Variation in Fitness Among Field Isolates of *Exserohilum turcicum* in Israel

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ABSTRACT

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Isolates of race 1 of *Exserohilum turcicum* collected from 15 fields in Israel varied considerably in three components of parasitic fitness when tested on the susceptible sweet corn hybrid cultivar Jubilee grown in growth chambers maintained at 20 C. Infection efficiency (percentage of sites inoculated with 10-μl droplets containing an average of 50 conidia each that developed lesions) ranged from 17 to 95%. Lesion size and sporulation 10 days after inoculation ranged from 0.3 to 9.3 cm^2 and from 833 to 14,000 spores per square centimeter, respectively. These two variables were significantly correlated ($r = 0.45$). Isolates did not differ significantly in spore germination. Variation among isolates from different locations was significantly greater than that among isolates from the same location.

Northern leaf blight of corn (*Zea mays* L.), incited by the fungus *Exserohilum turcicum* (Pass.) Leonard & Suggs, is prevalent in all regions of Israel where sweet corn is grown. Epidemics occur mostly from August through October. Investigations of the relationship between disease development and environmental conditions (5,8) found that moderate temperatures (20–25 C) and high relative humidity (90–100%) are essential for disease development. In many cases, however, incidence of northern leaf blight was low when environmental conditions were optimal, and severe epidemics sometimes occurred under suboptimal environmental conditions (7). Levy (7) recently reported the importance of the pathogen's parasitic fitness in determining the severity of epidemics caused by *E. turcicum*. The influence of weather conditions on disease severity decreases when fields are infested with a highly aggressive population of the pathogen (7). The objective of the present study was to survey the variation in infection efficiency, sporulation, germination, and lesion size among isolates of *E. turcicum* collected from various locations in Israel.

MATERIALS AND METHODS

Sweet corn cultivar Jubilee was used in all experiments. Jubilee was chosen because it is grown throughout Israel and is a susceptible genotype with a relatively short incubation period (8). Plants were grown in a greenhouse in 1-L plastic pots (two plants per pot) containing a mixture of soil, peat, and vermiculite (1:1:1, v/v). Five-week-old plants with five to six true leaves were inoculated.

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Twenty-five single-lesion isolates of *E. turcicum* were collected from each of 15 locations in Israel, for a total of 375 isolates (Table 1). A detailed map showing the locations is given by Abadi et al (1). Isolates were cultured on lactose-casein agar and maintained on potato-dextrose agar slants at 4 C. Isolates among and within locations were evaluated for variation in infection efficiency, lesion size, sporulation, and germination.

Conidial suspensions were prepared by washing the conidia from 10-day-old cultures that had been grown in an incubator at 25 C. Conidia were counted with a hemacytometer, and the suspension was adjusted to the desired concentration. Droplets (10 μl) of conidial suspension (50 conidia per droplet) were placed on the adaxial surface of the fourth and fifth leaves of plants (10 droplets per leaf). Inoculated plants were incubated for 14 hr in a mist chamber at 20 C in the dark and then transferred to growth chambers calibrated to 20 C with a light period of 12 hr/day at an intensity of 150 $\mu E \cdot m^{-2} \cdot s^{-1}$.

The latent period of *E. turcicum* depends on host susceptibility, temperature, dew period, and inoculation density. At high inoculation density, when temperature and dew period are optimal, 80% of the lesions appear 7 days after inoculation (6). Therefore, 10 days after inoculation, the number of lesions produced was counted, and lesion area was measured with a planimeter (Delta-t area meter system, Delta-t Device Ltd., Cambridge, England). Infection efficiency was calculated as the number of lesions formed as a percentage of the total number of inoculation sites.

Infection efficiency and lesion area experiments were conducted three times, each with at least 10 replications (10 plants for each isolate). Thus, in each experiment, 3,750 plants were inoculated (15 locations × 25 isolates × 10 replications). Plants were incubated in four growth chambers (500 pots in each, two plants per pot).

To measure germination, conidial suspensions were prepared by washing conidia from 10-day-old cultures on lactose-casein agar. Concentrations were adjusted to 10,000 conidia per milliliter based on counts made with a hemacytometer. A pipet was used to transfer 5 ml of the conidial suspension into a 9-cm-diam petri dish containing 3% water agar. Plates were incubated in the dark for 20 hr at 25 C, and percentage germination was determined from at least 200 conidia per petri dish. Five petri dishes were tested for each isolate.

For sporulation studies, infected leaves with lesions of known area were placed adaxial surface upward on moist filter paper in plastic trays. Trays were covered with polyethylene bags and incubated for 30 hr in darkness at 20 C. At the end of the sporulation period, spores were removed from individual lesions with a hairbrush, placed into a fixative solution (formaldehyde-acetic acid-ethanol, 90:5:5, v/v), and filtered through an 8-μm Millipore filter. Spores were counted with a microscope. Sporulation was measured for at least 10 lesions for each isolate. Sporulation experiments were conducted three times.

Analysis of variance was performed on each independent variable for isolates within and among locations. The Waller-Duncan k-ratio t test was used for mean separation of isolates when the overall F test was significant. Correlations among lesion size, sporulation capacity, and infection efficiency were calculated.

RESULTS

Locations varied considerably in the distribution of lesion area. Area of lesions caused by individual isolates ranged between 0.3 and 9.3 cm^2 10 days after inoculation. The largest lesions were measured in plants infected by isolates from Biqat haYarden 88 (range 4–9.3 cm^2) (Fig. 1 and Table 2), and the smallest lesions were measured in plants infected by isolates from Yad Mordekhay 86 (range 0.3–1.6 cm^2). Mean lesion area differed significantly among locations but not among isolates within locations. Variation among isolates within locations accounted for less than 1% of the total variance (Table 3).

The number of spores produced per square centimeter of infected leaf area ranged from 833 to 14,000 (Fig. 2).

Lesions caused by isolates from Newe Ya'ar 88 sporulated most profusely (10,333–14,000 spores/cm^2), and those caused by isolates from Yad Mordekhay 87 sporulated least abundantly (833–8,000 spores/cm^2). Variation among isolates within locations accounted for 3.1% of the total variance, while variation among locations accounted for 88.3% of the total variance (Table 3).

Infection efficiency of individual isolates varied from 17 to 95% (Fig. 3). Isolates from Biqat haYarden 88 had the highest infection efficiency (46–93%, mean 74%), and isolates from Yad Mordekhay 86 had the lowest infection efficiency (22–95%, mean 49%) (Table 2 and Fig. 3). Analysis of variance showed that variation among isolates and locations accounted for 16.5 and 24.9% of the total variance, respectively (Table 3).

Populations of *E. turcicum* from the 15 locations divided into eight distinct groups with regard to lesion area and sporulation and into three significant groups with regard to infection efficiency, according to the Waller-Duncan multiple range test analysis (Table 2). The greatest variation was noted in lesion area. The least fit population of *E. turcicum,* isolates collected in Yad Mordekhay in 1986, was characterized by markedly low values for all three test variables. Germinability of spores, however, did not differ significantly among isolates from all locations and ranged between 80 and 90%.

Correlations among infection efficiency, lesion area, and sporulation were significant but relatively low. The highest correlation computed was between lesion size and sporulation ($r = 0.45$, $P = 0.0001$). The lowest correlation was between infection efficiency and sporulation ($r = 0.15$). The correlation coefficient between lesion size and infection efficiency was 0.34. Table 4 shows that correlations among the location components of variance were all greater than 0.75, while correlations among the isolate components of variance were less than 0.15, indicating that location had similar effects on all three fitness criteria. Results of all experiments were reproduced in each of the three runs.

Table 1. Location and date of collection of isolates of *Exserohilum turcicum* used in this study

Location		Local grid coordinates	Collection date
BH88	Biqat haYarden	1908917056	April 1988
BS89	Bet She'an	1907121030	April 1989
BH89	Biqat haYarden	1908917056	April 1989
GB86	Giv'at Brenner	1302514026	September 1986
E88	Erez	1008810078	September 1988
NY88	Newe Ya'ar	1607023051	August 1988
N85	Na'an	1306614046	September 1985
S86	Sa'ad	1006209082	July 1986
NG86	Nir Gallim	1200313071	September 1986
BI88	Bar Ilan	1306516038	August 1988
BI87	Bar Ilan	1306516038	July 1987
GH85	Giv'at Hayyim	1404420004	September 1985
AH87	Ayyelet haShahar	2005026081	October 1987
YM87	Yad Mordekhay	1007511016	August 1987
YM86	Yad Mordekhay	1007511016	August 1986

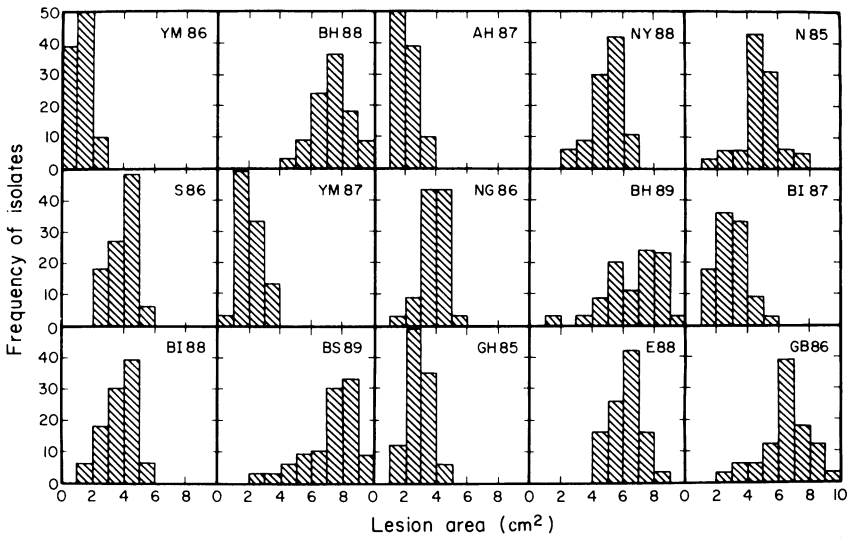

Fig. 1. Distribution of area of lesions on sweet corn cultivar Jubilee plants 10 days after inoculation with 25 isolates of *Exserohilum turcicum* collected at each of 15 locations in Israel. Location designations are given in Table 1.

Table 2. Mean lesion area, sporulation, and infection efficiency of 15 field populations of *Exserohilum turcicum* from Israel

Location	Lesion area[w,x] (cm^2)	Sporulation[x,y] (spores/cm^2)	Infection efficiency[x,z] (%)
BH88	7.0 a	12,000 b	74 a
BS89	6.9 a	12,242 b	74 a
BH89	6.3 b	10,545 c	72 a
GB86	6.1 bc	11,242 bc	69 a
E88	6.0 c	12,091 b	73 a
NY88	4.7 d	13,394 a	59 b
N85	4.4 d	5,121 e	58 b
S86	3.6 e	5,242 f	58 b
NG86	3.5 e	4,121 f	53 bc
BI88	3.3 e	3,909 f	67 a
BI87	2.6 f	3,848 f	70 a
GH85	2.4 f	2,297 gh	58 b
AH87	1.5 g	7,303 d	58 b
YM87	1.5 g	1,797 h	49 c
YM86	0.9 h	2,600 g	49 c

[w] Ten days after inoculation.
[x] Values in a column followed by a common letter do not differ significantly according to the Waller-Duncan k-ratio t test at $P = 0.05$.
[y] Number of spores produced per square centimeter of infected leaf area 10 days after inoculation.
[z] Number of lesions formed, expressed as a percentage of the number of sites inoculated.

DISCUSSION

It is generally believed that some biotypes of a pathogen are more fit than others (3,4). The results obtained in this study show that for *E. turcicum* in Israel, differences among populations from different locations were significantly greater than differences among isolates from the same field population. Populations differed significantly in three features affecting pathogenesis: lesion area, sporulation, and infection efficiency. Although no differences were detected in germinability of spores, isolates varied in the number of lesions produced with a standard inoculum level (infection efficiency), indicating that differences were expressed during appressorium formation, penetration, or the first steps of colonization.

One possible problem in measuring fitness components in a single environment is the possibility of the existence

of ecological races of the pathogen adapted to that specific environment. Levy (7) showed that the optimal temperature for the development of *E. turcicum* from different locations is similar, indicating that variation in fitness components probably does not result from ecological adaptations. Furthermore, the same patterns of variation in sporulation, lesion area, and infection efficiency of isolates within three locations (YM, BH, and BI) were noted over 2 yr, indicating some stability within local populations from year to year.

Table 3. Location and isolate components of variance in fitness characteristics of *Exserohilum turcicum*

Effect	df[a]	SS[b]	Variance component[c]	Percentage of variance
Lesion size				
Total variance	3,743	297,924.29	79.9681	100
Location	14	20,666.96	5.614	7.4
Isolate	360	26,912.36	0.044	0.56
Error	3,369	250,344.96	74.3	92.9
Sporulation				
Total variance	3,743	6.24×10^{10}	1.7×10^{7}	100
Location	14	5.47×10^{10}	1.5×10^{7}	88.3
Isolate	360	2.5×10^{9}	5.5×10^{5}	3.1
Error	3,369	5.0×10^{9}	1.5×10^{6}	8.5
Infection efficiency				
Total variance	3,743	120.2	0.032	100
Location	14	29.5	0.0081	24.9
Isolate	360	26.3	0.005	16.5
Error	3,369	64.3	0.01	58.4

[a] Degrees of freedom.
[b] Sum of squares.
[c] Calculated with a nested procedure (11).

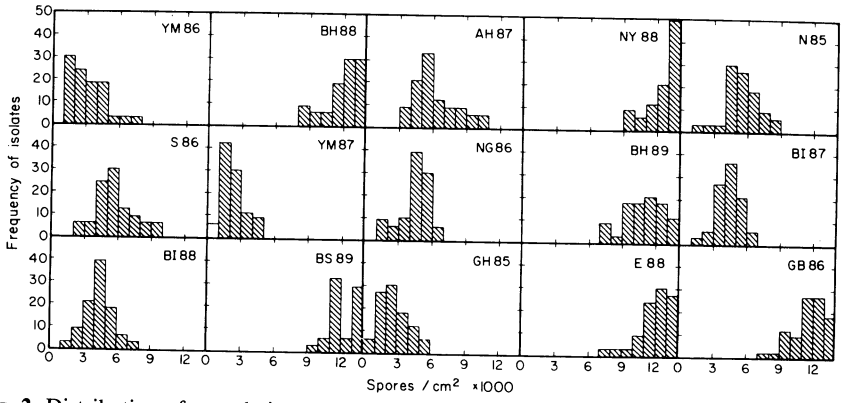

Fig. 2. Distribution of sporulation on 10-day-old lesions on sweet corn cultivar Jubilee plants inoculated with 25 isolates of *Exserohilum turcicum* collected at each of 15 locations in Israel. Location designations are given in Table 1.

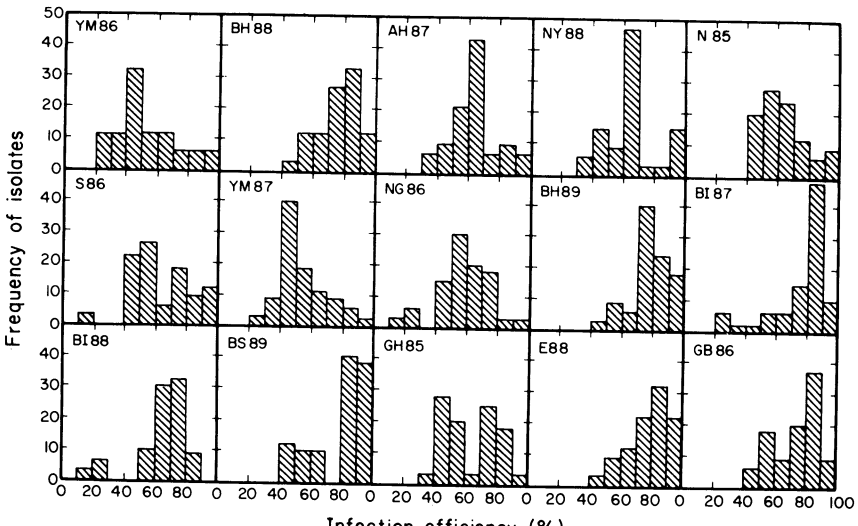

Fig. 3. Distribution of infection efficiency of spores produced on 10-day-old lesions on sweet corn cultivar Jubilee plants inoculated with 25 isolates of *Exserohilum turcicum* collected at each of 15 locations in Israel. Location designations are given in Table 1.

Table 4. Correlation coefficients between the isolate and location components of variance in lesion size (LS), infection efficiency (IE), and sporulation (S) of *Exserohilum turcicum*

		Location		
		LS	IE	S
Isolate	LS	0	0.86	0.85
	IE	−0.28	0	0.76
	S	0.14	−0.085	0

The differences among field isolates in fitness components may partly explain the lack of correlation between severity of epidemics and weather conditions favorable for the development of northern leaf blight (7). The findings also suggest that initial infection of cornfields by *E. turcicum* resulted from local sources of inoculum with similar genetic backgrounds rather than from airborne inoculum from other areas. Several studies have shown that the spread of *E. turcicum* in cornfields is very limited (9,10). A study of airborne conidia of *E. turcicum* in Nebraska indicated that conidia trapped in the spring were probably of local rather than distant origin (9). Boosalis et al (2) showed that conidia that overwintered on corn residue were the source of initial inoculum in a field. In Israel, initial inoculum may be provided both by chlamydospores carried by corn residue and by conidia produced on *Sorghum halepense* (L.) Pers., a perennial host of the pathogen (6). Note that initial symptoms of the disease appear on the lower leaves of plants, further suggesting the local origin of primary inoculum (6). Local origin of initial inoculum may also explain the low variation among isolates at a given location compared with the variation among isolates from different locations.

The accuracy of models designed to predict the severity of epidemics of northern leaf blight may be greatly improved by taking into account the pathogenicity of *E. turcicum* as reflected by infection efficiency, sporulation, and lesion size.

ACKNOWLEDGMENT

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