Inheritance of Pathogenicity in Setosphaeria turcica

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ABSTRACT

Of 47 field isolates of *Setosphaeria turcica,* 18 were virulent only to the host species from which each was isolated; whereas the remaining 29 were virulent to at least one other host. Virulence to corn and sorghum were inherited independently, as indicated by a 1:1:1:1 segregation for virulence to corn alone, sorghum alone, both corn and sorghum, and avirulence to both hosts from a cross between a corn specific isolate and an isolate virulent to both sorghum and Johnsongrass. Common occurrence of field isolates with virulence to both corn and sorghum, combined with independent inheritance of virulence to these two hosts, suggest that these could be treated as a third specialized form, *S. turcica* f. sp. *complexa.* Among ascospore isolates virulent to sorghum, approximately 75% were also virulent to Johnsongrass, suggesting that factors conditioning virulence to these two hosts are linked. On the basis of this apparent linkage and the congeneric relationship of these hosts, isolates virulent to Johnsongrass should be included in *S. turcica* f. sp. *sorghi.* Compatibility was inherited independently of virulence to corn in two crosses. In a third cross, compatibility was independent of virulence to sorghum and Johnsongrass also.

Among ascospore isolates avirulent to corn, three reactions (viz. chlorosis, fleck, and nonpathogenicity) were observed. Ascospore isolates avirulent to sorghum either incited flecks, or were symptomless on sorghum.

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Setosphaeria turcica (Luttrell) Leonard & Suggs and *Exserohilum turcicum* (Pass.) Leonard & Suggs are new taxonomic combinations for the ascigerous and conidial stages respectively, of the northern corn leaf blight organism (4). Since Mitra's report (8) of specialization in *E. turcicum* (= *Helminthosporium turcicum*) on corn or sorghum, there have been several others (1, 2, 3, 7, 12, 13) which also included Johnsongrass and Sudan grass as hosts. Although there was general agreement among these authors regarding the concept of specialization in *E. turcicum,* considerable differences in pathogenicities and racial affinities were demonstrated among the isolates collected from each of the four hosts. After studying field isolates of *S. turcica* (= *Trichometasphaeria turcica*) from corn, sorghum, and Johnsongrass and conducting hyphal-tip analyses of presumed heterokaryons, Masias and Bergquist (7) concluded that there were three stable host-specific forms in Hawaii. Two of these were

subsequently (1) referred to as *S. turcica* f. sp. *zeae* and *S. turcica* f. sp. *sorghi*. Those isolates which were virulent to both corn and sorghum were apparently placed in ff. sp. according to the host from which each was originally obtained.

There is a paucity of information regarding inheritance of pathogenicity to host species in *S. turcica*. Rodriguez and Ullstrup (13) reported on inheritance of pathogenicity in *S. turcica*, but because of low numbers of ascospore progenies, a clear pattern did not emerge. Nelson et al. (11) obtained nearly 2,000 ascosporic isolates from 35 crosses to evaluate genetic potential in *S. turcica;* however, since their research objective was to estimate pathogenic limits of the fungus rather than to study inheritance mechanisms, their findings did not clarify the genetics of host specialization in *S. turcica*. The present study was undertaken to elucidate the inheritance of host specificity and compatibility in *S. turcica*.

MATERIALS AND METHODS.—Monoconidial isolates of *S. turcica,* obtained from infected leaves of corn, sorghum, and Johnsongrass were collected from various islands in Hawaii. Cultures were also obtained from Masias and Bergquist. Isolates were grown on vegetable juice agar (10% Campbell's V-8 juice, 0.2% $CaCO_3$) at 24 C for five days under continuous fluorescent illumination (Cool-White, approximately 2,700 lx at the levels of the plates), then transferred to darkness at the same temperature for an additional two days to induce maximum sporulation. Inocula were prepared from seven-day-old cultures which were adjusted to 1.0 to 2.0 $\times 10^3$ spores/ml, in 1:2,000 Tween 20.

Inoculations of 23 corn, 13 sorghum, and 11 Johnsongrass isolates were made on Hawaii sweet corn hybrid H38(AA8 × P39), sorghum (NK125), and a Johnsongrass clone. All test hosts were highly susceptible to *S. turcica* isolates obtained from the respective host species. Seedlings at the five-leaf stage were inoculated with spore suspensions with an aerosol sprayer and kept in a moist chamber (25-28 C) for 24 hours, then transferred to greenhouse benches (21-32 C). Host reaction was considered susceptible, and thereby the test isolate was virulent, if large lesions appeared two weeks after inoculations; if only flecks, chlorotic spots, or no reactions were visible within this time period, the isolates were classified as avirulent.

For perithecial production Luttrell's method (5) was modified by pairing isolates at each end of autoclaved, 2-cm-long Johnsongrass culms, partially submerged in modified Sachs' agar (supplemented with 0.5% glucose and 0.5% starch). All 47 conidial isolates were tested for compatibility against standard mating type testers, R58(*A*) and 13a(*a*), at 24 C under continuous fluorescent illumination.

TABLE 1. Summary of mating types and pathogenicity of *Setosphaeria turcica* isolates from Hawaii

Forma specialis	No. of isolates	Mating types	
		A	*a*
S. turcica f. sp. *zeae*	15[a]	6	8
S. turcica f. sp. *sorghi*			
sorghum-specific	1	0	1
sorghum and Johnsongrass	17	1	16
Johnsongrass-specific	2	0	2
S. turcica f. sp. *complexa*			
corn and sorghum	5	2	3
corn, sorghum, and Johnsongrass	7	1	6
corn and Johnsongrass	0	0	0
Avirulent	0	0	0
Total	47[a]	10	36

[a]Mating type undetermined for one isolate.

In these studies, *S. turcica* genes for virulence to corn, sorghum, and Johnsongrass and their avirulent alleles were designated as *zeaA*$^+$, *zeaA1*;*sorA*$^+$, *sorA1* and *sorB*$^+$, *sorB1*, respectively. The presumed genotypes of the parents used in these studies were: K3 *(zeaA1 sorA*$^+$ *sorB*$^+$ *A);* C7 *(zeaA*$^+$ *sorA*$^+$ *sorB*$^+$ *a);* ascospore isolate B47 *(zeaA1 sorA*$^+$ *A);* ascospore isolate B65 *(zeaA*$^+$ *sorA*$^+$ *a);* and C2 *(zeaA*$^+$ *sorA1 sorB1 a).* A total of 366 single-ascospore progenies were isolated from three crosses and tested for compatibility. The ascospore isolates from the first two crosses were tested for pathogenicity to corn and sorghum. In the third cross, 160 ascospore progenies were examined for pathogenicity to all three hosts.

RESULTS.—Both mating types, *A* and *a,* were represented among conidial field isolates collected from Oahu, Kauai, and Hawaii. Of 23 corn isolates, nine were *A,* 13 were *a,* and one was undetermined (Table 1). Except for isolate K3, which was obtained from sorghum, all sorghum and all Johnsongrass isolates were compatibility *a*. All intragroup matings and selfs failed to produce perithecia after 21 days. The mating types of ascospore

TABLE 2. Inheritance of pathogenicity to corn and sorghum in *Setosphaeria turcica*

cross[b]	*zeaA*$^+$[a]		*zeaA1*		df	χ^2	P
	sorA$^+$	*sorA1*	*sorA*$^+$	*sorA1*			
K3 × C7	52 (22:30)[c]	0	58 (32:26)	0	1	0.3	0.5-0.7
B47 × B65	41 (16:25)	0	55 (26:29)	0	1	2.0	0.1-0.2
K3 × C2	42 (23:19)	33 (18:15)	39 (22:17)	46[d] (22:23)	3	2.2	0.7-0.9

[a]*zeaA*$^+$ *zeaA1* = gene for virulence to corn.
sorA$^+$, *sorA1* = gene for virulence to sorghum.
[b]Genotypes of parents: K3, B47 = *zeaA1 sorA*$^+$ *A;* C7, B65 = *zeaA*$^+$ *sorA*$^+$ *a;* and C2 = *zeaA*$^+$ *sorA1 a.*
[c]Figures in parentheses are mating type distributions *(A:a).*
[d]One isolate with undetermined mating type.

TABLE 3. Inheritance of pathogenicity to sorghum and Johnsongrass in *Setosphaeria turcica*[a]

		Johnsongrass		
		virulent *(sorB⁺)*	avirulent *(sorB1)*	Total
Sorghum virulent	*(sorA⁺)*	61 (35:26)[b]	20 (10:10)	81 (45:36)
avirulent	*(sorA1)*	0	79[c] (40:38)	79[c] (40:38)
Total		61 (35:26)	99[c] (50:48)	160[c] (85:74)

[a]Progeny from K3 *(sorA⁺ sorB⁺ A)* and C2 *(sorA1 sorB1 a)* cross.

[b]Figures in parentheses are mating type distributions *(A:a)*.

[c]One isolate with undetermined mating type.

progeny from all three crosses were 170 *A*, 195 *a*, and one was undertermined.

Each of the 47 conidial isolates from corn, sorghum, and Johnsongrass was virulent to the host from which it was isolated (Table 1), forming well-defined, wilt type lesions. Of 23 isolates from corn, 15 were virulent only to corn, five infected sorghum as well and three were virulent to all three hosts. Of 13 isolates from sorghum, one was pathogenic only to sorghum, eight infected Johnsongrass as well as sorghum, and four were pathogenic to all three hosts. Two of the 11 Johnsongrass isolates were virulent only to Johnsongrass, although inducing flecks on sorghum, whereas the other nine isolates also induced susceptible lesions on sorghum seedlings; none of the Johnsongrass isolates was virulent to all three hosts.

Susceptible lesions on corn and sorghum produced by 110 ascospore progeny from the K3 × C7 cross, and 96 ascospore progeny from the B47 × B65 cross, were well-defined wilt lesions. Noncompatible host reactions appeared either as flecks or were symptomless. Of the 110 ascospore isolates from K3 × C7 cross, 52 were virulent to corn and sorghum. Segregations for mating types among these 52 isolates were 22 *A* and 30 *a*. The remaining 58 ascospore isolates, virulent to sorghum only, segregated 32 *A* and 26 *a*. Similarly, 41 of the 96 ascospore progeny from B47 × B65 cross were virulent to both corn and sorghum (Table 2). Mating type segregations among these 41 isolates were 16 *A*:25 *a*. Among the 55 isolates virulent only to sorghum, segregations for compatibility were 26 *A* and 29 *a*.

The virulence of 160 monoascosporic isolates obtained from K3 × C2 cross segregated 33:39:42:46 based on reactions to corn and sorghum: first, virulent only to corn, second, virulent only to sorghum, third, virulent to both corn and sorghum, and fourth, avirulent to both hosts (Table 2). Among the ascospore progeny virulent to sorghum, including those which were also virulent to corn, approximately 75% were also virulent to Johnsongrass (Table 3). There were no isolates with virulence to Johnsongrass without virulence to sorghum. This was also the situation with all *S. turcica* field isolates of this study, with the exception of two Johnsongrass isolates, which caused flecking on sorghum.

Resistance and susceptibility in host-pathogen interactions among these 160 ascospore isolates were classified as for the field isolates and 206 ascospore progenies from the first two crosses. Some of the ascospore progeny from K3 × C2 cross produced an unusual reaction on corn seedlings which was classified as resistant. This was characterized by water-soaked, restricted, persistent chlorosis that did not develop into normal, wilt-type lesions 14 days after inoculation. Despite being non-necrotic, there was abundant in vitro sporulation on this tissue after surface sterilization and 24-hour incubation on water agar plates. Of the 85 ascospore isolates from this cross classified as avirulent to corn, 39 isolates induced water-soaked chlorosis, 28 induced flecks, and 18 were symptomless. The two mating types were distributed approximately equally among all categories. Among 79 ascospore isolates from the K2 × C2 cross which were avirulent to sorghum, 63 induced flecks, whereas 16 were symptomless.

DISCUSSION.—Five races of *S. turcica* were described by Masias (6) in Hawaii based on cross inoculations of isolates from corn, sorghum, and Johnsongrass on these hosts. Cross inoculations of 23 corn, 13 sorghum, and 11 Johnsongrass isolates demonstrated that all these races are represented in this study (Table 1) as well as a sixth type, pathogenic to both sorghum and Johnsongrass as represented by eight of 13 isolates from sorghum and nine of 11 isolates from Johnsongrass.

Compatibility of 366 monoascosporic isolates from two conidial crosses and one ascosporic cross segregated into 170 *A* and 195 *a*. Segregations did not deviate significantly from a 1:1 ratio in each of the three crosses or when the data are pooled, in conformity with Luttrell (5) and Nelson (9) that compatibility in *S. turcica* is controlled by a single gene.

The 1:1 recovery of both parental types with respect to corn pathogenicity in 110 and 96 ascospore progenies derived from K3 × C7 and B47 × B65 crosses respectively, demonstrated control by a single gene *zeaA⁺*, *zeaA1*. The distribution of 160 ascospore progeny obtained from K3 × C2 cross also showed that pathogenicity to sorghum is controlled by a single gene, *sorA⁺*, *sorA1* which is inherited independently of *zeaA* as well as compatibility. This is similar to crosses of *Cochliobolus carbonum* with *C. victoriae* in which pathogenicity and compatibility were inherited independently (10, 14).

The higher frequency of ascospore progenies virulent to both sorghum and Johnsongrass (parental) in contrast to those virulent only to sorghum (nonparental) suggests that factors conditioning virulence to those two *Sorghum* spp. are distinct, but genetically linked (Table 3). The absence of Johnsongrass-specific ascospore isolates was unexpected, particularly in light of recovery of two field isolates which were Johnsongrass-specific and many sorghum-virulent ascospore isolates which were avirulent to Johnsongrass. There were 20 isolates of the latter pathotype *sorA⁺ sorB1* and 61 of the parental type *sorA⁺ sorB⁺*, suggesting a crossover percentage of approximately 25%. In order to explain the absence of Johnsongrass-specific progeny, two alternatives are offered, viz. *sorA1 sorB⁺* is a combination in which Johnsongrass virulence is suppressed or is lethal. These alternative explanations can be reconciled with the occurrence of Johnsongrass-specific field isolates by the fact that the field isolates induce flecks on sorghum,

which suggest that their genotype could be other than *sorA1 sorB*$^{+}$.

All the monoconidial isolates of the present study produced either normal, wilt type lesions, resistant flecks, or were symptomless on H38 sweet corn hybrid. Similarly no chlorosis was produced on the sweet corn hybrid by any of the 206 ascospore progenies of the first two crosses. Thus, the induction of chlorosis as an expression of a noncompatible host reaction by numerous ascospore isolates of the third cross on this hybrid corn, warrants further investigation.

In an attempt to resolve the complexities of host specialization in *S. turcica,* Bergquist and Masias (1, 7) distinguished virulence of isolates into the specialized forms, *S. turcica* f. sp. *zeae* and *S. turcica* f. sp. *sorghi*. We suggest that the concept of *S. turcica* f. sp. *sorghi* be expanded to include those isolates virulent to Johnsongrass, due to the high frequency of isolates pathogenic to both sorghum and Johnsongrass (Table 1), the apparent linkage of sorghum virulence to Johnsongrass virulence, and the fact that the two hosts are congeneric.

The common occurrence of field isolates pathogenic to both corn and sorghum (1, 2, 3, 7, 8, 12, 13), and independent inheritance of virulence to these two hosts, suggests that a third grouping is warranted. It is therefore proposed that those *S. turcica* isolates which are virulent to corn as well as sorghum and/or Johnsongrass be named *S. turcica* f. sp. *complexa*.

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