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Disease severity, incidence and races of *Setosphaeria turcica* on sorghum in Uganda

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Abstract In order to understand the underlaying causes of new severe turcicum leaf blight outbreaks in East Africa, a survey was undertaken in Uganda to examine the sorghum—*Setosphaeria turcica* interaction in terms of disease severity and incidence, the overall fungal population structure, and new resistant resources. Highest disease severities were recorded on caudatum accessions, whereas kafir genotypes were most resistant. The disease was more severe in the most humid farmlands compared to moderately dry agro-ecologies. In districts with wide adoption of the Epuripur variety a very high incidence (100%) of turcicum leaf blight was found. The two *S. turcica* mating type genes *MAT1-1* and *MAT1-2* assessed on fungal isolates deriving from both sorghum and maize diseased leaves were found in 20 of 23 districts sampled and in equal proportions. Upon cross inoculation on maize differential lines, four *S. turcica* isolates were identified as race 1, two as race 2, and one isolate corresponded to race 0 and race 3, respectively. The remaining 10 *S. turcica* isolates did not cause any disease symptoms on the maize lines assessed. Highly resistant accessions originating from a regional collection were found among the five sorghum races (kafir, guinea, caudatum, bicolor and durra), and are now implemented in new sorghum disease resistance programs.

Keywords *Exserohilum turcicum* · Northern corn leaf blight · Sorghum

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Introduction

Maize (*Zea mays* L.) and sorghum (*Sorghum bicolor* Moenc) are the most important staple cereals for sub-Saharan Africa (SSA). While maize is an introduced crop (Miracle 1965), sorghum is believed to have been domesticated in SSA particularly in the Nile basin or Ethiopia as recently as 1,000 BC (Kimber 2000). The genus Sorghum is very diverse; all cultivated sorghum crops belong to *S. bicolor* ssp. *bicolor*, which is divided, based on morphology into five races, bicolor, caudatum, guinea, durra, and kafir (Harlan and de Wet 1972). All five races are grown in SSA. The sorghum crop is generally cultivated in drier and marginal regions of the continent and is a

key component of more than 50% of Africa's rural household livelihood strategies. In spite of its pivotal role in the region's agricultural development agenda, the current production level of sorghum is low, at less than one ton per hectare (FAO 2010). This is because sorghum cultivation is still mainly characterized by traditional farming practices, with low inputs and use of unimproved landraces (ICRISAT 1996; De Vries and Toenniessen 2001). With the recent unpredicted rainfall patterns, and reduced water quantities, there is increased interest in sorghum as one of the climate change adaptation strategies. Accordingly, the crop is now prioritized from national to sub-regional levels as a key target for improvements.

The main biotic constraints on sorghum and maize in East Africa include several insect pests represented by stem borers (*Lepidoptera* spp.) and sucking bugs (*Homoptera* and *Hemiptera* spp.), together with turcicum leaf blight (*Setosphaeria turcica*), grey leaf spot (*Cercospora zeae-maydis*, *Cercospora sorghi*), and maize streak virus disease (Adipala et al. 1993a; Ceballos et al. 1991; De Vries and Toenniessen 2001; Nkonya et al. 1998; Pingali and Pandey 2001; Tilahun et al. 2001). The heterothallic ascomycete *Setosphaeria turcica* Leonard & Suggs (anamorph: *Exserohlium turcicum*, former *Helminthosporium turcicum*) causes turcicum or northern leaf blight disease on maize. This fungal pathogen also attacks sorghum and related grasses, for example Johnson grass (Hamid and Aragaki 1974; Chiang et al. 1989). In geographic regions where high humidity and moderate temperatures prevail during the growing seasons, and infection occurs before silking, the reduction of photosynthetic potential may lead to yield losses of up to 80% in both maize and in sorghum (De Vries and Toenniessen 2001; FAO 2010). *S. turcica* reproduces exclusively asexually through conidial production with the sexual stage only induced under laboratory conditions (Leonard 1988; Moghaddam and Pataky 1994). Population genetic studies on *S. turcica* based on RAPDs and mating type tests have however demonstrated high genotypic diversity and even distribution of the two mating types on maize grown under tropical conditions compared to *S. turcica* populations collected in temperate regions (Borchardt et al. 1998). The cause of this high genetic diversity is unknown but presence of a sexual stage has been suggested. Turcicum leaf blight has earlier been considered of minor importance in Uganda until 1988 when it caused extensive yield losses on maize (Adipala et al. 1993a). By introducing improved resistance in new varieties the threat posed by the disease was subsequently reduced. Severe and sporadic outbreaks of turcicum leaf blight have reappeared in East Africa and elsewhere in the world (Ebiyau and Oryokot 2001; Pratt and Gordon 2006). Under conditions suitable for epidemics both maize, and the indigenous sorghum crop, which in SSA share agro-ecologies, are heavily affected (De Vries and Toenniessen 2001). A change in the *S. turcica* population has been suggested to be the main cause of this shift in disease pattern. In order to detect potential new changes of the *S. turcica* pathogen and the turcicum leaf blight disease, a survey was undertaken in Uganda to examine the sorghum—*S. turcica* pathosystem in terms of disease severity and incidence, race patterns and new resistant resources. The latter is not least important since host resistance is the most economical and environmental friendly way to control this plant disease.

Materials and methods

Agro-ecologies

Field surveys, repeated over three growing seasons were carried out in eight major sorghum growing agro-ecologies of Uganda to assess the incidence and severity of turcicum leaf blight on diverse sorghum races. The agro-ecological zones are based on differences in farming systems, edaphic factors, weather and climatic, altitude and major vegetation cover (Wortmann and Eledu 1999; Ebiyau and Oryokot 2001) shown in Supplementary Table S1 and Figure S1 (Additional material on line). The different farming systems tend to somewhat overlap between the agro-ecological zones. The same holds true for the different sorghum races. The most predominant are caudatum and guinea, with scattered presence of kafir. Durra and bicolor are uncommon in Uganda and occur in dry areas. The agro-ecologies include: The North-Eastern-Central Grass-Bush Farmlands characterized by one long rain season where the major cereals are sorghum and millets. The Southern and Eastern Lake Kyoga Basin agro-ecologies have predominately banana-millet-sorghum and cotton farming systems. The two farming systems in either

of Lake Victoria Crescent and Mbale Farmlands agro-ecologies of the banana-millet-cotton system and the banana-coffee lake shore system where the major cereal crops are maize, sorghum, and millets. The banana-millet-cotton system is more common on the Central Wooded Savanna, with minor presence of sorghum. Among cereals, finger millet, maize and sorghum are grown in Northern Moist Farmlands, West Nile Farmlands and North-Western Farmlands-Wooded Savanna, together with various cattle systems. Finally, in the Western Mid-Altitude Farmlands and the Semliki Flat region, banana, coffee, and cereals like maize are common but sorghum is also grown in some areas.

Field observations and material collection

A hierarchical sampling structure was used to collect *S. turcica* diseased sorghum leaf samples from fields in 23 districts found within eight agro-ecological zones. The sampling structure consisted of two hierarchical levels; agro-ecological zones and districts within agro-ecological zones. From each district, at least five fields, each averaging one-hectare in size were visited. In each field, disease incidence was assessed as the proportion of plants showing symptoms in a field. Twenty plants in middle of each field were randomly selected and the number of plants having *S. turcica* disease symptoms counted after whole plant basis and expressed as a percentage of the plant population. Disease severity on whole plant basis was rated using a scale of 0, 0.5, 1.5, 10, 25, 50 and >75% leaf area affected (Adipala et al. 1993a). At the same time 196 sorghum accessions were collected in all 8 Ugandan agro-ecologies visited and sorghum races identity classified according to Bantilan et al. (2004). In parallel, infected maize leaves were collected and 105 single spores were isolated and grown as described below.

Fungal isolates, culture and DNA isolation

The leaf samples were air dried for 4 to 7 days. A lesion was cut out from each leaf sample, surface sterilized using 0.5% sodium hypochlorite solution for 1 min, 70% ethanol for 30 s, rinsed three times in sterile H_2O, placed on moist paper in a Petri dish, and incubated for 48 h in a dark at 25°C to promote sporulation. Conidia were picked using a sterile needle and transferred to potato dextrose agar (PDA; Difco, Sparks, MD, USA) plates, supplemented with 0.1 mg/l streptomycin and 0.1 mg/l ampicillin (Astra-Zeneca, London, UK) and cultured under light for 16 h at 22°C and in darkness for 8 h at 16°C. Fungal mycelia were collected from the PDA plates and DNA isolated by using a modified CTAB method (Okori et al. 2004).

S. turcica species-specificity

All fungal isolates in the study were screened by PCR using the sequence information from the internal transcribed spacer 1 and 2 (ITS) of the 5.8S ribosomal RNA gene (GenBank accession number AF163067). The following primers were designed, forward: 5′-GCAACAGTGCTCTGCTGAAA-3′, reverse: 5′-ATAAGACGGCCAACACCAAG-3, generating a 344 bp fragment. PCR was carried out using the following conditions: 10 ng of template DNA was added to a 24 µl mix consisting of H_2O, 2.5 mM $MgCl_2$, 2.5 µl *Taq* buffer (Fermentas, Hatfield, South Africa), 0.2 mM of each dNTP, 0.25 µM of forward and reverse primers and 1 U of *Taq* polymerase (Fermentas). The PCR conditions used were 95°C for 4 min, 35 cycles of 30 s at 94°C, 30 s at 58°C, 1 min at 72°C and a final extension at 72°C for 10 min. The PCR products were separated on 1% agarose gels to confirm fragment size.

Mating-type assessment

S. turcica DNA sequences flanking the alpha box in *MAT1-1* and the HMG domain present in *MAT1-2* were sequenced and deposited in GenBank under the accession numbers, GU997138 (*MAT1-1*), and GU997137 (*MAT1-2*). Primers designed were *MAT1-1* forward: 5′-GCAAGTAAGCGAGCCTCAAC-3, reverse: 5′- AGTCCATGGGATACG CTACG -3; generating a 154 bp fragment, and *MAT1-2* forward: 5′- CAAACATCTCAAGGCGGAAT-3, reverse: 5′-ACGCAGGTGTTCTTCTTTCG-3 generating a 197 bp fragment. PCR was carried out using the following conditions: 10 ng of template DNA was added to a 24 µl mix consisting of H_2O, 2.5 mM $MgCl_2$, 2.5 µl *Taq* buffer (Fermentas) 0.2 mM of each dNTP, 0.25 µM of forward and reverse primers and 1 U of *Taq* polymerase (Fermentas). The PCR

conditions used were 95°C for 4 min, 35 cycles of 30 s at 94°C, 30 s at 55°C, 1 min at 72°C, and a final extension at 72°C for 10 min. The Mann–Whitney *U*-test (Conover 1999) and Pearson chi square test were applied to verify mating type distribution.

Perfect stage analysis

To identify the perfect stage of *S. turcica*, an additional set of diseased maize and sorghum leaves was collected from four regions (Kiboga, Iganga, Tororo, Soroti) where the two mating types (*MAT1-1* and *MAT1*-2) were found to be frequent on both crop species (Ramathani 2009; Martin unpublished). Approximately 700 leaf samples were examined for presence of dark brown/black globose and immersed pseudothecia with ascospores. Single spore isolates were retrieved from pseudothecia formed in the disease lesions, checked for compatibility mating using tester strains (Luttrell 1958; Ferguson and Carson 2007), and analysed by PCR, for species identity.

Screening of maize differential lines

To confirm fungal race identity, virulence of isolates was assessed on maize differential lines. The materials used were A619, A619*Ht1*, A619*Ht2*, and A619*Ht3* obtained from the Maize Genetics Resource Centre (USDA/ARS University of Illinois Urbana-Champaign, Urbana, Illinois, USA). A mixture of sterilized forest loam soil was used to grow the plants in the greenhouse at Makerere University Agricultural Research Institute in Kabanyolo (MUARIK). The plants were spray-inoculated 12 days after planting using solutions of 2.5×10^4 spores/ml of 18 individual isolates. The plantlets were subsequently covered for 48 h with a polythene sheet to increase the relative humidity and enhance infection. Each pot contained 3 plants and the experiments were replicated three times. The maize lines were evaluated for disease severity 15 days after inoculation as earlier described (Hooker 1963; Adipala et al. 1993a) Sporulating lesions without evidence of chlorosis was considered as susceptible types and non-sporulating lesions bearing chlorosis were the resistant types. Race nomenclature according to Leonard (1989), excluding the *HtN* genotype, was employed.

Assessment of sorghum germplasm to *S. turcica*

Five *S. turcica* isolates (Pak3, Ig23, Sor30, Ho6 and Ap) showing different response to maize differential lines, were grown on PDA plates together with autoclaved sorghum kernels for 10 days. Inoculation using individual isolates was done at the four- to six-leaf stage by placing 6 seeds per isolate of dry *S. turcica* colonized sorghum kernels into each leaf whorl. Inoculation was done in the evening to allow successful infection when temperature conditions were optimal (Carson 1995). The screening of the 196 accessions collected was done in a randomized complete block design with two replications for two consecutive seasons. Assessment of disease severity commenced 51 days after planting and continued on weekly basis for 6 weeks. A disease scoring scale ranging from 0 = no disease (no lesions identifiable on any of the leaves) to 5 = >75% of leaf surface diseased were used (Adipala et al. 1993a).

Data analysis

Disease severity data were used to compute area under disease progress curves (AUPDC) according to Broers et al. (1996) and subjected to analysis of variance (ANOVA). The disease severity ratings were subsequently subjected to nested-analysis of variance at two levels of hierarchy in which the sampling was done (agro-ecologies and districts within agro-ecologies) to determine the impact of *S. turcica* variability on disease severity. Where significant differences were detected, means were compared using Turkey's (Turkey-Kramer) simultaneous tests for unbalanced data at $P \leq 0.05$ (Steel et al. 1997). All statistical analyses were performed using GenStat 7 version 3.2, 2007 (Lawes Agricultural Trust: Rothamsted Experimental Station, UK) and MINITAB release 14 versions 14.20, 2005 (Minitab Inc, Pennsylvania, USA).

Results

Incidence and severity of turcicum leaf blight in eight Ugandan agro-ecologies

Turcicum leaf blight was observed in all agro-ecologies visited and found on all sorghum races but in varying proportion. ANOVA revealed highly significant influ-

ence of agro-ecology ($P \leq 0.001$) on disease severity. Within each agro-ecological zone, there was a significant influence of district on both disease severity ($P = 0.006$) and disease incidence ($P \leq 0.001$; Table 1). Disease incidence was highest in the Southern and Eastern Lake Kyoga basin (89%) and lowest in the northern—central-grass-bush farmlands (66%; Table 1). At the second level of hierarchy—the districts, disease incidence ranged from 100% in Pallisa (Southern and Eastern Lake Kyoga Basin) to 20% in Tororo and the associated severities were 42% and 26%, respectively (Table 1; Fig. S1). The lowest severity was recorded in districts of Mbale (14%), Tororo (20%) and Wakiso (13%) which are located in Lake Victoria crescent and Mbale Farmland agro-ecologies. In Pallisa district a very high incidence of turcicum leaf blight was found largely due to wide adoption of the Epuripur variety. In general, districts in the Northern moist farmlands and North-Western farmland agro-ecologies had very high incidence of turcicum leaf blight, for example Kaberamaidio, Gulu and Kumi all had incidences of over 75%. Nested ANOVA performed at two hierarchical levels (agro-ecology and districts within agro-ecology) confirmed the significant role of agro-ecology and districts within agro-ecologies ($P \leq 0.05$) on turcicum leaf blight disease severity (Table 2). As expected the disease was more severe in the most humid farmlands as compared to moderately dry agro-ecologies.

Prevalence and distribution of mating types

Assays based on mating type specific PCR primers of 89 randomly selected sorghum isolates and 105 maize derived isolates, representing all the 8 agro-ecologies under study, revealed the presence of both mating types (*MAT1-1* and *MAT1-2*) of *S. turcica* in Uganda. The distribution among sorghum isolates was; 48 of *MAT1-1* type and 41 isolates with *MAT1-2*. Among maize derived isolates 60 harboured *MAT1-1* and 45 showed a presence of *MAT1-2*. The mating type distributions in the two populations were not significantly different from an even distribution (sorghum isolates: $\chi^2 = 0.55$, maize isolates: $\chi^2 = 3.21$, $P < 0.05$). The Mann–Whitney *U*-test test also revealed no significant difference between the mating type medians in the two populations studied (sorghum isolates: $U = 66$; maize isolates: $U = 99.5$ $P > 0.05$). Overall, the two mating type genes were found in 20 of 23 districts sampled.

Genetic diversity of *S. turcica* isolates based on race differential assay

Eighteen sorghum derived *S. turcica* isolates were used to inoculate the differential inbred maize lines carrying the *Ht* genes and their reactions were recorded. The resistant maize plants showed small chlorotic spots while susceptible plants had elliptical grey necrotic lesions, and fungal sporulation was observed 15 days post infection (Table 3). Interestingly, all isolates were avirulent on the A619 genomic background whereas some isolates were found to be virulent on maize lines carrying *Ht* genes. Four isolates were determined as race 1, two were race 2, and one isolate corresponded to race 0 and race 3, respectively. The remaining 10 *S. turcica* isolates did not cause any disease symptoms on the maize lines assessed.

Table 1 Mean of disease severity and incidence of turcicum leaf blight in major sorghum growing agro-ecologies in Uganda

Agro-ecological zones	Severity[a]	Incidence[a]
Central Wooded Savanna	16.5	86.1
North-Eastern–Central Grass-Bush-Farmlands	22.1	65.8
Northern Moist Farmlands	30.5	73.7
North-Western Farmlands–Wooded Savanna	30.0	84.1
Western Mid-Altitude Farmlands and the Semliki Flat	37.0	76.4
West Nile Farmlands	37.6	67.0
Lake Victoria Crescent and Mbale Farmlanda	14.8	66.2
Southern and Eastern Lake Kyoga Basin	23.3	89.1
LSD	7.9	12.6
CV	57.3	34.6

[a] Disease severity and incidence were computed as percentage leaf area damaged and proportion of plants showing symptoms, respectively

LSD Fisher's Protected Least Significant Difference test at $P \leq 0.05$ (Steel et al. 1997)

Table 2 Nested analysis of variance for severity of turcicum leaf blight on sorghum

Source	DF	SS	MS	F[a]
Agro-ecology	7	28.69	5.16	9.45
District	22	10.69	1.79	3.26
Error	200	109.13	0.55	
Total	229	148.50		

[a] Statistical significant differences = $P \leq 0.05$
DF degrees of freedom; *SS* sum of squares
MS Mean square

Reaction of sorghum accessions to *S. turcica* infection

Analysis of variance revealed highly significant variation ($P \leq 0.001$) in disease severity and AUDPC data. The classification of disease reaction type using cluster analysis based on final severity and AUDPC values revealed four major groupings ranging from highly resistant to highly susceptible (Table 4). No significant variation of host response to the five *S. turcica* isolates was found. Highly resistant accessions were found in all five sorghum races included in the collection.

Table 3 Race identification of *S. turcica* isolates derived from sorghum based on responses on differential maize lines carrying different resistance genes

	Maize race differential				
Isolate	A619	A619/*Ht1*	A619/*Ht2*	A619/*Ht3*	Race
Pak 3	R	R	S	R	2
Ig 23	R	S	R	R	1
Ar 4	R	R	R	R	
Ar 6	R	R	S	R	2
Km 22	R	R	R	R	
Ser 9	R	R	R	R	
Dok 11	R	R	R	R	
P19	R	R	R	R	
Kabe 5	R	R	R	R	
Ar 8	R	R	R	R	
Sor 30	R	R	R	R	
Lr	R	R	R	R	
Wks 2	R	S	R	R	1
Kb 30	R	S	R	R	1
Gu 25	R	R	R	R	
Kat 9	R	S	R	R	1
Ho6	R	R	R	S	3
Ap	S	R	R	R	0

Discussion

In Eastern Africa, sorghum is by tradition composed of three major sorghum races caudatum, kafir and guinea, whereas durra and bicolor are more common towards the west (Gopal et al. 2002). More recent investigations confirmed the presence of all five sorghum races in Uganda, albeit at various proportions in the different agro-ecologies (Ramathani 2009; Mbeyagala 2010). *S. turcica* was pathogenic on all five major races of sorghum in this study, with varying levels of virulence. In a parallel survey on temporal aspects of turcicum leaf blight epidemics in Uganda it was found that disease development on sorghum was delayed by about 3 weeks compared to maize (Ramathani 2009). Furthermore, the disease severity, incidence and disease progress on sorghum was quite low compared to maize suggesting higher resistance in the sorghum genotypes grown compared to the commercial maize varieties. The turcicum leaf blight disease in this study was found to be more severe in the most humid farmland and in districts where the Epuripur cultivar (bicolor) was introduced. Epuripur is resistant to *Colletotrichum sublineolum* causing sorghum anthracnose but very susceptible to *S. turcica*. Concomitant occurrence of *C. sublineolum* and *S. turcica* foliar pathogens on sorghum is very common in East Africa (Esele 1995; Ngugi et al. 2001) and Epuripur is among the few varieties that differ in this respect.

The mix of maize varieties and local sorghum genotypes in the farming systems together with related and wild plant species growing within or adjacent to agricultural fields, may represent an important source of new fungal inoculum or form reservoirs leading to a constant presence of the pathogen, but could also comprise of plant genotypes with variable resistance genes. The latter would in theory slow down the disease progress (Dangl and Jones 2001; Stukenbrock and McDonald 2008) but that is not seen in this case. The potential for evolution of novel pathotypes or resistance genesis however dependent on selection pressure against both pathogen and host. Eastern Africa provides a suitable

Table 4 Phenotypic responses to *S. turcica* (isolate Pak3, Ig23, Sor30, Ho6, and Ap) on five *S. bicolor* races. The classification of the 196 accessions is based on cluster analysis of final disease severity and AUDPC values resulting in four groups: resistant = 0–2, moderate resistant = 2–3, susceptible = 3–4 and highly susceptible >4

Sorghum races	Resistant	Moderately resistant	Susceptible	Highly susceptible
kafir	35	0	29	3
guinea	31	0	29	0
caudatum	26	2	23	2
bicolor	8	0	1	0
durra	3	0	4	0

environment for this to happen. It has been proposed that *S. turcica* either co-evolved with maize in Mexico or on sorghum in East Africa but support favouring one of the two hypotheses has hitherto not been presented (Borchardt et al. 1998). Sorghum was domesticated in East Africa approximately 3,000 years ago (Kimber 2000) and rich genetic resources are still present in the region. Maize on the other hand, is believed to have reached the East African coast in the mid-1600 s and the crop was probably introduced in Uganda 200 years later (Miracle 1965). Turcicum leaf blight on maize was first reported in Uganda 1924 but did not cause any major damage until 64 years later (Adipala et al. 1993a). Hitherto, we have no data supporting whether *S. turcica* was present in East Africa before the introduction of maize but moving crops to new regions has been shown to impact migration and host range expansion of pathogens (Banke and McDonald 2005; Couch et al. 2005).

In order to detect changes in the *S. turcica* population, mating type distribution was assessed. The equal distribution of the two mating types suggests that sexual recombination cannot be excluded. Previous research has suggested that tropical climates may be most suitable for sexual reproduction of *S. turcica* (Borchardt et al. 1998). Thus, we put large efforts into identifying sexual fruiting bodies, pseudothecia, containing ascospores on both diseased maize and sorghum leaves, but no such typical fungal structures were observed and putative candidates could not be verified to be *S. turcica* by molecular analysis. The wealth of information from fungal genome sequences has revealed that known asexual fungi can retain the machinery to undergo sexual reproduction during evolution (Dyer 2007; Galagan et al. 2005). Consequently, a presence of *MAT* genes does not automatically lead to a functional sexual reproduction. However, new discoveries of sexual stages of former defined asexual pathogens like *Candida albicans* and *Aspergillus fumigatus* are now arising (Butler et al. 2009; O'Gorman et al. 2009). The finding of extensive variability in the *Candida* mating type locus (*MTL*) and the lack of all four mating-type genes in the sexual species *Lodderomyces elongisporus* (Butler et al. 2009) further highlights the complexity of sexual life cycles and that most likely several independent evolutionary events have contributed to the plethora of pathways leading to sexual reproduction and transmission of genetic material.

Resistance in maize to turcicum leaf blight has hitherto been conferred by major race-specific genes *Ht1*, *Ht2, Ht3* or *HtN* or via partial, polygenic resistance, reviewed by Welz and Geiger (2000). Since the establishment of a gene-for-gene system by Leonard et al. (1989), additional resistance traits have been characterized adding to the complexity of the classification (Ogliari et al. 2005). *S. turcica* epidemics on maize in Uganda has in the past been attributed to race 0 of the pathogen (Adipala et al. 1993b; Bigirwa et al. 1993). Our investigation showed that also race 1, 2 and 3 are present and maybe others since unfortunately no seed from other maize differential lines were available at the time of assessment. The presence of multiple races on maize in East Africa has been suggested in other surveys (Weltz 1998; Muiru 2008) and race proliferation is also reported from China (Dong et al. 2008). The susceptible responses on the maize genotypes challenged with our set of *S. turcica* isolates were unexpected, especially as the control lines carrying no *Ht* resistance remained uninfected. The variable resistance response compared to earlier reports on maize deriving isolates is difficult to explain and may involve one or more factors such as a different recognition system, temperature sensitivity that erodes the resis-

tance response as seen in other pathosystems (Gregory et al. 2009), or disease susceptibility conferred by resistance genes (Lorang et al. 2007; Faris et al. 2010).

The origin of these races is still speculative and it is unclear if they represent an emergence of new races or if existing races are now being identified through a more extensive survey. It has been suggested that these new races may have evolved from race 0, which has been observed in the East African region for a long time (Adipala et al. 1993a). In the USA, race 0 seems to have been present for a long period and predominant in many states, but for example race 3 and 4 were identified as early as 1957 (Ferguson and Carson 2004) and race 2 was reported 1978 by Leonard making the race evolution ambiguous. It has later been shown based on shared haplotypes and phenetic analysis that race 1 most likely originated from race 0 in USA (Ferguson and Carson 2007). In the latter study the sorghum derived isolates formed a separate subgroup compared to maize isolates. Whether the *S. turcica* race evolution is driven by deployment of host resistance genes and/or is linked to an undiscovered sexual stage is not known. However, in Africa *Ht*-based resistance has not been extensively used. We hypothesize that presence of sorghum genotypes on which the *S. turcica* is less adapted increase selection pressure and may account for the development of the new races observed. Under the assumption that the *MAT* idiomorphs of *S. turcica* play major roles in its life cycle, the common occurrence of both mating type genes in many locations may support sexual reproduction and emergence of such novel races. Indeed compatible mating of both maize and sorghum derived isolates can be made under controlled laboratory conditions (Ferguson and Carson 2007). Overall, both this change in *S. turcica* race pattern and the fact that isolates from sorghum readily infect maize demand precautious measures, for example shows that regular monitoring is needed to identify potential new aggressive races that could threaten maize and sorghum production worldwide. Further studies on evolution of these new fungal races under multiple cropping systems as well as the introduction of resistance traits from sorghum accessions, identified in this study, into regional breeding programs, is a further very important development.

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