Advanced Breeding, Development, and Release of High Biomass Energy Cane Cultivars in Florida

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ABSTRACT

Research into alternative energy sources has been on the rise since the 1970s. Novel sources of carbon-neutral energy are currently in high demand, but can pose different challenges in their development. Energy cane is a relatively new generation crop being bred as a source for biofuel feedstock and ethanol production. Though originating from sugarcane (*Saccharum* spp.), energy cane breeding strategies have diverged from the traditional goal of increasing sugars to maintaining a focus on selecting high biomass hybrids. These hybrids are derived from wide crosses between commercial sugarcane cultivars and *S. spontaneum*; a species within the *Saccharum* family characterized by high stalk counts and fiber content, excellent ratooning ability, and tolerances to abiotic and biotic pressures.

A cooperative energy cane cultivar development program was established in 2007 between the USDA-ARS Sugarcane Field Station (Canal Point, Florida), and the University of Florida-EREC (Belle Glade, Florida) to produce high-yielding, and disease-resistant energy cane clones. After completing four years of multi-location field trials, disease screening, and fiber component analyses, the first-ever five energy cane cultivars developed in Florida were released in October 2014 (i.e., UFCP74-1010, UFCP78-1013, UFCP-82-1655, UFCP84-1047, and UFCP87-0053). Yields of the new cultivars are comparable to, or exceed the commercial check, L79-1002 (released in 2008). Disease data, derived from both field trials and artificial inoculation, indicate very low smut susceptibility when compared to the check; with no significant differences between the new releases. Fiber composition was comparable between the five energy cane clones and L79-1002. UFCP74-1010, UFCP78-1013, UFCP-82-1655, UFCP84-1047, and UFCP87-0053 have been released publically, and are intended for cultivation on the mineral soils within Florida.

Improved strategic matings incorporating a wide range of germplasm, rigid disease screening protocols, and well-designed field trials have led to rapid improvement in clone cultivar development; with several cultivars currently in Stage II far exceeding the yields and disease ratings of both L79-1002 and some of the 2014 releases. Florida provides the most desirable location in the Unites States to breed sugarcane, and thereby energy cane as a specialized derivative product. Current cultivar development foci include: increasing genetic diversity by optimizing matings with related genera; incorporation of abiotic stress tolerances; continuing the trend in yield increases and disease tolerances; and evaluating cultivars in diverse environments across the country to help target alternate locations where energy cane is desired and can be grown as a biofuel feedstock.

INTRODUCTION

High biomass feedstocks, specifically in the form of fast-growing perennial grasses, are desirable as a replacement for fossil fuels because of their neutral/negative carbon footprints, net energy yields, minimal input requirements, and ability to grow on land which does not compete with food crops. C4 grasses are more suitable for lignocellulosic biofuels than their C3 counterparts as their high photosynthetic efficiencies typically yield more biomass.

In addition to contributing higher fiber : sugar ratios, thinner stalks, and increased plant-density population traits (i.e., as compared to sugarcane) to energy cane, clones of *S. spontaneum* have long been used as sources of disease and pest resistances, ratooning performance, cold tolerance, and vigor in sugarcane breeding programs for commercial sucrose production. It was not, however, until 2007 that L79-1002 was released specifically for energy feedstock purposes in the United States. Since then, L79-1002 has shown promising characteristics, but has demonstrated an increasing susceptibility to smut disease in the fields in Louisiana and Florida. Smut is the most deleterious disease in energy cane; causing poor plant regrowth and reduction in biomass. Thus, a need for improved germplasm was evident. Over the past few years, the USDA-ARS Sugarcane Research Unit (SRU) in Houma, LA released three new energy cane cultivars [i.e., HoCP91-0552 (Tew et al., 2011), Ho00-0961 (White et al., 2011), and Ho02-0113 (Hale et al., 2013)] for commercial production in Louisiana.

The genetic diversity in energy cane is still very low as compared to other crops. To that end, UFCP74-1010, UFCP78-1013, UFCP82-1655, UFCP84-1047, and UFCP87-0053 were bred and released (2014) as the first-ever energy cane cultivars for use in Florida. Additionally, a robust energy cane program collaboration between the USDA-ARS Sugarcane Field Station Canal Point (USDA CP) and the University of Florida-EREC (UF-EREC) has shown significant promise in its ability to provide a ready supply of ever-improving, and genetically diverse, genotypes as feedstock for cellulosic ethanol production. This collaboration is being expanded to include the University of Illinois; where distinct cold tolerances have been seen in miscanthus germplasm, and is being actively crossed into the energy cane cultivars at CP.

MATERIALS AND METHODS

Initial Genotype Selection – Initial energy cane clones (n=53) were chosen from a 24-year recurrent selection and breeding program population of *S. spontaneum* x commercial sugarcane at the USDA CP. After preliminary testing, five clones were chosen for further commercial field testing.

Yield Trials – The 5 cultivars were planted in a randomized complete block design trial, with four replications at three farms [i.e., (Tecan; southern Florida), (Citra; northern Florida), and Lykes Bros.; central Florida)] in 2007, 2008, and 2009. All farms have mineral soil with low organic matter (i.e., "marginal soils"). Manual (Citra and Lykes Bros) and mechanical (Tecan) harvesting methods were employed to assess fresh biomass yields. A 10-stalk subsample from each plot was used to calculate dry biomass yields, brix, and fiber compositional analysis.

Disease Assessment – Field plots were rated for natural infection of smut, brown and orange rusts, leaf scald, and SCMV. Genotypes were also artificially inoculated with smut, leaf scald, and SCMV to assess performance against commercial sugarcane checks for these diseases (Comstock et al., 1999; Sood et al., 2009).

Molecular Genotyping – 20 simple sequence repeat (SSRs), originating from a large pool of molecular probes used to genotype sorghum for disease resistances, were selected based upon their distribution across all 10 chromosomes and high polymorphic information content (PIC) scores in sorghum (James et al., 2012; Singh et al., 2010; and Parida et al., 2010). Amplicons were processed on an ABI 3730 Genetic Analyzer.

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RESULTS

Annual Productivity of High Yielding Biomass Crops (Fig.1) – Sugarcane and miscanthus are both low input high output biomass crops which can produce up to 500% more ethanol /acre than corn (2105 vs. 495 gallons/acre, respectively). As a derivative of sugarcane, energy cane breeding focuses on increasing the fiber and biomass of progeny, and is selected to grow on marginal soils.

Yield Trials (Fig. 2) – Statistical analysis for cane harvested (C) over a four-year cycle (i.e., plant cane, 1st ratoon, 2nd ratoon, and 3rd ratoon) revealed that the new genotypes have high biomass yields in mineral soils, which exceeded L79-1002. The higher yields were not different enough, however, to make the results significant.

Disease Assessment (Fig. 3) – The newly released energy cane cultivars were screened for smut (A), leaf scald (LS), and Sugarcane Mosaic Virus (SCMV). The clones have a very low susceptibility to natural smut infection (B; avg = 0.6-1.7 whips/m^2) as compared to the L79-1002 commercial check (avg = 26.7 whips/10m^2). Data for the new cultivars was collected over twelve location years with no significant differences seen among them. Artificial inoculation data displayed the same trend (i.e., cultivar avg = 11-18% infection versus check avg = 38% infection). The new Florida energy cane cultivars also have lower infection rates of LS and SCMV than the susceptible commercial checks for these diseases, and had moderate/full resistance to both brown and orange rusts.

Compositional Analysis (Table 1) – Fiber analysis indicated that the proposed genotypes and commercial check (L79-1002) had similar concentrations for cellulose, hemicellulose, lignin, and ash composition.

Fresh Biomass of Energy Cane Clones in Stage II and Stage III of the Canal Point Program (Fig. 4) – Energy cane clones in the pipeline display the efficacy of breeding efforts through both increased biomass, as well as the number of clones surpassing the commercial energy cane checks. Stage III fresh biomass was averaged over two years (2013 and 2014). Clones possessing high sugar + high biomass have been identified, and will be used to split the program into two-tiered objectives. Additionally, these clones will be crossed back into the traditional sugarcane program to introduce genetic diversity, disease resistances, and abiotic tolerances.

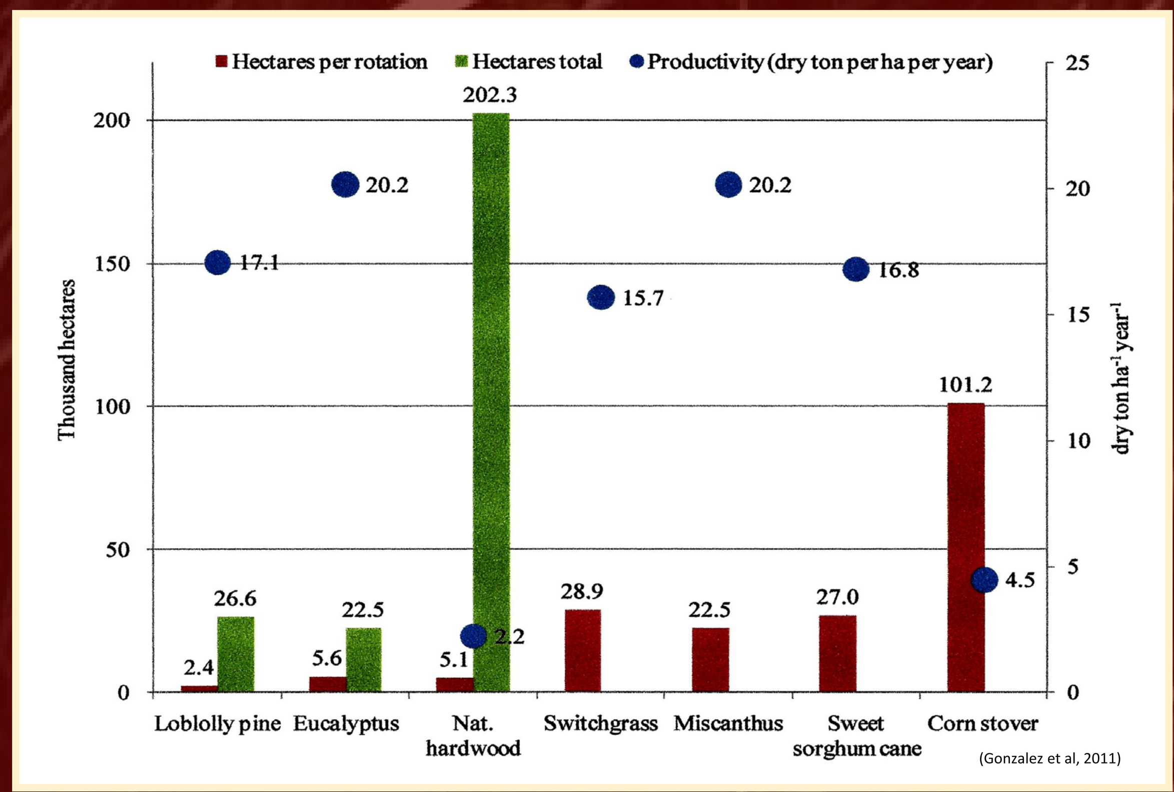

Figure 1. Annual productivity of high-yielding bioenergy crops. Miscanthus provides the highest yield whilst using ~ 20% of corn stover acreage. Input to output ratio of sugarcane first generation ethanol production is ~1:10; sweet sorghum, 1:8; miscanthus, 1:5; switchgrass, 1:4; sugarbeets, 1:2; and corn, 1:1.4.

(Goldemberg, 2008; Ibeto et al, 2011)

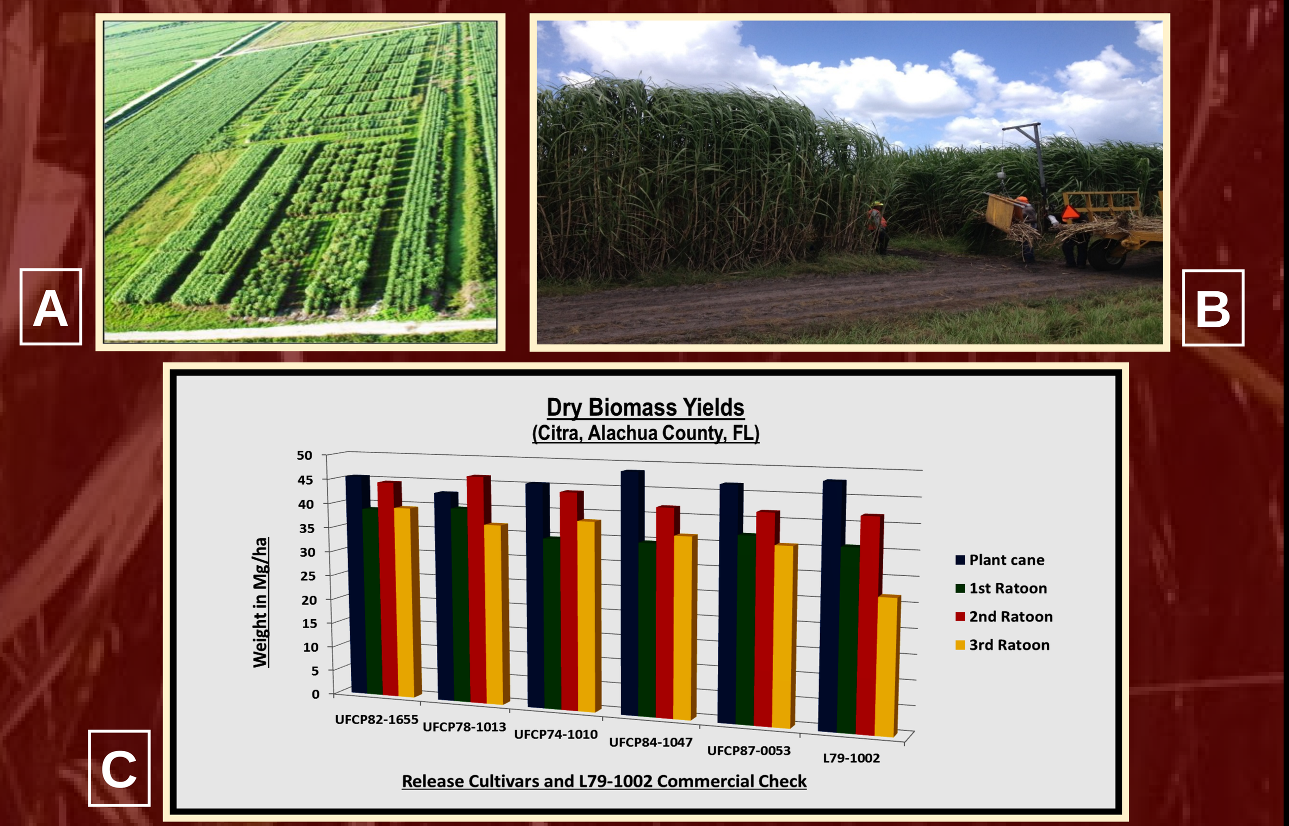

Figure 2. Field design (A) and harvesting (B) in an energy cane field. Mean dry biomass yields were taken for the 2014 energy cane releases and the L79-1002 commercial check. Biomass yields were acquired over a four year period at Citra, Florida (C), a three-year period at Lykes Bros, Highland, and a two-year period at Tecan, Florida. The four-year data shows no significant differences between the new and check cultivars.

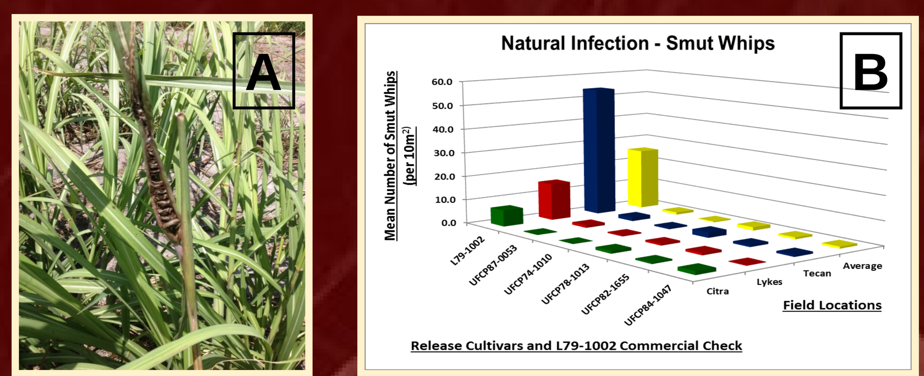

Figure 3. Disease screening of released Florida energy cane cultivars. (A) Smut; (B) Field infection over all locations ($P < 0.001$).

Location	Genotype	Cellulose (%)	Hemicellulose (%)		Lignin (%)	Ash (%)	
		Glucan	Xylan	Arabinan		Structural	Non-structural
Citra	2014 Releases	42.5	24.9	3.3	22.4	2.7	2.3
	L 79-1002	42.3	25.1	3.4	21.9	2.8	2.3
Lykes Bros.	2014 Releases	41	24.7	3.1	22.9	2.5	1.9
	L 79-1002	41.6	24.5	3	22.8	2.5	1.8

Table 1. Average percentages of dry biomass fiber composition collected from the 2014 Florida energy cane cultivars and L79-1002 check variety.

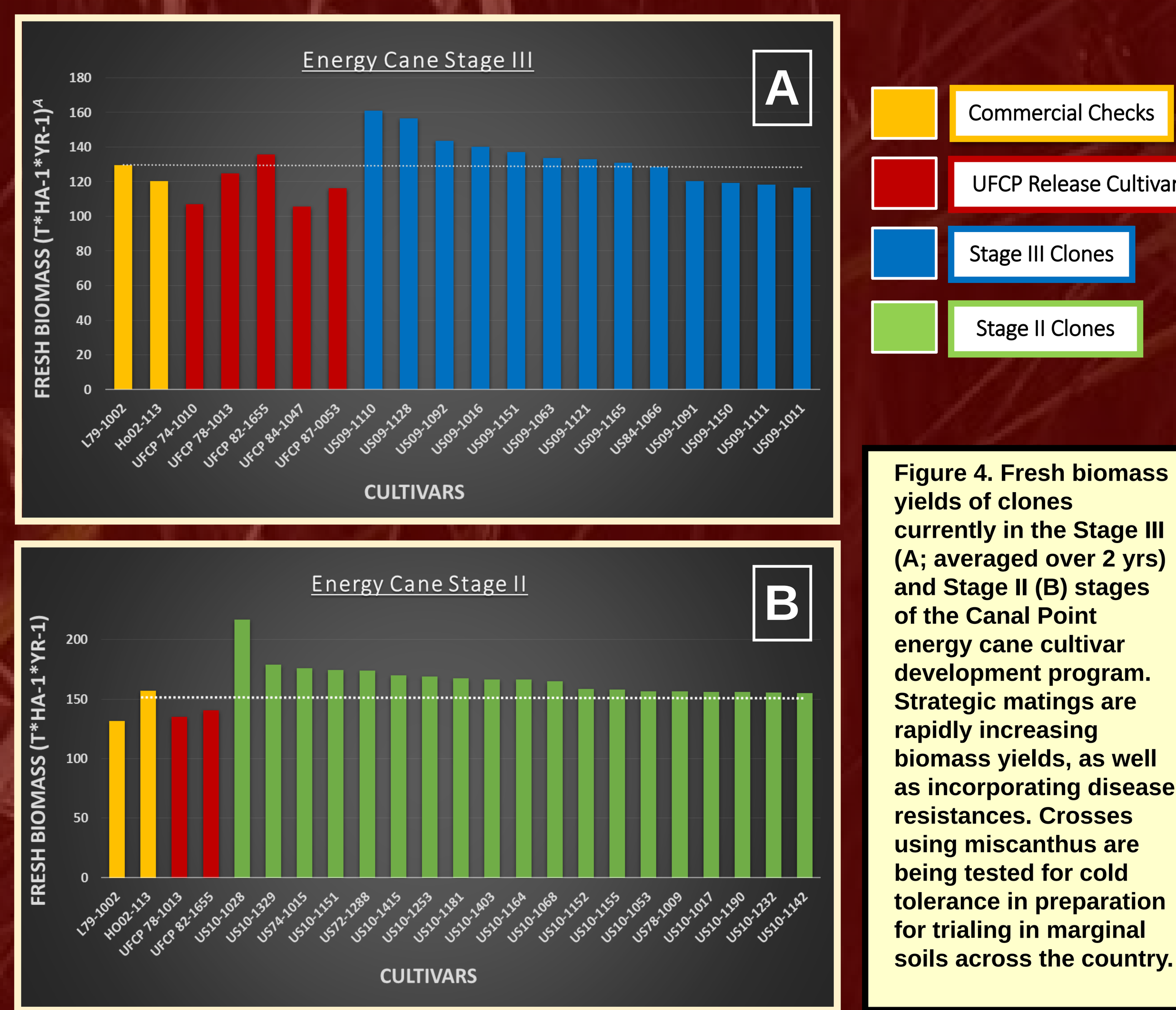

Figure 4. Fresh biomass yields of clones currently in the Stage III (A; averaged over 2 yrs) and Stage II (B) stages of the Canal Point energy cane cultivar development program. Strategic matings are rapidly increasing biomass yields, as well as incorporating disease resistances. Crosses using miscanthus are being tested for cold tolerance in preparation for trialing in marginal soils across the country.

DISCUSSION

Research and advanced breeding have demonstrated that energy cane possesses all of the attributes desirable in a biofuel feedstock: extremely good biomass yield in a small farming footprint; negative/neutral carbon footprint; maximum outputs from minimum inputs; well-established growing model for farming; and it is bred for cultivation on marginal soils, avoiding competition with croplands dedicated to food commodities. The USDA-ARS Canal Point field station is the premiere sugarcane breeding station in the United States. As demand for lignocellulosic sources increases, Canal Point will become a major source of energy cane cultivars in this country. It is anticipated that when the biochemical processing mechanisms for extractions from the feedstock have been solved and optimized, a rapid demand for energy cane germplasm will follow. One of the missions of the CP energy cane program is to create high yielding biomass clones which can resist abiotic stresses, and carry the robust disease tolerances typically found in our breeding programs. As energy cane can respond differently to varying environments, it is the goal of CP to identify and collaborate with industry and academic sources across the country. This would facilitate trialing, preparation, identification of geo-specific clones, and compilation of a germplasm portfolio tailored for diverse growing areas.

References - A list of references is available upon request as supplementary information.