Pathogenicity of Soil-Borne Bipolaris sorokiniana on Seed and Roots of Three Perennial Grasses

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ABSTRACT

Seed and root inoculations of *Agrostis tenuis, Festuca rubra,* and *Poa pratensis* with *Bipolaris sorokiniana* resulted in increased seed and plant mortality, reduced rate of seed germination, and influenced the development of leaf lesions. Seed inoculation had a negligible effect on *A. tenuis,* markedly slowed the germination of *P. pratensis,* and severely reduced germination of *F. rubra.* Inoculation of uninjured roots caused no mortality among any of the grass species; inoculation of injured roots caused some mortality in *A. tenuis* and severely reduced the stand of *P. pratensis,* but had no effect on *F. rubra.* The incidence of leaf lesions increased on all plants growing in *B. sorokiniana*-infested soil, but was most severe on root-inoculated plants with injured root systems.

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Agrostis tenuis Sibth. (colonial bentgrass), *Festuca rubra* L. (red fescue), and *Poa pratensis* L. (Kentucky bluegrass) are important perennial grasses of the North-Central United States. *Bipolaris sorokiniana* (Sacc. in Sorok.) Shoemaker (= *Helminthosporium sorokinianum* Sacc. in Sorok. = *H. sativum* P.K. & B.) is a pathogen on *F. rubra* (3, 8, 10) and *P. pratensis* (1, 2, 3, 4, 5, 6, 11), but it is not known to infect *A. tenuis.* Most research with *B. sorokiniana* on *P. pratensis* has been with leaf infection (1, 5, 6, 11), but *B. sorokiniana* also has been isolated from roots of *P. pratensis* (4, 7, 9, 10) and *F. rubra* (9, 10). The extent to which *B. sorokiniana* can infect the roots of *P. pratensis* is not clear; some suggest that roots of *P. pratensis* are resistant to infection (7, 8), and others that root infection of *P. pratensis* can reduce seedling stands by about 30% (2). The inherent resistance or susceptibility of *A. tenuis* and *F. rubra* roots to infection by *B. sorokiniana* is unknown. The research reported herein was initiated to evaluate the influence of soil-borne *B. sorokiniana* on germination of seed and infection of roots

of *A. tenuis, F. rubra,* and *P. pratensis.*

MATERIALS AND METHODS.—*Bipolaris sorokiniana* was isolated from leaf lesions on *P. pratensis.* All cultures were grown on V-8 Juice agar [20% (v/v) V-8 Juice and 3% Bacto agar in distilled water] in 30-ml disposable plastic culture flasks at 22 C under 2,152 lx (200 ft-c) of continuous light. Conidia for inoculations were collected from 20- to 40-day-old cultures (5) by placing 15 ml of distilled water in each culture flask and shaking to suspend the conidia. The suspension was adjusted to 500 conidia per ml with an automatic particle counter (High Accuracy Products Corp.).

Seed and plants initiated from seed of *A. tenuis* 'Holfior', *F. rubra* and *P. pratensis* 'Park' were used for all inoculations. Fifty seeds, established plants with uninjured roots, and transplanted plants with injured roots each (of each species) were established individually in a steamed 1:1 sand-loam soil mixture in compartmentalized plastic flats (3.8 × 6.0 × 2.5 cm), and the soil in each compartment was infested with 10 ml of conidial suspension (totalling 5,000 conidia). Surface-sterilized seeds (15-20 minutes, 10% Clorox) were washed in sterile distilled water and placed in soil infested with conidia (poured on soil surface) at the time of planting, and maintained under mist propagation until germination occurred. Rate of germination, total germination, and subsequent leaf-lesion development was observed for 5 weeks after planting.

The uninjured root systems of plants established in compartmentalized flats were inoculated after development of the third leaf; inoculation was done by infesting the root-zone soil with conidia by means of a hypodermic syringe. Inoculation of injured roots of transplanted plants was accomplished by germinating surface-sterilized seed on filter paper (Whatman No. 1) in petri dishes; seedlings were grown on filter paper to the three-leaf stage of development and then transplanted to compartmentalized flats and the soil was infested with conidia (poured on roots and soil) at the time of planting. The process of removing plants from filter paper provided relatively uniform injury to root hairs and roots. All root-inoculated plants were observed 5 weeks for mortality and subsequent leaf lesion development among surviving plants. Controls were maintained for all inoculations.

RESULTS. — *Seed inoculations.* — *Bipolaris sorokiniana* had little effect on germination of *A. tenuis;* germination of inoculated seed was slowed, but total germination was only 6% below that of controls (Fig. 1). Total germination of inoculated seeds of *F. rubra* was 46% below that of controls (Fig. 2). Among inoculated seed of *P. pratensis,* rate of germination was slowed, but it progessively increased to within 8% of controls at the end of 5 weeks (Fig. 3). Leaf-lesion development on plants produced from seed inoculations was minimal. After 5 weeks, 0, 2, and 3 plants produced from inoculated seed of *A. tenuis, F. rubra,* and *P. pratensis,* respectively, showed leaf lesion development. No lesions occurred on control plants.

Root inoculations.—All plants of each grass species survived inoculation of uninjured roots, and all plants of *F. rubra* survived inoculation of injured roots. Inoculation of injured roots of *A. tenuis* and *P. pratensis* resulted in the death of 5 (10%) and 15 (30%) plants, respectively, within the 5-week observation period.

The incidence of leaf lesions increased on all grass species after root inoculation. Leaf lesions appeared on root-inoculated plants of *A. tenuis* and *F. rubra* between 2-5 weeks after inoculation. Inoculation of uninjured and injured root systems of *A. tenuis* resulted in 17 (34%) and 21 (42%) plants, respectively, with leaf lesions (Fig. 4). Inoculation of uninjured and injured root systems of *F. rubra* produced 6 (12%) and 18 (36%) plants, respectively, with leaf lesions (Fig. 5). Leaf-lesion development on *P. pratensis* started 7 to 12 days after root inoculations; after 5 weeks, 46 (92%) and 44 (89%) plants, respectively, from inoculations of uninjured and injured roots showed leaf lesions (Fig. 6). Some leaf lesions also occurred on control plants of all species (Fig. 4, 5, 6).

DISCUSSION.—Seed mortality in the presence of *B. sorokiniana* varies with the grass species; total germination of inoculated seed of *A. tenuis, F. rubra,* and *P. pratensis,* was reduced 6%, 46%, and 8%, respectively, below that of their controls (Fig. 1-3). Previous research showed that 80% of the seed of *P. pratensis* germinated in

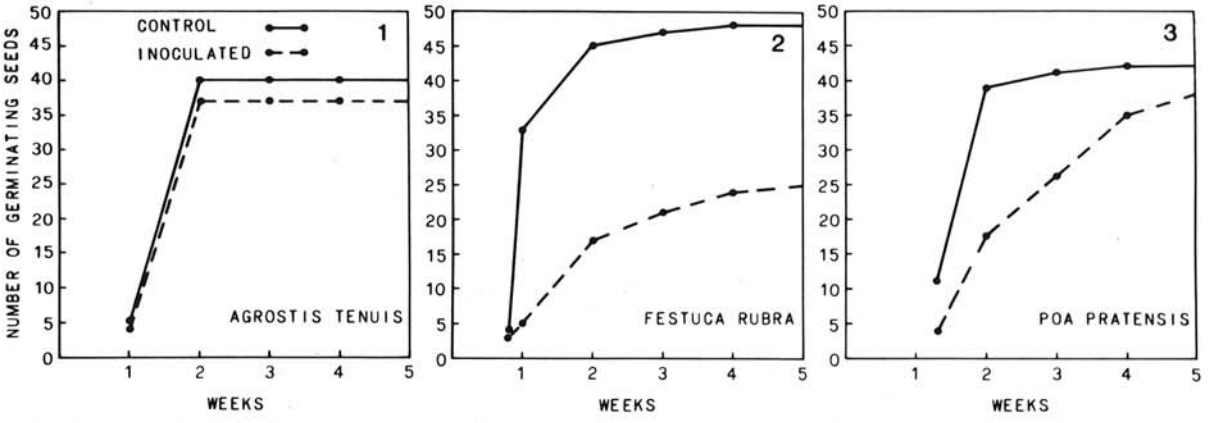

Fig. 1-3. Rate of germination and total germination of seed of *Agrostis tenuis* 'Holfior' (colonial bentgrass), *Festuca rubra* (red fescue), and *Poa pratensis* 'Park' (Kentucky bluegrass) inoculated with conidia of *Bipolaris sorokiniana.* **1)** *A. tenuis.* **2)** *F. rubra.* **3)** *P. pratensis.*

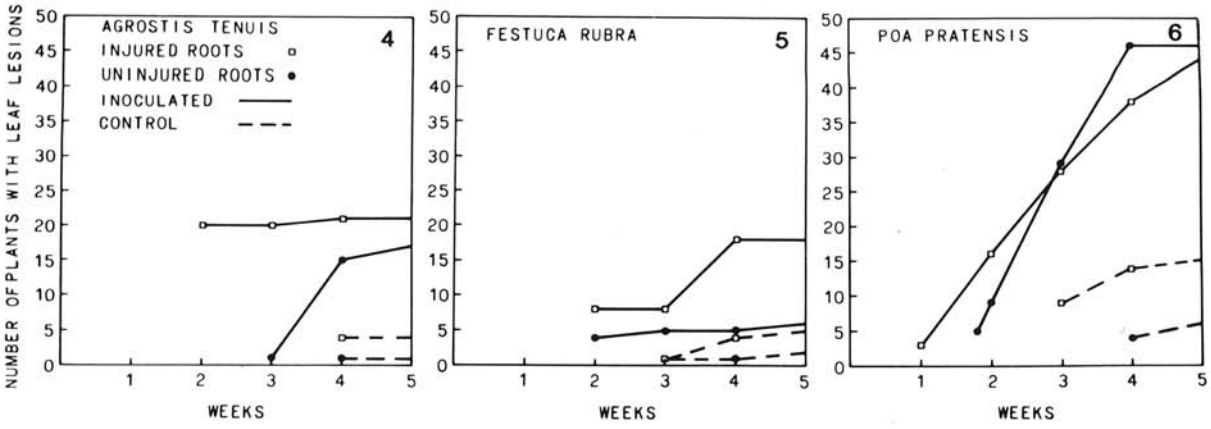

Fig. 4-6. Development of leaf lesions on plants of *Agrostis tenuis* 'Holfior' (colonial bentgrass), *Festuca rubra* (red fescue), and *Poa pratensis* 'Park' (Kentucky bluegrass) following inoculation of injured and uninjured root systems with conidia of *Bipolaris sorokiniana*. **4)** *A. tenuis*. **5)** *F. rubra*. **6)** *P. pratensis*.

the absence of *Helminthosporium* species (including *B. sorokiniana*), compared with 50% in their presence (2). In the present study, 84% of *P. pratensis* control seeds germinated compared with 76% of the inoculated seeds (Fig. 3). Differences in the previous (2) and present studies probably are attributable to the duration of the respective studies. In the previous study (2), observation of germination was terminated 2 weeks after seeding; in the present study, observation was extended to 5 weeks. After 2 weeks, 78% of the control seeds had germinated, compared with 36% of the inoculated seeds (Fig. 3); thus, at 2 weeks, the present and previous studies (2) are in agreement. At 5 weeks, however, germination of inoculated seed increased to 76% (Fig. 3). This suggests that, in the presence of *B. sorokiniana,* the rate of *P. pratensis* seed germination may be slowed, and that the failure of seed to establish seedlings may not be due solely to root infection as indicated by previous research (2).

Failure of seed to germinate and establish seedlings could result from seed rot, primary root infection, or coleoptile infection. Other research has characterized the seedling-infection phase of *B. sorokiniana* as a coleoptile rot that eventually spreads to the cortex of the roots (7). The slowing of the rate of seed germination (Fig. 2, 3) might further suggest the presence of substances from *B. sorokiniana* that inhibit seed germination without infection.

It is evident that the presence of *B. sorokiniana* in soil has a direct influence on leaf-lesion development. In that no direct leaf inoculations were made in this study, the inoculum for lesion development came primarily from the infested soil (probably via splashing). With the exception of *P. pratensis,* inoculation of injured roots resulted in plants with a higher incidence of leaf lesions than plants with inoculated uninjured roots (Fig. 4, 5). This suggests that root injury can influence the susceptibility of leaves to infection. Leaf lesion development on *P. pratensis* was equally severe after inoculation of injured and uninjured roots (Fig. 6). Most lesions occurred on leaves of tillers originating from the crowns at or below the soil surface where their exposure to *B. sorokiniana* was maximized. Lesions on control plants were from airborne contaminants; this is supported in that few lesions occurred, and that they were not present until the third or fourth week after root inoculation (Fig. 4-6).

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