

# Effect of Cachaza (Filter Press Cake)<sup>1</sup> and Sodium Chloride on Infection of Sugarcane by *Thielaviopsis paradoxa* in Puerto Rico<sup>2</sup>

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## INTRODUCTION

*Thielaviopsis paradoxa* (de Seynes) von Hohn., the causal agent of pineapple disease of sugarcane, has been observed affecting sugarcane seedpieces in the humid areas of Puerto Rico, especially in the Caño Tiburones area where the organic soil, Tiburones muck, is dominant. Liu noted recently that pineapple disease of sugarcane was very prevalent in darker soils (gray to black) of the Dominican Republic (1). The causal agent of the disease has been isolated very rarely from diseased sugarcane seedpieces in the saline soils along the southern coast of Puerto Rico. Patrick and Toussoum (4) reported in 1968 that organic matter amendments increased the virulence of certain soil-borne pathogenic organisms. Recaud in 1971 (5) reported that in Mauritius such amendments increased the incidence of chlorotic streak.

Although Liu and Cortés-Monllor (2) reported in 1972 that the highest rate of infection of sugarcane by *T. paradoxa* was obtained at the lowest soil moisture, information concerning the effect of "cachaza" as well as salt on growth and pathogenicity of this fungus is not available.

## MATERIALS AND METHODS

The pure filter press cake used in this study was obtained from a 1-year deposit at the Gurabo Substation. The same isolates of *Thielaviopsis paradoxa* (T<sub>1</sub>, T<sub>2</sub> and PA), used in previous studies (2,3), were employed in the present one. T<sub>1</sub> carries an American Type Culture Collection (ATCC 24269) and T<sub>2</sub> an American Type Culture Collection ATCC 24270. Potato dextrose agar was used throughout as a growing medium.

In the studies on the effect of soil amendment (filter press cake) on infection, the cut ends of single-eyed seedpieces of sugarcane variety P.R. 1059 were inoculated separately with a small, uniform round disc of mycelium (5 mm in diameter) of the light strain (T<sub>1</sub>) of *T. paradoxa* from sugarcane. The inoculated seedpieces were planted in enamelled trays con-

<sup>1</sup> Filter press cake or "cachaza" is a residual product from sugarcane manufacture.

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taining 0-, 30-, 50-, 70-, and 100-percent "cachaza"<sup>4</sup> mixed with steam-sterilized sand (the mixture having a 30-percent moisture content) and left in a shed covered with a 70-percent shade Saran screening for 13 days (from October 13, 1971 through October 26, 1971). At the end of the incubation period, the length of the discolored (infected) areas of the sugarcane seedpieces were measured.

In the studies on the effect of filter press cake on *in vitro* mycelial growth, isolates of *T. paradoxa* (T<sub>1</sub>, T<sub>2</sub>) from sugarcane and the isolate of *T. paradoxa* (PA) from pineapple were grown in potato dextrose agar medium. An extract (200 g cachaza/200 cc water) of cachaza was added to a water agar medium in the following proportions: 0-, 19-, 33-, 46-, and 66-percent cachaza. For each percentage of cachaza, eight petri dishes containing 20 ml of the above-mentioned medium were seeded separately with small, uniform, round discs of mycelium (5 mm in diameter) of each of the three isolates of *T. paradoxa*. 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 in the precision incubator at 32° C for 72 hours. The increase in the diameter of colonies was measured at the end of the incubation period.

In the studies on the effect of salt-amended sand on infection, the cut ends of single-eyed seedpieces of P.R. 1059 were inoculated separately with small, uniform, round cultural discs of mycelium (5 mm in diameter) of the dark strain (T<sub>2</sub>) of *T. paradoxa* from sugarcane. The inoculated seedpieces were planted in enamelled trays containing sterilized sands (30-percent moisture content) with the following salt (NaCl) content: 0-, 0.25-, 0.5-, 1.0-, 2.0-, and 4-percent.<sup>5</sup> The trays were then left at room temperature (22° to 25° C) for 12 days. For each salt content level, three trays containing five inoculated seedpieces of sugarcane were maintained at the above-mentioned temperatures. At the end of the incubation period, the length of the infected areas was measured.

The effect of salt (NaCl) content *in vitro* on mycelial growth was studied. Isolates of *T. paradoxa* (T<sub>1</sub>, T<sub>2</sub>) from sugarcane and the isolate of *T. paradoxa* (PA) from pineapple were grown in potato dextrose agar medium. Five-mm cultural discs containing the mycelium were placed in a petri

<sup>4</sup> 0-percent soil organic-substance content means that sands were washed and steam sterilized and no cachaza was added; the moisture content of the seedpieces was determined at approximately 75 percent; and the relative humidity during the period of the experiment ranged from 80 to 90 percent.

<sup>5</sup> 0-percent salt content means that sands were washed and steam-sterilized and no salt (NaCl) was added; the moisture content of the seedpieces was determined at approximately 75 percent; the moisture content of the sands was maintained at 30 per cent; and the relative humidity during the period of the experiment ranged from 75 to 80 percent.

dish containing water agar medium with the following sodium chloride (NaCl): 0-, 0.15-, 0.30-, 0.6-, 1.2-, and 2.4-percent. For each salt content level, ten petri dishes containing the above-mentioned medium were seeded separately with each of the three isolates of *T. paradoxa*. 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 in the precision incubator at 32° C for 72 hours. The increase in the diameter of colonies was measured at the end of the incubation period.

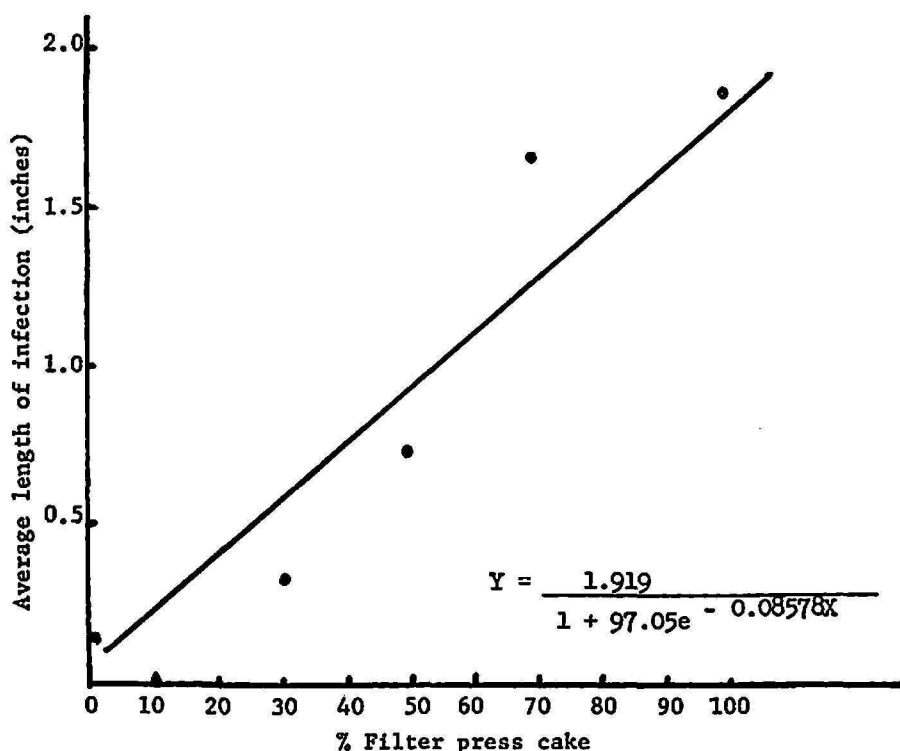


FIG. 1.—Effect of soil amendment (filter press cake) on infection of sugarcane by *Thielaviopsis paradoxa* (light strain T<sub>1</sub>, American Type Culture Collection ATCC 24269).

## RESULTS

### EFFECT OF FILTER PRESS CAKE ON INFECTION

The results (fig. 1) show that the rate of infection of sugarcane seedpieces by *T. paradoxa* increased with an increase of the percentage of filter press cake when inoculated seedpieces were planted in the enamelled trays containing the above-mentioned percent (0, 30, 50, 70 and 100) mixture of cachaza for 13 days. A study of the correlation coefficient between "cachaza" content of the soil and length of infection area of sugarcane seedpieces by *T. paradoxa* revealed that the regression coefficient ( $r$ ) was positive. The logistic equation determined for the average length of infection of sugarcane by *T. paradoxa* (light strain T<sub>1</sub>) is:

$$Y = \frac{1.919}{1 + 97.05e^{-0.08678x}}$$

$Y$  = expected rate of infection;  $X$  = percent cachaza content. The coefficient of determination ( $r^2$ ) = .90\*\* is highly significant at the 1-percent level.

#### EFFECT OF FILTER PRESS CAKE EXTRACT ON IN VITRO MYCELIAL GROWTH

The results obtained (table 1) show that the optimum filter press cake extract mixture content for mycelial growth of all the three isolates was 19 percent. Total filter press cake extract content higher than 19 percent did not additionally increase mycelial growth of the fungus.

TABLE 1.—Effect of "cachaza" (filter press cake) on mycelial growth of *Thielaviopsis paradoxa* in vitro<sup>1</sup>

"Cachaza" concentration	Average diameter of mycelial growth (mm)		
	T <sub>1</sub> <sup>2</sup>	T <sub>2</sub> <sup>2</sup>	PA <sup>2</sup>
Percent			
0	46	41	42
19	58	59	65
33	58	59	65
46	58	58	65
66	57	58	65

<sup>1</sup> Average of 8 replications.

<sup>2</sup> T<sub>1</sub> = *Thielaviopsis paradoxa* (light strain) from sugarcane.

T<sub>2</sub> = *Thielaviopsis paradoxa* (dark strain) from sugarcane.

PA = *Thielaviopsis paradoxa* from pineapple.

#### EFFECT OF SODIUM CHLORIDE ON INFECTION

The results (fig. 2) show that the rate of infection of sugarcane seedpieces by *T. paradoxa* (dark strain) was lower with a higher salt content of the soils when inoculated at temperatures of 22° to 25° C. A study of the correlation coefficient between salt content of the soils and length of infection area of sugarcane seedpieces by *T. paradoxa* revealed that the correlation coefficient ( $r$ ) at a temperature range of 22° to 25° C was negative with a value of  $-0.84177$ . The linear equation determined for the average length of infection of sugarcane seedpieces by *T. paradoxa* is:

$$Y = 61.42 - 0.9431X$$

$Y$  = expected rate of infection;  $X$  = percent salt content of the soils.  $F$ -value of the equation, 432.81, is highly significant at the 1-percent level.

## EFFECT OF SODIUM CHLORIDE ON IN VITRO MYCELIAL GROWTH

The results obtained (table 2) show that the optimum concentration of salt for mycelial growth in a water agar medium is between 0.3 and 0.6 percent.

## DISCUSSION

A number of surveys were made to record the incidence of pineapple disease of sugarcane at various times in different areas of Puerto Rico.

Pineapple disease of sugarcane was found to be widespread on B.49119,

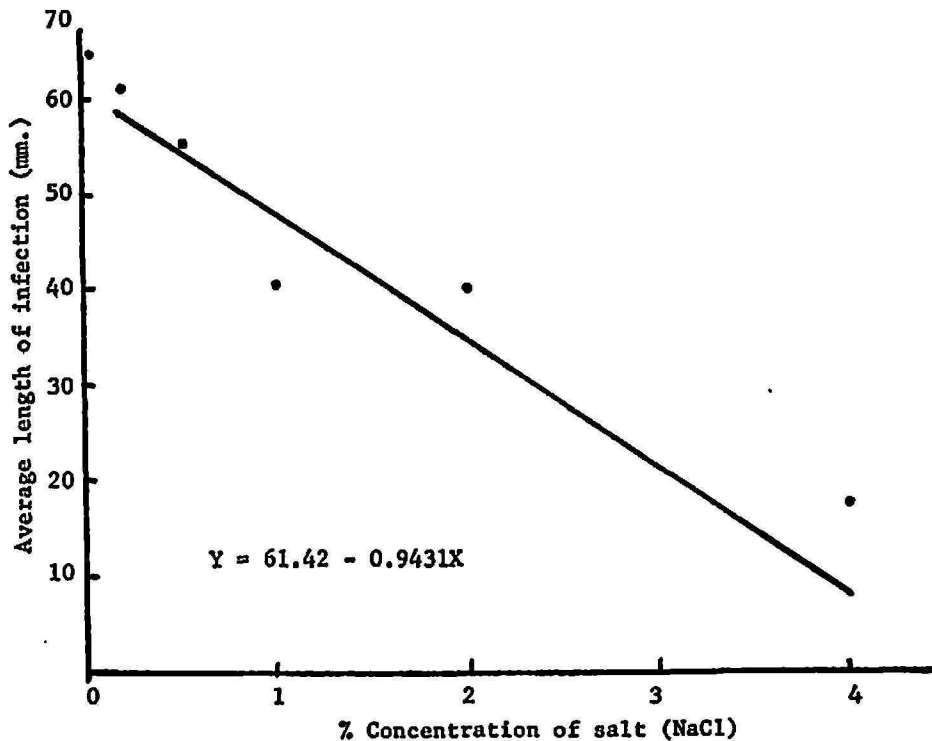


FIG. 2.—Effect of soil salt (NaCl) content on infection of sugarcane by *Thielaviopsis paradoxa* (dark strain T<sub>2</sub>, American Type Culture Collection ATCC 24270).

H. 32-8560, P.R. 980, and B. 4262 in winter at Caño Tiburones where muck soil predominates.

The results obtained in this study generally agree with those of Patrick and Toussoum (4) who reported that soil organic amendments increased severity of damage caused by certain soil-borne pathogenic organisms. The highest infection of sugarcane by chlorotic streak also occurred in soils with greater amounts of organic substances (5).

It is of interest to note that high salt content of the soil (1.20 to 4.0 percent) decreased infection of sugarcane seedpieces by this fungus when inoculated seedpieces were planted in the sands with the above-mentioned salt content.

## SUMMARY

The optimum filter press cake extract mixture content for mycelial growth of the various isolates of *Thielaviopsis paradoxa* from sugarcane and pineapple was found to be 19 percent.

Pathogenicity of *T. paradoxa* to sugarcane variety P.R. 980 was favored by high proportions of filter press cake. The rate of infection of sugarcane by this fungus increased with an increase of the percentage of filter press cake.

The optimum concentration of sodium chloride for mycelial growth in a water agar medium was between 0.3 and 0.6 percent.

The rate of infection of sugarcane seedpieces by *T. paradoxa* (dark strain) was lower with a higher sodium chloride content of the sand when inoculated at temperatures of 22° to 25° C.

TABLE 2.—Effect of salt on mycelial growth of *Thielaviopsis paradoxa* in vitro<sup>1</sup>

Salt (NaCl) concentration	Average diameter of mycelial growth (mm)		
	T <sub>1</sub> <sup>2</sup>	T <sub>2</sub> <sup>2</sup>	PA <sup>2</sup>
<i>Percent</i>			
0	63	48	64
.15	74	57	72
.30	81	61	80
.60	82	57	88
1.20	65	36	87
2.40	61	27	60

<sup>1</sup> Average of 10 replications.

<sup>2</sup> T<sub>1</sub> = *Thielaviopsis paradoxa* (light strain) from sugarcane.

T<sub>2</sub> = *Thielaviopsis paradoxa* (dark strain) from sugarcane.

PA = *Thielaviopsis paradoxa* from pineapple.

## RESUMEN

El contenido óptimo de extracto de cachaza en una mezcla para promover el crecimiento del micelio de varias cepas de *Thielaviopsis paradoxa*, aislada de la caña de azúcar y de la piña, fue 19 por ciento.

Las altas proporciones de cachaza propiciaron la patogenicidad de *T. paradoxa* en la variedad de caña P.R. 980. El grado de infección de la caña de azúcar por este hongo aumentó al aumentar el porcentaje de cachaza.

La concentración óptima de cloruro de sodio en un medio de agar en agua para promover el crecimiento del micelio fue entre 0.3 y 0.6 por ciento.

El ritmo de infección de los pedazos de caña de azúcar con *T. paradoxa* (cepa obscura) fue menor cuanto más alto fue el contenido de cloruro de sodio en la arena, al inocularse aquéllos a una temperatura entre 22° y 25°C.

## LITERATURE CITED

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