Curvularia Leaf Spots of Yams (*Dioscorea* spp.)[1]

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ABSTRACT

Leaf spots caused by *Curvularia geniculata*, *C. eragrostidis* and *C.* sp. were found and identified on *Dioscorea rotundata* and *D. alata* yams in Puerto Rico. In pathogenicity tests and field trials, *C. eragrostidis* caused more lesions than either *C. geniculata* or *C.* sp. *C. geniculata* and *C. eragrostidis* caused round to oval spots with irregular margins (3 × 3 mm) scattered on yam foliage. *C.* sp. caused blotches (42 × 27 mm) on leaf margins. *C. geniculata* spores are falcate with five cells measuring 18 × 8 μ (min), 27 × 14 μ (max), and 25.3 × 11.1 μ (mean); those of *C. eragrostidis* are oval with four cells and measured 18 × 11 μ (min), 37 × 20 μ (max) and 26.1 × 15.4 μ (mean); and those of *C.* sp. spores are oval to round with four cells measuring 20 × 9 μ (min), 24 × 11 μ (max) and 21.9 × 9.8 μ (mean). In field trials, the *D. rotundata* cultivars Guinea Blanco and Guinea Negro and the *D. alata* yams Florido, Binugas, Forastero and Purmay were found susceptible, whereas Gemelos, Gunung, Kinampay and Moresby were resistant to *Curvularia* leaf spots. *Curvularia* spp. were found to be tuber borne, infecting both cortex and internal tissues. In Guinea Blanco cultivar more than half of the plants were infected 240 days after planting, with a maximum foliar necrosis of 31%.

INTRODUCTION

Recently, the importance of yam diseases has been intensively studied (4, 5). Traditionally, only virus and anthracnose, also known as "candelilla," were considered serious foliar diseases of yams (6, 7). During the 1980–81 growing season, a high incidence of *Curvularia* leaf spots was observed (9) on *D. rotundata* cv. Guinea Blanco plants approaching senescence. In 1982 a severe leaf-spotting appeared early during tuber bulking, and leaf lesion outbreaks were observed at high severity in yam plantations distributed throughout the east west central slopes.

Leaf spots by *Curvularia eragrostidis* on *D. alata* yam have been reported in India (2), Hong Kong (3), and recently (1980) in Guadeloupe, French West Indies (8). *Curvularia* leaf spots have not previously been reported from yams in Puerto Rico. This paper covers the host susceptibility, etiology and symptomatology of *Curvularia* leaf spots on yams in Puerto Rico.

The true yam (*Dioscorea* spp.) is an important food crop of high market value in Puerto Rico, the Caribbean, Polynesian and African countries. In Puerto Rico, yams are usually planted on the humid mountain slopes

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on small farms of 1 to 3 acres (4). In 1982, sales at the local markets contributed $4.9 million to the Island's economy (1).

MATERIALS AND METHODS

SURVEY AND SAMPLING

Leaves and vines showing characteristic leaf spots were collected from yam plantations in the municipalities of Corozal, Barranquitas, Mayagüez, San Sebastián and Caguas. At Mayagüez, a disease and pest nursery was established, and yam cultivars, Gemelos, Gunung, Forastero, Kinampay, Moresby, Binugas, Purmay, Florido, Guinea and Guinea Negro, were evaluated for leaf spots. Collected leaf samples were kept in an insulated box in plastic bags and brought to the laboratory for routine isolation and bioassay. Leaf spots were removed with a cork borer. Samples were disinfected in a 0.5% sodium hypochloride solution for 4 min. and then placed in a sterile moist chamber. The moist chambers were large petri plates (15 cm) with wet cellulose pads. These were incubated at 28° C, 90% RH with a photoperiod of 12 h of artificial light for 64 h. Other leaves and vines with spots were pressed and dried for future reference. Symptom descriptions and lesion dimensions were taken from fresh material.

To make pure culture isolates, we disinfected necrotic tissue as previously described and placed it on potato dextrose agar (PDA). Plates were incubated in the dark at 28° C. After 4 days, fungal colonies were purified by hyphal tip transfers to fresh PDA. Pure cultures were sent to the Commonwealth Mycological Institute in Surrey, England, for identification. The growth, appearance, and spore characteristics were described from pure cultures. Pure cultures were also used in completing Koch's postulates and other inoculations.

INOCULUM PRODUCTION AND APPLICATION

We used six-day-old pure cultures of each fungus grown on PDA to prepare the inoculum. Two plates each were blended with 1 liter of distilled water in an electric blender, previously disinfected with 0.5% sodium hypochloride, ethanol and then rinsed twice with sterile water under aseptic conditions. The inoculum suspension was applied to test plants and to detached leaves with a sterile atomizer bottle.

PATHOGENICITY TESTS

Two tests were conducted on whole plants in a shade house. In the first we inoculated six 5-month-old healthy plants of Guinea by spraying the foliage with the inoculum. Two plants, sprayed with a solution of

PDA served as check. Plants were covered with plastic bags and incubated for 48 h in the shade house at 31° C. Daily observations were made to record symptom development.

The second test consisted on inoculating plants of Guinea and Guinea Negro of different ages under the same conditions in the shade house since it was noticed that the disease occurred on plants approaching senescence. Groups of four plants each of the following ages were inoculated as previously described: newly emerged vines; 5-month-old, and 9-month-old plants. Two plants of each age group per cultivar served as checks.

A third test was conducted with detached leaves in vitro. Healthy leaves from Guinea and Guinea Negro cultivars were detached and surface disinfected with 0.5% NaOCl solution for 4 min and rinsed under tap water for 20 min. We tested five treatments in which individual groups of three surface-sterilized leaves from each cultivar were placed in separate sterilized humid chambers for inoculation. The inoculated organisms were *Curvularia geniculata, C. eragrostidis, C.* sp, a mixture of the three species, and a check (inoculated with a sterile agar solution). Plates were incubated at 28° C with 90% RH for a 12-h photoperiod.

A similar test was performed simultaneously with three whole intact plants per treatment of the same cultivars. These plants were incubated in a glass house at 33° C and 85% RH under diffuse light. A sprinkle system provided 10 s of mist every 6 min for a period of 4 h per day. Observations on both tests were made 3, 6, 10 and 15 days after inoculation.

ISOLATION OF FUNGI FROM TUBERS

Tuber and tuber-seed pieces that were either treated[3] or not treated with Mertect 340 F (thiabendazole) before storage and/or before planting were bioassayed for microorganisms. Both the storage parenchyma and the cortex tissues of Guinea and Guinea Negro tubers were examined. In another examination only the cortex from cultivars Florido (*D. alata*) and Guinea Blanco were bioassayed. In all cases, the corresponding tissues were surface disinfected by submersion in a solution of 0.5% $Ca(OCl)_2$. These were plated on PDA and incubated at 28° C for 5 to 7 days, at which time organisms isolated from the tissues were identified.

DISEASE DEVELOPMENT

Plants of Florido and Guinea yams were monitored for *Curvularia* leaf spots in replicated plots at the College of Agriculture Farm, Mayagüez

[3] Tubers and seed-pieces treated with fungicide were submerged for 1 h in a 2000 p/m a.i. solution of the fungicide.

Campus. Plots consisted of 4 rows, 20 plants/row, replicated four times for each variety. Examinations were conducted from 90 to 240 days after planting. Incidence was determined by counting the number of plants per row having *Curvularia* spots. Severity was determined visually by estimating the percentage per plot of foliage with spots.

EVALUATION OF CULTIVARS

A disease and pest nursery consisting of 10 yam cultivars was established with 4-row plots of 20 plants each, replicated four times. The following *D. alata* cultivars: Binugas, Florido, Forastero, Gemelos, Gunung, Kinampay, Moresby and Purmay, and of *D. rotundata*, Guinea Blanco and Guinea Negro, were evaluated. Diseases were assessed monthly determining the incidence and severity of *Curvularia* leaf spots.

RESULTS

Two types of lesions were caused by *Curvularia* spp. on yams in Puerto Rico (fig. 1). The most prevalent lesions were round to oval with irregular borders, sometimes with a yellow halo. Their mean size is 3 × 3 mm and their color brown to black. *C. eragrostidis* and *C. geniculata* caused this type of lesions (table 1). The species were identified by Dr. A. Sivanessan of the Commonwealth Mycological Institute. Other lesions were irregular, brown necrotic blotches measuring 42 × 27 mm and located on leaf margins (table 1). A different unidentified *C.* sp. caused these symptoms. All three species of *Curvularia* were found to be pathogenic to yams in laboratory and greenhouse tests. Symptoms associated with each were reproduced and Koch's postulates completed.

Lesions developed 3 days after inoculation on detached leaves and at 6 days after inoculation on attached leaves (table 2). Inoculations of detached leaves resulted on more spots per leaf than were found on attached inoculated leaves. This was the trend found for all three fungi with either a single or mixed inoculations. Chlorotic halos were found only on leaves inoculated with *C. eragrostidis* and with a mixture of species (table 1). Symptoms occurred earlier and more severe with *C. eragrostidis* than with the other two *Curvularia* species.

In both yam species, *D. rotundata* and *D. alata*, *Curvularia* spp. were found to be tuber borne (tables 3 and 4). Isolates were obtained from the cortex and the storage parenchyma tissues of treated and nontreated tubers and seed pieces of *D. rotundata* yams. No information is available regarding detection of *Curvularia* in the storage parenchyma of *D. alata* cv Florido. There was a lower incidence in the Florido and Guinea Negro cultivars when yams were treated with the fungicide, but not in cultivar Guinea, in which a higher incidence was detected. It is known that porosporic dematiaceous hyphomycetes such as *Curvularia* are not controlled by benzimadazole fungicides.

FIG. 1.—Foliage of *Dioscorea rotundata* plant with a high severity of leaf spots caused by *Curvularia eragrostidis* (ce), *C. geniculata* (cg) and C. sp. (c). Notice: a) The round to oval lesions caused by Ce and Cg: b) the blotches caused by another species of *Curvularia* (c).

The first leaf spots were detected on Guinea 90 days after planting (1.5% severity) in the field. Incidence ranged from 4 to 8 plants per plot. At 150 days, about half of the plants of both Guinea and Florido had the disease ranging from 48 to 57 plants/plot. At that time severity range was 1.0 to 6.2% of the foliage. At 240 days, susceptibility differences between *D. alata* cv Florido and *D. rotundata* cv Guinea were evident (table 5) Severity was 8 times higher for Guinea than for Florido. In Guinea it ranged from 5.8 to 31.3%, whereas Florido had only 1.3 to 3.8% of the foliage with *Curvularia* spots.

TABLE 1.—*Description of species of* **Curvularia** *and the symptoms they cause on yam leaves*

Fungus species	Spores				Colony appearance on PDA	Type of lesions on leaves
	Description	Length × width (μ)				
		Min	Max	Mean		
C. eragrostidis	4 cells, ovoid	18 × 11	37 × 20	26.1 × 15.4	Black mycelium, adheres to the substrate, velvet appearance. Rapid growth.	Dark brown to black, round to oval spots, sometimes with a yellow halo. (3 × 3 mm).
C. geniculata	5 cells, falcate shape	18 × 8	37 × 14	25.3 × 11.1	Black mycelium with lighter sectors, cottony fluffy colony. Slow growth.	Same as for *C. eragrostidis* without halo.
C. sp.	4 cells	20 × 9	24 × 11	21.9 × 9.8	Black mycelium, fluffy.	Brown irregular blotches (42 × 2.7 mm) with irregular margins usually at leaf margin.

TABLE 2.—*Leaf lesion incidence on attached and detached leaves of two cultivars of* **Dioscorea rotundata** *inoculated with 3 species of* **Curvularia**

Yam cultivar	Inoculum treatment	Mean lesions (no.)/leaf							
		Attached leaves				Detached leaves			
		3[1]	6	10	15	3	6	10	15
Guinea	*C. geniculata*	0	0	1	3	0	2	4	4
	C. eragrostidis	0	1	2	4	2	3	4	6
	C. sp.	0	0	2	2	0	1	3	3
	Species mixture	0	1	3	4	3	4	5	6
	Check	0	0	0	0	0	0	0	0
Guinea Negro	*C. geniculata*	0	0	2	3	0	2	4	5
	C. eragrostidis	0	2	2	4	3	5	6	8
	C. sp.	0	0	1	2	0	1	2	3
	Species mixture	0	0	2	3	4	4	5	6
	Check	0	0	0	0	0	0	0	0

[1] Days after inoculation.

TABLE 3.—*Isolation of* **Curvularia** *spp. from fungicide-treated and non-treated tubers in* **D. rotundata** *yams*

Preplant seed pieces treatment[1]	Tuber tissue	*D. rotundata* cultivars	
		Guinea	Guinea Negro
Thiabendazole	Internal	37.5[2]	15.0
No Fungicide	Internal	18.0	20.0
Thiabendazole	Cortex	20.0	8.0
No Fungicide	Cortex	5.0	16.0

[1] Yam seed pieces were submerged for 1 h in a 2000 p/m a.i. thiabendazole (Mertect 340 F) 7 days prior to bioassaying for microorganisms.
[2] Percentage of isolates.

TABLE 4.—*Isolation of* **Curvularia** *spp. from the cortex of tuber seed pieces of* **D. rotundata** *and* **D. alata** *treated with combination of pre-storage and preplant fungicide treatments*

Time of application[1] of fungicide		Incidence of *Curvularia* (%) on cortex tissue	
Pre-storage	Preplant	Guinea	Florido
+	+	8.0	4.0
+	−	7.0	0.0
−	+	5.0	4.0
−	−	3.0	11.0

[1] At pre-storage, whole tubers were immersed in a thiabendazole (2000 p/m) bath for 1 h and stored for 44 days. Preplant fungicide at the same rate was applied to the corresponding seed pieces 5 days before bioassaying. Twenty-five pieces were obtained from 5 different yam tuber seed pieces per treatment and plated on PDA after surface disinfection.

TABLE 5.—*Severity of* **Curvularia** *leaf spots on* **D. alata** *and* **D. rotundata** *yams during 1983–84 growing season*

Experimental plot number	Foliar[1] sprays	Severity (%)[2]	
		Florido (*D. alata*)	Guinea (*D. rotundata*)
1	+	1.5	22.5
2	+	2.8	20.0
3	+	2.3	11.5
4	+	2.0	21.3
5	+	2.8	12.5
6	+	1.8	10.0
7	+	2.8	14.3
8	+	2.8	11.3
9	+	3.8	22.5
10	+	1.3	12.5
11	+	2.0	9.3
12	+	1.8	15.5
13	+	2.0	31.3
14	+	2.8	20.0
15	+	2.8	18.3
16	+	3.5	5.8
Mean		2.4	16.2

[1] Foliar sprays with thiabendazole were applied 216, 233, 275 days after planting at 2 g a.i./L to control yam anthracnose (*Colletotrichum gloeosporioides*).

[2] Severity was determined visually by estimating the percentage of foliage with spots. Mean of four replications of 20 plants each 240 days after planting.

Among the *D. rotundata* cultivars examined at the disease and pest nursery plots, as well as on farms, Guinea Negro was observed to be more severely affected by the disease than Guinea. However, in the pathogenicity tests these differences were not as obvious. In the nursery, some cultivars of *D. alata* such as Florido, Bonugas, Purmay and Forastero were found to be susceptible, whereas Gemelos, Gunung, Kinampay and Moresby showed resistance to the disease.

DISCUSSION

A leaf spot on yams caused by *C. eragrostidis* was reported for the first time in the Caribbean basin in Guadeloupe in 1980. *Curvularia* leaf spot on yams was prevalent in commercial plantations in Puerto Rico in 1982. Three species of *Curvularia* causing the spots were identified and their pathogenicity determined. Differences in virulence among the three species of *Curvularia* were also detected. In vitro pathogenicity tests, detached leaves were more sensitive to inoculations than whole plants. To our knowledge two of the species, *C. geniculata* and *Curvularia* sp. had not been reported as leaf spot pathogens of yams. Lesions of all three

Curvularia spp. were sometimes found on the same leaf. Moreover, *Cercospora* leaf spots were also present on leaves infected by *Curvularia* spp. These findings reveal the complex etiology of yam leaf spots. Our research has provided descriptions of the three species, which should help workers monitor foliar diseases of yams. On the basis of the prevalence and severity of the disease, a research program to develop control methods for yam leaf spot is warranted. In our studies, resistance was detected on certain *D. alata* varieties which could be used in areas where *Curvularia* leaf spot is especially damaging. Use of *D. alata* cultivars with anthracnose resistance should be considered in areas where anthracnose is damaging. Gunung and Kinampay have good combined resistance to both foliar diseases (Mignucci et al., unpublished). *D. rotundata* cultivars were generally very susceptible to *Curvularia* leaf spots.

Since *Curvularia* was found to be tuber borne, special precautions should be taken in buying tubers for seed. Research is also needed to develop a control program that could include screening of pre-plant fungicides to control the pathogen at the tuber level, combined with screening fungicides for foliar applications. This program should include the testing of varieties of different susceptibility to the fungus.

RESUMEN

Se identificó a *Curvularia geniculata*, *C. eragrostidis* y *C.* sp. como causantes de manchas foliares en *Dioscorea rotundata* y *D. alata* en Puerto Rico. En pruebas de patogenicidad y de campo, las manchas causadas por *C. eragrostidis* y *C. geniculata*, las cuales estaban dispersas por el follaje, son de redondas a aovadas con márgenes irregulares (3 × 3 mm). *C.* sp. causaba manchas grandes (42 × 27 mm) usualmente en los márgenes de las hojas. Las esporas de *C. geniculata*, que miden 18 × 8 μ (mín), 27 × 14 μ (máx) y 25.3 × 11.1 μ en promedio, son en "forma de luna" y tienen 5 células. Las causadas por *C. eragrostidis*, que son ovaladas y tienen cuatro células, miden 18 × 11 μ (mín), 37 × 20 μ (máx) y 26.1 × 15.4 μ en promedio; y las de *C.* sp., que son de ovaladas a redondeadas y tienen 4 células, miden 20 × 9 μ (mín), 24 × 11 μ (máx) y 21.9 × 9.8 μ en promedio. En pruebas de campo resultaron susceptibles a *Curvularia* las cultivares Guinea y Guinea Negro (*D. rotundata*) y Florido, Binugas, Forastero y Purmay (*D. alata*). Gemelos, Gunung, Kinampay y Moresby (*D. alata*) mostraron resistencia a la mancha foliar causada por *Curvularia*. *Curvularia* spp se aisló de la corteza y tejido interno de tubérculos. En el ñame de Guinea (*D. rotundata*) más de la mitad de las plantas estaban infectadas a los 240 días de sembrados, cuando la necrosis foliar máxima alcanzó un 31%.

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