Pathogenicity of Fungi Associated with Crown Rot of Bananas in Latin America on Grande Naine and Disease-Resistant Hybrid Bananas

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ABSTRACT

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Fungi associated with crown rot of bananas were isolated and identified from fruit obtained from Mexico, Guatemala, Costa Rica, and Ecuador in October and November 1993. *Fusarium semitectum* and *Penicillium* spp. were isolated most frequently. In vitro and in vivo growth of five fungi isolated from Costa Rican bananas (*F. semitectum*, *F. moniliforme*, a *Penicillium* sp., *Gliocladium roseum,* and a *Gliocladium* sp.) was determined. The optimum temperatures for growth of *F. moniliforme*, *F. semitectum*, a *Penicillium* sp., a *Gliocladium* sp., and *G. roseum* were 24.3, >28.0, 21.8, 24.1 and 29.6°C, respectively. All fungi, except the *Penicillium* sp., grew profusely on the surface of crowns. After inoculation of crowns, *F. moniliforme* and *F. semitectum* caused the greatest amount of rot. Hybrids recently released by the Honduran Foundation of Agricultural Research (Fundación Hondureña de Investigación Agrícola, FHIA), FHIA 1 (Goldfinger) and FHIA 2, were partially resistant to the crown rot fungi. Isolates of *F. semitectum* and *Penicillium* sp. from Costa Rica grew on potato dextrose agar amended with 10 mg liter^{-1} of thiabendazole, which may indicate a reduced sensitivity of these species to thiabendazole.

Additional keywords: *Musa* AAA, postharvest disease

The practice of cutting banana (*Musa* AAA) hands from the stalk to package fruit for the commercial market provides an infection court for microorganisms to enter the crown tissue and cause a decay known as crown rot (7,9,15,16,23). It is the most important postharvest disease of bananas (14,21,22,26). Decay is usually confined to the crown but may spread into the pedicels of the fingers (23,25). Crown rot is unevenly distributed among the hands; healthy and rotted crowns are usually present in the same box. Crown rot is frequently seasonal, and has been associated with both hot, dry weather (7,23), and periods of wet weather (6). The disease increases with transit time, and spreads rapidly through the crowns during ripening (6,7,21) as fruit are transferred from 14 to 17°C. Many fungi and other microorganisms have been isolated from decaying crowns (7,9,11,13–16,21–23,25, 26). The most frequently isolated fungi include *Colletotrichum musae*, *Botryodiplodia theobromae*, *Cephalosporium* sp., *Ceratocystis paradoxa*, *Verticillium theobromae,* and *Fusarium semitectum* (6,8, 13,15,16,21–23,25,26). The frequency of the different pathogens as well as their importance as primary agents of decay may change according to the country of origin. To develop an effective control program it is important to know the causal agents of crown rot of bananas within a region and determine their potential development under shipping and ripening conditions.

Crown rot incidence increased with substitution of the variety Gros Michel for the Cavendish varieties in 1960. This variety was much more susceptible to mechanical damage and required boxing of individual hands to maintain quality. The removal of the fruit from the main stalk at the crown rendered these tissues susceptible to the invasion of various pathogens (20). The Honduran Foundation of Agricultural Research (Fundación Hondureña de Investigación Agrícola, FHIA) has released new banana hybrids with superior agronomic characteristics and resistance to Black Sigatoka (*Mycosphaerella fijiensis* Morelet) (3). However, little information about their susceptibility to crown rot and other postharvest diseases is available.

Crown rot control begins in the field with good sanitation; however, postharvest treatment with fungicides, such as thiabendazole (TBZ) and imazalil, is widely used in most banana-growing areas (7,20). Recently, there has been an important increase in the incidence and severity of crown rot in Costa Rica in spite of fungicide treatment (R. Romero, personal communication).

The objectives of this research were (i) identify the principal crown-rot fungi associated with bananas from Costa Rica, Ecuador, Guatemala, and Mexico, (ii) determine the rate of growth and pathogenicity of crown-rot fungi associated with Costa Rican bananas at storage and ripening temperatures, and (iii) determine the pathogenicity of crown-rot fungi on newly released banana hybrids with resistance to Black Sigatoka.

MATERIALS AND METHODS

Isolation and identification of microorganisms. Fruit were harvested in October and November, 1993 from banana plantations (*Musa* AAA, Cavendish subgroup, cv. Grande Naine) in Mexico, Guatemala, Costa Rica, and Ecuador and packed in commercial packing houses. Two boxes (18.14 kg each) from each country were shipped by overnight delivery from ports of entry in the U.S. to the Department of Horticultural Science, North Carolina State University, Raleigh. The time from harvest to arrival in Raleigh was 7 to 14 days. On arrival, the incidence of crown rot was nearly 100% in boxes received from all countries. The incidence of crown rot was higher than reported in most commercial shipments because we considered a cluster to have crown rot if any lesion, regardless of size, was observed. Six to eight banana clusters with moderate to severe crown rot were selected from each box from all countries for each shipment date. Affected tissue was surface sterilized with a 0.5% NaOCl solution for 30 s. Four small pieces of tissue per cluster were aseptically removed, placed on potato dextrose agar (PDA) (Difco, Detroit, MI), and incubated at 20°C for 1 to 3 days. When fungal growth from the tissue was visible, fungi were subcultured onto PDA to obtain pure cultures for identification. Isolations from some crowns yielded two or more fungi; crowns were scored as affected by all fungi isolated. Fungi were identified by comparing colony and spore morphologies to published descriptions (2,17,24). Isolates suspected to be *Fusari-*

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um spp. were sent to the Fusarium Institute, Pennsylvania State University, for identification.

In vitro growth. Five-millimeter-diameter agar plugs were removed from the edges of monosporic cultures of one representative isolate each of the *Penicillium* sp., *Fusarium moniliforme*, *F. semitectum*, *Gliocladium roseum,* and a *Gliocladium* sp., isolated from bananas from Costa Rica, and transferred to dishes containing PDA. Culture dishes were incubated in growth chambers in the dark at 12, 16, 20, 24, or 28°C. Two measurements of colony diameter were made at 90° angles daily for 7 days for each of three dishes per treatment. The design of the experiment was a randomized complete block with five replicates. The experiment was repeated once over time. Regression analyses were performed using SAS (SAS Institute Inc., Cary, NC, release 6.08).

In vivo growth at storage and ripening temperatures: Preparation of inoculum. Conidial suspensions of isolates of the *Penicillium* sp., *F. moniliforme*, *F. semitectum*, *G. roseum,* and the *Gliocladium* sp., used in the in vitro study, were prepared in sterile distilled water by gently scraping the surfaces of 7- to 10-day-old cultures grown on PDA and adjusting in the spore concentration to 10^5 conidia/ml. Filter paper disks (8 mm diameter) were autoclaved at 121°C for 15 min and soaked in the conidial suspension of each test fungus.

Inoculation. The crown tissue of single fingers that had not been treated with any chemical and which exhibited no visible crown rot symptoms was surface sterilized with a 0.25% NaOCl solution for 30 s and then rinsed in sterile distilled water. Just before inoculation, the crown face was recut aseptically and one filter paper disk, soaked in a conidial suspension of the appropriate fungus, was applied to the center of freshly exposed crown tissue and covered with Parafilm (American Can Co., Greenwich, CT) to retain moisture. Each fungus was inoculated to each of five crowns (single finger replicates) of bananas (*Musa* AAA, Cavendish subgroup, cv. Grande Naine). Controls consisted of fruit with filter paper disks soaked in sterile distilled water applied to the crown and wrapped as described above. Fruit were incubated in moist chambers at 14 and 17°C. These temperatures correspond to shipment/storage and ripening temperatures, respectively.

Prior to incubation, fruit were gassed with ethylene (300 mg liter^{-1}) to initiate ripening. Crown rot evaluations were made 10 days after inoculation. The amount of rot was determined by cutting the crown longitudinally and measuring the depth of rot into the pedicels, from the original cut surface (inoculation point).

The design of the experiment was a randomized complete block with five replicates. The experiment was repeated once over time. Analysis of variance and least square differences were performed using SAS (SAS Institute Inc., Cary, NC, release 6.08).

Pathogenicity on FHIA 1 and FHIA 2. Development of crown rot caused by isolates of *F. semitectum*, *F. moniliforme*, *G. roseum,* and *Gliocladium* sp., isolated from bananas from Costa Rica and used in previous studies, was determined following the same methodology described previously for the in vivo experiment. The *Penicillium* sp. was not included in this study since it was not pathogenic at either storage or ripening temperatures. Filter paper disks, soaked in the appropriate conidial suspensions, were applied to five crowns of FHIA 1, FHIA 2, and Grande Naine. FHIA 1 and FHIA 2 fruit were obtained from FHIA (La Lima, Honduras). Inoculated fruit were incubated in a chamber at the Southeastern Plant Environmental Laboratory, Raleigh, NC, maintained at 24°C and 90% relative humidity for 10 days. The design of the experiment was a randomized complete block with five replicates. The experiment was repeated once over time. Analysis of variance and least square differences were performed using SAS (SAS Institute Inc., Cary, NC, release 6.08).

Sensitivity to TBZ. The same isolates of fungi used in the in vitro and in vivo experiments were cultured on PDA amended with five different concentrations of TBZ (Mertect 340F): 0, 0.01, 0.1, 1.0, and 10.0 mg a.i. liter^{-1}. Five-millimeter diameter agar plugs were removed from the edges of monosporic cultures and transferred to dishes of PDA amended with the fungicide. Culture dishes were incubated in growth chambers in light at 24°C. Colony diameters were recorded 4 days after inoculation for each of 10 dishes per treatment. Two measurements of each colony were made at 90° angles. The percentage of growth reduction was determined, and the EC_{50} (50% effective concentration) was calculated by regressing the natural logarithm of the colony growth reduction (dependent variable, in percent) on the concentration of a.i. of TBZ (independent variable) and solving for x when $y = \log_e 50$. The design of the experiment was a randomized complete block with four replicates. The experiment was repeated once over time.

RESULTS AND DISCUSSION

Isolation and identification. The most frequently isolated fungi were *F. semitectum* Berk. & Ravenel and *Penicillium* spp. (Table 1). *Fusarium semitectum* was the most frequently isolated fungus from Costa Rica and Guatemala. *Penicillium corylophilum* Dierckx was isolated most frequently from bananas shipped from Ecuador. The *Penicillium* spp. isolated from other countries were not identified to species; however, growth of most isolates obtained from a country were similar in appearance on PDA indicating that only one

Table 1. Relative frequency of fungi associated with crown rot of bananas originating from Costa Rica, Ecuador, Guatemala, and Mexico

	Country of origin				
	Costa Rica[y]				
Fungi isolated	1	2	Ecuador	Guatemala	Mexico
Curvularia sp.				6.0[z]	
Colletotrichum sp.					10.0
Fusarium moniliforme	28.5	4.5	19.0	12.5	
G. roseum	3.5	13.5			
Gliocladium sp.	14.5	17.0			19.0
F. semitectum	50.0	39.0	4.0	63.5	33.0
Gliomastix sp.			4.0		
Penicillium spp.	3.5	17.0		6.0	33.0
P. corylophilum			50.0		
Pestalotia sp.				6.0	5.0
Acremonium sp.		4.5	8.0		
Nectria sp.			15.0		
Unidentified		4.5			

[y] 1 and 2 refer to different shipments from Costa Rica.
[z] Frequency based on isolations from 6 to 8 crowns from each of two boxes of bananas received from each site in October and November 1993.

Table 2. Depth of lesions (in mm) caused by fungi associated with crown rot in bananas (*Musa* AAA, cv. Grande Naine) incubated at 14 and 17°C after 10 days of inoculation

	Temperature (C)[y]	
Fungal species	14	17
Control (water only)	0.0 a[z]	0.0 a
Penicillium sp.	0.0 a	0.2 a
Fusarium moniliforme	1.3 b	4.8 bc
Gliocladium roseum	0.8 b	3.1 b
Gliocladium sp.	1.4 b	3.7 b
F. semitectum	2.6 c	6.1 c

[y] 14 and 17°C represent the shipment/storage and ripening temperatures, respectively.
[z] Means followed by the same letter in each column are not statistically different using protected least significant difference ($P < 0.05$). Each value is the mean of 25 crowns.

or a few species are associated with crown rot. Equal frequencies of *F. semitectum* and a *Penicillium* sp. were recorded from bananas of Mexican origin. Different *Fusarium* spp. have been reported as a primary cause of crown rot in many countries (6,8,9,11,13–16,21–23,25,26). *Penicillium* spp. have been associated only occasionally with crown rot disease (11,16) although they are very important postharvest pathogens in other crops such as oranges and apples (1). Other fungi, such as *Colletotrichum musae*, *Botryodiplodia theobromae*, *Cephalosporium* sp., *Ceratocystis paradoxa* and *Verticillium theobromae*, that have been reported to play an important role as causal agents of crown rot in other countries (6,8,13,15,16,19,21–23,25,26), were not isolated during this study.

In vitro experiment. Most isolates grew poorly at 12°C. However, colony diameter generally increased linearly with temperature from 12 to 20°C. The optimum temperatures for fungal growth, determined using the first derivative of the quadratic regression line of the colony diameter versus temperature on day 4, for the different fungal isolates were 24.3°C (*F. moniliforme*), 29.6°C (*G. roseum*), 24.1°C (*Gliocladium* sp.), and 21.8°C (*Penicillium* sp.). The optimum growth of *F. semitectum* could not be determined because it continued to increase in linear growth rate beyond 28°C, the highest temperature tested.

In vivo experiment. This experiment allowed us to determine not only the relative effect of storage and ripening temperatures on fungal growth in the banana crowns but also the pathogenicity of the genera and species used in the experiment. Depth of the lesions in crowns varied with the fungus used (Table 2); *F. semitectum* caused the most rot at both temperatures tested.

The amount of crown rot caused by different fungal isolates at 14°C was significantly less than at 17°C, indicating that shipment and storage temperatures are important in delaying the development of crown rot. Higher temperatures used in ripening as well as the change in physiological status of the fruit associated with ripening provide more favorable conditions for development of crown rot (6,7, 21). Additionally, the growth rate of all the fungi increased with temperature. In vitro tests indicated that the severity of crown rot is likely to increase as bananas are removed from ripening rooms to the supermarket shelf and finally to the consumer.

Both species of *Fusarium* and *Gliocladium* were pathogenic to bananas and both isolates of each genus grew profusely over the crown surface; *F. semitectum* caused the most serious rot. Additional samples are needed throughout the growing season to confirm that these two genera are the primary cause of crown rot in Latin America. In this study, samples were taken only in October and November, near the end of the rainy season. The *Penicillium* sp. tested from Costa Rica did not produce any symptoms or show any growth on the surface of the crowns at the temperatures used. Studies should be conducted with *P. corylophilum*, the most frequently isolated fungus from bananas from Ecuador, as well as other *Penicillium* spp. to determine their possible role as causal agent of crown rot. In addition, the effects of inoculations with combinations of two or more fungi should be evaluated because of the possibility of synergism or antagonism among them.

Pathogenicity on FHIA 1 and FHIA 2. Crown rot fungi caused more rot on Grande Naine than on the new FHIA hybrids (Table 3); all fungi grew profusely in Grande Naine at 24°C. The greater depth of lesions caused by these fungi in Grande Naine compared with FHIA hybrids indicates that the hybrids have some resistance to crown rot (Table 3). This result confirms previous experiments performed by FHIA in Honduras, which showed resistance of these new genotypes to Honduran isolates of the crown rot fungal complex (M. Rivera, personal communication). Additional reports also confirm resistance of FHIA hybrids to the crown-rot fungi (4,18).

Sensitivity of fungi to TBZ. The EC_{50}s for *F. moniliforme, G. roseum,* and *Gliocladium* sp. were 8.9, 7.2, and 6.4 mg liter^{-1}, respectively (Table 4). Only *F. semitectum* and the *Penicillium* sp. grew at 10 mg a.i. liter^{-1} of TBZ. The EC_{50} for *F. semitectum* was 11.9 mg a.i. liter^{-1}. The EC_{50} for the *Penicillium* sp. could not be determined because colony growth of this fungus was inhibited very little at 10 mg a.i. liter^{-1}, the highest concentration tested. TBZ at 10 mg liter^{-1} has been reported to completely inhibit colony growth of *F. moniliforme* and *F. pallidoroseum* as well as *Penicillium* sp. isolated from bananas (11). In other studies, different *Fusarium* species were considered sensitive to TBZ when no growth was observed at 5 to 10 mg a.i TBZ liter^{-1} (5,10,12). *Fusarium semitectum* was one of the most aggressive fungi isolated, and had the lowest sensitivity to TBZ. These results suggest that decreased sensitivity to TBZ could be responsible for the reported increase in crown rot in Costa Rica.

Currently, there are no other fungicides registered by the U.S. Environmental Protection Agency with a different mode of action to control crown rot in bananas, except imazalil, which has an import tolerance. Additional research should consider potential fungicides that are close to approval by EPA, to determine if fungi less sensitive to TBZ have cross or multiple resistance to those fungicides. The ergosterol-biosynthesis inhibitor fungicides (EBIs) imazalil and prochloraz are effective in controlling benzimidazole-resistant strains of some crown rot fungi (11). Imazalil alone and in mixture with TBZ has been used in Costa Rica with success to decrease incidence and severity of crown rot (R. Romero, personal communication).

Table 3. Depth of lesions (in mm) caused by fungi associated with crown rot in three different varieties of bananas incubated at 24°C for 10 days

Fungal species	Variety		
	Grande Naine	FHIA 1	FHIA 2
Control (water only)	0.0	0.0	0.0
Fusarium moniliforme	9.7 NS[z]	2.6 NS	3.2 NS
G. roseum	15.1	1.7	1.3
Gliocladium sp.	10.0	0.6	2.0
F. semitectum	11.4	1.1	2.2

[z] NS = no statistical difference ($P = 0.05$). Control was not included in the analysis of variance.

Table 4. Percentage of colony growth reduction and 50% effective concentration (EC_{50}) on thiabendazole (TBZ) of fungi causing crown rot in bananas

Fungal species	Reduction at 10 mg a.i TBZ liter^{-1} (%)	EC_{50} (mg liter^{-1})
Penicillium sp.	9.9	>10.0
Fusarium moniliforme	56.1	8.9
G. roseum	69.4	7.2
Gliocladium sp.	79.7	6.4
F. semitectum	42.9	11.9

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