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Phylogenetic Affinity in the Potential Antagonism of *Trichoderma* spp. against *Moniliophthora roreri*

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Abstract: Frosty pod rot, caused by the fungus *Moniliophthora roreri*, is one of the most important diseases of cacao in Peru. Sustainable alternatives to control it include timely cultural practices such as pruning, and the application of biological control agents such as *Trichoderma* spp. We isolated 234 *Trichoderma* strains native to the department of Amazonas in Northern Peru from soil samples in cacao farms. These strains belong to at least eighteen species within four phylogenetic clades in the genus (Harzianum, Longibrachiatum, Hamatum, and Brevicompactum clades). We aimed to assess the in vitro biocontrol potential of these strains against *M. roreri*. We evaluated their mycoparasitism, antibiosis, and potential antagonism to select candidate strains for efficient biocontrol of *M. roreri*. We found evidence (Kruskal–Wallis test, $p < 0.005$) that strains belonging to the Harzianum clade tend to have higher mycoparasitism, antibiosis, and potential antagonism levels than strains in the Longibrachiatum and Hamatum clades. Additionally, this study constitutes the first report for antagonistic behavior against *M. roreri* for *T. parareesei*, *T. lentiforme*, *T. orientale*, *T. asperelloides*, *T. inhamatum*, *T. zelobreve*, *T. afarasin*, *T. ghanense*, *T. rifaii*, and *T. breve*. These results will be foundational for further *M. roreri* biocontrol studies.

Keywords: antagonism; biological control; mycoparasitism; *Trichoderma*

Citation: Leiva, S.; Rubio, K.; Díaz-Valderrama, J.R.; Granda-Santos, M.; Mattos, L. Phylogenetic Affinity in the Potential Antagonism of *Trichoderma* spp. against *Moniliophthora roreri*. *Agronomy* **2022**, *12*, 2052. https://doi.org/10.3390/agronomy12092052

Academic Editors: Ivo Toševski and Luca Ruiu

Received: 1 June 2022
Accepted: 24 August 2022
Published: 28 August 2022

1. Introduction

Moniliophthora roreri is the causal agent of frosty pod rot (FPR), one of the most destructive diseases of cacao (*Theobroma cacao* L.) in Latin America [1]. It can cause 100% yield losses under poor management conditions, which has led to the abandonment of cacao plantations [2]. In Peru, it was first reported in Quebrada Seca district, Utcubamba province, Amazonas department, in 1989, becoming the main cacao pathogen of the country since then [1]. Frosty pod rot is a disease twice as destructive as the black pod rot caused by *Phytophthora* spp., and more difficult to control than witches' broom, caused by *M. perniciosa* [3]. Therefore, its control is a major challenge in cacao cultivation [4]. To achieve sustainable cacao production, environmentally friendly control strategies must be implemented to overcome the problems caused by traditional chemical control methods [5]. Multiple strategies have been evaluated and recommended for the control of FPR. Cultural management practices such as purges, pruning, and timely harvests are very effective [6–12]. Additionally, the use of natural enemies of *M. roreri* such as *Trichoderma* spp. has been shown to be a sustainable alternative to mitigate the impacts of FPR [2,13,14].

Trichoderma spp. are filamentous fungi that live naturally in the soil in a close relationship with plant roots [15]. They are considered beneficial for agriculture because of their ability to protect the crops by acting as biological control agents to fungal pathogens [5,16–19]. Various plant diseases such as root rot, wilt, pod rot, and others can be mitigated by *Trichoderma* spp. [20–25].

In cacao, *Trichoderma* spp. has an important biocontrol potential against *M. roreri* and other major pathogens [2,9,13,14,19,26–28]. *Trichoderma* biocontrol of fungal pathogens can be achieved through mycoparasitism or antibiosis. Mycoparasitism is the ability of the fungus to grow and feed on other fungi [29]. Antibiosis is related to the production of metabolites that inhibit the growth of other organisms [30]. Most studies evaluating the *Trichoderma* spp. activity against *M. roreri* have identified strains and species with stronger biocontrol potential. However, they have only considered either mycoparasitism or antibiosis separately in their analysis. The integration of both biocontrol mechanisms, referred to as potential antagonism, has been proposed to more accurately evaluate the biocontrol capacity of *Trichoderma* spp. [13,14,31].

The objective of this study was to evaluate the in vitro potential antagonism against *M. roreri* of 234 strains in four phylogenetic clades of *Trichoderma* native to the Amazonas department in Northern Peru, based on their levels of mycoparasitism and antibiosis.

2. Materials and Methods

2.1. Acquisition of Native Strains of Trichoderma spp.

We evaluated 234 strains of *Trichoderma* recovered from the rhizosphere soil of *T. cacao* from farms growing the native fine aroma cacao (CNFA, Spanish acronym for "*cacao nativo fino de aroma*") from the provinces of Condorcanqui, Bagua, Utcubamba, Chachapoyas, Luya, and Rodríguez de Mendoza in the department of Amazonas, Peru (Table S1). All strains were isolated using the dilution technique [32]. Monosporic cultures were then obtained from each identified colony [33]. Two hundred and sixteen strains belonged to eighteen species [34], while the identification of eighteen strains remained ambiguous based on the identification protocol proposed by Cai and Druzhinina [35]. However, all strains belonged to four major *Trichoderma* phylogenetic clades previously described [35,36] and interactively presented at © 2022 Trichokey (https://trichokey.com/; last accessed on 1 July 2022) (Table S1). One hundred and sixteen strains belonged to the phylogenetic clade 1 (also known as Harzianum clade), one hundred and four to clade 3 (Longibrachiatum clade), thirteen to clade 5 (Hamatum clade), and one strain to clade 6 (Brevicompactum clade) (Table S1). The strains are conserved in the fungal collection of the plant health laboratory of the Universidad Nacional Toribio Rodríguez de Mendoza de Amazonas (LABISANV).

2.2. Acquisition of Moniliophthora roreri

A fully characterized strain of *M. roreri* (MBG_01) was used for the assays in this research. This strain was isolated from cacao pods with visible *M. roreri* mycelia, collected from Quebrada Seca, Utcubamba, Amazonas (UTM: 17M 779480 9368166). This strain was identified, characterized, and conserved in the LABISANV fungal collection.

2.3. Mycoparasitism Test

Mycoparasitism was evaluated by using the method of pre-colonized Petri plates [37]. A five-mm-diameter disc from 12-day-old *M. roreri* colonies were placed on the periphery of a 90-mm Petri plate containing potato dextrose agar (PDA) medium (Figures 1 and S1). Cultures were incubated in a bioclimatic chamber at 28 °C with a 12 h light/day regime (all incubations were conducted at these conditions unless specified). When *M. roreri* colonized 100% of the plate (three to five weeks later), a 2.5 × 0.5 cm^2 *Trichoderma* inoculum strip, taken from the edge of a three-day-old freshly sporulating colony, was placed on the opposite side from where *M. roreri* was originally seeded (Figure 1). The cultures were then further incubated for two weeks. Then, ten 5-mm discs were evenly extracted from each plate, including the original *M. roreri* disc that was originally inoculated. The discs were placed in two Petri plates with 20% PDA culture medium (five discs per plate) and further incubated. We monitored the growth of either *Trichoderma* or *M. roreri* for two weeks (Figure 1). Five repetitions of the test per *Trichoderma* strain were performed. The

percentage of mycoparasitism was estimated by using the following formula from Evans et al. [37]:

$$PP = (TG \times 100) / N;$$

where:

PP = Parasitism (%),
TG = Number of discs with *Trichoderma* growth,
N = Total number of discs taken for each repetition (plate).

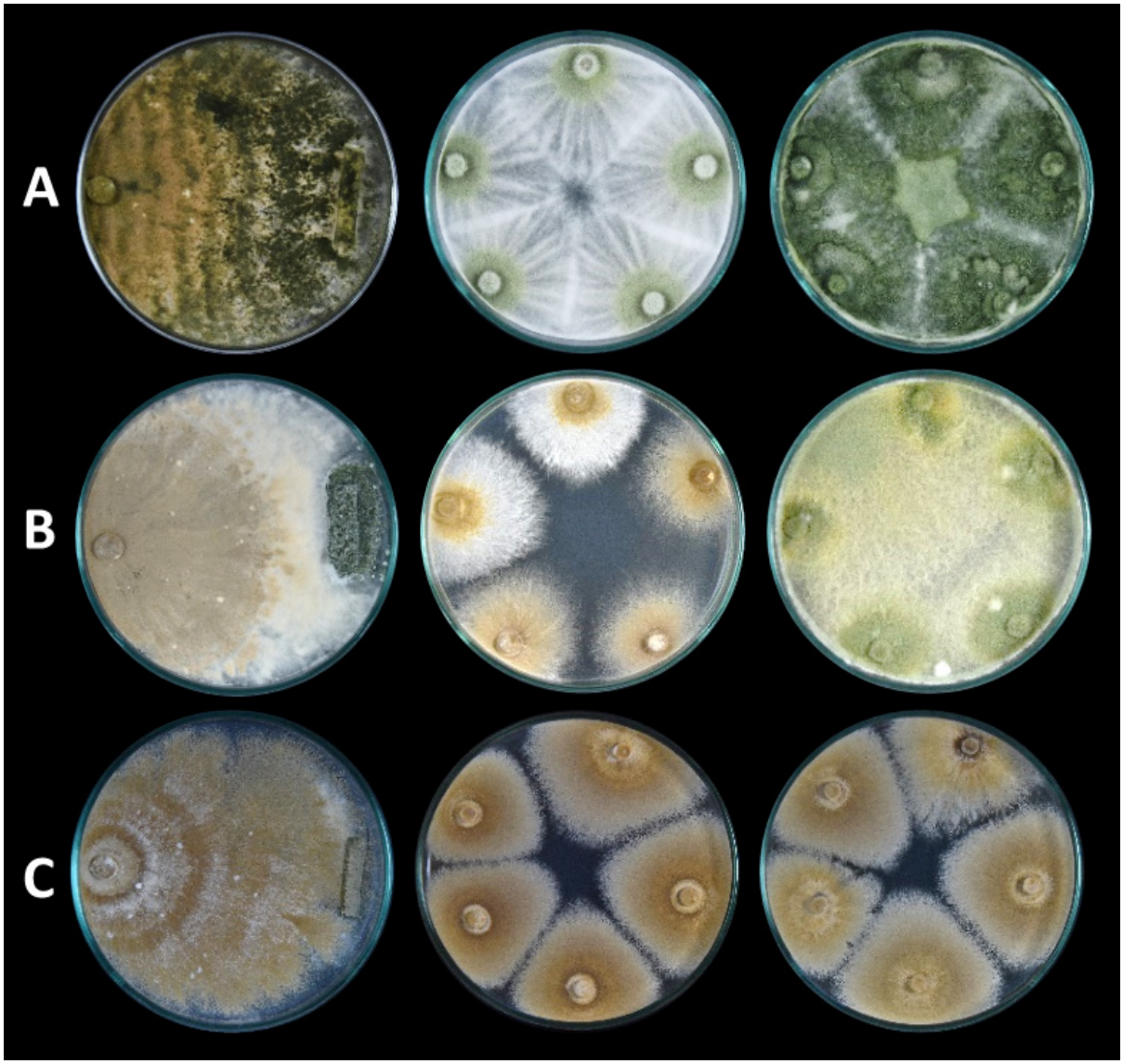

Figure 1. Mycoparasitism tests against *Moniliophthora roreri*. (**A**) Example of a test with a high mycoparasitism level (*Trichoderma longibrachiatum* strain F14M3) (100%). A_Left: dual culture at 21 days after inoculation. A_Center and A_Right: discs taken from A_left plate showing *Trichoderma* growth. (**B**) Example of a test with a medium mycoparasitism level (*T. parareesei* strain CRSF3_C2) (50%). B_Left: dual culture at 21 days after inoculation. B_Center: discs with *M. roreri* growth taken from B_left plate. B_Right: discs taken with *Trichoderma* growth from B_left plate. (**C**) Example of a test with low mycoparasitism level (*T. longibrachiatum* strain F21M5) (0%). C_Left: dual culture at 21 days after inoculation. C_Center and C_Right: discs with *M. roreri* growth taken from C_left plate.

2.4. Antibiosis Test

The antibiosis of *Trichoderma* strains was evaluated using the paired culture method [38]. Here, 5-mm discs of *M. roreri* (previously grown on PDA for 7 days) were placed on the periphery of a 90-mm PDA Petri plate and incubated for 10 ± 1 days or until a 1.3–3.3 cm-wide colony was established. From this colony, 5-mm *Trichoderma* discs from 3–4-day old PDA cultures were collected and transferred on the opposite side of the *M. roreri* colony previously established (Figure 2). Then, the confrontation plates were incubated. Five replicates were established for each *Trichoderma* strain, with five controls (non-confronted *M. roreri* plates). Daily measurements of the *M. roreri* radial growth were taken, until mycelia from both fungi came into contact in one of the five replicates. Finally, the inhibition of radial growth (i.e., antibiosis) of the phytopathogen was evaluated using the following formula from Holmes et al. [38]:

$$PA = [(RG - RGT)/RG] \times 100;$$

where:

PA = Antibiosis (%),
RG = Radial growth of *M. roreri* in control plates (mm),
RGT = Radial growth of *M. roreri* confronted with *Trichoderma* spp. (mm).

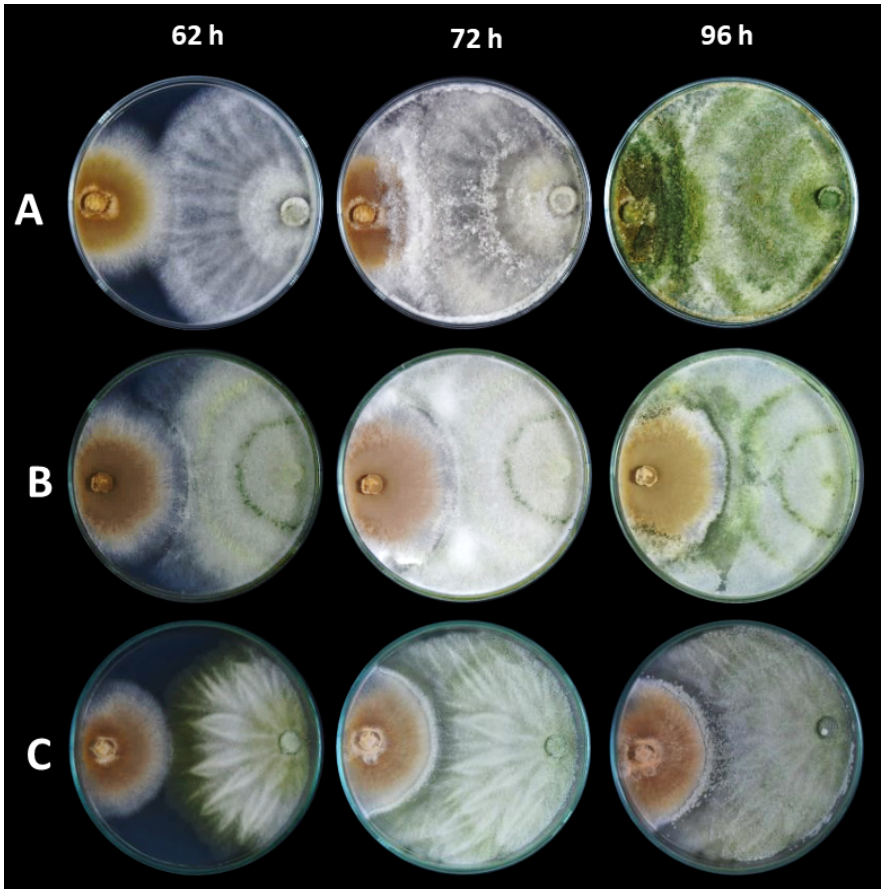

Figure 2. Antibiosis test against *Moniliophthora roreri*. (**A**) Example of a test with high antibiosis level (*Trichoderma afroharzianum* strain F13M4 in a dual culture with *M. roreri* at 62, 72, and 96 h of confrontation). (**B**) Example of a test with medium antibiosis level (*T. reesei* strain UCF7_M2 in a dual culture with *M. roreri* at 62, 72, and 96 h of confrontation). (**C**) Example of a test with low antibiosis level (*T. afroharzianum* strain F12M3 in a dual culture with *M. roreri* at 62, 72, and 96 h of confrontation).

2.5. Potential Antagonism

The potential antagonism was calculated by averaging both the mycoparasitism and antibiosis results, as proposed by Reyes-Figueroa et al. [14]:

$$AP = (PP + PA)/2;$$

where:

AP = Potential antagonism,
PP = Mycoparasitism of *Trichoderma* against *M. roreri* (%),
PA= Antibiosis of *Trichoderma* against *M. roreri* (%).

2.6. Data Analysis

The mycoparasitism, antibiosis, and potential antagonism levels of strains were analyzed under a completely randomized experimental design using ANOVA and the Scott Knott test for separation of means, when ANOVA detected significant differences, in Infostat V.2020 software [39]. The Scott Knott test allowed us to classify the strains into groups with different levels of mycoparasitism, antibiosis, and potential antagonism (starting from "a", which grouped strains at the highest levels of these antagonistic features). Additionally, a Kruskal–Wallis test to compare the mycoparasitism, antibiosis, and potential antagonism levels of the strains, according to the phylogenetic clade they belong to, was performed using the R package *agricolae* [40]. Finally, these data were integrated (Table S3) and used to perform a minimum spanning network analysis with R package *poppr* [41] to investigate the association of strains according to their potential antagonism.

3. Results and Discussion

3.1. Mycoparasitism

We found highly significant differences (Scott Knott test, $p < 0.0001$) in the mycoparasitic behavior of *Trichoderma* strains against *M. roreri*. The 234 strains were distributed in six groups significantly different to each other (Table S2).

All species had strains with some parasitic behavior against *M. roreri*. One hundred and forty strains belonging to seventeen species reached parasitism percentages above 50%, and more remarkably, fifty-nine strains reached 100% parasitism against *M. roreri* (Table S2). These strains belonged to: *T. afarasin* (1 strain), *T. afroharzianum* (22 strains), *T. asperelloides* (1 strain), *T. brevicompactum* (1 strain), *T. ghanense* (1 strain), *T. inhamatum* (4 strains), *T. lentiforme* (4 strains), *T. longibrachiatum* (3 strains), *T. orientale* (4 strains), *T. parareesei* (5 strains), *T. reesei* (4 strains), *T. zelobreve* (1 strain), *Trichoderma* spp. (ambiguous) (5 strains), and *Trichoderma* sp. 4 (3 strains) (Table S2). On the other hand, only 25 strains did not have parasitic behavior on *M. roreri*. These belonged to: *T. afroharzianum* (6 strains), *T. asperelloides* (1 strain), *T. lentiforme* (1 strain), *T. longibrachiatum* (3 strains), *T. parareesei* (5 strains), *T. reesei* (2 strains), *Trichoderma* sp. 1 (3 strains), and *Trichoderma* spp. (ambiguous) (4 strains) (Table S2).

In general, the interspecific mycoparasitism levels found in this study were variable, which was also reported in previous studies. Hoyos-Carbajal et al. [42] and Reyes-Figueroa et al. [14] found *T. harzianum* strains with high and low mycoparasitism levels against *Sclerotinia* spp. and *Rhizoctonia* spp. Reyes-Figueroa et al. [14] reported mycoparasitism levels against *M. roreri* ranging between 10% and 100% for strains of *T. harzianum*, *T. virens*, *T. spirale*, *T. brevicompactum*, *T. koningiopsis*, and *T. asperellum*. Evans et al. [37] reported a 29% colonization level on other cacao pathogen, *M. perniciosa*, by a *T. longibrachiatum* strain, and 100% by *T. virens* and *T. spirale* strains. Galarza et al. [27] reported mycoparasitism of 100% on various phytopathogenic fungi by *T. asperellum* strains. Additionally, strains of *T. asperelloides*, *T. lentiforme*, and *T. reesei* have been reported as parasitic on other pathogens, such as *Phytophthora palmivora* in palm crops [43], and *T. koningiopsis*, on *Macrophomina phaseolina* in peanuts [44]. In this study, we report for the first time the parasitic activity against *M. roreri* of *T. parareesei*, *T. longibrachiatum*, *T. lentiforme*, *T. orientale*, *T. asperelloides*, *T. inhamatum*, *T. zelobreve*, *T. afarasin*, *T. ghanense*, *T. rifaii*, and *T. breve*.

When we looked at these results in terms of the phylogenetic clades the strains belong to, we found that the Harzianum clade had strains with higher mycoparasitism levels than the Longibrachiatum and Hamatum clades (Figure 3). Galarza et al. [27] found that *T. asperellum* (Hamatum clade) and *T. harzianum* (Harzianum clade) had similar levels of mycoparasitism against *M. roreri*. They also found that the only strain of the Longibrachiatum clade they worked with (*T. virens*) had no mycoparasitism and a very low antibiosis capacity against the pathogen. The different mycoparasitic levels of strains within the different *Trichoderma* clades depend on their enzymatic matrix production capability. This enzymatic matrix includes cellulases, glucanases, lipases, proteases, and chitinases that help them to parasitize *M. roreri* with greater or lesser aggressiveness [2,14,45]. The production of this enzymatic repertoire may have phylogenetic patterns that future studies must investigate.

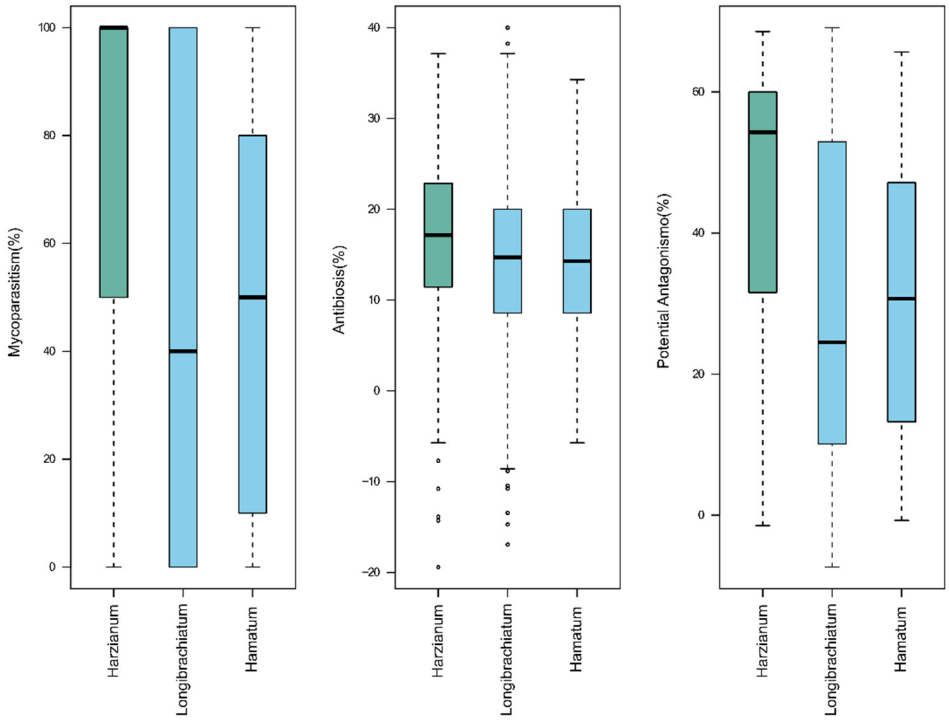

Figure 3. Mycoparasitism, antibiosis, and potential antagonism levels of the 234 *Trichoderma* strains grouped by phylogenetic clade. Boxplots with different colors in each plot indicate that the ranked median of mycoparasitism, antibiosis, or potential antagonism of strains within a phylogenetic clade differ significantly ($p < 0.05$) according to the Kruskal–Wallis test.

3.2. Antibiosis

We also found highly significant differences ($p < 0.0001$) in the antibiosis activity of *Trichoderma* strains against *M. roreri* (Table S2). All species had at least one strain with antibiosis behavior. Only two strains of *T. parareesei* and *T. afroharzianum* did not show antibiosis levels, UJF2-C2 ($-1.98\% \pm 5.23\%$) and UCF21_M2 ($-3.45\% \pm 2.81\%$), respectively.

The antibiosis levels of the 234 strains ranged from -3.45% to 31.09%. Sixty strains belonging to: *T. afarasin* (2 strains), *T. afroharzianum* (15 strains), *T. asperelloides* (4 strains), *T. brevicompactum* (1 strain), *T. inhamatum* (5 strains), *T. lentiforme* (10 strains), *T. longibrachiatum* (8 strains), *T. orientale* (1 strain), *T. parareesei* (9 strains), *T. reesei* (1 strain), *T. zelobreve* (2 strains), *Trichoderma* sp. 4 (1 strain), and *Trichoderma* spp. (ambiguous) (1 strain), had antibiosis levels greater than 20%, while strains F13M4 (*T. afroharzianum*), UCF5A-C1 (*T. longibrachiatum*), and F9M3 (*T. parareesei*) outperformed the others, reaching antibiosis levels greater than 30% (Table S2).

The analysis of antibiosis by phylogenetic clade revealed that the Harzianum clade had significantly higher antibiosis levels than strains in the Longibrachiatum and Hamatum clades (Figure 3). Galarza et al. [27] found that strains of *T. asperellum* (Hamatum clade) had, for the most part, significantly higher antibiosis levels against *M. roreri* than strains of *T. harzianum* (Harzianum clade). Additionally, *T. asperellum* was found to have higher antibiosis levels than *T. harzianum* and *T. longibrachiatum* (Longibrachiatum clade) against other pathogens, such as *Fusarium solani* [46]. However, these studies have no more than 15 isolates analyzed in total. Our study presents antibiosis information of more than 100 strains for Harzianum and Hamatum clades, providing statistical evidence of their biocontrol capacity against *M. roreri* (Figure 3). Other cacao pathogens, such as *M. perniciosa*, were also confronted against potential *Trichoderma* strains, finding no significant differences between *T. asperellum* and *T. harzianum* strains [27]. This may suggest that the antagonistic mode of action of *Trichoderma* spp. against *M. perniciosa* may be different than *M. roreri*.

We identified three strains, BIF7_C1 (*T. asperelloides*) in the Hamatum clade, and BMF19_C4 (*T. parareesei*) and CNF3_C1 (*T. parareesei*) in the Longibrachiatum clade, showing high levels of antibiosis (all in the Scott Knott group "a", Table S2) without mycoparasitism levels against *M. roreri.* This situation is not rare as similar findings were reported by Bailey et al. [2] and Reyes-Figueroa [14], in which strains within the Hamatum (*T. koningiopsis* and *T. ovalisporum*) and Longibrachiatum (*T. longibrachiatum* and *T. reesei*) clades had high levels of antibiosis but low levels of mycoparasitism against *M. roreri*. Moreover, this situation can extend to strains in the Harzianum clade, as Reyes-Figueroa [14] found that strains of *T. pleuroticola* had antibiosis but no mycoparasitism activity on *M. roreri*. The opposite situation is also possible. For example, we found strains F21M2 (*T. harzianum*) and BIF27_C2 (*Trichoderma* sp. ambiguous), both in the Harzianum clade, with 96% of mycoparasitism, but 3.94% and 1.71% of antibiosis, respectively. A similar scenario was reported by Bailey et al. [2], in which they found that strains within the Harzianum clade (mostly *T. harzianum*) had both the highest mycoparasitism levels and the lowest antibiosis levels [2].

3.3. Potential Antagonism

The potential antagonism in terms of phylogenetic clade of strains revealed a similar scenario of significance, in which strains in the Harzianum clade tended to have higher potential antagonism levels than strains in the Longibrachiatum and Hamatum clades (Figure 3). Due to the known intraspecific variability of *Trichoderma* species, we might expect that the phylogenetic affinities towards the potential antagonism against *M. roreri* of strains in the Harzianum clade does not apply to all species evaluated. However, when we dissected the data by species, we surprisingly found that *T. afarasin, T. breve, T. inhamatum, Trichoderma* sp. 2, *Trichoderma* sp. 3, *T. zelobreve*, *Trichoderma* sp. 4, *T. lentiforme,* and *T. afroharzianum*, which all belong to the Harzianum clade, had strains with high potential antagonism, mostly above 50% (Figures 4and S2). Conversely, the species *T. ghanense*, *T. orientale*, *Trichoderma* sp. 1, *T. longibrachiatum*, *T. parareesei*, and *T. reesei* (all in the Longibrachiatum clade), as well as *T. asperelloides* and *T. koningiopsis* (both in the Hamatum clade), had strains with potential antagonism mostly below 50% (Figures 4 and S2). This provides another perspective to visualize the data and to corroborate the phylogenetic affinity of the evaluated *Trichoderma* species in the Harzianum clade to potentially have a higher antagonistic behavior against *M. roreri* than at least the evaluated species in the Longibrachiatum clade. We cannot draw the same conclusion for species in the Hamatum clade because we only worked with 13 strains in 2 species, which is far less than the number of strains within the other two clades (over 100 each).

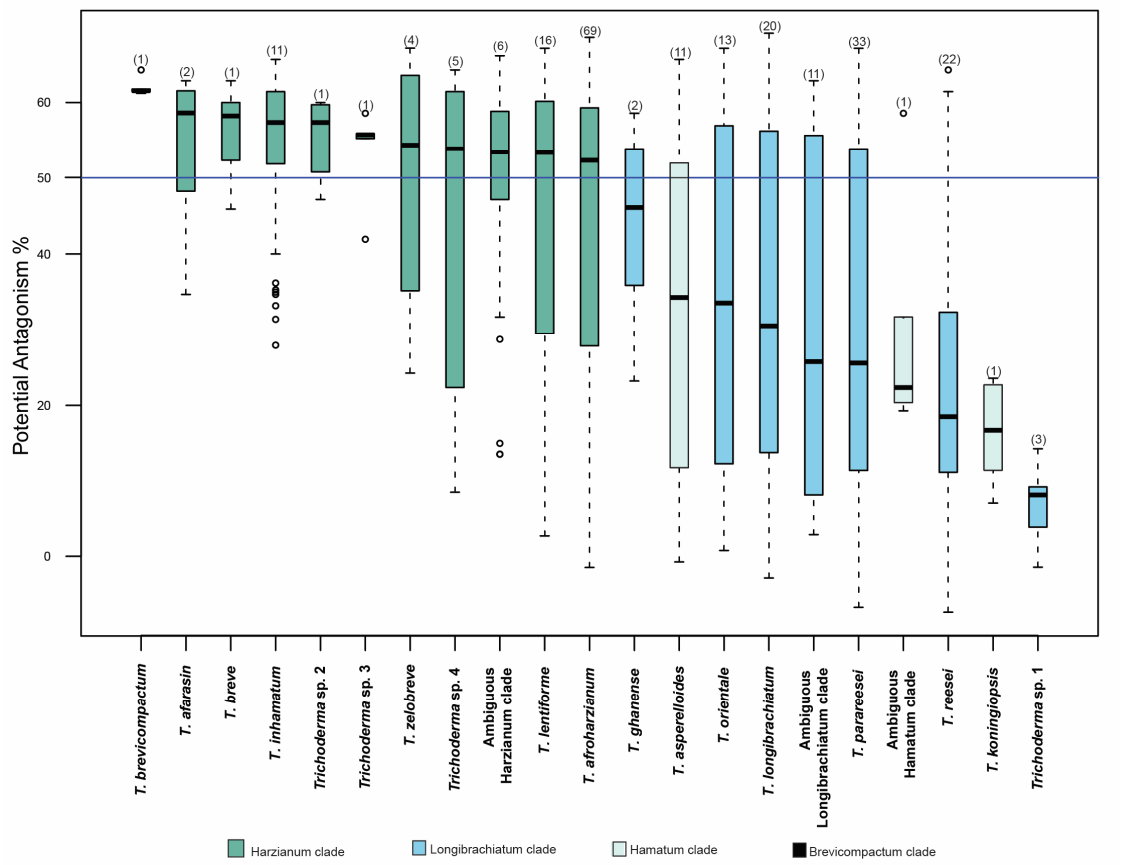

Figure 4. Boxplots displaying the potential antagonism of *Trichoderma* spp. evaluated in this study against *M. roreri* (strain numbers for each species).

Even though our only strain of *T. brevicompactum* (BBF1_C3) outperformed all other strains, we cannot conclude much about the species and the phylogenetic clade it belongs to (Brevicompactum) (Figure 4). Reyes-Figueroa et al. [14] evaluated four *T. brevicompactum* strains, finding variable levels of potential antagonism, ranging between 36.6% and 57.0%. Therefore, the phylogenetic affinity of *T. brevicompactum* in terms of *M. roreri* biocontrol needs further revision. However, the potential use of the strain BBF1_C3 must be considered in future biocontrol studies.

3.4. Association Analysis

The association analysis considering mycoparasitism, antibiosis, and potential antagonism of the 234 strains also showed that proportionally, strains with higher potential antagonism (Scott Knott group "a") were composed mostly from strains in the Harzianum clade and were more intimately interconnected in terms of their antagonistic features against *M. roreri* (Figure 5). Conversely, strains with lower potential antagonism (Scott Knott groups "d–g") were composed mostly from strains in the Longibrachiatum clade (Figure 5). These results confirmed our findings in terms of the phylogenetic affinities of *Trichoderma* clades to control *M. roreri.*

Phylogenetic affinities in *Trichoderma* spp. regarding their parasitic behavior have been previously reported in human and animal clinical samples [47]. Strains belonging to the Longibrachiatum clade were more commonly isolated from human respiratory tract samples, as opposed to strains in the Harzianum clade, which mainly came from superficial animal tissues [47]. To the best of our knowledge, this is the first study to report a phylogenetic pattern in the *Trichoderma* genus regarding the antagonistic behavior against the cacao pathogen *M. roreri*.

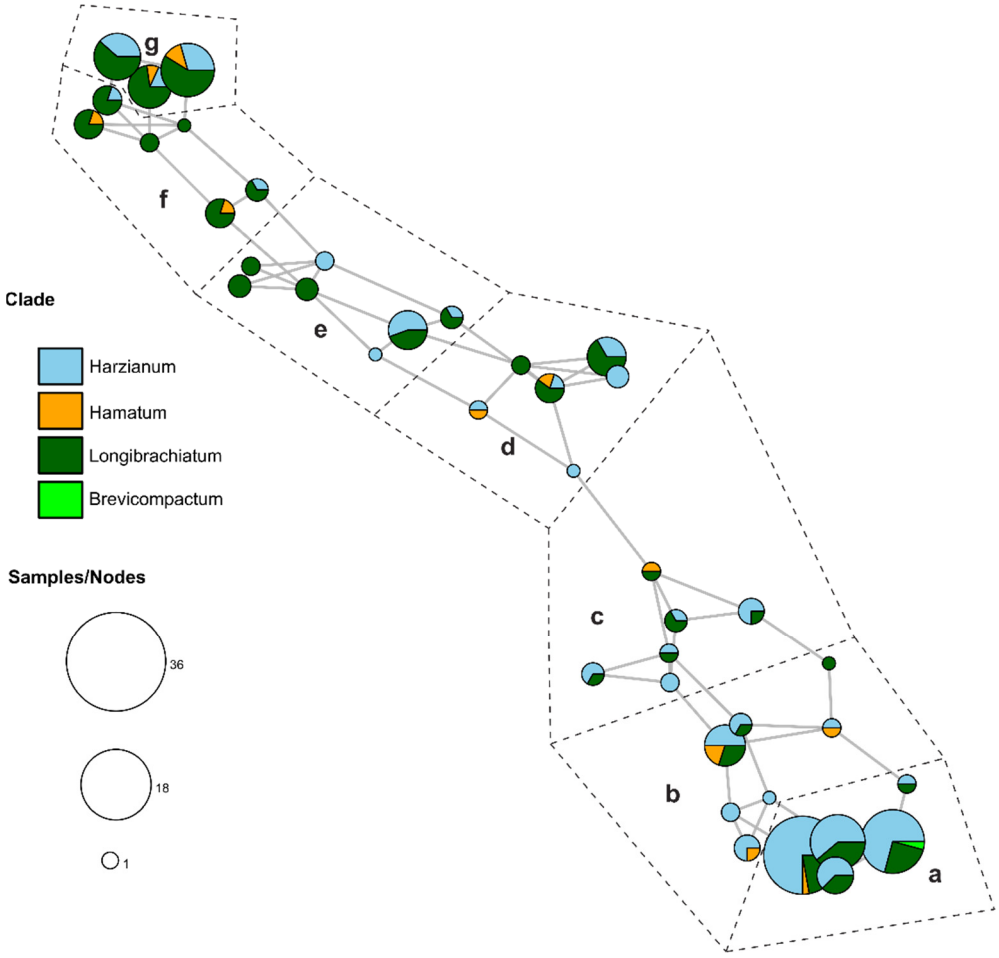

Figure 5. Minimum spanning network analysis of *Trichoderma* strains based on the mycoparasitism, antibiosis, and potential antagonism levels data. Nodes (circles) represent one single strain or multiple strains belonging to the exact same Scott Knott groups (Tables S2 and S3) for mycoparasitism, antibiosis, and potential antagonism combined. Node size indicates the number of samples, colors indicate the phylogenetic clades to which strains belong to, and connecting lines indicate the relatedness of strains (line lengths are arbitrary). Nodes are also framed with broken lines based on the potential antagonism Scott Knott group ("a–g") to which strains belong to.

The high variability observed on the mycoparasitism and antibiosis between and within species confirms that it is necessary to calculate the potential antagonism (i.e., the average of mycoparasitism and antibiosis) when selecting *Trichoderma* strains as biocontrol agents [30]. In this study, we combined mycoparasitism and antibiosis, following the procedures of previous studies [13,14,31], to evaluate mycoparasitism and antibiosis simultaneously, which have been used separately in many other studies [2,27]. This allowed us to establish a better approach to select strains with stronger biocontrol potential. Then, we identified several strains, mainly in the Harzianum clade, as the most promising biocontrol agents against *M. roreri*. Finally, the phylogenetic pattern we found in this study has implications for future studies to test *Trichoderma* strains in the field, either individually or as a consortium.

4. Conclusions

The results observed for mycoparasitism and antibiosis against *M. roreri* support the existence of a high intra- and inter-specific variability in *Trichoderma*. However, despite this variability, we found evidence of a phylogenetic pattern in terms of the antagonistic behavior of *Trichoderma* strains against *M. roreri.* The majority of strains in all evaluated species in

the Harzianum clade had levels of potential antagonism greater than 50% as opposed to the species in the Longibrachiatum and Hamatum clades, confirming the phylogenetic affinity of the Harzianum clade towards the antagonism against *M. roreri*. Finally, we demonstrated that the in vitro evaluation of the biocontrol potential of *Trichoderma* strains, even within the same species, is variable and should be assessed integrating the mycoparasitism and antibiosis levels, in the form of potential antagonism. This process is a necessary step for the selection of biocontrol agents of plant pathogens.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy12092052/s1, Figure S1: Location of the ten agar discs taken from the dual culture plate that were sub-cultured into two new PDA plates (Figure 1) to evaluate the mycoparasitism levels. Figure S2: Boxplot displaying the potential antagonism of 234 strains of *Trichoderma* spp. against *M. roreri* evaluated in this study. Table S1: Geographical data of the 234 strains of *Trichoderma* spp. evaluated against *Moniliophthora roreri* in Amazonas, Peru. Table S2: In vitro potential antagonism, mycoparasitism, and antibiosis percentage of 234 native strains of *Trichoderma* from Amazonas, Peru, assessed against *Moniliophthora roreri* (± standard error, averages with the same letter are not statistically different (Scott Knott, $\alpha = 0.05$)). Table S3: Data arrangement to conduct the minimum spanning network analysis (the numbers in the columns mycoparasitism, antibiosis, and potential antagonism correspond to the groups obtained in the Skott Knott analysis shown in Table S2: a = 1, b = 2, c = 3, d = 4, e = 5, f = 6, and g = 7).

Author Contributions: Conceptualization, S.L. and L.M.; methodology, S.L. and M.G.-S.; formal analysis, K.R. and J.R.D.-V.; investigation, S.L. and M.G.-S.; data curation, K.R.; writing—original draft preparation, S.L. and K.R.; writing—review and editing, K.R., J.R.D.-V., and L.M.; supervision, L.M.; project administration, S.L.; funding acquisition, S.L. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by FONDECYT, grant numbers: "CONTRATO N° 10-2018-FONDECYT-BM-ADT-AV", and "SNIP no. 352641 (CEINCACAO)".

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available in the article and the supplementary material.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Díaz-Valderrama, J.R.; Leiva-Espinoza, S.T.; Catherine Aime, M. The history of cacao and its diseases in the Americas. *Phytopathology* **2020**, *110*, 1604–1619. [CrossRef] [PubMed]
2. Bailey, B.A.; Bae, H.; Strem, M.D.; Crozier, J.; Thomas, S.E.; Samuels, G.J.; Vinyard, B.T.; Holmes, K.A. Antibiosis, mycoparasitism, and colonization success for endophytic *Trichoderma* isolates with biological control potential in *Theobroma cacao*. *Biol. Control* **2008**, *46*, 24–35. [CrossRef]
3. Phillips-Mora, W.; Aime, M.C.; Wilkinson, M.J. Biodiversity and biogeography of the cacao (*Theobroma cacao*) pathogen *Moniliophthora roreri* in tropical America. *Plant Pathol.* **2007**, *56*, 911–922. [CrossRef]
4. Zin, N.A.; Badaluddin, N.A. Biological functions of *Trichoderma* spp. for agriculture applications. *Ann. Agric. Sci.* **2020**, *65*, 168–178. [CrossRef]
5. Brotman, Y.; Gupta, J. Trichoderma. *Curr. Biol.* **2017**, *20*, 390–391. [CrossRef]
6. Torres de la Cruz, M.; Ortiz García, C.F.; Téliz Ortiz, D.; Mora Aguilera, A.; Nava Díaz, C. Temporal progress and integrated management of frosty pod rot (*Moniliophthora roreri*) of cocoa in Tabasco, Mexico. *J. Plant Pathol.* **2011**, *93*, 31–36. [CrossRef]
7. Krauss, U.; Soberanis, W. Rehabilitation of diseased cacao fields in Peru through shade regulation and timing of biocontrol measures. *Agrofor. Syst.* **2001**, *53*, 179–184. [CrossRef]
8. Soberanis, W.; Ríos, R.; Arévalo, E.; Zúñiga, L.; Cabezas, O.; Krauss, U. Increased frequency of phytosanitary pod removal in cacao (*Theobroma cacao*) increases yield economically in eastern Peru. *Crop Prot.* **1999**, *18*, 677–685. [CrossRef]
9. Bateman, R.P.; Hidalgo, E.; García, J.; Arroyo, C.; Ten Hoopen, G.M.; Adonijah, V.; Krauss, U. Application of chemical and biological agents for the management of frosty pod rot (*Moniliophthora roreri*) in Costa Rican cocoa (*Theobroma cacao*). *Ann. Appl. Biol.* **2005**, *147*, 129–138. [CrossRef]

10. Gidoin, C.; Avelino, J.; Deheuvels, O.; Cilas, C.; Bieng, M.A.N. Shade tree spatial structure and pod production explain frosty pod rot intensity in cacao agroforests, Costa Rica. *Phytopathology* **2014**, *104*, 275–281. [CrossRef]
11. Ortíz-García, C.F.; Torres-de la Cruz, M.; Hernández-Mateo, S. del C. Comparación de dos sistemas de manejo del cultivo del cacao, en presencia de *Moniliophthora roreri*, en México. *Rev. Fitotec. Mex.* **2015**, *38*, 191–196. [CrossRef]
12. Leandro-Muñoz, M.E.; Tixier, P.; Germon, A.; Rakotobe, V.; Phillips-Mora, W.; Maximova, S.; Avelino, J. Effects of microclimatic variables on the symptoms and signs onset of *Moniliophthora roreri*, causal agent of moniliophthora pod rot in cacao. *PLoS ONE* **2017**, *12*, e0184638. [CrossRef]
13. Leiva, S.; Oliva, M.; Hernández, E.; Chuquibala, B.; Rubio, K.; García, F.; Torres de la Cruz, M. Assessment of the potential of *Trichoderma* spp. strains native to Bagua (Amazonas, Peru) in the biocontrol of frosty pod rot (*Moniliophthora roreri*). *Agronomy* **2020**, *10*, 1376. [CrossRef]
14. Reyes-Figueroa, O.; Ortiz-García, C.F.; Torres de la Cruz, M.; Lagunes-Espinoza, L.D.C.; Valdovinos-Ponce, G. Especies de *Trichoderma* del agroecosistema cacao con potencial de biocontrol sobre *Moniliophthora roreri*. *Rev. Chapingo Ser. Cienc. Y Del Ambient.* **2016**, *22*, 149–163. [CrossRef]
15. Vinale, F.; Sivasithamparam, K.; Ghisalberti, E.L.; Marra, R.; Woo, S.L.; Lorito, M. Trichoderma–Plant–Pathogen interactions. *Soil Biol. Biochem.* **2008**, *40*, 1–10. [CrossRef]
16. Harman, G.E.; Howell, C.R.; Viterbo, A.; Chet, I.; Lorito, M. Trichoderma species—Opportunistic, avirulent plant symbionts. *Nat. Rev. Microbiol.* **2004**, *2*, 43–56. [CrossRef]
17. Poveda, J. Trichoderma as biocontrol agent against pests: New uses for a mycoparasite. *Biol. Control* **2021**, *159*, 104634. [CrossRef]
18. De Palma, M.; Docimo, T.; Guida, G.; Salzano, M.; Albrizio, R.; Giorio, P.; Ruocco, M.; Tucci, M. Transcriptome modulation by the beneficial fungus *Trichoderma longibrachiatum* drives water stress response and recovery in tomato. *Environ. Exp. Bot.* **2021**, *190*, 104588. [CrossRef]
19. Hanada, R.E.; Pomella, A.W.V.; Costa, H.S.; Bezerra, J.L.; Loguercio, L.L.; Pereira, J.O. Endophytic fungal diversity in *Theobroma cacao* (cacao) and *T. grandiflorum* (cupuaçu) trees and their potential for growth promotion and biocontrol of black-pod disease. *Fungal Biol.* **2010**, *114*, 901–910. [CrossRef]
20. Yassin, M.T.; Mostafa, A.A.-F.; Al-Askar, A.A. In vitro antagonistic activity of *Trichoderma harzianum* and *T. viride* strains compared to carbendazim fungicide against the fungal phytopathogens of *Sorghum bicolor* L. moench. *Egypt. J. Biol. Pest Control* **2021**, *31*, 118. [CrossRef]
21. Begum, M.F.; Rahman, M.A.; Alam, M.F. Biological control of alternaria fruit rot of chili by Trichoderma species under field conditions. *Mycobiology* **2010**, *38*, 113. [CrossRef] [PubMed]
22. Soytong, K.; Srinon, W.; Rattanacherdchai, K.; Kanokmedhakul, S.; Kanokmedhakul, K. Application of antagonistic fungi to control anthracnose disease of grape. *J. Agric. Technol.* **2005**, *1*, 33–41.
23. Mbarga, J.B.; Ten Hoopen, G.M.; Kuaté, J.; Adiobo, A.; Ngonkeu, M.E.L.; Ambang, Z.; Akoa, A.; Tondje, P.R.; Begoude, B.A.D. *Trichoderma ssperellum*: A potential biocontrol agent for *Pythium myriotylum*, causal agent of cocoyam (*Xanthosoma sagittifolium*) root rot disease in Cameroon. *Crop Prot.* **2012**, *36*, 18–22. [CrossRef]
24. El-Komy, M.H.; Saleh, A.A.; Eranthodi, A.; Molan, Y.Y. Characterization of novel *Trichoderma asperellum* isolates to select effective biocontrol agents against tomato fusarium wilt. *Plant Pathol. J.* **2015**, *31*, 50–60. [CrossRef]
25. Manzar, N.; Singh, Y.; Kashyap, A.S.; Sahu, P.K.; Rajawat, M.V.S.; Bhowmik, A.; Sharma, P.K.; Saxena, A.K. Biocontrol potential of native *Trichoderma* spp. against anthracnose of great millet (*Sorghum bicolour* L.) from Tarai and Hill regions of India. *Biol. Control* **2021**, *152*, 104474. [CrossRef]
26. Carvajal, V.; Enrique, J.; Valbuena, B.; Orlando, J.; Rosero, V.; Edgar, S.; Facultad, R.; Agronomía, N. Evaluación in vitro de microorganismos nativos por su antagonismo contra *Moniliophthora roreri* Cif & Par en cacao (*Theobroma cacao* L.). *Rev. Fac. Nac. Agron. Medellín* **2012**, *65*, 6305–6315.
27. Galarza, L.; Akagi, Y.; Takao, K.; Kim, C.S.; Maekawa, N.; Itai, A.; Peralta, E.; Santos, E.; Kodama, M. Characterization of Trichoderma species isolated in Ecuador and their antagonistic activities against phytopathogenic fungi from Ecuador and Japan. *J. Gen. Plant Pathol.* **2015**, *81*, 201–210. [CrossRef]
28. Crozier, J.; Arroyo, C.; Morales, H.; Melnick, R.L.; Strem, M.D.; Vinyard, B.T.; Collins, R.; Holmes, K.A.; Bailey, B.A. The influence of formulation on Trichoderma biological activity and frosty pod rot management in *Theobroma cacao*. *Plant Pathol.* **2015**, *64*, 1385–1395. [CrossRef]
29. Zeilinger, S.; Gruber, S.; Bansal, R.; Mukherjee, P.K. Secondary metabolism in Trichoderma—Chemistry meets genomics. *Fungal Biol. Rev.* **2016**, *30*, 74–90. [CrossRef]
30. Monte, E. Understanding Trichoderma: Between biotechnology and microbial Ecology. *Int. Microbiol.* **2001**, *4*, 1–4. [CrossRef]
31. Garcia Simoes, M.L.; Tauk-Tornisielo, S.M.; Niella, G.R.; Tapia, D.M.T. Evaluation of *Trichoderma* spp. for the biocontrol of *Moniliophthora perniciosa* subgroup 1441. *J. Biol. Life Sci.* **2011**, *3*, 18–36. [CrossRef]
32. Nelson, P.E.; Marasas, W.F.O.; Toussoun, T.A. *Fusarium Species an Illustrates Manual for Identification*; The Pennsylvania State University Press: University Park, PK, USA, 1983.
33. Estrada, M.N.; Vélez, P.E.; López, J.C. Estandarizacion de una metodologia para obtener cultivos monoesporicos del hongo *Beauveria bassiana*. *Cenicafé* **1997**, *48*, 59–65.

34. Leiva, S.; Aguilar, V.; Rubio, K.; Díaz-Valderrama, J.R.; Granda-Santos, M.; Mattos, L. Instituto de Investigación para el Desarrollo Sustentable de Ceja de Selva, Universidad Nacional Toribio Rodríguez de Mendoza de Amazonas, Chachapoyas 01001, Peru. *Manuscript in preparation*.
35. Cai, F.; Druzhinina, I.S. In honor of John Bissett: Authoritative guidelines on molecular identification of Trichoderma. *Fungal Divers.* **2021**, *107*, 1–69. [CrossRef]
36. de Respinis, S.; Vogel, G.; Benagli, C.; Tonolla, M.; Petrini, O.; Samuels, G.J. MALDI-TOF MS of Trichoderma: A Model system for the identification of microfungi. *Mycol. Prog.* **2010**, *9*, 79–100. [CrossRef]
37. Evans, H.C.; Holmes, K.A.; Thomas, S.E. Endophytes and mycoparasites associated with an indigenous forest tree, *Theobroma gileri*, in Ecuador and a preliminary assessment of their potential as biocontrol agents of cocoa diseases. *Mycol. Prog.* **2003**, *2*, 149–160. [CrossRef]
38. Holmes, K.A.; Schroers, H.; Thomas, S.E.; Evans, H.C.; Samuels, G.J.; Samuels, J. Taxonomy and biocontrol potential of a new species of Trichoderma from the Amazon basin of South America. *Mycol. Prog.* **2004**, *3*, 199–210. [CrossRef]
39. Di Rienzo, J.A.; Casanoves, F.; Balzarini, M.G.; Gonzalez, L.; Tablada, M.; Robledo, C.W. InfoStat Version 2020. Available online: https://www.infostat.com (accessed on 1 June 2022).
40. De Mendiburu, F.; Simon, R. Agricolae—Ten years of an open source statistical tool for experiments in breeding, agriculture and biology. *PeerJ PrePrints* **2015**, *3*, e1404v1.
41. Kamvar, Z.N.; Tabima, J.F.; Grünwald, N.J. Poppr: An R Package for genetic analysis of populations with clonal, partially clonal, and/or sexual reproduction. *PeerJ* **2014**, *2014*, e281. [CrossRef]
42. Hoyos-Carvajal, L.; Duque, G.; Orduz, S. Antagonismo in vitro de *Trichoderma* spp. sobre aislamientos de *Sclerotinia* spp. y *Rhizoctonia* spp. *Rev. Colomb. Ciencias Horticolas* **2011**, *2*, 76–86. [CrossRef]
43. Sarria, G.; Garcia, A.; Mestizo, Y.; Medina, C.; Varón, F.; Mesa, E.; Hernandez, S. Antagonistic interactions betwwen *Trichoderma* spp. and *Phytophthora palmivora* (Butler) from oil palm in Colombia. *Eur. J. Plant Pathol.* **2021**, *161*, 751–768. [CrossRef]
44. Martínez-Salgado, S.J.; Andrade-Hoyos, P.; Parraguirre Lezama, C.; Rivera-Tapia, A.; Luna-Cruz, A.; Romero-Arenas, O. Biological Control of charcoal rot in peanut crop through strains of *Trichoderma* spp., in Puebla, Mexico. *Plants* **2021**, *10*, 2630. [CrossRef] [PubMed]
45. Infante, D.; Martínez, B.; González, N.; Reyes, Y. Mecanismos de acción de Trichoderma frente a hongos fitopatogenos. *Rev. Protección Veg.* **2009**, *24*, 14–21.
46. Filizola, P.R.B.; Luna, M.A.C.; De Souza, A.F.; Coelho, I.L.; Laranjeira, D.; Campos-Takaki, G.M. Biodiversity and phylogeny of novel Trichoderma isolates from mangrove sediments and potential of biocontrol against Fusarium strains. *Microb. Cell Fact.* **2019**, *18*, 89. [CrossRef] [PubMed]
47. Sandoval-Denis, M.; Sutton, D.A.; Cano-Lira, J.F.; Gené, J.; Fothergill, A.W.; Wiederhold, N.P.; Guarro, J. Phylogeny of the clinically relevant species of the emerging fungus Trichoderma and their antifungal susceptibilities. *J. Clin. Microbiol.* **2014**, *52*, 2112–2125. [CrossRef] [PubMed]