# AGRICULTURAL ABSTRACTS

## The Louisiana Basic Breeding Program-Past, Present, and Future

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With the extraordinary success of LCP85-384, a *Saccharum spontaneum* $BC_4$ derivative, released in 1993, and the release of HoCP85-845, also a S. *spontaneum* $BC_4$ derivative, it is obvious that the USDA-ARS Basic Breeding Program at Houma, LA has provided tremendous dividends to the Louisiana sugar industry. Both clones were bred during the year 1980, and both involved *S. spontaneum* clone, US56-15-8. Some questions we need to address now are "What has happened during the past 20 years of crossing with basic germplasm that would give us reason to believe that further benefits can be expected from the basic breeding program?" "Where are we today in our basic breeding program?" "What must we do to maximize the likelihood of success in the future?" A review of our own program along with other breeding programs, particularly in Argentina, indicate that, with an intensified effort and some modifications in our breeding and selection approach based on lessons learned from the past, we should expect to see further substantial genetic improvement through basic breeding. Topics discussed will include: 1) number of BC generations needed to obtain commercial cultivars, 2) years needed between BC generations, 3) need for recombination between BC generations to exploit desirable recessive traits, 4) use of marker-assisted selection, 5) formation of complex *S. spontaneum* crosses, and 6) greater focus on populations rather than individuals.

## Assessment of Stalk Cold Tolerance of Louisiana Varieties During the 2000-2001 Crop Year

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The exposure of sugarcane to damaging frosts occurs in over 20 of the 79 sugarcane-producing countries of the world, but is most frequent on the mainland of the United States. The frequent winter freezes in the sugarcane area of Louisiana forced the industry to adapt to a short growing season (7-9 months) and a short milling season (about 3 months). Field experiments consisting of 3-row plots (18 ft) by 45 ft long are routinely planted at the Ardoyne Farm of the USDA-ARS, SRRC at Houma, Louisiana, for the estimating stalk cold tolerance of commercial and candidate varieties. For the 2000-2001 crop-year study, two commercial varieties, CP

70-321 and CP 79-318, with known cold tolerance were planted in the test as controls. Other commercial varieties included LHo 83-153, LCP 85-384, HoCP 85-845 and HoCP 91-555.

Freezing temperatures that affected the Louisiana Sugar Industry during the 2000-2001 crop-year occurred on December 20, 2000, when the minimum temperature recorded in the field at the Ardoyne Farm was 24˚F, and again on December 21, December 30 through January 5, 2001 and January 9 and 10. The lowest temperature of 22˚F was recorded on January 4. Freezing conditions prevailed for 8-15 hours during each freeze incident. Stalks of all varieties were frozen to the ground following the initial freeze with freeze cracks evident only after the January 4 freeze.

Samples were taken the date of the first freeze and again at 7, 14, 22 and 30 days after the first freeze. Criteria used to measure overall stalk cold tolerance included changes in Brix, sucrose, purity, yield of theoretical recoverable sugar per ton of cane, pH, titratable acidity, dextran by both the Rapid Haze and ASI II Methods and fiber content of juice and/or cane and mean stalk weight. On each date of harvest, 15-stalk samples were collected from each of the four replications of all varieties and were divided into two sub-samples on four of the five sampling dates to compare the analyses of juice extracted by the conventional 3-roller mill (10 stalks) and the pre-breaker/press method (5 stalks). On the remaining sampling date, juice was extracted from all 15 stalks by the 3-roller mill. Significant changes were noted in all criteria for all varieties, with the exception of mean stalk weight, at 22 and 30 days after the first freeze. Further, significant differences were also noted between varieties on each sampling date. Overall, the ranking of varieties for stalk cold tolerance, from best to worse, when considering all criteria was as follows: CP 70-321, LHo 83-153, LCP 85-384, HoCP 85-845, HoCP 91-555 and CP 79-318. Accordingly, the classification of stalk cold tolerance (post-freeze resistance) for these varieties based on the results obtained during the 2000-2001 crop year is as follows: Very Good - CP 70-321; Good - LHo 83-153; Good to Moderate - LCP 85-384; Moderate - HoCP 85-845; Moderate to Poor - HoCP 91-555; and Poor - CP 79-318. The stalk cold tolerance for both CP 70-321 and CP 79-318 is well documented from previous studies. There were only slight differences in the pH and titratable acidity of the juice when comparing extraction methods. Although the concentration of dextran in the juice as an average of all varieties and all dates of sampling was considerably different between the two methods of analyses (1,592 and 4,102 ppm for the Rapid Haze and ASI II Methods, respectively), the ranking amongst varieties was similar when comparing the two methods ($r = 0.98$).

## Post-Freeze Performance of 16 Sugarcane Cultivars Following the December 31, 2000 Freeze Event in Florida

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Freezing temperatures occurred for an extended period of time on the night of December 31, 2001 and morning of January 1, 2000. Temperatures below –2°C occurred for more than four hours in much of the Everglades Agricultural Area. The performance of 16 cultivars planted in six experiments planted at five locations was characterized by determining sugar content per gross ton of cane. Replicated variety trials at five locations were sampled serially on two-week intervals following the freeze event until March 20, 2000 and ground for sugar yield. Four of the five locations were exposed to freezing temperatures for more than 10 hours while one location received no freeze injury. Sucrose content of the 16 cultivars occurring at least at two of the freeze damaged experiments were contrast with sucrose content at the freeze protected location. CP89-2143 had the highest sugar per ton of cane at 80-days post-freeze and demonstrated relative losses comparable to CP72-2086, a known "freeze-tolerant" cultivar. CP85-1308 showed the greatest relative losses following the freeze event. CP80-1743, CP84-1198, CP85-1382 and CP88-1762 demonstrated relative losses similar to CP70-1133, a known "freeze-susceptible" cultivar.

## Sugarcane Tissue Phosphorus Concentration as Affected by P Rates Applied to a Florida Histosol

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Approximately 85% of the sugarcane (*Saccharum officinarum L*) acreage in Florida are located in the Everglades Agricultural Area, where soils are typically organic in nature. Phosphorus, K, and several micronutrients are commonly applied to histosols to produce acceptable yields. Because of increasing environment concerns, P application to all agricultural crops has been receiving increased attention. Though many studies on sugarcane response to P

fertilizer have been carried out worldwide, little information is available on the effects of P fertilization, especially with respect to seasonal tissue P concentration, for sugarcane grown on Florida's histosols. The objective of this field study was to assess tissue P concentration of sugarcane varieties at the different growth stages in response to increasing P rates. Five P rates (0, 34, 67, 101, 135 kg $P_2O_5$ $kg^{-1}$) and four sugarcane varieties (CP70-1133, CP72-2086, CP78-1628, and CP80-1827) were evaluated in a randomized complete block design (RCBD), in six replications at two sites. Top visible dewlap (TVD) leaf samples were collected at the early, grand growth, and late crop stages. Results indicated increases in tissue P concentration as P rate increased, especially in the early stages of crop growth. Phosphorus concentration was also highest in the early stages and lowest in late stages, nearing harvest date. First year, i.e., plant, sugarcane had higher tissue P concentration than first ratoon cane. Variety CP80-1827 presented the highest tissue P concentration in all the samplings. Interpretation and utilization of sugarcane tissue P concentrations for determining plant nutritional status and fertilizer recommendation should take into account time of sampling, P rate applied, and variety planted.

## Sugarcane Root and Soil Microbial Responses to Intermittent Flooding

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Sugarcane is one of the most environmental friendly agricultural crops grown in the Everglades Agricultural Area because it can tolerate short periods of flooding and has been reported to have less soil organic matter oxidation compared to other agricultural crops. Soil oxidation results primarily from aerobic microbial activity. Since flooding reduces soil oxygen levels, flooding as well as growing sugarcane may reduce soil organic matter oxidation. One concern regarding flooding of sugarcane is that mechanical harvesters would reduce yields of subsequent ratoons by pulling entire stools from the soil due to weakened root systems caused by the flooding. An experiment was conducted to determine the combined effect of water-table depth and intermittent flooding on soil organic matter oxidation potential and sugarcane root growth. Sugarcane was grown in 1.5 X 2.6 X 0.6 (wide, long, and deep, respectively) m polyethylene lysimeters out doors. Lysimeters were filled with a Pahokee muck soil. After plants reached an 8-cm height, intermittent flooding treatments were imposed consisting of 7 days flooding followed by 14 days drained to 16, 33, and 50-cm depths. A continuous 50-cm water table was used as a control. Starting July 10, soil samples were taken during the drain period on day 0, 3, 7, and 14 and analyzed for oxidation potential. Soil sampling continued over 5 consecutive cycles. On Jan. 19, 2001 sugarcane was harvested and shortly afterwards, root samples were taken. Root samples were extracted by taking four-6.4-cm cores to 0 to 15-, 15 to 30-, and 30 to 45-cm depths at a distance about 5 cm from the rows of sugarcane. Roots were washed and analyzed for dry wt, length, volume, surface area, and diameter. Soil organic matter

oxidation potential averaged over 5 drain cycles indicated that soil oxidation started increasing immediately after drainage and reached its maximum activity about one week later. Also, there appeared to be a residual effect of flooding as the oxidation potential of the flooding treatments was less than the continuously drained treatment over the 14-day drain cycle. The 16-cm water table had soil oxidation potentials that were less than half those of the other flooding treatments. Average root dry wt, length, surface area, and volume from high water table treatments in the sampled area were about twice those from continuously drained treatment. It appears that with intermittent flooding, roots around the sugarcane stool can compensate for unfavorable root environments by developing more roots in the less aerated soil compared to continuously drained soil. Combining raised water tables with intermittent flooding should improve both soil conservation and sugarcane root growth.

## Effect of Nitrogen Fertilizer Rates on Producer Economic Returns of Variety LCP 85-384 on a Heavy-Textured Soil in Louisiana

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Recommended nitrogen fertilizer rates for "strong" stands of sugarcane (*Saccharum* spp.) on heavy-textured soils in Louisiana are 112 to 135 kg N/ha for plant cane, and 157 to 179 kg N/ha for stubble cane. The high sugar yields (20% higher than the next best variety) obtained with variety LCP 85-384 raise questions about whether this variety has different nitrogen fertilizer requirements than other recommended varieties grown in Louisiana. To answer this question, twelve site-years of yield data from nitrogen rate studies with LCP 85-384 on a Baldwin silty-clay loam (thermic Vertic Ochraqualf) soil were used to determine economic returns (based on $0.42/kg of sugar, $0.66/kg of N, and the producer giving half of his crop to the sugar mill and landlord) to producers. The best economic returns for plant cane in five studies were at 0, 56, 67, 135, and 157 kg N/ha, respectively, compared to the recommended nitrogen application rate of 112 to 135 kg/ha. The highest economic returns for first-stubble cane in five studies were 67, 112, 112, 112, and 135 kg N/ha compared to the recommended rate of 157 to 179 kg N/ha. Consequently, the recommended N application rate for LCP 85-384 first-stubble cane appears to be too high and better economic yield responses could be obtained if it were fertilized like plant cane. There was only one site-year of data for second- and

third-stubble cane. In both cases, highest economic returns were obtained at 202 kg N/ha compared to the 135 kg N/ha rate.

## Production Trends of the Major Cane Sugar Producing Countries in the World

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Over 130 countries produce sugar about 134 million Mg sugar in 1999 to 2000 crop, of which 27 of them produced over one Mg sugar. Six countries, Brazil, India, China, USA, Australia, and Thailand generated 61% of the word cane-sugar production (97 million Mg) in 1999 to 2000. Total cane-sugar production from these six countries plus South Africa, the major cane sugar producer in Africa, has significantly increased in recent decades. Approximately 60% of the increase was due to expanded growing area.

The highest sugar production per area in the world is and has been in Hawaii with average production over 11 Mg sugar $ha^{-1}$. Thailand and Louisiana demonstrated the largest increases in total sugar production (244% and 145% Mg sugar) and per area production (145% and 87% Mg sugar $ha^{-1}$) in the last 20 years. Australia has maintained without significant change the highest average sucrose content (14 sucrose %cane) in the world since the 1920s. In the last 12 years sugar production per area (Mg sugar $ha^{-1}$) increases have been due mostly to improvements in cane yield production with little to no change in sucrose content. Perhaps we have reached a genetic plateau for sucrose content.

## Potential Effect of Yellow Leaf Syndrome on the Louisiana Sugarcane Industry

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A three-year field study was conducted to determine the effect of sugarcane yellow leaf virus (SCYLV) on two cultivars of sugarcane (LCP 82-89 and LHo 83-153). Yield loss (sugar

per unit area) was observed in LCP 82-89, with the greatest loss in the second-ratoon crop (23%). Quality components, % Brix, % sucrose, % purity, and starch concentration, of the stalks did not differ between SCYLV-infected and uninfected; however, in the tops, leaves and the immature portion of the stalk, % Brix, % sucrose, % purity, and starch concentration were higher in SCYLV-infected plants of both cultivars. Dextran content was inconsistent. Tops of stalks are normally removed by the mechanical harvester; however, they may not be removed if the cane is lodged and/or during wet weather harvesting. Green leaves and immature tissue containing elevated levels of starch delivered to the mill may reduce processing efficiency.

A collection of 407 parental sugarcane clones grown at Canal Point, Florida and used for making crosses for the Louisiana Industry were assayed for infection by SCYLV. As a result of natural spread, SCYLV infection was found in approximately 50% of the cultivars, indicating a high level of susceptibility to infection within the Louisiana germplasm.

Although visible symptoms of yellow leaf syndrome (YLS) caused by SCYLV are rarely observed in Louisiana, yield loss was observed in SCYLV-infected LCP 82-89 in the absence of symptoms and the virus in both cultivars affected quality components in leaves. With the recent discovery of *Melanaphis saccharalis* in Louisiana, a demonstrated vector of SCYLV, and the demonstration of yield and quality effects on sugarcane even in the absence of symptoms, YLS is a potential problem to the Louisiana industry.

## Feeding Effects of Yellow Sugarcane Aphid on Sugarcane

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Feeding by yellow sugarcane aphid, *Sipha flava* (Forbes), can cause reddening, premature yellowing and death of sugarcane leaves. Prolonged feeding by large populations of this aphid can lead to plant death. We report here the results of experiments using a susceptible sugarcane cultivar (CP80-1827) to quantify the growth and yield effects of early season *S. flava* feeding. Two-month old plants grown from single-eye setts in 5-gallon buckets were first subjected to yellow sugarcane aphid feeding for 8 to 10 weeks. Plant damage was rated on the number of leaves (0, 1, 2, 3, and 4) below the TVD on the primary stalk with <50% *S. flava* damage symptoms. These ratings were used to group plants for comparison of growth and yield effects against plants grown without aphid exposure (controls). Aphids were then removed and the plants transplanted into the field where they were maintained aphid-free for 7 months until harvest. *S. flava* feeding resulted in the production of longer, faster growing leaves and internodes, but also thinner, lighter stalks compared to the controls. Each leaf and internode that was produced after aphids were removed from the plants expanded slightly less than the previous one and gradually approached the length of these structures on control plants, but node diameters remained thinner on previously infested stalks. Internode volumes were reduced an average of 21% on plants in the highest damage category. Aphid-damaged stalks with thin internodes at their bases were more likely to lodge from wind and rat damage than controls.

Apparent sucrose was lower in juice from plants previously infested by *S. flava* than from those not exposed to the aphids. When combined with the reductions in internode volume and weight, even light *S. flava* damage (i.e., two out of six leaves below TVD with >50% damage) resulted in a 6% reduction in sugar yield. Heavy damage (i.e., six out of six leaves below TVD with >50% damage) to sugarcane plants from yellow sugarcane aphid feeding early in the season reduced sugar yield by 19%.

## Relative Abundance and Diversity of Aphid Species Collected in Traps Adjacent to Sugarcane Fields in Florida

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Even with the rapid expansion of the state's sugarcane industry during the 1960s, sugarcane mosaic, caused by the sugarcane mosaic virus potyvirus (SCMV), remained a disease of minor importance in Florida for nearly four decades. Although detected in sugarcane and weeds, disease incidence rarely exceeded several percent. Since the late 1990s, however, observers have noted a marked increase in SCMV incidence, particularly in the variety CP72-2086. A mainstay of the Florida industry, presence of SCMV in this variety could have serious repercussions. For even though CP72-2086 has demonstrated yield tolerance, it could serve as a significant pathogen reservoir, facilitating the spread of SCMV to other susceptible, but less tolerant varieties. In nature, SCMV is transmitted mechanically (i.e. planting of infected seed pieces) and by aphid species in a semi-persistent manner. With a paucity of baseline information on aphid diversity and populations in the Everglades Agricultural Area, investigations were conducted using standard yellow sticky traps to monitor aphid activity adjacent to sugarcane fields. Five traps were positioned for a 14-day period at monthly intervals along transects paralleling sugarcane fields located in areas representative of the western, central, and eastern cane-growing areas of the EAA. Cumulative numbers of aphids trapped peaked in March and then again in November. A total of 23 identifiable species were collected, representing 12 genera. Two of these species, *Rhopalosiphum maidis* and *Schizaphis graminium*, have been demonstrated to be capable of transmitting SCMV in nature. Two aphid species that commonly colonize sugarcane, *Sipha flava* and *Melanaphis sacchari*, were trapped relatively infrequently. Possible associations of the recent surge in SCMV in Florida and aphid populations will be discussed.

## Fifteen Years of Recurrent Selection for Sugarcane Borer Resistance

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The sugarcane borer, *Diatraea saccharalis* (F.), is an important insect pest of sugarcane in the Americas and the key insect pest of sugarcane in Louisiana. Long managed in Louisiana using an IPM program primarily relying on insecticides, there is increasing economic and environmental pressures to reduce the management program's dependency on insecticides. Plant resistance is an attractive alternative to insecticides.

In 1986 we began a satellite recurrent selection program to increase levels of borer resistance among parental lines used in the Louisiana Commercial Breeding Program. Following the initial crosses in 1985 among resistant parents identified from the USDA's 1983 Series, approximately 75,000 seedlings have been evaluated. Fifty-one selections were given the in-house designation RSB (recurrent selection borer). Of these 51 selections, 33 were assigned permanent numbers (US) and 18 were identified as having commercial potential. A total of 17 selections were registered with the Crop Science Society of America as germplasm clones. Biparental crosses have been made among these resistant clones and selections are being made to advance a new generation of recurrent selection.

## Mexican Rice Borer on Sugarcane and Rice: Significance to Louisiana and Texas Industries

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The sugarcane borer *Diatraea saccharalis* (F.) is the most common stem borer in the upper Texas rice belt, but the Mexican rice borer (MRB) *Eoreuma loftini* is becoming an increasing problem, particularly in the southern region of the Texas Rice Belt – Calhoun, Jackson, Victoria, and Matagorda Counties. The MRB was introduced prior to 1980 from Mexico into the Lower Rio Grande Valley where it immediately became a serious pest of sugarcane. In 1987, the MRB was first detected in the Texas Rice Belt in Jackson and Victoria Counties. In 2000, pheromone traps were set out in most Texas Rice Belt counties around

sugarcane in East Texas, and in Southwestern Louisiana sugarcane producing parishes to determine the spread of this insect since 1987. County Extension Agents , farmers, and Texas and Louisiana Agricultural Experiment Station scientists helped monitor the traps. In addition, personnel from both state departments of agriculture participated. The traps used were baited with synthetically produced MRB pheromone. Results of the 2000 trapping program showed the MRB had moved north into five new Texas Rice Belt counties – Wharton, Brazoria, Colorado, Waller, and Fort Bend. No MRB were collected in counties east of Harris where Houston is located.

About 1000 acres of sugarcane are now grown in Texas east of Houston near Beaumont, which is the eastern region of the Texas Rice Belt. Based on pheromone trapping, sugarcane grown in this area is free of MRB. Sugarcane farmers in Southeast Texas and Southwest Louisiana are concerned about the possible introduction of the MRB, which could become a serious pest of sugarcane in these regions. In the Lower Rio Grande Valley, the MRB is the number 1 pest of sugarcane; in fact, some fields are not harvested due to heavy damage. Consequently, the MRB has the potential to become a threat to rice and sugarcane in Southeast Texas and Southwest Louisiana.

Data from the Lower Rio Grande Valley suggest that drought stresses sugarcane is far more susceptible to MRB damage than healthy sugarcane. Thus, the pest potential in irrigated sugarcane is less compared to rain fed sugarcane, which represents over 95% of sugarcane in Louisiana.

Data from 1999 and 2000 indicate MRB is the predominant borer attacking rice in Jackson County (and possibly Calhoun and Matagorda Counties). MRB damage is similar to that of the sugarcane borer. The larvae cause deadhearts and whiteheads. Replicated small plot studies in Jackson County in 1999 showed that a combination of MRB and a small percentage of sugarcane borers reduced rice yields 3000 lb/acre. These are exceedingly high yield losses which may not be representative of the entire area but do show the potential for damage. Research by Texas A&M and LSU AgCenter scientists is currently being conducted to determine rice and sugarcane varietal susceptibility to MRB, gain additional biological knowledge of the MRB in order to better time control tactics, and evaluate selected insecticides using an integrated pest management approach. This research is partially funded by grants from the USDA CSREES Critical Issues, Rice Research Foundation, and the American Sugarcane League.

## Economically Optimal Crop Cycle Length for Major Sugarcane Varieties in Louisiana

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The widespread adoption of the high-yielding variety LCP85-384 has resulted in two significant changes in the production sector of the Louisiana sugarcane industry. Plant

characteristics of this variety make it very suitable for combine harvesting and helped to promote the conversion from whole stalk harvesting to combine harvesting in the state. Secondly, the variety is also an excellent Stubbling variety, resulting in the expansion of standard sugarcane crop cycles beyond harvest of second stubble. Outfield trials yield data over the 1996-2000 period for major sugarcane varieties produced in Louisiana was used to determine the optimal crop cycle length, which would maximize the net present value of producer returns. Cane yield and sugar per ton data for plant cane through third stubble was used to estimate the annualized net return of crop cycles through harvest of second and third stubble and to determine the breakeven level of fourth stubble yields which would justify production and harvest. Analysis of yield and net return data for the varieties CP 70-321, LCP 85-384, and HoCP 85-845 indicated that minimum yield levels necessary to keep older stubble in production for harvest depend directly upon the yields of the prior crop cycle phases and differ significantly across varieties.

## Optimum Maturity of CP Sugarcane Clones for Harvest Scheduling in Florida

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Variety maturity tests were conducted on 16 Canal Point (CP) clones at 5 locations over 3 years in the Everglades Agricultural Area in Florida. Cane sugar quality was measured at biweekly intervals during the October to March harvest season in each year. A quadratic response function of lbs. sucrose per gross ton of cane (SPT) vs. sampling date was calculated for each clone using the entire 3-year data set, and date and magnitude of maximum SPT calculated. CP89-2143 and CP72-2086 had the highest predicted SPT at 305 and 285 on Feb 9 and Feb 13, respectively. Model fit varied greatly between clones, with $R^2$ values ranging from 0.23 – 0.72. In general, clones with higher $R^2$ values tended to have maximum SPT after February 1. The SPT data was then divided into "early", "middle", and "late" maturity classes and the CP clones ranked based on average SPT within a given class. Results of this analysis will be discussed in terms of a harvest scheduling aid for Florida growers.

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# Protox Inhibitor Herbicide Effects on *Pythium* and Root Rot of Sugarcane

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A complex of root pathogens contributes to yield decline of sugarcane. Pythium root rot, caused by *P. arrhenomanes*, is one component of the disease complex. Root rot control would increase yield and could allow additional ratoons to be obtained. Herbicides can have non-target effects, such as enhancing or reducing root disease severity. Protoporphyrinogen oxidase (protox) inhibitor herbicides may reduce fungal disease severity in other crops by inducing host resistance. In addition, visual growth increases in sugarcane early growth following application of one protox inhibitor herbicide have been observed. Therefore, lab and greenhouse experiments were conducted to determine protox inhibitor herbicide effects on *Pythium*, root rot severity, and sugarcane growth.

Three protox inhibitor herbicides, Milestone (azafeniden), Spartan (sulfentrazone), and Valor (flumioxazin) were evaluated for their effects on *in vitro* mycelial growth rate of *P. arrhenomane*s, *P. ultimum*, and *P. aphanidermatum* and Pythium root rot and growth of sugarcane in two greenhouse experiments. Effects on sugarcane growth and root rot were evaluated after herbicide leaf or soil application at the recommended rate and 1/10 and 1/20 the recommended rate. Three types of soil were used, field soil (FS), sterilized field soil (SFS), and sterilized field soil infested with *P. arrhenomanes* (SFS+P).

All three herbicides strongly reduced *Pythium* mycelial growth *in vitro*. No growth of *P. arrhenomanes* occurred when rate one or above was applied in the growth medium. Mycelial growth inhibition still occurred at a 200-fold dilution of the recommended rate. Milestone had the strongest effect followed by Spartan and Valor. In the greenhouse, all three herbicides reduced *P. arrhenomanes* root colonization in some cases, but results were erratic between experiments. Milestone and Valor were phytotoxic in sterile and nonsterile soils, and with a short duration experiment, the damage may have made it difficult to detect effects on root rot severity and plant growth. No treatment clearly reduced visual root rot symptoms. Only 1/10 rate Spartan applied to leaves significantly reduced *P. arrhenomanes* colonization in SFS+P and increased plant growth. In field soil, more treatments reduced *Pythium* root colonization, but only leaf-applied Spartan at rate one and 1/10 rate Valor increased some component of sugarcane growth.

No consistent effects on disease severity and plant growth were shown. However, the greenhouse experimental system may not have been sufficient to clearly demonstrate the effects of the protox inhibitor herbicides on sugarcane root rot. Although variable, the results suggest these herbicides may be capable of reducing *P. arrhenomanes* infection and increasing plant growth through reduced root rot severity. The slight increases in plant growth following leaf application of herbicide suggest an indirect effect through induced resistance.

## Irrigation of Sugarcane on Clay in a High-Rainfall Environment

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Variable yield responses to irrigation of sugarcane, *Saccharum spp.*, in Louisiana's humid climate have made it difficult to evaluate its economic soundness. Nevertheless, the occurrence of several droughts during the past decade in southern Louisiana has intensified the interest in supplemental irrigation. During the severe drought of 2000, a study to evaluate the response of LCP 85-384 plant cane to irrigation was conducted on an Alligator clay soil (thermic Vertic Haplaquept), a soil textural class that tends to restrict root development under drought conditions. Irrigation was scheduled when stalks elongated 5 cm or less per week. Supplemental water was supplied in furrows on May 5, May 25, July 21 and August 28 for a cumulative total of 1130 $m^3$. The experimental site received a total of only 50.5 cm of rain from May through October, a rainfall deficit of 38.4 cm when compared to a 25-yr average for the same period. Height difference at harvest between the irrigated and non-irrigated plots was 50 cm. Yields mirrored the plant height disparity, with irrigated plots producing 44% higher cane ($P = .06$) and sugar ($P = .08$) yields than the control plots. The magnitude of the yield responses to irrigation in this experiment, 22.6 Mg $ha^{-1}$ of cane and 2.41 Mg $ha^{-1}$ of sugar, was comparable to that observed elsewhere under similar dry conditions.

## Effect of Tissue Culture Method on Sugarcane Yield Compnents

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Vegetative propagation is conducive to the spread of systemic sugarcane diseases, such as ratoon stunting disease (RSD). This important disease is now controlled in Louisiana largely by planting commercial seed-cane initially produced through tissue culture. Kleentek® seed-cane has been available to farmers since the late 1980s. In the early years, farmers sometimes noted that tissue culture derived plants had smaller stalk diameter and weight and a higher stalk population. The tissue culture method used at that time was leaf roll callus culture. Since then, the method has been changed to direct regeneration from the apical meristem to

attempt to reduce or eliminate differences between tissue culture derived plants and the original varieties.

To determine whether tissue culture method affects yield or its components, three varieties, CP 70-321, LCP 85-384, and HoCP 85-845, were compared in three successive crops, plant cane through second ratoon, at three locations. Experiments were planted with stalks from three sources: Kleentek plants derived from callus (undifferentiated cells) produced from the leaf roll above the apical meristem, Kleentek plants directly regenerated from an apical meristem, and original plants from conventional bud propagation. Stalks of plants derived from both tissue culture methods were typical of Kleentek seed-cane farmers would purchase for planting that had been rogued for phenotypic variants (off-types) and increased by bud propagation. Yield components compared included stalk diameter, length, weight, sucrose content, and population; cane tonnage; and sugar yield. Plants were visually inspected for off-types in May, August, and at harvest.

Differences in yield components between the two tissue culture methods and bud-propagated cane only occurred in CP 70-321. Stalk diameter and stalk weight were lower and stalk population was higher for plants derived from leaf roll callus compared to bud propagated cane. However, all yield components were similar for plants derived from apical meristem and bud propagation. Individual plant off-types were not observed in cane produced by either tissue culture method. In summary, variety and tissue culture method affected persistent, uniform variation in plant growth habit resulting from tissue culture that changed some yield components. However, apical meristem culture was suitable for production of seed-cane, as sugarcane derived by meristem culture of all three varieties did not differ significantly from the original germplasm for any measured trait.

## Genes Expressed During Regeneration in Tissue Culture

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Regeneration from tissue culture by way of somatic embryogencis is common in many varieties of sugarcane, but many economically important varieties of sugarcane are recalcitrant. Better understanding of the genetic control of embryogenesis could lead to the ability to transfer this trait to important varieties lacking it. This could assist in the rapid progation of these varieties and in the construction of beneficial transgenic varieties. We used differential display techniques to compare genes expressed in mRNA samples from non-cmbryogenic, proembryogenic, and embryogenic callus from variety CP 72-1210 and from non-embryogenic callus from the recalcitrant variety TCP 87-3388. Several novel sequences were identified. One codes for a hypothetical protein containing several phosphorylation sites. Another codes for a hypothetical protein with a glycosylation site and a camp controlled phosphorylation site. The

third codes for a hypothetical protein with a 37% homology to extension in canola. The last codes for a hypothetical protein that has a 93% homology to a putative glucose-6-phosphate/phosphate translocator in rice. Whether these sequences are unique to a specific tissue type is still under investigation.

## A Technique to Breed for Ratoon Stunting Disease in Sugarcane

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Ratoon stunting disease (RSD) caused by *Clavibacter xyli* subsp. xyli is one of the most important sugarcane (interspecific hybrids of *Saccharum* spp.) diseases in Florida. The objective of this study was to evaluate the effectiveness of stubble inoculation and determine if it could be used in a program to breed for RSD resistance. Field grown seedling sugarcane plants were inoculated at maturity by cutting with knives dipped in juice infected with ratoon stunting disease bacteria (RSD). The regrowth from these stools was sampled at the base of the mature stalks and RSD susceptibility was based on the number of colonized vascular bundles determined using the tissue blot immunoassay. After selection based on vegetative characteristics in Seedlings, the average RSD rating of 12 crosses with 658 selections was 1.52. When resampled as mature plants in Stage I, the average rating was 4.15. The plants were reinoculated and replanted into a Stage I sized plot. There were 67 clones selected for advancement to Stage II. They had an average RSD rating of 1.75. One major advantage of this system is that it requires no special planting in which to evaluate RSD resistance. The major disadvantage of this system from our standpoint in Florida is that it requires that seedling selection be done in the ratoon crop and that all clones in the breeding program would potentially be infected with RSD. In all probability very high yielding susceptible clones would be dropped with this selection scheme. Growers in Florida now manage RSD with a combination of genetic resistance and clean seed cane. Therefore, our industry is not willing to lose those potentially high yielding clones that are susceptible but could be profitable when grown without RSD.

## Progress in the Development of Transgenic Disease-Resistant Sugarcane

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Efforts are underway to develop sugarcane with transgenic resistance to the sugarcane yellow leaf luteovirus (SCYLV), leaf scald disease (LSD), and ratoon stunting disease (RSD). Genetic constructs containing the SCYLV coat protein in the sense (pFM395) and antisense (pFM396) orientations were obtain from T. E. Mirkov (Texas A&M, Weslaco). A genetic construct (pMBP39-22) containing a modified cecropin gene (MB39) was obtained from Lowell

Owens (USDA, Beltsville, MD). In vitro growth inhibition assays indicated that MB39 should be highly active against the RSD and LSD pathogens, *Clavibacter xyli* subsp. xyli and *Xanthomonas albilineans*, respectively. A number of other DNA constructs were made including those with the cecropin gene under control of the maize ubiquitan promoter (pZY-C), and the antisense SCYLV gene fused with the cecropin gene both under control of the ubiquitan promoter (pZY-CSA). Sugarcane callus cultures were co-bombarded with the individual constructs and another construct containing the NPT II gene as a selectable marker. Genetically transformed plants were regenerated from these materials and are being tested further.

## Potential Impact of DNA Marker Technology on Sugarcane Breeding

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At the turn of the new millennium, breeders have begun to realize how DNA marker technology may potentially impact traditional sugarcane breeding programs. Sugarcane is a tropical grass with both male and female organs within each tiny flower. Self-pollination may occur even after a male-sterility treatment such as the immersion of tassels in hot water or alcohol. The use of DNA marker technology may allow breeders to eliminate progeny from unwanted selfs early in the basic and commercial programs. At least five classes of DNA markers are available to use, each having its strong and weak points. These are restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), polymerase chain reaction (PCR), simple sequence repeat (SSR) or microsatellites, and amplified fragment length polymorphism (AFLP). Unlike the morphological traits, DNA fingerprints constructed with these classes of markers are quite reliable and not influenced by the environment. A few PCR (*Eri3*/*Eri4* and *GigI*/*PII*), RAPD (*OPA11-366*), and SSR (*SMC334BS*, *SMC336BS* and *MCSA068G08*) markers, that prove to be species-specific, have been developed to assist in the basic selection program at the Sugarcane Research Unit at Houma, Louisiana. Multi-disciplinary studies are underway to identify and clone RAPD or AFLP markers that are tightly linked to genes contributing to important agronomic traits. Multi-institutional collaborations are also being sought to construct microsatellite linkage maps from several genetic populations (F1, F2, BC1) of sugarcane.

## In Vivo Viability Assay of Sugarcane Pollen Stored at Ultra Low Temperature Following Preservation Treatments

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Storage of sugarcane pollen is desirable for enhancing germplasm because of the different flowering time. The viability of *Saccharum spontaneum* pollen can be significantly prolonged under low temperature after being properly air dried to reduce its moisture content.

The information on pollen viability of commercial cultivars (CP 70-1133, CP 98-1301, and CP 98-1654) were used to examine their viability after being stored at low temperature. Pollen samples were collected in the early morning after anthesis and divided into two sets: the first was dried in a cool dehumidified room for three hours and the second set was treated with cryoprotectants. Both sets of pollen were stored immediately at –80°C for 1 to 4 months. Cryoprotectants included 0.25 – 0.5 M solutions in various combinations of dimenthyl sulfoxide, glycerol, sorbitol, and sucrose. An in vivo assay was used to measure the pollen viability. Pollen was applied onto the tassels of green canes, CP 65-357 and Green German (*S.* officinarum), in the morning during the flowering season. Fuzz was harvested about 30 days after pollination for germination test. Seedlings were transplanted to field. Seedlings from crosses derived from stored *S.* officinarum pollen were classified based on the gross plant morphology at 4-month-old while seedlings derived from crosses with stored pollen of commercial cultivars were classified based on stalk colors. Stalk color was determined by one internode from each of 12-month-old seedlings that was cut and dipped vertically in 5% sulfurous acid solution for 3-4 days to eliminate chlorophyll pigment. Loss of pollen viability (%) due to preservation treatments was estimated by [1 – (seed set from stored pollen)/(seed set from fresh pollen)]100. Results showed that pollen of neither *S. spontaneum* nor commercial cultivars produced viable seedlings when they were stored at –80°C after being treated with cryoprotectants. After being exposed to air drying, pollen of both *S. spontaneum* and commercial cultivars produced viable seedlings ranging from poor to good seed set when the stored pollen was used to cross with CP 65-357 or Green German. Average losses of pollen viability were 50% (1997/98) and 88% (1999/00) for CP 98-1654. In addition to the use of the pollen storage for germplasm enhancement, this study suggests that stored pollen with genetic marker may be used to help identify hybrids for genetic and breeding investigations.