Specificity and Plant Extract Inhibition of qPCR for *Xanthomonas albilineans*

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Leaf scald, caused by *Xanthomonas albilineans*, is one of the major diseases of sugarcane worldwide. Leaf scald can cause high losses in cane tonnage and reduce juice quality in susceptible varieties. Host plant resistance is the primary control measure. Traditionally, resistance evaluation has been based on rating symptom severity after inoculation with the pathogen; however, erratic symptom expression makes visual rating an inconsistent method to measure resistance to the disease. For that reason, there is a need for a more sensitive and rapid method for quarantine and resistance research. Previously, a quantitative polymerase chain reaction (qPCR) was developed with demonstrated potential for resistance screening. However, only four varieties with extreme reaction against the disease (two highly susceptible and two highly resistant) were compared. Therefore, additional research was needed to demonstrate assay specificity and determine whether inhibitors of pathogen amplification are present following the extraction of DNA from plant tissue. The qPCR was tested against DNA from nine bacteria isolated from sugarcane sap, a common fungus epiphytic on sugarcane, *Cryptococcus albidus*, and two other bacteria, *Xanthomonas oryzae* and *Burkholderia gladioli*. The sugarcane associated bacteria included two pathogens, *Leifsonia xyli* subsp. *xyli* and *Herbaspirillum rubrisubalbicans*, and three unidentified bacteria that grew on the semiselective media used for the *X. albilineans* isolation. The results showed high specificity; the qPCR amplified only *X. albilineans* isolates. The plant extract inhibition test included sap from four varieties of sugarcane and five concentrations of *X. albilineans* DNA diluted in sap from each variety. Changes in the critical cycle threshold (CT) values were always lower than one demonstrating that extracts obtained from sugarcane leaves did not inhibit the qPCR. The high specificity and absence of inhibition provide additional evidence that qPCR can be a useful method for pathogen quantification and the study of leaf scald resistance.

Seedling Inoculation for Cross Appraisal of Brown Rust Resistance

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Brown rust, caused by *Puccinia melanocephala*, is an important disease of sugarcane. The primary control measure is breeding, selection, and cultivation of resistant cultivars. Selection is based on ratings determined from comparisons of disease severity resulting from natural infection. However, accurate determination of resistance levels in clones in response to natural infection can be problematic due to variability in disease severity between seasons and erratic exposure to inoculum within seasons. Selection for resistance at the seedling level is uncommon in breeding programs. Natural infection is unreliable for the identification of susceptibility
because rust severity tends to be low in seedling blocks, and seedlings are not uniformly exposed to inoculum. Inoculation of seedlings under controlled conditions was evaluated as a potential method for cross appraisal of brown rust resistance. The focus of the study was seedling inoculation methodology to determine if it could provide accurate ratings for individual seedlings and resistant progeny frequencies useful for cross appraisal. Sixteen crosses between parents with different levels of resistance were inoculated with increasing concentrations of urediniospores from \(10^3\) to \(10^6/\text{ml}\). Plants grown in the greenhouse were inoculated under moisture and temperature conditions highly favorable for infection then placed on lighted shelves at 23 C. A resistance rating was assigned to each seedling using a modified 1 to 9 scale based on a visual estimation of the number of lesions per leaf. Inoculation with a urediniospore concentration of \(10^5\) with disease severity ratings assigned at 2 weeks provided segregation of resistant and susceptible seedlings closest to what would be expected from the cross type. However, inoculum concentration strongly affected disease severity and the frequency of progeny rated resistant in crosses. Brown rust resistance is a moderately heritable trait. However, it was possible to overwhelm resistance in seedling populations with inoculation under controlled conditions, and cross type was not a consistent predictor of progeny distribution across resistance categories even under optimized conditions. These results suggest that seedling inoculation under controlled conditions is not suitable for cross appraisal for brown rust resistance.

**Development of Orange Rust of Sugarcane in Louisiana**

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Orange rust of sugarcane was observed for the first time in the Americas in 2007 in Florida. Urediniospores of *Puccinia kuehnii*, the causal agent of orange rust, were collected in aerial traps located in Louisiana in 2010; however, the first observation of disease symptoms in the state was not made until June 2012 on cultivar HoCP 05-961. The identity of *P. kuehnii* was verified using the species-specific qPCR assays. Seed cane increase plots of this newly released cultivar were surveyed, and orange rust was detected in 17 of 38 (45%) fields. Initially, disease incidence and severity were low, increasing gradually throughout the growing season, becoming severe in November at two locations. Observations of orange rust in 2012 were limited to HoCP 05-961. Studies have been designed to monitor the development of orange rust throughout the 2013 growing season and to determine what effect orange rust has on sugarcane yield.
Sugarcane Orange Rust in Florida: Its History, Current Status, and Management

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Orange rust, incited by \textit{Puccinia kuehnii}, was initially observed in Florida in 2007, the first report of this disease in the Western Hemisphere. During that initial growing season, it was notable only on commercial varieties CP80-1743, CP72-2086, and CL85-1040. Together, these varieties accounted for approximately 34\% of the Florida acreage. By 2008, two more important varieties, CP78-1628 and CP83-2143, had succumbed to the disease, and in 2009, CP88-1762 demonstrated susceptibility. Thus, percentages of susceptible acreages rose from 34 to 52, to 71, over the space of three years. Replicated yield losses trials demonstrated losses as high as 53\% on the extremely susceptible CL85-1040, with the industry completely dropping this variety from commercial production. Orange rust has since spread throughout the entire Western Hemisphere, being reported in a majority of sugarcane producing countries, including Brazil, the world’s largest sugarcane producer. In Florida, observations have revealed that the duration and severity of orange rust epidemics seems to be correlated with the occurrence or absence an area-wide frost event during the winter months. Such events significantly limit the availability of viable inoculum. While the Florida industry is presently struggling to replace susceptible varieties with more resistant varieties, registrations for fungicides belonging to two different fungicide classes, the strobilurins and triazoles, have provided growers with an interim management tool. Currently, breeding efforts at the USDA Sugarcane Field Station at Canal Point have incorporated a rapid screening technique for orange rust into their program, with the aim of limiting the influence of this important disease.

Developing Disease Resistance in CP-Cultivars

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Disease resistance is an important selection criterion in the Canal Point (CP) Sugarcane Cultivar Development Program. Ratoon stunt (RSD, caused by \textit{Leifsonia xyli} subsp. \textit{Xyli} Evtsuhenko \textit{et al.}), leaf scald (caused by \textit{Xanthomonas albilineans} Ashby, Dowson), mosaic (caused by \textit{Sugarcane mosaic virus} strain E), smut (caused by \textit{Sporisorium scitamineum} (Syd.) M. Piepenbr., M. Stoll & Oberw.), brown rust (caused by \textit{Puccinia melanocephala} H. & P. Sydow) and orange rust (caused by \textit{Puccinia kuehnii} E.J. Butler), are economically important diseases in Florida. Reactions to natural infection to all diseases except RSD begin in the Seedling (first) selection stage and continue in subsequent stages. Artificial inoculations begin in the third selection stage (Stage 2, with 1500 clones) and Stage 3 (135 clones) for RSD, brown rust, and orange rust. Artificial inoculations for leaf scald, mosaic, and smut are applied to the most promising clones in the final year of Stage 3 (40 clones) and continued on all clones in Stage 4 (18 clones). Selections of clones for disease resistance and tolerance are based on both natural infections and artificial inoculations. For most diseases, only resistant and moderately resistant...
clones are advanced to the next stage, except that sometimes clones with high sucrose yields and moderately susceptible ratings for RSD and other diseases with no natural infection in the fields are also selected. Also, there is an increased willingness among growers to advance clones that are moderately susceptible to brown or orange rust from Stage 3 to Stage 4 and Stage 4 to release, because growers have fungicides to control the spread of these two diseases. Data of artificial inoculations for different diseases from several years are compiled and compared to determine if inoculations techniques and/or selection practices require improvements. Data for RSD show that the CP 06 series in Stage 4 had more resistant clones than the CP 07 and CP 08 series. However, the CP 08 series in Stage 4 had more moderately resistant clones and less susceptible clones than the CP 06 and CP07 series. The CP 08 series in Stage 4 had a total of 70% resistant and moderately resistant clones compared to 50% in the CP 06 series and 47% in the CP 07 series. These variations could be results of escapes. All the series in Stage 4 had improved resistance to leaf scald than in Stage 3. The percentage of mosaic and smut resistant clones in Stage 3 increase and Stage 4 had no significant (P = 0.05) difference. This complex program of disease evaluation enables the CP program to continuously monitor the disease status of its new cultivars and to breed for improved disease resistance and tolerance while maintaining high yields.

A Historical Perspective on Cold Tolerance in the Canal Point Sugarcane Breeding Program

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Developing sugarcane cultivars with greater freeze tolerance is an important objective of the USDA-ARS Canal Point breeding program, as temperatures around -3°C (27°F) cause serious sugar yield losses during the harvest season. To estimate if progress was made in freeze tolerance by breeding for cane and sucrose yields, a set of 58 historical sugarcane cultivars, released from 1920 to 2008, were planted on 27 Jan. 2011 at Hague, FL (lat:29°45’0”/long:82°25’48”)) and exposed to subzero temperatures (T_C). A non-replicated 5x15 augmented α-lattice design was adopted, with six replications of CP 89-2143 as a reference for freeze tolerance. The sucrose (commercially recoverable or CRS, in g kg^{-1}) profile was monitored four times in the plant cane and first-ratoon crop-years, with the first samples taken before a freeze occurred in each year. The two-year dataset was analyzed in two steps, with variance components and adjusted cultivar means estimated first for individual sampling dates and later submitted in a combined analysis to assess the cultivar x environment interaction (CEI). Adjusted means were also regressed on years of release to obtain the gains in freeze tolerance due to breeding. Starting with the 2nd sampling, the cultivars were exposed to -2°C/28°F (6hrs), -7.7°C/18°F (4hrs), and -3°C (7hrs) in the plant cane, and to -1.5°C/29°F (7hrs), -3°C (8hrs), and to -1.5°C (8hrs) in the first-ratoon crop. The CEI effect was highly significant but not substantial, with the E and C main effects contributing 45% and 17% more to the variation in CRS, respectively. Gains in CRS improved at a rate of 0.41 g kg^{-1} yr^{-1} (P<0.001) after exposure to T_C ≥-3°C and at a rate of 0.46 g kg^{-1} yr^{-1} (P<0.001) to T_C < -3°C. These results indicate that efforts to increase sucrose and cane yields in the CP program have been accompanied with a significant improvement in adaptation to mild and harsh freeze conditions. The genetic component and its implications for further progress will be
Traditionally, the cooperative sugarcane (Saccharum spp.) breeding program located at Canal Point has selected genotypes exclusively on muck soils in the early to middle stages of the program. Only about 0.20% of genotypes are ever tested on sand, resulting in the possibility that many sand-adapted genotypes are discarded. The objective of this study was to determine the presence of genotype by soil interaction on muck and sand soils, amongst sugarcane genotypes in the second clonal selection stage. A significant genotype by soil interaction for important yield traits would warrant the selection of genotypes on sand soils at earlier stages.

The trials were planted on a Torry muck soil at the USDA-ARS Sugarcane Field Station in Canal Point, Florida in 2006 and 2007 and on a Margate sand soil at U.S. Sugar Corporation’s Townsite farm near Clewiston, Florida in 2007 and 2008. Limited seedcane availability necessitated planting the same series (2006 and 2007) in different years at different sites, resulting in a confounding of location with year for each series. The new genotypes were planted in unreplicated plots, except for a small subset which had two replications. Each trial included replicated plots of the check cultivars CP72-2086, CP78-1628, and CP89-2143. Check cultivars and new clones were analyzed for average stalk weight, number of stalks per acre, tons of cane per acre, theoretical recoverable sucrose, tons of sucrose per acre, commercially recoverable sucrose, Brix, and percent sucrose.

Genotype by soil interaction was modeled using the check cultivars. Within either location, a significant genotype by year effect was present for only two of the eight traits measured. In 2007, the year when checks were grown in both locations, a significant genotype by location effect was detected for five of the eight traits. These results suggest that significant genotype by soil interactions amongst the new genotypes are due more to differences between the soils than the years.

Significant interactions were detected amongst the replicated new clones for five of eight traits in the 2006 series, but only three traits in the 2007 series, likely due to the reduced power of a smaller number of clones. For all new clones, the Spearman (rank) correlation was calculated for each trait across locations. All correlations were statistically significant, but the values were low, ranging from 0.17 to 0.38. The exception was stalk weight, which was moderately correlated (0.50) between locations in both sets. These data indicate that clones that are highly ranked for a trait in one location may not be highly ranked in another. Taken together, our results strongly suggest that the Canal Point breeding program should begin genotype selection on sand at earlier stages.
stages in order to increase the likelihood of identifying superior cultivars for these soils.

**CP 89-2143  New Sugarcane Cultivar for the Rio Grande Valley of Texas**


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2USDA-ARS, Sugarcane Field Station, Canal Point, FL  
3Florida Sugar Cane League, Clewiston, FL  
4Rio Grande Valley Sugar Growers Inc., Santa Rosa, TX

The Florida cultivar CP 89-2143 has proven to be better in tons sugar per acre (TSA) than the commercial checks CP 72-1210 and TCP 87-3388 in replicated plant cane outfield studies. In the combined analysis of three outfield plant cane studies, CP 89-2143 yielded 7.78 TSA, which was significantly better than CP 72-1210 and TCP 87-3388, yielding 6.55 TSA and 6.87 TSA, respectively. Grower plant cane of CP 89-2143 (132.57 acres/13 fields) averaged 6.68 TSA compared to CP 72-1210 (139.30 acres/13 fields) at 5.89 TSA and TCP 87-3388 (138.60 acres/3 fields) at 6.05 TSA. A grower field harvested the first week of March 2013 provided a good comparison. CP 89-2143 (9.20 acres) yielded 9.44 TSA, while CP 72-1210 (19.91 acres) yielded 7.17 TSA. Growers are rapidly increasing the acreage of CP 89-2143 with the 425.42 acres in 2012-2013 expanding to 1688.50 acres in 2013-2014. Details of the comparative studies will be presented.

**Core Selection from the Miami World Collection of Sugarcane and Related Grasses using Phenotypic Markers**


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Sugarcane (Saccharum spp.) breeders are looking for new sources of germplasm with desirable genes to overcome abiotic and biotic stresses and to increase the sugar content and yield potential for developing cultivars for sucrose and bioenergy. One untapped source of desirable genes would be the germplasm collection referred to as the “World Collection of Sugarcane and Related Grasses” (WCSRG) located at the USDA-ARS Subtropical Horticulture Research Station in Miami, FL, which is one of two sugarcane germplasm collections in the world. It is a collection of approximately 1,200 accessions from 45 countries including Saccharum germplasm with 20 different species, the most abundant being S. spontaneum, S. officinarum and S. hybrids. The objectives of this research were to evaluate accessions in the WCSRG phenotypically and then combine this phenotypic data with the genotypic data of colleagues to create a core collection representing most of the diversity of the WCSRG. By intense phenotypic evaluation of
each accession in the WCSRG, we would then have a reasonable means of identifying the full phenotypic potential of the full WCSRG. Phenotypic measurements taken of all accessions in the collection were: stalk height, stalk diameter, internode length, stalk color, percent flowering, leaf sheath pubescence, inner stalk aerenchyma and pith, Brix, and presence of Sugarcane yellow leaf virus. Phenotypic observations revealed high Brix in 18 S. spontaneum accessions, with a mean Brix of 14.7% compared with 9.0% for all of the spontaneums in the collection that contained extractable juice. We also found 5 Erianthus and one Miscanthus accessions with high Brix (mean = 14.2%). There were 649 plants (57.3% of the population) that had no serological detectable levels of Sugarcane yellow leaf virus as compared to > 99% of commercial sugarcane cultivars in Florida being susceptible to this disease. For core collection selection, data were analyzed using the M strategy and Shannon coefficients with MStrat software. There were 300 accessions, including at least one accession representing all the known species present in the WCSRG that were selected into the core collection. The Shannon Score for the selected core collection was 31.30 and the Shannon score for the complete WCSRG was 32.17. Thus, according to the Shannon scores, our core collection retains 97.30% (100x31.30 /32.17) of the diversity of the WCSRG. To learn more about the potential of the WCSRG to contribute to improvements in bioenergy and sugarcane breeding and to identify molecular markers associated with important traits, we made minor modifications to the core collection (deleted some distant species and replaced them with S. officinarums) to form a mapping panel. In the next years, extensive measurements will be made on the mapping panel. Based on yield, stress tolerance, and flowering information in the core collection, immediate attention will be given to identifying S. spontaneums with high Brix as useful parents and other parents among all the species for use as sources of resistance to Sugarcane yellow leaf virus.

Core Selection from the Miami World Collection of Sugarcane and Related Grasses Using Genotypic Markers-Seeking Alleles for Biomass

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Sugarcane (Saccharum spp.) architecture affects sugarcane biomass, which is an important component of sucrose yield. Sugarcane cultivar improvement programs have not yet systematically explored the genetic sources of yield potential that may exist in the Saccharum germplasm. The World Collection of Sugarcane and Related Grasses at the Subtropical Horticulture Research Station in Miami, FL (WCSRG) presumably contains numerous genes for sugarcane biomass improvement. The objectives of this study are to: 1) genotypically evaluate the WCSRG, 2) select accessions in the collection that plant breeders can utilize as a representative core collection, and 3) discover alleles contributing to biomass in Saccharum. In total, 1186 accessions in the collection, comprising 16 species, were evaluated using microsatellite markers. From 24 polymorphic SSR markers, 128 alleles were obtained, and diversity analysis showed that the WCSRG has a gene diversity of 0.276. The highest gene
diversity was found in *S. spontaneum* followed by *S. robustum*. Phylogenetic analysis of the 1186 samples indicated 8 clusters. *S. spontaneum* accessions tended to cluster together. *Saccharum hybrids* mostly clustered with *S. officinarum* indicating that the *S. hybrids* were more closely related to *S. officinarum*. A core collection of 300 accessions was selected that represented most of the genetic diversity of the WCSRG. This core collection will not only be useful in sugarcane breeding programs but also for genetic studies such as association mapping and genomic selection. Chromosome numbers of the *Saccharum* accessions in the core collection will be evaluated using flow cytometry. About 30 candidate genes involved in plant architecture have been selected for biomass allele discovery, mainly based on a literature search for keywords such as tillering, vegetative growth, leaf morphology, etc. In the near future, the candidate gene allele variance will be evaluated in the core collection for association analysis to identify desirable alleles contributing to sugarcane biomass yield.

**Developing a Reference Genomic Map for Sugarcane Resistance Gene Mapping**

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Genetic maps with positions of markers and known genes based on recombination frequency are an important tool for gene mapping and cloning, marker-trait linkage analysis, marker assisted selection, and other studies. Though efforts have been made towards sugarcane map construction for over a decade, the existing genetic maps are still far from saturated due to the features of large genome size, polyploidy, and heterozygosity of sugarcane. The objective of this research is to establish a high density genomic map for sugarcane resistance gene mapping, which can be utilized for efficiently mapping disease resistance and other traits for a marker-assisted selection approach in sugarcane breeding program. In this study, the complete sorghum genome was used as a reference to align available sugarcane SSR primers and plant disease resistance genes (\(R\) genes) for the reference genomic map construction. In total, 6,149 unique SSR primers were identified from a literature search, which were successfully amplified in sugarcane. In addition, 104,910 \(R\) genes were downloaded from the Plant \(R\) Gene Data Base, including 112 manually curated \(R\) genes. Of the 6,149 pairs of SSR primer sequences, 1,124 were aligned to the sorghum reference genome with less than two base pair mismatch. Of the 104,910 \(R\) genes, 35,926 were mapped to the sorghum genome and matched to 1,924 sorghum gene models. This reference genomic map of 10 chromosomes each has an average of 112 SSR markers and 192 \(R\) genes. With this map, SSR markers and \(R\) gene derived markers can be used to screen any population segregating for disease resistance, to quickly locate corresponding gene loci, and more markers in the targeted region can be designed for fine mapping the candidate genes. With this map, a population of 130 clones with segregating reactions to sugarcane orange rust will be used to associate markers with the resistance.
Silicon Fertilization: Soil Availability, Plant Uptake, Crop Yield and Stalk Borer Damage

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Silicon is a beneficial element that can enhance sugarcane production. The objectives of this study were to evaluate the effect of Ca and Mg silicate application on available Si in the soil, sugarcane yield and stalk borer (Diatraea saccharalis) damage in two different sugarcane cultivars. The experiments were carried out on plant cane from March, 2009 through August, 2010 and first ratoon from August, 2010 through August, 2011. The soil was a Rhodic Haplustox (soluble Si in 0.5 mol L\(^{-1}\) acetic acid and 0.01 mol L\(^{-1}\) Si-CaCl\(_2\) were 6.9 and 2.9 mg kg\(^{-1}\)Si, respectively). The experiment was a complete randomized factorial design with four replications, four Si rates (0, 55, 110 and 165 kg ha\(^{-1}\)Si) and two cultivars: IAC 86 3396 (tolerant to stalk borer) and SP 89 1115 (susceptible to stalk borer). All plots received the same amount of Ca and Mg using additional lime and/or MgCl\(_2\) when necessary. The silicate application increased soluble Si in the soil (0-25cm), and reduced stalk borer damage in plant cane. Si extracted by both extractants and stalk yield on SP 89 1115 cultivar were also increased with Si application whereas borer damage was reduced in the same cultivar on first ratoon. Silicon fertilization applied in furrow showed to be effective on increasing Si in soil, sugarcane yield and reducing stalk borer damage.

Sugarcane Yield and Water Quality Impact of Vinasse from Ethanol Production Used for Sugarcane Fertilization

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Sugarcane (\textit{Saccharum} spp.) is potentially a good energy crop for Florida because it is a tall-growing C4 perennial grass and it is the highest yielding biomass crop currently grown in Florida. Before sugarcane or other energy crops can be used as biofuel feedstocks, the impact of application of by-products of biofuel production on soil and water quality must be determined. In ethanol production, up to 20 liters of stillage (vinasse) may be generated for each liter of ethanol produced. Vinasse has high concentrations of organic matter, nitrogen (N), potassium (K), and salt. Vinasse can be a fertilizer source of N and K if applied at rates not adversely impacting land or water. The objectives of the study were to 1) compare sugarcane production using vinasse as the K source with that using a typical commercial source of K, and 2) evaluate soil leachate for water quality following application of vinasse and commercial fertilizers. Twelve large lysimeters (3 × 4 m) were constructed at each of two locations and filled with Immokalee fine sand (siliceous, hyperthermic Arenic Haplaquods) at the Immokalee location (Southwest FL Research and Education Center) and Lauderhill muck (euic, hyperthermic Lithic Haplosaprist) at the Belle Glade location (Everglades Research and Education Center). Sugarcane was planted with two rows per lysimeter (1.5 m apart) with CP 78-1628 and CP 89-2143 planted at
Immokalee and Belle Glade, respectively. The experimental design was a RCBD with four replications and three treatments: 1) control with dry soluble fertilizer, 2) vinasse as the K source at 100% of the soluble K rate (150 lb K₂O/acre) and dry soluble P and micronutrients, and 3) vinasse as the K source at 150% of the soluble K rate (225 lb K₂O/acre) and dry soluble P and micronutrients. Soluble N was also applied to the mineral soil (180 lb N/acre for treatment 1) in split applications, with treatments 2 and 3 being adjusted based on the N content of the vinasse. Vinasse from fermenting sugarcane molasses (Florida Distillers) was applied in two applications each year for two years at each location. For plant cane and first ratoon crops there were no significant differences in tons cane/acre or tons sugar/acre among treatments indicating that vinasse is an effective nutrient source and that there were no negative effects of the vinasse treatment on sugarcane growth or sucrose yield at the rates used. Soil leachate nutrient concentrations at each location were not greater than acceptable surface water standards for any treatment. There were no significant differences in leachate N, P, or K among treatments at either location. Yield of sugarcane using vinasse was comparable to that with soluble nutrient sources at both locations indicating short-term use of vinasse as a nutrient source is acceptable. Yield and water quality during repeated application of vinasse needs to be monitored to determine long-term viability of this practice.

Bermudagrass: Spring Weed Control Programs and Biotype Research

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Research conducted from 2008 through 2012 evaluated bermudagrass control with Sencor (metribuzin) and Command (clomazone) plus Direx (diuron). Averaged across experiments, bermudagrass was controlled 54, 41, and 43% four weeks after Sencor application at 3 lb/A in mid-February, early-March, and mid-March, respectively; Command plus Direx at 3.3 pt + 2.5 qt/A controlled bermudagrass an average of 75, 58, and 55%, respectively. Six weeks after the mid-February application, control was 34% for Sencor and 61% for Command plus Direx. Sencor did not injure sugarcane, but whitening/bleaching and height reduction was observed when Command plus Direx was applied after sugarcane had emerged. Sugarcane yield when herbicides were applied in mid-February was greater for Command plus Direx compared with Sencor (41.5 vs. 40.5 ton/A). For application in early March, sugarcane and sugar yield were greater for Sencor compared with Command plus Direx (45.4 vs. 41.5 ton/A and 11,330 vs. 10,350 lb/A). Variability was observed for bermudagrass control among experiments and was expected due to the inherent ineffectiveness of the herbicides and differences in bermudagrass infestation at application.

Observations from the weed control study in respect to variability in time of bermudagrass emergence following the winter dormant period and in growth characteristics prompted additional research. Bermudagrass was collected from 13 outfield locations used by the LSU AgCenter and the USDA-ARS for sugarcane variety field trials, from four additional farm sites, and from three LSU AgCenter research stations. Collected plants, designated as biotypes, were potted for use as planting material. In April of 2011, plants established in 2-in pots were
transplanted into field plots 5 × 5 feet in size. Two plants spaced 2 feet from one another were planted in the center of each plot. At 84 days after planting, bermudagrass ground cover was less than 40% for biotypes J (Pointe Coupee Parish), N (Iberia Parish), and T (Tensas Parish), but greater than 80% for biotypes A (St. Martin Parish), C and D (St. Mary Parish), F (St. James Parish), Q (West Baton Rouge Parish), and R (Iberville Parish). Bermudagrass biotypes with the greatest plant height were A, B (Iberia Parish), E (St. John Parish), and Q, R, and S (Rapides Parish) (8.1 to 12.8 inches); plant height for biotype I (Terrebonne Parish) was 3.7 inches. Total dry weight over two years was greatest for biotype A (657 g/plot) and biotype Q (623 g/plot); lowest dry matter yield was observed for biotypes J and T. In April of 2012, seedhead emergence was greatest for biotypes G (Assumption Parish), H (Lafourche Parish), I, and P (St. Mary Parish). Differences observed among the bermudagrass biotypes in growth characteristics and ability to establish may help explain variability in control and competitiveness observed in sugarcane fields in Louisiana.

Alternatives to Organophosphates for Wireworm Control in Sugarcane

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The organophosphate insecticides phorate and ethoprop are the current standards for wireworm control in the EAA. During the past six years, 2 pyrethroids and 2 neonicotinoids have been screened against wireworms in container studies. Thiamethoxam is the only alternative chemical screened that reduces damage to the levels seen when using phorate. However, the mortality rates of wireworms in the thiamethoxam treated containers were much lower than those in phorate treated containers.

Woody Host Plants of the Sugarcane Root Weevil (Coleoptera: Curculionidae) in Florida Sugarcane

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Laboratory and greenhouse studies were conducted to evaluate adult sugarcane root weevil (Diaprepes abbreviatus) residence (location), feeding damage, and oviposition on sugarcane and woody plant species in Florida. In the laboratory, more adults survived feeding on lime (Citrus aurantifolia) and Brazilian peppertree (Schinus terebinthifolius) foliage compared to sugarcane (variety CP 04-1252) in a no choice feeding test. Four sugarcane varieties and three woody plant species were evaluated in a free choice test in the greenhouse. Sugarcane varieties were CP 89-2143, CP 88-1762, CP 80-1743, and CP 78-1628, and three woody plant species were Brazilian peppertree, castorbean (Ricinus communis), and citrus (Citrus reticulata ‘Sunki’ × Poncirus trifoliata ‘Flying Dragon’). Adult residence was highest on Brazilian peppertree compared to the other species. Brazilian peppertree was the preferred adult feeding choice compared to the other species although the feeding damage was not significantly different from citrus. There was little feeding damage on castorbean and sugarcane cultivars. Oviposition was observed on all
sugarcane varieties and woody plant species with exception of castorbean. Brazilian peppertree had the highest number of egg masses followed by citrus. Leaf tissue analysis showed that feeding preference of adult weevils for Brazilian peppertree was due to higher tissue concentration of N, Ca, Mg, and Mn compared to the other species. Woody plant surveys showed that Brazilian peppertree and castorbean had the highest frequency of detection in Florida sugarcane. These results show that woody plant species, particularly Brazilian peppertree found associated with Florida sugarcane is suitable as a food source and oviposition site for adult weevils. Similarly, citrus is also preferred as a food source and oviposition site. In contrast, castorbean is not a preferred feeding and oviposition choice. Sugarcane is generally more preferred for oviposition compared to feeding by adult weevils. Therefore, prevention of encroachment of Brazilian peppertree near sugarcane fields should be a front-line of defense against the weevil in Florida.

**Mexican Rice Borer: Five Potential Mechanisms for Resistance Discovered**

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Studies have shown that there are at least five modalities for potentially achieving resistance to the Mexican rice borer (MRB) in sugarcane. 1) Drought stressed sugarcane accumulates 7 of the 9 detectable essential free amino acids for insect growth and development, thus increasing the nutritional value of the crop to the Mexican rice borer. As a result of these findings, the role of irrigation in MRB management has become widely accepted and efforts by several research institutions are underway to identify drought tolerant sugarcane varieties to use against the pest. 2) Soil quality was also shown to affect levels of MRB infestation whereby, although soil enriched with compost high in N increased numbers of stalks produced per sett, MRB infestation intensified to such a degree that this advantage was offset and yield declined. Hence, fertilization of soil with N should be monitored carefully in order to moderate the attractiveness of MRB to the crop. 3) Analyses of sugars in preferred host plants (both weeds and crops) of the MRB showed that preference is associated with relatively high levels of fructose rather than sucrose and glucose. Decreasing fructose content could diminish the crop’s vulnerability. 4) While it is widely believed that MRB prefer to lay eggs on dry leaves, folded or curled leaves are an overriding preference. This indicates that sugarcane breeding aimed at developing varieties that do not have curling leaf edges during drying will likely confer a high degree of resistance. 5) Transgenic Bt corn with the VT3Pro gene expressing the Cry1A.105, Cry2Ab2, and Cry3Bb1 insecticidal proteins was found to be almost completely resistant to MRB injury, conferring antibiotic protection. Use of transgenic Bt sugarcane with the same or similarly effective proteins might become a useful tool for sugarcane resistance.
New Findings for Improving Survey and Control of the Mexican Rice Borer

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The Mexican rice borer (MRB) was reported to infest 22 species of crop and noncrop plant hosts. Of five tested noncrop host plants of the Mexican rice borer, sudangrass was preferred for oviposition, followed by johnsongrass and barnyardgrass. Preference was associated with fructose, stem diameter, and numbers of numbers of senescing leaves. In terms of crop hosts, stem diameter and senescing leaves (with curls at the edges) were not associated with preference, but concentration of fructose appeared to be a factor. Corn is preferred to sugarcane by at least 5-fold, and as such, it might provide an avenue for the rapid dissemination of the pest from region to region. Also, when corn is harvested, typically months earlier than sugarcane, the MRB moves from the harvested corn into sugarcane and levels of infestation were shown to increase by 5-fold. Knowledge of alternate host preferences and the interaction with corn and sugarcane provide useful information for monitoring MRB populations as well as possible strategies for control if corn harvest can be accompanied by well-directed MRB control tactics. Further, a commercial field study showed that pheromone-based trap counts of adult MRB are associated with the short window of exposure of MRB larvae on the external surfaces of sugarcane plants, making them vulnerable to otherwise ineffective insecticide applications. The study demonstrated that MRB can, using this combination of a novel survey method and insecticide application, be maintained below the economic injury threshold using only one application of an insect growth regulator per season.

Preparing for the Mexican Rice Borer: Proactive IPM Research

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In preparation for the establishment of the Mexican rice borer (MRB), Eoreuma loftini (Dyar), in Louisiana sugarcane, the LSU AgCenter in collaboration with Texas A&M University and USDA-ARS has been working for greater than 10 years to develop an effective IPM strategy and monitor range expansion of this invasive pest. Recent research progress includes aerial application insecticide trials, varietal resistance evaluations, and pheromone trap monitoring. A two-year large plot insecticide trial in the Rio Grande Valley of Texas in 2009 and 2010 demonstrated the potential to use pheromone trap assisted scouting to improve timing of insecticide applications. Pheromone trap captures were directly correlated to larval infestations.
(r² = 0.71), and when timed according to our threshold, a single application of novaluron reduced MRB injury and increased sugar yield. The importance of application timing was further supported by greenhouse studies which suggest that larval exposure on plant surfaces is less than 1 wk. Results from an aerial application study in 2012 indicate that new chemistries, flubendiamide and chlorantraniliprole, may further improve insecticidal efficacy. Assessment of varietal resistance in Beaumont, TX revealed MRB injury varies considerably between cultivars with percentages of bored internodes ranging from 1.9–17.2% and 6.0–26.5% for 2011 and 2012, respectively. Pheromone traps are currently being used to document MRB range expansion and population distribution in Texas and Louisiana. A study which examined the effect of inter-trap distance on trap capture revealed these traps are capable of attracting moths from distances of up to 250 m. Based on the current MRB range as documented by pheromone trap captures, it is anticipated that the first larval infestations in Louisiana sugarcane will be observed in 2013.
Improvements of Raw Sugar Quality Using Double Purge of C-Massecuites

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Double purge of C – magma offers an opportunity to improve color of Raw Sugar with small capital and energy investments by reducing the recycle of final molasses to the first crystallization stage. The system can be implemented with minor changes to the traditional 3-boiling scheme, by addition of a centrifugation stage after the first centrifugation of C – massecuite. Little or no water is applied at this stage obtaining 79 – 81 magma purity. In the second stage, the magma is affinated producing 92 – 94 magma purity. The 64 to 67 wash molasses purity can be blended with B or A molasses. The higher purity magma is the footing for A and B massecuites. A comparison of results obtained during the 2012 season for two Louisiana factories processing cane from the same sources showed that the double purge system improved the raw sugar color approximately 50% (1,400 compared to 3,000, colors at pH 8.5 using 1.2 µm GF filter). Compared with previous years, the factory performance was not affected even though their grinding rates were higher.

Preliminary Investigation of Filter Station Operations

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The two important issues regarding filter cake in the production of raw sugar are that of pol losses and capacity. With the recent introduction of belt filters to the Louisiana sugar industry, there was a desire to review filter operations in general and compare belt filters with that of the traditional rotary drum filters. Preliminary results show that filter operations are highly variable. The capacity of belt filters is substantially higher than drums while sugar losses are comparable to those of drums.

Design and Implementation of a Very Short Retention Time Filtrate Clarifier

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Filtrate, which accounts for almost 15 to 20% of the mixed juice, is usually recirculated resulting in: increased sugar inversion and reduction in sugar quality and clarifier performance. Previous attempts on filtrate clarification have shown limitations for its implementation due to the long residence times of the clarifiers, complex operations and low quality of clarified filtrate. A 100 gpm filtrate clarification pilot plant using a very short residence time clarifier (10 minutes) was designed and operated during the 2012 Louisiana harvesting season. Louisiana Low Turbulence (LLT) technology that was used successfully for mixed juice has been utilized. The results show
that when clarified filtrate and the clear juice of the main factory clarifiers were mixed in a proportion of 1:5, no significant difference was found between the clear juice and the mix and, suspended solids removal was as high as 95%. Additionally, the filtrate clarifier performance was compared to jar tests done on site. Finally, the operating results and design details will be discussed as well as scale-up opportunities.

**Cooling Crystallisation of Low-Grade Massecuite: Theoretical Background and Practical Experience with its Application**

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High sugar yields are a primary technological objective of a sugar factory, and a low molasses purity is decisive in achieving this objective. The final desugarisation step is cooling crystallisation, the end of which is first of all determined by temperature and viscosity. The cooling crystallisation process is determined by the parameters of the massecuite that enters the process, and the way the temperature is controlled. The effects on the expected development of supersaturation and the viscosity produced by the temperature conditions are discussed. The desugarisation level that can theoretically be achieved can be determined and is used for assessing the achieved purity of the mother syrup.

In conceiving the oscillating vertical cooling crystalliser (OVC) BMA has consistently developed a cooling crystallisation system for low-grade massecuite. Essential results are (a) the processing of high-viscosity massecuites, (b) the prevention of encrustations, (c) a constant temperature difference between massecuite and cooling water, and (d) a high massecuite volume (1100 ST) per unit.

Practical experience gained from various installations are presented, and aspects of how cooling crystallisers can be optimally integrated into the factory concept between vacuum pans and centrifugals are discussed.

**How to Manage Sugarcane in the Field and Factory Following Damaging Freezes**

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Exposure of sugarcane to damaging frosts occurs in approximately 25% of the sugarcane producing countries world-wide. A series of damaging freezes, -2.6, -3.3 and -2.1°C, occurred in Morocco on 4, 5 and 13 February 2012, respectively, only 2 weeks after the commencement of the harvest season. Furthermore, the sugarcane had not reached maturity with factory sugar recovery yields under 8.0%. The use of pH (litmus) paper in the field is considered a good indicator of possible deterioration of the juice when the pH is 5.0 or less, and can be used to define the level of topping to remove the deteriorated portion of the stalk prior to milling. In all,
thirty fields of the three leading varieties, CP 70-321, CP 66-346, and L 62-96, were inspected. Only 13% of the fields had a juice pH of 5.0 or less which was generally limited to the uppermost internodes. The worst damage occurred in fields with cane yields of < 40 t/ha, regardless of variety, and which had received no irrigation water and were harvested after June 2011, the previous year. Concentrations of mannitol and/or dextran in the juice are much more reliable indicators of sugarcane *Leuconostoc* deterioration. Because of high levels of brown leaves (>10%) in delivered cane, there were high levels of polysaccharide found in the juice that contributed to the measurement of high haze dextran levels; however, the absence of mannitol confirmed little or no deterioration had occurred from the growth of *Leuconostoc*. The key to success in reducing significant losses through deterioration when freezes occur are good management and close cooperation between growers and processors. Final recommendations on how to manage a freeze in both the field and factory are described.

**Enzymatic Analysis of Mannitol as a Measure of Post-Freeze Juice Degradation in Sugar and Energy Cane**

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When sugarcane (*Saccharum spp.*) is grown in subtropical climates, varieties selected for resistance to freezing temperatures are important. Damaging freezes late in the season can cause damage to the cane and juice degradation causing high juice acidity, low purity, and polysaccharide formation, which can result in slow processing, reduced sugar recovery, reduced profits, and at times, factory shutdown. Concentration of mannitol in the juice is used as an indicator of juice degradation and can be used to determine which varieties are the most resistant to juice degradation following a freeze. Twenty-five varieties were planted in St. Joseph Louisiana and exposed to damaging freezes in the plant-cane and first-ratoon crops. Varieties included early generation material from USDA-ARS sugarcane breeding programs in Houma, LA and Canal Point, FL as well as energycane and sugarcane clones which were not selected for cold tolerance. Significant differences in juice degradation were observed between varieties as well as years. When the last harvest date was compared between years, there was no significant genotype by year interaction, but significant differences were observed for varieties and years. Ho 02-146 was the most cold tolerant varieties, with mean ppmMannitol/Brix of 38,779. The least tolerant variety was US87-1006 with a mean ppm Mannitol/Brix of 142,365. When analyzed by type, varieties selected for cold tolerance in Houma, LA were significantly more cold tolerant than the energycane and commercial sugarcane varieties as well as those selected in Canal Point. Progress is being made toward breeding more late-season cold-tolerant varieties of *Saccharum*. 
Changes in Juice Sugar Components During Sugarcane Ripening

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Sugarcane is usually harvested from late October through early April in Florida. Although juice sucrose content and extractable sugar composition are directly associated with sucrose yield and quality, little is known about changes in juice sugar components (sucrose, glucose, and fructose) during ripening among sugarcane cultivars. The objectives of this study were to determine dynamics of these sugar components in juice during ripening and to identify their variation among cultivars. Eight sugarcane cultivars (CP78-1628, CP 88-1726, CP89-2143, CP00-1101, CP01-1372, CP05-1526, CPCL97-2730, and CPCL02-6848) were planted on a sand soil at the PPI Farm of the Florida Crystals Corp in December 2010. The experimental design was a randomized complete block with four replications. Juice samples were collected monthly from three stalks each time in all plots using a handheld punch from September through February in the plant-cane (2011) and first-ratoon (2012) crops. Brix was determined using a digital refractometer. Juice sucrose, glucose, and fructose were quantified using the microplate enzymatic method. As sugarcane ripened from September through December, juice Brix and sucrose content rapidly increased while glucose and fructose contents sharply declined. Thereafter, no substantial changes in any of these variables were detected. Overall, the first-ratoon crop had 15% lower juice sucrose and 44% lower hexose (glucose + fructose) contents than the plant-cane crop. Juice hexose contents were also significantly different among the cultivars. CP01-1372 had the highest (9.11 mg/ml juice) and CPCL02-6848 (1.77 mg/ml) had lowest juice hexose contents averaged across sampling dates and crops. Additionally, CPCL02-6848 had the highest sucrose content. These results of juice sugar components can be useful for evaluation of cultivars in juice quality and maturity.

FilterKwik Raw Sugar Filterability

B. Ton

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Test methods have been introduced into the global raw cane sugar manufacturing community to be proposed as candidates to potentially become the new uniform standard for raw sugar filterability. Since a filtration step is standard among most if not all cane sugar processing refineries, the notion of a test method with standardized metrics and test protocol is appealing, as various other tests have successfully been standardized internationally. The International Commission for Uniform Methods of Sugar Analysis (ICUMSA), a recognized global authority concerned solely with analytical methods related to sugar, has yet to establish a standard method of analysis in its proceedings to date. Nonetheless, several methods have been actively applied in different parts of the world. These various methods and theories have differing levels of complexity, rigor and practicality. This paper shall cover one method originating from the Sugar Milling Research Institute of South Africa and will discuss some of the relevant initial
considerations of its application at US Sugar.”

Near Infrared (NIR) Spectroscopy of Whole Biomass as a Rapid Characterization Tool for Biorefinery Feedstock Development and Quality Control

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Research initiatives in the development of sustainable biofuels have resulted in the need for rapid compositional analysis of biomass feedstock such as sweet sorghum and energy cane. Traditional methods of analysis require a significant amount of time and technical ability. While thorough, these methods are not ideal for the quantity or speed of analysis necessary in breeding feedstock varieties to exhibit agronomic and fiber quality traits specific to biofuel production. Near infrared (NIR) spectroscopy is a tool widely used in the agricultural industry to predict various parameter concentrations in both plant matter and process stream samples; however, it requires a separate chemometric calibration for each parameter and type of sample. In developing a calibration model, peak changes in the combination and overtone bands of the near infrared spectral regions are correlated with changes in concentration of the parameters of interest as determined by lab analysis. Such models are being developed using NIR spectra obtained from a SpectraCane FT-NIR located at the LSU AgCenter Sugar Research Station in Saint Gabriel, LA to predict the juice and fiber composition of whole stalk sweet sorghum and energy cane. Along with calibration models for fiber and juice percent stalk, parameters of interest include Brix, sucrose, glucose, fructose, ash, cellulose, hemicellulose, and starch. Whether due to variety, maturity, or environmental conditions, a significant amount of variance occurs in the component concentrations of both sweet sorghum and energy cane. The most pronounced differences occur in the amount of fermentable sugars present in juice, particularly sucrose. Since determining the efficiency and economic feasibility of biomass to biofuel conversion depends greatly on feedstock input quality, the ability to rapidly analyze whole stalk feedstock samples is a necessity for targeted varietal development and determination of individual crop quality.
In-field Assessment of Ratoon Cold Tolerance of *Pennisetum* and *Saccharum*

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Perennial bioenergy grasses of subtropical origin could be more widely grown in the southeast United States if they possessed ratoon cold tolerance. Our objective was to assess ratoon cold tolerance, and plant-cane dry mass yield and feedstock quality composition (acid detergent lignin, cellulose, combustible energy, hemicellulose, in vitro dry matter disappearance, N, and total nonstructural carbohydrates (TNC)) of elephantgrass (*Pennisetum purpureum*), Old World Erianthus (*Saccharum arundinaceum*, formerly *E. arundinaceus*), subsequently referred to as erianthus, and sugarcane (*Saccharum* spp. hybrids). The experiment was conducted near Booneville, Arkansas (35.08°N latitude) and consisted of three clones each of elephantgrass and erianthus, and six clones of sugarcane, evaluated in plant-cane (2008) and first-ratoon (2009) crops (test 1). The test was repeated in 2009-2010 (test 2). Absolute minimum air temperatures were -12.7 and -17.3°C in test 1 and 2, respectively. Species differed in subclone emergence in test 1 in the order sugarcane (85%) > erianthus (42%) > elephantgrass (10%). Only sugarcane exhibited first-ratoon emergence in test 2, and its emergence was poor. Elephantgrass had twice the plant-cane yield of the other species. Feedstock quality was generally similar among species, although sugarcane had greater TNC (220 g kg⁻¹) than other species (100 to 131 g kg⁻¹). None of the species were well suited as perennial bioenergy feedstocks given the generally poor ratooning at this latitude. Clonal selection for ratoon cold tolerance was possible, so future breeding efforts could result in improved ratooning ability for temperate climates.

Yields of Giant Miscanthus, Giant Reed, and Sugarcane as Bioenergy Feedstocks on an Upland Soil

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Perennial grasses have potential as renewable sources of cellulose-derived bioenergy. We compared yields of the species giant miscanthus (*Miscanthus x giganteus*), giant reed (*Arundo donax*), and sugarcane (complex hybrid of *Saccharum* sp.) when grown with or without irrigation. Species (giant miscanthus proprietary clone Q4264, giant reed, and sugarcane clone US 84-1028) were spaced-planted on an upland silt loam soil near Booneville, Arkansas in March 2007 and measured annually for yield in fall 2007-2009. Giant reed is locally common in the southeast and west-central United States where it is often planted as an ornamental, but it is potentially invasive and not recommended for cultivation. The test was carefully managed to minimize the risk of accidental escape of potentially invasive giant reed. Monthly irrigation rates ≥ 580 mm plant⁻¹ increased volumetric soil water and benefited growth of the plant-cane crop.
more than that of ratoon crops since rainfall usually exceeded potential evapotranspiration for ratoon crops. Irrigated giant reed significantly decreased soil water compared to sugarcane, reflecting its riparian adaptation. Regardless of irrigation, giant reed usually had greater dry weight per stalk, stalk diameter, stalk length, and yield than giant miscanthus and sugarcane, while giant miscanthus produced more stalks than giant reed and sugarcane. Giant miscanthus and giant reed either increased or maintained stalk numbers across crops, while sugarcane stalk number decreased across crops. Giant miscanthus was the best choice for a perennial, high yielding (> 22 Mg ha⁻¹), non-invasive (as currently classified) bioenergy grass on similar upland sites.

Identifying a New Causal Agent of Mosaic in Louisiana Sugarcane


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*Sugarcane mosaic virus* (SCMV) is a pathogen of economic concern that infects maize, sorghum, and sugarcane worldwide. It is a member of the genus *Potyvirus* in the family *Potyviridae* and contains a linear, positive sense ssRNA genome 10 kb long. It is transmitted non-persistently via aphids and causes stunting, chlorosis, and yield losses of 7 to 21% (over a three-year crop cycle) in susceptible varieties. Prior to the use of molecular techniques to characterize plant viruses, differences in cultivar response and variations in symptom expression during infection were attributed to different strains of SCMV. However, the sequence of the virus coat protein amino terminus differed between two groups of isolates. This difference was sufficient to separate the groups into two distinct species: SCMV and *Sorghum mosaic virus* (SrMV). Due to the widespread occurrence and ability of SCMV and SrMV to cause significant yield losses in susceptible cultivars, breeding and cultivating resistant varieties remains an important control method. Though SCMV is more widespread internationally than SrMV, ongoing research reveals that SrMV is responsible for causing the majority of mosaic symptoms seen in sugarcane in Louisiana. In recent years, strain I of SrMV has become the dominant strain isolated in Louisiana, whereas SCMV strain E remains prevalent in Florida. No nucleic acid product was amplified from a small number of samples collected in Louisiana sugarcane displaying mosaic symptoms when analyzed with SCMV- and SrMV-specific PCR primer sets. However, a product was amplified when *Potyvirus*-specific primers were used instead. This poses the question whether the mosaic symptoms expressed in these samples are caused by another species of *Potyvirus* or by a new strain of SCMV or SrMV. Consequently, gathering sequence data on these unidentified isolates is critical to the continued protection of the Louisiana sugarcane industry from this important pathogen.
Effect of Potassium Fertilizer Application on Sugarcane Yields in Louisiana

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In recent years the high cost of potassium fertilizers have forced many Louisiana sugarcane producers to eliminate this nutrient from their soil fertility programs. The potential consequences that this action may have on cane and sugar yields are largely unknown due to a lack of current research on potassium. In 2011, we initiated four potassium fertility studies in plant-cane and first-ratoon fields of HoCP 96-540 and L 99-226 in an attempt to provide this information. These studies were continued in 2012, in the first- and second-ratoon fields and two new plant-cane studies in fields of HoCP 96-540 and L 99-226 were initiated. Soil samples were collected prior to fertilizer application and after harvest. All of the sites selected for these studies tested low or very low for potassium. Five potassium rates were applied to each field ranging from 0 to 160 lbs K₂O/A. All plots were harvested with a chopper harvester and weigh wagon equipped with a billet-sampler to collect samples for juice quality analysis. In 2011, in plant-cane, HoCP 96-540, a significant increase in cane yield was observed in response to potassium (P=0.2) and a trend showed an increase in sugar yield. In first-ratoon, HoCP 96-540, a significant response to potassium was not observed in cane or sugar yields, but a trend was observed showing an increase in TRS. In plant-cane, L 99-226, a significant increase in both cane (P=0.05) and sugar (P=0.1) yields in response to potassium was observed. In first-ratoon, L 99-226, a significant response to potassium was not observed in either cane or sugar yields, although a trend showed an increase in both cane (P=0.3) and sugar yields. Results from 2012, with first-ratoon L 99-226 showed a significant response to potassium in both cane (P=0.15) and sugar (P=0.05) yields. In second-ratoon, L 99-226, a significant response in sugar yield (P=0.1) was observed. In first-ratoon, HoCP 96-540, a significant response in cane yields was observed (P=0.15). There was not a significant response in cane or sugar yields in second-ratoon HoCP 96-540 or in either plant-cane trial, although positive trends were observed in both plant-cane trials. Soil samples that were collected in 2012 from the first year of these trials to determine residual potassium showed an increase in potassium levels in all trials. Results from these studies demonstrate that significant increases can be achieved with potassium fertilizer application in both plant-cane and ratoon fields of two of the major Louisiana sugarcane varieties. Louisiana sugarcane producers that have eliminated potassium from their soil fertility programs may experience decreases in their cane and sugar yields as potassium levels fall to limiting levels.
Use of SSR Markers for DNA Fingerprinting and Diversity Analysis of Pakistani Sugarcane (Saccharum spp. hybrids) Cultivars

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In recent years SSR markers have been used widely for genetic analysis. The objective of this study was to use an SSR-based marker system to develop the molecular fingerprints and analyze the genetic relationship of sugarcane cultivars grown in Pakistan. Twenty-one highly polymorphic SSR markers were used for DNA fingerprinting and genetic diversity analysis of 20 Pakistani sugarcane cultivars. Using a capillary electrophoresis-based genotyping platform with fluorescently-labeled primers, the 21 SSR markers amplified through PCR a total of 144 DNA fragments (alleles) that were polymorphic. The number of alleles per SSR marker ranged from three to 11. The polymorphism information content (PIC) and resolving power (RP) values were also investigated. This study indicated that by selecting SSR markers with high PIC values, it was possible to identify sugarcane cultivars with a smaller number of markers. We also correlated PIC and RP values with marker effectiveness for sugarcane cultivar identification. Three SSR markers, namely, SMC31CUQ, mSSCIR3, and SMC597CS, were able to identify and distinguish the 20 Pakistani sugarcane cultivars. A homology tree generated by DNAMAN indicated that the 20 cultivars shared a genetic similarity greater than 58%. The SSR genotyping technology is a very useful tool for sugarcane breeders to use in the identification and genetic relationship analysis of their cultivars.

Frequency of Brown Rust Resistance Gene Bru1 in the Germplasm Collection of the Louisiana Sugarcane Breeding Program

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Brown rust, caused by the fungus Puccinia melanocephala, is the most challenging disease of sugarcane (Saccharum spp.) posing an increasing threat to sugarcane industries worldwide. Molecular breeding of rust resistant varieties requires identification of DNA markers tightly linked to or residing within resistance gene(s). A major breakthrough has recently occurred in sugarcane with the discovery of a major brown rust resistance gene Bru1 and the development of diagnostic molecular markers linked to Bru1. Bru1 has been shown to contribute a significant proportion of brown rust resistance in several sugarcane industries. We monitored the frequency of Bru1 in the Louisiana sugarcane gene pool with the objective of understanding the molecular basis of inheritance of brown rust resistance and selecting parents in the breeding program to optimize the use of Bru1. The frequency of Bru1 is very low in the current Louisiana breeding population. Only five (CP77-310, Ho09-827, L01-299, L10-146 and NCo310) out of 115 (4.3%) commercial varieties and breeding lines were positive for Bru1. Important Louisiana varieties,
such as LCP85-384, HoCP85-845, L97-128 and HoCP96-540, and their S. spontaneum progenitor US56-15-8, all tested negative. Similarly, among the basic crosses only 14 out of 211 (6.6%) clones involving diverse ancestry tested positive for Bru1. No definitive ancestral origin was inferred from the pedigree record of the positive clones. However, Bru1 occurred at a considerably higher frequency (50 out 188 i.e. 26.6%) in the wild and foreign/exotic germplasm collection maintained at the USDA sugarcane research unit at Houma, which suggests the opportunity and option of using novel donor parents for Bru1 breeding. Varieties that do not contain Bru1 gene have been shown to be resistant to rust in Louisiana. Preliminary studies have also indicated that the pathogen may be changing. Therefore, our ongoing research is aimed at identifying additional, novel resistance genes, which can be integrated with the Bru1 gene for durable resistance in sugarcane without over reliance on Bru1.

Effects of Tillage on Sugarcane Growth and Arthropod Ground Predators in Florida Sugarcane

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Soil sustainability is very important in muck soils of southern Florida, which have very high organic matter and affected by carbon oxidation. No-tillage or minimum tillage is considered to be one of the best practices to reduce soil carbon oxidation and to improve soil sustainability. A field trial has been established to determine the effects of no-tillage, minimum tillage, and conventional tillage on the plant cane growth of three commercial sugarcane (Saccharum sp.) cultivars (CP 88-1762, CP 89-2143, and CP 00-1101) and population of arthropod ground predators. Our initial data analysis indicated that the plant emergence was delayed in no-tillage compared to minimum and conventional tillage. At six weeks after planting, plants per acre in no-tillage (15,135) were significantly lower than minimum tillage (18,482) and conventional tillage (20,330). However, at 10 weeks after planting, there was no significant difference among the tillage levels, and the number of plants per acre in conventional, minimum and no-tillage were 23,077, 22,403, and 20,705, respectively. Interactions between tillage and varieties were not significant in either of these sampling dates. It indicates that the sugarcane plant emergence in no-tillage can be slow or delayed initially, which can be compensated with the progress of sugarcane growth season. Arthropod ground predators (collected with pitfall traps) primarily consisted of ants, centipedes, earwigs, rove beetles, and spiders.