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Effects of shoot girdling on photosynthetic capacity, leaf carbohydrate, and bud abscission in pistachio (*Pistacia vera* L.)

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Abstract

The effects of shoot girdling on stomatal conductance (g_s), leaf photosynthesis (P_N), concentrations of carbohydrates, nitrogen and chlorophyll (Chl) in leaves, areal leaf mass (ALM), the diameter and length of shoots, and bud abscission in pistachio were investigated. Girdling individual shoots at the base of the current year's shoot (girdle I), separating inflorescent buds on the terminal current year's shoot from the developing fruits on the previous year's shoot, reduced inflorescent bud abscission by 70% in comparison to nongirdled controls. Girdle I significantly reduced concentrations of nitrogen in leaves but increased those of nonstructural carbohydrates particularly of starch. Shoot diameter increased by 13.1% and 26.4% at 33 and 81 days after girdling (DAG), respectively, compared to 1% and 3.4% in the control, respectively. Both the leaf dry mass/fresh mass ratio and ALM were increased significantly by girdle I from 12 DAG. The concentrations of Chl *a*, Chl *b*, Chl (*a+b*), as well as the ratio of Chl *a/b*, all decreased with girdle I. The greatest negative effect of girdle I was on g_s and P_N. P_N was reduced by 55% of its initial value and was 44% less than in the control leaves at 10 DAG, and fell to approximately 30% that of the control from 21 DAG. In contrast, girdling at the base of one-year-old shoots (girdle II), thus not separating fruits from the inflorescent buds, did not significantly affect g_s or P_N. The effect of girdling on P_N and the possible factors that are involved in the reduction of photosynthesis in pistachio are discussed.

Additional key words: areal leaf mass; biennial bearing; chlorophyll; net photosynthetic rate; nitrogen; starch; stomatal conductance; sugars.

Introduction

Alternate bearing in pistachio (*Pistacia vera* L.) has been extensively researched; however, the causal mechanisms are still unknown, thus it remains a significant problem in commercial production. Crane and Nelson (1971) reported that the presence of fruit, and in particular seed growth, is responsible for inflorescent bud abscission and for inducing a biennial bearing pattern. The more fruit present on shoots or branches, the more buds drop (Porlingis 1974). Experiments with fruit removal resulted in inflorescent bud retention (Porlingis 1974, Wolpert and Ferguson 1990, Caruso *et al.* 1992, Vemmos 2005). It was initially suggested that competition between inflorescent buds and the developing ovaries and seeds for nutrients and assimilates was responsible for bud abscission (Crane and Nelson 1972). Later studies, however, showed that nutrient deficiency is not the primary agent of bud abscission, although the role of N and K needs further investigation (Crane and Al-Shalan 1977, Porlingis 1974, Vemmos 1999a, Wolpert and Ferguson 1990, Vemmos 2005).

The possible role of hormones such as abscissic acid (ABA) and gibberellin-like substances (Takeda and Crane 1980, Lin *et al.* 1984), ethylene production by inflorescent buds (Vemmos *et al.* 1994) and auxin application (Crane and Nelson 1972, Pontikis 1990) have also been studied, but none of these plant hormones have been directly implicated in bud abscission.

The role of carbohydrates in flower bud abscission has been extensively investigated. Although Crane *et al.* (1976) and Crane and Al-Shalan (1977) suggested there was no relationship between carbohydrates and bud drop, Takeda *et al.* (1980) found that inflorescent buds on defruited trees accumulated twice as much ^{14}C-photosynthate as those on fruiting trees, indicating that carbohydrate deficiency is possibly responsible for bud drop. Nzima *et al.* (1997) found that more starch was accumulated in current and one-year-old wood and inflorescent buds of nonfruiting ('off') than fruiting ('on')

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Abbreviations: ALM – areal leaf mass; Chl – chlorophyll; C_i – intercellular CO_2 concentration; DAG – days after girdling; DM – dry mass; FM – fresh mass; g_s – stomatal conductance; N – nitrogen; P_N – net photosynthetic rate; T_{leaf} – leaf temperature.

year trees. Similarly, Vemmos (1999b) found that inflorescent buds of defruited trees accumulated much more starch and sucrose than those of fruiting trees, suggesting a possible role of sucrose in bud drop. The importance of sucrose has been confirmed by Baninasab and Rahemi (2006), who also found a negative correlation between carbohydrate content and bud abscission. The important role of carbohydrates was also shown by Spann *et al.* (2008), who found that mobilization of carbohydrates from stem wood of 'on' trees during the primary period of kernel fill corresponded with the period of inflorescent bud abscission.

Girdling of shoots or branches causes a series of physiological changes above the ring, such as reduced growth, accumulation of carbohydrates or other organic substances, and affect the net photosynthetic rate (P_N) and the hormonal balance of the shoot (Goldschmidt and Huber 1992, Hartman *et al.* 2002, Zhou and Quebedeaux 2003). Thus girdling has often been used as a horticultural practice to manipulate tree growth, flower initiation, fruit set and development, fruit size, yield and quality (Goldschmidt *et al.* 1985, Schaper and Chanko 1993, Goren *et al.* 2003). The effect of girdling on carbohydrate concentration, however, is variable, depending on the size of branches, the presence of fruit, the time of application, the size of the girdle, as well as the kind of tree (Goldschmidt *et al.* 1985, Schaffer *et al.* 1985, Mataa *et al.* 1998, Zhou and Quebedeaux 2003).

Girdling has been used to control biennial bearing in olive (Lavee *et al.* 1983) and in mandarin (Goldschmidt *et al.* 1985, Mataa *et al.* 1998). In pistachio, girdling between inflorescences and current shoots before bud abscission resulted in a significant reduction of bud drop (Crane and Nelson 1972, Vemmos 2005). In contrast, girdling at the base of one-year-old shoots had only little or no effect on flower bud retention, suggesting that girdling of shoots or branches with fruit on cannot be used for the control of biennial bearing in pistachio (Vemmos 2005).

Girdling usually reduces P_N; the reduction is dependent on the size of the branches and the girdle, as well as the time of application, but mainly depends on the presence and size of photosynthetic 'sinks', such as flowers or fruits (Proietti and Tombesi 1990, Schaper and Chacko 1993). Roper and Williams (1989) found that trunk girdling of grapevine reduced P_N and increased the carbohydrate content in leaves. Similarly, Schechter *et al.* (1994a,b) found an accumulation of starch and low P_N in girdled nonfruiting apple limbs, but the reduction of P_N in girdled fruiting limbs was much smaller (only 10%). They concluded that feedback inhibition of photosynthesis was not true for apple. Ferree and Palmer (1982) found a negative effect of girdling on apple leaf P_N but only for 15 days after its application (during the flowering period) – there was no effect after this time. Zhou and Quebedeaux (2003) found a significant decrease in P_N (by 3-fold) in girdled shoots of apple within 7 days after the treatment. Simultaneously, starch concentrations increased 10-fold, while soluble sugars showed a smaller increase.

The regulation of photosynthesis by girdling has been investigated to an extent in several types of plants but there is no consensus as to the mechanism(s) involved (Neales and Incoll 1968, Goldschmidt and Huber 1992, Krapp and Stitt 1995, Zhou and Quebedeaux 2003). In the case of pistachio, data are particularly lacking.

The purpose of this study was to investigate the effect of shoot girdling on: (*1*) some morphological characteristics of leaf and shoot in pistachio, (*2*) Chl concentrations and P_N in leaves, (*3*) soluble sugar, starch and nitrogen concentrations in leaves, and (*4*) the possible relationship between leaf morphology and changes in other measured parameters caused by girdling in relation to P_N and bud abscission.

Materials and methods

The experiments were carried out at the orchard of the Agricultural University of Athens on pistachio (*Pistacia vera* cv. Aegenes) grafted on *Pistachia terebinthus* cv. Tsikoudia rootstock. All trees used were over 40 years of age and were in an 'on' year.

Experiment 1

Five trees were randomly selected in the spring of 2001 and eight shoots on each tree were chosen for uniformity in length, diameter and number of fruitescences. Two treatments were applied to four shoots each: (a) control; (b) girdle (I) – a ring was removed from the base of the current year's shoot to separate it from the fruitescences (Fig. 1). Current year's shoots were selected to have no lateral shoots and a ring of bark 8–10 mm wide was removed using a sharp knife. The cut areas were immediately covered with a mixture of wax and resin and sealed with plastic tape. The treatment was applied on 4th June prior to the onset of the second phase of bud abscission. Four replications of each treatment were applied to each tree. One control shoot and a girdled shoot of each replicate were selected very close to each other to comprise a pair of shoots on which P_N and bud abscission were measured.

Net photosynthetic rate (P_N), stomatal conductance (g_s), and intracellular CO_2 (C_i) measurements: P_N, g_s, and C_i were measured in the field on six different dates. The first measurement was taken before girdling on 4th June and the rest at 10, 21, 36, 57, and 78 days after girdling (DAG). P_N was measured by using a portable closed infrared gas analysis system (*model LI-6200*; *LiCOR Inc.*, Lincoln, NE, USA). Air flow rate into the infrared gas analysis system was 500 µmol s^{-1}, photosynthetically active radiation was 1,570 ± 130 µmol m^{-2} s^{-1}. Air and leaf temperature (T_{leaf}), as well as relative humidity (RH), were also recorded. All measurements were taken in the same top leaflet of the middle leaf node of each shoot between 10:00 and 14:00 (Vemmos 1994).

Leaf sampling: One leaflet per experimental shoot was randomly collected from each control and girdled shoot for each tree between 09:00 and 10:00 h (to minimize the effect of diurnal changes) on 0, 10, 21, 57, and 81 DAG and placed in an ice-box for transfer to the laboratory. Samples were not taken from leaves on which P_N was to be measured. The four leaflets collected for each treatment from each tree were considered as one sample per tree for analysis for Chl, carbohydrates, and nitrogen.

Chl (*a*+*b*) and areal leaf mass (ALM): Ten discs (0.23 cm^2) were taken from each leaflet, weighed and ground with purified sand in 5 ml of 80% acetone/water solution (v/v), using a pestle and mortar. After filtration through Whatman No. 1 paper, the extraction was repeated with another 5 ml of acetone solution. The combined filtrates were made up to 50 ml with the same 80% acetone solution. Chl *a*, Chl *b* and Chl (*a*+*b*) were determined by Arnon's (1949) method using a *Spectronic 70* spectrophotometer (*Bausch and Lomb,* Rochester, NY, USA). Another 10 leaf discs (0.23 cm^2) from the same leaflets were taken, weighed and dried for three days at 75°C. The leaf discs were then reweighed and ALM was calculated.

Nonstructural carbohydrate analysis in leaves: The remainder of the leaflets were cut in slices, weighed, put in Petri dishes and immediately transferred to a freezer (−30°C). After 24 h, they were put in a freeze-dryer for 2–3 days. After this time, leaves were reweighed, ground using a pestle and mortar and stored at −30°C until analysed.

Extraction, separation and detection of sugars using HPLC: Dry material (40–50 mg) was put into a centrifuge tube, then washed with 5 ml petroleum ether (40–60°C) and the sugars extracted with 80% ethanol according to Vemmos (1999b). HPLC-grade water (1.0–2.0 ml; *BDH*, Poole, Dorset, UK) was added to the residue in a centrifuge tube to dissolve the sugars. Charcoal (10–12 mg) was added to decolourize the solution, which was mixed well and then centrifuged at 3,000 rpm (×1,408 *g*) for 5 min. The supernatant was removed for HPLC analysis. A sample of 20 µl was injected onto a 250 × 4 mm *HC-75* Ca^{2+} column (*Hamilton Company*, NV, USA) at 65–70°C. The mobile phase of HPLC-grade water was supplied by a *Hewlett-Packard 1050* isocratic pump (*Hewlett Packard*, *Walbronn Analytical Division*, Germany) at 0.8 ml min^{-1}. After leaving the column, separated sugars were detected using a refractive index detector (*Hewlett-Packard HP 1047A*; *Hewlett Packard*, *Walbronn Analytical Division*, Germany).

Extraction of starch and glucose estimation: The solid residue left in the centrifuge tube after the removal of soluble material was washed and recentrifuged four times using 80% ethanol (3, 3, 3, and 5 ml; respectively). Starch extraction was performed using the method of Dekker and Richards (1971) as modified by Vemmos (1999b). One millilitre of 0.5 M NaOH was added to the dry material for starch gelatinisation, and starch was finally converted to glucose with 1.0 ml amyloglucosidase solution (*Sigma Chemical Inc.,* St. Louis, MO, USA) in acetate buffer (pH 4.5).

Glucose concentration was determined using the glucose oxidase (GOD)-peroxidase (POD) method of Barham and Trinder (1972) as modified by Vemmos (1999b). An aliquot (0.5 ml) of the supernatant containing the extracted glucose solution was transferred to a test tube and 2.0 ml GOD-POD reagent (*GOD/PAP*; *Biosis*, Athens, Greece) added. The solution was mixed and placed in a water bath at 37°C for 15 min. The absorbance of the red colour produced was measured at 510 nm using a *Spectronic 70* spectrophotometer (*Bausch and Lomb,* Rochester, NY, USA).

Total sugar concentrations were estimated by summing the concentrations of the individual sugars detected by HPLC. Total carbohydrate concentrations, *i.e.* total nonstructural carbohydrate concentrations, were estimated by summing total sugar and starch concentrations.

Nitrogen analysis in leaves: Nitrogen was determined from 0.5 g of dried leaflet sample using the Kjeldahl procedure and the total N concentration was measured using a Kjeltec *Auto 1030 Analyzer* (*Foss Tecator* AB, Hoganas, Sweden).

Bud abscission, shoot length and diameter: The percentage of flower buds abscised from the trees was recorded on all experimental shoots (control and girdled) at intervals to estimate the percentage bud abscission. Bud abscission was recorded once every 10 or 15 days. Shoot diameter was measured between the first and second flower bud from the base of the shoot by using callipers, and shoot length with a ruler. The first measurement was taken before the application of the girdle, the second at 33 DAG, and the third 81 DAG for diameter and 64 DAG for shoot length.

Experiment 2

In order to test whether the presence of fruit above the girdle had any influence on P_N, the following experiment was carried out; only parameters regarding photosynthesis were taken (namely, P_N, g_s, C_i, and RH). The same trees as those used in Experiment 1 were used. Six shoots on each tree were chosen for uniformity in length,

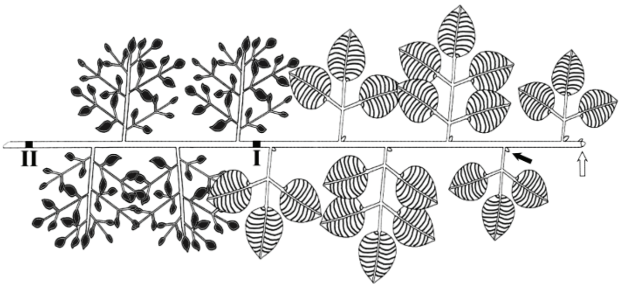

Fig. 1. Schematic representation of the location of girdle I (at the base of current year's shoot) and girdle II (at the base of one-year-old wood) on branches of pistachio. The closed arrow indicates an inflorescent bud, the open arrow indicates the terminal bud.

diameter and number of fruitescences. Two treatments were applied to three shoots each: (*1*) control; (*2*) girdle (II) – a ring at the base of one-year-old wood was made using the same method as that for girdle I (Fig. 1). Thus, in contrast to experiment 1, fruitescences were not separated from the inflorescent buds on the same shoot. The treatment was applied on 26th of July. Three replications of each treatment were applied to each tree. Measurements were taken in the same top leaflet of the middle leaf node of each shoot between 10:00 and 14:00 h. The first measurement was taken on 26th of July and just before the application of girdle II. Another three measurements followed (5, 15, and 26 DAG, respectively).

Statistical analysis: The study was carried out using a randomized blocks design and mean differences were examined for statistical significance using the paired *t*-test; differences were examined at P=0.05 05 (indicated in figures by asterisk). Appropriate transformation (arcsin squared) was performed on data as necessary to provide a normal distribution before analysis of variance was carried out. Linear regression analysis for the correlation of measured parameters with P_N and with bud retention was carried for data regarding girdle I. Statistical analyses were carried out using *JMP 8.0* (*SAS Institute*, Cary, NY, USA).

Results

Effect of girdle I on shoot growth and bud abscission: Fig. 2*A* shows that shoot diameter increased significantly in girdled shoots, while that of the control shoots initially increased slightly and then decreased. The increase in diameter was 13.1% at 33 DAG and 26.4% at 81 DAG, when it became significantly greater than in the control shoots. Shoot length was not affected by girdling (data not shown).

Shoot girdling significantly reduced the rate of bud abscission compared to the control shoots after the application of girdle I (Fig. 2*B*). Thus, the percentage of bud abscission was 29% on girdled shoots at 81 DAG compared to 96% on the untreated shoots at the same time.

Effect of girdle I on leaf characteristics related to the photosynthetic capacity: Fig. 3*A* shows that ALM on treated shoots increased rapidly after girdling until 57 DAG and then remained stable. In contrast, it increased slightly on the control shoots until 21 DAG but increased rapidly from 57 DAG on. Thus, after girdling, ALM on girdled shoots was significantly higher than that on the control shoots. Similarly to ALM, the ratio of DM/FM in leaves of girdled shoots increased rapidly after the treatment and became significantly higher than that of the control shoots (Fig. 3*B*).

The Chl (*a*+*b*) concentrations in leaves decreased in both treated and control shoots (Fig. 4*A*). However, the reduction was much higher in leaves of girdled shoots and the Chl (*a*+*b*) concentrations became significantly lower than those in the control leaves. Similar results were obtained for the individual concentrations of Chl *a* and Chl *b* (data not shown).

The ratio of Chl *a/b* also decreased after girdling in both treated and control shoots, but the reduction was higher in leaves of girdled shoots. This resulted in a lower Chl *a/b* ratio in girdled shoots compare to control shoots (Fig. 4*B*).

Effect of girdling on P_N, g_s, and C_i: P_N decreased rapidly in leaves of girdled shoots after the application of girdle I (Fig. 5*A*). Thus, P_N decreased by 55% from initial values and became 44% less than in the untreated leaves at 10 DAG. P_N continuously decreased in leaves of girdled shoots, reaching a third one of the corresponding control leaves at 21 and 36 DAG. P_N in the control leaves was significantly higher from 10 DAG, continuing to be so throughout the whole period of measurements. Similarly, g_s decreased rapidly in girdled shoots (girdle I), while that of the control remained almost stable throughout the experimental period (Fig. 5*B*). For the treated leaves, g_s fell by 57% and 76% at 10 and 21 DAG, respectively, remaining relatively stable thereafter; g_s of leaves of the control shoots was much higher than that of the girdled shoots. Girdle I significantly enhanced C_i in treated leaves (Fig. 5*C*) but this effect was apparent later than that on P_N and g_s (at 36 DAG).

In contrast to girdle I, girdle II did not significantly affect P_N (Fig. 6*A*) or g_s (Fig. 6*B*). However, P_N of leaves of treated shoots was slightly lower than of leaves of the control shoots at 5 and 15 DAG. Differences in C_i between treated and control shoots were also not significant (Fig. 6*C*).

RH measurements: The RH measured in the leaf chamber used for photosynthesis measurements was significantly lower for shoots treated with girdle I than

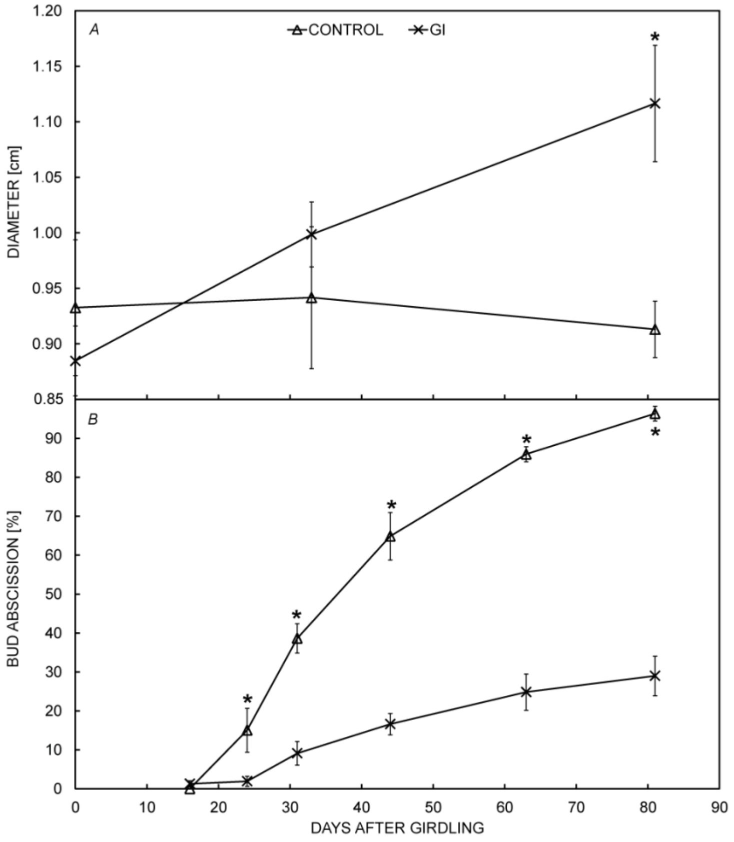

Fig. 2. The effect of girdle I on shoot diameter (*A*), and inflorescent bud abscission (*B*). Data are the mean values ± SE (*n* = 5).

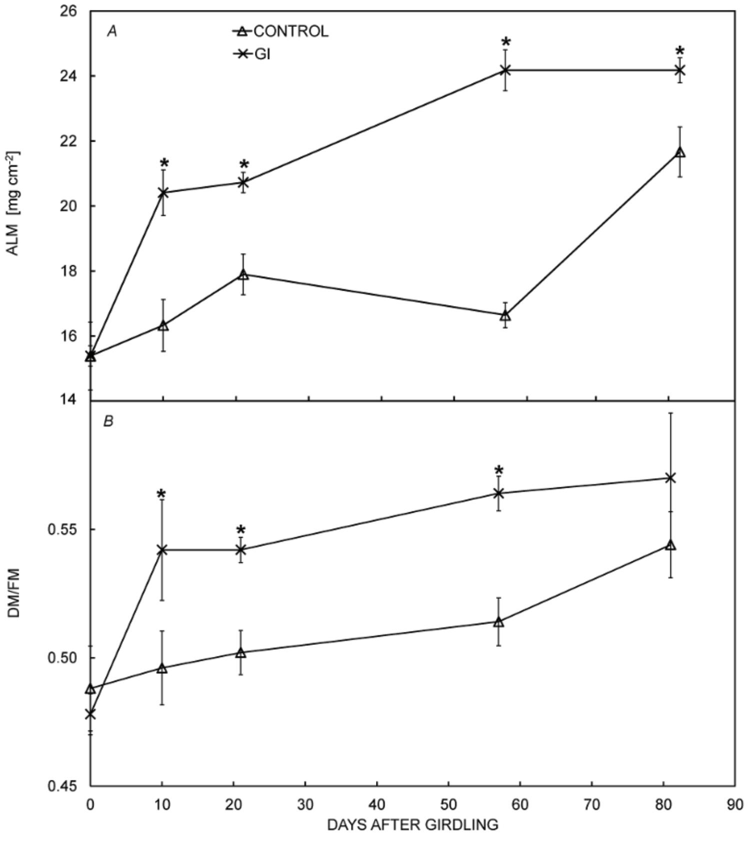

Fig. 3. The effect of girdle I on areal leaf mass (ALM) (*A*), and the ratio of DM/FM (*B*). Data are the mean values ± SE (*n* = 5).

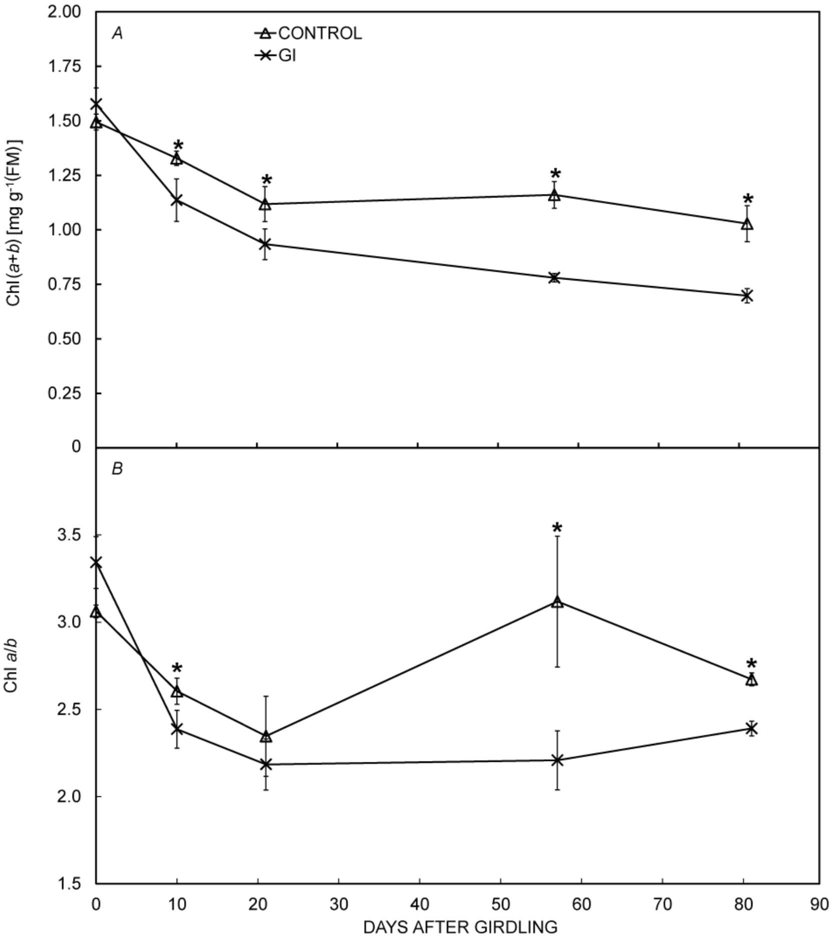

Fig. 4. The effect of girdle I on Chl (*a*+*b*) concentration (*A*) and Chl *a/b* ratio (*B*) in leaves. Data are the mean values ± SE ($n = 5$).

for the control shoots (Fig. 7*A*). In contrast with girdle I, girdle II did not affect RH, with the exception of the last date of measurement (Fig. 7*B*).

Effects of girdle I on N and carbohydrate concentrations in leaves: The concentration of N in leaves decreased in both girdled and control shoots but the reduction was higher in leaves of girdled shoots (Fig. 8*A*). Thus, the N concentrations were significantly lower in leaves of girdled shoots at 10 and 21 DAG but they became similar to those of the control shoots from that time on.

Initially, the concentrations of sucrose were significantly higher in leaves of girdled shoots at 10 and 21 DAG compared to those in the control shoots, becoming similar from then on (Fig. 8*B*). Glucose and fructose concentrations in leaves were much lower than those of sucrose (0.5–1.1% DM) and were very similar in girdled and control shoots throughout the period of measurements (data not shown). Total sugar concentrations followed the same pattern of changes as those of sucrose (Fig. 8*C*). Thus, the leaves on girdled shoots had significantly higher concentrations than the control shoots at 10 and 21 DAG.

Starch concentrations in leaves of girdled shoots increased rapidly after girdling, becoming 2- and 3.5-fold higher at 10 and 21 DAG than their initial values (Fig. 8*D*). In contrast, starch concentrations in leaves of the control shoots remained relatively stable throughout the experimental period. Thus, the leaves in girdled shoots had significantly higher starch concentrations (2.5- and 3.2-fold higher at 21 and 57 DAG) compared to the control. The changes in the concentrations of total nonstructural carbohydrate in leaves followed the same pattern as those for sucrose concentrations (data not shown). The concentrations after girdling, however, were always higher in leaves of girdled shoots than those of the control shoots.

Regression analysis: The analysis of linear regression between P_N and various parameters (Table 1) showed that P_N was significantly and positively correlated with g_s, Chl *a*, Chl *b*, Chl (*a*+*b*), Chl *a/b*, and N concentration in leaves. In contrast, significant negative correlations of P_N with C_i, T_{leaf}, and starch concentration were found. Bud retention was only significantly associated positively with starch concentration and percentage increase in shoot diameter (Table 2).

Discussion

The main purpose of this study was to investigate the effects of girdling on some leaf characteristics and physiological parameters and the possible relation of these changes to the photosynthetic capacity of leaves. It is well known that girdling affects carbohydrate concentrations and P_N in leaves above the ring. The effect, however, is markedly dependent on the presence of fruit and on shoot size. Thus, girdling has been used for the control of alternate bearing in some fruit trees (Schechter *et al*. 1994b, Mataa *et al*. 1998, Goren *et al*. 2003, Li *et al*. 2003). Unfortunately, this is not the case of pistachio, since girdling for pistachio shoots below the fruit position (*i.e.* girdle II) did not affect flower bud abscission or biennial bearing (Vemmos 2005). In contrast, girdling individual shoots above the fruit position (girdle I) resulted in significant bud retention

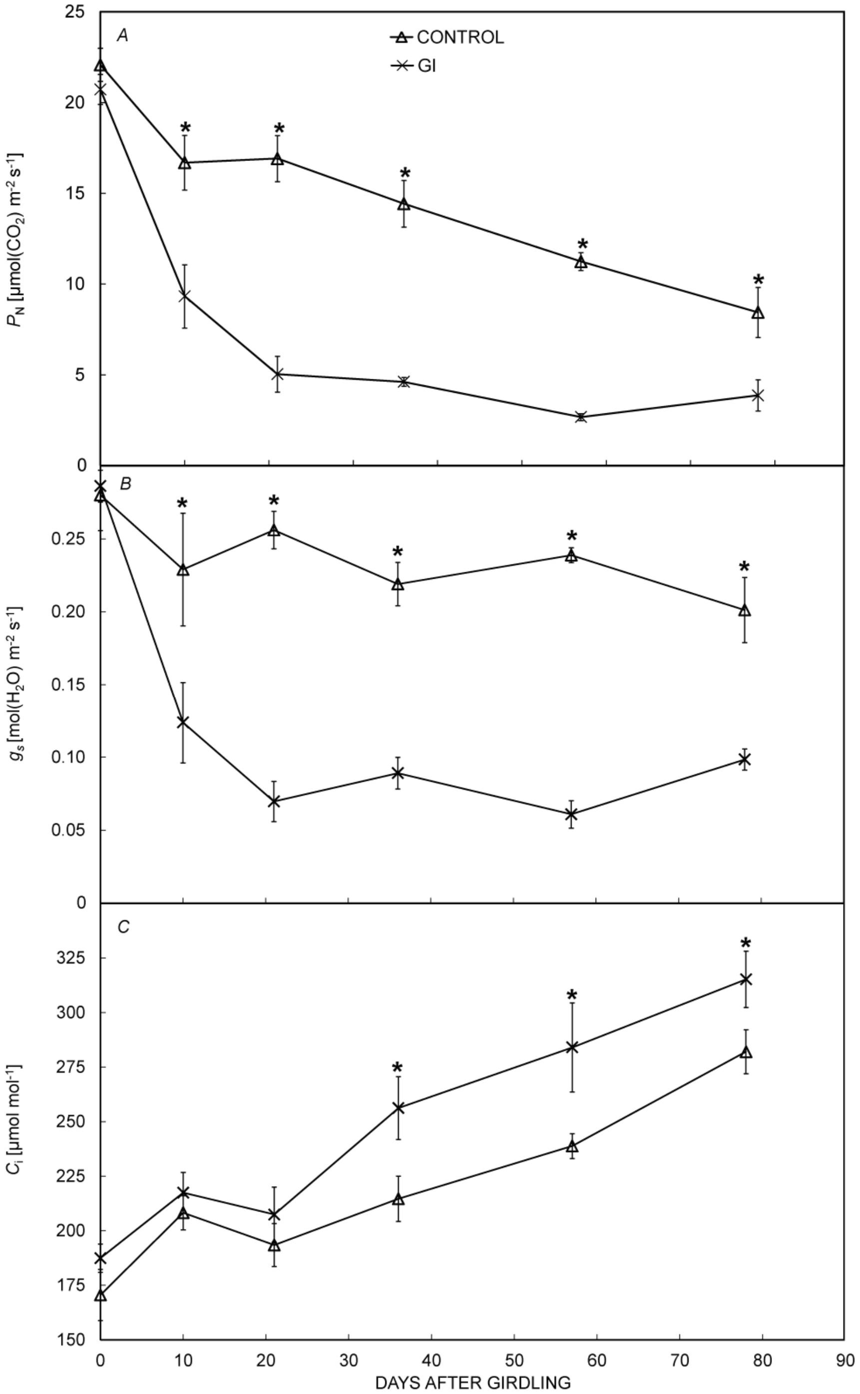

Fig. 5. Effect of girdle I on net photosynthesis (P_N) (*A*), stomatal conductance (g_s) (*B*), and intracellular CO$_2$ concentration (C_i) (*C*) in leaves. Data are the mean values ± SE ($n = 15$).

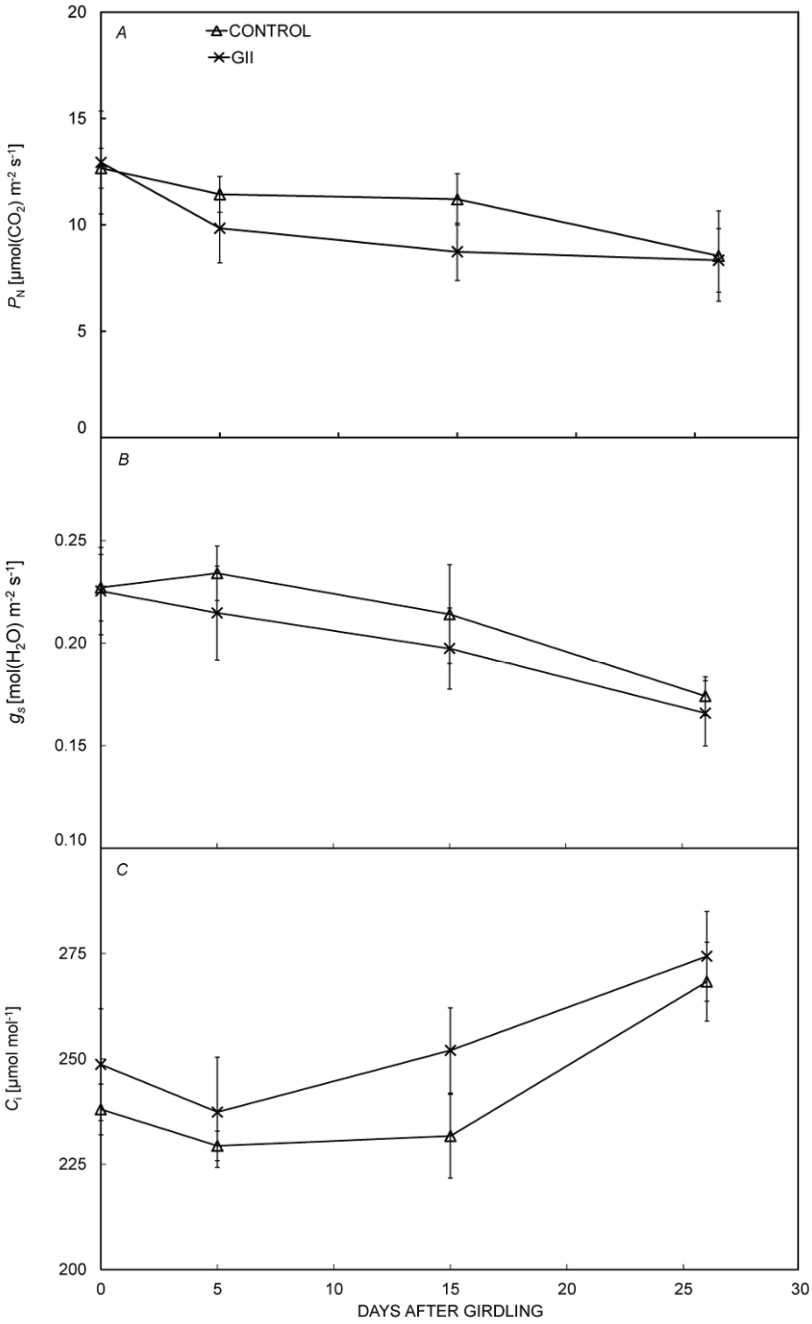

Fig. 6. Effect of girdle II on net photosynthesis (P_N) (*A*), stomatal conductance (g_s) (*B*), and intracellular CO_2 concentration (C_i) (*C*) in leaves. Data are the mean values ± SE (*n* = 15).

(Crane and Nelson 1972, Vemmos 2005). These results were confirmed in this study (Fig. 2*B*). However, this treatment is very difficult, if not impossible, to apply in a commercial setting for large trees such as pistachio in order to actually control biennial bearing.

Since it has been found that carbohydrate concentrations increased in all tissues above girdle I (Vemmos 2005), it was interesting to investigate the effect of girdling on the photosynthetic capacity of leaves. The results of the present experiments showed that leaf characteristics that might affect the photosynthetic capacity in leaves were all affected by girdle I. Thus, nitrogen, Chl *a*, Chl *b*, Chl (*a*+*b*) concentrations and Chl *a*/*b* were reduced by girdle I. In contrast, ALM and the DM/FM ratio in the same leaves increased after girdling, due possibly to nonstructural carbohydrate accumulation, in particular of starch and sucrose. Similar results for Chl and ALM after girdling have been found by other researchers (Schechter *et al.* 1994b, Prioetti and Tombesi 1990). Chls are directly related to the capacity of leaves for CO_2 assimilation (Schaper and Chacko 1993) and their reduction in leaves could directly affect P_N. Additionally, leaf senescence is usually related to a decline in Chl, protein, and hormone levels (Andersen and Brodbeck 1988, Schaper and Chacko 1993). This is reflected in the present study, where girdle I accelerated

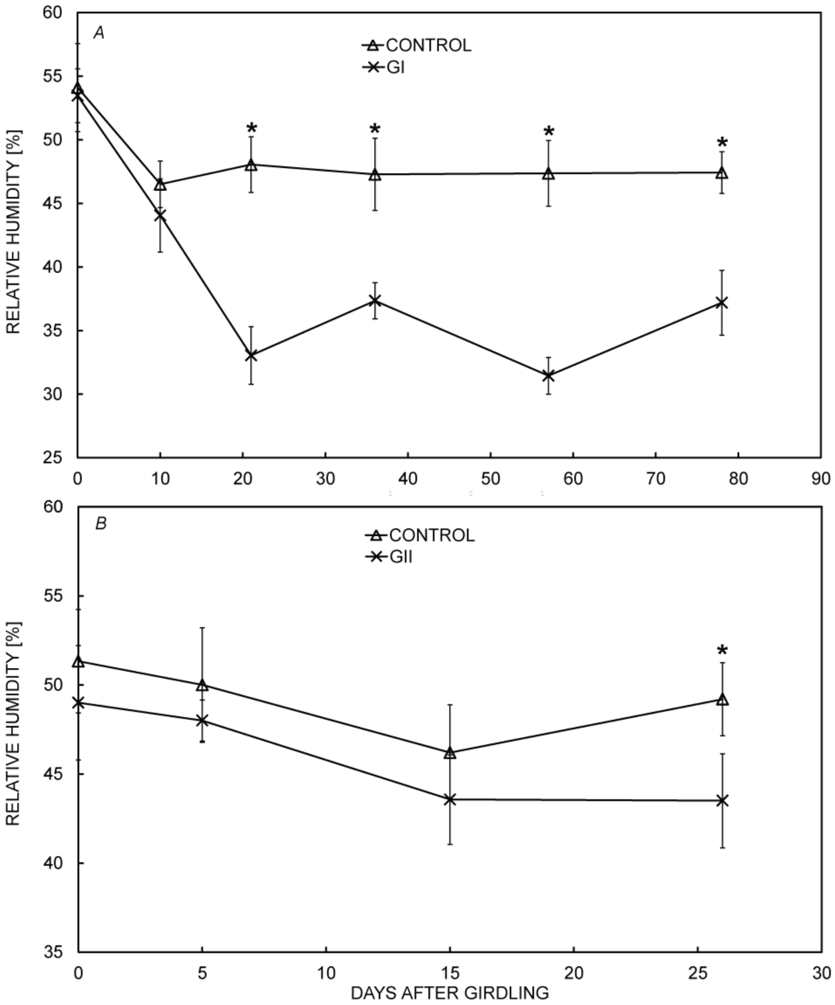

Fig. 7. Relative humidity (RH) during measurement of P_N for leaves of shoots treated with girdle I (*A*) and those treated with girdle II (*B*). Data are the mean values ± SE ($n = 15$).

leaf senescence, as shown by the reduction of Chl and the discolouration of leaf blades in leaves of girdled shoots (change from green to yellow) that was observed.

The relation of ALM and the DM/FM ratio to P_N has not been extensively investigated. However, it has been reported that ALM was negatively correlated with P_N in apple (Monselise and Lenz 1980, Schechter *et al*. 1994b. In contrast, a positive correlation of ALM with P_N has been reported for shade treatments in apple (Barden 1977), peach (Nii and Kuroiwa 1988), and olive (Gregoriou *et al.* 2007). The results of this study (Table 1) showed a negative correlation of ALM with P_N in pistachio. This differential effect of ALM on P_N may be due to simultaneous changes in leaf morphology and physiology induced by the different treatments (shading and girdling) in these studies. The increase in shoot diameter in girdled shoots can also be attributed to the accumulation of nonstructural carbohydrate in leaves and consequently in shoots (Vemmos 2005). The fact that shoot length was not influenced by girdle I is probably due to the time of girdling (June), since the shoots had already reached their final size.

In contrast to ALM, N concentrations decreased in leaves of girdled shoots. This is likely due to the increased ALM in the same leaves. Similar results for N have been reported for apple (Priestley 1976 and Schechter *et al.* 1994b), citrus (Schaffer *et al.* 1986), and pistachio (Vemmos 2005). A positive linear correlation between N concentrations and P_N was found in this study ($r = 0.793$, $P \leq 0.001$, Table 1). This indicates the importance of N for photosynthesis and shows it to be one possible factor affecting P_N after girdling.

The increased starch and sucrose concentrations in leaves of girdled shoots confirm previous findings in pistachio (Vemmos 2005) and are similar to those of Goldschmidt *et al.* (1985), Schaffer *et al*. (1985) and Mataa *et al.* (1998) in citrus, Schechter *et al*. (1994a) and Zhou and Quebedeaux (2003) in apple, Li *et al*. (2003) in nonfruiting shoots of citrus, and Proietti and Tombesi (1990) in olive. However, they are not in agreement with those found in fruiting shoots or limbs of various other kinds of trees (Garcia-Luis *et al.* 1995, Mataa *et al.* 1998, Li *et al*. 2003, Urban *et al*. 2004), indicating the high demand for photosynthates by the growing pistachio fruits, which appears to inhibit carbohydrate accumulation in leaves.

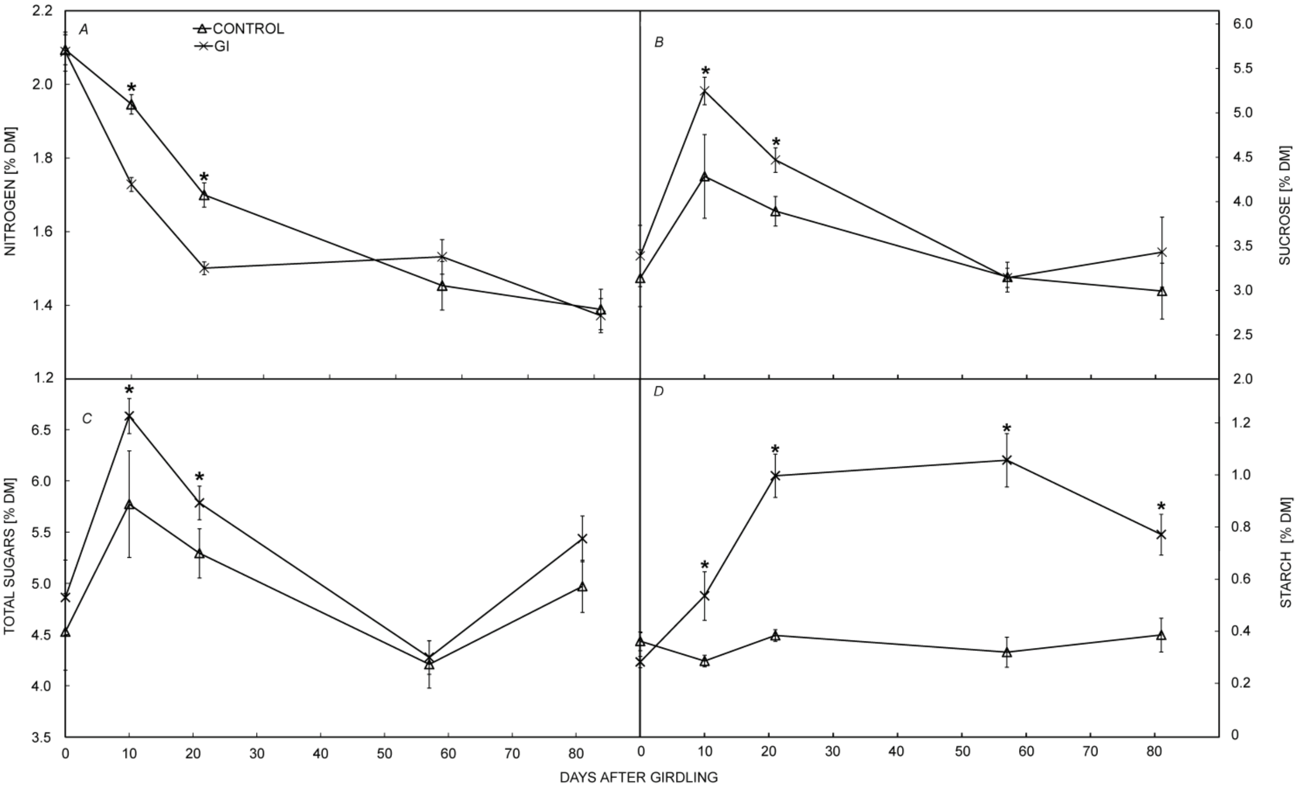

Fig. 8. Concentrations of nitrogen (*A*), sucrose (*B*), total sugar (*C*) and starch (*D*) in leaves of shoots treated with girdle I. Data are the mean values ± SE ($n = 5$).

Table 1. Regression analysis between net photosynthetic rate (P_N) and other parameters. r – correlation coefficient.

P_N *vs*.	r	P-value	Equation	n
g_s	0.905	<0.0001	$P_N = -0.0513 + 60.266 \times g_s$	180
C_i	−0.627	<0.0001	$P_N = 34.842 - 0.101 \times C_i$	180
T_{leaf}	−0.547	<0.0001	$P_N = 70.785 - 1.784 \times T_{leaf}$	180
RH	0.815	<0.0001	$P_N = -16.788 + 0.658 \times \text{RH}$	180
Chl *a*	0.818	<0.0001	$P_N = -6.721 + 22.729 \times \text{Chl } a$	30
Chl *b*	0.652	<0.0001	$P_N = -8.138 + 64.397 \times \text{Chl } b$	30
Chl (*a* + *b*)	0.810	<0.0001	$P_N = -8.706 + 18.235 \times \text{Chl } (a + b)$	30
Chl *a*/*b*	0.503	0.0002	$P_N = -5.462 + 6.565 \times \text{Chl } a/b$	30
Nitrogen	0.793	<0.0001	$P_N = -20.494 + 19.237 \times \text{N}$	30
ALM	−0.808	<0.0001	$P_N = 41.922 - 1.5617 \times \text{SLW}$	30
Sucrose	−0.038	0.7912	$P_N = 12.871 - 0.285 \times \text{sucrose}$	30
Starch	−0.714	<0.0001	$P_N = 20.245 - 15.490 \times \text{starch}$	30
Total sugars	−0.078	0.5887	$P_N = 14.746 - 0.567 \times \text{totalsug}$	30
Total carbohydrates	−0.294	0.0383	$P_N = 23.128 - 1.979 \times \text{totalcarb}$	30

Table 2. Regression analysis between percentage bud retention (BR) and other parameters. r – correlation coefficient.

BR *vs*.	r	P-value	Equation	n
Final diameter	0.162	0.4938	BR = 23.923 + 23.104 × final diameter	20
Increased diameter	0.710	0.0004	BR = 21.656 + 1.9967 × increased diameter	20
Starch	0.866	<0.0001	BR = 0.0212 + 69.337 × starch	20
Total carbohydrate	0.311	0.1822	BR = −0.024 + 12.729 × totalcarb	20
Sucrose	0.182	0.4413	BR = 15.023 + 10.448 × sucrose	20
Total sugars	−0.021	0.9286	BR = 53.081 − 1.031 × totalsug	20

P_N and g_s in leaves were significantly reduced by girdle I at 10 DAG and remained very low, and much lower compared to the control until 78 DAG (Fig. 5*A*,*B*). In contrast, girdle II, although reducing P_N and g_s slightly at 5 and 15 DAG, did not have a significant effect; the values for control and girdled shoots became very similar at 26 DAG (Fig. 6*A*,*B*). The initial sharp drop of P_N and g_s (at 10 DAG) with girdle I coincides with the high increase in sucrose and in particular starch concentration in leaves. This indicates that girdle I leads to carbohydrate accumulation in leaves (Fig. 8*A*,*B*) and other parts of the shoot above the ring (Vemmos 2005) as the result of the prevention of carbohydrate movement to the growing fruit and other parts of the tree below the girdle. It is known that when the rate of assimilate production is higher than their consumption, it might result in increased source/sink ratio, with a consequent reduction in P_N. This is possibly one of the main reasons for P_N reduction by girdle I. A similar negative effect of girdling on P_N and g_s has been found for other species (Roper and Williams 1989, Proietti and Tombesi 1990, Schechter *et al.* 1994a,b; Proietti 2003, Zhou and Quebedeaux 2003) on girdled shoots or branches. Girdle II, although preventing the carbohydrate movement from leaves to other parts of the shoot below, does not separate the fruit from the leaves. Since the fruits are very strong sinks of assimilates, they can inhibit carbohydrate accumulation in leaves and keep P_N at a similar rate to that of the control by consuming the majority of carbohydrates produced by leaves. Unfortunately, no carbohydrate analysis was carried out in leaves for girdle II. Takeda *et al.* (1980), however, found that the majority (>50%) of ^{14}C fed to leaves was moved and accumulated in the developing fruit, with very little going to the other parts of the shoot, while a significant amount of ^{14}C was incorporated into other parts of the shoot when the fruit had been removed. Proietti and Tombesi (1990) and Proietti (2003) found similar results in olive (with no reduction in P_N and g_s) after girdling with the presence of fruit on the shoots, which was attributed to the inhibition of assimilate accumulation in leaves. It might be concluded that the presence of fruit is the main reason that girdle II did not significantly reduce P_N and that the presence of developing fruit is one of the main factors that regulates photosynthesis in leaves after girdling. The high demand of pistachio fruits for assimilates was also shown by Vemmos (1994) who found that the presence of fruit increased the photosynthetic activity in leaves. P_N and g_s values found in this study for the control shoot are very similar to those found earlier for pistachio by Vemmos (1994), Guerrero *et al.* (2005), and Ghazvini *et al.* (2007). Similar values for g_s were also found in olive (Proietti 2000) and in *Mangifera indica* L. (Urban *et al.* 2005).

In contrast to P_N and g_s , C_i was unaffected by girdle I until 21 DAG and significantly increased from 36 DAG on for both treatments, becoming significantly higher in leaves of girdled shoots. Girdle II also increased C_i, although the effect was not significant. The higher C_i in leaves of girdled shoots might be explained by the smaller quantity of CO_2 used for photosynthesis after girdling. However, the fact that C_i generally increased during the period of measurements for both types of girdling is possibly due to the decreased P_N and g_s at the same period. The correlation of P_N with g_s was strongly positive for girdle I (Table 1) and positive correlation was also found for girdle II (data not shown). In contrast, the correlation of P_N with C_i was negative for both girdling treatments. It is well known that P_N is usually affected by g_s and a reduction in g_s is followed by a decrease in P_N but not always to the same extent. However, our results have shown that a reduction in P_N was correlated with an increase in C_i independently of girdle treatment. Thus, the decrease in P_N found in this study cannot be attributed to stomatal closure and to limitation of g_s. It seems that the reduction in g_s is possibly caused by the decrease of photosynthesis rather than *vice versa.* The possible factors that are related to P_N reduction after girdling are extensively discussed below. The reduced capacity of the mesophyll for CO_2 assimilation (Sharkey 1985) is possibly another reason for the increased C_i. Similar results for the increased C_i after girdling have been found by other researchers (Proietti and Tombes 1990 and Proietti 2003 in olive; Schechter *et al.* 1994a,b in apple; Krapp and Stitt 1995 in spinach). In contrast, Zhou and Quebedeaux (2003) found that C_i decreased after girdling in apple, in parallel with the reduction in P_N and g_s, indicating that the reduction in g_s reduced C_i and that stomatal limitation was mainly responsible for P_N reduction. Urban *et al.* (2004), however, did not find any effect of girdling or the presence of fruit on C_i of *M. indica* L. Conclusively, girdle I resulted in a sharp and considerable decrease of P_N, while girdle II only slightly reduced P_N and this is mainly due to the effect of fruit not being separated from the leaves and flower buds in this case. This is also the reason for the nonsignificant effect of girdle II on bud abscission compare to girdle I (Vemmos 2005).

The mechanisms by which girdling regulates photosynthesis in pistachio are still unknown; however, there are a few hypotheses for the mechanisms involved in other fruit trees. The first hypothesis maintains that end-product (carbohydrate) accumulation above the ring is a possible reason for the reduction of leaf P_N above the ring (Neales and Incoll 1968). Goldschmidt and Huber (1992), however, suggested that this regulation is possibly related to the nature of sugar metabolism in the cytosol that results in down-regulation of the Calvin cycle. Krapp and Stitt (1995) suggested down-regulation of genes for proteins involved in photosynthesis. More recently, Zhou and Quebedeaux (2003) suggested that inhibition of photosynthesis induced by girdling is mainly due to stomatal limitation rather than to carbohydrate accumulation *per se*.

Starch accumulation may alter chloroplast structure and function (Schaffer *et al.* 1986), resulting in a significant reduction in photosynthesis. The important role of starch in feedback regulation of photosynthesis has been well emphasized (Goldschmidt and Huber 1992, Paul and Foyer 2001). It seems that in pistachio, starch plays an important role in regulating photosynthesis after girdling as the main end-product accumulated in leaves. However, the mechanisms that are involved in this process need further investigation. This study showed a direct increase of starch concentration in pistachio leaves after girdle I and a strong negative correlation between starch accumulation and P_N (Table 1, $r = -0.714$). In contrast, no correlation between sucrose and P_N was found. A strong correlation between high starch content and low P_N has been reported for other species (Goldschmidt and Huber 1992, Paul and Foyer 2001). The much higher starch accumulation than sucrose indicates that starch is the main end-product accumulated in pistachio leaves. Thus, it seems that pistachio, similarly to citrus (Li *et al.* 2003), is a starch storer rather than a sucrose storer. It has been found that feedback inhibition of photosynthesis was greater in starch-storing species, such as cotton and cucumber, than in those species that favoured sucrose synthesis (Goldschmidt and Huber 1992). However, feedback inhibition of photosynthesis has also been reported in low-starch-accumulating species such as wheat (Paul and Foyer 2001). The same authors proposed that only under extreme conditions which combined high CO_2 concentrations, low nitrogen, and water stress is there direct evidence that starch accumulation restricts CO_2 diffusion. The higher C_i and the lower N concentrations in leaves with girdle I found in this study, in combination with the significantly lower RH found in the leaf chamber during measurement of photosynthesis (Fig. 7*A*), and the lower relative water content in the same leaves (as apparent from DM/FM; Fig. *3B*), create conditions that possibly restrict CO_2 diffusion.

It has also been reported that carbon metabolism is linked to N metabolism and changes in carbohydrate abundance impact N metabolism and *vice versa* (Paul and Foyer 2001). Since N content was found to be significantly lower in leaves of girdled pistachio shoots, the mechanisms by which the carbohydrate–N balance might regulate photosynthesis must also be considered. The possibility that carbohydrates act indirectly by repressing the expression of the genes that encode protein needed for photosynthesis (Krapp and Stitt 1995) must also be investigated further in pistachio. For example, Li *et al.* (2003) found that the accumulation of starch in citrus leaves and bark above the girdle was related to high RNA levels and they hypothesized that changes in starch concentration are possibly regulated by genes involved in starch synthesis.

Apart from the factors discussed above that affected P_N in this study, external factors such as RH and leaf temperature may also have been involved in reducing photosynthesis. The RH in the leaf chamber was significantly lower for leaves from shoots after girdle I than for the control leaves (Fig. 7*A*). This indicates a lower transpiration rate in treated leaves, which might have affected P_N. In addition, leaf temperature was significantly higher (1–2°C) in leaves of these girdled shoots (data not shown), probably due to the lower RH in the chamber and lower transpiration rate, as well as the lower water content in these leaves. In contrast, girdle II only slightly reduced RH, with exception of the last measurement, at which RH increased (Fig. 7*B*).

The important role of carbohydrates in bud abscission of pistachio has been demonstrated by several researchers (Takeda *et al.* 1980, Nzima *et al.* 1997, Vemmos 1999b). Baninasab and Rahemi (2006) also found a negative correlation between carbohydrate content and bud abscission. Spann *et al.* (2008) recently found that mobilization of carbohydrates from current season and one- and two-year-old stem wood of ‘on’ trees during the primary period of kernel fill corresponded with the period of inflorescent bud abscission. In this study, it was shown that girdle I promoted bud retention and this was strongly correlated with starch accumulation in leaves ($r = 0.87$, Table 2). It can be concluded that girdle I inhibited carbohydrate mobilization from current season to one-year-old shoots, resulting in carbohydrate accumulation. The fact that bud retention was significantly correlated with increased shoot diameter might also be attributed to carbohydrate accumulation in shoots, as was shown earlier (Vemmos 2005). In agreement with the previous reports, these results indicate once again the major role of carbohydrate, particularly of starch, in bud abscission and in controlling biennial bearing in pistachio.

It was shown that the presence of fruit is a strong assimilate sink in pistachio and therefore regulates the source–sink balance of assimilates within the shoot and controls leaf P_N. Carbohydrate accumulation (especially of starch) in leaves and current wood caused by girdle I increased shoot diameter and reduced inflorescent bud abscission. A feedback mechanism caused by the accumulation of starch in leaves rather than stomatal closure and g_s limitation is possibly the main factor regulating photosynthesis after girdling in pistachio. The possibility that starch accumulation is caused by the restriction of sucrose synthesis or that starch acts indirectly by down-regulating genes that encode proteins needed for photosynthesis, as well as N metabolism and the N/carbohydrate ratio in leaves of girdled shoots, need further investigation in pistachio.

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