

Moderate shade can increase net gas exchange and reduce photoinhibition in citrus leaves[†]

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Summary Daily variations in net gas exchange, chlorophyll fluorescence and water relations of mature, sun-acclimated grapefruit (*Citrus paradisi* Macfady.) and orange (*Citrus sinensis* L. Osbeck) leaves were determined in tree canopies either shaded with 50% shade screens or left unshaded (sunlit). Mean daily maximum photosynthetic photon flux density (PPFD) under shade varied from 500 to 700 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and was sufficient to achieve maximum net CO_2 assimilation rates (A_{CO_2}). Responses of grapefruit and orange leaves to shading were remarkably similar. At midday, on bright clear days, the temperatures of sunlit leaves were 2–6 °C above air temperature and 1–4 °C above the temperatures of shaded leaves. Although midday depressions of stomatal conductance (g_s) and A_{CO_2} were observed in both sunlit and shaded leaves, shaded leaves had lower leaf-to-air vapor pressure differences (D) along with higher g_s , A_{CO_2} and leaf water-use efficiency than sunlit leaves. Estimated stomatal limitation to A_{CO_2} was generally less than 25% and did not differ between shaded and sunlit leaves. Leaf intercellular CO_2 partial pressure was not altered by shade treatment and did not change substantially with increasing D . Radiation and high temperature stress-induced non-stomatal limitation to A_{CO_2} in sunlit leaves was greater than 40%. Reversible photoinhibition of photosystem II efficiency was more pronounced in sunlit than in shaded leaves. Thus, non-stomatal factors play a major role in regulating A_{CO_2} of citrus leaves during radiation and high temperature stress.

Keywords: chlorophyll fluorescence, grapefruit, leaf temperature, orange, photosynthesis, stomatal conductance, vapor pressure deficit.

Introduction

Leaves of broadleaf, evergreen citrus trees are characterized by relatively low maximum rates of CO_2 assimilation (A_{CO_2} ; typically < 12 $\mu\text{mol m}^{-2} \text{s}^{-1}$) compared with leaves of other C_3 plants (20–30 $\mu\text{mol m}^{-2} \text{s}^{-1}$) (Kriedemann 1971, Syvertsen and Lloyd 1994). The mechanisms underlying the low A_{CO_2} are not well understood, but low A_{CO_2} is thought to be one of the major factors limiting growth and productivity of citrus

trees (Goldschmidt 1999). Before improving photosynthetic efficiency, it is essential to identify the relative importance of the processes limiting A_{CO_2} , such as light, temperature, CO_2 supply, carbon source–sink balance and other interacting environmental factors.

In Florida, maximum photosynthetic photon flux density (PPFD) during midsummer is about 1500–2200 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Maximum A_{CO_2} of sun-acclimated leaves in the outer surfaces of citrus canopies is light-saturated at about one-third of full sunlight (600 to 700 $\mu\text{mol m}^{-2} \text{s}^{-1}$; Sinclair and Allen 1982, Syvertsen 1984). Citrus is considered shade-tolerant (Reuther 1977) and sun-exposed leaves routinely acclimate to shaded conditions as canopies develop (Syvertsen 1984). In addition, shade-acclimated leaves can acclimate to high radiation environments (Syvertsen and Smith 1984).

Sunlit citrus leaves in outer canopy positions can be as much as 9 °C warmer than leaves from shaded canopy positions, or 9 °C above air temperature, during spring and summer (Syvertsen and Albrigo 1980). The excess radiant energy and high temperatures of leaves and fruit frequently cause water deficits and reduce light-use efficiency, leading to reduced A_{CO_2} , growth, fruit yield and quality (Goldschmidt 1999). Large leaf-to-air temperature differences create steep leaf-to-air vapor pressure differences (D ; Jones 1992, Jifon and Syvertsen 2000). Citrus leaf stomatal conductance (g_s) is particularly sensitive to changes in D ; g_s decreases as leaf temperature (T_{lf}) and D increase (Sinclair and Allen 1982, Syvertsen and Salyani 1991). This might enable trees to limit water loss, and thereby increase water-use efficiency and productivity in semi-arid environments (Syvertsen and Lloyd 1994). Khairi and Hall (1976b) found that citrus leaf g_s and A_{CO_2} both decreased when T_{lf} and D increased above 30 °C and 2.5 kPa, respectively, but gas exchange recovered within 1 h after reducing T_{lf} to 26 °C. Similar results have been reported for orange (*Citrus sinensis* (L.) Osbeck cv. Pineapple), grapefruit (*Citrus paradisi* Macfady cv. Marsh) (Sinclair and Allen 1982, Brakke and Allen 1995) and tea (*Camelia sinensis* (L.) O. Kuntze) (Mohotti and Lawlor 2002), particularly on bright warm days characterized by large midday vapor pressure deficit.

[†] As originally published, there was a transposition of text at Line 8 of Paragraph 1 of the Introduction. The error has been corrected here. A notice of the correction will appear in a forthcoming issue of the print journal.

The strong correlations between D , g_s and A_{CO_2} suggest a major role for gas phase limitations to A_{CO_2} and productivity (Wong et al. 1979, Lu et al. 1998). Even in relatively thick, hypostomatous citrus leaves, however, conductance to CO_2 diffusion through intercellular air spaces is much greater than through mesophyll cell wall surfaces to sites of CO_2 fixation (Lloyd et al. 1992, Syvertsen et al. 1995). The relative importance of stomatal and non-stomatal limitations of citrus A_{CO_2} under field conditions has not been investigated.

The leaf intercellular CO_2 partial pressure (C_i) of plants growing under optimum conditions is generally maintained at about 70% of ambient CO_2 partial pressure (C_a) (Jones 1992, Drake et al. 1997). Thus, C_i can be used as an index of the gas-phase (stomatal) limitation to A_{CO_2} (L_s). If low A_{CO_2} were caused primarily by reduced g_s , then C_i would be expected to decline with declining A_{CO_2} , but this rarely occurs (Farquhar and Sharkey 1982). The relative reduction in A_{CO_2} below the potential rate that would occur if g_s were infinite can be used as a measure of L_s (Farquhar and Sharkey 1982, Long and Hällgren 1993; and see Jones (1998) for a discussion of the relative merits of various approaches to estimating L_s).

This study was designed to characterize stomatal and non-stomatal limitations on A_{CO_2} of sunlit and shaded citrus leaves under field conditions. We tested the hypothesis that reducing midday PPF over sun-acclimated leaves by about 50% would reduce T_{if} and D , and thereby increase g_s , A_{CO_2} and leaf water-use efficiency (WUE). Gas exchange analyses and chlorophyll a fluorescence techniques were used to investigate the relative importance of stomatal and non-stomatal factors causing midday depressions of citrus leaf g_s and A_{CO_2} .

Materials and methods

This study was conducted during the spring–summer (April to August) and fall (October, November) seasons of 1999–2001 at the University of Florida, Citrus Research and Education Center, Lake Alfred, FL (28.09° N, 81.37° W, elevation 51 m a.s.l.). Measurements were made on 12-year-old bearing Hamlin orange (*Citrus sinensis*) trees growing in the field along with potted 4-year-old Hamlin trees and Ruby Red grapefruit (*Citrus paradisi*) trees growing outdoors in 15-l plastic containers. All trees were well watered and fertilized. The shade treatment over field trees was achieved by individually draping four trees for 24 to 48 h with Aluminet (Polysack Plastic Industries, Nir Yitzhak, Israel), a spectrally neutral, aluminized polypropylene shade screen with a mesh size of 6 × 3 mm, which transmits about 50% of incident light. For the potted trees, which were about 1.6 m tall, shade screens were placed on top of 2.2-m tall PVC frames constructed over the trees. Four trees of each species were placed under 50% shade and four trees served as sunlit controls.

Photosynthetic photon flux density above and below the shade screens, air temperature and relative humidity were recorded continuously with a multi-sensor weather station (Apogee Instruments, Logan, UT). Leaf temperatures were measured with 30-gauge copper-constantan fine-wire thermocouples (Model 5TC-GG-30, Omega Engineering, Stamford,

CT) connected to a data logger (CR21X, Campbell Scientific, Logan, UT). The thermocouple junctions were pressed against the abaxial surfaces of mature leaves, selected from exterior canopy positions and held in place by lightweight clips. Leaf-to-air vapor pressure differences were calculated from air temperature, relative humidity and T_{if} based on the equations of Buck (1981).

Gas exchange measurements

Fully expanded, sun-acclimated leaves, about 3 to 5 months old, in exterior canopy positions were used for gas exchange measurements on selected clear days within 48 h after shading. Net A_{CO_2} , g_s and transpiration rate (E) were measured with a portable photosynthesis system (LI-6200, Li-Cor, Lincoln, NE) equipped with a well-stirred 0.25-l leaf chamber with constant-area inserts (12 cm²). Leaf internal CO_2 partial pressure and photosynthetic water-use efficiency (WUE = A_{CO_2}/E) were calculated automatically by the internal program of the LI-6200, based on the equations of von Caemmerer and Farquhar (1981). Measurements were conducted throughout the day at ambient $C_a = \sim 36$ Pa except for the A_{CO_2} versus C_i experiments (described below).

Stomatal limitation to A_{CO_2} was estimated from the relationships between A_{CO_2} and C_i for leaves on potted grapefruit trees following the procedures of Long and Hällgren (1993), McDermitt et al. (1989) and Li-Cor (1991). Specifically, L_s was calculated as:

$$L_s(\%) = ((A_0 - A)/A_0)100,$$

where A is assimilation rate at ambient C_a (~ 36 Pa) and A_0 is the assimilation rate at $C_i = 36$ Pa (obtained by interpolation from the A_{CO_2} versus C_i curve). That is, A_0 is the rate that would occur if g_s were infinite (no stomatal limitation). Because sunlit leaves had lower g_s than shaded leaves, the difference between A_0 values of shaded and sunlit leaves indicated the relative non-stomatal limitation to A_{CO_2} (L_{ns}) in sunlit leaves, and was calculated as:

$$L_{ns}(\%) = ((A_{0\text{shade}} - A_{0\text{sun}})/A_{0\text{shade}})100,$$

where $A_{0\text{shade}}$ and $A_{0\text{sun}}$ are the A_0 values of shaded and sunlit leaves, respectively. Natural daylight (PPFD range 1200–1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$) was used during all gas exchange measurements. Between measurements, the leaf chamber was placed in shade, and during measurements, the outside of the chamber was ventilated with an external fan to minimize increases in T_{if} . Leaf temperatures ranged from 29 to 40 °C and were comparable with those measured with thermocouples in situ.

Leaf water potential and tree water use

Leaf water potentials (Ψ_l) were measured with a Scholander-type pressure chamber (PMS Instruments, Corvallis, OR) during the course of two consecutive cloudless days in November 2001, using four replicate leaves from each of four potted grapefruit trees in both sunlit and shaded treatments. Leaves

used for Ψ_1 measurements were collected from the same shoots as leaves used for gas exchange measurements. Whole-tree water use was measured during the same period by weighing pots at the beginning and end of the photoperiod. Each pot was covered with a white plastic bag to minimize water evaporation from the soil.

Chlorophyll *a* fluorescence measurements

Chlorophyll *a* fluorescence characteristics were measured with a pulse modulated fluorometer (Model OS1-FL, Opti-Sciences, Tyngsboro, MA) and used to estimate the extent of photoinhibition. Throughout the gas exchange measurement days, at least 10 leaves per treatment including the leaves used for gas exchange measurements were dark-adapted for 0.5 h using leaf clips (FL-DC, Opti-Sciences) prior to fluorescence measurements. The chlorophyll fluorescence parameter F_v/F_m , which represents the maximum efficiency of photosystem II (PSII) photochemistry, was determined following the procedures of van Kooten and Snell (1990) and Maxwell and Johnson (2000). The F_m is the maximal fluorescence intensity, F_0 is the minimal (ground) fluorescence intensity and F_v is the variable fluorescence ($F_v = F_m - F_0$). The degree of photoinhibition was quantified as the ratio of F_v/F_m during the day relative to the value at dawn.

Following the gas exchange and chlorophyll *a* fluorescence measurements, two 1-cm² leaf discs were collected from the measurement leaf for chlorophyll determination. Chlorophyll was extracted with dimethylformamide in the dark and quantified by the equations of Wellburn (1994) after recording absorbance at 647 and 664 nm with a spectrophotometer (Model UV2401PC, Shimadzu, Columbia, MD).

Experimental design and data analysis

The experiments were set up in a completely randomized design with at least four trees of each species per shade or sunlit control treatment. Five independent experiments were conducted over 3 years (1999–2001). During each experiment, the shade or control treatments were randomly assigned to each tree. All measurements were carried out on selected clear days and within 48 h of shading to avoid light acclimation responses (Syvertsen 1984). The interactive and main effects of shade, measurement date (experiment) and time of day were analyzed with a three-factor analysis of variance in a completely randomized split-plot design (Little and Hills 1978, Moser et al. 1990). The significance of shade (main plot) was tested with the main plot experimental error (error a), whereas the effects of measurement date and time of day (subplots) were tested with the subplot experimental error (error b) (Steel and Torrie 1980). Where appropriate, regression models were fitted to the response variables.

Results

Mean maximum PPFD at midday on selected warm clear days was about 1900 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (daily mean = 38 $\text{mol m}^{-2} \text{s}^{-1}$)

and was reduced by about 50% as a result of shading (Figure 1a). Mean maximum air temperatures varied from 30 to 37 °C (daily mean = 27 °C) during summer months. Shading significantly reduced T_{lf} , resulting in lower D compared with sunlit leaves (Figures 1b and 1c). Sunlit leaves were, on average, 1 to 4 °C warmer than shaded leaves around midday. Shading did not significantly alter air temperature and relative humidity. However, D increased rapidly with T_{lf} . On dry days with mean air vapor pressure (VP) < 2 kPa, D was nearly 50% higher than on humid days with mean VP > 2 kPa (Figure 2a). Relationships between D and T_{lf} on dry and humid days paralleled each other.

Gas exchange

Physiological responses of grapefruit and orange leaves to shading were similar even though they were sometimes measured on different days. For brevity, only data from grapefruit leaves are presented in some cases. Stomatal conductance was relatively high and more responsive to changes in T_{lf} on humid

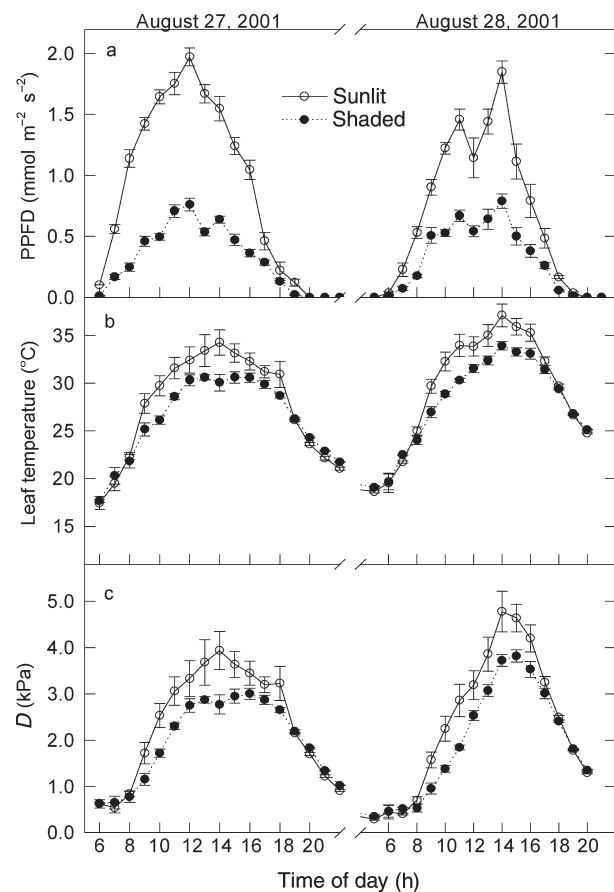


Figure 1. (a) Diurnal courses of incident photosynthetic photon flux density (PPFD) above and below shade screens, (b) leaf temperature and (c) leaf-to-air vapor pressure difference (D) of sunlit (○) and shaded (●) leaves of potted grapefruit trees grown outdoors on two representative days (August 27 and 28, 2001). Each value is the mean \pm SE of 4–10 single leaf measurements.

days than on dry days (Figure 2b). The responses of g_s to T_{lr} in shaded and sunlit leaves followed similar patterns but differed on dry versus humid days. Such shade-induced reductions in T_{lr} and D were associated with substantially higher A_{CO_2} and g_s in shaded grapefruit and orange leaves than in sunlit leaves, particularly around midday (Figures 3a–3d). Intercellular CO_2 partial pressure generally decreased during the day, but C_i did not differ between shaded and sunlit leaves (Figures 3e and 3f). Leaf WUE also decreased during afternoons, but shaded leaves had higher WUE than sunlit leaves during midday periods (Figures 3g and 3h). Differences in WUE between sunlit and shaded leaves were due mainly to differences in A_{CO_2} , because E was not significantly altered by 50% shading (data not shown).

Based on pooled grapefruit data, the relationship between A_{CO_2} and g_s was linear for g_s values from 0.05 to 0.3 $mol\ m^{-2}\ s^{-1}$ (Figure 4); thereafter, the relationship became asymptotic. Shading did not alter the A_{CO_2} versus g_s relationship; data from both shaded and sunlit leaves formed a continuous response.

Parameters A_{CO_2} , g_s and WUE all decreased with increasing D (Figures 5a, 5b and 5d). Again, relationships between gas

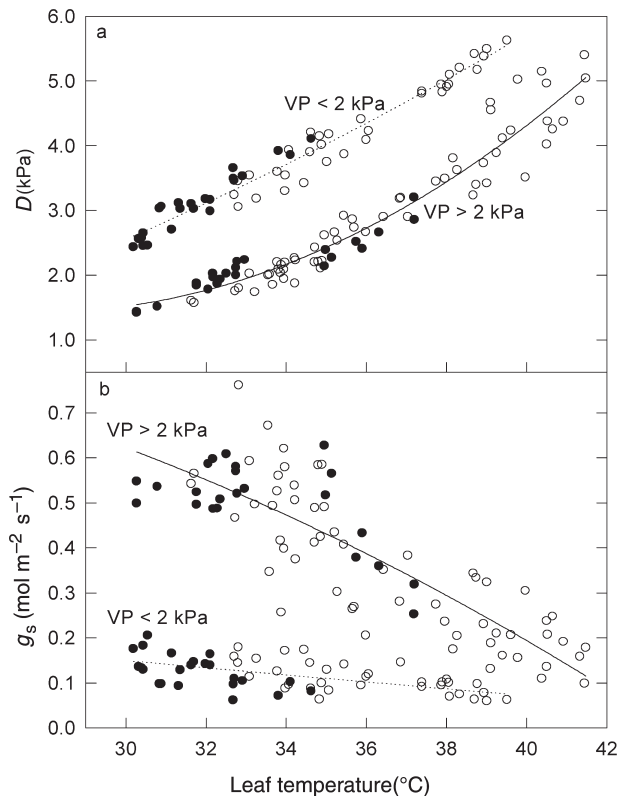


Figure 2. Relationships between leaf temperature and (a) leaf-to-air vapor pressure difference (D) and (b) stomatal conductance (g_s) for sunlit (○) and shaded (●) grapefruit leaves under field conditions. Dotted regression lines correspond to dry days when mean air vapor pressure (VP) was < 2 kPa (April 9 and 27, 2000; August 27, 2001; October 2, 2001; and November 8, 2001) and solid lines correspond to humid days with mean VP > 2 kPa (June 22, 1999; May 23, 2000; and August 27, 2000).

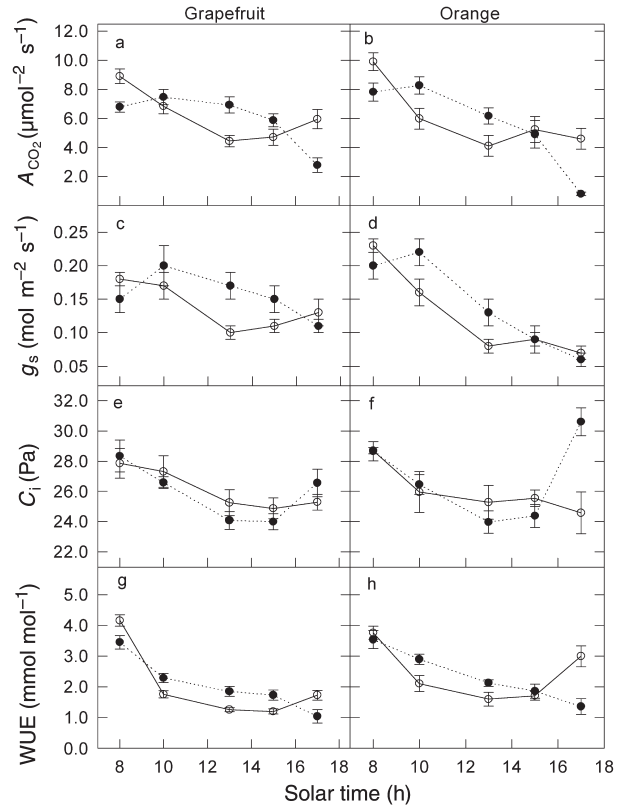


Figure 3. Diurnal courses of (a, b) net CO_2 assimilation (A_{CO_2}), (c, d) stomatal conductance (g_s), (e, f) leaf internal CO_2 partial pressure (C_i) and (g, h) water-use efficiency (WUE) of sunlit (○) and shaded leaves (●) of potted grapefruit and orange trees grown outdoors. Each value is the mean \pm SE of 4–10 single leaf measurements on August 27 and 28, 2001.

exchange characteristics and D from both shaded and sunlit leaves were parallel on dry (VP < 2 kPa) or humid (VP > 2 kPa) days. An increase in D from 1.5 to 5 kPa resulted in a larger reduction in g_s (~75%) than in A_{CO_2} (~40%), C_i (~13%) or WUE (~40%). Although C_i decreased slightly (~13%) with increasing D , it varied little between sunlit or shaded leaves (Figure 5c).

Shaded (50% PPFD) leaves had higher A_{CO_2} than sunlit leaves across the entire range of C_i (Figure 6). These relationships were used to calculate L_s and L_{ns} (Farquhar and Sharkey 1982, Long and Hällgren 1993) for grapefruit leaves. Stomatal limitation to A_{CO_2} was not significantly different between shaded ($18.5 \pm 1.7\%$) and sunlit leaves ($23.3 \pm 4.3\%$; Figure 6). The reduction in A_0 of sunlit leaves, relative to shaded leaves (L_{ns}), was $42.4 \pm 8\%$. The A_{CO_2} of shaded leaves was more responsive to increases in C_a above ambient pressure (from 36 to about 70 Pa) than A_{CO_2} of sunlit leaves. Although increasing C_a resulted in reduced g_s at every C_i (data not shown), C_i was similar in shaded and sunlit leaves over the range of 23 to 30 Pa.

Chlorophyll *a* fluorescence and photoinhibition

Total chlorophyll concentration ranged from 0.4 to 0.6 $g\ m^{-2}$

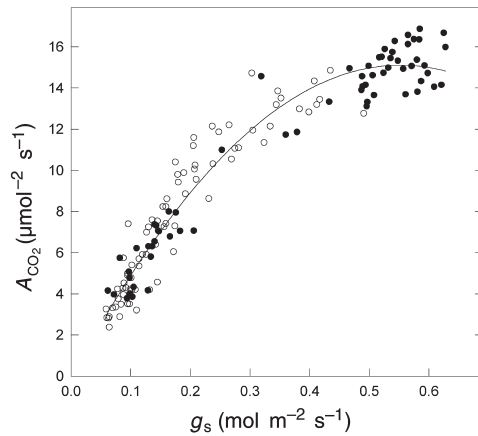


Figure 4. Relationship between stomatal conductance (g_s) and net CO_2 assimilation (A_{CO_2}) of sunlit (○) and shaded (●) grapefruit leaves. Data were pooled from measurements on field-grown and potted trees grown outdoors (June 1999; April, May and August, 2000; and June, August, October and November, 2001).

and chlorophyll a/b ratios ranged from 2.5 to 3.7, but neither was affected by the short-term shading of grapefruit and orange leaves (data not shown). With increasing PPFD and T_{if} , the F_0 of sunlit leaves often increased sharply at midday but recovered to values similar to those of shaded leaves in the late afternoon (Figure 7a). Midday photochemical efficiency decreased about 7% more in sunlit leaves than in shaded leaves, but both recovered in the late afternoon (after 1600 h; Figure 7b). The degree of photoinhibition, calculated as the daytime F_v/F_m ratio relative to the value at dawn, was about 16% at midday in sunlit leaves and only 5% in shaded leaves (Figure 7c).

Leaf water potential and tree water use

Leaf water potentials reached a minimum by early afternoon (1300 h), but there were no differences in Ψ_1 between shaded (-1.1 ± 0.06 MPa) and sunlit leaves (-1.2 ± 0.07 MPa). Whole-tree water use was unaffected by shading ($P = 0.21$; data not shown); however, shaded trees tended to use slightly more water (8%) than sunlit control trees.

Discussion

The observed depressions in g_s and A_{CO_2} with increasing T_{if} and D during the day are consistent with earlier observations for citrus (Kriedemann 1971, Khairi and Hall 1976a, Sinclair and Allen 1982, Cohen et al. 1997, Jifon and Syvertsen 2001) and other woody plants (Sandford and Jarvis 1986, Teskey et al. 1986, Mohotti and Lawlor 2002). At midday, g_s and A_{CO_2} of cool shaded leaves were consistently greater than those of sunlit leaves. Because D and T_{if} were strongly correlated (Figure 2a), the response of A_{CO_2} to D (Figure 5a) reflected the effect of temperature on A_{CO_2} . The observed midday decrease in A_{CO_2} could have been a result of stomatal or non-stomatal factors, or both. A large midday D could directly decrease g_s ,

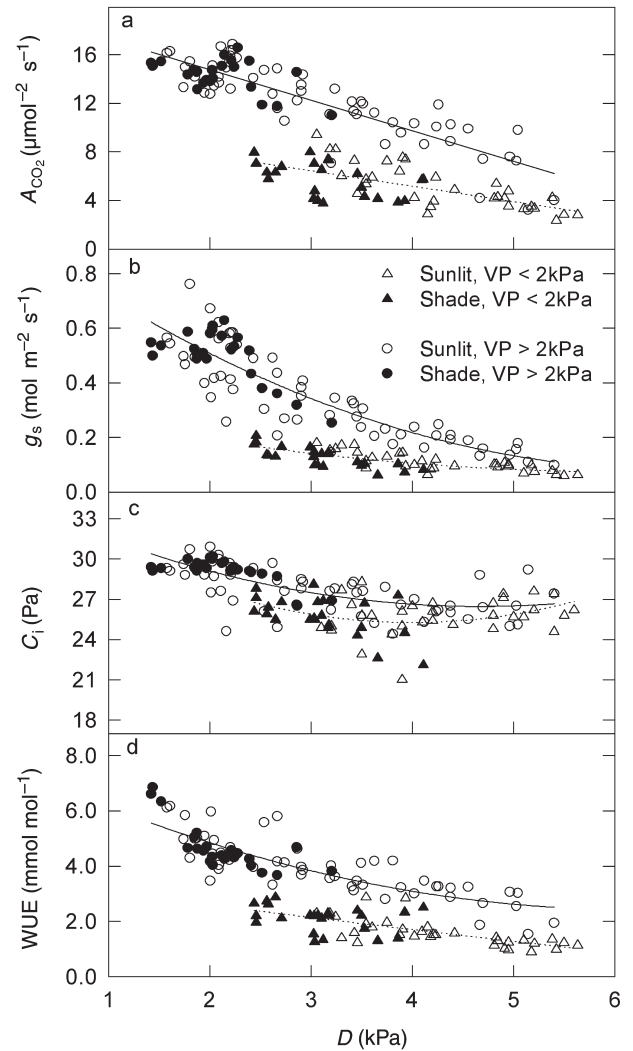


Figure 5. Relationships between leaf-to-air vapor pressure differences (D) and (a) net CO_2 assimilation (A_{CO_2}), (b) stomatal conductance (g_s), (c) leaf internal CO_2 partial pressure (C_i) and (d) water-use efficiency (WUE) for sunlit (○) and shaded (●) leaves of field-grown grapefruit trees. Dotted regression lines correspond to dry days when mean air vapor pressure (VP) was < 2 kPa and solid lines correspond to humid days with mean VP > 2 kPa. Data are from the same days as in Figure 2.

and thereby restrict CO_2 diffusion flux into the leaf (Figures 3e and 3f). Photoinhibition (Figure 7c) resulting from high PPFD and high T_{if} could also inhibit carboxylation and metabolism (Law and Crafts-Brandner 1999).

The strong correlation between g_s and A_{CO_2} (Figure 4) suggests a causal relationship between these parameters. If CO_2 diffusion were the major limitation to A_{CO_2} , a decrease in C_i would occur at the same time (Farquhar and Sharkey 1982). Although C_i generally decreased during the day (Figures 3e and 3f), it did not differ between shaded and sunlit leaves. In addition, the $A-C_i$ analyses revealed that L_s was not significantly different between shaded and sunlit leaves. Our values of L_s for citrus are similar to those reported for *Pinus taeda* L.

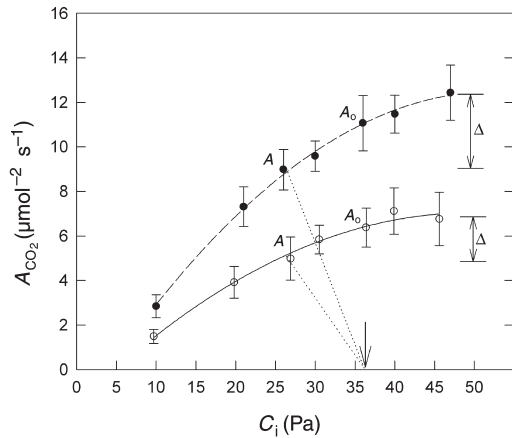


Figure 6. Response of net CO_2 assimilation (A_{CO_2}) to leaf internal CO_2 partial pressure (C_i) of sunlit (○) and shaded (●) leaves of field-grown grapefruit trees (October 2, 2001). Data at A are the A_{CO_2} values measured at ambient CO_2 partial pressure ($C_a \sim 36$ Pa) and at the actual stomatal conductance of 0.14 and 0.19 $\text{mol m}^{-2} \text{s}^{-1}$ for sunlit and shaded leaves, respectively. The A_0 values are the A_{CO_2} values that would be achieved if there were no stomatal restriction (i.e., at $C_i = C_a$ and $g_s = \infty$, arrow on C_i axis). The degree of stomatal limitation was calculated as $L_s = (A_0 - A)/A_0$. The Δ symbols indicate the response of A_{CO_2} to an increase in C_a from ~ 36 to ~ 70 Pa. Each value is the mean \pm SE of 4–10 single leaf measurements.

(Teskey et al. 1986) and cotton (Hutmacher and Krieg 1983), and support the argument that stomata usually impose a relatively small limitation on A_{CO_2} (Farquhar and Sharkey 1982). The similarity in C_i between shaded and sunlit leaves suggests that non-stomatal factors had a more important influence on A_{CO_2} than g_s . These observations were supported by the relatively high L_{ns} (42.4%) compared with L_s (23.3%) of sunlit leaves.

Several factors probably contributed to the high L_{ns} in sunlit leaves, including direct and indirect effects of supra-optimal T_{lf} around midday. Overall, A_{CO_2} in trees of all treatments decreased with increasing temperature, but the decrease was greater in sunlit leaves than in shaded leaves. The optimum temperature for citrus A_{CO_2} varies from 15 to 30 °C depending on humidity (Kriedemann 1968, Khairi and Hall 1976a). Air temperatures on clear warm days during our study typically exceeded 30 °C while D exceeded 2 kPa. On such days, sunlit leaf temperatures were 2–6 °C higher than air temperatures and T_{lf} occasionally exceeded 40 °C. The ensuing heat stress could have limited photosynthesis by disrupting the integrity of photosynthetic membranes, deactivating Calvin cycle enzymes (Law and Crafts-Brandner 1999) and inhibiting photoassimilate metabolism (reducing sink strength). Reduced sink strength can inhibit A_{CO_2} indirectly by reducing the rate at which inorganic phosphate (Pi) is recycled to support electron transport and carbon fixation in the chloroplast (Sharkey 1994). The relatively low responsiveness of A_{CO_2} to elevated CO_2 partial pressure in sunlit leaves (Δ , Figure 6) in-

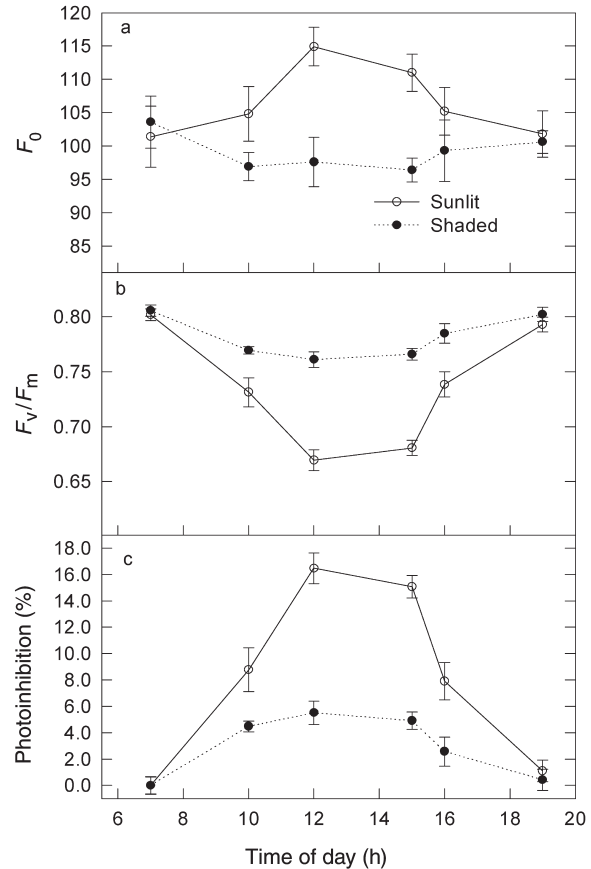


Figure 7. (a) Diurnal course of ground fluorescence (F_0), (b) maximum photosystem II photochemical efficiency (F_v/F_m) and (c) photoinhibition of sunlit (○) and shaded leaves (●) of field-grown grapefruit trees (August 27, 2001). The extent of photoinhibition was calculated as the ratio of F_v/F_m during the day to the value at dawn. Each value is the mean \pm SE of 10 single-leaf measurements.

dicated that Pi limited A_{CO_2} in sunlit leaves. For shaded leaves, however, A_{CO_2} increased significantly after switching C_a from 36 to 70 Pa CO_2 , indicating that A_{CO_2} of shaded leaves was not greatly limited by Pi. Heat-stress-induced limitations on photoassimilate utilization can lead to carbohydrate accumulation in leaves (Azcón-Bieto 1983) and reduced A_{CO_2} as a result of damage to grana and other membrane structures (Nafziger and Koller 1976). Also, stimulation of photorespiration and daytime dark respiration by higher T_{lf} would increase C_i , and thereby decrease g_s and carboxylation.

Although C_i is an important parameter in diagnosing non-stomatal regulation of A_{CO_2} , its calculation is subject to several potential errors, particularly heterogeneous (patchy) stomatal conductance (Terashima et al. 1988). We recognize that this could have led to an overestimation of C_i values, but we believe this effect to be of limited importance because citrus leaves are homobaric (Lloyd et al. 1992, Romera-Aranda et al. 1997), allowing lateral diffusion and homogenization of C_i in the mesophyll (Terashima et al. 1988).

Overestimation of C_i from gas exchange measurements has also been attributed to cuticular water loss, which contributes to measured g_s , and so can affect the calculation of C_i when g_s is low (Boyer et al. 1997, Meyer and Genty 1998). When g_s of *Alocasia macrorrhiza* (L.) G. Don. was $0.008 \text{ mol m}^{-2} \text{ s}^{-1}$, failure to consider cuticular water loss resulted in overestimation of C_i (Kirschbaum and Pearcy 1988). When g_s was $0.037 \text{ mol m}^{-2} \text{ s}^{-1}$, however, cuticular water loss did not significantly affect the calculated C_i . Cuticular water loss from citrus leaves is relatively low (Schönherr 1982), and in the present study, g_s values were 0.14 and $0.19 \text{ mol m}^{-2} \text{ s}^{-1}$ for sunlit and shaded leaves, respectively. Thus, cuticular water loss probably had little influence on the calculated C_i from A_{CO_2} versus C_i determination.

Depression of A_{CO_2} at midday could also be the consequence of excess excitation energy under conditions of high PPFD, leading to photoinhibition of PSII photochemistry (Ort 2001). As a measurement day progressed, photoinhibition was observed in all treatments, but was exacerbated in sunlit leaves as indicated by the large decrease in F_v/F_m at midday (Figure 7b). Reduction in F_v/F_m can result from a decrease in F_m or an increase in F_0 , because F_v/F_m is derived as $(F_m - F_0)/F_m$ (Maxwell and Johnson 2000). Decreased F_m and increased F_0 were observed on most days (Figure 7a). Daytime changes in PSII photochemistry were reversible, however, suggesting a protective mechanism for photoinhibition that perhaps involved xanthophyll cycle pigments (Gilmore and Ball 2000).

Although citrus is shade-tolerant, leaves can acclimate to high radiation environments (Syvertsen 1984) and apparently suffer little permanent photodamage as indicated by the high chlorophyll a/b ratios (2.5 to 3.7) observed in this study. Based on the lack of an effect of shading on leaf chlorophyll concentrations, it is unlikely that there was short-term acclimation of sun leaves to shade (Syvertsen and Smith 1984). Thus, the results of this study relate to sun-acclimated leaves and should not be extrapolated to leaves inside the canopy of mature citrus trees where there is much mutual shading as a result of high leaf area indices (Syvertsen and Lloyd 1994).

We have found that longer term (6–8 weeks) moderate shade can increase total fruit yield and juice content of mature *C. paradisi* trees, but can also reduce the total soluble solids content of individual fruit (Jifon and Syvertsen 2001). Shade screens not only reduce the direct radiation on outer canopy leaves, but can also increase the fraction of diffuse radiation on shaded leaves (Cohen et al. 1997). The resulting distribution of light within the canopy has been shown to increase dry matter production in tomato (Aikman 1989). Because the shade-reduced PPFD was still above that required for saturation of photosynthesis of sun-acclimated citrus leaves, it is possible that A_{CO_2} and WUE of whole trees could increase. Long-lived mature citrus leaves can acclimate to changing light environments (Syvertsen 1984, Syvertsen and Smith 1984), so effects of long-term moderate shade on leaf size, display and longevity as well as branch development and fruit yield characteristics in citrus warrant further study.

All study trees were well watered, so it is unlikely that water deficits contributed to the midday depression of A_{CO_2} . Shading increased leaf WUE, largely as a result of increased A_{CO_2} and not by altering E . Leaf water potentials, leaf transpiration and whole-tree water use were unaffected by shading. Although stomata were more open in shaded than sunlit leaves, the driving force for transpiration (the leaf-to-air vapor pressure difference, D) associated with shaded leaves was lower than that of sunlit leaves. Stomatal closure in response to increasing D is well documented in citrus (Syvertsen and Salyani 1991) and may be an adaptive physiological mechanism that allows citrus to survive in semi-arid environments with high evaporative demands (Syvertsen and Lloyd 1994). Maintenance of stable E values over a wide range of D has also been reported in other species of citrus (Camacho-B et al. 1974, Hall et al. 1975) and supports the concept that citrus can conserve water by limiting transpiration at some maximal rates (Sinclair and Allen 1982).

In summary, field-grown citrus under full sunlight and high temperature suffered midday depression of g_s and A_{CO_2} . Shading (50%) significantly reduced midday T_{if} and D , resulting in higher g_s and A_{CO_2} compared with sunlit leaves, but there were no differences in the morning hours. Although g_s of both shaded and sunlit leaves was sensitive to D , g_s played only a minor part in the observed reduction in A_{CO_2} . Non-stomatal factors induced by high light and temperature stress were more important in limiting A_{CO_2} than stomatal factors. Excess excitation energy in high light and the associated increase in T_{if} induced photoinhibition that persisted during the afternoon, but recovered by late afternoon. Shading decreased the degree of photoinhibition, thereby maintaining higher A_{CO_2} in shaded leaves than in sunlit leaves. The diversity of observed responses to radiation and temperature stress reported here supports the suggestion that no single mechanism can account for the responses of A_{CO_2} in the field (Jones 1998). In warm citrus producing regions, the beneficial effects of shading could improve leaf carbon assimilation, especially in young trees or trees with small canopies where most of the leaves are exposed to direct sunlight.

Notes

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