PATHOLOGY RESEARCH

Jeffrey W. Hoy, Carolyn F. Savario, Raghuwinder Singh, Adam F. Bigott, and Jancee Rice
Department of Plant Pathology and Crop Physiology

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 2016 included brown rust resistance evaluation; determining the distribution, incidence, and resistance to mosaic; providing support for healthy seedcane programs to manage ratoon stunting and other systemic diseases; evaluating disease resistance in the variety selection program; evaluating changes in the soil microbial community associated with long-term sugarcane cultivation; and billet planting. Research results on billet planting are reported separately.

Brown Rust

Mild winter and warm spring weather conditions resulted in a brown rust epidemic during Spring 2016. This permitted field research on brown rust. L 99-233 is one of the few varieties that did not become susceptible while under commercial cultivation. In controlled conditions inoculations, it exhibits low infection levels regardless of the brown rust pathogen isolate used for inoculation suggesting it has a quantitative type of resistance that could be more durable. Therefore, research has been focused on determining the expression of resistance in L 99-233 and its progeny to develop molecular markers in cooperation with Dr. Niranjan Baisakh. Natural infection was used to characterize a L 99-233 self-population for brown rust resistance during 2016. Clones exhibiting either high resistance or susceptibility are being included along with the parent and grandparents in a bulk segregant analysis to identify markers associated with rust resistance. Progeny from a bi-parental cross between L 99-233 and HoCP 96-540 (rust susceptible) intended for a molecular marker validation study were in plant cane seedlings during 2016. Approximately 200 seedling progeny from this cross will be increased and planted for rust resistance phenotyping during 2017. Details of the molecular genetics research for brown rust resistance are reported separately.

Orange Rust during 2016

Orange rust caused by the fungus *Puccinia kuehnii* was found for the first time in Louisiana during 2012 in the newly released variety Ho 05-961. Orange rust was observed at low severity in some fields of Ho 05-961 during 2016. Surveys for orange rust will continue during 2017.

Mosaic Distribution, Incidence, and Resistance

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 for multiple clones in breeding program variety tests and in variety increase plots at multiple locations. The research is focused on determining the current distribution and incidence of the disease in the variety selection and release programs and evaluating resistance levels in the commercial breeding program parent and selection populations and basic breeding program parent population. An extensive survey was conducted during May of clones in the Louisiana cooperative sugarcane breeding program Outfield Tests and Primary and Secondary Stations of the American Sugar Cane League Variety Release Program located on commercial farms.

Plants exhibiting symptoms of mosaic were observed in an experimental variety, HoCP 09-804, that was under consideration for release during 2016. Therefore, variety increase plots of HoCP 09-804 were surveyed for mosaic on the Secondary Increase Stations during May. No infected plants had been observed at the Primary Station in the Bayou Teche region, and apparently no infected seedcane was distributed as no mosaic infected plants were detected at any Secondary Stations receiving seedcane from that Primary Station (Table 1). Mosaic infected plants had been observed previously in HoCP 09-804 at the two Primary Stations on Bayou Lafourche, and mosaic infected plants were detected at some but not all Secondary Stations that received seedcane from those stations (Table 1). Adjacent fields of commercial varieties were surveyed, and no plants with mosaic symptoms were observed. The incidence of mosaic in HoCP 09-804 was very low or absent on some stations in all industry areas. Symptomatic plants were tagged and rogued in fields with low incidence. The decision to release HoCP 09-804 was made, and seedcane was distributed from Secondary Stations with very low (incidence estimates less than 0.5% before rogueing) or no incidence of mosaic.

During the survey, the numbers of plants exhibiting symptoms of mosaic were counted on different rows in each field, and the occurrences of runs (two or more infected plants directly adjacent to each other on a row) were recorded. The extensive occurrence of symptomatic plants in runs of varying lengths suggested that the mosaic infection was due to planting infected seedcane. An estimate of the plant population in fields was used to calculate the percentage of plants exhibiting mosaic symptoms in each field. Leaves were collected from symptomatic plants to confirm virus infection by reverse transcriptase polymerase chain reaction (RT-PCR). In addition, leaves were collected from asymptomatic plants, and RT-PCR will be used to determine whether any asymptomatic plants were virus infected. Mosaic can be caused by strains of two closely related viruses: *Sugarcane mosaic virus* and *Sorghum mosaic virus.* In recent times, strains of *Sorghum mosaic virus* have been detected in mosaic surveys conducted annually by the USDA-ARS Sugarcane Research Unit, but in recent surveys, a few virus isolates have not matched known strains. The virus infected samples collected from the survey will be evaluated to determine whether there has been a change in the occurrence of strains of either virus.

Experimental varieties were surveyed for mosaic at the three Primary Stations during May. Plants with mosaic symptoms were observed in clones at both stations located in the Bayou Lafourche region (Table 2). Variable numbers of mosaic infected plants were observed in four clones at the Little Texas station and three clones at the Palo Alto station. The highest incidence of mosaic was detected in Ho 11-532 at both locations.

Surveys of the breeding program Outfield Tests detected plants with mosaic symptoms in four experimental varieties in introduction plots (Table 3). Mosaic was detected in introduction plots at a single location for three clones and six locations for one clone, L 10-147. Mosaic was not detected in any clones in the actual outfield tests. Mosaic infected plants were detected in three experimental variety increase plots at the Sugar Research Station (Table 3).

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 14 clones (Table 4).

Table 1. Mosaic survey results for HoCP 09-804 at American Sugar Cane League Variety Release Program Secondary Increase Stations during May 2016.

Region	Crop year	Location	Plot area (acres)	Counted area (%)	Infected stools	Percent infection
Bayou Teche	Plant cane	Harper	5.0	30%	0	0%
	First ratoon	Berard	0.3	100%	0	0%
	First ratoon	Levert	0.4	100%	0	0%
	First ratoon	W. Judice	0.2	100%	0	0%
	First ratoon	R. Hebert	0.3	100%	0	0%
	First ratoon	Duplantis	0.4	100%	0	0%
	First ratoon	Domingues	0.5	67%	0	0%
	First ratoon	Sterling	0.6	100%	0	0%
	First ratoon	Adeline	1.4	56%	0	0%
	First ratoon	North Side	0.2	100	0	0%
	First ratoon	Breaux Brothers	0.4	90%	0	0%
Upper river	First ratoon	Beaud	0.7	100%	15	<0.1%
	First ratoon	Pearce	0.9	52%	0	0%
	First ratoon	Landry	0.7	67%	0	0%
	First ratoon	St. Louis	0.8	100%	0	0%
	Plant cane	LaCour	1.9	44%	55	0.2%
	Plant cane	Morris	2.5	50%	74	0.2%
	Plant cane	Alma	1.6	23%	569	2.5%
Lower river	First ratoon	Bon Secour	0.5	100%	0	0%
	Plant cane	Blackberry	2.4	30%	171	0.5%
	Plant cane	Glendale	1.4	28%	188	0.9%
	Plant cane	Martin and Poche	1.6	25%	8	<0.1%
Bayou Lafourche	First ratoon	Lula	1.0	100%	0	0%
	Plant cane	Raceland	1.5	44%	264	1.2%
	Plant cane	G. Knight	1.8	33%	21	<0.1%
	Plant cane	Glenwood	0.4	57%	4	<0.1%
	Plant cane	Glenwood	0.5	30%	21	0.3%
	Plant cane	Glenwood	4.0	100%	411	1.4%
	Plant cane	Cedar Grove	1.2	27%	228	1.3%
	Plant cane	McCloud	4.5	33%	334	0.6%
	Plant cane	Naquin	1.5	29%	54	0.2%

Table 1 continued.

Region	Crop year	Location	Plot area (acres)	Counted area (%)	Infected stools	Percent infection
Bayou Lafourche	Plant cane	Thibodaux French	1.1	100%	553	3.5%
	Plant cane	Thibodaux Gold Mine	1.6	48%	214	0.9%
	Plant cane	Belle Alliance	1.6	45%	0	0%
	Plant cane	Belle Alliance	2.9		12	<0.1%
	Plant cane	New Hope	1.4	48%	0	0
	Plant cane	Palo Alto	1.5	29%	34	<0.1%
	Plant cane	Little Texas from plant cane	1.1	24%	1,627	10.4%
	Plant cane	Little Texas from stubble	1.0	100%	1,231	9%

Table 2. Detection of mosaic-infected plants of clones during May 2016 in the American Sugar Cane League Variety Release Program Primary Increase Stations.

Clone	Primary Station	No. of infected plants
L 10-147	Little Texas	14
L 11-183	Little Texas	65
Ho 11-512	Palo Alto	7
Ho 11-532	Little Texas	228
	Palo Alto	95
Ho 12-626	Little Texas	2
	Palo Alto	2

Table 3. Detection of mosaic-infected plants of clones during May 2016 in the Louisiana Cooperative Breeding Program Outfield Test on-site introduction plots and increase plots for next year's introductions at the Sugar Research Station.

Clone	Plot type	Location	No. of infected plants
L 10-147	Introduction	Brunswick	40
	Introduction	Harper	33
	Introduction	Landry	39
	Introduction	Levert St. John	44
	Introduction	Naquin	3
	Introduction	Raceland	16
L 13-263	Introduction	Alma	5
L 13-242	Introduction	Landry	2
Ho 13-769	Introduction	Glenwood	6
L 14-266	Increase	Sugar Research Station	6
L 14-275	Increase	Sugar Research Station	8
L 14-294	Increase	Sugar Research Station	1

Table 4. Detection of mosaic-infected plants in field experiment evaluating mosaic resistance via natural infection at the Sugar Research Station during May 2016.

Clone	No. mosaic infected plants in 3 replicates	Clone	No. mosaic infected plants in 3 replicates
Ho 05-1201	2, 2, 1	HoCP 12-667	4, 0, 0
Ho 05-1526	3, 5, 0	L 13-242	4, 2, 0
L 10-147	4, 0, 0	L 13-263	5, 9, 0
L 11-183	1, 2, 0	L 14-266	5, 2, 2
L 12-201	1, 0, 0	L 14-275	1, 0, 0
Ho 12-626	2, 5, 0	L 14-285	2, 0, 0
Ho 12-630	3, 0, 0	L 14-297	2, 0, 0

Healthy Seedcane Program Support

Disease testing was conducted by the Sugarcane Disease Detection Lab for the 21st year during 2016. Kleentek and SugarTech seedcane production was monitored for ratoon stunt disease (RSD), and no disease was detected (Table 5). A total of 5,587 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 promising experimental varieties 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 24 sampled fields (Table 6). A total of 7,345 leaf samples were tested for *Sugarcane yellow leaf virus* (Table 7). 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. 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.

Evaluating Disease Resistance in the Variety Selection Program

Resistance to smut was evaluated for experimental varieties in the Variety Selection Program in an annual inoculated test at the Sugar Research Station, and a range of resistance was detected among the clones (Table 8). Multiple treatments were included for commercial variety L 01-299 to evaluate the effect of the leaf sheath on smut infection severity. The pathogen infects through germinating buds, so leaf sheaths are removed from stalks of clones to expose the buds during the dip inoculation. L 01-299 exhibits high disease severity in inoculated tests, but severe smut infection is uncommon in commercial fields. Therefore, the leaf sheath was left in place for an inoculated treatment, and non-inoculated treatments with the leaf sheaths left in place or removed were included. Smut severity was 71% for inoculated plants with the leaf sheath removed. However, only 7% infection occurred when the leaf sheaths were left in place. No

infection occurred for either non-inoculated treatment. The results demonstrate that the leaf sheath is an effective barrier against smut infection in L 01-299.

A second study to develop potential parents with resistance to smut was conducted. Two hundred ninety-four clones from the increase stage of the variety selection program were dip-inoculated with smut in a non-replicated test. Infection was recorded as +/-, and 54% of the clones did not develop any smut infection. All negative clones were again dip-inoculated and were replanted. Smut infection will be recorded during 2017.

Table 5. Ratoon stunt disease testing summary for 2016.

Source	Location	No. of fields	No. of varieties	No. of samples
Louisiana growers	State-wide	24	6	517
Variety Release Program	1° & 2° stations	-	27	1,616
Helena SugarTech®	Foundation stock	-	-	-
Kleentek®	Foundation stock	-	-	75
Kleentek®	Other than foundation	-	-	2,935
Local Quarantine	LSUAC	-	28	100
Research	LSUAC	-	-	334
Totals		24	-	5,587

Table 6. Ratoon stunt disease field and stalk infection testing results in different crop cycle years for all varieties combined during 2016.

Crop year	Total number of fields	Average field infection (%)	Total number of stalks	Average stalk infection (%)
Plant cane	2	0	52	0
First stubble	4	0	79	0
Second stubble	6	0	106	0
Older stubble	12	0	280	0
Totals/Averages	24	0	517	0

Table 7. Sugarcane yellow leaf virus testing summary for 2016.

Source	Location/type	No. of fields	No. of varieties	No. of samples
LDAF	Seed Certification	143	9	4,500
Helena SugarTech®	Foundation Stock	-	-	-
Kleentek®	Foundation Stock	-	-	75
Kleentek®	Other than foundation	-	-	2,326
Local Quarantine	LSUAC	-	28	100
Research	LSUAC	-	-	334
Totals		143	-	7,345

Soil Microbial Communities Associated with Long-Term Sugarcane Cultivation

Previous research has shown the continuous 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 is being conducted in cooperation with Dr. Lisa Fultz.

DNA was extracted from bulk and rhizosphere (root zone) soil samples and sent for sequencing at Argonne National Laboratories. DNA was amplified for the 16S ribosomal DNA and internal transcribed spacer (ITS) to identify bacteria and fungi, respectively, and sequencing was performed on the Illumina Miseq platform. 16S ribosomal DNA sequencing of the samples collected in 2014 and 2015 produced 18,272,694 sequences, and ITS sequencing produced 17,136,859 sequences.

Raw sequencing data was analyzed using the QIIME pipeline. Stringent quality filtering of 16S data sorted and identified 10,350,669 (56.6%) sequences into 179,092 groups known as operational taxonomic units (OTUs) at a level of 97% sequence similarity. 10,114,701 ITS sequences (59%) were sorted into 37,047 OTUs. Sequencing data was converted into sample dissimilarity matrices and used for analysis of variance (ANOVA). ANOVA revealed significant differences ($P > 0.001$) between short and long term sugarcane cultivation for both bacterial and fungal communities. Further, differences between paired sites were also found to be significant ($P > 0.001$), as well as the interaction between these two factors, suggesting the overall effects of continuous sugarcane cultivation on fungal and bacterial communities varies among sites.

Future research priorities include identification of individual taxa frequently associated with recent or long-term sugarcane cultivation, the use of soil nutrient data as explanatory variables for differences in microbial communities, and processing of DNA sequencing data from soils

sampled in 2016 to address the succession of soil microbial communities with exposure to sugarcane.

Table 8. Smut infection means and resistance ratings determined in an inoculated test for commercial and experimental sugarcane varieties during 2016.

Variety	Mean percent[1]	Rating[2]	Variety	Mean percent[1]	Rating[2]
CP 73-351	78	9	HoCP 12-673	9	3
LCP 85-384	12	4	L 13-234	3	2
HoCP 89-846	40	7	L 13-242	7	3
HoCP 96-540	11	4	L 13-243	0	1
L 99-226	21	5	L 13-251	1	2
L 01-283	10	3	L 13-257	0	1
L 01-299	71	9	L 13-260	1	2
L 01-299NSI	7	3	L 13-263	0	2
L 01-299NSNI	0	1	L 14-264	56	9
L 01-299SNI	0	1	L 14-265	31	6
Ho 02-6848	0	1	L 14-266	8	3
Ho 05-1102	0	1	L 14-267	0	1
Ho 05-1201	28	6	L 14-270	10	3
Ho 05-1526	0	1	L 14-271	24	5
Ho 05-1791	8	3	L 14-273	80	9
HoCP 09-804	32	6	L 14-274	22	5
Ho 09-840	26	6	L 14-275	73	9
L 10-147	16	4	L 14-276	66	9
L 11-183	7	3	L 14-282	71	9
L 12-201	10	3	L 14-285	21	5
L 12-202	19	5	L 14-288	76	9
Ho 12-615	3	2	L 14-289	44	9
Ho 12-626	13	4	L 14-294	63	9
Ho 12-630	3	2	L 14-295	72	9
HoCP 12-667	0	1	L 14-297	18	4
HoCP 12-671	2	2			

[1]Resistance ratings assigned on a 1-9 scale in which 1-3 = resistant, 4-6 = moderately susceptible, and 7-9 = highly susceptible. Means for smut infection calculated from the percentage of smut infected shoots in each of three replicates. Four treatments were included for one clone, L 01-299. Additional treatments included non-striped (leaf sheaths left in place) with inoculation (NSI), non-striped and non-inoculated (NSNI), and striped with inoculation (NI).