Effect of Temperature and Moisture on Various Aspects of Development, Growth, and Pathogenicity of *Thielaviopsis paradoxa* from Sugarcane in Puerto Rico[1]

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INTRODUCTION

Thielaviopsis paradoxa (De Seynes) Von Höhn, the causal agent of pineapple disease of sugarcane, has been observed frequently affecting sugarcane seedpieces during the cooler months of the year in the humid areas of Puerto Rico. This same fungus, however, also has been isolated frequently from diseased seedpieces of sugarcane in the dry areas during hot summer months. Several investigators (2,5,6) have provided evidence to indicate that the development of certain plant diseases is correlated closely with temperature and precipitation. In many instances, there is a certain degree of correlation between the relationship of temperature of certain fungi in pure culture to their distribution and seasonal occurrence. Kiryu (3) in 1939 in Formosa found that development of *T. paradoxa* occurred on potato sucrose agar at a temperature range of 13° to 34° C., with an optimum at 25° to 31° C. Chi (1) in 1949 also reported that the optimum temperature for the growth of the fungus occurred at 28° C., with a range from 10° to 34° C. These investigators, however, did not report studies concerning the effect of temperature and moisture on infection of sugarcane by this fungus. Liu and Mignucci (4) in Puerto Rico reported recently on the formation of the sexual stage of *T. paradoxa* in vitro.

This investigation was conducted for the purpose of securing data showing the effect of temperature and moisture upon the growth and infectivity of several isolates of *T. paradoxa* in Puerto Rico, and the effect of these factors on spore germination. No previous information has been assembled concerning the effect of moisture and temperature on infection of sugarcane by this fungus. The following report thus constitutes the first concerning these factors.

METHODS AND RESULTS

EFFECT OF TEMPERATURE ON SPORE GERMINATION

Washed macro- and micro-conidial spores of *T. paradoxa* (both dark and light strains (4)) were used to inoculate water-agar plates (three for each

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temperature) which had been maintained for 1 day in precision incubators at temperatures ranging from 8° to 40° C. with 4° C. intervals. The plates were then transferred back to the incubators at their corresponding temperatures and kept for 6, 12, and 24 hours. After each interval, counts of germinated and non-germinated spores were made with the aid of a dissecting microscope.

The results (table 1) show that the optimum range for germination of both strains of *T. paradoxa* on the medium used lies between 28° and 32° C.

EFFECT OF TEMPERATURE ON MYCELIAL GROWTH

Isolates of both strains of *T. paradoxa* from sugarcane were grown in potato dextrose agar medium at the following nine temperatures: 8°, 12°,

TABLE 1.—*Effect of temperature on spore germination of* **Thielaviopsis paradoxa** *(both light and dark strains) from sugarcane*[1]

Temperature	Light strain (T_1)				Dark strain (T_2)			
	Microspore		Macrospore		Microspore		Macrospore	
	Spore germination at the end of—				Spore germination at the end of—			
	6 hours	12 hours	12 hours	24 hours	6 hours	12 hours	12 hours	24 hours
°C.	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
8	0	0	0	0	0	0	0	0
12	0	0	0	42	21	20	11	22
16	23	32	21	42	42	61	22	40
20	34	76	18	33	43	81	24	40
24	26	66	19	34	64	82	25	43
28	42	87	22	42	66	84	36	66
32	63	74	33	65	87	74	44	86
36	48	68	24	48	43	61	21	42
40	0	0	10	20	0	0	0	0

[1] Average of 3 replications.

16°, 20°, 24°, 28°, 32°, 36°, and 40° C. For each temperature, five petri dishes containing 15 ml. of the above-mentioned medium were separately inoculated with small, uniform, round discs of mycelium (2 mm. in diameter) of both strains. 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 incubators at the different temperatures for 2 days. The increase in the diameter of colonies was measured at the end of the incubation period.

The optimum temperature range for mycelial growth of both strains was found to be above 28° C., with the maximum 36° C. and the minimum below 12° C. No mycelial growth was obtained either at 8° or at 40° C. (fig. 1).

EFFECT OF TEMPERATURE ON INFECTION

The cut ends of single-eyed seedpieces of sugarcane variety P.R. 980 were separately inoculated with small, uniform, round discs of mycelium (2 mm. in diameter) of the light strain (T_1) of *T. paradoxa* from sugarcane. The inoculated seedpieces were planted in enamelled trays containing sterilized sand and incubated in precision incubators for 7 days at the following temperatures: 8°, 12°, 16°, 20°, 24°, 28°, 32°, and 40° C. At the end of the in-

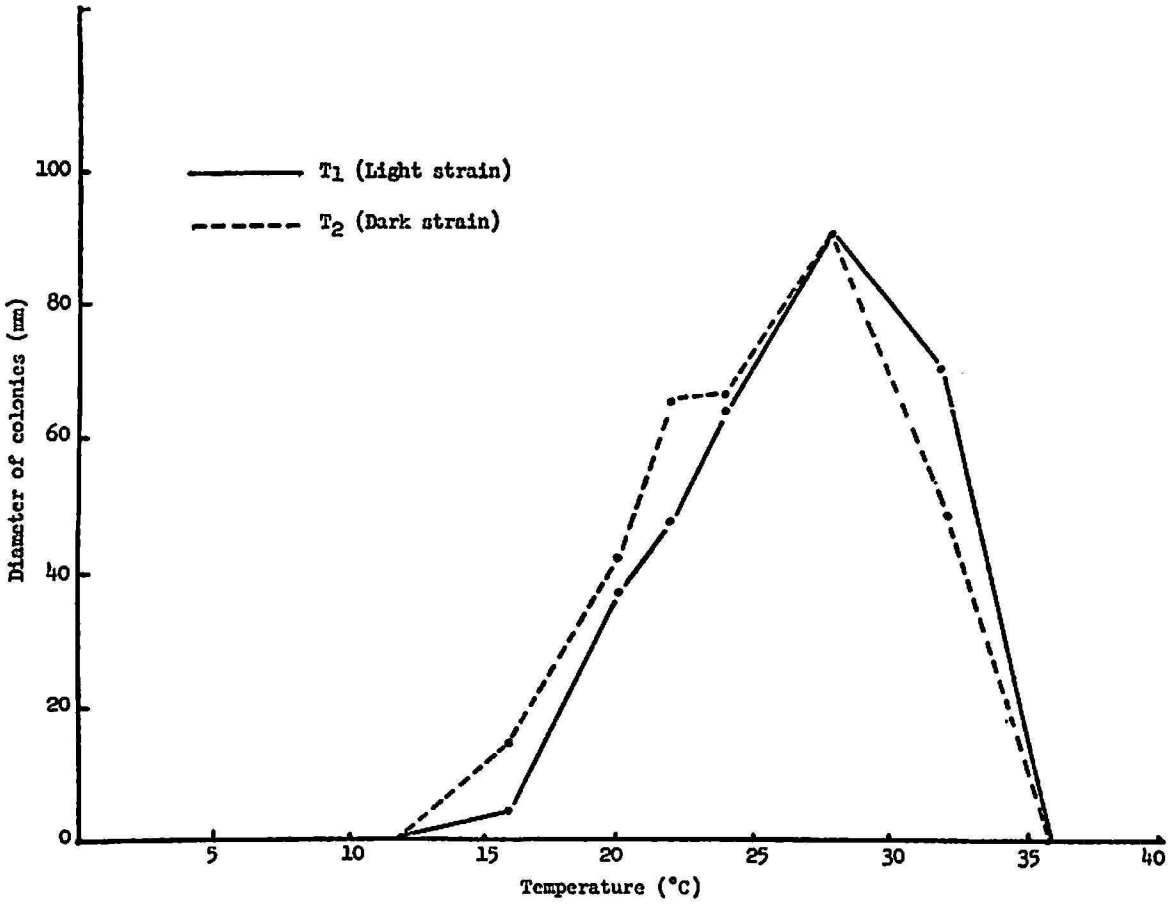

FIG. 1.—Diameter of colonies of *Thielaviopsis paradoxa* on potato dextrose agar at different temperatures.

cubation period, the length of the discolored (infected) areas of the sugarcane seedpieces was measured.

The results (fig. 2 and 3) show that the highest rate of infection of sugarcane seedpieces by *T. paradoxa* (light strain) occurred between 28° to 32° C. when inoculated seedpieces were incubated for 7 days at the above-mentioned temperatures. No infection of sugarcane by *T. paradoxa* was obtained at temperatures higher than 36° C. or lower than 8° C.

EFFECT OF MOISTURE ON INFECTION

The cut ends of single-eyed seedpieces of P.R. 1059 were separately inoculated with small, uniform, round discs of mycelium (2 mm. in diame-

ter) of the dark strain (T_2) of *T. paradoxa* from sugarcane. The inoculated seedpieces were planted in enamelled trays containing sterilized sand with the following moisture content: 75, 50, 25, 12.5, 6.25, and 0 percent.[3] The trays were then maintained in precision incubators for 10 days at the following temperatures: 22°, 28°, 32°, and 36° C. For each moisture content, three trays (each containing five inoculated seedpieces of sugarcane) were maintained at each of the above-mentioned temperatures. After incubation the length of the infected areas was measured.

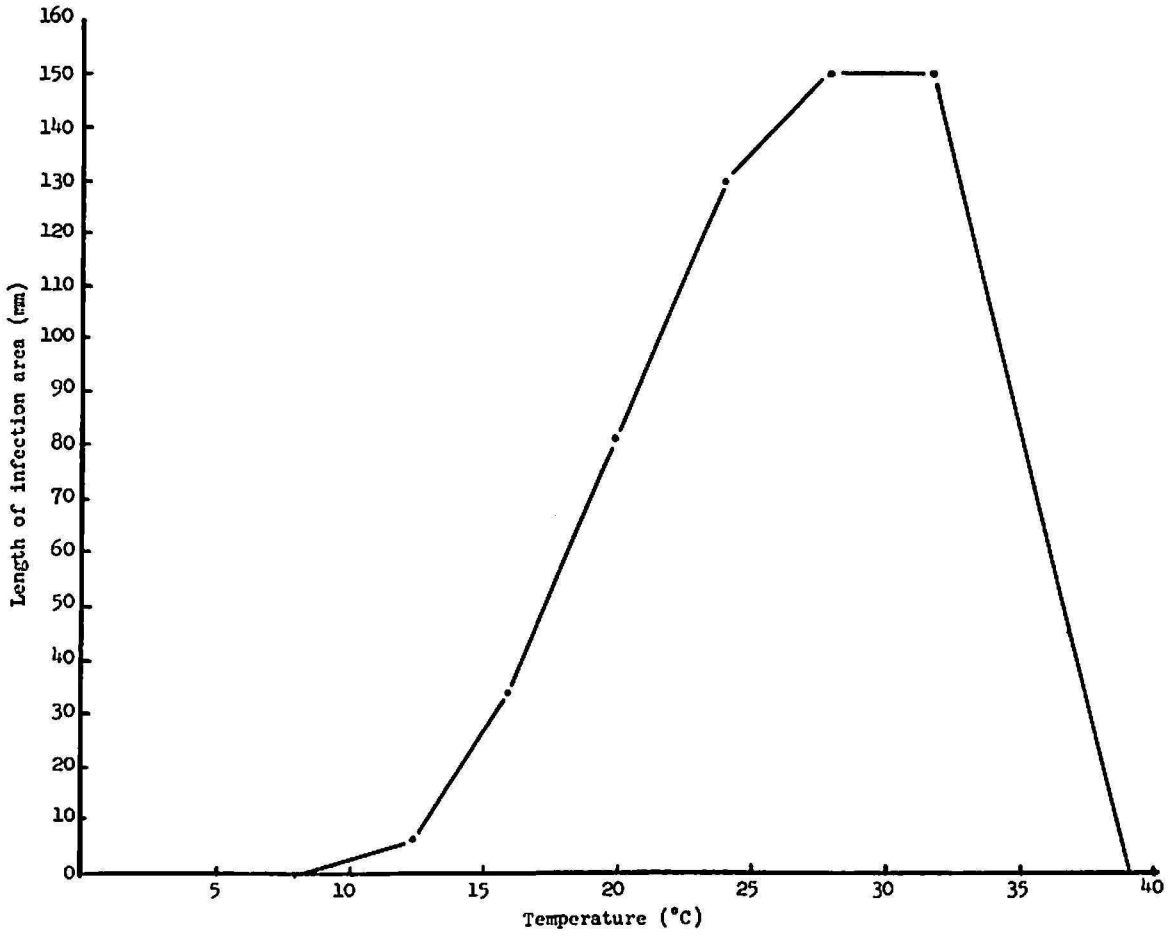

FIG. 2.—Effect of various temperatures on infection of sugarcane seedpieces by *Thielaviopsis paradoxa*.

The results (table 2 and fig 4) show that the highest rate of infection of sugarcane seedpieces by *T. paradoxa* (dark strain) occurred at 0-percent moisture content of the soils when incubated at temperatures of 22°, 28°, and 32° C. However, this same result was not obtained when the inoculated

[3] 0-percent moisture content means that sands were dried to 0 percent and no water was added; the moisture content of the seedpieces was determined at approximately 76.6 percent; and the air relative humidity during the period of the experiment ranged from 60 to 70 percent.

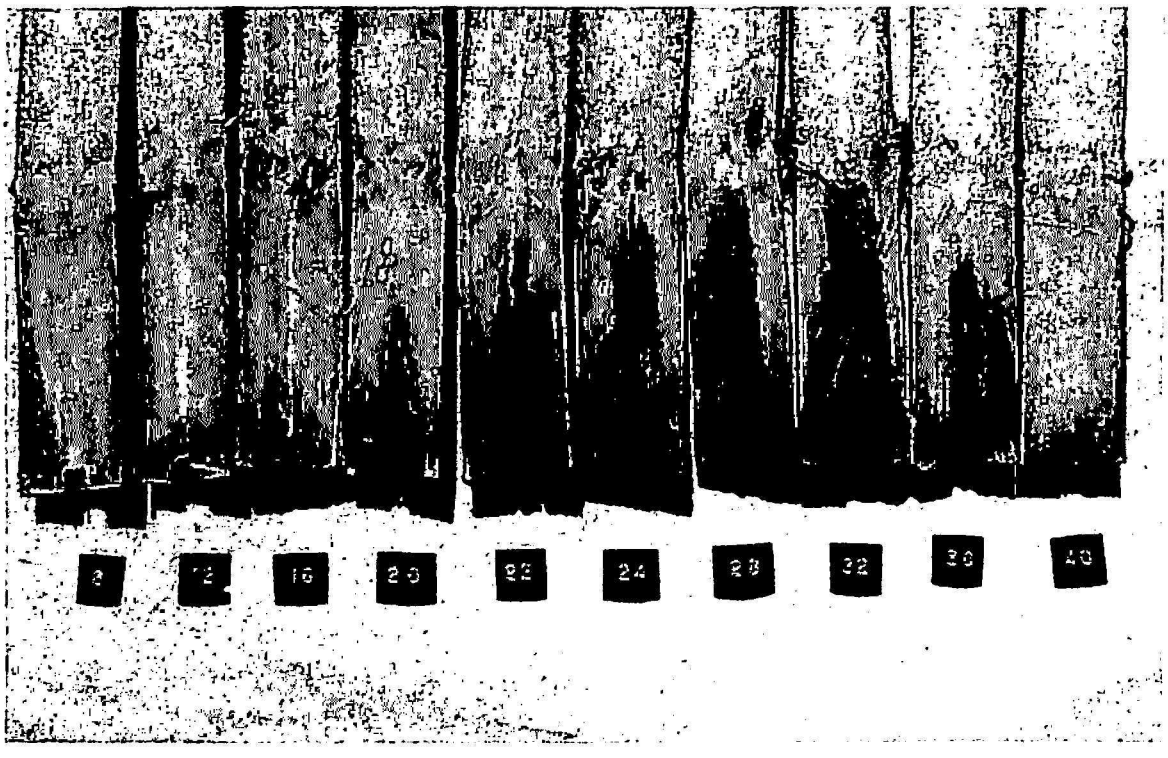

FIG. 3.—Differences in rate of infection of sugarcane seedpieces of sugarcane variety P.R. 980 by *Thielaviopsis paradoxa* at various temperatures; from left to right, 8°, 16°, 20°, 22°, 24°, 28°, 32°, 36°, and 40° C., respectively.

seedpieces were incubated at 36° C. The highest rate of seedpieces infection at 36° C. occurred between 25- to 50-percent moisture content of the soils.

A study of the correlation coefficient between moisture content of the soil and length of infection area of sugarcane seedpieces by *T. paradoxa* revealed that the regression coefficients (r) at temperatures of 22°, 28°, and 32° C. were negative, while positive at 36°. Table 3 shows the functional relationship worked out and gives the coefficient of correlation. The F-value was highly significant for the above-mentioned temperatures, and significant for 36° C. This suggests that there is an interaction between the

TABLE 2.—*Differences in length of infection areas (expressed in inches) in sugarcane seedpieces by* **Thielaviopsis paradoxa** *at various levels of soil moisture content*

	Treatment	Moisture content	Incubated at—			
			22°C.	28°C.	32°C.	36°C.
		Percent				
A	0 water	0	1.71	2.14	3.50	0.14
B	Added 125 ml. water	6.25	1.29	1.59	2.84	.29
C	do. 250 ml. do.	12.5	1.12	1.30	2.86	.51
D	do. 500 ml. do.	25	.84	.62	2.56	1.54
E	do. 1,000 ml. do.	50	.70	.26	2.44	1.68
F	do. 1,500 ml. do.	75	.00	.00	1.60	1.47

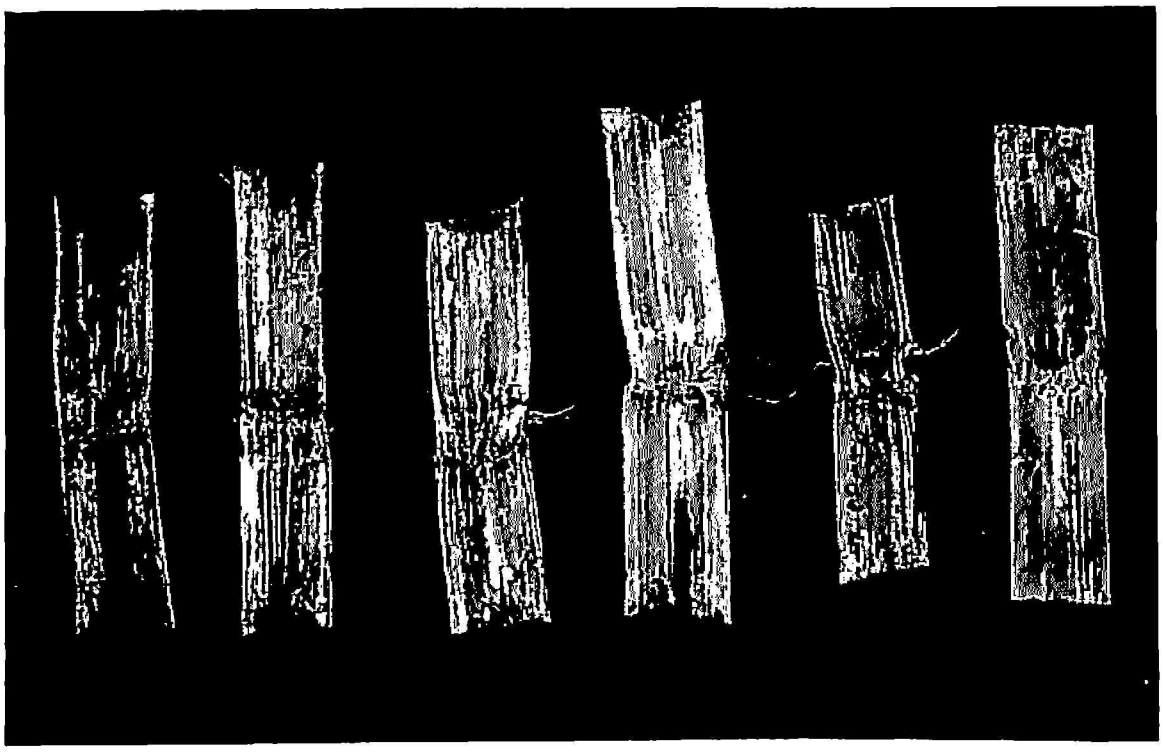

FIG. 4.—Rate of infection of sugarcane seedpieces of P.R. 1059 by *Thielaviopsis paradoxa* at 28° C. under various levels of soil moisture content; from left to right, 0, 6.25, 12.5, 25, 50, and 75 percent, respectively.

influences of temperature and soil moisture on the rate of infection of sugarcane seedpieces by *T. paradoxa*, there being a decrease of infection with soil moisture at 22 and 32° C., and an increase of infection with soil moisture at 36° C.

EFFECT OF TEMPERATURE AND MOISTURE ON DISTRIBUTION AND SEASONAL OCCURRENCE

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

TABLE 3. *Soil moisture content in relation to degree of infection of sugarcane seedpieces by* **Thielaviopsis paradoxa**

Temperature	Functional relationship[1]	Correlation coefficient (r)	F-value[2]
°C.			
22	$Y = 1.4868 - 0.0193\ X$	−0.9604	47.61**
28	$Y = 1.7319 - .0266\ X$	−.9295	25.42**
32	$Y = 3.2018 - .0202\ X$	−.9365	28.56**
36	$Y = .3851 + .0197\ X$	.8166	8.00*

[1] Y = Average length of infection areas of sugarcane seedpieces to be estimated (inches); X = soil moisture content (ml. in 2000 g. of soil).

[2] * = Regression is significant at 5-percent level; ** = regression is highly significant at 1-percent level.

TABLE 4.—*Relation of temperature and rainfall to distribution and seasonal occurrence of pineapple disease of sugarcane in Puerto Rico*

Ecological zones	Locality	Variety	Extent of pineapple disease observed	Season observed
1. Low temperature zone—with average temperature 71° F. during January to March and 76°–77° F. during June to August.	Central Cambalache	B. 49119	Widespread	Winter, 1969
	Central Roig	H. 32-8560	do.	do.
	Central Monserrate	P.R. 980	Occasionally observed	Spring, 1970
		B. 4362	do.	do.
	Central Coloso	B. 49119	do.	do.
		H. 32-8560	do.	do.
2. Moderate temperature zone—with average temperature 72°–75° F. during January to March and 78°–79° F. during June to August.	Central Igualdad	B. 49119	do.	Autumn, 1970
	San Sebastián	H. 32-8560	do.	do.
3. High temperature zone—with average temperature 76°–79° F. during January to March and 82°–83° F. during June to August.	Central Mercedita	B. 49119	Widespread	Summer, 1970
	Central Guánica	B. 49119	do.	do.

Pineapple disease of sugarcane was found to be widespread (table 4) on B. 49119 and H. 32-8560 at Central Cambalache and Central Roig in winter, when the average temperature ranged from 63° to 78° F. (17°–26° C.). At Central Guánica and Mercedita, it was also found to be widespread on B. 49119 in the summer when the average temperature ranged from 82° to 83° F. (27°–28° C.).

DISCUSSION

The results obtained in this study agree generally with those of Kiryu (3) and Chi (1) in Taiwan, who found the optimum temperature for mycelial growth and spore germination of *T. paradoxa* from sugarcane to be between 28° and 32° C. The highest degree of infection of sugarcane seedpieces by this fungus also occurred within this temperature range.

It is interesting to note that high moisture content of the soil (50 to 75 percent) did not increase infection of sugarcane by this fungus when inoculated seedpieces were incubated at temperatures of 22° to 32° C. The highest rate of infection of sugarcane seedpieces by *T. paradoxa* occurred at the lowest moisture content of the soil (0 percent).

In the northern and western part of Puerto Rico, temperatures during the winter months range from 71° to 75° F. (22° to 24° C.). These temperatures are not the most favorable for infection of sugarcane by *T. paradoxa*. Low rainfall during this period, however, may contribute to severe infection of sugarcane seedpieces by this organism.

In the southern part of Puerto Rico (Central Mercedita area) temperatures during the summer months range from 82° to 83° F. (22° to 28° C.). These temperatures are ideal for infection of sugarcane by *T. paradoxa*. Drought during this period tends to aggravate the situation.

SUMMARY

It was found that the optimum temperature range for mycelial growth and spore germination of *Thielaviopsis paradoxa* lies between 28° and 32° C., with the maximum above 36° C. and the minimum below 12° C. Neither mycelial growth nor spore germination was obtained at 8° or at 40° C.

Pathogenicity of *T. paradoxa* to sugarcane variety P.R. 1059 was favored by temperatures between 28° and 32° C. No infection of sugarcane by this fungus occurred at 8° or at 40° C.

The highest rate of infection of sugarcane by *T. paradoxa* was obtained at the lowest soil moisture with a maximum temperature up to 32° C. It thus appears that low soil moisture has a profound effect on rate of infection during summer months in Puerto Rico.

RESUMEN

Se determinó que la temperatura óptima para el crecimiento del micelio y la germinación de las esporas de *Thielaviopsis paradoxa* fluctúa entre 20° y 32° C., siendo el

nivel máximo mayor de 38° C. y el mínimo menor de 12° C. No se logró que el micelio creciera ni que las esporas germinaran a los niveles de 8° y 40° C.

Las temperaturas de 28° a 32° C. propiciaron la patogenicidad de *T. paradoxa* en la variedad de caña de azúcar P.R. 1059, pero no se logró la infección de la caña por el hongo a los niveles de 8° y 40° C.

El grado de infección de la caña por *T. paradoxa* fue mayor cuando más bajo era el contenido de humedad en el suelo hasta una temperatura máxima de 32° C. Tal parece, por tanto, que un bajo contenido de humedad en el suelo afecta marcadamente el grado de infección durante los meses del verano en Puerto Rico.

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