

10.1071/FP17268_AC

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Supplementary Material: *Functional Plant Biology*, 2018, 45(8), 865–876.

Supplementary Material

Diurnal variation in gas exchange and nonstructural carbohydrates throughout sugarcane development

Amanda P. De Souza^{A,B}, Adriana Grandis^A, Bruna C. Arenque-Musa^A and Marcos S. Buckeridge^{A,C}

^ALaboratory of Plant Physiological Ecology, Department of Botany, Institute of Biosciences, University of São Paulo, São Paulo, 05508-090, SP, Brazil.

^BCurrent address: Carl R. Woese Institute for Genomic Biology, University of Illinois at Urbana-Champaign, Urbana, 61801, IL, USA.

^CCorresponding author. Email: msbuck@usp.br

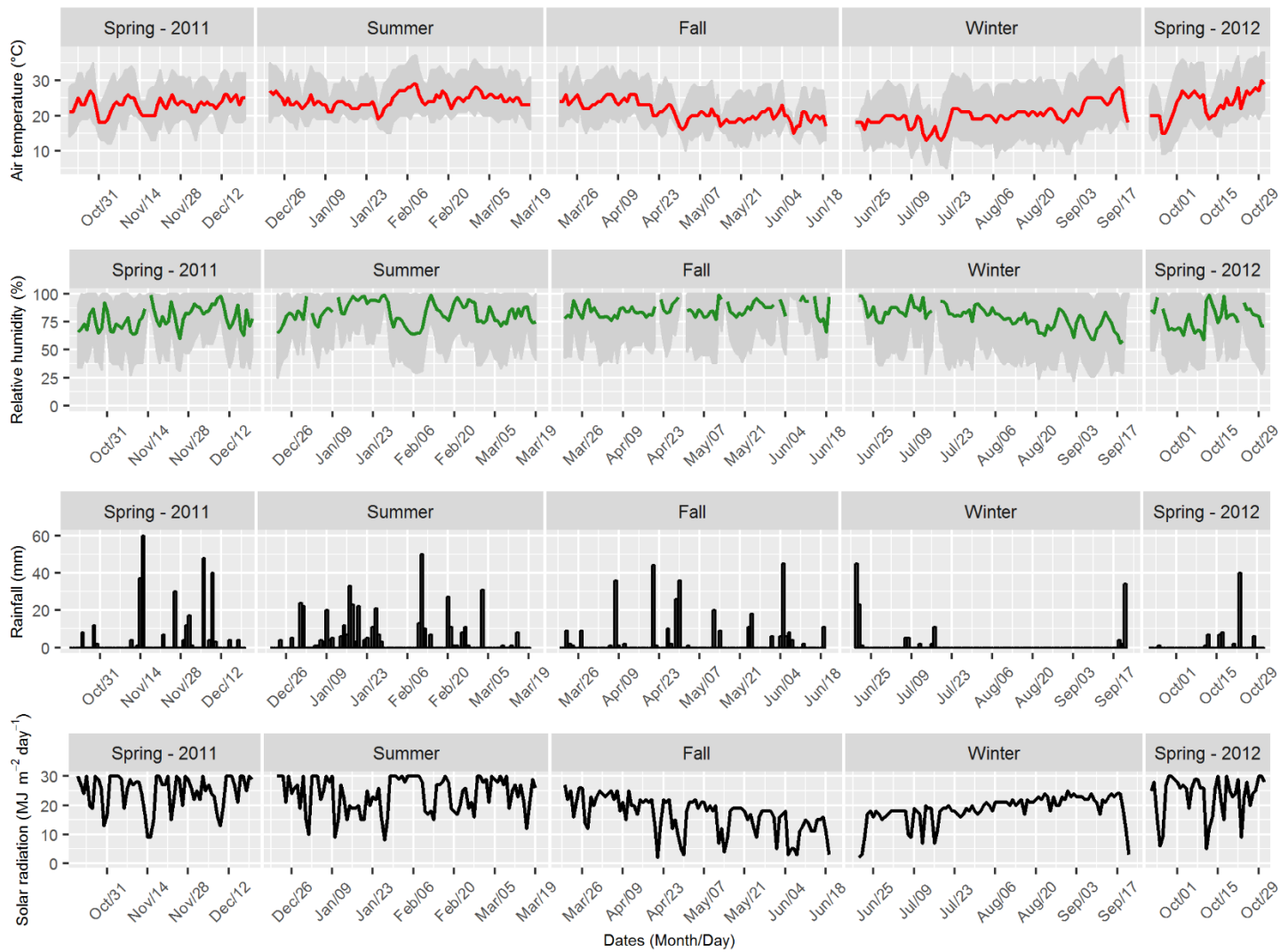


Fig. S1. Meteorological data at Piracicaba, SP, Brazil during the experiment in 2011-2012. Arrows represent the dates of data collection. Shaded areas represent maximum and minimum values for air temperature and relative humidity.

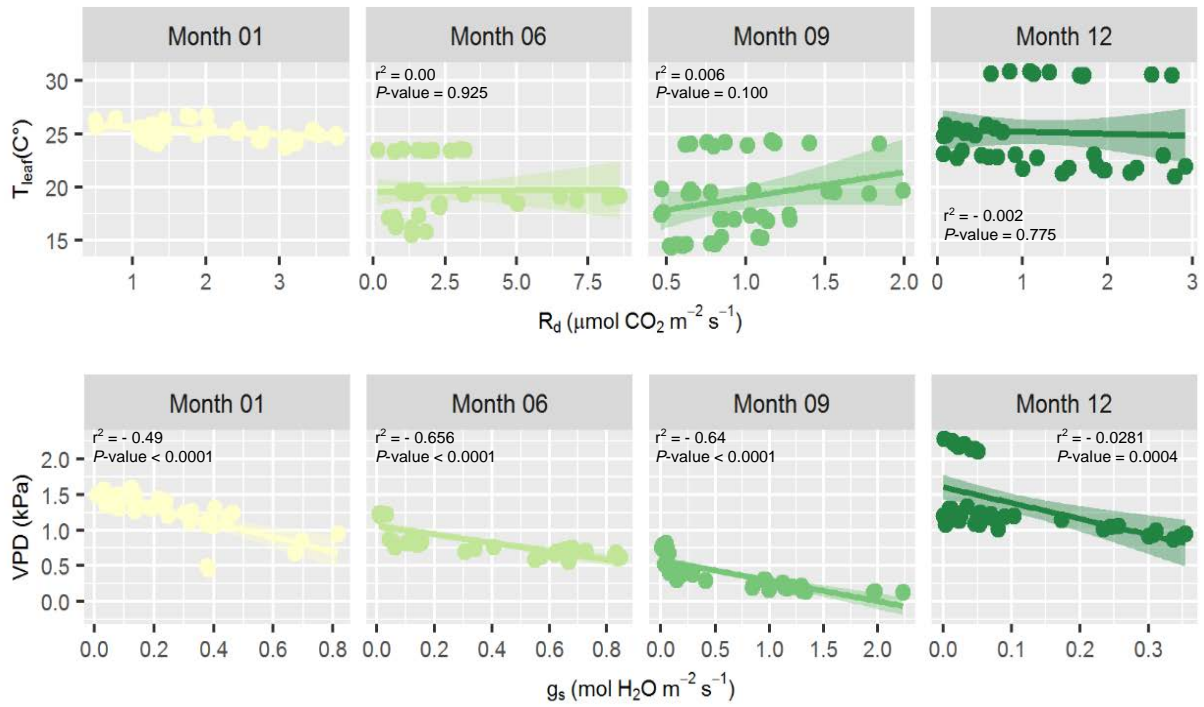


Fig. S2. Pairwise Pearson's correlation between leaf respiration (R_d) and leaf temperature (T_{leaf}), and between stomatal conductance (g_s) and vapor pressure deficit (VPD) in sugarcane plants during the night after 1, 6, 9 and 12 months of growth. Shaded areas represent the level of confidence interval equals to 0.95.

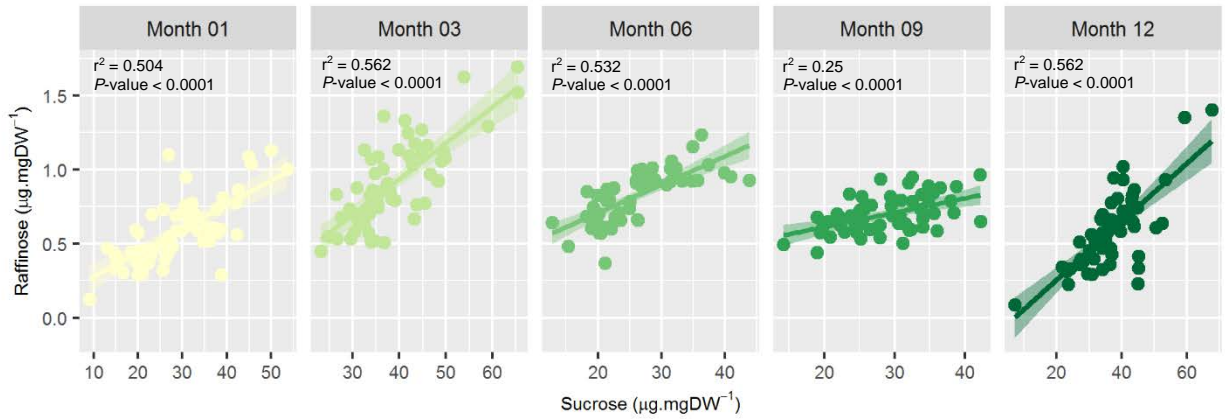


Fig. S3. Pairwise Pearson's correlation between sucrose and raffinose ($\mu\text{g.mgDW}^{-1}$) in leaves of sugarcane plants during a diurnal cycle after 1, 3, 6, 9 and 12 months of growth. Shaded areas represent the level of confidence interval equals to 0.95.

Table S1. Average \pm s.d. of carbon assimilation (A , $\mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$), stomatal conductance (g_s , $\text{mol H}_2\text{O m}^{-2}\text{s}^{-1}$), intrinsic water use efficiency ($i\text{WUE}$, $\mu\text{mol CO}_2 \cdot \text{mol H}_2\text{O}^{-1}$), photosynthetic active radiation (PAR , $\mu\text{mol m}^{-2}\text{s}^{-1}$), leaf temperature (T_{leaf} , $^{\circ}\text{C}$), vapor pressure deficit (VPD , kPa) and leaf respiration (R_d , $\mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$) for each month, considering all the time points. Different letters represent statistically significant differences ($P < 0.05$). **(A)** Values corresponding to measurements performed during the day ($n = 40$); **(B)** Values corresponding to measurements performed during the night ($n = 20$).

(A)

Month	A	g_s	$i\text{WUE}$	PAR	T_{leaf}	VPD
01	34.41 ± 11.07 a	0.32 ± 0.09 a	105.74 ± 16.36 ab	1254.45 ± 412.87 a	26.76 ± 1.44 a	1.20 ± 0.16 a
03	18.45 ± 12.72 b	0.10 ± 0.06 bd	164.96 ± 25.96 b	1534 ± 767.57 a	35.84 ± 3.12 b	3.12 ± 0.85 b
06	14.86 ± 7.81 b	0.16 ± 0.06 c	91.90 ± 26.35 ab	1271.43 ± 253.17 ab	26.14 ± 2.92 a	1.43 ± 0.42 a
09	8.89 ± 4.84 b	0.14 ± 0.08 bc	74.30 ± 39.84 a	999.73 ± 512.56 a	27.11 ± 1.60 a	0.82 ± 0.23 a
12	13.38 ± 2.37 b	0.07 ± 0.02 d	180.93 ± 30.36 c	1749.82 ± 51.39 b	39.74 ± 1.10 c	4.17 ± 0.35 c

(B)

Month	R_d	g_s	T_{leaf}	VPD
01	2.10 ± 1.00 a	0.29 ± 0.20 ac	25.23 ± 0.84 a	1.21 ± 0.29 a
06	$2.30^* \pm 2.07$ a	0.35 ± 0.31 a	19.61 ± 2.55 b	0.85 ± 0.23 b
09	0.96 ± 0.39 b	0.59 ± 0.63 b	18.92 ± 3.50 b	0.41 ± 0.23 c
12	1.20 ± 0.86 b	0.10 ± 0.11 c	25.15 ± 3.49 a	1.38 ± 0.48 a

* This average \pm s.d. comes down to 1.39 ± 0.70 if the value observed at 2 h, which was remarkably high, is not taken into account. The decrease in R_d values from 1–6 months to 9–12 months is 104% when 2.30 is considered as the average value of R_d ; this same reduction is 62% when 1.40 is considered as the average.

Table S2. Average \pm s.d. ($n=10$) of non-structural carbohydrates in leaves of sugarcane ($\mu\text{g}\cdot\text{mg}^{-1}$ dry weight) during a diurnal cycle after 1, 3, 6, 9 and 12 months of growth. Different letters represent statistically significant differences ($P < 0.05$)

NON-STRUCTURAL CARBOHYDRATES IN LEAVES						
Month	Hour	Glucose	Fructose	Sucrose	Starch	Raffinose
1	6	14.84 \pm 3.29 a	8.45 \pm 2.82 a	26.37 \pm 6.86 a	4.92 \pm 1.79 a	0.45 \pm 0.13 a
	10	14.08 \pm 6.33 a	7.38 \pm 3.99 a	28.6 \pm 10.93 ab	9.13 \pm 4.84 ab	0.54 \pm 0.25 ab
	14	17.59 \pm 6.53 a	9.29 \pm 4.44 a	39.6 \pm 8.52 b	17.6 \pm 4.32 c	0.81 \pm 0.23 b
	18	12.58 \pm 3.96 a	7.07 \pm 2.92 a	28.64 \pm 5.67 ab	26.3 \pm 8.80 d	0.56 \pm 0.14 ab
	22	11.47 \pm 3.99 a	5.37 \pm 2.12 a	24.84 \pm 8.54 a	18.23 \pm 4.79 c	0.61 \pm 0.28 ab
	2	13.51 \pm 5.24 a	6.91 \pm 3.46 a	24.92 \pm 9.21 a	13.41 \pm 4.15 b	0.56 \pm 0.11 ab
3	6	16.66 \pm 4.53 ab	5.72 \pm 1.71 ab	34.63 \pm 3.88 a	6.17 \pm 1.79 a	0.74 \pm 0.16 ac
	10	12.05 \pm 2.80 a	3.92 \pm 1.14 a	32.47 \pm 4.80 a	10.78 \pm 5.28 ab	0.68 \pm 0.21 a
	14	18.29 \pm 2.95 b	5.86 \pm 0.78 b	51.01 \pm 9.13 c	14.49 \pm 7.25 bc	1.26 \pm 0.21 b
	18	13.34 \pm 3.95 ab	4.29 \pm 1.82 ab	42.77 \pm 3.59 bc	20.50 \pm 8.29 c	1.00 \pm 0.19 bc
	22	15.44 \pm 4.26 ab	5.12 \pm 1.55 ab	37.9 \pm 7.59 ab	15.16 \pm 5.81 bc	0.98 \pm 0.34 bc
	2	15.91 \pm 4.44 ab	4.27 \pm 1.03 ab	33.26 \pm 6.75 a	13.46 \pm 4.17 abc	0.76 \pm 0.12 ac
6	6	6.37 \pm 1.53 ab	4.65 \pm 1.49 ab	20.72 \pm 4.28 a	10.42 \pm 4.34 a	0.83 \pm 0.13 ab
	10	5.81 \pm 1.13 b	4.12 \pm 1.02 b	26.31 \pm 4.94 ab	9.86 \pm 2.99 ab	0.90 \pm 0.15 b
	14	7.69 \pm 1.48 a	5.27 \pm 1.19 ab	34.66 \pm 5.86 c	14.68 \pm 2.97 c	0.95 \pm 0.04 b
	18	6.86 \pm 0.76 ab	4.53 \pm 0.63 ab	30.80 \pm 3.45 bc	16.93 \pm 2.54 c	0.95 \pm 0.13 b
	22	7.93 \pm 0.99 a	5.33 \pm 0.78 ab	22.08 \pm 2.74 a	13.84 \pm 3.93 abc	0.64 \pm 0.04 c
	2	7.69 \pm 1.66 a	5.69 \pm 1.34 a	21.16 \pm 2.69 a	13.02 \pm 4.08 abc	0.67 \pm 0.17 ac
9	6	4.67 \pm 0.75 a	1.84 \pm 0.47 a	25.06 \pm 5.09 a	10.94 \pm 4.74 a	0.71 \pm 0.13 ab
	10	7.03 \pm 1.42 b	2.79 \pm 1.15 b	32.88 \pm 4.63 b	12.72 \pm 1.71 a	0.73 \pm 0.12 ab
	14	7.77 \pm 3.08 b	2.94 \pm 0.64 b	33.93 \pm 5.02 b	22.27 \pm 5.05 b	0.75 \pm 0.09 b
	18	5.68 \pm 1.22 ab	2.48 \pm 0.52 ab	33.01 \pm 3.13 b	21.07 \pm 3.75 b	0.79 \pm 0.13 b
	22	5.06 \pm 1.28 ab	2.00 \pm 0.49 ab	23.74 \pm 2.58 a	18.54 \pm 3.74 b	0.60 \pm 0.05 a
	2	5.33 \pm 1.19 ab	2.10 \pm 0.30 ab	23.41 \pm 4.23 a	12.19 \pm 2.45 a	0.61 \pm 0.07 a
12	6	4.35 \pm 0.81 ab	2.23 \pm 0.79 ab	32.63 \pm 7.12 a	33.08 \pm 3.41 a	0.44 \pm 0.13 a
	10	4.02 \pm 0.92 abc	2.36 \pm 0.59 ab	36.94 \pm 8.19 ab	34.20 \pm 1.68 a	0.57 \pm 0.14 ab
	14	4.61 \pm 1.20 a	2.70 \pm 0.75 a	45.22 \pm 11.17 b	31.85 \pm 4.67 a	0.86 \pm 0.33 c
	18	3.78 \pm 1.04 abc	2.04 \pm 0.80 ab	40.41 \pm 5.09 ab	30.37 \pm 2.63 ab	0.72 \pm 0.16 bc
	22	3.18 \pm 0.91 bc	1.69 \pm 0.34 b	35.27 \pm 5.62 ab	27.35 \pm 3.98 b	0.48 \pm 0.13 ab
	2	2.95 \pm 0.99 c	1.61 \pm 0.62 b	27.02 \pm 9.42 a	31.98 \pm 2.73 a	0.37 \pm 0.2 a

Table S3. Average \pm s.d. ($n=10$) of non-structural carbohydrates in culm of sugarcane ($\mu\text{g}\cdot\text{mg}^{-1}$ dry weight) during a diurnal cycle after 1, 3, 6, 9 and 12 months of growth

Different letters represent statistically significant differences ($P < 0.05$). n.d. = not detected

NON-STRUCTURAL CARBOHYDRATES IN CULM						
Month	Hour	Glucose	Fructose	Sucrose	Starch	Raffinose
1	6	13.3 \pm 6.66 a	12.40 \pm 7.07 a	73.74 \pm 14.32 a	1.8 \pm 0.69 a	0.25 \pm 0.17 ab
	10	7.75 \pm 4.28 a	6.73 \pm 3.42 a	63.6 \pm 10.31 a	1.64 \pm 0.49 a	0.00 \pm 0.00 a
	14	12.63 \pm 10.01 a	11.85 \pm 10.43 a	63.12 \pm 12.02 a	2.00 \pm 0.63 a	0.46 \pm 0.31 b
	18	10.42 \pm 3.02 a	4.46 \pm 3.96 a	79.16 \pm 7.48 a	1.71 \pm 0.68 a	0.39 \pm 0.31 b
	22	8.76 \pm 3.66 a	7.95 \pm 3.50 a	66.79 \pm 18.35 a	1.92 \pm 0.71 a	0.25 \pm 0.12 ab
	2	11.12 \pm 3.31 a	9.49 \pm 2.59 a	70.87 \pm 15.99 a	1.97 \pm 0.69 a	0.33 \pm 0.09 ab
3	6	82.24 \pm 15.34 a	67.27 \pm 12.91 a	172.39 \pm 56.56 a	0.84 \pm 0.20 a	n.d.
	10	67.1 \pm 21.91 a	57.38 \pm 18.69 a	212.22 \pm 61.65 a	1.05 \pm 0.22 a	n.d.
	14	85.65 \pm 45.35 a	79.87 \pm 30.24 a	178.63 \pm 94.8 a	0.94 \pm 0.25 a	n.d.
	18	95.8 \pm 27.90 a	81.00 \pm 25.14 a	173.47 \pm 59.82 a	1.06 \pm 0.20 a	n.d.
	22	81.2 \pm 25.12 a	69.67 \pm 23.02 a	153.94 \pm 60.27 a	1.12 \pm 0.24 a	n.d.
	2	92.81 \pm 29.66 a	76.59 \pm 24.33 a	168.28 \pm 68.71 a	1.06 \pm 0.16 a	n.d.
6	6	2.13 \pm 0.96 a	2.29 \pm 0.65 a	443.88 \pm 18.37 a	0.80 \pm 0.26 a	n.d.
	10	1.70 \pm 1.15 a	1.66 \pm 0.86 a	474.35 \pm 40.39 a	0.79 \pm 0.14 a	n.d.
	14	6.24 \pm 3.22 b	6.36 \pm 3.12 b	451.12 \pm 35.12 a	0.91 \pm 0.27 a	n.d.
	18	10.58 \pm 4.78 c	10.08 \pm 2.96 c	447.9 \pm 21.86 a	0.78 \pm 0.27 a	n.d.
	22	2.71 \pm 1.93 ab	3.31 \pm 2.40 ab	429.02 \pm 21.01 b	0.62 \pm 0.04 a	n.d.
	2	1.69 \pm 0.70 a	2.54 \pm 1.62 a	477.94 \pm 20.31 a	0.86 \pm 0.4 a	n.d.
9	6	0.71 \pm 0.19 a	0.31 \pm 0.40 a	436.28 \pm 28.43 ab	1.09 \pm 0.26 a	n.d.
	10	0.92 \pm 0.35 ab	0.60 \pm 0.57 ac	426.51 \pm 38.84 ab	0.94 \pm 0.24 ab	n.d.
	14	1.08 \pm 0.31 ab	0.98 \pm 0.24 abc	440.11 \pm 27.99 ab	0.68 \pm 0.26 b	n.d.
	18	1.39 \pm 0.54 b	1.39 \pm 0.77 bc	450.25 \pm 52.12 ab	0.89 \pm 0.30 ab	n.d.
	22	1.30 \pm 0.43 ab	1.19 \pm 0.54 c	477.26 \pm 38.62 a	0.82 \pm 0.25 ab	n.d.
	2	1.30 \pm 0.72 ab	1.10 \pm 0.66 c	414.95 \pm 40.72 b	0.77 \pm 0.41 ab	n.d.
12	6	2.39 \pm 1.12 a	2.04 \pm 0.84 a	556.28 \pm 235.43 a	1.55 \pm 0.81 a	n.d.
	10	3.42 \pm 1.33 a	3.26 \pm 1.16 a	399.99 \pm 98.65 a	1.58 \pm 0.56 a	n.d.
	14	3.24 \pm 1.20 a	2.84 \pm 1.11 a	346.00 \pm 75.00 a	0.56 \pm 0.40 b	n.d.
	18	3.75 \pm 1.62 a	3.13 \pm 1.89 a	462.88 \pm 173.94 a	0.00 \pm 0.00 b	n.d.
	22	4.16 \pm 2.62 a	3.72 \pm 1.85 a	391.26 \pm 151.02 a	0.00 \pm 0.00 b	n.d.
	2	3.88 \pm 1.84 a	3.79 \pm 1.75 a	413.32 \pm 145.30 a	0.28 \pm 0.56 b	n.d.

Table S4. Average \pm s.d. ($n=10$) of non-structural carbohydrates in roots of sugarcane ($\mu\text{g}\cdot\text{mg}^{-1}$ dry weight) during a diurnal cycle after 1, 3, 6, 9 and 12 months of growth

Different letters represent statistically significant differences ($P < 0.05$). n.d. = not detected

NON-STRUCTURAL CARBOHYDRATES IN ROOTS						
Month	Hour	Glucose	Fructose	Sucrose	Starch	Raffinose
1	6	1.00 \pm 0.54 ab	0.56 \pm 0.45 ab	6.90 \pm 4.75 ab	1.19 \pm 0.27 ab	n.d.
	10	1.09 \pm 0.47 ab	1.20 \pm 1.37 ab	9.15 \pm 3.02 a	1.55 \pm 0.60 a	n.d.
	14	1.72 \pm 0.97 a	0.73 \pm 0.54 ab	10.17 \pm 1.77 a	1.04 \pm 0.22 b	n.d.
	18	1.05 \pm 0.55 ab	1.56 \pm 1.50 a	6.35 \pm 2.53 ab	1.16 \pm 0.39 ab	n.d.
	22	1.16 \pm 0.89 ab	0.49 \pm 0.24 ab	7.90 \pm 1.27 ab	1.2 \pm 0.26 ab	n.d.
	2	0.59 \pm 0.26 b	0.35 \pm 0.20 b	5.21 \pm 3.13 b	1.29 \pm 0.20 ab	n.d.
3	6	3.56 \pm 1.10 a	5.36 \pm 1.71 a	12.54 \pm 2.58 ab	1.14 \pm 0.13 a	0.15 \pm 0.11 a
	10	3.49 \pm 1.43 a	4.61 \pm 2.56 a	10.36 \pm 2.97 a	1.22 \pm 0.13 a	0.07 \pm 0.05 a
	14	2.78 \pm 1.02 a	5.2 \pm 2.14 a	13.48 \pm 2.44 b	1.19 \pm 0.11 a	0.15 \pm 0.14 a
	18	2.84 \pm 0.96 a	5.25 \pm 2.79 a	14.13 \pm 2.04 b	1.33 \pm 0.12 a	0.05 \pm 0.03 a
	22	3.41 \pm 0.99 a	5.00 \pm 1.45 a	11.94 \pm 1.00 ab	1.31 \pm 0.22 a	0.08 \pm 0.11 a
	2	2.72 \pm 0.97 a	4.19 \pm 2.02 a	12.87 \pm 2.25 ab	1.33 \pm 0.18 a	0.16 \pm 0.09 a
6	6	1.32 \pm 0.83 a	1.23 \pm 0.82 a	15.03 \pm 8.04 ab	0.47 \pm 0.14 a	n.d.
	10	2.02 \pm 1.02 ab	1.92 \pm 1.00 a	24.49 \pm 3.42 bc	0.73 \pm 0.18 ab	n.d.
	14	2.23 \pm 0.84 ab	2.02 \pm 0.79 a	18.00 \pm 6.26 abd	0.52 \pm 0.19 a	n.d.
	18	2.99 \pm 1.72 b	2.15 \pm 2.70 a	25.91 \pm 4.62 c	0.61 \pm 0.17 a	n.d.
	22	3.19 \pm 1.36 b	1.82 \pm 2.80 a	22.84 \pm 2.93 cd	0.69 \pm 0.28 ab	n.d.
	2	2.26 \pm 1.03 ab	0.08 \pm 0.05 a	21.78 \pm 5.82 abc	0.90 \pm 0.29 b	n.d.
9	6	1.87 \pm 0.40 a	1.91 \pm 0.43 a	39.8 \pm 7.39 a	0.71 \pm 0.22 a	n.d.
	10	1.97 \pm 0.69 a	2.06 \pm 0.63 a	42.46 \pm 9.34 a	0.68 \pm 0.23 a	n.d.
	14	2.61 \pm 0.75 ab	2.24 \pm 0.51 a	34.35 \pm 7.91 a	0.41 \pm 0.07 b	n.d.
	18	3.05 \pm 0.85 b	3.12 \pm 0.71 bc	36.07 \pm 7.45 a	0.43 \pm 0.08 b	n.d.
	22	2.43 \pm 0.74 ab	2.38 \pm 0.72 ab	41.7 \pm 8.56 a	0.55 \pm 0.18 ab	n.d.
	2	3.38 \pm 0.82 b	3.36 \pm 0.79 c	39.07 \pm 9.35 a	0.46 \pm 0.22 ab	n.d.
12	6	0.47 \pm 0.18 a	0.50 \pm 0.23 a	27.37 \pm 17.40 a	0.48 \pm 0.17 a	n.d.
	10	0.42 \pm 0.23 a	0.32 \pm 0.17 a	40.33 \pm 17.36 a	0.52 \pm 0.17 a	n.d.
	14	0.64 \pm 0.25 a	0.53 \pm 0.14 a	45.40 \pm 15.36 a	0.54 \pm 0.13 a	n.d.
	18	0.68 \pm 0.36 a	0.72 \pm 0.52 a	37.49 \pm 16.24 a	0.56 \pm 0.14 a	n.d.
	22	0.64 \pm 0.29 a	0.51 \pm 0.19 a	33.36 \pm 8.76 a	0.51 \pm 0.18 a	n.d.
	2	0.66 \pm 0.29 a	0.64 \pm 0.22 a	35.69 \pm 21.43 a	0.56 \pm 0.13 a	n.d.