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Survey of Sugarcane Yellow Leaf Virus in the Canal Point Breeding and Cultivar Development Program

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Abstract: Sugarcane yellow leaf virus (SCYLV), a *Polerovirus* in the family *Luteoviridae*, causes yellow leaf disease (YLD). Yield losses from YLD have been reported from several countries in both symptomatic and asymptomatic sugarcane cultivars. The breeding nursery at Canal Point (CP) in 2016 and primary and secondary seed increases in the CP cultivar development program at grower's farm from 2015 to 2019 were surveyed for SCYLV infection by the tissue-blot immunoassay using polyclonal antibodies raised against SCYLV. More than 32% of varieties in the CP breeding nursery were infected with SCYLV in 2016. The SCYLV data of primary and secondary seedcane increases from 2015 to 2019 showed that out of 54 varieties screened at different locations, 12 had no SCYLV-positive plants, 24 had less than 5%, 5 had 6% to 12%, and 13 had 20% to 75% of the plants infected with SCYLV. The SCYLV screenings in varieties in the primary and secondary seed increase plantings provide growers an opportunity to acquire virus-free clean seedcane by apical meristem propagation to minimize the spread of the SCYLV and avoid yield losses.



Citation: Sood, S.; Davidson, W.R.; Baltazar, M. Survey of Sugarcane Yellow Leaf Virus in the Canal Point Breeding and Cultivar Development Program. *Agronomy* **2021**, *11*, 1948. <https://doi.org/10.3390/agronomy11101948>

Academic Editor: Ryan Whitford

Received: 28 August 2021

Accepted: 27 September 2021

Published: 28 September 2021

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Keywords: sugarcane yellow leaf virus (SCYLV); germplasm; tissue-blot immunoassay; survey

1. Introduction

Sugarcane is an important crop, contributing to 80% of the sugar consumed in the world. It had a positive annual economic impact of more than \$647.5 million in Florida in 2018 [1]. Sugarcane grows on approximately 161,874.3 ha in Florida [2] and provides approximately 20% of the total sugar consumed in the USA. Sugarcane is also used for biofuel production [3,4] in several countries. More than 100 pathogens including bacteria, fungi, phytoplasmas, and viruses impact sugarcane production. One of the viral diseases, yellow leaf disease (YLD) caused by sugarcane yellow leaf virus (SCYLV) a *Polerovirus* [5] in the family *Luteoviridae* [6], is a major threat to sugarcane production worldwide [7]. YLD exhibit various symptoms such as mild to severe yellowing of the midribs, smaller leaves with clustering (fan-like shape) at the crown region of the plant along with the shortened internodes, necrosis of leaves from tip to the base of leaves [8]. A majority of the SCYLV-infected varieties in the Canal Point breeding and cultivar development program (CP program) and commercial cultivars in Florida are asymptomatic [9,10]. Many visual symptoms that resembled YLD symptoms may be caused by other biotic and abiotic stresses or plant senescence [11,12], making the survey of YLD difficult. The detection of SCYLV is, therefore, dependent on immunological assays, reverse transcription (RT)-polymerase chain reaction (PCR), quantitative (q) RT-PCR and other molecular detection methods. For this study, we used the tissue-blot immunoassay for SCYLV detection in more than five thousand samples every year. It has been reported that low SCYLV titer in some older leaves may fall below the sensitivity threshold of the immunoassay [12]. Another study reported that all samples from leaves number 1 to 3 showed a reliable positive reaction [13] by TBIA. We used the top visible dewlap leaf for SCYLV detection by TBIA. Comstock et al. [14] reported that RT-PCR and TBIA were sensitive in SCYLV detection.

The SCYLV has a single-stranded positive-sense RNA genome [15,16] and eight different genotypes—BRA (Brazil), CHN1 and CHN3 (China), CUB (Cuba), HAW (Hawaii), IND (India), PER (Peru), and REU (Reunion Island)—that have been reported from different countries based on the phylogenetic analysis of partial and/or full-length genome sequences [15–18]. The two genotypes, BRA and CHN1, are present in sugarcane in Florida [9]. The SCYLV is reported to alter the metabolism and transport of sucrose and photosynthesis [19,20]. These alterations limit plant development and cause losses in sugarcane productivity [21,22]. In the United States of America, 11–14% yield losses were reported from Florida and Louisiana [10,23]. However, Lockhart and Cronje [24] reported yield losses of 15% and up to 50% from the United States. Growing resistance varieties is the most efficient, economic, and environmentally friendly strategy to control any disease but due to lack of efficient inoculation technique and absence of visual symptoms [25], breeding for YLD resistance is a challenge. Several studies to identify loci associated with YLD resistance using biparental progenies [26,27] or genome-wide association mapping [28–30] could be helpful in breeding for SCYLV-resistant varieties. SCYLV is transmitted and spread semi-persistently by aphids, mainly *Melanaphis sacchari*, *Rhopalosiphum maidis*, and *R. rufiabdominalis* [31], depending on cultivar susceptibility and weather conditions [32,33]. However, a recent study showed that the transmission of SCYLV by aphid *M. sacchari* is not efficient in Florida [34]. Other aphids including yellow sugarcane aphid (*Sipha flava*) and *Ceratovacuna lanigera* have been reported to transmit SCYLV from infected to healthy plants [35,36]. In the field, SCYLV spread readily by the use of infected seedcane (setts) for plantings [33]. The spread of SCYLV can be reduced by the use of virus-free seedcane produced by apical meristem culture [37–40]. The CP program has been developing disease-resistant sugarcane cultivars with CP prefixes for Florida under a cooperative agreement between the USDA-ARS, the University of Florida, and the Florida Sugar Cane League, Inc. (Clewiston, FL, USA) since 1960. CP cultivars are grown in more than 95% of sugarcane acreage in Florida and in many Central American countries. The CP program uses germplasm collection from different countries and breeding programs as well as many promising CP varieties and cultivars to breed and develop sugarcane cultivars. The varieties are not bred or selected for SCYLV resistance in the CP program due to the lack of a mechanical inoculation protocol to screen a large number of varieties; however, the varieties in its germplasm were screened every five years and in seedcane increase plantings every year. The CP program has six stages of breeding and selection: crossing, seedlings, stages I, II, III, and IV [41]. It takes at least eight years to release a cultivar from the time a cross is made [42]. The potential varieties to be released for commercial production are advanced to primary seedcane increase followed by secondary seedcane increase. The objectives of this study were to evaluate the prevalence of SCYLV infection in the germplasm of the CP program and SCYLV status of the varieties in the primary and secondary seedcane increases to provide breeder and growers the SCYLV status of the varieties used in crossing and commercial production.

2. Materials and Methods

2.1. Collection of Samples

The top visible dewlap leaf was randomly collected for each variety from five, thirty, and fifty plants, respectively, in the CP breeding nursery, primary and secondary seedcane increase fields. The leaf samples were collected from plant cane. Approximately 80% (1195 varieties) of the CP breeding nursery (Table 1) was surveyed for SCYLV infection in 2016. The CP breeding nursery was planted in February 2015 and each plot was ~3.7 m in length with an 1.8 m alley. The varieties in the seed increases (primary and secondary) of the CP program were also tested annually for the SCYLV infection from 2015 to 2019. Each plot in the primary increase consisted of 3 rows ~135 m in length and each plot in the secondary increase consisted of 2 rows ~777 m in length. The primary and secondary increases are planted at different locations throughout South Florida. The varieties for primary and secondary increases in seedcane on muck soil were planted at seven commercial growers'

farms (Area 4 of US Sugar Corporation (A4, 26°51'34.7'' N 80°36'23.3'' W), A. Duda & Sons, Inc. (Belle Glade, FL, USA) (DU, 26°35.93' N, 80°37.81' W), Eastgate Farms, Inc. (Pahokee, FL, USA) (EG, 26°47.67' N, 80°39.97' W), Knight Management, Inc. (Sixmile Bend, FL, USA) (KN, 26°38.53' N, 80°27.21' W), Okeelanta Corporation (South Bay, FL, USA) (OK, 26°34.35' N, 80°49.72' W), Sugar Farms Cooperative North-Osceola Region (Pahokee, FL, USA) (SF, 26°50.53' N, 80°31.93' W), and Wedgworth Farms, Inc. (Belle Glade, FL, USA) (WD, 26°40.73' N, 80°34.37' W) and on sand soils in four commercial growers' farms (Hilliard Brothers of Florida Ltd. (Montura, FL, USA) (HI, 26°42.14' N, 81°2.31' W), Pahokee Produce Inc. (Westlake, FL, USA) (PP, 26°47.26' N, 80°25.09' W), Perry Farms/Lykes Bros. (Moore Haven, FL, USA) (PF, 26°45.7' N, 80°23.7' W), and Townsite (Clewiston, FL, USA) (TS, 26°44.37' N, 80°58.95' W) in November each year. Since 2016, primary and secondary seed increase fields on sand soil were planted at Shawnee farm (Shawnee, FL, USA) (SH, 26°46.59' N, 80°58.02' W) (Figure 1). The primary and secondary seed increase trials started at the SH location in 2016 and the PF location the following year. In 2015, nine varieties in primary increase and five varieties in the secondary increase were surveyed. In 2016, the primary and secondary increases had eight varieties in each trial but only two varieties were on muck soil. In 2017, 2018, and 2019, the primary and secondary increase trials together, respectively, consisted of 17, 9, and 11 varieties.

Table 1. Number of varieties in Canal Point breeding nursery in 2016.

Tested for SCYL V		Not Tested for SCYL V	
¹ CL	143	CL	0
CP	311	CP	37
CPCL	159	CPCL	11
Ho	43	Ho	64
HoCP	59	HoCP	49
L	14	L	28
Q	23	Q	7
TCP	87	TCP	9
US	246	US	60
Other	110	Other	

¹ Prefixes: Clewiston (CL); Canal Point (CP); Canal Point × Clewiston (CPCL); Houma (Ho); Houma × Canal Point (HoCP); Louisiana (L); Queensland (Q); Texas × Canal Point (TCP); Varieties from Canal Point wide crosses (US).

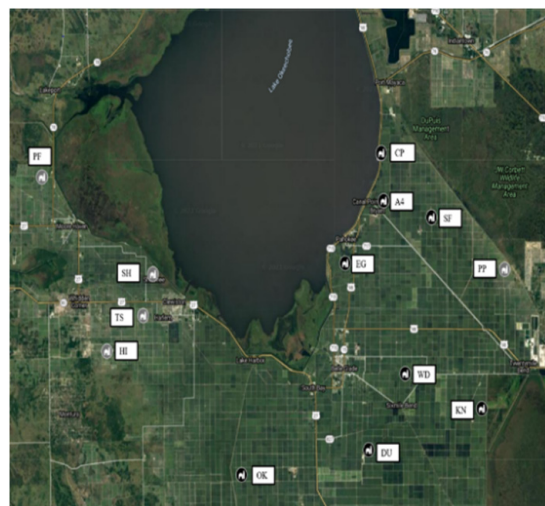


Figure 1. Sugarcane seedcane primary and secondary increase fields located at growers' farm during 2015 to 2019. Muck soil fields were located at Area 4 (A4), Duda (DU), Eastgate (EG), Knight (KN), Okeelanta (OK), South Florida (SF), and Wedgworth (WD). Sand soil fields were located at Hilliard brothers (HI), Pahokee Produce (PP), Perry Farm (PF), Shawnee Farm (SH), and Townsite (TS).

2.2. Tissue-Blot Immunoassay (TBIA)

Tissue blots were made from the midribs of the first-dewlap leaves that were transversely cut with a razor blade and immediately pressed onto a 0.45 µm nitrocellulose membrane (Biorad Laboratories, Hercules, CA, USA). The tissue-blot membranes (membranes) were kept at 4 °C until processed as described by Schenk et al. [43]. All the steps (1 to 9) described below were performed on a shaker at 75 rpm. (1). Membranes were blocked in 2% non-fat dry milk (2% milk) dissolved in a TBIA buffer (100 mM Tris-HCl, pH 7.5, and 150 mM NaCl) for 1 h at room temperature. (2). Membranes were rinsed once in a TBIA buffer for 1 min. (3). Membranes were placed into a SCYLV polyclonal antibody IgG solution (Dr. B. E. L. Lockhart, University of Minnesota, St Paul, MN, USA) diluted (1:8000) in 1% dry milk in TBIA buffer for 3 h at room temperature. (4). Membranes were rinsed three times in TBIA buffer for 5 min each. (5). Membranes were incubated in alkaline phosphatase-conjugated goat anti-rabbit IgG (Sigma-Aldrich, St. Louise, MI, USA) diluted (1:2000) in 1% dry milk in TBIA buffer for 3 h at room temperature. (6). Membranes were rinsed twice in TBIA buffer for 15 min each. (7). Membranes were incubated for 30 min in the dark in a substrate solution made of substrate buffer (300 mM Tris base, pH 9.1; 6% *w/v* solution of Naphthol in dimethylformamide); Fast Blue BB salts (0.1% *w/v*) and 0.1M MgCl₂ (0.5% *v/v*). (8). The membrane was soaked in 20% household bleach for 5 min. (9). Finally, membranes were rinsed in distilled water and dried on a paper towel. The membranes were inspected for blue color development in the phloem tissue (Positive reaction to SCYLV, Figure 2) with a stereomicroscope (100×). Positive reactions were determined by comparing them with resistant controls (CP 57-603). A plant was considered infected by SCYLV when at least one vascular bundle of one leaf showed a positive reaction.

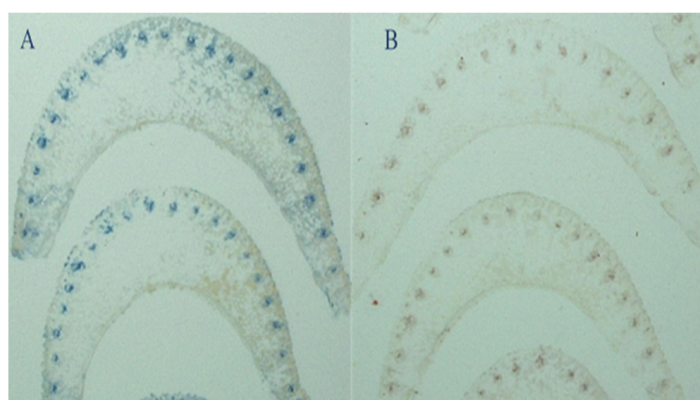


Figure 2. Tissue-blot immunoassay of midrib of top visible dewlap leaves. (A) Infected leaves; (B) healthy leaves. The blue dots are stained phloem cells where the virus lives.

2.3. Data Collection and Analysis

Percent SCYLV incidence was calculated to determine the SCYLV infection in large and small seed increase plantings and the CP breeding nursery.

$$\text{Percent incidence in CP breeding nursery} = \frac{\text{Number of varieties with SCYLV-positive results with the same prefix}}{\text{Total number of varieties tested with the same prefix}} \times 100$$

$$\text{Percent incidence in primary and secondary seedcane increase trials} = \frac{\text{Number of leaves with SCYLV}}{\text{Total number of leaves tested}} \times 100$$

Analyses of variance were performed using SAS/GLIMMIX in SAS 9.4 (SAS Institute, 2011) to determine statistically significant differences among clones, locations, and years. Varieties were clustered based on their percent incidence to SCYLV by K-mean clustering analysis using RStudio.

3. Results

3.1. Percent Incidence in the CP Breeding Nursery

The survey of varieties in the CP breeding nursery conducted in 2016 showed that 37.23% of the tested varieties were infected with the SCYLV. The percent SCYLV incidence in the varieties from different breeding programs ranged from 13.95% to 62.68% (Table 2). The highest percentage (62.68%) of CL varieties were infected with SCYLV followed by L (57.14%). The CP breeding nursery had varieties with a series as early as 27 (1927) and as late as 11 (2011). Some varieties could be older than 1927 (data not shown).

Table 2. Percent incidence of sugarcane yellow leaf virus in the Canal Point breeding nursery.

Prefixes	Breeding Program	Percent Infected Varieties
CL	Clewiston	62.68
CP	Canal Point	46.18
CPCL	Canal Point Clewiston	27.22
Ho	Houma	13.95
HoCP	Houma Canal Point	27.11
L	Louisiana	57.14
Q	Queensland	30.43
TCP	Texas Canal Point	35.63
US	Canal Point wide crosses	27.07
Others	Several programs	29.41

3.2. Percent Incidence in the Primary and Secondary Seedcane Increase Trials

The data collected from the primary and secondary seedcane increase plantings from 2015 to 2019 showed that out of 55 varieties screened at different locations, 12 were free of SCYLV, 24 had less than 5% SCYLV incidence, 5 had approximately 15%, and 13 had 20% to 75% of the plants infected with SCYLV (Table 3). The SCYLV incidence was not significantly ($p < 0.05$) different between varieties with 0 to 2.4% SCYLV incidence (Table 3). The percentages of disease incidence ranged from 1.09% to 90.92% in the CP 06 series in 2015. In addition, no variety was SCYLV negative. In contrast, The CP 07 series had only two varieties with 1.30 and 9.67% of SCYLV incidence (Table 3). The two siblings CP 09-1132 and CP 09-1137 from a cross between SCYLV-free female CPCL 97-0393 and SCYLV-infected male CP 00-2188 had 0% and 30% plants infected, respectively (Table 3). In contrast, another two sets of siblings had the same level of SCYLV infection (Table 3). One set CP 09-1807 and CP 09-1822 originated from a cross between two SCYLV-free parents (CP 01-2459 and CPCL 02-8021), and another set of siblings CP 09-1385 and CP 09-1390 originated from SCYLV-free female (CP 01-2459) and SCYLV-infected male (CP 00-2188). Most parents (Table 3) of the varieties in the primary and secondary increase were SCYLV infected (data not shown). Some varieties in the primary and/or secondary increases had SCYLV-infected plants at several locations, while some varieties had SCYLV-infected plants only at one location (Supplementary Table S1). The varieties in the primary and secondary increases from 2015 to 2019 were divided into three clusters by K-mean analysis (Figure 3). The red circle represents cluster 1, blue circle cluster 2, and purple circle cluster 3. The resistant varieties were grouped in cluster 1, somewhat resistant in cluster 2, and susceptible varieties in cluster 3 (Figure 3).

Table 3. Mean percent incidence of sugarcane yellow leaf virus in primary and secondary seedcane increases from 2015 to 2019.

Variety	Female	Male	Percent Incidence *
CP07-1313	TCP00-4519	CP84-1198	0.00 ^a
CP09-1132	CPCL97-393	CP00-2188	0.00 ^a
CP09-1236	CP84-1198	CL89-5189	0.00 ^a
CP09-1512	CPCL00-4027	CP01-2390	0.00 ^a
CP09-1894	CPCL00-4027	CP01-2459	0.00 ^a
CP09-2002	CP01-2390	Poly06-32	0.00 ^a
CP09-2392	CP 01-2390	POLY 07-04	0.00 ^a
CP10-1132	CP00-1074	Mix07G	0.00 ^a
CP10-1938	CP01-2390	Mix07 S	0.00 ^a
CP11-1314	CP05-1451	Mix08-B	0.00 ^a
CP11-1325	CP01-2390	Poly08-19	0.00 ^a
CP12-2441	HoCP05-923	HoCP96-540	0.00 ^a
CP10-2195	CP01-2390	POLY07-04	0.30 ^a
CP08-1110	CP01-2459	CP00-2188	0.40 ^a
CP10-1734	CP98-1029	Mix07 Q	0.56 ^a
CP10-2002	CP98-1029	Mix07 U	0.56 ^a
CP09-1807	CP01-2459	CL02-8021	0.67 ^a
CP10-1208	CP01-2390	TCP98-4454	0.67 ^a
CP10-1716	CP00-2180	Mix07 Q	0.67 ^a
CP10-2149	CP98-1029	US02-0099	0.89 ^a
CP08-2022	CP02-2103	CP80-1743	1.00 ^a
CP09-2067	CP88-1762	Poly06-35	1.09 ^a
CP06-2964	CP94-2095	CP97-1387	1.11 ^a
CP10-1189	CP03-1912	Mix07H	1.11 ^a
CP09-1390	CP01-2459	CP00-2188	1.13 ^a
CP06-2042	CP96-1252	01P04	1.20 ^a
CP07-2137	CP01-2390	CP84-1198	1.33 ^a
CP09-1822	CP01-2459	CL02-8021	2.00 ^a
CP09-1952	CP01-2390	Poly06-30	2.00 ^a
CP10-2413	CP98-1029	TCP00-4518	2.48 ^a
CP10-1313	CP98-1029	Mix07 I	2.78 ^{ab}
CP09-1430	CPCL00-6756	CP01-2390	2.92 ^{ab}
CP09-1385	CP01-2459	CP00-2188	3.89 ^{ab}
CP10-1620	CP00-1100	CP05-1740	4.58 ^{ab}
CP09-1906	CPCL96-4974	CP01-2459	4.67 ^{ab}
CP10-1134	CP00-1074	Mix07G	4.67 ^{ab}
CP11-2423	CPCL06-3272	HoCP96-540	6.00 ^{ab}
CP10-1619	unknown	unknown	7.25 ^{abc}
CP12-2213	CPCL05-1102	CP80-1743	7.50 ^{abc}
CP07-2320	CP02-2065	CP96-1865	11.00 ^{abc}
CP12-1417	CP02-2065	Poly09-16	12.07 ^{abc}
CP10-1177	CP03-1912	Mix07H	20.78 ^{abc}
CP12-2035	CP99-1896	Poly09-26	26.73 ^{bcd}
CP11-2248	CP06-2664	Poly09-24	29.57 ^{cdef}
CP09-1137	CPCL97-393	CP00-2188	30.00 ^{cdef}
CP11-1956	CP03-2188	CP01-2459	39.45 ^{defg}
CP11-1640	CP01-2390	Poly08-19	40.00 ^{defg}
CP12-2479	unknown	unknown	40.42 ^{defg}
CP09-1266	CP02-1143	CP92-1167	43.33 ^{defg}
CP09-1098	CP02-1143	CP03-1939	47.14 ^{efgh}
CP10-1296	CP98-1029	Mix07 I	51.13 ^{efgh}
CP09-1874	CP03-1026	CPCL00-6756	70.83 ^{hi}
CP10-1717	CP00-2180	Mix07 Q	75.00 ⁱ

* Mean of SCYLV data from all the locations, years, stages and including leaves without SCYLV. Percent incidence values with different lower case letters are significantly different ($p < 0.05$).

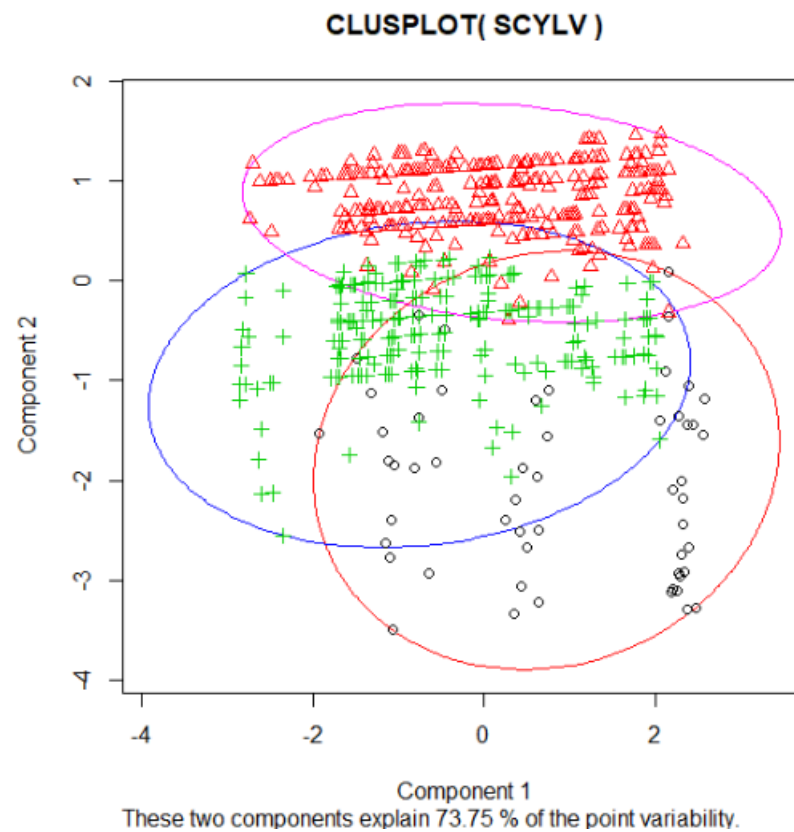


Figure 3. Varieties were divided into three clusters based on percent incidence of SCYLV analyzed by k-means clustering. The red circle represents cluster 1, blue circle cluster 2, and purple circle cluster 3. o, varieties in the cluster 1, +, varieties in the cluster 2 and Δ , varieties in the cluster 3.

The varieties planted in the primary and secondary increase trials in 2015 had the lowest SCYLV incidence followed by the varieties planted in 2018 but the SCYLV incidence in 2015 and 2018 was not significantly ($p < 0.05$) different. Similarly, no significant difference in disease incidence was detected between 2016 and 2017. The percent SCYLV incidence was the highest in 2019 and was significantly ($p < 0.05$) higher than all three years (Figure 4).

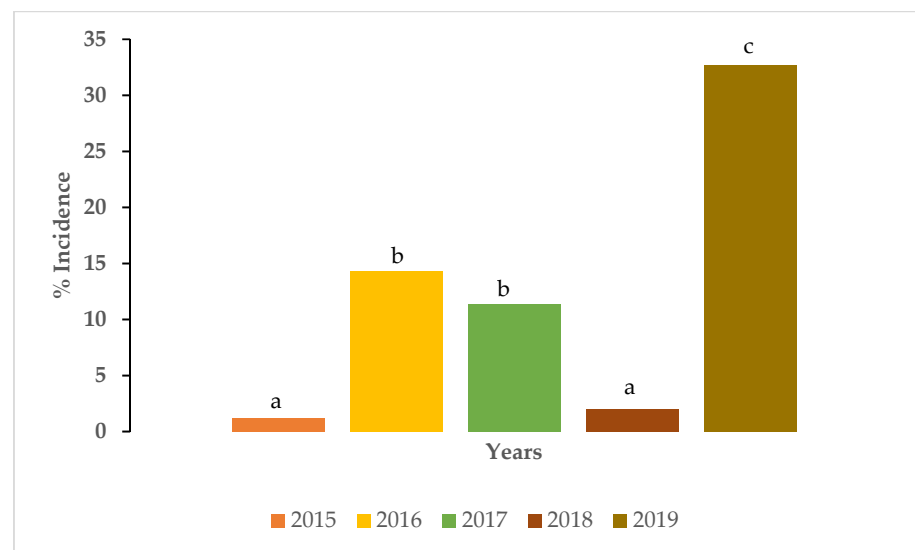


Figure 4. Percent incidence of sugarcane yellow leaf virus in varieties in the primary and secondary increase trials in different years. Percent incidence values with different lower case letters are significantly different ($p < 0.05$).

The SCYLV incidence was different at each location. In general, Pahokee produce had the lowest incidence of SCYLV, and Duda had the highest incidence of SCYLV. The SCYLV incidence at Pahokee produce was not significantly ($p < 0.05$) different from the other four sand locations (HI, PF, SH, and TS) and at one muck location (SF). In addition, the SCYLV incidence at the Wedgeworth location was not significantly ($p < 0.05$) different from the SCYLV incidence at Shawnee and South Florida locations. Similarly, SCYLV incidence was significantly ($p < 0.05$) similar at Area4, East gate, Knight, and Okeelanta locations. The SCYLV incidence at DUDA was significantly ($p < 0.05$) higher than all the sand locations and SF muck location (Figure 5).

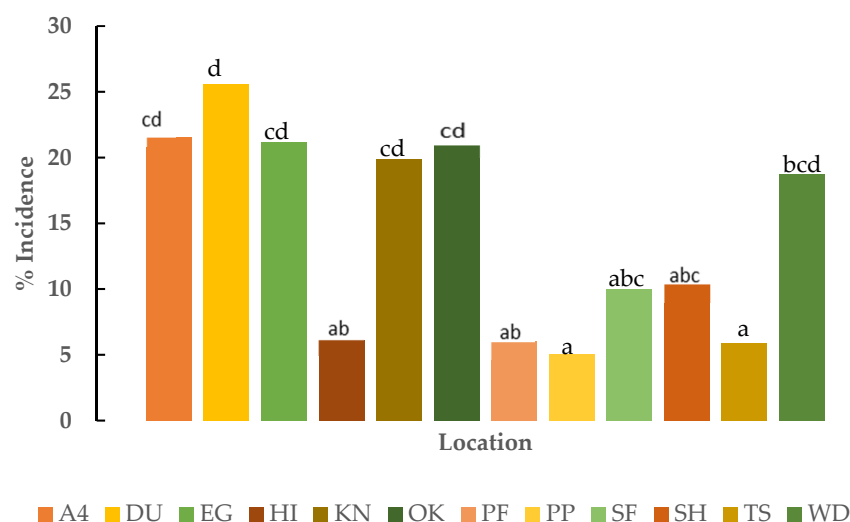


Figure 5. Percent incidence of sugarcane yellow leaf virus in varieties in the primary and secondary increase trials at different locations. Locations are Area 4 (A4), Duda (DU), Eastgate (EG), Hilliard (HI), Knight (KN), Okeelanta (OK), Pahokee Produce (PP), Perry Farm (PF), Shawnee (SH), South Florida (SF), Townsite (TS), and Wedgeworth (WD). Percent incidence values with different lower case letters are significantly different ($p < 0.05$).

The effect of variety, location, and year had a very high significant ($p < 0.0001$) effect on SCYLV incidence. The interaction of the variety \times year and interaction of all three components had a significant ($p < 0.0001$) effect on SCYLV incidence (Table 4). The interaction between variety and location had no significant ($p = 0.1575$) effect on SCYLV incidence, whereas the interaction between location and year had some significant ($p = 0.015$) effect on SCYLV incidence (Table 4).

Table 4. F statistics for variety, location, year and their interaction effects on sugarcane yellow leaf virus incidence.

Trait	DF	F	<i>p</i>
Variety (V)	54	6.52	<0.0001
Location (L)	11	2.86	<0.0001
Year (Y)	4	10.3	<0.0001
V \times L	368	1.23	0.158
L \times Y	42	1.58	0.015
V \times Y	108	5.51	<0.0001
V \times L \times Y	421	181.29	<0.0001

4. Discussion

We surveyed 1195 varieties in the CP breeding nursery in 2016 and 54 varieties in the primary and secondary seedcane increase plantings at six and four locations, respectively, on muck and sand soils over 2015 to 2019. The results of this survey showed that the SCYLV is widespread in Florida. Similar findings were reported in other studies [14,44].

A metagenomics study of *Saccharum* germplasm from Miami, Florida detected SCYLV in more than 80% of samples [14]. A total of 37.25% of the 1195 varieties surveyed in the CP breeding nursery were infected with the SCYLV. A higher SCYLV incidence in the germplasm in Florida was reported in 2003 [44]. The current germplasm at the CP is different than in 2003. Twenty-one percent of the varieties (Table 1) tested were US varieties and only 27% of the US varieties were susceptible to SCYLV (Table 2). In addition, the Florida sugarcane industry has been using SCYLV-free seeds for commercial production for approximately two decades; the lower SCYLV incidence in the breeding nursery could be due to lower SCYLV titer available for the infection of the newer varieties. It has been reported recently that the main vector (*M. sacchari*) of SCYLV was not able to transmit SCYLV efficiently in Florida [34]. More than 40% of CP varieties in the breeding nursery were infected with SCYLV (Table 2); which was also lower than the earlier report [44]. The CP breeding nursery includes varieties released for the commercial production and other promising varieties from the advance stages of the CP cultivar development program each year and, therefore, the varieties that were positive in the 2003 report [44] contributed to a smaller ratio of varieties in 2016 in the CP breeding nursery. The varieties in CP breeding nurseries are used as parents for crossing to develop cultivars for the Florida sugarcane industry. To develop disease-resistant cultivars, identification of the source of resistance is the prerequisite. Several breeding programs worldwide survey their germplasm for yellow leaf disease symptoms on a disease rating scale developed for their breeding program. SCYLV resilient genotypes were recognized, and disease-resistant progenies were developed successfully [12,45]. Most of the varieties in Florida are asymptomatic and, therefore, we used TBIA to identify SCYLV-negative varieties in the local collection of the germplasm and the primary and secondary seed increases. TBIA was shown to be as sensitive as RT-PCR [14], only three cultivars out of 71 cultivars that were determined positive to SCYLV by RT-PCR were negative by TBIA, similarly, five cultivars that were negative to SCYLV by RT-PCR were positive by DAS-ELISA [14]. Another study compared the detection of SCYLV by RT-PCR and viral metagenomic-based screening and found that 80% of the samples had the same results by both tests but 20% of the samples were tested positive by either one or other, not by both [9]. When highly reliable detection of SCYLV is critical then using at least two detection methods have been recommended [46]. To test more than 5000 samples every year, the TBIA is a suitable diagnostic technique to survey varieties in our breeding program. We often randomly perform a qRT-PCR test on varieties that had a negative TBIA reaction to confirm the specificity of SCYLV antibodies to the SCYLV genotypes present in Florida. The selection of sugarcane parents for crossing is a critical decision for breeders so the knowledge of the SCYLV infection status of varieties in the CP germplasm is useful information for future crossing efforts to develop SCYLV resistant/tolerant varieties. Lack of SCYLV-resistant germplasm (Table 2), as well as a mechanical inoculation technique, make it difficult to develop and select SCYLV-resistant cultivars in Florida and, therefore, the use of clean seedcane for plantings is the best alternative to grow SCYLV-free sugarcane. The survey of SCYLV infection in the varieties in the primary and secondary seedcane increases (Table 3) provides the status of SCYLV in the varieties released to sugarcane growers for commercial production. This allows growers to acquire SCYLV-free seedcane. The varieties negative to SCYLV by TBIA can be further tested by RT-PCR and/or RT-qPCR so they can be used immediately without micropropagation to save time and resources. There were 25 varieties with SCYLV incidence up to 2%, these varieties can be used by the growers who do not use SCYLV clean seedcane but they should avoid repropagation of infected seeds. Schenck and Lehrer [33] found that within a year 0 to 90% of virus-free plants were re-infected in commercial plots. An increase in SCYLV incidence from 30% to 55% within 3 years in the CP sugarcane cultivar development program was reported by Comstock and Miller [44]. The aphid vector *M. sacchari* and other aphids may disseminate the virus among the plants [33,47], but the speed of infection propagation from plant to plant is only a few meters per year [48]. A recent study showed that the *M. sacchari* is not efficient to transmit SCYLV in Florida [34].

However, this aphid has been reported to be sufficiently fast to infect susceptible varieties within a few years in Hawaii [48]. Several reports [47,49,50] found that yellow leaf spread by aphids depends on cultivar susceptibility, epidemiological conditions, and aphid predator populations. This could be a reason for variation in SCYLV incidence at the different locations and years. The varieties with higher than 2% SCYLV incidence should be cleaned by the micropropagation of the meristem tip. SCYLV-free plants had 44% more stalks, contributing to a 35% increase in sugar yield than SCYLV-infected plants [7].

5. Conclusions

In conclusion, this survey of SCYLV incidence in the primary and secondary seedcane increases allowed us to identify very promising SCYLV-resistant varieties. These varieties could be tested by RT-qPCR or another detection method for SCYLV infection. These resistant varieties will be a good resource for SCYLV resistance for the CP program because these varieties have already been selected for good agronomic and yield traits.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/agronomy11101948/s1>, Table S1: Sugarcane yellow leaf virus in varieties in the primary and secondary seedcane increases at different locations and years.

Author Contributions: Conceptualization, S.S.; methodology, S.S.; validation, S.S.; writing—original draft preparation, S.S.; writing, review and editing, S.S.; planting fields and sample collection, W.R.D.; planting fields and sample collection, M.B. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Acknowledgments: We would like to acknowledge the contributions of Miriam Baltazar, B.J. Cruz, Velton Bank, Johnny Tajeda, Wang Tajeda and Mathew Paige.

Conflicts of Interest: The authors declare no conflict of interest.

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