

Segregation of Pathogenicity Types and Host-Specific Toxin Production in Progenies of Crosses between Races T and O of *Helminthosporium maydis* (*Cochliobolus heterostrophus*)

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ABSTRACT

Randomly isolated ascospore progeny of certain crosses between race T and race O of *Helminthosporium maydis* segregated in a 1:1 ratio for race T-type and race O-type pathogenicities. Progeny of other crosses segregated in a ratio of approximately seven ascospores with race O-type pathogenicity to one ascospore with race T-type pathogenicity, suggesting either that more than one gene controls race T-type pathogenicity, or that segregation of a

single gene can be complex in certain crosses. In all cases involving cultures derived from 202 ascospores isolated from race T × race O and race T × race T crosses, race T-type pathogenicity was associated with ability to produce T-toxin. This association suggests that T-toxin production is required for race T-type pathogenicity.

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Additional key words: southern corn leaf blight, genetics of pathogenicity.

Substantial numbers of fungal plant pathogens are highly specific in their host-ranges. For several of these fungi, convincing evidence shows that host specificity is based upon the ability to produce one or more specific toxic metabolites called host-specific toxins (12). The requirement of these toxins for pathogenicity has been established by the observation that the same single gene appears to control both pathogenicity and production of the host-specific toxin (12). In two cases, involving *Helminthosporium victoriae* and *H. carbonum* (the sexual stage for both is *Cochliobolus*), the correlation between pathogenicity and toxin production among ascospore progeny has no exceptions. Each ascospore isolate which was pathogenic also produced toxin; each nonpathogenic ascospore isolate did not produce toxin (11).

A host-specific toxin produced by *Helminthosporium maydis* Nisikado & Miyake race T (sexual stage, *Cochliobolus heterostrophus* Drechs.), the cause of southern corn leaf blight, has recently been described (1). Both race T and the toxin (T-toxin) are specific for corn

containing Texas and certain other male-sterile cytoplasms (1, 2). Although the structure of the toxin is not known, preliminary evidence suggests that *H. maydis* race T is capable of producing at least four toxins (molecular weight range from 350 to 600) which are specific for corn with Texas male-sterile cytoplasm (3). In a study of genetic control of pathogenicity and toxin production, Lim and Hooker (6) found that ability to produce T-toxin was usually associated with race T-type pathogenicity, but several apparent exceptions occurred. The initial purpose of the investigation reported here was to reexamine *H. maydis* to determine whether race T-type pathogenicity is always associated with production of host-specific toxin. During the course of the work, we confirmed the observation of Lim and Hooker (6) that when certain toxin-producing isolates are crossed with nontoxin producers the progeny segregate in a 1:1 ratio for ability to produce toxin. However, in crosses involving other isolates, the progeny segregated in a ratio approximating 7:1 rather than the 1:1 ratio expected if a single gene controls toxin production.

TABLE 1. Description of the *Helminthosporium maydis* isolates used in this study

Isolate	Source	Type of pathogenicity	Production of T-toxin ^a	Mating type
NY9	New York	race T	+	A
NY338	New York	race T	+	a
1014	ascospore ^b	race T	+	a
644	ascospore ^b	race T	+	A
811	ascospore ^b	race T	+	a
Hm80	North Carolina	race T	+	A
Hm92	North Carolina	race T	+	a
D	Illinois	race O	-	a
U	Illinois	race O	-	A

^a+ = production of T-toxin in culture; - = no detectable T-toxin produced in culture.

^bCultures derived from these three ascospores produced high titers of T-toxin. Ascospore 1014 was from a cross of NY9 and NY338; 644 was from a cross of NY9 and 1014. Ascospore 811 was from a cross of 1014 and an apparent spontaneous albino mutant of NY9 (ascospore 811 gave rise to an albino culture). From the cross of NY9al × 1014, 3 complements of eight ascospores per ascus were isolated. In each ascus, four ascospores were mating type A and four were a; four ascospores produced cultures with normal pigment and four produced albino cultures. Mating type and albinism segregated independently of each other. A total of 107 ascospores were isolated from the three crosses of race T × race T; all of the ascospores gave rise to cultures which had T-type pathogenicity and all produced T-toxin in culture.

MATERIALS AND METHODS.—The isolates of the two known races of *H. maydis* used in this study are described in Table 1. The two race O isolates were opposite mating types and therefore could not be mated to the same race T tester isolate. Isolates from New York, Illinois, and North Carolina were supplied by C. W. Boothroyd (Cornell University), S. M. Lim (University of Illinois), and K. J. Leonard (North Carolina State University), respectively. The race T isolates were recognized by their specificity for corn with Texas male-sterile cytoplasm, and by their ability to produce T-toxin in culture; the race O isolates showed no specificity for corn cytoplasm, and did not produce T-toxin in culture (2).

Ascospores were produced by the method of Nelson (8); this method does not permit reciprocal crosses. Mature perithecia were examined under a dissecting microscope to determine whether or not asci and ascospores were present. As reported by Nelson (8), we found that infertile matings or matings with low fertility were common in *Cochliobolus heterostrophus*. Although some of the race T × race T crosses produced asci which contained up to eight ascospores, the race T × race O crosses examined in this study produced asci containing up to four ascospores. Ascospores from four-spored asci were selected when possible, but all ascospores obtained in this study must be considered to be chosen at random (Table 3, footnote^b). Each ascospore was transferred to a 9-cm diameter petri dish containing potato juice agar (no glucose), and incubated under fluorescent light for 1 week at 24 C. For toxin production, blocks of agar bearing mycelium and conidia were transferred from each petri dish to 125-ml flasks (two flasks/isolate) containing 25 ml modified Fries' medium (10) and incubated at 24 C in darkness for 12 to 15 days. To test for pathogenicity, the conidia remaining in each petri dish were suspended in water containing Tween 20 (1 drop/100 ml) and atomized onto corn plants (2,000 conidia/ml, 1 ml/plant).

Pathogenicity tests were conducted with plants (2 weeks old, three- to four-leaf stage) grown in 10.2 cm (4-inch) diameter pots (five plants/pot) in a controlled

environment chamber at 23 C and 80% relative humidity with 14 hours of light, 19,368 lx (1,800 f.c.) per day. After inoculation, plants were held in a fog chamber for 24 hours, then returned to the controlled environment chamber for 7 days before data were recorded. A set of four corn types (Table 2), representing two nuclear and two cytoplasmic backgrounds, was used for each isolate (one pot of each corn type per ascospore isolate). Inbred B14A was more susceptible to both race T and race O than was Mo17 [nuclear genes are known to affect susceptibility of corn to both race T and race O; (2)]. Both inbreds are more susceptible to race T if they contain Texas male-sterile cytoplasm, than if they contain normal cytoplasm. The criteria by which race T-type and race O-type pathogenicities were determined are summarized in Table 2. Assays using one inbred genome in Texas and normal cytoplasm gave ambiguous results with certain ascospore isolates; this confusion was avoided by using two inbred genomes, each in two different cytoplasm. Data for all pathogenicity tests were recorded independently by two different observers. The corn seed was supplied by John Brayton, Clyde Black & Son Inc., Ames, Iowa.

To detect host-specific toxin (T-toxin) activity, the culture filtrate of each ascospore isolate was diluted 1:4 with water and placed in the whorls (0.2 ml/whorl) of 2-week-old corn plants (five plants/isolate) using a 1.0-ml hypodermic syringe equipped with a 26-gauge needle. Prior to injection of the filtrate, the needle was passed through one side of the stem to create a small wound. Data were recorded 5 days after filtrates were applied to plants. The presence of T-toxin was detected by chlorotic and/or necrotic streaks extending up the leaf (2 to 6 cm) from the points of wounding on corn with Texas male-sterile cytoplasm; no streaks occurred on corn with normal cytoplasm. Filtrate without T-toxin activity either had no effect on corn or caused slight chlorosis (1 to 2 mm) at points of wounding on corn with both Texas male-sterile and normal cytoplasm.

RESULTS.—The results of six different crosses are summarized in Table 3. Race O isolate U was crossed with

TABLE 2. Distinguishing symptoms caused by races T and O of *Helminthosporium maydis* on a set of corn differentials in a controlled environment chamber

Type of pathogenicity ^a	Symptoms on corn inbred			
	B14A		Mo17	
	Texas male-sterile cytoplasm	Normal cytoplasm	Texas male-sterile cytoplasm	Normal cytoplasm
Race T	Gray-tan lesions (2-6 cm long) with diffuse margins; extensive chlorotic streaking	Red-brown lesions (0.5 - 1.5 cm long) containing concentric rings, and with discrete margins	Brown to dark gray lesions (0.5 - 1.5 cm long) with wide chlorotic halo or streaks	Orange-brown lesions (0.1 - 0.5 cm long) with discrete margins
Race O	Same as race T on B14A, normal cytoplasm	Same as race T on B14A, normal cytoplasm	Same as race T on Mo17, normal cytoplasm	Same as race T on Mo17 normal cytoplasm

^aEach ascospore isolate was recognized as having race T-type pathogenicity or race O-type pathogenicity by the set of reactions produced on the corn differentials. The race T and race O parents were included in each test of ascospore progeny; none of the progeny appeared to be nonparental types.

TABLE 3. Segregation of pathogenicity type and ability to produce T-toxin in ascospore progenies from crosses between races T and O of *Helminthosporium maydis*

Cross	Parents		Number of ascospores			
	Race O	Race T	Isolated	With race T-type pathogenicity	With race O-type pathogenicity	With ability to produce T-toxin ^a
1	U	Hm92	38	19	19	19
2	U	811	16 ^b	8	8	8
3	U	811	28 ^c	15	13	15
Totals			82	42	40	42
4	D	Hm80	34	5	29	5
5	D	644 ^d	36	6	30	6
6	D	644	50	5	45	5
Totals			120	16	104	16

^aIn every case the ascospores which produced T-toxin are those which had race T-type pathogenicity. None of the ascospores with race O-type pathogenicity produced detectable T-toxin.

^bThese 16 ascospores represent four asci, each containing four ascospores. Ten of the ascospores produced normally pigmented cultures and six produced albino cultures, suggesting that four ascospores per ascus do not represent the four products of meiosis (in eight-spored asci, the albino marker segregates as a single gene; see Table 1, footnote^b).

^cThese 28 ascospores were isolated at random; 12 produced normally pigmented cultures, 16 produced albino cultures.

^dMating types were determined for ascospores from this cross; 18 were A, 16 were a, and two failed to mate with any of the four tester isolates which were used.

race T isolates Hm92 and 811. Of 82 ascospores isolated, 42 exhibited race T-type pathogenicity and produce T-toxin in culture; 40 showed race O-type pathogenicity and produced no detectable T-toxin in culture. This segregation fits a 1:1 ratio ($\chi^2 = 0.049$, $P = 0.85$) and agrees with the ratio reported by Lim and Hooker (6). No exceptions were found in the correlation between race T-type pathogenicity and ability to produce T-toxin in culture.

In another series of crosses, race O isolate D was crossed with race T isolates Hm80 and 644 (Table 3). Of

120 ascospores isolated, 16 exhibited race T-type pathogenicity, and produced T-toxin in culture; 104 possessed race O-type pathogenicity and produced no detectable T-toxin in culture. This segregation does not fit a 1:1 or a 3:1 ratio, but it closely resembles a 7:1 ratio ($\chi^2 = 0.076$, $P = 0.80$). Again, exceptions to the correlation between race T-type pathogenicity and ability to produce T-toxin did not occur.

DISCUSSION.—The data are important for two reasons. First, analysis of 202 ascospores from race T × race O crosses (Table 3) and 107 ascospores from race T

× race T crosses (Table 1, footnote^b) showed no exceptions to the correlation between race T-type pathogenicity and ability to produce T-toxin. This result suggests that ability to produce T-toxin is required for race T-type pathogenicity. If T-toxin is the cause of race T-type symptoms, the mechanism by which these symptoms occur can be elucidated by a study of the mechanism of action of T-toxin. Second, the genetic control of race T-type pathogenicity and T-toxin production may be more complex than it first appeared. In certain crosses between race T and race O, progeny segregated in a 1:1 ratio with respect to race T and race O types of pathogenicity, suggesting that a single gene controls race T-type pathogenicity and production of T-toxin. However, in crosses with other isolates, the segregation ratio differed from 1:1, which raises the possibility that more than one (perhaps three or more) gene(s) is involved in control of race T-type pathogenicity and toxin production. If the control of pathogenicity does have a complex basis, it will have implications in studies of T-toxin biosynthesis, the number of host-specific molecules involved in the mechanism of disease caused by race T (3), the uncertainty over the origin of race T and its relationship to race O (4, 9), and the possibilities of new races appearing on agricultural crops in the future. However, since the ascospores analyzed in this study were isolated at random (in no case were complements of eight ascospores per ascus found in crosses of race T and race O), the apparent 7:1 ratios may have other explanations, such as linkage of a single (T) gene to a lethal gene, an effect of modifying genes on the action of a single controlling gene (11), interaction between nuclear and cytoplasmic genes, or complexities caused by chromosomal aberrations or gene conversions. For instance, Leonard (5) has observed nonrandom recovery of ascospores in asci of *C. carbonum* which contained less than eight ascospores. Our results cannot be explained by nonrandom selection of ascospores during the isolation procedure because mating types [as Nelson (7) has reported] and albinism segregated in 1:1 ratios, even when pathogenicity types and toxin production did not (Table 3). We are now attempting to identify the genetic mechanism which is responsible for the observed segregation ratios.

LITERATURE CITED

1. HOOKER, A. L., D. R. SMITH, S. M. LIM, and J. B. BECKETT. 1970. Reaction of corn seedlings with malesterile cytoplasm to *Helminthosporium maydis*. *Plant Dis. Rep.* 54:708-712.
2. HOOKER, A. L., D. R. SMITH, S. M. LIM, and M. D. MUSSON. 1970. Physiological races of *Helminthosporium maydis* and disease resistance. *Plant Dis. Rep.* 54:1109-1110.
3. KARR, A. L., JR., D. B. KARR, and G. A. STROBEL. 1974. Isolation and partial characterization of four host-specific toxins of *Helminthosporium maydis* (race T). *Plant Physiol.* 53:250-257.
4. LEONARD, K. J. 1973. Association of mating type and virulence in *Helminthosporium maydis*, and observations on the origin of the Race T population in the United States. *Phytopathology* 63:112-115.
5. LEONARD, K. J. 1973. Nonrandom survival of *Cochliobolus carbonum* ascospores in asci bearing less than eight ascospores. *Mycologia* 65:1229-1233.
6. LIM, S. M., and A. L. HOOKER. 1971. Southern corn leaf blight: genetic control of pathogenicity and toxin production in race T and race O of *Cochliobolus heterostrophus*. *Genetics* 69:115-117.
7. NELSON, R. R. 1957. A major gene locus for compatibility in *Cochliobolus heterostrophus*. *Phytopathology* 47:742-743.
8. NELSON, R. R. 1959. Genetics of *Cochliobolus heterostrophus*. I. Variability in degree of compatibility. *Mycologia* 51:18-23.
9. NELSON, R. R., J. E. AYERS, H. COLE, and D. H. PETERSEN. 1970. Studies and observations on the past occurrence and geographical distribution of isolates of race T of *Helminthosporium maydis*. *Plant Dis. Rep.* 54:1123-1126.
10. PRINGLE, R. B., and R. P. SCHEFFER. 1963. Purification of the selective toxin of *Periconia circinata*. *Phytopathology* 53:785-787.
11. SCHEFFER, R. P., R. R. NELSON, and A. J. ULLSTRUP. 1967. Inheritance of toxin production and pathogenicity in *Cochliobolus carbonum* and *Cochliobolus victoriae*. *Phytopathology* 57:1288-1291.
12. SCHEFFER, R. P., and O. C. YODER. 1972. Host-specific toxins and selective toxicity. Pages 251-272 in R. K. S. Wood, A. Ballio, and A. Graniti, eds. *Phytotoxins in plant diseases*. Academic Press, London. 530 p.