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Control Alternatives for Dactylonectria Torresensis in Blackberry

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Black foot disease (Dactylonectria torresensis) in blackberry cultivation (Rubus glaucus) is a recent problem in Ecuador, where its management has based on agrochemicals, which can be harmful to health and the environment. This study aimed to evaluate more sustainable alternatives for controlling the disease caused by this pathogen. Initially, different propagules were evaluated to induce the disease in plants of blackberry Castilla. Subsequently, biological products (three strains of Trichoderma spp.), organic products (flavonoids and Myrtaceae extract), and chemical products (azoxystrobin, propiconazole, carbendazim, and copper sulfate pentahydrate) were tested under greenhouse conditions. The main results indicated that carbendazim, Myrtaceae extract, Trichoderma spp., propiconazole, and copper sulfate pentahydrate were effective in reducing the pathogen severity, showing root collar necrosis percentages of 21.25%, 35.50%, 43.58%, 44.92%, and 47.92%, respectively, which was, significantly lower than the untreated control, which presented 73.75%. This study provides valuable guidance for developing more sustainable and effective management strategies for controlling black foot disease in blackberries, integrating the identified products into a fungicide rotation scheme as a component of integrated crop management.
Keywords: PLA, Al2O3, Nano foils, Bio-degradation,Thermal Stability.

1. Introduction

The blackberry (Rubus glaucus Benth) is a native fruit of the Andes with economic importance in Ecuador, producing 29,920 tons per year. It is cultivated in the highlands, mainly in Bolívar (1,423 ha), Cotopaxi (956 ha), and Tungurahua (775 ha) (SIPA, 2023).

Fresh and processed fruit offers many marketing opportunities at both national and internationally; however producers face several problems in its cultivation, primarily phytosanitary issues, which reduce the yield and fruit quality (Saldarriaga et al., 2017). Among the diseases affecting blackberries is black foot disease. In 2017, in the provinces of Tungurahua and Bolívar, this disease had an incidence of 13.3%, causing significant economic losses for blackberry farmers. Dactylonectria torresensis is a fungal species identified as one of the main causes of black foot in blackberry. It is characterized by necrotic lesions in the vascular tissue, leading to loss of turgidity, wilting of basal leaves, foliar chlorosis, collar and root rot, and lack of sprounts; all these symptoms hinder plant development and affect productivity (Sánchez et al., 2019).

D. torrescensis can also affect other fruit crops such as vineyards, apple trees (Manici et al., 2021), strawberries, and raspberries (Weber & Entrop, 2017). The incidence of this disease can lead to significant economic losses, and in the absence of effective control measures, farmers often apply more aggressive and frequent phytosanitary treatments, which negatively impact the blackberry value chain. In addition, the fruit contains high levels of agrochemical residues, which not only reduce export opportunities but are also harm health and the environment (Cardona et al., 2017). For this reason, it is important to find products that control the disease to develop effective management strategies.

In vitro studies showed that carbendazim, azoxystrobin, and Myrtaceae extract had a fungistatic effect on Dactylonectria torresensis, because they prevented the fungal mycelial growth (Racines-Oliva et al., 2019). The application of biological products such as Trichoderma spp. is also a valid option for management this disease, due to they compete with other microorganisms for space and nutrients, inducing plant defense mechanisms through the secretion of volatile antibiotics and/or diffusible metabolites that modify soil conditions, and promote plant nutrition and growth, while contributing to environmental conservation and encourage the sustainable of blackberry cultivation (Bleach, 2013; Hernández et al., 2018; Martínez et al., 2019).

Consequently, the objective of this study was to evaluate the effectiveness of organic, biological and chemical products for managing D. torresensis in blackberries, under controlled greenhouse conditions.

2. Materials and methods

Study location and strain used

The study was conducted at the Santa Catalina Experimental Station of INIAP, located in the Mejía canton, Pichincha province, Ecuador. The average temperature at the laboratory was 17°C with an average relative humidity of 71%. The strain of Dactylonectria torresensis (UFAH00033) used in this study was isolated from symptomatic blackberry plants collected in the Tungurahua province and previously identified by Sánchez et al. (2019).

Planting conditions and symptom evaluation in blackberry plants under greenhouse conditions

All blackberry plants were planted in black polyethylene bags containing 2 kg of sterilized soil substrate with mixture of black soil and pumina in a 3:1 ratio. The plants were maintained in a greenhouse at an average temperature of 18°C and relative humidity of 62% for sixteen weeks. After inoculation, symptoms were evaluated both above-ground and at the root collar. The variables assessed included above-ground and root dry weight, root length, chlorophyll concentration index in middle and basal leaves, percentage of wilting in basal leaves, and percentage of necrosis in the root collar.

Evaluation of D. torresensis inoculation methods

Under greenhouse conditions, six inoculation methods of the pathogen were tested on three-month-old blackberry plants. The methods included: chlamydospores, mycelium (Probst, 2011), and conidia (Ferro, 2008), applied with and without wounding of the roots, along with their respective controls. A suspension of 1 x 10^6 conidia or chlamydospores per mL were used for the inoculation. A completely randomized design (CRD) was employed with 9 plants per treatment, in total 108 plants. The variables evaluated were the percentage of wilting in basal leaves and the percentage of necrosis at the root collar using the 0 to 4 scales mentioned by Sánchez et al. (2019). Due to the lack of normality and homogeneity in the data, the non-parametric Kruskal-Wallis test was used. The inoculation method that presented the highest incidence of the disease was used in subsequent tests to evaluate the effectiveness of biological, chemical, and organic products.

Evaluation of the efficacy of commercial products based on Trichoderma spp.

Three commercial products based on Trichoderma spp. (B1, B2, B3) were tested, which met the quality control parameters established by the INIAP - ESSC Biological Control Laboratory (Báez et al., 2019). Their antagonistic capacity was evaluated using the dual culture technique. D. torresensis was taken from a 15-day-old monosporic culture, while Trichoderma spp. was taken from 8-day-old monosporic cultures, derived from the commercial products. The strains were planted with an 8-day difference to adjust the experimental conditions to the specific growth characteristics of each fungus. Daily evaluations of the growth radius of D. torresensis were conducted, and the percentage of radial growth inhibition (PICR) was calculated (Fernández & Suárez, 2009). Products that showed an percentage of inhibition ≥ 60% (García, 2015) were selected for subsequent evaluation on blackberry Castilla plants under greenhouse conditions.

Each biological product was tested at three concentrations (10^3, 10^4, and 10^5 CFU.mL^{-1}) and at two application times (15 days before and 15 days after pathogen inoculation) to evaluate their effectiveness. Twelve treatments were implemented, corresponding to the interaction of the studied factors, plus two controls (with and without D. torresensis inoculation), using a completely randomized design in a 232+2 factorial arrangement with 12 observations per treatment. An analysis of variance was used to determine statistical differences between treatments, and Tukey test 5% to find differences between mean.

Evaluation of the efficacy of organic and chemical products for managing D. torresensis

Two organic fungicides were evaluated: flavonoids (1 $L.ha^{-1}$) and myrtaceae extract (2 $L.ha^{-1}$), along with four chemical fungicides: carbendazim (1.5 $L.ha^{-1}$), azoxystrobin (0.1 $L.ha^{-1}$), propiconazole (125 $g.ha^{-1}$), and copper sulfate pentahydrate (1.3 $L.ha^{-1}$) (Racines-Oliva et al., 2019). These fungicides were applied as drench directly to the soil. A completely randomized design with 12 observations per treatment was used. An analysis of variance was used to determine statistical differences between treatments, and Tukey test 5% to find differences between means.

3. Results and Discussion

Evaluation of D. torresensis inoculation methods

The three types of propagules used in this research were capable of inducing black foot disease in R. glaucus (Andean blackberry), resulting in both aerial and root symptoms. However, no statistically significant differences were found between conidia, chlamydospores, and mycelium; this is because these structures enable the pathogen to disperse in the soil and survive for extended periods (Pecchia et al., 2023). The percentage of wilting in basal leaves was 83.33% for conidia, 82.35% for chlamydospores, 55.56% for mycelium, and 20.28% for the control. Meanwhile, the percentage of infection at the root collar was 56% for conidia, 47% for chlamydospores, 33.3% for mycelium, and 0% for the control. This is consistent with the study by Probst (2022), which found no statistically significant differences in disease incidence caused by conidia, chlamydospores, and mycelium, with means of 82.3%, 84.4%, and 65.5% respectively, compared to the control at 20.5%.

On the other hand, no significant differences were found between treatments with root wounds and those without root wounds. Therefore, conidia inoculation without root wounding was chosen, as inoculation method because it mathematically showed the highest percentage of infection (56%) at the root collar. This might be related to the fact the emergence of secondary roots, soil particles abrasion, and even the presence of nematodes create small openings that facilitate pathogen entry into the plant (Menkis & Burokiene, 2012; Guzmán et al., 2012), making mechanical wounding unnecessary.

In vitro evaluation of the efficacy of commercial products based on Trichoderma spp.

The bioproducts containing Trichoderma spp., under in vitro conditions, inhibited pathogen growth from day 4 to day 31. The strain from product B3 showed 68.97% inhibition (Figure 3), even growing over the surface of the D. torresensis colony. These interactions often correspond to mycoparasitic actions, including adhesion and wrapping of Trichoderma spp. hyphae around pathogen hyphae at the microscopic level (Hebbar & Samuels, 2015). These results are slightly higher than those obtained by Van Jaarsveld et al. (2019), who evaluated 10 Trichoderma spp. strains and reported inhibition percentages of D. torresensis (isolated from grapes) ranging from 8.3% to 66.94%.

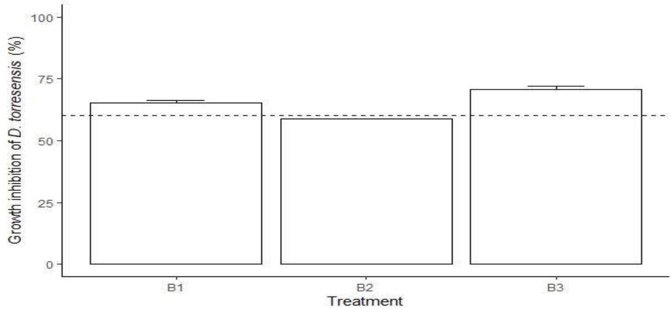

Figure 3. Percentage of inhibition of D. torresensis growth at 31 days of in vitro evaluation using Trichoderma spp. (the dashed line represents the 60% selection criterion according to García, 2015).

Competitive growth and antibiosis through the production of volatile and diffusible compounds are modes of action of some Trichoderma species to inhibit the growth of other microorganisms, which can be observed by confronting pathogens and antagonists on Petri dishes (Hebbar & Samuels, 2015).

Greenhouse evaluation of the efficacy of biological, organic, and chemical products for the managing D. torresensis

The application of all treatments with Trichoderma spp. significantly reduced root collar necrosis caused by the inoculation of D. torresensis, with means ranging from 39.82% to 52%, compared to the untreated inoculated control (73.75%) (Figure 4). Although there were no significant differences between the treatments (biological products and concentrations), all showed statistically differences compared to the |control; which suggests that all treatments were effective compared to the control. The results would be indicating that as the concentration of the control agent increased, the percentage of vascular tissue damage decreased. This is possibly due to greater root colonization and the antagonistic activity of Trichoderma against pathogens, which may contribute to the protection of vascular tissue (Van Jaarsveld et al., 2019).

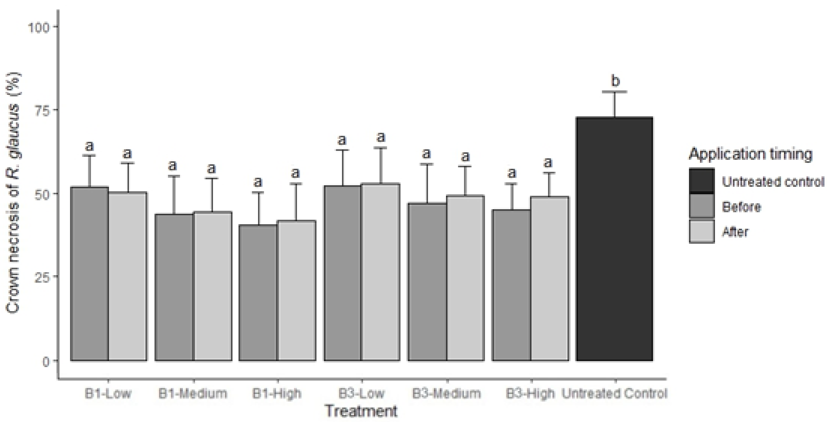

Figure 4: Effect of two commercial products based on Trichoderma spp., applied at three

concentrations for the control of root collar necrosis in R. glaucus Benth 16 weeks after D. torresensis inoculation. Letters in common are not significantly different, Tukey's post hoc test ($p<0.05$).

Although the Trichoderma spp. products were not sufficient to prevent infection caused by D. torresensis, a degree of protection was achieved as the percentages of root collar necrosis were significantly lower compared to the control. These results were similar to those obtained by Martínez-Diaz et al. (2020), where the incidence percentage of blackleg disease in grapevine treated with Trichoderma spp. was lower (40.2%) compared to the control (61.5%). On the other hand, Van Jaarsveld et al. (2019) reported disease incidence percentages in grapevines ranging from 1.00% and 2.50% in plants treated with Trichoderma spp. in contrast to the control (6.50%).

Studies conducted by Ahmed & El-Fiki (2017) demonstrated that the inoculation of T. harzianum in strawberry plants reduced the incidence of root rot disease under field conditions and increased nitrogen percentage (3.36) and total chlorophyll (32.62) compared to the control, which had 0.98 and 22.03, respectively. In the present study, it was also shown that the biological products B1 and B3 contributed to the increase in chlorophyll concentration index in middle leaves, with averages of 13 and 13.7, respectively, compared to the control (10.68) (Table 2).

Table 2: Effect of biological, organic, and chemical products on the phenology and biomass of Rubus glaucus Benth 16 weeks after inoculation with D. torresensis.

Treatments	Shoot Weight (g)	Root Weight (g)	ICM	Wilting (%)
Trichoderma 1	11.81 ± 3.33 a	4.18 ± 1.23 a	13.70 ± 2.63 c	61.17 ± 23.80 cd
Trichoderma 2	11.30 ± 3.40 a	4.17 ± 1.18 a	13.00 ± 2.14 bc	57.83 ± 21.57 cd
Myrtaceous extract	12.67±1.41 ab	4.37±0.70 ab	11.82±1.99 abc	46.92±14.33 bc
Flavonoids	15.37±1.56 bc	5.28±1.34 ab	12.16±1.49 abc	66.83±22.38 cd
Azoxystrobin	11.95±1.92 a	4.75±1.40 ab	11.02±1.04 ab	62.25±13.04 cd
Carbendazim	16.02±3.40 c	5.29±1.37 ab	11.06±1.86 ab	34.83±12.77 ab
Propiconazole	11.36±2.19 a	3.97±1.29 a	10.98±1.62 ab	44.25±11.19 bc
Copper sulphate	10.22±0.95 a	3.83±0.81 a	11.13±1.86 ab	63.92±20.52 cd
Inoculated control	11.36 ±1.65 a	4.43±0.99 a	10.68±1.51 a	69.25±26.20 d
Witness	15.76±2.93 c	5.19±0.81 b	10.83±2.11 abc	21.67±11.09 a

ICM: Chlorophyll index in middle leaves. Letters in common are not significantly different, Tukey's post hoc test ($p<0.05$).

Regarding organic synthetic products, the Myrtaceae extract showed better results than flavonoids, presenting 35.5% of root collar necrosis and 46.92% basal leaf wilt (Table 2).

Regarding the chemical synthetic products, the application of carbendazim was the most effective for controlling D. torresensis, showing a root collar necrosis percentage of 21.25% and a basal leaf wilt percentage of 34.83% compared to the control that showed 73.75% of necrosis and 69.25% wilt (Table 2 and Figure 5).

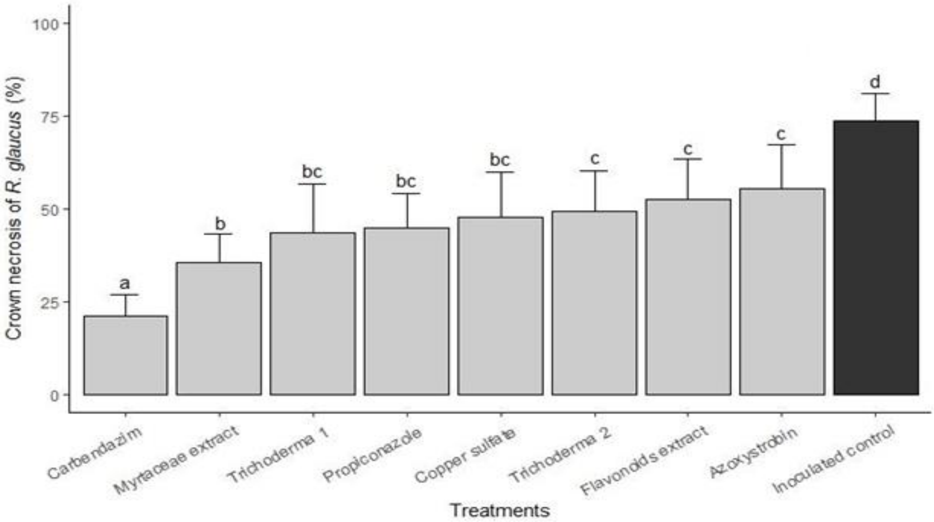

Figure 5: Effect of biological, chemical, and organic products on the percentage of root collar necrosis in Rubus glaucus Benth 16 weeks after D. torresensis inoculation. Letters in common are not significantly different, Tukey's post hoc test ($p<0.05$).

Although carbendazim achieved better disease management, it is important to note that it belongs to the benzimidazole group and is classified by FRAC as a fungicide with a very high risk of resistance due to its specific or single-site mode of action (FRAC, 2023). Studies by Sun et al. (2010), Di et al. (2015), and He et al. (2020) have shown that continuous use of carbendazim can led to pathogen resistance.

Nevertheless, despite the superiority of chemical control, it is crucial to promote an integrated pest management (IPM) approach that incorporates sustainable practices. The combination of chemical, organic, and biological product applications has been reported by various researchers to be more effective in disease management (Rego et al., 2006; Gaviria-Hernández et al., 2013; Bleach, 2013; Halleen & Fourie, 2016). Therefore, combining biological, organic, and chemical control methods not only maximizes effectiveness against the management of D. torresensis but also minimizes environmental impact and promotes long-term ecosystem health.

4. Conclusions

This study showed that the biological, organic, and chemical treatments evaluated could reduce the D. torresensis infection in blackberry plants because all treatments showed lower disease infection than the control, showing their potential to be included in a management strategy for this disease. Therefore, it is recommended to implement an integrated management approach that allows a sustainable cultivation, optimizing the use of chemical inputs alongside with eco-friendly alternatives.

However, these treatments should be evaluated under commercial cultivation conditions and over an extended growing period to observe their performance under open-field conditions.

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