Influence of Light on Maize Anthracnose in the Greenhouse

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ABSTRACT

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When maize seedlings were inoculated in the greenhouse with *Colletotrichum graminicola* and incubated in the dark for 18 hr, the percentage of leaf tissue affected was influenced by light. Solar radiation was measured over an 8-day period from 2 days before the day of inoculation through 6 days after inoculation. Leaves of seedlings exposed to relatively low levels of solar radiation (1,234 gm cal/cm^2) exhibited a significantly greater percentage of lesion coverage than did those of seedlings exposed to higher levels of light (2,530 to 3,361 gm cal/cm^2). Among the 22 corn cultivars studied, lesion coverage under low light intensity ranged from 42.2 to 97.5% whereas under conditions of high light intensity, lesion coverage ranged from 0.2 to 57.2%. When seedlings were incubated in the dark for 42 hr following inoculation, the lesion coverage ranged from 53.2 to 100% regardless of the subsequent amount of solar radiation the plants received. Thus, the critical period for illumination was during the day after inoculation. The data demonstrate that high disease ratings may be obtained when light is limiting, such as during periods of extensive cloud cover. Though lesion coverage was variable, the host reaction type did not change under the variable light conditions. Evalution of plants for resistance should be done on the basis of host reaction type as well as extent of lesion coverage.

Additional key words: Zea mays, corn hybrids, resistance, phenols.

The leaf blight and stalk rot phases of maize anthracnose have become a significant problem on both sweet and dent corn (*Zea mays* L.) (3,4,9,11,12,17,18,26,28–31). The increased occurrence of the disease has prompted screening of germplasm stocks for resistant genotypes. Studies have been reported for plants grown in the greenhouse, the growth chamber, and the field (8,14,19,20,22,24, 25,27,30). Seedlings were used in most investigations of disease development (6,7,13,21) and resistance screening (14,19,22,25) that have been reported. The use of seedlings is appropriate since the pathogen, *Colletotrichum graminicola* (Ces.) Wils., causes a seedling leaf blight (8,19) and the leaf reaction of seedlings is the same as that of adult (post-anthesis) plants in the field (14).

Several disease rating schemes have been employed for measuring the extent of lesion coverage (14,19,22,25). These schemes yield similar estimates of lesion coverage (8). Although lesion coverage has been used as a measure of resistance and susceptibility, Nicholson and Warren (19) argued that the type of lesion may be more important than lesion size or coverage. They described reaction types of host plants based on characteristic leaf symptoms. Color and shape of lesions, and patterns of chlorosis differentiated susceptible, resistant, and hypersensitively resistant reaction types.

Evaluation of plants on the basis of lesion coverage yielded discrepancies in categorizing plants as susceptible or resistant (22,27). These discrepancies may result from differences in virulence of the *C. graminicola* isolates (19) or may be based on host physiology as affected by light, temperature, and maturity. For example, Poneleit et al (22) suggested that inconsistency in their greenhouse ratings for anthracnose resistance may have been attributable to differences in temperature or light intensity. Wheeler et al (30) subsequently demonstrated that low light intensity increased disease severity.

Thompson and Leonard (25) grew plants in a Phytotron chamber under high illumination (40,000–50,000 lux) and used lesion length as a measure of resistance in seedlings. They noted the problem of lesion size as a measure of resistance when they subsequently reported (13) that lesion elongation was significantly affected by temperature and host maturity.

Hammerschmidt and Nicholson (6) also evaluated the influence of light on lesion area. Lesions on plants grown under low light intensity (9,800 lux) were significantly larger than those on plants grown under high light intensity (37,600 lux). They also demonstrated that under low light intensity, lesions on resistant or hypersensitively resistant plants were not significantly smaller than those on susceptible plants. Plants that could not be distinguished on the basis of lesion size could still be distinguished by lesion type. Thus, host reaction type (19) appears to be a valid means for rating disease resistance of seedlings, especially when environmental conditions of light and temperature are not easily controlled.

The greenhouse is often the appropriate place for screening large populations for resistance to disease. Often such facilities are poorly equipped for supplemental lighting or supplemental lighting is not used to avoid unacceptable increases in temperature. When the experimenter does not use artificial light, the effect of solar radiation is critical to disease development (2,23). Light affects the development of individual anthracnose lesions, but it is not known how much it affects overall lesion coverage. The purposes of this investigation were to determine the importance of variation in solar radiation in screening for anthracnose resistance in the greenhouse, and to determine when exposure to light is most important for expression of resistance.

MATERIALS AND METHODS

Experiments were conducted during the winter. Plants were grown without supplemental lighting in a greenhouse from which the whitewash had been removed. Plants were exposed to sunlight prior to and during disease development. Throughout each experiment solar radiation was measured at the Purdue Agronomy farm, 11 km northwest of the greenhouse, by the National Weather Service. Radiation was measured as energy per unit area and was expressed in Langley units accumulated per hour. Langley units were totaled for each day and expressed as gm cal/cm^2/24 hr (16).

Corn cultivars included 19 open-pedigree hybrids and three inbred lines (Table 1). The inbreds were used because the host reaction types are known. Inbreds Mo940, H91, and 33-16 are susceptible, resistant, and hypersensitively resistant to *C. graminicola,* respectively (6,7,19). Thus, the inbreds could be compared with the hybrids for extent of lesion coverage at various light intensities. An isolate of *C. graminicola* obtained from

diseased corn leaves was maintained on oatmeal agar under constant fluorescent light (3,500 lux) at 24 C (6). Spore suspensions for inoculations were prepared from 2-wk-old cultures. Spore suspensions were filtered through cheesecloth and their concentration was adjusted to 1.5×10^6 spores per milliliter. One drop of Tween-20 wetting agent was added per 100 ml of inoculum suspension.

Seedlings were inoculated at the three- to four-leaf stage by spraying the inoculum onto their leaves with an atomizer pressurized at 0.5 atmosphere. Following inoculation, plants were incubated in the dark at 100% relative humidity for either 18 or 42 hr. The 18-hr incubation experiment was done four times and the 42-hr incubation experiment was done twice. Plants were inoculated at 1700 hours, at the end of measurable radiation for the day. The 18-hr incubation period lasted until 1100 hours the following day, so that plants only lost 3 hr (0800-1100 hours) of measurable radiation. The 42-hr incubation period lasted until 1100 hours a full day later, so that plants were in the dark during one full day and the first 3 hr of measurable radiation the next day.

Following the incubation period, the moisture chambers were opened and leaf surfaces were allowed to dry. Pots were then randomly distributed on the greenhouse bench and symptom development was recorded. Ten plants of each hybrid were evaluated for lesion coverage and for host reaction type in each experiment. For the inbreds, the sample sizes differed among the experiments (Table 1). The extent of lesion coverage was estimated 12 days after inoculation by determining the percentage of lesion coverage for each plant on the upper three leaves that had been fully exposed to the inoculum. The plumular leaf was not included in disease ratings since in the greenhouse it typically senesces within 3 – 4 wk after planting. The percentage of leaf area covered with lesions was determined according to the southern corn leaf blight assessment key described by James (10). An average value for lesion coverage was calculated for each corn cultivar based on the total plant population of that cultivar. Host reaction type was evaluated according to the criteria described by Nicholson and Warren (19).

TABLE 1. Percentage of lesion coverage and host reaction type on corn hybrids and inbreds inoculated with *Colletotrichum graminicola* (18-hr dark incubation)

Corn hybrid or inbred[b]	Lesion coverage (%)[a] Grand mean	DMRT[c]	Host reaction type[d]
1. $Mo17_{Ht} \times A634_{Ht}$	15.8	a	R
2. 33-16	17.1	ab	HR
3. H98 × B73	20.9	abc	R
4. Oh545 × $B73_{Ht}$	23.7	abcd	R
5. B73 × H99	24.2	abcde	R
6. H98 × $A632_{Ht}$	26.6	abcdef	R
7. H60 × B73	26.8	abcdef	R
8. Ag23 × $A634_{Ht}$	27.6	abcdefg	R
9. $Mo17_{Ht}$ × H100	28.1	abcdefgh	R
10. $N28_{Ht} \times Mo17_{Ht}$	28.2	abcdefgh	R
11. $Mo17_{Ht} \times B73_{Ht}$	28.8	abcdefgh	S
12. H60 × $A632_{Ht}$	29.3	abcdefgh	S
13. (Ag23 × $Mo17_{Ht}$) × H100	31.4	bcdefgh	S
14. (Ag23 × $Mo17_{Ht}$) × B73	35.5	cdefgh	S
15. H91	36.4	defgh	R
16. $Mo17_{Ht}$ × B79	37.8	defgh	S
17. $A632_{Ht}$ × H99	39.2	efgh	R
18. Ag23 × $B73_{Ht}$	40.6	fgh	S
19. $C123_{Ht}$ × B73	40.9	fgh	S
20. $A632_{Ht} \times A619_{Ht}$	42.0	gh	R
21. $A632_{Ht}$ × H95	43.5	h	S
22. Mo940	60.7	i	S

[a] Percentage of lesion coverage reported as the grand mean across four experiments.
[b] The grand mean for inbreds 33-16, H91, and Mo940 is based on 29, 35, and 30 plants, respectively.
[c] DMRT, Duncan's Multiple Range Test. Numbers followed by the same letter in the column do not differ significantly. ($P = 0.05$).
[d] Host reaction type for each line was consistent across the four experiments. S, R, and HR = susceptible, resistant, and hypersensitively resistant host reaction types, respectively.

RESULTS AND DISCUSSION

Table 1 presents the percentage of lesion coverage for each of the 22 corn cultivars averaged (grand mean) across the four experiments with 18-hr incubation periods. Host reaction type (19) for each corn cultivar was consistent across the four experiments and separated the cultivars into the discrete categories of susceptible, resistant, and hypersensitively resistant. However, the corn cultivars were not classifiable in discrete categories of susceptibility and resistance based upon percent lesion coverage, although a substantial range in percentage of lesion coverage was observed.

Significant differences in lesion coverage were observed among the three inbreds in each of the four 18-hr incubation experiments (Table 2). For example, in experiment 1 lesion coverage averaged 3% on the hypersensitively resistant inbred 33-16, which differed significantly from the 20.4% of the resistant inbred H91, which in turn differed significantly from the 48.3% of the susceptible inbred Mo940.

Plants exposed to relatively low light intensities in experiment 4 had significantly more lesion coverage than did plants exposed to higher light intensities in experiments 1–3 (Table 2). The values for total solar radiation to which plants had been exposed are for an 8-day period of 2 days before inoculation, the day of inoculation, and 5 days after inoculation. In experiments 2 and 3, an intermediate level of solar radiation occurred and lesion coverage was statistically different from that in experiment 4 but not different from that in experiment 1 in which plants had been exposed to the highest level of solar radiation.

Lesion coverage on the 22 corn cultivars is presented in Fig. 1A for experiments 1 and 4, in which the level of solar radiation was highest and lowest, respectively. Lesion coverage for each of the 19 hybrids and three inbred lines was significantly greater at the lower level of light in experiment 4 (1,234 gm cal/cm^2). Experiments 2 and 3 are not shown on the graph since, as with the inbreds (Table 2), no significant difference in lesion coverage was observed for any hybrid across experiments 1 through 3.

The data in Fig. 1A suggest that plants exposed to less than some threshold amount of light do not readily inhibit lesion coverage. Hammerschmidt and Nicholson (6,7) demonstrated that the restriction of lesion enlargement is associated with phenolic compound synthesis and that increases in phenols are evident 24–48

TABLE 2. Comparison of percent anthracnose lesion coverage on inoculated (18-hr dark incubation) seedlings of corn inbreds maintained in the greenhouse and exposed to solar radiation as their only source of light

Inbred line[y]	Cumulative solar radiation[x] in four experiments: 3,361 gm cal/cm^2	2,780 gm cal/cm^2	2,530 gm cal/cm^2	1,234 gm cal/cm^2
33-16	3.0 a (a)[z]	2.5 a (a)	12.7 a (a)	42.2 a (b)
H91	20.4 b (a)	22.4 b (a)	28.4 b (a)	67.3 b (b)
Mo940	48.3 c (a)	-	56.5 c (a)	97.5 c (b)

[x] Solar radiation expressed as that accumulated over an 8-day period from 2 days before inoculation through 5 days after incubation.
[y] Inbreds 33-16, H91, and Mo940 are hypersensitively resistant, resistant, and susceptible to *Colletotrichum graminicola*, respectively.
[z] Numbers represent the average percent lesion coverage. Values in a column (within an experiment) are statistically different ($P = 0.05$) if followed by a different letter. Values in a row (across experiments) are statistically different ($P = 0.05$) if followed by a different letter in parentheses.

hr after inoculation. Since phenol synthesis is light-mediated (5,15), the most critical period for exposure to a high level of light may be within 24 – 48 hr after inoculation. This hypothesis was tested by subjecting plants to a 42-hr dark incubation following inoculation rather than the standard 18-hr dark incubation period. The results of two experiments are presented in Fig. 1B. Corn genotypes 1 through 22 are the same in both Fig. 1A and 1B and the numbering corresponds to the specific cultivars listed by number in Table 1. Extensive lesion coverage occurred on each line in both 42-hr experiments (Fig. 1B). Significantly, lesion coverage on a given corn cultivar did not differ greatly between the experiments, even though the level of solar radiation before and after incubation differed substantially (3,933 vs 2,618 gm cal/cm^2). It also is important that lesion coverage was significantly greater in both 42-hr dark incubation experiments than in the 18-hr incubation experiment in which the accumulation of light was greatest (3,361 gm cal/cm^2, experiment 1, Fig. 1A). This occurred even though the accumulation of light in one of the 42-hr incubation experiments was greater than the highest accumulation which occurred in any of the 18-hr incubation experiments (3,933 vs 3,361 gm cal/cm^2). Thus, the total exposure to light over 8 days did not necessarily affect inhibition of lesion coverage. Plants had to be exposed to high levels of light at some time during the 24 hr following the normal 18-hr incubation period to reduce percent lesion coverage.

This assertion is reinforced by the data in Table 3 which shows that the highest percentages of lesion coverage were associated with the lowest levels of light on the day after inoculation. The high levels of solar radiation both before and after the 42-hr incubation period in experiment 1 did not compensate for the absence of light on the first day after inoculation. This is consistent with the fact that phenol synthesis in corn is light-mediated (5,6) and that phenolic compounds inhibitory to *C. graminicola* begin to accumulate in leaf tissue between 24 and 48 hr after inoculation when plants are kept in a 14-hr photoperiod (7). Furthermore, Anthenill (1) demonstrated that when plants are placed in the dark, phenols do not accumulate in response to inoculation. Thus, maintaining plants in the dark would temporarily prevent the synthesis of phenolic compounds which presumably help to restrict lesion development.

This investigation demonstrates that the results of screening for resistance to anthracnose in the greenhouse can be significantly influenced by periods of cloudy weather as Poneleit et al (22) suggested. This is especially a problem when lesion size or lesion coverage is used as a sole criterion for evaluation of resistance. The problem can be avoided by evaluating resistance on the basis of host reaction type as well as lesion coverage (19) since reaction type does not vary under conditions of low light in the greenhouse (6). To avoid the problems of light fluctuation we regularly use a 12-hr

TABLE 3. Daily solar radiation during greenhouse screening trials for corn anthracnose disease development

Experiment conditions and no.	Solar radiation, gm cal/cm^2									Lesion coverage range (%) across 22 genotypes
	Days before inoculation		Day of inoculation	Days after inoculation					Total	
	2	1		1	2	3	4	5		
Dark incubation 18-hr										
1	446	156	249	338	623	622	565	362	3,361	3.0–48.3%
2	463	462	485	527	103	312	107	321	2,780	1.5–56.5
3	256	344	375	370	275	337	382	191	2,530	0.2–57.2
4	171	154	404	68	78	71	143	145	1,234	42.2–97.5
Dark incubation 42-hr										
1	598	620	673	-[a]	347	320	684	691	3,933	58.0–100
2	636	635	474	-	36	377	58	402	2,618	53.2–100

[a] Plants were kept in the dark the day after inoculation.

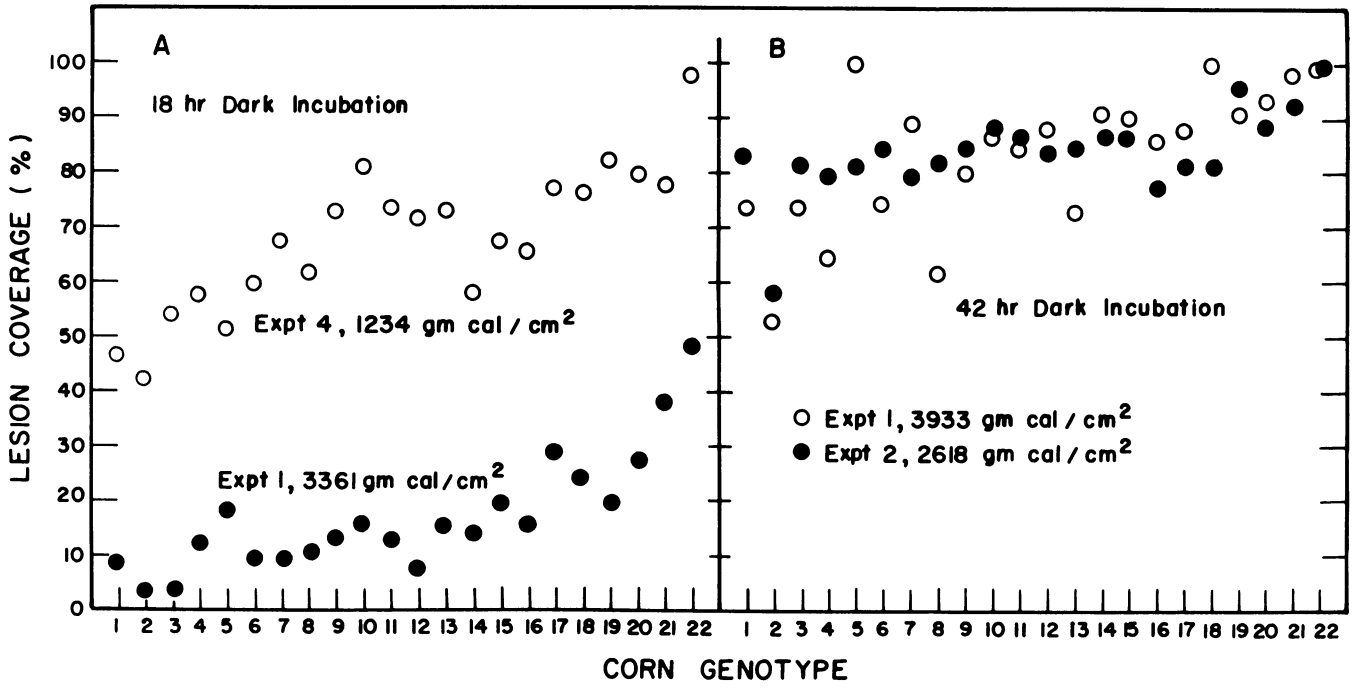

Fig. 1. Influence of light on percent anthracnose lesion coverage on 22 corn genotypes in the greenhouse. **A,** Plants incubated in the dark for 18 hr following inoculation. **B,** Plants incubated in the dark for 42 hr following inoculation. Corn genotype numbers correspond to numbered genotypes listed in Table 1. Light values represent total solar radiation to which plants were exposed over an 8-day period from 2 days before the day of inoculation through 6 days after inoculation.

photoperiod of supplemental light (two Sylvania Cool White FR96T12/SW/VHO/135 fluorescent tubes positioned 30 cm above the plants). Inoculating plants when weather predictions indicate bright sunny days should also reduce the problem. The most critical time for plants to be exposed to light is during the day after inoculation.

LITERATURE CITED

1. ANTHENILL, F. J. 1978. Effect of light on disease development and the accumulation of phenols in corn (*Zea mays* L.) during infection with *Colletotrichum graminicola* (Ces.) Wils. and *Helminthosporium carbonum* Ullstrup. Ph.D. Thesis, Purdue Univ. 146 pp.
2. BEN-YEPHET, Y. 1979. Isolate source and daylight intensity effects on the pathogenicity of *Verticillium dahliae* in watermelon seedlings. Phytopathology 69:1069-1072.
3. COOPERATIVE EXTENSION SERVICE. 1977. Corn anthracnose in Indiana: the situation, its economic implication, alternatives for 1978. Purdue University, W. Lafayette, IN 47907.
4. DALE, J. L. 1963. Corn anthracnose. Plant Dis. Rep. 47:245-249.
5. DUKE, S. O., and A. W. NAYLOR. 1976. Light control of anthocyanin biosynthesis in Zea seedlings. Physiol. Plant. 37:62-68.
6. HAMMERSCHMIDT, R., and R. L. NICHOLSON. 1977. Resistance of maize to anthracnose: Effect of light intensity on lesion development. Phytopathology 67:247-250.
7. HAMMERSCHMIDT, R., and R. L. NICHOLSON. 1977. Resistance of maize to anthracnose: Changes in host phenols and pigments. Phytopathology 67:251-258.
8. HOOKER, A. L. 1977. Corn anthracnose leaf blight and stalk rot. Pages 167–182 in: Proc. Thirty-first Annu. Corn Sorghum Res. Conf., Chicago, IL.
9. HOOKER, A. L., and D. G. WHITE. 1976. Prevalence of corn stalk rot fungi in Illinois. Plant Dis. Rep. 60:1032-1034.
10. JAMES, W. C. 1973. A manual of assessment keys for plant diseases. Publication 1458, Agriculture Canada, Canada Dept. of Agriculture, Ottawa.
11. LEONARD, K. J. 1974. Foliar pathogens of corn in North Carolina. Plant Dis. Rep. 58:532-534.
12. LEONARD, K. J., and D. L. THOMPSON. 1969. Corn stalk rot fungi in North Carolina. Plant Dis. Rep. 53:718-720.
13. LEONARD, K. J., and D. L. THOMPSON. 1976. Effects of temperature and host maturity on lesion development of *Colletotrichum graminicola* on corn. Phytopathology 66:635-639.
14. LIM, S. M., and D. G. WHITE. 1978. Estimates of heterosis and combining ability for resistance of maize to *Colletotrichum graminicola*. Phytopathology 68:1336-1342.
15. McCLURE, J. W. 1979. The physiology of phenolic compounds in plants. Pages 525–556 in: T. Swain, J. B. Harborne, and C. F. Van Sumere, eds. Recent Advances in Phytochemistry; Vol. 12, Biochemistry of Plant Phenolics. Plenum Press, New York, NY. 651 pp.
16. McINTOSH, D. H. 1972. Meteorological Glossary. Chemical Publishing Co., New York, NY. 319 pp.
17. MORGAN, O. D., and J. G. KANTZES. 1971. Observations of *Colletotrichum graminicola* on T corn and blends in Maryland. Plant Dis. Rep. 55:755.
18. NICHOLSON, R. L. 1975. The potential of corn anthracnose. Pages 41–49 in: Proceedings of the 1974 Purdue Corn Disease Conference, Dept. of Botany and Plant Pathology, Purdue Univ., W. Lafayette, IN 47907.
19. NICHOLSON, R. L., and H. L. WARREN. 1976. Criteria for evaluation of resistance to maize anthracnose. Phytopathology 66:86-90.
20. PERKINS, J. M., and A. L. HOOKER. 1979. Effects of anthracnose stalk rot on corn yields in Illinois. Plant Dis. Rep. 63:26-30.
21. POLITIS, D. J., and H. WHEELER. 1973. Ultrastructural study of penetration of maize leaves by *Colletotrichum graminicola*. Physiol. Plant Pathol. 3:465-471.
22. PONELEIT, C. G., D. J. POLITIS, and H. WHEELER. 1972. Resistance to corn anthracnose. Crop Sci. 12:875-876.
23. SCHUMANN, G., and H. D. THURSTON. 1977. Light intensity as a factor in field evaluations of general resistance of potatoes to *Phytophthora infestans*. Phytopathology 67:1400-1402.
24. SMITH, D. R. 1976. Yield reduction in dent corn caused by *Colletotrichum graminicola*. Plant Dis. Rep. 60:967-970.
25. THOMPSON, D. L., and K. J. LEONARD. 1974. Anthracnose resistance of corn inbreds. Research Report N-51. Dept. of Crop Science and Dept. of Plant Pathology, North Carolina State University, Raleigh, NC. 24 pp.
26. WARREN, H. L., and R. L. NICHOLSON. 1975. Kernel infection, seedling blight, and wilt of maize caused by *Colletotrichum graminicola*. Phytopathology 65:620-623.
27. WARREN, H. L., R. L. NICHOLSON, and M. T. TURNER. 1975. Field reaction of corn inbreds to *Colletotrichum graminicola*. Plant Dis. Rep. 59:767-769.
28. WARREN, H. L., R. L. NICHOLSON, A. J. ULLSTRUP, and E. G. SHARVELLE. 1973. Observations of *Colletotrichum graminicola* on sweet corn in Indiana. Plant Dis. Rep. 57:143-144.
29. WARREN, H. L., and P. L. SHEPHERD. 1976. Relationship of *Colletotrichum graminicola* to foliar and kernel infection. Plant Dis. Rep. 60:1084-1086.
30. WHEELER, H., D. J. POLITIS, and C. G. PONELEIT. 1974. Pathogenicity, host range, and distribution of *Colletotrichum graminicola* on corn. Phytopathology 64:293-296.
31. WILLIAMS, L. E., and G. M. WILLIS. 1963. Disease of corn caused by *Colletotrichum graminicolum*. Phytopathology 53:364-365.