

Mating Types, Virulence, and Cultural Characteristics of *Exserohilum turcicum* Race 2

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ABSTRACT

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Sixty-five isolates of *Exserohilum turcicum* race 2 were obtained from 13 locations in the U.S. corn belt. Twenty-seven isolates mated with the *A* mating type, but 38 isolates failed to mate with either *A* or *a* mating types. In the greenhouse, isolates from Florida, Pennsylvania, and West Virginia produced significantly ($P=0.05$) longer lesions on the corn hybrid A632×A619 than isolates from Iowa, Illinois, and Indiana. There were no differences among isolates for incubation period. Isolates from Delaware, Florida, Pennsylvania, and West Virginia had significantly larger radial growth on lactose-casein hydrolysate agar after 10 days at 20 C than isolates from Iowa, Illinois, and Indiana. Conversely, isolates from Champaign and Iroquois counties in Illinois and La Grange County in Indiana had significantly larger radial growth diameters at 28 than at 20 C.

Additional key words: host-parasite interaction, northern leaf blight, parasitic fitness, *Setosphaeria turcica*

Race 2 of *Exserohilum turcicum* (Pass.) Leonard & Suggs (teleomorph: *Setosphaeria turcica* (Luttrell) Leonard & Suggs) was first reported in Hawaii in 1974 (1). In 1979, race 2 was identified near Brook, IN (14), and from several locations in Ohio in 1980 (6). Jordan et al (3) confirmed race 2 was present in Florida, Iowa, Illinois, Indiana, Minnesota, New York, and Pennsylvania in 1982. When determining the races of isolates from the seven states, differences

in lesion lengths on B37 were observed (W. L. Pedersen and J. M. Perkins, *unpublished*). Field evaluation of corn germ plasm for resistance to *E. turcicum* race 2 identified inbreds that were resistant (chlorotic-type lesions) in Iowa and Illinois but susceptible in Pennsylvania, whereas other inbreds were susceptible in Iowa and Illinois but resistant in Pennsylvania (10). Because the chlorotic-type lesion resistance was effective against isolates of *E. turcicum* from various hosts around the world (2), the differences among locations may have been due to differences among isolates of *E. turcicum*.

Northern corn leaf blight (NCLB), caused by *E. turcicum*, is generally favored by moderate temperatures and heavy dews during the growing season (11). Leath (4) observed disease severities

of 58% on a susceptible corn hybrid in 1983 at Urbana, IL, despite high temperatures (average daily maximum temperature for July was 33 C) and an extreme drought (<1 cm of rainfall during July). Therefore, isolates from varying locations appear to differ in their ability to grow at higher temperatures.

The objectives of this study were to determine the mating type, virulence characteristics (incubation period and lesion length), and growth on lactose-casein hydrolysate agar (LCH) (13) at 20 and 28 C of isolates of *E. turcicum* race 2 from 13 locations in the U.S. corn belt.

MATERIALS AND METHODS

Leaves from corn plants carrying the *Ht1* gene for resistance and infected with *E. turcicum* were obtained from counties in Delaware, Florida, Iowa, Illinois, Indiana, Pennsylvania, and West Virginia (Table 1). All samples were collected in 1982, except one sample from Newton County, Indiana, was collected in 1979. Leaf tissue was dried and stored at 5 C. Leaf lesions were surface-disinfested with 0.5% NaOCl for 1 min, rinsed in distilled water for 5 min, and incubated in glass petri dishes (9 cm in diameter) lined with moist filter paper for 72 hr at 20 C. Five conidia from each sample, each from a different lesion, were transferred to individual plates of LCH and incubated at 20 C for 14 days. These plates were used as parental cultures for determining the race, mating type, incubation period, lesion length, and radial growth on LCH. Race determinations were done as

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described previously (3), using B37, B37*Ht1*, B37*Ht2*, and Oh43*Ht3* as differential cultivars. Resistance and susceptibility were based on the presence or absence of chlorotic-type lesions. Each isolate was tested for mating type by crossing with both *A* and *a* mating types (obtained from K. J. Leonard, Raleigh, NC) using techniques described by Luttrell (7). Dry, sterile barley straw with the leaf sheath attached was embedded in Sach's agar (13) at 45 C, and the agar was allowed to solidify. Crossing involved transferring plugs (0.5 mm) of LCH agar from the parental cultures of each isolate and either of the isolates designated as *A* or *a* mating type to opposite sides of the barley straw. All cultures were mated with both mating types. Control matings between known *A* and *a* mating types also were done. The plates were incubated at 25 C for 21 days, and the presence of perithecia on the barley straw was considered a compatible cross.

Incubation period and lesion length on the corn hybrid A632 × A619 were determined using the techniques described by Leath and Pedersen (5). A 10- μ l drop of a conidial suspension (38,000–45,000 conidia per milliliter) was placed on the adaxial surface of the third leaf of a 35-day-old corn seedling and allowed to air-dry. The plants were placed in a mist chamber at 100% relative humidity for 12 hr, then moved to the greenhouse (22 ± 3 C) for 21 days. A total of 12 lesions per isolate were measured (one lesion per plant, six plants per replicate, and two replicates).

Agar plugs (0.05 cm in diameter) obtained from the margins of the parent colonies were transferred to the centers of six LCH plates, and three plates per isolate were incubated at both 20 and 28 C. Diameters of colonies were measured after 10 days, and the experiment was repeated twice.

Data from the incubation period, lesion length, and radial growth studies were analyzed using an analysis of variance for a randomized complete block design. Comparisons among and within locations and isolates were made with Fisher's least significant differences ($P = 0.05$ (12)).

RESULTS

All isolates of *E. turcicum* were identified as race 2; B37 and B37*Ht1* were susceptible and B37*Ht2* and Oh43*Ht3* were resistant (chlorotic-type lesions). All isolates were of the *a* mating type or they did not mate with either mating type (Table 1). All control matings between *A* and *a* mating types produced perithecia.

Isolates (five monoconidial isolates per location) and repetition terms from the analysis of variance for incubation period, lesion length, and radial growth were not significant ($P = 0.05$), so they were combined into replicates ($n = 30$). Incubation period ranged from 8 to 10 days, but no significant differences were

observed among isolates. Isolates from Florida, Pennsylvania, and West Virginia produced significantly longer lesions (5.2–8.5 cm) than isolates from Iowa, Illinois, and Indiana (2.5–4.0 cm). The isolates from Delaware were intermediate in lesion length (4.5 cm).

Radial growth after 10 days was significantly greater at 20 than at 28 C for isolates from New Castle County in Delaware, Centre and Franklin counties in Pennsylvania, and Jackson County in West Virginia (Table 1). Colonies of isolates from Delaware, Florida, Pennsylvania, and West Virginia (except Lancaster County, Pennsylvania) were significantly larger at 20 C than isolates from all locations in Iowa, Illinois, and Indiana. Isolates from three locations, Iroquois and Champaign counties in Illinois and La Grange County in Indiana, had significantly larger radial growth diameters at 28 than at 20 C.

DISCUSSION

All isolates evaluated in this study were identified as race 2. This was expected because lesions were obtained from corn plants with the *Ht1* gene for resistance to *E. turcicum*, and neither race 1 nor race 3 produces susceptible lesions on corn plants with the *Ht1* gene. The absence of *A* mating types from the 65 isolates tested in this study indicates the potential for sexual recombination with *E. turcicum* is relatively low. The lack of successful matings with either mating type may be due to different physiological requirements for crossing or perithecial production, incompatibility factors (similar to those found in *Cochliobolus carbonum* [9]), or the mating type of *Setosphaeria turcica* may not be a simple bipolar type (7).

Leath and Pedersen (5) used a microdrop inoculation technique to detect the *HtN* resistance gene in several

corn inbreds. In this study, the technique was used to compare components of parasitic fitness (8) (incubation period and lesion length on a susceptible hybrid) of several isolates of *E. turcicum* race 2. The isolates from Florida, Pennsylvania, and West Virginia produced significantly longer lesions on the hybrid A632 × A619 than isolates from Iowa, Illinois, and Indiana. These results suggest that if lesion length on greenhouse-grown plants is positively correlated with greater parasitic fitness (8) in the field, the incidence and severity of NCLB should be higher in Florida, Pennsylvania, and West Virginia than in Iowa, Illinois, and Indiana. However, the isolates from Florida, Pennsylvania, and West Virginia were obtained from fields or research plots with high levels of disease, whereas the isolates from Delaware, Iowa, Illinois, and Indiana were from hybrid seed production fields or research plots with relatively low levels of disease. Therefore, the source of the samples may have resulted in the selection of isolates from Florida, Pennsylvania, and West Virginia that produce longer lesions.

E. turcicum is generally favored by moderate temperatures (17–27 C) and heavy dews during the growing season (11). However, inoculation with an isolate from Champaign County, Illinois, resulted in final disease severity of 58% at Urbana in 1983, a very hot and dry year (4). When evaluated for growth on LCH, isolates from one location each in Champaign and Iroquois counties in Illinois and La Grange County in Indiana had significantly ($P = 0.05$) larger radial growth at 28 than at 20 C. Conversely, isolates from New Castle County in Delaware, Centre and Franklin counties in Pennsylvania, and Jackson County in West Virginia produced significantly larger radial growth diameters at 20 than

Table 1. Source, mating type, lesion length, and radial growth on lactose-casein hydrolysate agar at 20 and 28 C for isolates of *Exserohilum turcicum* race 2 from 13 locations in the U.S. corn belt

Source (county and state)	Mating type	Lesion length (mm)	Radial growth (mm)	
			20 C	28 C
New Castle, DE	<i>a</i> (2) ^a	45.0	85.0	59.0* ^b
Indian River, FL	<i>a</i> (1)	80.0	76.0	65.0
Marshall, IA	<i>a</i> (5)	25.0	38.0	42.0
Champaign, IL	<i>a</i> (5)	25.0	46.0	66.0*
Champaign, IL	...	33.0	25.0	36.0
Iroquois, IL	<i>a</i> (3)	35.0	33.0	48.2
La Grange, IN	...	40.0	46.0	74.0*
Newton, IN	<i>a</i> (4)	35.0	39.0	43.0
Centre, PA	...	53.0	85.0	59.0*
Franklin, PA	<i>a</i> (1)	52.0	68.5	49.0*
Lancaster, PA	...	55.0	45.0	49.0
Jackson, WV	<i>a</i> (2)	66.0	85.0	60.0*
Jefferson, WV	<i>a</i> (4)	55.0	60.0	56.0
LSD (0.05) ^d		1.1	13.3	13.3
C.V. (%)		14.6	19.6	19.6

^a Number in parentheses indicates the number of monoconidial isolates of five tested from each sample location that produced perithecia when mated with *A* mating type of *E. turcicum*.

^b * = Significantly different from radial growth at 20 C based on LSD for comparing isolates at different temperatures; LSD (0.05) = 15.7.

^c Indicates no isolates mated with either *A* or *a* mating types.

^d LSD (0.05) for comparing isolates at the same temperature.

at 28 C. Similar to lesion length, radial growth diameters at 20 C of isolates from all counties in Florida, Pennsylvania, and West Virginia (except Lancaster County in Pennsylvania) were significantly larger than isolates from all counties in Iowa, Illinois, and Indiana. Although radial growth on a culture medium might not be related to the ability of a pathogen to cause disease in the field, the isolates from the northeastern United States had larger radial growth diameters at 20 C and produced longer lesions in the greenhouse than isolates from Iowa, Illinois, and Indiana.

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