Reactions of Isolates from Matings of Races 1 and 23N of *Exserohilum turcicum*

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ABSTRACT

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Races 1 and 23N of *Exserohilum turcicum* isolated from corn leaves infected with northern leaf blight that were collected in 1990 from Belle Glade, Florida, were crossed in culture on johnsongrass stems. Pseudothecia, which formed after 21–25 days in darkness, were crushed and streaked on water agar. Ascospores were isolated and transferred to lactose-casein hydrolysate agar. Parental isolates and isolates from 112 ascospore progeny were assayed twice in the greenhouse for resistant and susceptible reactions on corn inbred lines with the genes *Ht1, Ht2, Ht3,* and *HtN* and susceptible inbred checks. Reactions of the 112 isolates were not independent (1:1 ratio of similar:dissimilar reactions) on plants with the genes *Ht2* and *Ht3* or the genes *Ht1* and *HtN*, i.e., reactions were similar on *Ht2* and *Ht3* genotypes for 189 of 224 assays and different on *Ht1* and *HtN* genotypes for 159 of 224 assays. Eight combinations of virulence were observed in both greenhouse trials; however, reactions of 72 of the 112 isolates differed between trials. Races 1, 23N, and 123 were most frequent among the 112 isolates based on greenhouse assays. When seven isolates that were virulent on *Ht1, Ht2, Ht3*, and/or *HtN* in the greenhouse were assayed in the field, only four were virulent on *Ht1* and all isolates were avirulent on *Ht2, Ht3*, and *HtN*.

Exserohilum turcicum (Pass.) K.J. Leonard & E.G. Suggs (teleomorph = *Setosphaeria turcica* (Luttrell) K.J. Leonard & E.G. Suggs) is a heterothallic ascomycete with mating types *A* and *a*, conditioned by alleles at the mating type locus (13,15). The asexual stage of the fungus causes northern leaf blight (NLB) of corn (*Zea mays* L.). *E. turcicum* overwinters as conidia and/or mycelia on infected plant debris and has been isolated from other grass species, including Sudan grass (*Sorghum vulgare* Pers. var. *sudanense* (Piper) (A. Hitchc.), broomcorn (*S. v. technicum* (Körn.) Jáv.), and johnsongrass (*S. halepense* (L.) Pers.) (4). Johnsongrass is an overwintering site for *E. turcicum* (17). The sexual stage can be produced in culture using natural substrates and special media (19).

Resistance of corn to NLB has been grouped into two categories: partial and *Ht*. Partial resistance is conditioned by a few to several genes (11). Populations of field corn were improved for partial resistance to NLB in some of the first applications of recurrent selection procedures (10). Partial resistance usually is expressed as fewer lesions of smaller size. Infection efficiency, latent period, and other components of the infection cycle also may be affected by partial resistance (2). *Ht* resistance is conditioned by single dominant genes. Four dominant genes—*Ht, Ht2, Ht3*, and *HtN*—have been identified and incorporated into elite lines of maize. The gene *Ht*, usually referred to as *Ht1*, is expressed as chlorotic lesions with smaller size and a reduced amount of sporulation (5–7). The genes *Ht2* and *Ht3* condition a chlorotic reaction that usually is more extensive than that associated with *Ht1* (8,9). The expression of chlorotic reactions on hosts carrying *Ht2* or *Ht3* can be affected by light intensity and temperature (14,27). An isolate of race 23 produced avirulent, chlorotic lesions on plants with *Ht3* when temperature and light intensity were high (26/22 C day/night regimes and 647 $\mu E \cdot m^{-2} \cdot s^{-1}$), and an isolate of race 0 produced susceptible, necrotic reactions on plants with *Ht2* when temperature and light intensity were low (22/18 C day/night regimes and 162 $\mu E \cdot m^{-2} \cdot s^{-1}$) (14). The gene *HtN* is expressed initially as small flecks from which necrotic lesions develop after prolonged incubation and latent periods (3).

Several races of *E. turcicum* have been reported. Race 0, with the virulence formula *Ht1,Ht2,Ht3,HtN/0* (16), is prevalent in many parts of the world. Race 1 (*Ht2,Ht3,HtN/Ht1*), first reported from Hawaii in 1974 (1), was reported from the U.S. corn belt in 1978 (29). The gene-for-gene relationship was described for *E. turcicum* races 0 and 1 and the gene *Ht1* (18). Race 23 (*Ht1, HtN/Ht2,Ht3*) was collected from South Carolina in 1976 (26). Race 23N (*Ht1/Ht2,Ht3,HtN*) was observed first in Texas in 1980 (27). Race 2N (*Ht1,Ht3/Ht2,HtN*) was collected in Hawaii on an inbred carrying *HtN* (31). Recently, several new races were detected among isolates of *E. turcicum* collected from maize grown in China, Mexico, Uganda, and Zambia (30).

Resistance to NLB conferred by *Ht1* has been used extensively in field and sweet corn hybrids grown commercially in the United States. The genes *Ht2, Ht3*, and *HtN* have had limited commercial use. Thus, the selection pressure on populations of *E. turcicum* has been relatively severe for virulence against the gene *Ht1* but relatively minor for the other *Ht* genes.

In North America, races 0 and 1 are most prevalent (12,25). Race 23N is relatively rare. The frequency of race 23 has been low compared with that of races 0 and 1; however, about 8–9% of the isolates collected in the United States during surveys in 1979 and 1981 were race 23 (25). Corn leaves collected in 1990 from fields near Belle Glade and Zellwood, Florida, were infected with races 0, 1, 23, and 23N (21). Race 1 was most frequent (78%), probably because of the hectarage in Florida planted with supersweet (*sh2*) sweet corn hybrids that carry the gene *Ht1*.

The existence of two compatible mating types of *E. turcicum* in a geographic area could result in the recombination of virulence genes. Matings of races 1 and 23N in Florida, or elsewhere, could produce new combinations of virulence. New races of the pathogen occurring at very low frequencies probably would go undetected because of the lack of selection pressure in favor of these races. Conversely, extensive deployment of the genes *Ht2, Ht3*, and *HtN* is likely to increase the frequency of previously unobserved races if recombination of virulence occurs. The objective of this experiment was to evaluate the reactions of progeny from crosses of compatible isolates of *E. turcicum* races 1 and 23N.

MATERIALS AND METHODS

Isolation and preparation of single-conidium cultures. Two isolates of race

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1 of *E. turcicum* (SF-R1 and ES1) were obtained from NLB-infected corn leaves collected in 1990 from Urbana, Illinois, and Belle Glade, Florida, respectively. Two isolates of race 23N (ES41 and RH18-10) were obtained from NLB-infected leaves collected in 1990 from Belle Glade. Leaf lesions were cut in small pieces and surface-sterilized for 1 min in 0.5% sodium hypochlorite, rinsed in sterilized water, and placed on lactose-casein hydrolysate agar (LCA) (28). After 12–15 days, conidia were gently removed with a transfer loop and transferred to new plates. Ten single-conidium isolates were cultured from each of the four original isolates. Prior to mating, isolates were tested in the greenhouse for their reactions on two sets of maize differentials: B37, B37*Ht1*, B37*Ht2*, B37*Ht3*, B37*HtN*, A619, A619*Ht1*, A619*Ht2*, and A619*Ht3*. Conidia were transferred to LCA and placed under fluorescent light at room temperature (25 C). After 10 days, sporulating cultures were flooded with water and conidia were dislodged with a rubber policeman. Suspensions of 3,000–4,000 conidia per milliliter were prepared for each isolate. Corn seedlings at the four- to six-leaf stage were inoculated by placing 2–3 ml of the conidial suspension into whorls. Greenhouse conditions are described later. Fourteen days after inoculation, plants were evaluated for resistant or susceptible reactions based on the production of necrotic lesions on lines without an *Ht* gene; chlorotic or necrotic reactions on lines with *Ht1, Ht2*, and *Ht3*; and resistant or susceptible lesions on lines with *HtN*.

Crosses in culture. Barley seed and johnsongrass stalks were used as substrates for matings of isolates. Johnsongrass stalks were collected from plants that had flowered. Stalks were cut in pieces about 2–4 cm long, cut in half longitudinally, and autoclaved for 10 min.

Two pieces of autoclaved johnsongrass stalks were half immersed in molten Sach's agar about 1 cm apart in petri plates. After the agar cooled, a plug (0.5 mm in diameter) of a 10-day-old conidial isolate of race 1 was placed on the surface of one johnsongrass stem and a plug of a 10-day-old conidial isolate of race 23N was placed on the other stem. Single-conidium isolates of race 1 and race 23N were crossed in all combinations. Cultures were observed 10–15 days later for the presence of pseudothecia. Cultures containing pseudothecia were placed in a dark incubator at 25 C for 21–25 days. The same procedures were used to cross isolates of race 1 and race 23N with tester isolates of race 0 mating types *A* and *a* obtained from W. L. Pedersen (University of Illinois, Urbana) (22).

Individual ascospores were isolated. Sterilized microscope slides were put in sterilized petri plates. Ascospore suspensions were prepared by crushing pseudothecia in drops of sterilized water placed on microscope slides (19). Loops of the suspension were streaked on water agar plates. Cultures from single ascospores were isolated by taking small sections of water agar containing single ascospores and placing them on LCA, where the conidial stage formed. A total of 180 single-ascospore cultures were obtained from crosses of races 1 and 23N. Ascospore isolates were maintained under the same conditions as the parental isolates. Similar procedures were followed when matings were done on barley seed.

Maize differentials. Approximately 15 seeds each from eight ears of several maize differentials (A619, A619*Ht1*, A619*Ht2*, A619*Ht3*, A632*HtN*, B14*HtN*, B37, B37*Ht1A*, B37*Ht1B*, B37*Ht2*, B37*Ht3*, B37*HtN*, B68*HtN*, Oh43, Oh43*Ht1A*, Oh43*Ht1B*, Oh43*Ht2*, Oh43*Ht3*, Pa91, Pa91*Ht1*, Pa91*Ht2*, Pa91*Ht3*, Va26, Va26*Ht1*, Va26*Ht2*, and Va26*Ht3*) were planted ear-to-row at the Agronomy/Plant Pathology South Farm, Champaign, Illinois. Plants at the five- to seven-leaf stage were inoculated with a conidial suspension of race 0 of *E. turcicum*. Plants with susceptible lesions (i.e., necrotic lesions) were rogued except for lines that did not carry an *Ht* gene. Plants with resistant reactions were self-pollinated. Seed used in greenhouse assays were produced on resistant plants from rows that did not segregate for reactions to race 0.

Greenhouse evaluation of single-ascospore isolates. Isolates derived from single-ascospore progeny were evaluated twice on a set of differentials. Flats containing five maize differentials (*ht, Ht1, Ht2, Ht3*, and *HtN*) were inoculated with a conidial suspension of approximately 4,000 conidia per milliliter. Each flat contained five rows equally spaced. Each row had eight plants with a different *Ht* gene or the susceptible check (*ht*). Each row consisted of four plants each of two different inbred lines (e.g., four plants each of A619*Ht1* and B37*Ht1*) so that reactions of isolates could be observed on at least two different seed sources of the *Ht* genes. Eight ascospore isolates and the two parental isolates were evaluated in each trial by inoculating 10 flats, each flat with an individual isolate. Inoculated plants were maintained under greenhouse conditions at about 22/18 C with a light regime of 12-hr day and night. Resistant and susceptible reactions were assessed 2 wk after inoculation as described previously. Isolates representing new combinations of virulence were evaluated a third time in greenhouse trials. Isolates of particular interest, such as races 123N and 123, also were tested on plants grown in growth chambers at 22/18 C and 320 $\mu E \cdot m^{-2} \cdot s^{-1}$ (14). Some of the isolates representing new combinations of virulence also were sent to D. R. Smith (DeKalb Plant Genetics, DeKalb, IL) and M. L. Carson (USDA-ARS, North Carolina State University, Raleigh) for further evaluations. Lesions of isolates representing new combinations of virulence were collected and stored.

Field evaluations of selected isolates. Seven isolates with new combinations of virulence were evaluated in the summer of 1993 on plants grown in an isolated field. Seed of corn inbreds B37, B37*Ht1*, B37*Ht2*, B37*Ht3*, and B37*HtN* were grown in isolation. Plants were inoculated as previously described at the six- to eight-leaf stage and inspected daily for lesion development so that infected plants could be immediately destroyed by burning if isolates exhibited new combinations of virulence. Each differential also was inoculated with a conidial suspension of race 0. Reactions were recorded 12–14 days after inoculation as previously described and recorded weekly until plants reached harvest maturity.

Data analysis. Segregation ratios among progeny from crosses between races 1 and 23N were analyzed by chi-square to test for independence of reactions to pairs of *Ht* genes. The analysis was based on the production of resistant (R) or susceptible (S) reactions of isolates on hosts with *Ht* genes. Segregation ratios were based on the number of isolates that produced similar or dissimilar reactions on hosts with two different resistance genes. For example, isolates that produced similar reactions on *Ht1* and *Ht2* (i.e., resistant-resistant [R1R2] or susceptible-susceptible [S1S2]) were compared with those that produced dissimilar reactions (i.e., resistant-susceptible [R1S2] or susceptible-resistant [S1R2]) on *Ht1* and *Ht2*.

RESULTS

The 10 single-conidium isolates designated race 1 produced susceptible, necrotic lesions on B37, B37*Ht1*, A619, and A619*Ht1*. These isolates produced chlorotic lesions on B37*Ht2*, B37*Ht3*, A619*Ht2*, and A619*Ht3* and a resistant fleck on B37*HtN*. The 10 isolates designated race 23N produced susceptible lesions on B37, B37*Ht2*, B37*Ht3*, B37*HtN*, A619, A619*Ht2*, and A619*Ht3*.

Pseudothecia production was greatest on johnsongrass for all crosses. A few mature pseudothecia formed on exposed surfaces of the substrate after 21–25 days, but the majority were on substrate that was immersed in Sach's agar (Fig. 1A). Production of pseudothecia was inconsistent and varied within and among plates. Only a few pseudothecia formed on barley seed, and pseudothecia were more difficult to remove from barley seed than from johnsongrass stems. Only a few pseudothecia formed when isolates of races 1 and 23N were crossed to the mating-type testers. Isolates of race 1 were of mating type *A* and isolates of race 23N were of mating type *a* based

on the limited number of pseudothecia formed. No pseudothecium produced a full complement of eight ascospores in an ascus (Fig. 1B), and many pseudothecia failed to produce ascospores. Of 180 ascospores isolated from crosses of races 1 and 23N, 112 germinated.

Eleven of the 16 possible combinations of virulence were observed in the first trial among the isolates from 112 ascospore progeny, but only eight of these combinations were observed in the second trial. Reactions included those that would be classified as races 0, 1, 2, 12, 13, 1N, 23, 23N, 12N, 123, and 123N (Table 1). Combinations of virulence constituting races 2, 12, 13, 1N, 12N, 123, and 123N have not been reported previously from North America. Isolates that would be classified as races 3, N, 2N, 3N, and 13N were not recovered.

Reactions of 72 of the 112 isolates differed between the two trials. In the first trial, races 1 and 123 were most frequent; races 23, 23N, and 12 were common; and races 12N, 1N, and 13 were relatively rare (Table 1). In the second trial, races 123, 23N, and 23 were most frequent. About 30% of the progeny in both trials had the same reactions of the parental isolates, 1 and 23N.

Segregation among ascospore progeny for avirulent and virulent reactions on hosts with the four *Ht* genes was different from the expected 1:1 ratio except on *Ht1* in trial 2 and *Ht3* in trial 1 (Table 2). Virulent reactions were greater than expected on *Ht2* in both trials, on *Ht3* in trial 2, and on *Ht1* in trial 1. In both trials, avirulent reactions were greater than expected on *HtN*.

In each trial, segregation for similar or dissimilar reactions fit the hypothesized 1:1 ratio for four of the six pairwise combinations of *Ht* genes: *Ht1-Ht2, Ht1-Ht3, Ht2-HtN*, and *Ht3-HtN* (Table 3). Segregation of reactions deviated significantly from a 1:1 ratio for the combination of *Ht1* and *HtN* and for the combination of *Ht2* and *Ht3*. Reactions on hosts with *Ht2* or *Ht3* were similar for 85 and 104 of the 112 isolates in trials 1 and 2, respectively (Table 3). Reactions on hosts with *Ht1* or *HtN* were dissimilar for 72 and 87 of the 112 isolates in trials 1 and 2, respectively (Table 3).

The amount of necrosis and chlorosis varied among the greenhouse trials. Light intensity of about 325 $\mu E \cdot m^{-2} \cdot s^{-1}$ in the growth chamber produced more distinct symptoms than in the greenhouse. On differentials A619*Ht2* and A619*Ht3*, symptoms sometimes were similar in appearance to those produced by the gene *HtN*, with flecking that became necrotic.

Only four of seven isolates evaluated in the field produced susceptible reactions on B37*Ht1* and none produced susceptible reactions on B37*Ht2*, B37*Ht3*, or B37*HtN* (Table 4). These isolates would be classified as races 0 or 1 based on field reactions, whereas greenhouse assays would have identified them as races with new combinations of virulence.

DISCUSSION

The results of our study were inconsistent with respect to reactions of individual isolates but indicated that virulence of *E. turcicum* against *Ht* gene resistance in maize evaluated in the greenhouse was not independent and that field reactions of isolates differed considerably from greenhouse reactions. Previously, Lim et al (18) reported 1:1 segregation for virulence against the gene *Ht1* among 92 ascospore progeny from crosses of *E. turcicum* races 0 and 1. They concluded that virulence against *Ht1* was controlled by a single gene in *E. turcicum*. Our results from trial 2 corroborate those of Lim et al (18). Although the segregation ratio of resistant and susceptible reactions on *Ht1* in trial 1 was slightly different from 1:1, we agree with Lim et al (18) that virulence against *Ht1* is conditioned by a single gene in *E. turcicum*. If virulence in *E. turcicum* against all *Ht* genes for resistance in maize is inherited as single independent genes, crosses of races 1 and 23N should produce progeny with 16 combinations of virulence in equal frequency (Table 5). Only 11 of the 16 possible combinations were observed in our greenhouse evaluations, and 70% of the 112 isolates were classified in one of five races: 1, 23, 23N, 123, or 123N. Furthermore, seven isolates representing new combinations of virulence based on greenhouse and growth chamber trials were classified as races 0 or 1 in field trials.

In our study, reactions on plants with *Ht2* and *Ht3* and reactions on plants with *Ht1* and *HtN* were not independent. Reactions of progeny from crosses of races 1 and 23N segregated differently from the expected 1:1 ratio of similar:dissimilar reactions on plants with the genes *Ht2* and *Ht3* and plants with *Ht1* and *HtN*. These associations could have resulted from linkage of virulence genes in *E. turcicum*. Virulence against the genes *Ht1* and *HtN* would be in the repulsion phase for crosses of races 1 and 23N, and 65 and 78% of the progeny in trials

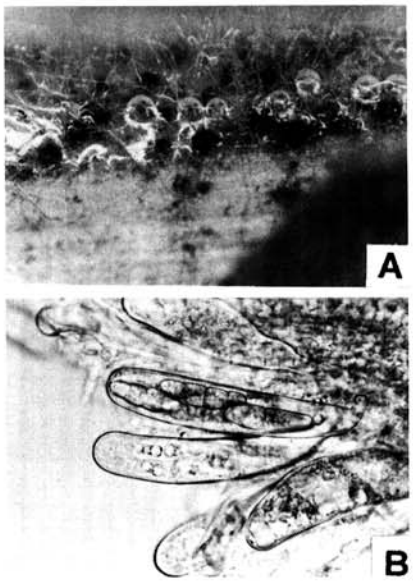

Fig. 1. Formation of pseudothecia of *Setosphaeria turcica* from matings of races 1 and 23N of *Exserohilum turcicum*: **(A)** Mature pseudothecia on johnsongrass stems immersed in Sach's agar and **(B)** uneven numbers of ascospores in asci.

Table 1. Reactions of isolates from 112 ascospore progeny of crosses between races 1 and 23N of *Exserohilum turcicum* on a set of host differentials in two greenhouse assays

	Number of progeny recovered	
Race[a]	Trial 1	Trial 2
0	4	0
1	23	4
2	8	3
3	0	0
N	0	0
12	13	4
13	4	1
1N	1	0
23	14	15
2N	0	0
3N	0	0
23N	10	32
12N	2	0
13N	0	0
123	22	46
123N	11	7

[a] Races based on *Ht* genes for which susceptible reactions were observed.

Table 2. Chi-square analysis of resistant and susceptible reactions among 112 conidial isolates from ascospore progeny of crosses of races 1 and 23N of *Exserohilum turcicum*

	Trial 1			Trial 2		
Host gene	Observed ratio[a]	Chi-square[b]	*P*[c]	Observed ratio	Chi-square	*P*
Ht1	36:76	14.29	<0.001	50:62	1.29	0.3–0.2
Ht2	32:80	20.57	<0.001	5:107	132.89	<0.001
Ht3	51:61	0.89	0.5–0.3	11:101	72.32	<0.001
HtN	88:24	36.57	<0.001	73:39	10.32	0.01–0.001

[a] Observed ratio of resistant:susceptible reactions on hosts with *Ht* genes.
[b] Chi-square values for observed ratios with expected ratios of 56:56.
[c] Probability of exceeding the chi-square value when the hypothesis of single-gene assortment is true.

Table 3. Chi-square analysis of segregation for similar and dissimilar (resistant and susceptible) reactions among 112 conidial isolates from ascospore progeny of crosses of races 1 and 23N of *Exserohilum turcicum*

Combination of *Ht* genes	Comparison of similar and dissimilar reactions[a]	Trial 1			Trial 2		
		Observed ratio[b]	Chi-square[c]	*P*[d]	Observed ratio	Chi-square	*P*
Ht1-Ht2	R1R2 + S1S2 vs. R1S2 + S1R2	52:60	0.57	0.5–0.3	57:55	0.36	0.7–0.5
Ht1-Ht3	R1R3 + S1S3 vs. R1S3 + S1R3	49:63	1.75	0.2–0.1	57:55	0.36	0.7–0.5
Ht1-HtN	R1RN + S1SN vs. R1SN + S1RN	40:72	9.14	0.01–0.001	25:87	34.32	<0.001
Ht2-Ht3	R2R3 + S2S3 vs. R2S3 + S2R3	85:27	30.04	<0.001	104:8	82.29	<0.001
Ht2-HtN	R2RN + S2SN vs. R2SN + S2RN	54:58	0.14	0.9–0.7	44:68	5.14	0.5–0.2
Ht3-HtN	R3RN + S3SN vs. R3SN + S3RN	69:43	6.04	0.05–0.01	50:62	1.29	0.3–0.2

[a] Resistant (R) and susceptible (S) reactions on differentials with *Ht* genes, e.g., R1R2 = resistant reactions of hosts with *Ht1* and *Ht2*, R1S2 = resistant reaction of host with *Ht1* and susceptible reaction of host with *Ht2*.
[b] If virulence of *E. turcicum* is monogenic and independent, expected ratios of similar (RR, SS) and dissimilar (RS, SR) reactions would be 56:56 for each combination of *Ht* genes.
[c] Chi-square values for observed ratios with expected ratios of 56:56.
[d] Probability of exceeding the chi-square value when the hypothesis of independent assortment is true.

Table 4. Reactions of selected isolates of *Exserohilum turcicum* on a set of maize differentials grown in an isolated field

Isolate	Greenhouse reactions				Reactions on maize differentials in the field				
	Ht1	*Ht2*	*Ht3*	*HtN*	B37	B37*Ht1*	B37*Ht2*	B37*Ht3*	B37*HtN*
27	1/4[a]	3/4	2/4	3/4	S[b]	S	R	R	R
62	1/3	2/3	1/3	3/3	S	R	R	R	R
67	1/3	1/3	1/3	3/3	S	R	R	R	R
84	3/3	3/3	3/3	3/3	S	S	R	R	R
94	3/3	3/3	3/3	3/3	S	S	R	R	R
100	3/3	3/3	3/3	0/3	S	S	R	R	R
133	3/3	3/3	3/3	3/3	S	NR[c]	R	R	R

[a] Number of susceptible reactions/number of trials.
[b] S = susceptible (necrotic reaction), R = resistant (chlorotic reaction), except *HtN*.
[c] No reaction.

Table 5. Sixteen possible combinations of virulence in *Exserohilum turcicum* for four host genes (*Ht1*, *Ht2*, *Ht3*, and *HtN*) that condition resistance to northern leaf blight

No. of host genes on which isolates are virulent	Races[a]
0	*0*
1	*1* *2* 3 N
2	*12* *13* *1N* *23* 2N 3N
3	*123* *12N* 13N *23N*
4	*123N*

[a] Combinations of virulence in italics were observed at least once among the 112 progeny from crosses of races 1 and 23N.

1 and 2, respectively, had a different reaction on hosts carrying the gene *Ht1* from that on plants with *HtN*. Conversely, virulence against the genes *Ht2* and *Ht3* would be in the coupling phase for crosses of races 1 and 23N, and 85 and 93% of the progeny in trials 1 and 2, respectively, had similar reactions on hosts with the gene *Ht2* or *Ht3*. Another explanation for these associations of reactions is that host resistance genes conditioned the same or similar mechanisms of resistance. There is some evidence that *Ht3*, which was introgressed into maize from *Tripsacum floridanum* (7), is a *Tripsacum* homolog of *Ht2*, which is on the long arm of maize chromosome 8 (K. D. Simcox, *personal communication*). If *Ht2* and *Ht3* are homologs, all isolates should have similar reactions on these genes and the 7–15% of isolates with dissimilar reactions would result from the variability of greenhouse assays. Also, we cannot exclude the possibility that variation in temperature and light intensity may have similar effects on reactions on *Ht2* and *Ht3*, as suggested by Leath et al (14), but dissimilar effects on *Ht1* and *HtN*.

Although associations between *Ht1* and *HtN* and between *Ht2* and *Ht3* were consistent between the two trials, many isolates had different reactions in the trials. Also, many of the new combinations of virulence were not consistent between trials. Similarly, none of the new combinations of virulence were confirmed on plants grown in isolation in the field. We could not attribute the inconsistency of these results to a single factor, although we suspect that variability of reactions on *Ht2, Ht3*, and *HtN* is the cause of much of the problem. Evidence that different light intensities and temperatures can cause different symptoms on differentials carrying *Ht* genes has been observed by others (14) and may have been the major cause of variation among individual isolates in our trials even though we attempted to control these conditions. Conversely, in these and previous trials (21), the parental isolates were consistent in reaction. The technique used to obtain single-ascospore isolates also could have resulted in variable reactions caused by cultures with a mixture of virulence. Likewise, the lack of a full complement of eight ascospores per asci and pooling of progeny from crosses of different parental strains of races 1 and 23N may have resulted in deviations from expected 1:1 ratios of virulent:avirulent reactions. Nevertheless, none of these explanations accounts for the complete lack of virulence on *Ht2, Ht3*, and *HtN* among the isolates tested in the field. Possibly, virulence against these three genes is expressed more easily under greenhouse conditions than in the field, and therefore reactions from greenhouse trials must be interpreted cautiously. If greenhouse evaluations are more variable than field reactions, virulence against these genes under field conditions may be less prevalent than previously believed (21,25–27, 30).

Mating type frequency among isolates of *E. turcicum* in different geographic areas has been investigated previously (19,20,22), and genetic recombination can be expected when compatible mating types are present. New combinations of *E. turcicum* have been discovered (4,22,26, 27,29–31) from several areas where maize is grown, even though the sexual state has not been observed. The formation of psuedothecia in culture on grass hosts other than maize (4,19,20,24) and the ability of the fungus to infect and overwinter on some of these hosts (4,17) afford the possibility of sexual recombination occurring naturally on hosts other than maize. Other hosts also may

be exerting selection pressure that has resulted in the rather unexpectedly high frequency of races 23 and 23N (25,27) for which little selection pressure has been exerted in maize. The gene *Ht3*, originally transferred to *Z. mays* by an interspecific cross with *T. floridanum* (7), and possibly the resistance conveyed by the gene *Ht2* (if, in fact, *Ht2* and *Ht3* are homologs) may exist in other species that are hosts for *E. turcicum*.

The importance of the sexual stage, *Setosphaeria turcica*, in the epidemiology of northern leaf blight appears primarily to be the ability of the pathogen to genetically recombine, resulting in new combinations of virulence and other traits. The inability to detect, in nature, some of the races observed in this study (e.g., races 2, 13, 1N, 12N, 123, and 123N) may result from a lack of selection pressure placed on the pathogen population due to the infrequent use of the genes *Ht2, Ht3*, and *HtN*. Nevertheless, undetected genetic diversity within populations of *E. turcicum* and/or new combinations of virulence resulting from crosses may limit the successful deployment of new combinations of resistance genes. The gene *HtN* has been favored recently by sweet corn breeders (23) and is being deployed on a limited basis in combination with the gene *Ht1* in *sh2* sweet corn hybrids grown primarily in Florida. If the recombination of virulence that we observed in the greenhouse portion of this study occurs in nature, and virulence is expressed under field conditions (which we did not observe), the deployment of the combination of *Ht1* and *HtN* in Florida should increase the frequency of race 1N to detectable levels. If, however, our observations from field evaluations are indicative of normal field situations, the genes *Ht2, Ht3*, and *HtN* may have a more prolonged usefulness than would be expected from the results of our greenhouse trials. Thus, a reasonable strategy of control is to deploy *Ht2, Ht3*, and *HtN* in areas where NLB is severe (e.g., Florida), with the likelihood of selecting for virulence in the next decade and, concurrently, improving the levels of partial resistance to *E. turcicum* in sweet corn hybrids grown in areas where populations of *E. turcicum* are diverse for virulence.

The difficulties we encountered in crossing isolates of *E. turcicum* and the inconsistent reactions of individual isolates in greenhouse and field trials are further evidence that this pathosystem is not ideally suited for studying the genetics of host:pathogen interactions. Reports of new combinations of virulence, such as those observed in our greenhouse trials, may be limited to the conditions under which the trials were conducted. Further studies are needed to corroborate reactions among greenhouse and field situations.

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