

Ribonucleic Acid from Yeast by Counter Current Distribution. *J. Biol. Chem.*, 240, 2129-2135.

Morrison, J. C., Morrison, W. C., Whybrew, W. D., Wiser, W. L., and Bucovaz, E. T. (1975). Alteration in Coenzyme A-Synthesizing Protein Complex Activity Related to Cyclic Hydrocarbon Conjugation. *IRCS, Med. Sci.*, 3, 23.

Morrison, W. C., Whybrew, W. D., Morrison, J. C., Fryer, J. E., Sobhy, C. M., and Bucovaz, E. T. (1975). Alternant Functions of the Coenzyme A-Synthesizing Protein Complex of Baker's Yeast. *Amer. Chem. Soc. 170th National Meeting*.

Sobhy, C. M., Whybrew, W. D., Morrison, J. C., Morrison,

W. C., Wiser, W. L., Fish, S. A., Fryer, J. E., and Bucovaz, E. T. (1975). Coenzyme A-Synthesis by a Protein Complex of Baker's Yeast. *Fed. Proc.*, 34, 599.

Stevens, D. P., Mackey, I. R., and Cullen, K. J. (1975). Carcinoembryonic Antigen in an Unselected Elderly Population: A Four Year Follow Up. *Br. J. Cancer*, 32, 147-151.

Thomson, D. M. P., Krupcy, J., Freedman, S. O., and Gold, P. (1969). The Radioimmunoassay of Circulating Carcinoembryonic Antigen of the Human Digestive System. *Proc. Nat. Acad. Sci.*, 64, 161-167.

JOURNAL OF THE TENNESSEE ACADEMY OF SCIENCE

VOLUME 52, NUMBER 1, JANUARY, 1977

VARIATION OF PATHOGENICITY TO CORN SEEDLINGS OF ISOLATES OF *HELMINTHOSPORIUM MAYDIS*

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ABSTRACT

Four single spore isolates of *H. maydis* Race T were used to study pathogenic variability in greenhouse studies. Seedlings of Ky21 inbred corn produced with normal (N) and six different cytoplasm (T, C, G, M, W, K) conditioning male sterility were inoculated with spore suspensions by atomization. Pathogenicity of the isolates was determined by measuring size of lesions with a planimeter. Variation in pathogenicity of the isolates indicates the existence of biotypes within Race T.

INTRODUCTION

Mercado and Lantican (1961) first noted increased susceptibility of cytoplasmic male-sterile corn to *H. maydis*, the pathogen causing southern corn leaf blight. Villareal and Lantican (1965) and Scheifele, et al. (1970) made similar observations. Following the 1970 southern corn leaf blight epidemic, Hooker, et al. (1970b) differentiated Race O and Race T of *H. maydis*. Resistance to Race T has been reported in corn lines with normal (N) cytoplasm and in several produced with male-sterile cytoplasm (Hooker, 1970a; Smith, 1971; Bergquist, 1972).

Studies of pathogenic variability have been made with *H. victoriae*, *H. carbonum*, and *H. sativum* on corn and oats (Nelson and Herbert, 1960; Nelson and Ullstrup, 1961; Wood, 1959). Prior to the 1970 epidemic, Yu (1933) and Orillo (1952) reported varying degrees of pathogenicity of *H. maydis* on corn.

The objective of this study was to determine the pathogenic variation among isolates of *H. maydis* Race T to corn seedlings with six male-sterile and normal cytoplasm.

MATERIALS AND METHODS

Four single spore isolates of *H. maydis* Race T isolated from lesions in the field were used to study pathogenic variability in a greenhouse test. Test plants were seedlings of corn inbred Ky21 produced with N and male-sterile cytoplasm T, C, G, M, W, and K. Sporulating fungal cultures on potato dextrose agar (PDA) were flooded with a 5% sucrose, 0.2% Tween 20 solution and the spores were suspended by scraping the surface of the plate. A hemacytometer was used to make spore counts, and spore suspensions for each isolate were adjusted with sterile water to 20,000 spores/ml.

Six seedlings of each cytoplasm type in 2-4" pots were inoculated in the 3-4 leaf stage. All combinations of cytoplasm and fungus isolate were made. Inoculum was applied to leaf surfaces with an atomizer. The plants were then placed in a darkened mist chamber for 24 hours, then removed from the mist to a greenhouse bench. After 72 hours the second and third leaves of each were removed by clipping with a razor blade at the sheath. The leaves were placed in a plant press and dried for two weeks. At this time, the leaves were photographed and the photographs enlarged to 5 x 7 inches. A total area for each dried leaf was determined using an area meter; the diseased area was determined by planimetry of the photographs, and percentage of diseased area computed for each leaf. A Duncan's analysis was performed to determine significance among the isolates within each cytoplasm (Steel and Torrie, 1960).

RESULTS AND DISCUSSION

The high percentage of diseased leaf area on seedlings with T cytoplasm, as shown in Table 1, indicates that all isolates were Race T. There were no significant differences in pathogenicity among isolates for C, W, and T cytoplasm. There was significant variation in pathogenicity of isolates on other cytoplasm. Isolates 3 and 4 were the most pathogenic on N cytoplasm with

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3.5 and 2.5 percent of the leaf area diseased, respectively, and isolate R4 was the least pathogenic. Isolates 3, 4, and 1 were not significantly different on G cytoplasm; isolate 2 was significantly less pathogenic than the others on plants with G cytoplasm. Isolate 4 was the most pathogenic and 1 was the least pathogenic on M cytoplasm. On plants with K cytoplasm, isolate 1 was significantly more pathogenic than other isolates. Isolates 2, 3, and 4 were not different on K. These results indicate that within *H. maydis* Race T there are biotypes that are more virulent to particular male-sterile cytoplasm than to others.

The susceptibility of male-sterile cytoplasm C and W reported here may have been due, in part, to effects other than the male sterile cytoplasm. There may have been genotypic differences among the seedlings due to the lack of complete homozygosity in the inbred used. In addition, Bergquist and Peverly (1972) have suggested that variation in the degree of resistance and susceptibility to *H. maydis* is conditioned by the interaction of nuclear and cytoplasmic factors. However, Smith et al. (1971) reported cytoplasm C and W resistant to *H. maydis* Race T in greenhouse studies using Ky21. The discrepancy between the cytoplasm reaction reported here for cytoplasm C and cytoplasm W and that of previous works may have been due to the high concentrations of spores used in the greenhouse inoculations. The 20,000 spore/ml concentration used in this study is large in comparison to those of other similar studies (Bergquist, 1972; Hooker, 1970a).

TABLE 1. Pathogenicity of 4 isolates of *Helminthosporium maydis* Race T on Ky21 corn seedlings with normal and 6 male-sterile cytoplasm.

Isolate	Percentage diseased leaf area/cytoplasm type						
	T	N	C	G	M	W	K
1	49.75a ¹	0.75 c	46.50a	6.00a	1.00 b	19.75a	22.25a
2	49.75a	1.25 bc	48.25a	2.75 b	2.25ab	28.25a	1.75 b
3	48.00a	3.50a	6.75a	5.25a	2.50ab	47.50a	1.50 b
4	39.25a	2.50ab	15.00a	7.75a	4.75a	22.25a	5.00ab

¹The small letters indicate Duncan's multiple range groupings. Treatments followed by the same letter do not differ significantly at the 5% level.

ACKNOWLEDGMENTS

The authors wish to thank Dr. L. M. Josephson, Department of Plant and Soil Science, University of Tennessee, Knoxville, for providing the seed used in this study.

LITERATURE CITED

- Bergquist, R. R., and G. Peverly. 1972. Reaction of corn inbreds and hybrids with different cytoplasm and genotypes to *Helminthosporium maydis*, Race T. *Plant Disease Repr.* 56: 112-114.
- Hooker, A. L.; D. R. Smith; S. M. Lim; and J. B. Beckett. 1970a. Reaction of corn seedlings with male-sterile cytoplasm to *Helminthosporium maydis*. *Plant Dis. Repr.* 54:708-712.
- Hooker, A. L.; D. R. Smith; S. M. Lim; and M. D. Musson. 1970b. Physiological races of *Helminthosporium maydis* and disease resistance. *Plant Dis. Repr.* 54:1109-1110.
- Mercado, A. C., and R. M. Lantican. 1961. The susceptibility of cytoplasmic male-sterile lines of corn to *Helminthosporium maydis* Nisikado and Miyake. *Philippine Agr.* 45:235-243.
- Nelson, R. R., and T. T. Herbert. 1960. The inheritance of pathogenicity and mating type in crosses of *Helminthosporium carbonum* and *Helminthosporium victoriae*. *Phytopathology* 50:649 (Abstr.)
- Nelson, R. R., and A. J. Ullstrup. 1961. The inheritance of pathogenicity in *Cochliobolus carbonum*. *Phytopathology* 51: 1-2.
- Orillo, F. T. 1952. Leaf spot of maize caused by *Helminthosporium maydis*. *Philippine Agr.* 36:327-392.
- Scheifele, G. L.; W. Whitehead; and C. Rowe. 1970. Increased susceptibility to southern leaf spot (*Helminthosporium maydis*) in inbred lines and hybrids of maize with Texas male-sterile cytoplasm. *Plant Dis. Repr.* 54:501-503.
- Smith, D. R.; A. L. Hooker; S. M. Lim; and J. B. Beckett. 1971. Disease reaction of thirty sources of cytoplasmic male-sterile corn to *Helminthosporium maydis* Race T. *Crop Sci.* 11:772-773.
- Steel, R. B. D., and J. H. Torrie. 1960. Principles and Procedures of Statistics. McGraw-Hill Book Co., New York. p. 481.
- Villareal, R. L., and R. M. Lantican. 1965. The cytoplasmic inheritance of susceptibility to *Helminthosporium* leaf spot in corn. *Philippine Agr.* 49:294-300.
- Wood, L. S. 1959. Genetic variation of *Helminthosporium sativum* in relation to seedling blight of small grains. *Diss. Abstr.* 20:1136.
- Yu, T. F. 1933. Studies on *Helminthosporium* leaf spot of maize *Sinensia* 3:273-318.