Heterogeneous Reaction of Shattercane to Periconia circinata and its Host-Specific Toxin

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ABSTRACT

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A collection of shattercane (*Sorghum bicolor*) from southeastern Nebraska contained a portion (35 to 40%) of seeds that was susceptible to the causal agent of milo disease, *Periconia circinata*, and to its host-specific toxin. The shattercane was not affected by inoculation with an isolate of the fungus nonpathogenic to grain sorghum nor by culture filtrates of the nonpathogenic isolate processed by the same methods used to prepare toxin from the pathogen. The level of susceptibility to the toxin was quantitatively intermediate between that of homozygous susceptible and heterozygous (F_1) grain sorghum genotypes. The remaining seeds (60 to 65%) as well as those of two other shattercane collections were resistant. Implications of these findings relative to the single-gene source of resistance to *P. circinata* are discussed.

Additional key words: pathotoxin, Periconia root rot, wild cane, *Pc* gene, sorghum.

Susceptibility of grain sorghum *Sorghum bicolor* (L.) Moench to *Periconia circinata* (Mangin) Sacc. is determined by the incompletely dominant *Pc* gene (9), which also conditions sensitivity to the host-specific toxin produced only by pathogenic isolates of *P. circinata* (8). Sources of resistance were discovered in the late 1930s (3), and the disease has been controlled effectively by eliminating the dominant gene pair (*PcPc*) from commercial sorghum hybrids. Apparently, the pathogen has a very limited capacity for genetic shifts from nonpathogenic to pathogenic strains or for the evolution of new races (5). Thus, the source of resistance, in the form of the homozygous recessive condition (*pcpc*), remains effective nearly 40 yr following its discovery. In recent years, however, *P. circinata* has been associated with sorghum plants exhibiting symptoms of root and stalk rot in Arizona (10), Texas (7), California (D. C. Erwin and R. A. Khan, *personal communication*), Kansas (L. K. Edmunds, *personal communication*), and Nebraska (L. D. Dunkle and G. N. Odvody, *unpublished*) and the possible existence of a new race has been suggested (11). These recent isolates have not been the pathogenic, toxin-producing strains typical of the original pathogen.

Analysis of the population of *P. circinata* in a milo disease nursery indicated that all isolates of the fungus from roots of resistant genotypes were not toxin producers and were only weakly pathogenic (5). Planting of resistant sorghum hybrids in the United States sorghum belt, where milo disease first was found, apparently has resulted in a reduction in the proportion of pathogenic strains of *P. circinata*. However, in the presence of susceptible sorghum genotypes, the population of pathogenic strains could increase again. A potential reservoir of susceptibility to *P. circinata* may be in shattercane (*Sorghum bicolor*; wild cane), which is interfertile with cultivated sorghums and is a major, persistent weed throughout much of the U.S. sorghum- and corn-growing region. The presence of susceptibility to *P. circinata* in collections of shattercane was investigated, and the level of susceptibility was compared with that of genotypes having known quantitative responses to the toxin and known reactions to the pathogen.

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MATERIALS AND METHODS

Plant material. Near-isogenic lines of Colby milo susceptible (S) and resistant (R) to *P. circinata* were used as standard genotypes for quantifying toxin response and for determining reaction to the pathogen. Seeds of the heterozygous (F_1) genotype (provided by K. F. Schertz, USDA-SEA, Texas A & M University) were obtained by crossing the resistant male-sterile line A Tx 3197 with S-Colby (9). Collections of shattercane seeds were taken from two sorghum fields in southeastern Nebraska and from a corn field in southern Indiana.

Toxin preparation. Cultures of toxin-producing (tox^+) or non-toxin-producing (tox^-) isolates of *P. circinata* were grown from single conidia produced on roots of S-Colby (5). Stock cultures were maintained on potato-dextrose agar (PDA). For toxin production, the fungus was grown at 24 to 26 C for 10 days in bottles containing 100 ml of liquid modified-Fries (MF) medium containing 0.1% yeast extract (6). The culture filtrate then was replaced with MF medium lacking yeast extract. After an additional 14 days, the culture filtrates (usually 2 to 5 L) were collected, concentrated 20-fold in vacuo at 35 C, deproteinized with one volume of methanol at 4 C for 48 hr, and the methanol-soluble material was concentrated to 50-fold with respect to the original volume of culture filtrate. This solution was adsorbed to activated charcoal (3 to 5 g per 2 L of original culture filtrate), and the charcoal was washed thoroughly with about 2 L of water until the effluent was devoid of yellowish pigment. Toxic activity was eluted from charcoal with 500 ml of 10% (v/v) pyridine (6). The pyridine eluate was concentrated to 300-fold, adjusted to 0.1 N acetic acid with glacial acetic acid and to pH 5.0 with redistilled pyridine, and loaded onto a column (3 × 40 cm) of QAE-Sephadex (Q25-120) anion exchange resin (Sigma Chemical Co., St. Louis, MO 63178). The resin was washed with 500 ml of 0.1 N acetic acid pyridine buffer (APB), pH 5.0, and then with 500 ml of APB, pH 3.0. Toxic activity was eluted with 1.0 N acetic acid, and the acetic acid was removed by concentrating the eluate at 35 C to 2,000-fold. Toxin preparations at this stage of purification contained five ninhydrin-positive spots separated by thin-layer chromatography on silica gel G. Host-specific activity was associated with one of those spots (Wolpert and Dunkle, *unpublished*). Unless stated otherwise, toxin

concentrations referred to in the experiments are concentrations of this refined preparation.

Bioassays. Susceptibility to the host-specific toxin was determined using three bioassay systems, and each experiment was repeated at least twice. Seedling bioassays (5) were used to determine the qualitative response of shattercane collections to toxin. Seedlings were grown in Stender dishes (5 cm diameter) containing 20 ml of nutrient solution (4) and incubated at 24 to 26 C under continuous fluorescent light (10,000 lux). Five-day-old seedlings (six per dish) were treated with toxin preparation diluted with water and incubated in light. Symptoms exhibited by susceptible seedlings were evident within 24 hr at concentrations of 20 μg toxin/ml. The symptoms consisted of rolled and flaccid leaves that initially were gray-green and later became necrotic. After 48 hr, seedlings were rated for the extent of these symptoms on a 0 (no symptoms) to 4 (dead seedlings) scale. More precise quantification of response to toxin by the leaf wilting angle criterion of Bronson and Scheffer (1) was not possible with young seedlings having short leaves.

Quantitative estimates of shattercane susceptibility to the toxin relative to that of susceptible sorghum genotypes were determined using root growth inhibition bioassays. Seeds were germinated for 24 to 30 hr at 26 C, and 25 seedlings with primary roots 2-3 mm long were incubated in the dark at 26 C for 48 hr in 9-cm-diameter dishes containing 15 ml of 0.01 M KH_2PO_4 (control) or of toxin diluted with 0.01 M KH_2PO_4.

Electrolyte leakage bioassays were conducted on individual seedlings grown in Stender dishes (3.8 cm diameter) containing 5 ml of nutrient solution. Roots of 5-day-old seedlings were washed thoroughly in distilled water to remove excess ions, and the seedlings were transferred to Stender dishes containing 5 ml of distilled water (conductivity = 1.4 to 1.8 μmhos) or toxin in distilled water. Conductivity of the solution surrounding the roots was measured at hourly intervals using a flow-through pipette-type conductivity cell (k = 0.316).

Seedling inoculation. Susceptibility to *P. circinata* was determined by inoculating 5-day-old S, R, or shattercane seedlings grown under continuous light in 15-cm-diameter petri dishes containing 200 ml of nutrient solution. Seedlings (50 to 60 per dish) were incubated at 24 to 26 C in 150 ml of a conidial suspension (300 to 500 conidia/ml of water). Conidia of tox^+ and tox^- isolates of *P. circinata* were produced as described (2). Inoculated seedlings were transferred after 3 days in the conidial suspension to fresh nutrient solution without inoculum. Seedling reaction was evaluated 11 days later when the experiment was terminated.

RESULTS AND DISCUSSION

Initial screening for susceptibility to *P. circinata* in the shattercane collections was done by the seedling bioassay method. Seedlings in two of the collections were not affected by relatively high concentrations (20 μg/ml) of the toxin preparation. The population of seedlings in the other shattercane collection (Lancaster County, Nebraska) exhibited a heterogeneous response to toxin; in two separate assays 25 and 45% of the seedlings, respectively, had died within 48 hr after exposure to toxin.

TABLE 1. Amounts of electrolytes lost from single seedlings of susceptible (S) or resistant (R) Colby milo or of shattercane when exposed to toxin from *Periconia circinata*[a]

Sorghum type	Treatment	Conductivity (μmhos)[b]	Symptoms[c]
R-Colby	Water	0.1–1.3	0
R-Colby	Toxin	0.2–2.8	0
S-Colby	Water	0.3–3.6	0
S-Colby	Toxin	72.4–75.4	4
Shattercane	Water	3.3–7.0	0
Shattercane (75%)	Toxin	0.9–7.8	0
Shattercane (25%)	Toxin	41.5–65.0	3

[a]Individual seedlings (6S, 6R, 19 shattercane) were grown in Stender dishes containing 5 ml of nutrient solution and then transferred to 5 ml of water or toxin solution (14 μg/ml of water).

[b]Conductivity measurements of the solution surrounding the seedling roots were made at hourly intervals and corrected for the zero time measurements. Values given for the S, R, and water-treated shattercane seedlings are the range of the 8-hr measurements of three seedlings. The values given for the toxin-treated shattercane seedlings are the range of measurements made at 8 hr on 12 seedlings that exhibited no symptoms or on four seedlings that were susceptible to toxin and later exhibited symptoms.

[c]Seedlings were rated after 48 hr on a scale of 0 (no symptoms) to 4 (killed by toxin).

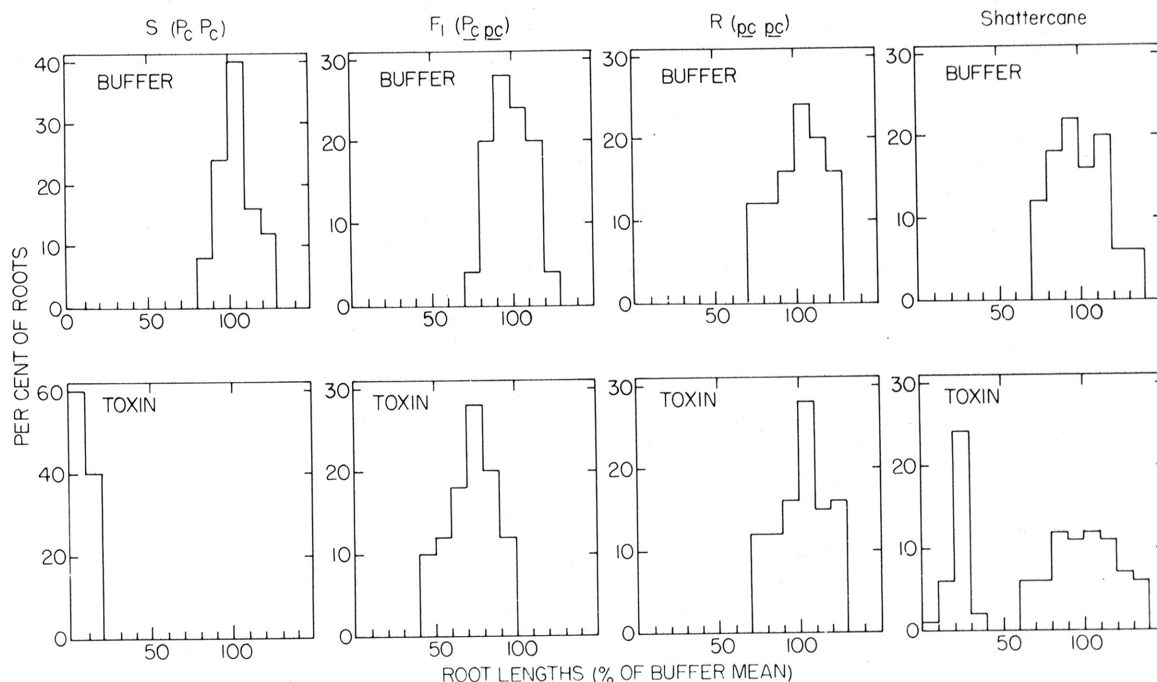

Fig. 1. Distribution frequency of root lengths of susceptible (S), heterozygous (F_1), and resistant (R) sorghum genotypes and of shattercane in root growth inhibition bioassays using toxin produced by *Periconia circinata.* Germinated seeds (100 seeds/treatment) were incubated 48 hr in 0.01 M KH_2PO_4 (BUFFER) or in 0.01 M KH_2PO_4 containing 7 μg toxin/ml (TOXIN).

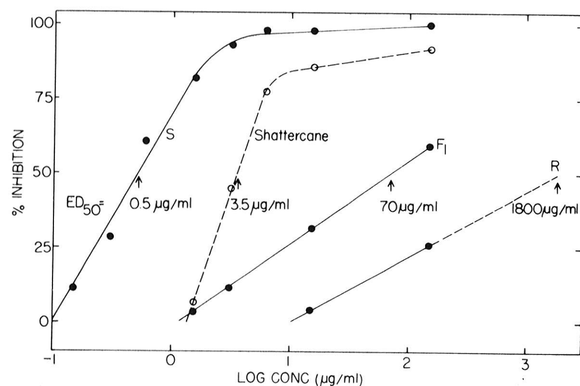

Fig. 2. Root growth inhibition bioassay of sorghum genotypes susceptible (S), heterozygous (F_1), and resistant (R) to toxin produced by *Periconia circinata* and of the portion of shattercane seedlings exhibiting susceptibility to toxin. Concentrations of toxin preparation required to inhibit root growth by 50% (ED_{50}) are indicated. Each data point is the average of ten measurements.

TABLE 2. Response of susceptible (S) and resistant (R) Colby milo and shattercane seedlings to inoculation with *Periconia circinata* conidia[a]

Sorghum type	Expt. no.	Inoculum	Seedlings killed (no./total)	Seedlings killed (%)
R-Colby	1	tox^+	0/61	0
	2	tox^+	0/50	0
	2	tox^-	0/50	0
S-Colby	1	tox^+	36/42	86
	2	tox^+	45/50	90
	2	tox^-	0/50	0
Shattercane	1	tox^+	20/60	33
	2	tox^+	21/51	42
	2	tox^-	0/50	0

[a]Seedlings grown in petri dishes containing 200 ml of nutrient solution for 5 days were inoculated with 300-500 conidia of a toxin-producing (tox^+) or non-toxin-producing (tox^-) isolate of *P. circinata* per 1 ml of water. Three days later, the seedlings were transferred to fresh nutrient solution and the number of seedlings killed by the inoculum was determined 11 days after that when the experiment was terminated.

Electrolyte leakage bioassays confirmed the heterogeneity in the population of shattercane seedlings (Table 1). In 25% of the seedlings, electrolyte leakage was induced by toxin (14 μg/ml) and symptoms developed only in those seedlings, whereas the majority (75%) of the seedlings was not affected by toxin. The extent of electrolyte loss from susceptible shattercane seedlings was less than that from S-Colby seedlings, and the rate of symptom development was slightly lower. In two other experiments different toxin preparations were tested; electrolyte leakage and symptom development occurred in 25 and 37% of the shattercane seedlings, respectively.

In root growth inhibition bioassays, shattercane seedlings responded as two distinct populations when exposed to toxin at 7 μg/ml (Fig. 1). This concentration of toxin inhibited root elongation of S-Colby by 95% and that of the heterozygous (F_1) genotype by 20 to 25% (Fig. 1). Roots of approximately 35% of the shattercane seedlings became reddish-brown, as did roots of S-Colby, and their growth was inhibited 75 to 80%. Root growth in the remaining portion was not affected. Similar results were obtained with a preparation of toxin purified to electrophoretic homogeneity and bioassayed at 50 ng/ml. Preparations of culture filtrates from a nonpathogenic (tox^-) isolate processed by methods used to purify toxin did not affect root growth, seedling growth, or electrolyte loss in the shattercane or grain sorghum genotypes.

To quantify the degree of susceptibility in the shattercane and relate it to known genotypes, root growth inhibition bioassays were conducted over a range of toxin dilutions (Fig. 2). Concentrations of toxin preparations required to inhibit root elongation by 50% (ED_{50}) in S-Colby, shattercane, F_1, and R-Colby seedlings were (in μg/ml) 0.5, 3.5, 70, and 1,800, respectively. Thus, in root growth inhibition bioassays, the susceptible shattercane seedlings were considerably more sensitive than the heterozygous seedlings and less sensitive than were seedlings homozygous dominant (susceptible) for the *Pc* gene. According to these results, the shattercane seedlings apparently were not heterozygous at the *Pc* locus. The possibility cannot be eliminated, however, that a modifier gene or a gene locus different from *Pc* is responsible for susceptibility in the shattercane population studied. Resistant mutants of S-Colby milo that have been analyzed genetically apparently are allelic at the same (*Pc*) locus (K. F. Schertz, *unpublished*), and susceptibility in shattercane may be due to another allele at that locus.

Seedlings were inoculated with conidia of *P. circinata* to determine whether the heterogeneous response to toxin was correlated with susceptibility to pathogenic isolates of the fungus. Of the S-Colby seedlings inoculated with the tox^+ isolate, 86 to 90% were killed within 14 days after inoculation (Table 2), and the remainder were stunted and chlorotic. The population of shattercane seedlings clearly segregated into susceptible (33 to 42%) and resistant portions comparable to those distinguishable by toxin bioassays. Growth of seedlings was not detectably influenced by inoculation with the tox^- isolate. Thus, the specificity in response to the pathogen corresponded qualitatively and quantitatively to the specificity of toxin reaction.

Because susceptible genotypes generally have not been available in commercial sorghum hybrids during the last two decades, it is difficult to account for the presence of susceptibility to *P. circinata* in shattercane, a noncultivated sorghum which has persisted as a weed and has acquired deciduous grain characteristics either through mutation or outcrossing to cultivated sorghums. Attempts are underway to develop pure susceptible lines of shattercane in order to establish whether susceptibility is due to: (i) a locus other than the *Pc* locus, (ii) modifier or suppressor genes, (iii) another allele at the *Pc* locus, or (iv) the same (*Pc*) allele that was present initially in the milo germplasm expressing susceptibility to *P. circinata*. If the latter is responsible for susceptibility, then the *Pc* locus apparently has persisted in the sorghum genome and has pleiotropic functions that are essential for normal growth. Accordingly, the recessive allele must determine not an absence of function or structure but an altered product, which, in absence of the toxin, has the same functions as the product of the dominant allele.

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