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PRELIMINARY STUDIES ON THE ECOLOGY AND MANAGEMENT OF BANANA CROWN ROT FUNGI IN THE DOMINICAN REPUBLIC

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ABSTRACT: Banana crown rot (CR) reduces the quality of exporting banana (AAA) in the Dominican Republic. Annual losses of organic bananas are estimated about US$ 200,000 due to fruit damages caused by CR. So far, CR has been attributed to biotic as well as abiotic factors. To determine the relationship among fungi from washing water and CR symptoms on organic banana we conducted samplings of water in washing stations in organic banana plantation in the Southwest Region of the Dominican Republic. Water samples were collected, processed and isolated on selective Mathur's medium. Isolates of *Colletotrichum* spp., *Fusarium* spp. and *Curvularia* spp. were identified. Isolates of these fungi were inoculated on unripe banana fruits to test for pathogenicity. Isolates of *Colletotrichum* spp. and *Fusarium* spp. caused symptoms similar to CR developed under natural conditions. Currently, the efficacy of organic acids products and other alternative control is being investigated.

Key words: Ecology, Banana crown rot, Management, Fungi, Dominican Republic.

INTRODUCTION

The Dominican Republic is the most important exporter of organic banana (Cavendish AAA) to the European Community Countries, exporting an average of 150,000 boxes accounting for an income of US$ 55 millions for organic banana growers (Proyecto Mejoramiento de la Calidad del Banano, 2002).

The banana growers, associated to the European Fairtrade Market face numerous problems that cause them to meet only 55% of the market demand. Most of their losses are brought about by shipment rejection at arriving ports, mainly due to Crown Rot (CR) and Fruit Ripening (FR). Growers are at risk of losing the European market if these problems are not corrected promptly (Proyecto Mejoramiento de la Calidad del Banano, 2002).

Banana crown rot has been previously associated to several fungi: *Fusarium roseum, Colletotrichum musae, Botryodiplodia theobromae, Verticillium theobromae, Ceratocystis paradoxa* and *Phomosis* spp., among others (Burden 1967, 1968; Green and Goss, 1963; Lukezic and Kaiser, 1966; Stover, 1972; Snowdon, 1990; Sommer and Donald, 1992; Ploez et al., 1994). Research conducted in Central America by Green and Goos (1963) found that *Fusarium roseum, Verticillium theobromae* and *Colletotrichum musae* were the most prevalent fungi.

Research conducted in the French Antilles concluded that isolates *C. musae*, associated to banana flower also caused post harvest diseases of export banana (De Lapeyre et al., 1997, 1998, 2000). Under conventional agriculture, banana crown rot is managed with fungicides, such as thiabendazole, imazalil and bitertanol, among others. With time, fungi associated with banana diseases have developed resistance to most of them (De Lapeyre and Dubois, 1997). Work conducted in the Dominican Republic by White (2000), provided information on the occurrence

of post harvest damage to export banana and attributed that to deficient post harvest management and further infection by species of *Fusarium*, *Colletotrichum*, *Aspergillus*, *Penicillium*, *Alternaria*, *Pestalotia*, *Trichoderma*, *Geotrichum*, *Nigrospora*, and *Curvularia*.

Because of the restrictions and regulations established for organic banana management, growers do not use post harvest chemical treatment but wash the fruits prior to packing with tap water amended with natural latex removers and organic acids. This practice however, has not been experimentally tested to determine if it prevents the growth and dissemination of fungi associated with crown rot.

The objective of this study was to sample, isolate and identify fungi present in the water in washing stations of organic banana fruits, previous to packing and exporting, in the province of Azua, Dominican Republic. As well as determine if these fungi are capable of causing crown rot by pathogenicity test.

MATERIALS AND METHODS

Sampling

Sample collections were conducted from May to October, 2003. Four main washing stations, with a capacity of about 5000-10,000 gallons of water, were sampled. Samples were collected every eight days, during four months. Five hundred ml of water per sample were collected in sterilized glass vials. Samples were collected at four points in the station, these were 1) main pipe that feeds the washing tank, 2) tank of latex washing, 3) washing tank with $AL_2(SO_4)_3$ and 4) pre-packing washing tank. Samples were kept at 15^0 C.; processed after 48 hours and poured into Petri dishes with Mathur's medium.

Fungal Isolation

Fungal isolations were performed using a modified method by De Lapeyre et al., (2000), who characterized *Colletotrichum* sp. population from banana flowers. Sub samples of twenty-five ml water were taken from the original sample and centrifuged at 4,000 rpm. Aliquots of 200 µl of precipitates were evenly dispersed on the surface of Petri dishes with Mathur's medium ($(MgSO_47H_2O$ 2.5 g, 2.7 g KH_2PO_4 , peptone 1 g, yeast extract 1g, sucrose 10 g, agar 15 g, rifampicin 100 mg, ketoconazole 0.8 g, in 1L of distilled water), there were four replicates per sub sample. Plates were incubated for seven days at 25-27^0 C for colony growth counts and identification of fungi.

After populations were counted, we selected representing the predominant cultural types that grew on the medium for further pathogenicity test.

Pathogenicity Test

To test pathogenicity, we selected five isolates. These isolates were C1 = *Colletotrichum* spp. (lacking setae), C2 = *Colletotrichum* spp. (with setae), F1 = *Fusarium* spp. (with macro and microconidia), F2 = (macroconidia only) and CV1 = *Curvularia* spp. These isolates were transferred to Mathur's medium, for inoculum production. These were inoculated on unripe banana crowns collected at packing sites a day before inoculations. Inocula as conidia suspensions were prepared by scraping the surface of the plate and mixing it in 100cc of sterile

distilled water One hundred µl of the suspension was used for inoculations. The average conidia concentration inoculated varied with the treatment, therefore, values ranged from 1.5 x 10^6 conidia /ml for *Curvularia* to 9.1x 10^7 millions for *Fusarium.*, whereas isolates of *Colletotrichum* (with and without setae) averaged values of 3.6 and 6.5 x 10^6 conidia/ml, respectively.

Before inoculation, banana crowns were surface disinfected with 50% alcohol and then the surface tissue aseptically removed. Each fungal isolate was inoculated on individual banana crowns. After inoculation the surface was covered with plastic polyetilene film (cling wrap) to keep moisture as well as avoid contamination. A randomized complete block design was used with 7 treatments and 5 replications: C1, C2, F1, F2, and CV1. An additional treatment with a mixture of all five fungi was also inoculated. Control treatments were inoculated with sterilized distilled water and covered as indicated above. Inoculated fruits were incubated at 25-27^0 C and >80 % moisture in the dark for 18 days.

Evaluations were conducted after 12 and 18 days; which is a period similar to the shipment from the farms in the Dominican Republic to the European ports. A rating scale developed by De Lapeyre (personal communication, 2003) was used, where 0 = no symptoms, 1 = less than 25 % colonized surface, 2 = less than 50 % of colonized surface, 3 = less than 75 % colonized surface and 4 = more than 75 % colonized surface. Other criteria used were necrotic tissue extension, yellow halo around the crown, premature ripening of fruits, presence or absence of fungal mycelia and exudates on crown surface.

RESULTS

Samplings and fungal isolations

Fungi belonging to the species *Colletotrichum* spp., *Fusarium* spp. and *Curvularia* spp. were isolated from different water sources and washing stations sampled.

Isolates of *Colletotrichum* spp. (C1) on Mathur's medium, presented salmon orange spore masses, abundant conidia and acervuli lacking setae. These C1 isolates were the most common. Other isolates of *Colletotrichum* spp. (C2) showed abundant white mycelia, and dark at the center of the colony and with pink borders and acervuli with setae.

Isolates of *Fusarium* spp. (F1) developed yellow pigmented colonies, and abundant macroconidia as well as white mycelia on the surface of the colony. F2 isolates of *Fusarium* spp. developed few macroconidia, abundant microconidia, and yellow to brown color at the center of the colony.

On the other hand, isolates of *Curvularia* spp. (CV1 and CV2) developed few conidia and colonies were dark brown with gray borders and abundant dark mycelia. Table 1 summarizes the results of population counts and fungi isolated for each sample date.

Pathogenicity Test

Isolates C1 and C2 of *Colletotrichum* spp. and isolate F2 of *Fusarium* spp. caused crown rot on inoculated banana fruits. Symptoms started to develop six days after inoculation. After twelve days, treatments with isolates C1 and C2 were the most affected inducing typical symptoms of crown rot, reaching up to grade 4, the highest level of the scale (Table 2). Furthermore, these isolates produced white mycelium on crown surface, yellow halo around the crown, as well as peduncle and crown necrosis (Figure 1).

Likewise, isolates of *Fusarium* spp. presented crown surface necrosis and fruit yellowing. Nevertheless, for *Curvularia* spp. expressions of symptoms were not clear. Besides, the isolate mixture showed a light crown surface necrosis; while the control treatment produced dark brown exudates on crown surface without yellowing or necrosis, of the adjacent tissue.

Eighteen (18) days after inoculation, isolates C1 and C2 induced complete fruit ripening and necrosis of the crown with abundant white mycelia developing on the surface (Figure 2). The F2 treatment also developed fruit ripening, crown necrosis and white mycelia on the surface. Isolate CV1 did not cause crown rot symptoms but slight necrosis. The mixture of fungal isolates did not cause fruit ripening, but surface rot and yellowing of the peduncles. Banana fruits in control treatment remained green even after eighteen days.

DISCUSSION

The most predominant isolates sampled at the washing stations in Azua, Dominican Republic were *Colletotrichum* spp. Our results agree with those by De Lapeyre et al., (2000), which indicated that isolates of *Colletotrichum* spp. were the most abundant fungi associated with crown rot and banana flowers. According to literature reports, *Colletotrichum musae* produces abundant conidia and acervuli lacking setae on culture. Isolates C1 obtained in our study had similar cultural characteristics; however, the identity of the species needs to be accurately determined.

According to De Lapeyre et al., (2000) *C. musae* is the most important fungus causing banana post-harvest diseases in the French Antilles.

In many countries, *C. musae*, as well as other fungal species has been reported as an important causal agent of severe banana crown rot (Green and Goos, 1963; Lukezic and Kaiser, 1966; Ogawa, 1968). It is important to notice that fungi isolated were only found in the washing tanks but not from the main pipe that feeds the tanks, which suggests that contamination may occur during transportation of the fruits from the field to the stations. In our study banana crown rot was associated with premature ripening of fruits.

Further research will be conducted to determine the occurrence of crown rot causing fungi in other banana growing areas of the Dominican Republic where crown rot has been associated to shipment losses.

Currently, the efficacy of organic acid products and other preventive treatment is being investigated.

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Table 1. Fungal genus and populations collected at the washing stations in organic banana farms. Azua. Dominican Republic.

Sample dates	Washing station	Section	Cfu/ml		
			Colletotrichum spp.	*Fusarium* spp.	*Curvularia* spp.
28/07/2003	Finca 6	2	$2x10^2$	0	0
	La Tina	3	"	0	0
10/08/2003	Finca 6	2	$2x10^2$	$2x10^2$	0
	La Tina	3	"	"	0
	La Tina	4	$6x10^2$	0	0
	La Tina 3	2	$2x10^2$	$4x10^2$	0
	La Tina 3	4	$6x10^2$	0	0
26/08/2003	Finca 6	3	0	0	$2x10^2$
	La Tina	3	$4x10^2$	0	0
	La Tina	4	0	$4x10^2$	$2x10^2$
	La Tina 3	3	0	0	"
	La Tina 3	4	0	0	0
	Estebanía	2	0	$2x10^2$	0
	Estebanía	3	$4x10^2$	0	0
14/09/2003	Finca 6	2	$2x10^2$	0	0
	La Tina	4	$4x10^2$	0	0
	Estebanía	2	0	$2x10^2$	0

Section:
2: Tank of latex washing
3: Washing tank with $Al_2(SO_4)_3$
4: Pre – packing washing tank

Table 2. Disease Reaction based on scale by De Lapeyre (2003).

Treatments	Average
C1	4
C2	3.8
F1	3.2
F2	3.6
CV1	1
Mixture of all	3
Control	0

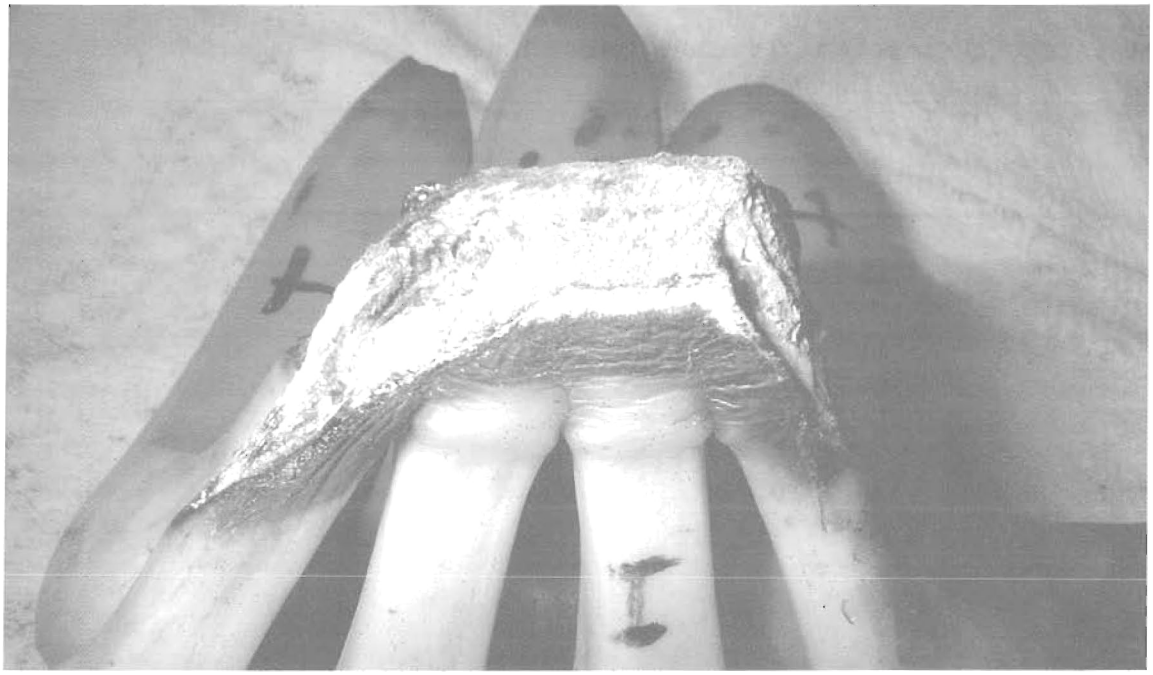

Figure 1. Typical symptoms of crown rot, developed twelve days after inoculation with *Colletotrichum* spp. (C1).

Figure 2. Crown rot development after 18 days. Control treatment is shown at the center of picture.