

## **INCIDENCE AND SPREAD OF SUGARCANE YELLOW LEAF VIRUS IN SUGARCANE CLONES IN THE CP-CULTIVAR DEVELOPMENT PROGRAM AT CANAL POINT**

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### **ABSTRACT**

The incidence of sugarcane yellow leaf virus (SCYLV) in sugarcane clones increased the longer the clones were in the CP-cultivar development program and exposed to natural infection. During 1998 to 2002, the average incidence of SCYLV in Stage II clones was 30.1 %, while SCYLV incidence in Stage IV clones, in the program 3 years longer, was 55.6 %. A few clones had an incidence of SCYLV below 25 % by the time they were advanced to Stage IV. These clones may have partial resistance to the virus. The results have implications for breeding and selecting for resistance to the virus.

### **INTRODUCTION**

Sugarcane yellow leaf syndrome was recognized in Hawaii in the 1980s and was subsequently observed in numerous countries (Comstock *et al.*, 2002b; Izaguirre-Mayoral *et al.*, 2002; Lockhart *et al.*, 1996; Lockhart and Cronje, 2000; Vega *et al.*, 1997; Viswanathan, 2002). Two different pathogens, sugarcane yellow leaf phytoplasma and sugarcane yellow leaf virus (SCYLV) have been associated with the sugarcane yellow leaf syndrome symptoms (Cronje *et al.*, 1998; Lockhart *et al.*, 2000; Scagliusi and Lockhart, 2000). In Florida, only SCYLV has been reported (Comstock *et al.*, 1998). Disease losses of 25 % in Brazil in SP 71-6163 have been attributed to SCYLV (Vega *et al.*, 1997). Yield losses of 15 to 20 % also have been reported due to yellow leaf virus in Louisiana (Grisham *et al.*, 2002). Elevated Brix readings of juice extracted from the midribs of symptomatic leaves have been reported (Comstock *et al.*, 1994). Differences in leaf area, total reducing sugars, chlorophyll content, and sugar transport were observed between symptomatic and asymptomatic plants infected with SCYLV (Izaguirre-Mayoral *et al.*, 2002; Viswanathan, 2002). All reported changes negatively impact sugar yield.

Symptoms of SCYLV are more evident in mature and stressed plants (Lockhart and Cronje, 2000). Only isolated plants exhibit symptoms in Florida before the start of the harvest season that begins in mid-October. Symptoms start as the weather turns cooler in October-November, initially with the lower midrib of leaves 3 to 6 (counting from the top expanding leaf downward) becoming yellow. The yellowing then expands into the leaf blade with necrosis starting from the leaf tip and progressing down the leaf blade becoming most evident in December until the end of the harvest season in March. During January through March, entire fields may appear yellowish.

This paper addresses SCYLV in the CP-cultivar development program in Florida. Symptoms of the syndrome were observed in 1994 in clones that were used in crossing at the USDA Sugarcane Field Station at Canal Point, Florida (Comstock *et al.*, 1994). The presence of SCYLV was confirmed by a serological tissue blot assay using a SCYLV specific antibody (Comstock *et al.*, 2002a; Comstock *et al.*, 1999) and a reverse transcriptase polymerase chain

reaction assay using primers to detect the virus (Comstock *et al.*, 1998). There are no reports of the sugarcane yellow leaf phytoplasma in Florida.

The objectives of this paper are: 1) to determine the variability of incidence of SCYLV in clones in the CP-cultivar development program at Canal Point, Florida, 2) to determine if the incidence of SCYLV increases in the clones with time, 3) to determine if resistance exists in the current selection program and 4) to determine if natural infection can be used to select clones resistant to the virus.

## MATERIALS AND METHODS

### Surveys

Plants of sugarcane clones in Stages II through IV (four sequential years) of the CP-cultivar development program (USDA-ARS Sugarcane Field Station, Canal Point, Florida) were surveyed for the presence of SCYLV for 5 years, during 1998 through 2002. The number of clones, plants sampled, and locations of plots in the cultivar development program that were sampled during 1998 to 2002 are presented in Table 1. The incidence of SCYLV infection of the clones in each CP Series was an average of the incidence of all the clones based on the number of infected leaf samples divided by the total number of leaves sampled and assayed in that year and selection stage.

### Tissue Blot Immunoassays

SCYLV infection was determined by assaying for the presence of the virus in the youngest fully emerged leaf by a tissue blot immunoassay using antibodies specific for the virus. Briefly, the leaf was removed from a plant and the leaf blade tissue was removed from the midrib. The basal portion of the midrib was cut with a sharp, razor-blade scalpel, and the freshly cut midrib was firmly pressed on a nitrocellulose membrane, leaving a clear impression of the leaf midrib on the membrane. One impression per leaf midrib was made. The membrane was serologically developed using SCYLV specific antibodies developed by B. E. Lockhart, University of Minnesota (Minneapolis) according to Schenck *et al.* (1997) except that Fast Blue was used as the enzyme substrate (Comstock *et al.*, 1998). A stereo-microscope was used to examine the leaf prints. Because SCYLV is located in the phloem, a sample was positive for the presence of the virus when the phloem bundles within the leaf print stained blue.

## RESULTS AND DISCUSSION

The incidence of SCYLV infection among clones for each CP Series in Stage II through IV for years 1998 through 2002 is shown in Table 1. For each CP Series, the incidence of samples with SCYLV generally increased the longer the series was in the cultivar development program. The average yearly incidence of SCYLV infected clones in Stage II ranged from 25.6 to 32.0 % during the five years that they were sampled. The incidence of SCYLV infection among all clones that were advanced to Stage IV during the same period ranged from 41.2 to 66.8 % (Table 1). The average incidence of SCYLV in Stage II was 30.1 % for years 1998-2002 and increased to 55.6 % in Stage IV. These results plus the fact that the incidence of SCYLV among plants in grower's fields in Florida exceeds 85% clearly indicates a possible

threat of SCYLV in Florida. The virus is present in essentially all commercial CP-cultivars. The high incidence of infection in the selected population indicated that there is little resistance among CP sugarcane clones. Almost all parental clones used for crossing in the cultivar development program are infected with the virus or have symptoms indicating a lack of SCYLV resistance for the crossing program (Comstock *et al.*, 1998; Miller *et al.*, 1994).

In Venezuela, there were clear reductions in yield parameters between symptomatic and asymptomatic plants that are infected with the virus. However, without severe symptom development, the yield losses were not dramatic (Izaguirre-Mayoral *et al.*, 2002). In India, in similar comparisons of yield parameters between symptomatic versus asymptomatic plants, reduced stalk diameter, lower Brix readings, and lower photosynthetic rates were associated with symptomatic plants. SCYLV infection was based on visual symptoms and not on detecting the virus in test plants. However, serological tests confirmed the presence of the virus in most plants suspected of being infected in a separate diagnostic test (Viswanathan, 2002).

The incidence of SCYLV in the CP 95 through CP 98 Series clones is shown at each stage as they moved through the program from Stage II to Stage IV trials (Tables 2-5). Six individual clones (CP 96-1865, CP 97-1164, CP 97-1850, CP 97-1944, CP 97-1989 and CP 97-2068) had an incidence of SCYLV infection of 20 % or less in Stage IV. These clones presumably have some resistance to SCYLV infection, since there was equal opportunity for infection with other clones in field trials during the 7 years of testing after being derived from true seed. These clones with less than 20 % incidence of SCYLV infection apparently had a partial resistance. The clones had no common parentage.

The high increase in incidence of SCYLV in the cultivar development program indicates that little resistance has been incorporated using the present parental clones. An effort to introduce resistance from sources other than the CP clones presently used for breeding would assist in the development of SCYLV resistant clones. Clones of *Saccharum spontaneum* appear to be a good choice, since only seven of 100 clones surveyed in the World Collection at Miami were infected with SCYLV compared to 75 % of the *S. officinarum* clones (Comstock *et al.*, 2002a). Others have reported *S. spontaneum* clones as having a low incidence of infection (Schenck *et al.*, 1997). An alternative breeding option would be to use imported commercial clones that are reported resistant. Eight Hawaiian varieties (H varieties) with SCYLV resistance have been imported via the USDA quarantine for use in crossing. Additionally, several clones that appear to have partial resistance, since less than 25 % of the plants sampled were SCYLV infected in Stage IV, will be evaluated on their potential to produce resistant progeny. Their progeny also would be more commercially acceptable and therefore, more desirable than using wild *S. spontaneum* clones and imported commercial clones as parents.

A major restriction in incorporating resistance is a lack of an efficient method of inoculating plants to evaluate resistance. Although the spread of SCYLV is relatively fast, it is not fast enough to allow efficient screening of populations for the incorporation of resistance into a cultivar development program. Several years are required to insure adequate exposure of plants relying on natural infection by aphids. A period of 3-5 years to evaluate resistance restricts the cultivar development program. The low number of virus-free clones or clones with a low incidence of infection that remains after a 3-5 year exposure period is totally inadequate.

Methodology to inoculate massive numbers of plants using insectary aphids is needed but probably not feasible since the numbers of clones that can be evaluated will still be limited. Once the plants are inoculated, virus detection in plants is not a limitation since the tissue blot immunoassay allows the rapid determination of the presence of SCYLV in thousands of plants.

As an alternative to detecting resistant plants, a project to associate molecular markers with the resistance is in progress. If marker assisted selection can be developed for SCYLV resistance, the process for the development of resistant cultivars would be greatly enhanced.

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**Table 1.** Incidence of SCYLV in clones in the CP-cultivar development program.

	1998	1999	2000	2001	2002	Overall mean
<b>Stage II</b>						
Series	CP 97	CP 98	CP 99	CP 00	CP 01	
No. clones	1008	957	854	463	1423	
Leaves/clone	1 (2 dates)	1	1	3	1	
Location	CP Station	CP Station	CP Station	CP Station	CP Station	
% Positive <sup>a</sup>	25.6 %	38.3 %	27.8 %	32.0 %	27.0 %	30.1 %
<b>Stage III</b>						
Series	CP 96	CP 97	CP 98	CP99	CP 00	
No. clones	130	130	130	130	130	
Leaves/clone	20	10	10	10	10	
Location	Sugar Farms 46.7	Sugar Farms	Sugar Farms	--	--	
% Positive <sup>a</sup>	%	24.0 %	35.4 %	--	--	35.4 %
Location	--	Duda	Duda	Duda	Duda	
% Positive <sup>a</sup>	--	23.9 %	31.3 %	36.4 %	55.6 %	36.8 %
<b>Stage III Inc.</b>						
Series	CP 95	CP 96	CP 97	CP98	CP99	
No. clones	40	40	40	40	28	
Leaves/clone	20	10	10	10	10	
Location	Sugar Farms	Sugar Farms	Duda	Duda	Duda	
% Positive <sup>a</sup>	55.3 %	49.3 %	26.6 %	48.8 %	51.4%	46.3 %
<b>Stage IV</b>						
Series	CP 94	CP 95	CP 96	CP 97	CP 98	
No. clones	11	11	11	14	14	
Leaves/clone	80	40	40	40	40	
Location	Sugar Farms	Sugar Farms	Duda	Duda	Duda	
% Positive <sup>a</sup>	66.8 %	54.8 %	54.8%	41.2 %	60.2 %	55.6 %

<sup>a</sup> % positive is the number of leaves tested positive divided by the total number of leaves tested.

**Table 2.** Incidence of SCYLV in CP 95 Series clones during their advancement to Stage IV.

Clone	Stage II/1996*	Stage III/1997	Stage III Increase/1998	Stage IV/1999	Stage IV /2000
CP 94-2203	–	ND	0	2.5	42.7
CP 95-1039	+	100	92	100	82.0
CP 95-1076	ND	ND	ND	15	71.0
CP 95-1429	–	0	25	42.5	71.0
CP 95-1446	ND	100	ND	100	90.9
CP 95-1569	–	40	95	15	47.5
CP 95-1570	–	0	30	47.5	78.3
CP 95-1712	–	40	30	52.5	80.0
CP 95-1726	+	0	100	95	90.7
CP 95-1834	+	0	100	87.5	70.0
CP 95-1913	–	100	45	45	84.5

\* A single leaf assayed per clone: + is positive and – is negative. ND = no data.

**Table 3.** Incidence of SCYLV in CP 96 Series clones during their advancement to Stage IV.

Clone	Stage II 1997*	Stage III 1998	Stage III Inc. 1999	Stage IV 2000	Stage IV ratoon/2001
CP 96-1161	++	80	70	52.5	90
CP 96-1171	–	75	100	ND	100
CP 96-1252	–	40	60	95	95
CP 96-1253	+++	100	100	100	100
CP 96-1288	+	55	90	47.5	100
CP 96-1290	–	20	10	27.5	32.5
CP 96-1300	+++	80	70	90	100
CP 96-1350	–	7	ND	55	75
CP 96-1602	–	45	50	35	100
CP 96-1686	–	50	30	100	42.5
CP 96-1865	+	10	0	0	17.5

\* Each + or – indicates the number of leaves sampled per clone: + is positive and – is negative. ND = no data.

**Table 4.** Incidence of SCYLV in CP 97 Series clones during their advancement to Stage IV.

Clone	Stage II/ 1998 *	Stage III/1999	Stage III Inc./2000	%	
				Stage IV/ 2001	Stage IV ratoon/ 2002
CP 97-1068	--	70	80	47.5	67.5
CP 97-1164	--	10	0	0	2.5
CP 97-1362	--	0	ND	47.5	80
CP 97-1387	++	90	ND	95	22.5
CP 97-1433	--	10	50	72.5	ND
CP 97-1777	--	30	0	20	47.5
CP 97-1804	-+	100	70	100	100
CP 97-1850	+-	0	ND	2.5	12.5
CP 97-1928	-+	100	ND	50	97.5
CP 97-1944	--	0	40	0	2.5
CP 97-1979	-	0	10	7.5	27.5
CP 97-1989	--	0	ND	10	20
CP 97-1994	--	0	0	97.5	42.5
CP 97-2068	--	10	ND	26.7	7.5

\* Each + or - indicates the number of leaves sampled per clone: + is positive and - is negative. ND = no data.

**Table 5.** Incidence of SCYLV in CP 98 Series clones during their advancement to Stage IV.

Clone	Stage II/ 1999 *	Stage III/ 2000	%	
			Stage III Inc. 2001	Stage IV/ 2002
CP 98-1029	+	80	-	100
CP 98-1107	-	0	10	40
CP 98-1118	-	0	30	55
CP 98-1139	-	0	-	22.5
CP 98-1325	-	0	10	0
CP 98-1335	ND	0	-	100
CP 98-1417	-	70	-	15
CP 98-1457	+	-	100	95
CP 98-1481	-	-	-	12.5
CP 98-1497	-	10	-	65
CP 98-1513	ND	40	60	85
CP 98-1569	+	10	-	65
CP 98-1725	+	80	-	95
CP 98-2047	ND	-	80	92.5

\* A single leaf assayed per clone: + is positive and - is negative. ND = no data.