HoCP 00-950: an Early Maturing Variety for the Louisiana Sugar Industry

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The Agricultural Research Service of the United States Department of Agriculture, the Louisiana Agricultural Experiment Station of the Louisiana State University Agricultural Center, and the American Sugar Cane League of the U.S.A., Inc., working cooperatively to develop improved sugarcane varieties, have jointly evaluated and released the new variety, HoCP 00-950, for commercial planting in the summer of 2007. HoCP 00-950 is a progeny of the cross HoCP 93-750 x HoCP 92-676 made at Canal Point (CP) Florida in 1995 and selected at Houma (Ho), Louisiana, in 1997. Genetically, HoCP 00-950 is not closely related to LCP 85-384, Ho 95-988, HoCP 96-540, or L 97-128, but is closely related to L 99-226. Yield data from a total of 51 mechanically harvested trials in environments representative of the sugar industry in Louisiana indicate that HoCP 00-950 produces similar gross cane yields as HoCP 96-540 (31.7 vs. 32.3 tons/acre = 98%) but because of its high sugar content at harvest (296 vs. 276 lbs sugar/ton of cane = 107%), its recoverable sugar per acre has been slightly higher (9380 vs. 8940 lbs sugar/acre = 105%) in combined plant, first-ratoon and second-ratoon crop harvests.

HoCP 00-950 is resistant to sugarcane mosaic virus (strains A, B, and D) and sorghum mosaic virus (strains H, I, and M). The variety is resistant to smut (*Ustilago scitaminea* Sydow) and rust (*Puccinia melanocephala* H. and P. Syd.) and is moderately susceptible to leaf scald [*Xanthomonas albilineans* (Ashby) Dowson]. It is more susceptible to ratoon stunting disease (*Leifsonia xyli* subsp. *xyli*) than most sugarcane varieties. HoCP 00-950 is susceptible to the sugarcane borer [*Diatraea saccharalis* (Fabricius)]. Hence, it should not be grown in areas where insecticides cannot be applied. It is anticipated that HoCP 00-950 will make its greatest contribution in on-farm ripener management. If growers are unable to timely ripen cane due to inclement weather or if ripener efficacy is diminished due to environmental conditions, HoCP 00-950 could be used to correct the ripener schedule and/or to allow other varieties adequate time for optimal artificial ripening.

Starch Content among Sugarcane Germplasm and Prospects of Breeding for Low Starch Content

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Characterization of sugarcane germplasm for juice quality traits such as starch content could encourage their use for germplasm enhancement. Starch content among sugarcane germplasm was evaluated in three experiments. Experiment I had 49 accessions including 5 *Saccharum spontaneum, 13 S. barberi, 11 S. robustum, 8 S. sinense, 9 S. officinarum* and 1 each of *Erianthus* sp., *Miscanthus* sp. and *S. bengalense*. Experiment II had 52 *S. spontaneum* and one *S. officinarum* accessions. Experiment III had 76 clones including 6 cultivars and 70 unselected clones of F_1 and BC_1 origin derived from crosses between *S. spontaneum* and cultivars. Experiments I and II revealed significant differences in starch levels among the *Saccharum* species, and significant differences among clones within species. Generally, the cultivated *Saccharum* species produced less starch than their wild relatives. *Saccharum* species with > 5 stalks per stool could be ranked into high starch (*S. spontaneum*), medium starch (*S. barberi*, *S. sinense* and *S. robustum*) and low starch (*S. officinarum*) content. In Experiment III starch content ranked as cultivars < BC1 < F_1 clones. Thus, parents low in starch content could be selected even from among high starch species such as *S. spontaneum* for use in germplasm enhancement.

QTL Analysis for Sugar Related Traits in a *Saccharum* interspecific Cross

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In Louisiana, the USDA-ARS Sugarcane Research Laboratory at Houma has operated an active basic breeding program along side the commercial program for the last four decades. Strategies to identify and introgress novel alleles from the basic germplasm into cultivated sugarcane could benefit from implementing a marker assisted selection approach. In this study, we report the identification of markers associated with sugar related QTLs using an F_1 population derived from a *S. officinarum* ‘La Striped’ x *S. spontaneum* ‘SES 147B’ cross. Genetic linkage maps of *S. officinarum* and *S. spontaneum* were produced using AFLP along with two new molecular marker techniques namely, SRAP and TRAP. SRAP and TRAP markers preferentially detect polymorphisms in gene rich regions of the genome. The *S. officinarum* map comprised of 146 linked markers forming 49 linkage groups (LG) and the *S. spontaneum* map comprised of 121 linked markers forming 45 LG. The mapping population, comprising of 100 F_1 clones, was evaluated for Brix (B) and sucrose (S) at the early (E) and at late (L) plant growing seasons in 2004 (04EB, 04LB, 04LS) and 2005 (05EB, 05ES). Composite interval mapping (CIM) QTL analysis was performed to identify marker-trait associations for Brix and sucrose using the PLABQTL software. In *S. officinarum*, the five individual trait-season combinations gave a total of 46 QTLs with individual LOD scores ranging from 2.51 to 7.64. The phenotypic variation (adjusted R^2) explained by all the QTLs in each of the trait-season combination ranged from 28.4 % to 47.8 %. In *S. spontaneum*, a total of 22 QTLs were found with individual LOD scores ranging from 2.69 to 7.51. Similarly, the phenotypic variation (adjusted R^2) ranged from 23.0 % to 43.5 %. Thirty-three digenic interactions (*i*QTL) were observed in *S. officinarum* whereas only three were observed in *S. spontaneum*. Most of the *S.*

officinarum QTLs were positive although a few negative QTLs were found. Conversely, *S. spontaneum* QTLs were mostly negative, although a few positive ones were also found. There was evidence of transgressive segregation in the F_1 population. Genotypic and phenotypic correlations among the five trait-season combinations were positive and significant ($P < 0.05$) and broad-sense heritability values were in the moderate (0.69) to high (0.88) range. A few QTLs were unique and consistent for individual traits and seasons (04EB and 05EB) whereas, most were consistent for Brix and sucrose regardless of the season or year. Two marker-QTLs (AF1286 on LG L6 and AF475 on LG L8) in *S. officinarum* and one (PM0983 on LG S5) in *S. spontaneum* were consistent across traits, seasons and crop-years. The results also indicated that SRAP and TRAP markers could potentially be used for QTL tagging as several marker-trait associations were found for these markers. Markers identified in this study could be used to monitor the introgression of sucrose related alleles, including those related to early maturity, from *S. officinarum* and *S. spontaneum* germplasm.

Genetic Diversity and Phylogenetic Relationships among Sugarcane and Related Species Determined from Microsatellite DNA Data

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Genetic diversity and phylogenetic relationships were assessed among 105 clones of commercial sugarcane hybrids and related *Saccharum* species using 22 microsatellite (SSR) DNA markers. These included 17 sugarcane cultivars from the U.S. mainland, 23 *S. officinarum* clones, 16 *S. robustum* clones, 15 *S. barberi* clones, 10 *S. sinense* clones, 22 *S. spontaneum* clones, and two *S. edule* clones. Total genomic DNA samples extracted from leaf tissue were subjected to PCR with fluorescence-labeled SSR primers. Amplified SSR DNA fragments were fractionated by capillary electrophoresis (CE) along with DNA size standards, and resulting CE run files were analyzed with the GeneMapper™ software (www.appliedbiosystems.com). Five hundred and sixteen polymorphic SSR fragments were identified and presence or absence of each of the 516 fragments was recorded for each clone. The 516 data matrices were subjected to cluster analysis using NTSYSpc (www.exetersoftware.com) and DNAMAN® (www.lynnon.com) programs. Both analyses produced almost identical homology trees, but the DNAMAN® program also produced a phylogenetic tree with bootstrap values. Six major groups/clusters of clones were identified that mostly supported the classical *Saccharum* taxonomy. One exception was that the two *S. edule* clones did not group together but were found within the *S. robustum* cluster. The greatest diversity was displayed among *S. spontaneum* clones, which were also more distantly related to the other species, suggesting greater potential for their utilization in sugarcane improvement through breeding. Less diversity was found both within and between the other four species (*S. officinarum*, *S. robustum*, *S. barberi*, and *S. sinense*) as well as the cultivars. As for the U.S. cultivars, the Florida cultivars were generally separated from the Louisiana and Texas cultivars, which appeared to be similar to one another genetically. The results placed 10 wild species clones (about 10%) outside their normal groups/clusters, due either to taxonomical misclassification or mis-labeling during shipment and handling.

Can Barn Owls Reduce Rodent Abundance in Sugarcane?

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Rodents are capable of inflicting severe damage to sugarcane crops. In the Everglades Agricultural Area (EAA), estimates of the financial impact of these pests range from $35-238/ha. Rodenticides currently are used to control rats, but these chemicals are costly, must be re-applied regularly, and may have unintended ecological side-effects. Over the past decade, members of the EAA community have installed nest boxes throughout the area to increase regional abundance of barn owls. It is hoped that these predators reduce, or possibly eliminate, the need for rodenticides. The nest box program has successfully increased owl abundance, but until our study no quantitative data have been collected to ascertain if this artificially inflated population has led to fewer rats.

To determine if barn owls are impacting pest rodent abundance in the EAA, we conducted live-trapping surveys of rats three times per year for two years in areas with high densities of barn owls, with no barn owls, and where the density of owls was increased after the first year by installing nest boxes. In all study sites, rodent abundance was low following harvest and increased as the growing season progressed. Peak rodent abundance varied among study areas, but was not attributable to the presence of barn owls. It is likely that weeds and unharvested sugarcane stalks in and adjacent to field ditches contribute to high rodent densities. These habitat features serve as refugia for rodents following the harvest of sugarcane fields. While it is unlikely that barn owls alone are capable of lowering rodent abundance, they may play a role in an integrated pest management strategy that includes the elimination of rodent refugia habitat. This strategy may lessen the need for rodenticide application.

Soil Organic Matter and Macronutrient Levels after Mill Mud Application to Sugarcane Growing in a Sand Soil

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Mill mud contains high nutrient levels and is often applied to sugarcane grown in sand soils at high rates. The potential for nutrient leaching into the subsoil is not known. An experiment was conducted to assess soil organic matter and macronutrient movement into three soil depths (0- to 6-, 6- to 12-, and 12- to 18-in depths) after three sugarcane harvests. Treatments

consisted of all combinations of three cropping system practices prior to planting sugarcane (fallow, soybean green manure, and soybean forage removed), two mill mud rates (none and 110 tons acre^{-1} applied one time before planting), and two fertilizer practices (low-enough to keep the plants alive and adequate-extension service recommendations). Cropping system practices generally had little effect on soil nutrient levels. Also, fertilizer effects were small compared to effects due to mill mud. After 3 years, mill mud application increased soil organic matter (OM), total N, and total C compared with no mill mud by 1.2, 0.02, and 0.52%, respectively, in the 0- to 6-in depth. In the 6- to 12-in soil depth, OM, N, and C increases of mill mud treatments compared to no mill mud treatments were smaller (0.2, 0.01, 0.1%, respectively). In the 12- to18-in depth after 3 years, changes in OM, N, and C were minor. Extractable P, K, Ca, and Mg were also increased by 117, 16, 1154, and 53 ppm in the surface soil after 3 years when comparing mill mud to no mill mud treatments. But in the 6- to 12-in depth, only P, Ca, and Mg were increased slightly (8.4, 146, 8.2 ppm, respectively) due to mill mud application. There was little effect of mill mud on P, K, Ca, and Mg in the 12- to 18-in soil depth. Extractable Si was little affected by mill mud application at any soil depth. Soil pH was increased by mill mud application after 3 years at all 3 soil depths. Soil pH increases of mill mud treatments above no mill mud were 1.1, 0.8, and 0.8 pH units for 0- to 6-, 6- to 12-, and 12- to 18-in depths, respectively. The data suggest that when mill mud is applied to sugarcane on a sand soil, organic matter is conserved and macronutrients are not readily leached into subsoil. Mill mud is an effective liming material for sand soils that can increase soil pH to an 18-cm depth.

Screening for Brown Rust Resistance in Sugarcane by Whorl Inoculation

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Brown rust, caused by *Puccinia melanocephala,* is an agronomically important disease of sugarcane in Florida. Cultivar resistance is the best mean of managing the disease. Unfortunately, natural infection is not always efficient in determining resistant cultivars and a more reliable screening method is a necessity for effective selection of resistant genotypes. *P. melanocephala* is an obligate pathogen; this makes the artificial inoculation of large numbers of cultivars difficult since a large quantity of rust spores is required along with environmental conditions that favor infection and disease development.

A whorl inoculation technique was evaluated and optimum concentration of inocula was determined on ten varieties of known rust reaction. The disease reaction was comparable to historical rust reaction data of the variety by using inocula containing 10^5 spores ml^{-1}. This technique was used to screen for rust resistant cultivars in the field. A 0.1 ml of spore suspension containing10^5 spores ml^{-1} was used to inoculate 43 varieties in Stage III increase and 18 varieties in Stage IV of Canal Point cultivar development program. Inoculations were performed by placing the spore suspension in the leaf whorl of three individual stalks per stool using a pipette. Four replicates per variety were used; inoculated stalks were identified by cutting the tops off the leaves. The field was planted in late November 2006 and inoculated in March 2007 during the morning hours. Field temperature ranged from 61-82 °F, relative humidity was between 47-91% and wind speed was 1-20 mph. Symptoms appeared on leaves (of susceptible cultivars) as a band of pustules. Plants were rated for their reaction to rust two and four weeks after inoculation. Resistant cultivars showed either flecks or no symptoms while susceptible cultivars had sporulating pustules. The whorl inoculation technique enabled rapid screening of a large number of cultivars in field plantings, requiring small amount of inocula, less time and labor. Moreover, the reliability of whorl inoculation technique is high.

Can Fungicides Provide an Alternative Control Measure for Brown Rust?

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Brown rust, caused by *Puccinia melanocephala*, is an important disease of sugarcane worldwide that has traditionally been controlled through the development and cultivation of resistant cultivars. However, the durability of resistance to brown rust is uncertain, as the pathogen possesses the ability to adapt and overcome host plant resistance. This results in what has been termed a “boom and bust cycle” in which the disease periodically causes epidemics of sufficient severity to cause significant yield loss. One factor affecting this aspect of the host/pathogen interaction is the extent of cultivation of a resistant cultivar. Extensive cultivation of one cultivar can create a selection pressure on the pathogen population and lead to a more rapid emergence of a genetic variant capable of successfully infecting the resistant cultivar. In Louisiana, the cultivar LCP 85-384 was ultimately grown on 90% of the production area. In 2000, when the LCP 85-384 acreage had increased to over 70%, a severe epidemic of brown rust occurred in what had previously been rated as a resistant cultivar. A sudden, severe disease outbreak in a major cultivar can have serious economic consequences. The multiple year crop cycle of sugarcane does not allow for the rapid replacement of a major cultivar, even if an acceptable substitute is available. The susceptibility of LCP 85-384 prompted an evaluation of fungicides to determine whether they could provide an effective, economic control measure to use during periodic outbreaks of brown rust.

Strobilurin and triazole fungicides were evaluated singly and in combinations in field experiments for the ability to control natural infections of brown rust during 2006. Some fungicides, particularly some combinations of a strobilurin and triazole fungicide, were effective in reducing brown rust symptom severity. These fungicide treatments resulted in large numerical yield increases compared to the non-treated control, but these increases were mostly non-significant. However, the largest yield differences occurred in the treatments that significantly reduced symptoms, and the same treatments produced the highest yields in two experiments. These consistent associations suggest that certain fungicides may be able to provide a useful alternative control measure for brown rust. Additional research is needed to identify the most effective fungicide treatments and conclusively determine the potential benefits resulting from treatment.

Rating Sugarcane Varieties for Susceptibility to RSD with Real-time PCR

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Tissue-blot enzyme immunoassay (TB-EIA) is commonly used to rank sugarcane varieties for susceptibility to infection by *Leifsonia xyli* subsp. *xyli*, the bacterium that causes ratoon stunting disease. The ranking is based on the number of vascular bundles in the stalk that are colonized (CVB) with the bacterium -- the higher the number of CVB, the more susceptible the variety. For a reliable TB-EIA, stalk tissue from plants older than 5-months is required because the low number of bacteria found in younger stalk tissue is below the detectable limit of the assay. Recently, a real-time PCR (polymerase chain reaction) assay was developed to detect and quantify low concentrations of *L. xyli* subsp. *xyli* found in leaf tissue. Real-time PCR offers the ability to perform qualitative and quantitative analysis of *L. xyli* subsp. *xyli* infection throughout the growth cycle of sugarcane. Twenty-seven varieties were ranked for susceptibility to *L. xyli* subsp. *xyli* infection by analyzing leaf tissue from 2-3 months old plants with real-time PCR and stalk tissue from 7-9 months old plants with TB-EIA. The susceptibility rankings of the varieties by the two methods were in close agreement indicating that real-time PCR is a viable method for early detection of susceptibility to *L. xyli* subsp. *xyli*. With real-time PCR, evaluations can, therefore, be made in a timelier manner, and the number of cultivars that can be efficiently evaluated increased.

Effect of Fallow Period Weed Control on Wireworm Populations in Sugarcane

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During the fallow period between the final ratoon crop and replanting, some Florida sugarcane growers use tillage, herbicides, or a combination of both to control weeds while others allow weeds to grow uncontrolled. Damage from wireworms, the larval state of the click beetle, can result in non-uniform patchy stands. Based on research in sweet potato, it is speculated that wireworm populations can be reduced by keeping fields free of weedy vegetation. The objective of our study was to evaluate the effect of chemical and mechanical weed management systems on wireworm populations in fallow sugarcane fields. A field experiment was established in April 2006 at the University of Florida/IFAS Everglades Research and Education, Belle Glade, FL following harvest of a second ratoon sugarcane crop. After harvest, the field was disked 3 times to destroy the remaining sugarcane stubble and prepare the site for fallow. Treatments were mechanical fallow, chemical fallow, and a non-treated weedy control. Mechanical fallow was achieved with a single pass of a disk harrow. Chemical fallow consisted of broadcast foliar applications of glyphosate at 1.0 lbs ae/A + 2,4-D at 0.25 lbs ae/A. When weed groundcover exceeded 10% in mechanical and chemical fallow plots, weeds were controlled with disking or herbicide treatment. Pre-treatment samples for weeds and wireworms were taken in all plots during April 2006. All plots were again sampled for weeds and wireworms in July and October 2006. In the pre-treatment samples, the average number of wireworms per sample ranged from 0.35 to 0.4 and was not different among plots. In July, the number of *Melanotus* wireworms per sample was significantly greater in the weedy control (1.1 per sample) compared to the chemical and mechanical fallow which had 0.4 and 0.35 wireworms per sample, respectively. The number of *Conoderus* wireworms per sample was highest (1.45 per sample) in the weedy control, which was statistically different than the mechanical fallow (0.65 per sample), but not the chemical

fallow (0.8 per sample). In the final sampling (October), the weedy control plots had 1.25 wireworms per sample) compared to 0.35 per sample in the chemical and 0.4 per sample in the mechanical fallow plots. Initial weed populations were not different among treatments. At the July sampling, weed density was 75.1 per m^2 in the weedy control plot compared to 5.5 and 17.8 per m^2 in the chemical and mechanical fallow, respectively. By October, weed density remained high (110.3 per m^2) in the weedy control compared to chemical (5.8 per m^2) and mechanical fallow (7.3 per m^2) treatments. At both the July and October sampling dates, higher wireworm counts coincided with greater weed densities. This indicates that controlling weeds during fallow periods can result in reduce wireworm populations.

Abundance and Spatial Variation of Wireworms in Florida Sugarcane Fields on Muck versus Sandy Soil

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Fourteen commercial sugarcane fields in southern Florida were sampled for wireworm populations. Seven fields were located on muck soils and seven on sandy soils to compare wireworm populations on these two soil types. *Melanotus communis* was the most common wireworm in both soil types, although more were found in muck soils. *Conoderus* spp. were six times more abundant on sandy soils than muck soils. Similar numbers of *Glyphonyx bimarginatus* were found in both soil types. Overall, more wireworms of all species combined were found on muck soils than sandy soils. However, variation in wireworm numbers between fields was high so that some sandy fields had more wireworms than some muck fields.

Supplementing Native Sugarcane Borer Infestations by Artificial Infestation

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When conducting assessments of the response of sugarcane varieties to feeding by the sugarcane borer (*Diatraea saccharalis*), we routinely intercrop sugarcane (interspecific hybrids of *Saccharum* spp.) rows with a row of corn (*Zea mays*) and infest these corn plants with laboratory reared, first-instar sugarcane borer larvae. The justification for this practice is the assumption that artificial infestation is required to obtain consistent and uniform insect pressure needed for varietal screening and yield-loss studies. On occasion, native infestations of the borer have been virtually non-existent or slow-developing and during those years it seems certain that infesting intercropped corn allowed evaluations to be conducted that would not have been possible with native infestation alone. We have not, however, previously attempted to quantify infestation intensities or determine the consistency of borer infestations resulting from the artificially infested intercropped corn. During the 2004, 2005, and 2006 growing seasons we collected infestation data from the corn intercropped among plots of sugarcane in our yield

reduction studies with the objective of determining the uniformity and intensity of sugarcane borer infestations derived from artificially infested corn. Corn (Pioneer hybrid 3085) was intercropped with sugarcane in a 3:1 sugarcane to corn skip-row pattern on the following dates: 3 March 2004, 24 March 2005, and 27 March 2006. Planting in this manner resulted in each three rows of sugarcane being bordered by one row of corn. The corn averaged 5,300 plants per acre. We infested the corn using a hand-held inoculator the first week of May; a time corresponding to the first generation of sugarcane borer in Louisiana. We collected data on number of larvae per inoculation, percent of corn plants infested, number of adults emerging from each corn plant, percent of sugarcane stalks infested, and percent sugarcane internodes bored. Percent of plants infested (inoculated with 10 larvae per plant) averaged 65% producing an average of 145 adults per acre. By the third week in June, 30% of the sugarcane stalks in the yield reduction study were infested by sugarcane borer larvae. Year to year variability was high for infestation intensity, but bored internode counts revealed that, on average, the percentage of the first five internodes bored was 20% (18% in 2006, 21% in 2005, and 22% in 2004). Our descriptive data indicate that we are being successful in initiating infestations of borer in our field evaluations and that these infestations occur early in the season, a time that the most economically important internodes are being formed. We will continue the practice of supplementing native infestations of sugarcane borer in our evaluation plots as the assurance of having a successful evaluation justifies the time and expense required to implement this practice.

Storm Surge from Hurricane Rita Affects Sugarcane Borer Pest Management in Louisiana

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Between 30,000 and 40,000 acres of sugarcane production in St. Mary, Iberia, and Vermilion Parishes was substantially impacted from salt water flooding from Hurricane Rita storm surge, causing both an increase in soil salt residue and affecting the insect pest management system. A four treatment, 12-replication study comparing storm surge flooded and non-flooded plant and stubble fields was utilized to assess arthropod (neutral and predatory species) abundance and diversity, sugarcane borer pest severity, and pest control actions during the summer of 2006. A 40% ($P<0.05$) reduction in imported fire ants due to storm surge enhanced arthropod species diversity and relative abundance of other species. Even with an overall 2.4-fold increase ($P<0.05$) in the average number of insecticide applications used per field, farmers still had a strong trend for higher sugarcane borer injury ($P=0.08$). Though the hurricane enhanced a greater balance among soil-associated arthropods, this new ecological balance disturbed the pest management stability between beneficial and pest arthropods requiring additional insecticide use.

Studies of Confirm® Insecticide Resistance in the Sugarcane Borer

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Under different insecticide selection pressures, varying levels of Confirm® resistance in sugarcane borer, *Diatraea saccharalis* (F.) are occurring at several locations in the Louisiana sugarcane industry. Technical grade Confirm®-incorporated into diet bioassays have indicated 3.78- and 7-fold increase in LC_{50} and LC_{90} values, respectively, for borers collected from an area with higher selection pressure (Duson area) when compared to the susceptible standard (borers collected from a non-insecticide use area - Alexandria). Ten selections with Confirm® over 12 generations of the Duson cohort in the laboratory resulted in 27.1- and 83.3-fold increases in the LC_{50} and LC_{90} values, respectively. Several life parameters including pupal weight, days to pupation, days to emergence, fecundity, and hatchability were significantly compromised in the highly resistant cohort when compared to the susceptible and moderate level resistant cohorts. Both male and female pupae of the highly resistant cohort (Duson Selection) weighed 33% less than the untreated susceptible standard. The length of the time to pupation was increased by 32 and 21% for males and females, respectively, and days to emergence were prolonged by 26 and 55% for the male and female, respectively. Only 84.5 eggs per female were laid from the resistant group as compared to 260 by the susceptible standard cohort. Egg hatchability in the Alexandria cohort was 65.8% whereas only 26 % eggs of the Duson Selection cohort were able to hatch. These results indicate that resistance does have an ecological fitness cost for the sugarcane borer, however, in the absence of other environmentally friendly chemistries, it is suggested that use of tebufenozide must be alternated with other chemistries (pyrethroids) to avoid industry wide buildup of resistance and additional control failures.

Comparison of Mill Mud, Soybean Cropping System, and Fertilizer Nutrient Sources for Sugarcane on a Sandy Soil

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Improving soil organic matter and soil fertility are important factors in the sustainability of sugarcane (*Saccharum* spp.) production on mineral soils. A 3-year field trial was established in 2004 on a sandy Alfisol in Florida to compare the effect of organic and inorganic nutrient sources on soil fertility and sugarcane production. The three nutrient sources, compared in a 2x2x3 factorial experiment in a split-split plot arrangement, were: 1) mill mud (filter cake, cachaza) applied at sugarcane planting (0 or 224 tons/ha), 2) fertilizer (0 or commercial rate), and 3) cropping system prior to sugarcane (soybean (*Glycine max* L. Merr.) as green manure, soybean with above-ground biomass removed for forage, and weedy fallow). Addition of soybean green manure increased sucrose yield (TSH) 20%, whereas the below-ground biomass in the soybean forage treatment did not provide a sugarcane yield benefit in plant cane. A

significant mill mud x fertilizer interaction indicated that fertilization was not required if mill mud was added to plant cane.

In contrast to plant cane results, soybean green manure did not improve sugarcane ratoon crop yields, and there were no significant interactions in the ratoon crops. The application of mill mud resulted in a 49% TSH increase in first ratoon and a 167% increase in second ratoon compared to treatments without mill mud, whereas inorganic fertilizer application increased TSH by 31 and 49% in first and second ratoon, respectively compared to treatments without fertilizer. Over the 3-year crop cycle addition of mill mud alone led to 4.1 TSH greater than inorganic fertilizer alone whereas soybean green manure alone produced 2.6 TSH less. However, combinations of nutrient sources with mill mud had additive effects in the ratoon crops, leading to highly significant 3-year yield increases when mill mud + fertilizer or mill mud + green manure were applied compared to either source alone. Our results indicate that mill mud was more effective than soybean green manure or inorganic fertilizer in increasing sugarcane yields on a sandy soil. However, growers should fertilize ratoon crops when mill mud has been applied to achieve maximum sugarcane yields.

Sugarcane Plant Crop Response to Mill Mud, Compost, and a Water Absorbent Polymer on a Mineral Soil in Florida

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Approximately 20% of the sugarcane grown for sugar in Florida is grown on mineral soils in or near the Everglades Agricultural Area. These soils are very low in organic matter content and have no silt or clay in the root zone, and so the cation exchange capacity and water-holding capacity are very low. Small increases in organic matter content can result in substantial improvements in crop production, but application of an organic material may or may not be cost-effective. Previous research has shown substantial responses in sugarcane production to broadcast applications of 100 tons/acre or more of mill mud. This small-plot study is designed to examine the response of sugarcane to a furrow application of mill mud (7.5, 15, and 30 yd^3/ac), yard waste compost (7.5, 15, and 30 yd^3/ac), or a water absorbent polymer (40 lb/ac). Treatments also include a control and separate broadcast applications of mill mud and compost at a rate of 120 yd^3/ac. The experiment was established in the fall of 2005 on Margate sand (siliceous, hyperthermic Mollic Psammaquent). There were significant increases in tons sugar/acre with the broadcast mill mud application and the furrow polymer application in the first year. There were trends of higher tons sugar/acre with some of the furrow mill mud and compost treatments, particularly at 15 yd^3/ac with each material, but results with each material were variable. This experiment will be continued for two more years and we are planning to establish a second test with selected treatments on another mineral soil location.

The Influence of Combine-residue Management on Surface Water Quality and Sugarcane Productivity

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Louisiana sugarcane growers either burn or sweep to the row furrows the residue deposited by combines. Retention of this plant material can result in considerable loss of sugar in the subsequent stubble crop. The development of a management system that utilizes the residue for protection against soil erosion and to enhance soil fertility, but is benign to re-growth would be ideal. An EPA-sponsored project was initiated in fall of 2005 to evaluate the effects of four combine-residue management treatments on the quality of surface runoff water and sugarcane productivity at two locations in the Vermilion-Teche watershed, Jeanerette and Youngsville. Post-harvest treatment of the residue includes 1) burning (RB), 2) retention (RR), 3) spraying with compost tea/urea (CU) and 4) shredding to reduce particle size (SR). A randomized block experimental design is being used to statistically evaluate treatment effects. Two replications only are being used for water quality sampling, but four replications are being employed for cane and sugar yield determination. End-of-field collections are made using H-flumes and automated samplers instrumented with submerged probe flow modules. Samplers were calibrated to composite 300 ml samples at 50 gal/min flow. Analyses are made for TSS, TDS, turbidity, TKN, nitrite and nitrate nitrogen, chloride, bromide, sulfate, total P and BOD5. Field determinations are made for EC, pH and DO. Data for only the Jeanerette location are being presented. During 2006, sufficient flow for sampling occurred for 18 rainfall events, but two events were subject to water back flow into the flumes and, therefore, only 16 of the 18 collection events are being considered for this presentation. Total suspended solids (TSS) for RB, RR, CU and SR were 2.20, 1.85, 2.70 and 2.84 tons/acre, respectively. Though the differences in sediment removal between the treatments were not significant ($P = .26$), seasonal differences did occur. Soil loss for the RB treatment was consistently higher and double (1,408 vs. 712 lb/acre) that of the RR treatment for collections to early-summer, suggesting that burning of the residue exposed the soil surface to accelerated erosion levels prior to full canopy protection. The quality of water samples for BOD and total P was good to excellent for all collections. Nitrate content, however, was relatively high, especially for the treatment that received an application of fall-applied urea. The two residue management treatments designed to mitigate the adverse effects of residue retention, CU and SR, did not positively influence either rate of residue disappearance or soil loss. Sugar yield differences for the four treatments were not significant and ranged from 8,555 to 9,246 lb sugar/acre.

Sugarcane Post-harvest Residue Retention and Certain Ripener Applications Reduce First and Second Ratoon Yields

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Retention of sugarcane (interspecific hybrids of *Saccharum* spp.) post-harvest residue and certain glyphosate ripener application regimes have independently been shown to reduce yields of the subsequent ratoon crop. The objective of this experiment was to determine the combined effects of post-harvest residue retention, ripener application, and ripener treatment-to-harvest intervals (THI) on yields of the treated first ratoon and the subsequent non-treated second ratoon. Whole plots consisted of either a non-treated control or a 0.21 kg a.e./ha application of glyphosate to first ratoon LCP 85-384 in 2003 and 2004. Split-plots consisted of THI of 40, 50, and 60 days for the first ratoon. Split-split plot treatments consisted of mechanical repositioning of post-harvest residue into the wheel furrow compared with complete retention of residue in the second ratoon. At a 40 and 60 day THI, sucrose yield was not significantly increased due to ripener application, but at a 50 day THI glyphosate application increased sucrose yield by 740 kg/ha compared to the control in the first ratoon. In the second ratoon, there was a glyphosate by THI interaction on sucrose yield indicating that these stresses do compound each other. For glyphosate-treated cane, harvesting at a 60 day THI in the first ratoon reduced cane yield of the second ratoon by 4.9 and 6.0 Mg/ha and sugar yields by 900 and 950 kg/ha compared to the 40 and 50 THI, respectively. Furthermore, waiting until a 60 day THI reduced sucrose yields by 300 kg/ha compared to the non-treated control in the second ratoon. The fact that there were no interactions between post-harvest residue retention and ripener application on yields of the subsequent second-ratoon crop indicates that these stresses act independently of each other. Full residue retention reduced cane and sucrose yield by 2.3 Mg/ha and 300 kg/ha compared to partial removal. Until residue tolerant varieties are identified, producers should remove post-harvest residue to mitigate yield losses in subsequent ratoons. In addition, delaying the harvest of ripener-treated cane due to uncontrollable climatic conditions until a 60 day THI will negate the yield advantage associated with a ripener application in the harvested crop and will decrease yields in the subsequent crop. The use of multiple varieties in a harvesting management program could eliminate some of the harvesting of cane at non-optimal THI. The use of naturally high sucrose varieties such as L 97-128 and HoCP 00-950 without a ripener application could be used to buffer against non-optimal THI for ripener-treated cane.

The Use of Tiger 90CR™ Sulphur as a Carrier for Low Analysis Micronutrients Formulations

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Tiger 90CR™ Sulphur is the industry leading degradable elemental sulphur that is used for soil amending as well as a season long release of plant available sulphur (sulphate). The oxidation of Elemental sulphur by sulphur oxidizing microbes produces sulphuric acid (H2SO4=) which is beneficial to many soils that have a high pH. The use of Tiger 90CR™ Sulphur (Elemental Sulphur) as a carrier for low analysis micronutrients provides a new delivery system that has unique characteristics that improves micronutrient distribution and performance. Micronutrient availability is affected by several factors including soil pH. On mineral soils pH above 7.0 can greatly restrict micronutrient availability. The use of Tiger 90CR™ Sulphur as a carrier for micronutrients improves micronutrient performance on these soils by acidifying the micro site that contains the micronutrient source. The unique micro site acidification zone improves micronutrient performance by reducing the pH in the micro site lowering the pH as well as acidifying the oxide micronutrient source, converting it over a period of time to the sulphate form. Lab experiments of Tiger Copper and Tiger Zinc formulations showed that 48%

of the copper and 68% of the Zinc micronutrient metal oxides were converted to the sulphate metal form in a 30 day period. Research on the Tiger Micronutrient formulations also showed the value of the Elemental Sulphur carrier as Nitrogen Use Efficiency is improved. Studies conducted at Arise Research & Discovery at Martinsville Illinois on Tiger 22% Iron from 2002 to 2005 showed that nitrogen uptake in the plant increased by 21- 27% and nitrate leaching was reduced by 27-32%. A similar reduction in nitrate leaching was found on a field corn study in a 2006 Arise Research & Discovery trial showing a 30% reduction with the application of Tiger 90CR™ Sulphur. The Tiger Micronutrient™ fertilizer formulations also provide many benefits over conventional micronutrient sources such as improved product quality, and uniform sizing. From these studies, Tiger Micronutrient™ fertilizers provide many improved efficiencies over conventional micronutrients, particularly on environmentally sensitive agricultural zones prone to nutrient leaching, as well as improved performance on high pH calcareous soils.

Estimation of Sugarcane Sucrose and Biomass Levels with Remote Sensing Techniques

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Remote sensing techniques were used to predict sucrose levels (TRS) and gross cane yield in field-grown sugarcane. To estimate sucrose levels, leaves were collected from plant-cane and first-ratoon sugarcane plants from the variety maturity studies conducted at the USDA-ARS, Sugarcane Research Laboratory's, Ardoyne Research Farm in Schriever, LA. Leaf samples were collected monthly between September 25 and November 20, 2006, from 10 varieties in the plant-cane crop, and bi-weekly between August 28 and December 5, 2006, from nine varieties in the first-ratoon crop. There were four replications in each test and four leaf samples were collected from each plot. High resolution, hyperspectral leaf reflectance measurements were made in the laboratory, shortly after sampling, using an Ocean Optics SD-2000 fiber optic spectrometer and a halogen light source. Whole-stalk samples were collected on each sampling date and analyzed for sucrose levels using the core press method. Reflectance data were condensed into 10 or 20-nm intervals and then with sucrose data were subjected to analysis of variance and linear discriminant analysis to predict TRS levels. Biomass of two first-ratoon commercial sugarcane fields was estimated using aerial imagery acquired on November 6, 2006. To provide a ground truthing data set, selected rows, from each field, were harvested in 15-meter increments with a single-row, chopper harvester, with weights determined using a weigh wagon. A total of 176 plots were harvested in this manner. Yield data were subjected to variogram analysis and block kriging to construct yield maps of each field for comparison with biomass estimates generated from the acquired aerial imagery.

Multivariate analysis of leaf reflectance and TRS data from the plant-cane maturity study resulted in a 73% correct classification of TRS levels when all varieties were combined. When varieties were considered separately TRS levels could be classified correctly in 100% of the cases examined. In the first-ratoon maturity study TRS levels were correctly classified in 55% of the cases in the combined data set and in 100% of the cases when varieties were considered separately. Finally, kriged maps of sugarcane gross-cane yield, demonstrated a close agreement with estimated biomass levels for both of the fields examined. Successful implementation of

these remote sensing technologies would allow producers to estimate both sucrose and gross cane yields in the field prior to harvest. This information could then be used to more effectively schedule harvest operations and identify yield limiting crop stress situations. Finally, biomass maps could also be potentially used to develop variable-rate ripener application systems.

Allelopathic, Autotoxic, and Hormetic Effects of Post-harvest Sugarcane Residue

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With green sugarcane (interspecific hybrids of *Saccharum* spp.) harvesting, 6 to 24 tonne ha^{-1} of post-harvest residue is deposited on the field surface covering the sugarcane stubble that must reemerge for several ratoon crops. The objectives of this research were to: 1) determine if post-harvest residue possesses allelopathic, autotoxic, and hormetic properties, 2) determine the interaction of soil type with possible autotoxic effects, and 3) identify a reliable indicator species. In order to determine the effects of post harvest residue on the growth of sugarcane and other species, a leachates were extracted from residue using water. Extract concentrations consisted of 0, 0.1, 10, 25, and 100% of the original solution of a 1:28 tissue to water extract. The higher concentrations of residue extracts exhibited autotoxicity by delaying early leaf development. The lower extract concentration of 10% increased sugarcane bud germination by 45% compared to the control indicating hormetic effects. Allelopathic activity on tall morninglory (*Ipomoea hederacea*) was more pronounced on a light soil; germination and radical length were reduced by all concentrations by an average of 42% and 8mm, respectively, compared to the control. Seedling dry weights were reduced by an average of 10 mg by the 10, 25, and 100 % extract concentrations relative to the control. On the heavy soil, only the 100% concentration reduced radical length and weight by 5 mm and 4 mg, respectively, relative to the control. Allelopathic effects on oat (*Avena nuda*), rye (*Secale cereale*), and tomato (*Lycopersicon esculentum*) showed poor correlation with the autotoxic effects on sugarcane. Chemical analysis by GC/MS indicated the extract contained benzoic acid. Further studies are needed to establish the impact of benzoic acid in natural settings. The presence of both hormetic and autotoxic effects of post-harvest residue may explain why yield effects are sometimes inconsistent. Environmental conditions will influence the level of residue decomposition and therefore the level of benzoic acid present in the germinating bud zone. Moreover, autotoxicity may explain why sometimes mechanical removal of the residue from the row-top into the wheel furrow produces lower yields than a field burn.

Identification of Molecular Markers Associated with Sugar Related Traits in *Saccharum* Species Using Discriminant Analysis

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Traditional QTL analysis utilizes linkage mapping populations created to maximize polymorphism. Often, such mapping populations are not representative of the type of germplasm commonly used in breeding. Moreover, the ability to detect marker-trait associations depends on the linkage map saturation and the magnitude of the QTL effects. Discriminant analysis (DA), a multivariate statistical procedure, has been proposed as an alternative platform to QTL analysis where ideal mapping populations and *apriori* linkage maps are not available as in sugarcane. In this study, a previously mapped population consisting of 100 F_1 progeny derived from a *S. officinarum* 'La Striped' x *S. spontaneum* 'SES 147B' cross was used for DA. The population was evaluated for Brix and sucrose at the early and late plant growing seasons in 2004 and 2005. Molecular marker profiles were generated for each of the 100 individuals using the AFLP, SRAP and TRAP techniques. The population was divided into three groups (low, medium and high levels of sucrose and Brix) based on 2-standard deviation. The marker data were separated into two sets; one for each parent based on the origin of the marker and DA was performed using PROC STEPDISC and DISCRIM options of SAS for each set. In both cases, DA identified markers associated with all traits and the identified markers correctly classified (> 99%) the population into three groups. Two DA-identified markers in *S. officinarum* were identical to QTLs whereas most of the DA-identified markers were in the vicinity of QTLs. In addition, DA identified several new markers including unlinked markers pointing to previously unidentified regions on the linkage map. These results suggest that DA could be useful as an alternative approach to QTL analysis. Our future focus is to validate DA using a random set of diverse clones.

Effectiveness of Reduced Phorate Rates for Wireworm Control in Sugarcane Grown in Mineral vs. Organic Soils

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Wireworm (larval Elateridae) feeding on seed pieces, roots and shoots of seed cane leads to reduced sugarcane (*Saccharum officinarum* L.) stands directly through feeding damage and indirectly through disease introduction. Granular insecticide treatments (i.e., phorate and ethoprop) are regularly applied during sugarcane seed planting for wireworm control. The United States Environmental Protection Agency is considering reducing the maximum allowed rates for these soil-applied insecticides. The purpose of this research was to evaluate the

effectiveness of reduced phorate application rates for wireworm control in sugarcane in mineral and in organic soils.

Four rates of phorate and a no treatment check, 0, 13.7, 16.4, 19.2 and 21.9 kg/ha (0, 12.2, 14.6, 17.1 and 19.5 lb/ac), were evaluated in simulated field experiments within 18.9 L plastic buckets. Soil (organic soil from Belle Glade or mineral soil from Clewiston, FL) beneath the seed pieces was compacted to field density to a depth of 17.8 cm. Ten wireworm larvae (*Melanotus communis* (Gyllenhal)) were added to the soil in each bucket 2 d before the introduction of sugarcane seed pieces. Three to four 2- to 3-node sugarcane (CP82-1628) billets were placed on top of the compacted soil (7 to 8 nodes/ bucket) followed by the addition of recommended rates of granulated fertilizer and the phorate (Thimet 20G, AMVAC) treatments. Soil above the seed pieces was compacted to a level 2.5 cm from the lip of the buckets (i.e., final depth of 15.2 cm soil above seed pieces). Ten buckets were set up for each of the five treatments and for each soil type. Additional 10-replicate-treatments for each soil type were added for sugarcane without either phorate or wireworms, and for sugarcane with phorate (19.2 kg/ha) and no wireworms. The experiment was conducted within a fan and pad cooled greenhouse maintained between 20 and 30 °C. Shoot counts were made weekly. After 60 days, the soil in each bucket was extensively searched for wireworm larvae, pupae and adults. Seed pieces were examined for eye germination and shoot growth, and shoots and seed pieces dissected for detection of wireworm and disease damage.

Bioassays of phorate soil residues in each bucket were conducted weekly for the first four weeks using fresh wireworms placed in 240 ml plastic cups along with ca 150 cm^3 of soil collected from the top 15.2 cm of soil from each bucket using a soil probe. Wireworm morbidity was evaluated after 48 hr continuous exposure.

Emergence of primary shoots was improved by <50% over the wireworm-only treatments with the addition of phorate. Stand counts increased slightly with increased phorate rates. No wireworm damaged or killed shoots were observed above the soil level with the two highest phorate rates. Wireworm damage rates were less than 5% in with the two lowest phorate rates, but greater than 75% in the wireworm-only treatments. There was a slight reduction in seed piece damage by wireworms with an increase in phorate rates. Stand counts were slightly less in the mineral than in the organic soil at all phorate treatment rates. There was less seed piece and shoot damage in the mineral than in the organic soils at the 13.7 and 16.4 kg/ac phorate rates. Wireworm mortality in the buckets increased with increased phorate rates. Phorate soil residues above the seed pieces remained highly toxic (>90% mortality) to wireworms for at least 4 wk in mineral soil with no observed rate response. Wireworm mortality in organic soil collected above the seed pieces was significantly less than in mineral soil and increased with increased phorate rates from 10% at 13.7 kg/ac to 40% at 21.9 kg/ac.

Using Microsatellite DNA Markers to Determine the Genetic Identity of Parental Clones used in the Louisiana Sugarcane Breeding Program

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Sugarcane propagates asexually through vegetative cuttings. To validate the genetic identity of sugarcane clones during shipping and handling, we produced molecular fingerprints based on 21 microsatellite (SSR) DNA markers for 116 Louisiana parental clones that were included in the crossing programs at Canal Point, Florida and Houma and St. Gabriel, Louisiana. Of the 116 clones, 48 were planted at more than one location and the identity of these clones was verified across the locations. DNA was extracted from leaf tissue and subjected to PCR amplification and capillary electrophoresis (CE) using a liquid-handling station, 384-well reaction plates, and fluorescence-labeled SSR primers. The genotyping files were recorded automatically during the CE and were then analyzed using GeneMapper™ software. A total of 144 distinctive SSR alleles were designated in a sequential order. The presence (A) or absence (C) of these alleles in designated sequential order constituted a DNA sequence, which represented the molecular characteristic or SSR genotype of that clone. An alignment of all SSR genotypes using the DNAMAN® software produced homology and phylogenetic trees. Analysis among different samples of 39 of the 48 clones planted at multiple locations produced identical SSR genotypes. For the other 9 clones, SSR genotypes from one or more locations did not group together with the original on the phylogenetic trees. These plants/clones were considered to be mis-labeled and were removed from the crossing program. The SSR genotypes produced from this study were stored in a local molecular database for future use in clonal verifications, cross fidelity assessments, and polycross paternity determinations.

The Impact of Breeding and Selection on Genetic Parameters in the Canal Point Sugarcane Breeding Program

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Understanding the role of breeding in shaping the genetic diversity patterns among sugarcane (*Saccharum* spp.) breeding clones is pertinent to sustaining future genetic gains. The purpose of this study was to assess the level, pattern and structure of genetic diversity among the Canal Point (CP) breeding population using parameters such as temporal changes in allelic richness, percent polymorphism (%P), expected (H_E) heterozygosity, and linkage disequilibrium (LD; I_A^S) and also to detect the signatures of selection. Sixty-one sugarcane genotypes (six wild progenitors, eight early hybrid founders, and 47 cultivars and /or breeding clones developed during the 1930-2000 period), were divided into seven decades and characterized with 24 target-region amplification polymorphism (TRAP) primer combinations. Mean gene diversity (H_E) averaged across decades was 0.22 (range 0.21-0.23) which is typical in out crossing species. Reductions in H_E were slower (0.002 decade^{-1}) than reductions in %P (6%) and in allelic richness (21 decade^{-1}), indicative of a population bottleneck that initiated with the founder population. Although there was a 0.07 decade^{-1} (range 0.13-0.58) increase in coefficient of relatedness, AMOVA revealed that 96% of the genetic variance was among individuals, with decades accounting for only 4%, an evidence of homogenization by constant gene exchange among decades. While phylogenetic analysis generated a star-like tree, suggestive of a panmictic population, a Bayesian-based clustering method grouped the individuals into six clusters that

were in agreement with the *S. officinarum*, *S. sinense*, and the *S. spontaneum* (Java and Indian forms) lineages, with known contribution to the background of the CP germplasm. Highly significant LD (I_A^S; $P \leq 0.0002$) was detected within each decade and at the population level. Fluctuations in I_A^S suggested an approach to equilibrium with a large drop (from 0.0121 to 0.0026) occurring from the progenitor to the founder population (1930's), a sharp increase to 0.0139 in the 1951-1970, and a decrease thereafter to 0.004 in the 1980-1990's. Coalescent simulations yielded 15 loci as putatively influenced by selection, by falling below the lower limit of the 95% quartile that encompasses the expected distribution of neutral loci. Balancing selection (simultaneous selection for multiple traits), neutral loci (loci not under selection pressure) and the buffering effects of polyploidy were responsible for maintaining polymorphisms in this breeding program. The continued realization of genetic gains in the CP program implies that, the breeding population still harbors sufficient genetic variability and that, the relevant genomic regions are being effectively targeted during selection.