Sexual Compatibility, Morphology, Physiology, Pathogenicity and *in vitro* Sensitivity to Fungicides of *Thielaviopsis paradoxa* Infecting Sugarcane and Pineapple in Puerto Rico[1]

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INTRODUCTION

Pineapple disease of sugarcane is caused by *Thielaviopsis paradoxa* (De Seynes) v. Hohn. (5). This disease, reported as important to the sugarcane industry in Puerto Rico during the period 1932 to 1933 by Cook (1,2), is still important (5) on the northern coast of the Island. It also attacks pineapples while in transit from Puerto Rico to mainland markets. Improper control measures of this fungus recently created a serious temporary problem to the local pineapple industry. A possibility also exists that a new virulent race of *T. paradoxa* may have developed under our conditions capable of attacking both sugarcane and pineapple.

T. paradoxa was found originally in 1866 by De Seynes (3) in France on rotten fruit of pineapple. Although he believed this fungus also caused pineapple disease of sugarcane, he did not make pathogenicity tests nor study sexual compatibility between the two causal organisms. The recent recovery of sexually compatible strains of *T. paradoxa* from sugarcane by Liu and Mignucci in Puerto Rico (4) made the determination of such a relationship possible for the first time. The following report is thus the first of its kind. Also included in this report is the identification of a race of *T. paradoxa* from pineapple pathogenic to sugarcane, its physiological characteristics and its sensitivity in vitro to various fungicides.

PROCEDURE AND RESULTS

SYMPTOMS OF BLACK ROT ON PINEAPPLE

In the early stage, the affected pineapple shows a soft and juicy water-soaked appearance. Black spores are produced over the entire surface of the diseased tissue when the affected fruit has been exposed to the air (fig. 1, A). This black formation consists of the dark colored macrospores of the fungus. In the advanced stage, the whole fruit is covered with these black spores and becomes so completely disintegrated that it yields to the slightest pressure. Early internal symptoms consist of darkened areas

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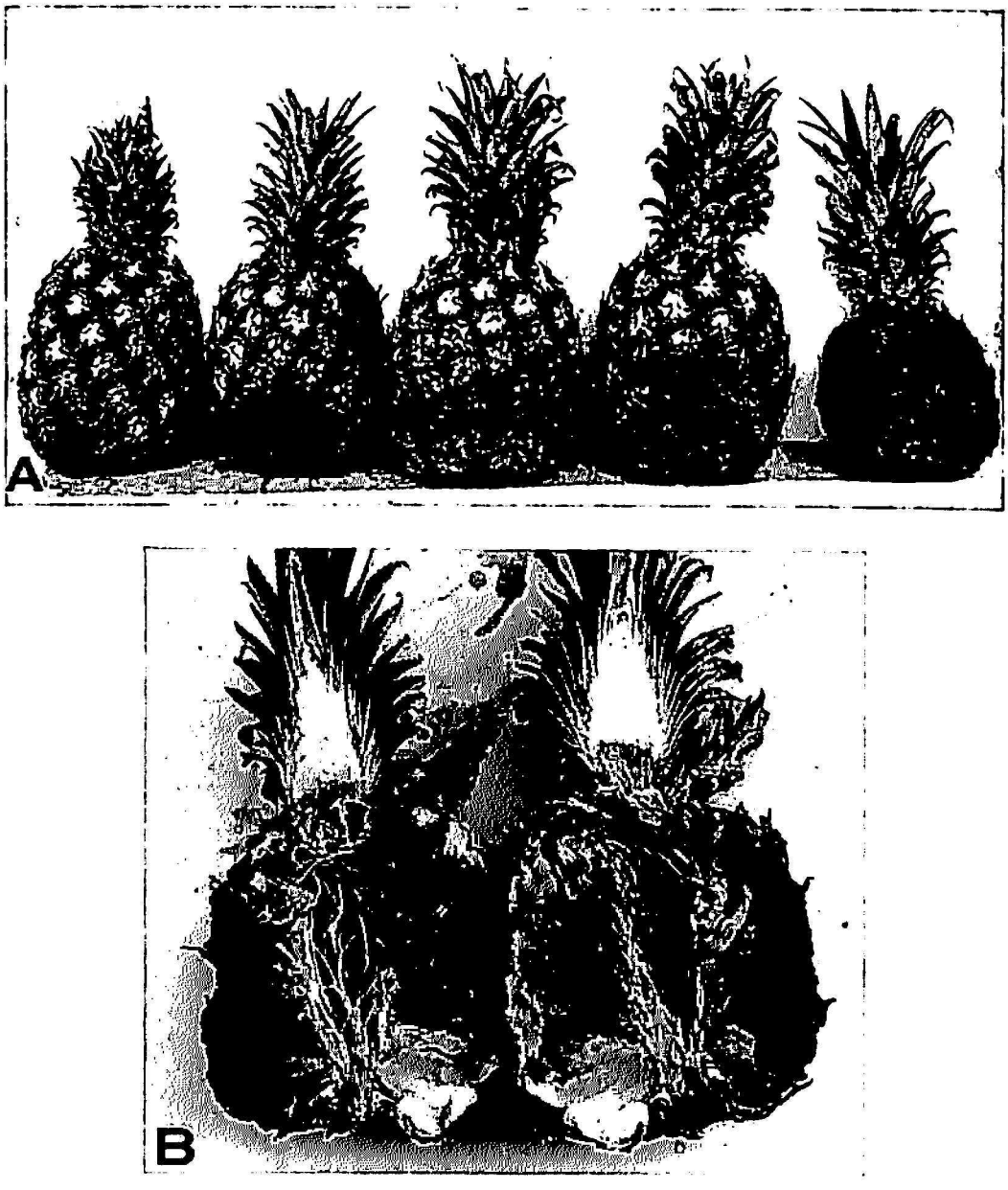

FIG. 1.—Pineapples affected by *Thielaviopsis paradoxa*: A, External and B, internal symptoms of black rot disease of pineapple.

under the skin which may later spread until the flesh of the pineapple becomes totally black-rotted (fig. 1, B).

FUNGUS MORPHOLOGY AND SEXUAL COMPATIBILITY

Fruits of pineapple (variety Red Spanish) were obtained from the Land Authority of Puerto Rico farm at Manati. *Thielaviopsis* species were isolated from the affected fruits. The fungus was isolated by planting discolored fruit tissues on potato dextrose agar (PDA). Subcultures of the isolate were grown on PDA. The morphology of the fungus was studied with the aid of the microscope and the use of cultures grown on PDA.

Two days after seeding, black colonies appeared on the medium. Monosporic isolations were made. An examination of conidia produced by the monoconidial colonies revealed that all were typical of the genus *Thielaviopsis*. The macroconidia of the pineapple of the pineapple isolate (PA) were 16.80 to 19.59 μ long $\times$ 8.26 to 11.20 μ wide and the microconidia 5.50 to 10.20 μ long $\times$ 2 to 5.20 μ wide. Two strains (light and dark or T_1 and T_2) of *T. paradoxa* isolated by Liu and Mignucci (4) from sugarcane, were included in this study. As indicated in table 1, there is no significant difference in length between macroconidia of the isolates (T_1 and T_2) from sugarcane and those from pineapple (PA). However, there is a significant

TABLE 1.—*Size of macro- and microconidia of three isolates of* **Thielaviopsis paradoxa** *from sugarcane and pineapple*[1]

Source	Size of conidia (μ)							
	Macro—				Micro—			
	Length	T-value	Width	T-value	Length	T-value	Width	T-value
Isolate from sugarcane (T_1)	17.58[2]		8.85		10.29		4.67	
Isolate from sugarcane (T_2)	17.58	0.01	10.82	8.82**	9.00	7.23**	4.59	2.40*
Isolate from pineapple (PA)	17.42	.52	9.67	3.65**	9.88	2.40*	5.12	5.28**
				5.17**		4.83**		7.69**

[1] T-value 8.82** corresponds to the comparison of T_1 with T_2, 3.65** to the comparison of T_1 with PA, 5.17** to the comparison of T_2 with PA, and likewise for the other T-value's in the table.

[2] Average of 200 spores.

* Significantly different at 5-percent level.

** Significantly different at 1-percent level.

difference in width of macroconidia between isolates of sugarcane also. Both the length and the width of the microconidia differed significantly between the isolates from sugarcane and those from pineapple. Perithecia were not obtained when the dark strain (T_2) from sugarcane was crossed with the same pineapple isolate (PA). When the light strain (T_1) was grown together with the isolate from pineapple (PA), fertile perithecia formed in 3 to 7 days at 24° to 28° C. at the zone where the mycelium of the two isolates merged (fig. 2). The perithecia obtained from this cross are indistinguishable from those obtained by crossing the light and the dark strains ($T_1 \times T_2$) from sugarcane. Perithecia are partially to completely immersed in the substratum. The base of the perithecia are pale brown, globose, 245 to 280 μ in diameter and ornamented with numerous, irregularly shaped, knobbed appendages (fig. 3, A) dark brown to black

at the tips. The necks of the perithecia are black, pale brown at the apex, stout, 1,200 to 1,365 μ long $\times$ 45 to 65 μ wide in diameter at the tip (fig. 3, B). Ostiolar hyphae are hyaline to pale brown, erect, numerous, tapered to a point and measure 105 to 160 μ long $\times$ 2 to 3 μ wide (fig. 3, C). Ascospores are hyaline, ellipsoid, 8 to 12 μ long $\times$ 4 to 5 μ wide. The culture of *T. paradoxa* from pineapple (PA) are much darker than either T_1 or T_2. Macroconidia of PA are borne in chains (fig. 3, D) and are dark, ovoid to oval, thick walled and smooth. Microconidia are also borne in chains, hyaline to brown.

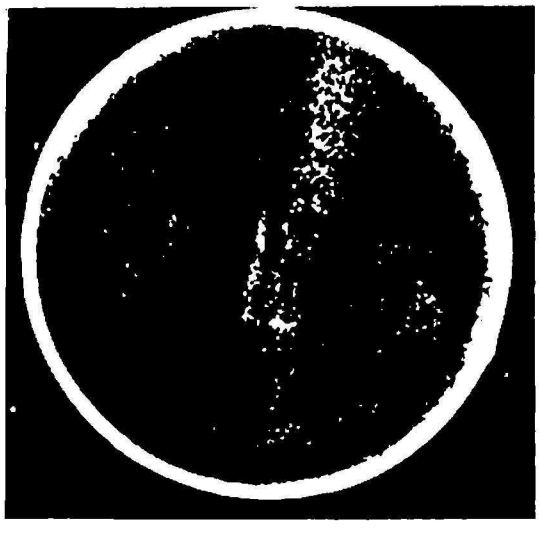

FIG. 2.—Formation of fertile perithecia in potato dextrose agar 7 days after crossing the isolate of *Thielaviopsis paradoxa* from pineapple.

PHYSIOLOGIC CHARACTERISTICS

I. Effect of Various Cultural Media on Growth

Several culture media, i.e., potato dextrose agar (PDA), bean pod agar (BPA), malt agar (MA), czapek dox agar (CD), nutrient agar (NA) and corn meal agar (CMA) were found to be excellent for culturing the various isolates of *T. paradoxa*.

Monoconidial isolates of *T. paradoxa* from sugarcane (T_1 and T_2) as well as PA from pineapple were grown separately in potato dextrose agar. Seven petri dishes containing approximately 15 ml. of each of the above-mentioned media were seeded separately with 2-mm. culture discs of the three isolates. The discs were cut with a sterile cork borer from the advancing margin of colonies kept in potato dextrose agar. The dishes containing the inoculum were incubated at 28° C. for 48 hours. The increment in the diameter of the colonies was measured at the end of a 2-day incubation period.

The data (fig. 4) indicated that PDA and MA were the best media for radial growth determinations. T_1 showed the fastest rate of growth in all the media tested and T_2 showed the slowest rate of growth in the same media except for BPA. Moderate rate of growth of PA was obtained in all media tested except in the case of BPA. The colonies of PA were consistently darker in all the media tested than those of T_1 or T_2.

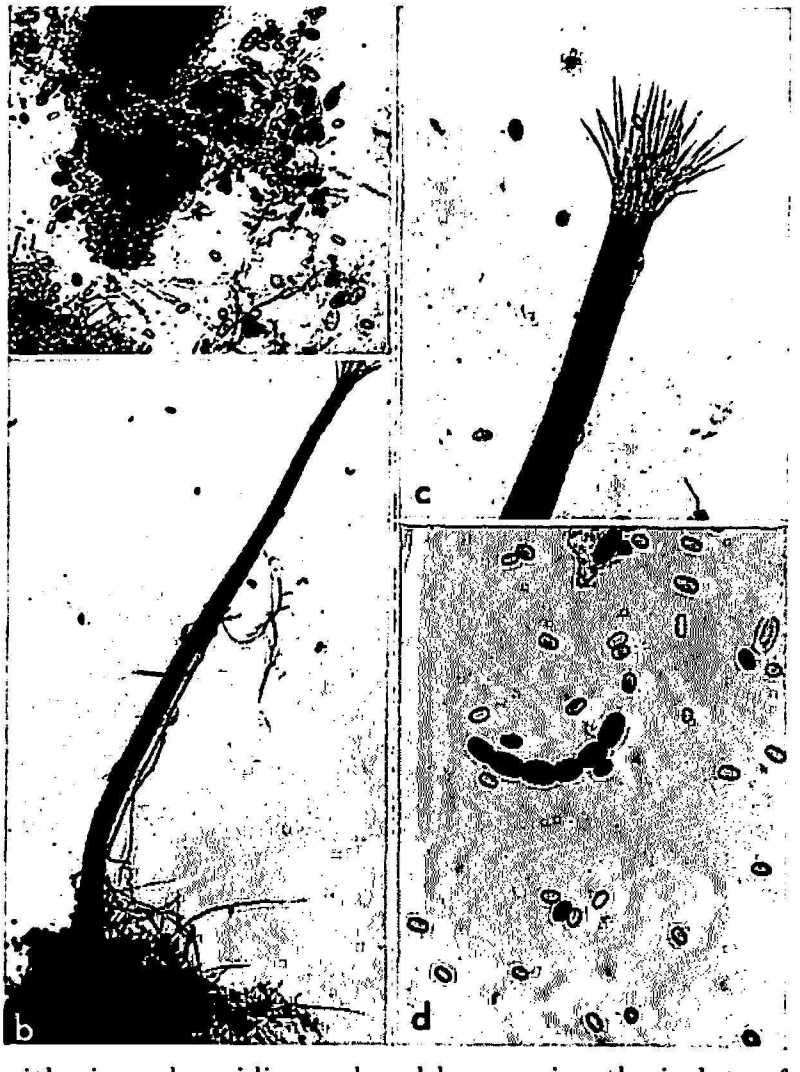

FIG. 3.—Perithecia and conidia produced by crossing the isolate of *Thielaviopsis paradoxa* from pineapple (PA) with the isolate from sugarcane (T_1): A, Knobbed appendages B, perithecia C, ostiolar hyphae and D, conidia.

II. Effect of Temperature on Mycelial Growth

Monoconidial isolates of *T. paradoxa* from sugarcane (T_1 and T_2) as well as those from pineapple (PA) were grown in PDA at 8°, 12°, 16°, 20°, 24°, 28°, 32°, 36°, and 40° C. For each temperature, six petri dishes containing approximately 15 ml. of the above-mentioned medium were seeded separately with 2 mm. culture discs of each of the three isolates. The discs were cut with a sterile cork borer from the advancing margin of colonies

maintained on PDA. The dishes containing the inoculum were incubated at the different temperatures for 2 days. The increment in the diameter of colonies was measured at the end of the 2-day incubation period.

The results (fig. 5) show that the optimum temperature range for mycelial growth of all the three isolates (T_1, T_2 and PA) of *Thielaviopsis* lies between 24° and 28° C. on the medium used. T_1 from sugarcane was the fastest growing isolate. T_2 was found to grow at a very slow rate. PA maintained a moderate rate of spread at the same temperatures.

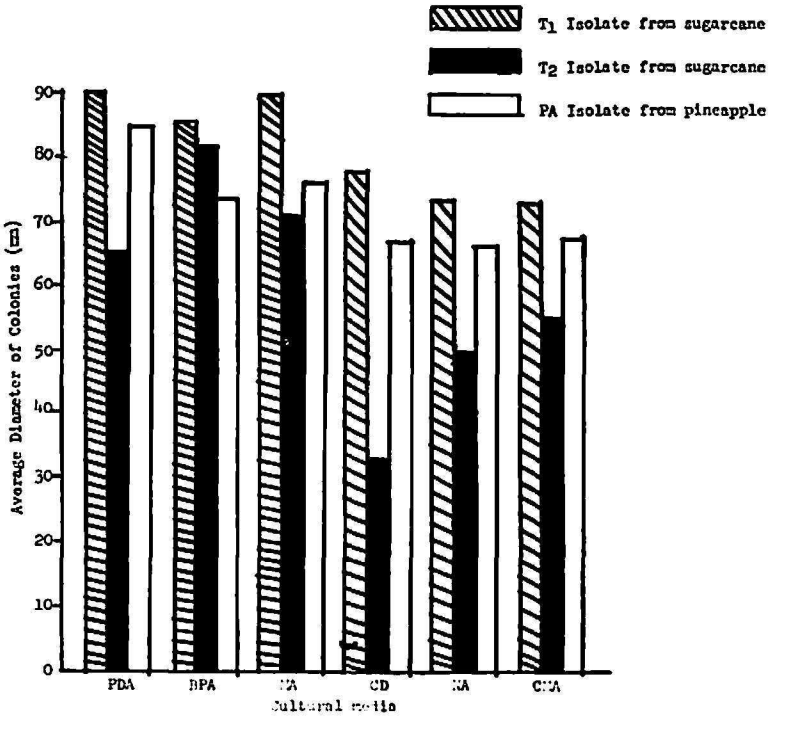

FIG. 4.—Size of colonies of three isolates of *Thielaviopsis paradoxa* after 2-day incubation on various cultural media (PDA = potato dextrose agar, BPA = bean pod agar, MA = malt agar, CD = czapek dox agar, NA = nutrient agar and CMA = corn meal agar).

III. Effect of pH on Mycelial Growth

The conidia of all the three isolates of *Thielaviopsis* (T_1, T_2 and PA) were grown separately on PDA at the following pH: 3, 4, 5, 6, 7, 8, 9, 10, and 11. For each pH, five petri dishes containing 15 ml. of the above-mentioned medium were seeded separately with 2 mm. culture discs of the three isolates of *Thielaviopsis*, cut with a sterile cork borer from the advancing margin of colonies kept in the same medium. The dishes containing the inoculum were incubated at 28° C. for 30 hours. The increment in the diameter of the colonies was measured at the end of the 30-hour incubation period.

The data (fig. 6) indicated that the optimum pH range for mycelial growth of all the three isolates of *Thielaviopsis* (T_1, T_2 and PA) lies between

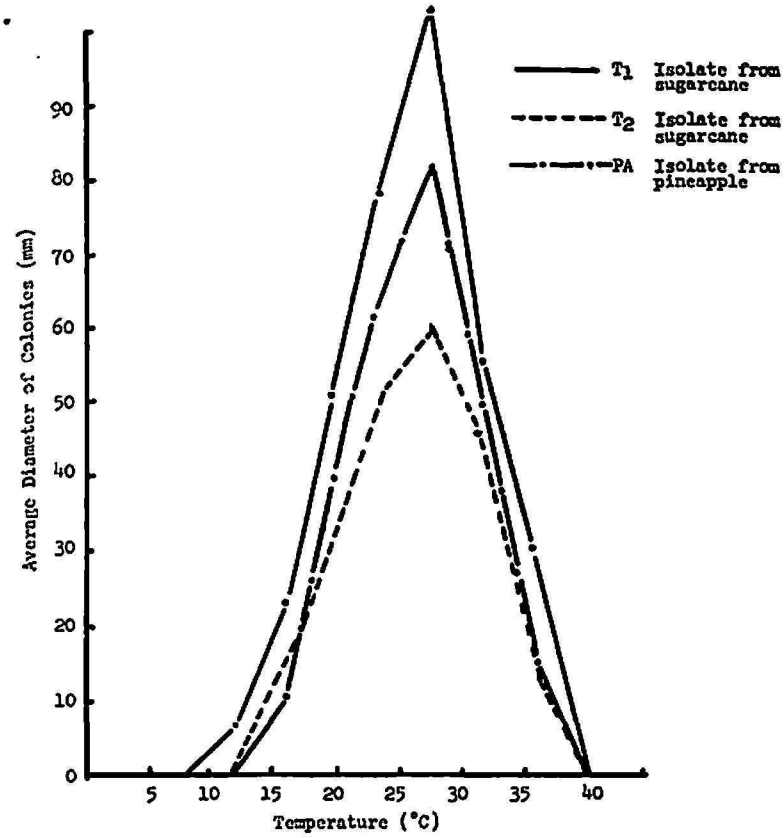

FIG. 5.—Size of colonies of three isolates of *Thielaviopsis paradoxa* after 2-day incubation on potato dextrose agar at different temperatures.

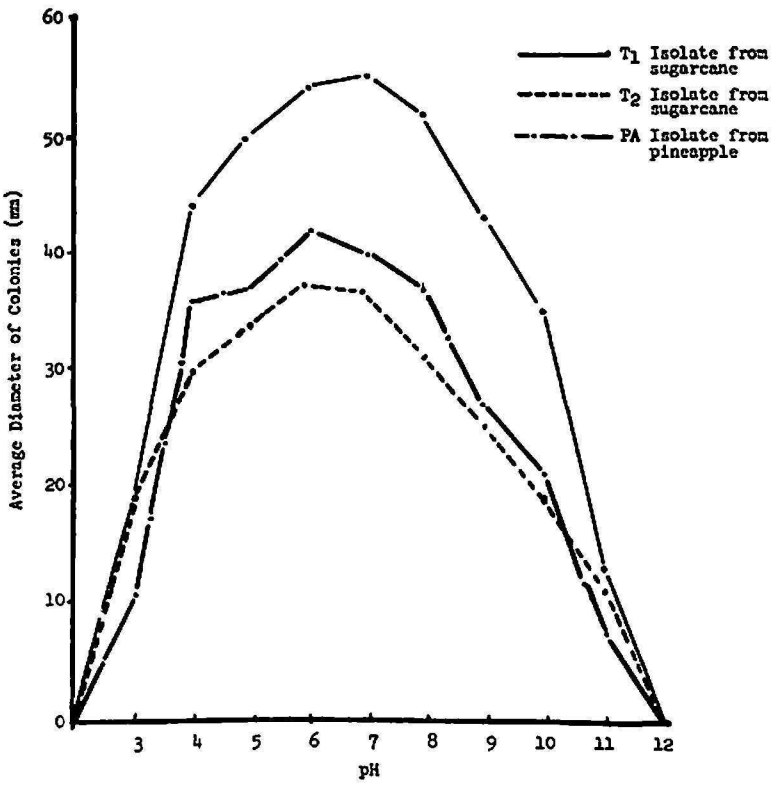

FIG. 6.—Size of colonies of three isolates of *Thielaviopsis paradoxa* after 2-day incubation on potato dextrose agar at different pH values.

6 and 7. However, T_1 was the fastest growing on all the pH tested while T_2 showed the slowest rate of growth. PA maintained a moderate rate of growth on the same pH.

PATHOGENICITY TESTS

The cut ends of single-eyed seedpieces of P.R. 1059 were separately inoculated with small, uniform, round discs of mycelium (2 mm. in diameter) of the isolates of *Thielaviopsis* (T_1, T_2 and PA). The inoculated seedpieces were planted in enamelled trays containing sterilized sand with 30-percent moisture content The trays were then left in room temperature (22–25° C.) for 12 days. At the end of the incubation period, the length of the infected area of the sugarcane seedpieces was measured.

The results (table 2) indicated that the rate of infection varied significantly depending on the isolate used. In addition, the tissues of the inocu-

TABLE 2.—*Average length of infection area of sugarcane seedpieces by three isolates of* **Thielaviopsis paradoxa** *from sugarcane and pineapple*

Source	Length of infection area	T-value	
	Mm.		
Isolate from sugarcane (T_1)	36.00		
Isolate from sugarcane (T_2)	38.00	0.30	
Isolate from pineapple (PA)	51.00	2.25*	1.95

* Significantly different at 5-percent level.

lated sugarcane seedpieces were much more severely affected by PA than either by T_1 or T_2. The tissues affected by PA also were darker in appearance (fig. 7).

IN VITRO FUNGICIDE TESTS

Five fungicides, benzoic acid, Dowicide A, Benlate, Tecto 60 (TBZ) and Dithane M-45, were used in this study. *T. paradoxa* was grown on PDA at 28° C. For each concentration (0.25, 0.5, 1.0, 1.5, and 2.0 percent) of the above-mentioned fungicides, four petri dishes containing 20 ml. of PDA were employed in each case. Washed conidia of *T. paradoxa* were evenly spread on the surface of the medium. A small disc of paper (10 mm. in diameter) presoaked in each of the various concentrations of the fungicides was placed on the center of the medium. The plates were transferred to incubator (28° C.). In each case the relative diameter of the inhibiting zone was measured at the end of 72 hours.

The results obtained (table 3) showed that benzoic acid as well as Dowi-

cide A seemed to be the most effective for inhibiting mycelial growth of *T. paradoxa.*

A study of the correlation coefficient (Spearman rank) between concentration of the fungicide and diameter of inhibiting zone of mycelial growth of *T. paradoxa* by these fungicides revealed that the correlation coefficients (r) with fungicides, benzoic acid, Dowicide A and Dithane M-45 were all significant at the 1-percent level and, non-significant with Benlate and Tecto 60. Table 4 shows the Spearman rank correlation coefficient values. The T-value was highly significant for benzoic acid, Dowicide A and Dithane M-45. These results indicate that the non-systemic fungicides employed significantly affected the growth of the various isolates of *T.*

FIG. 7.—Sugarcane seedpieces of P.R. 1059 12 days after inoculation with various isolates of *Thielaviopsis paradoxa:* A, Isolate from sugarcane (T_1), B, isolate from sugarcane (T_2), and C, isolate from pineapple (PA).

paradoxa. This relationship was not in evidence, however, in the case of the systemic fungicides Benlate and Tecto 60.

DISCUSSION AND CONCLUSION

The results of this study indicate in general that macro- and microconidia of the pineapple isolate of *T. paradoxa* (PA) resemble in appearance the isolates obtained from sugarcane (T_1 and T_2), as described by Liu and Mignucci (4). Perithecia were formed when PA was crossed with the light strain (T_1) of *T. paradoxa* from sugarcane in vitro. No perithecia were obtained when the same PA was crossed with the dark strain (T_2) from sugarcane. Because the perithecia and ascospores produced by $T_1 \times$ PA are morphologically indistinguishable from those produced by $T_1 \times T_2$, it is concluded that they belong to the same species, i.e., *Ceratocystis paradoxa,* the perfect stage of *Thielaviopsis paradoxa.* However, the rate

TABLE 3.—*Effect of various fungicides on growth in vitro of* **Thielaviopsis paradoxa** *(sugarcane and pineapple isolates)*

Fungicide	Concentration	Diameter of inhibition zones		
		T_1*	T_2*	PA*
	Percent	*Mm.*	*Mm.*	*Mm.*
Benzoic acid	0.25	41.25**	40.00**	32.50**
	.50	62.50	57.50	53.25
	1.0	68.75	62.00	58.75
	1.5	67.00	66.25	60.25
	2.0	75.00	72.00	63.25
Dowicide A	.25	50.00	39.00	37.50
	.50	65.00	45.00	52.50
	1.0	64.00	50.00	61.75
	1.5	75.00	60.00	63.25
	2.0	73.00	60.00	68.00
Benlate	.25	55.00	47.50	52.00
	.50	53.75	48.25	53.00
	1.0	53.75	51.75	51.25
	1.5	51.50	51.50	50.75
	2.0	60.75	51.50	56.25
Tecto 60	.25	47.50	38.25	38.75
	.50	46.75	39.25	41.00
	1.0	47.00	39.00	41.00
	1.5	47.50	39.50	42.50
	2.0	45.00	38.75	43.00
Dithane M-45	.25	0	0	0
	.50	0	0	30.00
	1.0	0	0	32.25
	1.5	30.00	18.75	32.50
	2.0	0	25.00	36.25

* T_1 = light colored strain isolated from sugarcane.
T_2 = dark colored strain isolated from sugarcane.
PA = dark colored strain isolated from pineapple.
** Average of 4 replications.

TABLE 4.—*Concentration of fungicides in relation to rate of inhibiting mycelial growth of* **Thielaviopsis paradoxa** *in vitro by benzoic acid, Dowicide A, Tecto 60, Benlate and Dithane M-45*

Fungicide	Spearman rank correlation coefficient (r)	T-value[1]
Benzoic acid	0.70	7.57*
Dowicide A	.74	8.59*
Tecto 60	.14	1.10
Benlate	.18	1.45
Dithane M-45	.51	4.57*

* Correlation coefficient is significant at 1-percent level.

of growth of PA under various temperatures, pH, and cultural media was consistently different from those characterizing other strains of the fungus. The colonies produced by PA were consistently darker in appearance. In addition, PA also attacks seedpieces of sugarcane more virulently.

Results obtained from fungicide tests in this study agree with those of Liu et al. (6); Benlate is very effective in inhibiting mycelial growth of *T. paradoxa*. However, in this study benzoic acid as well as Dowicide A seemed more effective than Benlate.

SUMMARY

A previously unreported race of *Thielaviopsis paradoxa* was isolated from pineapple fruits with symptoms of black rot disease. Macro- and microconidia resemble in appearance those of *T. paradoxa*, the causal agent of the pineapple disease of sugarcane. Perithecia were produced in PDA (24°–28° C.) when the pineapple isolate was crossed with the light strain of *T. paradoxa* from sugarcane. Perithecia are characteristic of *Ceratocystis paradoxa* (horn-like appendages on the base of the perithecia and long, pointed ostiolar hyphae) and are morphologically indistinguishable from those obtained by crossing two sexually compatible strains from sugarcane. However, the rate of growth of the pineapple isolate, under various temperatures, pHs, and cultural media, was consistently different from those characterizing other strains of the fungus. The pineapple strain attacks sugarcane seedpieces more virulently than isolates obtained from sugarcane. In vitro tests were conducted in the laboratory to determine the relative toxicity of the fungicides benzoic acid, Dowicide A, Benlate, Tecto 60 (TBZ), and Dithane M-45 on pineapple and sugarcane isolates of the fungus. Benzoic acid and Dowicide A appeared to be the most effective of these fungicides for inhibiting mycelial growth of *T. paradoxa*.

RESUMEN

Se aisló una cepa del hongo *Thielaviopsis paradoxa*, no informada antes, de frutas de piña que presentaban síntomas de la enfermedad conocida como la podredumbre negra de la fruta. Las macroconidias y microconidias se asemejan a las de *T. paradoxa*, agente causativo de la llamada enfermedad de la piña en la caña de azúcar. Los peritecios se produjeron en un medio nutritivo de papa azucarado (potato dextrose agar), a una temperatura entre 24° y 28° C., al cruzarse la cepa aislada de la piña con una cepa clara de *T. paradoxa*, obtenida de la caña de azúcar. Los peritecios son típicos de *Ceratocystis paradoxa* (con apéndices en forma de cuernos en su base e hifas ostiolares puntiagudas) y morfológicamente indistinguibles de los que se obtienen al cruzar dos cepas sexualmente compatibles que se originen en la caña de azúcar. No obstante, el ritmo de crecimiento de la cepa bajo condiciones diversas de temperatura, valores diversos de pH y diversidad de medios nutritivos, fue siempre diferente a los que caracterizan otras cepas del mismo hongo. La cepa que se obtuvo de la piña afecta la semilla de caña (pedazos del tallo) con mayor virulencia que las obtenidas de la caña misma. En el laboratorio se hicieron pruebas in vitro para determinar el

grado de toxicidad relativa de varios fungicidas (ácido benzoico, Dowicide A, Benlate, Tecto 60 (TBZ) y Dithane M-45) a las cepas de este hongo aisladas de la piña y la caña de azúcar. Entre los fungicidas que se probaron, el ácido benzoico y el Dowicide A fueron los más efectivos para evitar el crecimiento del micelio de *T. paradoxa*.

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