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Effect of different leaf-to-fruit ratios on photosynthesis and fruit growth in olive (*Olea europaea* L.)

P. PROIETTI, L. NASINI, and F. FAMIANI

Dipartimento di Scienze Agrarie e Ambientali, University of Perugia, 06121, Perugia, Italy

Abstract

The influence of different leaf-to-fruit (l-t-f) ratios on leaf net photosynthetic rate (P_N) and fruit characteristics in *Olea europaea* L. cv. Frantoio was evaluated in 2001 and 2002. In both years, at the end of June, at the end of July, and in mid-September (first, second, and third time of treatment, respectively), defoliation or fruit thinning were performed to give l-t-f ratios of 1/1, 3/1, 5/1, and 7/1 (about 5.1, 15.3, 25.6, and 35.8 cm^2 of leaf area per fruit, respectively) on girdled and ungirdled peripheral shoots. P_N showed substantial seasonal and diurnal variations. In ungirdled shoots, no differences due to the different l-t-f ratios were observed, whereas in girdled shoots P_N tended to be lower in shoots with a high l-t-f ratio. In general, the values of leaf transpiration rate (E), stomatal conductance (g_s), sub-stomatal CO_2 concentration (C_i), and dark respiration rate (R_D) were associated with those of P_N. The starch and reducing sugar contents and area leaf dry mass (ADM) tended to be higher in leaves on girdled shoots with high l-t-f ratio, whereas in ungirdled shoots no differences related to the different l-t-f ratios were observed. The higher saccharide content in the leaves and the lower P_N, in the presence of a high C_i, observed in girdled shoots with a high l-t-f ratio suggests that the depression in P_N in these shoots may be the result of a feedback inhibition of the photosynthetic mechanism that regulates such a process. The l-t-f ratio did not have a substantial effect on fruit drop. In ungirdled shoots, the different l-t-f ratios did not produce significant differences in terms of fruit growth and leaf dry matter and saccharide contents, whereas in girdled shoots fruit growth increased as the l-t-f ratio increased, particularly when treatments were applied at the initial stage of fruit development. The percentage of oil in the pulp, on a dry matter basis, was not substantially influenced by girdling and l-t-f ratio. The abundant availability of assimilates seemed to cause earlier fruit ripening and, at the same time, retard fruit senescence (fruit detachment force). Shoot growth was slightly reduced by girdling. The abundant availability of assimilates, induced by girdling associated with high l-t-f ratio, stimulated flower induction.

Additional key words: fruit size; gas exchange; girdling; oil content; ripening; source-sink relationships.

Introduction

In order to intensify olive cultivation, a good knowledge of the physiological aspects related to fruit development and oil synthesis is required. In central Italy, blooming, fruit-set, fruit growth, and ripening in olive trees occur from June to November–December on shoots that grew the previous year. The developing fruit is a strong sink that requires a continuous supply of building materials (Proietti 2000). It competes successfully with shoot growth for newly assimilated materials as well as reserves previously accumulated in different tree tissues. Most assimilates are supplied by the leaves on the same shoot where the fruit is attached (Rallo and Suarez 1989, Márquez *et al.* 1990, Proietti and Tombesi 1996). The olive fruit is a drupe in which the mesocarp (pulp), endocarp (pit), and seed accumulate different quantities of dry matter, depending on assimilate availability, during the period of fruit development. The dry matter of the endocarp increases until the end of August and then remains constant, while the mesocarp, after having a limited growth until mid-August, increases in dry matter until mid-December. The seed grows at a constant rate until the end of October and then remains constant (Proietti *et al.* 1994). The relative growth rate (RGR) of the endocarp is faster than that of the mesocarp until mid-August, after which it becomes noticeably slower. Oil synthesis begins in the fruit about 60 d after full bloom and continues without interruption for about 4 months (Duran Grande and Izquierdo Tamayo 1964, Proietti *et al.* 1994). Most oil synthesis occurs in the mesocarp and the final fruit oil content depends on the size of the mesocarp and its percent oil content. Changes in source level at different stages of fruit development can influence the assimilate

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Fax: ++39-075-5856255, e-mail: first@unipg.it

distribution among the different fruit components. The availability of a large quantity of assimilates in July primarily affects endocarp growth, whereas in August through November it affects mesocarp growth and oil synthesis (Proietti 2003). Differences in assimilate availability, due to the crop load on the tree, influence the fruit ripening pattern (Inglese *et al.* 1999). Different branches of the same tree may have different fruit loads and this may contribute to the non-uniform growth and ripening patterns that occur in many olive cultivars (Shulman and Lavee 1979, Inglese *et al.* 1999).

Very few studies have been conducted on the effects of source-sink level on net photosynthetic rate (P_N), fruit growth, and oil synthesis in olive. In particular, little information is available about relationships between leaf number per fruit and fruit growth and characteristics. More information is needed to better understand the biological and physiological processes that control cropping and to optimise cultural practices, particularly pruning.

The aim of the present research was to study the effects of a localised leaf-to-fruit ratio (l-t-f) at different stages of fruit growth in either girdled or ungirdled shoots, on fruit development, oil synthesis, and related processes such as assimilate translocation and P_N. Girdling is used to study the effects of assimilate accumulation in the shoot because, by blocking the phloem continuum, the translocation of photosynthates produced in the leaves is also blocked. In this way, the developing fruit can derive assimilates only from its shoot. If girdling regards only a few and very small branches, like in the present work, it does not cause collateral significant effects on tree general growth and reserve accumulation.

Materials and methods

The trial was carried out in 2001 and 2002 in central Italy (Deruta, 43°N latitude, altitude about 220 m a.s.l.), under natural conditions, in a 15-year-old non-irrigated olive grove of Frantoio cultivar. The soil is clay loam and the trees are trained to the vase system (a trunk 1 m high and 3–4 main branches) with a spacing of 5×6 m. The weather during the summer is usually characterised by little rainfall and high temperatures. In 2001-2, from June to September, the annual average cumulated rainfalls were about 290 mm, the average maximum temperature was 28.5 °C, the average minimum temperature was 14.5 °C.

In both years, at the end of June (initial stage of fruit development), at the end of July, and in mid-September (first, second, and third time of treatment, respectively), defoliation or fruit thinning were performed to give l-t-f ratios of 1/1, 3/1, 5/1, and 7/1 (about 5.1, 15.3, 25.6, and 35.8 cm^2 of leaf area per fruit, respectively) on girdled and ungirdled peripheral shoots. The leaves used to establish the different l-t-f ratios were one-year-old and young fully expanded leaves. Twenty-one shoots, homogeneous in vegetative and productive appearance, were selected each time on three olives (7 shoots per tree) with high fruit load [about 109 g(yield) cm^{-2}(trunk cross-sectional area at 40 cm from the ground)], and were used as replicates for each treatment. Twenty-one girdled and ungirdled shoots, with their natural l-t-f ratios (about 2.1, 2.4, and 2.8 at the first, second, and third time of treatment, respectively) were selected as the control. The girdling (10 mm wide) was made at the shoot base with a two-edged girdling knife; the wound was covered with black PVC tape. The girdlings were maintained for the entire growing season by removing any scar tissue at one-month intervals. All twenty-one replicates were maintained until harvest in order to make all the observations on the fruits. For determining P_N, E, g_s, C_i, and R_D and other laboratory analyses, the leaves had to be detached, which modified the different l-t-f ratios. Therefore extra shoots for each treatment were selected (24 for the first time of treatment application and 4 for the second and third times of treatment).

For each treatment tested in the first time of treatment, about one week after the treatment, at the end of August, and at the end of September, the P_N, E, g_s, C_i, and R_D were measured on 1-year-old leaves selected on the middle part of the shoots (4 replications per treatment). Measurements were taken on cloudless days, from 09:00 to 11:00 in the morning and from 16:00 to 18:00 in the afternoon. One week after the first time of treatment (beginning of July), P_N, E, g_s, and C_i were also taken on young leaves. At the end of September, in the morning, all gas exchange measurements were also taken on leaves of the shoots of the second and third time of treatment.

Leaf P_N, E, g_s, C_i, and R_D were measured using an *LCA-2* portable gas exchange analyser (*Analytical Development Co.*, Hoddesdon, Herts, U.K.) and a Parkinson leaf chamber type *PLC(n)*. The detached leaf was immediately enclosed in the chamber and exposed perpendicularly to sunrays (incoming PPFD 1 400–1 600 μmol m^{-2} s^{-1}). The flow rate of air passing through the chamber was kept at 5 cm^3 s^{-1} [about 10 000 cm^3 s^{-1} m^{-2}(leaf area)]. During gas exchange measurements, the external CO_2 concentration was about 360 cm^3 m^{-3} and the air temperature inside the leaf chamber was 2–4 °C higher than that in the atmosphere (varying from 28 to 35 °C in July and August and from 24 to 29 °C in September). R_D was measured by covering the chamber with a black cloth screen. All measurements were taken under steady-state conditions.

After the gas exchange measurements, the one-year-old leaves were immediately taken to the laboratory in a portable refrigerator for other determinations. Leaf area was measured using a leaf area meter (*Hayashi Denkoh Co.*, model *AAM-7*, Tokyo, Japan). Half of the leaves were then used to determine the area leaf dry mass

(ADM) and water content by drying them to a constant mass in a forced air oven at 90 °C. With the remaining leaves, the reducing sugar and starch contents (Morris 1948) as well as the chlorophyll (Chl) content (Bruinsma 1963) were determined. Chl content was only measured in September.

At harvest (end of October), the fruit detachment force was determined on 100–150 fruits per treatment using a *Carpo* dynamometer. Then the following measurements were made on 3 olive samples per treatment: fresh and dry matter of the whole fruit, pulp, and pit; pulp firmness using an *Effegi* penetrometer with 1.5 mm tip diameter; colour (evaluated by sight by scoring from 1 = epicarp completely green to 6 = beginning of mesocarp pigmentation); pulp/pit ratio; oil content using a *Foss-let 1531* apparatus (*Foss Electric Denmark*).

Fruit-drop was estimated by taking the difference between the number of fruits on the shoot at the time of treatment application and at harvest. The influence of l-t-f and girdling on the current season shoot growth was evaluated by measuring the length of the shoots in October. Flower bud differentiation was estimated by determining the number of inflorescences per node in June 2002 and 2003.

As in the two years taken into account the results of the different treatments showed a similar pattern, to simplify the presentation, the values obtained during the two years of sampling were averaged. Data were subjected to analysis of variance and the means were compared by least significant differences at $p=0.05$.

Results and discussion

Leaf P_N showed substantial seasonal and diurnal variations (Tables 1 to 3). In ungirdled shoots, no differences due to the different l-t-f ratio were observed, whereas, in general, the P_N in girdled shoots tended to be lower in shoots with a high l-t-f ratio. In general, the E, g_s, C_i, and R_D were associated with P_N. The seasonal and diurnal trends of P_N, E, g_s, and C_i were similar to those obtained by Proietti (2000) with the same olive cultivar. The fact that the lower P_N observed in girdled shoots with a high l-t-f ratio were obtained in the presence of a high C_i suggests that the depression in P_N in these shoots could be the result of a feedback inhibition of the photosynthetic mechanism for regulating such a process, as has been observed in some other fruit species (Flore *et al.* 1988, Famiani *et al.* 2000). According to several authors, when the source and sink are manipulated resulting in an increased source/sink ratio, the P_N decreases because, when the production of assimilates is markedly greater than the capacity for their consumption, a sort of stress is produced by the saccharide accumulation in the leaves (Neales and Incoll 1968, Claussen and Biller 1977, Daie 1985, Foyer 1988, Proietti and Tombesi 1990, Wang *et al.* 1998, Iglesias *et al.* 2002, Zhou and Quebedeaux 2003). The lack of differences in P_N at the end of August and in the afternoon could be due to the general depression of P_N determined by the high summer temperatures and little rainfall. In other studies on olive, girdling on branches with fruits did not result in a lower P_N (Proietti and Tombesi 1990, 1996, Proietti *et al.* 1999); however, in these studies branches and not shoots were girdled and therefore the situation was different. In fact, besides having fruits, the branches also had several growing shoot apexes and reserve tissues that were larger than those in shoots. These factors could have reduced the general source/sink ratio and thus avoided a reduction of P_N. In ungirdled shoots with high l-t-f ratio the P_N did not diminish probably due to the demand for assimilates from other sinks, such as roots, shoot apices, fruits on near shoots, and reserve tissues.

In ungirdled shoots the starch and reducing sugar contents and ADM in one-year-old leaves did not show any differences related to the different l-t-f ratio applied, whereas in shoots girdled in the first and second time of treatment application, the starch and reducing sugar contents and ADM tended to be higher in leaves on shoots with high l-t-f ratios (Table 4). This is consistent with the occurrence of a feedback inhibition of P_N in girdled shoots. The lack of substantial differences in ungirdled shoots due to the l-t-f ratio may indicate a high translocatability of saccharides.

The Chl content in the one-year-old leaves was not influenced by girdling or l-t-f ratio. Evidently the increase in starch content, produced by girdling and high l-t-f ratio, was not high enough to reduce the Chl content and, consequently, lower the P_N as observed by Proietti (2003) for the Leccino olive cultivar in branches girdled at the end of fruit growth.

Girdling did not substantially influence the leaf water content and this seems to indicate that water stress was not associated with girdling.

The fruit-drop from girdling time to harvest (end of October) was influenced by girdling. Fruit drop in the control was 13.6 %; it increased to about 20 % with the first time of treatment application, but decreased with the second and third time of treatment application (about –7 and –13 %, respectively). The l-t-f ratio in the first and second time of treatment did not have any substantial effects on fruit-drop, whereas in the third time of treatment the fruit-drop tended to decrease as the l-t-f ratio increased in both girdled and ungirdled shoots (data not shown).

In the shoots girdled in the first and second time of treatment (end of June and July), fruit dry matter was positively correlated with l-t-f ratio; the matter increase tended to reduce when the ratios were greater than 5/1, for the girdling applied at the beginning of fruit growth, or 3/1, for the girdling applied at the end of July (Fig. 1). The first time of treatment had a greater effect than the

Table 1. Effect of first treatment (end of June), on diurnal net photosynthetic rate (P_N) [µmol(CO_2) m^{-2} s^{-1}], transpiration rate (E) [mmol(H_2O) m^{-2} s^{-1}], stomatal conductance (g_s) [mmol(H_2O) m^{-2} s^{-1}], sub-stomatal CO_2 concentration (C_i) [cm^3 m^{-3}], and respiration rate (R_D) [µmol(CO_2) m^{-2} s^{-1}] in one-year-old leaves on girdled and ungirdled shoots during the vegetative season. For each time, in each column means followed by different letters are significantly different at $p \leq 0.05$.

Time		Leaves	Morning					Afternoon			
		per fruit	P_N	E	g_s	C_i	R_D	P_N	E	g_s	C_i
Beginning of July	Girdled	1	7.4 b	4.8 c	123 b	341 a	–1.1 a	3.2 a	3.6 a	102 a	342 a
		2.14	6.6 ab	3.9 abc	100 a	341 a	–1.3 a	4.2 a	4.4 a	124 a	340 a
		3	6.4 ab	3.2 a	82 a	345 a	–1.3 a	2.9 a	3.8 a	103 a	345 a
		5	6.5 ab	3.3 ab	96 a	341 a	–1.2 a	2.9 a	3.4 a	106 a	347 a
		7	5.8 a	3.1 a	85 a	349 a	–1.0 a	3.0 a	4.0 a	102 a	347 a
	Ungirdled	1	7.1 ab	4.7 c	128 b	337 a	–1.5 a	3.8 a	4.5 a	123 a	340 a
		2.14	7.6 b	4.5 c	114 b	336 a	–0.9 a	4.1 a	4.0 a	108 a	337 a
		3	7.7 b	4.4 c	116 b	341 a	–1.0 a	3.5 a	4.0 a	109 a	340 a
		5	6.8 ab	3.9 abc	96 a	345 a	–0.9 a	4.1 a	4.6 a	125 a	338 a
		7	7.0 ab	4.2 bc	119 b	334 a	–1.1 a	3.7 a	4.6 a	122 a	343 a
End of August	Girdled	1	5.3 a	4.8 a	77 a	228 a	–2.0 a	3.2 a	5.8 a	71 a	253 a
		2.14	4.8 a	4.9 a	70 a	225 a	–1.9 a	2.6 a	4.0 a	52 a	265 a
		3	5.4 a	4.7 a	77 a	217 a	–2.1 a	2.7 a	4.3 a	55 a	233 a
		5	4.7 a	3.7 a	65 a	250 a	–2.2 a	3.1 a	4.2 a	63 a	237 a
		7	4.4 a	4.1 a	65 a	245 a	–1.9 a	2.9 a	4.1 a	73 a	240 a
	Ungirdled	1	4.5 a	3.8 a	58 a	246 a	–2.2 a	2.6 a	4.2 a	53 a	269 a
		2.14	4.9 a	3.7 a	59 a	218 a	–2.3 a	2.5 a	4.2 a	55 a	265 a
		3	4.4 a	3.6 a	64 a	216 a	–2.1 a	2.9 a	4.0 a	54 a	242 a
		5	5.4 a	4.8 a	76 a	210 a	–2.2 a	3.0 a	5.7 a	72 a	247 a
		7	5.2 a	4.5 a	78 a	205 a	–1.9 a	2.8 a	5.7 a	71 a	240 a
Mid September	Girdled	1	12.0 ab	5.8 ab	323 a	231 a	–2.1 a	5.7 a	5.0 a	122 a	259 a
		2.14	12.6 b	5.6 ab	309 a	237 a	–1.4 a	6.4 a	5.6 a	124 a	249 a
		3	11.8 ab	5.6 ab	325 a	238 a	–2.2 a	5.5 a	5.3 a	119 a	255 a
		5	12.3 ab	5.6 ab	337 a	229 a	–1.6 a	5.6 a	5.3 a	115 a	249 a
		7	10.5 a	5.3 a	297 a	243 a	–1.7 a	6.3 a	5.4 a	125 a	254 a
	Ungirdled	1	11.5 ab	6.2 b	323 a	244 a	–1.9 a	6.8 a	5.8 a	135 a	248 a
		2.14	11.7 ab	5.9 ab	290 a	247 a	–2.2 a	6.9 a	6.1 a	130 a	249 a
		3	12.2 ab	5.9 ab	331 a	231 a	–1.5 a	5.8 a	6.0 a	125 a	242 a
		5	12.1 ab	5.6 ab	302 a	240 a	–1.6 a	5.5 a	5.2 a	119 a	256 a
		7	12.2 ab	6.0 ab	324 a	238 a	–2.1 a	5.9 a	5.9 a	122 a	251 a

Table 2. Effect of first treatment (end of June), on diurnal net photosynthetic rate (P_N) [µmol(CO_2) m^{-2} s^{-1}], transpiration rate (E) [mmol(H_2O) m^{-2} s^{-1}], stomatal conductance (g_s) [mmol(H_2O) m^{-2} s^{-1}], sub-stomatal CO_2 concentration (C_i) [cm^3 m^{-3}], and respiration rate (R_D) [µmol(CO_2) m^{-2} s^{-1}] in young leaves on girdled and ungirdled shoots one week after treatment. In each column means followed by different letters are significantly different at $p \leq 0.05$.

Time		Leaves	Morning				Afternoon			
		per fruit	P_N	E	g_s	C_i	P_N	E	g_s	C_i
Beginning of July	Girdled	1	8.0 b	4.2 abc	115 c	337 a	3.6 a	3.9 a	107 a	340 a
		2.14	8.0 b	4.1 abc	109 abc	335 a	4.1 a	4.1 a	117 a	340 a
		3	7.1 ab	3.8 ab	97 abc	341 a	2.8 a	3.3 a	97 a	343 a
		5	7.1 ab	3.7 ab	91 ab	340 a	3.0 a	3.8 a	103 a	344 a
		7	6.9 a	4.0 abc	89 a	343 a	4.2 a	4.7 a	119 a	340 a
	Ungirdled	1	8.0 b	4.5 bc	115 c	331 a	3.5 a	4.3 a	107 a	340 a
		2.14	7.0 ab	4.6 c	108 abc	342 a	3.4 a	3.6 a	93 a	341 a
		3	7.4 ab	3.7 ab	96 abc	340 a	3.1 a	3.7 a	91 a	344 a
		5	7.1 ab	3.6 a	94 abc	347 a	3.3 a	3.6 a	94 a	344 a
		7	7.9 ab	4.6 c	112 bc	334 a	3.9 a	4.5 a	114 a	337 a

Table 3. Effect of second and third treatments (end of July and mid September, respectively), on net photosynthetic rate (P_N) [μmol(CO$_2$) m^{-2} s^{-1}], transpiration rate (E) [mmol(H$_2$O) m^{-2} s^{-1}], stomatal conductance (g_s) [mmol(H$_2$O) m^{-2} s^{-1}], sub-stomatal CO$_2$ concentration (C_i) [cm^3 m^{-3}], and respiration rate (R_D) [μmol(CO$_2$) m^{-2} s^{-1}] in one-year-old leaves on girdled and ungirdled shoots at the end of September. For each treatment, in each column means followed by different letters are significantly different at $p \leq 0.05$.

Treatment		Leaves per fruit	P_N	E	g_s	C_i	R_D
Second	Girdled	1	11.1 ab	6.0 b	275 ab	242 a	–2.9 a
		2.4	10.1 ab	5.7 ab	263 ab	241 a	–2.7 a
		3	9.8 ab	5.6 ab	256 ab	242 a	–2.5 a
		5	9.5 ab	5.5 ab	277 ab	246 a	–2.7 a
		7	9.1 a	4.9 a	246 a	248 a	–3.1 a
	Ungirdled	1	11.7 b	5.9 ab	290 b	247 a	–2.7 a
		2.4	9.9 ab	5.6 ab	284 b	258 a	–2.3 a
		3	10.5 ab	5.8 ab	262 ab	252 a	–3.1 a
		5	9.3 ab	5.4 ab	246 a	243 a	–2.7 a
		7	9.8 ab	5.2 ab	246 a	242 a	–2.5 a
Third	Girdled	1	11.0 b	5.3 ab	250 bc	248 a	–3.0 a
		2.8	11.0 b	6.2 bc	251 bc	253 a	–2.4 a
		3	10.9 b	6.2 bc	247 bc	250 a	–2.9 a
		5	9.6 a	4.5 a	210 a	266 b	–2.3 a
		7	9.2 a	4.9 a	225 ab	266 b	–2.8 a
	Ungirdled	1	11.9 b	5.9 bc	273 cd	248 a	–2.0 a
		2.8	11.7 b	5.9 bc	290 d	247 a	–2.7 a
		3	12.3 b	6.5 c	293 d	250 a	–2.2 a
		5	12.3 b	6.6 c	268 cd	254 a	–3.3 a
		7	12.2 b	5.8 bc	267 cd	243 a	–3.3 a

second one. Girdling applied in September did not substantially influence fruit dry matter. In the ungirdled shoots the fruit dry matter increased slightly passing from 1/1 to 3/1 l-t-f ratio, but then remained almost constant. Compared to ungirdled shoots, girdlings applied at the end of June and July increased fruit dry matter with the l-t-f ratio equal to or higher than 3/1 and *vice-versa*. This, according to the results obtained in other fruit species such as *Actinidia deliciosa* (Snelgar and Thorp 1988, Famiani *et al.* 1997) and chestnut (Famiani *et al.* 2000), confirms that under reduced assimilate supply in the shoot, the developing fruit can attract substances from other tree parts and that above a certain assimilate availability in the shoot, fruits on the same shoot are not the strongest sink and assimilates can be attracted by other sinks (fruits in near shoots, vegetative apexes, reserve tissues, *etc.*).

Compared to ungirdled shoots, the pulp dry matter in girdled ones, similarly to the fruit dry matter, was higher when the l-t-f ratio was equal to or higher than 3/1 and *vice-versa*, with the exception of treatments applied in the third time in which girdling was not associated with an increased pulp dry matter (Fig. 1). Evidently, the effect of girdling is reduced after the major period of pulp growth. The influence of increasing the l-t-f ratio on pulp growth was similar to that observed for fruit dry matter. The pit dry matter increased with the higher l-t-f ratios only when girdling was applied in the first time of treatment. The subsequent girdlings did not influence pit growth because it slows down earlier than pulp growth during fruit development (Proietti *et al.* 1994, Farinelli *et al.* 2002). The l-t-f ratio had very little effect on pit growth and was limited to treatments applied in the first time in both girdled and ungirdled shoots.

The different effects of treatments on pulp and pit growth resulted in strong variations in the pulp/pit ratio on a dry matter basis (Fig. 2). In girdled shoots, the pulp/pit ratio increased substantially passing from 1/1 to 5/1 l-t-f ratio and then increased slightly with further increases. The higher pulp/pit ratios were obtained with the 5/1 and 7/1 l-t-f ratios applied at the end of July, when pulp growth was still intense and pit growth was reduced. In the ungirdled shoots, the pulp/pit ratio, in general, was lower than in the girdled shoots and only increased on passing from a 1/1 to 3/1 l-t-f ratio, after which it remained almost constant. The main increase was again observed with the second time of treatment.

The percentage of oil in the fruit, on a dry matter basis, in response to the treatments was similar to that of the pulp/pit ratio because the treatments did not substantially influence the percentage of oil in the pulp on a dry matter basis (Fig. 2). This is in agreement with Lavee and Wodner (2004), who found that oil content in the mesocarp of olive fruits is not dependent on yield or fruit-size.

At harvest the olives were darker and pulp firmness was poorer on the girdled shoots, with respect to the ungirdled ones, with the exception of a 1/1 l-t-f ratio (Fig. 3). The increase of l-t-f ratio induced strong increases in pigmentation and decreased pulp firmness in both ungirdled and girdled shoots. These variations were

Table 4. Effect of girdling and l-t-f ratio on leaf area dry mass (ADM) [g m^{-2}] and contents of reducing sugars, starch, chlorophyll (Chl) [g m^{-2}], and water [%(f.m.)] in one-year-old leaves. For each treatment and time, in each column means followed by different letters are significantly different at $p \leq 0.05$.

Treatment	Time		Leaves per fruit	ADM	Reducing sugars	Starch	Chl	Water content
First	Beginning of July	Girdled	1	189 a	274 abc	72 b		52.2 a
			2.14	194 ab	275 bc	79 c		52.9 a
			3	197 ab	273 abc	85 d		53.5 a
			5	197 ab	291 c	109 e		53.6 a
			7	205 b	290 c	111 e		53.1 a
		Ungirdled	1	196 ab	258 ab	64 a		51.4 a
			2.14	189 a	249 ab	65 a		52.3 a
			3	185 a	243 a	63 a		53.9 a
			5	197 ab	261 abc	61 a		53.1 a
			7	194 ab	254 ab	66 a		53.2 a
	Mid August	Girdled	1	218 ab	290 abc	117 ab		47.5 a
			2.14	205 ab	2868 abc	116 ab		48.4 a
			3	225 b	301 c	121 b		50.4 a
			5	275 c	298 bc	129 b		51.5 a
			7	272 c	302 c	128 b		50.9 a
		Ungirdled	1	196 ab	285 abc	92 a		47.7 a
			2.14	202 ab	278 abc	95 a		47.4 a
			3	191 a	270 a	94 a		51.8 a
			5	206 ab	277 abc	94 a		47.3 a
			7	209 ab	275 ab	95 a		48.1 a
	End of September	Girdled	1	250 a	401 abc	324 a	9.7 a	49.7 a
			2.14	249 a	411 cd	310 a	10.2 a	50.4 a
			3	240 a	397 abc	316 ab	9.7 a	49.0 a
			5	276 b	430 d	355 c	11.1 a	49.3 a
			7	272 b	426 d	358 c	9.7 a	49.4 a
		Ungirdled	1	251 a	390 ab	311 a	8.7 a	52.0 a
			2.14	248 a	397 abc	324 ab	11.0 a	51.6 a
			3	241 a	384 a	309 a	9.5 a	52.7 a
			5	244 a	392 ab	312 a	10.4 a	50.6 a
			7	250 a	404 bc	317 ab	10.2 a	49.8 a
Second	End of September	Girdled	1	243 a	444 c	375 b	9.2 a	51.2 a
			2.4	256 abc	445 c	396 b	10.0 a	48.5 a
			3	267 bc	437 bc	377 b	8.9 a	48.9 a
			5	269 c	463 c	412 c	9.5 a	48.2 a
			7	266 bc	461.c	401 c	9.7 a	49.1 a
		Ungirdled	1	242 a	414 ab	323 a	9.5 a	50.2 a
			2.4	248 a	397 a	324 a	11.0 a	51.6 a
			3	248 a	417 ab	337 a	10.9 a	49.3 a
			5	254 ab	395 a	320 a	9.3 a	48.2 a
			7	248 a	394 a	310 a	9.8 a	48.8 a
Third	End of September	Girdled	1	237 a	434 a	309 a	10.9 a	52.6 a
			2.8	244 a	435 a	319 a	10.1 a	50.7 a
			3	233 a	437 a	317 a	10.2 a	50.6 a
			5	238 a	439 a	327 a	9.0 a	50.3 a
			7	246 a	431 a	312 a	11.4 a	48.8 a
		Ungirdled	1	242 a	414 a	326 a	10.5 a	51.7 a
			2.8	248 a	397 a	324 a	11.0 a	51.6 a
			3	237 a	417 a	330 a	10.9 a	51.0 a
			5	250 a	405 a	318 a	9.8 a	49.9 a
			7	248 a	404 a	313 a	10.6 a	48.5 a

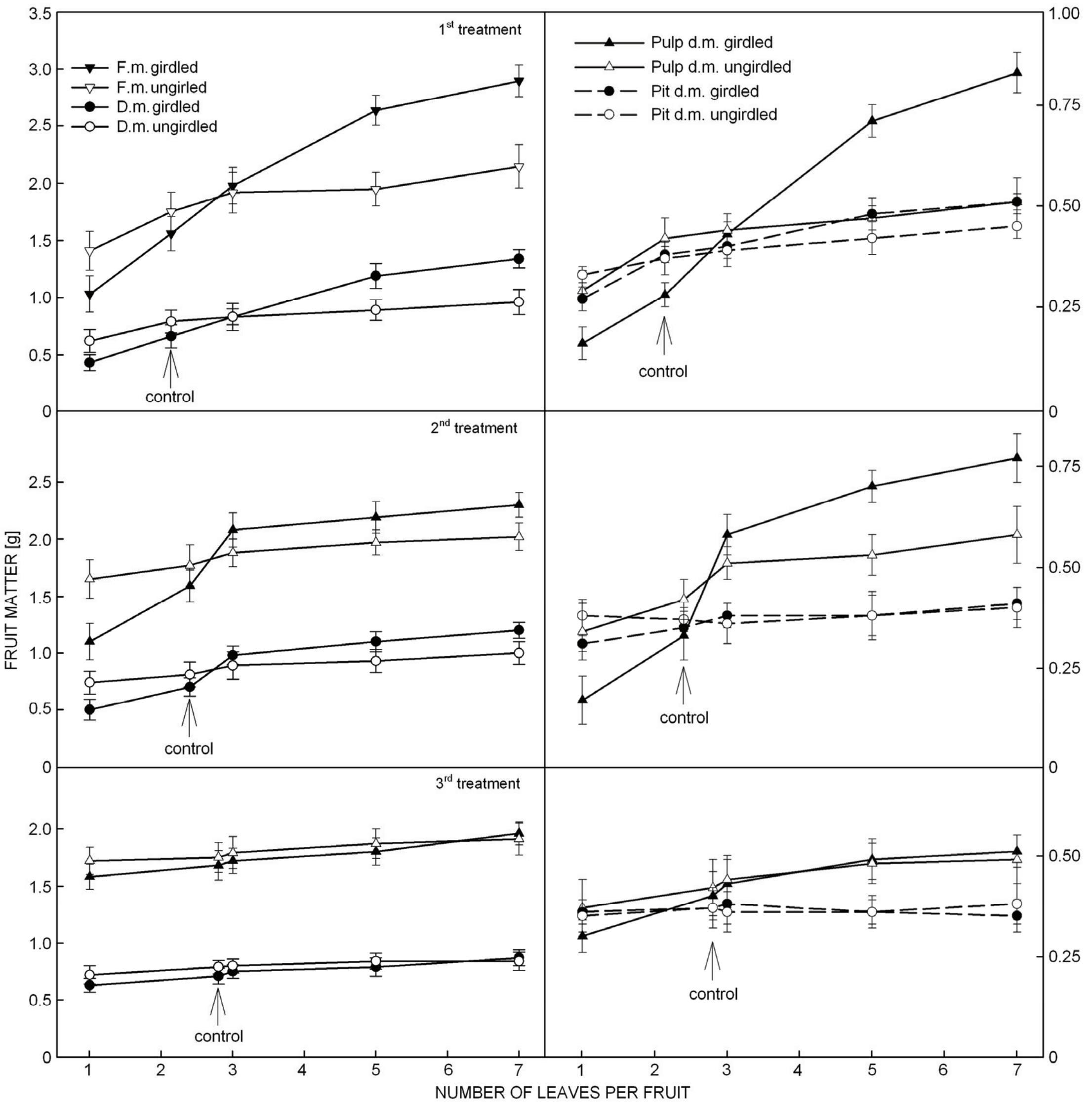

Fig. 1. Effects of treatment time, girdling, and leaf-to-fruit (l-t-f) ratios on fruit (*left*) or pulp and pit (*right*) matter. Means of 3 replicates ± standard error.

great passing from 1/1 to 3/1 l-t-f ratio in the ungirdled shoots, whereas they were moderate for higher l-t-f ratios; in girdled shoots the variations were great also with higher ratios. According to Proietti (2003), the abundant availability of assimilates, particularly beginning with the first phase of the fruit growth, seems to induce earlier fruit ripening. However, an increased l-t-f ratio was associated with a higher detachment force particularly in the girdled shoots (Fig. 4). According to these results, an abundant assimilate availability seems to retard fruit senescence, whereas a low assimilate availability seems to accelerate it. Under low nutrient availability, fruit drop may occur earlier, thus preventing excessive impoverishment of the nutrient reserves.

The effects of the availability of variously located assimilates on fruit ripening was also observed in Leccino and Carolea olive cultivars (Inglese *et al.* 1999, Proietti 2003) and in other fruit tree species (Allan *et al.* 1993, Augusti *et al.* 1998). In accord with the results obtained in other olive cultivars and species (Allan *et al.* 1993, Piller *et al.* 1998, Wilton 2000, Proietti 2003), girdling reduced the current season shoot growth by about 35, 17, and 10 % in treatments applied in the first, second, and third time, respectively. The generally small shoot growth was probably responsible for the lack of differences between the different l-t-f ratios (data not shown).

In girdled shoots, with treatments applied at the end of June, the results regarding the effects of different l-t-f ratios on fruit dry matter at harvest show that the production and allocation of dry matter in the fruits per unit leaf area decreased as the l-t-f ratio increased. On a seasonal basis, in shoots with the l-t-f of 1/1, the amount of dry matter produced and allocated to the fruits by 1 cm^2 of leaf surface was ~84 mg, with the l-t-f ratio of 3/1 ~54 mg, with the l-t-f ratio of 5/1 ~47 mg, and with the l-t-f ratio of 7/1 ~37 mg. The decrease in the allocation of dry matter per unit of leaf surface on the fruit as the l-t-f ratio increases could be explained by a diversion of photosynthates towards the reserve tissues of the shoots, and/or the higher maintenance costs of the fruits, that were bigger in the shoots with a high l-t-f ratio, and/or a depression of leaf P_N. The lower allocation of dry matter to the fruits in shoots with a high l-t-f ratio is not due to a greater elongation of the shoots, since no differences in growth were registered among the different l-t-f ratios applied. Also in the ungirdled shoots, the amount of dry matter allocated to the fruits per unit of leaf surface decreased as the l-t-f ratio increased. In this case, the lower allocation could also be explained considering that assimilate can also be translocated to other shoots and parts of the tree.

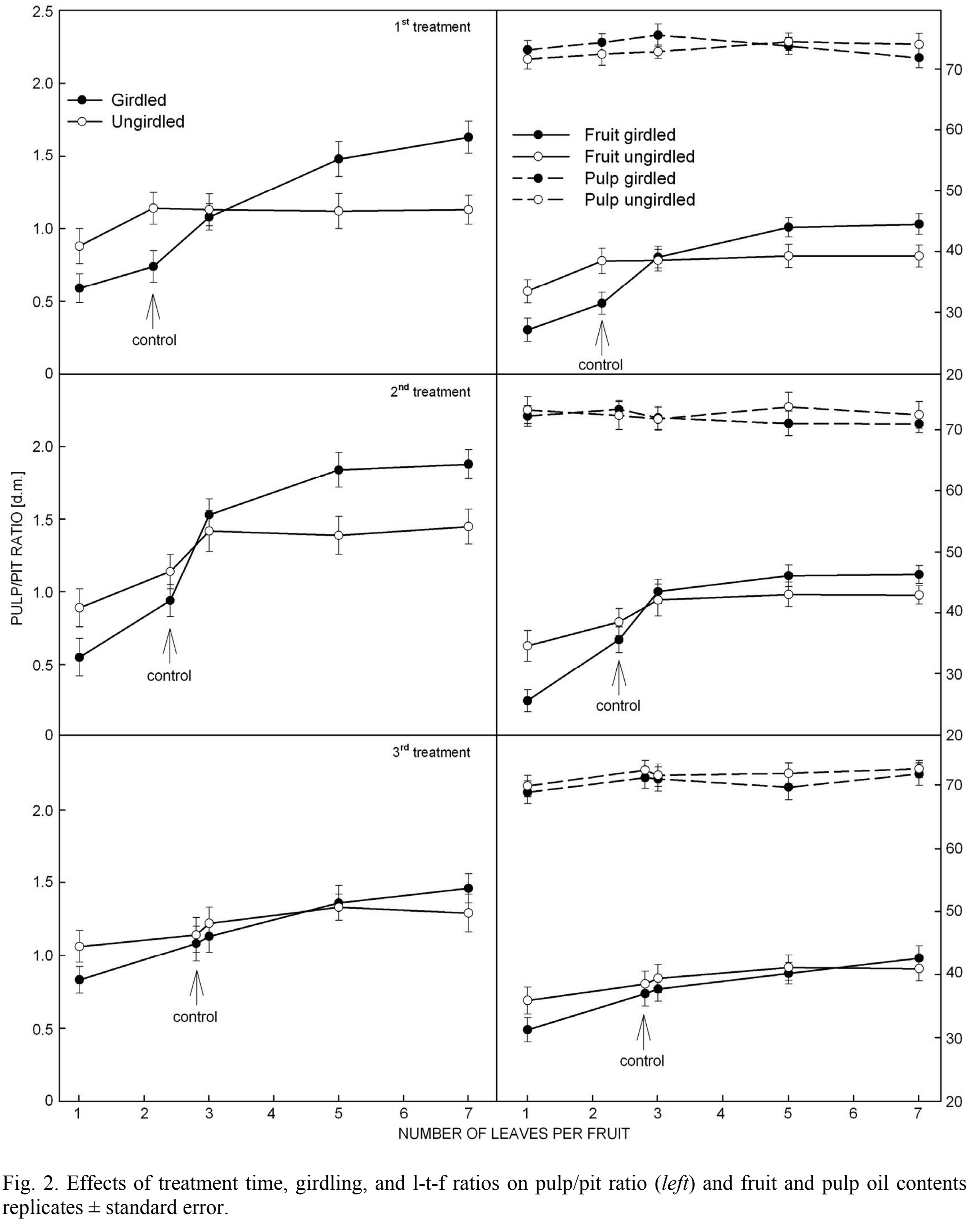

Fig. 2. Effects of treatment time, girdling, and l-t-f ratios on pulp/pit ratio (*left*) and fruit and pulp oil contents (*right*). Means of 3 replicates ± standard error.

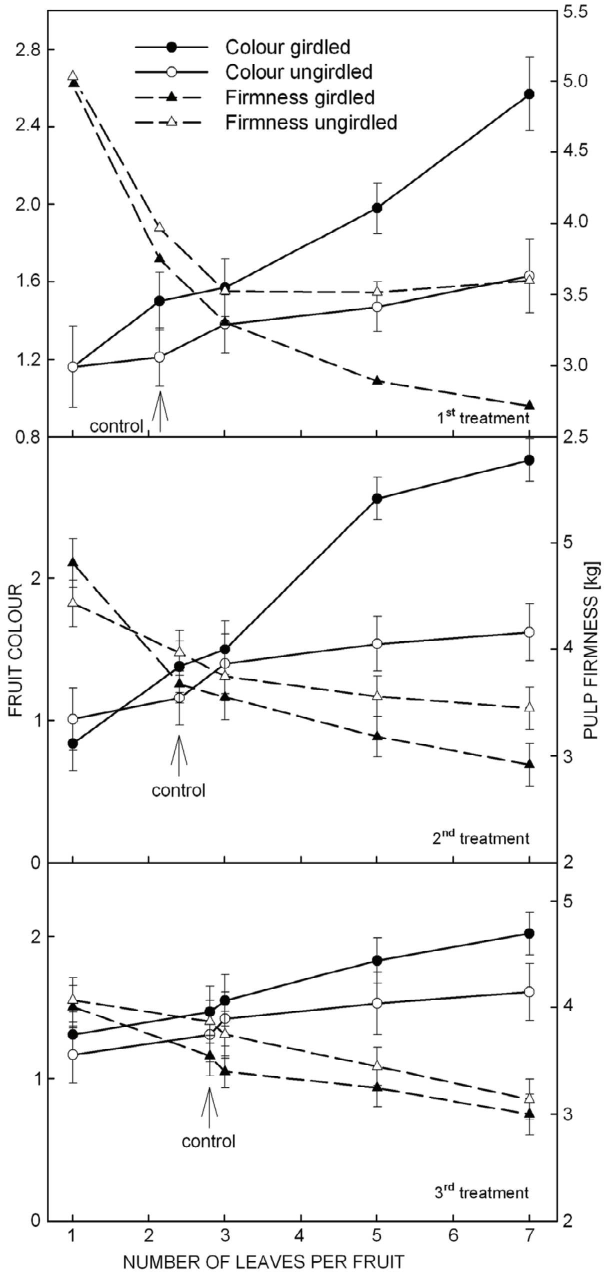

Fig. 3. Effects of treatment time, girdling, and l-t-f ratios on fruit colour and pulp firmness. Means of 3 replicates ± standard error.

In shoots girdled at the beginning and particularly at the end of July, the increase of l-t-f ratio stimulated flower bud differentiation (Fig. 4). This confirms that the abundant availability of assimilates influences flower induction (Sarmiento *et al.* 1976). This effect, however, was small probably because the tree was impoverished by the high production. No effect due to l-t-f ratio was observed on shoots girdled in September and on ungirdled shoots. In the ungirdled shoots, the absence of l-t-f ratio effect on blooming can be attributed to the great competition caused by the high fruit load that impeded a good availability of assimilate in the shoot even with a high l-t-f ratio (Proietti and Tombesi 1996).

As far as basic mechanisms involved in source-sink regulation are concerned, the results mainly confirm the existence of active feedback systems between source and sink tissues, also observed in whole plants, as demonstrated by the fact that a high sink demand may increase P_N and phloem transport of assimilates from source leaves to sinks (Dickson 1991).

In conclusion, in olive trees with a high fruit load, the localised alteration of the assimilate availability in ungirdled shoots, due to the different l-t-f ratio, starting one week after full bloom onwards, had little effect on fruit growth and leaf dry matter and saccharide content. In girdled shoots fruit growth increased as the l-t-f ratio increased but the effect changed in relation to the time of assimilate availability alteration: with more than 5 leaves per fruit, for the treatment starting from one week after fruit-set, and more than 3 leaves per fruit, for the treatments starting at the end of July and mid-September, the increase in fruit dry matter was very small. The results concerning the ungirdled shoots indicate an easy translocability of assimilates. Consequently, high l-t-f ratios make it possible to accumulate more starch in reserve tissues for fruiting in the following year.

In the conditions and cultivar considered, three leaves per fruit (about 15 cm^2 of leaf area) that were well irradiated gave a fruit growth that was similar to control, whereas seven leaves per fruit (about 35 cm^2 of leaf area) almost guaranteed the maximum potential of fruit growth. All the phases of fruit growth were affected by assimilate availability, but the effect of the l-t-f ratio applied at the beginning of fruit growth was stronger than those applied later.

Changes in the availability of assimilates at different times during fruit development caused variations in assimilate distribution between different components of the fruit. In girdled shoots, a higher pulp/pit ratio was obtained with the 5/1 and 7/1 l-t-f ratios. In the ungirdled shoots, where the pulp/pit ratio was lower than in the girdled shoots, it increased passing from 1/1 to 3/1 l-t-f ratio and then remained constant. In every case, the main increase occurred if the treatment was applied when the pulp was growing most actively and the pit growth had slowed down.

The percentage of oil in the pulp, on a dry matter basis, was not substantially influenced by girdling and l-t-f ratio. So the oil production per tree was determined mainly by fruit yield and pulp/pit ratio that are strongly related to fruit size.

The abundant availability of assimilates, particularly if starting from the beginning of the fruit growth, seems to cause an earlier fruit ripening, but also slows fruit senescence and *vice-versa*.

The l-t-f ratio did not have a substantial effect on fruit drop. Therefore, one week after fruit-set onward, the fruit matter more than fruit number was influenced by the

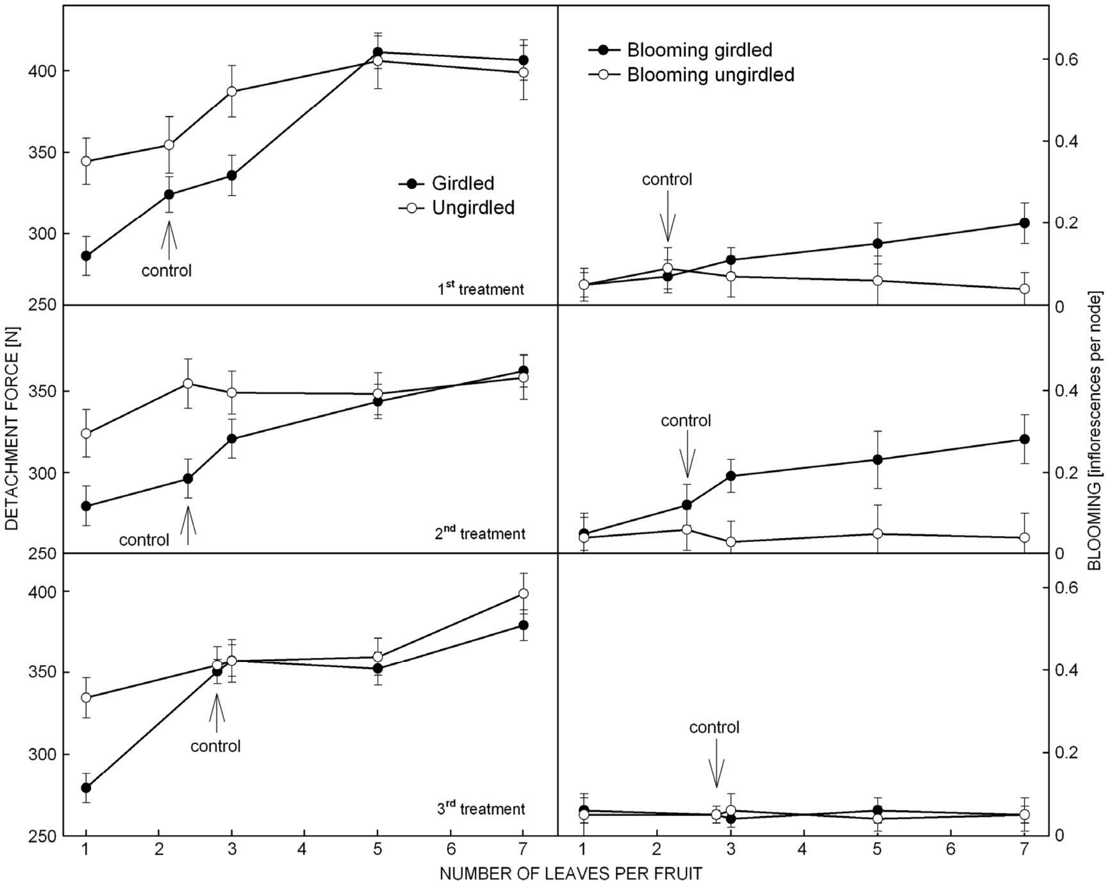

Fig. 4. Effects of treatment time, girdling, and l-t-f ratios on fruit detachment force (*left*) and blooming (*right*). Means of 3 replicates ± standard error.

availability of leaf assimilates. Shoot growth was slightly reduced by girdling whereas the abundant availability of assimilates, induced by girdling associated with a high l-t-f ratio, stimulated flower induction. The lower allocation of assimilates to the fruits (dry matter produced and allocated to the fruits by 1 cm^2 of leaf surface) in girdled shoots with a high l-t-f ratio can be explained not only by a different use of the produced assimilates but also by the lower P_N registered in these shoots, which may be the result of a feedback inhibition. The fact that this effect was not observed on ungirdled shoots with a high l-t-f ratio was probably due to the demand for assimilates by other sinks, such as fruits on other shoots, roots, shoot apices, and reserve tissues. However, considering that in other studies girdling of fruiting branches did not cause a variation in the P_N and that in this study the differences were not always significant, it seems that in olive a feedback inhibition occurs in the presence of high source/sink ratios. Moreover, the results also suggest that the effects of the source/sink ratio on P_N could depend on the interaction between the phenological phase of the trees (the activity of fruits, reserve tissues, vegetative apexes as sinks, changes according to the phase of development) and the climate that varies during the season.

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