A Variable-Spored Isolate of *Drechslera dactylidis* Pathogenic on Orchardgrass and Corn

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ABSTRACT

ZEIDERS, K. E., 1980. A variable-spored isolate of *Drechslera dactylidis* pathogenic on orchardgrass and corn. Plant Disease 64:211-213.

Drechslera (*Helminthosporium*) *dactylidis* causing severe leaf spot and blotch was isolated from orchardgrass (*Dactylis glomerata*) near State College, Pennsylvania. The fungus regularly produced a certain proportion of abnormally curved, branched, or staurate conidia in culture and on leaves of inoculated orchardgrass plants. Information on cultural characteristics, morphology, and taxonomy of *D. dactylidis* is presented and discussed. In greenhouse inoculations, the pathogenicity of *D. dactylidis* was tested on 18 gramineous species. Only orchardgrass and corn (*Zea mays*) were susceptible. Twelve forage grasses and four small grains were nonsusceptible, thus indicating a very narrow host range among Gramineae for this pathogen. Results indicate that *D. dactylidis* is a potential threat to orchardgrass and possibly corn, especially during the warm summer months. The ability of this fungus to attack corn was demonstrated for the first time.

A dematiaceous hyphomycete identified as *Drechslera* (*Helminthosporium*) *dactylidis* Shoem., perfect state *Pyrenophora dactylidis* Ammon (1), was isolated from orchardgrass (*Dactylis glomerata*) with severe leaf spot and blotch in August 1975 near State College, Centre County, Pennsylvania. In greenhouse inoculations, *D. dactylidis* produced severe leaf blotch and blight symptoms on orchardgrass comparable to symptoms observed in the field (Fig. 1). The fungus was readily reisolated from the inoculated leaves. The symptoms on orchardgrass closely resembled those reported by Graham (3) as being caused by *Pleospora phaeocomes* (Rab.) Wint., with an unnamed *Helminthosporium* conidial state. Isolates of *D. dactylidis* regularly produced a certain proportion of branched, tripointed, or staurate spores in culture (Fig. 2) and on diseased leaf tissue of inoculated orchardgrass plants. This phenomenon is apparently rare within the genera *Drechslera* and *Bipolaris* (*Helminthosporium*).

Turner and Millard (11) reported that a variant tripointed spore form of *H. avenae*, whose basic spore form is straight-cylindrical, was common on natural and artificial media. Stevens (10) reported that this form occurred frequently in certain *Helminthosporium* spp. from barley (*Hordeum vulgare*) and corn (*Zea mays*) when grown on rich carbohydrate agar but not when transferred to cornmeal agar.

Because of the unusual spore morphology of this fungus and its virulence on orchardgrass, studies were made of its morphology, pathogenicity, and host range among forage grasses and cereal crops representing 11 tribes of Gramineae.

Contribution No. 511, U.S. Regional Pasture Research Laboratory.

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Accepted for publication 10 October 1979.

MATERIALS AND METHODS

The culture of *D. dactylidis* (1239 of the U.S. Regional Pasture Research Laboratory collection and ATCC 38281 of the American Type Culture Collection) used in most of the studies was isolated from orchardgrass adjacent to The Pennsylvania State University Wastewater Renovation and Conservation Project near State College. Another culture of *D. dactylidis* (483), isolated from orchardgrass in 1955 by J. H. Graham in Centre County, Pennsylvania, was used in some cultural, perfect state, and host range studies.

For all inoculations and most cultural experiments, *D. dactylidis* was grown on 20% V-8 juice agar (V8A) in petri dishes in an incubator at 22 C with alternating

12-hr periods of fluorescent light and darkness. For cultural comparisons, the two isolates were grown in three replicates on Difco potato-dextrose agar (PDA), cornmeal agar (CMA), and V8A. For perfect state studies, 3-mm plugs from cultures of *D. dactylidis* 1239 and 483 that had been stored at 5 C for 8 mo were transferred to fresh plates of V8A, PDA, CMA, and a fortified PDA containing 0.5 g per liter each of malt extract, yeast extract, and casein hyrolysate. The cultures were incubated at 22 C for 6 days, stored at 5 C for 4 days, then transferred back to 22 C for 7 days. In another test, diseased leaves of orchardgrass inoculated with *D. dactylidis* 1239 were placed in petri dish moist chambers and stored at 5 C for 6 wk. They were then incubated as described for agar cultures and examined for perfect state development several times over a period of 17 days.

In mating studies testing the effect of light on perfect state formation, 3-mm plugs of the two *D. dactylidis* isolates were paired on 12-mm squares of sterile corn leaf tissue on plates of Sach's agar (5) and incubated for 17 days at 20 C under continuous light, continuous darkness, or alternating 12-hr periods of light and darkness.

Conidial-mycelial suspensions of *D. dactylidis* were prepared for inoculations by flooding 7- to 8-day-old cultures with distilled water. The conidia and mycelia were brought into suspension by gentle agitation with a rubber policeman, then blended in distilled water with two drops of Tween 20 surfactant per liter. Inoculum concentration was about 2,500 spores per milliliter. For host range studies, plants of 19 species were grown from seed in a mixture of peat moss and vermiculite (1:1, v/v) in 10-cm pots. Plants were 6–7 wk old when inoculated, with the exception of corn and sudangrass, which were 4 wk old. Three replicate pots of each species were sprayed with a water suspension of *D. dactylidis* until thoroughly wet, then immediately placed in a large incubation chamber at 21 C without light. Plants were transferred to a greenhouse bench 48 hr later. Plants were examined for initial symptom development 2 days after inoculation. Disease severity was rated at 5 and 8 days after inoculation.

RESULTS

The pathogen. *D. dactylidis* isolates 1239 and 483 produced circular colonies in which the mycelium was dark gray in the center and white and fluffier toward the margin. About 8 days were required for colonies of isolate 1239 to cover an 85-mm petri dish; isolate 483 was slightly slower in radial growth rate. Growth rates were slower on PDA than on V8A and slower on CMA than on PDA. On V8A, daily growth rings were more pronounced in isolate 483 than in isolate 1239. Growth rings were not evident on PDA and CMA.

Light was required for sporulation by both *D. dactylidis* isolates. Without light, no sporulation by either fungus was observed on V8A, PDA, or CMA over a 14-day period. After 5 days of growth, sporulation by both isolates was profuse on V8A, less profuse on PDA, and absent on CMA. After 7 days, sporulation by both isolates on CMA was sparse.

Spores were olive to light brown; most were straight, but some were curved or branched and Y-shaped (Fig. 2). Curved spores were usually five- or six-celled, with the third cell from the distal end swollen in a manner similar to spores of *Curvularia* spp. Branched spores developed on V8A and PDA but not on CMA. A few spores of each isolate on CMA were curved, however, with the third cell from the distal end swollen. The basal cell and sometimes both the basal and the tip cells of spores were lighter in color and often slightly smaller in diameter than interior cells. The cells within a spore often varied considerably in length, some being two or even three times longer than others. Sometimes the cross septa were not precisely transverse but set at oblique angles. Straight spores measured 46–81 × 16–30 μm. The average of 50 spores was 58 × 15 μm. Spores had five to eight cross septa, with an average of six. Conidiophores were dark and bore one to four, but most commonly three, spores. Average conidiophore size was 105 × 5 μm with three cross septa.

On water agar, spores of both isolates began germinating after 3 hr. After 6–8 hr, spore germination by both isolates was mostly from end cells. Germ tubes from the basal cell were nearly always lateral, at or near right angles to the long axis of the spore. Sometimes there were two lateral germ tubes from the basal cell. Germination from the apical cell was usually straight out from the tip or at a slight angle. In some spores, the germ tubes from both the basal and apical cells were lateral. Curved or branched spores of *D. dactylidis* germinated in a manner similar to that of straight-cylindrical spores. After 24 hr, about 15% of the

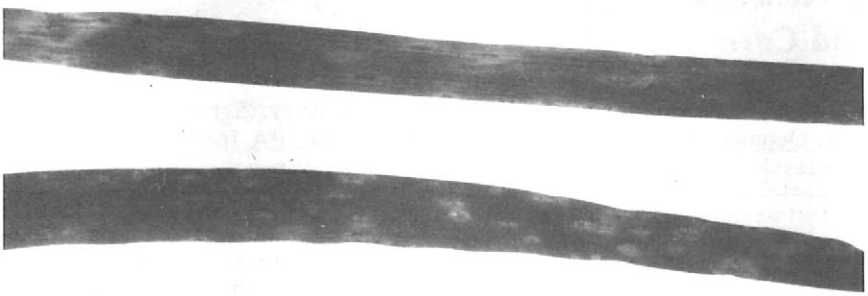

Fig. 1. Symptoms of leaf blotch on orchardgrass leaves 8 days after inoculation with *Drechslera dactylidis* isolate 1239.

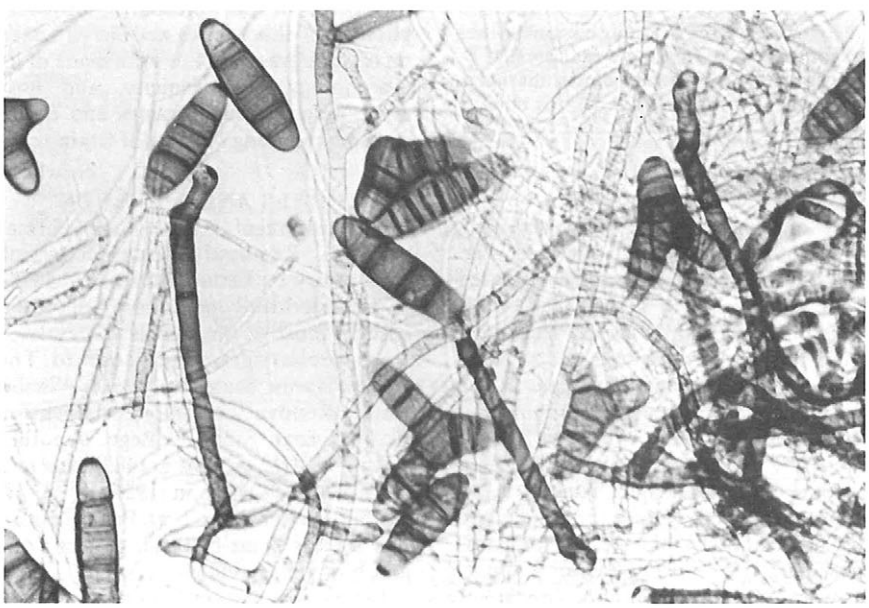

Fig. 2. Morphological variability in conidia of *Drechslera dactylidis* isolate 1239 from orchardgrass. From culture on V-8 juice agar. (×400)

spores usually had one germ tube from an interior cell.

In perfect state studies, dark sclerotial bodies, or protothecia, of *D. dactylidis* developed on V8A, PDA, and CMA, on sterilized corn leaf tissue on Sach's agar, and on inoculated orchardgrass leaves. Asci did not develop under any of the experimental conditions.

Host range. Pathogenicity of *D. dactylidis* 1239 was tested on 18 gramineous species representing 11 tribes. Only orchardgrass and corn, representing the tribes Festuceae and Tripsaceae, respectively, were susceptible. Severe leaf spot and blotch symptoms developed on both species by 5 days after inoculation. The following 16 species were nonsusceptible: *Phleum pratense, Festuca arundinacea, Bromus inermis, Phalaris arundinacea, Lolium perenne, Poa pratensis, Cynodon dactylon, Andropogon gerardi, Panicum virgatum, Sorghastrum nutans, Bothriochloa ischaemum, Sorghum sudanense, Avena sativa, Triticum aestivum, Hordeum vulgare,* and *Oryza sativa.*

D. dactylidis 483 from orchardgrass was also highly pathogenic on orchardgrass but only mildly pathogenic on corn. It was nonpathogenic on sudangrass (*Sorghum sudanense*), deertongue grass (*Panicum clandestinum*), and flat pea (*Lathyrus sylvestris*).

DISCUSSION

In 1959, Shoemaker (7) proposed a taxonomic revision of some graminicolous species of *Helminthosporium*, based on method of spore germination and conidial morphology, into two new genera, *Drechslera* and *Biploaris*. *Drechslera* produces cylindrical conidia that germinate from all cells, whereas *Bipolaris* conidia are fusoid and normally germinate only from end cells. The perfect states of *Drechslera* spp., when known, belong in *Pyrenophora* Fries (8) or *Pleospora* (Rab.) Ces. & de Not.; the perfect states of *Bipolaris* spp., when known, are in *Cochliobolus* Drechs. (2) or *Trichometasphaeria* Munk (4). *Drechslera* corresponds to the *Cylindro-Helminthosporium* group and *Bipolaris* corresponds to the *Eu-Helminthosporium* group of Nisikado (6). In both genera, conidia are produced only terminally on the conidiophore (acrogenous).

Graham (3) reported that conidia of the *Helminthosporium* state of *Pleospora phaeocomes* usually were 50–70 × 11–15 μm, with four to six septa. These dimensions are comparable to those reported by Shoemaker (8) and also to those reported in this paper. This study and Graham's report (3) indicate that germination of conidia is usually from end cells. These similarities suggest that the fungi are probably the same species except that Graham's *Helminthosporium* sp. did not produce curved or branched conidia. The similarity in disease symptoms produced on orchardgrass by the two fungi also suggest that they are the same species.

The fact that germination of conidia was usually from end cells suggests that the fungus was a *Bipolaris*. In the case of *D. dactylidis* isolates 1239 and 483, the germ tubes from the basal cell usually came out laterally, at or near right angles to the long axis of the spore, which is typical of *Drechslera* spp. (R. A. Shoemaker, *personal communication*). The nearly cylindrical shape of most of the conidia also indicates *Drechslera* and not *Bipolaris,* whose conidia are normally fusoid. Other characteristics of these fungi that agree with published descriptions of *D. dactylidis* include the lighter color of the basal cell and its slightly smaller diameter compared with interior cells, the variability in cell length, and the frequent irregularly oblique orientation of the cross septa (1,8). Branched or tripointed spores occurred regularly on V8A and PDA, moderately rich carbohydrate media, but not on CMA, a low carbohydrate medium. Stevens (10) reported similar results when *Helminthosporium* spp. from barley and corn were transferred from rich carbohydrate agar to CMA. The presence of aberrant curved, branched, or tripointed spores in *D. dactylidis* has not been reported previously. The significance and inheritance of this characteristic are not known.

Two of Stevens' (10) *Helminthosporium* cultures that developed aberrant tripointed spores were isolated from corn. That the tripointed *D. dactylidis* 1239 was isolated from orchardgrass growing adjacent to a corn field for two consecutive years may be significant. *Bipolaris* spp. are causal agents of several important diseases of corn (9). With the exception of a previous report by Zeiders (12), *Drechslera* segregates of *Helminthosporium* have not been reported on corn.

Of the 18 gramineous species representing 11 tribes on which pathogenicity of *D. dactylidis* was tested, only orchardgrass and corn, representing the tribes Festuceae and Tripsaceae, respectively, were susceptible. Twelve forage grasses and four cereal grains. representing nine tribes, were nonsusceptible, indicating a very narrow host range among Gramineae. Results indicate that this unusual isolate of *D. dactylidis* is a potential threat to orchardgrass and possibly corn, especially during the warm summer months. The ability of this fungus to attack corn was demonstrated for the first time.

ACKNOWLEDGMENT

I thank R. A. Shoemaker for assistance in identifying the fungus.

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