American Journal of Agricultural and Biological Sciences 5 (3): 403-414, 2010
ISSN 1557-4989

Sugarcane Response to Water-Deficit Stress during Early Growth on Organic and Sand Soils

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Abstract: Problem statement: Approximately 20% of sugarcane (*Saccharum* spp.) is grown on sand soils in south Florida, USA. Sugarcane yields in the region linearly increased in last 33 years on organic (muck) soils, but not on sand soils. Water deficit during the formative growth phase on sand soils probably limits sugarcane yields. **Approach:** A greenhouse study was conducted in 2009 and 2010 to evaluate the physiological and growth responses of sugarcane to water-deficit stress during formative growth. Treatments included organic (muck) and sand soils and two water regimes Well Watered (WW) and Water-Deficit Stress (WS). Sugarcane cultivar CP 80-1743 was planted in pots and fertilized with N, P and K based on soil analyses. All pots were well watered until 58 days after planting, when water was withheld from the WS pots. During the WS treatment, plant growth rate, leaf Relative Water Content (RWC), proline content and photosynthesis components were measured. Final tillers, Green Leaf Area (GLA) and shoot biomass were determined 27 (in 2009) or 22 (in 2010) days after initiating the WS treatment. **Results:** Stress symptoms of sugarcane plants appeared 7-10 days earlier on sand soil than on muck soil. Water stress reduced stomatal conductance (gs), Photosystem II Photochemical Efficiency (ΦPSII), leaf Photosynthesis rate (Pn), the number of tillers and GLA, resulting in reduced shoot biomass, especially on sand soil. Neither leaf RWC nor proline content was a sensitive WS indicator. **Conclusion:** Nondestructive measurements of physiological traits of gs, ΦPSII and Pn during the formative stage may be useful for early detection of water stress in sugarcane.

Key words: Formative growth phase, photosynthesis components, physiological traits, soil properties, sugarcane

INTRODUCTION

Sugarcane (a complex hybrid of *Saccharum* spp.) is an important crop in Florida, USA with a total of 157,074 ha in 2008 (Rice *et al*., 2009). Approximately 20% of this sugarcane was grown on sand soils. A major goal of the Canal Point (CP) sugarcane cultivar selection program in Florida is to develop high-yielding cultivars with disease resistance and tolerance to abiotic stresses for organic (muck) and sand soils (Glaz and Kang, 2008). Edme *et al*. (2005) reported that, for a 33-year period, about 69% of the sugar yield gain in south Florida was from genetic improvements attributable to the CP cultivar selection program, but these yield gains were mainly associated with muck rather than sand soils. Based on these findings, scientists in Florida are conducting a comprehensive review of the CP program to identify breeding and management strategies that will improve sugarcane yields for sand soils without compromising the progress being made for muck soils (Glaz and Kang, 2008).

Unlike the muck soil stresses, such as excessive nitrogen and frequent flooding conditions due to shallow water tables (Glaz *et al*., 2008), sugarcane grown on sand soils is often subjected to environmental stresses such as nutrient deficiencies (Ezenwa *et al*., 2005) and water deficit (Silva *et al*., 2007) due to low soil organic matter and low soil water content (Ezenwa *et al*., 2005). Thus, we propose that a stronger focus on genotypic tolerance to the abiotic factors that challenge sugarcane grown on sand soils will contribute to improved genetic potential for yields. In addition to improve irrigation management, development of stress-tolerant (especially to water-deficit stress) genotypes may improve sugarcane production on sand soils. However, it is unclear that which physiological traits can be used to efficiently and early detect sugarcane plant water-deficit stress. An improved understanding

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of growth habits and physiological traits that arise in response to water stress will improve sugarcane genotype selection efficiency for sand soils.

It is well known that water-deficit stress alters a variety of physiological processes such as stomatal conductance, transpiration rate, leaf temperature, photo-chemical electron transport, photosynthesis, respiration and photo-assimilate partitioning (Gardner *et al*., 1984). These physiological traits are directly or indirectly associated with crop growth and yields (Tollenaar and Aguilera, 1992; Zhang *et al*., 2001; Silva *et al*., 2007). There is genetic variation among crop species and genotypes within species in response to water-deficit stress that is also affected by developmental growth stage. In sugarcane, four distinct growth stages (i.e., germination, tillering, grand growth and maturity) have been characterized (Gascho and Shih, 1983). The tillering and grand growth stages, known as the sugarcane formative phase, have been identified as the critical water demand period (Ramesh, 2000). This is mainly because 70-80% of cane yield is produced during this phase (Singh and Rao, 1987). Therefore, quantifying plant water status and leaf photosynthetic components during the formative phase may be useful for identifying sugarcane plant response to water-deficit stress.

Sugarcane physiological and morphological traits responsible for improved cane yield, sucrose content and resource use remain poorly understood (Edmeades *et al*., 2004; Inman-Bamber *et al*., 2005). Stress symptoms of sugarcane on sand soils in Florida are generally not so extreme that they are detected visibly. However, based on the low yields and lack of genetic gain on sand soils (Edme *et al*., 2005), we hypothesized that physiological processes are negatively affected by abiotic stresses, especially water-deficit stress during sugarcane formative growth on the Florida sand soils. Therefore, identification of physiological and growth responses in sugarcane to water-deficit stress should aid to better understand physiological mechanisms and improve cultivar selection and field management for sand soils in Florida.

In the present study, we conducted a greenhouse pot experiment to investigate growth and physiological characteristics of sugarcane during its formative growth phase (i.e., 60-90 days after planting) during development of water-deficit stress on muck and sand soils. The specific objectives were to: (i) determine leaf Relative Water Content (RWC), proline content, photosynthesis components, plant growth and dry matter production and (ii) identify growth and physiological traits that can be used to effectively evaluate status of water stress in sugarcane.

MATERIALS AND METHODS

Plant culture and treatments: A pot study was conducted in a greenhouse at the USDA-ARS Sugarcane Field Station, Canal Point, Florida, USA in 2009 and 2010. Pots were 38 cm in both diameter and depth with four small holes (1.5 cm diameter) at the base. Treatments included two soils, Pahokee muck (euic, hyperthermic Lithic Haplosaprist) and Margate sand (siliceous, hyperthermic Mollic Psammaquent) and two water regimes, Well-Watered (WW) and Water-deficit Stress (WS). The muck and sand soils were collected from sugarcane production fields near South Bay and Clewiston, FL, respectively. Pots were filled with the respective soils and placed into individual containers that were used for desired water treatments. Greenhouse temperatures ranged from 30-35°C during the day and 20-25°C at night throughout the experiment. The night temperature in the greenhouse during the coldest period (Jan. to mid March) was relatively lower in 2009 than in 2010 because a heating system was added in the greenhouse in 2010. The greenhouse relative humidity ranged from 40-60% depending on weather conditions during the experiment. The Photosynthetically Active Radiation (PAR) in the greenhouse was approximately 90% of ambient level without any supplemental lights.

Single-bud stalk sections of a commercial sugarcane cultivar 'CP 80-1743' (Deren *et al*., 1991) in the region were planted in pots on 27 Jan. 2009 and 2 Feb. 2010. Fertilization with P (37 kg ha^{-1} for muck and 20 kg ha^{-1} for sand), K (140 kg ha^{-1} for muck and 186 kg ha^{-1} for sand) and micro nutrients was performed at planting based on soil test results and based on recommendations for sugarcane nutrient management in Florida (Gilbert and Rice, 2009). No N fertilizer was used for the muck soil because annually approximately 900 kg N ha^{-1} is made available through soil organic matter mineralization (Glaz and Gilbert, 2006), but the sand soil received a rate of 100 kg N ha^{-1} based on the soil test and recommendations. Nitrogen (ammonia nitrate) fertilizer for the sand soil treatment was applied on 19 Feb. 2009 and 22 Feb. 2010. All pots were well watered by adding water in containers daily before initiation of water treatments. The water regime treatment started on 24 Mar. 2009 and 27 Mar. 2010, when plants averaged 6.5 (in 2009) to 8.2 (in 2010) leaves on their main stalks. Water was withheld from the WS treatment pots and the shortage of soil water gradually developed the water stress, while the WW pots still received water daily to always keep a depth of 2 cm water in the containers.

Measurements: Soil samples were collected from all pots prior to planting and before application of any fertilizers. Prepared soil samples were sent to the University of Florida's Everglades Soil Testing Laboratory at the Everglades Research and Education Center in Belle Glade, FL for analyses of pH (Daroub *et al*., 2008), water extractable P (Daroub *et al*., 2008), acetic acid extractable P and K contents (Korndorfer *et al*., 1995). Additionally, soil organic matter content was determined using the loss on ignition from 105-600°C. Soil total N and C contents were analyzed using a VarioMax CNS Macro Elemental Analyzer (Elementar Americas, Inc., Mt. Laurel, NJ). Soil bulk density and water holding capacity were also determined prior to planting. To estimate soil water holding capacity and bulk density, six additional pots were filled with muck or sand soils and transported a laboratory. Exceed water was added in the pots three times to insure soil completely saturate and extra water drained slowly from small holes at the base of pots. Soil cores were collected in each pot 24 h after water was added using a 0200 soil core sampler (Soilmoisture Equipment Corp., Santa Barbara, CA). The wet soil samples were thoroughly transferred to alumina soil cans from the brass cylinders and weighed. Then, the wet soil samples were dried at 105°C for 24 h and weighed. Soil water holding capacity and bulk density were calculated based on soil wet and dry weights and core volume.

During the WS treatment, leaf samples were collected between 10:30 and 11:30 h to measure leaf Relative Water Content (RWC) and proline content weekly. Leaf RWC was determined according to Dhopte and Manuel (2002) and leaf proline content was assayed based on the method of Bates *et al*. (1973) using the leaf immediately below the Top Visible Dewlap (TVD) leaf.

Leaf SPAD index and leaf photosynthesis components, such as Photosynthetic rate (Pn), stomatal conductance (gs), intercellular CO_2 Concentration (Ci), photosystem II photochemical efficiency (ΦPSII) and leaf transpiration rate (Tr), were measured every 3 or 4 days between 10:30 and 13:00 h from the TVD leaves. SPAD index was measured with a Minolta SPAD-502 chlorophyll meter (Minolta Co., LTD., Japan). A LI-6400XT portable photosynthesis system (LI-COR Inc., Lincoln, NE) was used to measure leaf photosynthetic components. When measuring leaf photosynthesis components, PAR in the leaf chamber, provided by the 6400-02 LED light source, was set to 1500 μmol m^{-2} sec^{-1}, relative humidity was adjusted to near ambient level (50-60%) and leaf chamber CO_2 concentration was set to 380 ppm.

The number of nodes (or leaves) and stalk length on the primary stalk and the number of tillers were recorded when the WS treatment was initiated and completed. Mean increment rates for main stalk elongation, nodes of the main stalk and number of tillers were estimated based on the following formula:

$$\text{Mean Increment Rate} = \frac{(G_2 - G_1)}{(t_2 - t_1)}$$

Where:

G_2 and G_1 = One of several growth variables (i.e., the number of nodes, stalk elongation or the number of tillers) measured at the ending and beginning, respectively

t_2 and t_1 = The ending and beginning dates of the WS treatments, respectively

When leaves from the WS-treated plants rolled or wilted permanently on sand soil (April 20 for both years) and the symptom showed from 11:00-16:00 h on muck soil, plants in all pots were cut near the soil surface and immediately separated into green leaves, brown leaves and stalks (stalks + leaf sheaths). The numbers of large tillers (tiller stalk length ≥20 cm) and small tillers (length <20 cm) were recorded. Green Leaf Area (GLA) was measured using a LI-3100 leaf area meter (LI-COR Inc., Lincoln, NE). The separated plant parts were dried in a forced-air oven at 60°C and weighed until their weights were stable.

Experimental design and data analysis: The experiment was a two-factor factorial using a Randomized Complete Block (RCB) design with seven (2009) or eight (2010) replications. Data were analyzed separately each year due to differences between years in plant size and the duration of the stress treatments. To test soil type, water regime and their interactive effects on plant physiological and growth variables measured, significance of each fixed effect was analyzed using the PROC MIXED procedure of SAS (SAS Institute, 2003). Block was considered as a random effect and soil type and water regime were considered as fixed effects. If the hypothesis of equal means between treatments were rejected by the F test, trait means were separated with the LSD at $p = 0.05$. The LSD values were calculated with the SE values generated by the Diff option in the SAS MIXED procedure.

RESULTS

Soil properties: The sand soil had significantly higher bulk density, lower water holding capacity and lower pH values than the muck soil (Table 1).

Table 1: Characteristics of muck and sand soils used in this study in 2009 and 2010. All measurements were taken at planting time before fertilizer application

	2009			2010		
Soil property	Muck	Sand	SE	Muck	Sand	SE
Bulk density (g cm^{-3})	0.38	1.25***	0.021	0.35	1.41***	0.003
Water holding capacity (%)	176.10	12.10***	0.990	182.70	21.80***	2.610
pH	7.60	6.60**	0.070	7.80	7.20***	0.010
Organic matter (%)	76.70	1.50***	0.030	77.40	1.50***	0.090
Carbon (g kg^{-1})	422.20	7.50***	1.590	316.20	8.00***	0.060
Nitrogen (g kg^{-1})	29.10	0.70***	0.150	21.40	0.70***	0.120
Carbon/nitrogen ratio	14.50	10.60**	0.380	14.80	11.60***	0.100
Acetic acid extractable P (mg kg^{-1})	25.40	25.70^{NS}	NS	40.70	39.50^{NS}	NS
Water extractable P (mg kg^{-1})	0.84	2.50**	0.170	1.00	4.00***	0.010
Potassium (mg kg^{-1})	42.80	27.00*	3.370	44.50	13.90***	0.170

*,** and *** indicate the significances at p≤0.05, 0.01 and 0.001 levels, respectively between the two soils within a year and NS = Not Significant. Degree of Freedom (DF) = 2 for both years

Table 2: Changes in Relative Water Content (RWC) and proline content of sugarcane top dewlap leaves during development of water-deficit stress in 2009 and 2010 for the Well-Watered (WW) and Water-Stressed (WS) plants as affected by soil type and sampling date

	2009					2010				
Days after water	Muck soil		Sand soil		SE	Muck soil		Sand soil		SE
treatment	WW	WS	WW	WS	(DF = 6)	WW	WS	WW	WS	(DF = 6)
RWC (%)										
2	88.0	87.0	87.8	91.9	NS	88.1	90.0	89.5	87.5	NS†
8	85.2	87.0	87.8	88.3	NS	87.5	87.2	86.0	86.3	NS
15	84.2	83.5	84.6	81.4	NS	89.8	85.2	91.4	60.8	3.22
22	90.2	87.1	88.9	69.0	4.09					
SE (DF = 26)	NS	NS	NS	3.34		NS	NS	NS	2.66	
Proline content (µg g^{-1} FW)										
2	10.8	13.0	9.7	8.2	NS	13.0	13.2	11.7	14.9	NS
8	13.8	11.6	11.7	12.7	NS	15.5	15.5	14.8	19.1	NS
15	12.5	12.8	9.8	7.3	NS	9.3	10.9	4.9	656.0	20.74
22	12.1	14.2	13.8	385.1	112.74					
SE(DF = 26)	NS	NS	NS	67.21		NS	NS	NS	12.73	
	2009, p > F					2010, p > F				
	RWC		Proline			RWC		Proline		
Soil (S)	0.188		0.062			<0.001		<0.001		
Water (W)	0.028		0.046			<0.001		<0.001		
S × W	0.102		0.048			<0.001		<0.001		
Date (D)	0.020		0.008			<0.001		<0.001		
S × D	0.006		0.008			<0.001		<0.001		
W × D	0.003		0.010			<0.001		<0.001		
S × W × D	0.033		0.011			<0.001		<0.001		

† NS: Not Significant

Sand soil also had much lower organic matter, Carbon (C), total N and K contents and C:N ratio than muck soil before fertilizers were used. Additionally, the sand soil showed significantly greater water extractable P content, but comparable acetic acid extractable P content, as compared with the muck soil. Although soil N, P and K differences of the two soils were eliminated by using fertilizers at planting based on the soil test results and production recommendations, the differences in these other physical and chemical properties between muck and sand soils might partially explain treatment differences within the same water regime.

Leaf RWC and proline content: Overall, water regime and sampling date significantly affected leaf RWC and leaf proline content in 2009 (Table 2). The two-and three-way interactions of sampling date with soil type and water regime were also significant (p<0.05-0.001). Leaf RWC of the WW plants ranged from 84-91% during the experiment and did not differ between muck and sand soils or among the measurement dates (Table 2). In the first 15 days of the WS treatment in 2009, there were no differences between the WS and WW treatments in leaf RWC for either muck or sand soil.

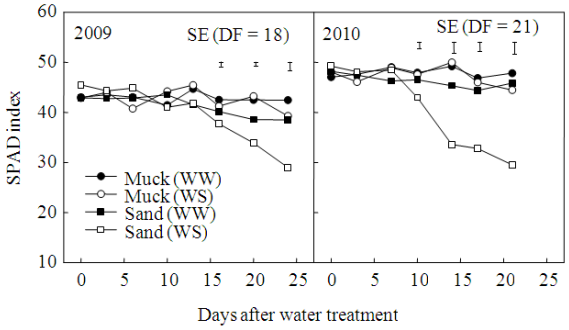

Fig. 1: SPAD index of the top visible dewlap leaf measured with a SPAD meter as affected by measurement date, soil type and water-deficit stress. Vertical bars indicate Standard Errors (SE) of mean across treatments at the individual dates if treatment difference is significant at p≤0.05. Well-Watered (WW), Water-Deficit Stress (WS)

At 22 days after implementing the water deficit, only the WS-treated plants (RWC = 69%) on sand soil had significantly lower leaf RWC than plants in other treatments (RWC = 87-90%). Leaf RWC in 2010 had similar responses to soil type and water regime as compared with results in 2009, but the WS-treated plants on sand showed significantly low leaf RWC a week earlier in 2010 than in 2009.

Similar to leaf RWC, neither water regime nor soil type affected leaf proline content in the first 15 (in 2009) or 8 (in 2010) days after initiating the WS treatment (Table 2). The difference in leaf proline content was only detected when sugarcane plants were subjected to severe water-deficit stress (i.e., leaves rolled up). At 3 or 2 weeks after initiating the stress treatment, leaf proline content of the WS plants on muck soil did not differ from the WW plants. However, proline contents of the WS plants on sand soil in 2009 and 2010 were approximately 30 and 80 times higher, respectively, than those recorded for other treatments (Table 2).

Leaf SPAD index: Leaf SPAD index was affected significantly by measurement date ($p<0.001$), soil type ($p<0.01$-0.001) and water regime ($p<0.001$). Except for soil × water in 2009, all other two-and three-way interactions of date, soil and water on leaf SPAD index were also significant ($p<0.05$-0.001). Under the WW conditions for both soils and during the WS treatment for muck soil, leaf SPAD index changed little with the measurement dates. Leaf SPAD index did not differ among treatments in the first 10 days after initiating the WS treatment (Fig. 1). Thereafter, leaf SPAD index of the WS plants sharply declined on sand soil, but not on muck soil. When the WS plants on sand soil were permanently wilt 24 (in 2009) or 22 (in 2010) days after the WS treatment, leaf SPAD index of the WS-treated plants was only 71-75% of the WW plants. Averaged across measurement dates, leaf SPAD indexes of the WW-and WS-treated plants on muck soil did not differ in either year, but the WS-treated plants had significantly lower SPAD index than the WW plants ($p<0.05$-0.001) on the sand soil and the mean leaf SPAD index for the WW and WS plants on sand soil were 41.9 and 39.7, respectively, in 2009 and 46.3 and 41.1, respectively, in 2010.

Leaf photosynthesis components: Main effects of soil type, water regime and measurement date on leaf Pn were significant ($p<0.05$-0.001) except for soil type in 2010. There was no soil × water interaction, but interactions of soil × date, water × date and soil × water × date on leaf Pn were significant ($p<0.05$-0.001). Under the WW conditions, leaf Pn of plants grown on muck and sand soils ranged from 29-35 and 28-33 µmol m^{-2} sec^{-1}, respectively, in 2009 and 30-40 and 29-37 µmol m^{-2} sec^{-1}, respectively, in 2010 (Fig. 2a). Averaged across measurement dates, leaf Pn of the WW plants on sand soil was about 12% lower than that of the WW plants on muck soil in both years. Leaf Pn did not differ between the WW and WS plants on either muck or sand soil in the first 10 days after initiating the WS treatment (Fig. 2a). Thereafter, the WS-treated plants had significantly lower leaf Pn than the WW plants for sand soil. For muck soil, leaf Pn of the WS-treated plants became significantly lower than that of the WW plants 24 days after initiating the WS treatment in 2009 (15.4 vs. 30.0 µmol m^{-2} sec^{-1}) and 17 days after initiating WS in 2010 (10.7 Vs 40.1 µmol m^{-2} sec^{-1}). At the same date, leaf Pn of the WS plants on sand soil dropped to almost zero.

Leaf gs responded similarly to leaf Pn to soil and water treatments (Fig. 2b). In contrast, Ci increased significantly when plants exposed to severe stress (Fig. 2c) which accompanied with greatly decreased leaf Pn. Responses of leaf Tr (Fig. 2d) and ΦPSII (Fig. 2e) to soil type and water regime during development of the WS were also similar to those of leaf Pn (Fig. 2a) and gs (Fig. 2b). Decreased leaf Pn during moderate WS was mainly associated with decreased gs. The significant increase in Ci (Fig. 2c) during the severe WS (20-24 days in 2009 and 10-17 days in 2010 after initiating the stress treatment) suggests that reduced photosynthesis enzyme activities also contributed to low leaf Pn. When plants grew under the water deficit condition, gs, Tr and ΦPSII declined, resulting in low leaf Pn (Fig. 2).

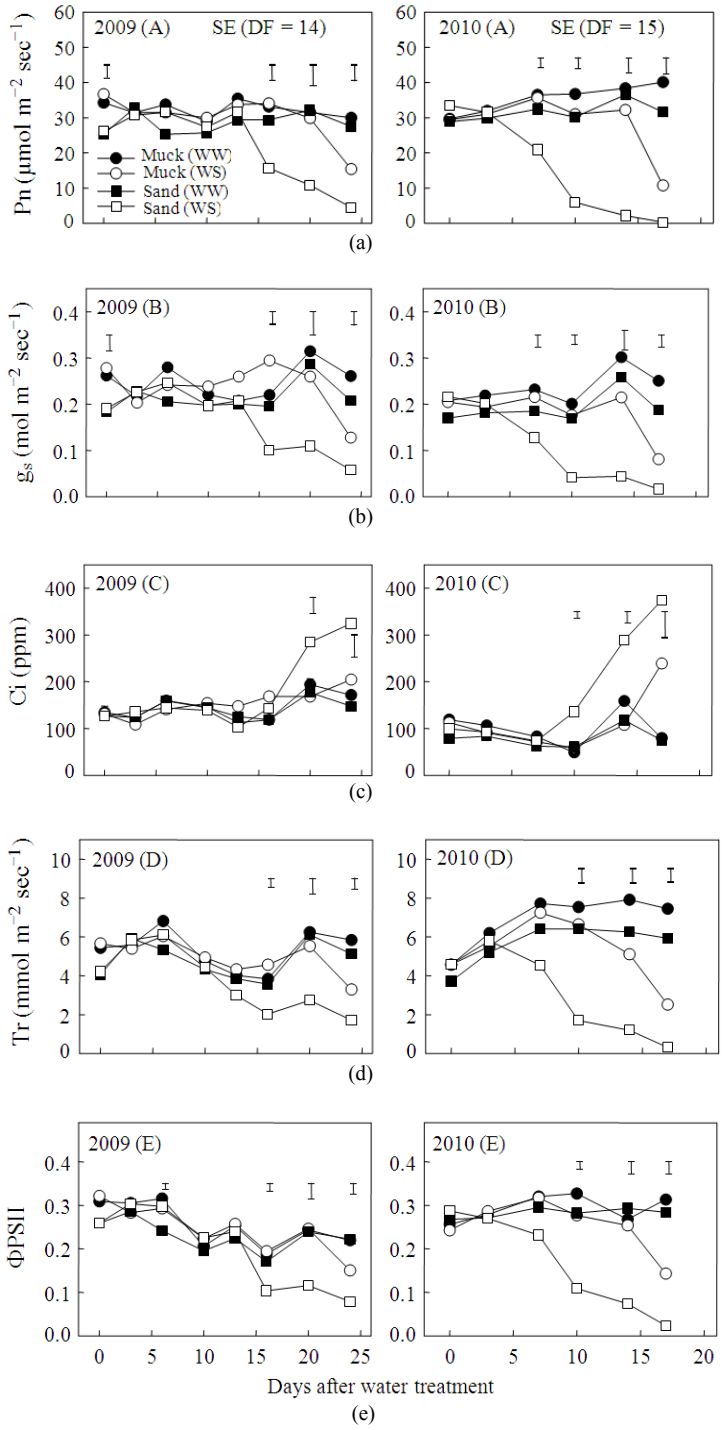

Fig. 2: (a) photosynthetic rate (Pn); (b) stomatal conductance (g_s); (c) intercellular CO_2 concentration (C_i); (d) transpiration rate (Tr) and (e) photosystem II photochemical efficiency (Φ_{PSII}) of top visible dewlap leaf during the water-deficit stress treatment for Well-Watered (WW) and Water-Stressed (WS) plants grown on the muck and sand soils. Vertical bars indicate Standard Errors (SE) of mean across treatments at the individual dates if treatment difference is significant at $p \leq 0.05$

Table 3: Increment rates for main stalk elongation, nodes of the main stalk and tillers of the Well-Watered (WW) and Water-Stressed (WS) sugarcane plants during the development of water-deficit stress in 2009 and 2010 as affected by soil type

		2009			2010		
Soil type	Water regime	Stalk elongation (cm day^{-1})	Node (No. day^{-1})	Tiller (No. day^{-1})	Stalk elongation (cm day^{-1})	Node (No. day^{-1})	Tiller (No. day^{-1})
Muck	WW	0.548	0.161	0.137	1.056	0.196	0.160
	WS	0.503	0.125	0.113	0.380	0.098	0.110
Sand	WW	0.488	0.155	0.101	1.416	0.165	0.084
	WS	0.369	0.113	0.048	0.220	0.075	0.044
SE		0.055	0.014	0.024	0.112	0.015	0.029
DF		18	18	18	21	21	21
p>F							
Soil		0.022	0.385	0.008	0.219	0.019	0.002
Water		0.048	0.001	0.034	<0.001	<0.001	0.040
Soil × Water		0.348	0.770	0.393	0.003	0.683	0.810

Table 4: Numbers of large tillers, small tillers and total tillers and green leaf area at harvest (i.e., 28 (in 2009) or 22 (in 2010) days after initiation of water stress treatment) as affected by soil type and water treatments

		2009				2010			
Soil type	Water regime†	Large tiller (No.pl^{-1})	Small tiller (No.pl^{-1})	Total tiller (No.pl^{-1})	Green leaf area (cm^2 pl^{-1})	Large tiller (No.pl^{-1})	Small tiller (No.pl^{-1})	Total tiller (No.pl^{-1})	Green leaf area (cm^2 pl^{-1})
Muck	WW	2.57	2.00	4.57	2343	4.25	3.13	7.38	3908
	WS	1.71	3.14	4.86	1464	2.63	4.00	6.63	2340
Sand	WW	1.43	1.71	3.14	1172	4.25	2.25	6.50	3511
	WS	0.29	1.57	1.86	269	2.50	3.25	5.75	679
SE		0.44	0.61	0.67	331	0.43	0.63	0.68	362
DF		18	18	18	18	21	21	21	21
p>F									
Soil		<0.001	0.044	<0.001	<0.001	0.839	0.083	0.082	<0.001
Water		0.005	0.258	0.348	0.001	<0.001	0.048	0.132	<0.001
Soil × Water		0.653	0.150	0.147	0.958	0.839	0.889	0.989	0.022

† WW: Well Watered, WS: Water Stress

Rates of stalk elongation and node and tiller formations: Soil type significantly affected stalk elongation rate in 2009 and node addition in 2010 and tiller formation in both years (Table 3). Water regime affected all three growth traits ($p<0.05$-0.001), but there were no soil × water interactions except for stalk elongation rate in 2010 (Table 3). Among the measured growth variables, tiller formation was most sensitive to soil type and their responses to WS were slightly different between two years. Averaged across the two water treatments, plants grown on sand soil had a 19% lower stalk elongation rate and a 46% lower tiller formation rate in 2009 and an 18% lower node addition rate and a 53% lower tiller formation rate in 2010 than plants grown on muck soil. When averaged across soil types, the WS treatment reduced rates of stalk elongation, node increment and tiller formation by 15, 25 and 33%, respectively, in 2009 and by 76, 52 and 37%, respectively, in 2010 as compared with the WW treatment (Table 3).

Tillers and green leaf area: In 2009, soil type did significantly affect numbers of large tillers, small tillers and total tillers of sugarcane plants at harvest time, but WS only reduced the number of large tillers (Table 4). In 2010, soil type did not affect numbers of tillers, but the WS significantly reduced the number of large tillers and increased the number of small tillers. There was no interaction of soil × water on any of tiller variables in both years. Soil type and water regime mainly affected large tillers rather than smaller tillers. Averaged across soil types, the WS-treated plants in 2009 and 2010 produced 50 and 40% less large tillers, respectively, than the WW plants.

Green leaf area was sensitive to soil type and water regime. Both sand soil and water-deficit stress significantly reduced GLA (Table 4). Averaged across water treatments within a year, GLAs of plants grown on sand soil in 2009 and 2010 were 62 and 33% lower, respectively, than those of plants grown on muck soil. The WS plants in 2009 and 2010 had 51 and 59% lower GLA, respectively, than the WW plants when averaged across soil treatments (Table 4). Compared with the WW plants, the WS-treated plants on sand soil had greater reduction (77-80%) in GLA than on muck soil (38-40%), an indication that water stress was much more severe on sand soil than on muck soil.

Table 5: Total shoot dry matter at harvest and shoot dry matter partitioning in green leaves, brown leaves and stalks as affected by soil type and water treatments in 2009 and 2010

		2009 (g plant^{-1})				2010 (g plant^{-1})			
Soil type	Water regime†	Green leaf	Stalk	Brown leaf	Total	Green leaf	Stalk	Brown leaf	Total
Muck	WW	13.33	9.96	0.30	23.59	27.50	20.84	4.54	52.88
	WS	12.75	9.94	0.86	23.56	18.11	15.97	5.67	39.75
Sand	WW	9.39	7.72	0.82	17.93	27.29	29.61	5.74	62.65
	WS	2.87	3.60	1.94	8.41	7.20	16.02	13.08	36.30
SE		1.63	1.28	0.46	3.01	2.77	2.84	0.94	5.62
DF		18	18	18	18	21	21	21	21
p>F									
Soil		<0.001	<0.001	0.024	<0.001	0.010	0.039	<0.001	0.435
Water		0.006	0.035	0.018	0.038	<0.001	<0.001	<0.001	<0.001
Soil × Water		0.019	0.037	0.398	0.039	0.012	0.042	0.001	0.111

† WW: Well Watered, WS: Water Stress

Shoot biomass: Both soil type and water regime and their interaction significantly affected total shoot biomass in 2009 (Table 5). Under the WW and WS conditions, plants grown on sand soil had 24 and 64% less shoot biomass, respectively, than plants grown on muck soil and a 27 day WS treatment did not affect shoot biomass on muck soil, but it significantly reduced total shoot biomass by 53% on sand soil. Similar responses were recorded for green leaf biomass and stalk biomass, to soil type and water regime, while the reversed pattern was found for brown leaf biomass in response to the treatments. The water-deficit stress on muck soil did not significantly affect biomass of any plant component, but significantly reduced biomasses of green leaves and stalks and increased brown leaf biomass on sand soil (Table 5). Compared to the WW plants on sand soil, the WS-treated plants had 69% less green leaf biomass and 53% less stalk biomass, but 137% greater brown leaf biomass.

In 2010, shoot biomass was not affected by soil type, but WS reduced shoot biomass significantly on both soils and the WS-treated plants on muck and sand soils had 25 and 42% less total shoot biomass, respectively, than the WW plants (Table 5). The main effects of soil and water as well as their interaction on biomasses of green leaves, stalks and brown leaves were also significant. The WS reduced green leaf biomass and stalk biomass, increase brown leaf biomass. The decrease/increase in biomass of plant components was much more on sand soil than on muck soil. Plants on sand soil had less green leaf biomass, but greater biomasses of stalks and brown leaves than plants on muck soil, resulting in no difference in total shoot biomass between the two soils in 2010 (Table 5).

DISCUSSION

Overall, the stress symptoms appeared on the WS-treated plants earlier in 2010 than in 2009. This was probably because plants in the first year were smaller than in the second year when the stress treatment was initiated, with an average of 6.5 and 8.2 nodes on the main stalks in 2009 and 2010, respectively. Greater shoot biomass for each treatment in 2010 than in 2009 at harvest (Table 5) also supports this explanation. The large plants in 2010 compared with 2009 at the same date after planting were mainly caused by the difference in temperature after planting as described earlier. Although the duration of the WS treatment was different, the responses of plant growth and physiological traits measured in this study to soil type and WS were consistent in the two years.

Drought is one of the most important environmental stress factors limiting sugarcane production worldwide (Venkataramana *et al*., 1986). Water-deficit stress alters a variety of growth and physiological processes in sugarcane, which cause decreased yields (Zhang *et al*., 2001; Silva *et al*., 2007). Therefore, early detection of water-deficit stress is important for irrigation management and could serve as a selection mechanism for drought tolerance. Studies on other crops have indicated that leaf RWC and/or proline content are useful indicators for early detecting plant water-deficit stress (Bates *et al*., 1973; Aspinall and Paleg, 1981; Ilahi and Dorffling, 1982; Levy, 1983; Claussen, 2005; Gonzalez *et al*., 2008; Umebese *et al*., 2009; Paknejad *et al*., 2009). Ilahi and Dorffling (1982) investigated changes in proline of four maize varieties differing in drought resistance during a prolonged water stress period and found that proline levels increased continuously during the stress period in all the four varieties, but to different amounts with higher level of proline in the drought-susceptible varieties than the drought-resistant varieties. In contrast, our results revealed that sugarcane leaf RWC and proline content did not differ among treatments at 15 or 8 days after initiating water stress (Table 2), although the WS-treated plants on sand soil had significantly lower gs

and leaf Pn than other treatments (Fig. 2). Therefore, unlike early reports in other crops (Ilahi and Dorffling, 1982; Levy, 1983; Gonzalez *et al*., 2008), it appeared that leaf RWC and proline content might not be sensitive to water-deficit stress in sugarcane.

Plant leaf N concentration is correlated with N fertilizer application rate and leaf chlorophyll content in corn (Scheepers *et al*., 1992; Zhao *et al*., 2003) and sorghum (Zhao *et al*., 2005). Leaf chlorophyll content has long been used for detecting plant N status (Scheepers *et al*., 1992; Kantety *et al*., 1996; Gholizadeh *et al*., 2009) and therefore to guide crop N fertilizer application rates. When sugarcane plants grew under the WW condition in the present study, leaf SPAD index, an indicator of leaf chlorophyll level, did not differ between the muck and sand soils (Fig. 1), indicating that plant N status should be similar during the experiment between the two soils by N fertilizer application in sand soil. Other stress environment may also influence leaf chlorophyll content. For instance, water deficit reduced leaf chlorophyll content of field-grown soybean (Paknejad *et al*., 2009) chlorophyll degradation as a consequence of drought stress may result in photo-inhibition and photo-bleaching (Long *et al*., 1994). Silva *et al*. (2007) found that drought caused a decline in sugarcane leaf chlorophyll level, but this reduction varied among genotypes. Drought tolerant sugarcane cultivars have higher level of chlorophyll than drought susceptible cultivars (Jangpromma *et al*., 2010). In the present study, leaf SPAD index did not change among measurement dates and the two water regime treatments on muck soil. In contrast, leaf SPAD index of the WS-treated plants on sand soil sharply declined when plants were subjected to water-deficit stress 10-15 days after the treatment (Fig. 1), indicating plants already faced severe water-deficit stress at the time. The differences in response of SPAD index to the WS treatment on the two soils in our study were probably associated with soil physical properties as described in Table 1. The sand soil had much less organic matter and lower water holding capacity than the muck soil. Therefore, plants showed water-deficit stress based on leaf SPAD index on sand soil much earlier than on muck soil.

Water deficit often limits crop growth, physiology and yields (Gardner *et al*., 1984). Therefore, the ability to maintain key physiological processes, such as photosynthesis during moderate water stress, is crucial to sustain productivity. Our results indicated that leaf Pn of the WS-treated plants declined much earlier on sand soil than on muck soil (Fig. 2a). Decreased leaf Pn under the moderate water-deficit stress was likely associated with reduced gs (Fig. 2b) rather than leaf RWC or leaf proline content (Table 2). For instance, leaf Pn of the WS plants on sand soil declined almost 50% at 15 days after initiating stress treatment compared with the WW plants in 2009 (Fig. 2a), but no differences were detected in either leaf RWC or proline content at that time (Table 2). Similarly, neither leaf RWC nor proline content differed between the WW and WS treatments on sand soil at 8 days after initiating water stress treatment although leaf Pn of the WS plants was 36% lower ($p<0.05$) than that of the WW plants at 7 days after treatment initiation in 2010. Therefore, leaf Pn and gs are better and faster physiological traits than leaf RWC and proline content for early detection of sugarcane plant water-deficit stress.

Saliendra and Meinzer (1991) found that leaf water potential did not differ between sugarcane under irrigation and drought treatments even though the irrigated plants had significantly higher shoot growth rate and gs than the plants subjected to drought. They suggested that signals originating in the roots rather than in the leaves may regulate growth and stomatal behavior in sugarcane during soil drying. Although leaf water potential was not measured in the present study, our results that leaf RWC and proline content did not differ between the WW and WS treated plants on sand soil although significantly low gs and Pn were detected, are consistent with the findings of Saliendra and Meinzer (1991).

When sugarcane was subjected to the moderate water-deficit stress of no water added for 15 days on sand soil and 24 days on muck soil in 2009 or 7 days on sand soil and 13 days on muck soil in 2010, stomatal conductance declined (Fig. 2b), resulting in decreased transpiration rates (Fig. 2d). O'Neill *et al*. (2006) suggested that ΦPSII measurements can be used to distinguish tolerant from susceptible corn hybrids under drought conditions. Our results indicated that changes in ΦPSII during development of water stress (Fig. 2e) were similar to changes in leaf Pn (Fig. 2a), indicating that ΦPSII is also a useful physiological trait for early detection of sugarcane water stress. Significant increase in Ci for the WS plants on sand soil (Fig. 2c) between 20 and 25 days in 2009 or from 10-17 days in 2010 after initiating the WS treatment suggested that under severe water stress, decreased leaf Pn was mainly caused by non-stomatal limitation, such as photo-assimilate enzyme activities (Du *et al*., 1998).

Sugarcane stalk elongation is sensitive to water stress (Nable *et al*., 1999). Our results revealed that a 22- or 27-day water stress treatment during a portion of the sugarcane formative growth phase significantly reduced rates of stalk elongation and tiller formation on sand soil, but had less effect on these growth traits on

muck soil (Table 4). Similarly, WS significantly reduced sugarcane shoot biomass accumulation. The degree of the negative effect of WS depended on soil type and experimental year (Table 5). Reduced shoot biomass under the WS condition was associated with both low leaf Pn (Fig. 2a) and decreased green leaf area (Table 4). The formative phase (i.e., tillering and grand growth) has been identified as the critical water demand period and the phase during which sugarcane is most sensitive to drought (Ramesh, 2000). Water deficit decreased mean stalk weight and the number of stalks, resulting in low cane yields (De Silva and Costa, 2004). Ramesh (2000) suggested that measurements of growth variables during the formative phase may help predict sugarcane total biomass at final harvest. However, destructive measurements of biomass for a large number of samples are cost and time consuming. Nondestructive measurements of physiological traits, such as gs, ΦPSII, Pn during the formative growth should be useful for early detection of water stress. In south Florida, the sugarcane formative phase usually occurs from February through July. Precipitation in south Florida in spring is low compared with summer. Our results and weather characteristics in the region further suggest that water deficit during the formative phase may limit sugarcane plant growth and yields on sand soils. Thus, selecting cultivars more tolerant to water deficit based on physiological and growth traits during the formative phase, while working to improve irrigation management, may improve sugarcane yield on sand soils in Florida.

CONCLUSION

Results of this study indicated that termination of irrigation resulted in measurable effects of water-deficit stress sooner on sand than on muck soils. Soil type, weather characteristics and plant growth stage must be considered when scheduling irrigation for sugarcane. Leaf proline and RWC were not successful at early detection of plant water-deficit stress in the formative growth phase of sugarcane. However, water-deficit stress significantly decreased leaf gs, ΦPSII, Pn, the number of large tillers and GLA, which collectively contributed to reduced shoot biomass. Using some of these nondestructively physiological measurements, especially leaf photosynthesis components accompanied with growth traits, may help in early detection of sugarcane water stress during the formative growth phase, thereby improving sugarcane production on sand soils. They may also be used to develop improved irrigation management of sugarcane on sand soils.

ACKNOWLEDGEMENT

We thank Philip Aria for his valuable technical assistance and skilled data collection and management. Use of trade or product names is for informational purpose only and does not imply endorsement by the United States Department of Agriculture to the exclusion of any other product that may be suitable.

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