Characterization of the Periconia circinata
Population in a Milo Disease Nursery

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ABSTRACT

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The toxin-producing ability and pathogenicity of isolates of *Periconia circinata* isolated from soil and sorghum in a milo disease nursery were investigated. In culture, 13% of isolates from soil, 34% from roots of susceptible sorghums, and none from resistant sorghums produced a toxin that was active against susceptible sorghum seedlings. None of the isolates produced a toxin with detectable activity against sorghum genotypes considered to be resistant. Conidia of *P. circinata* persisted in field soil and, in laboratory studies, functioned as infective propagules. Conidia from both toxin-producing and non-toxin-producing isolates germinated on host roots, formed apparent infection structures, and incited cortical lesions on roots of susceptible and resistant sorghums. However, extensive vascular infections and eventual death occurred only on susceptible sorghums inoculated with toxin-producing isolates. Non-toxin-producing isolates of *P. circinata* were weak parasites of sorghum in the laboratory. The possible origin and pathogenic potential of *P. circinata* are discussed.

Additional key words: root exudates, host-specific toxin, pathotoxin, root rot.

Periconia circinata (Mangin) Sacc. causes milo disease or Periconia root rot of certain sorghum genotypes. Milo disease was prevalent on those genotypes during the 1920's and 1930's. Symptoms include a scalded or wilted appearance of plants that are usually stunted and often fail to head (4, 8). Severely infected plants develop a crown rot and die prematurely. Since only milo and milo derivatives of sorghum were affected, control of the disease was achieved readily by growing other sorghum types (5, 8).

Positive identification of the causal agent was not reported until 1948 (4), more than 20 yr after the disease first appeared and more than 10 yr after the advent of resistant sorghums (8). Before *P. circinata* was identified as the pathogen, other organisms, most notably *Pythium arrhenomanes,* were implicated as casual agents of milo disease (3, 12). Apparently many workers had overlooked fruiting bodies of *P. circinata* on roots of infected plants or had considered the fungus to be a saprophyte. Leukel (4) noted the abundance of conidia produced by *P. circinata,* but he was unable to induce them to germinate and did not speculate on their function. Conidia probably act as inoculum in soil, but only recently

has their ability to germinate been demonstrated (2). Isolation of *P. circinata* directly from soil has not been reported. Usually, the fungus is isolated as single conidiophores or mycelial growth from infected roots.

Isolates of *P. circinata* pathogenic to milo and milo derivatives were shown to produce a toxin specific for those genotypes (10). Host susceptibility to the pathogen and its toxin is conferred by the dominant gene *Pc*. Homozygously recessive *(pc pc)* plants are resistant and heterozygous plants (*Pc pc*) are intermediate in their response to the pathogen and its toxin (11). The *Pc* allele of the gene is relatively unstable and mutations of *Pc* to *pc* are common, occuring in one of approximately 8,000 gametes (11). Instability of this gene was probably responsible for the frequent appearance of resistant plants among those of susceptible genotypes and contributed to rapid development of isogenic resistant sorghum lines from susceptible lines (8, 11). Mutations from resistant *pc* to susceptible *Pc* have never been detected (K. F. Schertz, *personal communication*). Absence of this mutation, which could be easily detected in heterozygous plants, and apparent absence of other pathogenic strains of *P. circinata* account for the longevity (over 40 yr) of the *pc* gene as an effective means of disease control. Continued longevity of this resistance is vital because the only male-sterile-inducing cytoplasm currently available for hybrid seed production comes from milo genotypes.

In recent years, close association of *P. circinata* with roots of sorghum plants having poorly developed root systems, small heads, or small seed has been noted in most sorghum-growing areas of the United States, including Arizona, Texas, California, Kansas, and Nebraska (9, 13; Edmunds, Dunkle, and Odvody, *unpublished*). We have also observed *P. circinata,*especially late in the season, on roots of apparently healthy sorghum plants with well developed heads and root systems. The present and potential role of *P. circinata* as a pathogen of sorghum is unknown and the subject of controversy. We have insufficient knowledge of its biology to assess the possible existence of more than one pathogenic strain. For example, it has not been determined whether *P. circinata* prevails as a pathogen or as a saprophyte on sorghum roots, or more specifically, if *P. circinata* can act as a pathogen without producing a host-specific toxin.

We attempted to make those determinations and re-evaluate the potential of *P. circinata* as a sorghum pathogen by studying isolates of the fungus originating principally from the milo disease nursery at Garden City, Kansas. That nursery has been maintained continuously since the disease first appeared there in 1926; the pathogenic strain of *P. circinata* has been perpetuated in the nursery by growing susceptible Colby milo in alternate rows with test entries. Our specific objectives were (i) to determine the proportion of toxin-producing and non-toxin-producing isolates of *P. circinata* from nursery soil and from susceptible (S) or resistant (R) sorghums in the nursery and (ii) to determine the pathogenic potential of those isolates on S and R sorghums in the laboratory.

MATERIALS AND METHODS

Isolation of Periconia circinata from sorghum roots. — Root systems of 70 resistant (R) sorghums plus suceptible Colby milo (S) were collected from the milo disease nursery at Garden City, Kansas, in the fall of 1975. Only S Colby plants displayed typical milo disease symptoms, but *P. circinata* was visible on roots of a few R plants. Roots from S and R plants were surface sterilized for 2 min in 1% NaOCl, rinsed in distilled water, and incubated for 2 wk at room temperature on moist, sterile filter paper in petri dishes. Roots with sporulating *P. circinata* (Fig. 1-A) were held above potato-dextrose agar (PDA) plates and then tapped lightly to distribute mature conidia across the agar surface. Inoculated plates were incubated at 30 C for 18 to 24 hr and single-spore isolates were obtained by transferring individual germinated conidia to PDA plates. Five isolates from each genotype were obtained from single plants of 40 R genotypes. Due to the smaller number of S plants collected, 50 single-spore isolates were obtained from each of the S Colby plants (10 roots per plant and five isolates per root).

Isolation of Periconia circinata from soil. — Recently tilled soil was collected from the milo disease nursery in April 1976. Approximately 10 g of air-dried, pulverized soil was added to 50 ml of a saturated sucrose solution and stirred for 5 min to suspend conidia of *P. circinata*. The heavy soil particles were allowed to sediment and 10-ml aliquots of the liquid suspension were pipetted into 30-ml centrifuge tubes. This suspension was overlayed with 10 ml of a 40% (w/v) sucrose solution and centrifuged in a swinging-bucket rotor for 10 to 15 min at 6000 g. Conidia of *P. circinata* remained at the interface of the 40% and saturated sucrose solutions and were removed with a 10-ml soil pipette, washed through a 0.047-mm pore size (300-mesh) screen and collected on a 5μm Millipore filter. Individual conida were transferred onto 5-mm plugs of PDA containing 100 μg streptomycin sulfate/ml. When conidia had germinated (usually <5%), the individual PDA plugs were transferred to PDA plates until 100 single-spore isolates were obtained.

Sorghum culture in the laboratory. — Sorghum seeds were germinated on moist paper towels in an upright position for 48 hr at room temperature to obtain small seedlings with long, straight radicals. Seedling radicals were inserted through holes in an aluminum foil cover over Stender dishes containing 20 ml of nutrient solution. The seedlings were incubated at 26 to 28 C under continuous fluorescent light (10,750 lux) until 1-wk-old (third leaf in whorl). Each Stender dish contained sorghum plants of two genotypes (three S and three R Colby) for toxin bioassays or one genotype (five S or five R Colby) for pathogenicity studies. Nutrient solution was washed from sorghum roots with distilled water prior to toxin bioassays or other experiments. Plants were exposed to continuous fluorescent light and 26 to 28 C during all experiments.
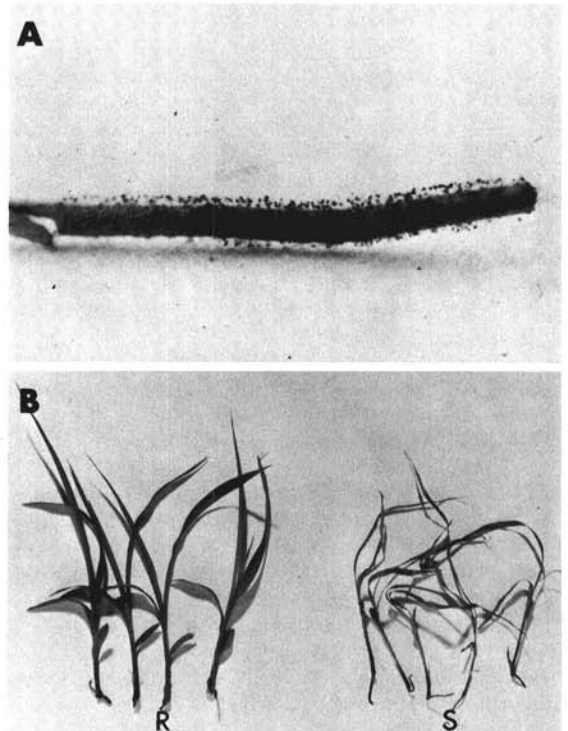

Fig. 1-(A,B). A) Conidia and conidiophores of *Periconia circinata* on an infected sorghum root after 2 wk of incubation at room temperature on moist, sterile filter paper. **B)** Sorghum seedlings of S and R Colby after 2 days of exposure to the diluted culture filtrate of a toxin-producing isolate of *P. circinata*.

Evaluation of the toxin-producing ability of isolates. — Isolates of *P. circinata* were grown on liquid modified Fries' medium (7) for 14 days at 25 to 30 C and the culture filtrates were collected. Toxin activity of filtrates was evaluated by immersing roots of near-isogenic susceptible and resistant sorghums (S and R Colby) in diluted filtrates. If toxin was present, symptoms (foliar wilting and plant death) developed on S Colby within 24 to 48 hr but R Colby was unaffected (Fig. 1-B). Bioassays were terminated after 3 days. Isolates with and without toxin-producing ability were designated tox+ and tox–, respectively. In an attempt to detect a toxin active against genotypes considered to be resistant, several filtrates representing isolates from resistant plants, but with no toxin activity against S or R Colby were tested against several common lines and hybrids.

Pathogenicity tests. — Conidia of tox+ and tox– isolates were used as inoculum to test pathogenicity and virulence of these isolates on S and R Colby. Conidia were produced by removing 5-mm plugs from PDA cultures of each isolate and incubating them on water agar (WA) for 2 wk at 30 C. Conidia were formed on the periphery of the plugs and easily suspended in liquids, with very little mycelial contamination. One-week-old plants (S and R Colby) were inoculated by spraying roots with a suspension of conidia or by dipping them directly into the suspension. Inoculated sorghum plants were placed in Stender dishes containing distilled water or nutrient solution and were incubated under continuous light at 26 to 28 C. Conidial germination, root infection, lesion development, and disease development were monitored daily. Re-isolation of *P. circinata* from inoculated, infected roots was achieved as previously described except that roots were surface sterilized for 30 sec.

RESULTS

Evaluation of toxin production by field isolates of Periconia circinata. — Only 13% of the isolates of *P. circinata* from soil and 34% of the isolates from S Colby were tox+ (Table 1). Of the S Colby roots from which isolates of *P. circinata* were obtained, 24% yielded both tox+ and tox– isolates, 24% yielded only tox+ isolates, and 52% yielded only tox– isolates. All isolates obtained from R genotype plants were tox–. None of the tox– isolates tested produced a toxin with detectable activity against several genotypes considered to be resistant. In seedling bioassays, toxin activity against S Colby was usually detectable at 1/500 dilution of filtrates from tox+ isolates. Since culture filtrates were routinely bioassayed at a 1/5 dilution, the amount of toxin in filtrates of isolates considered tox– must have been at least 100-fold less than that normally present in filtrates of tox+ isolates. Toxin activity could be predicted by pigment differences in culture filtrates. Tox+ isolates had yellow-green filtrates; tox– isolates had light-yellow filtrates.

Pathogenicity of field isolates. — Conidia of the tox+ or tox– isolates of *P. circinata* germinated on the root surfaces of S and R Colby plants within 24 to 48 hr after inoculation. Appressoria-like structures were formed by germ tubes on host roots after 48 hr (Fig. 2-A) and subsequently (3 to 5 days) were associated with small, red, cortical lesions that developed on roots of S and R Colby

inoculated with either isolate type (Fig. 2-B). However, extensive vascular infections of roots with disintegration of most cortical tissue and plant death occurred by 9 days post inoculation (PI) only in the S Colby/tox+ (host/isolate) combination (Fig. 2-C). Cortical lesions that formed on the R Colby/tox+ and S Colby/tox– combinations seldom developed into vascular lesions; disintegration of cortical tissue and foliar symptoms never occurred. Vascular lesions were readily distinguished from cortical lesions by the rapid emergence of mycelia from the cut ends of vascular tissue. Following surface sterilization treatments to kill conidia on the roots, *P. circinata* was re-isolated from all host/isolate combinations. Within 48 to 72 hr PI, *P. circinata* could be re-isolated from the S Colby/tox+ combination; more than 72 hr PI were required before the fungus could be re-isolated in all other combinations. Cultures of *P. circinata* reisolated from roots of all host/isolate combinations exhibited no change in their ability to produce toxin.

Conidia of tox+ or tox– isolates adjacent to roots of S or R Colby seedlings in distilled water germinated better (88%) than they did in distilled water alone (nil) or on PDA (15%). In a separate experiment, root exudates (concentrated × 100 from about 1 liter of root washings) stimulated conidia to greater germination (22%) than did distilled water with no exudates (6%).

The appressoria-like structures produced by conidial germ tubes on host roots prior to development of lesions were induced in response to hard, inert surfaces. Conidia germinating on glass or plastic surfaces formed structures similar to those formed on roots, but germinated conidia on WA or Millipore filters developed only branched or unbranched germ tubes.

DISCUSSION

Of the isolates representing conidia that overwintered during 1975-1976 in soil continuously cropped to suceptible sorghum for at least 50 yr, only 13% were tox+. Thus, natural populations of the fungus must be largely non-toxin producers, or if toxin producers, we did not have a sorghum genotype that permitted their detection. Percentage germination of isolated conidia was low (<5%) but no techniques other than incubation on PDA were used to induce greater germination. Each 10-g sample of soil yielded approximately 100 conidia of *P. circinata* and,

TABLE 1. Evaluation of isolates of *Periconia circinata* for toxin-producing ability[a]

Source of isolates[b]	tox–	tox+	% tox+
Soil	87	13	13
S Colby	99	51	34
R genotypes	200	0	0

[a] Toxin-producing ability was determined by growing isolates on liquid modified Fries' medium for 14 days and testing diluted filtrates for activity against S and R Colby seedlings.

[b] Isolates were obtained from roots of 40 resistant sorghums (five isolates per plant) and three S Colby plants (10 roots per plant and five isolates per root) collected in the milo disease nursery at Garden City, Kansas, in October 1975, or from soil collected in the nursery during April 1976.

although not quantitative, the isolation method probably did not discriminate between tox+ and tox– strains. If our soil data are representative of the soil inoculum potential, the roots of S and R genotypes in the milo disease nursery were exposed to a population of conidia with strain-type proportions of 13% tox+ and 87% tox–. On S Colby, despite the prevalence of tox– strains, we would expect selection pressure to favor root infections and colonization by the tox+ strain because of host susceptibility to toxin. Neither strain should be favored on R genotypes (insensitive to toxin) and, therefore, the proportion of
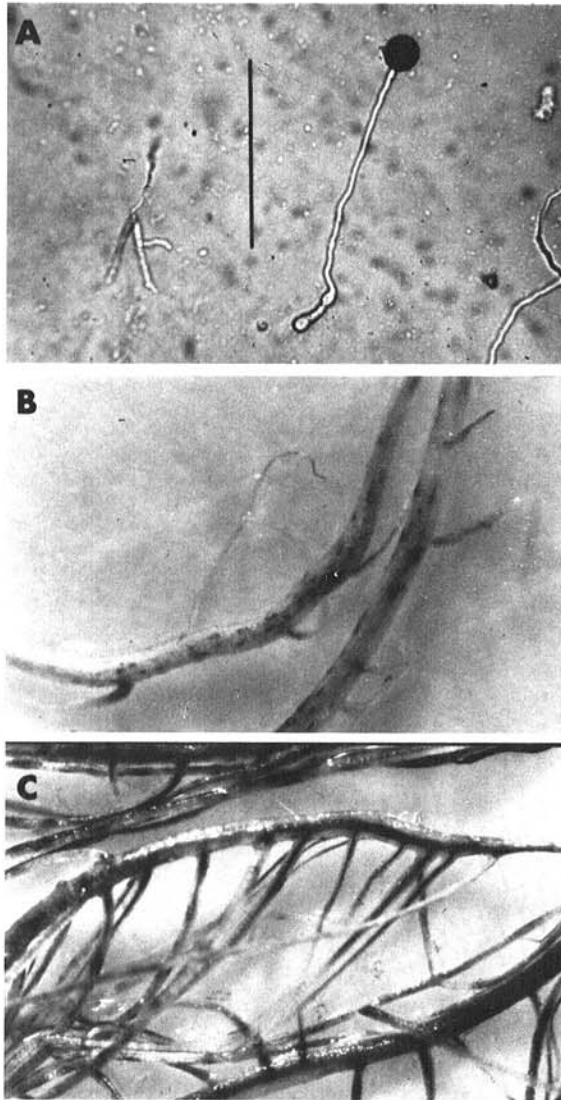
Fig. 2-(A to C). Infection processes and disease development of *Periconia circinata* in laboratory pathogenicity tests. **A)** Germinated condium of *P. circinata* which has formed an appressorium-like structure at the tip of the germ tube. The marker represents 100 μm. **B)** Cortical root lesions incited by a non-toxin-producing isolate of *P. circinata* on R Colby sorghum. **C)** Extensive vascular root lesions caused by a toxin-producing isolate of *P. circinata* on S Colby.

tox+ and tox– isolates from roots of those genotypes should reflect ratios of the fungal strains in field soils. Although tox– isolates predominated, our data show that some selection for tox+ infections occurred on S Colby (Table 1). Similar ratios of tox+ and tox– isolates from S genotypes have been reported (6). If tox+ and tox– isolates differed only in their ability to produce toxin, the prevalence of tox– strains on S Colby may be due to its greater soil inoculum potential, and to its multiplication in toxin-affected tissue near lesions caused by the tox+ strain. A similar phenomenon was reported for *Helminthosporium victoriae* and *H. carbonum* (1, 14). Non-toxin-producing (non-pathogenic) isolates of those species can colonize host tissue only if it is susceptible to and treated with the homologous host-specific toxin from pathogenic isolates. The absence of tox+ isolates of *P. circinata* from R genotypes was unexpected and may indicate that under field conditions tox+ propagules of the fungus are unable to infect or saprophytically colonize root tissue of R plants. The pigmentation differences between filtrates of tox+ and tox– isolates are also indicative of qualitative differences other than toxin production. Pathogenicity tests in the laboratory, however, indicated that tox+ isolates could infect and incite cortical lesions on roots of R plants. If the tox+ strain can infect only susceptible milo and milo derivatives in the field, an increase or even maintenance of its population in soil seems unlikely without regular presence of those genotypes.

The origin of the toxin-producing strain of *P. circinata* has remained an enigma ever since milo disease was first noted in 1924 (3). Introduction as seed-borne inoculum was implied in one report (12), but that seems unlikely because root infections with *P. circinata* rarely proceed past the crown (8). Sensitivity of conidia and conidiophores to desiccation and other environmental factors reduces the possibility of external, seed-borne inoculum (Odvody and Dunkle, *unpublished observations*). Pathogen dispersal by irrigation water and wind have been suggested (8) but they cannot account for the sporadic and sometimes localized occurrence of milo disease. Finally, milo disease has been associated with sorghum only in North America.

Prior to the introduction of sorghum, *P. circinata* probably existed in North America as a soil saprophyte or weak parasite of native plants. Since milo disease became apparent at specific locations only after S genotypes had been grown for more than one season (3, 8), the tox+ strain probably comprised a minute proportion of the soil population of *P. circinata* before that time. During the 1920's and 1930's, monoculture of susceptible sorghums apparently increased very rapidly the proportion of the tox+ strain in soil. A mutational event resulting in the ability of *P. circinata* to produce a pathotoxin seems unlikely, because milo disease developed over a wide geographical area in a very short period of time, and because no apparent mutation in the pathogen during the past 50 yr has resulted in other groups of sorghum being affected.

Renewed interest in *P. circinata* is concerned in part with the possible evolution of a new pathogenic strain of the fungus and the production of a toxin active against genotypes that presently are resistant. Milo disease represents activity by the kind of pathogen that could

expose current genetic vulnerability of our grain sorghums in the normally semi-arid climate of the Great Plains. But the role of *P. circinata* in current root rot problems of sorghum is uncertain. Our isolates of *P. circinata* from roots of sorghum grown at various locations in Nebraska did not produce typical milo disease on S or R genotypes in greenhouse tests, and we could not demonstrate production of a toxin active against sorghum of any genotype tested. Similar results have been reported by other workers (9, 13). Under laboratory conditions we determined that tox⁻ isolates from the milo disease nursery were weak parasites of S and R plants. The tox+ isolates were highly virulent only on S Colby and weakly virulent on R Colby. Another report suggests that *P. circinata* is a weak parasite of R sorghums (9).

The presence of tox⁻ *P. circinata* in many sorghum-growing areas may indicate an ongoing selective change in the soil populations that began with the advent of sorghums resistant to the tox+ strain. Unlike the rapid increase of the tox+ strain in soil planted to S genotypes, the increase in and selection for virulent, non-toxin- or low-toxin-producing strains of *P. circinata* may be relatively slow. If new races are being selected, they seem to be of low virulence and disease development may be more readily influenced by environment.

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