RESEARCH / INVESTIGACIÓN

Pathogenicity of *Moniliophthora roreri* isolates from selected morphology groups in harvested cacao pods and *in vitro* sensitivity to compost tea

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Abstract: *Moniliopthora roreri* is the frosty pod rot disease (FPD) and one of the most devastating cacao pathogens worldwide. However, *M. roreri* pathogenicity on harvested cacao pods and sensitivity to compost tea have not been fully described. Monosporic cultures of *M. roreri* from different morphology groups were obtained. The isolates' pathogenicity was tested by inoculation onto harvested cacao pods, and symptoms were evaluated at 3-day intervals during 16 days before estimating the area under the disease progress curve (AUDPC). The sensitivity of *M. roreri* to compost tea was evaluated on potato dextrose agar (PDA) amended with 1 to 5 % compost tea. All morphology groups could infect harvested cacao pods during the 16 days with a disease severity index abode 75 %. Compost tea completely inhibited the growth of *M. roreri* when used at 4.5 % or higher. Results suggest a shortened biotrophic phase during the infection in harvested pods and a medium to high sensitivity of *M. roreri* to compost tea.

Key words: Moniliasis, cacao national, biol, biotrophic, necrotrophic.

Introduction

Cacao (*Theobroma cacao*) trees are usually categorized as "fine flavor" or "bulk/ordinary" depending on the aroma released by the fermented beans[1]. Ecuador is the top delicate flavor cacao producer worldwide[2] with extensive production areas for the 'Nacional' and 'CCN51' types of cacaos. The Nacional cacao is preferred by consumers[3] but is more susceptible to the frosty pod rot disease (FPD) than CCN51[4]. The FPD has caused significant losses in the cacao production of Latin American countries[1], and the production of Nacional cacao is partially being replaced by CCN51 in Ecuador[4] mostly because of FPD.

Moniliophthora roreri—the causal agent of FPD—has been classified into 14 morphology groups, and various characteristics, including growth rate and genetic patterns of each group, were recently described[5]. To cause the disease, the pathogen infects plant-attached cacao pods and undergoes a biotrophic phase of up to 3 months before causing necrosis and spore masses over the pod's surface[6]. During the biotrophic phase, the pod is usually symptomless but, in some cases, may develop malformations before entering the necrotrophic phase[6,7]. The pathogenicity of *M. roreri* strains from different origins has been assessed in plant-attached pods of various cultivars in which the necrosis was evidenced[8].

In addition to plant-attached pods, various diseases, including *Phytophthora* Pod Rot[9,10] and *Botryodiplodia theobromae* rot[11] can affect harvested cacao pods causing pod damage while becoming an inoculum source for spreading the disease. However, the pathogenicity of *M. roreri* from the different morphology groups has not been evaluated on harvested cacao pods.

In Ecuador, a significant proportion (about 7600 ha in 2007) of the cacao production is organic[12,13], and fungicides or other chemicals cannot be applied in the fields. As an alternative, the use of biological control agents[14] and the application of compost teas[15] have been proposed to control plant pathogens in organic plantations. Compost teas are usually obtained through fermentation of agricultural waste and effectively control several plant pathogens, including *M. roreri*[16]. However, the sensitivity to compost teas of *M. roreri* isolates belonging to the different morphology groups has not been reported.

This research aimed to assess harvested cacao pods' pathogenicity and the sensitivity to compost tea of *M. roreri* isolates from selected morphology groups.

Materials and methods

Isolation and classification of *M. Roreri*.

Isolates of *M. Roreri* were obtained as indicated in previous reports[5]. Briefly, mature cacao pods showing FPD symptoms, including pod lesions with white to creamy mycelium, and internal pod tissues showing necrosis[17], were collected from plantations located on Ecuador's coast. Healthy pods were also randomly collected and analyzed as controls. Sampled pods that showed symptoms of other diseases were not considered for this study.

Sampled pods were cut into small sections and placed onto MEA (malt extract agar, Oxoid) and incubated at 27°C for 48 h to allow the growth of *M. roreri*. Mycelia were then transferred to fresh MEA plates and incubated at 27°C for 7 days. Meiospores formed on the MEA media were then mixed in water (10^6 meiospores/mL), and 10 µL of the mix were transferred to WA (water agar, Oxoid) and incubated at 27°C for 24 to 48 h. Using a stereomicroscope, germinated meiospores were selected and transferred to MEA plates followed by incubation at 27°C for 20 days. Mycelia samples were then transferred to MEA and incubated at 27°C for 15 days. The identity of each isolate was confirmed by PCR amplification and sequencing of the ITS region. All of the obtained isolates were classified into one of the 14 morphology groups defined in previous reports[5] using the classification scale shown in Table 1.

Pathogenicity assessment

Pathogenicity tests were conducted on harvested pods using the dry conidia method[18]. Briefly, *M. roreri* meiospores were attached to a pin's head and deposited onto a two square centimeters area of a cocoa pod previously marked and moistened

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Morphology group	PDA colony description	Isolates obtained
1	Cream-salmon only	11
2	Intercalated Dark brown and cream-salmon	11
3	Cream-salmon to light brown to white	6
4	Dark brown to light brown to white	3
5	Dark brown to cream-salmon to white	9
6	Dark brown to cream-salmon	8
7	Dark brown only	7
8	Light brown to white	2
9	Cream-salmon to white	3
10	Cream-salmon to light brown	3
13	Dark brown to light brown	7
14	Cream-salmon to white	12
Total samples		82

*Samples from groups 11 and 12 were not obtained.

Table 1. Isolates sampled from each of the morphology groups defined by Maridueña-Zavala (2016)*.

with sterile water. Inoculated pods were placed in a moist chamber to promote the germination of conidia. External severity of symptoms was assessed using the disease severity scale (SS) from 0 to 5 based on previous studies[8] and shown in Table 2.

The disease severity index (DSI) was calculated using Equation 1[19].

$$DSI = \sum_{i=0}^{5} SS_i \times n_i / N \; x \; 100\ \% \qquad 1$$

Where n_i is the number of pods showing symptoms at the severity scale i, and N is the total number of pods inoculated.

Values of DSI were recorded at 0, 3, 7, 12, and 16 days after inoculation, and the area under the disease progress curve (AUDPC) was estimated using Equation 2[20]:

$$AUDPC = \sum_{i=1}^{N_i - 1} \frac{DSI_i + DSI_{i+1}}{2} (t_{i+1} - t_i) \qquad 2$$

Where the DSI values were recorded at the time intervals ti, all the experiments were run in triplicate.

Sensitivity to compost tea

A compost tea with antifungal properties was prepared exactly as described in previous reports[15]. Compost tea doses of 0, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5 and 5 % were prepared in PDA. Three-millimeter disks of *M. roreri* mycelia from morphology groups 3, 8, and 14 were transferred onto the PDA containing the different compost tea concentrations. The plates were then incubated at 27 °C, and the mycelial diameter was measured at 3, 6, 9, and 12 days after inoculation. Other morphology groups were not tested as the preliminary data suggested no significant differences in the sensitivity to compost tea among the different groups.

Statistical analyses

Analysis of variance ANOVA compared values of AUDPC obtained by each morphology group tested, and significance was reported for $P < 0.05$. Inhibitory concentrations 50 (IC 50) were estimated from the mycelial growth data obtained at the different compost teas concentrations using Prism 7 software (GraphPad, La Jolla, CA 92037 USA). T-test was used to compare the means of IC50, and significance was reported at $p < 0.05$.

Results

A total of 82 isolates were obtained and the identity was

Scale	Symptoms
0	No symptoms
1	Few and small oil spot
2	Plenty and clear Oil spot, deformation or irregular mature.
3	Necrosis without sporulation
4	Necrosis and sporulation in less than the quarter part of the fruit.
5	Necrosis and sporulation in more than the quarter part of the fruit.

Table 2. Severity scale of cocoa pods infected with *M. roreri*.

confirmed as *M. roreri* by the DNA sequence of the ITS region.

Pathogenicity test

All isolates could infect the harvested cacao pods and cause identical symptoms to FPD reported in plant-attached pods (Figure 1). Isolates from morphology groups 1, 2, 4, 6, 8, 10, 13, and 14 caused the first FPD symptoms 3 days after pod inoculation, whereas pods inoculated with isolates from groups 3, 5, 7 and 9 showed FPD symptoms from day 7 onwards (Figure 2). Similarly, all isolates produced DSI of 75 % or higher within 16 days after the pathogen's inoculation on the harvested pods (Figure 2) and yielded AUDPC values of 25 or above (Figure 3). The lowest AUDPCs were caused by groups 3 and 5 ($P < 0.05$) while the rest of the groups produced similar AUDPC values (Figure 3), but no correlation was found between the AUDPC values and the morphology groups.

Compost tea significantly inhibited the growth of *M. roreri* at concentrations of 1 % or above and was able to fully inhibit the growth of all isolates tested at 4.5 % or higher (Figure 4). The IC50 values were above 4 % for all isolates tested (Figure 5), but no significant differences were found when comparing the IC50 values of the different morphology groups.

Discussion

Most isolates caused the first FPD symptoms on day 3 af-

Figure 1. Typical FPD symptoms produced on harvested cacao pods inoculated with *M. roreri*.

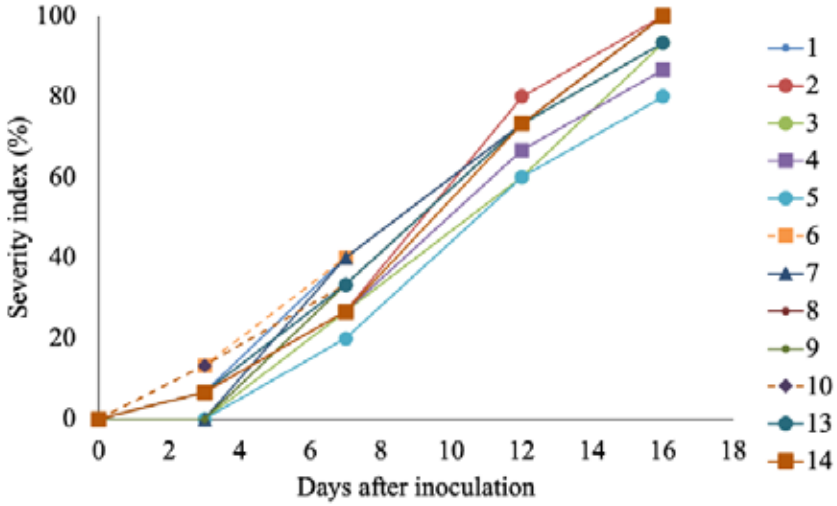

Figure 2. FPD disease progression on harvested cacao pods inoculated with *M. roreri* isolates belonging to the morphology groups described in Table 1.

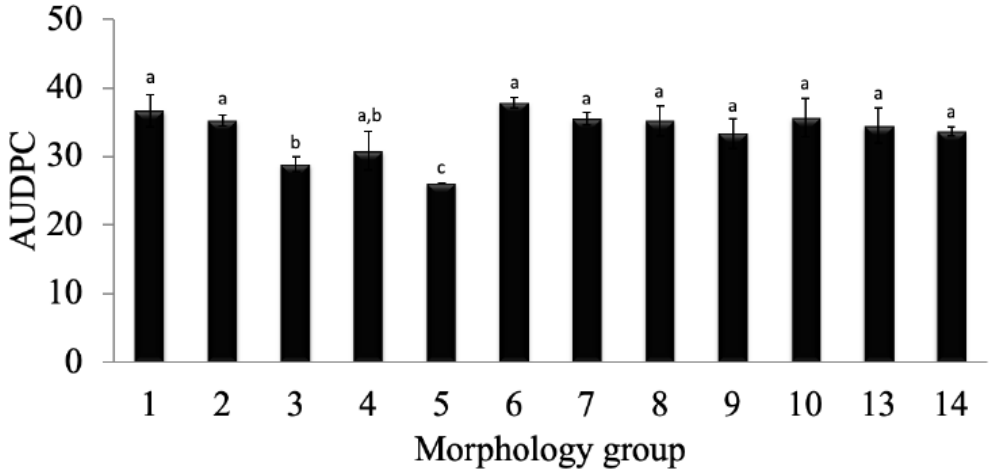

Figure 3. Values of the area under the disease progress curve (AUDPC) for *M. roreri* groups are described in Table 1. Different letters represent significant differences at $p < 0.05$

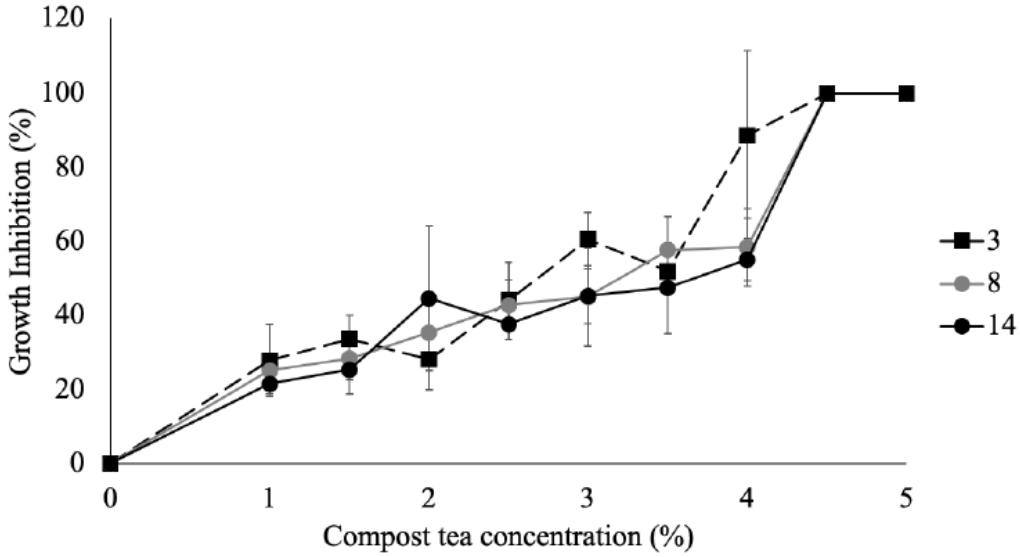

Figure 4. Growth inhibition of *M. roreri* from morphology groups 3, 8, and 14 by compost tea.

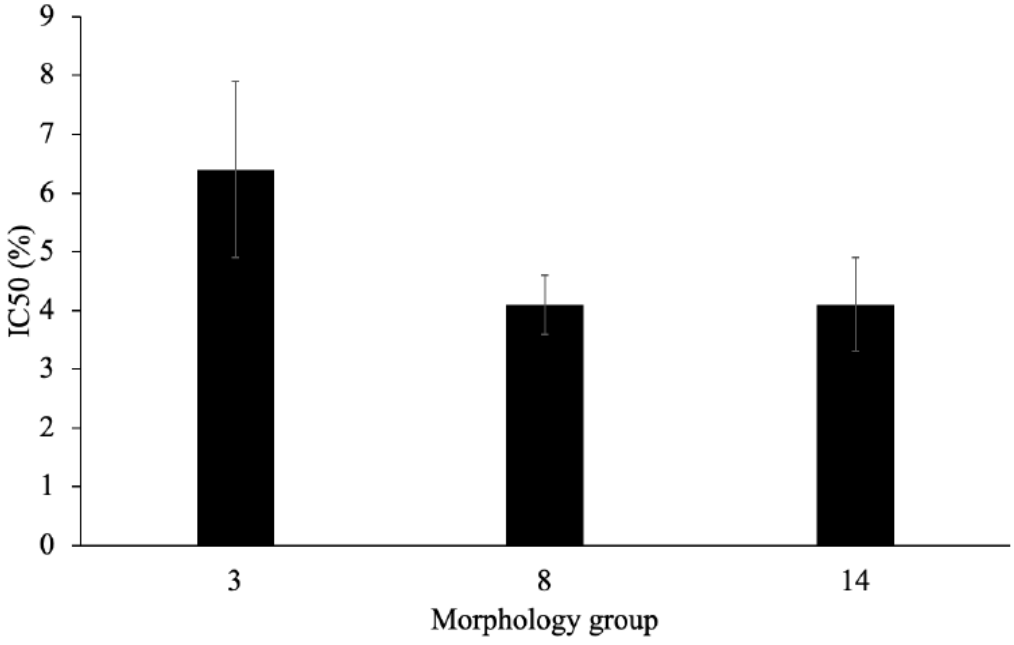

Figure 5. Inhibitory concentration 50 (IC50) values of *M. roreri* from morphology groups 3, 8, and 14 by compost tea.

ter inoculation and all isolates were able to fully produce the FP symptoms on the harvested cacao pods within 16 days after inoculation. This disease progression is significantly faster than that observed for plant-attached pods, in which progression times of 9 weeks[7,8] to 3 months[6] have been reported. The data suggest that the asymptomatic-biotrophic phase of FPD was absent or significantly shortened in harvested ripe cacao pods inoculated with *M. roreri*. Results are similar to those observed in other fungal pathogens such as *Colletotrichum spp.* When infecting ripe harvested fruits, *Colletotrichum spp.* can produce fruit rot with a shortened biotrophic phase. However, this phase can be extended if *Colletotrichum spp.* infects unripe harvested fruits[21]. Further research is needed to assess the pathogenicity of *M. roreri* in harvested cacao pods of different ripening levels.

No correlation between pathogenicity and fungal morphology was observed. Results agree with previous reports in which isolates of *M. roreri* with different levels of aggressiveness showed no correlation with genetic variation[8]. Additionally, various fungal species' pathogenicity can be dissociated from morphological switching and in vitro growth rate[22].

The compost tea significantly inhibited the growth of all *M. roreri* isolates at concentrations of 1 % or above, reaching full inhibition at concentrations of 4.5 % or greater. The compost tea concentrations effective against *M. roreri* were lower than the 5 to 20 % needed for the control of other fungal pathogens such as *Alternaria solani*[23], *Alternaria alternate*, *Botrytis cinereal*, and *Pyrenochata lycopersici*[24] but higher than the 2.5 % required to inhibit the growth of *M. perniciosa*[25], suggesting an intermediate to high sensitivity of *M. roreri* to compost tea. Further research is needed to assess the sensitivity of *M. roreri* to compost tea in the field.

Conclusions

All the morphology groups of *M. roreri* were able to produce FPD necrotrophic symptoms in harvested pods during the 16-day evaluation period. Therefore, the biotrophic phase of the disease was likely absent or significantly shortened. Compost tea effectively controlled the pathogen's growth *in vitro* at concentrations from 1 to 4.5 %, showing the potential for applications in organic plantations. This is the first report presenting M. roreri isolates' pathogenicity from different morphology groups on harvested cacao pods.

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Not applicable.

Competing interest

There are no competing or financial interest associated to this research.

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