Use of Helminthosporoside to Select Sugarcane Seedlings Resistant to Eye Spot Disease

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Published with approval of the Director as Journal Series Paper No. 299 of the Experiment Station, Hawaiian Sugar Planters' Association.

Accepted for publication 9 November 1971.

ABSTRACT

The host-specific toxin produced by *Helminthosporium sacchari* was evaluated for its use in determining the reaction of sugarcane (*Saccharum* sp. hybrids) seedlings to eye spot disease. Progeny reactions to spray applications of toxin were related to parental eye spot disease ratings. Seedling response to toxin was indicative of their adult plant reactions. The concentration of toxin influenced the number of seedlings showing symptoms and symptom intensity. Seedlings susceptible to eye spot can be removed from a population by spray applications of toxin.

Phytopathology 62:466-470.

Eye spot disease of sugarcane (*Saccharum* sp. hybrids) caused by *Helminthosporium sacchari* (van Breda de Haan) Butler can cause considerable losses when susceptible clones are grown (2). Epiphytotics have not occurred recently in Hawaii because resistant clones are grown commercially. In the past, severe outbreaks of eye spot on susceptible clones contributed to discontinuance of their planting (2).

New clones are continually being tested as possible candidates for commercial use. Eye spot disease resistance is one of many criteria that determines the possible usefulness of a new clone. Steiner & Byther (6) demonstrated the use of two host-specific toxins produced by *H. sacchari* for determining the reaction of clones to eye spot disease. The most active toxin has been identified as 2-hydroxylcyclopropyl-α-D-galactopyranoside by Steiner & Strobel (7), who named it "helminthosporoside". The reaction of sugarcane clones to the pathogen and its toxins were closely correlated, thus enabling use of the toxins to screen clones for eye spot disease resistance (6). Four- to six-month-old plants were used in these studies.

Reaction of host-specific toxins produced by other pathogens differentiated between resistant and susceptible seedlings (1, 3, 8). The toxin produced by *Helminthosporium victoriae* has been used to select oat seedlings resistant to Victoria blight (8). The reaction of sugarcane seedlings to helminthosporoside has not been investigated previously.

The purpose of this study was to test the reaction of sugarcane seedlings to partially purified helminthosporoside, and to determine if their reaction as young seedlings would be indicative of their reaction as adult plants. Results would reveal the possibility of eliminating eye spot-susceptible clones at the seedling stage. A preliminary report has been published (5).

MATERIALS AND METHODS.—Toxin preparations were made according to Steiner & Byther (6), omitting the butanol and Sephadex treatment. Culture filtrate of *H. sacchari* was concentrated, and partially purified by methanol and chloroform treatments. The final aqueous phase was concentrated to one-fiftieth the original volume. At this stage of purification, the preparation is stable and host-specific, and both toxins are present, but a majority of activity is attributed to helminthosporoside. This partially purified preparation was used to spray seedlings and to determine adult plant reactions to eye spot disease.

Seedlings used in these tests were plants derived directly from true seed, not to be confused with those derived from vegetatively propagated seedpieces or setts. Seed was obtained from polycrosses whose female parents were known but had pollen supplied by several unknown male clones. Three- to four-week-old seedlings were sprayed with various concentrations of the toxin preparation, using an atomizer. They were immediately covered with a polyethylene bag and incubated 40 hr at room temperature. Symptoms induced by toxin were evaluated 2 to 4 days after removal from the bags.

Reactions of the seedlings to toxin applications were categorized as resistant, intermediate, or susceptible (Fig. 1). Resistant plants showed no reactions to the toxin, whereas susceptible plants were either killed or showed extensive systemic symptom development. Intermediate plants responded with localized symptom development on a portion of their leaves.

Seedlings having a known reaction to toxin were transplanted to wooden greenhouse flats, and later individually planted outdoors into 1-gal cans or into field plots.

Eye spot ratings on 5-month-old transplants were determined, using the method of Steiner & Byther (6). Two-tenths ml of partially purified toxin diluted 1:10 was injected into a hole made with a cork borer in the upper portion of the stalk. Reactions were rated on a scale of 1 to 9: 1, no visible reaction; 9, all leaves of the plant showing extensive symptoms. Intermediate ratings were based on extent (number of leaves affected) and intensity of symptoms.

RESULTS.—Three-week-old seedlings derived from female parents having various eye spot ratings were sprayed with a toxin solution diluted 10 times.

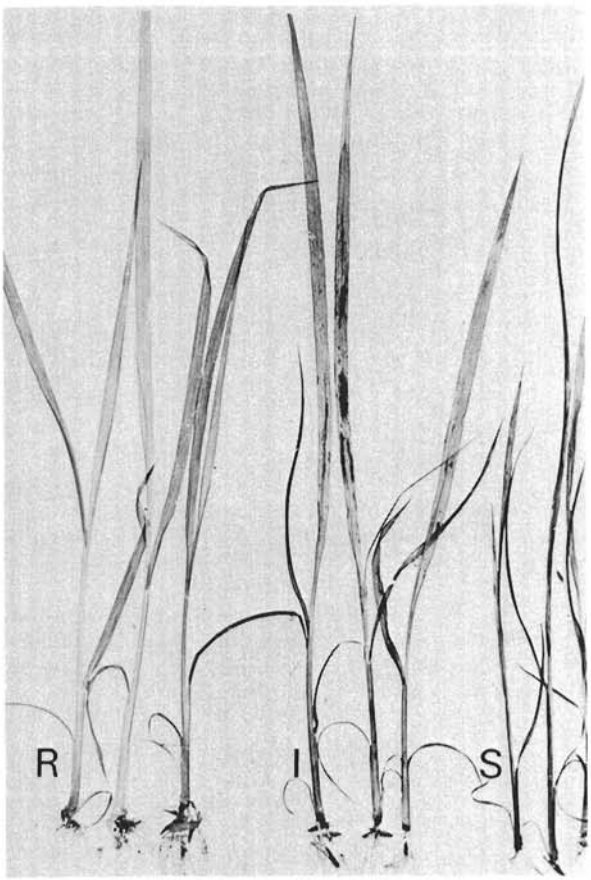

Fig. 1. Resistant (R), intermediate (I), and susceptible (S) reactions of 4-week-old sugarcane seedlings to toxin produced by *Helminthosporium sacchari.*

Progeny reaction to the toxin was generally correlated with their parental eye spot ratings (Table 1). A majority of seedlings derived from resistant female parents were resistant to toxin, and a high percentage of seedlings derived from susceptible female parents were susceptible to toxin. Among progeny derived from intermediate female parents, reactions to toxin were variable and probably were influenced to a greater extent by the disease reaction of the male parents involved in the polycrosses.

The influence of the male parent in determining the reaction of progeny to eye spot susceptibility was determined for several polycrosses. Seed lots from several different polycrosses having a common female parent were obtained. Limited seed of this type was available. Significant differences occurred in progeny reactions to toxin from these seed lots, indicating that male parents in these polycrosses did affect eye spot susceptibility (Table 2). Tests were replicated 4 times, and no significant differences were observed between replications. An average coefficient of variation of 9.6% for these tests indicated the constancy of the spray applications.

Reactions of the seedlings varied according to the dilution of the toxin (Table 3). Ninety-five per cent of the progeny from a resistant female parent (H 59-1923) had no reaction to the toxin diluted 100 times, whereas concentrations of 1:10 and nondiluted failed to produce symptoms on 80 and 56%, respectively. Progeny from a susceptible population (H 54-2514) were more sensitive to toxin concentration. Fifty-five per cent of the seedlings were severely affected by a toxin dilution of 1:10; none was susceptible to a 1:100 dilution. Fourteen and 47% of these seedlings were resistant to toxin concentrations of 1:10 and 1:100, respectively.

The association between the parental eye spot disease rating and their progeny reaction to toxin suggested that the seedlings were reacting in a manner indicative of what their adult plant reaction would be. To verify this supposition, progeny from four polycrosses whose parents had eye spot ratings of 3, 5, 7, and 8 were sprayed with various toxin dilutions. Plants having resistant, intermediate, and susceptible reactions were transplanted and later individually grown in 1-gal cans. Due to the severe reaction of susceptible seedlings, few survived transplanting. At 5 months, the plants were tested for eye spot susceptibility with a 1:10 dilution of partially purified toxin. Adult plants having a rating of 1-3 were considered resistant; 4-6, intermediate; and 7-9, susceptible.

Adult plant reactions to toxin were similar to their reactions as seedlings. Eighty-six per cent of the

TABLE 1. Reaction of sugarcane seedlings to toxin produced by *Helminthosporium sacchari* compared to eye spot disease rating of female parent[a]

Female parent	Parent eye spot disease rating	Seedling reaction[b]		
		Resistant	Intermediate	Susceptible
		%	%	%
H 45-2608	2	92	8	0
H 59-1923	3	80	18	2
H 52-663	5	42	49	9
H 55-8248	5	10	33	57
H 49-823	6	24	66	10
H 54-2514	7	14	31	55

[a] Seedlings derived from polycrosses. Male parents unknown.
[b] Determined from 100 seedlings sprayed with a 1:10 toxin dilution under laboratory conditions.

TABLE 2. Variation in toxin reaction of seedlings from different polycrosses having a common female parent

Female parent	Parent eye spot disease rating	Seedling lot	Seedling reaction[a]		
			Resistant	Intermediate	Susceptible[b]
			%	%	%
H 56-4511	5	A	48.4**	48.6	3.4
		B	35.8	54.3	7.9
H 63-733	5	A	42.5**	53.0	4.5**
		B	30.1	57.6	12.3
H 58-421	3	A[c]	79.7 x**	20.3 x **	0.0 x*
		B	60.4 y	36.5 y	3.1 y
		C	48.4 y	50.6 z	1.2 xy

[a] Average of four replications averaging 97 seedlings in each.
[b] ** = 1% level of significance; * = 5% level of significance.
[c] Means followed by different letters (x, y, z) are significantly different (Duncan's multiple range test).

TABLE 3. Reaction of sugarcane seedlings to various concentrations of toxin produced by *Helminthosporium sacchari*

Female parent[b]	Eye spot disease rating	Toxin dilution	Seedling reaction[a]		
			Resistant	Intermediate	Susceptible
			%	%	%
H 59-1923	3	1	56	36	8
		1:10	80	18	2
		1:100	99	1	0
H 52-663	5	1:10	42	49	9
		1:100	93	7	0
H 54-2514	7	1:10	14	31	55
		1:100	47	53	0

[a] Determined from 100 seedlings.
[b] Seedlings derived from polycrosses. Male parents unknown.

seedlings which showed no reaction to a 1:10 toxin dilution were resistant at 5 months of age (Table 4); none was susceptible. All transplants from seedlings resistant to undiluted toxin were resistant to a 1:10 toxin dilution at 5 months of age.

To further test the hypothesis that seedling reaction to toxin is indicative of adult plant reaction, and also to determine if plants could be treated under greenhouse conditions, progeny from female parents having an eye spot rating of 5 or 6 were sprayed with a 1:10 toxin dilution. Plastic bags were not used to cover the seedlings after spraying in the greenhouse because of high temperatures. The seedlings were again divided according to their reaction to toxin, and transplanted to field plots where their eye spot reaction was determined at 5 months.

In a population of plants that did not receive a toxin spray, 48% were resistant; 20%, intermediate; and 32%, susceptible (Table 5). This is contrasted with those seedlings which were resistant to a 1:10 toxin spray in the laboratory, of which 94% were resistant and none was susceptible in the field.

Greenhouse spraying was not generally as effective as laboratory spraying in differentiating resistant plants (Table 5). Resistant progeny (in greenhouse testing) from clone H 52-663 had a similar distribution of resistance to the nonsprayed normal population. However, plants that did show a reaction in the greenhouse were susceptible when field-tested; 91% were susceptible as compared with 32% in the normal population.

Greenhouse spraying of a more susceptible population derived from another parent (H 49-823) was effective in selecting resistant seedlings. Only 15% of the adult plants in the nontreated population were resistant, and 70% were susceptible as contrasted with 50% resistant and 14% susceptible from the resistant portion of the toxin-sprayed population.

Because of the inconsistency associated with toxin spraying in the greenhouse and the need for a large scale application, other techniques were evaluated. Consistent responses of seedlings to toxin were achieved by spraying outdoors in shaded areas during the late afternoon, thus avoiding the overheating experienced in the greenhouse. Seedling flats were grouped and covered with a polyethylene tarpaulin after spraying. To exclude contact between the

TABLE 4. Adult plant reactions to toxin produced by *Helminthosporium sacchari* compared with their reaction as seedlings when sprayed with various dilutions of toxin

Seedling reaction[a]	Toxin dilution sprayed on seedlings	Adult reaction[b]			No. tested
		Resistant	Intermediate	Susceptible	
		%	%	%	
Resistant	1	100	0	0	15
Intermediate	1	57	36	7	14
Resistant	1:10	86	14	0	57
Intermediate	1:10	53	40	7	68
Resistant	1:100	68	31	1	38
Intermediate	1:100	7	57	36	14

[a] Determined when 21 days old. Seedlings originated from polycrosses whose female parents had eye spot ratings of 3, 5, 7, and 8.
[b] Determined when 5 months old with a 1:10 dilution of toxin.

TABLE 5. Comparison of seedling reactions to their reactions as adult plants when treated with toxin produced by *Helminthosporium sacchari* under laboratory and greenhouse conditions[a]

Female parent	Eye spot disease rating	Toxin application (sdlg. stage)	Seedlings reaction[b]	Adult plant reaction[c]			No. tested
				Resistant	Intermediate	Susceptible	
				%	%	%	
H 52-663	5	None		48	20	32	54
		Laboratory	Resistant	94	6	0	33
		Laboratory	Intermediate	7	56	37	27
		Greenhouse	Resistant	41	18	41	17
		Greenhouse	Intermediate	0	9	91	12
H 49-823	6	None		15	15	70	26
		Greenhouse	Resistant	50	36	14	22
		Greenhouse	Intermediate	16	16	68	25

[a] Seedlings derived from polycrosses. Male parents unknown.
[b] Sprayed when 21 days old with a 1:10 toxin dilution.
[c] Determined when 5 months old with a 1:10 toxin dilution.

tarpaulin and the seedlings, inverted empty wooden flats were placed over each seedling flat. The tarpaulin was removed the next day. To determine the number of seedlings that escaped spraying, seedlings showing symptoms after one spray were removed and the population resprayed. In seedling flats having a density of one plant/cm^2, a 16 to 25% error occurred due to escapes. Thus, it was necessary to make two spray applications when seedling density was high.

DISCUSSION.—Results indicate that a seedling reaction to spray application of toxin is indicative of its reaction as an adult plant. Srinivasan (4) found the same principle true for seedling reaction to red rot of sugarcane. Four- to six-week-old seedlings resistant to foliar inoculation of *Collectotrichum falcatum* were also resistant to stalk rot at a later stage. In contrast, Wismer (9) found no correlation between reaction of juvenile and older plants to inoculations with the brown spot pathogen, *Cercospora longipes*.

Host-specific toxins have been used successfully by others to screen for disease resistance at the seedling stage. Wheeler & Luke (8) drenched young oat seedlings with toxin produced by *Helminthosporium victoriae* to eliminate seedlings susceptible to Victoria blight. The reaction of corn to *H. maydis* could be determined by using a host-specific toxin according to Hooker et al. (1). Toxin inhibition of primary root growth of corn seedlings was correlated with resistance to *H. maydis*.

Laboratory testing using polyethylene bags was more effective in determining seedling reaction to toxin than greenhouse spraying without bags. Using bags in greenhouse testing did not improve the response (*unpublished data*). However, by placing greenhouse seedling flats in shaded areas outside and covering them with polyethylene tarpaulins, successful treatment of large numbers of seedlings was accomplished. In 1971, 200 flats involving ca. 50,000 seedlings were treated in this manner. It was necessary to make a second application 2 to 3 days after the first to reduce the number escaping selection.

There are several reasons why populations from two parents having a similar eye spot rating showed differences in their susceptibility distribution (Table

1). The parental disease rating was determined under field conditions which are subject to some variation. Progeny derived from polycrosses can show considerable variation depending upon (i) the pollen production, fertility, and eye spot rating of the male parent(s) (Table 2); and (ii) the genetic makeup of the female parent. Resistance may be controlled by a multigenic system derived from five different species of *Saccharum*. For similar reasons, variations occur in progeny from different seedling lots derived from the same female parent.

The desired degree of eye spot disease resistance in a population can be achieved by varying the concentration of the toxin spray. If a high degree of resistance was desired in order to obtain breeding material, undiluted toxin preparations could be used. If only field resistance was required, the very susceptible could be eliminated by more dilute toxin concentrations, leaving intermediate and resistant seedlings to propagate for further field selection.

Eye spot-susceptible parents were shown to produce higher percentages of susceptible progeny than do resistant parents. Although many of the clones used for breeding at this Experiment Station now have good resistance to eye spot disease, certain susceptible clones are still used because of their other outstanding characteristics. It is now possible to eliminate susceptible progeny produced from these clones by toxin applications. An early screen for eye spot susceptibility will eliminate carrying many of the susceptible seedlings through the selection procedure. The quantity of seedlings used in the breeding program at this Experiment Station has normally precluded screening for disease resistance until 7 years after propagation.

Elimination of susceptible progeny early in the selection process with the use of helminthosporoside will result in valuable savings in time and money.

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