Host-Specific Forms of Trichometasphaeria turcica in Relation to Homokaryons and Heterokaryons in Nature

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ABSTRACT

Trichometasphaeria turcica (Helminthosporium turcicum) isolates from nature pathogenic to only one host species of corn, sorghum, or johnson grass were homokaryons. *T. turcica* isolates from nature pathogenic to both corn and sorghum were heterokaryons, with one exception which is postulated to be a recombinant from a heterokaryon. Heterokaryon analysis suggests physiological distinctness of genes that condition pathogenicity to each host species. Genes which conditioned pathogenicity to corn and sorghum functioned in an additive manner in a heterokaryon. No evidence for recessiveness or dominance was detected in heterokaryons pathogenic to more than one host.

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Physiologic specialization in *Trichometasphaeria turcica* (Pass.) Luttrell *(Helminthosporium turcicum* Pass.) was observed among corn and sorghum isolates tested on their respective host sources (11). As early as 1922, Mitra (8) recognized *T. turcica* on corn and sorghum as two separate strains or races in India. Robles (12) identified two races of *T. turcica* on South American strains of corn. Compatibility tests revealed that isolates of the fungus from corn and sorghum were cross-compatible and that pathogenicity was under genetic control (13).

Demonstration that pathogenicity for two hosts resulted from heterokaryosis would be prima facie evidence that two genes are involved, each specific to a particular host. Heterokaryons have been observed in several species of the genus *Helminthosporium* (4, 5, 9, 10). We report here the characterization of heterokaryotic and homokaryotic forms of *T. turcica* in relation to host-specificity in nature.

MATERIALS AND METHODS. – Monoconidial isolates of *T. turcica* were obtained from typical lesions incited by this fungus on corn, sorghum, and Johnson grass, collected from islands of Hawaii, Kauai, Molokai, and Oahu. Inoculum was increased on "complete" medium modified from Beadle and Tatum (1, 2). All possible inoculations and successive

reisolations were made with 60 cultures each from corn and sorghum and 10 cultures from Johnson grass on seedlings of two cultivars each of sorghum (B-6202, I.S. 8750), corn (Oh 43, Oh 43-*Ht*$_1$A) and Johnson grass (Jg-1, Jg-2). On the basis of three successive pathogenicity tests with each isolate to these hosts, cultures were selected for tests of heterokaryosis.

Inoculations were made by spraying 2-wk-old seedlings with an aqueous suspension of conidia (1-2 × 10^3 conidia/ml) to which Tween 20 had been added at two drops/100 ml. Inoculated plants were maintained in a humid chamber for 14-16 h, then, removed to greenhouse benches. Plants with large lesions appearing 15 days after inoculation were classified as susceptible, while plants with flecks or chlorotic-lesions were rated as resistant.

The method utilized for separating component nuclear mixtures of presumed heterokaryons pathogenic to two hosts from nature consisted of successive passage of monoconidial isolates through corn or sorghum by successive inoculations and reisolation of hyphal tips of single germinated conidia on water agar. The purpose of host passage through both corn and sorghum was to prevent the inadvertent selection of host-specific isolates from a presumed heterokaryon. Hyphal tips were utilized for isolation from single germinated conidia after each passage through a host so as to isolate with certainty, the nuclear components of each single conidium. Johnson grass was not included in these tests.

Isolates of *T. turcica* pathogenic to a single host species were designated "host-specific" isolates while those pathogenic to more than one host species were "nonhost-specific" isolates. A wild-type pathogenic allele is designated by a "+" and a superscript lower-case letter to designate the host the fungus can attack (i.e., pathogenic to corn is $+^c$). A nonpathogenic allele is indicated by an italicized lower-case letter (i.e., nonpathogenic to corn is *c*).

RESULTS. – Among the monoconidial cultures from corn, all were nonpathogenic to Johnson grass (Jg-1, Jg-2), eight were pathogenic to both corn (Oh 43, Oh 43-*Ht*) and sorghum (B-6202, I.S. 8750), and 47 cultures incited typical lesions only in corn (Oh 43, Oh 43-*Ht*). Five cultures were nonpathogenic to all three hosts.

Of the monoconidial isolates from sorghum, six were pathogenic to both corn (OH 43, Oh 43-*Ht*) and sorghum (B-6202, I.S. 8750); two were pathogenic to corn, sorghum, and Johnson grass (Jg-1); 47 incited typical lesions only in sorghum (B-6202, I.S. 8750); and five were nonpathogenic to three hosts.

Six of the 10 isolates from Johnson grass were pathogenic only to this host (Jg-1) and four were nonpathogenic to all three hosts. Johnson grass clone Jg-2 was resistant to *T. turcica* isolates from all hosts.

Isolates pathogenic only to their original host, corn ($+^c$ *s jg*), sorghum (*c* $+^s$ *jg*), or Johnson grass (*c s* $+jg$), were stable through three cycles of inoculation and hyphal tip reisolation from single germinated conidia. In contrast, those pathogenic to both corn and sorghum were unstable in that reisolates were pathogenic to either corn alone, sorghum alone, both hosts, or neither host.

A nonhost-specific isolate $+^c$ $+^s$ (S-1) from sorghum was passed through corn in three cycles of successive inoculation/reisolation by hyphal-tip transfer of single germinated conidia. Four mono-conidial sub-isolates from S-1 examined for pathogenicity to corn and sorghum were stable $+^c$ $+^s$ forms; a fifth isolate segregated into $+^c$ $+^s$ and $+^c$ *s* forms. No *c* $+^s$ or *c s* forms were recovered from reisolations from corn or sorghum. Hyphal tip isolations showed monoconidial isolates 1-4 to be homokaryons. Stable nonhost-specific ($+^c$ $+^s$) forms were recovered from sorghum isolates S-1 following a series of reisolations from corn. Stability of this $+^c$ $+^s$ isolate was noted by inability to obtain from hyphal tip sources, isolates pathogenic to only one host after three additional host passages. By contrast, a nonhost-specific ($+^c$ $+^s$) isolate (C-1) from corn passed through corn in three successive cycles of reisolation, yielded only stable host-specific $+^c$ *s* sub-isolates. A third study involving nonhost-specific isolate (C-2) from corn when reisolated from corn yielded three $+^c+^s$, one $+^c s$, no *cs*, and no c^{+s} forms. However, when it was reisolated from sorghum, it yielded five $+^c$ $+^s$, no $+^c s$, one *c s*, and two c^{+s} forms.

DISCUSSION. – In preliminary tests with *T. turcica* isolates from hosts in nature, three stable host-specific forms were identified, based on pathogenicity tests to corn, sorghum, and Johnson grass. Heterokaryotic isolates were pathogenic to corn and sorghum or to corn, sorghum and Johnson grass. Isolates from Johnson grass were pathogenic only to this host, which is in agreement with data of Rodriguez and Ullstrup (13). Lefebvre and Sherwin (7) and Robert (11) did not recover isolates from nature pathogenic to both corn and sorghum or both corn and Johnson grass hosts. Rodriguez and Ullstrup (13) failed to obtain ascosporic progenies pathogenic to both corn and sorghum, corn and Johnson grass, sorghum and Johnson grass, or to all these hosts when host-specific isolates of *T. turcica* from these hosts were crossed. Larger numbers of progenies from crosses of isolates of multi-host forms may have revealed such forms.

It is postulated that $+^c$ and $+^s$ had undergone somatic recombination and mitotic segregation to form a stable homokaryon for $+^c$ and $+^s$. The hypothesis that somatic crossing-over had occurred would have to be substantiated by the recovery of four mitotic products, assuming that $+^c$ and $+^s$ are non-allelic and at separate loci. Four recombinant types were recovered from isolate C-2 in hyphal tip isolations of germinated conida. These were pathogenic to corn and sorghum, only corn, only sorghum and nonpathogenic to both hosts, $+^c$ $+^s$, $+^c$ *s*, *c* $+^s$ and *c s*, respectively.

A heterokaryon pathogenic to both corn and sorghum was identified by isolating nuclear components by hyphal tips from different germinating conidia. Evidence obtained indicates no dominance or recessiveness for factors conditioning pathogenicity to corn and sorghum. It is concluded that $+^c$ and $+^s$ are physiologically distinct genes, and

that they function in an additive manner in a heterokaryon. Genetic evidence of heterokaryons of conidial origin supports the cytological evidence of Knox-Davies and Dickson (6) that several nuclei pass into each conidium during ontogeny.

Stability of host-specific forms could assist disease control by crop rotation. It is conceivable that alternate cropping of corn and sorghum may disrupt the disease cycle in tropical locations. An abrupt change from corn to sorghum may result in a sufficient lag before inoculum could build up to sufficient levels to incite epidemics on the other crop host. Limited observations in our experimental field studies on the island of Kauai support this assumption of delayed epidemics in corn and sorghum rotations. Flor (3) found races of *Melampsora lini* (Pers.) Lev. possessing the least number of virulent genes needed to attack a widely grown variety predominated in nature. Races of flax rust carrying unnecessary virulence genes, that is, genes enabling them to attack cultivars not grown commercially, decreased. Current studies with *T. turcica* indicate a trend toward a loss from heterokaryons of an unneeded gene for pathogenicity. This extends Flor's hypothesis to nuclear mixtures in cells. For *T. turcica,* in the absence of the sexual stage, which has not been observed in nature, there remain mutation, the parasexual cycle, heterokaryosis, and a combination of these which would lead to the development of "races" of wider pathogenicity to different hosts.

We propose that isolates of *T. turcica* pathogenic only to a single host species be called "formae speciales" while the term "race" be reserved for those isolates of the fungus formae speciales virulent to a specific cultivar within a host species that carries a specific gene for resistance.

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