38'21,2"	Basidiomycota	Coriolopsis polyzona	33,30	3	Neutra MR	9,72	5	Neutral MP	No Patog_L	Pa
Ec056	Guayas	Naranjal2	2o40'35,2"	79o38'21,2"	Ascomycota	Colletotrichum gloeosporioides	2,78	5	E lose MR	-3,51	7	Neutral MP	No Patog_L	Pa
Ec057	Guayas	Naranjal2	2o40'35,2"	79o38'21,2"	Ascomycota	Colletotrichum gloeosporioides	35,82	7	Neutra MR	-6,73	5	Neutral MP	No Patog_L	Pa
Ec059	Guayas	Naranjal2	2o40'35,2"	79o38'21,2"	Ascomycota	Xylaria feejeensis	-33,30	4	E win MR	-1,38	5	Neutral MP	No Patog_L	Pa
Ec060	Guayas	Naranjal2	2o40'35,2"	79o38'21,2"	Ascomycota	Pestalotiopsis microspora	-33,30	3	E lose MR	10,45	5	Neutral MP	No Patog_L	Pa
Ec063	Guayas	Naranjal2	2o40'35,2"	79o38'21,2"	Ascomycota	Pestalotiopsis microspora	-20,39	7	Neutra MR	1,73	5	Neutral MP	No Patog_L	Pa
Ec065	Guayas	Naranjal2	2o40'35,2"	79o38'21,2"	Ascomycota	Colletotrichum gloeosporioides	-29,78	6	E lose MR	-4,2	4	Neutral MP	No Patog_L	Pa
Ec066	Guayas	Naranjal2	2o40'35,2"	79o38'21,2"	Ascomycota	Colletotrichum gloeosporioides	9,32	6	E lose MR	-4,33	9	Neutral MP	No Patog_L	Pa
Ec067	Guayas	Naranjal2	2o40'35,2"	79o38'21,2"	Ascomycota	Lasiodiplodia theobromae	8,35	3	Neutra MR	6,07	4	Neutral MP	No Patog_L	Pa
Ec069	Guayas	Naranjal2	2o40'35,2"	79o38'21,2"	Ascomycota	Pestalotiopsis microspora	-53,70	4	E lose MR	-8,42	5	Neutral MP	No Patog_L	Pa
Ec070	Guayas	Naranjal2	2o40'35,2"	79o38'21,2"	Ascomycota	Trichothecium roseum	-29,73	5	Neutra MR	5,95	7	E win MP	No Patog_L	Pa
Ec071	Guayas	Naranjal2	2o40'35,2"	79o38'21,2"	Ascomycota	Colletotrichum gloeosporioides	25,00	3	Neutra MR	8,47	5	Neutral MP	No Patog_L	Pa
Ec072	Guayas	Naranjal2	2o40'35,2"	79o38'21,2"	Ascomycota	Endomelanconiopsis endophytica	18,47	7	Neutra MR	-4,7	8	Neutral MP	No Patog_L	Pa
Ec073	Guayas	Naranjal2	2o40'35,2"	79o38'21,2"	Ascomycota	Phomopsis sp.	57,12	6	E lose MR	-8,31	5	Neutral MP	No Patog_L	Pa
Ec075	Guayas	Naranjal2	2o40'35,2"	79o38'21,2"	Ascomycota	Colletotrichum gloeosporioides	-59,95	5	E lose MR	-9,42	6	Neutral MP	No Patog_L	Pa
Ec076	Guayas	Naranjal2	2o40'35,2"	79o38'21,2"	Basidiomycota	Psathyrella sp.	5,85	7	Neutra MR	-1,34	5	Neutral MP	Patog_L	Pa
Ec078	Guayas	Naranjal2	2o40'35,2"	79o38'21,2"	Basidiomycota	Pseudolagarobasidium belizense	46,52	6	Neutra MR	-6,59	5	Neutral MP	No Patog_L	Pa
Ec081	Guayas	Balao1	2o30'29,5"	79o46'34,8"	Ascomycota	Colletotrichum gloeosporioides	-8,15	5	Neutra MR	-0,32	5	Neutral MP	No Patog_L	Pa
Ec082	Guayas	Balao1	2o30'29,5"	79o46'34,8"	Ascomycota	Colletotrichum gloeosporioides	-40,08	5	Neutra MR	-3,3	6	Neutral MP	No Patog_L	Pa

Ec083	Guayas	Balao1	2o30'29,5"	79o46'34,8"	Ascomycota	Colletotrichum gloeosporioides	18,20	6	Neutra MR	-0,32	5	Neutral MP	No Patog_L	Pa
Ec084	Guayas	Balao1	2o30'29,5"	79o46'34,8"	Ascomycota	Daldinia eschscholtsii	12,97	4	Neutra MR	2,79	5	Neutral MP	No Patog_L	Pa
Ec085	Guayas	Naranjal1	2o30'49,2"	79o26'11,2"	Ascomycota	Fusarium solani	-31,73	5	Neutra MR	-13,17	8	Neutral MP	No Patog_L	Pa
Ec089	Guayas	Naranjal1	2o30'49,2"	79o26'11,2"	Ascomycota	Xylaria sp.	11,34	6	Neutra MR	-7,16	6	E win MP	No Patog_L	Pa
Ec090	Guayas	Naranjal1	2o30'49,2"	79o26'11,2"	Ascomycota	Fusarium solani	-41,65	5	Neutra MR	-11,13	6	Neutral MP	No Patog_L	Pa
Ec091	Guayas	Naranjal1	2o30'49,2"	79o26'11,2"	Ascomycota	Didymosphaeria futilis	-44,86	6	E lose MR	7,66	7	Neutral MP	No Patog_L	Pa
Ec095	Guayas	Naranjal1	2o30'49,2"	79o26'11,2"	Ascomycota	Fusarium decemcellulare	-16,08	5	E lose MR	-18,54	5	Neutral MP	No Patog_L	Pa
Ec098	Guayas	Naranjal2	2o40'35,2"	79o38'21,2"	Ascomycota	Lasiodiplodia theobromae	41,65	3	Neutra MR	12,58	5	Neutral MP	No Patog_L	Pa
Ec099	Guayas	Balao2	2o51'51,8"	79o37'20,8"	Ascomycota	Xylariaceae sp.	25,00	3	Neutra MR	6,01	8	Neutral MP	No Patog_L	Pa
Ec100	Guayas	Balao2	2o51'51,8"	79o37'20,8"	Ascomycota	Xylaria berteri	-18,47	10	Neutra MR	5,71	10	E win MP	No Patog_L	Pa
Ec104	Guayas	Balao2	2o51'51,8"	79o37'20,8"	Ascomycota	Fusarium thapsinum	-33,90	5	Neutra MR	-5,77	6	Neutral MP	No Patog_L	Pa
Ec107	Guayas	Balao2	2o51'51,8"	79o37'20,8"	Ascomycota	Colletotrichum gloeosporioides	8,15	5	Neutra MR	8,47	5	Neutral MP	No Patog_L	Pa
Ec108	Guayas	Balao2	2o51'51,8"	79o37'20,8"	Ascomycota	Colletotrichum gloeosporioides	1,44	6	Neutra MR	-15,37	5	Neutral MP	No Patog_L	Pa
Ec109	Guayas	Balao2	2o51'51,8"	79o37'20,8"	Ascomycota	Colletotrichum gloeosporioides	19,41	8	Neutra MR	-7,64	8	Neutral MP	No Patog_L	Pa
Ec110	Guayas	Balao2	2o51'51,8"	79o37'20,8"	Ascomycota	Xylariaceae sp.	-24,32	7	Neutra MR	-6,59	5	Neutral MP	Patog_L	Pa
Ec112	Guayas	Balao2	2o51'51,8"	79o37'20,8"	Ascomycota	Hypoxylon investiens	7,80	7	Neutra MR	-6,59	5	Neutral MP	No Patog_L	Pa
Ec113	Guayas	Balao2	2o51'51,8"	79o37'20,8"	Ascomycota	Hypoxylon investiens	-22,20	4	Neutra MR	1,44	4	E win MP	No Patog_L	Pa
Ec115	Guayas	Balao1	2o30'29,5"	79o46'34,8"	Ascomycota	Colletotrichum gloeosporioides	-9,57	8	Neutra MR	0,99	7	Neutral MP	No Patog_L	Pa

Ec116	Guayas	Balao1	2o30'29,5"	79o46'34,8"	Ascomycota	Phomopsis sp.	-39,02	7	Neutra MR	-15,37	5	Neutral MP	Patog_L	Pa
Ec117	Guayas	Balao1	2o30'29,5"	79o46'34,8"	Ascomycota	Curvularia verruculosa	-16,70	3	Neutra MR	-11,13	6	Neutral MP	No Patog_L	Pa
Ec120	Guayas	Balao1	2o30'29,5"	79o46'34,8"	Ascomycota	Lasiodiplodia theobromae	33,30	3	Neutra MR	6,64	3	Neutral MP	No Patog_L	Pa
Ec123	Guayas	Balao1	2o30'29,5"	79o46'34,8"	Ascomycota	Eutypa sp.	-54,73	7	Neutra MR	-2	7	Neutral MP	No Patog_L	Pa
Ec124	Guayas	Balao1	2o30'29,5"	79o46'34,8"	Ascomycota	Nigrospora sphaerica	7,90	6	Neutra MR	-0,32	5	Neutral MP	Patog_L	Pa
Ec129	Azuay	Molleturo	2o30'49,2"	79o26'11,2"	Ascomycota	Nigrospora sphaerica	-20,37	4	E lose MR	7,74	4	Neutral MP	No Patog_L	Pa
Ec130	Azuay	Molleturo	2o30'49,2"	79o26'11,2"	Ascomycota	Phomopsis sp.	25,90	4	Neutra MR	-8,49	7	Neutral MP	No Patog_L	Pa
Ec132	Azuay	Molleturo	2o30'49,2"	79o26'11,2"	Ascomycota	Corynespora cassiicola	18,47	7	Neutra MR	-18,28	5	Neutral MP	No Patog_L	Pa
Ec133	Azuay	Molleturo	2o30'49,2"	79o26'11,2"	Ascomycota	Colletotrichum gloeosporioides	17,00	8	E lose MR	-19,8	8	Neutral MP	No Patog_L	Pa
Ec134	Azuay	Molleturo	2o30'49,2"	79o26'11,2"	Ascomycota	Fusarium equiseti	39,42	6	E lose MR	-0,32	5	Neutral MP	No Patog_L	Pa
Ec135	Azuay	Molleturo	2o30'49,2"	79o26'11,2"	Ascomycota	Nectria pseudotrichia	16,88	5	Neutra MR	6,07	4	Neutral MP	No Patog_L	Pa
Ec136	Azuay	Molleturo	2o30'49,2"	79o26'11,2"	Ascomycota	Nigrospora oryzae	-1,80	7	E lose MR	1	7	Neutral MP	No Patog_L	Pa
Ec138	Azuay	Molleturo	2o30'49,2"	79o26'11,2"	Ascomycota	Fusarium equiseti	-41,92	6	E lose MR	0,68	7	Neutral MP	No Patog_L	Pa
Ec139	Azuay	Molleturo	2o30'49,2"	79o26'11,2"	Ascomycota	Nectria pseudotrichia	16,67	4	Neutra MR	-6,59	5	Neutral MP	No Patog_L	Pa
Ec146	Guayas	Naranjal2	2o40'35,2"	79o38'21,2"	Ascomycota	Colletotrichum gloeosporioides	5,18	7	Neutra MR	-5,17	7	Neutral MP	No Patog_L	Pa
Ec149	Guayas	Naranjal2	2o40'35,2"	79o38'21,2"	Ascomycota	Pestalotiopsis microspora	-53,70	4	Neutra MR	-1,51	6	Neutral MP	No Patog_L	Pa
Ec151	Guayas	Naranjal2	2o40'35,2"	79o38'21,2"	Ascomycota	Lasiodiplodia theobromae	-25,00	3	Neutra MR	6,64	3	Neutral MP	No Patog_L	Pa
Ec152	Guayas	Naranjal1	2o30'49,2"	79o26'11,2"	Ascomycota	Fusarium decemcellulare	-31,41	8	Neutra MR	-12,68	7	Neutral MP	No Patog_L	Pa

Ec153	Guayas	Naranjal1	2o30'49,2"	79o26'11,2"	Ascomycota	Fusarium decemcellulare	-15,49	9	Neutra MR	-21,7	5	Neutral MP	No Patog_L	Pa
Ec156	Guayas	Naranjal1	2o30'49,2"	79o26'11,2"	Basidiomycota	Ceriporia lacerata	-29,60	4	E lose MR	-1,56	3	E win MP	No Patog_L	Pa
Ec157	Guayas	Naranjal1	2o30'49,2"	79o26'11,2"	Ascomycota	Lasiodiplodia theobromae	41,65	3	E win MR	10,05	3	E win MP	No Patog_L	Pa
Ec159	Guayas	Naranjal2	2o40'35,2"	79o38'21,2"	Ascomycota	Pestalotiopsis microspora	-34,08	7	Neutra MR	1,04	7	Neutral MP	No Patog_L	Pa
Ec160	Guayas				Ascomycota	Colletotrichum gloeosporioides	25,90	4	Neutra MR	-7,59	5	Neutral MP	No Patog_L	Pa
Ec161	Guayas	Balao1	2o30'29,5"	79o46'34,8"	Ascomycota	Fusarium decemcellulare	-67,38	6	E lose MR	0,8	7	Neutral MP	No Patog_L	Pa
Ec162	Guayas	Naranjal2	2o40'35,2"	79o38'21,2"	Ascomycota	Pestalotiopsis microspora	47,76	8	Neutra MR	-4,5	5	Neutral MP	No Patog_L	Pa
Ec163	Guayas	Naranjal2	2o40'35,2"	79o38'21,2"	Ascomycota	Colletotrichum gloeosporioides	10,26	8	E lose MR	8,47	5	Neutral MP	No Patog_L	Pa
Ec164	Guayas	Naranjal2	2o40'35,2"	79o38'21,2"	Ascomycota	Colletotrichum gloeosporioides	-25,86	6	E lose MR	-3,7	8	Neutral MP	No Patog_L	Pa
Ec166	Azuay	Molleturo	2o30'49,2"	79o26'11,2"	Ascomycota	Fusarium decemcellulare	5,14	10	E lose MR	-3,53	6	Neutral MP	No Patog_L	Pa
Ec167	Azuay				Ascomycota	Fusarium decemcellulare	-16,08	5	Neutra MR	1,98	10	Neutral MP	No Patog_L	Pa
Ec168	Azuay				Ascomycota	Fusarium decemcellulare	-11,14	10	Neutra MR	4,03	10	Neutral MP	No Patog_L	Pa
Ec170	Guayas				Ascomycota	Phomopsis sp.	43,63	8	Neutra MR	-15,15	8	Neutral MP	No Patog_L	Pa
Ec173	Guayas	Balao2	2o51'51,8"	79o37'20,8"	Ascomycota	Xylaria sp.	-29,08	6	Neutra MR	9,39	9	Neutral MP	No Patog_L	Pa
Ec174	Guayas				Ascomycota	Hypoxylon investiens	71,62	6	Neutra MR	-2,88	5	Neutral MP	No Patog_L	Pa
Ec175	Guayas	Balao1	2o30'29,5"	79o46'34,8"	Basidiomycota	Ceriporia lacerata	-29,73	5	Neutra MR	3,39	5	E win MP	No Patog_L	Pa
Ec177	Guayas	Balao1	2o30'29,5"	79o46'34,8"	Ascomycota	Pestalotiopsis microspora	47,04	6	E lose MR	-2,08	6	Halo >1cm	No Patog_L	Pa
Ec178	Guayas	Balao1	2o30'29,5"	79o46'34,8"	Ascomycota	Xylaria sp.	-8,73	9	E lose MR	1,47	8	E win MP	No Patog_L	Pa

Ec180	Guayas	Balao2	2o51'51,8"	79o37'20,8"	Ascomycota	Xylariaceae sp.	-23,88	7	Neutra MR	-13,55	3	Neutral MP	No Patog_L	Pa
Ec183	Azuay	Molleturo	2o30'49,2"	79o26'11,2"	Ascomycota	Nectria pseudotrichia	56,93	5	Neutra MR	3,89	4	Neutral MP	No Patog_L	Pa
Ec185	Azuay	Molleturo	2o30'49,2"	79o26'11,2"	Ascomycota	Lasiodiplodia theobromae	-12,70	9	E win MR	-8,54	3	Neutral MP	No Patog_L	Pa
Ec186	Azuay	Molleturo	2o30'49,2"	79o26'11,2"	Ascomycota	Lasiodiplodia theobromae	25,00	3	Neutra MR	3,23	3	Neutral MP	No Patog_L	Pa
Ec187	Azuay	Molleturo	2o30'49,2"	79o26'11,2"	Ascomycota	Fusarium equiseti	-33,30	7	E lose MR	3,39	5	E lose MP	No Patog_L	Pa
Ec189	Guayas	Balao1	2o30'29,5"	79o46'34,8"	Ascomycota	Xylaria sp.	-21,03	10	E lose MR	-5,19	3	E lose MP	No Patog_L	Pa
Ec190	Guayas	Balao1	2o30'29,5"	79o46'34,8"	Basidiomycota	Ceriporia lacerata	-25,00	3	Neutra MR	3,39	5	E win MP	No Patog_L	Pa
Ec192	Guayas				Ascomycota	Nigrospora sphaerica	-35,30	5	Neutra MR	-2,78	7	E win MP	No Patog_L	Pa
Ec193	Guayas				Basidiomycota	Rigidoporus sp.	-28,87	7	Neutra MR	-1,68	6	Neutral MP	No Patog_L	Pa
Ec194	Guayas	Naranjal1	2o30'49,2"	79o26'11,2"	Ascomycota	Xylaria sp.	-1,38	7	E win MR	-19,97	5	E win MP	No Patog_L	Pa
Ec196	Guayas	Naranjal1	2o30'49,2"	79o26'11,2"	Ascomycota	Fusarium decemcellulare	4,38	9	E lose MR	-12,1	8	Neutral MP	No Patog_L	Pa
Ec197	Guayas	Balao1	2o30'29,5"	79o46'34,8"	Ascomycota	Pestalotiopsis microspora	38,62	7	Neutra MR	-16,96	3	Halo >1cm	No Patog_L	Pa
Ec201	Guayas	Balao1	2o30'29,5"	79o46'34,8"	Ascomycota	Xylaria sp.	7,49	8	E win MR	-15,6	4	E win MP	No Patog_L	Pa
Ec203	Guayas	Balao2	2o51'51,8"	79o37'20,8"	Ascomycota	Xylaria sp.	37,28	7	E win MR	-11,61	6	E win MP	No Patog_L	Pa
Ec205	Guayas	Balao2	2o51'51,8"	79o37'20,8"	Ascomycota	Pestalotiopsis microspora	-40,34	6	Neutra MR	-18,16	4	Neutral MP	No Patog_L	Pa
Ec207	Guayas	Naranjal1	2o30'49,2"	79o26'11,2"	Ascomycota	Fusarium decemcellulare	-14,76	6	Neutra MR	-10,83	9	Neutral MP	No Patog_L	Pa
Ec212	Azuay				Ascomycota	Fusarium decemcellulare	-6,23	9	Neutra MR	-8,86	6	Neutral MP	No Patog_L	Pa
Ec217	Azuay				Ascomycota	Fusarium solani	14,44	9	Neutra MR	-2,98	6	Neutral MP	No Patog_L	Pa

Ec220	Azuay				Ascomycota	Fusarium graminearum	30,32	6	Neutra MR	-13,31	4	Neutral MP	No Patog_L	Pa
Ec222	Azuay				Ascomycota	Fusarium equiseti	-15,27	10	Neutra MR	7,98	10	Neutral MP	No Patog_L	Pa

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