Effect of Ratoon Stunting Disease on Yield of Sugarcane Grown in Multiple Three-Year Plantings

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ABSTRACT

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Yield losses in sugarcane caused by ratoon stunting disease were observed for seven of eight cultivars tested in four to six crop cycles of 3-yr plantings. In the seven cultivars, average 3-yr losses ranged from 29 to 60 t/ha; sugar losses ranged from 2,627 to 7,369 kg/ha. Losses tended to increase with the number of years a planting was cropped. Cane losses averaged 14% in the first-year crop and increased to 27% in the third-year (second-ratoon) crop. For a given cultivar, the magnitude of losses varied among plantings; however, the planting × disease treatment interaction was not significant. Losses from ratoon stunting disease were observed for six cultivars that currently occupy approximately 93% of the sugarcane hectarage in Louisiana.

Additional keywords: *Clavibacter xyli* subsp. *xyli*, *Saccharum*.

Ratoon stunting disease of sugarcane (*Saccharum* interspecific hybrids) was first observed in Queensland, Australia, during the summer of 1944–1945 (19). The causal agent later was determined to be the xylem-limited bacterium *Clavibacter xyli* subsp. *xyli* (5,6,14). Diseased plants show no external symptoms. Internal symptoms may include a salmon pink discoloration just below the growing point of young cane and an orange red discoloration of the vascular bundles at the nodes of mature cane. Sugarcane is a vegetatively propagated plant, which often is ratooned to produce multiple crops from a single planting. The pathogen is readily transmitted mechanically to cane cut for propagation or to stubble cut by contaminated harvest equipment (9,19).

Estimates of yield losses from ratoon stunting disease vary depending on host tolerance and environment. In Australia (9), yield losses in replicated tests ranged from 11 to 51% in cultivars Q 57 and Q 28, respectively, and in Taiwan, yields in plant cane from diseased cuttings of cultivar NCo 310 were 13 to 25% less than those from hot water-treated cuttings (4). Bailey and Fox (2) estimated a 3% reduction in yield of the annual crop in South Africa during a normal year. Adequate irrigation may mask the expression of ratoon stunting disease in plant cane and first-ratoon crops, but if the crop is under moisture stress, a significant reduction in yield can be expected (17). Hughes (8) in 1974 suggested that ratoon stunting disease causes yield losses in practically every sugarcane-growing country in the world. There is little to indicate that the situation has changed significantly. The results of most studies conducted in tropical sugarcane-growing areas reflected yield effects on cane that was older and more mature than the cane in temperate Louisiana where the growing season is much shorter.

The first report of ratoon stunting disease in Louisiana was by Abbott in 1953 (1). Several reports subsequently have been published indicating yield losses caused by ratoon stunting disease in the state (10–13,18,19). Each report contained examples of cultivars resistant to ratoon stunting disease that suffered no significant loss and intermediate and susceptible cultivars that may have had losses exceeding 40%. A comparison of cultivars that were common among the tests or that were entered in more than one test of a study indicates that external environmental conditions influence yield losses.

Most studies done in Louisiana report the results of only the first-year crop or only the first-year and second-year (first-ratoon) crops (10,11,18,19). Several studies have indicated that the highest yield losses occur in the ratoon crops (8,9,11,12,19). A typical Louisiana planting includes two-ratoon crops. Other studies that included the results of three crops were done with only a single planting (12,13). The objective of this study, therefore, was to obtain a reliable estimate of yield loss for the most widely planted cultivars in Louisiana for multiple, 3-yr plantings.

MATERIALS AND METHODS

Field experiments were arranged in a split-plot design with four replications. Main (treatment) plots had either plants infected with *C. x. xyli* or uninfected plants, and the subplots were sugarcane cultivars. Plots were 5 m long and 6 m (three rows) wide. Between 1978 and 1986, the experiment was repeated six times. Each planting consisted of three annually harvested crops, first-year crop, first-ratoon crop, and second-ratoon crop; the crops were treated as repeated measures on the same plots. Up to eight cultivars were included in each experiment.

To manage the experiment as consistently and uniformly as possible, the stalks for propagation were produced in a nursery 1 yr earlier so that plants would be grown on the same soil type and stalks would be of similar maturity. To establish diseased stock plants, single-node cuttings of each cultivar were submerged for 10 min in juice expressed from stalks of mature plants infected with *C. x. xyli*. The inoculated nodes were planted in the greenhouse, and the plants were grown for approximately 5 mo before they were transplanted to the field nursery. Once plants infected with *C. x. xyli* were established, subsequent nurseries were planted with cuttings from diseased plants. Uninfected plants were established from uninoculated stalks treated with hot water at 50 C for 2 hr (3) and were planted directly in the nursery. Uninfected cuttings for subsequent nurseries were treated with hot water each time. Random samples of mature stalks from the nurseries at planting and from the experiments at harvest were examined

visually. The characteristic orange red discoloration of the vascular bundles of the basal nodes was observed among all samples from plants in the ratoon stunting disease nurseries and in the diseased treatments of the experiments, and no visible symptoms were observed among the control plants. A random sample of stalks from diseased and control treatments of each experiment was collected near harvest, and expressed juice was examined by phase contrast microscopy for the presence or absence of the disease-causing bacteria. The expected results were obtained from each sample. Stalks visually free of sugarcane mosaic symptoms were selected for use in planting nurseries and experiments.

Each field plot was planted with 18 stalks in the fall. The 1979–1981 test was planted in Mhoon silty clay loam; the other experiments were planted in Sharky clay. Standard plantation practices of cultivation, fertilization, and herbicide and insecticide application were used (15). Each spring the percent stalks infected with sugarcane mosaic was recorded for each plot. The sugarcane was harvested in three successive years. Cane from each plot was cut with a mechanical harvester between mid-October and mid-November, burned to remove dead leaves, and weighed. The bottom blade of the harvester was disinfected with a 15% solution of a phenolic disinfectant (Lysol cleaner, Lehn and Fink Products Division of Sterling Drug, Inc., Montvale, NJ) before moving the harvester from diseased to control plots. A random, 15-stalk subsample was taken from each plot and milled. From the mill analysis, theoretical recoverable sugar per plot was calculated. Yield was expressed as cane (t/ha) and as sugar (kg/ha) for the first-year and two-ratoon crops (second and third years) individually and as a total of the 3-yr planting.

Three analyses were performed for a split-plot design. First, the total yield (first-year crop + first-ratoon crop + second-ratoon crop) was calculated, and each planting was analyzed separately. The remaining analyses were performed on data averaged over replicates. In these analyses, each planting was considered a replicate. The second analysis was performed on total yield combined across all plantings. The third analysis was on the yield of each crop (first-year crop, first-ratoon crop, and second-ratoon crop) separately. Mean comparisons between infected and uninfected plants were performed in each of the above analyses using Fischer's least significant difference method.

An additional analysis was performed using yields for each

TABLE 1. Differences in yield (kilogram of sugar per hectare) between control and sugarcane plants infected by *Clavibacter xyli* subsp. *xyli* for six 3-yr plantings as affected by cultivar

	3-yr planting												Mean loss per planting	
	1978–1980		1979–1981		1980–1982		1981–1983		1982–1984		1984–1986			
Cultivar	kg/ha	%	kg/ha	%	kg/ha	%	kg/ha	%	kg/ha	%	kg/ha	%	kg/ha	%
L 60-25	...	...	703 ns[a]	3	1,920 ns	10	3,506 *	15	−1,670 ns[b]	−9	...	...	1,115 ns	5
CP 65-357	2,949 ns	12	678 ns	2	4,706 **	22	3,345 *	12	3,031 **	13	2,535 ns	11	2,874 **	12
CP 70-321	5,673 **	28	5,306 **	18	7,494 **	33	9,111 **	36	7,908 **	31	8,743 **	44	7,372 **	31
CP 70-330	6,417 **	27	6,075 **	21	6,336 **	27	6,456 **	24	3,123 **	15	...	...	5,681 **	23
CP 72-355	4,080 *	15	1,318 ns	5	6,820 **	27	7,846 **	30	...	...	...	...	5,016 **	19
CP 72-356	2,795 ns	12	3,176 *	12	6,194 **	29	5,542 **	23	4,447 **	17	...	...	4,431 **	18
CP 72-370	6,329 **	27	3,265 *	11	5,133 **	23	...	...	3,497 **	14	...	...	4,556 **	18
CP 74-383	...	...	...	...	2,192 ns	11	1,318 ns	6	2,887 *	12	4,111 *	19	2,627 *	12
LSD 0.01	4,760		3,585		3,258		4,508		2,970		5,785		2,775	
0.05	3,003		2,495		2,318		3,268		2,175		3,563		2,012	

[a] Differences in mean yield between control and ratoon stunting disease-affected plants were not significant (ns), significant at $P = 0.05$ (*), or significant at $P = 0.01$ (**) based on Fischer's least significant difference (LSD) method.
[b] The yield (kilogram of sugar per hectare) was higher in the diseased treatment.

TABLE 2. Mean yield of control and sugarcane with ratoon stunting disease (RSD) for the first-year crop, first-ratoon crop, second-ratoon crop, and the total for the planting cycle

			First-year crop			First-ratoon crop			Second-ratoon crop			Total for the 3-yr planting		
	Cultivar	Number of plantings	Control	RSD	Difference (%)	Control	RSD	Difference (%)	Control	RSD	Difference (%)	Control	RSD	Difference (%)
Cane yield (t/ha)[a]	L 60-25	4	65	63 ns[b]	3	67	63 ns	6	52	48 ns	8	184	174 ns	5
	CP 65-357	6	76	67 *	12	71	63 *	12	58	48 **	17	206	177 **	14
	CP 70-321	6	68	57 **	16	66	45 **	32	52	24 **	54	186	126 **	32
	CP 70-330	5	72	63 *	12	69	54 **	22	53	29 **	55	194	146 **	25
	CP 72-355	4	69	56 **	19	67	52 **	22	65	47 **	28	201	155 **	23
	CP 72-356	5	70	60 **	20	68	53 **	22	62	47 **	24	200	156 **	22
	CP 72-370	4	70	60 **	14	64	57 *	11	55	40 **	27	189	156 **	18
	CP 74-383	4	69	57 **	17	63	57 *	10	60	49 **	18	192	162 **	16
	LSD 0.01			10			9			10			20	
	0.05			7			6			7			14	
Sugar yield (kg/ha)[a]	L 60-25	4	8,162	7,799 ns	4	8,079	7,746 ns	4	5,613	5,194 ns	8	21,854	20,739 ns	5
	CP 65-357	6	9,734	8,890 ns	9	8,595	7,576 *	12	6,365	5,354 *	16	24,694	21,820 **	12
	CP 70-321	6	9,097	7,913 *	13	8,585	5,774 **	33	6,112	2,734 **	55	23,794	16,422 **	31
	CP 70-330	5	9,363	8,650 ns	8	9,086	7,107 **	22	6,413	3,423 **	47	24,862	19,181 **	23
	CP 72-355	4	9,524	8,079 **	15	8,770	6,906 **	21	7,807	6,100 **	22	26,101	21,085 **	19
	CP 72-356	5	8,765	7,572 *	14	8,294	6,706 **	19	7,372	5,722 **	22	24,431	20,000 **	18
	CP 72-370	4	9,609	8,208 **	15	8,365	7,350 ns	12	6,930	4,789 **	31	24,903	20,347 **	18
	CP 74-383	4	8,144	7,102 *	13	7,413	6,933 ns	6	6,406	5,301 *	17	21,963	19,336 *	12
	LSD 0.01			1,326			1,152			1,388			2,775	
	0.05			942			844			996			2,012	

[a] Mean of four to six plantings.
[b] Difference between control plants and sugarcane with ratoon stunting disease was not significant (ns), significant at $P = 0.05$ (*), or significant at $P = 0.01$ (**) by Fischer's least significant difference (LSD) method.

crop, planting, treatment, and cultivar. This analysis was for a split-split-plot where the main unit was treatment, the planting was the block, the cultivar was the subunit, and the crop (repeated measure) was a sub-sub unit. The crop was treated as a quantitative treatment (year 1, 2, and 3); its effect was explained as a linear trend (16). Cultivar and crop interaction was evaluated by individual *F*-test for homogeneity of crop slopes between cultivars.

RESULTS

Significant losses in cane and sugar per hectare were observed in plants infected with *C. x. xyli* for the 3-yr planting for each cultivar except cultivar L 60-25 (Table 1). The highest yield loss was observed in cultivar CP 70-321 where losses were 32 and 31% in cane tonnage and in sugar, respectively. The remaining cultivars lost 14–25% in cane tonnage and 12–23% in sugar when infected with *C. x. xyli.*

Yield losses generally were greatest in the second-ratoon crop for all cultivars, and in most cultivars, yield losses were least in the first-year crop (Table 2). Ratoon stunting disease caused no significant loss of sugar in the first-ratoon crops of cultivars CP 72-370 and CP 74-383, even though there had been significant losses in the first-year crops. When crop was treated as a linear trend, the yield-loss slopes of cultivars CP 70-321 and CP 70-330 were significantly greater than those of the other cultivars (Fig. 1).

DISCUSSION

In a single-planting experiment, Koike et al (13) reported that L 60-25 is a resistant cultivar because the juice from its diseased stalks had low bacterial counts and there was no significant reduction in yield of plants infected with *C. x. xyli.* The cultivar L 60-25 also is designated a resistant cultivar in the studies reported here. Cultivar CP 70-321 is designated as susceptible to ratoon stunting disease because of the high yield losses observed among the diseased plants. The remaining cultivars, which lost moderate levels of tonnage and sugar when infected with *C. x. xyli*, are classified as being intermediate in resistance.

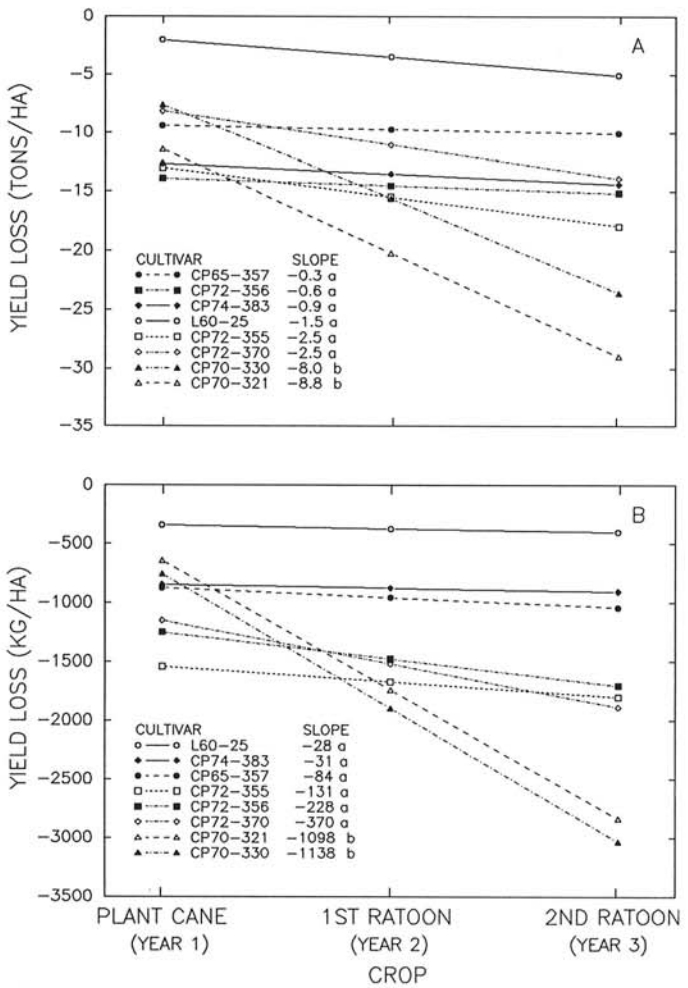

Fig. 1. Effect of crop on sugarcane yield loss caused by *Clavibacter xyli* subsp. *xyli.* **A,** Cane yield loss (tons per hectare). **B,** Sugar yield loss (kilograms per hectare). Slopes followed by the same letter did not differ according to an *F*-test for homogenenity ($P = 0.01$).

Yield losses from ratoon stunting disease have been difficult to demonstrate with a single, 3-yr planting. After one planting, Koike et al (13) demonstrated a significant reduction in sugar per hectare in four cultivars classified as susceptible, but among the 12 cultivars considered to be intermediate because of the levels of bacterial counts in the juice, significant sugar losses were recorded in only three. Similar problems in interpretation of results were encountered in this study when the yield loss of a cultivar was considered using only the results from one planting (Table 1). For example, the yield of diseased plants of cultivar CP 65-357 was not significantly less than the yield of healthy control plants in three of six plantings. The mean loss of 12% over the course of the study, however, was highly significant. In cultivar CP 70-321, loss of sugar was significant in each of the six, 3-yr plantings, but the losses varied from 18 to 44%, reflecting the influence of the varied environmental conditions that occurred during the study. Planting × treatment interaction was not significant (data not shown).

The sugarcane plant is stressed by ratooning in Louisiana where the cane stubble is exposed to subfreezing temperatures during the winter. The greatest loss of cane yield was in the second-ratoon crop after the severe winter of 1983–1984 in which the minimum temperature at the soil surface was −10.5 C (data not shown), suggesting that infected plants were more sensitive to injury by cold temperature than healthy plants.

Koike (10,12) demonstrated a synergistic effect rather than an additive effect on yield loss when plants were infected by both *C. x. xyli* and sugarcane mosaic virus in some cultivars. Among the plants in the present yield-loss experiment, the incidence of sugarcane mosaic virus infection was low, less than 20%, except for the 1982–1984 planting in which percentages were between 40 and 55% for cultivars L 60-25, CP 65-357, and CP 72-356. Yield losses of these cultivars in the 1982–1984 planting were near or below the mean yield losses for all plantings (Table 1), suggesting that sugarcane mosaic virus did not increase yield loss.

Seven of the cultivars in this study have been grown as commercial cultivars in Louisiana. L 60-25 is no longer grown as a commercial cultivar but was included as a standard for resistance. Although not commercially released, cultivar CP 72-355 was included in four experiments pending its possible release. The other six cultivars of this study occupy approximately 93% of the planted sugarcane land in Louisiana (7). These studies have shown the need for multiple plantings to increase the understanding of yield loss by ratoon stunting disease in sugarcane within individual cultivars. The primary method for control of ratoon stunting disease is hot-water treatment of stalks to be planted, followed by sanitary cultural practices (3). The practices require a continuous commitment by the growers to be effective. The demonstration of significant yield losses by ratoon stunting disease should encourage use of these practices.

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