Effects of Fusarium spp. on Germination and Stem Rot of Sugarcane in Puerto Rico

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INTRODUCTION

Fusarium spp. were reported first in Puerto Rico by Johnston and Stevenson in 1917 (*6*)[2] on sugarcane trash, material in damp chambers, cultures of cane soil and in one instance as the apparent cause of a red rot of cane stalks. In 1920 Edgerton and Moreland (*5*), working with a white strain of *Fusarium* in Louisiana, got consistent reductions in the germination of cuttings from both top and bottom halves of stalks of the sugarcane variety D. 74. In 1935 Abbott (*1*) found that a purple strain of *Fusarium* reduced the germination of P.O.J. 213 in Louisiana as much as 41 percent below that of the control. While studying a *Fusarium* disease in the form of a top rot on the North Coast of Java, Van Dillewijn (*4*) found in 1948 that a 30-percent stalk mortality of P.O.J. 2878 occurred in October, the last month of the dry monsoon. In 1953, Bourne (*3*) reported that *Fusarium* stem rot caused major economic losses during 1951 in the Florida Everglades, with an estimated damage of 15 to 20 percent in some fields of F. 31-436. In Puerto Rico, internodal discoloration or top rot presumably caused by *Fusarium moniliforme* Sheldon were frequently observed in the fields, especially with the variety P.O.J. 2878. Although Tucker (*7*) reported the presence in Puerto Rico of the Pokkah Boeng disease of sugarcane, caused by *F. moniliforme*, he did not study the effect of this fungus on seedpiece germination and stem rot of sugarcane.

Because *F. moniliforme* is known to cause reduction in germination of setts in Louisiana and elsewhere (*5*) and that uniform, good germination is essential for good crops, studies were undertaken to determine whether these isolates of *Fusarium* have contributed to the poor germination and depressed growth of cane under local conditions.

This paper presents data on the pathogenicity of two *Fusarium* isolates on sugarcane, as well as on the morphology and physiology of the isolates. The effects of various fungicides on the *Fusarium* isolates and *Fusarium*

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[2] Italic numbers in parentheses refer to Literature Cited, p. 434.

seedpiece rot of sugarcane, including that of the systemic fungicide Benlate,[3] also are discussed.

PROCEDURE AND RESULTS

FUNGUS MORPHOLOGY, PHYSIOLOGY AND IDENTIFICATION

Diseased seedpieces of sugarcane showing depressed growth were collected in June 1968 from fields at Central Monserrate and Cambalache in the north and Central Fajardo and Roig in the eastern part of the Island. *Fusarium* spp. were isolated from the affected canes. The fungi were isolated by planting discolored root fragments on potato dextrose agar containing pentachloronitrobenzene (PCNB) and streptomycin sultate. Subcultures of the isolates were grown in potato dextrose agar (PDA). The morphology of the fungi was studied by microscopic observation of cultures grown on PDA. Five days after inoculation, both purple and pinkish colonies appeared on the medium. Monoconidial isolations were made. An examination of the conidia produced by the monoconidial colonies revealed that all were typical of the genus *Fusarium*. The macroconidia produced from the purple colonies were 20.5 to 36.9 μ long $\times$ 4 to 5 μ wide, and the microconidia 8.2 to 16.4 μ long $\times$ 4 to 5 μ wide. Macroconidia produced from the pinkish colonies were 24.6 to 53.3 μ long $\times$ 4 to 5 μ wide (fig. 1). The isolates were sent for identification of species to Dr. William C. Snyder, University of California, Berkeley, California. He identified the purple isolates as *F. moniliforme* and the pinkish as *F. roseum*.

Monoconidial isolates *F. moniliforme* and *F. roseum* were grown in potato dextrose agar and cornmeal agar at 8°, 12°, 16°, 20°, 24°, 28°, 32°, and 39° C. For each temperature, five petri dishes containing 15 ml. of the above-mentioned medium were inoculated with a 2 mm. culture disc. The discs were cut with a sterile cork borer from the advancing margin of colonies kept in cornmeal agar. The discs were incubated at the different temperatures for 5 days. The increment in the diameter of colonies was measured at the end of 2-day, 3-day, 4-day, and 5-day incubation periods.

The results show (table 1) that the optimum temperature range for mycelial growth of *F. moniliforme* lies between 24° and 28° C. on the two media used. The optimum temperature range for *F. roseum*, however, lies between 28° and 32° C. on the same media.

PATHOGENICITY TRIALS

Infection by Dipping

Forty seedpieces of sugarcane variety P.O.J. 2878 were dipped separately for 6 hours in spore suspensions of *F. moniliforme* and *F. roseum*, then

[3] Benlate -benomyl (methyl 1-butylcarbamoyl) 2-benzimidazole-carbamate.

planted in metal flats containing steam-sterilized soils. Seedpieces soaked in tap-water for the same time period were used as controls. The inoculum was prepared by transferring the monoconidial isolates to Czapek Dox Broth and by blending the broth 2 weeks after incubating at 28° C. The density of the conidia was estimated at approximately 1,000 per ml.

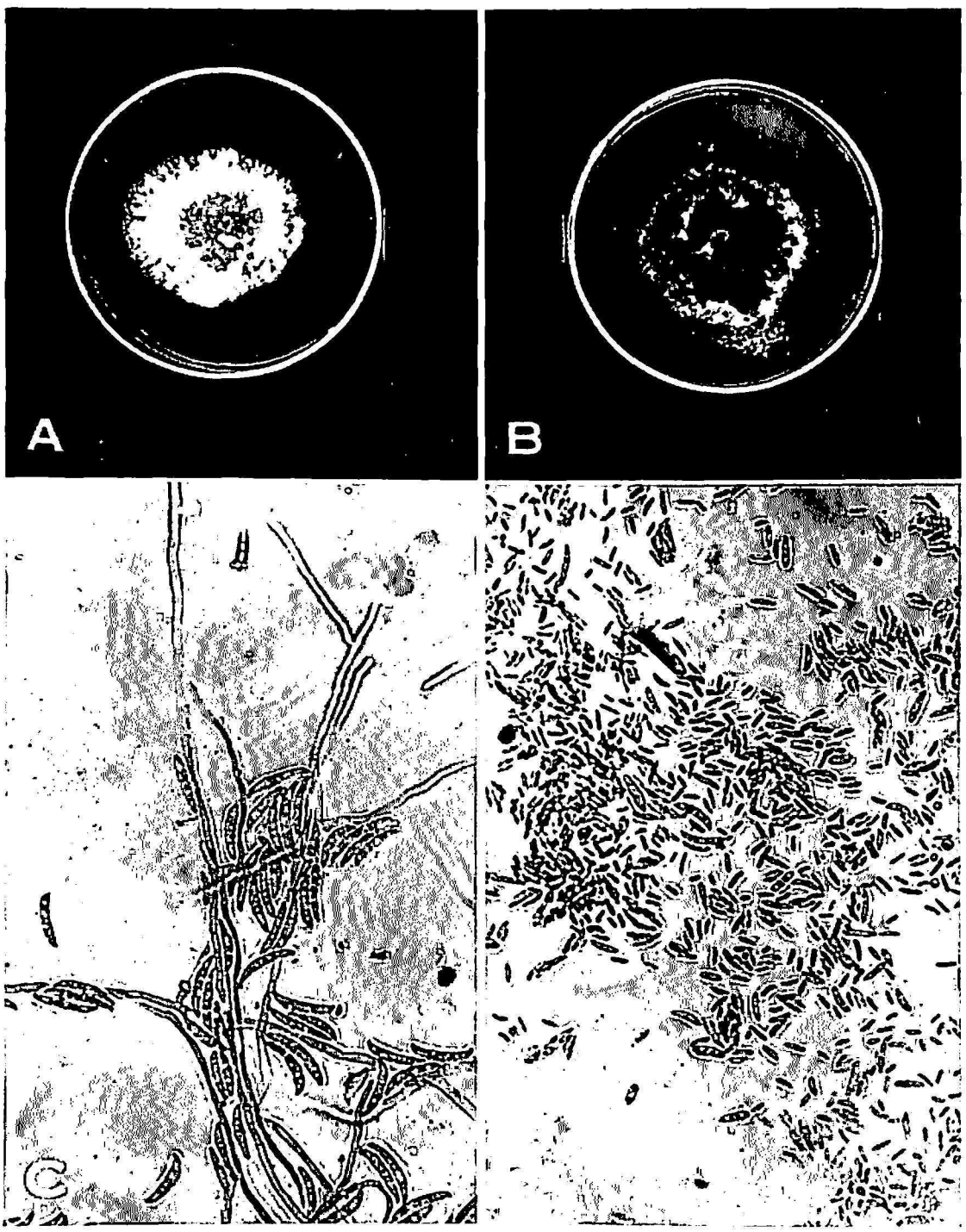

FIG. 1. Cultural appearance and conidia of *Fusarium* spp.: A, Cultural appearance of *F. roseum* and B, *F. moniliforme*; C, conidia of *F. roseum*; D, conidia of *F. moniliforme*.

Six weeks after inoculation by dipping, seedpiece germinations were recorded. The results obtained indicated that *F. moniliforme* reduced the germination of P.O.J. 2878, 40 percent below that of the control. Additional tests were made with P.R. 1059, P.O.J. 2878, P.R. 980, P.R. 1117, N: Co. 310 and P.R. 1085 using the same inoculation method as described for the first test. The results obtained indicated that *F. moniliforme* reduced

TABLE 1.—*Effect of various temperatures, ranging from 8° to 39° C., on increment (expressed in mm.) of diameter of colonies of* **Fusarium moniliforme** *and* **F. roseum** *from sugarcane*

Fungus	Cultural media	Days after inoculation	Temperature in centigrades						
			8°	16°	20°	24°	28°	32°	39°
F. moniliforme	CMA[1]	2	4.0[2]	4.4	6.4	8.1	9.1	9.8	7.2
		3	4.0	7.5	14.6	19.4	23.7	22.9	7.3
		4	4.0	13.9	25.4	32.5	39.8	35.5	7.3
		5	4.0	32.2	56.9	72.2	81.0	63.2	7.3
	PDA[3]	2	4.0	4.2	6.2	9.6	11.0	12.1	4.0
		3	4.0	7.3	17.5	22.0	25.0	23.4	5.5
		4	4.0	15.6	28.2	36.5	41.0	35.5	5.5
		5	4.0	36.9	61.4	78.9	88.9	59.9	5.5
F. roseum	CMA	2	4.0	4.0	4.9	6.6	8.2	10.0	4.0
		3	4.0	4.7	11.2	15.0	19.8	22.9	4.0
		4	4.0	6.6	18.5	25.5	33.2	35.3	4.0
		5	4.0	14.8	41.2	57.5	68.8	75.0	4.0
	PDA	2	4.0	4.0	4.5	7.2	8.1	10.8	4.0
		3	4.0	4.7	11.0	17.4	19.3	25.8	4.5
		4	4.0	7.7	18.8	27.7	34.6	40.0	5.5
		4	4.0	14.9	42.3	60.4	74.3	79.8	7.8

[1] CMA—corn meal agar.
[2] Average of five replications.
[3] PDA—potato dextrose agar.

germination of all varieties tested, while *F. roseum* had little or no effect on germination (table 2).

Infection by Substrate Inoculation

Forty seedpieces of each variety were planted per flat in steam-sterilized soils. The canes were cut back 3 months after planting. The cut surfaces of the canes were inoculated and the soils were infected immediately with spore suspensions of *F. moniliforme* and *F. roseum*, separately. Canes inoculated with tapwater were used as controls. Four weeks after inoculation, seedpiece germination was recorded. The results indicated that inoculation with *F. moniliforme* greatly reduced stubble germination of all

varieties tested, while inoculation with *F. roseum* had no effect on stubble germination (table 2).

Infection by Stem Inoculation

Thirty stalks each of sugarcane varieties P.R. 980, P.R. 1059, P.R. 1085, P.R. 1117, N: Co. 310 and P.O.J. 2878 were inoculated with spore suspensions of *F. moniliforme* using the stem-puncture technique. The inoculum was increased in Czapek Dox Broth. Seven days after inoculation, the inoculated stalks were split with a knife for examination. As indicated by the lengths of the internal lesions in the stalks (table 3), the results obtained show that P.R. 980 was the most resistant variety to *F. moniliforme*, and that P.R. 1085, P.O.J. 2878 and P.R. 1059 were comparatively most susceptible (fig. 2).

TABLE 2.—*Effect of* **Fusarium** *spp. on germination of sugarcane*

Variety	Percent germination of plant cane			Percent germination of stubble[1]		
	Control	*F. moniliforme*	*F. roseum*	Control	*F. moniliforme*	*F. roseum*
P.R. 980	73[2]	46	53	100	52	91
P.R. 1059	13	3	10	62	30	82
P.R. 1085	60	27	60	90	79	91
P.R. 1117	57	33	40	85	10	66
P.O.J. 2878	50	17	53	92	70	90
N: Co. 310	30	6	30	95	59	91

[1] Canes were cut back at 3 months of age.
[2] Average of five replications.

CHEMICAL CONTROL

Four chemicals, Benlate, Busan 72,[4] Daconil,[5] and Dithane M-45[6] were tested in the laboratory at 0.5-, 1.0- and 1.5-percent concentrations for their toxicity against *F. moniliforme*. *F. moniliforme* was grown on potato dextrose agar medium, to which four 2-mm. paper discs containing varying concentrations of technical grade Benlate, Busan 72, Daconil and Dithane M-45 were added. Paper discs containing no fungicides were added to the plates used as controls. As shown in figure 3, Busan 72 and Benlate appeared to be most effective fungicides for inhibiting mycelial growth of *F. moniliforme*.

[4] Busan 72–80 percent of 2-(thiocyanomethylthio) benzothiazole.
[5] Daconil 2787—tetrachloro isophthalonitrile.
[6] Dithane M-45—coordination product of zinc ion and manganese ethylene bisdithiocarbamate.

Benlate and Busan 72 also were studied under greenhouse conditions. Steam-sterilized soils, infected with a spore suspension of *F. moniliforme*, were used. Seedpieces of sugarcane variety P.O.J. 2878 were dipped in

TABLE 3.—*Effect of* **Fusarium** *spp. on stem rot of sugarcane*

Variety	Length of internal lesion	
	Fusarium moniliforme	*F. roseum*
	Inches	*Inches*
P.R. 980	1.92[1]	1.62
P.R. 1059	2.15	2.03
P.R. 1085	3.11	2.21
P.R. 1117	2.31	2.00
N: Co. 310	2.09	1.89
P.O.J. 2878	2.01	1.37

[1] Average of 28 to 30 stalks.

FIG. 2. Sugarcane stalks inoculated with spore suspension of *Fusarium moniliforme* via stem-puncture technique (from left to right P.R. 980 P.O.J. 2878, N: Co. 3.10, P.R. 1059, P.R. 1117, and P.R. 1085.

varying concentrations of Benlate and Busan 72 before planting. Seedpieces dipped in sterile water before planting were used as controls. Germination of P.O.J. 2878 was greatly improved when seedpieces were dipped in in-

creased concentrations of both Benlate and Busan 72, with 80 percent and 70 percent germination respectively (table 4).

A field trial with Benlate was established at the Isabela Substation. The

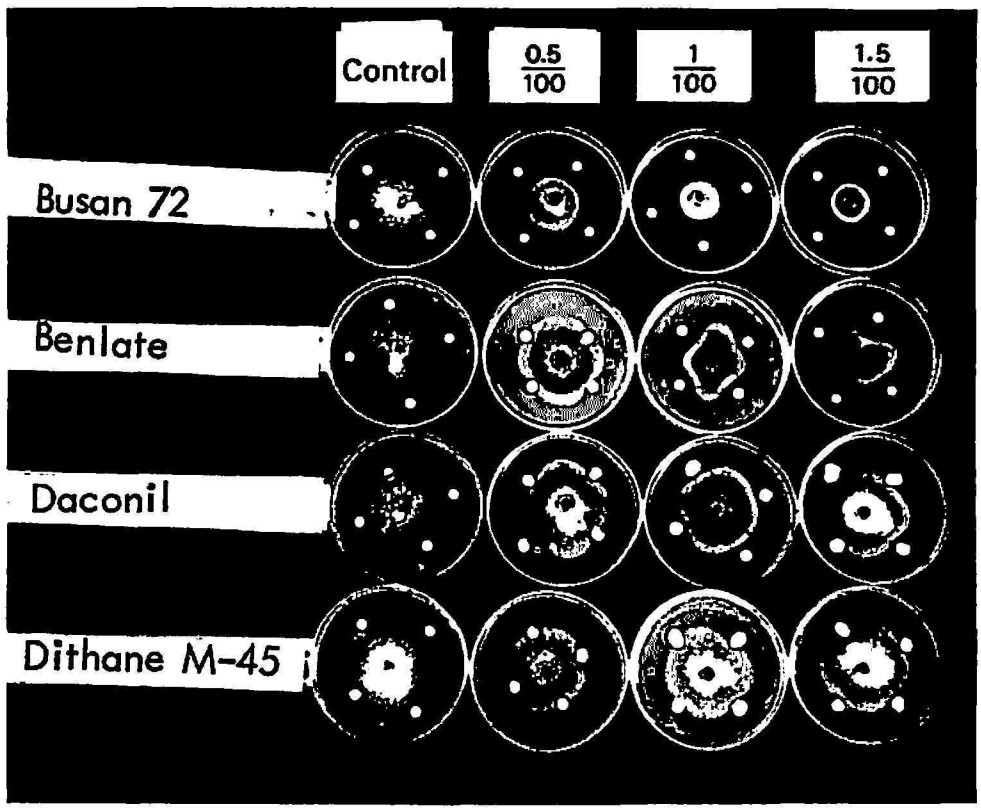

FIG. 3.- Effect of various concentrations of fungicides: Busan 72, Benlate, Daconil and Dithane M-45 on mycelial growth of *Fusarium moniliforme*.

TABLE 4.—*Effect of various fungicides on seedpiece germination of sugarcane variety P.O.J. 2878 planted in* **Fusarium moniliforme**-*infected soils under greenhouse conditions*

Chemical	Concentration	Germination
	Percent	*Percent*
Benlate	0.5	65[1]
	1.0	65
	1.5	80
Busan 72	1.0	40
	2.0	55
	3.0	70
Control	0	40

[1] Average of three replications.

following four treatments were used: Seedpieces of P.R. 980 were dipped before planting in: 1, tap water (control); 2, a spore suspension of *F. moniliforme*; 3, a 1.0-percent solution of Benlate; 4, a 1-percent solution of Benlate

but inoculated the night before with a spore suspension of *F. moniliforme*. A complete randomized block design with three replications was used for this trial.

As shown in table 5, seedpieces inoculated with *F. moniliforme* had a poorer germination than healthy ones, but their germination was better when treated with a 1-percent solution of Benlate.

DISCUSSION

The results obtained in these studies indicate that *F. moniliforme* affects seedpiece and stubble germination of sugarcane. These results agree with the findings of Edgerton and Moreland (*5*) and Abbott (*1*) in Louisiana. This is the first report on the incidence and effects of *Fusarium* spp. on germination of sugarcane in Puerto Rico.

TABLE 5.—*Effect of Benlate on seedpiece germination of sugarcane variety P.R. 980 infected with* **Fusarium moniliforme** *under field conditions*

Treatment		Percent Germination
O	Seedpieces dipped in tap water	71[1]
B	Seedpieces dipped in 1-percent solution of Benlate	65
FB	Seedpiece dipped in 1-percent Benlate but inoculated with spore suspension of *F. moniliforme* the night before planting	65
F[2]	Seedpieces dipped in a spore suspension of *F. moniliforme* the night before planting	25

[1] Average of three replications.

[2] Without dipping in 1-percent solution of Benlate.

The results obtained under greenhouse conditions also indicate that both *F. moniliforme* and *F. roseum* are capable of causing stem rot of sugarcane when conditions are favorable.

Although Bartels and MacNeill (*2*) reported that several mutants of *Fusarium* were resistant to Benlate, results obtained in these studies indicate that Benlate not only inhibits mycelial growth of *F. moniliforme* in vitro but also protects seedpieces of sugarcane against the fungus under both greenhouse and field conditions.

SUMMARY

Fusarium moniliforme and *F. roseum* were isolated from the diseased seedpieces of sugarcane plants. Various methods were employed to inoculate canes with the different isolates, i.e., seedpiece-dipping, addition of inoculum to the substrate, and stem-puncture. *F. moniliforme* reduced germination of variety P.O.J. 2878 as much as 40 percent when seedpiece-dipping and substrate infection methods were used. Inoculation with

F. roseum had little effect. Sugarcane varieties P.O.J. 2878, P.R. 1117, P.R. 980, P.R. 1059, P.R. 1085 and N: Co. 310 proved susceptible in varying degrees when inoculated with *F. moniliforme* by the stem-puncture method. Among the chemicals tested, Benlate, a systemic fungicide, seemed to be most promising for protecting sugarcane seedpieces against *Fusarium* rot.

RESUMEN

De pedazos del tallo de caña de azúcar enferma, usados para semilla, se aislaron el *Fusarium moniliforme* y *F. roseum*. Se usaron varios métodos para inocular caña sana con los organismos aislados, a saber, el de inmersión de la semilla, infección por inoculación del substrato y punzadura del tallo. Cuando se usaron los métodos de inmersión de la semilla e inoculación del substrato, el *F. moniliforme* redujo la germinación de la variedad P.O.J. 2878 hasta en un 40 por ciento. La inoculación con el *F. roseum* surtió poco efecto. Las variedades P.O.J. 2878, P.R. 1117, P.R. 980, P.R. 1059, P.R. 1085 y Na. Co. 310 variaron en su grado de susceptibilidad, cuando se inocularon con el *F. moniliforme* punzando el tallo. Entre los agentes químicos que se probaron, el fungicida sistémico Benlate pareció ser el más prometedor para proteger los pedazos de caña para semilla contra la podredumbre causada por el *Fusarium*.

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