

## DIFERENȚELE PRIVIND SCHIMBUL DE GAZE DIN FRUNZĂ ȘI CARACTERISTICILE FRUNZEI DINTRE DOUĂ SOIURI DE MIGDAL DIFFERENCES IN LEAF GAS EXCHANGE AND LEAF CHARACTERISTICS BETWEEN TWO ALMOND CULTIVARS

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### Abstract

Leaf chlorophyll content, specific leaf weight (SLW), photosynthetic and transpiration rates, stomatal functioning, water use efficiency and quantum yield were assessed during the kernel filling period for two consecutive years in order to understand tissue-centered physiological profile differences between two commercial almond cultivars, 'Ferragnès' and 'Texas'. Similar SLWs were observed on the studied cultivars; however, chlorophyll content, net photosynthetic and transpiration rates and stomatal functioning demonstrated statistically significant differences. In both cultivars, an overall decline in the examined parameters towards fruit maturation (i.e. end of the summer) was recorded. 'Ferragnès' leaves were found to be more efficient in leaf photosynthesis related performance during kernel filling, when irrigated sufficiently, in comparison to 'Texas' leaves. Low average values of leaf conductance during summer in 'Texas' leaves revealed its potential for adaptation in cool climates and increased carbon assimilation therein for high kernel yield.

**Cuvinte cheie:** *Prunus dulcis*, clorofilă, fotosinteză, transpirație

**Keywords:** *Prunus dulcis*, chlorophyll, photosynthesis, transpiration

### 1. Introduction

Almond [*Prunus dulcis* (Mill.) D.A. Webb] is one of the oldest tree nut crops cultivated extensively in the Mediterranean region with global economic interest not only for their nutritive value (nut content in edible lipids, proteins and carbohydrates) but also to some emerging pharmacological and anticancer uses (Takeoka et al., 2000; Amico et al., 2006). Photosynthetic efficiency and functional stomatal differences between cultivars comprise a major clustered set of parameters to achieve higher kernel production yield (De Herralde et al., 2003). Stomata as cellular epidermal valves on almond leaves are involved in gas exchange and directly affect CO<sub>2</sub> assimilation and water use efficiency (Bergmann and Sack, 2007). Differences in stomatal structure and function between cultivars may have pronounced effects on plant productivity.

The two widely used cultivars in the Mediterranean area are 'Ferragnès' and 'Texas' (Alonso-Segura et al., 2007). Although both cultivars demonstrate excellent trends in nut yield, 'Ferragnès' kernels are commercially oriented for fresh consumption, while 'Texas' almonds are mainly used for food processing (Wirthensohn et al., 2010). Therefore, both cultivars are covering in a balanced way, consumer and industrial needs at local and global level (Drogoudi et al., 2013).

'Ferragnès' was established in France in 1960 with a physiological profile of a vigorous tree, self-sterile, late blooming with satisfactory yield potential, good kernel features and absence of doubles (Grasselly and Crossa-Raynaud, 1980). 'Texas' (also called 'Mission') was introduced to California in 1891 and, during the last decades, exhibits stable high market demand, due to its late blooming and high nut yield for food industry in the Mediterranean area (Asai et al., 1996). In the Mediterranean region, 'Texas' trees bloom seven days earlier and ripen fruit later than 'Ferragnès' trees (harvest begins almost 10 days after 'Ferragnès') (Vasilakakis, 2004). Differences in kernel productivity between the two cultivars were not always significant (Godini and Palasciano, 1998); however 11% lower productivity for 'Texas' was observed in southern Italy (Godini, 2002).

Leaf photosynthetic characteristics are considered to be a basic approach to understand the plant's response to environmental conditions (DeJong, 1986). Gas exchange measurements on almond trees allow us to predict the proper environmental conditions for maximum yield for each variety (Raven, 2002). Size variation of stomata in wild and domesticated almond varieties has been recorded for Mediterranean climate conditions (Palasciano et al., 2005); however, their photosynthetic functionality was not measured when plant needs maximum water (July-August) due to environmental conditions. Herein, two almond cultivars, Ferragnès and Texas, were studied for their distinct leaf photosynthetic efficiency profile during the highest water demanding period of each annual cropping cycle, which includes the kernel filling period.

## 2. Material and methods

Two-year experiment was conducted during July and August in mature almond orchard located in Dimini area, Central Greece. The trial comprised two almond cvs, 'Ferragnès' (pollenizer cv. 'Ferraduel') and Texas (polliniser the local cv. Retsu) grafted on GF-677 almond-peach hybrid rootstock. The two cultivars were planted in alternate five-row blocks with scattered polliniser trees. Trees were irrigated regularly close to 100% ETc using a subsurface drip irrigation system, while fertilization was based on leaf analysis and nut production.

Twelve trees of each cultivar with same age, similar nut yields and plant growth vigour were selected for leaf physiological parameter assessment in different plantations in the same geographical area. Leaf characteristics, including leaf chlorophyll content and leaf dry matter content, were measured at three periods during the growing season (early July, late July and late August). Chlorophyll and leaf dry matter content were determined on leaf samples taken from fully expanded sun-exposed leaves from the middle of well developed shoots (12 leaves per selected tree, two 6-leaf replicates per tree). Immediately after the transfer to the laboratory, leaf disks were taken with 9 mm diameter borer from the collected leaves, chlorophyll was extracted with 95% ethanol and chlorophyll (Chl) a and b concentrations were determined spectrophotometrically (Wintermans and Motts, 1965). Total Chl and ratio of Chl a to Chl b (Chl a/b) were then calculated. Dry matter content was measured using similar leaf disks of known surface area after weighing the leaf disks immediately after removing (fresh mass) and after drying at 80 °C to constant weight (dry mass). Specific leaf weight (SLW) was then calculated.

In both experimental years, leaf physiological parameters were recorded with a portable photosynthesis system LCpro<sup>+</sup> (ADC BioScientific Ltd., Hoddesdon, Herts, U.K.). The values of net photosynthetic rate (Ps), transpiration rate (E), leaf stomatal conductance (g<sub>s</sub>), leaf temperature and internal CO<sub>2</sub> concentration (IntCO<sub>2</sub>) were measured directly from the photosynthetic unit.

Water use efficiency (WUE) and quantum yield (QY) were estimated with the above parameters and GASEX program (Postl and Bolhar-Nordenkamp, 1993).

All leaf photosynthesis related measurements were conducted July and August (i.e. throughout the kernel-filling period) in the morning hours (09:00 to 12:00), thus before early afternoon when high temperatures significantly reduce photosynthetic activity (Marsal et al., 1997) and in four fully expanded sun-exposed leaves per selected tree.

Data were analyzed using ANOVA from SPSS package (version 17.0, SPSS, Chicago, IL). Analysis of variance was conducted with two factors, cultivar and time. Values shown are means from 24 or 48 replicates per cultivar. Overall least significant difference (LSD<sub>0.05</sub>) was calculated.

## 3. Results and discussions

During the first experimental year, leaves from 'Ferragnès' trees had similar SLW, Chl a, Chl b and total Chl content, and ratio of Chl a/b to leaves from 'Texas' trees (Table 1). SLW decreased from early to late July, and remained stable until late August for both cultivars. Leaf Chl a and b and total Chl contents and the ratio Chl a/b gradually decreased from early July to late August for both cultivars (Table 1).

In the second experimental year, leaves from 'Ferragnès' trees had higher Chl a, Chl b and total Chl content than leaves from 'Texas' trees, but the differences were significant only in late July. No significant differences were found between the two cultivars in SLW and Chl a/b ratio. SLW decreased from early July to late July and remained stable until late August for both cultivars. Leaf Chl a content decreased in late August, and Chl b content increased from late July to late August leaving total Chl content unchanged over time (Table 2). Due to the above changes, the ratio Chl a/b decreased in late August for both cultivars.

There were significant differences between the two cultivars in all photosynthetic parameters in the first experimental year. At the end of July, 'Ferragnès' leaves had higher Ps, E, g<sub>s</sub> and QY and lower WUE and IntCO<sub>2</sub> compared to 'Texas' leaves (Table 3). At the end of August, 'Ferragnès' leaves had lower leaf Ps and E rates and g<sub>s</sub> and higher QY, WUE, and IntCO<sub>2</sub> than 'Texas' leaves (Table 3). Although the two measurement dates were statistically analyzed separately, we can conclude some differences between the two dates. In 'Ferragnès' leaves, Ps, E and g<sub>s</sub> decreased and WUE and IntCO<sub>2</sub> increased in August compared to July. Almost always the opposite was true for Texas' leaves, as Ps, E and g<sub>s</sub> increased in August compared to July.

In the second experimental year, 'Ferragnès' leaves exhibited higher leaf Ps and E values than 'Texas' leaves. There were no differences between the two cultivars in g<sub>s</sub> rates, WUE, QY, and IntCO<sub>2</sub> except from late August when 'Ferragnès' leaves displayed higher g<sub>s</sub> rates and QY than 'Texas' leaves (Table 4). All photosynthetic parameters gradually decreased from July to late August in both cultivars with the exception of leaf E and IntCO<sub>2</sub>, which were not significantly different during the summer months in 'Ferragnès'.

In both almond cultivars SLW reached high average values in early summer and significantly decreased thereafter reflecting the large amounts of assimilated carbon used for vegetative growth until

early July, when air temperatures are modest, and kernel filling thereafter during the warmest months. Actually, from early July to the end of August (depending on the ripening period for each cultivar), photosynthetic products are mainly transferred to the fruit for kernel filling (Marsal et al., 1997). The fact that 'Ferragnès' leaves had similar (first experimental year) or more chlorophyll (second experimental year) over the summer than 'Texas' leaves suggests that 'Ferragnès' trees could be more capable to form photosynthetically efficient leaves, which could support CO<sub>2</sub> assimilation and kernel filling during the summer.

Net photosynthetic rate and stomatal conductance declined during the summer for both cultivars, changes which are in accordance to Marsal et al. (1997) and Matos et al. (1997). The significant relationship between Ps and stomatal functioning is well documented: as stomata close under drought or high temperature stressful conditions, Ps decreases (Girona et al., 1993; Marsal et al., 1997; Klein et al., 2001; Romero et al., 2004). In our study, soil water availability wasn't a stress factor; reductions in stomatal opening and Ps could be associated with changes in carbohydrate translocation rate to fruit tissues due to fruit maturation or with summer heat stress as it was found in olive (Proietti, 2003).

In addition, the reduction of chlorophyll (Chl a & b) concentration in August may have partially affected leaf photosynthetic ability (Gikloo and Elhami, 2012). As no macroscopic senescence symptoms were observed in the leaves during the experimental period, the decrease mainly in Chl a concentration was possibly due to the extensive summer heat period or the decline of sun radiation (as the Chl a/b ratio decreased, a shade effect) from July to August.

Romero et al. (2004) found in almond leaves that, under water stress conditions, IntCO<sub>2</sub> and WUE increased in order to maintain high photosynthetic rates during moderate stress and excessive water loss, while Matos et al. (1997) stated that the changes in Ps, E, gs and WUE were the result of the effect of soil and environmental factors including light, CO<sub>2</sub>, humidity, wind velocity and temperature. High air temperatures during the summer will cause leaf stomatal closure and reduction in net photosynthesis in various tree species common to areas with hot dry summer period (Schulze et al., 1974; Schulze et al., 1975; Tenhunen et al., 1981; Tenhunen et al., 1984). In our two years study, 'Ferragnès' and 'Texas' trees were regularly irrigated, but the air temperature was considerably high (mean air temperature during July and August was 25.9 and 25.1 °C for the first, and 27.7 and 26.4 °C for the second experimental year, respectively; meteorological data were collected from the closest meteorological station, Magnesia, Greece). So, air temperature decreased in August compared to July but, also, the second summer study period was warmer than the first summer study period. This was reflected in the Ps rates of both cultivars, which declined in the second year, despite the similar fruit load between the two years.

The above differences between the two years were reflected in the two cultivars in a different way. In 'Ferragnès' probably due to fruit maturation (i.e. weak translocation carbon rates) at the end of August, during the first year under favourable summer conditions, leaf functioning decreased but remained in relatively high rate. This possibly means that excess carbohydrates may be allocated to storage pools for next year's crop and vegetative growth as it was found in other *Prunus* species (Gucci et al., 1991). In the same cultivar, during the warmer second study year, leaf functioning (mainly Ps) was reduced compared to the first year, but remained in acceptable levels. These data show the superior photosynthetic efficiency of 'Ferragnès' leaves, independently from constraining weather conditions, when irrigated properly.

In 'Texas', during the first year, with the cooler late summer period, leaf functioning increased to cover the final kernel filling requirements towards fruit maturation, as 'Texas' almonds ripen almost 10 days later than 'Ferragnès' almonds. During the second year, with high summer temperatures, 'Texas' leaves always had lower leaf functioning than 'Ferragnès' leaves. This possibly demonstrates the sensitivity of this cultivar to high air summer temperatures despite the ample irrigation water availability.

#### 4. Conclusions

'Ferragnès' was more efficient in using sun radiation (as depicted by QY values) during the hot summer period in comparison to 'Texas', when ample irrigation water was available. This explains the high vegetative growth and yield of this cultivar under the Mediterranean conditions when irrigated efficiently.

The lower performance of 'Texas' found in our study may be related to its limited capacity to function during the hot summer months, even when irrigation water is available. This trait of 'Texas' relates the lower productivity found with hot summer period among cropping years.

## References

1. Alonso-Segura J.M., Socias I., Company R., 2007. Negative inbreeding effects in tree fruit breeding: self-compatibility transmission in almond. *Theor. Appl. Genet.* 115:151-158.
2. Amico V., Barresi V., Condorelli D., Spatafora C., Tringali C., 2006. Antiproliferative terpenoids from almond hulls (*Prunus dulcis*): identification and structure-activity relationships. *J. Agric. Food Chem.* 54:810-814.
3. Asai W.K., Micke W.C., Kester D.E., Rough D., 1996. The evaluation and selection of current varieties. In: Micke W.C. (Ed). *Almond Production Manual*. U.C. D.A.N.R., Publ. 3364, pp 52-60.
4. Bergmann D.C., Sack F.D., 2007. Stomatal development. *Annu. Rev. Plant Biol.* 58:163-181.
5. De Herralde F., Biel C., Savé R., 2003. Leaf photosynthesis in eight almond tree cultivars. *Biol. Plant* 46: 557-561
6. DeJong T.M., 1986. A whole plant approach to photosynthetic efficiency in tree crops. In: Lakso AN, Lenz F (Eds). *Photosynthesis in Fruit Trees*. Cornell University, Ithaca, New York, pp 18-22.
7. Drogoudi P.D., Pantelidis G., Bacchetta L., De Giorgio D., Duval H., Metzidakis I., Spera D., 2013. Protein and mineral nutrient contents in kernels from 72 sweet almond cultivars and accessions grown in France, Greece and Italy. *Int. J. Food Sci. Nutr.* 64:202-209.
8. Gikloo S.T., Elham B., 2012. Physiological and morphological responses of two almond cultivars to drought stress and cycocel. *Int. Res. J. App. & Basic Sci.* 3:1000-1004
9. Girona J., Marsal J., Cohen M., Mata M., Miravete C., 1993. Physiological, growth and yield responses of almond (*Prunus dulcis* L.) to different irrigation regimes. *Acta Hort.* 335:389-398.
10. Godini A., 2002. Almond fruitfulness and role of self-fertility. *Acta Hort.* 591:191-203.
11. Godini A., Palasciano M., 1998. Growth and yield of four self-fruitful and four self-unfruitful almonds on three rootstocks: a thirteen-year study. *Acta Hort.* 470:200-207.
12. Grasselly C., Crossa-Raynaud P., 1980. *L' Amandier*. Maisonneuve et Larose, Paris (in French).
13. Gucci R., Xiloyannis C., Flore J.A., 1991. Gas exchange parameters, water relations and carbohydrate partitioning in leaves of field-grown *Prunus domestica* following fruit removal. *Physiol. Plant* 83: 497-505.
14. Klein I., Esparza G., Weinbaum S.A., DeJong T.M., 2001. Effects of irrigation deprivation during the harvest period on leaf persistence and function in mature almond trees. *Tree Physiol.* 21:1063-1072.
15. Marsal J., Girona J., Mata M., 1997. Leaf water relation parameters in almond trees during a deficit irrigation period. *J. Amer. Soc. Hort. Sci.* 122:582-587.
16. Matos M.C., Matos A.A., Mantans A., Cordeiro V., Vieira da Silva J.B., 1997. Photosynthesis and water relations of almond tree cultivars grafted on two rootstocks. *Photosynthetica* 35:249-256.
17. Palasciano M., Camposeo S., Godini A., 2005. Stomatal size and frequency in wild (*A. webbii*) and cultivated (*A. communis*) almonds. *Options Méditerranéennes* A63:305-310.
18. Proietti P. 2003. Changes in photosynthesis and fruit characteristics in olive in response to assimilate availability. *Photosynthetica* 41:559-564.
19. Postl W.F., Bolhar-Nordenkamp H.R., 1993. 'GASEX': A program to study the influences of data variations on calculated rates of photosynthesis and transpiration. In: Hall DO, Scurlock JMO, Bolhar-Nordenkamp HR, Leegood RC, Long SP (Eds). *A Field and Laboratory Manual: Photosynthesis and Production in a Changing Environment*. Chapman & Hall, London, pp 488-455.
20. Raven J., 2002. Selection pressures on stomatal evolution. *New Phytol.* 153:371-386.
21. Romero P., Navarro J.M., Garcia F., Ordaz P.B., 2004. Effects of regulated deficit irrigation during the pre-harvest period on gas exchange, leaf development and crop yield of mature almond trees. *Tree Physiol.* 24:303-312.
22. Schulze E.D., Lange O.L., Evenari M., Kappen L., Buschborn U., 1974. The role of air humidity and leaf temperature in controlling stomatal resistance of *Prunus armeniaca* L. under desert conditions. I. A simulation of the daily time course of stomatal resistance. *Oecologia* 17:159-170.
23. Schulze E.D., Lauge O.L., Kappen L., Evenari M., Buschborn U., 1975. The role of air humidity and leaf temperature in controlling stomatal resistance of *Prunus armeniaca* L. under desert conditions. II. The significance of leaf water status and internal carbon dioxide concentration. *Oecologia* 18:219-233.
24. Takeoka G., Dao L., Teranishi R., Wong R., Flessa S., Harden L., Edwards R., 2000. Identification of three triterpenoids in almond hulls. *J. Agric. Food Chem.* 48:3437-3439.
25. Tenhunen J.D., Lange O.L., Braun M., 1981. Midday stomatal closure in Mediterranean type sclerophylls under simulated habitat conditions in an environmental chamber. II. Effect of the complex of leaf temperature and air humidity on gas exchange of *Arbutus unedo* and *Quercus ilex*. *Oecologia* 50:5-11.
26. Tenhunen J.D., Lange O.L., Gebel J., Beyschlag W., Weber J.A., 1984. Changes in photosynthetic capacity, carboxylation efficiency, and CO<sub>2</sub> compensation point associated with midday stomatal

closure and midday depression of net CO<sub>2</sub> exchange of leaves of *Quercus suber*. *Planta* 162:193-203.

27. Vasilakakis M., 2004. General and Specialized Pomology. Gartaganis Publications, Thessaloniki, Greece (in Greek).
28. Wintermans I.F., Mots A., 1965. Spectrophotometric characteristics of chlorophylls a and b and their pheophytins in ethanol. *Bioch. Biophys. Acta* 109:448-453.
29. Wirthensohn M.G., Chin W.L., Franks T.K., Baldock G., Ford C.M., Sedgley M., 2010. Investigation of flavour compounds from sweet, semi-bitter and bitter almond kernels. *Options Méditerranéennes* A94:117-122.

## Tables and figures

**Table 1. Specific leaf Weight (SLW) and chlorophyll (expressed as mg per m<sup>2</sup> leaf surface) of 'Texas' and 'Ferragnès' almond trees over time during the 1<sup>st</sup> experimental year.**

Cultivar	Date	SLW (cm <sup>2</sup> g <sup>-1</sup> DM)	Chl a (mg m <sup>-2</sup> )	Chl b (mg m <sup>-2</sup> )	Chl total (mg m <sup>-2</sup> )	Chl a/b
Texas	06-Jul	100.5	211.2	60.3	271.5	3.5
	28-Jul	83.2	180.2	67.6	247.8	2.7
	30-Aug	87.3	142.2	58.1	200.3	2.4
Ferragnès	06-Jul	96.5	208.6	66.7	275.3	3.1
	28-Jul	91.5	175.5	62.2	237.7	2.8
	30-Aug	87.4	146.6	53.5	200.0	2.7
<i>P</i> <sub>(Cultivar)</sub>		ns	ns	ns	ns	ns
<i>P</i> <sub>(Date)</sub>		***	***	***	***	***
Overall LSD <sub>0.05</sub>		8.8	18.5	4.5	23.2	0.16

ns = not significant, and \*\*\* = *P* < 0.001. Separation by Duncan's multiple range test, 95% confidence level.

**Table 2. Specific leaf weight (SLW) and chlorophyll (expressed as mg per m<sup>2</sup> leaf surface) of 'Texas' and 'Ferragnès' almond trees over time during the 2<sup>nd</sup> experimental year.**

Cultivar	Date	SLW (cm <sup>2</sup> g <sup>-1</sup> DM)	Chl a (mg m <sup>-2</sup> )	Chl b (mg m <sup>-2</sup> )	Chl total (mg m <sup>-2</sup> )	Chl a/b
Texas	03-Jul	110.0	186.8	82.8	269.6	2.3
	28-Jul	86.7	177.0	77.5	254.5	2.3
	24-Aug	88.6	161.9	94.5	256.4	1.7
Ferragnès	03-Jul	110.7	191.8	86.3	278.1	2.2
	28-Jul	87.7	212.2	95.0	307.2	2.2
	24-Aug	85.9	179.4	105.3	284.7	1.7
<i>P</i> <sub>(Cultivar)</sub>		ns	**	**	**	ns
<i>P</i> <sub>(Date)</sub>		***	**	***	ns	***
Overall LSD <sub>0.05</sub>		12.8	21.9	9.3	32.3	0.12

ns = not significant, \*\* = *P* < 0.01, and \*\*\* = *P* < 0.001. Separation by Duncan's multiple range test, 95% confidence level.

**Table 3. Leaf net photosynthetic rate (Ps), transpiration rate (E), stomatal conductance (g<sub>s</sub>), leaf quantum yield (QY), water use efficiency (WUE), and leaf internal CO<sub>2</sub> concentration (IntCO<sub>2</sub>) of 'Texas' and 'Ferragnès' almond trees during the 1<sup>st</sup> experimental year.**

Parameters/ Date	Texas	Ferragnès	<i>P</i>	LSD <sub>0.05</sub>	Texas	Ferragnès	<i>P</i>	LSD <sub>0.05</sub>
	July				August			
Ps (μmol m <sup>-2</sup> s <sup>-1</sup> )	12.4	14.3	*	1.40	14.2	12.6	**	1.30
E (mmol m <sup>-2</sup> s <sup>-1</sup> )	2.0	2.6	***	0.17	2.8	1.8	***	0.18
g <sub>s</sub> (mol m <sup>-2</sup> s <sup>-1</sup> )	0.13	0.22	***	0.03	0.20	0.10	***	0.04
QY (mol CO <sub>2</sub> / 100 photons)	0.8	1.0	*	0.15	0.7	1.1	***	0.17
WUE (mmol CO <sub>2</sub> / mol H <sub>2</sub> O)	6.2	5.5	**	0.40	5.1	7.0	***	0.50
IntCO <sub>2</sub> (μL L <sup>-1</sup> )	259	232	*	22	248	304	***	39

ns = not significant; \* =  $P < 0.05$ ; \*\* =  $P < 0.01$ ; and \*\*\* =  $P < 0.001$ . Separation by Duncan's multiple range test, 95% confidence level.

**Table 4. Leaf net photosynthetic rate (Ps), transpiration rate (E), stomatal conductance (g<sub>s</sub>), leaf quantum yield (QY), water use efficiency (WUE), and internal CO<sub>2</sub> concentration (IntCO<sub>2</sub>) of 'Texas' and 'Ferragnès' almond trees during the 2<sup>nd</sup> experimental year.**

Parameters/ Date	Texas	Ferragnès	<i>P</i>	LD <sub>0.05</sub>	Texas	Ferragnès	<i>P</i>	LD <sub>0.05</sub>
	July				August			
Ps (μmol m <sup>-2</sup> s <sup>-1</sup> )	7.51	9.01	**	1.20	4.46	7.00	***	1.21
E (mmol m <sup>-2</sup> s <sup>-1</sup> )	2.21	2.68	*	0.41	1.51	2.36	***	0.30
g <sub>s</sub> (mmol m <sup>-2</sup> s <sup>-1</sup> )	0.09	0.11	ns	0.03	0.04	0.08	***	0.01
QY (mol CO <sub>2</sub> / 100 photons)	0.75	0.79	ns	0.17	0.37	0.62	***	0.14
WUE (mmol CO <sub>2</sub> / mol H <sub>2</sub> O)	3.48	3.41	ns	0.47	2.94	2.98	ns	0.51
IntCO <sub>2</sub> (μL L <sup>-1</sup> )	196.91	196.00	ns	18.60	174.28	181.33	ns	21.18

ns = not significant; \* =  $P < 0.05$ ; \*\* =  $P < 0.01$ ; and \*\*\* =  $P < 0.001$ . Separation by Duncan's multiple range test, 95% confidence level.