

Biomass Production and Water Economy of Sugarcane and Energy Cane Genotypes Grown in Water-Deficient and Well-Watered Regimes

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ABSTRACT

Aims: Study designed to characterize water economy and biomass production of sugarcane and energy cane genotypes grown in a rain-shelter under well-watered and water-stressed conditions during plant cane and two consecutive ratoon cane phases.

Study design: Randomized complete block with 5 replications.

Place and Duration of Study: Texas A&M AgriLife Research and Extension Center near Corpus Christi during 2015 and early 2016.

Methodology: Stem cuts of sugarcane line TCP94-5753 and energy cane lines TUS56 and TUS59 were hand-planted in pots. There were three phases including a water-stressed initial plant cane phase and two sequential well-watered ratoon cane phases. Daily whole-plant transpiration was calculated from hourly pot weight changes measured by electronic loadcells. At the end of each phase, plants were harvested to determine above-ground biomass, partitioned into dry leaf blades biomass and stem dry biomass.

Results: No differences in above-ground dry biomass production or water economy among genotypes after the water-stressed plant cane or the first ratoon cane phase, but TUS56 and TUS59 produced 2.32 and 1.83 times more tillers than the sugarcane genotype, respectively. Cumulative transpiration of TUS56 at the end of this first ratoon cane phase was 17% higher than that of TUS59 and TCP94-5753. At the end of the second ratoon cane phase, total above-ground dry biomass were 60% higher in TUS56 than in the sugarcane genotype, but not different from those in TUS59. Cumulative whole-plant transpiration during this phase was about 88% higher for the energy cane genotypes.

Conclusion: Genotypes performed equally in above-ground biomass production and water economy after exposure to water deficits during the initial plant cane phase. No differences in biomass production were observed after the well-watered first ratoon cane phase among genotypes, but TUS56 exhibited more leafiness and transpired more than TUS59 and the sugarcane genotype, and both energy cane lines produced more tillers than the sugarcane.

Keywords: Water economy; plant water use; biomass production; water use efficiency; sugarcane vs energy cane.

1. INTRODUCTION

The use of crop species that have high efficiency of conversion of solar energy into biomass, such as grasses like sugarcane (*Saccharum* spp) that use the C4 photosynthetic pathway, have a great potential to be used as sources of biomass for the production of biofuels [1,2]. Energy cane (*Saccharum spontaneum*), a new type of cane with high fiber yield initially developed by breeding programs in Louisiana and Puerto Rico [3,4], is an important

alternative to sugarcane (*Saccharum officinarum*) as a source of biomass for the production of biofuels [5,6]. Other advantages of *S. spontaneum* over *S. officinarum* include its ability to produce more stalks, an abundant root system [1], and a stronger ratooning ability [7]. Furthermore, since soil water availability is a dominant factor affecting plant productivity and irrigation being the common practice to maintain biomass production [8,9], comparative evaluations of sugarcane and energy cane genotypes would benefit from characterizing the responses of these species' to contrasting soil water regimes.

While plants growing under plentiful soil water regimes exhibit unlimited transpiration, photosynthesis and growth rates, plants that are exposed to soil water deficits commonly exhibit decreased expansive growth (particularly leaf area and stem elongation), decreased transpiration and photosynthesis through stomata closure, and, due to the latter, decreased biomass production [10,11,12]. Plant exposure to soil water deficits can also increase water use efficiency as transpiration is decreased more than photosynthetic carbon uptake [10,11]. This is particularly notable in C4 species such as sorghum and sugarcane species. Substantial genotype-by-environment interactions have been repeatedly found in standard crop field tests, including sugarcane, conducted under naturally or managed variable soil water regimes. Sugarcane genotype-by-environment interactions have been reported in relation to leaf elongation rate and leaf senescence [13] and stomatal resistance [14] and biomass production [15]. A recent study designed to characterize water economy of a group of sugarcane transgenic lines grown in a rain-sheltered under well-watered and water-stressed conditions allowed to identify genotypes differing in water use efficiency, genotypes that were more water-prodigious or water-conservative when soil water was not limiting, as well as other genotypes that were less tolerant to water stress than their respective non-transgenic genetic backgrounds [16]. However, no reports have been found in relation to a simultaneous comparative characterization and quantification of the whole-plant water economy and growth of sugarcane and energy cane genotypes under both well-watered and water-deficient water regimes.

The objective of this study was to characterize and quantify the whole-plant water economy and above-ground biomass production of one sugarcane and two energy cane genotypes grown in a rain-shelter under well-watered and water-stressed conditions during plant cane and two consecutive ratoon cane phases.

2. MATERIALS AND METHODS

The study was conducted at the Drought Tolerance Laboratory at the Texas AgriLife Research and Extension Center near Corpus Christi from the spring of 2015 to winter of 2016. This facility consists of two joined greenhouse structures modified to operate as rain shelters housing a large number of electronic mini lysimeters capable of measuring continuous whole-plant transpiration under controlled watering regimes. Computerized systems monitored whole-plant plant water use and controlled watering with a nutrient solution.

The treatments consisted of three genotypes of sugarcane and energy cane (TCP94-5753, TUS56, and TUS59, respectively), three growth phases (plant cane, first ratoon cane, and second ratoon cane), and two water regimes (water-deficient and well-watered). Germinated stem cuts of sugarcane line TCP94-5753 and energy cane lines TUS56 and TUS59 supplied by Dr. J. Da Silva's Sugarcane Variety Improvement Program at the Texas AgriLife Research and Extension Center in Weslaco were hand-transplanted in 13.5-L pots on March 5, 2015. The soil medium consisted of fritted clay, which is known by its high water holding capacity and quick drainage of excess water [17]. Pots were uniformly filled with 11.4 L of the soil medium to minimize maximum soil water availability as a variable factor

affecting plant growth and plant water economy. Drained water holding capacity of pots was 4.1 L, of which about 60% (2.46 L) was available to plants. Upon transplanting, the soil surface was covered with aluminum foil to minimize soil evaporation but leaving an opening around the emerged shoots. Tiny holes were punctured in the aluminum foil to allow infiltration and uniform surface distribution of irrigation water. Five fairly uniform plants of the sugarcane line and of each of the two energy cane lines (a total of fifteen plants) were spatially arranged to conform a 5-replication randomized complete block experimental design. The fifteen potted plants were each permanently hanged from a weighing electronic loadcell for continuous measurement of whole-plant water use. All experimental plants were individually irrigated daily to excess with a modified Hoagland solution made up with purified city water from the post-transplant juvenile stage until the start of the first phase of the study on May 13, 2015. Pots were individually irrigated using a spout-based distribution system to secure a uniform application of water.

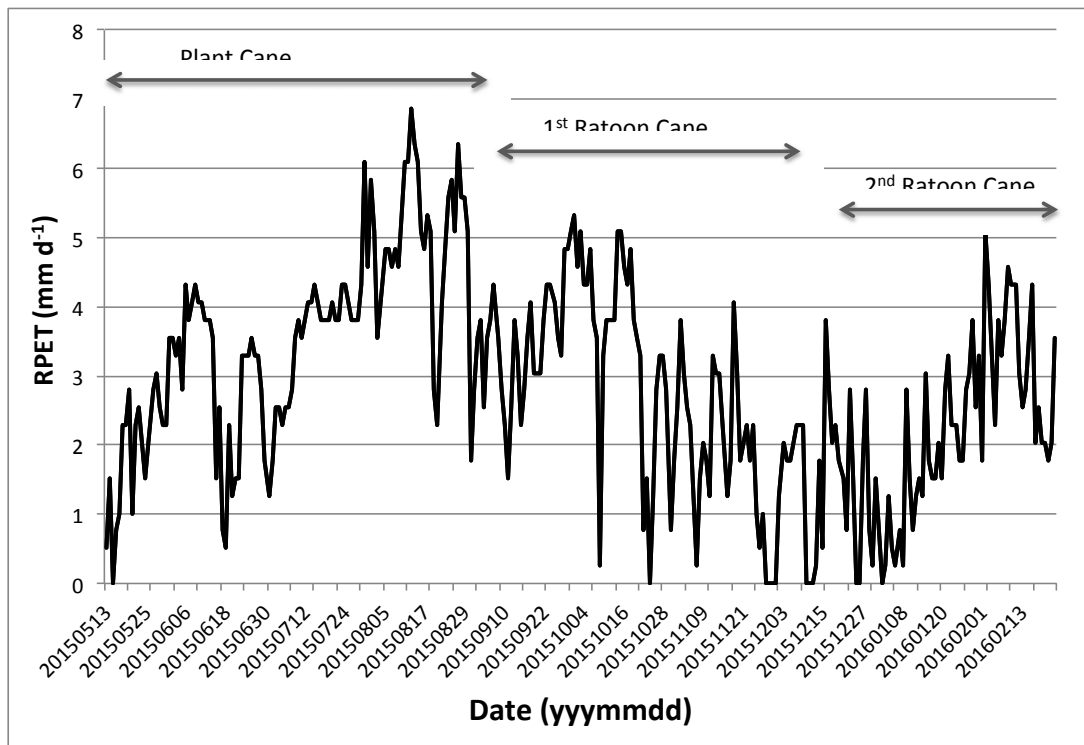
The study was designed in three growth phases, namely plant cane, first ratoon cane, and second ratoon cane. In the plant cane phase (13 May – 08 September, 2015), all three experimental lines were subjected to a moderate water deficit by limiting daily irrigation from the pre-study well-watered irrigation level to 1 min d⁻¹ at 650 L min⁻¹ (this irrigation flow rate was maintained throughout the length of the study). This procedure, which allowed for a slow field-like onset of water stress on the test plants, was set to study the responses of the experimental lines to drought. At the end of the first plant cane phase, above-ground plant parts were harvested in all pots for tillering and dry biomass measurements. Upon harvesting, the first ratoon cane phase (10 September – 09 December 2015) was initiated on September 10th and continued until December 9th, 2015, when ratoon cane plants harvested for above-ground dry biomass and tillering measurements. During this second phase of the study, the first ratoon cane plants were well watered, initially 3 min d⁻¹ and later 5 min d⁻¹, as plants increased in size. Upon harvesting the first ratoon cane phase, the third phase of the study (second ratoon cane) was initiated on December 23rd, 2015 and continued until February 23rd, 2016, when above-ground plant parts were harvested for biomass measurements. During this second ratoon cane phase, plants were also well watered. This procedure of exposing the experimental lines to a well-watered regime during the second and third phases of the study was set to assess their growth potential during each of two successive ratoon cane phases.

Harvested above-ground plant biomass was dissected into leaf blades and tiller stems, the latter including leaf sheaths, and their dry biomass measured. The tillering characteristics were assessed by counting the number of tillers, measuring their total length, and calculating their average specific stem length (total dry tiller biomass/total length).

Pot weights were measured continuously at 10-min intervals using a computerized automated system. This data was used to calculate the daily whole-plant transpiration (DWPT) as the 24-hr cumulative pot weight differences between consecutive hours. This method removed almost all interference of plant growth in the calculation of plant transpiration. The total cumulative whole-plant transpiration (CDWPT) during each of the three phases of the study was calculated for each pot as the sum of the daily whole-plant transpiration values. Cumulative whole-plant transpiration per unit leaf mass was also calculated to further characterize the leaf transpiration for each of the experimental lines. The experimental lines were also compared in terms of a nominal water use efficiency value (above-ground biomass/cumulative whole-plant transpiration).

Weather conditions during the experimental period are best summarized by the daily variation in reference potential evapotranspiration (RPET) calculated from hourly air temperature and humidity, solar radiation and wind speed measured by an automated field

123 weather station located approximately 100 m east of the Drought Tolerance Laboratory (Fig.
 124 1). Cumulative RPET (CRPET) was calculated for each of the three phases of the study.
 125 Linear regressions of CDWPT on CRPET were obtained for distinct sub-periods in all three
 126 phases of the study to advance the interpretation of whole-plant transpiration responses to
 127 the applied water regimes. Experimental data (sums, averages, standard deviations, and
 128 coefficients of variation) were summarized using Excel (Microsoft Corporation, Redmond,
 129 WA) and statistical analyses including ANOVA, mean separations, and contrasts were
 130 performed using JMP (SAS Institute, Cary, NC). Means were compared with Fisher's
 131 Protected LSD test at the 5% probability level.



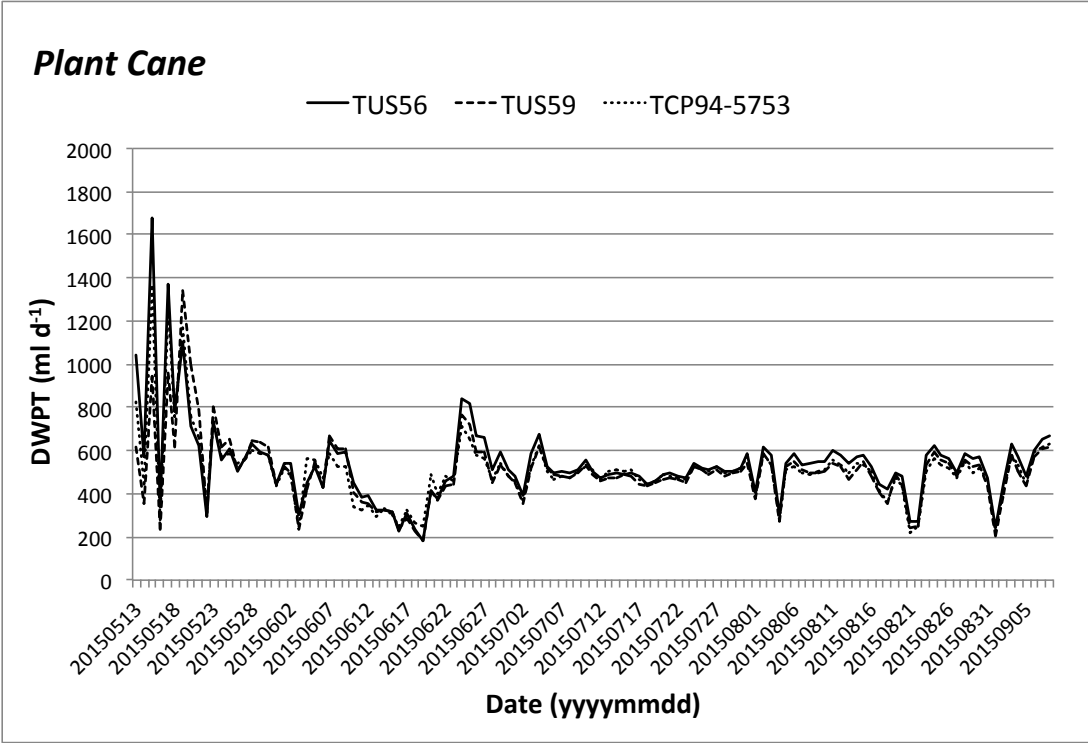
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 134 **Figure 1. Progression of daily reference potential evapotranspiration (RPET)**
 135 **throughout the three phases of the study. Data source: The Crop-Weather**
 136 **Program for South Texas available online at <http://cwp.tamu.edu>.**

137 138 3. RESULTS AND DISCUSSION

139 3.1 Phase 1 of study: Plant cane under water-deficient conditions

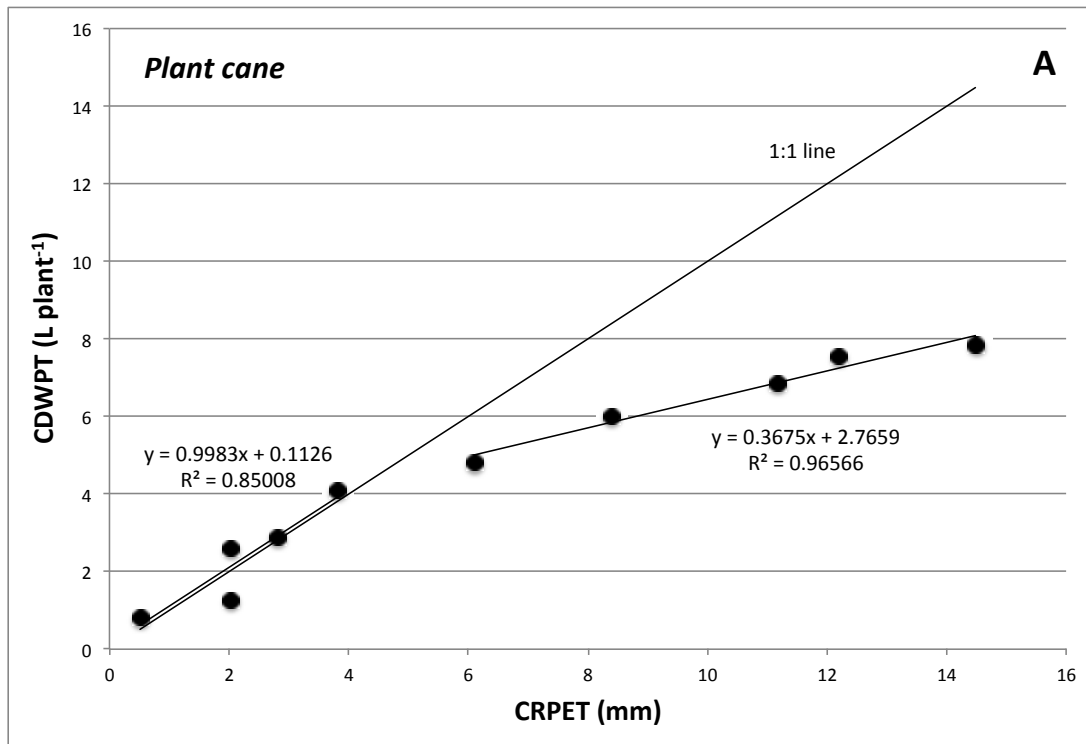
140 Day-to-day variation of daily whole-plant transpiration observed during the plant cane phase
 141 under moderate water-deficient conditions (Fig. 2) resulted from: a) normal day-to-day
 142 variation in weather conditions, b) progressive variations in leaf area per plant, including
 143 production of new leaves, leaf expansive growth, and leaf senescence, and c) progressive
 144 variations in leaf conductance leading to declines in leaf transpiration due to stomatal
 145 closure. Progression of daily RPET during phase 1 (Fig. 1) was characterized by the normal
 146 day-to-day variation and an overall increasing trend throughout the 99-day length of this first
 147 phase from about 1 to about 6.5 mm d⁻¹. This phase included three periods of decreased

148 atmospheric evaporative demand, one relatively long period (June 6th - July 13th) with low
 149 values of $2.8 \pm 1.04 \text{ mm d}^{-1}$ and two shorter periods (Aug 3rd to 9th and Aug 16th to 23rd) with
 150 values of $4.5 \pm 0.46 \text{ mm d}^{-1}$ and $4.3 \pm 1.15 \text{ mm d}^{-1}$, respectively.

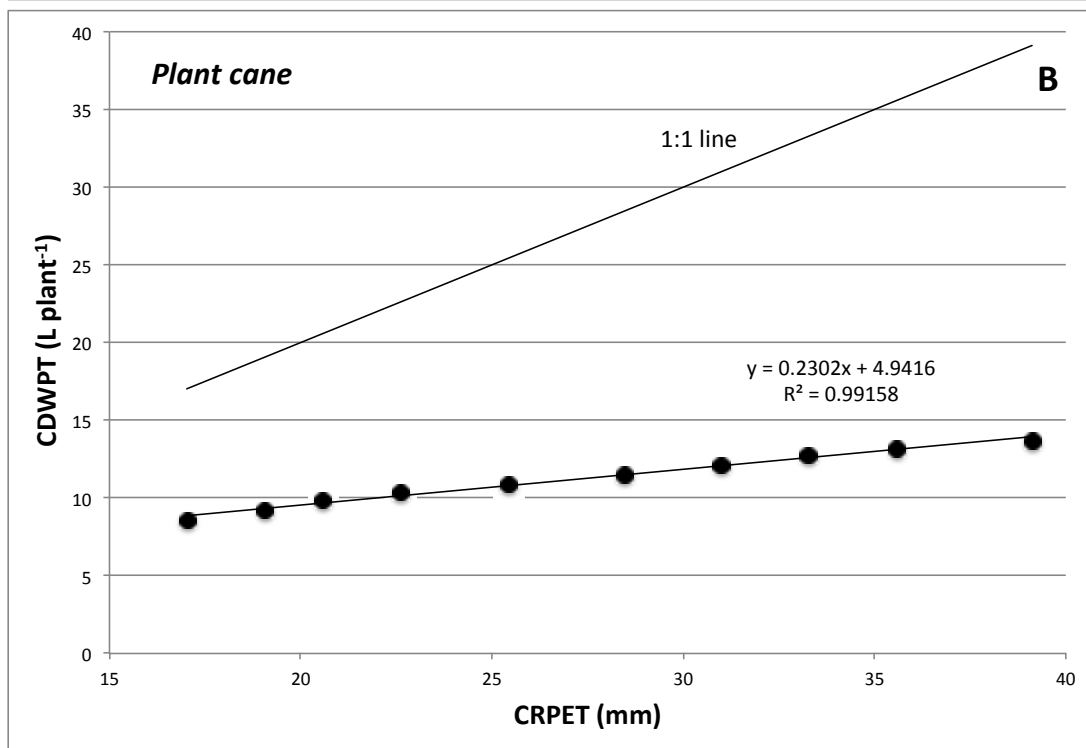


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 152
 153 **Figure 2. Progressions of the daily whole-plant transpiration (DWPT) of the three**
 154 **genotypes growing under a moderate water-deficient regime during the initial plant**
 155 **cane phase of the study.**

156 During the initial five days of phase 1 following the start of water deficits, the CDWPT across
 157 genotypes maintained a 1:1 relationship with CRPET (slope of the linear regression of
 158 CDWPT on CRPET was 0.9983) as plant leaf area continued to grow and leaf transpiration
 159 remained uninhibited (Fig. 3A). During the following five days (days 6-10), CDWPT averaged
 160 37% of CRPET (slope of linear regression was 0.3675), which resulted from decreasing
 161 DWPT likely due mainly to the inhibition of leaf area growth (Fig. 3A). As the restriction in
 162 daily irrigation led to further reductions in soil water content, CDWPT continued to decline
 163 averaging 23% of CRPET during the following 10 days (Fig. 3B). During the following 79
 164 days, CDWPT averaged 12% of CRPET (slope equal to 0.1229), which resulted in CDWPT
 165 being 19% of CRPET on day 99 (Fig. 3C). These declines in CDWPT from day 11 to day 99
 166 resulted likely through the combined effects of stomatal closure and leaf growth inhibition
 167 [10,11]. The almost flat progression of DWPT after day 55 (July 6, 2015) observed in Fig. 2
 168 appears to indicate that leaf area growth was fully inhibited from that point in time.



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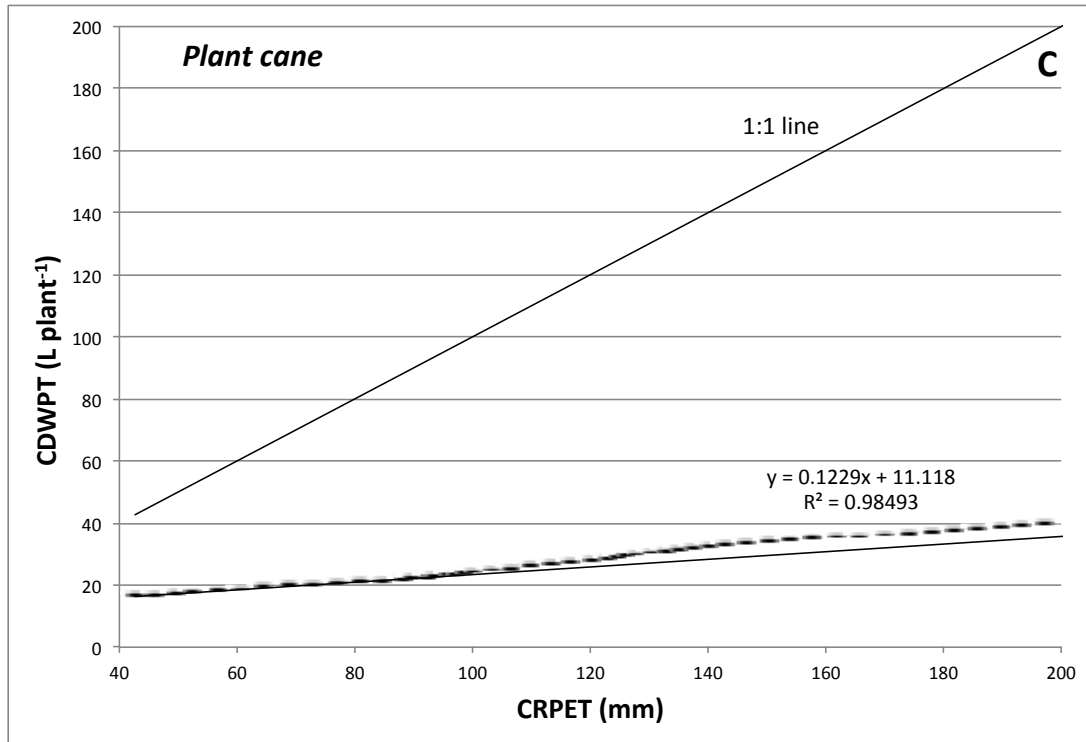


Figure 3. Linear regression of cumulative whole-plant transpiration (CDWPT) on cumulative reference potential evapotranspiration (CRPET) calculated for distinct periods within the plant cane phase of the study: 1 to 10 days (A), 11 to 20 days (B), and 21 to 99 days (C). Cumulative whole-plant transpiration values were calculated with daily whole-plant transpiration averaged across the three studied genotypes.

The genotypes during the plant cane first phase of the study under a water-deficient regime presented similar above-ground dry biomass production or its partitioning (Table 1). Total above-ground dry biomass averaged 437.6 g per pot across genotypes, comprised of 140.3 g per pot of dry leaf blades and 297.3 g per pot of dry stems including leaf sheaths.

The tillering characteristics were different among genotypes, particularly between the energy cane genotypes and the sugarcane genotype (Table 1). The number of tillers produced by the sugarcane genotype TCP94-5753 was about 42% lower than those produced by the two energy cane genotypes TUS56 and TUS59. The ability of energy cane to produce a larger number of stalks (tillers) was reported before [1]. Genotype TUS56 produced a larger length of tillers than the other two genotypes; 1.29 times more than TUS59 and 2.62 times more than TCP94-5753. These differences led to TCP94-5753 exhibiting a much higher specific stem length than the energy cane genotypes; 2.5 times higher than that of TUS56 and 1.87 times higher than that of TUS59.

Water economy among genotypes did not differ after exposure to water deficits during phase 1 (Table 1), as represented by CDWPT, transpiration per unit dry leaf mass, and a nominal water use efficiency. Cumulative whole-plant transpiration during this plant cane phase averaged 61.5 L per pot across genotypes. Transpiration per unit leaf mass, averaged 0.381

196 L g⁻¹ across genotypes. The nominal water use efficiency averaged 7.1 g L⁻¹ across
 197 genotypes.

198 **Table 1. Summaries of statistical analyses of state variables related to above-ground**
 199 **biomass partitioning, tillering characteristics, and water economy of two energy cane**
 200 **genotypes and one sugarcane genotype growing during the initial plant cane phase of**
 201 **the study under a water-deficient regime.**

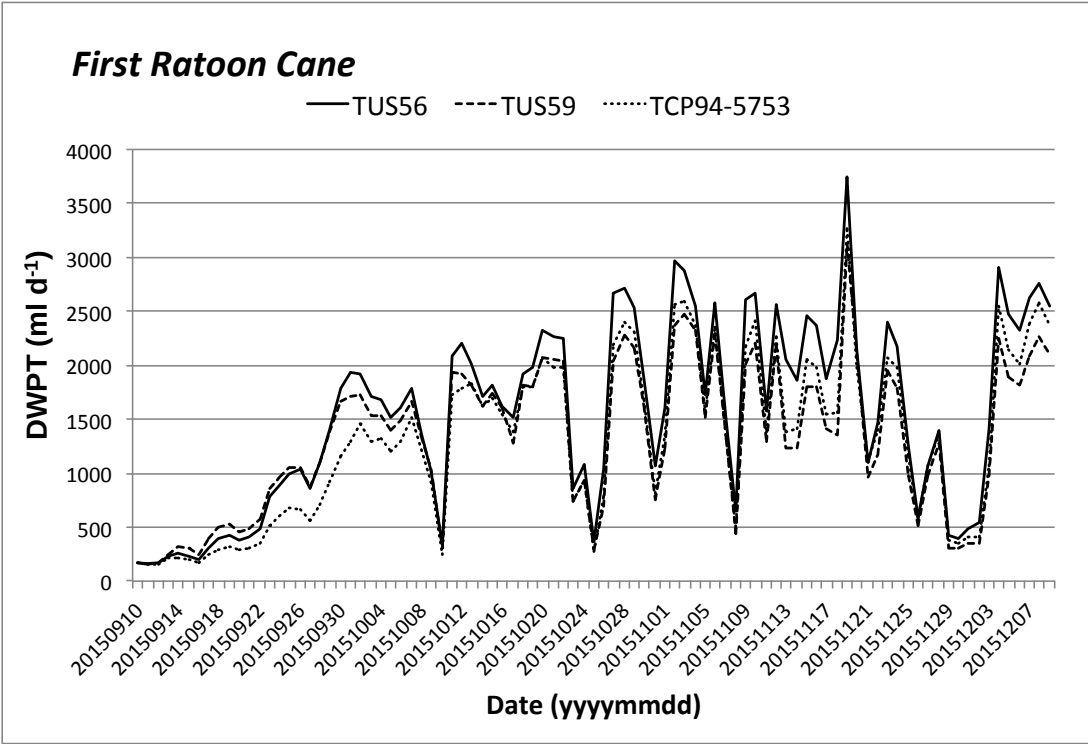
<i>Growth phase: plant cane</i>			
Above-Ground Dry Biomass Partitioning			
<i>Genotype</i>	Leaf blades (g per pot)	Stems (with leaf sheaths) (g per pot)	Total Above- Ground Biomass (g per pot)
TUS56	138.1 a	293.6 a	431.7 a
TUS59	153.3 a	307.5 a	460.7 a
TCP94-5753	129.6 a	290.7 a	420.3 a
LSD 0.05	25.7	95.9	93.9
Tillering Characteristics			
<i>Genotype</i>	Number of tillers (per pot)	Total length of tillers (m per pot)	Specific stem length (g m ⁻¹)
TUS56	11.6 a	0.76 a	39.0 b
TUS59	12.4 a	0.59 b	52.1 b
TCP94-5753	5.0 b	0.29 c	97.4 a
LSD 0.05	1.9	0.14	17.1
Water Economy			
<i>Genotype</i>	CDWPT (L plant ⁻¹)	CDWPT per Leaf Mass (L g ⁻¹)	Nominal Water Use Efficiency (g L ⁻¹)
TUS56	63.9 a	0.467 a	6.8 a
TUS59	59.9 a	0.395 a	7.7 a
TCP94-5753	60.6 a	0.472 a	6.9 a
LSD 0.05	4.7	0.0938	1.6

202 Means followed by a different letter are significantly different at 0.05 level. CDWPT:
 203 Cumulative Daily Whole-Plant Transpiration.

204 3.2 Phase 2 of study: First ratoon cane under well-watered conditions

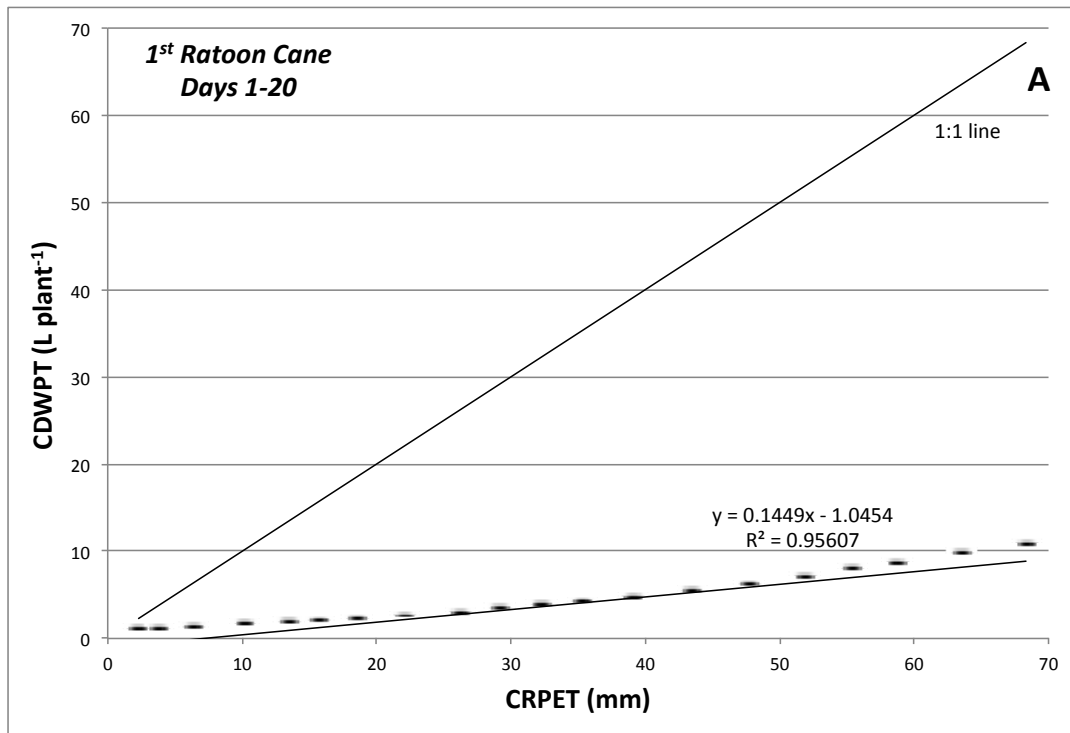
205 Day-to-day variation of daily whole-plant transpiration observed during the first ratoon cane
 206 phase under well-watered conditions (Fig. 4) resulted primarily from: a) normal day-to-day
 207 variation in weather conditions and b) progressive variations in leaf area per plant, including
 208 production of new leaves and leaf expansive growth. Progression of daily RPET during this
 209 second phase of the study, which extended for 91 d and occurred mostly during the fall
 210 season (Fig. 1) was characterized by the normal day-to-day variation and an overall

211 increasing trend during the first 24 d, followed by an overall declining trend accentuated
 212 from day 76 to day 85. The progressions of daily whole-plant transpiration for the three
 213 genotypes studied showed generally higher values for the energy cane TUS56 (Fig. 4).
 214 Since plants were grown under a well-watered regime, daily whole-plant transpiration values
 215 were much higher than those observed during the previous plant cane phase grown under a
 216 water-deficient regime during the summer season.

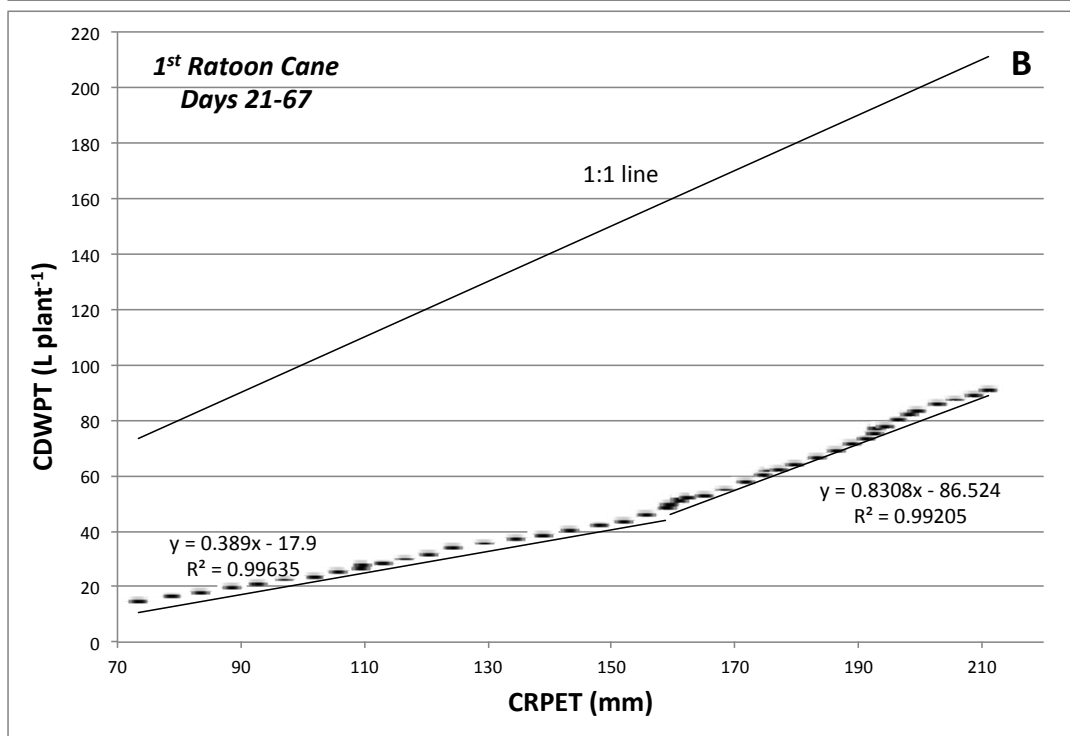


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 219 **Figure 4. Progressions of the daily whole-plant transpiration (DWPT) of the three**
 220 **genotypes growing under a well-watered regime during the first ratoon cane phase of**
 221 **the study.**

222 During the initial 20 days of the well-watered first ratoon phase, which followed above-
 223 ground harvest of the plant cane phase, the CDWPT across genotypes remained at about
 224 14% of CRPET (slope of the linear regression of CDWPT on CRPET was 0.1449, as shown
 225 in Fig. 5A), as above-ground plant growth slowly began developing leaves. During the
 226 following 46 days (days 21-67) as plants entered a rapid growth rate and plant leafiness
 227 increased, CDWPT averaged 39% of CRPET during the first half of this period and 83% of
 228 CRPET during the second half (slopes of linear regression were 0.389 and 0.8308,
 229 respectively, as shown in Fig. 5B). From day 68 to 91, the average CDWPT paralleled the
 230 average CRPET (slope of linear regression was 0.9953), as plant foliage continued to
 231 increase (Fig. 5C).



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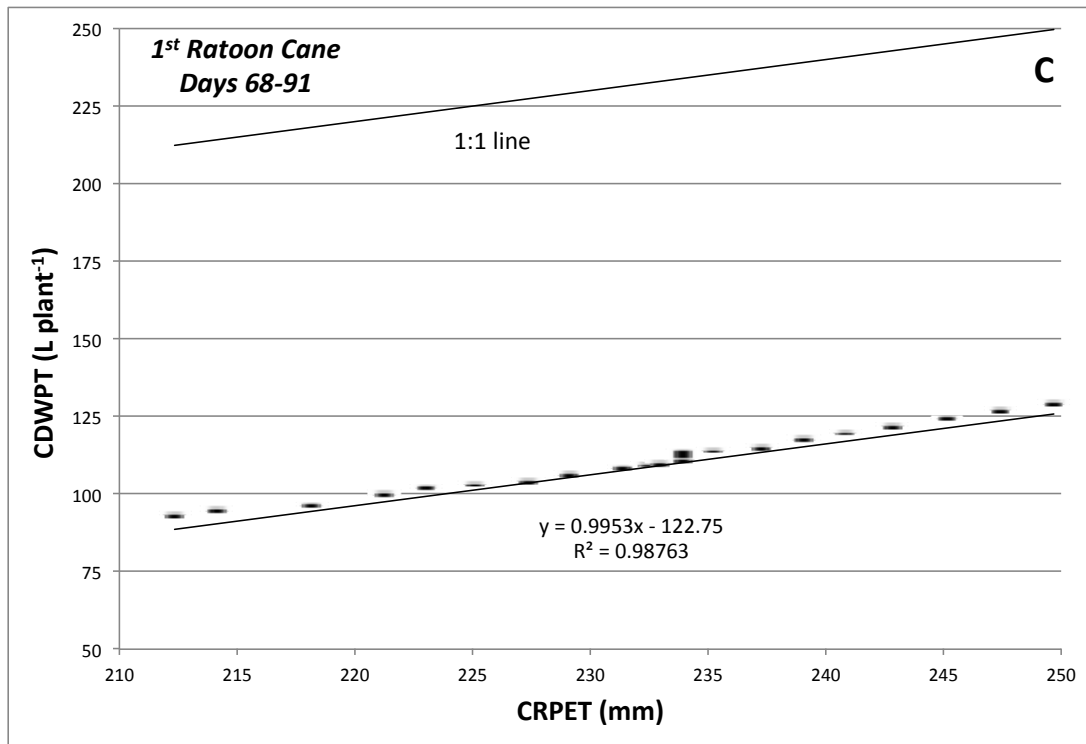


Figure 5. Linear regression of cumulative whole-plant transpiration (CDWPT) on cumulative reference potential evapotranspiration (CRPET) calculated for distinct periods within the first ratoon cane phase of the study: 1 to 20 days (A), 21 to 67 days (B), and 68 to 91 days (C). Cumulative whole-plant transpiration values were calculated with daily whole-plant transpiration averaged across the three studied genotypes.

The above-ground dry biomass production or stems dry biomass (including leaf sheaths) among genotypes during the first ratoon cane phase of the study under a well-watered regime were not different (Table 2). Total above-ground dry biomass and stems dry biomass averaged, respectively, 886 and 552.9 g per pot across genotypes. There were differences among genotypes, however, in leaf blades dry biomass. TUS56 produced 15 and 10% more leaf blades biomass than TUS59 and TCP94-5753, respectively.

Tillering characteristics among genotypes were not different at the end of the well-watered first ratoon cane phase (Table 2). The energy cane genotypes TUS56 and TUS59 produced 2.32 and 1.83 times more tillers than the sugarcane genotype TCP94-5753, respectively, while TUS56 produced 27% more tillers than TUS59. As was reported earlier [1], and as results showed during the previous plant cane phase under a water-deficient regime, the energy cane genotypes growing under a well-watered regime during this first ratoon cane phase also exhibited the ability to produce a larger number of stalks (tillers) than the sugarcane genotype. The high tillering ability of energy cane genotypes has been linked to their rhizomatous rooting system [18]. Similarly, the energy cane genotypes TUS56 and TUS59 produced 2.66 and 2.14 times more length of tillers than the sugarcane genotype TCP94-5753, respectively. Genotype TUS56 produced 25% more length of tillers than TUS56. These differences led to TCP94-5753 exhibiting about 2.18 times higher specific stem length than the energy cane genotypes. There was no difference in specific stem

length between the energy cane genotypes TUS56 and TUS59. These results indicate also that the tillering potential of energy cane genotypes may differ substantially.

Water economy among genotypes did change during in the well-watered first ratoon phase (Table 2). Cumulative whole-plant transpiration during this phase was 17% higher for TUS56 than for TUS59 and TCP94-5753, which appears to be closely related to the higher leafiness of TUS56 relative to the other genotypes. Transpiration per unit leaf mass was not different between the energy cane genotypes, but was about 6.4% higher than that of the sugarcane genotype. The nominal water use efficiency was similar for TUS59 and TCP94-5753, but 12.3% lower for TUS56, as this energy cane genotype was the most prodigal in cumulative transpiration.

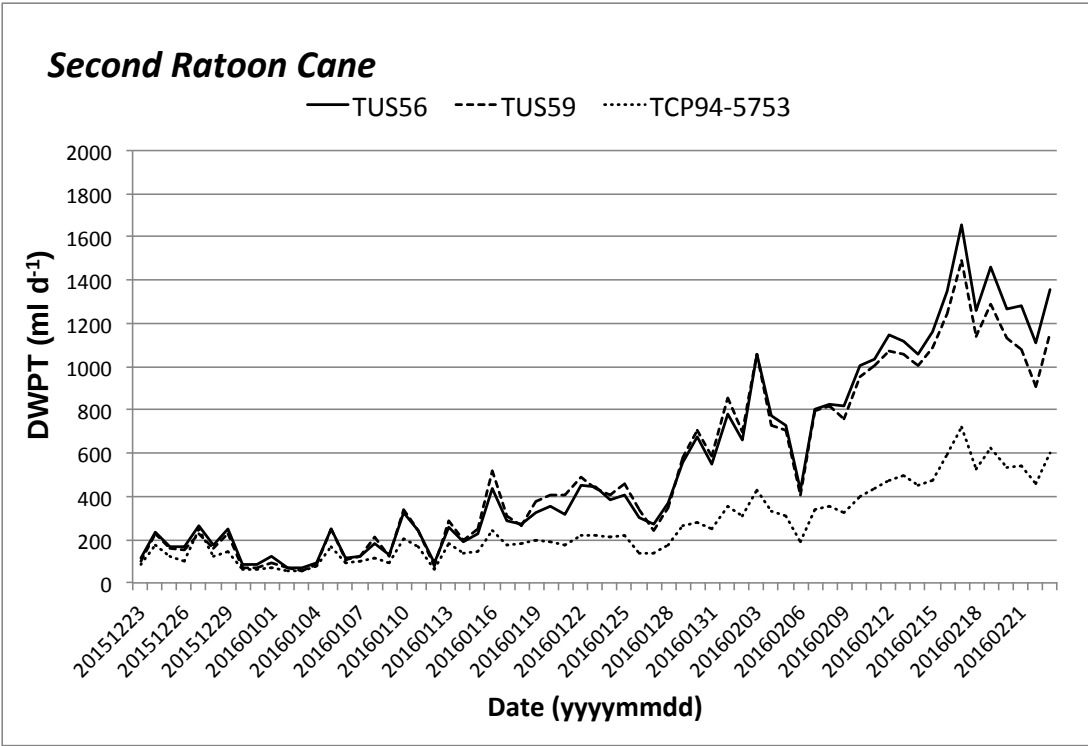
Table 2. Summaries of statistical analyses of state variables related to above-ground biomass partitioning, tillering characteristics, and water economy of two energy cane genotypes and one sugarcane genotype growing during the first ratoon cane phase of the study under a well-watered regime.

<i>Growth phase: first ratoon cane</i>			
Above-Ground Dry Biomass Components			
Genotype	Leaf blades (g per pot)	Stems (with leaf sheaths) (g per pot)	Total Above-Ground Biomass (g per pot)
TUS56	359.8 a	536.5 a	896.3 a
TUS59	313.0 b	594.0 a	907.0 a
TCP94-5753	326.7 b	528.1 a	854.7 a
LSD 0.05	20.4	76.0	85.5
Tillering Characteristics			
Genotype	Number of tillers (per pot)	Total length of tillers (m per pot)	Specific stem length (g m ⁻¹)
TUS56	46.0 a	3.30 a	16.3 b
TUS59	36.2 b	2.65 b	22.6 b
TCP94-5753	19.8 c	1.24 c	42.4 a
LSD 0.05	8.4	0.33	6.3
Water Economy			
Genotype	CDWPT (L plant ⁻¹)	CDWPT per Leaf Mass (L g ⁻¹)	Nominal Water Use Efficiency (g L ⁻¹)
TUS56	140.5 a	0.392 a	6.4 b
TUS59	120.5 b	0.385 a	7.5 a
TCP94-5753	119.4 b	0.365 b	7.1 a
LSD 0.05	4.7	0.019	0.4

Means followed by a different letter are significantly different at 0.05 level. CDWPT: Cumulative Daily Whole-Plant Transpiration.

277 **3.3 Phase 3 of study: Second ratoon cane under well-watered conditions**

278 As described for the first ratoon cane phase, the day-to-day variation of daily whole-plant
279 transpiration observed during the second ratoon cane phase under well-watered conditions
280 (Fig. 6) resulted primarily also from: a) normal day-to-day variation in weather conditions and
281 b) progressive variations in leaf area per plant, including production of new leaves and leaf
282 expansive growth. Progression of daily RPET during this third phase of the study, which
283 extended for 63 d and occurred mostly during the winter season (Fig. 1), was characterized
284 by a normal day-to-day variation and an overall increasing trend throughout its duration. The
285 progressions of daily whole-plant transpiration for the three genotypes studied showed much
286 lower values for the sugarcane genotype than both energy cane genotypes. Since
287 atmospheric evaporative demand was low at the start of this second ratoon phase although
288 it increased steadily throughout, daily whole-plant transpiration values were lower than those
289 observed in the fall during the previous first ratoon cane phase.



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291
292 **Figure 6. Progressions of the daily whole-plant transpiration (DWPT) of the three**
293 **genotypes growing under a well-watered regime during the second ratoon cane phase**
294 **of the study.**

295 During the initial 52 d of the well-watered second ratoon phase, which followed the above-
296 ground harvest of the first ratoon phase, the average CDWPT across genotypes remained at
297 15% of CRPET (slope of linear regression of CDWPT on CRPET was 0.1521), as above-
298 ground plant growth slowly began developing leaves (Fig. 7A). During the following 11 days
299 (days 53-63), CDWPT averaged only 40% of CRPET (Fig. 7B), which indicates a low rate of
300 foliage development. This is particularly the case for the sugarcane genotype, which shows
301 a much lower progression of DWPT than that of the two energy cane genotypes (Fig. 6).

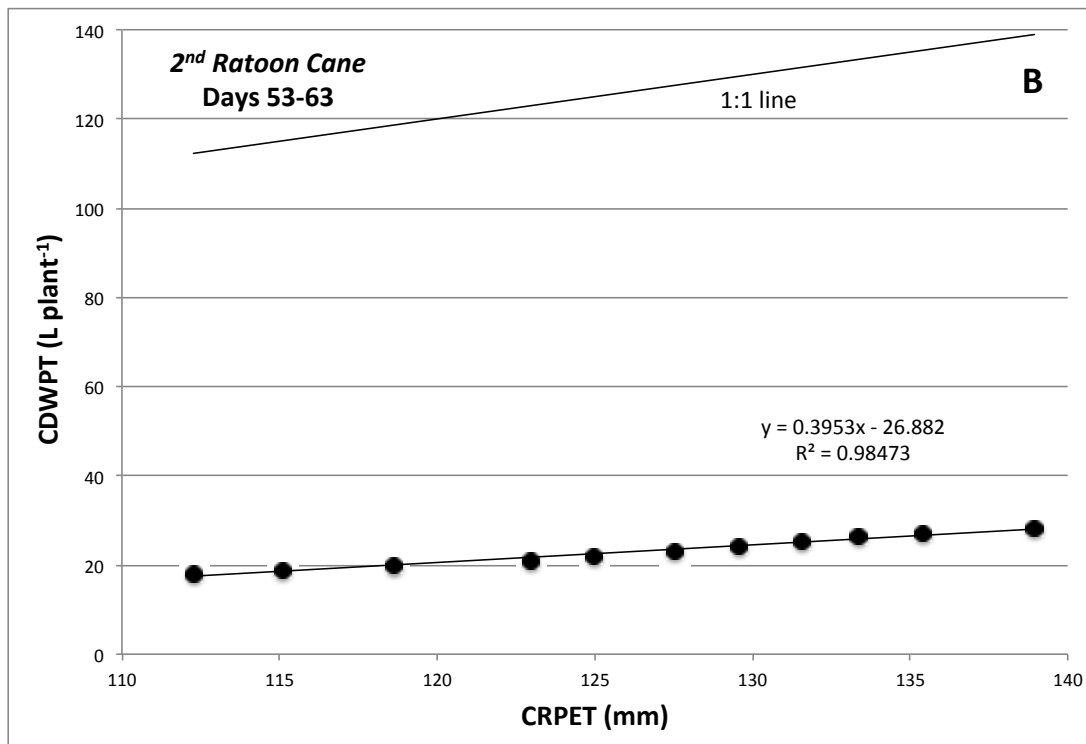
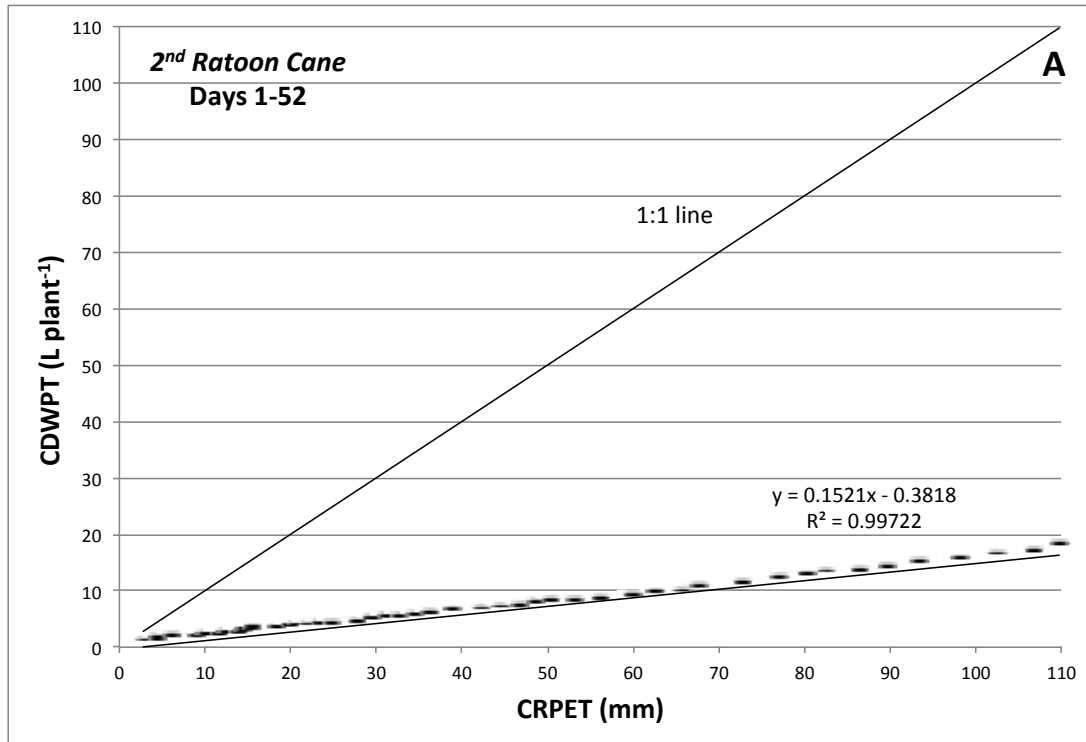


Figure 7. Linear regression of cumulative whole-plant transpiration (CDWPT) on cumulative reference potential evapotranspiration (CRPET) calculated for distinct periods within the second ratoon cane phase of the study: 1 to 52 days (A) and 53 to

308 **63 days (B).** Cumulative whole-plant transpiration values were calculated with daily
 309 whole-plant transpiration averaged across the three studied genotypes.

310 Differences in above-ground dry biomass production components among genotypes during
 311 the second ratoon cane phase of the study under a well-watered regime were noted (Table
 312 3). Total above-ground dry biomass, stems dry biomass, and leaf blades dry biomass were,
 313 respectively 60, 57, and 64% higher in TUS56 than in TCP94-5753, while these biomass
 314 components in TUS59 were intermediate and not significantly different from either TUS56 or
 315 TCP94-5753.

316 The water economy among genotypes during the well-watered second ratoon phase varied
 317 according to genotype (Table 3). Cumulative whole-plant transpiration during this phase was
 318 about 88% higher for the energy cane genotypes than for the sugarcane genotype. Most
 319 significant factors leading to this difference in cumulative whole-plant transpiration between
 320 the energy cane genotypes and the sugarcane genotype were a 64% higher leaf blades
 321 mass exhibited by TUS56 and a 29.5% higher transpiration per unit leaf mass exhibited by
 322 TUS59. There were no significant differences in transpiration per unit leaf mass between the
 323 two energy cane genotypes, nor between TUS56 and the sugarcane genotype TCP94-5753.
 324 The nominal water use efficiency of TCP94-5753 was about 25% higher than that of the
 325 energy cane genotypes, which resulted from the combined effect of producing an average
 326 33% less above-ground biomass but an average 47% less cumulative transpiration than the
 327 energy cane genotypes.

328 **Table 3. Summaries of statistical analyses of state variables related to above-ground**
 329 **biomass partitioning and water economy of two energy cane genotypes and one**
 330 **sugarcane genotype growing during the second ratoon cane phase of the study under**
 331 **a well-watered regime.**

<i>Growth phase: second ratoon cane</i>			
Above-Ground Dry Biomass Components			
Genotype	Leaf blades (g per pot)	Stems (with leaf sheaths) (g per pot)	Total Above- Ground Biomass (g per pot)
TUS56	212.0 a	381.2 a	593.2 a
TUS59	177.7 ab	335.5 ab	513.1 ab
TCP94-5753	129.3 b	242.5 b	371.8 b
LSD 0.05	61.4	122.3	177.7
Water Economy			
Genotype	CDWPT (L plant ⁻¹)	CDWPT per Leaf Mass (L g ⁻¹)	Nominal Water Use Efficiency (g L ⁻¹)
TUS56	35.8 a	0.170 ab	6.0 b
TUS59	32.3 a	0.180 a	5.7 b
TCP94-5753	18.1 b	0.139 b	7.3 a
LSD 0.05	6.5	0.0316	1.3

332 Means followed by a different letter are significantly different at .05 level. **CDWPT:**
 333 **Cumulative Daily Whole-Plant Transpiration.**
 334

335 4. CONCLUSIONS

336 Three sequential studies conducted during the plant cane growth phase and two subsequent
337 ratoon cane phases made it possible to characterize and comparatively evaluate the water
338 economy and growth performance of two energy cane genotypes (TUS56 and TUS59) and
339 one sugarcane genotype (TCP87-3388).

340 Exposing the genotypes to water deficits for 99 d during the initial plant cane growth phase
341 did not result in above-ground dry biomass production or water economy differences among
342 genotypes, however, the energy cane genotypes exhibited a high tillering ability producing
343 about 2.4 times more tillers than the sugarcane genotype.

344 The 91-d long well-watered first ratoon cane phase resulted in no differences in above-
345 ground dry biomass or stems dry biomass (including leaf sheaths) among genotypes.
346 However, energy cane genotype TUS56 produced 15 and 10% more leaf blade dry biomass
347 than TUS59 and TCP94-5753, respectively, which appears to largely explain why TUS56
348 had a 17% higher cumulative daily whole-plant transpiration than TUS59 and TCP94-5753.
349 Additionally, the energy cane genotypes TUS56 and TUS59 produced 2.32 and 1.83 times
350 more tillers than the sugarcane genotype, respectively. The 27% higher tiller production of
351 TUS56 compared to that of TUS59 indicates that the tillering potential of energy cane
352 genotypes may differ substantially.

353 The suboptimal environmental conditions (cool temperatures and low solar radiation) that
354 occurred during the 63-d long well-watered second ratoon cane phase did not prevent the
355 energy cane genotypes from exhibiting a ratooning ability stronger than that of the
356 sugarcane genotype. Total above-ground dry biomass, stems dry biomass, and leaf blades
357 dry biomass were not significantly different between the two energy cane genotypes, but
358 were 60% higher in TUS56 than in the sugarcane genotype. Because of this enhanced
359 growth, the cumulative daily whole-plant transpiration was about 88% higher for the energy
360 cane genotypes than for the sugarcane genotype.

361 The better tillering ability shown by the energy cane genotypes over the sugarcane genotype
362 during the water stressed plant cane phase and the first ratoon cane phase and the better
363 above-ground biomass production of energy cane over sugarcane in the second ratoon cane
364 phase are both consistent with previous reported findings.

365

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368 **COMPETING INTERESTS**

369

370 Authors have declared that no competing interests exist.

371

372 **AUTHORS' CONTRIBUTIONS**

373 This work was carried out in collaboration between all authors. Author CJF designed the
374 study, wrote the protocol of the study and the literature searches. Author JDS performed
375 selection and germinated the stem cuts of the genotypes studied. Author JCC managed the
376 rain-shelter facility and performed data collection and statistical analyses. Author CJF
377 developed and managed the computerized system to measure daily plant water use and
378 wrote the manuscript. Author WJG formatted the manuscript for publication. All authors read
379 and approved the final manuscript.

380

381 **REFERENCES**

382 [1] Johnson JMF, MD Coleman, R Gesh, A Jaradat, R. Mitchell, D Reicosky, and WW
383 Wilhelm. Biomass-bioenergy crops in the United States: a changing paradigm. *Am. J. Plant*
384 *Sci. Biotechnol.* 2007;1(Suppl 1):1-28.

385 [2] USDOE. Biomass: multi-year program plan. United States Department of Energy,
386 Washington. 2008.

387 [3] Giamalva MJ, Clarke SJ, Stein JM. Sugarcane Hybrids Of Biomass. *Biomass.* 1984;
388 6:61–68.

389 [4] Samuels G, Alexander AG, Rios CE, Garcia H. The production of energy cane in Puerto
390 Rico: The Hatillo Project. *J.AM. Soc. Sugarcane Technol.* 1984;3:14-17.

391 [5] Alexander AG. The energy cane alternative. Elsevier, Amsterdam. 1985.

392 [6] Carvalho-Netto OV, Bressiani JA, Soriano HL, Fiori CS, Santos JM, Barbosa GVS, Xavier
393 MA, Landell MGA, Pereira GAG. The potential of the energy cane as the main biomass crop
394 for the cellulosic industry. *Chem. Biol. Tech. Agric.* 2014;1:20.

395 [7] Ming R, Moore P, Wu KK, D'Hont A, Glaszmann JC, Tew TL, Mirkov TE, Da Silva J, Jifon
396 J, Rai M, Schnell RJ, Brumbley SM, Lakshmanan P, Comstock J, Paterson AH. Sugarcane
397 improvement through breeding and biotechnology. In: Janick J, editor. *Plant Breeding*
398 *Reviews.* John Wiley, New York.

399 [8] Inman-Bamber NG, Smith DM. Water relations in sugarcane and response to water
400 deficits. *Field Crop Res.* 2005;92:185–202.

401 [9] Jones, MR, Singels A, Ruane AC. Simulated impacts of climate change on water use and
402 yield of irrigated sugarcane in South Africa. *Agric. Syst.* 2015;139:260–270.

403 [10] McCree KJ, Fernandez CJ. A simulation model for studying physiological water stress
404 responses of whole plants. *Crop Sci.* 1989;29:353-362.

405 [11] McCree KJ, Fernandez CJ, Ferraz de Oliveira R. Visualizing interactions of water stress
406 responses with a whole-plant simulation model. *Crop Sci.* 1990;30:294-300.

407 [12] Ramesh P. Effect of different levels of drought during the formative phase on growth
408 parameters and its relationship with dry matter accumulation in sugarcane. *J. Agron. Crop*
409 *Sci.* 2000;85:83–89.

- 410 [13] Inman-Bamber NG, De Jager JM. The reaction of two varieties of sugarcane to water
411 stress. *Field Crops Res.* 1986;14:15–28.
- 412 [14] Inman-Bamber NG, De Jager JM. Effect of water stress on growth, leaf resistance and
413 canopy temperature in field-grown sugarcane. *Proc. South Africa Sugar Technol. Assoc.*
414 1986;60:156–161.
- 415 [15] Glaz B, Davidson RW, Milligan SB, Comstock JC, Edmé SJ, Gilbert RA. Evaluation of
416 new Canal Point sugarcane clones: 2005–2006 harvest season. 2007:ARS-167. U.S.
417 Department of Agriculture, Agricultural Research Service, Washington, DC.
- 418 [16] Fernandez CJ, Mirkov E, Dickman MB, Molina MF, Molina-Risco MD, Correa JC,
419 Grichar WJ. Characterization of the water economy of sugarcane transgenic genotypes. *J.*
420 *Exp. Agric. Int.* 2016; 14(3):1-13.
- 421 [17] VanBavel CHM, Lascano R, Wilson DR. Water relations of fritted clay. *Soil Sci. Soc.*
422 *Am. J.* 1978;42:657-659.
- 423 [18] Da Silva JA. The importance of the wild cane *Saccharum spontaneum* for bioenergy
424 genetic breeding. *Sugar Tech.* 2017; 19(3):229-240.