Further Physiologic Specialization in *Helminthosporium turcicum*

D. R. SMITH and J. G. KINSEY, Plant Pathologists, DEKALB AgResearch, Inc., DeKalb, IL 60115

ABSTRACT

SMITH, D. R., and J. G. KINSEY. 1980. Further physiologic specialization in *Helminthosporium turcicum*. Plant Disease 64:779-781.

A previously undescribed race of *Helminthosporium turcicum* is virulent to corn plants containing either gene *Ht2* or *Ht3*. This race is designated *H. turcicum* race 3 with the virulence formula *Ht1/ Ht2Ht3*.

Additional key words: corn pathogen monitoring project, gene *Ht*, physiologic races

Before 1961, the major sources of resistance in corn (*Zea mays* L.) to *Helminthosporium turcicum* Pass. (= *Exserohilum turcicum* (Pass.) Leonard & Suggs [17]) were polygenic in their inheritance (14). This resistance was expressed as reduced numbers of lesions on the infected plants (2).

0191-2917/80/08077903/$03.00/0

In 1961, a chlorotic-lesion resistance that inhibited fungal sporulation within the lesions was reported (5). Inheritance of this resistance is a single, completely dominant gene that was designated *Ht1* (=*Ht*) (6,7). This type of resistance has been very useful, and a large portion of the corn hybrids marketed in the U.S. and European corn belts possesses *HT1* (8). Many other sources of chlorotic-lesion resistance are now known to exist (10,11). One source designated NN14B developed from the Australian corn inbred NN14 has a gene designated as *Ht2* that is at a different gene locus from *Ht1* (9). Another chlorotic-lesion source was isolated from a cross of corn by *Tripsacum floridanum* (8), and the gene has been designated *Ht3* (13). Other sources of resistance include a chlorotic-fleck type of resistance from the African inbred B1138T (8), and a major gene source designated *HtN* that suppresses lesion development in adult plants (4).

The stability of *Ht1* was investigated, and 166 isolates of *H. turcicum* collected from various hosts around the world were avirulent to plants containing the *Ht1* gene (12). Also, virulence of *H. turcicum* isolates remained unchanged after successive passages onto plants with the *Ht1* gene (16). However, during 1972 an isolate of *H. turcicum* virulent to plants with the *Ht1* gene was observed in a seed production field in Hawaii (1). This

Table 1. Phenotypic response of four corn genotypes inoculated with three isolates of *Helminthosporium turcicum* 14 days after inoculation

Isolate	Genotype			
	H4460	H4460 *Ht1Ht1*	H4460 *Ht2Ht2*	*Ht3Ht3* source
74:105	N[a]	C	C	C
79:44	N	N	C	C
M76:689-816	N	C	N	N

[a] N = necrotic lesions, C = chlorotic lesions.

isolate was avirulent on plants containing the *Ht2* gene and was shown to be an undescribed physiologic race of *H. turcicum* that was designated race 2, represented by the virulence formula *Ht2/Ht1* (1,18).

During the 1979 growing season, either race 2 or a similar race was observed in production fields in Indiana (20). This is the first report of the occurrence of an isolate of *H. turcicum* in the conterminous U.S.A. that is virulent to plants containing *Ht1* resistance.

Since 1974, DEKALB AgResearch, Inc. has conducted a corn pathogen monitoring project (19) for the systematic collection and evaluation of various corn pathogen isolates. During the evaluation of *H. turcicum* isolates collected in the 1976 growing season, some conflicting results were obtained that suggested the possibility of another physiologic race of *H. turcicum*. These results were repeated in 1979 when a more discriminating source of *Ht2* resistance was developed and a corn source homozygous for *Ht3* became available.

Table 2. *Helminthosporium turcicum* conidia produced after 5-day incubation on lesion disks from greenhouse inoculations of single-spore isolates of 74:105 and M76:689-816 onto differential corn genotypes

Genotype	Estimated conidia (no.)	
	74:105	M76: 689-816
Hy-2	3400[a]	2600
Hy-2 *Ht1Ht1*	400	0
Hy-2 *Ht2Ht2*	200	2400
Hy-2 *Ht1ht1 Ht2ht2*	0	0
Oh43	2200	2000
Oh43 *Ht1Ht1*	200	0
Oh43 *Ht2Ht2*	0	2000
Oh43 *Ht1ht1 Ht2ht2*	400	0
Mo17	2400	2000
Mo17 *Ht1Ht1*	0	0
Mo17 *Ht2Ht2*	0	1600
Mo17 *Ht1ht1 Ht2ht2*	0	0
B14A	1600	1400
B14A *Ht1Ht1*	200	0
NN14B	0	1400

[a] Each value is the mean of five replications.

MATERIALS AND METHODS

Since 1974, samples of corn tissue infected with various pathogens routinely have been received at DeKalb, IL, from different locations within the U.S.A. The leaf samples were observed for symptoms, and portions of the infected tissue were placed under high humidity for 3 days in petri plates to allow fungal sporulation. Conidia of *H. turcicum* were picked from the conidiophores and placed onto potato-dextrose agar (PDA) in petri plates. These isolates were grown for 10 days at 20 C with 12 hr of darkness and 12 hr of fluorescent light (580 μ E/m^2/sec) in an incubator. They were then placed in a refrigerator at 4 C until plants in the greenhouse were ready to inoculate.

The *H. turcicum* isolates were of three groups. The first group was received from a monitoring plot near Estill, SC, during the 1976 growing season. The second group was a type isolate from central Illinois collected during the 1974 growing season. The third group was an isolate initially collected near Brook, IN, during the 1979 growing season (20). These isolates will be referred to as M76:689-816, 74:105, and 79:44 respectively.

The corn genotypes used in the greenhouse for routine evaluation of the first two groups of *H. turcicum* isolates were B37, B37 *Ht1Ht1*, B14A, and NN14B, which is homozygous for the *Ht2* gene. The original source of the *Ht1* gene used in these investigations was from GE440. When these plants were in the 4- to 6-leaf stage, they were inoculated with a suspension of 2,000 conidia per milliliter of the isolates. The conidial suspension was sprayed at 15 psi over the plants. The plants were placed in a humidity chamber without light at 100% RH and 20 C for approximately 16 hr and then removed to greenhouse benches. Notes on lesion type were recorded 14 days after inoculations in all experiments.

Two other experiments were done with single-spore isolates of the *H. turcicum* collections M76:689-816 and 74:105. The first consisted of inoculating 10 single-spore isolates of M76:689-816 and one single spore isolate of 74:105 onto the genotypes Oh43, Oh43 *Ht2Ht2*, B14A, and NN14B in the manner previously described. The genotypes used in the second experiment were Oh43, Oh43 *Ht1Ht1*, Oh43 *Ht2Ht2*, Oh43 *Ht1ht1 Ht2ht2*, Hy-2, Hy-2 *Ht1Ht1*, Hy-2 *Ht2Ht2*, Hy-2 *Ht1ht1 Ht2ht2*, Mo17, Mo17 *Ht1Ht1*, Mo17 *Ht2Ht2*, Mo17 *Ht1ht1 Ht2ht2*, B14A, B14A *Ht1Ht1*, and NN14B. A selected single-spore isolate of M76:689-816 and 74:105 was used for these evaluations.

Portions of lesions from the second experiment were studied for conidial production. A no. 2 cork borer was used to take 5-mm disks of infected leaf tissue from the lesions of plants 14 days after inoculation. These disks were immediately placed over moist filter paper in petri plates and were incubated for 5 days at 20 C. On the fifth day, the disks were placed in 1 ml of water and agitated for 1 min. The number of conidia per disk was estimated by using an improved Neubauer hemacytometer.

A final series of experiments involved a proprietary inbred line H4460, H4460 *Ht1Ht1*, H4460 *Ht2Ht2*, and a corn source homozygous for *Ht3*. The isolates of *H. turcicum* evaluated across these differentials included 74:105, M76:689-816, and 79:44. Inoculation and notes on lesion type were managed as previously described. Sporulation was not numerically estimated but rather was described for portions of infected leaf tissue from these inoculations.

RESULTS

Symptoms. From the first evaluations of *H. turcicum* isolate 74:105, two reaction types resulted from the inoculations onto B37, B37 *Ht1Ht1*, B14A, and NN14B. B37 *Ht1Ht1* and NN14B were resistant and exhibited chlorotic lesions with little or no necrosis. B37 and B14A were susceptible and exhibited elliptical grayish green necrotic lesions.

The evaluations of *H. turcicum* isolate M76:689-816 also yielded two reaction types from the inoculations onto B37, B37 *Ht1Ht1*. B14A, and NN14B. B37 *Ht1Ht1* was resistant and exhibited chlorotic lesions. B37, B14A, and NN14B were susceptible and exhibited necrotic lesions. These results were identical to

Table 3. Relative number of *Helminthosporium turcicum* conidia on lesions after 2-day incubation following greenhouse inoculations of *H. turcicum* isolates onto differential corn genotypes

Genotype	Days of incubation								
	0			1			2		
	74:105[a]	79:44	M76: 689-816	74:105	79:44	M76: 689-816	74:105	79:44	M76: 689-816
H4460	0[b]	0	0	2	1	3	4	4	4
H4460 *Ht1Ht1*	0	0	0	0	2	0	0	4	1
H4460 *Ht2Ht2*	0	0	0	1	1	3	1	1	4
Ht3Ht3 source	0	0	0	0	0	2	0	0	4

[a] *H. turcicum* isolate.

[b] Relative sporulation: 0 = no conidia, 1 = 1–200 conidia, 2 = 201–500 conidia, 3 = 501–1,000 conidia, and 4 = more than 1,001 conidia.

those of the 74:105 evaluations except that NN14B exhibited a susceptible reaction to *H. turcicum* isolate M76:689-816.

The results of the inoculation of the single-spore isolates of 74:105 and M76:689-816 onto Oh43, Oh43 *Ht2Ht2*, B14A, and NN14B were consistent with the initial evaluations. Oh43 *Ht2Ht2* and NN14B exhibited chlorotic lesions from the 74:105 inoculations; Oh43 and B14A exhibited necrotic lesions. Oh43, Oh43 *Ht2Ht2*, B14A, and NN14B exhibited necrotic lesions from the single-spore inoculations of M76:689-816.

The results of the second experiment, including a single-spore isolate of M76:689-816 and 74:105, were in agreement with the other evaluations. Oh43, Hy-2, Mo17, and B14A exhibited necrotic lesions from the 74:105 inoculations, but Oh43 *Ht1Ht1*, Oh43 *Ht2Ht2*, Oh43 *Ht1ht1 Ht2ht2*, Hy-2 *Ht1Ht1*, Hy-2 *Ht2Ht2*, Hy-2 *Ht1ht1 Ht2ht2*, Mo17 *Ht1Ht1*, Mo17 *Ht2Ht2*, Mo17 *Ht1ht1 Ht2ht2*, B14A *Ht1Ht1*, and NN14B expressed chlorotic lesions. The M76:689-816 single-spore inoculations produced necrotic lesions in Oh43, Oh43 *Ht2Ht2*, Hy-2, Hy-2 *Ht2Ht2*, Mo17, Mo17 *Ht2Ht2*, B14A, and NN14B and chlorotic lesions in Oh43 *Ht1Ht1*, Oh43 *Ht1ht1 Ht2ht2*, Hy-2 *Ht1Ht1*, Hy-2 *Ht1ht1 Ht2ht2*, Mo17 *Ht1Ht1*, Mo17 *Ht1ht1 Ht2ht2*, and B14A *Ht1Ht1*.

The final series of experiments involving H4460, H4460 *Ht1Ht1*, H4460 *Ht2Ht2*, and a source homozygous for *Ht3* inoculated with *H. turcicum* isolates 74:105, 79:44, and M76:689-816 yielded the following results (Table 1). H4460 exhibited necrotic lesions to all isolates tested. H4460 *Ht1Ht1* exhibited necrotic lesions to isolate 79:44 and chlorotic lesions to isolates 74:105 and M76:689-816. H4460 *Ht2Ht2* and the *Ht3Ht3* source exhibited necrotic lesions to isolate M76:689-816 and chlorotic lesions to isolates 74:105 and 79:44.

Sporulation. In the evaluation of the single-spore isolates of 74:105 and M76:689-816, there were large differences in conidial production due to isolates and genotypes. Regardless of isolate, corn genotypes homozygous for *Ht1* had fewer conidia than the non-Ht normal counterparts (Table 2). Also, genotypes homozygous for *Ht2* had fewer conidia than their non-Ht normal counterparts from the 74:105 inoculations. However, there were no large differences in conidial production from genotypes possessing *Ht2*, compared with their non-Ht normal counterparts in the M76:689-816 inoculations. The genotypes possessing both *Ht1* and *Ht2* in a heterozygous condition did not exhibit conidial production much different from the genotypes homozygous for gene *Ht1*.

The final evaluations using H4460, H4460 *Ht1Ht1*, H4460 *Ht2Ht2*, and the source homozygous for *Ht3* resulted in the following sporulation information (Table 3). By the second day of sporulation, the necrotic lesions from the inoculations (74:105 onto H4460; 79:44 onto H4460 and H4460 *Ht1Ht1*; and M76:689-816 onto H4460, H4460 *Ht2Ht2*, and the source *Ht3Ht3*) each showed more than 1,001 conidia. However, all chlorotic lesions (74:105 onto H4460 *Ht1Ht1*, H4460 *Ht2Ht2*, and the *Ht3Ht3* source; 79:44 onto H4460 *Ht2Ht2* and the *Ht3Ht3* source; and M76:689-816 onto H4460 *Ht1Ht1*) had fewer than 200 conidia per lesion.

DISCUSSION

The evaluation of *H. turcicum* isolate M76:689-816 clearly shows a previously undescribed race of *H. turcicum* within the conterminous U.S.A. This race is uniformly avirulent on genotypes possessing the *Ht1* gene and virulent on genotypes possessing the *Ht2* gene and *Ht3* gene. Also, heterozygous plants with both *Ht1* and *Ht2* genes are not predisposed to susceptibility by the *Ht2* gene. Conidial production was consistently greater from necrotic lesions than from chlorotic lesions within isogenic comparisons.

We propose that the isolate M76:689-816 be designated *H. turcicum* race 3 with the virulence formula *Ht1/Ht2Ht3* (effective/ineffective genes). The identification of *H. turcicum* race 3 demonstrates the merits of a systematic corn pathogen monitoring project. It is also apparent that for any monitoring project to be effective, an evaluation of collected isolates is required.

Based on its virulence formula (*Ht2Ht3/Ht1*), 79:44 would be classified as a member of the *H. turcicum* race 2 group. Also, from these evaluations, *Ht2* apparently cannot be differentiated from *Ht3* with the isolates of *H. turcicum* used in this study. It might be that *Ht2* is identical in function to *Ht3*.

These results suggest that direct substitution of gene *Ht2* for *Ht1* to protect the corn crop in the conterminous U.S.A. would be unwise. If *Ht2* were substituted for *Ht1*, *H. turcicum* race 3 might increase rapidly and result in a "man-guided" epiphytotic similar to the cereal rust situation discussed by Johnson (15).

The most effective program for the utilization of monogenic resistances would be to combine genes *Ht1* and *Ht2* or *Ht1* and *Ht3* into elite corn inbred lines used in hybrid production. This would offer protection not only in the hybrid corn crop but also in seed production fields where hybrid seed is produced. It should be emphasized that since races presently exist that can negate the benefits of *Ht1*, *Ht2*, and *Ht3* singly, efforts to select for polygenic resistance in corn to *H. turcicum* should be strengthened.

Future investigations to better determine the distribution of race 2 and race 3, the effectiveness of other sources of monogenic resistance to race 3, and the effectiveness of polygenic resistance against race 3 and studies that might substantiate that the *Zea mays-H. turcicum* host-pathogen system would fit the gene-for-gene hypothesis proposed by Flor (3) are needed.

LITERATURE CITED

1. BERGQUIST, R. R., and O. R. MASIA. 1974. Physiologic specialization in *Trichometasphaeria turcica* F. sp. *zeae* and *T. turcica* F. sp. *sorghi* in Hawaii. Phytopathology 64:645-649.
2. ELLIOTT, C., and M. T. JENKINS. 1946. *Helminthosporium turcicum* leaf blight in corn. Phytopathology 36:660-666.
3. FLOR, H. H. 1956. The complementary genic systems in flax and flax rust. Adv. Genet. 8:29-54.
4. GEVERS, H. O. 1975. A new major gene for resistance to *Helminthosporium turcicum* leaf blight of maize. Plant Dis. Rep. 59:296-299.
5. HOOKER, A. L. 1961. A new type of resistance in corn to *Helminthosporium turcicum*. Plant Dis. Rep. 45:780-781.
6. HOOKER, A. L. 1963. Inheritance of chlorotic-lesion resistance to *Helminthosporium turcicum* in seedling corn. Phytopathology 53:660-662.
7. HOOKER, A. L. 1963. Monogenic resistance in *Zea mays* L. to *Helminthosporium turcicum*. Crop Sci. 3:381-383.
8. HOOKER, A. L. 1975. *Helminthosporium turcicum* as a pathogen of corn. Rept. Tottori Mycol. Inst. (Japan) 12:115-125.
9. HOOKER, A. L. 1977. A second major gene locus in corn for chlorotic-lesion resistance to *Helminthosporium turcicum*. Crop Sci. 17:132-135.
10. HOOKER, A. L. 1978. Additional sources of monogenic resistance in corn to *Helminthosporium turcicum*. Crop Sci. 18:787-788.
11. HOOKER, A. L., H. M. HILU, D. R. WILKINSON, and C. G. VanDYKE. 1964. Additional sources of chlorotic-lesion resistance to *Helminthosporium turcicum* in corn. Plant Dis. Rep. 48:777-780.
12. HOOKER, A. L., R. R. NELSON, and H. M. HILU. 1965. Avirulence of *Helminthosporium turcicum* on monogenic resistant corn. Phytopathology 55:462-463.
13. HOOKER, A. L., and H. SMILJAKOVIC. 1979. Maize breeding for disease resistance. Eucarpia Proc.
14. JENKINS, M. T., and ALICE L. ROBERT. 1952. Inheritance of resistance to the leaf blight of corn caused by *Helminthosporium turcicum*. Agron. J. 44:136-140.
15. JOHNSON, T. 1961. Man-guided evolution in plant rusts. Science 133:357-362.
16. KINSEY, J. G., and A. L. HOOKER. 1973. Changes in *Helminthosporium turcicum* populations following serial host passage. Plant Dis. Rep. 57:590-591.
17. LEONARD, K. J., and E. G. SUGGS. 1974. *Setosphaeria prolata*, the ascigerous state of *Exserohilum prolatum*. Mycologia 66:281-297.
18. LIM, S. M., J. G. KINSEY, and A. L. HOOKER. 1974. Inheritance of virulence in *Helminthosporium turcicum* to monogenic resistant corn. Phytopathology 64:1150-1151.
19. SMITH, D. R. 1977. Monitoring corn pathogens. Proc. 32nd Annu. Corn and Sorghum Res. Conf., Am. Seed Trade Assoc. pp. 106-121.
20. TURNER, M. T., and E. R. JOHNSON. 1980. Race of *Helminthosporium turcicum* not controlled by *Ht* genetic resistance in corn in the American corn belt. Plant Dis. 64:216-217.