

**COMPARISON OF CL AND CP GERMPLASM REACTIONS TO SUGARCANE
YELLOW LEAF VIRUS, LEIFSONIA XYLI SUBSP. XYLI AND PUCCINIA
MELANOCEPHALA**

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ABSTRACT

In 2004, germplasm from the terminated US Sugar Corporation cultivar development program based in Clewiston, FL (CL) was donated to the USDA-ARS Sugarcane Field Station based in Canal Point, FL (CP). The disease reactions of portions of these populations were evaluated to determine what the impact of CL germplasm may be on the future development of disease resistant cultivars at the Sugarcane Field Station. The disease reactions of germplasm when considered as a group from the CL and CP programs differed in their overall reactions to both yellow leaf (caused by *Sugarcane yellow leaf virus*) and ratoon stunting (caused by *Leifsonia xyli* subsp. *xyli*), but did not differ in their reaction to brown rust (caused by *Puccinia melanocephala*). Comparing the CL and CP 1970-1989 Series clones, the CL germplasm as a group had more clones free (18 of 53) of *Sugarcane yellow leaf virus* than the CP germplasm (3 of 58). In regard to ratoon stunting, the historic (prior to 1970) clones of both populations were either similar or the historic clones from the CP germplasm were more susceptible than the CL clones. However, the CP clones currently in Stage II as a population were more resistant to *L. xyli* subsp. *xyli* than the donated CL clones. The disease reaction information both as germplasm from each program and that of individual clones will aid in selecting resistant parental clones and making choices of crosses in the breeding program.

INTRODUCTION

In 2004, US Sugar Corporation at Clewiston, Florida closed its CL cultivar development program and donated its germplasm to the USDA-ARS Sugarcane Field Station for further selection or use as parents in the CP cultivar development program. A few proprietary CL cultivars were restricted for parental use only. In 2006, 16% of the Stage I, 72% of the Stage II and approximately half of the Stage III, Stage III increase, and Stage IV tests planting in 2005 included CL germplasm. Besides cultivars that were in the active CL cultivar development program, many clones in the historical germplasm nursery at US Sugar Corporation were also transferred to Canal Point. This included both older CL clones as well as older CP clones that were no longer available at Canal Point. The prefix of all donated CL germplasm entering a CP testing stage was changed to CPCL indicating that at least one stage of selection occurred in the CP program. The clones of the new germplasm needed to be evaluated both individually and as a group for their reaction to pathogens to fully determine their usefulness and impact on the CP program with respect to disease resistance.

Three pathogens were selected because of their importance to the Florida sugarcane industry: i/ *Sugarcane yellow leaf virus* (SCYLV), the causal agent of yellow leaf, a disease

which was previously called yellow leaf syndrome, ii/ *Leifsonia xyli* subsp. *xyli* (*Lxx*), the causal agent of ratoon stunting, and iii/ *Puccinia melanocephala*, which is the causal agent of brown rust.

SCYLV is widespread in growers' fields being transmitted by the aphid, *Melanaphis sacchari* (Lockhart and Cronje, 2000) and potentially causes significant economic yield losses (Comstock and Miller, 2004; Comstock et al., 1999). Unfortunately, there is a relative lack of *SCYLV* resistance in CP germplasm (Comstock et al., 1999). Sources of *SCYLV* resistance in *Saccharum* species have been primarily restricted to *S. spontaneum* (Comstock et al., 2005). By the time clones progress to Stage II of the CP program 30% are infected with *SCYLV* (Comstock et al., 1999). Over 85% of plants of CP cultivars grown commercially are infected by *SCYLV* in Florida (Comstock, unpublished data).

The CP program has been screening for resistance to ratoon stunting since the early 1990s while the CL program had not. If the CL germplasm was more susceptible it could negatively impact the breeding progress that has been made in developing ratoon stunting resistance.

The pathogen *Puccinia melanocephala* is present in Florida and multiple races of the pathogen make it difficult to develop cultivars with sustainable resistance. Historically, asymptomatic CL and CP cultivars or genotypes only showing low levels of rust, have been selected and commercially grown. More commonly, cultivars often begin to display increasing rust susceptibility after several years of commercial production. Such rust susceptibility often causes the early demise of an otherwise profitable cultivar.

The overall objective of this study was to determine reactions to *SCYLV*, *Lxx* and *P. melanocephala* of clones that were transferred from the US Sugar's cultivar development program and compare them to the reactions of CP clones at a comparable stage at Canal Point.

MATERIALS AND METHODS

The reaction of clones to *SCYLV* was determined based on data of sugarcane infection by the virus under natural conditions. In order to guarantee sufficient time for aphid transmission of *SCYLV*, only clones from the 1989 series or older were evaluated. The naming system for the CL and CP program were slightly different. The CL naming system assigned a series year one year before the CP system does. Thus the CL89 series is similar to the CP90 series with respect to the year it was first planted as a seedling. These clones were assumed exposed to *M. sacchari*, the aphid that transmitted *SCYLV* for at least 17 years in Florida. In total, the presence of *SCYLV* in 52 CL clones and 30 CP clones prior to the 1970 Series and 53 CL clones and 58 CP clones from the 1970 through 1989 series of both CL and CP programs was determined using a tissue blot immunoassay (Schenck et al., 1998) (using antiserum specific for *SCYLV* obtained from Ben Lockhart, University of Minnesota), and at least five leaf samples of each clone were assayed. The percent infection was determined by dividing number of positive *SCYLV* leaf prints by total number of leaf prints.

The reaction of sugarcane clones to *Lxx* was evaluated using the standard inoculation technique at Canal Point where seedcane is inoculated using knives dipped in cane juice obtained from ratoon stunting infected stalks of CP 53-1, a clone that harbors high populations of *Lxx*. The ratoon stunting reaction was based on the average number of colonized vascular bundles (CVB) determined by a tissue blot immunoassay (Harrison et al., 1986). Five stalks were tested per sugarcane cultivar and stalks were sampled ten months after planting a plot with a single inoculated stalk. Plants were grouped into resistant (CVB = 0), moderately resistant ($0 < \text{CVB} \leq 5$), moderately susceptible ($5 < \text{CVB} \leq 10$), susceptible ($10 < \text{CVB} \leq 20$) and very susceptible ($\text{CVB} > 20$) to *Lxx*.

A total of 78 CL clones and 54 CP clones in the 1970 through the 1989 Series were evaluated as well as the 318 CPCL clones and 881 CP clones in Stage II (planted 9 Dec 2004) of the CP cultivar development program. Additional Stage II clones (768 CPCL clones and 276 CP clones) were evaluated in September 2006 and these clones were planted on 17 and 25 November 2005.

Reactions to *P. melanocephala* were determined based on data of infection under natural conditions of clones in Stage II at Canal Point during June-July of 2006. Plants were rated on a 0 to 4 scale where 0 = no rust symptoms, 1 = very few small flecks, no sporulation, 2 = numerous flecks with no sporulation, 3 = very small pustules with a little sporulation primarily on lower leaves and occasional pustule(s) on upper leaves, and 4 = large numbers of sporulating pustules on upper leaves and coalescing pustules on lower leaves on the plants. A total of 979 CPCL clones and 379 CP clones were rated.

RESULTS AND DISCUSSION

Of the older pre-1970s clones, five CL clones (Table 1) and six CP clones (Table 2) were either *SCYLV*-free or showed a virus incidence lower than 25%. The CL clones had a slightly lower incidence of *SCYLV* than the CP clones (Figure 1). CL germplasm from the 1970 and 1989 series was more resistant to *SCYLV* than the CP germplasm since more of the CL clones were *SCYLV*-free (Figure 2). The CL germplasm had only recently been transferred from US Sugar Corporation at Clewiston and the question of a faster rate of spread by aphids in the eastern part of the industry can not be totally ruled out. However, there are numerous examples of cultivars such as CL 61-620, CP 72-1210, CP 84-1198 having similar incidences of *SCYLV* in both the eastern and western areas of the industry. Approximately 35% of the CL clones were in the 0 to 24.9% *SCYLV* infection group compared to only 10% of the CP clones. The CL clones averaged 55.0% *SCYLV* infection compared to 81.8% infection for the CP clones. More interestingly, there were 18 *SCYLV*-free CL clones (34%) in the clones from 76 to 88 series while only 3 CP clones (5%) were virus free (Tables 1 and 2). Presently, the level of *SCYLV* infected plants of CP cultivars grown commercially is over 85% unless the plantings or seedcane was derived from tissue-culture and was disease-free (Comstock, unpublished data). Since *SCYLV* causes yield losses, it is an economically important disease (Comstock and Miller, 2004; Flynn et al., 2005). The high percentage of *SCYLV* infection in the CP germplasm demonstrates the difficulty of developing resistance. The detection of resistance in the CL germplasm identifies possible sources of resistance and should benefit the overall program since many of these clones are commercial types.

Table 1. Percent *Sugarcane yellow leaf virus* (SCYLV) infection in older and historical CL clones.

CL Cultivar	% Infection	CL Cultivar	% Infection	CL Cultivar	% Infection
CL 41-0070	0.0	CL 65-0294	100.0	CL 78-1252	0.0
CL 41-0142	87.5	CL 65-0356	37.5	CL 78-1600	72.2
CL 41-0191	100.0	CL 65-0406	37.5	CL 81-1453	0.0
CL 41-0223	87.5	CL 65-0433	100.0	CL 81-1984	75.0
CL 47-0083	25.0	CL 65-0435	37.5	CL 82-2119	0.0
CL 47-0143	87.5	CL 65-0572	50.0	CL 83-1163	100.0
CL 49-0054	25.0	CL 66-0141	100.0	CL 83-2031	0.0
CL 49-0082	75.0	CL 66-1013	87.5	CL 83-3431	0.0
CL 49-0200	75.0	CL 66-1167	100.0	CL 83-3586	62.5
CL 50-0381	100.0	CL 66-1294	75.0	CL 83-4266	0.0
CL 52-0039	100.0	CL 67-1849	100.0	CL 84-3152	0.0
CL 54-0205	100.0	CL 68-0386	100.0	CL 84-3714	87.5
CL 54-0312	100.0	CL 68-0453	27.3	CL 84-4234	100.0
CL 54-0336	100.0	CL 68-0575	37.5	CL 84-4302	100.0
CL 54-0378	91.8	CL 68-1336	37.5	CL 85-1040	100.0
CL 54-0405	100.0	CL 69-0886	66.7	CL 85-1865	0.0
CL 54-0532	100.0	CL 69-0976	87.5	CL 85-2154	100.0
CL 54-1910	80.0	CL 72-0321	100.0	CL 85-2660	80.0
CL 57-0039	100.0	CL 72-0508	37.5	CL 85-4636	37.5
CL 58-0037	100.0	CL 72-0537	100.0	CL 86-4019	75.0
CL 58-0065	100.0	CL 72-0895	87.5	CL 86-4047	100.0
CL 59-0959	100.0	CL 73-0239	89.7	CL 86-4519	37.5
CL 59-1052	74.4	CL 73-0451	100.0	CL 86-4590	0.0
CL 59-1167	100.0	CL 74-0087	100.0	CL 87-1630	0.0
CL 59-1319	100.0	CL 74-0259	62.5	CL 87-2882	0.0
CL 59-1332	87.5	CL 75-0853	39.0	CL 87-2944	0.0
CL 61-0005	87.5	CL 75-0854	87.5	CL 87-4325	0.0
CL 61-0205	75.0	CL 76-0332	0.0	CL 88-1850	0.0
CL 61-0235	100.0	CL 77-0595	100.0	CL 88-2747	0.0
CL 61-0620	9.9	CL 77-0727	100.0	CL 88-4723	0.0
CL 64-0122	100.0	CL 77-0794	100.0	CL 88-4730	90.0
CL 65-0260	100.0	CL 77-0797	90.0	CL 88-4866	0.0
CL 65-0262	0.0	CL 77-1417	100.0	CL 89-4290	100.0
CL 65-0272	87.5	CL 78-0323	87.5	CL 89-4515	100.0
CL 65-0279	100.0	CL 78-1120	72.7	CL 89-5189	50.0

Table 2. Percent *Sugarcane yellow leaf virus* (SCYLV) infection in older and historical CP clones.

CP Cultivar	% Infection	CP Cultivar	% Infection	CP Cultivar	% Infection
CP 27-0035	100.0	CP 70-1133	98.0	CP 80-1743	100.0
CP 27-0108	87.5	CP 70-1527	100.0	CP 80-1752	100.0
CP 29-0103	100.0	CP 71-1086	100.0	CP 80-1827	100.0
CP 31-0294	100.0	CP 72-0374	99.1	CP 81-1238	87.3
CP 31-0511	0.0	CP 72-1210	100.0	CP 81-1254	77.5
CP 31-0588	37.5	CP 72-1312	85.1	CP 81-1302	87.2
CP 34-0079	60.0	CP 72-1370	100.0	CP 81-1384	100.0
CP 36-0111	0.0	CP 72-2086	96.9	CP 82-1172	57.3
CP 36-0211	62.5	CP 73-1547	21.8	CP 82-1592	100.0
CP 47-0193	100.0	CP 74-2005	78.2	CP 82-1596	100.0
CP 50-0028	100.0	CP 75-0308	100.0	CP 84-1062	40.9
CP 52-0048	100.0	CP 75-1082	88.4	CP 84-1185	100.0
CP 52-0068	100.0	CP 75-1091	100.0	CP 84-1198	97.7
CP 53-0017	100.0	CP 75-1553	97.5	CP 84-1322	99.1
CP 53-0018	100.0	CP 75-1632	6.8	CP 84-1591	95.3
CP 56-0059	100.0	CP 77-0407	61.5	CP 84-1861	95.5
CP 56-0063	100.0	CP 77-0413	100.0	CP 85-1308	42.3
CP 57-0603	0.0	CP 77-0414	27.3	CP 85-1432	98.7
CP 57-0621	100.0	CP 77-1776	88.1	CP 85-1491	23.5
CP 61-0037	100.0	CP 78-0317	100.0	CP 86-1664	100.0
CP 62-0374	100.0	CP 78-0348	37.5	CP 88-1450	100.0
CP 63-0588	100.0	CP 78-1247	100.0	CP 88-1508	100.0
CP 65-0059	80.0	CP 78-1263	100.0	CP 88-1762	98.1
CP 65-0357	97.3	CP 78-1628	99.4	CP 88-1834	100.0
CP 67-0500	0.0	CP 78-2114	100.0	CP 89-1509	0.0
CP 68-0350	0.0	CP 79-0348	100.0	CP 89-2143	64.4
CP 68-1022	100.0	CP 79-1380	0.0	CP 89-2376	93.3
CP 68-1026	100.0	CP 79-1540	0.0	CP 89-2377	100.0
CP 68-1067	0.0	CP 79-1697	100.0		
CP 69-1052	100.0	CP 80-1557	99.1		

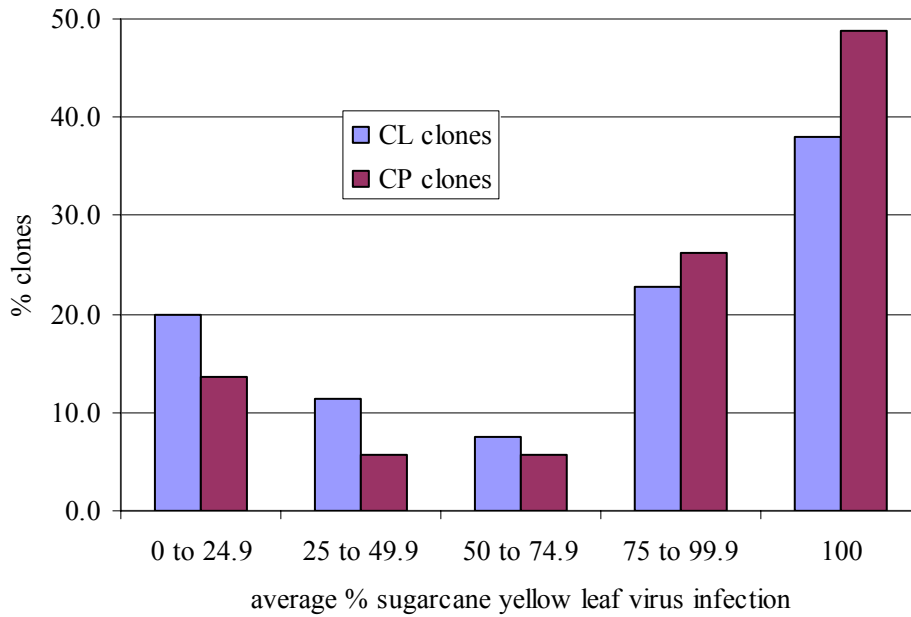


Figure 1. Percent of CL and CP clones from series prior to 1970 that are infected with *Sugarcane yellow leaf virus* (SCYLV) (grouped by average % infection).

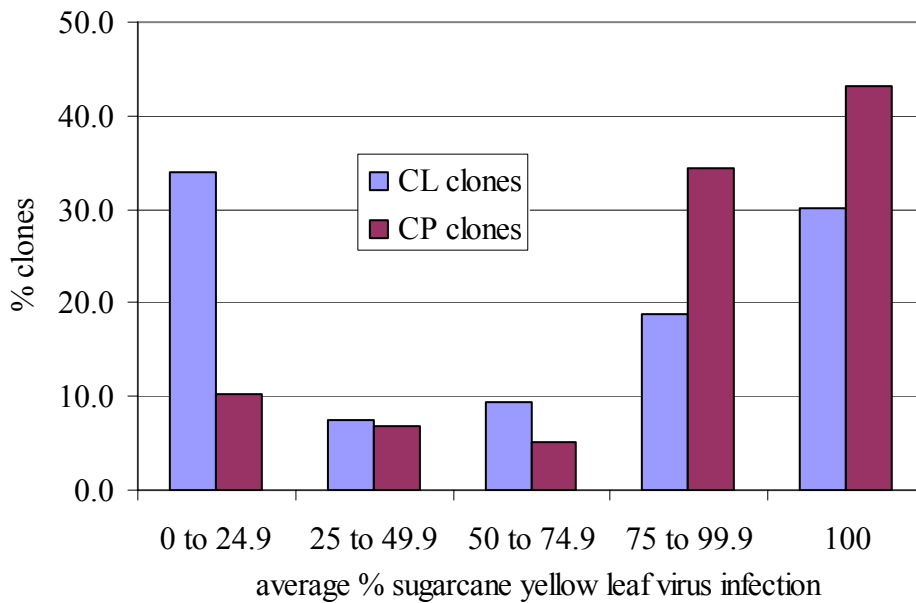


Figure 2. Percent CL and CP clones from the 1970 through 1989 series infected with *Sugarcane yellow leaf virus* (grouped by average % infection).

The reaction to *Lxx* of the historical clones (before 1970) indicated either the CL clones were either slightly more resistant or there was no difference between the CL and CP germplasm (Figure 3). The overall average of the infection (CVB) was 11.8 for the 78 CL clones tested and 16.1 for the 54 CP clones tested. Neither germplasm as a group has an advantage of developing resistance to ratoon stunting. Only specific clones that were resistant to *Lxx* as determined by their low CVB average should be exploited to enhance ratoon stunting resistance in the CP program unless progeny tests determine that certain susceptible parents produce high proportions of resistant progeny.

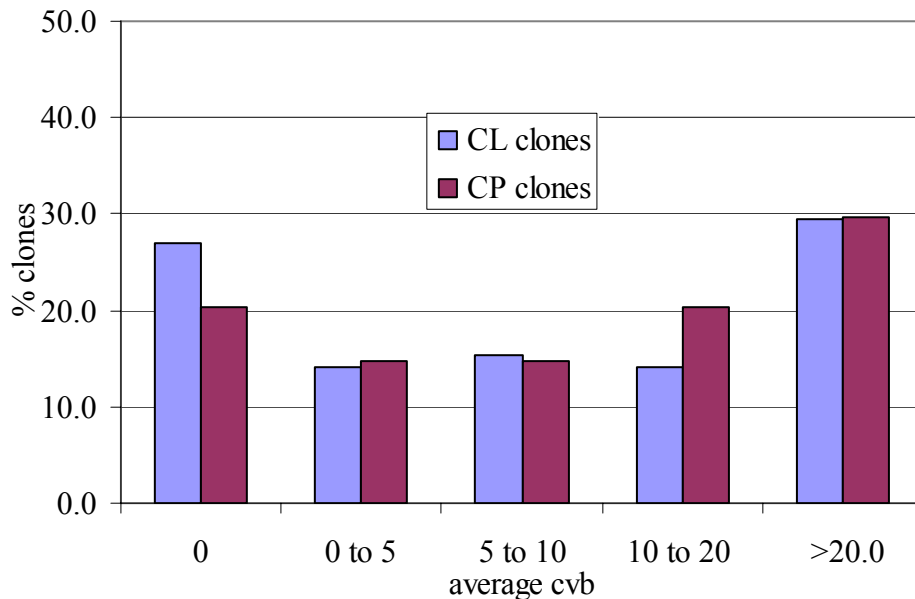


Figure 3. Percent of CL and CP clones older than the 1989 series grouped by their average colonized vascular bundle (CVB) reaction to *Leifsonia xyli* subsp. *xyli*.

Evaluation of the clones in Stage II indicated a distinct difference between the more recently developed CL and CP germplasm as far as resistance to ratoon stunting (Figure 4). The 2004-Stage II CPCL clones were overall more susceptible than the CP clones. These clones averaged 13.4 CVB and had a median of 9.6 CVB (the CVB value where an equal number of clones are above and an equal number is below) for the 318 clones evaluated. In comparison, the CP clones averaged 5.1 CVB and had a median of 0.4 for the 881 CP clones evaluated. Moreover, in both the zero and $0 < CVB \leq 5$ grouping the percentage of CP clones exceeded the CPCL clones. In the 2005-Stage II, CPCL clones were also more susceptible to *Lxx* than the CP clones (Figure 5). Approximately 10% more CP clones had zero CVB and $0 < CVB \leq 5$ than the CPCL clones. There were 15% more CPCL clones in the very susceptible group with > 20 CVB. The CVB averages for the CPCL and CP clones as groups were 15.0 and 8.1, respectively and the median values 9.8 and 2.6, respectively. Unrestricted use of CL germplasm without consideration of the ratoon stunting reaction of the parental clone will negatively impact developing resistant cultivars since the CPCL germplasm as a group is more susceptible to *Lxx* than the CP germplasm. Although the CP program has not practiced a high degree of selection for ratoon stunting resistance, approximately 70% of the CP clones were either resistant or

moderately resistant (CVB value below 5). In contrast, only 40% of the CPCL clones were in this resistance grouping. If in the future CVB values increase dramatically in the clones in Stage II or subsequent stages in the CP development program, because of a change in the parental clones, more selection pressure against ratoon stunting susceptibility may be necessary if resistance to this economically important disease is desired.

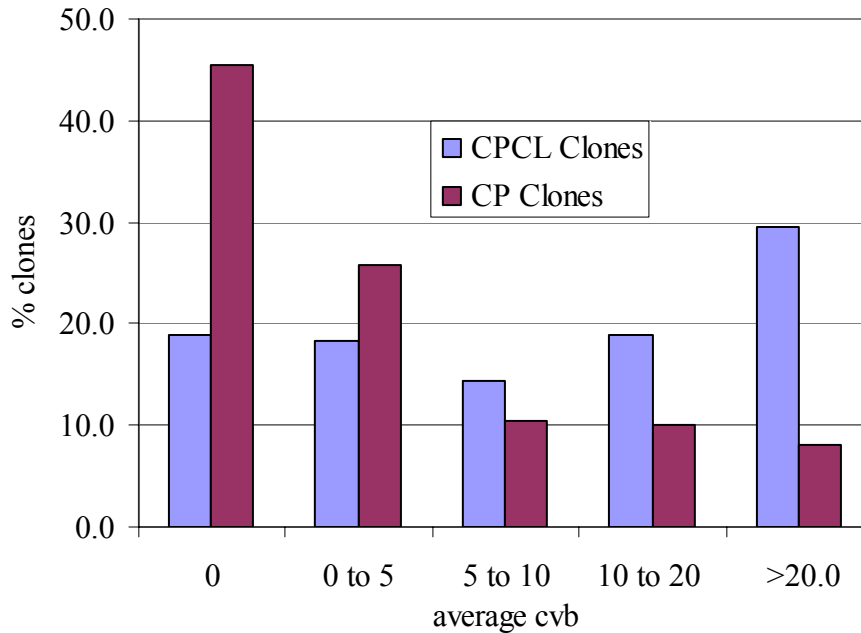


Figure 4. Percent CPCL and CP clones - 2004 Series Stage II clones grouped by their average colonized vascular bundle (CVB) reaction to *Leifsonia xyli* subsp. *xyli*.

The ratings for brown rust were similar because the average rating was 1.0 for the CPCL clones versus 1.1 for the CP clones. There was approximately 10% more CPCL clones in the zero to 1 rating and approximately 10% less in the 2 to 3 rating than the CP clones (Figure 6). As far as developing rust resistance, neither germplasm offers a distinct advantage as compared to the other. The brown rust reaction of each parental clone must be considered if rust resistance is continued to be developed.

In summary, the incorporation of the CL germplasm offers an opportunity to develop sugarcane yellow leaf resistant cultivars. However, the indiscriminant parental use of CL clones with respect to ratoon stunting resistance may hinder developing resistance to ratoon stunting. The wise use of parents is required to reach full potential in developing resistance to these three diseases. Furthermore, the maintenance of resistance standards is important to maintain and enhance the development of resistant cultivars.

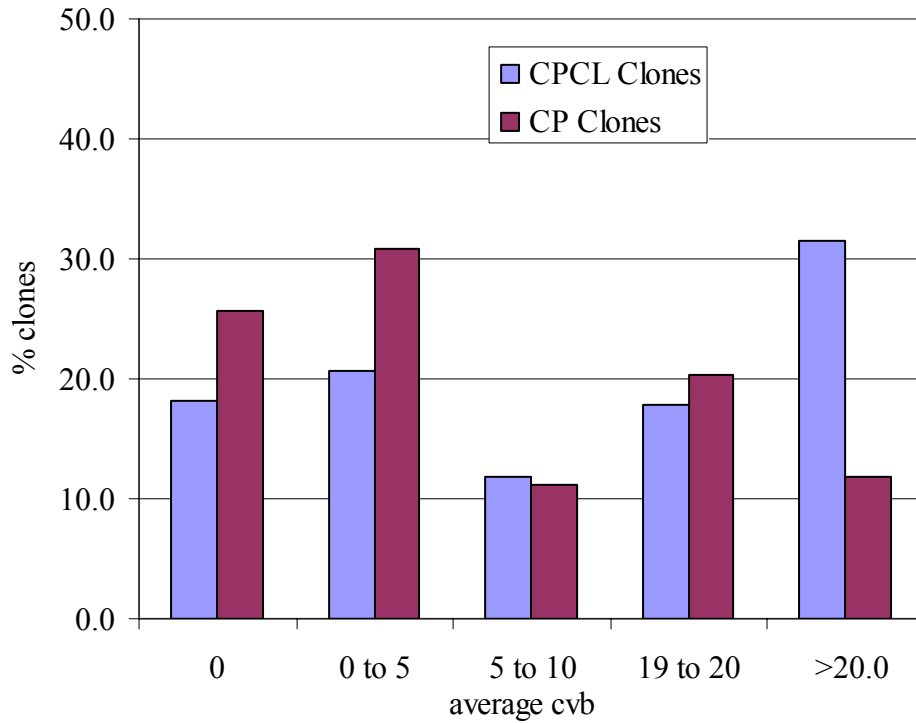


Figure 5. Percent CPCL and CP clones - 2005 Series Stage II clones grouped by their average colonized vascular bundle (CVB) reaction to *Leifsonia xyli* subsp. *xyli*.

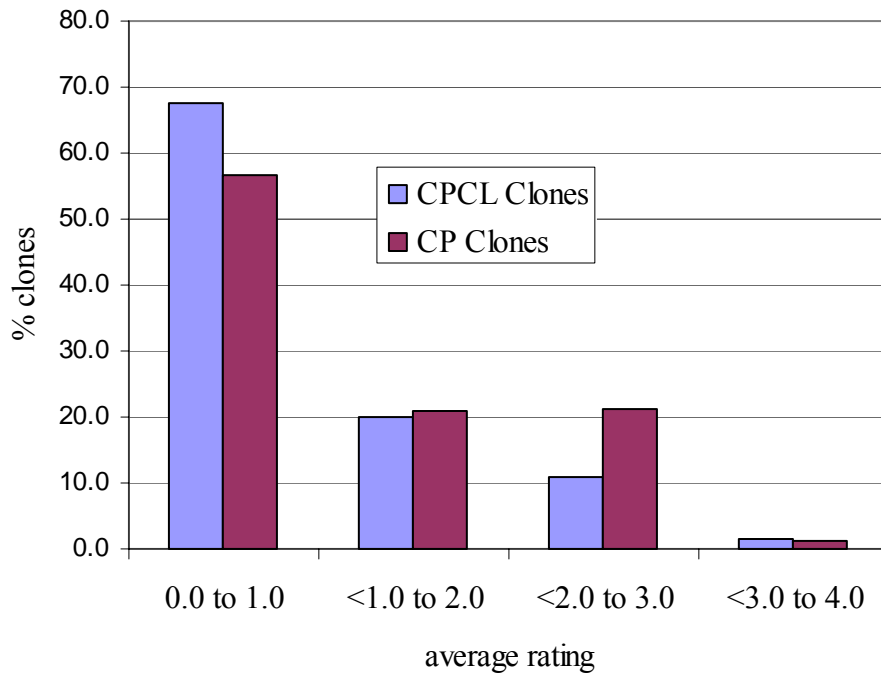


Figure 6. Percent CPCL and CP clones - 2005 Series Stage II clones at each of the rust grades rated on a 0 to 4 scale, with 0 having no rust and 4 severe rust symptoms .

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