Occurrence of *Exserohilum turcicum* on Maize in Uganda

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ABSTRACT

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Incidence and severity of northern leaf blight were assessed 3–4 wk after mid-silking on 20 maize plants in each of 115 maize fields in Uganda in 1989. Sampling dates for the maize fields ranged from 6 November to 31 December. Northern leaf blight was prevalent in all 115 fields but was most severe in the wet and humid zones around Lake Victoria. Maize differentials containing *Ht1, Ht2, Ht3*, or *HtN* genes were used to identify races of *Exserohilum turcicum* present in Uganda. Maize inbred lines containing *Ht1, Ht2, Ht3*, or *HtN* genes were resistant to 215 isolates tested, whereas those without *Ht* genes showed necrotic susceptible reactions, a virulence pattern recently designated as race 0. Results indicate that race 0 is in Uganda. The mating type (MAT) of the Ugandan isolates was determined by pairing the isolates from Uganda with tester isolates of the known mating types, A or a. Forty-four of the 189 isolates studied formed fertile pseudothecia; 36 were MATa and eight were MATA.

Northern leaf blight, incited by the fungus *Exserohilum turcicum* (Pass.) K.J. Leonard & E.G. Suggs (teleomorph: *Setosphaeria turcica* (Luttrell) K.J. Leonard & E.G. Suggs), on maize (*Zea mays* L.) was observed for the first time in Uganda in 1924 (4). Until 1988 it had been considered minor and sporadic, presumably because of resistance in open-pollinated cultivars. In 1988, however, it caused extensive damage on maize in central and western Uganda. Damage was most severe on maize genotypes EV8428-SR and EV8429-SR, introductions from Centro Internacional de Mejoramiento de Maiz y Trigo (CIMMYT), Mexico.

Gene-for-gene relationships have been demonstrated for *E. turcicum* on maize, and five physiological races have been identified (11,20,21). Race 0 has the virulence formula *Ht1, Ht2, Ht3, HtN*/ and is widely distributed in many parts of the world (1). Race 1, with the virulence formula *Ht2, Ht3, HtN*/*Ht1*, was first detected in 1974 in Hawaii (2) but is now widespread in the corn belt of the United States (6,7,12,15). Race 23, with virulence formula *Ht1, HtN*/*Ht2, Ht3*, and race 23N, with virulence formula *Ht1*/*Ht2, Ht3, HtN*, appears restricted to the continental United States (20). Race 2N, with virulence formula *Ht1, Ht3*/*Ht2, HtN*, was reported as recently as 1991 in Hawaii (21).

E. turcicum has a heterothallic sexual stage, with two mating types (MAT) (12,16–18). The sexual stage, however, has not been found in nature (10). There are no records of northern leaf blight investigations in Uganda, so little is known about the status of the disease or the range of virulence present in the population. Knowledge of physiological races and their mating patterns permits a partial prediction of the potential pathogenicity of a species and allows development of suitable breeding programs (16,17). The objectives of this study were to determine the prevalence and severity of northern leaf blight in the maize-growing regions of Uganda, to characterize the races present, and to determine their mating types.

MATERIALS AND METHODS

Prevalence and distribution of northern leaf blight. Uganda is divided into four agroecological zones. Most of the maize is grown in zone I (districts 2, 13, and 19) and zone IV (districts 3–10, 16, 17, 20, and 22), which are wet (1,400 mm annual rainfall), warm (20–25 C), and humid (>80% RH) during most days. Zone II (districts 4 and 21) and zone III (districts 1, 2, 11, 12, 14, 15, 18, and 23) are relatively dry (800–1,100 mm annual rainfall) with temperatures of 25–30 C and <70% RH during most days. Twenty-three of the 33 districts in Uganda were surveyed during November–December 1989 (Fig. 1). In each district, five fields, approximately 40 km apart, were sampled. Data were collected from plants in approximately the center of each field. A total of 20 plants, five each from east, west, north, and south of the field center and approximately 5 m apart, were assessed. Each plant was examined for number of infected leaves, number of lesions per plant, and disease severity for the whole plant, rated on a scale of 0, 0.5, 1, 5, 10, 25, 50, and >75% leaf area infected. The average ratings of the 20 plants in each field were calculated for statistical analysis. Analysis of variance was conducted to determine if district or zone significantly affected each variable. Means were compared using Fisher's least significant difference (LSD) at $P = 0.05$. Correlation coefficients also were calculated between variables, using all data points.

Preparation of single-spore cultures. Blighted leaves were removed from plants in each field, put in plastic bags in an insulated container to prevent heating, and transported to the Makerere University Agriculture Laboratory, Kampala, Uganda. Additional tissue collected during the 1988 epidemic and samples collected from nonsurveyed plots were also used. In 1988, infected leaf samples were collected from 22 fields in 14 districts (districts 3–9 and 16–22), pressed, and stored at room temperature (22 ± 2 C). For both 1988 and 1989 samples, lesions were excised, surface-disinfested for 30 sec with 0.1 HgCl in sterile distilled water, and then maintained for 12–24 hr in petri dishes with moistened filter paper. Single conidia were transferred with sterile needles onto Difco potato-dextrose agar (PDA). After 10 days, culture plugs were transferred to PDA in 10-ml vials and mailed, under quarantine conditions, to the Ohio Agricultural Research and Development Center, Wooster. Cultures were then transferred to fresh PDA.

Characterization of races of *E. turcicum* in Uganda. Virulence of 215 isolates was tested on maize differentials A619, A619*Ht1*, A619*Ht2*, A619*Ht3*, and B14*HtN*, supplied by W. L. Pedersen, University of Illinois, Urbana. Isolates of race 0 (ATCC 64837), race 1 (ATCC 64835), race 23 (ATCC 64836), and 23N (ATCC 64834) (11), obtained from American Type Culture Collection, Rockville, Maryland, were used for comparison. Inocula for virulence tests were prepared by washing conidia from 10- to 14-day-old cultures growing on PDA. Conidial suspensions were filtered through four layers of cheesecloth, and spore concentration was adjusted with a hemacytometer to 20,000 conidia per milliliter. Maize seedlings in the four- to six-leaf stage were inoculated by pipetting 1 ml

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of the conidial suspension into the whorl of each seedling. Seedling reactions were assessed 7–10 days after inoculation. All studies were conducted in growth chambers maintained at 22/18 C day/night temperatures and average light intensity of 280 ± 19 $\mu E \cdot m^{-2} \cdot s^{-1}$ (8,9,11).

The experiment was of a completely randomized design. Each experimental unit consisted of a pot of three plants. The experiment was conducted four times representing four replications in time.

Mating types. Mating type (MAT) and fertility of each isolate were detetermined by pairing each isolate from Uganda with a known mating type, NK 2 and NK 22 (MATA) and NK 19 and NK 27 (MATa) obtained from G. C. Bergstrom, Cornell University, Ithaca, New York. Autoclaved sections of senescent corn leaves (2–3 × 1 cm) were placed on modified Sacchs agar (5) in 9-cm disposable petri dishes. Mycelial plugs of 7- to 8-day-old monoconidial cultures of the Ugandan isolates and tester isolates were placed on the opposite sides of leaf sections (i.e., one or two culture plugs on either side, 1 cm apart). Plates were placed upside down to avoid condensation and were incubated at 25 C in the light. Plates were observed periodically for presence of pseudothecia. When formed, pseudothecia were left to mature for about 2 wk, then were examined for presence of ascospores. The experiment was repeated with all isolates. Ability to form both pseudothecia and ascospores confirmed fertility. Ascospores were not tested for viability. Fertile Ugandan isolates were not mated with each other except for those from Kiwenda (MATa), Luwero (MATA), and Kakinzi (MATA) in district 4. At least three isolates from each location surveyed in 1989 were studied (71 of the 115 isolates). In addition, 22 isolates collected during the 1988 epidemics and 96 isolates collected from nonsurveyed fields in 1989 were tested with known mating types. Tester isolates were mated with each other to determine whether isolates of one physiological race would cross with isolates of the opposite mating type of different physiological races. The race designations of tester isolates were also determined on maize differentials with *Ht* and *HtN* genes.

Fig. 1. Location of 115 maize fields in Uganda sampled for northern leaf blight in 1989. Districts surveyed were: 1 = Arua, 2 = Nebbi, 3 = Rakai, 4 = Luwero, 5 = Mukono, 6 = Mubende, 7 = Kampala, 8 = Masaka, 9 = Mpigi, 10 = Jinja, 11 = Iganga, 12 = Tororo, 13 = Mbale, 14 = Soroti, 15 = Kumi, 16 = Masindi, 17 = Mbarara, 18 = Rukungiri, 19 = Kabale, 20 = Bushenyi, 21 = Kasese, 22 = Hoima, and 23 = Kamuli.

RESULTS

Northern leaf blight was present in all 115 fields surveyed. Average disease severity ranged from 0.5 to 25% (Fig. 2). Disease levels were lower in drier areas, i.e., districts 2, 10–18, and 23 in zones II and III (Figs. 3 and 4). Northern leaf blight was more severe (>5%) in the humid (>80% RH), warm (24 C), and wet areas (>1,200 mm rainfall), i.e., zones I and IV (Fig. 3), especially in Mpigi (district 9) and Kabale (district 19) (Fig. 4). With the exception of Kabale, the highest disease levels were recorded in areas surrounding Lake Victoria, i.e., Rakai (district 3), Mukono (district 5), and Mpigi (district 9) (Fig. 4). The percentage of leaf area affected was highly correlated with the number of infected leaves ($r = 0.81$, $P = 0.01$) and with the number of lesions per plant ($r = 0.89$, $P = 0.001$).

All 215 Ugandan isolates were virulent (necrotic lesions) on A619 but avirulent on A619*Ht1*, A619*Ht2*, A619*Ht3*, and B14*HtN*, indicating that all isolates were race 0. Both MATA and MATa mating types were represented among the isolates studied, although MATa was more frequent than MATA (Fig. 5). Of the 189 isolates tested, only 44 formed fertile pseudothecia; 16 isolates formed pseudothecia with no ascospores. Of the five fertile isolates from 1988, four were MATa and one was MATA, whereas of those from 1989, 32 were MATa and seven were MATA. In some cases, both mating types were found in the same location (districts 4 and 17–19). The three MATA and MATa isolates from Luwero (district 4) formed fertile pseudothecia, but formation of pseudothecia by fertile crosses of Uganda isolates and known MATs was irregular. Only one to three pseudothecia were produced in each case.

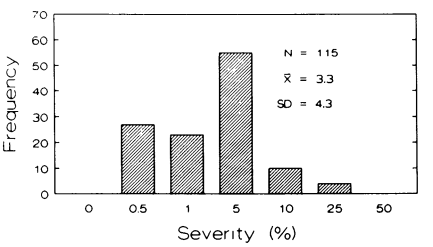

Fig. 2. Frequency distribution of northern leaf blight severity in 115 maize fields from 23 districts of Uganda in 1989. Severity on whole plants was rated on a scale of 0, 0.5, 1, 5, 10, 25, 50, and >75% leaf area affected 3–4 wk after mid-silking. N = number of fields sampled, $\bar{x}$ = sample mean, and SD = standard deviation.

Virulence tests showed that tester isolates NK2 (MATA) and NK19 (MATa) were race 0, NK22 (MATA) was race 1, and NK27 (MATa) was race 23 (Table 1). Fertile Uganda isolates (race 0) produced pseudothecia when crossed with opposite tester isolates of races 0, 1, and 23.

DISCUSSION

Development of northern leaf blight is favored by moderate temperatures (20–25 C) and high relative humidity

(90–100%) (13,14). These conditions are common in most of central and western Uganda agroecological zones I and IV, where the 1988 epidemic occurred. These zones also had higher severity of northern leaf blight than eastern and northern areas in 1989. Since about 1986, there has been intensive cultivation of maize in central and western Uganda. In zones I and IV, maize is often grown twice a year and fields are often planted fairly close together. Thus, there appears to be a ready supply of maize residue for survival of *E. turcicum*. The presence of readily available inoculum combined with conducive environmental conditions and a large number of fields planted to the susceptible cultivars EV8429-SR and EV8428-SR may account for the relatively high prevalence of northern leaf blight in some areas. In much of eastern and especially northern Uganda (zones II and III), where conditions are drier and less humid, there is usually one maize season and plantings are not extensive. These conditions are not as favorable for epidemics and may partly explain the low disease levels in these areas. Also, farmers grow mostly local cultivars, such as Kawanda Composite A (KWCA), that appear to be resistant to *E. turcicum*.

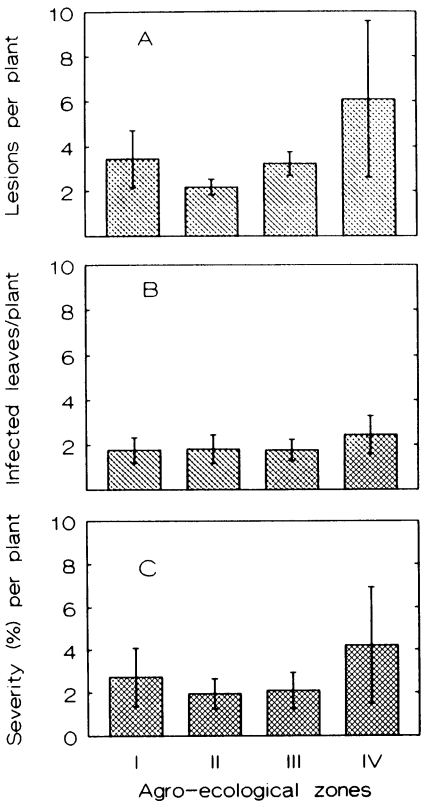

Fig. 3. (A) Lesions per plant, **(B)** number of infected leaves per plant, and **(C)** severity of northern leaf blight of maize in the four agroecological zones of Uganda in 1989. Severity on whole plants was rated on a scale of 0, 0.5, 1, 5, 10, 25, 50, and >75% leaf area affected 3–4 wk after mid-silking. Zone I = districts 2, 13, and 19; zone II = districts 4 and 21; zone III = districts 1, 2, 11, 12, 14, 15, 18, and 23; zone IV = districts 3–10, 16, 17, 20, and 22. Some districts (see Figure 1) fell in more than one zone. Bars represent standard errors.

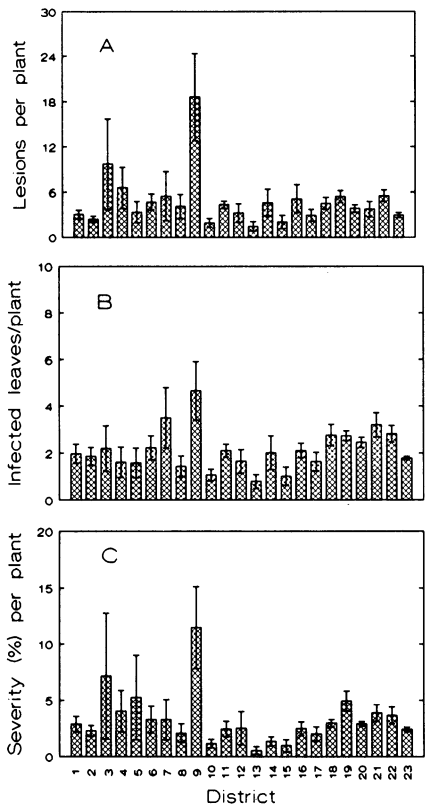

Fig. 4. (A) Lesions per plant, **(B)** number of infected leaves per plant, and **(C)** severity of northern leaf blight of maize in 23 districts (see Figure 1) surveyed in Uganda in 1989. Severity on whole plants was rated on a scale of 0, 0.5, 1, 5, 10, 25, 50, and >75% leaf area affected 3–4 wk after mid-silking. Bars represent standard errors.

Table 1. Virulence of mating type tester isolates on maize differentials with *Ht1*, *Ht2*, *Ht3*, or *HtN* resistance gene

Tester isolate	Mating type	Race[a]	Maize differentials				
			A619	A619*Ht1*	A619*Ht2*	A619*Ht3*	B14*HtN*
NK2	A	0	S[b]	R	R	R	R
NK22	A	1	S	S	R	R	R
NK19	a	0	S	R	R	R	R
NK27	a	23	S	R	S	S	R

[a] From Leonard et al (11).
[b] S = susceptible (necrotic lesions), R = resistant.

Maize breeding lines at CIMMYT's Subtropical Station at Tlaltizapan, Mexico, are not selected for resistance to *E. turcicum* because northern leaf blight does not develop under Tlaltizapan's environmental conditions, even after inoculations (3). Maize introductions EV8428-SR and EV8429-SR from CIMMYT are grown for higher yield potential and for their resistance to maize streak virus, which is endemic in Uganda. Under the conducive environment for development of northern leaf blight and the presumed high inoculum level of *E. turcicum* prevailing in Uganda, CIMMYT's EV8428-SR and EV8429-SR selections were adversely affected by northern leaf blight in 1988.

Although northern leaf blight was widespread in 1989, the average severity was relatively low (Fig. 2). Epidemics of northern leaf blight generally develop late in the season (1). Plants sampled in Uganda were basically at a similar growth stage (3–4 wk after mid-silking). The disease level probably would have been higher if the plants had been sampled later.

Our results suggest that race 0 is the most common, and probably the only, biotype of *E. turcicum* in Uganda. Levy (13) and Levy and Cohen (14) reported that epidemics of northern leaf blight sometimes occurred under suboptimal environmental conditions in the presence of aggressive populations of *E. turcicum*. Although no other races of *E. turcicum* were detected among the 215 isolates studied, it is possible that more aggressive populations of *E. turcicum* may have developed in Uganda.

In mating type studies, the frequency of isolates forming pseudothecia or ascospores was similar to those reported earlier (7,12,16,19). The majority of isolates were MATa, which is consistent with earlier studies (7,12,19). However, Nelson (17) was able to induce abundant formation of pseudothecia on sterilized barley seeds; 20 isolates were MATA and 13 were MATa. In our study, formation of pseudothecia was equally rare and inconsistent on sterilized barley seeds (*unpublished*).

Our data, and those of others, suggest predominance of MATa over MATA. The low occurrence of fertile pseudothecia, an indication of low frequency of recombination, may partly explain absence of the sexual stage of *E. turcicum* in nature. However, since MATA and MATa of the same physiological race and of different races can hybridize in culture, occurrence of new races of *E. turcicum* seems likely.

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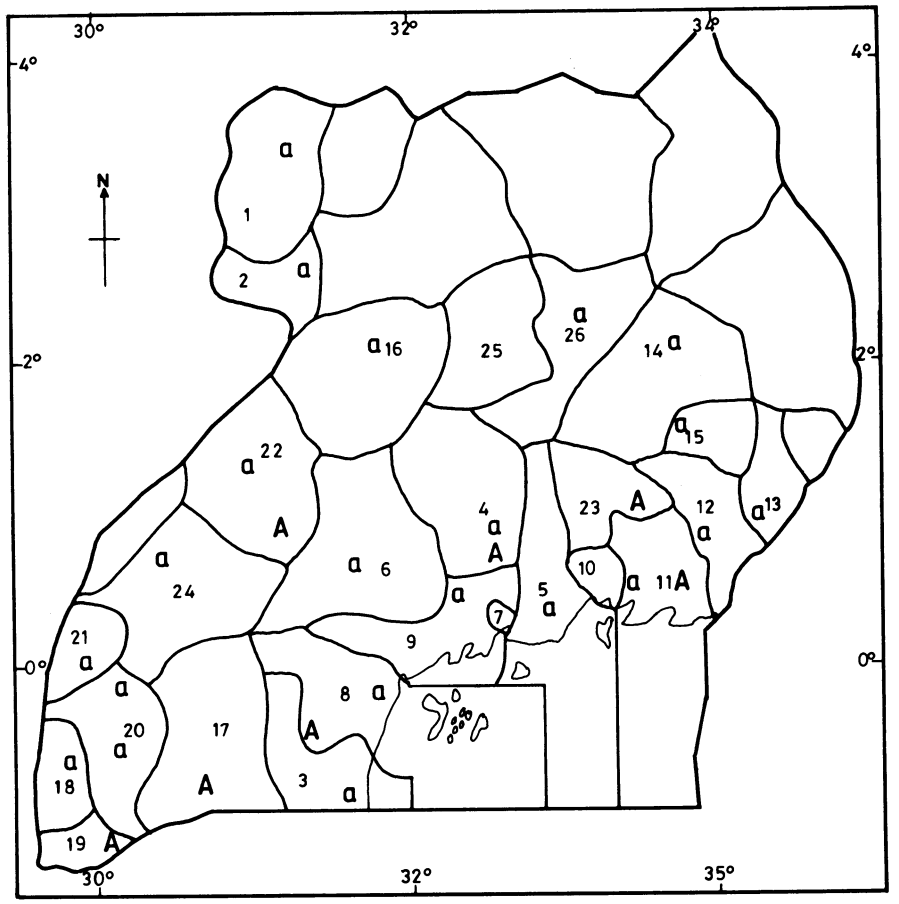

Fig. 5. Distribution of mating types MATA and MATa in maize fields in Uganda in 1988 and 1989. Districts surveyed were: 1 = Arua, 2 = Nebbi, 3 = Rakai, 4 = Luwero, 5 = Mukono, 6 = Mubende, 7 = Kampala, 8 = Masaka, 9 = Mpigi, 10 = Jinja, 11 = Iganga, 12 = Tororo, 13 = Mbale, 14 = Soroti, 15 = Kumi, 16 = Masindi, 17 = Mbarara, 18 = Rukungiri, 19 = Kabale, 20 = Bushenyi, 21 = Kasese, 22 = Hoima, 23 = Kamuli, 24 = Kabarole, 25 = Apach, and 26 = Lira.

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