Single-Stool Plots for Estimating Relative Yield Losses Caused by Ratoon Stunting Disease of Sugarcane

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ABSTRACT

Dean, J. L. 1983. Single-stool plots for estimating relative yield losses caused by ratoon stunting disease of sugarcane. Plant Disease 67:47-49.

In the most sensitive of three trials involving nine to 11 clones of sugarcane, the minimum detectable difference in average plant weight between healthy and ratoon-stunting-diseased plants was estimated to be 3.3 kg with 10 replicates (pairs of plants), 2.2 kg with 20 replicates, and 1.8 kg with 30 replicates; both type I and type II error rates were set at 0.05. These differences represented about 19, 13, and 10%, respectively, of the mean weight of all healthy plants in the trial.

Although ratoon stunting disease (RSD) of sugarcane (interspecific hybrids of *Saccharum*) is so nearly symptomless that it often cannot be diagnosed in the field (12), it is widely regarded as a major disease and may be responsible for more yield loss on a worldwide basis than any other disease of sugarcane (6). RSD can cause dramatic losses, especially under drought conditions (6), but in Florida it is more likely to take a small, usually unnoticed toll from a large acreage. Because RSD is prevalent in Florida and heat therapy is little practiced, highly susceptible clones are not likely to become commercially important. Furthermore, because of the unique system of water table control practiced in the agricultural area of the Everglades region, sugarcane rarely suffers drought stress.

RSD, long believed to be a virus disease (15), was recently shown conclusively to be caused by an unnamed, fastidious, xylem-inhabiting bacterium (4). The suspected mechanism of yield reduction is impaired xylem transport.

Replicated yield trials are the only proven method of evaluating clones for resistance to RSD. Because of the large plots required (7,10,16) and the sheer bulk and weight of plant material that must be handled and transported, sugarcane yield trials are very expensive. RSD trials are doubly expensive because each entry must be tested in both the healthy and the diseased state and because added expense is involved in establishing and maintaining known diseased and healthy stocks for testing.

Accepted for publication 18 May 1982.

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Wismer (18) used single-plant plots to estimate RSD losses and discussed the need for a method that is cheaper than the usual agronomic yield trial but did not show data. Matsuoka (9) used four-plant plots for the same purpose. Benda (1) explored the use of single-plant plots for determining losses due to sugarcane mosaic. However, the sensitivity of these trials for detecting losses was not reported.

In statistical tests of significance, two types of errors are possible. A type I error is false declaration of a significant difference, and a type II error is failure to detect a significant difference. The type I error rate (α) is traditionally set at 0.05 or 0.01, and the type II error rate (β) is traditionally ignored in plant pathology and other disciplines, although statisticians have called attention to the information lost by this omission (3,19). If other factors are constant, the probability of a type II error increases as the probability of a type I error decreases. Therefore α should be set only after a consideration of the relative seriousness of type I and type II errors in a particular experiment. Carmer (2) analyzed this problem in one kind of crop performance trial and concluded that an α between 0.2 and 0.4 is more appropriate than the traditional 0.01 or 0.05.

The implications of Carmer's analysis are not confined to crop performance trials. In RSD trials used as an aid to selection in a breeding program, a type I error results in rejection of a resistant clone and a type II error promotes release of a susceptible clone. Because both errors can lead to cultivation of an inferior clone, they are judged equally serious. This suggests that α and β should be about equal and that both should be low. It should be noted that determining the sensitivity of a testing procedure depends upon estimating β (the type II error rate).

The primary purpose of this research was to determine the sensitivity of single-plant plots in paired-comparison trials for the evaluation of resistance to RSD. A secondary aim was to evaluate a few clones of interest in the breeding program.

MATERIALS AND METHODS

Bud viability is reduced by heat treatments that cure RSD (15), and buds may be inhibited by immersion in sap from either healthy or infected stalks (20). These direct effects of treatment are usually avoided in RSD trials by a seed cane increase between treatment and installation of the trial. In these trials, the delay caused by a seed increase and the direct effects of treatment were avoided by another method. Single-budded cuttings were hot-water treated for 2 hr at 51 C, then half were immersed in sap from known healthy CL 41-223 stalks and half were immersed in sap from known infected stalks of the same clone. The cuttings were started in moist sphagnum moss, then transplanted to soil in peat pots (1978) or plastic pots (1979). After 4–6 wk in the greenhouse, healthy and diseased plants within clones were paired according to size and transplanted into the field. Paired plants were installed opposite each other in adjacent rows. Row width was 1.5 m. Healthy and diseased plants were distributed randomly down each row at 1-m intervals.

Two trials were planted, one in May 1978 and the other in June 1979. A plant crop was harvested from both trials and a ratoon crop from the second trial. At harvest, the plants were cut just above the soil line, and whole plants were weighed individually. The clones and the number of replicates in each trial are shown in Tables 1 and 2.

The standard, one-tailed t test for paired data was used to detect differences in plant weight between healthy and diseased plants within each clone. The one-tailed test is appropriate because RSD may decrease, but not increase, plant weight. Minimum detectable differences, which indicate the sensitivity of the trials, were calculated by the formula given by Zar (19).

RESULTS AND DISCUSSION

At the time of harvest of the first plant-cane trial, inoculated and control plants of CP 43-47 were spot-checked for vascular symptoms of RSD. This clone shows unusually strong symptoms when infected. Of 10 pairs of plants checked, all of the inoculated and none of the control plants showed symptoms.

CP 53-1, CP 43-47, and F 36-819 are known to be very susceptible to RSD (5; H. Koike, *personal communication*), and plant weights of all of these were significantly reduced by RSD (Table 1). CP 29-116, CP 52-68, and L 60-25 had previously been reported as resistant to RSD (5,14); plant weight loss was not detected in CP 29-116 or CP 52-68, but a significant loss was found in L 60-25. No explanation can be offered for this apparently anomalous reaction of L 60-25. No prior information was available on the remaining clones. Significant losses were found in CP 70-1133 and CP 68-1026.

No known highly susceptible clones were included in the second trial. CL 41-223 was known to be intermediate in response to RSD, and CP 70-1133 had shown a significant loss in the first trial. A significant loss was detected in CL 41-223 but not in CP 70-1133 (Table 2). CP 68-1067, which had shown no loss in the first trial, was affected significantly.

In the ratoon crop of the second trial, no significant losses were detected, probably because of the large increase in variance from plant crop to ratoon. Pooled variances for the three harvests were 14.14, 8.06, and 31.68 for the first plant crop, second plant crop, and ratoon crop, respectively. The variances of the

Table 1. Average plant weight of healthy and ratoon-stunting-diseased sugarcane plants in a paired comparison trial; plant crop, 1979 harvest

Clone	Plant weight (kg)		Loss[a] (%)	Replicates (no.)
	Healthy	Diseased		
CP 53-1	20.8	16.2	22.1**	20
L 60-25	19.3	16.8	13.0**	20
CP 43-47	24.6	18.9	23.2**	18
CP 56-59	15.3	15.3	0.0	20
CP 52-68	16.3	17.4	−6.8	20
CP 29-116	20.9	20.4	2.4	20
CP 63-588	15.4	13.4	13.0	18
CP 68-1026	18.3	14.9	18.6*	20
F 36-819	16.0	13.8	13.8**	20
CP 68-1067	15.3	14.7	3.9	20
CP 70-1133	22.2	19.4	12.6*	20

[a] * Indicates significant loss ($P < 0.05$); ** indicates significant loss ($P < 0.01$).

Table 2. Average plant weights[a] of healthy and ratoon-stunting-diseased sugarcane plants in a paired comparison trial; plant crop, 1980 harvest

Clone	Plant weight (kg)		Loss[b] (%)
	Healthy	Diseased	
CP 68-1067	13.7	11.5	16.1**
CP 72-1312	17.2	16.6	3.5
CP 71-1086	24.0	24.6	−2.5
CP 73-1311	16.1	17.3	−7.5
CP 63-588	16.9	16.4	3.0
CP 71-1027	19.4	19.5	−0.5
CP 56-59	18.2	18.8	−3.3
CL 41-223	10.7	9.0	15.9*
CP 70-1133	18.8	17.7	5.9

[a] The number of plant pairs (replicates) was 21 in all clones.
[b] * Indicates significant loss ($P < 0.05$); ** indicates significant loss ($P < 0.01$).

Table 3. Average minimum detectable difference (kg) between healthy and ratoon-stunting-diseased sugarcane plants at various levels of α, β, variance, and replication[a]

Number of replicates	Plant crop ($s^2 = 8.06$)[b]		Ratoon crop ($s^2 = 31.68$)[c]	
	$\alpha=\beta=0.05$	$\alpha=\beta=0.01$	$\alpha=\beta=0.05$	$\alpha=\beta=0.01$
5	4.41	9.51	10.73	18.86
10	3.29	5.07	6.53	10.04
20	2.20	3.22	4.35	8.04
30	1.76	2.55	3.49	5.06

[a] α = Probability of a type I error, β = probability of a type II error.
[b] Range of $s^2 = 18.1$.
[c] Range of $s^2 = 47.6$.

three harvests were not homogeneous as determined from a table of critical values of the maximum *F*-ratio distribution (13). The improvement from the first to the second plant crop probably resulted mainly from the change from peat to plastic pots, which avoided the variable injury caused by separation of entangled root systems that had grown through the peat pots. The great increase in variance from plant crop to ratoon in the second trial resulted in part from erratic natural spread of leaf scald and smut into the ratoons and in part from random stalk breakage caused by wind. However, there may be a general tendency for variance to increase in ratoons of paired comparison trials. Some of the uniformity gained by the original pairing of plants may not last through the ratoon crop.

Table 3 shows the minimum detectable difference between healthy and diseased plants at two levels of α and β, at several levels of replication, and at the variance levels obtained in the plant crop (most favorable case) and ratoon crop (least favorable case) of the second trial. Because of the large variance in the ratoon crop, the sensitivity of the trial was too low to be useful. In the plant crop, 30 plant pairs would have detected differences of 1.8 kg/plant or about 10% of the mean healthy plant weight in that trial. Greater sensitivity would be desirable, but this is in the useful range.

Economic data are not available for an exact comparison, but there is no doubt that it is cheaper to grow and harvest 30 pairs of plants by the methods used here than to grow and harvest large replicated plots (7,10). These single-stool plots, however, probably give a better estimate of the relative performance of clones than of the absolute losses due to RSD because of "representational" errors, as Vanderplank (17) named the failure of plots to represent fields accurately. Vanderplank described only those errors involving the movement of pests or pathogens. Such errors were not involved in these trials because RSD apparently is spread only by the cutting knife or by propagation of infected cuttings. Precautions were taken to avoid this kind of spread. However, the intrarow spacing in these plots (1 m) deviates sharply from field practice, and absolute losses measured in the plots probably misrepresented field losses, although this distortion may be small on a percentage basis. Lyrene et al (8) found a high correlation between stalk number in spaced sugarcane plants and in competitive plots, and stalk number is an important yield component (11).

It is apparent that gains from increasing replication above 30 would be very costly (Table 3), but further gains might be achieved through further reduction in variance. More research is needed to determine the best months for conducting such trials and the optimum length of growing season. The data in Table 3 are based on trials that were harvested at a normal harvest time in Florida, but the harvest was made many weeks after temperatures had been too low for good growth of sugarcane. It is likely that during this unfavorable growing period, plant weight differences did not change, whereas variance continued to increase because of random damage from rodents, lodging, and other causes.

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