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 2019 included screening for resistance to mosaic; providing phenotypic data for developing molecular markers for resistance to brown rust, mosaic, and smut; providing support for healthy seedcane programs to manage systemic diseases; and billet planting. Research results on billet planting are reported separately.

Mosaic Resistance Evaluations

A project addressing mosaic, a historically important disease for the Louisiana sugarcane industry, was continued during 2019. The goal of all of the recent mosaic research is to prevent it from re-emerging as a problem for the industry. An ongoing component of the project has been to evaluate resistance to mosaic for breeding program parents by utilizing mechanical inoculation of young plants grown in the greenhouse. The first inoculations were conducted during 2017. Two inoculations were then conducted during 2018 with USDA-ARS Sugarcane Research Unit commercial and basic breeding program potential parents and accessions from the basic germplasm collection, and one inoculation was conducted with parents of the LSU AgCenter breeding program. During 2019, two inoculations were conducted. The first screened 2017 and 2018 series USDA clones for resistance. The second was a repeat inoculation of clones that previously exhibited a resistant reaction in one inoculation and a susceptible reaction in another. Inoculum consisted of sugarcane leaves from multiple clones infected with SrMV.

Infection levels were determined by visual observation of systemic mosaic symptoms in newly-emerged leaves of the inoculated plants. Each parent had a minimum of four plants (six single-node cuttings planted) with 4-5 leaves grown in Styrofoam trays. Assigned ratings were HR = highly resistant (0% mosaic infection), MR = moderately resistant (1-24%), MS = moderately susceptible (25-49%), S = susceptible (50-74%), and HS = highly susceptible (75-100%). The infection intervals were based on a percentage range because of some variability in the number of surviving plants per clone. 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.

The first experiment included five 2017 series and 36 2018 series parents. Visual symptoms were evaluated at two times after inoculation (28 and 45 days) to evaluate the effect of time after inoculation on detection of symptoms. No symptomatic plants were observed (HR rating) for 14/41 (34.1%) of the parents for both visual ratings (Table 1). Three of those 14 (21.4%) had at least one plant test positive for SrMV by a polymerase chain reaction (PCR) assay (conducted by Kathy Warnke at the USDA-ARS Sugarcane Research Unit). Nine of 22 (40.9%) had symptomatic plants in at least one visual rating but all negative for SrMV by PCR. The overall agreement between visual symptoms and PCR results was 26/41 (63.4%). The explanation for

these discrepancies is not apparent. Visual ratings were in agreement for both times of evaluation (for both resistant and susceptible ratings) for 33/41 (81.5%) of the parents. Symptomatic plant frequencies for the check varieties were 0% for HoCP 96-540 (R), 55% for HoCP 09-804 (MS), and 82.3% and 33% for the HS checks, L 08-088 and Rio sorghum, respectively.

The second experiment included 42 parents from series ranging from 2008 to 2019 that had received contrasting resistant and susceptible ratings in previous experiments. Visual symptoms were again evaluated at two times after inoculation (32 and 46 days). No symptomatic plants were observed (HR rating) for 23/42 (54.8%) of the parents for both visual ratings (Table 2). Twelve of the 23 (47.8%) parents with a HR visual rating had at least one plant test positive by PCR assay (conducted by Kathy Warnke at the USDA-ARS Sugarcane Research Unit). In this experiment, only 1/19 (5.3%) had a susceptible visual rating but a negative PCR detection for SrMV. Visual ratings were in agreement for both times of evaluation (for both resistant and susceptible ratings) for 35/42 (83.3%) of the parents. Symptomatic plant frequencies for the check varieties were 0% for HoCP 96-540 (R), 16.5% for HoCP 09-804 (MS), and 50% and 67% for the HS checks, L 08-088 and Rio sorghum, respectively.

Annual inoculation of mosaic is being reinstituted as a component of the breeding program. However, in experiments conducted over the last three years, variability has been observed in the mosaic resistance reactions obtained in different inoculations. This variability raises questions concerning the interpretation of results obtained with mechanical inoculation.

Questions include:

- Is susceptibility indicated by the detection of infected plants in an inoculation (even if none are detected in another inoculation)? At this point, the best conclusion is yes. No infection has been detected in the resistant check variety.
- How much variability is there in susceptibility among genotypes? (Can varying levels of resistance/susceptibility be distinguished?) At this point, the best conclusion is that there is variability. In inoculated tests with other sugarcane diseases, degrees of susceptibility are evident. Also, moderately susceptible reactions show the highest degree of variability.
- How well do the results from six inoculated plants distinguish differences? At this point, the best conclusion is that the number is adequate. It allows different ratings to be assigned, and it would be difficult to include more reps logistically.
- How much variability is there in the success of an inoculation (in the expression of visible systemic symptoms of infection)? At this point, symptom expression can be erratic. Good plant growth is essential.
- When is the best time to evaluate visible systemic infection symptoms? A comparison of visible symptom results with PCR results suggests a later evaluation after topping the plants at 4-5 weeks might provide the best results, but there was good agreement between visual ratings done at two times after inoculation. However, it is more difficult to maintain the inoculated plants for longer periods.
- Ultimately, the most important question is whether mechanical inoculation in the greenhouse can reliably identify and distinguish resistant and susceptible parents.

Table 1. Mosaic mechanical inoculation results for 2017 and 2018 series parents in an experiment conducted at the USDA-ARS Sugarcane Research Unit during 2019.

	First visual rating		Second visual rating		PCR first rating	
Variety	Infection	Rating[a]	Infection	Rating[a]	Infection	Rating[a]
Ho2017-756	83%	HS	17%	MR	0%	HR
HoCP17-702	0%	HR	20%	MR	0%	HR
HoCP17-725	100%	HS	67%	S	0%	HR
HoCP17-734	100%	HS	33%	MS	0%	HR
HoCP17-768	17%	MR	83%	HS	50%	S
HoCP18-801	100%	HS	67%	S	100%	HS
HoCP18-803	100%	HS	100%	HS	100%	HS
HoCP18-806	50%	S	0%	HR	83%	HS
HoCP18-808	100%	HS	83%	HS	83%	HS
HoCP18-810	33%	MS	0%	HR	50%	S
HoCP18-811	0%	HR	0%	HR	0%	HR
HoCP18-815	17%	MR	0%	HR	0%	HR
HoCP18-817	0%	HR	0%	HR	0%	HR
HoCP18-818	17%	MR	0%	HR	0%	HR
HoCP18-822	0%	HR	0%	HR	0%	HR
HoCP18-823	0%	HR	0%	HR	0%	HR
HoCP18-824	0%	HR	0%	HR	0%	HR
HoCP18-826	0%	HR	0%	HR	0%	HR
HoCP18-827	100%	HS	40%	MS	0%	HR
HoCP18-829	0%	HR	0%	HR	100%	HS
HoCP18-834	100%	HS	80%	HS	50%	S
HoCP18-835	0%	HR	0%	HR	0%	HR
HoCP18-837	100%	HS	33%	MS	0%	HR
HoCP18-839	60%	S	0%	HR	0%	HR
HoCP18-840	0%	HR	0%	HR	0%	HR
HoCP18-842	0%	HR	60%	S	0%	HR
HoCP18-846	0%	HR	0%	HR	0%	HR
HoCP18-847	33%	MS	33%	MS	40%	MS
HoCP18-852	17%	MR	17%	MR	100%	HS
HoCP18-856	33%	MS	33%	MS	100%	HS
HoCP18-857	0%	HR	0%	HR	100%	HS
HoCP18-859	0%	HR	0%	HR	100%	HS
HoCP18-861	20%	MR	20%	MR	100%	HS
HoCP18-862	17%	MR	17%	MR	100%	HS
HoCP18-863	100%	HS	40%	MS	100%	HS
HoCP18-865	100%	HS	60%	S	0%	HR
HoCP18-866	0%	HR	33%	MS	0%	HR
HoCP18-869	17%	MR	0%	HR	0%	HR
HoCP18-872	50%	S	0%	HR	0%	HR
HoCP18-877	0%	HR	0%	HR	0%	HR
HoCP18-878	0%	HR	0%	HR	0%	HR

Table 2. Mosaic mechanical inoculation results for parents that had received both resistant and susceptible ratings in previous experiments

	First visual rating		Second visual rating		PCR first rating	
Variety	Infection	Rating[a]	Infection	Rating[a]	Infection	Rating[a]
Ho08-730	0%	HR	17%	MR	50%	S
Ho11-573	0%	HR	0%	HR	50%	S
Ho13-708	0%	HR	0%	HR	50%	S
Ho14-864	0%	HR	0%	HR	0%	HR
Ho15-962	0%	HR	0%	HR	0%	HR
Ho15-975	0%	HR	0%	HR	50%	S
Ho16-608	0%	HR	0%	HR	0%	HR
Ho16-619	50%	S	50%	S	83%	HS
Ho16-627	0%	HR	0%	HR	0%	HR
Ho16-636	0%	HR	0%	HR	50%	S
Ho16-641	0%	HR	0%	HR	50%	S
Ho16-648	0%	HR	0%	HR	0%	HR
Ho16-651	0%	HR	17%	MR	0%	HR
Ho16-652	0%	HR	0%	HR	0%	HR
Ho16-654	17%	MR	17%	MR	100%	HS
Ho16-666	0%	HR	17%	MR	100%	HS
Ho16-7061	0%	HR	0%	HR	83%	HS
Ho16-9014	0%	HR	0%	HR	100%	HS
Ho16-9018	25%	MS	0%	HR	33%	MS
Ho16-9020	100%	HS	0%	HR	33%	MS
Ho16-9054	0%	HR	0%	HR	100%	HS
Ho17-717	33%	MS	33%	MS	100%	HS
Ho17-775	0%	HR	50%	S	100%	HS
Ho17-9122	0%	HR	0%	HR	100%	HS
Ho17-9143	0%	HR	33%	MS	100%	HS
Ho17-9155	0%	HR	0%	HR	0%	HR
Ho17-9160	0%	HR	33%	MS	67%	S
Ho17-9161	0%	HR	17%	MR	100%	HS
Ho19-832	33%	MS	50%	S	100%	HS
HoCP13-723	17%	MR	50%	S	100%	HS
HoCP14-830	0%	HR	0%	HR	0%	HR
HoCP14-831	0%	HR	0%	HR	0%	HR
HoCP14-892	0%	HR	33%	MS	0%	HR
HoCP15-510	0%	HR	0%	HR	0%	HR
HoCP15-991	0%	HR	0%	HR	0%	HR
HoCP16-672	33%	MS	50%	S	100%	HS
HoCP17-709	100%	HS	100%	HS	100%	HS
HoL15-508	0%	HR	0%	HR	0%	HR
HoL15-993	17%	MR	0%	HR	0%	HR
L14-275	0%	HR	0%	HR	83%	HS
L15-311	0%	HR	0%	HR	33%	MS
L15-315	67%	S	100%	HS	83%	HS

The detection of mosaic susceptibility in parents in the variety selection program indicated that some level of susceptibility was going undetected in the crossing and selection programs. The reliance only on natural infection to detect susceptibility was not adequate to detect susceptible clones due to insufficient inoculum pressure. Susceptible clones were detected at all levels of the breeding program. The sources of susceptibility can now be eliminated or used with appropriate caution in future crossing.

The results over three years of mechanical inoculations has led to tentative conclusions:

- It is necessary to conduct more than one inoculation to determine mosaic resistance reactions.
- The majority of parents exhibit consistent reactions in multiple tests, and ratings can be assigned with a reasonable degree of confidence.
- For clones with split outcomes (resistant and susceptible ratings assigned in different experiments), assume a moderate level of susceptibility.
- More research is needed to improve the inoculation method to achieve greater repeatability (uniformity of reactions across experiments).
- Forging ahead with large scale screening by mechanical inoculation has provided extremely valuable information on resistance/susceptibility levels in parent populations but with a higher degree of uncertainty than is desirable for some clones.

Phenotypic Data for Developing Molecular Markers for Disease Resistance

Results are being obtained for resistance reactions (phenotypes) of clone populations selected for association of molecular markers for resistance to multiple diseases in studies being conducted in cooperation with Dr. Niranjan Baisakh. All of the consistent mosaic inoculation results for the commercial and basic parents will serve as an initial resistance marker association population. During 2019, natural infection levels were adequate to assign brown rust resistance ratings for a biparental cross population (L 99-233 resistant x HoCP 96-540 susceptible). The same biparental population with reversed ratings (L 99-233 susceptible x HoCP 96-540 resistant) was successfully screened for resistance to smut in an inoculated field experiment during 2019. Clones of the population were dip-inoculated and planted at the Sugar Research Station.

Healthy Seedcane Program Support

Disease testing was conducted by the Sugarcane Disease Detection Lab for the 24th year during 2019. Kleentek and SugarTech seedcane production was monitored for ratoon stunt disease (RSD), and no disease was detected (Tables 3 and 4). A total of 2,285 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 active experimental varieties from the 2015 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 21 sampled fields. A total of 6,842 leaf samples were tested for *Sugarcane yellow leaf virus* (Table 5). Commercial tissue-culture seedcane sources were tested as part of the LDAF seedcane certification program. No field failed to certify due to virus infection.

Table 3. RSD testing summary for 2019

Source	Location	No. of fields	No. of varieties	No. of samples
Louisiana growers	State-wide	21	6	429
Variety Release Program	1° & 2° stations	-	5	306
Helena SugarTech®	Foundation stock	-	-	-
Kleentek®	Foundation stock	-	-	68
Kleentek®	Other than foundation	-	-	215
Local Quarantine	LSUAC	-	26	73
Research	LSUAC	-	-	1,194
Totals		20	38	2,285

Table 4. RSD field and stalk infection frequencies in different crop cycle years for all varieties combined during 2019

Crop year	Total number of fields	Average field infection (%)	Total number of stalks	Average stalk infection (%)
Plant cane	0	0	0	0
First stubble	4	0	116	0
Second stubble	1	0	27	0
Older stubble	11	0	228	0
Unknown	5	0	58	0
Totals/Averages	21	0	429	0

Table 5. Sugarcane yellow leaf virus testing summary for 2019

Source	Location	No. of fields	No. of varieties	No. of samples
LDAF	Seed Certification	195	-	5,719
Helena SugarTech®	Foundation stock	-	-	-
Certis Kleentek®	Foundation stock	-	-	86
Certis Kleentek®	Other than foundation	-	-	954
Local quarantine	LSUAC	-	26	73
Research	LSUAC	-	-	10
Totals		195	26	6,842