An Inoculation Technique to Detect the *HtN* Gene in Inbred Lines of Corn Under Greenhouse Conditions

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ABSTRACT

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A 10-μl drop of inoculum containing about 500 conidia (50,000 conidia per milliliter) of *Exserohilum turcicum* was applied to the leaf surfaces of 11-day-old corn (*Zea mays*) seedlings. After 10 days, susceptible inbred lines could be distinguished from lines with either of the dominant resistance genes *Ht1* or *HtN* based on incubation periods and lesion characteristics. This technique was also effective in determining the portion of an inbred population carrying the *HtN* gene.

Additional key words: northern corn leaf blight

Northern leaf blight of corn (*Zea mays* L.) caused by *Exserohilum turcicum* (Pass.) Leonard & Suggs (Teleomorph: *Setosphaeria turcica* (Luttrell) Leonard & Suggs) has been effectively controlled for years by using the resistance gene *Ht1*, also designated *Ht*, from the inbred GE440 (5). This gene conditions a chlorotic-lesion type of resistance to *E. turcicum* and the degree of chlorosis is dependent upon the remainder of the plant's genotype (5). Race 2 of *E. turcicum*, which is virulent on corn carrying the *Ht1* gene for resistance (5), now exists throughout the northern Corn Belt (W. L. Pedersen, *unpublished*). For this reason, sources of resistance other than *Ht1* are being investigated. The gene *HtN*, identified in the Mexican variety Pepitilla, was reported in 1975 by Gevers (2). This gene was recently shown to be an effective source of resistance to *E. turcicum* under field conditions by effectively prolonging incubation time and decreasing inoculum availability by prolonging the latent period (5,6). This gene, however, has been difficult to use in a backcross breeding program because the detection of the *HtN* gene has required the use of full-season field tests because incubation periods may be prolonged up to 60 days (6). Field tests may be unreliable when environmental conditions favor poor disease development, and when lesions develop on plants with the *HtN* gene, they can not be readily distinguished from lesions on plants susceptible to *E. turcicum*. A technique that would permit rapid detection of the *HtN* gene in greenhouse-grown corn plants would be useful and would facilitate the rapid detection of the *HtN* gene in breeding material. This would eliminate backcrossing without prior selection and allow for the more rapid development of *HtN* inbreds.

MATERIALS AND METHODS

Ten-day-old cultures of *E. turcicum* grown on lactose casein hydrolysate medium (8) in 100-mm-diameter petri plates were flooded with 5 ml of distilled water. Conidia were loosened with a rubber policeman and the resulting suspension was filtered through four thicknesses of cheesecloth. Conidial concentrations were adjusted to about 500 conidia/10-μl drop (50,000 conidia per milliliter) and 0.05 ml of Tween 80 was added per 20 ml of inoculum. Three separate experiments were conducted and experiment 1 was repeated three times, whereas experiments 2 and 3 were repeated twice.

The inbred lines used in this study were chosen from different families of inbred lines to include different sources and levels of resistance to *E. turcicum*. The inbred lines W22HtN, Oh45RHtN, B57HtN, B68HtN, Mo12HtN, K64HtN, B14AHtN, B37HtN, W22, Oh45, B57, B68, Mo12, K64, B14A, B37, RW22Ht1, B68Ht1, RB14AHt1, and RB37Ht1 were planted in plastic cones 4 × 21 cm with Fertilimix Potting Soil (A. H. Hoffman, Inc., Landisville, PA 17538) as a potting medium and grown on greenhouse benches at 24 ± 3 C. Plants were inoculated at the three-leaf stage, stage 0.5 on Hanway's (3) growth scale. Cones were arranged randomly with 25 single plant replicates per inbred. Each plant was inoculated with three 10-μl drops, one on each side of the midrib on the second leaf and one on the emerging third leaf. Drops contained 538 ± 59 conidia with 93% viability as measured by the percentage of conidia germinated after 24 hr on lactose casein medium. Drops were allowed to dry to prevent runoff and then the plants were placed in mist chambers at 100% RH for 12 hr. After misting, plants were removed to greenhouse benches. Corn seedlings were evaluated on the 10th day after inoculation, 21 days after planting. Each plant was classified based on lesion type and the categories were: 1 = no lesion, 2 = chlorotic lesion typical of *Ht1* plants, 3 = susceptible lesion ≤2 cm long, and 4 = susceptible lesion >2 cm long.

A second experiment was conducted with five isolates of *E. turcicum* and six inbred lines: W22, Hy, B37, W22HtN, HyHtN, and B37HtN. This experiment was arranged with inbreds, condition of the *HtN* gene (dominant or recessive), and isolates as factors. Plants were grown in 17-cm-diameter clay pots with four plants per pot. Each pot contained two plants homozygous dominant for the *HtN* gene and two plants homozygous recessive for the *HtN* gene. Individual plants were considered replicates with

Table 1. The origin, race, inoculum concentration, and viability of five isolates of *Exserohilum turcicum* used in experiment two

Isolate origin	Race[a]	Conidia per 10-μl drop	Viability[b] (%)
1981 Dekalb Co., IL	1	493 ± 37	95.1
1981 Woodford Co., IL	1	543 ± 34	97.3
1981 Swift Co., MN	1	723 ± 102	96.9
1980 Newton Co., IN	2	785 ± 96	95.2
1979 Licking Co., OH	1	650 ± 42	98.4

[a] Race 1 is avirulent and race 2 virulent to corn lines carrying the *Ht1* gene.
[b] Viabilities were determined by plating dilutions of inoculum suspension on six replica plates of lactose casein medium and examining for conidial germination after 24 hr.

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two pots per treatment. Plants were inoculated and misted as described previously, except each plant received only one 10-μl drop of inoculum. Inoculum concentrations and viability of conidia for this experiment are shown in Table 1.

A third experiment was conducted to evaluate the effectiveness of this inoculation technique in determining the portion of plants carrying the dominant *HtN* allele in segregating lines or populations. Seeds of inbred lines B37 and B37HtN were combined to simulate lines or populations segregating in exact 3:1 or 1:1 (susceptible:resistant) ratios. Each population consisted of 48 seeds so a 3:1 ratio would have 36 B37 seeds and 12 B37HtN seeds. This study was also conducted with inbred lines Hy and HyHtN. Single seeds were planted in individual cones and plants were grown and inoculated as described for the first experiment. Conidia for the third experiment were obtained from leaf tissue of infected B37 plants from the previous test. This was done to obtain fresh mature conidia when the plants were at the three-leaf stage. Leaf tissue was placed on moist filter paper in 100-mm-diameter petri plates, incubated for four days, placed in a test tube with 10 ml of distilled water, agitated with a Vortex mixer, and diluted to 428 ± 65 conidia/10 μL (42,800 conidia per milliliter). Conidial viability was 94% on lactose casein medium.

Table 2. Classification of 25 plants of 20 corn inbreds 10 days after inoculation with 10-μl drops containing 538 ± 59 conidia of race 1 *Exserohilum turcicum*

Inbred	Category[a] 1	2	3	4	Mean[b]
W22HtN	18	0	6	1	1.6
Oh45RHtN	18	0	5	0	1.4
B57HtN	21	0	0	0	1.0
B68HtN	21	0	2	1	1.4
Mo12HtN	9	0	11	5	2.5[c]
K64HtN	25	0	0	0	1.0
RB14AHtN	17	0	6	2	1.7
B37HtN	25	0	0	0	1.0
RW22Htl	3	20	0	0	1.9
B68Htl	3	11	2	0	1.9
RB14AHtl	0	20	0	0	2.0
RB37Htl	0	25	0	0	2.0
W22	4	0	5	15	3.3
Oh45	8	0	10	7	2.6
B57	3	0	10	12	3.2
B68	4	1	8	8	3.0
Mo12	1	0	1	22	3.8
K64	0	0	2	22	3.8
B14A	0	0	12	12	3.5
B37	0	0	12	12	3.5

[a]Categories are based on leaf reactions of individual plants where 1 = no lesion, 2 = chlorotic-lesion typical of *Htl* plants, 3 = susceptible lesion ≤ 2 cm in length, and 4 = susceptible lesion > 2 cm long and 2–4 mm wide.
[b]Means were determined by assigning a value to each entry equal to the category in which it was entered and summing all entries for an inbred and dividing by the total entries.
[c]Lesions were less than 1 mm wide.

RESULTS AND DISCUSSION

Results of the first experiment indicated that inbreds could be distinguished as susceptible or resistant to *E. turcicum*, based on examinations of inoculation sites (Table 2). The inbreds with the *HtN* gene usually had no lesion development after 10 days because of the prolonged incubation time associated with this gene. The chlorotic-lesion response typical of *Htl* plants (4) was readily detected. In inbreds with low levels of background resistance to leaf blights (such as Mo12) symptom development was prolonged 4–6 days and necrotic lesions developed within 10 days. This apparent ineffectiveness of the *HtN* gene in some inbreds has also been observed in the field. Other inbreds having plants with lesions that were classified as susceptible were W22HtN, Oh45RHtN, B68HtN, and RB14AHtN. These lesions, however, were all very narrow and never widened beyond 1 mm, whereas susceptible lesions were 2–4 mm wide. The presence of these atypical lesions may have been due to low levels of background resistance or the heterozygous condition of the *HtN* gene. There were also a few plants from inbreds not carrying the *HtN* gene that did not show susceptible lesions and these were probably escapes or the result of high levels of background resistance to *E. turcicum* (1).

Table 3. Analysis of variance from four replicates of three corn inbreds with and without *HtN*, inoculated with conidia from five isolates of *Exserohilumm turcicum*

Source	df	Sum of squares	Mean squares[a]
Inbred	2	1.0	0.5
Isolate	4	3.6	0.9
Inbred/isolate	8	12.3	1.5**
Gene (*HtN* vs. *htN*)	1	106.4	106.4**
Inbred/gene	2	9.4	4.7**
Isolate/gene	4	10.5	2.6**
Inbred/isolate/gene	8	8.3	1.0*
Error	90	44.3	0.5
Total	119	135.8	

[a]** = Value significant at the 0.01 level and * = value significant at the 0.05 level.

In the second experiment, neither the three inbred pairs nor the five isolates of *E. turcicum* showed significant ($P = 0.05$) differences when seedlings were evaluated for symptoms of northern corn leaf blight (Table 3). However, the gene effect, *HtN* versus *htN*, was highly significant ($P = 0.01$), as were the four interactions. The significant ($P = 0.01$) inbred by isolate interaction may have been the result of the different genetic backgrounds of the inbreds or differences in parasitic fitness among isolates. The interaction between the inbreds and the condition of the *HtN* gene may be due to varying action of the *HtN* gene in different genetic backgrounds as has been reported previously (2,6). The isolate by gene interaction was probably related to the two previous interactions and all three of these interactions are also related to the significant ($P = 0.05$) inbred by isolate by gene interaction.

The third experiment confirmed that the 10-μl drop inoculation technique was effective in determining the frequency of plants carrying the *HtN* gene in segregating populations (Table 4). In all cases, the data confirmed the expected segregation ratios for both inbred pair tests.

With the increasing prevalence of race 2 and possibly race 3 (7) of *E. turcicum*, alternatives to the *Htl* gene for resistance to *E. turcicum* are being incorporated into elite inbreds. This microdrop inoculation method (10-μl drop) allows rapid detection, 21 days from planting to evaluation, of the *HtN* gene in individual plants and in segregating populations. At present, this technique is not suitable for differentiating between plants that are homozygous or heterozygous for *HtN*. Because of the uniformity of lesion development, this technique may also have applications in component analyses of rate-reducing types of resistance and in testing with more than one foliar pathogen on single plants.

Table 4. Observed reactions of segregating inbred populations of 21-day-old greenhouse-grown corn inbreds inoculated with race 1 of *Exserohilum turcicum*

Segregating inbred population[a]	Reaction: Susceptible	Reaction: Resistant	Expected ratio	X^2 value	X^2 probability
Hy:HyHtN	23	25	1:1	0.08	0.90–0.75
Hy:HyHtN	22	26	1:1	0.17	0.75–0.50
B37:B37HtN	24	24	1:1	0.00	1.0
B37:B37HtN	23	25	1:1	0.08	0.90–0.75
Hy:HyHtN	36	12	3:1	0.00	1.0
Hy:HyHtN	36	12	3:1	0.00	1.0
B37:B37HtN	32	16	3:1	1.83	0.25–0.10
B37:B37HtN	32	16	3:1	1.83	0.25–0.10

[a]Segregating inbred populations with expected ratios of 1:1 were 50% Hy or B37 and 50% HyHtN or B37HtN, respectively; populations with expected ratios of 3:1 were 75% Hy or B37 and 25% HyHtN or B37HtN, respectively.
[b]Chi-square values are not significant and indicate a good fit with expected segregation ratios.

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