CP-Cultivar Development Program: Challenges and Responses

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The goal of the CP-Cultivar Development Program is to release high-yielding, stress- tolerant cultivars for the Florida sugarcane industry. To meet this goal, two major challenges have recently emerged: 1. it has become increasingly apparent that brown and orange sugarcane rusts are having a major impact on the program, and 2. the program needs to improve its ability to identify productive cultivars for sand soils. The objective of this presentation is to describe the CP program's progress in addressing these challenges. More intense selection pressure for rust resistance has been implemented by modifying screening procedures in the earlier stages of the program. All clones in the first clonal selection stage (Stage I) are now rated for their reaction to rust in August and all clones with a rating of 2 and above on a 4 point scale are eliminated. Likewise, all Stage II, III, and IV clones are rated at least twice for their sugarcane rust reactions based on natural infection in the regular cultivar development plots and in separate plantings using whorl inoculation. For the first time since orange rust was detected in Florida in 2007, all clones advanced to Stage III in November 2009 had a rating of 1 or less for rust susceptibility. To improve the selection of clones for sand soils, one Stage IV test site on organic soil was moved to a sand location thereby increasing the number of sites with sand soil from two to three. Also, previously this program advanced the same clones from Stage III to all Stage IV locations, regardless of soil type. Now, selection is conducted independently for sand and muck soils. The result has been that about one-third of the clones in Stage IV are tested on both soil types, with one-third tested on sand only, and one-third tested on muck only. Early indications are that this is increasing the number of clones released for sand. Finally, a scientist has been added to the team who will spend substantial time studying how we can genetically improve sugarcane tolerance to the stresses when it is exposed to on sand soils, and other scientists have ongoing or recently completed research projects that aim at improving our selection on sand soils. The CP program modifications have been with the sugar industry partnership and cooperation.

Repeatability of Sugarcane Selection on Sand and Organic Soils

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The Canal Point (CP) Sugarcane Cultivar Development Program (a cooperative program between the USDA-ARS, the University of Florida and the Florida Sugarcane League) has been

more successful at breeding for cultivars adapted to organic soils (muck) than for those adapted to sand soils. Currently, only the last two stages of the selection process are tested on sand soils with a reduced set of genotypes that had previously been selected on muck. This strategy might not be identifying the best sand-adapted genotypes. To test this hypothesis, an intermediate selection stage (Stage 2), which is regularly planted exclusively on organic soil (Torry muck) at Canal Point, FL was also established and sampled for two years (2007-09) on a sand soil (Margate sand) at Townsite Farm of US Sugar Corporation near Clewiston, FL. The analysis of an identical set of genotypes for both soil types resulted in a genotype x environment interaction significant for all traits and years. Spearman rank correlations among traits for sand and muck were significant but low. Theoretical recoverable sucrose (TRS) had the highest correlation between locations (r=0.31 across years) while tons of cane per acre (TCA) and tons of sucrose per acre (TSA) had the lowest (r=0.23 and r=0.21, across years, respectively). Repeatability (R) between sand and muck was higher for TRS (0.31 across years) and lower for TCA and TSA (0.25 and 0.22 across years, respectively). Results indicated that there were a low number of genotypes with similar performance for both soil types. Therefore, some clones that were not selected in the Stage 2 on muck soil were selected in the Stage 2 on sand and vice versa. This may explain the lower efficiency of the current program in identifying genotypes adapted to sand soils.

Assessing the quantitative and molecular changes in the Florida sugarcane breeding program

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To ascertain the genetic gains obtained through breeding sugarcane (*Saccharum* spp.) for Florida, a comprehensive analysis was undertaken using cultivars released over a 50-year period. Fifty genotypes, including early progenitors (e.g. POJ, NCo310 used as the base population) and cultivars (13 CL and 32 CP released from 1950 to 2000) were field-tested at the USDA-ARS Sugarcane Field Station (Canal Point, FL) in a triple 5x11 α-lattice design and sampled in the plant cane and two ratoon crops (2006-2010). All sources of variation were significant for all traits, with crops contributing three times more to the total variation than genotype for stalk weight (SW), sucrose content (SC), and theoretical recoverable sucrose (TRS), and with genotype playing a major role for sucrose yield (SY) and the interaction being less important. Genetic gains for this 50-year period were 0.06% yr^{-1} for SC, 0.05 kg Mg^{-1} yr^{-1} for TRS, and 1.16 yr^{-1} for SY, with a tendency towards a non significant decrease for either of these traits. These gains were accompanied with significant genetic and phenotypic correlations between SC and SY (r_g = 0.37±0.17, r_p=0.58±0.04), between TRS and SY ((r_g = 0.39±0.17, r_p=0.60±0.04), and between SC and TRS (r_g = 0.97±0.01, r_p=0.98±0.002). The genetic and phenotypic relationships between SW and SC or between SW and TRS were negative but not significant ((r_{gSC} = -0.16±0.18, r_p=-0.10±0.06; r_{gTRS} = -0.123±0.20, r_p=-0.09±0.06). The correlations between SW and SY were positive and highly significant (r_g = 0.88±0.06, r_p=0.69±0.03). Highly significant and moderate heritability estimates supported these results, ranging from 0.45±0.08 for TRS, 0.52±0.07 for SC and SY, to 0.64±0.06 for SW, with higher values calculated on an entry means than on a plot basis. These results ascertained that genetic improvement in breeding

sugarcane for Florida rest on solid grounds with moderate heritability and strong genetic correlations among the traits. The evolution of the quantitative variation for the traits will be discussed and compared with that at the molecular level.

L 01-299 – A New Sugarcane Variety for Louisiana

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L 01-299 was released to the Louisiana sugar industry on August 28, 2009 by the LSU Agricultural Center in cooperation with the U.S. Department of Agriculture Research Service and the American Sugar Cane League. The cross (XL96-402) for L 01-299 was made at facilities located at the Sugar Research Station in St. Gabriel, Louisiana in 1996. Photoperiod facilities were used to induce flowering for the female and male parents used for the cross, L93-365 and LCP 85-384, respectively. Seedlings from the cross were planted in the field in April 1997 followed by single stool selection in September 1998. Early stage selection culminated in the assignment of a permanent varietal designation in 2001. The main criteria that growers use when selecting varieties to plant is yield. L 01-299 was released because of its high sugar and cane yields, erectness, and excellent ratooning ability. Cumulative data indicated that L 01-299 produced 14 percent higher sugar yield, 13 percent higher cane yield and 1 percent higher sucrose content than HoCP 96-540 when averaged across all crops. The variety is characterized as having excellent ratooning ability with a high population of medium sized diameter stalks, slightly lighter in weight compared to HoCP 96-540. Harvesting characteristics are important for sugarcane varieties. L 01-299 is an erect variety, having withstood the effects of many hurricanes during the time of variety testing. Disease resistance is another important component of variety selection. The new cultivar is resistant to common brown rust disease (*Puccinia melanocephala* H. and P. Sydow), susceptible to smut disease (*Ustilago scitaminea* Sydow & P. Sydow), moderately resistant to leaf scald [*Xanthomonas albilineans* (Ashby) Dowson], resistant to mosaic strains. The effect of yellow leaf disease and orange rust on the yield on L 01-299 is unknown. Insect pest management studies have been conducted that indicate L 01-299 is moderately resistant to the sugarcane borer (*Diatraea saccharalis* F.) and Mexican rice borer (*Eoreuma loftini* Dyar).

L 03-371 – A New Sugarcane Variety for the Louisiana Sugar Industry

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On May 5, 2010 the LSU Agricultural Center released a new sugarcane (*Saccharum* spp.) variety L 03-371 in cooperation with the U.S. Department of Agriculture Research Service and the American Sugar Cane League. The cross for the new variety was made at facilities located at the Sugar Research Station in St. Gabriel, Louisiana on October 9, 1998. Photoperiod facilities were used to induce flowering in the parental clones. The female and male parents used for the cross of L 03-371 were CP 83-644 and LCP 82-89, respectively. Seedlings from the cross were planted in the field in April 1999 followed by single stool selection in September 2000. Early stage selection culminated in the assignment of a permanent varietal designation in 2003. The main criteria that growers use when selecting varieties to plant is yield. To the sugarcane grower, sugar yield, cane yield and sugar per ton of cane are the main traits of interest. L 03-371 was released because of its high sugar and cane yields, ratooning ability and excellent disease attributes. L 03-371 had plantcane sugar yields and sugar per ton of cane yields that were significantly higher than HoCP 96-540 in Outfield Variety Testing conducted from 2007 - 2009. This new variety had significantly higher sugar per ton of cane than HoCP 96-540 in the first and second ratoon crops and sugar yields that were equal to that of HoCP 96-540. The variety is characterized as having excellent ratooning ability with a high population of medium sized diameter stalks.

Second-generation sequencing for marker development in sugarcane

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Second generation sequencing (also known as next-generation or massively parallel sequencing) involves the simultaneous generation of millions of short DNA sequences. The impact and applications of this technology are still emerging; however, strategies that reduce the complexity of the DNA sample prior to sequencing and the utilization of reference genome sequences are two important tools which greatly enhance the application of the technology. Here, we examined the utility of Restriction site Associated DNA (RAD) LongRead sequencing for the identification of Single Nucleotide Polymorphism (SNP) markers in sugarcane. RAD libraries were prepared for sugarcane clone CP 92-1167 and wild, *Saccharum spontaneum* accession IND 81-146 through a combination of enzyme digestion and physical shearing of genomic DNA. Sequencing consisted of paired end 54 bp sequence reads (2 x 54 bp) using the Illumina GAII platform. A total of 0.628 Gb of raw sequence was generated and assembled into approximately 6,500 contigs per genotype. Since no reference genome is currently available for sugarcane, the fully sequenced *Sorghum* genome was used as a framework for alignment of assembled sequences. Approximately 25% of the total number of contigs aligned with the sorghum genome, 500 of which were common to both genotypes and *Sorghum* at sequence identities of >90%. Mining the alignments revealed a total of 1,239 SNPs and 153 short insertion/ deletions with an average SNP frequency of 1 SNP per 127 bp. This study has shown the utility of SNP marker identification in sugarcane using complexity reduction sequencing and utilization of the fully sequenced *Sorghum* genome. Further exploration of sugarcane-sorghum synteny will be presented.

Development of a Genetic Linkage Map for Louisiana Sugarcane: New Microsatellite (SSR) DNA Markers Identified For LCP 85-384

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A genetic linkage map of sugarcane (*Saccharum* spp. hybrids) cultivar LCP 85-384 was recently developed using a mapping population of 300 selfed progeny of LCP 85-384 and a total of 773 single dose amplified fragment length polymorphism (AFLP), targeted region amplification polymorphism (TRAP), or microsatellite (SSR) markers. Application of this map has been limited due to the small number of DNA markers, in particular microsatellite (SSR) DNA markers. Continually adding DNA markers to the map will improve its usefulness in identifying associations between important agronomic traits and DNA markers through quantitative trait linkage (QTL) analysis. The objectives of this study were to search the public database for new SSR markers and evaluate their transferability to the U.S. sugarcane germplasm. A total of 376 SSR markers were found from two published papers, of which 305 markers were reportedly derived from expressed sequence tags (ESTs) and 71 were designed based on genomic DNA sequences. Although EST sequences are highly conserved, more genetic variability can be found in non-expressed regions of genomic DNA sequences. Conventional oligo-nucleotide primers of 81 ESTs and 71 genomic SSR markers were synthesized commercially and evaluated by polymerase chain reaction (PCR) using the genomic DNA of LCP 85-384 as the template. The PCR-amplified DNA products along with DNA size markers were separated in 1.5% agarose gels by electrophoresis, visualized under UV lights by ethidium bromide staining, and photographically documented. The results indicated that 71 (88%) ESTs and 39 (55%) genomic SSR markers were capable of producing DNA fragments from the genomic DNA of LCP 85-384. These transferable SSR markers will be labeled with fluorescent phosphoramidite dyes and used on a capillary electrophoresis-based high throughput genotyping platform to fingerprint the LCP 85-384 mapping population. This will produce more SSR data to increase the marker density of the linkage map. The potential utility of these newly transferred SSR markers in enhancing U.S. sugarcane germplasm evaluation and varietal genotyping will also be investigated.

Searching for DNA Markers Associated with Sugar Content in Sugarcane

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Sugar content is among the most important traits in sugarcane for its commercial production. A relatively stable genetic character in sugarcane breeding programs, sugar content requires direct instrumental measurements during selection. This process is labor intensive and must be done only when the crop reaches maturity. The objective of this study was to explore the possibility of identifying suitable DNA markers for marker-assisted selection of sugar content in sugarcane. We used the candidate gene approach for marker development. From the GenBank database, we fetched four genomic sequences encoding for *SuSy* (sucrose synthase) and *SPS* (sucrose phosphate synthase) and 76 expressed sequence tags (ESTs) including seven for *SPS*, 41 for *SuSy*, and 28 for *AInv* (acid invertase). These three enzymes play important roles in sugar metabolism in sugarcane. We also found homologous sequences of these three genes of *Sorghum*, *Oryza sativa*, and *Arabidopsis*. Sequences belonging to the same gene were aligned using DNAMAN® software to produce homology trees. Based on these homolog trees, 14 ESTs or whole genomic sequences were chosen for the design of 16 pairs of PCR primers. The primer pairs were used to amplify PCR products from the genomic DNA of six clones from a mapping population derived from selfing pollination of LCP 85-384. These clones were segregating for sugar content but were similar for other phenotypic traits. The amplified PCR products were separated by electrophoresis in 1.5% agarose gels, stained with ethidium bromide, visualized under UV lights, and photographically documented. Each of 14 primer pairs produced a single DNA band as expected and two primer pairs did not produce any band. The DNA products of crude PCR reaction mixtures were cloned directly into a pCR4-TOPO vector from Invitrogen. Four to six recombinant clones from each PCR reaction were sequenced. Analyses of the DNA sequence data demonstrated the following: 1) the amplified PCR products from any sample were mixtures of DNA fragments displaying sequence variability, very likely due to the aneu-polyploidy nature of sugarcane; and 2) the sequence variability of LCP 85-384 was transmitted to its progeny. We will further scrutinize the variability of these DNA sequences to determine if there is any variability associated with sugar content.

Genetic Analysis of the Sugarcane (*Saccharum* hybrids spp.) Cultivar 'LCP 85-384': II. Identification of Quantitative Trait Alleles for Starch Content

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Starch in sugarcane juice can impede the extraction of sugar during processing and also affect the quality of the refined sugar. The problem has been exacerbated by the widespread adoption of green cane harvesting in sugarcane. More starch is usually present in the leaves and young growing portions of the plant than the stalk although judging on a per weight basis a greater

proportion of starch is still being contributed by the stalk. The deliberate effort by breeders to broaden the genetic base of sugarcane using *Saccharum spontaneum* germplasm which is high in starch may also be contributing to the increasing levels of starch in recent cultivars. Alpha-amylase enzymes are used during sugar processing to hydrolyze starch but this practice is expensive and not always efficient. Developing sugarcane cultivars low in starch content is a more preventative, economical, and sustainable solution. Identifying quantitative trait alleles (QTAs) that associate with starch content and using them in marker-assisted selection during sugarcane improvement would be an efficient way to reduce the starch content. For this purpose, linkage and QTL mapping experiments were conducted in Louisiana using a selfed population of LCP 85-384. Linkage analysis led to the mapping of 717 AFLP, SSR, and TRAP single dose markers onto 108 co-segregating groups (CG) with a cumulative map length of 5,348 cM. Starch data collected from two replicated field plots of 227 individuals was used to identify QTAs by the composite interval mapping (CIM) method employed in MapQTL v 5.0. A total of 10 QTAs located on eight CGs were detected for starch. These QTAs explained 5.4% to 32.3% of the phenotypic variation with positive additive effects for nine of the ten QTAs. Tentative assignments of QTAs to the parental genomes of LCP 85-384 showed that six QTAs were contributed by both parents, one by the female (CP 77-310) and two by the male (CP 77-407) parent.

Towards the molecular characterization of lignin-related genes in the *Saccharum* complex

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Crude oil from fossil fuel is the predominant source of energy in the world today and its demand is on the rise to meet ever increasing consumption. The view that crude oil reserves are finite coupled with concerns of its use to long term environmental quality, economic growth and political stability has motivated interest into domestic and renewable sources of energy. The production of fuels from carbohydrates found in lignocellulosic biomass offers the potential to reduce global dependence on fossil fuel. As an herbaceous perennial crop with high biomass production potential and high carbohydrate content of its biomass, sugarcane can become a key component of the renewable energy supply in the US. However, because of its biological structure, lignocellulosic biomass is generally recalcitrant to bioconversion and sugarcane biomass is no exception. The crystalline structure of the main carbohydrate, cellulose, renders it highly insoluble and resistant to enzymatic action (hydrolysis) while the combination of hemicellulose and lignin provide a protective sheath around cellulose, which must be modified or removed (pretreatment) before efficient hydrolysis of cellulose can occur. Much research has been expended on finding novel pretreatment and hydrolysis processes, however, this effort would be greatly enhanced if accompanied by the availability of sugarcane biomass with characteristics that allow for easy bioconversion. Our long term goal is to improve the bioconversion (to energy) potential of sugarcane biomass through breeding and selection. To this end, we undertook to understand the characteristics of three major genes in the lignin biosynthesis pathway from two *Saccharum* species, *S. officinarum* and *S. spontaneum*, known to

differ in fiber characteristics. We also used these lignin-related genes to study genetic diversity among 63 genotypes in the *Saccharum* complex, including *S. officinarum*, *S. spontaneum*, *S. sinense*, *S. barberi*, *S. robustum*, *Erianthus* sp., and *Miscanthus* sp. using the target region amplification polymorphism (TRAP) markers. This paper presents the progress achieved so far in this project.

Phosphorus Soil Test Calibration for Sugarcane Grown on Everglades Histosols

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A calibrated soil test for P fertilizer application to sugarcane (*Saccharum* spp.) grown on organic soils in south Florida is an important best management practice for minimizing P loads in water draining to the Everglades. The current calibration uses water as the soil extractant which has the limitations of being very sensitive to pH and being most applicable to short-season crops. Past field studies were included with current studies and analyzed to develop an updated soil test P calibration for sugarcane on Histosols in the Everglades Agricultural Area. Five experiments (14 crop years total) included annual banded P rates of 0, 9, 18, 36, 72, and 144 kg P ha^{-1}. Two of these studies also included annual broadcast P applications at the same rates. Three additional studies (10 crop years total) included preplant broadcast applications of 0, 15, 29, 44, and 59 kg P ha^{-1}. Relative sucrose ha^{-1} without P application related strongly to Mehlich 3-extractable P, with strongest response to P fertilizer at soil test values $\leq$ 10 g P m^{-3}. Phosphorus extracted with water, acetic acid, and Bray 2 did not relate well to crop response overall because of specific locations at which crop response was measured but not predicted by extractable P. A new soil test calibration is proposed based on Mehlich 3 soil extraction. The highest recommended P rate is 36 kg P ha^{-1} for Mehlich 3-extractable P $\leq$ 10 g m^{-3} in preplant soil samples.

Identification of Physiological Traits for Early Detecting Water Deficit Stress in Sugarcane

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Sugarcane (*Saccharum* spp.) genotype selection has been more successful for organic (muck) than sand soils in Florida. Water deficit stress during its formative growth phase may limit sugarcane growth and yields on Florida sand soils. Therefore, identifying proper physiological traits will help scientists select genotypes with water stress tolerance and improve variety selection efficiency for sand soils. A greenhouse study was conducted to determine some physiological traits that may be used to select water deficit tolerant sugarcane genotypes. Treatments included muck and sand soils, two water regimes [well watered (WW) and water deficit stress (WS)], and two genotypes (CP 80-1743 and CP 01-2390). CP 80-1743 is a commercial cultivar for Florida muck soils and CP 01-2390 is an experimental line with superior agronomic performance on Florida sand soils but was not released due to susceptible to smut disease. These two genotypes were used to consider their performance on Florida muck and sand soils. The experiment was a split plot design with seven replications. Soil type and water regime were main plots and genotype was sub-plot. Single-bud stalk sections of sugarcane were planted in pots and fertilized with N, P, and K based on soil analyses. To ensure consistent conditions of soil water content and nutrients, the two genotypes were planted in each pot and labeled to indicate the genotypes. Thus, there were two plants per pot (one was CP 01-2390 and the other was CP 80-1743). All pots were well watered until 58 days after planting, when water was withheld from the WS pots. During the stress treatment, leaf relative water content (RWC) and proline content were measured weekly and leaf stomatal conductance (g_s), photosystem II photochemical efficiency (Φ_{PSII}), and leaf net photosynthesis rate (Pn) were determined every 3 or 4 days. Water stress significantly reduced leaf g_s, Φ_{PSII}, and Pn. In contrast, leaf RWC and proline content were not sensitive WS indicators in this study. Photosystem II photochemical efficiency did, but either g_s or leaf Pn did not differ significantly between the two genotypes. The effects of water stress on sugarcane plants were much greater on sand than on muck soils. These results suggest that physiological traits of leaf g_s, Φ_{PSII}, and Pn can be used for early detecting sugarcane plant water stress and for stress tolerant genotype screening. Improving genotypic resistance to water stress, in addition to improving irrigation management, will increase sugarcane yields on sand soils.

Effect of Residue Management on Atrazine Retention and Sugarcane Yield

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We investigated the effectiveness of residue on retention of applied atrazine on sugarcane. Variety L 97-128 was planted on August 15, 2006 in Commerce silt loam soil. The residue was collected randomly on November 7, December 8, 2008, January 8, February 4, and March 6 2009. Multiple 1 m^2 areas were collected within the plots, the residue was dried at 55C for 24 h and weighed. Our results are from the second stubble which was harvested November 19, 2009. Atrazine retention by the residue and the Commerce soil were measured in the laboratory using batch methods where radioactive isotope (14C-UL ring labeled) was used. The rate of decay of the sugarcane residue were also quantified. Sugarcane stalk population, yield, and amount of mulch residue left on the soil surface were measured for each treatment. The results indicated that the rate of atrazine retention by the residue was similar for the entire growing season with an average value of K_d = 17.9 L/Kg and standard errors ranging from 0.62 to72 L/Kg, and r^2 of 0.99. Earlier work on the same soil for sugarcane varieties LCP 85-384, showed the average atrazine retention rate was16.3±0.21 L/Kg . We found that retention capacity of the residue for atrazine did not change significantly with the age of the decaying residue over the growing season. Such a finding was consistent with earlier for sugarcane variety LCP 85-384. Therefore, we conclude that only one retention parameter (or *Kd)* is necessary to describe

herbicide retention, regardless of when atrazine application is made. We also present results of sugarcane yields for plant cane and first stubble (2007-2009).

Long-term Effects of Post-harvest Residue Management in Louisiana (Three Production Cycles)

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A study was initiated in 1997 to evaluate the long-term effects of harvest residue management on sugarcane (*Saccharum officinarum* L.). Objectives were to measure the benefits and consequences of combine-generated residue retention on subsequent crops in the production cycle. Residue management treatments include 1) pre-harvest burning, 2) post-harvest raking residue to the middles and 3) full retention of the residue. Treatments were established in the first stubble (ratoon) crop of production cycle number one in 1998 and maintained in place for the duration of three production cycles, ending with the third stubble crop in 2009. The amount of residue blanket remaining after harvest averaged 5.89 and 10.08 Mg ha^{-1} , respectively, for the pre-harvest burned and non-burned treatments. Retention of the residue, especially for the plots with residue raked to the furrow bottoms, resulted in difficulties for tillage operations and fertilizer applications in years when the residue was wet and/or plentiful. While retention of the residue did not diminish yield in all seasons, averaged over all the stubble crops in the three cycles pre-harvest burning resulted in higher sugar yields than both the raked-residue and the retained- residue management approaches. Difference in sugar yield for the other treatment comparison was not significant. It must be acknowledged, however, that differences in measured yield between the burned and non-burned treatments must factor in the direct effects of burning prior to harvesting as well as the effects of the retained residue. The adverse effects of residue retention appeared to be cumulative within cycles, with the most debilitating effects occurring in the more advanced stubble crops. By the last stubble crop in each of the three cycles, the stalk population for the cane with the retained residue possessed the lowest number of stalks. The good news about post-harvest retention of the residue is that the negative effects of retaining it did not carryover through the fallow period to subsequent production cycles, as indicated by the relatively high yields of the plant cane crops at the initiation of each production cycle.

Sampling for Wireworms at Sugarcane Planting

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Wireworms (Coleoptera: Elateridae) are important insect pests of newly planted sugarcane in Florida. Usually, newly planted fields receive a soil insecticide application to prevent wireworm damage. During a two year study, 38 fields were sampled for wireworms to determine the necessity of soil insecticide application at planting. Soil samples were taken for wireworms in a transect across each field using a new sampling method. The fields were paired so as to be similar except that one field received a soil insecticide application and one field did not. During the first year (2008), there was no significant difference in gross tons per acre, net standard tons per acre, % sucrose, or tons of sugar per acre in fields receiving insecticide application versus fields without insecticide application. During the second year (2009), there was again no significant difference in gross tons per acre, net standard tons per acre, % sucrose, or tons of sugar per acre in fields receiving insecticide application versus fields without insecticide application.

Sugarcane Genotype Tolerance to Wireworms

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Sugarcane (interspecific hybrids of *Saccharum* spp.) growers in Florida normally apply a soil insecticide at planting to limit wireworm *(Melanotus communis* Gyllenhall*)* damage to seed cane (vegetative plantings of stalks). The objective of this study was to measure the tolerance of eight commercial sugarcane cultivars and one promising genotype to severe wireworm pressure at planting. In a greenhouse experiment with five replications, either 0 or 5 wireworms were introduced into flats (61 cm wide x 112 cm long x 17 cm deep) filled with 14 cm of Pahokee muck soil. Bottom, middle, and top stalk sections (each 71 cm in length) from one genotype were planted in each flat. Treatments were planted on 3 Dec. 2009 in a randomized complete block design arranged in split plots with the genotype x wireworm treatment (each flat) as the main plot and stalk section (top, middle, and bottom) as subplot. The experiment was harvested on 22-23 Feb. 2010. Root, stalk, and leaf weights were all reduced ($P = 0.01$) by wireworms. There were also significant differences among genotypes averaged across both wireworm treatments, but there were no significant genotype x wireworm interactions for root, stalk, or leaf weights. Stalk section and the genotype x wireworm x stalk section interaction affected leaf and stalk weights ($P < 0.01$). Emergence was measured weekly and it was found that the linear emergence response differed ($P < 0.01$) for treatments with (27.9% emergence) and without wireworms (23.1% emergence), but there were no significant interactions between genotypes and wireworms. This preliminary analysis suggests that some genotypes may be more tolerant to wireworm damage than others. The analysis of final emergence by stalk sections will help

determine whether it is recommended to conduct field studies to confirm this preliminary conclusion.

Sugarcane Rust Mite, *Abacarus sacchari*: an Old World Pest Attacking Sugarcane in the New World

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The sugarcane rust mite, *Abacarus sacchari* Channabasavanna (Actinedida: Eriophyidae), was discovered on sugarcane in Canal Point, FL in September 2007. Though first observed in Belle Glade, FL on sugarcane in 1983, it was originally identified as *Abacarus officinari* Keifer. Described from Asian specimens, *A. sacchari* has now been recorded from Africa, Australia, India, Costa Rica, Venezuela, Brazil and Florida. Feeding by these mites results in an orange to rusty reddish-brown to purple discoloration on the underside of leaves that was originally masked by an outbreak of orange rust, *Puccinia kuehnii*. The orange symptom color is similar to that of orange rust, but the *A. sacchari* feeding symptom is more uniformly distributed over the leaves and does not result in the raised pustules characteristic of rust. Damage symptoms are variety specific. Beyond the range in discoloration among varieties, symptoms can be restricted to the mid vein, leaf blade, or spread across the leaf surface by variety. Significant differences in photosynthetic components were observed between sugarcane with and without *A. sacchari*. Leaves damaged by mite feeding displayed reduced photosynthesis associated with reduced chlorophyll content, transpiration, and stomatal conductance, but elevated intercellular CO_2 concentrations.

Effects of Sugarcane Harvest and Tillage Practices on Lesser Cornstalk Borer Damage and Yield

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Lesser cornstalk borer, *Elasmopalpus lignosellus* Zeller (Lepidoptera: Pyralidae), is an important sugarcane pest in southern Florida. It feeds on meristematic tissues of young sugarcane shoots and causes dead hearts, symmetrical rows of holes in the leaves, and plant death. Field studies were conducted in 2006 to determine the effects of harvest residue from green harvesting versus pre-harvest burning on *E. lignosellus* damage and sugarcane yield. Harvest residue removed from a green harvested field and placed in plots in a plant cane field resulted in significant reduction of *E. lignosellus* damage. Sugarcane (TCA) and sucrose (TSA) yields did not differ between plots with and without harvest residues in plant or ratoon sugarcane. Grower concerns of reduced water percolation and fertilizer availability in green harvested fields led to changes in supplementary field trials. In 2008 and 2009, three post-harvest tillage treatments to break down harvest residues and increase percolation were tested in combination with green and pre-harvest burned fields for their effects on *E. lignosellus* damage and sugarcane yield. Overall, significantly less *E. lignosellus* damage was again observed in green versus pre-harvest burned fields in both years. No- and intermediate-tillage significantly reduced *E. lignosellus* damage compared to conventional tillage in green harvested fields only. Tillage level had no effect on feeding damage in the pre-harvest burned fields. In both years, greater TCA was produced in intermediate than in the other tillage levels in green harvested sugarcane, whereas TCA and TSA were greater in conventional than in the other tillage levels in pre-harvest burned sugarcane. Results of field trials suggested that plants were compensating for *E. lignosellus* damage by increasing tiller production. A 2-yr experiment was conducted in a greenhouse to evaluate variety and age specific compensatory response in sugarcane to lesser cornstalk borer infestation. Infestation at the 3-leaf stage resulted in more dead hearts and dead plants than when infested at the 5- and 7-leaf stages. The sugarcane variety CP 89-2143 was more sensitive to *E. lignosellus* damage and resulted in reduced sugarcane and sucrose yield compared with CP 78-1628 and CP 88-1762. All varieties infested at the 3-leaf stage produced more yield than when infested at the 7-leaf stage. Comparison of yield reduction with lethal damage by *E. lignosellus* (dead hearts + dead plants) showed these varieties had equal ability to compensate for feeding damage, but that compensation ability declined with the delay in infestation time.

Eptam in Sugarcane: Incorporation Methods, Weed Control, and Economics

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Research was conducted in fallowed sugarcane fields to evaluate Eptam at 2, 3, 4, and 5 pints/A incorporated on pre-formed sugarcane beds using a Lilliston® rolling cultivator and a hipper/bedder. Eptam treatments were followed by Roundup Original Max (glyphosate) at 46.5 oz/A and weed control was compared with a glyphosate alone program consisting of two applications of Roundup Original Max at 46.5 oz/A. For bermudagrass, johnsongrass, and purple nutsedge, a significant interaction between Eptam rate and incorporation method was not observed. Averaged across Eptam rates 30 days after application, bermudagrass was controlled 62% when incorporated with a rolling cultivator compared with 43% for the hipper/bedder. For johnsongrass and purple nutsedge, incorporation implements were equally effective and control averaged 49 and 30%, respectively. Control of all weeds was greater 30 days after application for Eptam at 5 pt/A compared with 3 pt/A; 66% vs. 45% for bermudagrass, 67% vs. 57% for johnsongrass, and 47 vs. 33% for purple nutsdege. At 60 days after Eptam application, bermudagrass and johnsongrass control averaged across incorporation methods was at least 88% regardless of whether glyphosate was applied following Eptam or applied alone. For purple nutsedge, however, control was 63% when glyphosate was applied twice compared with once following Eptam (around 34% control). Using the current cost of $45.10/gallon for Eptam, cost of 2 pt/A would be $11.28. The current cost of Roundup Original Max at 46.5 oz/A is $13.08/A; the cost of generic glyphosate at 64 oz/A is $5.50/A. Total weed control cost where Eptam is followed by glyphosate would be $24.36 and $16.78/A, depending on glyphosate formulation. This compares with $26.16 and $11.00/A where the glyphosate products are applied twice.

Effect of Planting Techniques and Seeding Densities on Growth and Yield Performance of Spring Planted Sugarcane

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The studies were undertaken to see the effect of three planting techniques i.e. P_1 (sowing of sugarcane in 60 cm apart furrows), P_2 (sowing of sugarcane in 120 cm apart trenches) and P_3 (sowing of sugarcane in 150 cm apart trenches) and three seeding densities i.e. S_1 (60,000 DBS ha^{-1}), S_2 (75,000 DBS ha^{-1}) and S_3 (90,000 DBS ha^{-1}) at farm of Sugarcane Research Institute, Faisalabad during February 2006 and 2007 by using sugarcane cultivar HSF-240 in RCBD with three replications in plots measuring 3.6 x 6.5 m using standard fertilizer level of 168-112-112 NPK kg ha^{-1}. Maximum number of tillers m^{-2} (14.55) were obtained when crop was sown in 150 cm apart trenches while seeding density of 60,000 DBS ha^{-1} produced the highest number of tillers m^{-2}(14.46). Maximum millable canes ha^{-1}(99763) were produced when crop was sown in

120 cm apart trenches while seeding density of 90,000 DBS ha^{-1} produced highest millable canes ha^{-1} (117443). Highest stripped cane weight (1.00 kg) was produced by planting of sugarcane in 150 cm apart trenches. Seeding density of 75,000 DBS ha^{-1} produced canes with highest stripped cane weight of (1.01 kg). Highest cane yield of 92.26 t ha^{-1} was produced by the crop sown in 120 cm apart trenches. As regards seeding density, seed rate of 75,000 DBS ha^{-1} gave maximum cane yield of 93.57 t ha^{-1}.

Improving estimation of commercial sugarcane yields in south Florida

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Accurate estimation of sugarcane (*Saccharum* spp.) crop yield is crucial for the Florida industry for both individual field harvest scheduling and industry-wide crop forecasting. The objective of this research was to compare the prediction accuracy of three yield-estimating modeling approaches: 1) Canegro, a dynamic crop growth and development model; 2) Radiation Use Efficiency (RUE), a mechanistic model to estimate crop growth; and, 3) Ordinary least squares regression with dummy variables (OLS), a linear growth response for estimating sugarcane yield. Parameters were developed or obtained from the literature where needed to adapt the models to south Florida environmental conditions and use the commercial cultivars CP 72-2086, CP 80-1743, CP 88-1762 and CP 89-2143. The model estimates were compared to observed commercial yield on a field by field basis from 9,511 harvested fields over an 11 year period from 1993 – 2004. The prediction error was calculated using each method by crop harvest year, cultivar, ratoon age and land type. There was no significant difference between predicted and observed average cane yield across the aggregated data set using the OLS model. The Canegro model under-predicted overall cane yield by an average 5.2% over the 11 years and the RUE model under-predicted overall sugarcane yield by 9.2% over the same period. The OLS method over-predicted sugar yield by 1.6% on average over the 11 years while the Canegro method under-predicted sugar yield by 38% and the RUE method over-predicted sugar yield by 5.5%. The dynamic crop model Canegro and RUE model tended to predict yields of CP 72-2086 more closely than the other 3 cultivars. Our results indicate that the OLS model was more accurate than the dynamic crop models developed from independent datasets. A coupled model approach incorporating the dynamic crop model approach with OLS is being tested to improve model performance on an individual field basis. This research contributes to the knowledge of using mathematical approaches to estimate the sugarcane crop and will potentially have value for production management in the Florida sugarcane industry.

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The CASUPRO Sugarcane Simulation Model

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The application of simulation models in the sugar industry has constituted an important strategy in some cane-growing areas in the world. These have helped improve understanding of complex processes related to cane and sucrose production as a result of water-soil-plant-climate interactions. As the world-wide importance of the sugarcane crop grows, so does the incentive to improve production decision making. Researchers from CENICAÑA Colombia, EMBRAPA Brazil and the U.F. Agricultural and Biological Engineering crop simulation modeling lab have joined together in developing CASUPRO, a sugarcane simulation model based on the widely used DSSAT CROPGRO template, and incorporating methods and features not found together in other available sugarcane growth and development models:

- Primary focus on simulating the “plant" crop
- Stalk-based phenology and morphology
- Mechanistic approach to stalk (tiller) population, based on light interception and competition between tillers
- “Tons fresh cane” as a direct output
- Simulates stress from water, phosphorus and nitrogen limitations, and temperature extremes

The objective of CASUPRO is to be an effective tool for commercial sugarcane production management with regard to climate impacts, allocation of irrigation and fertilizers, analyzing yield variability, estimating costs of production, harvest scheduling and yield forecasts. To more readily achieve worldwide scientific and commercial acceptance and applicability, the CASUPRO project has been collaborative, multinational and non-proprietary. After several years of development, the first sequence of systematic parameterization is underway, using field experiment data from Florida, Colombia and Brazil. The purpose of this presentation will be to report the first results of the CASUPRO model calibration and validation.

Artificial Neural Network Model for Predicting Yield in Sugarcane Seedling Populations

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Artificial neural networks (ANN) are mathematical models based on biological neural networks. ANN is a supervised learning method and uses pattern learning from a training data set which is a sub-sample of the whole data to produce predictions of response variables. We demonstrate the potential of ANN models as a tool for selection in sugarcane. Cane-yield components, namely, stalk number, stalk height, and stalk diameter, were measured on individual seedlings and used as predictor variables to produce a selection decision (reject or select a seedling) based on the ANN model. Compared with the currently used visual method of selection, the difference in cane yield between the mean of the selected and rejected seedlings was greater for seedlings selected by the ANN model. The difference increased when similar selection intensity was applied to both selection methods. Compared to the visual method, the ANN model selected fewer seedlings with cane yield lower than the population mean, and rejected fewer seedlings with cane yield higher than the population mean. The ANN model compels the breeder to consider all traits simultaneously when deciding whether to select or reject a clone. This is likely more efficient than judging the merits of a clone by considering each trait independently or collectively in a serial manner. It would be impractical to collect data on each individual seedling before selection. These measurements could be taken on a subset of seedlings within each family and the ANN model used to determine selection rates to be applied in selecting each family.

Sugarcane modeling applied to Brazilian production systems: Parameterization and Evaluation of Predictions of DSSAT/CANEGRO

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Sugarcane has a great social importance in Brazil and is one of the most important commodities in Brazilian agribusiness, contributing to the energy and food security of the country, as sugar, ethanol and biomass energy are produced from sugarcane plantations. Crop simulation models may enhance our understanding of the effects of projected climate scenarios on sugarcane yield to improve crop monitoring activities, yield forecasting, and decision making. To utilize an existing model for this crop, the main physiological parameters controlling growth and development must be known, the model must be parameterized, and its predictions evaluated. The DSSAT/Canegro model was analyzed for Southern Brazilian production systems based on measurements on varieties SP83-2847 and RB72-454 in four different sites, one of which had both irrigated and rainfed treatments. The model was parameterized using the principle of data splitting by leaving-one-out cross-validation method in order to simultaneously include the full variability of environmental conditions and field measurements in the estimation of parameters and independent evaluation of model predictions. Eleven parameters were optimized using the Generalized Likelihood Uncertainty Estimation (GLUE) method using 3000 random samples in two steps: 4 phenological parameters (plant, tiller and canopy phenology), and 7 growth parameters (partitioning, sucrose accumulation and leaf expansion). Model predictions were evaluated in terms of the following output variables: LAI, stalk and aerial dry mass (STKH and AELH), sucrose content (SUCH) and number of green leaves (Leaf#), all analyzed in terms of modeling efficiency (ModEff), and Willmott agreement index (d). Base temperature and phyllocron interval were entered based on experimental data values. The ModEff values were STKH=0.769, SUCH=0.626, AELH=0.915, LAI=0.256, and Leaf#=-2.793, and d-index: STKH=0.936, SUCH=0.800, AELH=0.971, LAI=0.846, and Leaf#=0.528. The robust performance of the model, especially in simulating STKH and SUCH, confirmed that the DSSAT/Canegro model is capable of simulating sugarcane production in Brazil.

A High-Throughput *In Vitro* Propagation System for Sugarcane Using Apical Meristems

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Conventional propagation of sugarcane (*Saccharum spp.*) using seed cane pieces with bud sets as a starting material is limited by the slow propagation rate and the potential transmission of pathogens from the seedcane to the subsequent crop. Micro-propagation through tissue culture offers a competitive advantage over the conventional method because large quantities of disease-free planting material can be produced in a relatively short period of time. The present study provides a method of direct shoot organogenesis of sugarcane using shoot apical meristems as explants. Apical meristems are recovered from surface sterilized sugarcane tops and cultured in Murashige and Skoog (MS) liquid media supplemented with sucrose (20 g/L), and the growth regulators benzylaminopurine (BAP) (0.9 mg/L) and α-naphthylacetic acid (NAA) (1.86 mg/L) for shoot initiation. After 21 days of culturing, the initiated plantlets are transferred to shoot proliferation media containing MS, sucrose (20 g/L), kinetin (2.0 mg/L) and NAA (2.0 mg/L). Multiplying shoots are then separated and sub-cultured at 3-week intervals until enough plantlets are obtained. Plantlets are transferred to rooting media composed of MS basal salts, sucrose (20 g/L), and indole-3-butyric acid (IBA) (2.0 mg/L). The *in vitro* grown plantlets are later hardened in the greenhouse, where a survival rate of 81% has been recorded after 6 weeks. The present *in vitro* tissue culture system provides a simple, high-throughput method of sugarcane propagation. It enables the rapid and mass generation of disease-free planting stock, thus eliminating the need for field collection of plant materials.

Comparing the Natural Spread of *Sugarcane yellow leaf virus* in Florida to Its Natural Spread in Louisiana

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The incidence of sugarcane infected with *Sugarcane yellow leaf virus* (SCYLV) is reported to be higher in Florida than in Louisiana. Six Florida commercial cultivars (CP 72-1210, CP 72-2086, CP 78-1628, CP 80-1743, CP 88-1762, and CP 89-2143) and seven Louisiana commercial cultivars (CP 70-321, LCP 85-384, HoCP 85-845, HoCP 91-555, Ho 95-988, HoCP 96-540, and L 97-128) were planted in 2003 at two USDA, ARS research locations, the Sugarcane Field Station, Canal Point, Florida and the Sugarcane Research Laboratory, Houma, Louisiana, to compare the spread of SCYLV at one location to the other using the same experimental design and a common source of seed cane. Incidence of SCYLV infection was determined among leaf samples collected in plant-cane, first-ratoon, and second-ratoon crops in Florida and in plant-cane and first-ratoon crops in Louisiana using leaf print immunoassay and reverse transcriptase-polymerase chain reaction (RT-PCR), respectively. At the Florida location, the incidence (% of total samples analyzed) of SCYLV-infected plants among the Florida (13.4%) and Louisiana (12.9%) cultivars was similar. At the Louisiana location, the incidence of SCYLV-infected plants was greater in the Florida cultivars (6.8%) than in the Louisiana cultivars (1.8%). In a repeated experiment initiated in 2004 in Louisiana, the incidence of SCYLV-infected plants was very low (<2.0 % overall), even though samples were collected through the third-ratoon crop. The highest incidence of SCYLV-infected plants was among the Florida cultivars was CP 72-1210 and CP 88-1762 at the Florida location and CP 79-1210 and CP 78-1628 at the Louisiana location. The highest incidence among the Louisiana cultivars was LCP 85-384 and HoCP 96-540 across the two locations. Under Florida conditions, the overall susceptibility of the Louisiana set of cultivars does not appear to differ from that of the Florida set of cultivars suggesting that the Louisiana set is no less susceptible than the Florida set. Possible non-genetic explanations for why the incidence of SCYLV infections is greater in Florida than in Louisiana need to be explored including differences in environmental conditions and virus-vector efficiency.

Leaf Scald Resistance Screening in Sugarcane using a qPCR Approach

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Sugarcane is adversely affected by systemic diseases caused by fungi, viruses, and bacteria. One of the most important systemic bacterial pathogens of sugarcane is *Xanthomonas albilineans* (Xa), the causal agent of leaf scald. Severe losses of cane and juice quality and indirect costs associated with the elimination of promising cultivars in breeding programs have been reported. The best control method is the development and planting of resistant cultivars. However, erratic disease expression makes conventional screening a difficult task. It has been demonstrated that the bacterial population at the shoot apex is correlated with resistance. Therefore, a quantitative polymerase chain reaction (qPCR) assay was developed and evaluated as a potential alternative method for determining clone resistance levels. Xa populations determined with SYBR green qPCR for two resistant and two susceptible cultivars were found to be similar to results comparing disease incidence, severity and vascular infection. Differences in resistance levels were most obvious when the Xa population in the TVD-2 leaf was compared. Susceptible cultivars HoCP 89-846 and HoCP 85-845 contained a high bacterial population while the resistant cultivars, LCP 85-384 and Ho 95-988, had a low bacterial concentration in both greenhouse and field experiments. Subsequently, a Taqman probe qPCR was developed and used to determine and compare Xa populations in inoculated plants in the greenhouse for 16 cultivars with a range of resistance levels to leaf scald. Xa populations varied among the different cultivars, and bacterial populations were generally associated with the resistance ratings determined previously in field experiments with inoculated plants. The results will be compared with Xa populations determined in a field experiment. The findings suggest that bacterial population quantification by qPCR might be used to identify resistant and susceptible cultivars and might serve as an alternative method for leaf scald resistance screening using leaf tissue from new emergent leaves.

Evaluation of Brown Rust Resistance in the Louisiana Basic Breeding Program's First Clonal Trial

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Over the past decade, the Louisiana sugarcane industry has experienced increasing levels of pressure from brown rust (*Puccinia melanocephala)*. In 2000, an epidemic spread throughout the Louisiana industry, severely affecting the state's top yielding variety, previously resistant LCP 85-384, which at the time comprised roughly 71% of the state's sugarcane acreage. The variety was replaced by other resistant varieties, such as Ho95-988, HoCP 96-540, and L99-226. Unfortunately, most of the new varieties also became susceptible to brown rust within a few years after release. In the spring of 2009, a severe rust epidemic developed on the USDA, ARS, Sugarcane Research Laboratory's (SRL) research farm in Schriever, Louisiana. In an attempt to identify new, and more durable sources of brown rust resistance among our breeding population, 2125 wide crosses and early-generation backcrosses were rated for resistance to the disease. These clones were part of the first clonal stage of the SRL's basic breeding program and were diverse in ancestory. Clones were derived from 57 male parents, 81 female parents, and 144 cross combinations. Clones were rated for resistance on a scale of zero to nine (0=no rust visible; 9=completely brown leaves); the average rust score was 3.0. Commercial standards included in the trial were HoCP 96-540, L01-283, HoCP 00-950, and L99-226, and they had average rust ratings of 3.7, 1.8, 3.0, and 4.5, respectively. Ten percent of the basic breeding clones evaluated were rated a 1.0 for rust (very minor flecking), followed by 28.0, 26.5, 21.5,

8.2, 4.4, 1.0, 0.3 and 0 percent for ratings of 2 – 9, respectively. Significant differences (α=.05) were observed between different cross combinations. When advancements were made to the second clonal stage, rust ratings were taken into account. The average rust rating was shifted slightly toward resistance to brown rust in the next stage of selection, and individuals with rust ratings greater than 4 were dropped from further testing with few exceptions (if they contained another trait of interest). Percentage of individuals advanced to the second clonal stage of the program included 13.0, 35.8, 29.6, 14.7, 4.9, 1.6, 0, .3.0, and 0 for ratings of 1-9 respectively. Results from this study will help in the selection of parental material for breeding future rust-resistant varieties.

Isolation and Characterization of Resistant Gene Analogs from RSD Resistant and Susceptible Sugarcane Clones: A Progress Report

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Ratoon stunting disease (RSD) can decrease sugarcane yield by 5 to 15% if not properly managed. Currently, the disease is primarily managed by two approaches: breeding for RSD resistance and through sanitation, i.e., cleaning equipment and a clean seed program. Understanding the molecular basis of disease response would provide critical information for breeders and pathologists to efficiently develop cultivars resistant to RSD. Resistant gene analogs (RGAs) have been isolated from many crops and are found to be associated with disease resistance gene(s). The aims of this study were to isolate and characterize RGAs from the sugarcane cultivar LCP 85-384 and to identify single nucleotide polymorphism (SNP) DNA marker(s) among the RGAs that appear to be associated with RSD response. Based on real-time PCR and tissue-blot immune binding assays, we identified two distinctive groups of clones, one which was RSD resistant and the other RSD susceptible, from a mapping population that was produced by self-pollination of LCP 85-384. From these two groups, we selected five resistant and five susceptible clones that shared several other phenotypic traits, including plant height, stalk number, sugar and fiber content, etc., thereby maximizing the level of genetic similarity among these clones except RSD response. A total of 25 primer pairs were used, 19 of which were taken directly from RGA publications on four other crops, namely, rice, maize, soybean, and cotton. The remaining six were designed based on reported sugarcane RGAs. All primer pairs were used to amplify RGAs following a standard PCR procedure on genomic DNA samples of LCP 85-384 along with the five resistant and the five susceptible clones. Amplified RGA products, along with DNA size standards, were separated in 1.5% agarose gels by electrophoresis, stained with ethidium bromide, visualized under UV lights, and photographically documented. Discrete bands within the size range of 350 to 600 base pairs were gel purified and cloned into the Invitrogen's pCR4-TOPO vector, and the recombinant plasmids were sequenced. To date, we have isolated 10 RGAs from one resistant self progeny of LCP 85-384 and found that the DNA sequences of these RGAs were homologous to reported RGAs. Cloning of RGAs from LCP 85-384 and the rest of resistant and susceptible clones will allow us to determine the mode of transmission of RGA and to identify any potential SNP marker differentiating between RSD resistant and susceptible clones.

Identification of RAPD Marker Associated With Brown Rust Resistance in Sugarcane

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Susceptibility to brown rust caused by *Puccinia melanocephala* is a major reason for the withdrawal of sugarcane cultivars from production. An efficient way to control the disease is to breed cultivars with durable resistance. Our aim was to identify random amplified polymorphic DNA (RAPD) markers that associate closely with brown rust resistance using a mapping population derived from self-pollination of the Louisiana cultivar LCP 85-384. Two bulked DNA samples were generated, one from five clones that were rated highly resistant (R) and the other from five clones rated highly susceptible (S) to the brown rust based on field ratings of disease symptoms. Additionally, the 10 clones were selected because they shared several other phenotypic traits, i.e., plant height, stalk number, sugar and fiber content, etc. A total of 319 RAPD primers were screened by polymerase chain reaction (RAPD-PCR) on the bulked DNA samples along with the DNA of LCP 85-384 for the positive control and sterile water for the negative reaction samples. The amplified RAPD-PCR products were separated by electrophoresis in 1.5% agarose gels, stained with ethidium bromide, visualized under UV lights, and photographically documented. Although 312 primers produced similar banding patterns, seven primers, i.e., AM-14, AM-19, L-18, N-17, Y-5, AO-15, and N-6, produced DNA bands that were discriminative between the resistant and susceptible bulked samples. RAPD-PCR reactions were repeated three times on these seven primers to validate these findings. One of these discriminative RAPD-PCR products, a 450 base pair-long DNA band, from the primer N-06 was produced from all five resistant clones as well as LCP 85-384, but not from the five susceptible clones. We are in the process of cloning and sequencing this DNA band to begin designing a sequenced characterized amplified region (SCAR) marker that is reliable and reproducible.

A Comparison of Orange Rust Epidemics in Florida during 2008 and 2009 and Their Influence on Yield

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Orange rust, caused by the fungal pathogen *Puccinia kuehnii*, was first detected on sugarcane in Florida in June 2007. During 2008 and 2009, epidemiological studies were initiated to monitor orange rust and to determine its potential impact on yield. All observations were made on plant-cane in replicated field trials planted to the highly susceptible variety CL 85-1040. A comparison of epidemics during these two years revealed that orange rust reached a threshold

level of 5% on the top-visible-dewlap leaf minus four during May of 2008, but did not reach this threshold until July during the 2009 cropping season. During both years, rust severity levels of 30-40% were eventually reached and maintained throughout the duration of the crop. Analyses of yield loss components during the respective years indicate significant differences in levels of yield loss incited by orange rust. Stalk biomass and sucrose levels were significantly impacted during both 2008 and 2009, but stalk populations were affected only during 2008. Presumably, this was due to the delay in the rust epidemic during 2009. Potential factors influencing the initiation and duration of the respective epidemics will be discussed.

Evaluation of New Energy Cane Varieties for Louisiana

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Renewed interest in U.S bioenergy markets may offer an alternative source of income for Louisiana sugarcane growers. High-fiber sugarcane or energy cane varieties are currently being developed. As with traditional sugarcane varieties, energy-cane varieties need to be evaluated across the sugarcane belt on commercial farms with different soil types and agronomic practices. Three candidate energy cane varieties Ho 99-51, Ho 02-113 and Ho 03-48 were evaluated over four years, from plant cane (2005) through third ratoon (2009), at Schriever, LA and Welsh, LA. In a second experiment initiated in the fall of 2008, the experimental energy cane varieties US 72-114, Ho 01-07, Ho 02-144, Ho 02-147, Ho 06-9001 and Ho 06-9002 were evaluated in the first year (plant cane crop) at Centerville, LA and Vacherie, LA. For comparison, two commercial energy cane varieties were included in the experiments as standards; L 79-1002 at Schriever and Welsh and Ho 00-961 at Centerville and Vacherie. Net cane yields (tons/acre) were determined by harvesting cane with a chopper harvester whose extractor fan was turned off. A sample of 10 stalks was randomly collected from each plot prior to harvest to determine sugar and fiber yields. Ho 02-113 produced the greatest total fiber and sugar yield (21 dry tons/acre) averaged over four years at Schriever and Welsh. This variety also had the greatest average fiber (15 tons/acre) and sugar yield (6 tons/acre). In the second experiment, US 72-114 produced the greatest total fiber and sugar yields (25 tons/acre). Ho 01-07, Ho 06-9001 and Ho 02-147 had fiber and sugar yields of 24, 22 and 20 tons/acre, respectively. US 72-114 had the greatest fiber yield (17 tons/acre), but the greatest sugar yield (10 tons/acre) was produced by Ho 01-07. Of the varieties tested, Ho 02-113, US 72-114, Ho 01-07, Ho 02-147 and Ho 06-9001 appear to be the best varieties for biofuels production in Louisiana. Based on the results of the long term study, at least one variety, Ho 02-113, could be released in 2010.