Seasonal effects of deficit irrigation on leaf photosynthetic traits of fruiting and non-fruiting shoots in almond trees

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Summary We investigated seasonal trends in, and relationships between, leaf structural properties, leaf nitrogen concentration, and maximum (Amax) and potential (Ap) leaf net CO2 assimilation of 1-year-old fruiting (f) and current-year non-fruiting (nf) shoots in 5-year-old almond trees (Prunus dulcis (Mill.) D.A. Webb cv Marta). These trees had been subjected in the previous 4 years to either full irrigation (FI regime) or sustained deficit irrigation (DI) at 50% of standard crop evapotranspiration during the entire growing season (DI regime) in the semiarid climate of southeast Spain. Measurements were made during an entire growing season on sun-exposed leaves. Leaf dry mass per unit area (Wa), area and dry-mass-based leaf N concentrations (Na and Nw, respectively), and area and dry-mass-based Amax (Ama and Amw, respectively) were lower in f-leaves than in nf-leaves. Changes in leaf structural attributes induced by DI were more pronounced in nf-leaves than in f-leaves, the latter being little affected. Over the entire growth season, Ama and Ap were correlated negatively with Wa and positively with Na for both the leaf classes and the irrigation regimes. When calculated with respect to total leaf N concentration, maximum photosynthetic nitrogen-use efficiency (PNUEm) was significantly higher in f-leaves than in nf-leaves, with no significant differences between the leaf classes among the irrigation regimes. However, when PNUEm was calculated with respect to photosynthetic N, no significant effect of leaf class or irrigation regime was observed. Overall, our results showed that DI and FI trees exhibited similar seasonal patterns of leaf structural properties and maximum and potential leaf net CO2 assimilation rates, but there were distinct N-allocation patterns between f- and nf-leaves. In the DI treatment, leaf structural adjustments appeared to operate to maintain a high N status in the leaves of fruit-bearing shoots, to the detriment of N resources allocated to vegetative shoots.

Keywords: leaf N concentration, phenological stage, photosynthetic capacity, photosynthetic nitrogen-use efficiency, Prunus dulcis, specific leaf area.

Introduction

Deficit irrigation (DI) strategies can regularize or increase, or both, almond productivity when a limited amount of irrigation water is available (Goldhamer et al. 2006). These strategies, which include regulated DI (Chalmers et al. 1981), sustained DI (Girona et al. 2005, Goldhamer et al. 2006), and partial root-zone drying (Dry and Loveys 1999, Kang et al. 2003), can substantially reduce tree water consumption without causing a significant negative effect on tree productivity (Girona et al. 1997, Goldhamer and Viveros 2000, Esparza et al. 2001a, 2001b, Romero et al. 2004, Girona et al. 2005). One of the prerequisites for valuation and further implementation of best DI management practices is a detailed knowledge of plant physiologic responses to abiotic stresses, while accounting for leaf age, plant phenology (Dungan et al. 2003, Xu and Baldocchi 2003, Muroaka and Koizumi 2005) and fruit load (DeJong 1986, Palmer et al. 1997, Syvertsen et al. 2003).

Studies on the application of DI to the almond tree have provided valuable information, especially on leaf net CO2 assimilation (DeJong 1983, Wartinger et al. 1990, Higgins et al. 1992, Matos et al. 1998, Romero et al. 2004, Rouhi et al. 2007) and an influence of natural or imposed drought-induced stress on photosynthetic attributes (Klein et al. 2001, Heilmier et al. 2002, Romero and Botia 2006). Details related to nutrient deficiency (Basile et al. 2003), species, cultivar type and rootstocks (Matos et al. 1997, De Herralde et al. 2003, Rouhi et al. 2007) and leaf ontogeny (Wartinger et al. 1990, Matos et al. 1998, Klein et al. 2001, Romero and Botia 2006) are also available. Knowledge about how water stress can modify the functioning of different types of shoots in the almond tree is needed, because it is likely to affect almond yield determinants. Studies on fruit trees have shown differences in leaf structural and physiologic traits between shoot types (Fujii and Kennedy 1985, Syvertsen et al. 2003, Zhang et al. 2005), associated with the presence or absence of fruits on shoots. Recently, Heerema et al. (2008) reported that the
survival of almond rosette-like short shoots (spurs) over winter was strongly related to their leaf area per unit of leaf mass. Although a clear seasonal trend in leaf traits, which can vary considerably over time with leaf age, has been observed (Wilson et al. 2000), it has not been clearly established if leaf age dominates other factors such as soil water availability in the almond tree.

To our knowledge, the long-term effects of DI strategies on the response of leaf structural and physiologic traits of fruit-bearing shoots and vegetative shoots, and on the pattern of N allocation to these classes of foliage, have not been documented. Such responses might induce changes in the pool of carbohydrate reserves, which are known to affect the renewal of fruiting position, as well as the amount of reserves available at the onset of a new growth cycle, leading to subsequent yield reduction in the following year (Esparza et al. 2001a, 2001b). In early foliating species, growing shoots and developing fruits are strong sinks for assimilates, which compete for the reserves stored in woody plant parts during the previous growing season (Weinbaum et al. 1987, Millard 1996, Nii 1997, Rosecrance et al. 1998, Youssefi et al. 2000, Bi et al. 2003). The influence of fruits (sinks) in attracting assimilates from the proximal leaves (Nii 1997, Syvertsen et al. 2003) and competition for N resources between leaves and fruits (Youssefi et al. 2000) can lead to lower N concentration in leaves near the fruit, as observed in citrus trees (Syvertsen et al. 2003). In walnut trees, a net decrease in leaf N concentration as a result of protein degradation (resorption) can occur in response to the demands of developing fruits (Weinbaum et al. 1994), but this pattern has not clearly been demonstrated in almond trees (Weinbaum and Muraoka 1986). Stress conditions have been reported to affect leaf N concentration (Reich et al. 1989). Because leaves are an important source of N for tree reserves, restricting the water supply by DI might have a negative effect on N accumulation in woody structures (Bi et al. 2003). It has been observed that, after harvest, N storage in perennial tree parts depends much more on N recycling from leaves than on soil N uptake (Rosecrance et al. 1998, Esparza et al. 2001a), and this storage can provide up to 50% of the N used over the growing season (Weinbaum et al. 1987).

Romero et al. (2004) reported a significant reduction in the photosynthetic capacity of young branches of almond trees under pre-harvest conditions of mild to moderate soil water deprivation, whereas the accumulation of dry matter in the kernel was little affected compared with the fully irrigated trees. Because shoots appear to function as autonomous structures with respect to assimilation and use of carbon in many tree species (Sprugel et al. 1991), the sustained sink activity of fruits under conditions of water stress might indicate that the almond tree tends to preserve the photosynthetic capacity of fruit-bearing shoots to the detriment of the vegetative shoots. This suggestion is supported by the observed differences in nonstructural carbohydrate concentration between shoot types of dormant almond trees that had previously been subjected to water stress during the harvest period (Esparza et al. 2001a).

The results of the cited studies suggest that a DI strategy applied for several consecutive years could lead to significant changes in the morphologic and physiologic traits of almond leaves. We hypothesized that vegetative and fruit-bearing shoots exhibit a differential response to water stress through changes in leaf structural and physiologic traits. To test this hypothesis, we characterized the seasonal trend in leaf traits, and investigated whether they vary (1) with leaf age and class (fruiting versus non-fruiting shoots) and (2) between irrigation regimes (full irrigation (FI) versus sustained DI), and investigated to what extent the relationships between leaf traits are conserved or co-vary across leaf classes and irrigation regimes. We also examined the effect of irrigation treatment on the photosynthetic nitrogen-use efficiency (PNUE) of the two leaf classes.

Materials and methods

Site description

The study was conducted during the 2004 growing season in a 1-ha plot of 5-year-old almond (Prunus dulcis (Mill.) D.A. Webb cv Marta) trees that had been grafted on ‘Mayor’ rootstock and planted in December 1999 at a spacing of 6 m × 7 m in an almond orchard at the Agricultural Experimental Station of the University of Cartagena (37°35’ N and 0°59’ W). The climate at the site is Mediterranean with low cloudiness, high temperature, low humidity and virtually no rainfall during the summer. The winter is mild, with a mean air temperature of about 10 °C during the coldest month (January). Mean annual temperature was 18.2 °C in 2004 and precipitation was 325 mm. These values are close to climatic means determined over 20 years at a nearby weather station (mean air temperature was 18.3 ± 0.9 °C and mean precipitation was 355 ± 90 mm). The corresponding value of reference crop evapotranspiration (ET0), calculated with the FAO Penman–Monteith equation (Allen et al. 1998), is 1180 ± 60 mm. The soil texture was a deep silt-clay-loam with low available potassium and organic matter concentrations and high phosphorus concentration. Electrical conductivity (EC) of the saturation extract was 1.4 dS m⁻¹ in 2004. The bulk density of the surface layer was 1.4 ± 0.1 g cm⁻³ and available water-holding capacity of soil was about 0.18 mm⁻¹.

Irrigation regimes

In 2000, two irrigation treatments, FI and sustained DI were applied to the experimental plots until the end of 2004, following a completely randomized statistical design with three replicates per treatment and 12 trees per
replicate. Trees were irrigated by a single drip line equipped with six pressure compensated drippers (41 h⁻¹ per dripper) per tree.

In the FI plots, an amount of water corresponding to 100% of the standard crop evapotranspiration, ETc, was supplied weekly. We calculated ETc as the product of ETo and the crop coefficient for almond trees (Doorenbos and Pruitt 1977), and corrected with a localization factor (Fereres et al. 1982). During the first 3 years (2000–2002), the trees in the DI plots were irrigated following a sustained DI strategy, receiving 60% of ETc in the entire irrigation season. In 2003 and 2004, the water supply was further restricted such that the trees received about 40% of ETc.

The EC of irrigation water was 1.2 dS m⁻¹ with chloride and sodium concentrations of 4.6 and 5.41 meq l⁻¹, respectively. Trees were fertilized with 35, 35, 57 kg ha⁻¹ year⁻¹ of N, P, K and were managed according to current commercial practices (i.e., a routine pesticide program was maintained, pruning was applied manually in December, and all weeds were removed from the orchard).

**Measurement period**

In 2004, leaf characteristics were measured from the beginning of April (day of year (DOY) 97, when fruits had reached 92% of their final size) until post-harvest, corresponding to the period of reserve accumulation in woody structures. Fruit harvest occurred in mid-August (DOY 220) for trees in the DI treatment and about 10 days later for trees in the FI treatment.

Figure 1 gives a schematic representation of the stages of tree development as a function of DOY for trees in the FI regime. The curves of relative cumulative values of fruit growth, young branch growth and fruit dry mass accumulation (kernel-filling) were normalized against their maximum values. Before the experimental period (February 2004), mean trunk diameter of the trees was 14.08 ± 0.28 cm in FI and 12.02 ± 0.15 cm in DI, and canopy height was 3.56 ± 0.09 m in FI and 3.19 ± 0.07 m in DI. At the end of the 2003 growing season, crown leaf area was 68.11 ± 2.48 m² tree⁻¹ in FI and 41.94 ± 3.58 m² tree⁻¹ in DI.


**Measurements of leaf N concentration and leaf area**

Foliar N concentration was measured from April to September 2004 in the fully expanded leaves of non-fruiting branches (current-year branches) and fruiting branches (1-year-old branches) – hereafter nf- and f-leaves, respectively – in the two irrigation treatments. Samples consisted of at least 12 leaves in each class taken from the upper third of sun-exposed branches. Leaf area was measured with an area meter (Delta-T Image Analysis System (DIAS), Delta-T Devices, Cambridge, UK).

Mean leaf N concentration was determined after Kjeldahl digestion of leaves that had been oven-dried at 80 °C for 48 h, weighed and ground. For each class of leaves, leaf N concentration is expressed per unit leaf area (N_{a}, in g m⁻²) and per unit leaf dry mass (DW) (N_{w}, in g g⁻¹). Leaf dry mass per unit leaf area (W_{a}) is expressed in g m⁻².

**Gas exchange measurements**

Leaf gas exchange was measured from April to September on trees in the FI and DI irrigation regimes. The criteria adopted for selecting leaves on the two classes of branches were similar to those described for the leaf N concentration measurements. Only healthy branches were selected from the periphery of the tree crown. Area-based leaf net CO₂ assimilation (A, μmol m⁻² s⁻¹), transpiration rate (E, mmol m⁻² s⁻¹), stomatal conductance to water vapor (g_s, mmol m⁻² s⁻¹) and intercellular CO₂ concentration (C_i, μmol mol⁻¹) were measured with a portable gas exchange system CIRAS2 (PP Systems, Hitchin, Hertfordshire, UK). The desired photosynthetic photon flux (I, μmol m⁻² s⁻¹) was provided by an internal red/blue LED light source (PC069-1). A CIRAS2 injection system controlled the ambient CO₂ concentration (C_a, μmol mol⁻¹) in the chamber by adjusting the flow of CO₂ from a CO₂ cylinder, and also controlled the leaf temperature (T_l) in the chamber. Air temperature (T_a) and actual vapor pressure of the chamber air were continuously monitored by the CIRAS2. Measurements were made at periodic intervals (~ 7 to 10 days) throughout the growth season on at least six leaves (one leaf per branch, two branches per tree...
and three trees per irrigation treatment). All leaf gas exchange measurements were made between 1000 and 1300 h (local time).

Light-saturated leaf net CO₂ assimilation (A_{\text{na}}) and maximum stomatal conductance (g_{\text{sm}}) were measured at I = 1400 μmol m⁻² s⁻¹, near constant Cₐ (∼ 360 μmol mol⁻¹) and Tₖ (∼ 30 °C). Area- and dry-mass-based values of A_{\text{na}} are denoted as A_{\text{ma}} (μmol m⁻² s⁻¹) and A_{\text{mw}} (μmol g⁻¹ s⁻¹), respectively. Light-saturated, PNUE_{\text{ma}}, was computed as the ratio A_{\text{ma}}/W (μmol g⁻¹ s⁻¹).

Leaf potential net CO₂ assimilation or maximum metabolic capacity (A_{\text{p}}) was measured from April to September in trees in both FI and DI regimes, at saturating I (∼ 1400 μmol m⁻² s⁻¹) and saturating Cₐ (∼ 1400 μmol mol⁻¹), and at near optimum Tₖ (35 °C). This Tₖ value was derived in spring from the measurements of A_{\text{ma}} in FI leaves over a wide range of Tₖ (18–42 °C).

Tree water status

Every 2 weeks, we measured predawn leaf water potential (Ψ_{\text{pd}}) with a pressure chamber (Model 3000, Soil Moisture Equipment, Santa Barbara, CA) on mature leaves (12 leaves per irrigation treatment, i.e., six trees and two leaves per tree) taken before dawn from the upper third of the branches. Data for the f- and nf-leaves were pooled.

Statistical analysis

We analyzed (1) leaf N concentration (area- and dry-mass-based) as a dependent variable against Wₐ and (2) the photosynthetic parameters (area- and dry-mass-based) as dependent variables against Wₐ and against leaf N concentration (area- and dry-mass-based) by using linear regression. Significant differences between slopes and non-zero intercepts were evaluated by analyses of covariance, by comparing (1) FI and DI per each leaf class, (2) FI leaves and DI leaves with pooled data for the two leaf classes, (3) the two leaf classes with pooled data from FI and DI for each leaf class and (4) all datasets. To discriminate the main treatment effects on leaf structural properties and on photosynthetic parameters analysis of variance (ANOVA) was used. All analyses were performed using Statgraphics software (Statgraphics Plus for Windows Version 4.1).

Results

Climate conditions

Maximum absolute values of air temperature (Tₐ) and vapor pressure deficit (VPD) increased rapidly from spring (23 °C and 2.2 kPa, respectively) to summer (36.6 °C and 4.4 kPa, respectively) (Figure 2A), corresponding to the increase in the daily integral of global solar radiation over the same period (Figure 2C). During sunny days, mean values of Tₐ and VPD remained below 18 °C and 1.1 kPa, respectively, in spring and below 29 °C and 2.1 kPa, respectively, in summer (Figure 2B). The calculated reference crop evapotranspiration (ET₀) was typical for the location, with maximum daily values in summer of about 8 mm day⁻¹ (Figure 2D). Because of the high amount of precipitation in spring 2004 (Figure 2D), irrigation started on May 3 in all plots. Practically no rain occurred from the end of June until post-harvest (September). For the entire irrigation season (May until the end of November), the total amount of irrigation water was 410 and 170 mm for FI and DI, respectively, and rainfall was 355 mm.

Seasonal variation in predawn leaf water potential

In both irrigation treatments, predawn leaf water potential Ψ_{\text{pd}} = −0.30 ± 0.03 MPa during the active vegetative growth period (i.e., Stages II and III; Figures 1 and 3), indicating high soil water availability during this period. The FI trees maintained Ψ_{\text{pd}} within a small range from the onset of the kernel-filling stage (−0.35 ± 0.02 MPa) until post-harvest (−0.45 ± 0.01 MPa) compared with the range of variation observed in DI trees over the same period (from −0.47 ± 0.05 to −0.83 ± 0.06 MPa, respectively).

Seasonal trends in leaf structural traits

The seasonal trends in leaf dry mass per unit area (Wₐ) (Figure 4A) and area-based nitrogen concentration (Nₐ) (Figure 4B) were characterized by two seasonal peaks that were more marked in nf-leaves than in f-leaves. For both leaf traits, the first peak occurred near DOY 140–145 (onset of the kernel-filling stage), whereas the absolute maximum was reached just before harvest (DOY 218–220). Dry matter accumulated in the leaf blade except for two periods coinciding with the first half of kernel-filling and the onset of reserve accumulation (Figure 4A). Over time, Wₐ showed a greater increase in FI trees (about 36% in both leaf classes) than in DI trees (21% and 29% in f- and nf-leaves, respectively) (Figure 4A). Independently of irrigation treatment, Wₐ was generally greater in nf-leaves than in f-leaves. The FI trees maintained differences between the two leaf classes when Wₐ was averaged over the growth season (P = 0.02), whereas the corresponding differences in DI trees were significant only across Stages IV and V (P ≤ 0.04). Restriction of water supply moderately – but significantly – reduced Wₐ in nf-leaves (about 10%) during post-harvest compared with nf-leaves of FI trees (P < 0.01), but the DI treatment did not influence Wₐ in f-leaves during the growing season. Values of Wₐ were highly correlated between f- and nf-leaves within each irrigation regime (R² > 0.9), data not shown.

Area-based leaf N concentration (Nₐ) was significantly higher in nf-leaves than in f-leaves (Figure 4B) from the onset of kernel-filling stage onward in both irrigation treatments. In FI trees, Nₐ averaged over the measurement period was 27% higher in nf-leaves (3.1 ± 0.3 g m⁻²) than in f-leaves (P < 0.01), a greater difference than that observed for Wₐ (17%). In DI, the difference in Nₐ among leaf classes...
was much lower (about 13% higher in nf-leaves) \((P = 0.01)\) and was of the same order of magnitude as that observed for \(W_a\). Thus, on average over the growth season, the amount of N in nf-leaves was 15% higher in FI trees than in DI trees \((P < 0.01)\). The N concentration in f-leaves was not significantly affected by irrigation regime.

The seasonal variations in \(W_a\) and \(N_a\) resulted in a sustained decrease in \(N_w\) \(= N_a/W_a\) over time (Figure 4C). Differences in \(N_w\) between leaf classes were observed in FI trees only during Stages IV and V (14% higher in nf-leaves than in f-leaves, \(P = 0.03\)), i.e., about twofold lower than those observed for \(N_a\). The nf-leaves maintained a higher \(N_w\) over the growth cycle in the FI treatment \((0.0274 \pm 0.003 \text{ g g}^{-1})\) than in the DI treatment \((P = 0.02)\), a pattern similar to that observed for \(N_a\).

**Regression analyses: \(N\) versus \(W_a\) relationships**

The results of the regression analyses between leaf N concentration \((N_a\) or \(N_w)\) and \(W_a\) are shown in Table 1, for all combinations of leaf class and irrigation treatment over the entire growth cycle. There were significant relationships between \(N_a\) and \(W_a\) in nf-leaves in both FI and DI treatments \((R^2 = 0.61\text{ and }R^2 = 0.43,\text{ respectively})\), but not in f-leaves \((R^2 \approx < 0.01)\) (Table 1). For the pooled dataset, there was a highly significant relationship \((P < 0.001)\) between \(N_a\) and \(W_a\) \((R^2 = 0.37),\) as well as between \(N_w\) and \(W_a\) \((R^2 = 0.29).\) For each developmental stage, significant relationships \((P < 0.001)\) were obtained when
Seasonal effects of deficit irrigation on leaf and current-year non-fruiting (nf) crop evapotranspiration throughout the growing season. Irrigation and DI, sustained deficit irrigation at 50% of standard

Light-saturated photosynthetic attributes showed a non-uniform trend over the growth cycle (Figure 7A–C), with most of the attributes tending to reach a more or less clear maximum. Area-based $A_m$ was highest during Stages II and III, decreasing rapidly after the onset of Stage IV (Figure 7A). In both irrigation regimes, the differences in $A_m$ between leaf classes were not significant, except in the DI treatment during Stage IV when nf-leaves had significantly higher values than f-leaves (20.0 ± 1.4 versus 17.6 ± 0.5 μmol m⁻² s⁻¹) ($P < 0.01$). The f-leaves had similar $A_m$ in both irrigation treatments, whereas the nf-leaves had significantly lower $A_m$ in the DI treatment than in the FI treatment during Stages II, III and V (17% and 38%, respectively). Mean $A_m$ was not significantly affected by irrigation regime during the kernel-filling stage, regardless of leaf class.

Mass-based $A_m$ tended to decrease during the growing season (Figure 7B), declining in FI leaves during Stages II and III (from about 0.30 ± 0.05 to 0.22 ± 0.02 μmol g⁻¹ s⁻¹) and then reaching a plateau until the first half of the kernel-filling stage, coinciding with the decline in $W_a$ (Figure 4A). In contrast, $A_m$ was rather constant in DI leaves over the same period (≈ 0.20 μmol g⁻¹ s⁻¹); however, after harvest, it declined to low values (≈ 0.10 μmol g⁻¹ s⁻¹, pooled data for both irrigation regimes). The relative decline over the growing season was greater for $A_m$ (≈ 61%) than for $N_w$ (37%), and the differences in $A_m$ between leaf classes and between irrigation regimes were significantly reduced during the kernel-filling stage.

The seasonal trend in $g_{sm}$ (Figure 7C) showed maximum absolute values during the kernel-filling stage (232 ± 35 and 273 ± 20 mmol m⁻² s⁻¹ for nf-leaves in FI and DI trees, respectively). Peak values were more marked in FI trees than in DI trees, because of lower variability in $g_{sm}$ in the former. After reaching a maximum, $g_{sm}$ more or less paralleled the downward trend observed for $A_m$. The magnitude of this decrease was lower in FI trees than in DI trees, with a mean reduction in $g_{sm}$ for the two leaf classes of 85 mmol m⁻² s⁻¹ in the FI treatment and 153 mmol m⁻² s⁻¹ in the DI treatment for the period DOY 154–245. The greatest differences in $g_{sm}$ between irrigation regimes were observed during the post-harvest stage.
The ratio of intercellular CO₂ concentration to ambient CO₂ concentration ($C_i/C_a$) was lowest during the active vegetative growth period (data not shown). The ratio tended to increase from Stages II and III (mean value in FI leaves: 0.52 ± 0.07) until the second half of kernel-filling stage and then remained more or less constant (mean value in FI leaves beyond Stage V: 0.61 ± 0.04).

Area-based leaf potential net CO₂ assimilation ($A_{pa}$) exhibited a seasonal trend similar to that of $A_{ma}$, with highest rates during Stages II and III (Figure 7D) and a decrease over the growing season that was independent of leaf class and irrigation regime. The differences in $A_{pa}$ among all combinations of leaf class and irrigation treatment were similar to or lower than in $A_{ma}$.

**Analysis of the relationships between $A_{ma}$ and $W_a$ and between $A_{ma}$ and leaf N concentration**

Regression analyses revealed highly significant relationships between $A_{ma}$ and $W_a$, and between $A_{ma}$ and $N_a$ ($P < 0.001$) for all combinations of leaf class and irrigation treatment, as well as for the pooled datasets. Two examples of $A_{ma}$ versus $W_a$ relationships are given in Figure 8A and B. For the pooled data for $f$- and $nf$-leaves per irrigation treatment (relationships for FI leaves and DI leaves, Figure 8A), no significant differences between irrigation regimes were observed in slopes or in non-zero intercepts. For the pooled data for the FI and DI treatments per leaf class (relationships for $f$- and $nf$-leaves, Figure 8B), there were significant differences in slopes and non-zero intercepts between leaf classes. The relationships between $A_{ma}$ and $W_a$ (Figure 8B), and between $N_a$ and $W_a$ (Figure 6) were similar. For all pooled datasets, $W_a$ explained about two-thirds of the seasonal variability in $A_{ma}$ ($R^2 = 0.65$, $P < 0.001$). The regression analysis for $A_{ma}$ versus $W_a$ yielded lower $R^2$ values than for $A_{ma}$ versus $N_a$.

Seasonal changes in $A_{ma}$ and $N_a$ were poorly correlated to changes in $N_a$ ($R^2 < 0.10$), but were correlated with $N_w$ ($0.69 < R^2 < 0.90$) with $P < 0.001$ for the linear regressions between either $A_{ma}$ or $A_{ma}$ versus $N_a$, for all combinations of leaf class and irrigation regime. Two examples of $A_{ma}$ versus $N_a$ relationships are shown in Figure 9A ($A_{ma}$ versus $N_a$ for FI leaves and DI leaves, pooled data of $f$- and $nf$-leaves) and Figure 9B ($A_{ma}$ versus $N_a$ for $f$- and $nf$-leaves, pooled data of FI and DI).

**PNUE**

The seasonal trend in light-saturated PNUE$_m$ reached a plateau from Stages II and III until the second half of kernel-filling stage (Figure 10A) and then decreased dramatically, reaching a minimum after harvest. Regression analyses of the PNUE$_m$ versus $W_a$, PNUE versus $N_a$, and PNUE versus $N_w$ relationships indicated that PNUE$_m$ was strongly correlated with $W_a$ and $N_a$ but loosely correlated with $N_w$ (data not shown). The relationship between PNUE$_m$ and $N_w$ (Figure 10B) shows that, for a fixed value of $N_w$, PNUE$_m$ was generally higher in $f$-leaves than in $nf$-leaves, irrespective of irrigation regime. Pooling all the data revealed that PNUE$_m$ was more closely correlated with $W_a$ ($R^2 = 0.78$; Figure 10C) than with $N_w$ ($R^2 = 0.49$). The three outliers in Figure 10C correspond to the post-harvest period in the DI regime.

**Nitrogen-use efficiency versus photosynthetic N**

An estimate of the amount of leaf N concentration diverted to the photosynthetic apparatus (i.e., dry-mass-based photosynthetic N, $N_{wp}$) was derived from Eq. (1) (Field and Mooney 1983), based on the pooled data for FI and DI for each leaf class

$$A_{ma} = m(N_w - N_{wo}) = mN_{wp},$$

where $N_{wo}$ is the residual value of leaf N concentration when $A_{ma} = 0$ and the slope $m$ represents the mean PNUE related to photosynthetic leaf N concentration.
i.e., $\text{PNUE}_{\text{emp}} = A_{\text{nw}}/N_{w}$. Assuming that Eq. (1) is valid over the entire range of $N_{w}$, and that, for a given leaf class, $N_{w0}$ is constant throughout the growing season and is independent of the irrigation regime (Figure 9B), the regression analysis yielded for $f$-leaves: $N_{w0} = 0.0127 \pm 0.004 \text{ g g}^{-1}$ and $\text{PNUE}_{\text{emp}} = 15.62 \pm 1.78 \text{ mol g}^{-1} \text{ s}^{-1}$; and for $nf$-leaves: $N_{w0} = 0.0153 \pm 0.005 \text{ g g}^{-1}$, and $\text{PNUE}_{\text{emp}} = 16.14 \pm 2.38 \text{ mol g}^{-1} \text{ s}^{-1}$. Thus, the values of $N_{w0}$ differed significantly between leaf classes ($P < 0.05$), whereas $\text{PNUE}_{\text{emp}}$ did not differ significantly between leaf classes, in contrast to what was observed for $\text{PNUE}_{\text{mp}}$ (Figure 10B).

The trend in photosynthetic N as a fraction of total leaf N concentration ($f_{\text{PN}} = N_{\text{wp}}/N_{w}$) indicated that, in both irrigation regimes, the $f$-leaves had a high $f_{\text{PN}}$ during Stages II and III (~0.60 on DOY 90) compared with $nf$-leaves (~0.50 and 0.40 in FI and DI, respectively) (Figure 11). Thereafter, $f_{\text{PN}}$ tended to decline in both leaf classes, the greatest decrease occurring during the second half of the kernel-filling stage. In the post-harvest stage, $f_{\text{PN}}$ ranged from 0.25 ($nf$-leaves in DI) to 0.40 ($f$-leaves in FI).

**Discussion**

**Effects of irrigation treatment on leaf structural traits in fruiting versus non-fruiting shoots**

Our study highlighted two main characteristics of the seasonal pattern of leaf structural traits. First, there was a close similarity in the seasonal patterns of $W_{a}$, $N_{w}$ and $N_{a}$ between FI and DI trees (Figure 4A–C). The greatest similarity was in $N_{w}$, which exhibited a decrease of about 40% throughout the season, independently of leaf class and irrigation treatment. This reduction was greater than that reported for other temperate deciduous trees (Niinemets et al. 2004) but similar to that found in almond leaves by Weinbaum and Muraoka (1986). There are several possible explanations for this seasonal pattern: (1) dilution of N in the leaf blade, because of the accumulation of non-N compounds; (2) N allocation to fruits and woody structures and (3) changes in N partitioning between metabolic and structural components within the leaf (Yasumura et al. 2006). Second, independently of irrigation treatment, the $f$-leaves...
Figure 7. Seasonal trends in (A) area-based ($A_{\text{ma}}$) and (B) dry-mass-based ($A_{\text{mw}}$) light-saturated net CO$_2$ assimilation, (C) maximum stomatal conductance ($g_{\text{ma}}$) and (D) potential net CO$_2$ assimilation ($A_{\text{pa}}$) in FI (open symbols) and DI (closed symbols) regimes, in *P. dulcis* f-leaves (circles) and nf-leaves (triangles) as a function of DOY. Pooled standard deviations of the mean (not shown for clarity) were typically close to (A) ± 2.0 μmol m$^{-2}$ s$^{-1}$, (B) ± 0.052 μmol g$^{-1}$ s$^{-1}$, (C) ± 47 mmol m$^{-2}$ s$^{-1}$ and (D) ± 4.2 μmol m$^{-2}$ s$^{-1}$. Vertical dotted lines delimit the beginning and the end of each developmental stage (cf. Figure 1). Abbreviations: f- and nf-leaves were obtained from 1-year-old fruiting (f) and current-year non-fruiting (nf) shoots, respectively; FI, full irrigation and DI, sustained deficit irrigation at 50% of standard crop evapotranspiration throughout the growing season.

had lower $W_a$, $N_a$ and $N_w$ than the nf-leaves (Figure 4A–C). The DI regime did not significantly affect $W_a$ in f-leaves but decreased $W_a$ in nf-leaves by 10–15%, mainly from pre-harvest onward (Figure 4A). Stress conditions have been reported to affect leaf N concentration (Reich et al. 1989). This was confirmed in our study, but only for the nf-leaves, which had significantly lower $N_a$ in DI trees than in FI trees, whereas $N_a$ in f-leaves was little affected by the DI treatment (Figure 4B). A similar conclusion can be drawn for $N_w$ (Figure 4C). Variations in $N_a$ ($=W_aN_a$) may result from variations in $W_a$, in $N_a$, or in both. A single relationship characterized the whole dataset when the
Figure 9. Relationships (A) between area-based maximum leaf net CO₂ assimilation \((A_{\text{max}})\) and mass-based leaf N concentration \((N_w)\) in \(P.\ dulcis\) trees in the FI and DI regimes, using pooled data from \(f\)- and \(nf\)-leaves and (B) between dry-mass-based \(A_{\text{max}}\) \((A_{\text{max}})\) and \(N_w\) for the \(f\)- and \(nf\)-leaves, using pooled data from FI and DI regimes. Regression lines in (A) \(y = -4.01 + 868.91x\) \((R^2 = 0.76, P < 0.001)\) and \(y = -13.05 + 1217.9x\) \((R^2 = 0.80, P < 0.001)\) for FI and DI, respectively, and in (B) \(y = -0.199 + 15.62x\) \((R^2 = 0.89, P < 0.001)\) and \(y = -0.247 + 16.04x\) \((R^2 = 0.80, P < 0.001)\) for \(f\)- and \(nf\)-leaves, respectively. Abbreviations: \(f\)- and \(nf\)-leaves were obtained from 1-year-old fruiting \((f)\) and current-year non-fruiting \((nf)\) shoots, respectively; FI, full irrigation and DI, sustained deficit irrigation at 50% of standard crop evapotranspiration throughout the growing season.

\(N_w\) versus \(W_a\) relationship was analyzed separately for each stage of development (Figure 5A–C), but the slope value (giving the mean \(N_w\) over a stage) shifted as the growing season progressed, indicating that the \(N_w\) versus \(W_a\) relationship is stage-dependent in almond trees, in contrast to nectarine trees (Rosati et al. 1999).

Independently of irrigation treatment, the presence of fruits near the leaves increased the slope and the intercept of the \(N_w\) versus \(W_a\) relationship compared with the corresponding values in \(nf\)-leaves, implying that \(f\)-leaves experienced a greater decrease in \(N_w\) for a given \(W_a\) and had a lower nitrogen concentration at high \(W_a\) (Figure 6). This may be ascribed to a higher depletion of N in the leaf blade to cope with the demand of the fruits during the kernel-filling stage and that of the perennial organs after harvest. A unique linear \(N_w\) versus \(W_a\) relationship was found for \(f\)-leaves in the FI and DI treatments, whereas this relationship differed significantly between irrigation...
treatments for nf-leaves (Figure 6), suggesting that, in the DI regime, leaf structural adjustments operated to maintain a conservative N status in the leaves of fruit-bearing shoots, but to the detriment of N resources allocated to vegetative shoots.

**Effects of irrigation treatment on leaf physiologic traits in fruiting versus non-fruiting shoots**

Almond species are known to exhibit high rates of photosynthesis among woody Rosaceae (DeJong 1983, Wullschleger 1993, Higgins et al. 1992, Lakso 1994, González-Real and Baille 2000). We observed maximum absolute \( A_{\text{ma}} \) values close to 24 \( \mu \text{mol m}^{-2} \text{s}^{-1} \) (Figure 7A), consistent with the values reported for almond leaves (DeJong 1983, Higgins et al. 1992, Matos et al. 1997, De Herralde et al. 2003, Rouhi et al. 2007).

Independently of the irrigation regime, leaves of fruiting and non-fruiting shoots exhibited high \( A_{\text{ma}} \) during the active vegetative growth period when the values of \( g_{\text{sm}} \) were below their maximum (Figure 7A–C), therefore maintaining a high intrinsic water-use efficiency, as observed by Heilmieier et al. (2002). Low \( C_{l}/C_{a} \) ratios over this period (about 0.52) confirmed that highest photosynthetic activity occurred in spring. The finding that the decreases in both \( A_{\text{ma}} \) and \( g_{\text{sm}} \) from the second half of kernel-filling stage onward occurred concomitantly with the increasing values of \( C_{l}/C_{a} \) suggests that internal mesophyll limitations dominated over stomatal limitations (Jifon and Syvertsen 2003).

The decreasing trend in both \( A_{\text{ma}} \) and \( A_{\text{p}} \) (Figure 7A–D) during the stage of fruit dry mass accumulation was consistent with the results reported by Walcroft et al. (2002) for peach trees. However, this pattern is not a general characteristic of fruit-bearing trees. Several studies have reported a positive effect of fruit demand for assimilates on the photosynthetic rates of peach (DeJong 1986) and apple (Palmer et al. 1997) trees.

There was a steep reduction in \( A_{\text{ma}} \) (Figure 7B) from the second half of kernel-filling stage onward that was independent of irrigation regime and leaf class. However, after harvest, this declining trend slowed in the nf-leaves in the FI treatment but was still marked in the DI treatment. Because translocation of assimilates to perennial tree organs occurs post-harvest, the decrease in \( A_{\text{ma}} \) in DI trees during this stage indicates that DI trees could be subjected to carbohydrate shortage in the following growth season (Esparza et al. 2001a). This decrease probably explains the lower \( A_{\text{ma}} \) in DI trees than in FI trees, as well as their smaller trunk diameter at the beginning of the growing season (see section Materials and methods).

The lower \( A_{\text{ma}} \) observed in DI trees compared with FI trees, along with the sustained decrease in \( A_{\text{ma}} \) observed in trees in both treatments after Stages II and III, should be analyzed to determine to what extent \( g_{\text{s}} \) (Figure 7C) determines this pattern by restricting \( \text{CO}_2 \) availability in the mesophyll. Comparison of the seasonal trends in area-based \( A_{\text{p}} \) (Figure 7D) and \( A_{\text{ma}} \) (Figure 7A) indicated a rather similar behavior. Maximum \( A_{\text{p}} \) occurred in the spring but declined during the remainder of the growing season, independently of irrigation treatment. These results provided indirect evidence that, in both irrigation treatments, \( g_{\text{s}} \) was not the main determinant of the decrease in photosynthetic capacity (Jifon and Syvertsen 2003) observed in our almond trees.

A negative correlation was observed between \( A_{\text{ma}} \) (area- and dry-mass-based values) and \( W_{\text{a}} \) in both classes of leaves, which seemed to be independent of irrigation regime (Figure 8A and B). Higher values of \( A_{\text{ma}} \) in FI trees than in DI trees could be related to differences in \( W_{\text{a}} \) (Figure 4A), i.e., to differences in leaf thickness (\( W_{\text{a}} = \text{leaf thickness} \times \text{leaf density} \)). Thicker leaves are likely to have more chloroplasts per unit of leaf area (Rieger and Duemmel 1992, Evans and Porter 2001) and therefore higher photosynthetic capacity.

Our results demonstrated that maximum assimilation varied considerably over time with leaf age (Wilson et al. 2000), and that this seasonal effect dominated over other factors such as leaf class and soil water availability. This finding is supported by the data showing that \( N_{\text{ma}} \), which is often used to describe the effects of leaf age (Field and Mooney 1983, Reich et al. 1991), explained between 70% and 92% of the seasonal variability in both \( A_{\text{ma}} \) and \( A_{\text{mw}} \) (Figure 9A and B).

The marked decline of PNUE\(_{\text{ma}}\) throughout the growth season (Figure 10A), irrespective of the irrigation treatment, and the concomitant decrease in \( A_{\text{p}} \) (Figure 7D) together suggest the seasonal dependence of N allocation within the leaf. It is known that the allocation patterns of chlorophyll and Rubisco can vary with leaf ontogeny (Porter and Evans 1998, Rey and Jarvis 1998, Wilson et al. 2000). The finding that seasonal changes in \( A_{\text{ma}} \) and...
both leaf classes exhibiting rather similar patterns of between leaf class and irrigation regime was limited, with in photosynthetic capacity. Although the interaction DI trees were not responsible for the seasonal reduction of the active vegetative growth stage. The mild conditions of soil water deficit imposed on the leaves was unaffected by a lower photosynthetic capacity at the start of the growing season (Figure 11), which might support the hypothesis advanced by Wilson et al. (2000) of a seasonally dependent fractional allocation of leaf N to Rubisco.

**Effect of soil water status imposed by the irrigation strategy**

Lower $A_{ma}$ and $A_{nu}$ in f-leaves of DI trees (17% and 15%, respectively) than in FI trees prevailed throughout the spring (Stages II and III) despite several heavy rainfall (Figure 2) that maintained soil water near field capacity in both irrigation treatments. This pattern, along with the lower crown leaf area of DI trees compared with FI trees (see section Materials and methods), suggested a carryover effect of DI on whole-tree potential for $CO_2$ uptake and resource acquisition. Thus, after 4 years of sustained DI, the DI trees appear to have acclimated to water stress but at the cost of a lower photosynthetic capacity at the start of the active vegetative growth stage.

We found that the photosynthetic capacity of almond leaves was unaffected by a ~100% decline in predawn leaf water potential, likely indicating acclimation to the sustained conditions of mild water stress (minimum value of $ψ_{pd}$ about $-1$ MPa) imposed during the previous 4 years. The mild conditions of soil water deficit imposed on the DI trees were not responsible for the seasonal reduction in photosynthetic capacity. Although the interaction between leaf class and irrigation regime was limited, with both leaf classes exhibiting rather similar patterns of $A_{ma}$ and $N_a$ in both irrigation regimes, the f-leaves were more responsive to soil water deficit than the f-leaves, with the responses being mediated through leaf structural changes (see Figure 6).

We conclude that, under the DI regime we applied, leaf structural adjustments appeared to operate to maintain a similar allocation of N resources in the leaves of fruit-bearing shoots, but to the detriment of N resources allocated to vegetative shoots. Foliar structural changes, along with changes in nitrogen partitioning within the leaf – which might be related to concomitant decreases in photosynthetic leaf N concentration and the fractional allocation of leaf N to Rubisco – were probably responsible for the decrease in photosynthetic capacity throughout the growth cycle.

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**References**


Seasonal changes in almond photosynthetic parameters


