An Inoculation Apparatus for Evaluation of Bipolaris sorokiniana Lesion Development on Progressively Older Leaves of Poa pratensis

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ABSTRACT

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A method of inoculating progressively older leaves of individual shoots of *Poa pratensis* with *Bipolaris sorokiniana* was developed. This facilitated evaluation of lesion development and disease severity. Lesion numbers were based on leaf area measurements, and different lesion types were used to quantify disease severity. The third youngest leaf of a shoot was least susceptible to infection and its disease severity rating was lower than that of other leaves. Lesion development and disease severity were greatest on the oldest inoculated leaves. This technique should expedite the study of leaf age-facultative parasite interactions.

Additional key words: facultative parasites, foliar pathogens, techniques.

Researchers conducting leaf inoculation studies with *Bipolaris sorokiniana* (Sacc. in Sorok.) Shoem. (= *Helminthosporium sorokinianum* Sacc. in Sorok. = *H. sativum* P. K. & B.) on *Poa pratensis* L. have generally disregarded leaf age in evaluating lesion development (1, 4, 5). Most studies have evaluated lesion development on the basis of total lesion counts per tiller, plant, or turf area; these types of evaluations fail to show potential differences in infection on progressively older leaves. The progressive decline in photosynthesis and loss of assimilates in progressively older leaves of perennial grasses (3, 6) could directly influence the parasitic capabilities of *B. sorokiniana*. The purpose of this research was to develop an inoculation apparatus and procedure for making precise evaluations of *B. sorokiniana* lesion development on progressively older leaves of *P. pratensis*.

MATERIALS AND METHODS.—*Preparation of pathogen and plants.*—*Bipolaris sorokiniana* was isolated from leaf lesions on *P. pratensis* on 3% Bacto agar, hyphal-tipped, and transferred to V-8 juice agar (20%, v/v) V-8 juice in 3% Bacto agar in distilled water). The isolate of the pathogen was grown in 30-ml disposable plastic culture flasks at 24 C under approximately 2,150 lx of continuous light. Conidia for inoculations were from 14- to 28-day-old cultures to keep virulence near optimum (2). Conidia were suspended in distilled water and mycelial fragments removed by passing the suspension through a 61μm sieve. Conidia were diluted to 250 per ml in 500 ml of sterile distilled water with an automatic particle counter (High Accuracy Products Corp., Claremont, CA). Each inoculated leaf received 0.1 ml (25 conidia) of the conidia suspension.

Poa pratensis 'Newport' was used for all inoculations. Vegetatively propagated plants were grown in a (1:1:1, v/v) loam-peat -Perlite mixture in 7.6-cm (3-in) diameter pots for approximately 90 days. One shoot with four healthy leaves on each plant was inoculated. Four leaves of three separate lots of nine shoots each were inoculated. Sterile distilled water was applied to control plants.

Inoculation apparatus and procedure.—Components of the inoculation apparatus are illustrated in Fig. 1. Each of the four youngest, visible leaves selected for inoculation, on individual shoots, were placed in inoculating tubes (Fig. 1-D) with the upper epidermis directly under the inoculating ports. Leaf blades were held in position for inoculation with pieces of styrofoam stopper (Fig. 1-F). A standard tuberculin syringe was used for inoculation; 0.1 ml of distilled water containing 25 conidia was applied to each leaf surface, through each of the five inoculation ports, in aliquots of 0.02 ml each (five conidia/0.02 ml water/inoculation port). The inoculation apparatuses (Fig. 1-G) and plants were placed in refrigerator crispers (Fig. 1-A) on folded cheesecloth (Fig. 1-B) which was saturated with distilled water to maintain humidity. The refrigerator crispers were placed in polyethylene bags to reduce the loss of water and maintained in growth chambers at 22-24 C with an 18-hour daylength (26,900 lx) for 10 days; then plants were evaluated for lesion development.

Infection evaluation procedure.—Ten days after inoculation, leaves were excised on either side of the inoculation tubes (Fig. 1-D) to a length of 10 cm. Leaves were evaluated for infection and disease severity. Infection was determined on the basis of the number of lesions per cm^2 of leaf area (10 cm × width) and on the type of lesion. Lesion ratings were as follows: no lesion = 0, fleck lesion = 1 (Fig. 2-A); lesion without halo = 2 (Fig. 2-B); lesion with halo = 4 (Fig. 2-C); streaking lesions between inoculation ports or on margin of leaf = 8 (Fig. 2-D); and coalescing of large lesions between inoculation

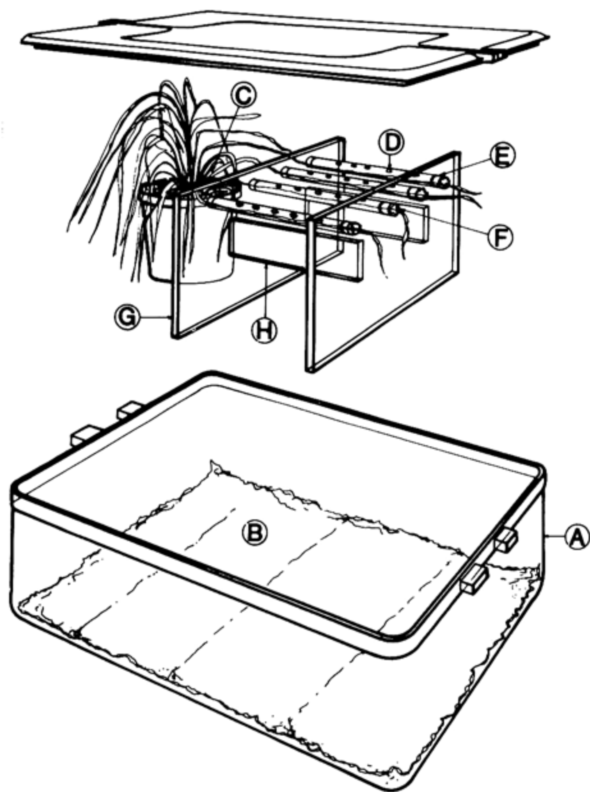

Fig. 1. Inoculation apparatus and component parts for evaluation of infection of progressively older leaves of *Poa pratensis* by *Bipolaris sorokiniana.* (A) Refrigerator crisper approximately 28L × 10H × 20W cm. (B) Folded cheesecloth (20 × 60 cm) saturated with distilled water to maintain humidity. (C) Shoot with four youngest, visible leaves mounted in (D) inoculation tubes (10 cm long, 8 mm I.D., pyrex) with five 2.0-mm inoculation ports (1 cm apart, 3 cm from ends of tube). Tubes mounted 1.5 cm from top of plexiglass holder. (E) Tygon tubing stops (approximately 1.0-cm long) placed on inoculating tubes to prevent slippage from holder. (F) Pieces of styrofoam plugs placed in tube ends to hold leaves in position. (G) Plexiglass (3.2 mm) holder for inoculation tubes (17L × 9H × 8W cm). (H) Plexiglass cross supports for holder (3.5 × 7.4 cm) mounted 3.5 cm from top.

ports = 16 (Fig. 2-E). The sum of the lesion-type ratings per leaf divided by the leaf area was used to provide a numerical value for disease severity. The data presented are the average of 27 leaves (three lots of nine leaves each) of each age.

RESULTS AND DISCUSSION.—The number of lesions per cm^2 of leaf area was similar on the first, second, and fourth leaf of inoculated plants; the third leaf, however, had significantly fewer lesions per leaf area (Table 1). Lesion-type ratings were almost the same on the two youngest leaves. The third leaf had the least severe lesion rating, but was not significantly different from the lesion ratings on the two youngest leaves (Table 1). The most severe lesion development occurred on the oldest

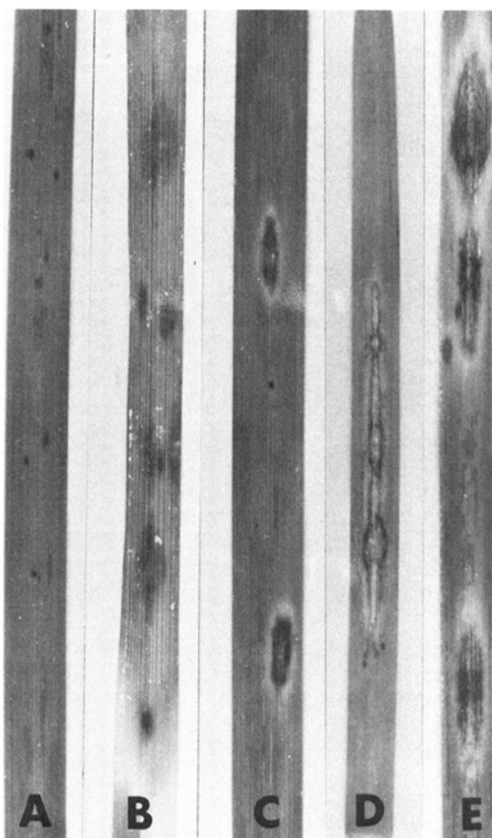

Fig. 2-(A to E). Lesion types and their ratings on leaves of *Poa pratensis* infected by *Bipolaris sorokiniana.* **A)** Fleck lesions = 1. **B)** Lesions without halos = 2. **C)** Lesions with halos = 4. **D)** Streaking lesions between inoculation ports = 8. **E)** Early stage of large lesions coalescing between inoculation ports = 16.

TABLE 1. Lesion and disease development on *Poa pratensis* leaves of different ages inoculated with *Bipolaris sorokiniana*

	Mean lesion number and type and disease severity[a]		
Leaf age[b]	Lesions per cm^2 of leaf area[c]	Lesion type rating[d]	Disease severity[e]
1	3.6	40.1	8.9
2	3.0	40.9	9.1
3	1.9	32.4	7.7
4	3.0	54.6	13.1
($P = 0.05$)[f]	1.1	19.2	4.5

[a]Mean of three lots of nine leaves each of each age group.
[b]Leaf one represents youngest visible leaf on shoot.
[c]Means of the number of lesions per leaf ÷ leaf area (10 cm long × width cm).
[d]Means of the sum of lesion ratings.
[e]Means of lesion type ratings ÷ leaf areas.
[f]Differences required for significance. Duncan's multiple range test.

leaves (leaf four). Disease severity was not significantly different among the three youngest leaves, but disease severity on the fourth leaf was significantly greater than that on the third leaf and nearly so than that on the first leaf (Table 1).

The inoculation technique provides a means of evaluating foliar pathogens on different aged leaves of a single shoot. Infection and disease development can be evaluated relative to leaf age and to cultural practices (cutting, fertilization, pesticides) that may influence the physiology and longevity of individual leaves of different ages. It is possible that measurement of the interactions between facultative parasites and leaf age may provide a greater understanding of the nature of facultative parasitism and the survival requirements of such organisms as parasites and saprophytes. Research is in progress in which this inoculation system is being utilized to determine the influence of nitrogen fertilization on the carbohydrate and nitrogenous constituents of different aged leaves and their subsequent infection by *B. sorokiniana*. Similar studies are also in progress with broadleaf herbicides.

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