

# A New Species of *Helminthosporium* Causing Leaf Spot Disease of Sugarcane in Puerto Rico

Lii-Jang Liu<sup>1</sup>

## INTRODUCTION

Many species of *Helminthosporium* cause leaf spot and seedling blights of grasses and corn. Only two species of *Helminthosporium*, however, have been reported on sugarcane in Puerto Rico: *H. sacchari*, causal agent of eye-spot disease and *H. stenospilum*, causal agent of brown stripe. Parris in 1950 (9)<sup>2</sup> isolated *H. rostratum* from brown stripe, as well as eye-spot leaf lesions. Bourne in 1956 (1) described a new species of *Helminthosporium* causing disease on sugarcane and named the fungus *H. purpurascens* Bourne. Recently, he also reported *Bipolaris rostrata* (Drechs.) Shoemaker to be the cause of a leaf spot disease in sugarcane and grasses (2).

Leaf spot caused by *Helminthosporium* spp. is one of the principal leaf diseases of sugarcane in Puerto Rico. High incidence of the disease along the northern coast of the Island has given reason for concern.

While cultural variants of *Helminthosporium stenospilum* were being studied in Puerto Rico, conidia of *H. rostratum* were isolated occasionally. Conidia of what appeared to be an undescribed species of *Helminthosporium* also were isolated from leaf-spotted plants of sugarcane varieties P.R. 980, P.R. 1028, P.R. 1059, P.R. 1048, and M. 336. Such plants were secured from the Caño Tiburones, Isabela, Igualdad, and San Sebastián areas. The new fungus species is described below and data is offered on its pathogenicity on sugarcane. New information concerning the morphology, as well as the physiology, of two related species of *Helminthosporium*, i.e., *H. rostratum* and *H. holmii*, also is presented.

## REVIEW OF THE LITERATURE

A small group of species, characterized by a protuberant conidial hilum, exists among the various species of *Helminthosporium*, subgenus *Euhelminthosporium* (7). These include *H. turcicum* Pass, *H. rostratum* Drechs., *H. monoceras* Drechs., *H. halodes* Drechs., and *H. halodes* var. *tritici* Mitra. *H. turcicum* is known to cause leaf spot disease on corn (8) and *H. monoc-*

<sup>1</sup> Phytopathologist, Agricultural Experiment Station, Mayagüez Campus, University of Puerto Rico, Río Piedras, P.R. Thanks are due to Dr. E. E. Butler, Professor of Plant Pathology, University of California, Davis, California for generously supplying the culture of *Helminthosporium holmii* and for his invaluable advice. Thanks also are due to Miss Gilda Vicente and Miss Julia Mignucci for assistance in the laboratory.

<sup>2</sup> Italic numbers in parentheses refer to Literature Cited, p. 21-2.

eras on grasses (3). *H. halodes* var. *tritici* occurs primarily on the roots of grasses (4). Their pathogenicity on sugarcane thus far has not been determined.

*H. rostratum* is known to incite ear rot of corn (8) and leaf spot of grasses (11). This fungus was reported recently as the cause of a leaf spot disease of sugarcane and grasses (2).

*H. rostratum* was described first by Drechsler in 1923 (3). Mature conidia measure 32 to 184  $\mu$  long X 14 to 22  $\mu$  wide with a conspicuous protruding hilum at the base. This fungus can be differentiated readily from other



FIG. 1.—Leaf spot on sugarcane leaves caused by *Helminthosporium portoricensis* n. sp.

graminicolous forms by its rostrate spores. Shoemaker (10) includes this fungus under the generic name *Bipolaris* because its conidia germinate only from each end. According to Luttrell (5,6), conidia of *H. holmii* are similar to those of *H. rostratum* except that the conidia of the former are greater in diameter and darker in appearance. No *Helminthosporium* species with conidia similar to those of *H. rostratum* (but without rostrate spores) have been reported on sugarcane.

## METHODS AND RESULTS

### DESCRIPTION OF SYMPTOMS

In the early stage of infection, affected plants show minute, watery spots. These spots enlarge gradually. The sites of initial infection turn dark brown

TABLE 1.—Frequency of distribution and size of conidia of three *Helminthosporium* species from sugarcane

	<i>H. portoricensis</i> n. sp.				<i>H. rostratum</i>				<i>H. holmii</i>			
	Length	Conidia	Width	Conidia	Length	Conidia	Width	Conidia	Length	Conidia	Width	Conidia
	Microns	Number	Microns	Number	Microns	Number	Microns	Number	Microns	Number	Microns	Number
14	17.60-29.50	79	8.60-10.50	7	17.60-29.50	18	8.60-10.50	46	17.60-29.50	0	8.60-10.50	
	29.60-39.50	97	10.60-12.50	49	29.60-39.50	14	10.60-12.50	102	29.60-39.50	0	10.60-12.50	2
	39.60-49.50	15	12.60-14.50	68	39.60-49.50	26	12.60-14.50	38	39.60-49.50	0	12.60-14.50	10
	49.60-59.50	5	14.60-16.50	30	49.60-59.50	86	14.60-16.50	10	49.60-59.60	0	14.60-16.50	70
	59.60-69.50	4	16.60-18.50	38	59.60-69.50	42	16.60-18.50	0	59.60-69.50	14	16.60-18.50	46
	69.60-79.50	0	18.60-20.50	8	69.60-79.50	14	18.60-20.50	0	69.60-79.50	38	18.60-20.50	52
	79.60-89.50	0	20.60-22.50	0	79.60-89.50	0	20.60-22.50	0	79.60-89.50	90	20.60-22.50	8
	89.60-99.50	0	22.60-24.50	0	89.60-99.50	0	22.60-24.50	0	89.60-99.50	26	22.60-24.50	10
	99.60-109.50	0	24.60-26.50	0	99.60-109.60	0	24.60-26.50	0	99.60-109.50	26	24.60-26.50	2
	109.60-119.50	0			109.60-119.60				109.60-110.50			

and become elongated. The maturing lesions turn into definite stripes which vary from 2 mm. to 6 mm. in length (fig. 1).

#### IDENTIFICATION OF CAUSAL ORGANISM

Conidia of the previously undescribed species of *Helminthosporium* are broad, obclavate with a protruding conical hilum when the fungus is cultured on potato dextrose agar. Basal and apical conidial cells are frequently lighter in color and set off by accentuated, thicker, darker septa. Conidia may be straight or curved, measuring 17.60 to 69.50  $\mu$  long X 8.60 to 20.50  $\mu$  wide with 7 to 10 septations (table 1).

Conidia of *H. rostratum* and *H. holmii* were also examined. Those of *H. rostratum* were 17.60 to 79.50  $\mu$  long X 8.60 to 16.50  $\mu$  wide while those of *H. holmii* were 59.60 to 119.50  $\mu$  long X 10.60 to 26.50  $\mu$  wide. Conidia of both *H. rostratum* and *H. holmii* are broad obclavate with protruding conical hilum (fig. 2).

#### PHYSIOLOGIC CHARACTERISTICS

##### *I. Effect of Temperature on Mycelial Growth*

Monoconidial isolates of the previously undescribed *Helminthosporium* were grown in potato dextrose agar, corn meal agar and nutrient agar at 8°, 12°, 16°, 20°, 24°, 28°, 32°, 36°, and 40° C. For each temperature, five petri dishes containing 15 ml. of the above mentioned media were inoculated with 2 mm. culture discs. The discs were cut with a sterile cork borer from the advancing margin of colonies kept in corn meal 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 and 5-day incubation periods.

The results (table 2) show that the optimum temperature range for mycelial growth of isolates of the new species of *Helminthosporium* lies between 28° and 30° C. on the media used.

Similar results were obtained with *H. rostratum* and *H. holmii*.

##### *II. Effects of pH on Mycelial Growth*

The conidia of the undescribed species of *Helminthosporium* were grown on potato dextrose agar with the following pH: 4, 5, 6, 7, 8, 9, 10, and 11. For each pH, five petri dishes containing 15 ml. of the above mentioned medium were inoculated with 2 mm. culture discs of the *Helminthosporium* species, cut with a sterile cork borer from the advancing margin of colonies kept in corn meal agar. The dishes containing the inoculum were incubated at 28° C. for 6 days. The increment in the diameter of the colonies was measured at the end of the 3-, 4-, 5-, and 6-day incubation periods.

The data (table 3) indicated that the optimum pH for mycelial growth of the undescribed species of *Helminthosporium* lies between 7 and 10. Similar results were obtained with *H. rostratum* and *H. holmii*.

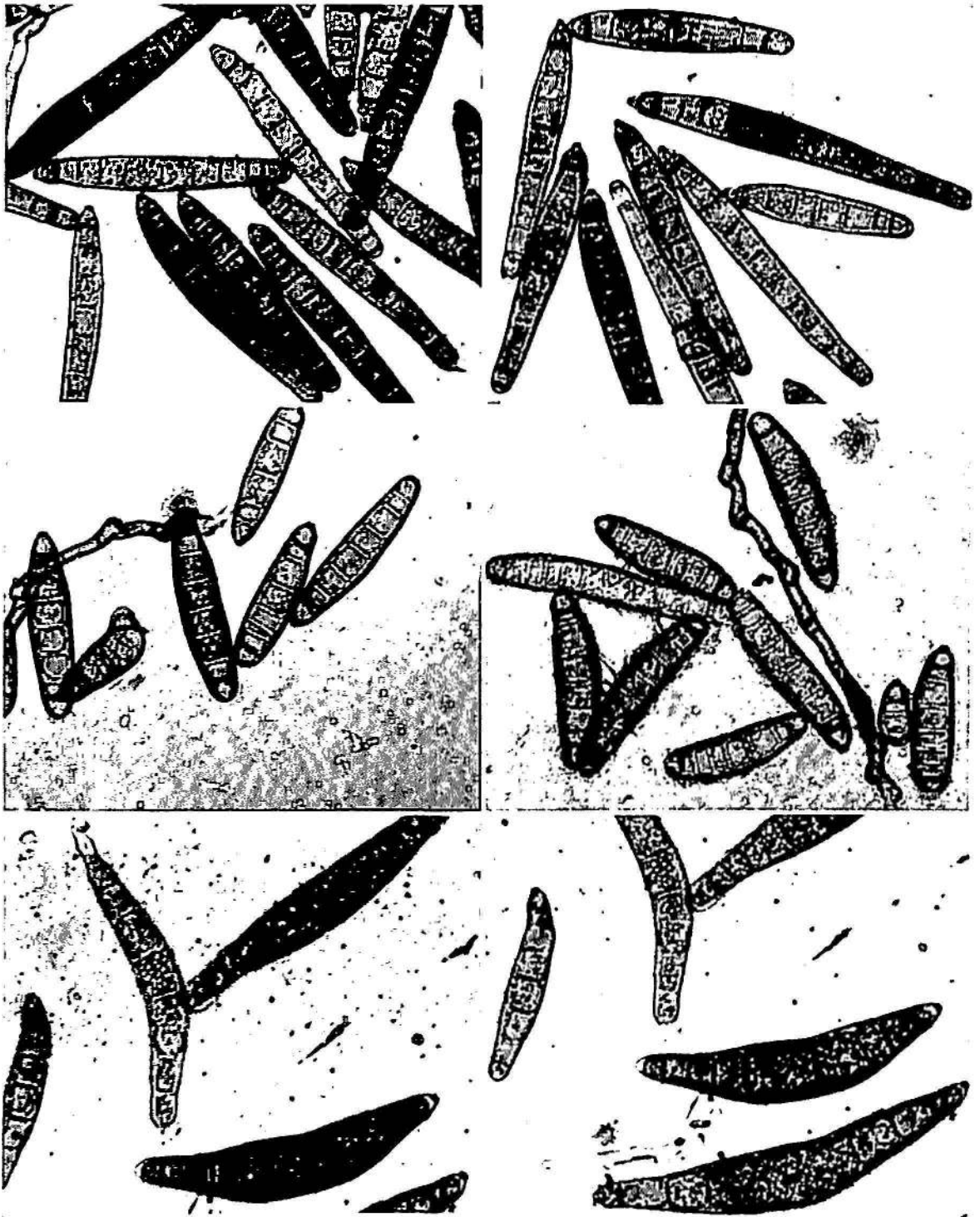


FIG. 2.—Conidia of *Helminthosporium* spp.: From top down, 1st row, *Helminthosporium rostratum*; 2nd row, *Helminthosporium portoricensis* n. sp.; 3rd row, *Helminthosporium holmii*.



TABLE 2.—Effect of various temperatures on growth (expressed in mm.) of three *Helminthosporium* species on potato dextrose agar (PDA) nutrient agar (NA) and corn meal agar (CMA)

Temperature	Incubation period	<i>H. portoricensis</i> n. sp.			<i>H. rostratum</i>				<i>H. holmii</i>	
		PDA	NA	CMA	PDA	NA	CMA	PDA	NA	CMA
	Days									
8° C	2	4.2*	4.2*	4.2*	4.2*	4.2*	4.2*	4.2*	4.2*	4.20*
	5	4.20	4.20	4.2	4.20	4.20	4.20	4.20	4.33	4.20
12° C	2	4.2	4.2	4.2	4.2	4.2	4.2	4.2	4.2	4.20
	5	8.71	6.80	7.50	8.02	6.00	6.45	7.02	6.00	5.24
16° C	2	7.00	5.15	6.50	6.00	4.00	5.40	8.93	6.80	6.76
	5	36.60	24.50	31.80	26.40	14.50	21.69	21.28	16.40	16.08
20° C	2	13.33	8.12	11.90	12.30	7.12	10.95	13.40	9.66	11.84
	5	63.41	43.20	54.40	58.41	39.10	49.95	40.20	27.00	33.60
24° C	2	18.40	12.96	17.00	16.40	10.96	14.65	19.10	13.65	15.61
	5	82.00	56.30	70.40	72.00	46.33	60.40	48.60	38.80	47.30
28° C	2	30.60	20.30	24.75	26.65	20.30	22.70	24.36	18.50	22.58
	5	85.50	65.56	84.00	75.45	60.56	74.10	43.25	46.50	61.80
32° C	2	29.68	20.28	25.70	25.08	18.28	23.75	25.50	19.60	22.20
	5	85.50	64.91	85.50	75.45	54.94	74.50	49.20	44.67	58.25
36° C	2	15.40	9.8	12.40	13.40	7.85	10.40	18.64	15.50	14.58
	5	27.00	25.60	32.00	25.00	23.40	28.40	28.90	28.50	28.08
40° C	2	5.70	6.10	4.20	5.20	5.80	3.98	6.66	5.96	5.80
	5	5.58	5.18	4.72	4.45	4.00	4.55	5.24	5.48	5.40

\* Average of five replications.

## PATHOGENICITY TESTS

Plants of sugarcane varieties P.R. 975, P.R. 980, P.R. 1013, P.R. 1016, P.R. 1028, P.R. 1048, P.R. 1059, P.R. 1085, P.R. 1116, P.R. 1117, P.R. 1137, P.R. 1148, P.R. 1191, P.R. 1197, P.R. 1207, B. 37-172, B. 41-227,

TABLE 3.—Effect of pH on growth of three species of *Helminthosporium* on potato dextrose agar

pH	Days after inoculation	<i>H. portoricensis</i>	<i>H. rostratum</i>	<i>H. holmii</i>
		Mm.	Mm.	Mm.
4	3	35.70	25.06	27.91
	4	44.33	34.05	34.50
	5	56.83	46.73	40.33
	6	70.33	60.35	44.33
5	3	36.16	26.06	28.90
	4	47.66	37.65	32.00
	5	61.91	51.19	41.16
	6	74.60	64.06	48.16
6	3	37.83	28.83	27.91
	4	48.50	38.05	34.76
	5	57.16	47.06	44.00
	6	78.25	68.15	49.41
7	3	40.83	30.54	30.83
	4	53.33	43.35	38.83
	5	71.66	60.65	49.40
	6	84.50	74.05	54.60
8	3	40.58	29.58	29.83
	4	52.16	41.26	35.83
	5	70.50	60.00	44.00
	6	83.00	74.00	47.80
9	3	40.08	30.08	29.91
	4	52.00	42.00	37.33
	5	70.66	60.56	47.16
	6	83.00	73.60	57.00
10	3	35.83	24.83	28.16
	4	45.83	35.38	35.75
	5	62.50	51.50	46.33
	6	76.33	66.32	57.50
11	3	27.91	21.92	20.41
	4	35.75	30.70	27.58
	5	47.83	37.53	36.83
	6	57.58	47.28	47.41

B. 42-63, B. 49-119, Co. 421, H. 32-8560, H. 44-3098, and P.O.J. 2878 were sprayed with a spore suspension of the undescribed species of *Helminthosporium*, *H. rostratum* and *H. holmii*. The inoculated plants were covered with polyethylene bags and left on benches under a shed covered with Saran cloth for 2 weeks. The infection rating used in this study consisted of a scoring of each plant on a 1 to 5 basis. In this system, a score of 1

TABLE 4.—Pathogenicity of three *Helminthosporium* species on sugarcane

Variety	Degree of infection when artificially inoculated with—		
	<i>H. portoricensis</i>	<i>H. rostratum</i>	<i>H. holmii</i>
P.R. 975	1*	1*	0*
P.R. 980	2	2	0
P.R. 1013	1	2	0
P.R. 1016	2	2	0
P.R. 1028	1	1	0
P.R. 1048	1	1	0
P.R. 1059	2	1	0
P.R. 1085	3	3	0
P.R. 1116	1	1	0
P.R. 1117	2	1	0
P.R. 1137	3	3	1
P.R. 1148	3	3	1
P.R. 1166	3	3	1
P.R. 1191	2	3	1
P.R. 1197	2	3	0
P.R. 1207	1	1	0
B. 37-172	2	2	0
B. 41-227	2	3	0
B. 42-63	1	2	0
B. 49-119	1	2	0
Co. 421	1	1	0
H. 328560	1	1	0
H. 443098	1	2	0
P.O.J. 2878	1	2	0

\* Infection types: 0, No symptoms; 1, very resistant—many] small, dot-like lesions; 2, moderately resistant—moderate amount of elongated, very rarely-united lesions; 3, moderately susceptible—many elongated, often united lesions.

indicates freedom from leaf spot. Scores advance with increasing severity of infection.

As shown in table 4, P.R. 1028, P.R. 1048 and P.R. 1207 were the varieties most resistant to the undescribed *Helminthosporium* species as well as to *H. rostratum* and *H. holmii*. P.R. 1085, P. 1137, P.R. 1148, P.R. 1166, P.R. 1191, P.R. 1197, and B. 41-227 were the varieties most susceptible to the undescribed *Helminthosporium* species and to *H. rostratum*, but resistant to *H. holmii*.



## DISCUSSION AND CONCLUSION

The results of this study indicate in general that the conidia of the undescribed species of *Helminthosporium* isolate resemble those of *H. rostratum* and *H. holmii*. The size and shape of the conidia of the various species are so variable, however, that neither Drechsler's key to *H. rostratum* (3) nor Luttrell's key for *H. holmii* (4) can be used to identify this fungus.

According to Drechsler, *H. rostratum* can be differentiated readily from other graminicolous forms by its rostrate spores. Although conidia of the *Helminthosporium* isolate resemble those of *H. rostratum*, the long, constricted, cylindrical rostrate conidia, characteristic of *H. rostratum*, have not been observed. According to Luttrell, conidia of *H. holmii* are similar to those of *H. rostratum* Drechs. except for their greater diameter and darker appearance. Specimens of *H. holmii*, obtained from Dr. E. E. Butler, University of California, Davis, were examined. Our results show that conidia of *H. holmii* are mostly more than 14.60  $\mu$  wide. The conidia of this *Helminthosporium* isolate are less than 14.60  $\mu$  wide as a rule and thus can hardly be considered the same species. Similarly, this undescribed species of *Helminthosporium* can not be identified as *H. halodes* var. *tritici* Mitra because conidia of *H. halodes* var. *tritici* generally are more than 14.60  $\mu$  wide and primarily occur on roots of grasses. Because of the variation in size and shape, and because of the absence of long, constricted, rostrate conidia characteristic of *H. rostratum*, the isolate is thus described and given the name *H. portoricensis* n. sp.

Because some varieties of sugarcane, such as P.R. 1028, P.R. 1048, and P.R. 1207, exhibited a high degree of resistance to the new *Helminthosporium* isolate, replacement of susceptible varieties with resistant ones in certain locations of the Island seems the best way to control the disease.

## SUMMARY

A previously undescribed species of *Helminthosporium* was isolated from leaves of P.R. 980 and P.R. 1059 showing lesions resembling those of brown stripe. The conidia of the new *Helminthosporium* isolate resemble those of *H. rostratum* but the long, constricted, cylindrical rostrate conidia, characteristic of this fungus, are absent. The conidia of *H. holmii*, another allied species, also were examined. The conidia of the new species are smaller in diameter than those of *H. holmii*. In view of the variations in size and shape and the absence of the characteristic rostrate conidia, the new fungus is given the name *Helminthosporium portoricensis* n. sp.

Results of the cultural studies indicated that the newly described species has the same optimum temperature and pH range as that of *H. rostratum*

and *H. holmii*. This new isolate grew comparatively faster, however, than the other two species on potato dextrose agar.

Among the varieties tested, P.R. 1207, P.R. 1028, and P.R. 1048 seemed to be particularly resistant to *portoricensis*.

#### RESUMEN

Se aisló, por primera vez, una especie de *Helminthosporium* aún no descrita, de hojas de las variedades de caña P.R. 980 y P.R. 1059 que demostraban lesiones parecidas a las de la raya morena. Los conidios de la nueva *Helminthosporium* que fue aislada se parecen a los de la *H. rostratum*, excepto en la forma alargada, constreñida y cilíndrica de los conidios rostrados que son rasgos característicos de ésta última. También se examinaron los conidios de la *H. holmii* que es otra especie aliada. Los conidios de la especie aparentemente nueva eran menores en diámetro que los de la *H. holmii*. En vista de las variaciones en cuanto al tamaño y forma de los conidios y considerando el hecho de que en la nueva no aparecen conidios rostrados, tan típicos de la *H. holmii*, el nuevo hongo se ha identificado tentativamente como *Helminthosporium portoricensis* n. esp.

Los resultados de los cultivos hechos indicaron que la nueva especie requiere la misma temperatura óptima e igual grado de pH que las *H. rostratum* y *H. holmii*. Sin embargo, creció comparativamente con mayor rapidez que las otras dos especies en un medio de papa azucarado (*potato dextrose agar*).

Entre las variedades de caña que se probaron, las P.R. 1207, P.R. 1028 y P.R. 1048 parecieron ser particularmente resistentes a la nueva especie de *Helminthosporium*.

#### LITERATURE CITED

1. Bourne, B. A., A new species of *Helminthosporium* causing disease in sugarcane and other grasses, *Proc. Int. Soc. Sugarcane Technol.* 9: 1079-88, 1956.
2. —, *Bipolaris rostrata* (Drechs.) Shoemaker, the cause of disease in sugarcane and other grasses, *Sugar y Azúcar*, pp. 19-24, August 1968.
3. Drechsler, C., Some graminicolous species of *Helminthosporium*, *J. Agr. Res.* 24(8): 641-739, 1923.
4. Luttrell, E. S., A key to species of *Helminthosporium* reported on grasses in the United States, *Plant Dis. Repr.*, ARS, USDA, Supplement 201, pp. 59-67, 1951.
5. —, A *Trichometasphaeria* perfect stage for a *Helminthosporium* causing leaf blight of *Dactyloctenium*, *Phytopathology* 53(3): 281-5, 1963.
6. —, *Leptosphaeria* (*Metasphaeria*) perfect stage for *Helminthosporium turcicum* and *H. rostratum* (Abstract), *Phytopathology* 47: 313, 1957.
7. —, Systematics of *Helminthosporium* and related genera, *Mycologia* 56(1): 119-32, 1964.
8. Nelson, R. R., and Kline, D. M., The pathogenicity of certain species of *Helminthosporium* to species of the Gramineae, *Plant Dis. Repr.*, ARS, USDA 45(8): 644-8, 1961.

9. Parris, G. K., The *Helminthosporia* that attack sugarcane, *Phytopathology* 40(1): 90-103, 1950.
10. Shoemaker, R. A., Nomenclature of *Drechslera* and *Bipolaris* grass parasites segregated from *Helminthosporium*, *Can. J. Bot.* 37: 879-87, 1959.
11. Whitehead, M. D., and Calvert, O. H., *Helminthosporium rostratum* inciting ear rot of corn and leaf spot of thirteen grass hosts, *Phytopathology* 49(2): 817-20, 1959.