

■ Actually close the avocado stomata during a daytime?

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ABSTRACT

The objective was to investigate the combined effect of irrigation regime and fruit load on the development 'Hass' avocado trees grown in lysimeters, at different phenological periods. The effects of the fruit sink on water uptake and gas-exchange properties were assessed by comparing the performance of fruiting and de-fruited avocado trees.

The role of leaf-carbohydrate concentrations was emphasized by fruit removal. Fruit presence provided a significant sink for carbohydrates, which vanished when they were removed. Rapid accumulation of carbohydrates in leaves of de-fruited trees induced a reduction in stomatal aperture early in the morning, which led to reductions in stomatal conductance (g_s) and photosynthesis per unit leaf area (A) rates in the course of the day, in spite of the increasing irradiance and vapor pressure deficit (VPD). The strong carbohydrates sink formed by the presence of the fruits slowed down carbohydrates accumulation, enabling additional factors, such as irradiance, VPD, and root-zone water regime to affect g_s and A . Higher stomatal aperture in fruiting tree induced a significant increase in water uptake of compared with de-fruited trees and consequently, their daily water uptake was 40% higher than that of de-fruited trees, thus despite the higher vegetative growth of de-fruited trees. The traditional 'KCET0' approach might be insufficient as a basis for proper irrigation management during the productive period and irrigation management should be adjusted therefore for the actual crop load.

Key words: Crop load, Irrigation frequency, *Persea Americana*, Photosynthesis, Stem diameter, Stomatal conductance.

ABBREVIATIONS

A – assimilation rate, photosynthesis per unit leaf area; EC - electrical conductivity; ET_0 - reference evapotranspiration; g_s - stomatal conductance to water vapour; KC – crop coefficient; LD - leaf conductance; LWP - leaf water potential; PPFD - photosynthetic photon flux density; SWP - stem water potential;

INTRODUCTION

The well-established and widely-applied approach to determine crop water needs relies on standard meteorological data and crop coefficients. This approach is widely adopted worldwide and commonly termed the 'KCET0' approach (Allen et al., 2006). In this approach the reference evapotranspiration ET_0 represents the effect of the weather on the water requirements of a reference crop and the crop coefficient KC represents the seasonal dynamics of the crop water requirements relative to the reference crop. The reference evapotranspiration is estimated by various methods being Penman-Monteith the most widely adopted. This method applies to a hypothetical crop of prescribed physical characteristics (height, surface resistance and albedo) basically corresponding to "an extensive surface of green grass of uniform height, actively growing and adequately watered". The crop coefficient KC accounts for the differences in crop canopy and aerodynamic resistance relative to the reference crop. Although it is stated that "The KC coefficient serves as an aggregation of the physical and physiological differences between crops" (FAO 56 report), the 'KCET0' approach favor the physical aspects of the soil-plant-atmosphere system and disregard the physiological part of the problem. The plant is conceptually addressed as a passive system that reacts to the combined effects of soil water availability and the atmospheric demand.

The basic assumption common to all plant-based methods is that the stomatal aperture is determined by the plant water status and by the atmospheric evaporative demand solely. However, such an assumption oversimplifies the complexity of the gas exchange processes at the leaf scale. It is commonly accepted that sink-source relationships frequently affect the photosynthesis process (Goldschmidt and Huber, 1992), and feedbacks from the end-product carbon metabolite have been hypothesized to control the net photosynthesis rate (Paul and Pellny, 2003). It suggests that crop load may affect the thresholds of the various plant-based water stress indicators. Lenz (1986) demonstrated that water uptake of fruiting trees was significantly higher than that of non-fruiting trees grown in lysimeters, despite the considerably lower total leaf area of the former. Defruiting reduced stomatal conductance (g_s), assimilation rate (A), SWP and LWP for various fruit trees, i.e. apple (Wibbe and Blanke, 1995; Reyes et al., 2006), avocado (*Persea americana* Mill.; Schaffer et al., 1987), plum (*Prunus domestica*, L.; Gucci et al., 1991), citrus (*Citrus unshiu* L.; Iglesias et al., 2002), peach (*Prunus persica* L.; DeJong 1986a, b; Naor et al., 1999; *Prunus persica* L.; Li et al., 2005), and coffee (*C. Arabica* L.; DaMatta et al., 2008).

The considerable effect of fruit load on stomatal aperture, SWP, LWP, g_s , and A clearly shows that thresholds of these plant-based indicators might be highly dependent on the current phenological stage of the crop. Therefore, proper seasonal protocol to account for the actual crop load is a major challenge. To address this fundamental question, we examined the response of 'Hass' avocado trees grown in lysimeters to different irrigation regimes, and monitored the plant water uptake at high temporal resolution during the successive growth stages. The effects of these irrigation treatments on tree development and yield have been reported elsewhere (Silber et al., 2012, 2013). The objectives of the present study were to assess the effects of the fruit sink on stomatal aperture and water uptake.

MATERIALS AND METHODS

The experiment was carried out at the Zemach Experimental Station, located in the northern part of the Jordan Valley, in Israel. The climate is Mediterranean, with mild, wet winters and hot, dry summers. Weekly mean daily values of global solar radiation, air temperature, vapour pressure deficit (VPD), and “class-A” pan evaporation from 2007 to 2010 were supplied by the Israeli Meteorological Service, and are detailed elsewhere (Silber et al., 2012). The rainfall season in the region extends from November through April, and annual precipitation at Zemach totalled 303, 237, 356, and 396 mm in 2006-7, 2007-8, 2008-9, and 2009-10, respectively. Detailed information on this experiment can be found in (Silber et al., 2012) and it will be described briefly in the following.

‘Hass’ avocado trees grafted on ‘Degania 117’, a West-Indian avocado rootstock, were planted in September 2006 in 250-L plastic containers (50-cm height × 80 cm diameter) containing 200-L of perlite of 2-mm grain size. The experimental design included three irrigation frequencies replicated five times in randomized blocks, each replicate consisted four trees. Three irrigation treatments were applied: daily pulsed irrigation (10-20 min every 30 min) throughout the daylight (Irg1); one daily irrigation event that started at night and terminated in the morning every day (Irg2); and one irrigation event every two days (Irg3). The daily amounts of water and nutrients were the same in all treatments and it included a leaching fraction of 0.4 that avoided electrical conductivity (EC) values of the draining solution to exceed 1.8 dS m⁻¹. Each container was irrigated via seven 3.5-L h-1 pressure-compensated drippers (Netafim Inc., Israel). The irrigation and fertilization was controlled by a computer. All other horticultural practices were similar in all treatments in accordance with the recommendations of the Israeli Extension Service. On 22 March 2010, all the fruitlets from two pulse-irrigated trees (treatment Irg1) in two different experimental blocks were removed. Two Irg1 trees, one adjacent to each of the defruited trees, in the same experimental blocks and having practically the same canopy area served as controls.

Daily cycles of instantaneous net assimilation rate (A) and stomatal conductance to water vapour (g_s) were measured with a CIRAS-2 portable open-path gas-exchange system (PP Systems, Amesbury, USA). Mature leaves facing the sun at the time of measurement were enclosed in the clear-top leaf chamber, which was supplied with air at ambient temperature and humidity, and containing 380 ppm of CO₂. After reaching stable readings of A and g_s (usually after about 1 min in the chamber) the values were recorded. Measurements were conducted at 2-h intervals, and only on days with clear skies.

On 24 August 2010, immediately after the gas exchange measurements, three mature leaves facing the sun were collected from trees with and without fruits for carbohydrate analysis, at three different times – 06:30, i.e., before sunrise, and consequently before gas exchange could be measured; 10:30, and 12:30. The leaves were excised, inserted into a liquid nitrogen container, and stored in deep freeze (-80°C) pending analysis. Sugars were extracted from leaf samples by re-suspending them in 5 mL of 80% ethanol in an 80°C water bath for 60 min. This procedure was repeated twice. The ethanol solutions were combined and completely evaporated at 40°C under continuous ventilation. The resulting dried sugars were dissolved in 1 mL of distilled water and were stored frozen at -80°C, pending analysis. The amounts of sugars were determined by the Anthrone reagent method, as modified for determination of reducing and non-reducing sugars (Van Handel, 1967, 1968). The ethanol-insoluble residue was used to determine the concentration of starch in the grafted segment of the stem. Starch digestion was carried out by incubating and autoclaving samples with 6 mL of water, adding 4 mL of buffer containing 200 units of amyloglucosidase, and incubating overnight at 55°C (Dinar et al., 1983). The amount of released glucose was determined with Sumner reagent (Sumner, 1921).

Statistical analyses were carried out with JMP(®) 9 software. All data were analysed for the effects of treatments and blocks by means of the general linear model procedure of SAS (SAS Institute, Cary, NC, USA). Differences among means were tested with the standard least squares mode of ANOVA, followed by Tukey’s HSD pairwise comparison of means. Differences with a probability larger than 95% were taken as significant. In all cases, the effect of blocks was not significant, therefore only the treatment effect is presented.

RESULTS

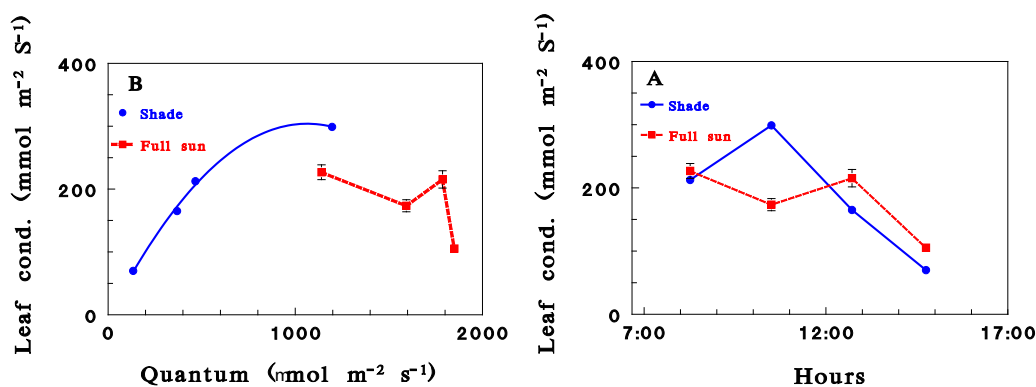


Figure 1. A: Leaf conductance (LD) of sun-facing and partially shaded leaves during a representative day (7 July 2011), non-bearing trees. B: Relationships between LD and light intensity photosynthetic photon flux density (PPFD)

The differing irrigation managements led to significant differences in medium moisture content, plant water uptake and accordingly, vegetative growth (Silber et al., 2012). The Diurnal variations of g_s and A reveal an apparent anomaly. Diurnal variations in irrigation treatments induced significant differences in water availability (data not presented) and, correspondingly, in water uptake (data not presented) but, nevertheless, none of the measurements of leaf g_s and A conducted during the two vegetative years responded either to irrigation treatments or to diurnal meteorological variations (Silber et al., 2013). However, a regular tree bears a mixture of leaves facing the sun in the external surface of its canopy and partially shaded leaves in the interior. To explore the possibility that the g_s of sun-facing leaves does not correlate properly the whole tree conductance, diurnal measurement of g_s of shaded and sun-facing leaves was performed on 7 July 2011 on trees bearing no fruit in treatment Irg1 (Fig. 1). Shaded leaves g_s was much higher than sun-facing leaves in late morning where it seems that the maximal g_s occurs at irradiance of $\sim 1000 \text{ mmole.m}^{-2}\text{s}^{-1}$ in the PAR wave. Measured values of g_s of leaves facing the sun exhibited a similar pattern to those presented for vegetative years, i.e., highest values in the morning (08:15), with subsequent decline (Fig. 1A), irrespective of the light intensity or climatic components such as global irradiance and VPD. Unlike leaves facing the sun, g_s values measured in partially shaded leaves were closely correlated with the photosynthetic photon flux density (PPFD), and a significant quadratic regression ($r^2 = 0.99$) was obtained between g_s and the PPFD (Fig. 1B). Note that under similar light intensity the g_s values of shaded leaves exceeded those of sun-facing leaves. Thus, the diurnal variation of stomatal conductance of sun-facing leaves does not necessarily reflect that which could characterize the mean value over all the leaves.

Diurnal variation of g_s and A during the flowering and the fruit-set periods (March until mid-May 2009 and 2010) resembled the patterns for the vegetative periods, i.e., small effects of irrigation managements and meteorological conditions (Silber et al., 2013). Interestingly, this trend changed as fruit growth became significant (end of May until mid-July), with the effects of irrigation treatments and climatic conditions becoming more pronounced (data not presented).

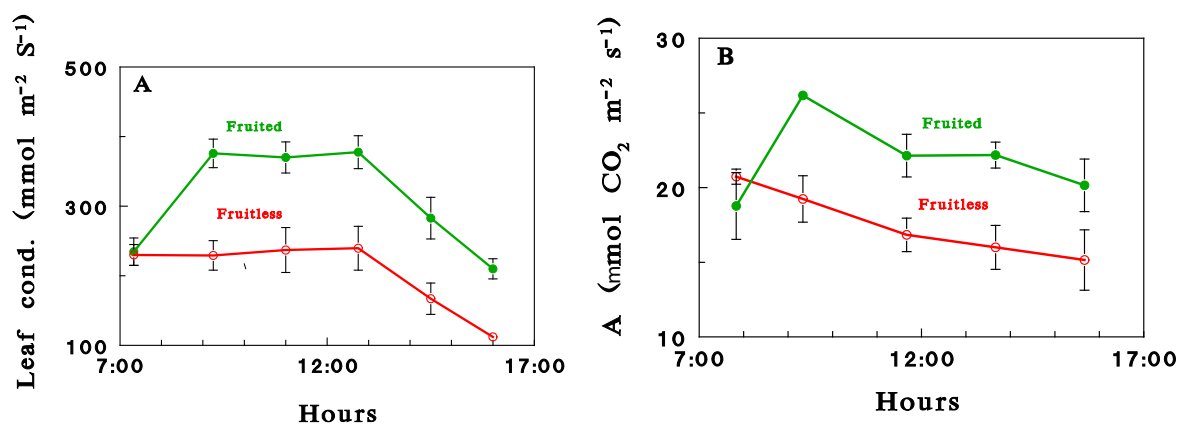


Figure 2. Effect of fruit removal during 30 May and 6 July 2010 on: A - The daily cycle of leaf conductance (g_s). B - Photosynthesis (A). Symbols represent experimental data \pm SE (not shown when smaller than the symbol).

On 22 March 2010 all fruits were removed from two trees in two experimental blocks of the pulsed irrigation treatment (Irg1). Fruit removal immediately induced a flush of trunk growth, which subsequently slowed down as flushes of new leaves appeared. The flushes of new leaf growth and trunk growth were alternated (practically no trunk growth when shoot growth occurs), similar to earlier report on alternated shoot and root growth in avocado (Ploetz et al., 1991). The dominant feedback between fruit presence and stomatal aperture, expressed in the measured g_s and A values (Silber et al., 2013), was confirmed by fruit removal: de-fruited diminished g_s and A , and also significantly altered their diurnal cycles (Fig. 2A, B). The daily cycles of g_s and A in de-fruited trees resembled the typical pattern of the vegetative periods or of the flowering period in the reproductive years (Silber et al., 2013), i.e., monotonic decrease from highest values in the morning, and weak influence of meteorological conditions (Fig. 2A, B). In fruiting trees A reached its highest value around 10:00 and then decreased slightly until 16:00, whereas the behavior of g_s was similar to that in fruited trees.

An additional aspect of the presence of fruits is its impact on leaf carbohydrate content: lower photosynthesis rates in the de-fruited trees (Figs 2B, 3) were associated with higher leaf carbohydrate concentrations (Fig. 4), which seems to be the trigger for the stomata closure in defruited trees (Gucci et al., 1991; Goldschmidt and Huber, 1992; Fan et al, 2010; Iglesias et al., 2002, Paul and Pellny, 2003). Leaf-carbohydrate concentrations in trees with and without fruits were at their lowest before sunrise and increased during the day, but their diurnal patterns differed: in leaves of defruited trees the concentration increased steeply and reached a maximum around 10.00, whereas in fruiting trees it increased monotonically until noon (Fig.4).

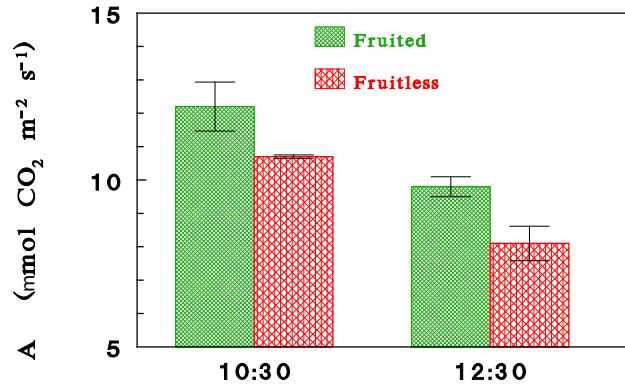


Figure 3. The net photosynthesis rate (A) of fruiting and de-fruited tree leaves on 24 August 2010

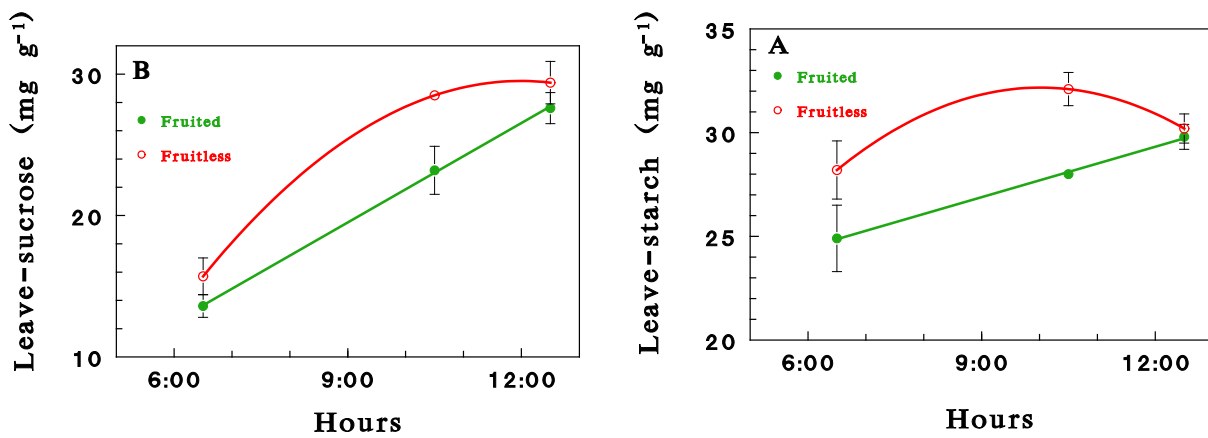


Figure 4. Effects of fruit removal on carbohydrates content on 24 August 2010: A – Leaf-starch. B – Leaf – sucrose. Symbols represent experimental data ± SE (not shown when smaller than the symbol).

The presence of fruits on trees affected their water uptake also: despite the higher vegetative growth of defruited trees (data not presented), their daily water uptake was 40% lower than that of fruiting trees (Fig. 5), in accordance with Lenz (1986) report for apple.

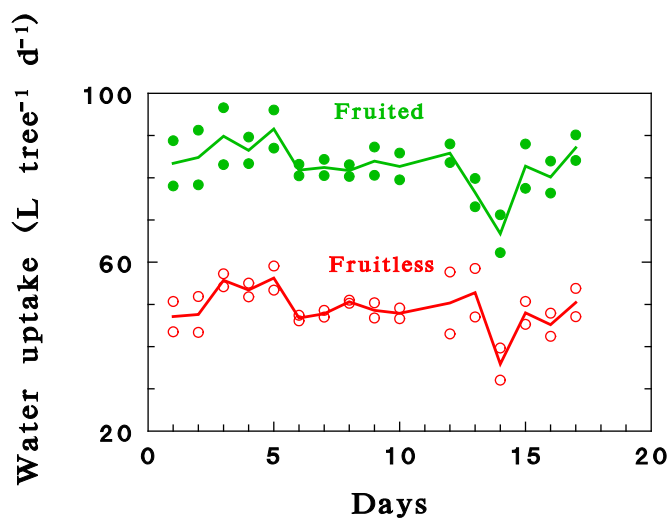


Figure 5. Daily water uptake of fruited and de-fruited trees during 17 days in July 2010.

DISCUSSION

Stomatal aperture in plants is controlled by complex biochemical processes involving enzymes, leaf-carbohydrate concentrations, water and nutrient availability, and weather conditions (Goldschmidt and Huber, 1992; Hetherington and Woodward, 2003; Paul and Pellny, 2003). The present findings clearly show that leaf-carbohydrate may play an important role in the complex framework of stomata aperture. The role of leaf-carbohydrate concentrations was emphasized by fruit removal. Fruit presence provided a significant sink for carbohydrates, which vanished when they were removed. The rapid accumulation of starch and sucrose in leaves of defruited trees (Fig. 2) induced a reduction in stomatal aperture early in the morning, which led to reductions in g_s values and A rates in the course of the day in spite of the increasing irradiance and VPD. The strong carbohydrates sink formed by the presence of the fruits slowed down carbohydrates accumulation, enabling additional factors, such as irradiance, VPD, and root-zone water regime to affect g_s and A . This conclusion is supported by the data presented in Fig. 1: partial shading of leaves possibly reduced the photosynthesis rates and, consequently, carbohydrate accumulation. It is likely, therefore, that the carbohydrate content of the partially shaded leaves was low and, therefore, did not limit their stomatal apertures as in the case of sun-facing leaves. Consequently, light intensity affected the g_s of shaded leaves much more significantly than that of sun-facing leaves (Fig. 1).

Irrigation treatments in the present study hardly affected vegetative growth, flowering intensity, or fruit setting (Silber et al., 2012). Nevertheless, the extent of fruitlet abscission in June (June drop) was considerably affected by the irrigation treatments and consequently, relative to the yield of Irg1 the irrigation treatments induced a decrease of 42 and 82% in numbers of harvested fruits in Irg2 and Irg3, respectively (Silber et al. 2012). It is likely that the very rapid fruit growth during this period created a high demand for carbohydrates (Whiley and Wolstenholme, 1990; Wolstenholme, 1981, Wolstenholme and Whiley, 1997). Consequently, the combination of water stress with insufficient carbohydrates supply to the developing fruits caused the severe fruitlet abscission. From the middle of July (i.e., in growth stage II; Silber et al., 2012), the rate of fruit growth diminished and, probably, so did the demand for carbohydrates, therefore, fruit sensitivity to stress events was also reduced.

The higher g_s in fruiting trees that resulted from their lower carbohydrate concentration increased their water uptake by ~60% compared with de-fruited trees (Fig. 5). This could have an important effect on irrigation scheduling, which traditionally has been based on weather parameters and crop factors such as canopy size, leaf area index and leaf resistance (Allen et al., 1998; Howell and Meron, 2007). In terms of the traditional 'KCETO' approach, flow in plants is inherently conceptualized as a passive process of water from the soil to the atmosphere. The notable effect of fruit presence on water uptake (Fig. 5) clearly demonstrates the limits of such approach and emphasized the role of the active component in the process which is related to plant physiological state. Irrigation management during the productive period should be adjusted therefore for the actual crop load (Naor, 2006).

CONCLUSIONS

Accumulation of leaf-carbohydrates in the absence of strong sink during the vegetative period or in de-fruited trees plausibly induced a reduction in stomata aperture early in the morning, in spite of the increasing irradiance and VPD.

The higher stomatal aperture in fruiting tree induced a significant increase in water uptake of compared with de-fruited trees. This could have an important effect on irrigation scheduling.

The traditional 'KCETO' approach might be insufficient as a basis for proper irrigation management during the productive period and irrigation management should be adjusted therefore for the actual crop load.

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