Physiologic Specialization in Trichometasphaeria turcica f. sp. zeae and T. turcica f. sp. sorghi in Hawaii

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ABSTRACT

A new race of *Trichometasphaeria turcica* f. sp. *zeae*, virulent to corn lines carrying *Ht* resistance genes (Ht_1^A, Ht_1^B, Ht^{Bw}, and Ht^{Mo1}) is currently reported from Hawaii. This race is designated race 2 to distinguish it from race 1, already known in Hawaii, that is avirulent on corn lines with these monogenic sources of resistance. Resistance gene Ht_2 conditioned a chlorotic-lesion reaction in seedlings and mature plants to both races. Non-host-specific and host-specific isolates of race 2 were identified from nature; i.e., pathogenic to both corn and sorghum or to corn alone, respectively. Races 1 and 2 of *T. turcica* f. sp. *sorghi* were identified on corn differentials. Corn isolates of *T. turcica* were generally characterized as having gray to green-white, profuse aerial mycelium whereas colonies of sorghum isolates were dark olivaceous, with scant, appressed aerial hyphae. *T. turcica* isolates from corn or sorghum could not be distinguished on the basis of conidial morphology or position of germ-tube protrusion. A phytoalexin-like substance obtained from corn and sorghum leaves of noncompatible, host-pathogen interactions was inhibitory to spore germination.

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Additional key words: physiological race, genes for resistance, resistance.

Cultural and pathological variation is a usual phenomenon in *Trichometasphaeria turcica* (Pass.) Luttrell (*Helminthosporium turcicum* Pass.) (6, 12). *T. turcica* from corn comprised two physiological races in Minnesota (11). Two physiological groups were reported in India by Bhowmik and Prasada (2) when isolates of the fungus from corn, sorghum, and Sudan grass were tested on these hosts. Masias and Bergquist (9) found heterokaryons of *T. turcica* from nature to be pathogenic to both corn and sorghum and homokaryotic isolates to be either host-specific and/or non-host-specific to corn, sorghum or Johnson grass. Masias and Bergquist (9) proposed *formae speciales* be used to designate isolates of *T. turcica* pathogenic to a single host species while the term "race" was to be reserved for those isolates of the fungus virulent to specific cultivar within a host species that carries a specific gene for resistance.

T. turcica from 26 states of the U.S.A. and 16 foreign countries in Central and South America, Europe, Africa, and Asia was unable to overcome resistance of corn seedlings carrying Ht_1^A gene (4). However, some ascospore progenies from crosses of isolates of *T. turcica* from restricted lesions on resistant corn inbreds produced typical susceptible lesions on resistant inbreds carrying the Ht_1^A gene (10).

We report here the results of studies on physiological specialization of *T. turcica* from corn and sorghum, morphological variation, and induction of a phytoalexin-like substance in noncompatible host-pathogen relationships. This is the first report of *T. turcica* f. sp. *zeae* and *T. turcica* f. sp. *sorghi* from nature virulent to *Ht* resistance genes in corn.

MATERIALS AND METHODS.—Methods of obtaining and propagating collections of *T. turcica*, culture media, of inoculating and incubating corn and sorghum plants, and of classifying host reactions and type of infection were described (9).

Five corn-specific isolates of *T. turcica* f. sp. *zeae*, designated Mol-1, Mol-2, C-1, C-3, and C-4 and one non-host-specific isolate (C-2) all isolated from corn; one sorghum-specific isolate of *T. turcica* f. sp. *sorghi* (S-2); and one non-host specific isolate (S-1) of *T. turcica*

isolated from sorghum were used. Mol-1 and Mol-2 of *T. turcica* f. sp. *zeae* were obtained from the island of Molokai while the remaining isolates were from the island of Kauai. An additional 60 monoconidial isolates from corn and sorghum were collected from the islands of Hawaii, Kauai, Molokai and Oahu. Corn host lines used were WF9 Ht_1^A, WF9, Oh 43, Ht_1^A, Oh 43, RB 37 Ht_1^A, RH 55 Ht_1^A, RH 55 Ht_1^B, RC 103 Ht_1^A, RC 103, RC 103 Ht_1^B, 14B Ht_2, Mol Ht^{Mol} and 14 Ht_1/Ht_1 Ht_2/Ht_2. The sorghum lines were: B 6202, I.S. 2663, 2680, 2687, 2760 and 8777.

Colony morphology was determined from 7-day cultures that had been maintained at 26 ± 2 C in continuous fluorescent irradiation. Five temp, 16, 20, 24, 28, and 31 ± 1 C with continuous cool-white fluorescent irradiation, were utilized for the study of growth rate. Conidial numbers were determined from fungal colonies after exposure to continuous darkness and continuous irradiation for 8 days at five temp, 16, 20, 24, 28, and 31 ± 1 C. In a further study of conidiation, cultures were exposed to continuous irradiation for 7 days at the above temp and subjected to darkness at 20 C for 24 h on the 8th day. Then conidial numbers were determined. Conidial germination was determined with 24-h-old conidia plated on 2% water agar and incubated 12 h at 16, 20, 24, 28, and 31 ± 1 C in continuous fluorescent light. Conidia, 24-h-old, were obtained by growing cultures for 7 days at 28 C under continuous light followed by exposure to darkness for 24 h at 20 C.

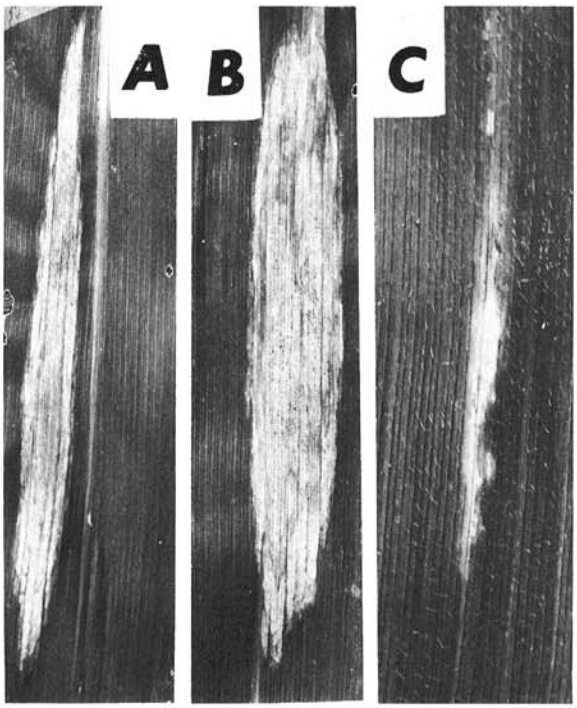

Fig. 1-(A to C). Lesion development indicated by races of *Trichometasphaeria turicica* in corn. **A)** *T. turcica* race 1 on susceptible Oh 43 $ht_1^A ht_1^A / ht_2 ht_2$ corn. **B)** Race 2 on susceptible Oh 43 $Ht_1^A Ht_1^A / ht_2 ht_2$ corn. **C)** Race 2 on resistant 14 B $ht_1^A ht^A / Ht_2 Ht_2$ corn.

Isolates S-2, S-1, C-3 and C-1 were examined for their ability to induce phytoalexin-like substances. A C-1; S-2; S-1 susceptible sorghum line (B 6202) and a C-1; C-3; S-1 susceptible corn line (RC 103), and a *Ht* resistant (RC 103 Ht_1^A) corn line were utilized. Three to four pieces of leaf 2-3 × 4-7 cm from 3-wk-old seedlings were placed in a solution of 15 percent sucrose and 20 ppm kinetin in 90-mm diam petri dishes. Drops of spore suspensions of each isolate were placed on the leaf surface of three hosts. Approximately 2 ml of diffusate from leaf surface were collected daily for 4 days with a micropipette and conidia and germ tubes removed by centrifugation. Effect of diffusates on conidial germination of four isolates was tested by suspending conidia in diffusates in a watch glass maintained in petri dish humidity chambers, and incubated at 24 C at continuous fluorescent irradiation for 24 h. Checks were drops of initially sterile water collected from leaves of each host after 1, 2, 3, and 4 days, and distilled sterile water not exposed to leaves.

RESULTS.—*Identification of physiologic races of T. turcica f.* sp. *zeae*.—Two physiologic races of *T. turcica* f. sp. *zeae* were distinguished among corn isolates tested. Isolates Mol-1, C-1, C-3, and S-1 were virulent to Oh 43 ht_1^A and avirulent to Oh 43 Ht_1^A and were designated race 1. Isolates Mol-2 andC-4 were virulent to both these hosts and were designated race 2. Race 1 reaction on Oh 43 Ht_1^A was a chlorotic lesion with no sporulation. Susceptible lesions incited by race 2 were indistinguishable on both lines (Fig. 1) and were similar in size.

Pathogenicity tests on corn lines RB 37 Ht_1^A, RB 37 RH 55 Ht_1^A, RH 55 Ht_1^B, RC 103 Ht_1^A, RC 103 Ht_1^B, Mol-Ht^{Mol}, and Ht^{Bw} with race 2 isolates of *T. turcica* f. sp. *zeae* revealed that these monogenic sources of resistance were susceptible to this race under field and/or greenhouse conditions. *T. turcica* resistant and susceptible infections also occurred on these monogenic sources of resistance in the field under natural conditions on the islands of Kauai and Molokai, indicating the presence of both races. Resistance gene Ht_2 in 14B ht_1/ht_1 Ht_2/Ht_2 and 14 Ht_1/Ht_1 Ht_2/Ht_2 conditioned a chlorotic lesion reaction to races 1 and 2 in seedlings and on mature plants (Fig. 1). Race 1 isolates were avirulent to these sources of resistance in seedlings and mature plants in both greenhouse and field tests. The infection type of race 1 on RC 103 was characterized as a chlorotic-lesion with reduced conidiation while race 2 conditioned a susceptible lesion and abundant conidiation. Resistant lesions incited by race 2 on seedlings carrying Ht_2 were characterized as an elongated chlorotic lesion which occasionally extended over the entire length of leaf in seedlings, while no lesions developed on upper foliage of mature plants at silking stage or maturity when exposed to natural infections of the fungus. Size of susceptible lesions on *Ht* resistant corn lines, excluding Ht_2, was identical to lesion size on *ht* susceptible counterparts.

Physiological specialization of T. turcica f. sp. sorghi.—*Sorghum bicolor* accessions I.S. 2663, 2680, 2687, 2760, and 8777 were resistant to isolates of *T. turcica* f. sp. *sorghi* and *T. turcica* f. sp. *zeae* from islands of Hawaii, Kauai, Oahu, and Molokai while inbred sorghum B 6202 was susceptible to host-specific isolate (S-2) and non-host-specific isolates (S-1 and C-1). The

above sources of resistance had similar hypersensitive-fleck reactions when tested with isolates of the fungus from corn and sorghum.

Non-host-specificity.—*T. turcica* isolate C-2 from corn was found to be pathogenic to Oh 43 Ht_1^A, Oh 43 ht_1^A and B 6202 sorghum while isolate S-1 from sorghum was pathogenic to Oh ht_1^A and B 6202. Fifty-five race 2 isolates were host-specific for corn while five were non-host-specific. Non-host-specificity relationship of isolates from corn and sorghum has been shown to be due to heterokaryons and/or homokaryons (9).

Colony morphology.—*T. turcica* colonies from sorghum were generally characterized by having a determinate margin, scant appressed aerial hyphae, whereas fungal colonies from corn had a determinate margin and profuse aerial hyphae about the center of a gray to green-white colony. Morphologically, colony characters of sorghum isolates were stable at 16, 20, 24, 28, and 31 C while colonies of corn isolates typical of *T. turcica* f. sp. *zeae* at 20-31 C appeared like sorghum isolates, *T. turcica* f. sp. *sorghi* at 16 C.

Growth rate.—Optimum temp for growth was 28 C for both corn and sorghum isolates of *T. turcica*. A slightly more rapid rate of growth was observed with fungal isolates from sorghum at 31 C.

Conidiation.—Conidiation was most abundant at 24 C for both corn and sorghum fungal isolates. Conidiation was inhibited at 28 C in continuous fluorescent light. Growth in continuous light at 28 C for 7 days followed by exposure to darkness at 20 C for 24 h resulted in abundant conidiation.

Conidial morphology.—Conidia of corn and sorghum fungal isolates were from 55-125 μ, 12.5-22.8 μ, and were three- to seven- septate. No differences in conidial morphology were observed in cultures grown under darkness from those grown in 12- to 14-h photoperiod, for either corn or sorghum isolates of *T. turcica*.

Conidial germination.—Optimum germination occurred at 24 C for corn and sorghum isolates. Germination of conidia of corn isolates was higher than that of sorghum isolates 6 h after incubation at 28 and 31 C. At higher temp, more lateral germination occurred in corn isolates than in sorghum isolates.

Production of phytoalexin-like substances.—Diffusates obtained from corn leaves inoculated with sorghum-specific isolate S-2 and sorghum leaves inoculated with corn-specific isolate C-3 inhibited conidial germination of host-specific and non-host-specific sorghum isolates (S-1 and S-2) and corn isolates (C-1 and C-3). Inhibition increased with incubation period, being slight on the first day and very high by the fourth day. Diffusates were produced more rapidly and in higher quantities by sorghum leaves inoculated with corn-specific isolate C-1 when compared to diffusate produced by corn leaves inoculated with sorghum-specific isolate S-2. Inhibition of conidial germination by diffusates produced by RC 103 Ht_1^A resistant corn leaves was very low during the first 2 days, becoming increasingly high on the 3rd and 4th day. The effect of these diffusates was similar on spore germination of four isolates tested. Slight inhibition by diffusates induced by S-1 and C-1 when inoculated to corn and sorghum, respectively, was observed 3 and 4 days after inoculation; none was noticed during the first two days. Germination of conidia in water previously maintained on the leaf of the hosts for 1, 2, 3, and 4 days was similar to that of distilled sterile water.

DISCUSSION.—Two colony types were clearly distinguishable among corn and sorghum isolates from nature. The colonies of isolates representing races 1 and 2 of *T. turcica* f. sp. *zeae* were identical and could not be distinguished on basis of colony morphology. These colony types were stable through several transfers and within a temp range of 21-31 C. Sorghum isolates of *T. turcica* f. sp. *sorghi* had a wider temp range for growth in culture. The occurrence of distinct fungal colony types of *T. turcica* isolates from corn and sorghum along with host-specificity provides basic characters for classifying isolates from corn and sorghum as "formae speciales" (9).

Lateral germination occurred for several corn isolates, whether or not this type of germination is significant as a morphological character of these isolates remains to be elucidated by further studies.

Development of *T. turcica* resistant cultivars of corn will be rendered more difficult by discovery of physiologic race 2 of *T. turcica* f. sp. *zeae* to which cultivars carrying *Ht* resistant alleles had previously been considered resistant (3). Race 2 is hereby utilized to designate those isolates of *T. turcica* f. sp. *zeae* virulent to corn lines carrying the Ht_1^A, Ht_1^B, Ht^{Bw} and Ht^{Mol} alleles for resistance. On the basis of these pathogenicity tests, the aforementioned resistance alleles could not be distinguished and thus they may be pseudo-alleles. Ht_2 conditioned resistance to both races of *T. turcica* and is clearly a different gene based on these pathogenicity studies, thus supporting Hooker's genetic data (A. L. Hooker, *personal communication*) of independence for Ht_1^A and Ht_1^B from Ht_2. Nelson et al. (10) noted that *T. turcica* isolates virulent to corn lines carrying Ht_1^A could be obtained from ascosporic progenies from crosses of isolates recovered from resistant lesions. Such isolates could have been utilized for evaluating corn selections for resistance to this potential biotype of the pathogen. The two races of *T. turcica* identified by Robles (11) in 1949 were not identified on differential hosts utilized in these studies and therefore could not be related to the present investigation which has utilized monogenic differentials of recent origin (3).

The current effectiveness of *Ht*-resistance gene(s) in continental U.S.A., Central and South America and Europe (4) suggests a marked uniformity of natural *T. turcica* populations with respect to reaction on the *Ht* resistance gene(s) on the continents. Hooker's digenic 14 Ht_1^A/Ht_1^A Ht_2/Ht_2 *T. turcica* resistant selection could not be distinguished on basis of disease reaction to race 2 from the monogenic corn line 14 B ht_1^A/ht_1^A Ht_2/Ht_2. In geographical locations where race 2 is prevalent, there probably is no advantage in utilizing this digenic combination. However, in areas of the world where race 2 has not been recovered from nature, corn lines for commercial use with these two resistance genes would be most useful. Hawaii with its year around subtropical environment is an ideal location for development and/or identification of digenic combinations for use in other agricultural areas that do not have race 2. The method suggested for development of two gene combinations

under Hawaii conditions would be to utilize the selective pathogenicity of races 1 and 2 to identify resistance genes in the progeny of hybrids between lines carrying single genes for resistance. The progeny with the desired combination of genes could then be backcrossed to either parent to reconstitute the desired inbred line that has two resistance genes. The advantage of the two-gene resistance line would be to reduce the possibility of a loss of usefulness of either gene in the host resulting from a chance mutation in the fungus. The chance that a race will become virulent from a digenic mutation is much less than the chance monogenic mutation.

In tests reported upon in this paper, race 2 isolates were recovered from the Hawaiian Archipelago. Race 1 was recovered from resistant lesions on corn cultivars carrying the Ht_1^A resistance allele from islands of Kauai and Molokai. The widespread distribution of race 2 of *T. turcica* f. sp. *zeae* in the Hawaiian Archipelago suggests this biotype may not be of recent origin. Masias and Bergquist (9) identified heterokaryons and homokaryons from nature to be non-host-specific and suggested the parasexual mechanism may be operative. This leads us to speculate that race 2 may have evolved as a result of mutation and mitotic recombination in the fungus, due to a lack of the sexual stage in nature here. Cultivation of many exotic tropical corn lines and recent development of an enterprising seed industry in Hawaii brings together many sources of resistance. Under such conditions in a suitable environment, the pathogen may have evolved a new form for existence. The occurrence of several variants of *T. turcica* f. sp. *zeae* in a limited area also suggests possible introduction of the organism to Hawaii from other locations. The well documented distribution *of Helminthosporium maydis* Nisikado & Miyake from contaminated seeds (7) may lead one to suggest possible seed transmission of *T. turcica.*

The breeding program, already in progress, for development of *T. turcica* resistant cultivars of sorghum, appears to be on a sound basis. Indian sorghum introductions 2663, 2680, 2687, 2760 and 8777 have been found to have several qualitative factors conditioning resistance to *T. turcica* (1). The presence of several resistance factors could account for the fact that no variability has been observed in *T. turcica* f. sp. *sorghi* populations when tested on these exotic sources of resistance. Thus far, there has been no evidence for existence of physiologic races of *T. turcica* f. sp. *sorghi* pathogenic to these sources of resistance in Hawaii. Nevertheless, it would be desirable to subject these resistant accessions to *T. turcica* infections in other parts of the world to determine whether forms capable of attacking them exist.

Corn line RC 103 Ht_1^A expressed a degree of resistance as indicated by the phytoalexin test, reduced sporulation in lesions incited by race 1, and a slow rate of build-up of the pathogen race 2 in secondary infections under field conditions. Previous reports have suggested that quantitative resistance factors are not necessary for adequate field protection (5). It may be that environment does not favor the pathogen in such areas and the simplest level of resistance may keep the pathogen under control. In wetter areas of the subtropics, qualitative resistance factors may not be effective alone and may have to be combined with quantitative resistance factors for effective disease control. Increased resistance of hybrid combinations could result from use of lines derived from unrelated sources of quantitative resistance.

Diffusates obtained from leaves of non-compatible host-pathogen systems were inhibitory to spore germination of four *T. turcica* isolates, suggesting production of some chemical substance which may play a role in pathogenic incompatibility of corn and sorghum. Bioassays with diffusates obtained from a resistant corn line were in agreement with results reported by Lim et al. (8). These results with phytoalexin-like substances extend the information to sorghum. Sorghum diffusates were produced in a shorter period of time when compared to results of Lim et al. (8) for corn diffusates. Of particular significance was induction of phytoalexin-like substance in RC 103 ht_1^A and RC 103 Ht_1^A when tested with a host-specific isolate of *T. turcica* from sorghum that was avirulent to corn. On the contrary a host-specific isolate from corn triggered the production of phytoalexin-like substances in sorghum B 6202 and corn RC 103 Ht_1^A. The substances from RC 103 ht_1^A and RC 103 Ht_1^A corn remain to be compared to phytoalexin-like substances from sorghum. It is not known with certainty whether quantitative or qualitative resistance factors are responsible for genetic control of phytoalexin-like substances induced by host-specific sorghum *T. turcica* isolates in RC 103 ht_1^A or if genetic factors other than resistance genes control this response. The results of non-compatible host-specific tests suggest this test may be utilized for identifying resistance factors non-detectable in in vivo studies with compatible host-specific forms of *T. turcica.* Further studies, including bioassays with extracts from corn and sorghum leaves inoculated with host-specific non-compatible isolates, characterization and identification of these substances from corn and sorghum and determination of their role in leaf blight resistance of corn and sorghum are suggested.

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