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

M. Veste
University of Bielefeld
Faculty of Biology
Department of Ecology
PO Box 100131
33501 Bielefeld
Germany

A. Ben-Gal
Arava Research &
Development Yotvata
MP Eilot 88820
Israel

U. Shani
Hebrew University of Jerusalem
Faculty of Agricultural, Food and
Environmental Quality Sciences
The Seagram Center for Soil and
Water Sciences, PO Box 12
Rehovot 76100, Israel

Additional index words: photosynthesis, photoinhibition, heat stress, temperature

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 AravaValley (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 irradation and stress conditions, which limit photosynthetic energy conversion by reducing CO_2 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, CO_2 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 CO_2 exchange and transpiration of fully expanded leaves were measured with a portable CO_2/H_2O porometer system (HCM-1000, Walz GmbH, Effeltrich, Germany) under ambient conditions according to Veste & Herppich (1995) and von Willert et al. (1995). Ambient CO_2 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 ($F_v/F_m = F_m-F_0/F_m$) was determined on dark-adapted (10 to 12 min) attached leaves (n = 7). F_m, F_v and F_0 denote the maximum, the variable and the initial fluorescence, respectively (cf. von Willert *et al*. 1995). Quantum yield of linear electron transport ($F'_m- F / F'_m = \Phi_{PSII}$) were analysed as summarized by von Willert *et al*. (1995) and electron transport rate (J_e) was calculated according to Krall & Edwards (1992) as $J_e = \Phi_{PSII} * 0.5 * 0.84 * PPFD$ (PPFD = photosynthetic photon flux density).

3. Results

Typical diurnal time courses of net CO_2 exchange (J_{CO2}), transpiration (J_{H2O}), leaf conductance (g_{H2O}) of *Citrus grandis* and the microclimatic conditions on a normal summer and winter day are shown in Fig. 1. Maximum net CO_2 exchange rates of *C. grandis* typically ranged between 8 and 12 $\mu mol\ m^{-2}\ s^{-1}$ throughout the year. Both photosynthetic electron transport (J_e, Fig. 2) and net CO_2 exchange (data not shown) were always light saturated at PPFD above 800 $\mu mol\ m^{-2}\ s^{-1}$. During winter month diurnal courses of leaf conductance, net CO_2 exchange and transpiration were very simimilar, and no midday depression of gas exchange occured (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}.
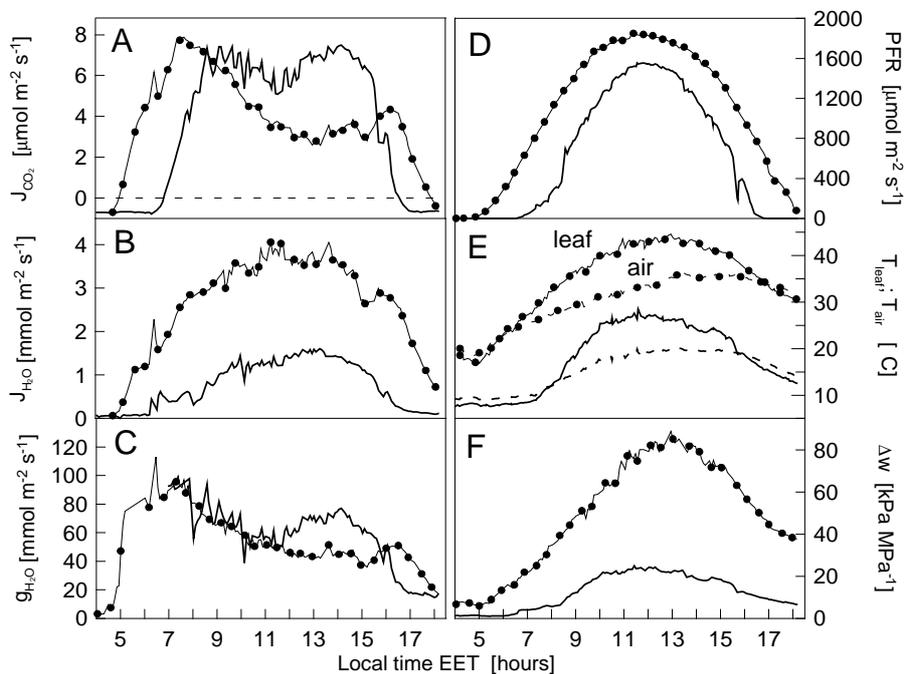

Figure 1: Typical diurnal courses of net CO_2 exchange (J_{CO2}, A), transpiration (J_{H2O}, B), leaf conductance (g_{H2O}, C), photon flux density (PPFD, D), leaf and air temperature (T_{air}, T_{leaf}, E) and air-to-leaf water vapour deficit (Δw, F) in June (circles) and December (solid line).
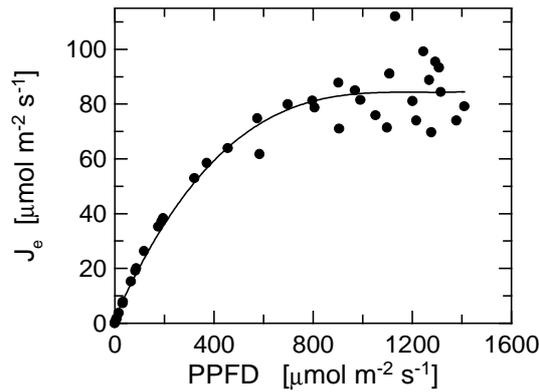
Figure 2: Light response of photosynthetic electron transport (J_e) of *Citrus grandis* in winter.

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, Δw increased to more than 85 kPa MPa^{-1} (Fig. 1). Due to the harsh 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).
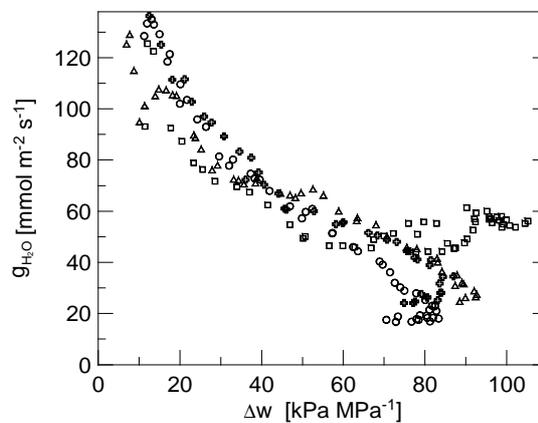

Figure 3: Plot of leaf conductance for water vapour over air-to-leaf water vapour deficit. Given are the results of four summer days (June).

   Air temperatures occasionally exceeded 40 °C. On these occassions 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 Δw (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).
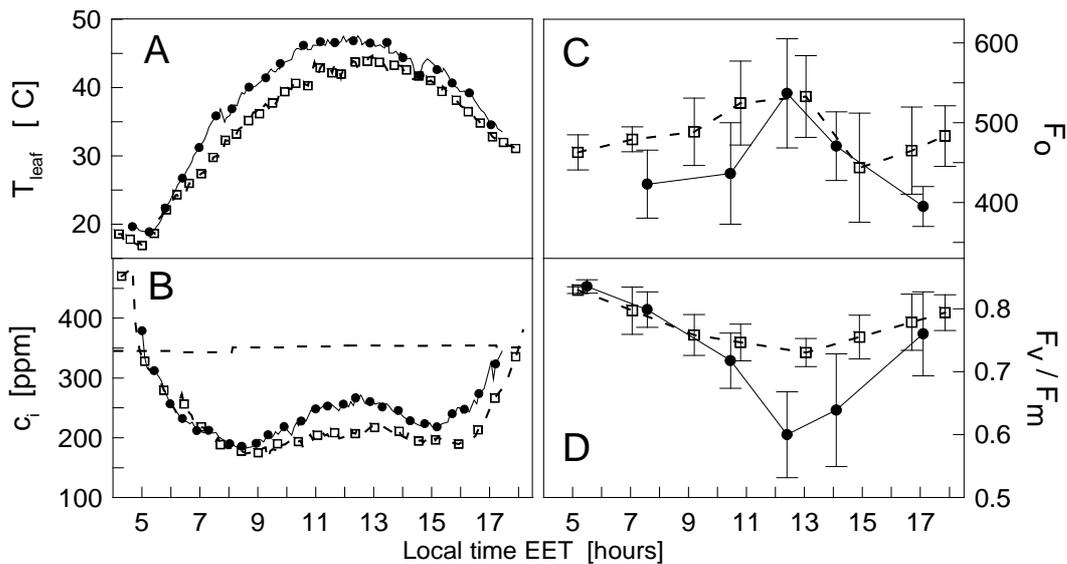

Figure 4: Diurnal courses of leaf temperature (T_{leaf}, A), internal CO_2 concentration (c_i, B), initial fluoresecence (F_o, C) and maximum quantum yield of PS II (F_v/F_m, D) on an normal summer day (squares) and under heat stress (circles).

4.   Discussion

Maximum net CO_2 exchange rates of *Citrus grandis* observed in this study under extreme desert conditions corresponded well with values (4 to12 µmol m^{-2} s^{-1}) obtained for for *Citrus sinesis* and *Citrus paradisci* 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 Δw 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 Δw on leaf conductance may explain similar transpiration rates under different environmental conditions. However in

146

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 CO_2 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 CO_2 uptake. If CO_2 uptake was only restricted by stomatal closure than the internal CO_2 concentration should decrease. However, under those high temperatures observed in our study c_i significantly 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 CO_2 supply (Baker 1993). Furthermore, photorespiration may increase under high light intensities and high temperatures thus contributing to the increase of c_i. In the investigation presented, PSII was not much affected in comparision to the investigations of Werner *et al*. (1999) and Herppich (2000). F_v/F_m declined only by 12% even at leaf temperatures above 42 °C. In addition, predawn values of F_v/F_m 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 F_v/F_m and the rise in F_0 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 F_v/F_m 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 & Björkmann, 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 occuring in the Arava Valley.

References

Baker, N.R., 1993. Light-use efficiency and photoinhibiton of photosynthesis in plant under environmental stress, In: Smith, J.A.C. & Griffiths, H. Water deficits, plant responses from cell to communitiy, Environmental Plant Biology Series, βios Scientific Publisher, Oxford, 221-235

Berry, J.A. & Björkmann, O., 1980. Photosynthetic response and adaptation to temperature in higher plants, Ann. Rev. Plant Physiol. 31: 491-543

Bielorai, H., Levin, I. & Assaf, R., 1973. Irrigation of fruit trees, In: Yaron, B., Danfors, E., Y. Vaadia, Arid Zones Irrigation, Ecological Studies 5, Springer, Stuttgart, 397-404

Bilger, W., Schreiber, U. & Lange, O.L., 1987. Chlorophyll fluorescence as an indicator of heat induced limitation of photosynthesis in *Arbutus unedo* L., In: Tenhunen *et al*. (eds.) Plant Response to Stress, 391-399

Blanke, M. M., 2000. repair mechnisms following photoinhibition in Citrus. Acta Horticulturae No. 531 (this volume): 155-158

Braake, M. & Allen, L.H., 1995. Gas exchange of Citrus seedlings at different temperatures, vapor-pressure deficits, and soil water contents, J. Amer. Soc. Hort. Sci. 120(3): 497-504

Björkman, O., Badger, M.R. & Armond, P.A., 1980. Response and adaptation of photosynthesis to high temperatures, In: Turner, N.C. & Kramer, P.J. Adaptation of plants to water and high temperatures stress, Wiley-Interscience Publication, New York, 233-249

Clifford, S.C., Kadzere, I. Jones, H.J. & Jackson, J.E., 1997. Field comparision of photosynthesis and leaf conductance in *Ziziphus maritiana* and other fruit tree species in Zimbabwe, Trees 11: 449-454

Cohen, S., Mareshet, S., Guillou, L.L., Simon, J.-C. & Cohen, M., 1997. Response of citrus trees to modified radiation regimes in semi-arid conditions, J. Exp.Bot. 48: 35-44

Hall, A.E., Camacho, B.S.E. & Kaufmann, M.R., 1975. Regulation of water loss by citrus leaves. Physiologia Plantarum 33: 62-65

Havaux, M., 1993. Rapid photosynthetic adaptation to heat stress triggered in potato leaves by moderatly elevated temperatures, Plant Cell Environm. 16: 461-467

Heber, U., Bligny, R., Streb, P. & Douce, R., 1996. Photorespiration is essential for the protection of the photosynthetic apparatus of C3 plants against photoinactivation under sunlight, Bot. Acta 109(4): 307-315

Herppich, M., Herppich, W.B. & von Willert, D.J., 1994. Influence of drought, rain and artificial irrigation on photosynthesis, gas exchange and water relations of the fynbos plant *Protea acaulos* (L.) Reich at the end of the dry season. Bot. Acta 107: 369-472.

Herppich, W.B., Flach, B. M.-T., von Willert, D.J. & Herppich, M., 1997. Field investigations of photosynthethtic activity, gas exchange and water potential at different ages in *Welwitschia mirabilis* during a severe drought, Flora 191: 59-66

Herppich, W.B., 2000. Interactive effects of light and drought stress on photosynthetic acitivity and photoinhibition under (sub-) tropical conditions, Acta Horticulturae No. 531 this volume: 137-146

Krall, J.P. & Edwards, G.E., 1992. Relationship between photosystem II activity and CO_2 fixation, Physiologia Plantarum 86: 180-187.

Kriedemann, P.E. & Baars, H.D., 1981. Citrus Orchards, In: Kozlowski, T.T.: Water deficits and plant growth, Academic Press, 325-417

Lange, O.L. & Meyer, A., 1979. Mittäglicher Stomataschluß bei Aprikose *(Prunus armeniaca)* und Wein *(Vitis vinifera)* im Freiland trotz guter Bodenwasser-Versorgung, Flora 168: 511-528

Larcher, W., Wagner, J., Neuner,G., Méndez, M., Jiménez, M.S. & Morales, D. 1991. Thermal limits of photosynthesic function and viability of leaves of *Persea indica* and *Persea americana*, Acta Oecologia 12 (4): 529-541

Moreshet, S., Cohen, Y., Green, G.C., & Fuchs, M., 1990. The partitioning of hydraulic conductances within mature orange trees, J. Exp. Bot. 41: 833-839

Singlair,T. R. & Allen, L.H., 1982. Carbon dioxide and water Vapour Exchange of Leaves on Field-Grown Citrus Trees, J. Exp. Botany 137: 1166-1175.

Spiegel, R. & Goldschmidt, E.E., 1996: Biology of Citrus, Biology of Horticultural Crops, Cambridge University Press, 230 p.

Veste, M. & Herppich, W.B., 1995. Influence of diurnal and seasonal fluctations in atmospheric CO_2 concentration on the net CO_2 exchange of poplar trees, Photosynthetica 31(3): 371-378

von Caemmerer, S. & Farqhuar, G.D., 1981. Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves, Planta 153: 76-387

von Willert, D.J, Mattysek, R. & Herppich, W.B., 1995. Experimentelle Pflanzenökologie, Grundlagen und Anwendungen, Thieme Verlag, Stuttgart

Yamane, Y., Kashino, Y. Koike, H. & Satoh, K., 1997. Increases in the fluorescence F_0 level and reversible inhibition of photosystem II reaction center by high-temperature treatment in higher plants., Photosynth. Res. 52: 57-64

Werner, C., Correia, O. & Beyschlag, W., 1999. Two different strategies of mediterranean machia plants to avoid photoinhibitory damage by excessive radiation levels during summer drought, Acta Oecologica 20 (1): 119-123

Weis, E. & Berry, J.A. 1988. Plants and high temperature stress, In: Long, S.F. & Woodward, F.I., eds., Plants and Temperature, Comp. Biol. Ltd., Cambridge, 329-346