Yield Comparisons: Disease-Free Tissue-Culture Versus Bud-Propagated Sugarcane Plants and Healthy Versus Yellow Leaf Infected Plants

J. C. Comstock and J. D. Miller
USDA-ARS, Sugarcane Field Station, Canal Point, Florida 33438

ABSTRACT

Yield parameters were compared for five sugarcane cultivars (CP 72-1210, CP 80-1827, CP 84-1198, CP 85-1382 and CP 89-2143) grown from tissue culture derived and clonally propagated seedcane that was virus-free or infected with S*ugarcane yellow leaf virus* (SCYLV), respectively. Yield parameters measured in plant cane and first ratoon were number of stalks per plot, plot weight, % sugar yield and kg sucrose per plot. In the first of two experiments, three yield parameters (number of stalks, weight and sucrose per plot) were significantly higher for tissue-culture derived plots than for plots planted with heat-treated seedcane in the plant crop for all five cultivars when analyzed individually and combined. In the subsequent first ratoon crop, the number of stalks and weight per plot of CP 72-1210, CP 84-1198 and CP 85-1382 were higher ($P \leq 0.05$) for the tissue culture derived plots than for the plots planted with heat-treated seedcane. Also in first ratoon, the kg sucrose per plot was higher for CP 72-1210 ($P \leq 0.10$) and CP 84-1198 and CP 85-1382 ($P \leq 0.05$) in the tissue culture derived plots than in plots planted with heat-treated seedcane. The number of stalks per plot of CP 89-2143 was also higher ($P \leq 0.10$) in the tissue culture plots than in the heat-treated plots. Results of CP 80-1827 in first ratoon were opposite the other cultivars with plots planted with heat-treated seedcane having higher yield (numbers of stalks and weight per plot) than plots planted with tissue culture derived seedcane ($P \leq 0.05$). In the second experiment, the plot weight and kg sugar per plot of a virus-free treatment for all cultivars combined were higher ($P \leq 0.05$) than in the SCYLV-infected treatment. Although the majority of the yield parameters were numerically higher for plants in the SCYLV-free plots for the individual cultivars, most were not statistically significant ($P \leq 0.05$). Kg sucrose per plot for CP 84-1198 and weight per plot for CP 89-2143 were higher ($P \leq 0.05$) for plants in the SCYLV-free plots than the plots planted using infected plants. The results indicate a benefit from using tissue culture derived seedcane that is free of SCYLV infection.

INTRODUCTION

Sugarcane yellow leaf virus (SCYLV) was reported to be associated with symptoms of sugarcane yellow leaf syndrome (12, 14). SCYLV has been reported in many countries around the world (2, 4, 7, 9, 14, 15). Recently, the name yellow leaf has been proposed for the disease (http:www.isppweb.org/names_common.asp) replacing the use of sugarcane yellow leaf syndrome. In Florida, the incidence of commercially grown sugarcane plants infected with SCYLV is high (4). The incidence of SCYLV infection of sugarcane clones in the CP cultivar development program increases the longer the clones are in the program. In the seedling stage, after the plants were in the field for 6 months, 2 % of them were infected. The clones that were advanced through the program to Stage IV, 4 years later, had an incidence of 55 % infection as an average of CP 96 to CP 99 series (4) with no selection for resistance. Plants of susceptible cultivars in commercial fields usually have an incidence of 85 % or higher in Florida (4).

There have been reports of SCYLV affecting yield parameters. In Brazil, the cultivar SP 71-6163 was withdrawn from commercial production because of losses of greater than 25 % in plants having yellow leaf syndrome that were subsequently shown to be infected with SCYLV (14). In Louisiana, preliminary results indicated a significant yield loss in SCYLV-infected plants (7). Stalk diameter and juice quality were negatively affected, and five of nine clones had reduced sucrose levels in asymptomatic but infected plants (9). The rate of photosynthesis was reduced in SCYLV-infected plants (15).

Sugarcane cultivars that are infected with SCYLV can be cleaned up via meristem tissue culture (10). This technique is used in Louisiana, Florida and in South American (Brazil and Columbia) countries where seedcane derived via tissue culture is planted. The tissue culture technique to produce seedcane may begin with either a small portion of apical meristem where disease-free plants are often obtained, or a larger piece of plant tissue including the apical area that does not free the plants of pathogens. The tissue culture practice in Louisiana was originally used as a means to control ratoon stunting disease, but it can also eliminate viruses if properly performed. The effect of using seedcane derived via tissue culture as a means to control SCYLV and increase yields has not been evaluated in Florida.

Single-plant plots have been used to estimate losses in sugarcane due to ratoon stunting disease, sugarcane bacilliform virus and rust (3, 5, 6). Single-plants or small plots provide a mechanism to accurately determine the disease status of each plant during the experiment. This is particularly important when no external symptoms are evident as in ratoon stunting disease. Only representative sampling can be taken in large plot tests. Since symptoms of SCYLV are not always expressed or appear late in the crop, it would be an advantage to be able to test each plant in a yield loss experiment to establish its disease state. Small plots also allow high replication of experiments that will permit detection of small differences (6).

The objectives of this study were to compare the yield parameters of plantings from seedcane derived through tissue culture and that of heat-treated, clonally propagated seedcane and to estimate the effect of SCYLV by comparing the yield parameters of SCYLV-free and SCYLV-infected plants.

MATERIALS AND METHODS

Yield Tests

Two yield tests were conducted to compare plants derived from tissue culture and clonal propagation and to determine the effect of SCYLV on sugarcane yield parameters. The first experiment at the Sugarcane Field Station in Canal Point, Florida was planted on March 7, 2001. A second test was planted at the University of Florida, EREC Experiment Station in Belle Glade, Florida on January 18, 2002. The Canal Point test was established using two different seedcane sources: Kleentek® (Certis) tissue culture derived seedcane obtained as plantlets that were virus-free for the SCYLV-free treatment and regular bud-propagated seedcane that was long-hot-water treated. The heat treatment normally eliminates the ratoon stunt pathogen, and it was assumed that all the seedcane was RSD-free. The heat does not eliminate SCYLV, and previous assays indicated over a 90 % infection. Tissue culture derived seedcane was similar to seedcane

commercially sold as Kleentek® except that it did not undergo a culling to remove off-types. In both cases, the seedcane used to establish the experiment was the first cutting from nurseries established with the different seed sources. The experiment at Belle Glade was established from seedcane taken from the Canal Point test plots that was planted with all tissue culture derived seedcane which was either cut from plots of SCYLV-free or plots naturally infected with SCYLV. Plant and first ratoon data were taken on the Canal Point experiment, and only plant crop data were taken on the test at Belle Glade.

Each experiment was a complete randomized block design with five sugarcane varieties (CP 72-1210, CP 84-1198, CP 85-1382, CP 80-1827 and CP 89-2143). Each 1 m plot was established by cutting in half a 2 m stalk of sugarcane and planting the two pieces side by side. The plots in the row were separated by a 2 m gap, and the rows were 1.5 m apart. The test at Canal Point had 40 replications, and the test at Belle Glade had 36 replications. Analyses of variance were conducted using PROC GLM in SAS version 6.10 (SAS Institute Inc. Cary, NC) on number of stalks per plot, plot weight, sucrose, % sugar yield and kg sucrose per plot.

Disease States

Disease status of SCYLV was determined prior to planting on stalks within a single plant for the Canal Point test and by plot for the Belle Glade test and three times during each crop using a tissue blot immunoassay (13). In the Canal Point experiment, one leaf from each 1 m plot was assayed in May and three leaves per plot in October and December of the plant crop (2001). One leaf per plot was assayed in April, July and September of the first ratoon crop (2002). In the Belle Glade experiment, one leaf per plot was assayed in May, October and December of the plant crop (2002).

Yield Parameters

The number of stalks per plot, plot weight, sucrose content, % sugar yield, and kg sucrose per plot were determined. A sample of five stalks was cut from each plot to determine stalk weight and for juice analysis. Juice analysis was conducted at the sugar laboratory at the Sugarcane Field Station, in Canal Point. Plot weight and kg sucrose per plot were determined from yield parameters of the five stalk bundle multiplied by stalks counts and % sugar yield of each plot.

RESULTS AND DISCUSSION

In the Canal Point experiment combining all cultivars, the number of stalks, stalk fresh weight, and kg sucrose per plot were significantly higher in plants in the tissue culture derived plots than plants in plots planted with heat-treated, bud-propagated seedcane for both the plant and first ratoon crops (Table 1). The kg stalk weight per plot difference between the two seedcane sources was 32 % in the plant crop and 8 % in first ratoon. The kg sucrose per plot difference between the two seedcane sources was 33 % in the plant crop and 12 % in the first ratoon crop. The plant crop difference was considerably higher than that obtained by growers. An average difference for both cane tonnage and sugar per hectare was 4 to 8 % comparing tissue culture (Kleentek®) versus long hot-water-treated seedcane for the crop cycle of a plant

and two ratoons evaluating multiple cultivars (R. Perdomo, personal communication). Although the plants developing from the long-heat-treated seedcane normally do not have ratoon stunt and were assumed RSD-free, there is no effect of the treatment on SCYLV. Previous assays indicated over 90 % SCYLV infection of the heat-treated, bud-propagated plants. Thus, there were two differences combined in the treatments, type of seedcane and SCYLV infection state. This complicated the interpretation of the results. These treatment differences, however, reflect two seedcane options that the growers have for planting. A third option, using field-run seedcane, is less desirable because of the high incidence of yellow leaf and possible infection with ratoon stunting disease.

In the plant crop of the Canal Point experiment, the number of stalks, plot fresh weight and kg sucrose per plot were significantly higher for all individual cultivars in plants in the tissue culture derived plots than from plants in plots planted with heat-treated seedcane cut (Table 1). Sucrose content was only higher for one cultivar.

In the first ratoon crop of the Canal Point experiment, the number of stalks per plot was higher in plots planted with tissue culture derived seedcane than in plots planted with regular heat-treated seedcane for CP 72-1210, CP 84-1198 and CP 85-1382 at $P \leq 0.05$ and for CP 89-2143 at $P \leq 0.10$. Comparing weights per plot, CP 72-1210, CP 84-1198 and CP 85-1382 were higher at $P \leq 0.05$ in the plots planted using tissue culture derived seedcane versus heat-treated seedcane. The average kg weight difference in the ratoon test per plot for the cultivars, excluding CP 80-1827, was 15 %. The kg sucrose per plot of CP 84-1198 and CP 85-1382 and the number of stalks for CP 89-2143 were higher at $P \leq 0.10$ in the plots planted with tissue culture derived seedcane compared to heat-treated seedcane. In contrast to the other cultivars and to plant cane data, plot weight for CP 80-1827 was significantly higher for plots planted using regular heat-treated seedcane than for plots planted using seedcane derived via tissue culture. There is no explanation for the higher yields of CP 80-1827 using regular seedcane. Although these results indicate cultivar differences, a general yield increase was obtained using seedcane derived via tissue culture. Although the differences were not always statistically different with individual cultivars, there is a definite trend. Dean (6) reported a tendency for variation of plant weight to increase in ratoons making it more difficult to detect yield differences.

Whether the response is caused by type of seedcane or its disease status or a combination of the two could not be determined by this single comparison. Therefore, the second experiment was conducted.

During the experiment at Canal Point, some plots established with virus-free seedcane became naturally infected with SCYLV (Figure 1). The rate of infection was cultivar dependent, with CP 72-1210 becoming the most rapidly infected in comparison to the other cultivars. At the end of the 2-year experiment, 86 % of the CP 72-1210 plots established with virus-free seedcane were infected. The incidence of infection at the end of the 2-year experiment for the other cultivars was 44 % for CP 89-2143, 38 % for CP 84-1198, 28 % for CP 80-1827 and 20 % for CP 85-1382. The incidence of SCYLV in leaves sampled from clones Stage II of the cultivar development program was 30 % after approximately 2.5 years from being transplanted in the field as seedlings and being exposed to natural infection by viruliferous aphids (unpublished

data). Based on the incidence of SCYLV in these cultivars and in Stage II clones, the use of virus-free seedcane would probably result in a lower incidence of the virus throughout a normal 3-year crop cycle compared to the over 85 % that is currently found in plants grown from regular seedcane in grower fields (4). Yield components were higher in first ratoon despite the increase in infection by SCYLV.

In the Belle Glade experiment for all cultivars, combined plot weight and kg sugar per plot were higher for the virus-free compared to the SCYLV-infected plots at $P \leq 0.05$ (Table 2). Both yield parameters were approximately 11 % higher in the virus-free plots than in the infected treatment. In the analysis of the data for the individual cultivars, only cane weight per plot of CP 89-2143 was higher at $P \leq 0.05$ for the SCYLV-free plots over the SCYLV-infected plots (Table 2). Furthermore, kg sucrose per plot for CP 89-2143 was significantly higher at $P \leq 0.10$ for the SCYLV-free over the SCYLV-infected plots. Cane weight per plot for CP 84-1198 and kg sucrose per plot for CP 89-2143 were significantly higher at $P \leq 0.10$ for the SCYLV-free plots. No other differences were detected in individual cultivars. Although the results indicate that the SCYLV-free plots were consistently higher in yield parameters, except % sugar yield, statistical differences were at the borderline of detection in this highly replicated experiment for comparisons of individual cultivars. Plant stress has been reported to accentuate the effect of SCYLV on yield (9). The plants in this test were not stressed, so there would not be any accentuation of yield differences caused by stress.

Several papers have compared planting material derived from the apical meristem and apical leaf whorl using tissue culture and regular seedcane for yield components, variation and genetic stability (1, 8, 11, 16). Generally, there is more phenotypic variability in plants obtained by shoot culture than in plants obtained by planting regular bud propagated seedcane (1, 8, 16). Also, molecular techniques detected more polymorphisms in plants derived from meristem culture than in plants obtained by normal vegetative propagation (16). Burner and Grisham (1) reported that little or no induced variability occurred in important yield parameters as a result of shoot tip culture. Stalk diameter may decrease and stalk population may increase for plants derived from tissue culture depending on the method used (8). No yield differences were detected between plants obtained using tissue culture derived from apical meristems and plants obtained using bud propagation (8). Only the report by Hoy et al. (8), discussed the disease status of the planting material. In that study, it was assumed that there were no differences in disease status in all the comparisons. In our studies, there were differences in SCYLV infection status between the two seedcane sources. This appears to explain at least part of the yield differences between the two seedcane sources we obtained.

The results indicate a benefit of using tissue culture derived seedcane that is free of SCYLV. Because there is a high incidence of SCYLV infection occurring in commercially grown plants, a definite problem is indicated. This is particularly true since yield losses can be increased by adverse environmental conditions in mineral soils. The use of tissue culture derived seedcane also would aid in the control of ratoon stunting disease, leaf scald, and mosaic.

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Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U. S. Department of Agriculture and does not imply its approval to the exclusion of other products that also may be suitable. This article reports the results of research only.

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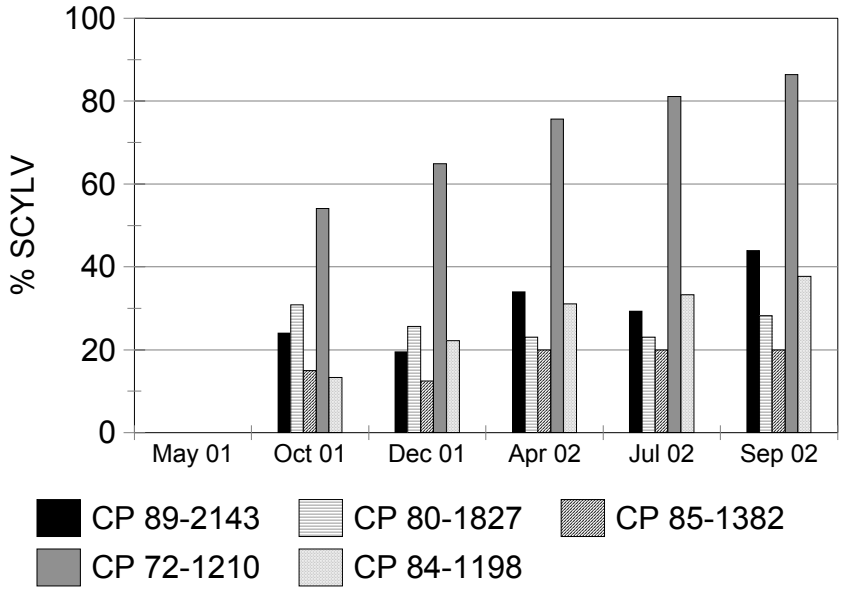

Figure 1. Increased incidence of SCYLV by plot with time in originally virus-free tissue culture derived seedcane during the Canal Point yield experiment.

Table 1. Yield parameters in plots planted with tissue culture derived seedcane versus plots planted with heat-treated seedcane in plant and first ratoon crops at Canal Point.

		Plant cane				First ratoon			
Cultivar	Seedcane source	Stalks/ plot	Plot wt (kg)	% yield	Kg sugar /plot	Stalks/ plot	Plot wt (kg)	% yield	Kg sugar /plot
All cultivars	TC	18.45	29.16	11.44	3.33	30.58	39.63	11.47	4.55
	HWT	12.84	19.76	11.23	2.22	25.73	36.47	11.08	4.03
LSD 0.05		**0.66** [a]	**1.14**	0.27	**0.14**	**1.41**	**2.35**	**0.24**	**0.28**
LSD 0.10		**0.55**	**0.96**	0.23	**0.12**	**1.18**	**1.97**	**0.20**	**0.23**
CP 72-1210	TC	21.24	32.35	11.87	3.82	34.82	43.09	11.78	5.08
	HWT	11.42	17.28	11.64	2.02	25.14	36.67	12.00	4.41
LSD 0.05		**1.64**	**2.87**	0.60	**0.37**	**3.41**	**5.82**	0.47	0.71
LSD 0.10		**1.36**	**2.38**	0.50	**0.30**	**2.84**	**4.83**	0.39	**0.59**
CP 80-1827	TC	15.42	29.53	11.40	3.35	25.69	36.99	11.04	4.09
	HWT	12.61	22.48	10.32	2.34	30.75	47.66	9.17	4.40
LSD 0.05		**1.16**	**2.77**	**0.70**	**0.34**	**3.02**	**4.48**	**0.60**	0.50
LSD 0.10		**0.96**	**2.30**	**0.58**	**0.28**	**2.50**	**3.72**	**0.50**	0.44
CP 84-1198	TC	17.55	26.90	11.55	3.10	31.41	38.64	11.82	4.58
	HWT	13.70	19.36	11.49	2.24	25.90	31.30	11.29	3.54
LSD 0.05		**1.37**	**1.58**	0.72	**0.25**	**3.71**	**5.57**	**0.50**	**0.70**
LSD 0.10		**1.14**	**1.31**	0.60	**0.21**	**3.08**	**4.64**	**0.42**	**0.58**
CP 85-1382	TC	18.38	26.44	10.73	2.82	32.76	44.79	11.05	4.96
	HWT	10.37	15.13	10.87	1.65	21.46	34.08	10.55	3.62
LSD 0.05		**1.66**	**3.11**	0.57	**0.38**	**2.90**	**5.97**	0.61	**0.78**
LSD 0.10		**1.38**	**2.59**	0.48	**0.31**	**2.41**	**4.97**	0.50	**0.58**
CP 89-2143	TC	19.90	30.80	11.68	3.60	29.05	35.52	11.69	4.18
	HWT	15.77	24.09	11.75	2.81	26.13	35.31	11.84	4.21
LSD 0.05		**1.00**	**2.11**	0.46	**0.29**	3.15	5.54	0.55	0.73
LSD 0.10		**0.83**	**1.75**	0.39	**0.24**	**2.63**	4.61	0.46	0.60

[a] Bold indicates a statistically significant difference at the probability listed in the left column.

Table 2. Yield parameters of SCYLV-infected and healthy plants derived from tissue culture.[a]

Cultivar	Disease state	Stalks/ plot	Plot wt (kg)	% yield	Kg sugar/ plot
All cultivars	–	17.24	25.21	12.06	3.04
	+	16.57	22.40	12.14	2.72
LSD 0.05		1.06	**1.68**[b]	0.20	**0.21**
LSD 0.10		0.89	**1.41**	0.17	**0.18**
CP 72-1210	–	18.59	25.16	11.55	2.89
	+	17.55	22.02	12.15	2.68
LSD 0.05		3.13	4.25	0.62	0.56
LSD 0.10		2.58	3.51	**0.51**	0.46
CP 80-1827	–	14.07	22.93	11.71	2.71
	+	12.31	20.85	11.88	2.46
LSD 0.05		2.18	4.36	0.39	0.55
LSD 0.10		1.78	3.57	0.32	0.45
CP 84-1198	–	16.39	25.65	12.51	3.21
	+	16.81	22.72	12.29	2.79
LSD 0.05		1.83	3.02	0.55	**0.39**
LSD 0.10		1.51	**2.49**	0.46	**0.33**
CP 85-1382	–	17.71	22.95	11.92	2.73
	+	17.63	21.55	11.92	2.57
LSD 0.05		3.41	5.65	0.55	0.67
LSD 0.10		2.81	4.66	0.45	0.55
CP 89-2143	–	20.00	30.14	12.32	3.71
	+	18.13	24.98	12.32	3.10
LSD 0.05		2.86	**4.89**	0.41	0.62
LSD 0.10		2.36	**4.02**	0.32	**0.51**

[a] Experiment planted at Belle Glade from virus-free and SCYLV-infected seedcane cut from the experiment at Canal Point.

[b] Bold indicates a statistically significant difference at the probability listed in the left column.