Field Screening of Sugarcane for Eye Spot Resistance

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ABSTRACT

Rapid field screening of sugarcane for eye spot resistance was accomplished by inoculation with power spray equipment. Inoculum consisted of homogenized, mycelial cultures of *Bipolaris sacchari* in 5% blackstrap molasses. Molasses added to a similar inoculum gave a five-fold increase in infection in a pot experiment. The eye spot resistance of the screened population was significantly higher than that of the original unscreened population.

Injury to plants in the field caused by the host-specific toxin of *B. sacchari* in the inoculum was significantly correlated with resistance to eye spot.

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Additional key words: Saccharum sp., inoculum additives, host-specific toxin.

In a typical sugarcane (*Saccharum* sp.) breeding program the elimination of inferior genotypes is accomplished in at least five selection stages (the seedling stage and four vegetatively propagated stages) over a period of eight or more years. Plot size, number of parameters measured, and number of replications increase as clones advance through the selection stages. Because of the increasing investment in clones with their advance in selection stages, it is important to eliminate disease-susceptible clones as early as possible. The earlier the selections are screened, the more clones there are to screen; thus, rapid methods are necessary for screening in the early selection stages.

Byther and Steiner (1) used the host-specific toxin of

Bipolaris sacchari (Butler) Shoemaker [formerly *Helminthosporium sacchari* (6)] to screen sugarcane seedlings for resistance to eye spot. They were able to increase the frequency of eye spot-resistant clones from 15% in an untreated population to 50% in a screened population. The purpose of this report is to describe a rapid field method for the elimination of eye spot-susceptible clones in early vegetatively propagated selection stages.

MATERIALS AND METHODS.—*Effect of molasses in inoculum.*—A factorial experiment with 12 sugarcane clones and four levels of molasses (0, 1, 5, and 10%) in the inoculum was arranged in a randomized-block design with eight replications. A plot consisted of a single potted plant.

Inoculum was increased on modified Fries medium (3) solidified with 1.5% agar. Two-week-old cultures of *B. sacchari* were homogenized in a blender and diluted with water or molasses solution to give 32 ml of inoculum from each ml of original culture medium. The inoculum consisted mainly of fragmented mycelia with very few conidia. Inocula were applied with a hand-operated sprayer to plants growing in the greenhouse. Immediately after inoculation, plants were moved into a high-humidity chamber at 20 C and left overnight. Then they were moved outdoors for the remainder of the test period. The total number of eye spot lesions on the two most heavily infected leaves of each plant was counted 2 weeks after inoculation.

Field inoculations.—Isolates of *B. sacchari* were selected for high pathogencity in greenhouse tests. Different isolates were used in different years.

Inoculum was increased in 2-liter, screw-capped jars containing 250 ml of modified Fries agar. Each jar was seeded with 10 ml of a suspension of fragmented mycelia. Four days later, when the agar was covered with a dense mat of mycelia, the cultures were shaken to break up the agar and increase the surface available for fungal growth. Ten days later, cultures were homogenized in a blender and diluted with a molasses solution to give 32 ml of inoculum per milliliter of original culture medium and a final molasses content of 5%.

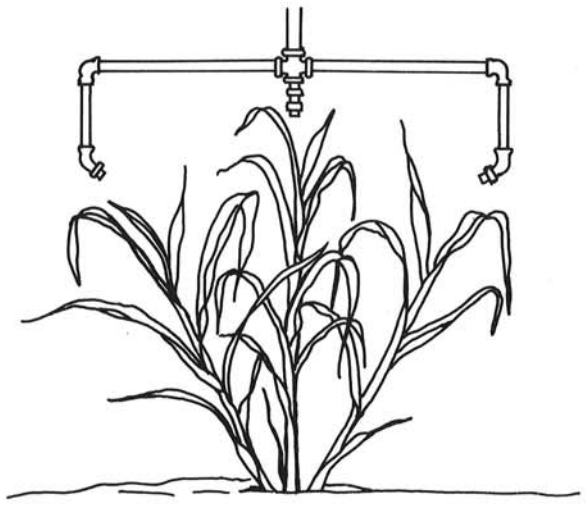

Fig. 1. Nozzle arrangement used for artificial inoculation of sugarcane field plots with *Bipolaris sacchari.*

All field plantings were inoculated between 1600 and 2200 hours in December or early January, when the plants were about 3 months old and 0.6-1.2 m tall. Inoculum was applied with a power sprayer through three No. 8004 nozzles directed as shown in Fig. 1. Each nozzle delivered 46 ml of inoculum per meter of row.

Inoculation of selections in stage I, 1972-73.—The sugarcane breeding program of the USDA in Florida has been described by Miller (4). Briefly, true seed are used to produce a highly heterozygous population of seedlings. Further selection stages are derived by vegetative propagation. Clones in stage I (one-row plots, 1.2 m long) and stage II (two-row plots, 4.6 m long) are unreplicated. Later selection stages are not involved in the work reported here.

In 1972-73, the stage I planting was composed of 5,486 clones planted in two separate fields. Interspersed at regular intervals throughout the test were plots of CP 63-588, a cultivar resistant to intermediate in reaction to eye spot. It served as a check on the uniformity of inoculation and as a standard of reference for disease severity ratings.

Plants were inoculated during a period when heavy dews were forming every night. About 6 weeks after the plants were inoculated, disease severity was rated on a scale from 0 to 6. A rating of 0 indicated no detectable disease development, and 6 indicated dead or severely affected plants. These data were transformed to the uniform disease rating system (scale from 0 to 9) as described by Hutchinson (2). All subsequent ratings were made on this scale.

Inoculation of selections in stage II, 1973-74.—The sugarcane inoculated in December 1973 was composed mainly of 584 stage II clones (selections from the stage I planting that was inoculated in 1972). Included in the same field were 21 plots of each of the check cultivars, CP 57-603 (susceptible), and CP 63-588 (resistant to intermediate).

Inoculation was repeated three times at biweekly intervals to increase the probability of obtaining adequate infection. One row of each two-row plot was inoculated. Disease severity was rated in late January.

Plants reacted to toxin in the inoculum 48 hours after inoculation, but the reaction was recorded 7 days after inoculation. Reddish streaks on the young leaves, was rated as 1 (reaction absent) or 2 (reaction present).

RESULTS.—*Effect of molasses in inoculum.*—The inoculum with 10% molasses produced about five times as many lesions as inoculum without molasses (Table 1). Analysis of variance revealed significance ($P = 0.01$) for molasses, for clones, and for the interaction of molasses × clones. The significant interaction occurred because the increase in the number of lesions varied greatly among clones. Molasses at any level, however, increased the number of lesions in all clones.

Screening selections in stage I.—The first symptom, discolored streaks on young leaves, appeared 24 hours after inoculation and persisted for several weeks. This appeared to be a reaction to the host-specific toxin in the inoculum. The symptoms were indistinguishable from those produced in previous tests (Dean and Miller, *unpublished*) by stalk injection of partially purified toxins obtained from G. W. Steiner and R. S. Byther or prepared

TABLE 1. The effect of molasses in inoculum on infection of sugarcane by *Bipolaris sacchari*[a]

Molasses (%)	Clone CP63 588	CP69 1056	CP69 1037	CP69 1087	CP68 1158	CP69 1016	CP69 1014	CP69 1062	CP69 1059	CP69 1052	CP69 1074	CP69 1061	Mean
0	9.9	5.0	13.4	13.5	7.5	5.1	12.6	9.6	0.9	7.4	18.8	17.9	10.1
1	19.3	42.0	71.3	43.5	24.4	40.1	23.4	45.8	3.3	31.3	25.5	39.4	34.1
5	44.1	36.6	36.8	34.1	32.0	53.1	53.4	37.3	6.1	17.9	47.0	48.8	37.3
10	44.4	56.9	87.9	53.1	42.0	141.0	96.1	31.3	6.0	26.6	38.4	38.6	55.2
Mean	29.4	35.1	52.3	36.0	26.5	59.8	46.4	31.0	4.1	20.8	32.4	36.2	

[a]Each amount is the number of eye spot lesions per plant as an average of eight replications.

from local isolates of *B. sacchari* by the method of Steiner and Byther (7).

Fungal lesions were noted 5 days after inoculation; secondary infection developed heavily on newly emerging leaves for many weeks. Growth of susceptible clones essentially stopped and did not resume until about 3 months later. Growing points of shoots were killed in many plots. An estimated 3% of the clones eventually were killed by eye spot.

Disease ratings of the check plots (CP 63-588) ranged from 3.0 to 6.0, with all but a few plots being rated 4.5.

Of 5,486 inoculated clones, 10% were rated resistant (rating classes 1-3), 35% intermediate (classes 4-6), and 55% susceptible (classes 7-9 (Fig. 2). The maximum rating of 9 was assigned to 19% of the clones; this indicates that essentially no green leaf tissue was left in these plots.

Screening selections in stage II.—There was much less disease in this test than in the stage I planting of the previous year, but there was enough to permit discrimination among several levels of eye spot resistance.

The average rating of CP 63-588 (intermediate resistance) was 2.9 with a range from 2 to 4 (Fig. 2). The average rating of CP 57-603 (susceptible control) was 6.9 with a range from 5 to 8. The difference between disease ratings of CP 63-588 and CP 57-603 was significant, $P = 0.01$.

The average rating was 4.9 for the 101 stage II clones responding to toxin in the inoculum, but 3.3 for those clones showing no reaction. This difference was significant, $P = 0.01$. The correlation coefficient between toxin reaction and disease severity was low ($r = 0.405$), but significant, $P = 0.01$.

Only 28 clones (all rated in the intermediate class in stage I) were identifiable for eye spot rating class in both stage I and stage II. The clones received a mean eye spot rating of 3.4 in stage II with a range from 2 to 5. This may be compared with the resistant-to-intermediate check cultivar, CP 63-588, which received a mean rating of 2.9 with a range from 2 to 4. Therefore, evidence based on these 28 clones implies good agreement between years in the classification of clones for eye spot resistance.

DISCUSSION.—The enhancement of pathogenicity of *B. sacchari* by inclusion of molasses in the inoculum was presumably a nutritional effect on the fungus. Other workers have reported increased pathogenicity of fungi when nutrients were included in the inoculum (5, 8).

Steiner and Byther (7) found that undiluted culture filtrates of *B. sacchari* showed a nonspecific toxicity to sugarcane, and failed to distinguish between resistant and susceptible clones. A ten-fold dilution of the filtrate seems to have differentiated between a resistant and a susceptible clone. In our work, the low but significant correlation between reaction to toxin in the inoculum and reaction to infection by the fungus shows that the crude toxin in the inoculum was specific.

In any program using artificial inoculation to screen for disease resistance, it should be shown that the plants react similarly to artificial and natural inoculation. In the stage I clones in 1972-73, disease severity ratings were based on secondary (natural) infection. In the stage II clones in 1973-74, the ratings were based on primary infection resulting from artificial inoculation. Thus, the evidence for agreement between years provided by the 28 clones that were identifiable in both years is evidence also for a common reaction to artificial and natural inoculation.

The mean eye spot severity rating was 6.6 for the stage I selections and 3.6 for the stage II selections. The decrease in severity appears to be about half genetic and half environmental. The evidence for this is the decrease in the average severity rating of the check cultivar, CP 63-588 (from 4.5 in stage I to 2.9 in stage II). This decrease in the rating of the check is roughly half of the decrease in the rating of the selections.

The genetic increase in resistance between stages I and II resulted from natural selection. Highly susceptible clones were killed; slightly less susceptible clones were severely stunted in the early part of the season. This early

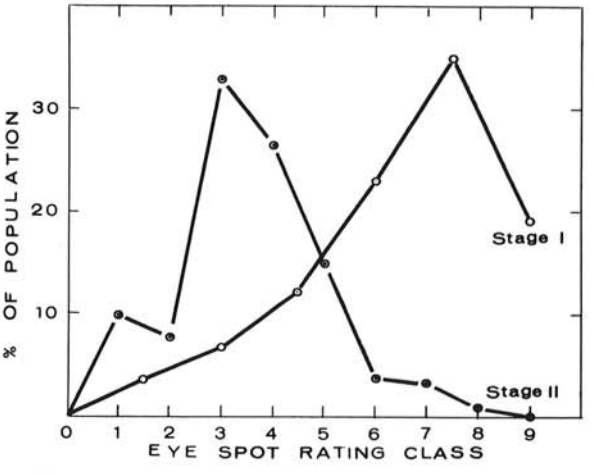

Fig. 2. The distribution (%) of stage I and stage II sugarcane clones among eye spot rating classes.

retardation resulted in less total growth for the season and caused the affected clones to be rejected for advancement to stage II. Since this "natural" selection would occur only in years highly favorable to eye spot development, consistent elimination of susceptible clones will require selection based on eye spot ratings.

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