PATHOLOGY RESEARCH

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Pathology research addresses the important diseases affecting sugarcane in Louisiana. The overall program goal is to provide farmers with practices to minimize losses to diseases in a cost-effective manner. Projects receiving emphasis during 2017 included brown rust resistance evaluation; determining the increase, recovery, yield impact, and resistance to mosaic; evaluating changes in the soil microbial community associated with long-term sugarcane cultivation; providing support for healthy seedcane programs to manage systemic diseases; evaluating disease resistance in the variety selection program; and billet planting. Research results on billet planting are reported separately.

Brown Rust

Cold winter and erratic spring weather conditions resulted in an erratic brown rust epidemic during spring 2017. This limited field research on brown rust. Research has been focused on determining the quantitative expression of resistance in L 99-233 and its progeny to develop molecular markers in cooperation with Dr. Niranjan Baisakh. Natural infection and inoculation have been used to characterize a L 99-233 self-population for brown rust resistance. Progeny (approximately 250) from a bi-parental cross between L 99-233 and HoCP 96-540 (rust susceptible) intended for a molecular marker validation study were planted at two locations during 2017 to evaluate resistance to natural infection. Details of the molecular genetics research for brown rust resistance are reported separately.

Mosaic Increase, Recovery, Yield Impact, and Resistance Evaluation

A new project addressing mosaic, an old foe of the Louisiana sugarcane industry, was initiated during 2016. The impetus for the project was the detection of virus-infected plants of multiple clones in breeding program variety tests and in variety increase plots at multiple locations. The research during 2017 was focused on determining the rates of mosaic increase in infected fields of HoCP 09-804, the recovery from symptoms and possibly from virus infection in two varieties, the impact of infection on yield, and screening the current commercial and basic breeding program parent and selection populations for resistance by mechanical inoculation and natural spread. In addition, surveys were conducted for 2015 series clones to be introduced to the breeding program outfield tests and American Sugar Cane League Primary Increase Stations, clones already planted at the Primary Stations, and initial plant cane increases of L 11-183 at some of the ASCL Secondary Increase Stations. All surveys were based on visual observation of mosaic symptoms, and a subsample of symptomatic and asymptomatic leaves were collected to test for infection by *Sorghum mosaic virus* (SrMV) by reverse-transcriptase polymerase chain reaction (rtPCR).

To determine rates of mosaic increase, follow-up surveys were conducted during May in seven first ratoon fields of HoCP 09-804. Rapid increases in mosaic incidence were not detected at the seven different locations. Disease incidence was higher in first ratoon compared to plant cane for three fields and lower for four fields (Table 1). Rates of increase ranged from 24-97% and rates of decrease ranged from 27-41%. Incidence in plant cane ranged from 0.5-2.5% with the

exception of the field at Little Texas (10.4%). After exposure to one season of aphid transmission, incidence levels ranged from 0.2-3.1% in six fields and decreased from 10.4 to 6.1% at Little Texas.

Table 1. Change in incidence of mosaic from plant cane to first ratoon in seven fields of HoCP 09-804 at different locations determined from visual surveys of symptomatic plants.

Location	Incidence of mosaic symptomatic plants in plant cane during 2016	Change in incidence of mosaic symptomatic plants in first ratoon
Alma	2.5%	+24%
Blackberry	0.5%	-62%
Cedar Grove	1.3%	+70%
Glendale	0.9%	-27%
Glenwood	1.4%	-32%
Little Texas	10.4%	-41%
Raceland	1.2%	+97%

Additional mosaic surveys were conducted during May at the three American Sugar Cane League Primary Increase Stations. These surveys did not detect any mosaic in Ho 11-573, L 12-201, L 13-324, L 13-251, L 13-257, Ho 13-708, HoCP 13-726, HoCP 13-737, HoCP 13-738, Ho 13-739, HoCP 13-740, HoCP 13-755, HoCP 13-758, and HoCP 13-775. Also, no mosaic was detected in the L 2015 series introductions at the Sugar Research Station that were to provide seedcane for outfield tests and primary increase stations.

The results from rtPCR testing indicated that the mosaic symptoms recorded during the surveys were the result of infection by SrMV and demonstrated that virus was present in leaves showing symptoms but not in leaves without symptoms. For HoCP 09-804 samples, 98.4% of 191 symptomatic leaf samples collected from different locations tested positive for SrMV, while none of 244 asymptomatic samples tested positive for SrMV. For samples collected from 31 other varieties, SrMV was detected in 98.9% of 90 leaf samples, while no SrMV was detected in 64 asymptomatic samples. These results demonstrate that surveys based on observation of visual symptoms can provide an accurate assessment of mosaic incidence in a field.

'Recovery' defined as the loss of viral infection symptoms and possibly virus accumulation within an infected plant was evaluated in plants showing mosaic symptoms of two varieties, HoCP 09-804 and L 10-147. Single stalk plots were planted with individual stalks of each variety that were showing leaf symptoms of mosaic (symptomatic) or not showing symptoms (asymptomatic) at the Sugar Research Station. The following spring, single stalk plots were evaluated to determine whether any asymptomatic plants had developed from buds on symptomatic stalks. The extent of recovery varied between the two varieties (Table 2). Plots (individual stalks) that had asymptomatic plants were higher for L 10-147 (32.1%) than for HoCP 09-804 (3.4%). The frequency of asymptomatic plants also was higher for L 10-147 (18.9%) compared to HoCP 09-804 (2%). There was variability in the extent of recovery within plots with asymptomatic plants with some exhibiting complete recovery (100% asymptomatic plants) and some exhibiting partial recovery (containing a mixture of asymptomatic and symptomatic plants) (Table 3). The occurrence of complete and partial recovery was similar between the two varieties.

Table 2. Comparison of mosaic 'recovery' evaluated as asymptomatic plants developing from single stalk plots of HoCP 09-804 and L 10-147 that were planted with individual mosaic symptomatic stalks.

Variety	Total number of plots	Plots with asymptomatic plants	Total number of plants	Asymptomatic plants
HoCP 09-804	58	2 (3.4%) b	152	3 (2.0%) b
L 10-147	81	26 (32.1%) a	291	55 (18.9%) a

Table 3. Comparison of extent of mosaic 'recovery' within single stalk plots of HoCP 09-804 and L 10-147 that were planted with individual mosaic symptomatic stalks.

Variety	Plots exhibiting 100% recovery from mosaic	Plots exhibiting less than 100% mosaic recovery	Average percent recovery	Range in percent asymptomatic plants
HoCP 09-804	1	1	66.7%	33.3-100%
L 10-147	10	16	61.8%	14.3-100%

Testing by rtPCR determined that SrMV was no longer detectable in the majority of recovered plants of both varieties (Table 4). The frequency of recovered plants in which SrMV could no longer be detected was lower for L 10-147 (5.5%) than for HoCP 09-804 (33.3%). The results with these two varieties demonstrate that recovery does occur for mosaic infected sugarcane plants under Louisiana conditions, and this phenomenon could affect disease incidence in the field over time (and may have been involved in the reductions in mosaic incidence observed in the two annual surveys). However, the rate of recovery can vary considerably among varieties.

Table 4. Positive detection of *Sorghum mosaic virus* (SrMV) by reverse transcriptase polymerase chain reaction in 'recovered" (asymptomatic) plants developing from single stalk plots of HoCP 09-804 and L 10-147 planted with individual mosaic symptomatic stalks.

Variety	Total plants tested	Number of plants positive for SrMV
HoCP 09-804	3	1 (33.3%)
L 10-147	55	3 (5.5%)

The recovery experiment also was used to estimate and compare the impact of mosaic on bud germination and yield of HoCP 09-804 and L 10-147. The number of buds was determined for each individual stalk planted. The percent germination could then be determined from the number of primary shoots developing from each stalk. Bud germination was adversely affected by mosaic in HoCP 09-804 but was unaffected for L 10-147 (Table 5). Shoot populations the following spring were lower in plots from symptomatic stalks compared to asymptomatic stalks for both varieties; the population was 61% lower for symptomatic stalks of HoCP 09-804 and 29% lower for L 10-147 (Table 5). The millable stalk population during August was then lower only for mosaic symptomatic stalk plots of HoCP 09-804 (Table 5).

Table 5. Comparison of bud germination, spring shoot populations, and millable stalk populations for single stalk plots of HoCP 09-804 and L 10-147 that were planted with individual mosaic asymptomatic or symptomatic stalks.

Variety	Mosaic status	Bud germination	Spring shoots	Millable stalks
HoCP 09-804	Asymptomatic	28% a	32,043 a	33,561 a
HoCP 09-804	Symptomatic	15% b	12,483 c	19,590 b
L 10-147	Asymptomatic	33% a	35,433 a	33,033 a
L 10-147	Symptomatic	33% a	25,195 b	30,985 a

Yield components stalk weight, sucrose per ton of cane (commercially recoverable sugar), cane tonnage, and total sucrose per acre were estimated and compared to evaluate the impact of mosaic on the yield of HoCP 09-804 and L 10-147. Stalk weight was similar for plots planted with symptomatic compared to asymptomatic stalks within each variety suggesting only a minor effect of mosaic on this yield component (Table 6). The effect of mosaic on sucrose content of stalks varied between the varieties. It was higher in symptomatic stalk plots compared to asymptomatic plots of HoCP 09-804, whereas sucrose content was similar for stalks from symptomatic and asymptomatic stalk plots of L 10-147 (Table 6). Both cane tonnage and total sucrose yield estimates were lower for symptomatic stalk plots of HoCP 09-804 compared to asymptomatic stalk plots but were similar for L 10-147 (Table 6). These results demonstrate the variable effect of mosaic on yield in different varieties and indicate that mosaic can cause significant yield loss in HoCP 09-804, while L 10-147 appears to have some tolerance to the disease. The reduction in yield for HoCP 09-804 was mainly due to a reduction in stalk population.

Table 6. Comparison of stalk weight, sucrose per ton of cane, cane tonnage, and total sucrose yield per acre estimated from single stalk plots of HoCP 09-804 and L 10-147 that were planted with individual mosaic symptomatic or asymptomatic stalks.

Variety[a]	Stalk weight (lbs.)	Sucrose per ton of cane (lbs.)	Cane tonnage	Sucrose per acre (lbs.)
HoCP 09-804 -	2.28 ab	219.7 b	37.9 a	8,365 a
HoCP 09-804 +	2.11 b	230.2 a	20.5 b	4,764 b
L 10-147 -	2.60 a	214.8 b	42.8 a	9,219 a
L 10-147 +	2.55 a	212.2 b	39.4 a	8,346 a

[a]Plots for two varieties were planted with a single stalk that was either mosaic asymptomatic (-) or symptomatic (+).

Resistance to mosaic was evaluated for breeding program parents and selections in two different ways. To evaluate resistance to natural infection, mosaic infected cane was planted in border rows and a row through the middle of the variety selection program inoculated test to evaluate resistance to smut and leaf scald. This approach utilizing virus “spreader rows” attempts to detect mosaic susceptible clones by controlled exposure to natural infection. A survey of the three replicate plots of all clones detected plants with mosaic symptoms in 15 clones (Table 7). Two infected plants were detected in a commercial variety, HoCP 04-838, that is considered to be resistant. Mosaic infected plants were detected in two of three replicates of HoCP 09-804.

Table 7. Detection of mosaic-infected plants in a field experiment evaluating mosaic resistance via natural infection at the Sugar Research Station during May 2017.[a]

Clone	No. mosaic infected plants in 3 replicates	Clone	No. mosaic infected plants in 3 replicates
HoCP 04-838	2,0,0	L 14-267	4,2,1
HoCP 09-804	2,1,0	L 14-274	2,1,0
L 13-324	2,0,0	L 14-285	1,1,0
Ho 13-708	3,0,0	L 14-288	2,0,0
HoCP 13-726	4,3,-	L 15-311	3,2,0
HoCP 13-737	4,1,-	L 15-317	2,0,-
HoCP 13-758	4,0,-	L 15-337	5,1,0
L 14-265	1,0,0	-	-

[a]Stalks from mosaic infected plants were planted in every fourth row and across the ends of the field to provide a source of inoculum to be spread by aphids into the experimental plots.

A second approach utilized mechanical inoculation to evaluate resistance to mosaic. Two inoculations were conducted with USDA-ARS Sugarcane Research Unit commercial and basic breeding program parents and selections. In the first inoculation, 212 potential parents from the 2014, 2015, and 2016 series were inoculated each with six plants with 4-5 leaves grown in Speedling trays in the greenhouse. Two highly susceptible checks, L 08-088 and Rio sorghum, one moderately susceptible check, HoCP 09-804, and one resistant check, HoCP 96-540, were included with three replicates of six plants inoculated and one replicate non-inoculated. Inoculum consisted of sugarcane leaves from CP 44-101 infected with SrMV. Infection levels were determined by visual observation of systemic mosaic symptoms in leaves of the six inoculated plants 5 weeks after inoculation.

No symptomatic plants were observed for 65, 72, and 69% of the 2014, 2015, and 2016 series, respectively (Table 8). The three series each had varieties with different amounts of infection for the six inoculated plants indicating variation in the level of susceptibility (Table 8). The infection intervals were calculated and reported as a percentage because of some variability in the number of surviving plants per clone, but each interval equates to 0, 1, 2, 3-4, and 5-6 plants infected (symptomatic) out of six inoculated per variety. Inoculation of the checks produced the expected results with 100% infection for L 08-088 and Rio sorghum, infection levels of 0, 33, and 40% for HoCP 09-804, and no infection for HoCP 96-540, indicating the inoculation was successful. Testing for SrMV by rtPCR was conducted for a subset of symptomatic and asymptomatic plants, and virus was detected in symptomatic but not asymptomatic plants.

Table 8. Results from first USDA mosaic mechanical inoculation of 212 potential commercial and basic breeding program parents from the 2014, 2015 and 2016 series.

Mosaic incidence intervals (% symptomatic plants)	Varieties in 2014 series	Varieties in 2015 series	Varieties in 2016 series
75-100	2 (6%)	4 (7%)	15 (12%)
50-74	4 (13%)	4 (7%)	10 (8%)
25-49	2 (6%)	4 (7%)	6 (5%)
1-24	3 (10%)	4 (7%)	7 (6%)
0	20 (65%)	39 (72%)	88 (69%)

A second inoculation of USDA commercial breeding program active selections from the 2011-2015 series and potential parents from the basic breeding program 2017 series was performed with 109 varieties with the same check varieties. Results are reported only for clones that had at least four plants. Some level of susceptibility was detected in over half (55%) of the active selections in the 2011-2015 series, while 77% of the 2017 series potential parents had no infection (Table 9). Inoculation of the checks produced the expected results with 100% infection for Rio sorghum, 67 and 100% infection for L 08-088, infection levels of 17 and 25% for HoCP 09-804, and no infection for HoCP 96-540, indicating the inoculation was successful.

Table 9. Results from second USDA mosaic mechanical inoculation of 109 varieties, including active selections from the 2011-2015 commercial breeding series and 97 clones from the 2017 series basic breeding program potential parents.

Mosaic incidence intervals (% symptomatic plants)	Active selections in 2011-2015 commercial series	Potential parents in 2017 basic series
75-100	5 (25%)	9 (7%)
50-74	1 (5%)	4 (3%)
25-49	3 15%)	9 (7%)
1-24	2 (10%)	8 (6%)
0	9 (45%)	98 (77%)

The detection of mosaic infected plants in multiple advanced selections in the variety selection program indicated that some level of susceptibility was going undetected in the crossing and selection programs. The recent reliance only on natural infection to detect susceptibility was apparently not adequate to detect susceptible clones due to insufficient inoculum pressure. The addition of a mosaic spread component to the smut and leaf scald inoculated test is now detecting susceptibility through natural infection resulting from more uniform exposure to mosaic infected plants. Valuable information on mosaic susceptibility was obtained from the inoculated tests. Susceptible clones were detected at all levels of the breeding program. Susceptibility was traced to common parentage in some cases. The sources of susceptibility can now be eliminated or used with appropriate caution in future crossing. Annual mechanical inoculation of mosaic will need to be reinstituted as a component of the breeding program. The goal of all of the mosaic research is to prevent this important disease from re-emerging as a problem for the Louisiana sugarcane industry.

Healthy Seedcane Program Support

Disease testing was conducted by the Sugarcane Disease Detection Lab for the 22nd year during 2017. Kleentek and SugarTech seedcane production was monitored for ratoon stunt disease (RSD), and no disease was detected (Table 10). A total of 1,622 stalk samples from research farms, variety increase plots, and grower fields were tested for RSD with no positives detected. The Local Quarantine supplied healthy plant material of 12 active experimental varieties from the 2013 series to the two seedcane companies to establish Foundation Stock plants that will provide apical meristems for tissue culture. Limited testing was conducted on commercial farms, and no RSD was detected in 39 sampled fields. A total of 7,436 leaf samples were tested for *Sugarcane yellow leaf virus* (Table 11). Commercial tissue-culture seedcane sources were tested

as part of the LDAF seedcane certification program. No field failed to certify due to virus infection.

Resistance to Leaf Scald

The primary control measure for leaf scald is host plant resistance. Currently, resistance is evaluated by visually rating disease severity in a breeding program annual inoculated test. However, in 2017, wet weather conditions after inoculation resulted in no systemic symptom development in the leaf scald susceptible check varieties, and it was not possible to evaluate and rate the resistance levels in the experimental varieties. This erratic symptom expression illustrates the need for molecular markers to predict leaf scald resistance. Research is on-going to develop molecular markers for leaf scald resistance in cooperation with Dr. Niranjan Baisakh. Details of the molecular marker research are presented separately.

Table 10. Ratoon stunting disease testing summary for 2016.

Source	Location	No. of fields	No. of varieties	No. of samples
Louisiana growers	State-wide	39	5	761
Variety Release Program	1° & 2° stations	-	3	180
Helena SugarTech®	Foundation stock	-	5	18
Kleentek®	Foundation stock	-	33	60
Kleentek®	Other than foundation	-	-	523
Local Quarantine	LSUAC	-	26	80
Research	LSUAC	-	-	-
Totals		39	-	1,622

Table 11. Sugarcane yellow leaf virus testing summary for 2016.

Source	Location/type	No. of fields	No. of varieties	No. of samples
LDAF	Seed Certification	181	9	5,953
Helena SugarTech®	Foundation Stock	-	5	73
Kleentek®	Foundation Stock	-	15	28
Kleentek®	Other than foundation	-	-	1,250
Local Quarantine	LSUAC	-	26	80
Research	LSUAC	-	-	52
Totals		181	-	7,436

Evaluating Disease Resistance in the Variety Selection Program
Resistance to smut is evaluated annually for experimental varieties in the Variety Selection Program in an annual inoculated test at the Sugar Research Station. A second study to develop potential parents with resistance to smut was conducted. One hundred sixty clones from the increase stage of the variety selection program were dip-inoculated with smut in a non-replicated test for the second time. However, due to adverse weather the experiment was not inoculated and planted until September, and extended dry weather followed. As a result of the unfavorable environmental conditions, smut did not develop in the susceptible check varieties, and it was not possible to rate the populations for smut resistance. All active selections and the resistant parent development population were again dip-inoculated and replanted. Smut infection severity will be recorded during 2018.

The same two populations were inoculated with the leaf scald pathogen during June 2017. The initial symptoms that developed in the inoculated leaves indicated a successful inoculation. However, the occurrence of extended wet weather during the 8 week period following inoculation to allow systemic symptoms to develop resulted in no systemic symptoms in the susceptible check varieties, and it was not possible to rate the populations for leaf scald resistance.

The active selections from the commercial breeding program were rated for resistance to brown rust based on visual observation of natural infection symptoms. The 127 clones were rated on a 1-9 scale with 1-3 = resistant, 4-6 = moderately susceptible, and 7-9 = highly susceptible. Ninety-six (72%) received a resistant rating, 28 (22%) received a moderately susceptible rating, and 3 (2%) received a highly susceptible rating.

Soil Microbial Communities Associated with Long-Term Sugarcane Cultivation
Previous research has shown long-term monoculture of sugarcane impacts soil microbial communities and results in reduced yield potential. Growers have often observed this phenomenon when comparing growth and yield for cane planted in soils with and without out a recent history of sugarcane cultivation. Between 2014 and 2015, six paired sites of plant cane of the same variety in "new ground" and "old ground" were sampled. In 2016, two of these sites were revisited and sampled in first ratoon in order to address questions of how rapidly the soil microbial community changes with exposure to sugarcane. This study was conducted in cooperation with Dr. Lisa Fultz.

DNA was extracted from bulk and rhizosphere (root zone) soil samples and outsourced for sequencing. DNA was amplified for the 16S ribosomal DNA and internal transcribed spacer (ITS) to identify bacteria and fungi, respectively. Amplicon-based metagenomics was then used to profile the diversity of soil prokaryotic and fungal communities associated with short and long-term sugarcane cultivation to better understand the etiology of yield decline related to detrimental soil microbiota. Principal coordinate analyses of soil prokaryotic β-diversity revealed differences based primarily on location, but cropping history also had a significant impact. Ordinations of fungal β-diversity revealed shared differences in microbial community structure based on cropping history across locations. Similar to previous research, these results suggest fungi play a major role in the detrimental effect soil microbial communities associated with long-term sugarcane cultivation have on yield. Correlations of environmental variables with principal

coordinate analyses suggest location-specific depletion of soil nutrients coincide with changes in fungal and, to a lesser extent, bacterial communities. However, additional research is required to determine if management of soil nutrients can prevent detrimental changes in microbial community structure associated with sugarcane monoculture. Analysis of taxonomic assignments identified large numbers of low abundance taxa associated with short and long-term cropping histories across all six locations that likely contribute to community-level effects on sugarcane yields. Notable genera more associated with “new ground” included *Bacillus*, actinobacteria, and *Fusarium*, while genera more associated with “old ground” included *Burkholderia* and *Trichoderma*.