

AGRICULTURAL ABSTRACTS

Target Region Amplification Polymorphism (TRAP) markers for Sugarcane Genotyping

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The DNA-based molecular marker technologies are attracting considerable interest among plant breeders as a means of supplementing conventional breeding approaches. Current molecular marker technologies such as RFLP, RAPD, AFLP, gSSR, and SRAP are very efficient in generating a large number of fragments or markers, however, these markers are randomly distributed across the genome and trait association is often based on distant linkage. Molecular markers for crop improvement, ideally, should be based on functionally characterized candidate genes which would increase the probability of direct trait association. The large genome size and high ploidy level of sugarcane makes it a complex and recalcitrant crop to study and improve using genetics approaches. Therefore, sugarcane would benefit greatly from a candidate gene approach to molecular markers. Access to increasing numbers of EST sequences obtained from diverse cDNA libraries coupled with freely available bioinformatics tools now allows us to explore new opportunities in sugarcane molecular marker technology.

The TRAP is a PCR-based marker technique that uses EST sequence information to generate markers. A fixed primer of 18 nucleotides is designed from the targeted EST sequence and an arbitrary primer is designed with either an AT- or GC-rich motif to anneal with an intron or exon, respectively. We have genotyped nine sugarcane cultivars, previously characterized with the AFLP marker technique, using 12 TRAP markers. Polymorphism was revealed for all four fixed primers, derived from functionally characterized candidate genes (sucrose synthase, sucrose phosphate synthase, pyruvate orthophosphate dikinase and soluble acid invertase), in combination with three arbitrary primers. Comparisons will be presented between the two marker systems in their ability to predict genetic diversity relative to the coefficient of parentage. An additional set of genotypes belonging to the *Saccharum* complex is also being genotyped for genetic diversity analysis using the TRAP marker technique. Future studies will utilize the TRAP marker technique for genome mapping and trait association.

Abbreviations: RFLP, Restricted Fragment Length Polymorphism; RAPD, Random Amplified Polymorphic DNA; AFLP, Amplified Fragment Length Polymorphism; gSSR, genome-derived Simple Sequence Repeats; SRAP, Sequence Related Amplified Polymorphism; EST, Expressed Sequence Tag; PCR, Polymerase Chain Reaction

Isolation and Molecular Characterization of Flowering Related Genes in Sugarcane

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Cross hybridization remains the foremost means through which sugarcane breeders create genetic variation for selection. Unfortunately, sugarcane does not flower naturally in Louisiana and the breeding program has to rely on artificial photoperiod treatment to achieve flowering among the parents used for crossing. Flowering can be erratic even after artificial photoperiod treatment, thus, a better understanding of sugarcane's response to photoperiod treatment would be helpful in predicting the flowering characteristic (free-flowering vs flowering shy; early- vs late flowering) of parents and in synchronizing crosses. Molecular mechanisms involved in the photoperiod control of flowering are well understood in the long day (LD) model species *Arabidopsis thaliana*. A body of evidence explaining the photoperiod control of flowering is now accumulating for rice, a short day (SD) model species for grasses. Recent progress in genome analysis coupled with the advent of bioinformatics has paved the way for molecular research in a non-model, complex polyploid species such as sugarcane. Here, we have adopted a homology based approach to isolate and clone the Phytochrome B gene in sugarcane by using information from orthologous sequences from rice and other members of the grass family. The phytochrome B (Phy B) gene is among a class of photoreceptor genes (Phy A to E) which is known to induce early flowering in LD plants and delay flowering in SD plants. Our specific aim is to corroborate the hypothesis that mutations in the Phy B gene result in early flowering in SD plants. We have sequenced partial clones of the gene from sugarcane which show remarkable similarity (85 – 98%) with known Phy B gene sequences from rice and other grass species. Our research is in progress to clone and characterize the full length sequence of this (Phy B) and other flowering related genes from sugarcane genotypes differing in their response (early- vs late-flowering) to similar photoperiod treatments. Eventually, we hope to develop Sequence Tagged-site (STS) markers to categorize response to photoperiod treatment of parents in the crossing program.

Ratoon Stunt Effects on Yields of Sugarcane

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The effect of *Leifsonia xyli* subsp. *xyli*, (Lxx), the causal agent of ratoon stunt, on yield was evaluated in three yield trials using eight CP-cultivars by harvesting the plant and first-ratoon crops. Plots were established by cutting a single 2-m disease-free seedcane stalk in half at planting. In the first test, the treatments were healthy (non-inoculated), and Lxx inoculated, by dipping the freshly cut ends in a container of expressed juice obtained from Lxx infected stalks

of CP 53-1, a cultivar known to harbor high populations of the pathogen. In the second trial, stalks were cut from plots of the first test confirmed healthy and Lxx-infected by a tissue blot immunoassay. The Lxx infected stalks were also inoculated at planting. In the third trial, stalks were only cut from the healthy plots of the first trial and inoculated by cutting with knives dipped in juice expressed from infected CP 53-1. Yield parameters were determined by stalk counts per plot and the fresh weight and sucrose analysis of a 5-stalk sample of each plot. The disease state of each stalk sampled for sucrose analysis was determined by tissue blot immunoassay using a 10 to 15 cm section cut from the base of each stalk and analysis of data was conducted on plots of confirmed disease status. Combining the data from all cultivars, the yield parameters were reduced by 3.5 % to 15.8 % in Lxx infected plants compared to healthy plants. In individual cultivars, yield losses were not always statistically significant. In the first trial, disease-free plots in 4 of 8 cultivars in the plant and 6 of 8 cultivars in the first ratoon had higher yields of sugar per plot. In this trial, the inoculations were not fully successful and there were fewer Lxx infected plots of some cultivars. In the second trial, the yield of sugar per plot for disease-free plots was higher in all 8 cultivars in the plant and 7 of 8 cultivars in the first ratoon crop. In the third trial, the yield of sugar per plot in disease-free plots was higher for 6 of 8 cultivars in the plant and for all cultivars in the first ratoon. Based on the overall results, Lxx infection causes yield losses of most CP sugarcane cultivars.

Comparison of Yield Parameters and Disease Incidence of Traditional Seedcane Sources and Kleentek®, a Commercial Tissue-culture Based Seedcane

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A commercial seedcane, Kleentek®, is currently available for disease management in Florida. This program uses micropropagation of meristematic tissue for virus elimination and mass propagation of healthy seedstock. Trials were established to determine disease levels, spread rates, and estimate yield response from the use of this seedcane. Two adjacent field sections located at Okeelanta Corporation near South Bay, Florida were planted in replicated field trials on December 7, 1999. One section, designated as the fallow trial, had been in rice cultivation for the previous cycle. The other, designated as the successive trial, had been in continuous sugarcane production. Four cultivars were planted in each trial: CP89-2143, CP84-1198, CP80-1743, and either CP70-2086 (fallow trial) or CP80-1827 (successive trial) using three seed sources: (1) Kleentek®, (KT), (2) progeny of hot water treated seedcane (HT), and (3) field run seedcane without any recent history of treatment (FR). Plots were harvested by hand with disinfected knives between plots for plant and first stubble to minimize spread of *Leifsonia xyli* subsp. *xyli* (*Lxx*), causal agent of ratoon stunting disease. Yield parameters and the incidence of *Lxx* and sugarcane yellow leaf virus (SCYLV) were determined for the plant, first ratoon and second ratoon crops.

Pathogen incidence varied between the seedcane sources. In the FR, the initial incidence of *Lxx* in CP80-1743 was > 90 % in the plant crop of both trials and in CP80-1827, it was 65 % in the successive trial. Although *Lxx* was initially absent in KT and HT plots, by second ratoon some spread of *Lxx* occurred in most cultivars. A higher incidence of *Lxx* occurred in the successive trial by the second ratoon. However, excluding the FR plots, the highest incidence of *Lxx* was only 22.5 % in the second ratoon. The incidence of SCYLV was initially >95 % in the plant crop of HT and FR plots. KT plots initially tested free of SCLYV but the incidence gradually increased to 16.9 % and 28.8 % by second ratoon harvest in the fallow and successive trials, respectively. Spread of SCYLV into CP80-1743 appeared to be slower than in the other cultivars.

In both trials, among seedcane sources, there were significant differences for tons of cane and sugar per acre but not percent theoretical sugar. Overall, KT plots produced > 6 % more stalks than HT and FR plots. KT plots produced > 12% more tonnage and sugar than FR plots. The KT plots produced > 7% and 4 % more cane tonnage and sugar than HT plots in the fallow trial and successive trial, respectively. Yield in HT plots was significantly higher for numbers of stalks, cane tonnage and sugar than FR plots in the successive trial. Although the trends were similar for all varieties, there was significant cultivar by seedcane source interactions. CP89-2143 and CP80-1743 gave the most consistent yield responses to seedcane source. Overall results suggest that using Kleentek to manage diseases can increase yields by relatively equivalent amounts in successive and fallow planted fields.

Field Evaluation of Agronomic Characteristics and Disease Resistance in Transgenic Sugarcane Lines in Florida

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New breeding techniques involving genetic transformation hold promise for increasing sugarcane (interspecific hybrids of *Saccharum* spp.) yields and disease resistance. However, somaclonal variation may produce undesirable field characteristics in transgenic sugarcane not readily identifiable in the laboratory. The objective of this study was to thoroughly evaluate variability in agronomic characteristics and field disease resistance of sugarcane genetically transformed for resistance to SCMV strain E. A total of 100 sugarcane plants from progenitors 'CP84-1198' and 'CP80-1827', consisting of 4 VR lines (VR1, 4, 14 and 18) which had been biolistically transformed with *nptII* and the untranslatable form of SCMV strain E coat protein gene (*Ubi-eut*), were planted in Florida in 2001, and data gathered from plant cane through the second ratoon harvest (Experiment 1). In 2002, 30 plants from the VR18 line were selected for replanting with data collected through first ratoon harvest (Experiment 2).

Transgenic plants from 'CP 84-1198' tissue had significantly greater stalk number and stalk weight than those from 'CP 80-1827' in plant cane, first ratoon and second ratoon crops. This contributed to significantly greater TSH in plant cane (73% greater), first ratoon (27%) and second ratoon (22%) for 'CP 84-1198' transgenic plants. These accessions also had lower incidence of SCMV disease in all 3 crops. Plants from the VR18 line ('CP 84-1198' parentage) had significantly greater economic indices than all other lines in plant cane, and lines VR 14 and 18 had significantly greater stalk number, stalk weight, TCH and TSH than other VR lines in all 3 crops. Accessions from the VR 18 line had lower SCMV disease incidence in plant cane, first ratoon and second ratoon. Somaclonal variation was very high in Experiment 1, with TCH ranging from 26 - 211 tons ha⁻¹ and TSH from 3.2 – 28.9 tons sucrose ha⁻¹ in the plant cane crop. Experiment 2 on the VR18 line identified 14 transgenic accessions with superior yield to the 'CP 84-1198' non-transgenic parent, combined with SCMV disease resistance in the field. Somaclonal variation decreased with increased selection pressure in experiment 2, with TCH ranging from 70 – 149 tons ha⁻¹ and TSH from 8.5 – 19.0 tons sucrose ha⁻¹ in plant cane. Based on a combined 5 crop-years of data, 2 VR18 accessions have been planted in 0.25 ha multiplication plots for potential release. The large variability in yield characteristics and disease resistance encountered in this study demonstrates the necessity of thorough field evaluation of transgenic sugarcane.

Water Table and Sugarcane: A Review of Recent Research

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Sugarcane (*Saccharum* Spp.) in the Everglades Agricultural Area (EAA) of Florida is intermittently exposed to high water tables and floods. This presentation reviews recent investigations of sugarcane genotypic, morphological, physiological, pathological, and agronomic responses to water table and flood. In field tests, CP 72-2086 and CP 82-1172 had no yield losses, whereas CP 80-1743 had sugar losses of 25% due to high water table. In a second field experiment, CP 72-2086 formed significantly more constitutive aerenchyma than CP 80-1743. In lysimeters, photosynthesis was not affected by 1-week floods and yields of one genotype (CP 95-1429), with constitutive aerenchyma, were not affected by water-table depth or floods. Yields of a genotype (CP 95-1376) without constitutive aerenchyma declined linearly with water-table depth, and by 0.6% per day of flood. In a summer experiment conducted in containers with young plants, stalk and root weights were reduced by 3.2 and 3.6%, respectively, per day of flood; in an autumn experiment, they were reduced by 1.0 and 1.1%, respectively, per day of flood. In pots, sugar weight was decreased by 7.2% by a 21-day flood initiated 42 days before harvest. In lysimeters, sugar weight increased by 25.6% with a 20-day flood initiated 41 days prior to harvest. Four pot studies identified no consistent relationship between flood-drain regimes and effect of ratoon stunting disease on sugarcane yields. Maximum sugarcane

emergence occurred at a water-table depth of 25 cm in a pot study. Areas of future work include attempts to use: (1) photosynthesis measurements to identify water-table effects, (2) molecular markers for genotype emergence and aerenchyma formation, and (3) economic and agronomic studies to quantify costs/benefits of management options that include compensation for ecological benefits.

Evaluation of Ho 95-988, HoCP 96-540, and L 97-128 for Susceptibility to Ratoon Stunting Disease

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The susceptibility of recently released sugarcane cultivars for Louisiana, HoCP 96-540 released in 2003 and Ho 95-988 and L 97-128 released in 2004, to ratoon stunting disease (RSD) was evaluated between 1999 and 2003. Single-bud cuttings were collected from each cultivar in the fall, dip inoculated in juice from stalks infected with *Leifsonia xyli* subsp. *xyli*, the bacterium that causes RSD, germinated in the greenhouse, then transplanted in the spring to a field nursery at the USDA, ARS research farm. Four mature stalks were collected from each cultivar in the plant-cane, first-ratoon, and second-ratoon crops of at least two complete crop cycles and susceptibility to infection was evaluated using tissue-blot immunoassay (TBIA). The average percent colonized vascular bundles (CVB) as determined by TBIA for Ho 95-988, HoCP 96-540, and L 97-128 was compared to the percent CVB of other cultivars currently or recently recommended for planting in Louisiana. The lowest percent CVB (2%) was found among samples of Ho 95-988. No infection was recorded for many of the stalk samples. Relatively low percent CVB was also found for L 97-128 (14%) and HoCP 96-540 (9%). The highest percent CVB was observed in Ho CP 91-555 (49%). Other cultivars with high percent CVB readings were CP 70-321 (41%), CP 72-370 (27%), and LCP 82-89 (48%), and those with intermediate levels were HoCP 85-845 (24%) and LCP 85-384 (23%). Because of the lower level of infection among the three most recently released cultivars, control of RSD among these cultivars should be more effective.

L 97-128 – A New Sugarcane Variety for Louisiana

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The Louisiana Agricultural Experiment Station of the LSU Agricultural Center, The Agricultural Research Service of the United States Department of Agriculture, and The American Sugar Cane League of the U.S.A., Inc., working cooperatively to develop improved sugarcane varieties, have jointly developed and hereby announce the release of a new variety, L 97-128, for commercial planting in the summer of 2004.

L 97-128 was derived from a cross (XL92-42) made in 1992 between LCP 81-10 as the female parent and LCP 85-384 as the male parent. Single stool seedling selection was done at the St. Gabriel Research Station located at St. Gabriel, Louisiana in 1994. The stalks of L 97-128 are greenish-brown (green predominates) and are covered with a heavy wax layer. In the sunlight the stalks have a more purplish hue. The new variety has an average population of large diameter stalks. Its stalk population is 86% and stalk weight is 124% of Louisiana's leading variety, LCP 85-384, averaged over plant-cane, first-stubble, second-stubble, and third-stubble crops. L 97-128 is a good stubbling variety.

Yield data from 51 mechanically harvested outfield tests that are replicated on both light and heavy textured soils indicate that L 97-128 produces approximately 9% greater recoverable sugar per acre than LCP 85-384 averaged across plant-cane, first-stubble, and second-stubble crops. L 97-128 is very early maturing and has produced 4% greater recoverable sugar per ton of cane than LCP 85-384 when averaged across all tests. Based on 20 tests, the fiber content of the new variety is 12.2%, which is only slightly higher than LCP 85-384. Field observations indicate that L 97-128 is an erect variety and well suited to both whole stalk and combine harvesting systems. The leaf sheaths of L 97-128 are less tightly held than LCP 85-384, which should aid in trash extraction during combine harvesting of green (unburned) cane.

L 97-128 is resistant to sugarcane mosaic virus and sorghum mosaic virus. The new variety is moderately susceptible to smut (*Ustilago scitaminea* Sydow), moderately resistant to rust (*Puccinia melanocephala* H. And P. Syd.) and moderately resistant to leaf scald [*Xanthomonas albilineans* (Ashby) Dowson] under natural field infection. The effect of yellow leaf syndrome on the yield of L 97-128 is unknown. Similar to all other varieties grown in Louisiana, L 97-128 may sustain significant yield loss in stubble crops from ratoon stunting disease (*Clavibacter xyli* subsp. *Xyli*). To realize the maximum yield potential of this variety, healthy seed cane free of this disease must be planted. L 97-128 is susceptible to the sugarcane borer [*Diatraea saccharalis* (Fabricius)] and should be scouted to ensure timely insecticide applications and should not be planted where insecticides cannot be applied. Field observations indicated that L 97-128 is not any more susceptible to herbicides commonly used for weed control than LCP 85-384.

Based on two years of maturity data obtained from the USDA-ARS Sugarcane Research Unit, L 97-128 is very early maturing and continues to accumulate sucrose throughout the harvest. The new variety had 14% higher recoverable sugar per ton of cane when harvested in

mid-September than LCP 85-384. When harvested in mid-December, L 97-128 had 4.2% higher recoverable sugar per ton of cane than LCP 85-384.

Seed cane of L 97-128 will be distributed by the American Sugar Cane League of the U.S.A., Inc.

Monitoring Adult Fall Armyworm (Lepidoptera: Noctuidae) Populations in Florida Sugarcane Using Pheromone Traps

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The fall armyworm (FAW), *Spodoptera frugiperda* J. E. Smith, is a pest of occasional importance in Florida sugarcane. Sporadic outbreaks of FAW often occur rapidly in fields of young sugarcane during the spring or fall and can result in severe defoliation. Two genetic FAW strains are known to occur in Florida, the 'corn' and 'rice' strains, but whether both of these strains occur in sugarcane was not known. This study was conducted to obtain preliminary information on monitoring FAW in sugarcane using traps baited with pheromone lures, and to investigate the genetic strain(s) of FAW associated with cane. Five different synthetic FAW pheromones available for purchase (Hercon, Trece, Scentry 2-component, Scentry 4-component, and Scenturion) were studied using universal moth traps at four different locations within the sugarcane-growing region during April – October 2003. The Scenturion lure attracted significantly greater numbers of FAW than the other lures. The largest numbers of moths were collected during late April and early May, peaking at an average of 23 moths per trap per night over all lures and locations with a maximum of 125 per trap per night at one location in a trap baited with the Scenturion lure. Large outbreaks of FAW larvae were discovered across approximately 113 hectares of young ratoon cane around 10 to 15 days after this peak of FAW activity, the closest infestation to any trap being 15 km away. Whether the peak period of activity of FAW at our traps was indicative of an area-wide peak remained unknown. No outbreaks of FAW larvae were reported in closer proximity to any trap, but some fields of young cane in the vicinity of traps may have had low levels of FAW larvae. The study indicated that traps baited with pheromone might hold potential as a method of identifying when and where infestations of FAW may develop in cane. PCR analyses indicated 99% of the moths collected during the study were 'rice strain' individuals. A small number of larvae from an infested field of young cane during the fall were identified as 'rice' strain individuals. These preliminary data indicated that FAW infestations in sugarcane might be comprised predominantly by 'rice' strain individuals.

Mention of trade names or commercial products in this abstract is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

A Survey of Temporal and Spatial Variability in Louisiana Sugarcane Production Systems

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This study reports results from our evaluations of the spatial variability in sugarcane yield and quality in relation to variation in soil chemical properties. Sugarcane cv 'LCP 85-384' was harvested in two untreated producers' fields for three consecutive years in Schriever and Patoutville, LA. In Schriever a plant-cane, first-, and second-stubble crop was harvested and in Patoutville a fourth-, fifth-, and sixth-stubble crop was harvested, in 2001, 2002 and 2003, respectively. Each field was harvested in a grid-cell pattern with cell dimensions of 10.6 x 15.2-m with a single-row, chopper harvester, with weights determined using a weigh wagon equipped with a billet sampler. Soil samples (0-15 cm) were also collected after harvest from each grid cell. Yield, quality, and soils data were analyzed by both univariate statistics and geostatistical techniques. The majority of properties describing sugar yield and quality at both locations were found to possess non-normal distributions in 2001. This number decreased in 2002 and 2003. The coefficients of variation were relatively stable between location and year varying from 5 to 20%. In Schriever in 2001, 2002, and 2003, all sugar yield and quality parameters were spatially correlated with the exception of TRS and Fiber in 2003. The ranges of spatial correlation varied from 26 to 187 m. In Patoutville in 2001, 2002, and 2003, all sugar properties were spatially correlated with ranges of spatial correlation varying from 27 to 133 m. Correlation analysis was performed to study the relation between soil properties and sugar parameters. In Schriever, the soil Ca:Mg ratio and soil S were correlated to all sugar parameters and in Patoutville, soil OM, and soil pH were correlated to all sugar parameters. Finally, kriged maps of sugarcane yield, quality, and soil properties illustrated the relations between soil variability and sugar yield and quality. These maps have also been utilized in our variable rate lime studies.

Effect of Zinc Fertilization on Sugar Cane (LCP 85-384) Yields

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Two field experiments were conducted to test the effects of zinc (Zn) fertilizer application on sugarcane yield. One acid and one calcareous soil each testing low in available Zn by DTPA test

method were chosen for the study. The fertilization consisted of 5 rates (0, 4, 8, 16, and 32 lb Zn /A) of soil-applied solid zinc sulfate ($ZnSO_4$), and one rate of foliar spray application (1.2 lb Zn/A using 0.5% liquid $ZnSO_4$). One sulfur rate (18 lb/A) as gypsum was applied to check the sulfur effect caused by $ZnSO_4$ application. Soil-applied Zn at 4-8 lb/A significantly ($P<0.05$) increased cane and sugar yields of LCP 85-384 by 27-32% in acid Dundee soil and by 23-26% in calcareous Jeanerette soil. The foliar zinc treatment increased yields at both sites but only statistically significant at acid soil site (by 23 and 29% for cane and sugar, respectively). Sulfur application also significantly ($P<0.05$) increased both cane and sugar yields by 27-31% in the acid soil and 19-21% in the alkaline soil. Overall the results suggest that zinc application as $ZnSO_4$ in Louisiana soils low in DTPA test-Zn benefit sugarcane production significantly. DTPA test, a common test used for alkaline soils, worked also for predicting Zn deficiency in acid soils for sugarcane.

Effect of Nitrogen on Sugarcane Plant Growth and Flowering Under an Artificial Photoperiod Treatment

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Sugarcane does not flower naturally in Louisiana because of cool fall temperatures. Therefore, the LSU AgCenter Sugarcane Breeding Program relies completely on a controlled photoperiod facility to induce flowering. Several factors including genotype, plant age, photoperiod treatment, temperature, moisture, and nutrient status affect flowering in sugarcane. Little information is available regarding the effect of plant nutrient management on flowering under controlled photoperiod treatment. This research was initiated to study the effect of nitrogen on sugarcane plant growth and flowering under an artificial photoperiod treatment.

The treatments consisted of three sugarcane varieties, two pot sizes, and two levels of nitrogen applied before the artificial photoperiod treatment followed by six levels of nitrogen applied during the artificial photoperiod treatment. The data collected included stalk height, stalk diameter, days to flowering, days to flag leaf formation, duration of flag leaf, percent flower, inflorescence length, inflorescence weight, and percent viable seed. This paper discusses the results of this study.

Root Characteristics and Dry Yields of Sugarcane Due to Water Table Depth

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Microbial decomposition of organic soils in the Everglades Agricultural Area (EAA) is resulting in a decrease in soil depth. Raising water tables reduces decomposition rates, but often reduces sugarcane (interspecific hybrids of *Saccharum* spp.) yields. An experiment was conducted to determine the interrelationships between shoot yield and root characteristics due to water-table depths. Twelve sugarcane genotypes were grown outside in 38-L plastic pots with water-table depths of 0 (saturated), 15, and 30 (drained) cm. At 10 months, sugarcane shoots and roots in the upper (0-15 cm) and lower (15-30 cm) layers were harvested. Shoot dry matter was reduced with high water table, but root dry matter within each soil layer was not affected by water-table depth. About 74% of the total root dry weight and length were confined to the upper soil layer regardless of water table. More than 50% of the total root length was less than 0.5-cm diameter in both soil layers. A greater percentage of root lengths in the 2.5- to 4.5-mm root diameter class in the upper soil layer were related to shoot yields. In the lower soil layer, root lengths in the 0- to 1.0-mm root diameter class were most related to shoot yield. Our data show that sugarcane tolerance of water tables <30 cm include increased root mass and length and reduced root diameter near the soil surface. Percentage root length in various root diameter classes may also serve as indicators for increased sugarcane dry yields.

Midrib Effect on Sugarcane Leaf Tissue Nutrient Concentrations

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Sugarcane (interspecific hybrids of *Saccharum* spp.) leaf tissue samples collected for nutrient status determination may or may not contain the midrib. Removal of the sugarcane midrib tissue adds an extra step to the sampling process and is especially time-consuming when plants are young since leaf surface area is small at that stage. Knowledge of nutrient concentration differences between leaf samples with (W) and without (WO) the midrib is limited. If growers and consultants have a better understanding of the impact the midrib has on nutrient values obtained, better use of published research may be achieved. The effects of sampling time (May and October) and method (W and WO midrib) on leaf-blade nutrient concentration of cultivar CP 78-1628 were investigated on Margate sand (siliceous, hyperthermic Mollic Psammaquents) in a split plot randomized complete block design. Nutrient concentrations were higher in the May samples than in those collected in October. The presence of midrib resulted in decreased concentrations of N, P, Ca, Mg, Zn, Mn, and Fe in the leaf sample to varying degrees, but increased concentration of K. Simple correlation coefficients between samples analyzed W and WO the midrib were positive and significant for P, Ca, Mg, and Mn in May. Since nutrient concentration differences between W and WO midrib samples have been documented, stating the sampling method with regard to presence or absence of the midrib must be included in technical and scientific reports. Comparing leaf nutrient data to critical values must take into account the sampling method used because critical values W the midrib are different from WO midrib, for most nutrients.

Nitrogen Fertilization of Sugarcane on a Sandy Soil: I. Yield and Leaf Tissue Nutrient Composition

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The determination of optimum N fertilization rates for sugarcane (interspecific hybrids of *Saccharum* spp.) on Florida sandy soils has been limited to a few studies. The objective of this study, conducted during 1999-2003 at the Southwest Florida Research and Education Center, UF-IFAS, at Immokalee, FL (27°25' N, 81°25' W), was to determine the response of CP 78-1628 grown on a sandy soil to varying N-rates. Twelve plots (13.6-m x 24.4-m; 1.5-m row spacing) were arranged in a randomized complete block design with three rates of N fertilizer (170, 280, and 390 kg N ha⁻¹ yr⁻¹) and four replications. The N fertilizer rates were provided by four split applications. The soil at the study site was identified as a Malabar fine sand (Grossarenic Ochraqualf), 98% sand (< 2% organic matter) with an argillic horizon at 1-m depth. Sugarcane leaf samples (10 top-visible dewlap laminae with midrib) were collected for nutrient analysis at 0900-1000 h from 10 mature stalks selected at random from each plot, between May and October. Samples were taken at 4.5 and 4.0 wk following the latter three-split applications of fertilizer in the first-ratoon and second-ratoon crops, respectively. Nitrogen concentration was obtained in Kjeldahl digests. Phosphorus, K, Ca, Mg, Zn, Mn, Cu, Fe, B, and Na were determined by ashing ground samples, dissolving ashes in HCl and filtering. Concentrations of elements in the filtrate were determined at the ARL by inductively coupled plasma (ICP) or atomic absorption spectrophotometry (AAS) in combination with colorimetric analysis for P. The data were treated as a repeated-measures experiment with the three N-fertilizer rates as the main plots and the sample date (sub plot) and crop (sub-sub plot) as the repeated-measure factors. Contrast analyses between N-fertilizer rates were tested. Leaf tissue macro and micronutrients were not affected by the N rates. Sugar yields tended to increase with increasing N rates but were not significantly different in either the plant or the ratoon crops. Only stalk weight in the second-ratoon crop approached a significant slope for N rate ($\text{g stalk}^{-1} = 0.51 \text{ kg N ha}^{-1} \text{ yr}^{-1} + 515$; $R^2 = 0.25$, $P=0.0962$). Lowering the quantity of N ha⁻¹ in each split and increasing the frequency of the splits may improve the efficiency of N utilization by sugarcane. This study suggests that the time interval between split applications from June to September should be between 2.5 and 6.5 wk.

Nitrogen Fertilization of Sugarcane on a Sandy Soil: II. Soil and Groundwater Analyses

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There is limited information on the impact of N fertilizer applied to sugarcane on sandy soils regarding soil and groundwater analyses. This study determined the response of soil and groundwater to varying N rates on a sandy soil planted to sugarcane cultivar CP 78-1628. Twelve plots (13.6-m x 24.4-m; 1.5-m row spacing) were arranged in a randomized complete block design with three rates of N fertilizer (170, 280, and 390 kg N ha⁻¹ yr⁻¹) and four replications. The N rates were divided into four split applications. After planting, piezometers were installed in the center of each plot to a soil depth of 1.3 m. Rainfall and temperature data were recorded by a weather station located <1 km from the study site. The data were treated as a repeated-measures experiment with the three N fertilizer rates as the main plots and the sample date (sub plot), post-split application date (sub-sub plot), and crop (sub-sub-sub plot) as the repeated-measure factors. Soil macro and micronutrients plus Al, Na, Cl⁻, pH, buffer pH, OM, and electrical conductivity were not affected by the N rates when sampled between the plant, first and second ratoon crops. Soil and groundwater N concentrations indicated a rapid loss of the applied N due to leaching after the split application. Lowering the quantity of N ha⁻¹ with each split application and increasing the frequency of the splits may increase the N utilization efficiency of sugarcane. The results also suggest that a time interval of 2.5 to 6.5 wk between split applications from June to September may reduce N movement to groundwater.

Application of high-throughput DNA marker technology in sugarcane breeding: Phase I – nucleic acids extraction

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Nucleic acid extraction is the primary limiting step in high-throughput (HT) application of the DNA marker technology in modern sugarcane breeding. A common method of extraction is to homogenize the sugarcane leaf tissue in a CTAB buffer with a Mini-Beadbeater followed by organic solvent fractionation and alcohol precipitation. Using this method, a technician can process approximately 60 DNA samples per day. Although the quantity and quality of the nucleic acids are sufficient for most PCR and RT-PCR applications, the process is too slow for high-throughput applications.

A high-throughput DNA extraction method developed by Xin et al. (BioTechniques 34:820-826) for the analysis of cotton, sorghum, and other plants was evaluated as an alternative DNA extraction method for possible use in marker-assisted sugarcane breeding program. In this procedure, a small piece (about 30 mm²) of tissue from the youngest fully expanded leaf is excised and placed into a well of a 96-well plate containing a denaturing buffer (100 mM NaOH/2% Tween-20). The plate is sealed and placed on a PCR thermal cycler. After incubation at 95°C for 10 minutes, the plate is placed on ice for three minutes and spun at 3000 rpm for 1

minute. Fifty microliters of a neutralization buffer (100 mM Tris-HCl/2 mM EDTA) are then added to each well and the plate re-sealed with aluminum sealing tape.

PCR amplifications of the HT products were comparable to those of the CTAB-Beadbeater method. Storage evaluations from the two methods of isolation also suggest that the HT-extracted nucleic acids can be stored in a refrigerator or freezer for later use with results from the stored material remaining consistent over time. With the HT method, a technician can process approximately 600 DNA samples per day, a ten-fold increase over the CTAB Beadbeater method of isolation. In addition the HT method uses neither organic solvent nor toxic compound.

An Analysis of Barn Owl Prey Diversity in the Everglades Agricultural Area of South Florida

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The common barn owl, *Tyto alba*, has recently been promoted as a form of biological control for rodent pests of sugarcane, rice and vegetable crops grown in the Everglades Agricultural Area of south Florida. In an effort to augment endemic populations of this predatory raptor, several hundred nesting boxes have been placed along fields and ditch banks. The vast majority of these boxes have been colonized, facilitating the successful rearing of two broods per year. Since barn owls regurgitate the undigested remains of their prey in the form of a pellet, the identification and analysis of these remains provide insight with regard to their prey preferences. During the 2003/2004 sugarcane harvest season, over 100 barn owl pellets were collected from each of four separate geographic locations within the EAA. The pellets were then dissected for prey analysis. Three pellet collection sites were located on Histosols, and one was located on sandy soils. Results reveal a wide range of small mammalian prey. Prey species included cotton rats (*Sigmodon hispidis*), rice rats (*Oryzomys palustris*), roof rats (*Rattus rattus*), Norway rats (*Rattus norvegicus*), round-tailed muskrats (*Neofiber alleni*), marsh rabbits (*Sylvilagus palustris*), house mice (*Mus musculus*), and southern short-tailed shrews (*Blarina carolinensis*). Frogs, lizards, small birds, and large insects made up the balance of identifiable prey, but consisted of less than 1 percent of identifiable prey. Southern short-tailed shrew remains were prevalent in pellets collected from the sand-land site, but were virtually absent from pellets collected from muck-land sites, showing the influence of local ecology on prey diversity.

Effects of Drought Stress and Sugarcane Cultivar on Mexican Rice Borer (Lepidoptera: Crambidae) Oviposition

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Greenhouse experiments at Weslaco, TX, involved Mexican Rice Borer, *Eoreuma loftini* (Dyar) (Lepidoptera: Crambidae), oviposition tests on Louisiana sugarcane cultivars (LCP 85-384 and HoCP 85-845) under drought and non-drought stressed conditions. Data included the total number of egg masses, eggs laid and number of dry leaves on each plant for each treatment. Differences were not detected among cultivars for number of egg masses and eggs laid. However, more dry leaves were found on LCP 85-384 than on HoCP 85-845 ($P < 0.05$). Drought stress significantly affected the amount of dry leaves, number of egg masses and eggs laid ($P < 0.05$). Oviposition occurred exclusively on dry leaves. The increased oviposition on both the resistant (HoCP 85-845) and the susceptible (LCP 85-384) cultivars under stress conditions could be explained substantially by the increase in the number of dry leaves under stress. A replicated field study of these same cultivars at Ganado, TX, showed that irrigation could reduce the degree of *E. loftini* infestation and damage on both resistant and susceptible cultivars.

Current Status of Tebufenozide Resistance in the Sugarcane Borer in Louisiana

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Insecticide resistance monitoring has been initiated due to the increasing use of the insect growth regulator tebufenozide (Confirm®) in the Louisiana sugarcane industry. During 2002 and 2003, sugarcane borer, *Diatraea saccharalis* (F.) population samples were collected in Iberville, Calcasieu/Jefferson Davis, Iberia/St. Mary, and Rapides/Avoyelles parish areas. Using previously established laboratory procedures, susceptibility baselines were compared among sample locations and to previously identified data collected in 1995 assessing the initial susceptibility of a culture collected throughout the industry. Statistically significant increases in resistance ratios (as compared to Louisiana Mixed) at the LC_{50} levels ranged from 1.77 (Iberia/St. Mary 2003) to 2.70 (Iberville 2002). Comparing the reduced sensitivity at the 90% mortality level appropriate to the normally expected control, LC_{90} resistance ratios ranged from 1.94 (Rapides/Avoyelles 2003) to 3.91 (Calcasieu/Jefferson Davis 2003). Even though major control failures have not been observed in the field, alternating insecticide chemistries is an essential component of sugarcane borer pest management in order to preserve the availability of the insecticide for as long as possible. Only with a carefully orchestrated balance of control tactics to minimize pest selection pressure, can we expect to have any permanency in our pest management program.

Johnsongrass Effects on Sugarcane Growth and Chopper Harvester Efficiency

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The species of weed and the duration of its interference can have a dramatic impact on sugarcane stalk size, number, and maturity, and ultimately cane and sugar yields. Very little is known about the compounding effects harvested-weed residues may have on theoretically recoverable sugar (TRS) levels and sugar yields. The impact of light (1 plant/9 row feet (rf)), medium (1 plant/6 rf), and heavy (1 plant/3 rf) rhizome johnsongrass infestations at the start of the crop year on TRS levels and cane and sugar yields was determined in a plant-cane (2002) and subsequent first-ratoon (2003) crop of 'LCP 85-384' sugarcane chopper-harvested green or burnt.

In the plant-cane crop, johnsongrass reduced stalk counts by 9% (light infestation), 11% (medium infestation), and 14% (heavy infestation) when compared to a weed-free check. Stalk height was not affected by the various johnsongrass infestation levels. Reductions in stalk counts associated with the various levels of johnsongrass interference in the plant-cane crop were not reflected as differences in cane yields at the $P \leq 0.05$ level, but cane yields were higher (20%) where the cane was burned prior to harvest. Fiber levels detected in the harvested plant-cane crop were not influenced by johnsongrass infestation levels regardless of whether the cane was harvested green or burnt, but fiber levels in the burnt cane were higher than in the green cane. Differences in fiber levels were not reflected as differences in TRS levels. As expected, sugar yields mirrored cane yields with sugar yields not being influenced by johnsongrass infestation level, but being higher where cane was burnt.

In the first-ratoon crop, late season panicle counts of 32 400, 41 800, and 55 400 per acre were obtained for the light, medium, and heavy johnsongrass infestation levels, respectively. Stalk counts decreased by approximately 5% at each increasing johnsongrass level while stalk heights were essentially equivalent at all johnsongrass levels and 4% lower than the weed-free check. In 2003, cane yields were 6% higher when the cane was harvested burnt. In contrast to the plant-cane crop, cane yields reflected johnsongrass infestation level with yields decreasing 8 to 20% as the infestation level increased. Fiber levels in the harvested billets were similar regardless of johnsongrass infestation and whether cane was harvested green or burnt, but fiber levels were actually lowest at the highest johnsongrass infestation level. This did not translate into higher TRS levels when compared to the weed-free check. Sugar yields were also affected independently by johnsongrass infestation levels and burning. Burnt cane yielded more cane and sugar while sugar yield decreased from 8 to 26% as the johnsongrass infestation increased.

Improved harvester/operator efficiency contributed to the higher yields in the burnt cane. In addition, higher cane yields and fiber levels in the burnt cane may be partially attributable to the presence of immature cane tops in the burnt cane. These tops were easily blown away under a green-cane harvesting scenario. Finally, the chopper harvester appears to be relatively effective in removing johnsongrass residues present at harvest in the standing cane even when the crop is harvested green.

Sugarcane Mulch Residue: Effect on Herbicide Retention and Runoff Losses

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A literature search revealed that there is no published research that correlates the effectiveness of mulch residue remaining on the soil surface, following sugarcane harvest, with sugar yield, retention of applied herbicides, leaching losses in the runoff, and their downward movement in the soil profile. Such information is a prerequisite in quantifying the role of mulch residue on sugarcane yield and in minimizing leaching losses of applied agricultural chemicals. Over the last five years, we quantified the effect of sugarcane residue (mulch cover) from combine harvesting on sugarcane yield and retention and runoff of applied herbicides. Two main treatments were investigated: a no-till treatment where the mulch was not removed and a no-mulch treatment where the mulch was raked off the plots. The amounts of extractable atrazine, metribuzin, and pendimethalin from the mulch residue and the surface soil layer were quantified during the 1999 and 2000 growing seasons. The presence of mulch residue did not adversely affect the sugarcane yield (tons/acre and lbs sucrose/acre). Significant amounts of applied herbicides were intercepted by the mulch residue, which were at least one order of magnitude higher than that retained by the surface soil layer. When the residue was not removed, a reduction in runoff-effluent concentration, as much as 50% for atrazine and pendimethalin, was realized. Ongoing research focuses on the effect of burning the residue versus mechanical removal of the residue with a three-row sweeper. Results from the 2003 plant cane crop indicated that the highest yield was for the burn treatment where 5 and 9% reductions in yield were obtained for the sweep and no-till treatments, respectively.

Breeding Sugarcane for Mexican Rice Borer Resistance

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We assessed the reaction to the Mexican rice borer (MRB) on a core population of 24 sugarcane genotypes, some with known resistance to sugarcane borer (SCB), that were planted in a field trial in an area with traditionally high populations of the pest. MRB is currently the most serious pest of sugarcane in Texas and a potentially serious threat to sugarcane and rice in both Louisiana and Florida. Damage of MRB was also assessed from the same varieties in the greenhouse with artificial inoculation. Additionally, lyophilized leaves of each variety were incorporated in a meridic diet to evaluate its effect on larvae development. Initial evaluations based on deadhearts, indicate L97-128 as the most susceptible variety. The occurrence of MRB on some elite genotypes, known to be highly resistant to SCB, suggests the presence of different mechanisms controlling resistance to the two pests. With the goal of studying the genetics of resistance we applied the molecular marker technique to search for major genes involved. A Quantitative trait allele (QTA) search was performed using association studies within the

population for the marker discovery, with one locus contributing to MRB reaction being identified. Future perspectives include 1) a more extensive marker discovery by applying Amplified Fragment Length Polymorphism (AFLP) on the same genotypes; 2) extend the core population by including additional Texas genotypes; and 3) identify differentially expressed genes under MRB attack, by applying the cDNA-AFLP technique. The long-term objective of our research is to develop a genetic breeding methodology for MRB resistance, involving research with gene expression.

Ho 95-988, a Product of the USDA-ARS Basic Breeding Effort in Louisiana

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The Agricultural Research Service (ARS) of the United States Department of Agriculture, the Louisiana Agricultural Experiment Station of the Louisiana State University AgCenter, and the American Sugarcane League of the USA, Inc., working cooperatively, have jointly evaluated and released the new cultivar, Ho 95-988, for commercial planting in 2004. Ho 95-988 is a progeny of a cross between CP 86-941 X US 89-12 that was made by ARS' Sugarcane Research Unit (SRU) at Houma (Ho), Louisiana in 1990. The maternal parent, CP 86-941, is a progeny of CP 76-356 (a BC₃ derivative of Hawaiian cultivar H 49-3646) X CP 78-304 (a BC₃ derivative of *S. spontaneum* clone US 56-15-8). The paternal parent US 89-12 is a progeny of CP 79-348 (a BC₃ derivative of *S. robustum* 28 NG 251) X US 80-24 (a BC₂ derivative of *S. spontaneum* clone US 56-15-8). Thus, all four grandparents of Ho 95-988 are products of the basic breeding effort that was initiated at the SRU some 40 years ago. Ho 95-988 has been genotyped with four microsatellite markers.

In yield trials, Ho 95-988 has produced about 7% greater total recoverable cane and sugar per hectare than LCP 85-384 in plant-cane, first-ratoon and second-ratoon crops. Ho 95-988 is considered to be an excellent ratooning cultivar. It has a high population of medium-sized stalks that turn purple when exposed to sunlight. Ho 95-988 is resistant to mosaic, rust, and leaf scald, and above average resistance to ratoon stunting disease. It has an intermediate reaction to smut, but is considered to be adequately resistant to be grown on large scale, without expectation of significant yield loss. Ho 95-988 is susceptible to the sugarcane borer and should not be grown in areas where insecticides cannot be applied. Overall, this cultivar will offer Louisiana producers some genetic diversification and potentially higher yields than LCP 85-384.

Ratooning Ability and Stalk Tolerance to Freezing Temperatures Among F₁ Hybrids Involving *Saccharum spontaneum* and Commercial-type Sugarcane

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Post-freeze ratooning ability and stalk tolerance were examined in clones of *Saccharum officinarum*, *S. spontaneum*, interspecific F₁ hybrids and commercial-type sugarcane. Ratooning ability of wild and commercial-type clones was evaluated by comparing stubble in 15 cm pots either exposed to -5°C for 22 days or not. Stubble clones not exposed to freezing temperatures survived and tillered based on shoot counts 30 days after exposure, but only F₁ hybrids from clone SES 234 (*S. spontaneum*) X LCP85-384 and TUCP77-42 tillered after exposure to the freeze treatment. The interspecific progeny of LA Stripe (*S. officinarum*) X SES147B (*S. spontaneum*), Badila (*S. officinarum*), and commercial clones, LCP85-384 and HoCP91-555, did not survive the freeze treatment. To determine stalk tolerance to freezing temperatures, we harvested stalks of LCP85-384, HoCP96-540, TUCP77-42 and nine F₁ hybrids of SES 234 X LCP85-384 from a field location that survived the mild winter of 2003-2004. Fifteen stalks were exposed to ~5°C for either none, one, four or eight days. Seventy-two eye pieces per clone were planted, and germination rates were determined after 14 days. After the one-day exposure, three hybrids of SES 234 X LCP85-384 had germination rates of 83 to 97% compared to 55% and 18% for LCP85-384 and TUCP77-42, respectively. After the four-day exposure, two of these same clones had 33% germination compared to 1% for LCP85-384 and 0% for TUC77-42. Furthermore, juice quality analysis of the stalks from the same bioassay showed differences in brix, acidity and post-harvest deterioration among the interspecific F₁ hybrids. Identification of cold tolerance using bioassays early in the breeding program should result in the release of commercial varieties with improved tolerance to the cold temperatures typically encountered during the winter months in Louisiana.

The Effect of Glyphosate and 2,4-D on Cane Ripening and Ratooning Ability, and the Comparison of Glyphosate Products in Ripening Performance

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In 2002, a large plot study was conducted to determine if the addition of 1 quart/acre 2,4-D antagonized the effects of 6 oz/A Polado L (glyphosate). Two non-herbicidal ripeners (potassium nitrate, potassium carbonate) were also evaluated in this experiment. The plots were 2.1 acres in size, and all treatments were randomized and replicated 4 times. The plots were harvested with a Cameco combine and all loads were core sampled at the mill. At 49 days after treatment (DAT), Polado at 6 oz/A reduced tonnage 3%, increased sugar/ton 34 lbs, and increased sugar yield 916 lbs/acre compared to the nontreated check. The addition on 2,4-D to Polado did not antagonize the ripening of cane in this experiment. Potassium nitrate and potassium carbonate did not increase sugar yield at 49 DAT. In the 2003 season, the entire test

area received a uniform standard application of 8 oz/A Polado L. The plots were harvested in the same manner as 2002. Plots treated the previous year with 6 oz/A Polado showed a significantly greater cane yield (+ 1.9 tons/A) than all other treatments, including the non-treated check and the Polado + 2,4-D treatments. It is possible that late season 2,4-D treatments may have affected germination of below ground lateral buds.

A separate study was conducted in 2003 to evaluate Polado L at the 6 oz/A and 4 oz/A rate as well as to compare a new glyphosate formulation, Touchdown IQ at 8oz/A. Due to differences in the glyphosate acid concentration and salt formulation, the 8oz/A rate of Touchdown IQ is equivalent to the 6oz/A rate of Polado. Because Polado L is not formulated with a surfactant, 0.25% v/v Hi-Yield 80/20 nonionic surfactant was added to these treatments. No surfactant was added to the Touchdown IQ treatment, as this product is formulated with a surfactant. Plots were 1.6 acres in size and all treatments were randomized and replicated four times. Plots were harvested with a commercial combine and all loads were core sampled at the mill.

Ripener applications were applied by helicopter at daybreak with heavy dew on the cane foliage. The sun never broke through the heavy cloud cover all day, and at 6 hours after application, a heavy rain was received as the cane lodged. When the plots were harvested at 38 DAT, Touchdown IQ was the only treatment that significantly increased sugar/ton (+ 40 lbs/ton) compared to the nontreated check. Due to inconsistencies in the cane stand, no differences were detected in cane or sugar yield. Hand cleaned twenty-stalk samples indicated that both Polado at 6oz/A and Touchdown IQ at 8oz/A significantly increased percent brix at 38 DAT. Polado L at 4oz/A did not significantly increase TRS or percent brix in this experiment.

The Response of LCP 85-384 to the Application of Silicate Slag in Louisiana

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The response of LCP 85-384 to the application of varying rates of silicate slag was monitored for the complete sugarcane production cycle on a light-textured soil (fine-silty, mixed, superactive, hyperthermic Aeric Epiaqualfs) with a water extracted silica content of 13.5 ppm and an acetic acid extracted silica content of 63 ppm. Equivalent rates, applied at 2.24 and 4.48 Mg ha⁻¹, of calcitic lime and silicate slag were included so that treatment responses could be attributed to either silica or to the effects of liming. Interpretations were based on multi-year averages for all variables because of non-significant year x treatment interactions. Mixing 2.24 and 4.48 Mg ha⁻¹ of silicate slag into the seedbed prior to planting or placing 2.24 Mg ha⁻¹ of slag under cane at planting resulted in higher (P = 0.02) cane tonnage than the check. For sugar yields, only the 4.48 Mg ha⁻¹ application rate was higher (P = 0.07) than the check. Significantly higher (P = 0.04) sugar yield for the 4.48 Mg ha⁻¹ slag rate compared to the equivalent calcitic lime rate suggests the yield response was due to the silica content of slag, though this was not the case when comparing the lower application rates for slag and lime (P = 0.27). The soil chosen

for this evaluation contained the lowest silica content of all the sites evaluated, resulting in a positive and significant response of sugarcane to the application of silicate slag. Preliminary data from other silicate slag application tests show that the response to slag decreases as soil silica levels increase. Research to refine silica soil tests and establish deficiency levels for soils in the Louisiana sugar belt is ongoing.

Combine Fan Speed and Ground Speed Effects on Cane Quality, Yield, Losses, and Economic Returns

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Cane quality is becoming more important to the Louisiana industry, with some mills offering premiums for high quality sugarcane. Operational settings on chopper harvesters are extremely important with green-cane harvesting, which is the predominant practice now in Louisiana. A split-plot experiment was harvested with main plots being ground speeds of 2.5, 3.0, and 3.5 mph and subplots being fan speeds of 650, 850, and 1050 rpm. Split plots were three rows wide and 46 m long, and treatments were replicated four times in a RCBD in a field of first stubble LCP85-384. Plots were harvested using a single-row chopper harvester and weighed with a weigh wagon. At this time, randomly collected billet and post-harvest residue samples were retained for further analysis. There was no fan speed by ground speed interaction for the parameters measured in this study. The 1050 rpm fan speed increased TRS by 10% but decreased cane tonnage by 15% compared to the two lower fan speeds. Furthermore, the residue data showed a doubling of the sugar loss behind the combine with the 1050 rpm setting. Sugar yields were not statistically different for the three fan speeds. On the other hand, the highest fan speed resulted in higher net income compared to the lowest speed, when one analyzed complete harvesting and shipping cost on a per acre basis. In conclusion, high quality cane, even without premium pay schedules, results in increased profits. Furthermore, producers that send high amounts of extraneous matter to the mill may increase their cane yield but not their profits.

Rearing Procedures for the Sugarcane Aphid with Results from Antibiosis and Initial Transmission Studies

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The sugarcane aphid, *Melanaphis sacchari*, was first reported in Louisiana in 1999 and is now found throughout the Louisiana sugarcane industry. Although at times, the sugarcane aphid

can be found in high numbers in Louisiana sugarcane fields, its status as an economic pest of sugarcane remains unclear. The greatest threat of the sugarcane aphid to cane yields appears to be as a vector of sugarcane yellow leaf virus (SCYLV). We initiated research to develop rearing procedures to produce sugarcane aphids for use in greenhouse SCYLV transmission studies and in studies to determine cultivar resistance to the aphid. In our procedures, aphids are cultured on sorghum grown in 20 cm plastic pots and restricted to individual pots by cages constructed of polycarbonate plastic. Sorghum (*Sorghum bicolor*) plants are required to be approximately 14 days old to support aphids and are capable of supporting an aphid colony for approximately 30 days. As plants reach a stage where they can no longer support aphids, a starter colony (2-3 infested leaves) is moved to a new and uninfected pot containing host plants to continue the aphid culture. Clip-on cages, also made of polycarbonate plastic, were designed to isolate a single aphid on a 15-cm leaf section of a potted sugarcane plant. These clip-on cages were used to investigate aphid reproduction among sugarcane varieties. In an evaluation containing nine of the most advanced varieties from the Louisiana Variety Development Program, we found that the variety Ho 95-988 produced significantly more aphids per female (14 nymphs; overall mean = 8 nymphs) and produced nymphs for a significantly longer period of time (8 days; overall mean = 3 days) than the other varieties tested. During our initial transmission studies we were able to successfully transmit SCYLV in the greenhouse, but have been unsuccessful in repeating transmission in subsequent studies. The reasons for subsequent failures in transmission are presently not known, but will be important considerations in future research.

MANUFACTURING ABSTRACTS

The Hidden Costs of Boiler Water Treatment – An Evaluation of Boilers in the Louisiana Sugarcane Industry

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If one considers the importance that steam plays in the production of sugar, the boilers should be considered one of the highest priorities in a sugar factory. In fact, some have said that a sugar factory boiler is the heart of the factory. But, do sugar factory personnel place a high priority on the condition of the boiler? To answer this question, a study was conducted on 43 sugar factory boilers in Louisiana at the end of the 2003 crop. Video borescope equipment was used to examine the internal condition of 222 different tubes and to document their condition. Additional data from analyzing boiler water and feedwater during the crop was evaluated to show the impact of boiler conditions and operating practices on the operating efficiencies of the factories. What is the true cost of a boiler water treatment program? It is far more than the cost of the treatment chemicals alone. Loss of production time and loss of recoverable sucrose are directly attributable to the availability of uninterrupted steam flow which is a function of the internal condition of the boiler. In most cases, the annual cost of repairs to the boiling house alone far exceeds the cost of the boiler chemical treatment program. The criteria for selecting a boiler water program should be placed on performance, not on lowest price. Based on the severity of conditions found, in terms of deposition and scaling, calculations were made to demonstrate how this impacts factory profits when supplemental fuels are required to achieve firing rates required for efficient factory operation. A discussion of how these adverse conditions occur and why the water treatment program is of utmost importance is also presented.

Dextran Dip-Stick, a Model for Assay Development

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Antibody technology provides highly specific reagents for analytical purposes. Typically the cost of these reagents has been high because of the requirements of cell culture for production and difficulty in eliciting antibody responses to many compounds. Phage display technology minimizes both of these problems and seems to be an ideal mechanism for the production of a wide variety of specific analytical test systems. In order to demonstrate the efficacy of this approach, a “library” was screened for anti-dextran phages. These were used to develop a paper-dip stick method for dextran detection. The test produced results comparable to the Midland sucrotestTM, and has potential for routine screening of sugar juices. The phage

library approach seems to be an ideal system for the production of a wide range of analytical tests and could be used to develop detection systems for many of the complex molecules found in sugarcane and its process streams.

Easy and Uniform Measurement of the Activity of Dextranase at the Sugarcane Factory or Refinery

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At the present time, the activities or strengths of commercial dextranases in the U.S. cannot be directly compared because there is no uniform method used by vendors to measure the activity. A very wide variation exists in the activities of commercial dextranases, and this is compounded by the fact that activities and prices can change regularly. Furthermore, the factory storage characteristics of commercial dextranases differ widely which further highlights the urgent need for not only a uniform method to (a) measure and economically compare the activities of different commercial dextranases, but (b) one which can be used easily at the factory or refinery. A dextranase method, based on simple titration, was identified and modified for easy use at the sugarcane factory/refinery. This method does not need any sophisticated equipment and there is no need for standards and a standard curve.

Optimization of Factory Applications of Dextranases in the U.S.

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The application of commercial dextranases to breakdown dextran in U.S. sugar manufacture is still not optimized. This is partly because of misinformation about where to add the enzyme and which enzyme to use. Furthermore, there is no uniform method to measure the activity of commercial dextranases by vendors, which has meant that direct comparison of activities is not possible. In this study, a simple titration method to measure activities and modified for easy factory use. All activities can be confirmed with IC-IPAD. Most commercial dextranases in the U.S. are from a fungal source: *Chaetomium gracile* or *erraticum*, and are available in “non-concentrated” or “concentrated” forms. An approximate 8-10 fold difference in activity exists between the two concentration forms, and activity variations exist within each form. In Louisiana only “non-concentrated” dextranases have been applied to either last evaporator bodies or juice. Dextranase activity, in last evaporator syrup temperature (~145°F) and Brix (~65) conditions, were dramatically reduced (activity began to decrease after 25-30 Brix). Overall juice applications were more efficient and economical than adding dextranases to evaporator syrups. Application of “non-concentrated” dextranase to evaporator syrup was uneconomical. However, “concentrated” dextranases can be applied to syrup as low as 10ppm/solids (equiv. to 45ppm/juice) which is useful to consider when severe dextran problems

occur. Heating juice to 120°F in the presence of dextranase dramatically removed more dextran from a juice (3380ppm/Brix) that at the current ambient temperature of application (90°F) and was much more economical, with the “concentrated” dextranase being more economical than the “non-concentrated” dextranase. Dextranase was shown to work in the presence of carbamate biocide. Under factory storage conditions, over a grinding season (90 days), the activity of the “concentrated” dextranase decreased only slightly (~9%) whereas “non-concentrated” dextranase activity had approximately halved (~46%), and even reduced in activity when stored under refrigeration.

Boiler Treatment Chemicals – Facts and Considerations

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As the boilers are the heart of any sugar cane mill, boiler water treatment chemicals are an important part of the effective operation of any mill. There are a number of options available to the mills when it comes to boiler water treatment chemicals, however, there are no “silver bullets”. This paper provides a generic, no nonsense discussion of boiler water treatment chemicals currently available to sugar cane mills. The paper discusses the various chemistries which are and have been in use in the mills and the advantages and disadvantages of each. Possibly more importantly, the paper discusses the limitations of any boiler water treatment chemical program. It details other considerations and recommendations to help insure the success of any program. These considerations are based on situations actually encountered routinely in the sugar cane mills in Louisiana and Florida.

A Method for Correction of Pol Error in Sugar and Juice

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The switch to non-lead clarification agents has increased the error in pol sucrose measurements. This is because the non-lead clarification agents precipitate neither dextran nor fructose. Pol sucrose assumes that measurements are directly accountable by the polarization of sucrose and that minor components (i.e. glucose and fructose) cancel out. In mixed juice, this is seldom the case as fructose can provide a negative rotation up to five times greater than the positive contribution made by an equivalent amount of glucose under similar conditions. The change in glucose to fructose ratios that occurs when dextran is synthesized further complicates the interpretation of data acquired by standard polarimetric means. A model system of equations was developed that allows the approximation of “true” (HPLC) sucrose from pol data provided that dextran concentrations are known. The model gave accurate results (95% confidence limits) with samples containing up to 2000ppm/bx dextran.

Introduction of Factory Laboratory Automation at Raceland Mill

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Raceland factory introduced and commissioned an automated factory laboratory system during the 2003 season. The system is called CaneLab and was developed on a Windows platform by InfoWave, a software company based in Durban, South Africa, which specializes in the sugar industry. The principle of the system is to capture analytical data automatically into an Oracle Database and then manipulate this data to generate customized reports for the factory. Software updates and maintenance of the CaneLab system is carried out by InfoWave in Durban and relevant changes are downloaded to Raceland Sugar via the internet through a firewall that ensures total confidentiality for the customer. CaneLab is a comprehensive program and offers infinite opportunities to develop and produce reports that are specific to the customer needs. Detailed data in the new Raceland Sugar reports facilitated the resolution of a number of problems that were not highlighted in the old reporting system.

Experience with CaneLab at Raceland Sugar has been extremely positive. After only one day of training, analysts were completely familiar with the system and accepted it with some enthusiasm. The benefits realized by Raceland Sugar include the elimination of transcriptions errors, the elimination of calculation and averaging errors and the insurance that the analyses are actually being completed and not fabricated.

Online Evaporator Heat Transfer Coefficient Measurement

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A measurement of the amount of scaling in evaporators can be very important for mill operations in determining when a vessel is performing inefficiently. A measure of the heat transfer coefficient shows how a vessel scales over time and can be used to predict when a vessel requires cleaning. In the past, heat transfer coefficients would have to be measured with temperatures (or pressures) and flow or Brix measurements on each vessel in order to perform the calculations. With the advancement of computer technology, advanced algorithms and iterative computations can now be performed in a few seconds with fewer measurements.

This presentation describes a simple and cheap method for calculating heat transfer coefficients using minimal flow and Brix data (two magnetic flow meters and one microwave density meter) along with temperature data (nine probes). The temperatures are recorded using resistance temperature devices, which are cheap and highly accurate. The computer iterates for a steady state mass and energy balance around the evaporator station and computes the heat

transfer coefficient. The St James sugar mill has a quadruple evaporator set with no vapor bleeds and no condensate flash recovery. The calculations are performed in Excel utilizing the solver function, the calculate function and macros. After the computation is performed in Excel, heat transfer coefficients are displayed on the process control system at the mill and the data is stored on a Honeywell UMC800 controller for further processing.

The results show that the fourth effect scaling is determining the need to clean the evaporators, and that the first three effects could be cleaned less frequently. Ways to reduce the rate of scaling in the last effect could have a considerable effect on required cleaning frequencies.

Compressed Air Energy Savings in Sugar Cane Processing

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This talk will cover compressed air system efficiency in sugar cane processing facilities and will include results of a study performed at a Florida Processor. Subjects covered will include: methodologies for conducting plant air system audits and efficiency evaluations, air leak quantification, compressor sequencing, pressure regulation and commonly found control problems. The presentation is focused on the fundamental, most often encountered, problems in Sugar Cane processing facilities. The talk will also discuss the basic tools available to help identify and mitigate air system deficiencies.

One of the greatest misperceptions is that compressed air is an inexpensive utility. It is not! Compressed air power is twice the cost of hydraulic power and 7-9 times the expense of direct electrical power. Waste and misapplication are costing manufacturing facilities hundreds of millions of dollars annually. Extensive audit data has been compiled from performance tests of over 1600 compressors over the past 10 years. Several common controls and system problems have been found to be pervasive in compressed air systems. Many of these problems are easily diagnosed and resolved if proper maintenance and diagnosis is applied. Finally, there are many tools available to help the plant engineer and maintenance personnel to diagnose and maintain their system at significantly lower operating costs. The goal of the presentation is to give plant personnel knowledge they can apply to their own system once they have returned to their facility.