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Fruit load governs transpiration of olive trees

AMNON BUSTAN[1,2], ARNON DAG[1], URI YERMIYAHU[1], RAN EREL[1], EUGENE PRESNOV[1], NURIT AGAM[3], DILIA KOOL[1], JOOST IWEMA [1,4,5], ISAAC ZIPORI[1], AND ALON BEN-GAL[1*]

[1]Gilat Research Center, Agricultural Research Organization, mobile post Negev 85280, Israel.
[2]Desert Agro-Research Center, Ramat-Negev R&D, D.N. Halutza 85515, Israel
[3] Jacob Blaustein Institutes for Desert Research, Ben-Gurion University of the Negev, 84990, Israel
[4] Soil Physics, Ecohydrology and Groundwater Management, Wageningen University, The Netherlands.
[5]Current affiliation: Department of Civil Engineering, University of Bristol, UK.

*corresponding: A Ben-Gal bengal@volcani.agri.gov.il, tel: 972 89928644, fax: 972 89926485

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Abbreviations: CER, carbon exchange rate; DOY, day of year; EFR, early-season fruit removal; ET, evapotranspiration; ETR, electron transport rate; HY, high yielding; LFR, late-season fruit removal; LY, low yielding; MFR, mid-season fruit removal; OLY, originally low yielding; STWP, midday stem water potential; SPWC, specific water consumption; TCSA, trunk cross sectional area.

Abstract

We tested the hypothesis that whole-tree water consumption of olives is fruit load dependent and investigated driving physiological mechanisms. Fruit load was manipulated in mature olives grown in weighing-drainage lysimeters. Fruit was thinned or entirely removed from trees at three separate stages of growth; early, mid and late in the season. Tree scale transpiration, calculated from lysimeter water balance, was found to be a function fruit load, canopy size, and weather conditions. Fruit removal caused an immediate decline in water consumption, measured as whole-plant transpiration normalized to tree size, which persisted until the end of the season. The later the execution of fruit removal, the greater was the response. The amount of water transpired by a fruit-loaded tree was found to be roughly 30% greater than that of an equivalent low- or non-yielding tree. The tree-scale response to fruit was reflected in stem water potential but was not mirrored in leaf-scale physiological measurements of stomatal conductance or photosynthesis. Trees with low or no fruit load had higher vegetative growth rates. However, no significant difference was observed in the overall aboveground dry biomass among groups, when fruit was included. This case, where carbon sources and sinks were both not limiting, suggests that the role of fruit on water consumption involves signaling and alterations in hydraulic properties of vascular tissues and tree organs.

Introduction

It is largely accepted and understood that the presence of fruit on plants influences source-sink carbon relationships and actively or passively affects water status and water consumption (Naor, 2014, Sade and Moshelion 2014). That said, quantification of how water consumption or water requirements are altered by fruit presence or fruit load has rarely been addressed (Guichard et al. 2005). Olive (*Olea europaea* L.) production has historical importance throughout the Mediterranean, where olive oil is a fundamental component of the regional diet (Serra-Majem et al. 2003). Traditionally, olives are not irrigated; however, in recent decades, water application has become recognized as being constructive and effective (Lavee 2011). Under typical Mediterranean climatic conditions (hot and dry summers), irrigation can enhance olive fruit and oil yields by as much as fourfold (Lavee et al. 1990, Grattan et al. 2006, Moriana et al. 2003).

Water is a limited resource in much of the Mediterranean basin as well as in newer regions of olive cultivation. Therefore, substantial efforts are made to optimize fruit and oil production by manipulating quantity and regime of irrigation water supply (Iniesta et al. 2009). However, understanding of olive tree water status and strategies for orchard water management typically ignore key intrinsic processes related to fruit development and oil accumulation that possibly lead to fruit load effects on water requirements. The olive is well adapted to the Mediterranean climate (Connor 2005), where seasonal phenological-physiological requirements for photosynthates and for water coincide with typical prevalent summertime drought-related environmental stresses. Having also a strong tendency for biannual bearing (Lavee 2006), the olive represents a particularly interesting case for the study of fruit load – water status and consumption interactions.

The seasonal reproductive process in fruit trees becomes the plant's dominant carbon sink, particularly in modern heavily-yielding orchards. Carbon demand has been found to spike during bloom (Bustan and Goldschmidt 1998) and, when an ample number of fruit is set almost simultaneously, carbon source limitation can cause significant fruit drop (Zucconi et al. 1978, Rapoport and Rallo 1991, Rivas et al. 2006). After retardation of fruit abscission mechanisms (Huberman et al. 1983, Castillo-Llanque and Rapoport 2009) and the final establishment of the ultimate number of fruit on a tree, the fruit which first rapidly grow and, in olives, consequentially accumulate substantial amounts of oil, present an increasing demand for carbohydrates (Bustan et al. 2011). These carbon demands can be met by enhanced utilization of stored carbohydrate reserves. In deciduous fruit trees, the early stages of reproductive growth and development rely on the remobilization of stored carbon (Körner 2003). In alternate bearing citrus cultivars, the concentration of non-structural carbohydrates may undergo extreme fluctuations due to differences in fruit load between years (Goldschmidt and Golomb 1982). In olive, in spite of a significant tendency to alternate bearing, the role of stored carbohydrates supporting the developing crop is less pronounced (Bustan et al. 2011).

An expansion of the foliage area, essentially increasing photo-assimilation capacity, can theoretically assist to bridge the carbon gap brought on by a heavy fruit load. However, concurrent vegetative growth is substantially inhibited by the developing fruit in many species of fruit trees. Particularly in olives, vegetative and reproductive growth seldom occur simultaneously (Lavee 2006, Dag et al. 2010). Thus, coping with the carbon challenge apparently involves a significant increase in daily primary production by either raising the carbon exchange rate (CER) or by expanding time of stomatal opening and gas exchange processes. Carbon source limitation has been suggested as the prevalent situation (Muller et al. 2011), in which CER is consistently maintained at the maximum level allowed by environmental factors such as solar irradiation, temperature, and humidity. Alternatively, assuming that sink limitations control carbon assimilation, CER would be up-regulated when sink demands increase and down-regulated when the demands decline. While most of the studies addressing fruit load effects on photosynthesis showed significant reduction in CER following fruit removal (Avery 1975, DeJong 1986, Berman and DeJong 1996, Naor et al. 1997, Syvertsen et al. 2003, Wünsche and Ferguson 2005, Haouari et al. 2013, Silber et al. 2013a), up-regulated CER by rising sink demands is difficult to prove. It may be postulated that, as long as sufficient sink demands are maintained in a tree, carbon supply would be limited by the current source capacity. However, declining sink demands might limit CER through feedback inhibition mechanisms (Gifford and Evans 1981). While some authors attributed CER decline to metabolic feedback inhibition by carbohydrate species accumulating in the source leaf (Goldschmidt and Huber 1996, Syvertsen et al. 2003, Silber et al. 2013a), others pointed to direct or indirect effects on stomatal conductance (g_s) (DeJong 1986, Naor et al. 1997, Martín-Vertador et al. 2011a, Silber et al. 2013b). If stomatal regulation is involved, reduced water consumption may be a natural consequence of decreasing g_s (Martín-Vertador et al. 2011b). The question whether trees are also capable (and by what means) of an opposite course - enhancing CER and water uptake in response to the intensity of their reproductive phase, remains open.

Crop water requirements are typically determined according to the 'K_CET_0' approach (Allen et al. 1998), relying on standard meteorological data and crop coefficients. The plant is conceptually addressed as a system passively responding to the combined effects of soil water availability and the atmospheric demand. Fruit load is known to significantly affect water status in many fruit tree species (Naor 2006, Intrigliolo and Castel 2007, Conejero et al. 2010, Silber et al. 2013b) but is not considered a factor in evaluating crop water requirements. Since negligible amounts of water are transpired or taken up by fruit compared to leaves, indirect explanations of fruit effects on water status and possible influences on water requirements are therefore necessary. One explanation is the ability of a species to move along an isohydric/anisohydric scale (Klein 2014), either in terms of the above mentioned consequences of increasing demands for carbohydrates or associated with mechanisms augmenting water availability to developing organs. Sade and Moshelion (2014) postulated that the presence of fruit might shift plants from isohydric to anisohydric stomatal behavior.

The majority of the experimental work to determine tree water requirements has been carried out under field conditions, where plant water uptake cannot be measured directly. In field experiments, indirect parameters such as stem or trunk diameter variations, stem water potential (STWP), g_s, or sap flow, are used as indicators of water consumption. In light of the complexity and difficulty in translating data from such parameters into quantified water consumption a direct holistic approach would seem more appropriate. In spite of inherent differences from field-grown trees due to innate boundary conditions, lysimeter-grown trees provide a unique opportunity to directly, accurately, and reliably complete the water balance and directly measure plant water consumption during successive growth stages along seasons and years (Ben-Gal et al. 2010, Agam et al. 2013, Silber et al. 2013a). We hypothesized that quantitative whole tree water consumption of olives is fruit load dependent. The objectives of the study were to test this hypothesis by a) directly and continuously determining the effects of fruit load on olive tree water consumption and; b) investigating the driving physiological mechanisms causing these effects.

Materials and methods

Lysimeters and water balance

Single 4-year old ‘Barnea’ olive trees were grown in fifteen 2.5 m^3 volume free-standing lysimeters at the Gilat Research Center in the northwestern Negev, Israel (31°20' N, 34°40' E) (Ben-Gal et al. 2010). Each lysimeter consisted of a polyethylene container (1.4 m high X 1.5 m diameter) filled with loamy sand soil, a bottom layer of highly conductive porous rockwool media in contact with the soil, and drainage piping filled with the rockwool extending downward from the lysimeter bottom. The rockwool drainage extension (Ben-Gal and Shani 2002) disallowed saturation at the lower soil boundary while permitting water to move out of the soil and be collected. The trees in lysimeters were automatically provided water and fertilizer and drainage water was automatically collected (Tripler et al. 2007). Each lysimeter's soil surface was covered by a water permeable geotextile (Non-Woven Geotextile, 500 g· m^{-2}, Noam-Urim, Israel) to minimize evaporation losses. The lysimeters were placed every 2.5 m, four to a row in four rows with 4 m spacing and were surrounded by border trees. The second lysimeter in the second row was treeless. Each individual lysimeter was positioned on a square weighing platform with load cells situated in each corner. By distributing load cell output current only over the relevant range of interest (4 to 5 tons) a resulting resolution of ± 15.5 g was reached. Evapotranspiration (ET) was calculated daily according to: $ET = I – D – \Delta W$; where I is irrigation (pre-determined), D is drainage (measured) and ΔW is change in soil water (derived from the change in lysimeter mass). There was no rainfall during the experimental period. The trees were irrigated daily, with quantities exceeding (by ~20%) the previous day's transpiration rates as calculated from the weight data of the lysimeters. In order to evaluate whether fruit load would particularly affect plant water status during times of water stress, all the trees were subjected to short term controlled moderate drought three times during the experimental period. Drought was induced by reducing irrigation to half of the previous day's measured ET. Drought periods were DOY 164-167 (13-15 June), DOY 207-209 (26-28 July) and DOY 262-264 (Sep 19-21). Nutrients were added to the irrigation solution as liquid commercial 7:3:7 ($N:P_2O_5:K_2O$) fertilizer (Fertilizers and Chemicals LTD, Israel) at a continuous concentration in irrigation solution of 50 ppm N.

Manipulations of fruit load

All trees received identical treatment from planting in June 2008 until the beginning of the current experiment (Spring 2011). At bloom, trees were randomly designated to five groups replicated three times: control; early (23-May, DOY 141, just after fruit set) fruit removal; early fruit thinning (also on 23-May, DOY 141, every second fruit); mid-season (7-Jul, DOY 186) fruit removal, during pit hardening; and late-season (7-Sep, DOY 248) fruit removal, during oil accumulation. Fruit thinning and removal were carried out manually and the fruit were weighed and counted for each tree. Final fruit harvest of control and thinned trees took place on 31-Oct, DOY 304. Subsequent to removal of all fruit, when the actual load of each tree became clear, the trees were retroactively regrouped according to status of fruit load. A summary of fruit load per tree throughout the experiment is given in Table 1. Trees initially carrying more than 10,000 fruits (12 trees) were considered high-yielders (HY), while trees with initially less than 10,000 fruits (3 trees) were termed as originally low-yielders (OLY). In each event of fruit load manipulation, trees were discarded from the HY group and designated to the early- (DOY 141), mid- (DOY 186), or late-season (DOY 248) fruit removal groups (EFR, MFR, and LFR groups, respectively). Some manipulated trees remained fruitless within the OLY group, or remained within the HY group, as fruit thinning was insufficient to send them below the threshold of 10,000 fruits per tree. Thus, the HY group decreased gradually from 12 to 4 trees at harvest, while the OLY, EFR, MFR, and LFR groups consisted of 3, 3, 2, and 3 trees, respectively (Table 1).

In further analyses of the results, trees were designated to only two groups, HY and LY, according to their current fruit load status (above and below 10,000 fruit per tree) at each of the four phases of the experiment along the season: I (DOY 100-140); II (DOY 141-185); III (DOY 186-247); and IV (DOY 248-304). Consequently, while the HY group decreased accordingly from 12 to 4 trees as described, the number of trees of the LY group gradually increased from 3 to 11 at the end of the experiment (Table 1).

Vegetative growth

Trunk cross sectional area was calculated using periodical measurement of trunk circumference. Circumference was measured at a marked point on the trunk approximately 50 cm above the soil. At the end of the experiment, after final harvest of fruit, trees were removed from the lysimeters, separated into leaves, branches, limbs and trunk, dried at 70^{o}C and weighed. Above ground biomass was measured and leaf area was calculated using a portable leaf area meter (Li-Cor Li-3000, NE, USA).

Physiology and water status

Measurements were conducted on stems and leaves 0.5-1.5 meters above the soil surface. Mid-day stem water potential (STWP) was measured weekly around solar noon, as described by Shackel et al. (1997) on single shoot terminal sections with 6-7 leaves covered at least 2 hours in advance by sealed aluminum-plastic bags. Shoot sections were taken from the northern (shaded) side of the trees' canopies. Gas exchange, stomatal conductance and fluorescence-based measurements were taken every 2-3 weeks around solar noon, on young but fully grown leaves between 5 and 20 cm from the shoot tip. For each tree, 5 replicate leaves, uniformly distributed over sun exposed canopy, were measured. Carbon exchange, stomatal conductance and electron transport rate (ETR) were measured with a portable gas exchange system (LI-6400, LI-COR Biosciences Lincoln, NE, USA). The chamber was set to mimic outside conditions. The mid-day physiological measurements were conducted between 12:30 and 13:30. On 4 August 2011, diurnal (predawn till sunset) patterns were evaluated as each of the physiological parameters was measured once an hour.

Data analysis

Relationships between leaf area and biomass to trunk cross sectional area and of water consumption to number of fruits per tree were tested using SigmaPlot (Systat Software, San Jose, CA). Linear regression lines were fitted to data. Effect of treatments on measurements of STWP and leaf scale carbon exchange, conductance and ETR was analyzed by one-way ANOVA (Tukey–Kramer multiple comparisons test) using JMP statistical software (SAS Institute, Cary, NC).

Results

Effect of fruit load on tree specific water consumption (SPWC)

Comparative analysis of net water consumption of each individual tree confirmed substantial variability among trees having similar fruit load, attributed to significant differences in canopy size (leaf area). Evaluation of results and effects of treatments therefore required methods for normalization of the data. The aboveground dry biomass of each tree was determined a month after final fruit harvest (Table 2). Trunk cross sectional area (TCSA) was calculated from the periodical measurement of trunk circumference throughout the reproductive season. A strong linear correlation was found between final TCSA and both the final aboveground dry biomass and the calculated total leaf area (Fig. 1). Thus, the recurrent TCSA measured on individual trees along the season was employed as a tree-size normalizing factor for water consumption, giving rise to the parameter of specific water consumption (SPWC), quantified as liters per TCSA (cm^2) per tree per day.

Figure 2 shows the average daily SPWC of individual trees during each of four experimental periods of the season. The basal SPWC, given by trees with no or low fruit loads increased with time, was indicated by the movement of the interception point upward from less than 0.4 at the beginning of the season to about 0.63 $L\ cm^{-2}\ d^{-1}$ at its end. Between bloom and final fruit set (100-140 DOY), SPWC was irresponsive to fruit load. During the second period (until 185 DOY), the weak increase of SPWC was hardly significant. From that point on, however, two distinct groups of trees were clearly distinguished by differing SPWC; high yielding trees had characteristically high SPWC, while low-yielding and defruited trees had lower SPWC values. Once defruited, trees moved from the higher to the lower SPWC group. The influence of fruit load on SPWC increased gradually along the season, as indicated by the significantly steeper slope of the correlation curve during periods III and IV (186-250, and 251-304 DOY, respectively) (Fig. 2).

Figure 3a presents full-season patterns of SPWC of the five groups of trees, sorted according to manipulations of their fruit yield. The HY trees with more than 10,000 fruit per tree, consistently displayed the highest SPWC. The OLY trees, with less than 10,000 fruit from the beginning, had significantly lower SPWC values quite early in the season and remained relatively low until the end. Early removal of fruit just after final fruit set differentiated this group from the

HY and sent it to the lowest SPWC level. The SPWC of EFR dropped by about 15-20% below its original HY group, and remained 5% below that of OLY trees (Fig. 3b). The effect of the mid-season fruit removal was more significant, causing an immediate drop of SPWC, again splitting the MFR trees from HY and causing them to replace the EFR trees as the group with the lowest SPWC. Within a week after fruit removal, the SPWC of the MFR trees dropped to 25% below HY. Their SPWC then fluctuated within a range of 25-40% below the HY trees and 10-25% below the OLY trees until harvest. The latest fruit removal also reduced SPWC rapidly and significantly below those of the HY and OLY groups. After harvest, SPWC of the high-yielding trees dropped steeply to converge with those of the other trees. Thus, extensive fruit thinning or defruiting was always associated with an immediate substantial decline in tree water consumption and its stabilization at a new, significantly lower level thereafter.

Direct measurements of leaf level physiology

Leaf activity, including carbon exchange rate (CER), stomatal water conductivity (g_s), and electron transport rate (ETR) fluctuated considerably, and responded with lower values during periods of water shortage. On an individual tree basis, fruit removal or thinning at any timing or severity, was not accompanied by significant changes in leaf activity, measured several days or weeks afterward. Diurnal hourly measurements, aimed at elucidating possible differences in the duration of leaf activity due to alteration of source-sink relationships, did not reveal any significant differences due to fruit level or removal (data not shown). The clustering of trees by their current fruit load and SPWC (Fig. 2) suggested that retrospective regrouping of the trees according to their up to date number of fruit, might provide a more consistent view. Clustering the trees by their current fruit number into high and low yielding categories (HY and LY, respectively), revealed a slight, seldom significant, tendency of higher CER, g_s, and ETR in HY trees between July and the final fruit harvest (Fig. 4).

Water potential

The retrospective regrouping approach was employed also to the weekly measurements of mid-day STWP. During most of the reproductive season, HY trees displayed lower STWP values compared to LY trees (Fig. 5). Nevertheless, STWP fluctuated considerably between measurements, and significant differences occurred more consistently only towards the end of season.

Fruit load and vegetative growth

TCSA was employed as an indicator for the vegetative growth of the whole tree during the season. Growth rate of HY trees was significantly lower than that of LY trees only during the third study period (186-250 DOY) (Fig. 6a). This observation was further confirmed using the periodic relative growth rate (RGR) of TCSA (Fig. 6b). This more definitive parameter, calculated as percent of growth added per tree per period and unaffected by initial differences in the absolute dimensions of the trunk, decreased significantly in the HY trees from about 0.11 during the first experimental period (90-141 DOY) to less than 0.055 during the third period, while the reduction in the LY trees was appreciably smaller. Noteworthy is the recovery of this parameter to about 0.12 during the fourth period (251-304 DOY), among both groups of trees.

The partition of dry matter between the major aboveground organs was examined about a month after harvest. HY and LFR trees had significantly less dry trunk and limb biomass, in comparison to LY, EFR, and MFR trees (Table 2). No significant differences occurred in the dry biomass of branches and leaves. The overall vegetative aboveground biomass was significantly greater for the LY, EFR, and MFR trees. However, no significant difference was observed in the overall aboveground dry biomass among groups, when fruit was included. A clear trade-off between fruit and vegetative growth was evident. At low fruit load or following fruit removal, vegetative growth, mainly of limbs and trunk, was stimulated. Note that under the condition of non-limiting water supply characterizing most of the present study, all trees maintained continuous growth of leaves and branches throughout the season.

Discussion

There is increasing evidence for the influence of developing fruit on the water status and water requirement of trees (Ben-Gal et al. 2011, Martín-Vertedor et al. 2011a, b, Naor 2014, Sade and Moshelion 2014). This has mostly been established from indirect measurements under orchard conditions, where restricted water availability surely plays a role in water allocation between various organs and in competition between vegetative and reproductive processes. In the present study, the challenging conditions of water shortage were primarily avoided by applying water daily such that climatic and leaching requirements were satisfied and secondarily manipulated with short-term controlled drought events.

The results of the present study confirm that the dominant parameter determining tree-scale water consumption is canopy (tree) size or leaf area. Initial variability in the size of the trees in the study, in spite of their identical histories, made normalization of this parameter necessary prior to investigation of the effect of fruit load. The TCSA parameter was found to correlate very well with tree and canopy biomass and leaf area index at the end of the experiment (Fig 1). The TCSA, easily determined using lysimeters, quantitatively represents a tree's transpiring canopy and allows analysis of dynamic water consumption independent of tree size reflecting only climate and plant physiological factors.

Atmospheric demand played the most important role in changes in SPWC seen over the season. Measured daily SPWC more than doubled between winter and summer (Fig. 3). Since the atmospheric demand was common to all the trees, concurrent differences in SPWC between trees must be due to differential physiological response. Unequivocally, the presence of developing fruit induced significantly greater tree-scale water consumption. This influence was not present at the beginning of the season, from flowering until final fruit set, became subsequently observable, and became stronger with the progress of fruit growth and development. From DOY 185, during the periods of intensive fruit growth and oil accumulation, a clear segregation occurred between trees displaying low and high SPWC, directly corresponding to low and high fruit loads, respectively (Fig. 2). Sudden removal of fruit brought about an immediate decline in tree water consumption, which persisted until the end of the season. The later the fruit removal was executed, the greater was the response (Fig. 3), indicating that factors such as fruit size or stage of development may specifically influence the governing of tree water consumption. The amount of water transpired by a fruit loaded tree was found to be roughly 30% greater than that of a low- or non-yielding tree. While solid physiological indications exist to support hypotheses regarding the influence of fruit on the tree water status (Naor et al. 1997, Tognetti et al. 2004, Trentacoste et al. 2010, Naor et al. 2013, Silber et al. 2013a), to the best of our knowledge, the direct quantitative evidence presented in the current study regarding water use of fruit trees is novel.

In olives, developing fruit are known to inhibit concurrent vegetative growth (Lavee 2006). Under field conditions, fruit removal promoted subsequent vegetative growth, unless executed later than pit-hardening (Dag et al. 2010). In the present study, vegetative growth was constitutive along the season, probably due to the relative young age of the trees and the non-limiting water supply. However, considerable trade-off between fruit load and vegetative development did occur, expressed by significantly greater growth rate of TCSA (Fig. 6) and by the larger dry biomass of the limbs (Table 2) among low-yielding trees. This trade-off is likely even more pronounced in commercial orchards, where, in spite of prevailing water restrictions, common irrigation practices seldom consider fruit load level. Under a uniform irrigation practice, high fruit load would inhibit vegetative growth from fruit-set throughout the season, during which time low-yielding trees might exhibit relatively vigorous vegetative growth. This scenario might accelerate alternate bearing. The current study joins a number of others and supports literature suggesting that fruit load must be included as a factor in irrigation scheduling (Ben-Gal et al. 2011, Dell’Amico et al. 2012, Moriana et al. 2012, Naor et al 2013) and that, in addition to contributing to significant water savings, irrigation practices that consider fruit load may be a useful means reducing irregular bearing in olives.

Beyond such practical considerations, the question of how developing fruit influence tree water requirements can be considered. Possible mechanisms include: stomatal response to water balance and alteration of the soil-plant-atmospheric continuum, influence on carbon source-sink relationships, dynamic progression from isohydric to anisohydric stomatal regulation, or signals from fruit promoting changes in hydraulic properties of vascular tissues and tree organs.

Unlike leaves that possess large surface to volume ratio and are rich with stomata, the fruit is a spheroid displaying much smaller specific surface area. Some few active stomata are indeed present on the fruit surface at an early stage of development, but these are quickly covered with a waxy cuticle. Thus, significant gas and water exchange between the fruit and its environment does not occur during most of the fruit development period (Proietti et al. 1999) and therefore fruit do not directly contribute to tree transpiration or tree-scale water balance.

Developing fruit function as a strong sink for photoassimilates. Theoretically, the demands by heavy fruit load may exert intensified foliar activity, exhibited by enhanced CER or extended periods of photosynthetic activity. Enhanced CER would require some increase in stomatal conductance (g_s), which might explain the escalated transpiration occurring under high fruit loads. Noteworthy however, is the rather weak relationships between g_s and CER at the upper range of g_s (Fernández 2014). Nevertheless, in the present study, CER values as well as g_s did not vary significantly between high and low fruit loads (Fig. 4). Also, diurnal examinations of these parameters (data not shown) did not provide evidence for extended foliar activity under high fruit load. These results are in agreement with previous studies in olive (Proietti 2001, Hagidimitriou and Pontikis, 2005, Proietti et al. 2006), who showed that leaf-to-fruit ratio scarcely affected CER and g_s. Conversely, Martín-Vertedor et al. (2011a) were able to show that under medium or high crop load, g_s increased by an average of 17% over trees that did not have fruits. We recognize that the data regarding leaf scale photosynthesis and transpiration in the current study, taken mid-day on diagnostic leaves, was not sufficient to absolutely negate possible fruit load influence on the processes and their diurnal dynamics.

There are several explanations for the difficulty in obtaining the expected differences in olive leaf activity. Discrete instantaneous g_s measurements would always be subject to many environmental and intrinsic influences, including the diurnal dynamics of exposure to sunlight, temperature, vapor pressure deficit (VPD), and leaf age. A mature olive tree carries a huge number of small leaves, the variability among which may be immense at any given moment. Elucidating the effect of a single factor under field conditions from only a few instantaneous measurements would be statistically rather challenging, due to the very low signal-to-noise ratio expected. Therefore, even if it exists, a direct influence of fruit on g_s may be difficult to capture via typical measurement methods. Additionally, Fernández et al. (2011a) showed that, under typical semiarid summer conditions, $g_{s\text{-max}}$ was usually reached in the morning, much earlier than the diurnal climax of plant transpiration (T_a). Similarly, maximum sap flow rates are recorded in the afternoon, while stomatal closure begins much earlier, in the morning (Moreno et al. 1996). This is because T_a, and consequently, the sap flow in the trunk, is driven mainly by VPD, following its daily pattern (Tognetti et al. 2009; Diaz-Espejo et al. 2012). While increasing VPD also induces earlier stomatal closure, the reducing effect of decreased g_s is smaller than the enhancement of T_a by high VPD (Fernández 2014). Thus, the linkage between g_s and T_a, especially concerning instantaneous measurements, was far from straightforward during the present study. Whole tree performance was therefore preferably evaluated by direct integrative measurement of T_a.

In the long-term however, g_s may play a significant role in adjusting tree water status. Tardieu and Simonneau (1998) distinguished between isohydric species, where stomatal regulation maintains a fairly consistent minimum leaf water potential (ψ_l) from day to day, and anisohydric species, where ψ_l markedly decreases with changes in evaporative demand. Klein (2014) recently suggested a continuum rather than a dichotomy between isohydric and anisohydric behaviors. Moreover, the mode of stomatal regulation (i.e., isohydric/anisohydric) has been shown to vary over the course of a growing season in a given species. Some grapevine cultivars, for instance, show dynamic stomatal sensitivity and can switch from isohydric-like behavior to anisohydric-like behavior in response to changing environmental conditions (Rogiers et al. 2012, Zhang et al. 2012).

Cuevas et al. (2010) reported that olives showed near-isohydric behavior, similar to that reported for other Mediterranean woody crops (Schultz 2003). Analogous to several other fruit tree species (e.g., grapevine, apple, and avocado) reported to change their ‘risk-management strategies’ (Palmer 1992, Naor et al. 1997, 2008, Silber et al. 2013a), olives have been shown to exhibit higher stomatal conductance and higher CO_2 assimilation rate under heavy crop load, although these effects were more pronounced under deficit irrigation than in well irrigated trees (Naor et al. 2013). Moreover, solid evidence exists concerning the influence of fruit load in olives on midday water potential, a widely accepted integrative parameter of tree water status. As shown here as well (Fig. 5), high crop load is significantly associated with a decrease in midday plant (stem) water potential (Sadras and Trentacoste 2011, Naor et al. 2013). This behavioral change implies a shift in hydraulic regulation as a function of sink demand.

Olives generally display low hydraulic conductivity (Larsen et al. 1989, Bongi and Pallioti 1994) and are able to withstand water potentials below turgor-loss point with minor seasonal xylem embolism (Torres-Ruiz et al. 2013). Subsequently, under different water regimes, olives display differences in xylem structure and function (López-Bernal et al. 2010, Rossi et al. 2013). In semiarid regions, these traits support survival of individual trees. Nevertheless, the emergence of the reproductive phase necessitates an opposite evolutionary strategy, in which water and nutrient availability should be enhanced to furnish the development of seeds and complete the reproduction process. In fact, the full-bloom and fruit-development phases have been found the most sensitive periods for water stress in olive trees (Tognetti et al. 2005, Moriana et al. 2012). Therefore, some aptitude to trade-off between high hydraulic conductance and avoidance of embolism (Martinez-Vilalta et al. 2002, Hacke et al. 2006) is required. Diaz-Espejo et al. (2012) suggested that regulating signals other than simple hydraulics were potentially involved in determining plant water conductance in olives, and that these signals were themselves controlled by something other than soil water status. Possibly these signals emerge from developing fruit.

Plant water channels, aquaporins (AQPs), are understood to play significant roles in controlling plant water status, hydraulic conductivity, membrane osmotic permeability and stomatal regulation (Kaldenhoff et al. 2007, Shatil-Cohen et al. 2011, Prado and Maurel 2013, Li et al. 2014, Moshelion et al. 2015). AQPs are subject to rapid, substantial, and stable shoot-to-root signals, regulating root hydraulic conductivity (Vandeleur et al. 2014). Similarly, developing fruit may govern AQP expression and activity in remote plant organs (Sade and Moshelion 2014). Developing fruit, via the excretion of plant hormones, provoke and govern the construction of supporting vascular systems (Nitsch 1952, Crane 1964, Aloni 1987, Bustan et al. 1995, Ozga and Reinecke 2003, Else et al. 2004). Hormonal factors may also regulate the functioning of the fruit vascular routes, ensuring sufficient supply of water and nutrients. Significant differences occurring in AQP expression between low- and high-yielding olive trees (Turktas et al. 2013) may support this view.

High turgor pressure is essential for the growth of plant organs, particularly of fruit. Under Mediterranean summer conditions, turgor pressure during the day tends to be very low. Therefore, fruit growth is commonly limited to periods after nocturnal water recovery and turgor pressure revival. Rapid reclamation of plant water status following midday decline would extend the prospective growth period, benefiting growing organs. The rate of nocturnal water recovery depends on environmental water status (soil water availability and VPD), plant capacity for water storage (Moreno et al. 1996, Fernández et al. 2006b) and on xylem water conductance. Sap flow at night is known to occur in olive, accounting for significant nocturnal water recovery (Fernández et al. 2008b). Developing fruit likely act, via hormones and AQPs, to enhance both xylem water conductance and plant capacity for water storage. While a clear benefit would be ascertained by fruit growth at night, enhanced xylem water conductance likely also leads to increased transpiration and consequent lower STWP.

Conclusions

Under the normally non-restrictive water conditions that prevailed in the present study, constitutive vegetative growth suggests that carbon sources were not limited. Symptoms of carbon sink limitation, such as declined CER and g_s, expected in response to fruit removal, were for the most part insignificant, possibly due to alternative sink demands. Nevertheless, fruit load had a significant effect on tree water potential and an even greater effect on tree-scale water consumption, which was about 30% higher in fruit-loaded trees and responded dramatically to fruit removal. Mechanisms explaining the role of fruit on water consumption likely involve signaling and changing hydraulic properties of vascular tissues and tree organs.

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Figure legends

Figure 1. Relationship between trunk cross sectional area (TCSA), tree biomass (a) and leaf area (b) at time of tree removal after final fruit harvest in November 2011.

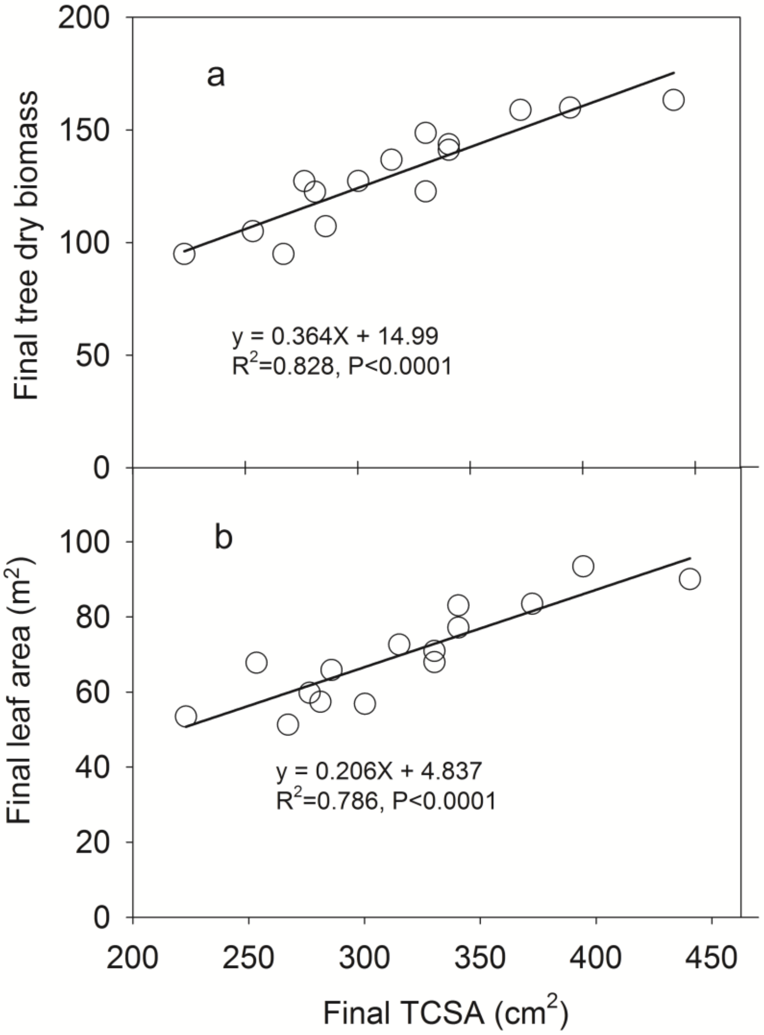
Figure 2. Periodical daily average of calculated specific water consumption (SPWC) for lysimeter grown olive trees as a function of current fruit load at four subsequent phenological periods from bloom to final harvest. Filled symbols present individual trees with current fruit load below 10,000 fruit, as follows: originally low-yielding (OLY), early (DOY 141) thinned or defruited (EFR), mid-season (DOY 185, MFR), and late-season (DOY 248, LFR) defruited trees. Empty symbols present trees with current fruit load higher than 10,000 (HY).

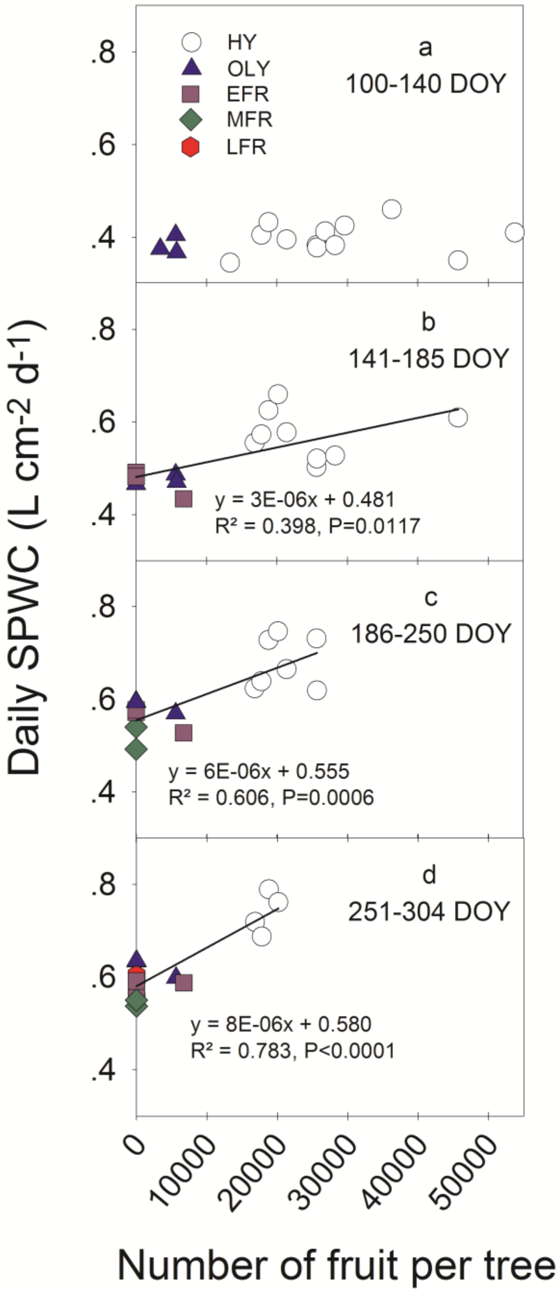
Figure 3. Time course of specific water consumption (SPWC) for olive fruit season in 2011 (a). SPWC calculated as tree-scale daily evapotranspiration (L) / trunk cross sectional area (cm^2). Lysimeter grown olive trees divided into treatment classes: HY (high yielding) more than 10,000 fruits/tree; OLY (originally low yielding) less than 10,000 fruits/tree; EFR (early fruit removal); MFR (mid fruit removal); LFR (late fruit removal). Relative SPWC (b) – SPWC normalized to the OLY group. Error bars are standard errors.

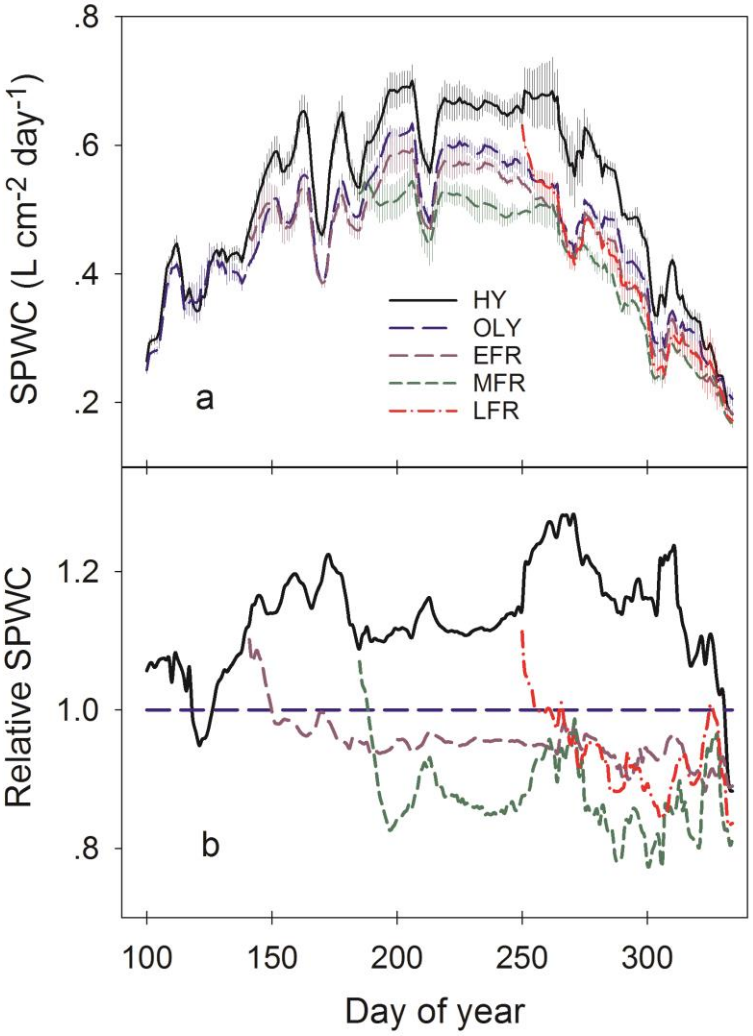
Figure 4. Time course of leaf-scale carbon exchange rate (CER, a), stomatal conductance (g_s, b) and electron transfer rate (ETR, c) for olives grown in lysimeters. HY are high yielding (>10,000 fruits) and LY are low or non-yielding trees (<10,000 fruits), respectively. Error bars are standard errors.

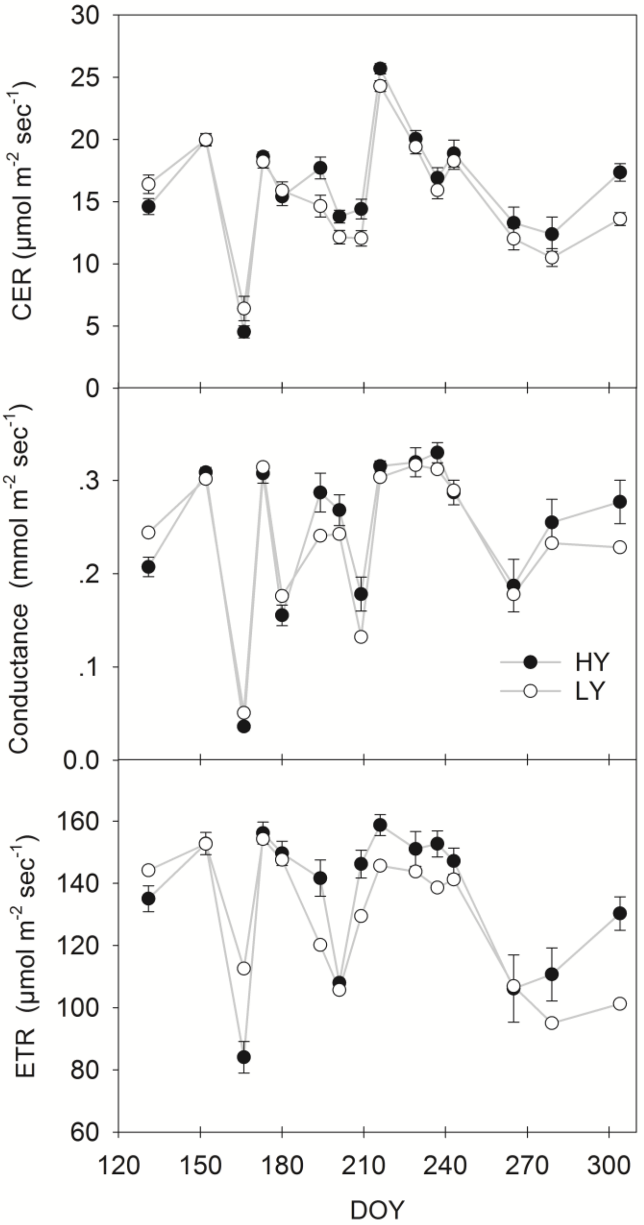
Figure 5. Time course of measured midday plant water potential (STWP) in olive trees grown in lysimeters with either current high crop load (HY, >10,000 fruits) or low/no crop load (LY, (<10,000 fruits). Error bars are standard errors. Stars indicate dates with significant differences between the treatments.

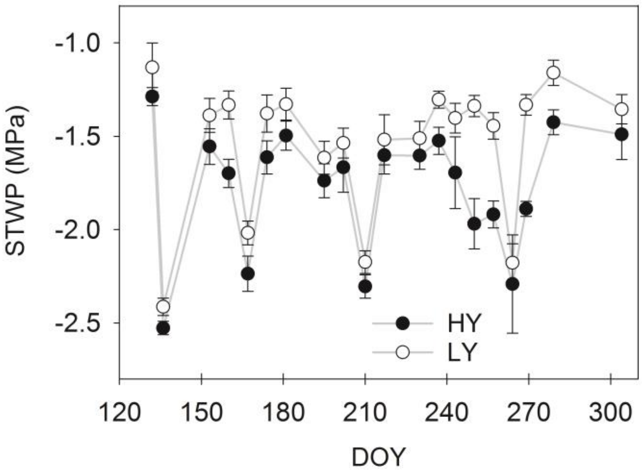
Figure 6. Growth rate of trunk cross sectional area (TCSA) in olive trees with either current high crop load (HY, >10,000 fruits) or low/no crop load (LY, <10,000 fruits), shown as absolute values (a) or as relative growth rate (TCSA RGR) per experimental period (b). Period I (100 - 140 DOY), period II (141 - 185 DOY), period III (186 – 250 DOY), and period IV (251-304 DOY). Error bars are standard errors.

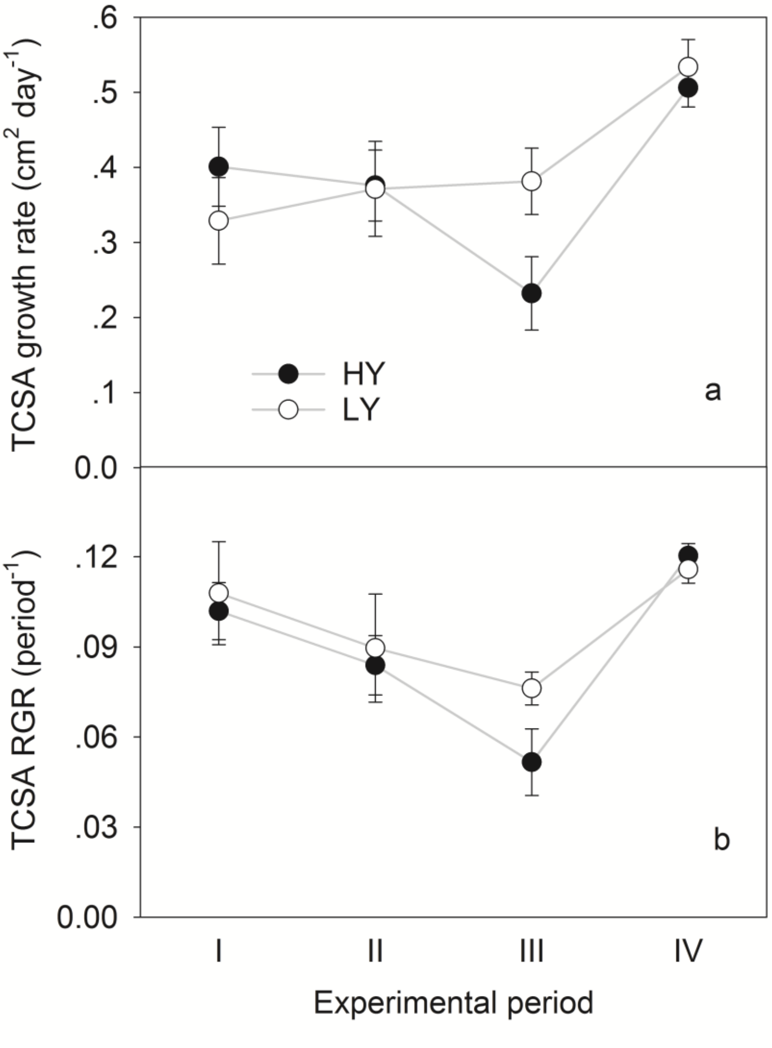

a
b
Final tree dry biomass
Final leaf area (m2)
Final TCSA (cm2)
y = 0.364X + 14.99
R2=0.828, P<0.0001
y = 0.206X + 4.837
R2=0.786, P<0.0001
0
50
100
150
200
0
20
40
60
80
100
200
250
300
350
400
450


HY
OLY
EFR
MFR
LFR
a
100-140 DOY
b
141-185 DOY
y = 3E-06x + 0.481
R² = 0.398, P=0.0117
c
186-250 DOY
y = 6E-06x + 0.555
R² = 0.606, P=0.0006
d
251-304 DOY
y = 8E-06x + 0.580
R² = 0.783, P<0.0001
.8
.6
.4
Daily SPWC (L cm-2 d-1)
0
10000
20000
30000
40000
50000
Number of fruit per tree

SPWC (L cm-2 day-1)
.8
.6
.4
.2
a
HY
OLY
EFR
MFR
LFR
Relative SPWC
1.2
1.0
.8
b
100
150
200
250
300
Day of year


CER (µmol m-2 sec-1)
30
25
20
15
10
5
0
Conductance (mmol m-2 sec-1)
.3
.2
.1
0.0
HY
LY
ETR (µmol m-2 sec-1)
160
140
120
100
80
60
120
150
180
210
240
270
300
DOY


STWP (MPa)
-1.0
-1.5
-2.0
-2.5
120
150
180
210
240
270
300
DOY
● HY
○ LY


.6
.5
.4
.3
.2
.1
0.0
TCSA growth rate (cm^2 day^{-1})
HY
LY
a
.12
.09
.06
.03
0.00
TCSA RGR (period^{-1})
b
I
II
III
IV
Experimental period