IMPACT OF THERMAL STRESS AND HIGH VPD ON GAS EXCHANGE AND CHLOROPHYLL FLUORESCENCE OF CITRUS GRANDIS UNDER DESERT CONDITIONS

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Abstract

The photosynthetic response of Citrus grandis to high light intensities, low air humidity and high temperature stress was investigated under desert conditions in the southern Arava Valley (Israel). During summer, a typical midday stomatal closure was observed even in well-watered trees due to the dramatic increase of the leaf-to-air water vapour deficit. As a result of the reduced transpirational cooling, leaf temperatures increased up to 11 °C above ambient air temperature. The combination of heat stress and photoinhibition resulted in a reversible decrease of photosynthetic activity of Citrus grandis under the extreme summer conditions.

1. Introduction

Citrus, native of subtropical and tropical regions in Eastern Asia became widely cultivated in areas with Mediterranean climate. In Israel, Citrus grandis, C. sinensis, C. limon and C. paradisi are major fruit crops of the coastal plain, where the mean annual rainfall ranges between 500 and 700 mm (Bielorari et al., 1973) and average temperature at midday in the August is 30 °C (Cohen et al., 1997).

During recent years cultivation of Citrus has substantially increased under desert conditions in the Negev and in the Arava Valley of Israel. In these arid areas crop production requires intensive irrigation. The climate of the Arava is characterized by mild winters and very hot summers with high air temperatures (> 42 °C), low air humidity and high radiation. Optimum temperature for Citrus photosynthesis varies from 25°C to 30°C, while temperatures above 35°C reduce photosynthetic activity (Spiegel & Goldschmidt, 1996). High temperatures combined with low air humidity may induce stomatal closure. In addition the combination of high irradiation and stress conditions, which limit photosynthetic energy conversion by reducing CO₂ supply, may result in enhanced photoinhibition (Baker, 1993; Blanke, 2000; Herppich 2000). Photosystem II is that component of the photosynthetic apparatus which is most sensitive to heat (Bilger et al., 1987) and high light stress (Baker, 1993). In the present study, the response of transpiration, stomatal opening, CO₂ uptake and chlorophyll fluorescence of Citrus grandis was investigated under extreme desert conditions.

2. Materials and methods

Three year old Citrus grandis trees were investigated during June and December 1997 at the Arava Research Station in Yotvata, Israel (29° 53´ N, 53° 3 ´E ), 40 km north of Eilat. The orchard was irrigated automatically by a drip system every second day during summer and every fourth day during winter. The soil was an Arava loamy sand.
Net CO₂ exchange and transpiration of fully expanded leaves were measured with a portable CO₂/H₂O porometer system (HCM-1000, Walz GmbH, Effeltrich, Germany) under ambient conditions according to Veste & Herppich (1995) and von Willert et al. (1995). Ambient CO₂ concentration was nearly constant at 350 ppm throughout the day. Area (projected leaf area) based gas exchange parameters were calculated after von Caemmerer and Farquhar (1981).

Simultaneously with gas exchange, chlorophyll fluorescence was monitored on the same plant with a Mini-PAM fluorometer (H. Walz GmbH, Effeltrich, Germany). Maximum photochemical efficiency of PSII (Fᵥ/Fₘ = Fₘ/F₀) was determined on dark-adapted (10 to 12 min) attached leaves (n = 7). Fₘ, Fᵥ, and F₀ denote the maximum, the variable and the initial fluorescence, respectively (cf. von Willert et al. 1995). Quantum yield of linear electron transport (F´ₘ/F´ₘ = ΦPSII) were analysed as summarized by von Willert et al. (1995) and electron transport rate (Jₑ) was calculated according to Krall & Edwards (1992) as Jₑ = ΦPSII * 0.5 * 0.84 * PPFD (PPFD = photosynthetic photon flux density).

3. Results

Typical diurnal time courses of net CO₂ exchange (JCₐO₂), transpiration (JH₂O), leaf conductance (gH₂O) of Citrus grandis and the microclimatic conditions on a normal summer and winter day are shown in Fig. 1. Maximum net CO₂ exchange rates of C. grandis typically ranged between 8 and 12 µmol m⁻² s⁻¹ throughout the year. Both photosynthetic electron transport (Jₑ, Fig. 2) and net CO₂ exchange (data not shown) were always light saturated at PPFD above 800 µmol m⁻² s⁻¹. During winter month diurnal courses of leaf conductance, net CO₂ exchange and transpiration were very similar, and no midday depression of gas exchange occurred (Fig. 1). Maximum air temperatures on cloudless winter days reached 20 to 22 °C and mean maximum Δw did not exceed 20 kPa MPa⁻¹.

![Figure 1](image-url)

Figure 1: Typical diurnal courses of net CO₂ exchange (JCₐO₂, A), transpiration (JH₂O, B), leaf conductance (gH₂O, C), photon flux density (PPFD, D), leaf and air temperature (Tₐir, Tₙₙ₉₉, E) and air-to-leaf water vapour deficit (Δw, F) in June (circles) and December (solid line).
In contrast, mean maximum air temperatures varied from 33 to 37 °C during summer days. The temperature of sun-exposed leaves raised up to 11 °C above air temperature during hours of high radiation. As a result, $\Delta w$ increased to more than 85 kPa MPa$^{-1}$ (Fig. 1). Due to the harsh conditions, $\Delta w$ substantially decreased after ca 9:00 even in well-watered plants.

Midday stomatal closure reduced net CO$_2$ uptake by nearly 65%. Additionally, high temperature in combination with high irradiance directly affected photosynthesis (Fig. 4). This was indicated by the increase of internal CO$_2$ concentration around noon (Fig. 4B). Furthermore, maximum photochemical efficiency ($F_v/F_m$) decreased by 12% from pre-dawn to noon (Fig. 4C). The initial fluorescence ($F_0$) was nearly unaffected in the early morning hours and tended to rise slightly when leaf temperature exceeded 27°C. At leaf temperatures of 43°C the initial fluorescence was 15% higher than pre-dawn levels (Fig. 4C).

Air temperatures occasionally exceeded 40 °C. On these occasions leaf temperatures of sun-exposed leaves increased up to 47.5 °C. Under these conditions, $J_{CO2}$ was seriously reduced by the pronounced stomatal closure. Interestingly, leaf conductance at maximum $\Delta w$ (max. $T_{leaf}$ = 47.6 °C) was higher on that hot day than on cooler days (max. $T_{leaf}$ ≤ 43 °C). Maximum transpiration rate increased from 3.57 to 5.9 mmol m$^{-2}$ s$^{-1}$.
This higher transpiration rate significantly contributed to the cooling of the leaf and might have prevented the increase of leaf temperature above a critical point. Heat stress above leaf temperatures of 45°C lead to a decrease in the photosynthetic activity (Fig. 4). Nevertheless, in these heat stressed leaves midday internal CO$_2$ concentration ($c_i$) was nearly 50 ppm higher ($c_i = 253$ ppm) as compared to leaves experiencing normal summer temperatures (Fig. 4B) and $F_v/F_m$ declined to a lower level (Fig. 4D). The mean initial fluorescence rose by 25% from 436 to 537 when leaf temperatures increased from 45°C to 47.6 °C indicating serious heat stress (Fig 4C). Both $F_0$ and $F_v/F_m$ recovered during the afternoon when leaf temperature and radiation declined again (Fig. 4D).

Figure 4: Diurnal courses of leaf temperature ($T_{leaf}$, A), internal CO$_2$ concentration ($c_i$, B), initial fluorescence ($F_o$, C) and maximum quantum yield of PS II ($F_v/F_m$, D) on a normal summer day (squares) and under heat stress (circles).

4. Discussion

Maximum net CO$_2$ exchange rates of Citrus grandis observed in this study under extreme desert conditions corresponded well with values (4 to 12 µmol m$^{-2}$ s$^{-1}$) obtained for for Citrus sinensis and Citrus paradisi under moderate environmental conditions (Singlair & Allen, 1982; Spiegel & Goldschmidt, 1996; Blanke, 2000). Photosynthesis of citrus was saturated at relatively low light intensities around 800 µmol m$^{-2}$ s$^{-1}$ in this study. Low light saturation between 600 to 800 µmol m$^{-2}$ s$^{-1}$ are well known for other Citrus species (Singlair & Allen, 1982; Syvertsen, 1984; Braake & Allen, 1995). This indicates that sun-exposed leaves are exposed to an excess of light during most of sunny cloudless days. This easily explains why maximum CO$_2$ exchange rates did not differ between summer and winter as PPFD were always above the point of light saturation.

A negative influence of $\Delta w$ on the stomatal opening was observed also in this study for well-irrigated citrus trees in the dry summertime as found for other Citrus cultivars (Hall et al., 1975; Kriedemann & Baars, 1981; Braake & Allen, 1995). A midday depression of stomata opening is typical for other well-irrigated fruit trees such as apricots (Prunus armeniaca) and grapes (Vitis vinifera) (Lange & Meyer, 1979). Syvertsen (1982) hypothesized that the direct effect of $\Delta w$ on leaf conductance may explain similar transpiration rates under different environmental conditions. However in
our case, leaf conductance in *Citrus grandis* was similar in winter and summer and transpiration rates were even higher in summer. Thus, it may be assumed that *Citrus* has to reduce the maximum transpiration rates because water uptake and transportation capacity are limited by a low hydraulic conductance (Kriedemann & Baars, 1981; Moreshet et al., 1990). The fact that the midday stomatal closure in the fruit trees is controlled by Δw has an important implication on the irrigation management. Increasing the soil water content by applying a greater irrigation volume will not result in higher leaf conductance and higher CO₂ uptake midday (Lange & Meyer, 1979). As a result of the low transpiration rates leaf cooling was reduced and leaf temperatures exceeded air temperatures up to 11 °C. This overheating of the leaves caused a drastic increase of Δw, thus negatively affecting leaf conductance and CO₂ uptake. If CO₂ uptake was only restricted by stomatal closure than the internal CO₂ concentration should decrease. However, under those high temperatures observed in our study cₗ increased. This clearly indicates photoinhibition and/or other down-regulating systems (Clifford et al., 1997; Herppich et al., 1997). High photon flux density may induce photoinhibition by overexcitation of photosystem II when stomatal closure limited CO₂ supply (Baker 1993). Furthermore, photorespiration may increase under high light intensities and high temperatures thus contributing to the increase of cₗ. In the investigation presented, PSII was not much affected in comparision to the investigations of Werner et al. (1999) and Herppich (2000). Fₐ/Fₘ declined only by 12% even at leaf temperatures above 42 °C. In addition, predawn values of Fₐ/Fₘ were relatively high if compared with other evergreen Mediterranean trees (Werner et al., 1999). Photorespiration provides an effective electron sink and may help prevent excessive reduction of electron transport chain and photoinactivation of electron transport during light stress (Heber et al., 1996).

An increase of photorespiration may explain the low reduction of the maximum photochemical efficiency of the photosystem II in *Citrus* leaves. Heber et al. (1996) showed that photorespiration is a useful physiological way to prevent photoinhibition under stomatal closure.

The dramatic decline of Fₐ/Fₘ and the rise in F₀ which was observed at temperatures above 47°C indicates the inhibition of the photosynthetic apparatus by heat stress (Bilger et al. 1987, Yamane et al., 1997; Herppich et al., 1994). However, as described for other mediterranean trees (Bilger et al., 1987), serious heat stress can be observed above 45°C. In our study, any inhibition was reversible and Fₐ/Fₘ increased to predawn values as leaf temperature decreased during the afternoon. Short term experiments indicated that acclimatization to heat stress may occur. Weis & Berry (1988) showed for bean leaves that a 3-hour pre-adaptation at 40°C caused an increase in the tolerance limit by about 4°C. In potatoes a moderate increase of temperatures in 2.5 hours from 25°C to 38°C caused rapid photosynthetic acclimatization and increased the critical temperature from 38°C to 43°C (Havaux, 1993). Also, growth temperatures may largely influence temperature optimum photosynthesis (Björkman et al., 1980). Under field conditions *Citrus* will be able to adapt to increasing temperature during the growing season. Desert evergreen species, which experience large seasonal changes in temperature show large changes in their thermal stability. The shift in thermal tolerance can be up to 10°C and even higher values have been reported (Berry & Björkman, 1980). Our experiments show for citrus that PSII acclimates to heat stress during the season. Thus, *Citrus grandis* seems to be well adapted to the high photon flux density and heat stress occasionally ocuring in the Arava Valley.

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