

## ALTERNATE BEARING AFFECTS NUTRITIONAL STATUS AND NET ASSIMILATION RATE OF AN IRRIGATED OLIVE GROVE UNDER ARID CONDITIONS

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### ABSTRACT

Alternate bearing is a typical behavior of various fruit tree crops and is common among olive cultivars (*Olea europaea* L. var. *sativa* Hoffm. e Lk.). Since this phenomenon affects yield and consequently oil production, it is a concern for olive oil industry in order to offer a constant olive oil amount each year. A 4-year field experiment was conducted on cv Chemlali olive trees in 'on' and 'off' years in order to study, under arid conditions, both annual macro-element balance and net photosynthetic activity. Shoots growth was much higher in the 'off' year with respect to the 'on' one (+11.70 cm vs. +2.60). Net photosynthesis rate was much higher in the 'off' year with respect to the 'on' year and the highest values were observed at spring, when there is an intense vegetative growth. Differences for the mineral elements were observed between the 'on' and 'off' years. Nitrogen accumulated in leaves mainly in spring in the 'off' year, whereas high N values were detected also at the end of summer in the 'on' year. Consequently, N fertilization can be accomplished in February–March and possibly after harvest (November–December) to be used at budburst in the successive season. A difference was found between leaf P concentrations in 'on' or 'off' years from flowering to fruit-set, with the lowest values in the 'on' year, in particular in June–July. A supply of P at autumn–winter and partially in spring–summer in particular in an 'on' year would be appropriate. K accumulated in leaves in 'on' year from spring to summer, thus a supply of K in January–February, before new vegetation, for trees either in 'on' or 'off' year can be necessary for supporting and implementing the root system activity and the successive shoot growth ('off' year) and fruit development ('on' year). These results should be useful to partially reduce, through an appropriate fertilization schedule, the alternate bearing in olive tree.

**Key words:** macronutrients; 'on' and 'off' year, mineral elements, fruit load

### INTRODUCTION

Olive is an important crop cultivated in arid and semi-arid regions with Mediterranean climate [Vossen 2007, Clodoveo et al. 2014]. Olive trees can

withstand long periods of drought and survive in very sparse plantings as very large size trees even in arid and semi arid climates [Famiani et al. 2014]. In re-

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cent years, the olive cultivation has been extended to irrigated and fertilized areas with super-intensive cropping systems [Camposeo and Godini 2010, Godini et al. 2011]. Many olive cultivars are characterized by alternate bearing which can be considered a homeostatic mechanism of individual trees to balance between reproductive efforts and survival requirements for vegetative growth [Goldschmidt 2005]. The year of high fruit load is defined as 'on', while the year with low fruit load is defined as 'off'. The developing seeds of olive are known to inhibit summer vegetative growth and to prevent floral induction in existing buds [Lavee 2006]; therefore, after an 'on' year, trees lack sufficient floral buds for the consequent reproductive cycle [Dag et al. 2010]. The alternate bearing causes wide yield fluctuations with consequent price variability in the olive oil market [Mili 2006]. In a more sustainable system, the goal should be to minimize alternate bearing by keeping constant and satisfactory yields while reducing inputs (water, fertilizers, fuel, etc.). In most cases, intensification of olive cultivation considerably reduced alternate bearing [Moriani et al. 2003, Connor and Fereres 2005, Theoris 2009, Caruso et al. 2014] but with more inputs. Pruning, harvesting time, fertilization and irrigation may help together in reducing alternate bearing [Bouaziz 1995, Marschner 1995, Bustan et al. 2013, Camposeo et al. 2013, Vivaldi et al. 2015]. In particular, an appropriate management of the fertilization revealed to be a powerful tool to partially overcome this problem [Fernández-Escobar et al. 1999, Soing 1999, Inglese et al. 2002].

In general, a rational fertilization schedule is influenced by many factors such as cultivar, soil and climatic conditions and whether trees are in an 'on' or 'off' year. The developing olive fruits either require a continuous supply of photosynthates or mineral elements [Proietti et al. 1999], in competition with leaves and stems. In the 'on' year, reserves stored in other tissues or organs are mobilized towards the flowers for the production of a large amount of pollen grains necessary for the pollination process [Poli 1979, Ferrara et al. 2007, Mazzeo et al. 2014].

Recent papers have studied the interactions between mineral elements and yield [Bustan et al. 2013]

or between mineral elements and irrigation with treated wastewater [Bedbabis et al. 2010, Segal et al. 2011, Bedbabis et al. 2014 a,] in 'Chemlali' and 'Barnea' and between mineral elements and tree age [Bedbabis et al. 2016]. Some authors investigated the effects of application of olive mill and municipal wastewater on soil chemical properties, yield [Bedbabis et al. 2014 b, 2015] and photosynthetic activity [Mechri et al. 2011, Ben Ahmed et al. 2012]. Unfortunately, no information are available about the effect of alternate bearing on nutrients concentration in leaves and stems or even on photosynthetic activity of olive trees in arid conditions. This should be crucial from an agronomic point of view for the fertilization management of cultivars with high alternate bearing.

The aim of the present 4-year trial was to study the effect of fruit load due to 'on' and 'off' year both on the annual macro-nutrient balance and net photosynthetic activity in a 'Chemlali' olive grove in order to reduce, through an appropriate fertilization schedule, the alternate bearing.

## MATERIAL AND METHODS

### Study area

The olive grove was located in Sfax (34°43N, 10°41E) in Central-Eastern Tunisia, a region with an average annual rainfall of about 200 mm and a mean annual temperature of 18.6°C. The study was carried out from 2002 to 2005 on 14-year old 'Chemlali' olive trees (*Olea europaea* L. var. *sativa* Hoffm. e Lk.) spaced 4.0 × 4.0 m. A randomized block design with two blocks was used. Each block consisted of 15 trees (3 repetitions of 5 trees each). All the selected trees were characterized by the same bearing condition, with two 'on' years (2002 and 2004) and two 'off' years (2003 and 2005). The grove received each year mature compost (30 tons/hectare) and was drip irrigated with four drip nozzles (two per side) set in a line along the rows (at 0.5 m from the trunk). The seasonal irrigation volume was around 500 mm.

### Soil characteristics

Soil samples were collected at 0–180 cm depth (six layers of 30 cm each), air-dried at room temperature, crushed to pass a 2-mm sieve, and mixed thoroughly for analyses. Soil texture was determined using the pipette method according to the methodology described by Gee and Or [2002]. Soil pH was determined with a pH meter (420A, Orient) in water ( $\text{pH}_{\text{H}_2\text{O}}$ ) and in 0.01M  $\text{CaCl}_2$  ( $\text{pH}_{\text{CaCl}_2}$ ). Soil/water ratio of the suspensions was 1:2.5 (w/v). The soil textural class was determined at the beginning of the trial (January 2002) according to the USDA soil texture classification.

Soil salinity was assessed by determination of electrical conductivity (EC) at 25°C on a saturated paste using a conductivity meter (MC 226). Soil organic carbon was measured with a Shimadzu TOC-5000 Analyzer, nitrogen (N) was measured with the Kjeldhal method and potassium (K) was determined on ammonium acetate soil extract [Richards 1954] using a JENWAY flame photometer. Phosphorous (P) was determined by the vanado-molybdate colorimetric procedure with a JENWAY 6405 UV/ Vis Spectrophotometer (Milan, Italy). Ca content was measured with an atomic absorption spectrophotometer (A Analyst 200, PerkinElmer).

### Vegetative growth

Vegetative growth was determined on 8 tagged shoots per tree. Shoots were sampled in the four cardinal directions, 4 in the outer canopy and 4 in the inner canopy. Shoots length was determined periodically each month.

### Mineral analysis of leaves and shoots

Fully developed leaves were taken from the middle portion of the current year shoots both in the outer and inner canopy in the four cardinal directions (8 shoots per tree). They were collected once per month, put in paper bags and stored in a portable cooler to be carried to the laboratory. Successively, stems and leaves were dried in an oven (60°C) until constant weight and analyzed according to the method described by Pauwels et al. [1992].

### Photosynthesis measurement

The measurements of net photosynthetic rate (Pn) were carried out on fully expanded leaves selected from the middle part of the shoots. Pn was measured on well-exposed ten leaves per tree from five trees per replicate from 10:00 to 13:00 pm following the method reported by Camposeo and Vivaldi [2011], using a portable gas exchange system (Li-COR 6200, Inc., Lincoln, Nebraska USA).

### Statistical analysis

Statistical analysis was conducted by one-way ANOVA using the program SPSS 10 statistical software (SPSS Inc., Chicago, IL, USA). LSD test ( $P \leq 0.05$  and  $P \leq 0.01$ ) was used to separate the mean values.

## RESULTS

### Soil characteristics

Soil of the experimental site was classified as sandy soil according to USDA classification (tab. 1). The average pH values ranged from 7.50 to 8.20. The average EC values ranged from 0.23 to 3.74  $\text{dS m}^{-1}$ ; however, EC values remained below the salinity threshold (4.00  $\text{dS m}^{-1}$ ). The concentration of K in the soil varied between 380 and 740  $\text{mg kg}^{-1}$  with a maximum of 740  $\text{mg kg}^{-1}$  at a depth of 80 cm, probably caused by salt leaching. Organic matter was 1.7% at the upper layer (because of the compost application) and  $\leq 1\%$  at the bottom layers (tab. 1).

### Vegetative growth

Vegetative growth was significantly higher in trees in 'off' year (from 15.8 up to 27.5 cm) with respect to trees in 'on' year (from 26.1 up to 28.7) from spring up to autumn (tab. 2). During the winter (rest period), the vegetative growth was weak in trees both in 'on' and 'off' year because of low metabolic processes; whereas, from March to June growth was higher for the trees in the 'off' year (2.90 cm) with respect to the trees in the 'on' year (0.70 cm). If we consider the period from January up to November, the shoots in the 'on' year had a growth of 2.60 cm whereas the shoots in the 'off' year of 11.70 cm (tab. 2).

**Table 1.** Some physical and chemical characteristics of the soil in the experimental olive grove

Parameter	Depth (cm)				
	0–30	30–60	60–90	90–120	120–150
Clay (g kg <sup>-1</sup> )	55	50	65	70	75
Silt (g kg <sup>-1</sup> )	50	20	45	55	60
Sand (g kg <sup>-1</sup> )	895	930	890	875	865
pH	7.50	7.55	7.52	8.00	8.20
EC (dS m <sup>-1</sup> )	0.23	0.30	2.35	3.68	3.74
N (g kg <sup>-1</sup> )	10.50	5.00	3.30	3.80	3.10
K (mg kg <sup>-1</sup> )	480	380	740	460	420
P (mg kg <sup>-1</sup> )	40	35	40	25	15
OM (g kg <sup>-1</sup> )	16.90	11.00	10.00	10.00	6.60

Abbreviations: N – nitrogen, K – potassium, P – phosphorus, EC – electrical conductivity, OM – organic matter

**Table 2.** Shoot length (cm) in ‘on’ and ‘off’ year (mean values of two years)

	January	February	March	May	June	August	September	November
‘on’	26.1 ±0.2	26.3 ±2.0	26.8 ±1.1	27.3 ±0.5	27.5 ±0.5	27.9 ±1.1	28.2 ±1.2	28.7 ±1.2
‘off’	15.8 ±0.3	15.9 ±1.1	16.5 ±3.3	19.3 ±7.5	19.4 ±2.3	20.0 ±1.3	23.2 ±1.3	27.5 ±1.4

Data represent the mean values of three repetitions for each month and each year

### Seasonal changes in the mineral composition of leaves and stems

Leaf N concentrations varied between 1.12 and 2.92% of dry matter (d.m.) in the four years (tab. 3). In particular, in the ‘on’ year the N concentration reached the highest values at March, September and October, with a range of 2.60–2.92% in 2002 and 1.84–2.20% in 2004. In the ‘off’ year, the highest values of N ( $\geq 2.20\%$ ) were measured at spring, in March and April. The lowest N concentration was found at spring (April) in the ‘on’ year and in sum-

mer (July–August) in the ‘off’ year. In the stems, N concentration ranged from 0.46 to 2.00%, with a significant peak in September for trees both in ‘off’ and ‘on’ year, and a decline to lower contents until February, with no differences between ‘on’ and ‘off’ years (tab. 4).

Leaf P concentrations ranged from 0.02 to 0.16% (tab. 3). As observed for nitrogen, P concentration in leaves in the ‘on’ year reached the highest values (0.09–0.11%) in spring and autumn (tab. 3), whereas in the ‘off’ year values up to 0.14–0.16% were meas-

ured only in one period, at April–May. In the stems (tab. 4), P concentration varied from 0.03 to 0.14%, with the highest values at autumn in both ‘on’ and ‘off’ year and the lowest ones in summer.

The mean K values in leaves ranged between 0.41 and 1.47% (tab. 3). K accumulated mainly from spring to summer with values  $\geq 1\%$ . The lowest values in both years were measured in January (0.41–0.97%). In the stems, K levels ranged from 0.45 to 1.40% and from 0.70 to 1.60%, in ‘on’ and ‘off’ year, respectively (tab. 4). K concentration increased at the

end of summer and dropped in December–January in both the ‘on’ and ‘off’ year.

Leaf Ca concentrations found in trees in ‘on’ and ‘off’ year were generally higher than the limit of sufficiency (1%) reported for olive (tab. 3) [Fernández-Escobar et al. 2009]. Ca in leaves was high at the end of summer (August) in the ‘on’ year and low at May, whereas in the ‘off’ year the highest values (2.86–2.92%) were measured in June–August and the lowest values in spring ( $< 2.0\%$ ). Stem Ca concentration varied between 0.40 and 1.99% (tab. 4)

**Table 3.** Leaf N, P, K and Ca contents in ‘on’ (2002 and 2004) and ‘off’ (2003 and 2005) years

Element	Year	J	F	M	A	M	J	J	A	S	O	N	D
N	on (2002)	2.30 <sup>c</sup>	1.90 <sup>e</sup>	2.60 <sup>b</sup>	1.50 <sup>h</sup>	2.18 <sup>d</sup>	1.70 <sup>g</sup>	1.68 <sup>g</sup>	1.65 <sup>g</sup>	2.92 <sup>a</sup>	2.70 <sup>b</sup>	2.20 <sup>d</sup>	1.80 <sup>f</sup>
	off (2003)	2.30 <sup>d</sup>	1.71 <sup>g</sup>	2.60 <sup>a</sup>	2.50 <sup>b</sup>	2.00 <sup>e</sup>	1.52 <sup>h</sup>	1.32 <sup>i</sup>	1.35 <sup>i</sup>	2.40 <sup>c</sup>	2.30 <sup>d</sup>	1.91 <sup>f</sup>	1.50 <sup>h</sup>
	on (2004)	1.70 <sup>d</sup>	1.58 <sup>e</sup>	2.20 <sup>a</sup>	1.12 <sup>f</sup>	1.88 <sup>c</sup>	1.71 <sup>d</sup>	1.65 <sup>d</sup>	1.65 <sup>d</sup>	1.84 <sup>c</sup>	2.10 <sup>b</sup>	1.60 <sup>e</sup>	1.58 <sup>e</sup>
	off (2005)	2.01 <sup>c</sup>	1.61 <sup>d</sup>	2.34 <sup>a</sup>	2.22 <sup>b</sup>	1.68 <sup>d</sup>	1.52 <sup>e</sup>	1.32 <sup>g</sup>	1.39 <sup>f</sup>	1.66 <sup>d</sup>	1.66 <sup>d</sup>	1.52 <sup>e</sup>	1.42 <sup>f</sup>
	Sig	*	*	ns	**	*	*	*	*	**	*	*	*
P	on (2002)	0.10 <sup>a</sup>	0.11 <sup>a</sup>	0.10 <sup>a</sup>	0.11 <sup>a</sup>	0.10 <sup>a</sup>	0.06 <sup>b</sup>	0.05 <sup>b</sup>	0.09 <sup>a</sup>	0.10 <sup>a</sup>	0.11 <sup>a</sup>	0.11 <sup>a</sup>	0.10 <sup>a</sup>
	off (2003)	0.11 <sup>b</sup>	0.11 <sup>b</sup>	0.11 <sup>b</sup>	0.13 <sup>b</sup>	0.16 <sup>a</sup>	0.07 <sup>c</sup>	0.07 <sup>c</sup>	0.05 <sup>c</sup>	0.10 <sup>b</sup>	0.12 <sup>b</sup>	0.11 <sup>b</sup>	0.10 <sup>b</sup>
	on (2004)	0.11 <sup>a</sup>	0.11 <sup>a</sup>	0.11 <sup>a</sup>	0.07 <sup>b</sup>	0.08 <sup>b</sup>	0.03 <sup>c</sup>	0.04 <sup>c</sup>	0.06 <sup>bc</sup>	0.09 <sup>a</sup>	0.07 <sup>b</sup>	0.09 <sup>a</sup>	0.08 <sup>b</sup>
	off (2005)	0.09 <sup>b</sup>	0.10 <sup>b</sup>	0.09 <sup>b</sup>	0.14 <sup>a</sup>	0.15 <sup>a</sup>	0.07 <sup>c</sup>	0.08 <sup>bc</sup>	0.02 <sup>d</sup>	0.07 <sup>c</sup>	0.08 <sup>bc</sup>	0.10 <sup>b</sup>	0.09 <sup>b</sup>
	Sig	ns	ns	ns	ns	*	ns	ns	*	ns	ns	ns	ns
K	on (2002)	0.53 <sup>de</sup>	0.75 <sup>c</sup>	0.85 <sup>b</sup>	0.99 <sup>a</sup>	0.95 <sup>a</sup>	0.50 <sup>e</sup>	0.62 <sup>d</sup>	0.60 <sup>d</sup>	0.24 <sup>f</sup>	0.50 <sup>e</sup>	0.57 <sup>d</sup>	0.78 <sup>c</sup>
	off (2003)	0.41 <sup>g</sup>	0.71 <sup>f</sup>	0.80 <sup>e</sup>	1.02 <sup>b</sup>	1.24 <sup>a</sup>	1.27 <sup>a</sup>	1.22 <sup>a</sup>	1.02 <sup>b</sup>	1.08 <sup>b</sup>	0.94 <sup>c</sup>	0.86 <sup>d</sup>	0.73 <sup>f</sup>
	on (2004)	0.97 <sup>f</sup>	1.15 <sup>cd</sup>	0.75 <sup>g</sup>	0.77 <sup>g</sup>	1.32 <sup>b</sup>	1.02 <sup>e</sup>	1.22 <sup>c</sup>	1.22 <sup>c</sup>	1.11 <sup>d</sup>	1.28 <sup>b</sup>	1.30 <sup>b</sup>	1.47 <sup>a</sup>
	off (2005)	0.47 <sup>g</sup>	0.68 <sup>f</sup>	0.85 <sup>d</sup>	1.15 <sup>b</sup>	1.17 <sup>b</sup>	1.19 <sup>b</sup>	1.25 <sup>a</sup>	1.17 <sup>b</sup>	0.97 <sup>c</sup>	0.95 <sup>c</sup>	0.91 <sup>cd</sup>	0.77 <sup>e</sup>
	Sig	*	ns	ns	ns	*	**	*	**	**	*	*	ns
Ca	on (2002)	1.62 <sup>g</sup>	1.49 <sup>gh</sup>	1.32 <sup>h</sup>	1.79 <sup>f</sup>	0.82 <sup>i</sup>	2.20 <sup>d</sup>	2.04 <sup>e</sup>	2.89 <sup>a</sup>	2.02 <sup>e</sup>	2.69 <sup>b</sup>	2.15 <sup>d</sup>	2.39 <sup>c</sup>
	off (2003)	2.37 <sup>b</sup>	2.38 <sup>b</sup>	1.23 <sup>f</sup>	1.88 <sup>cd</sup>	0.62 <sup>g</sup>	2.86 <sup>a</sup>	2.86 <sup>a</sup>	2.92 <sup>a</sup>	1.56 <sup>e</sup>	1.99 <sup>c</sup>	2.03 <sup>c</sup>	2.03 <sup>c</sup>
	on (2004)	1.99 <sup>e</sup>	1.90 <sup>f</sup>	1.85 <sup>g</sup>	2.14 <sup>d</sup>	1.12 <sup>i</sup>	2.51 <sup>b</sup>	1.76 <sup>h</sup>	2.89 <sup>a</sup>	2.30 <sup>c</sup>	2.37 <sup>c</sup>	1.95 <sup>ef</sup>	2.01 <sup>e</sup>
	off (2005)	1.76 <sup>f</sup>	1.95 <sup>e</sup>	2.82 <sup>a</sup>	1.78 <sup>f</sup>	2.09 <sup>d</sup>	2.86 <sup>a</sup>	2.86 <sup>a</sup>	2.92 <sup>a</sup>	2.01 <sup>de</sup>	2.20 <sup>c</sup>	2.42 <sup>b</sup>	1.88 <sup>e</sup>
	Sig	**	**	*	*	*	*	*	ns	*	**	*	*

Data represents the mean value of three replications for each month. Small letters indicate significant differences ( $P \leq 0.05$ ) among months for each year according to LSD test. Asterisks indicate significant differences among years for each month. Significant at  $P \leq 0.05$  (\*),  $P \leq 0.01$  (\*\*) and non significant (NS)

with different trend with respect to leaf concentration, the highest values measured from February to March (1.37–1.99%) and the lowest in May ('off' year) and August–September ('on' year).

#### NET photosynthetic rate

The net photosynthetic rate measured in trees in 'on' and 'off' year is reported in Figure 1. The highest values of photosynthesis were noticed during spring period in both 'on' and 'off' year, coinciding with the intense vegetative growth phase of the olive tree

(April–May). In general, values  $> 15 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$  were detected in the 'off' year, when the Pn values were significantly higher than in 'on' year.

#### DISCUSSION

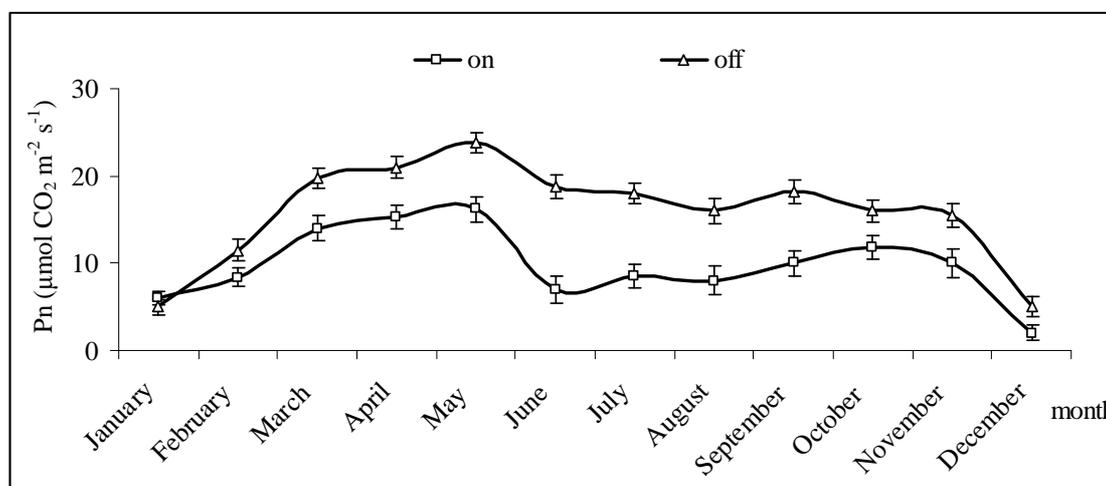
##### Soil characteristics

The pH values were within the range (7.00–8.50) for olive tree development [Gargouri 1998]. The soil presented a good N content at 0–30 cm of depth (tab. 1), probably due to either N fertilization (mine-

**Table 4.** Stem N, P, K and Ca contents in 'on' (2002 and 2004) and 'off' (2003 and 2005) years

Element	Year	J	F	M	A	M	J	J	A	S	O	N	D
N	on (2002)	1.30 <sup>d</sup>	1.10 <sup>f</sup>	1.60 <sup>b</sup>	1.40 <sup>c</sup>	1.30 <sup>d</sup>	1.00 <sup>g</sup>	1.10 <sup>f</sup>	1.60 <sup>b</sup>	1.90 <sup>a</sup>	1.20 <sup>e</sup>	1.20 <sup>e</sup>	1.10 <sup>f</sup>
	off (2003)	1.50 <sup>b</sup>	0.90 <sup>g</sup>	1.40 <sup>c</sup>	1.30 <sup>d</sup>	1.20 <sup>e</sup>	0.98 <sup>fg</sup>	1.06 <sup>f</sup>	1.00 <sup>f</sup>	2.00 <sup>a</sup>	1.30 <sup>d</sup>	1.30 <sup>d</sup>	1.50 <sup>b</sup>
	on (2004)	0.46 <sup>d</sup>	0.49 <sup>d</sup>	0.54 <sup>cd</sup>	0.65 <sup>b</sup>	0.62 <sup>b</sup>	0.56 <sup>c</sup>	0.57 <sup>c</sup>	0.60 <sup>b</sup>	0.81 <sup>a</sup>	0.50 <sup>d</sup>	0.48 <sup>d</sup>	0.46 <sup>d</sup>
	off (2005)	1.52 <sup>b</sup>	1.00 <sup>e</sup>	1.54 <sup>b</sup>	1.48 <sup>bc</sup>	1.18 <sup>d</sup>	0.98 <sup>e</sup>	1.06 <sup>e</sup>	1.28 <sup>d</sup>	1.65 <sup>a</sup>	1.23 <sup>d</sup>	1.18 <sup>d</sup>	1.42 <sup>c</sup>
	Sig	ns	ns	*	ns	ns	ns	ns	*	ns	ns	ns	*
P	on (2002)	0.11 <sup>a</sup>	0.09 <sup>ab</sup>	0.08 <sup>b</sup>	0.10 <sup>a</sup>	0.11 <sup>a</sup>	0.06 <sup>c</sup>	0.05 <sup>c</sup>	0.09 <sup>ab</sup>	0.08 <sup>b</sup>	0.11 <sup>a</sup>	0.08 <sup>b</sup>	0.10 <sup>a</sup>
	off (2003)	0.04 <sup>d</sup>	0.04 <sup>d</sup>	0.09 <sup>bc</sup>	0.08 <sup>c</sup>	0.14 <sup>a</sup>	0.07 <sup>cd</sup>	0.07 <sup>cd</sup>	0.05 <sup>d</sup>	0.08 <sup>c</sup>	0.10 <sup>b</sup>	0.11 <sup>b</sup>	0.08 <sup>c</sup>
	on (2004)	0.12 <sup>a</sup>	0.12 <sup>a</sup>	0.11 <sup>a</sup>	0.03 <sup>d</sup>	0.05 <sup>c</sup>	0.06 <sup>bc</sup>	0.05 <sup>c</sup>	0.09 <sup>ab</sup>	0.07 <sup>b</sup>	0.10 <sup>a</sup>	0.10 <sup>a</sup>	0.03 <sup>d</sup>
	off (2005)	0.05 <sup>c</sup>	0.03 <sup>d</sup>	0.03 <sup>d</sup>	0.07 <sup>bc</sup>	0.06 <sup>c</sup>	0.07 <sup>bc</sup>	0.07 <sup>bc</sup>	0.05 <sup>c</sup>	0.07 <sup>bc</sup>	0.10 <sup>a</sup>	0.10 <sup>a</sup>	0.08 <sup>bc</sup>
	Sig	*	ns	ns	ns	*	ns	ns	*	ns	ns	*	*
K	on (2002)	0.51 <sup>g</sup>	1.10 <sup>d</sup>	1.20 <sup>bc</sup>	1.18 <sup>c</sup>	1.10 <sup>d</sup>	1.02 <sup>e</sup>	1.22 <sup>b</sup>	1.27 <sup>b</sup>	1.40 <sup>a</sup>	1.00 <sup>e</sup>	0.92 <sup>ef</sup>	0.85 <sup>f</sup>
	off (2003)	0.80 <sup>f</sup>	1.10 <sup>d</sup>	0.95 <sup>e</sup>	1.19 <sup>c</sup>	1.59 <sup>a</sup>	1.02 <sup>de</sup>	1.07 <sup>d</sup>	1.47 <sup>b</sup>	1.58 <sup>a</sup>	1.49 <sup>b</sup>	0.92 <sup>e</sup>	0.70 <sup>g</sup>
	on (2004)	0.52 <sup>e</sup>	0.81 <sup>d</sup>	0.97 <sup>c</sup>	0.92 <sup>c</sup>	1.25 <sup>ab</sup>	1.02 <sup>c</sup>	1.22 <sup>c</sup>	1.27 <sup>ab</sup>	1.36 <sup>a</sup>	1.15 <sup>b</sup>	1.10 <sup>b</sup>	0.45 <sup>e</sup>
	off (2005)	0.82 <sup>fg</sup>	0.95 <sup>e</sup>	0.87 <sup>f</sup>	1.17 <sup>c</sup>	1.55 <sup>a</sup>	1.02 <sup>d</sup>	1.07 <sup>d</sup>	1.47 <sup>b</sup>	1.60 <sup>a</sup>	1.57 <sup>a</sup>	0.87 <sup>f</sup>	0.75 <sup>g</sup>
	Sig	*	ns	*	ns	*	ns	*	*	*	*	ns	ns
Ca	on (2002)	0.80 <sup>f</sup>	1.40 <sup>d</sup>	1.80 <sup>a</sup>	1.40 <sup>d</sup>	1.30 <sup>e</sup>	1.62 <sup>b</sup>	1.49 <sup>c</sup>	0.59 <sup>g</sup>	0.50 <sup>g</sup>	1.50 <sup>c</sup>	1.30 <sup>e</sup>	1.40 <sup>d</sup>
	off (2003)	1.10 <sup>e</sup>	1.90 <sup>a</sup>	1.60 <sup>b</sup>	1.40 <sup>c</sup>	0.40 <sup>f</sup>	1.08 <sup>e</sup>	1.08 <sup>e</sup>	1.41 <sup>c</sup>	1.40 <sup>c</sup>	1.30 <sup>d</sup>	1.30 <sup>d</sup>	1.10 <sup>e</sup>
	on (2004)	1.00 <sup>e</sup>	1.37 <sup>c</sup>	1.81 <sup>a</sup>	1.32 <sup>cd</sup>	1.22 <sup>d</sup>	1.70 <sup>b</sup>	1.36 <sup>c</sup>	0.65 <sup>f</sup>	0.59 <sup>f</sup>	1.35 <sup>c</sup>	1.42 <sup>c</sup>	1.39 <sup>c</sup>
	off (2005)	0.75 <sup>d</sup>	1.99 <sup>a</sup>	1.60 <sup>b</sup>	1.33 <sup>c</sup>	0.37 <sup>e</sup>	0.85 <sup>d</sup>	0.82 <sup>d</sup>	1.41 <sup>c</sup>	1.38 <sup>c</sup>	1.36 <sup>c</sup>	1.38 <sup>c</sup>	0.73 <sup>d</sup>
	Sig	*	*	*	ns	ns	*	*	**	**	ns	ns	*

Data represents the mean value of three replications for each month. Small letters indicate significant differences ( $p \leq 0.05$ ) among months for each year according to LSD test. Asterisks indicate significant differences between years for each month. Significant at  $p \leq 0.05$  (\*),  $p \leq 0.01$  (\*\*), and non significant (ns)



**Fig. 1.** Net photosynthetic rate (Pn,  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) in 'on' (square) and 'off' years (triangle). Data represent mean values of two years. Bars indicate standard error

ral nitrogen) or organic matter mineralization (mature compost), whereas at the bottom layers N content resulted poor. The content of P resulted adequate for olive tree growth and development in the layer where the root system is generally more abundant. The lower level of salinity registered at a soil depth of 0–0.30 m, in comparison with the layer of 1.20–1.30 m, suggested that salts were transported and accumulated at deeper layers as a consequence of the soil texture, i.e., a sandy soil.

### Vegetative growth

The lower growth of shoots in the 'on' years with respect to that in 'off' years was the consequence of the more abundant fruit load, since fruits compete for resources and limit the growth of the shoots [Pastor et al. 1998, Fernández-Escobar et al. 1999]. Lavee [2006] reported that, during an 'on' year, the developing fruits usually restrain the vegetative growth until harvest. In the 'on' year, photosynthates are allocated towards a stronger sink, the fruits, and therefore the growth of the shoots resulted more limited as also observed in our trial (tab. 2).

### Seasonal changes in the mineral composition of leaves and shoots

The mean N values detected in this 4-year trial were higher than values reported by other authors

[Segal et al. 2011, Bustan et al. 2013] for 'Barnea' and 'Leccino', probably because of the cultivar, age of the trees and soil characteristics. However, the high values measured in March and September can be explained by the uptake occurring in stems-leaves, which are strong sinks at that time of the vegetative season. Nitrogen will be used for shoot growth ('off' year) and fruit development ('on' year). Leaf N concentration in trees in 'on' year is low during summer, when the element is used by the growing fruits, as previously reported by several studies [Jorado and Lietao 1990, Fernández-Escobar et al. 2004]. Fernández-Escobar et al. [2011] indicated that during the 'on' year, most of N (more than 60%) was accumulated in the fruit at the end of the year, indicating that the fruit is the most powerful sink for this element, and our results showed a significant reduction of N both in leaves and stems at that time of the season. The N contents showed significant seasonal trends in all the years. At autumn, high N concentrations in leaves in 'on' and 'off' year were measured, with general higher N concentration in leaves of trees in 'on' year (tab. 3). Fernández-Escobar et al. [2004] reported that leaf N concentration increased in trees in 'off' year at autumn and were steady during winter. Trees in 'off' year showed a decrease of leaf N concentration in June–August (tab. 3), probably because the demand of N for shoots growth still oc-

curing at this period, whereas this reduction was more limited for the trees in the 'on' year (tab. 2). Vegetative growth and flower formation may cause a decrease of leaf N concentration at this period since N is required by these two strong sinks [Bouat et al. 1964, Brahem 1997, Fernández-Escobar et al. 1999]. Consequently, N application is suggested to support an adequate vegetative growth and tree balance with application of organic and/or mineral N fertilizers at spring to allow a good absorption of the element.

According to Fernández-Escobar et al. [2004], N stored in leaves (and permanent organs) during the 'off' year is mobilized at the beginning of the spring of the next ('on' year) to support the new growth. N application at autumn can be recommended for the storage of nitrogen to be used at budburst. On the basis of these results, in the 'on' year N fertilization can be accomplished in February–March until pit hardening [Morales-Sillero et al. 2008], and possibly after harvest (November–December) to be used in the successive season. During an 'off' year, an autumn and/or spring application of N would be appropriate to stimulate a good vegetative growth for the production in the successive year. The higher leaf N concentration reported at autumn (tab. 3) in trees in 'on' year compared to trees in 'off' year indicated a nitrogen accumulation after the harvest for the successive vegetative growth ('off' year). This suggests N fertilization at that time of the season to prepare trees for the 'off' year, when larger amounts of N are necessary to sustain the growth of the shoots.

With regards of P, our values were generally higher than those reported by Brahem and Mehri [1997] (0.05 to 0.074%) and Gargouri [1998] (0.06 to 0.08%) on the same cultivar, but in these latter researches trees were not irrigated and fertilized and soil characteristics were different. High values of P were measured in leaves and stems in spring with a reduction to lower levels in summer in trees in both 'on' and 'off' year, as also reported by Fernández-Escobar et al. [1999]. Our data confirmed that flowering, fruit-set and fruit growth require more P than vegetative activity and this element is mobilized from leaves to flowers and fruitlets in the 'on' year more than towards shoots in the 'off' year. A supply of P either for trees in 'on' and 'off' year at autumn-

winter could better sustain the growth of reproductive structures that can reduce the alternate bearing. The amount and time of P application should be adjusted to the expected fruit load each year [Bustan et al. 2013], so a larger amount when a higher fruit load is expected ('on' year) and a lower amount in the 'off' year. Such adjustments would carefully consider local constraints of P uptake [Dag et al. 2009], P remobilization [Schachtman et al. 1998, Vance et al. 2003, Lynch and Ho 2005], and the metabolic consequences of P that are unique to oil-producing species [Chesworth et al. 1998] such as olive.

Potassium concentrations were similar to those reported by Gargouri [1998] in the same cultivar grown in Enfidha (central Tunisia). In Spain, Fernández-Escobar et al. [1999] reported value limit of leaf K concentration in olive from 0.40 to 0.80%. At ripening, 60% of the total K was found in the fruit and then remained steady until the time of harvest [Lavee 1997]. From a practical approach, a supplement of K at the beginning of summer in 'on' year can be necessary for fruit growth and subsequent oil biosynthesis. However, Erel et al. [2011] showed that increased K availability had no effect on fruit-set and fruit number or yield in bearing olive trees. Moreover, Dag et al. [2009] demonstrated a wide range of K levels in fruit flesh (1.50–3.50%), with no significant influence on any measured parameter of olive oil quality. The concentration of K was higher and stable in 'off' year during the summer, as compared to 'on' year concentration, probably because a lesser mobilization towards fruits occurred. The leaf K concentration in summer was even higher than the threshold values of 0.70–0.80% considered as adequate [Freeman et al. 2005, Theoris 2009] in trees in 'off' year, indicating that K supply was not necessary. In the 'on' year, a reduction of K concentration was probably the consequence of the transport of this element towards the fruits for oil synthesis. At the end of summer, newly grown leaves and stems become the main substitute sink for K, and they subsequently turn to be the major source of this mineral with a consequent decrease of K during autumn-winter in trees in 'off' year (tabs 3 and 4). The root system activity that precedes vegetative growth may be also the cause of the decline of leaf K concentration at this

time [Lavee 1997]. Indeed, Ben Rouina et al. [1999] showed that the formation and the extension of the long-haired roots seemed to mobilize an important quantity of K. Our results are also similar to those reported in pistachio trees by Rosecrance et al. [1998], who reported a rapid decline of K in leaf after harvest as consequence of K translocation from leaves to other (permanent) organs like trunk and roots for winter storage. A supply of K in January–February, before new vegetation, for trees in ‘on’ and ‘off’ year can be necessary for supporting and implementing the root system activity and the successive shoot growth (‘off’ year) and fruit development (‘on’ year).

Values of Ca were higher in leaves of trees in ‘off’ year than in trees in ‘on’ year and were similar to those reported by Gargouri [1998] for the same cultivar. Leaf Ca concentration reached low values in May (tab. 3) probably because of the high demand of Ca for flowering, fruit-set and growth processes in trees in ‘on’ year and for the vegetative growth in trees in ‘off’ year. In particular, arabinan rich pectic polysaccharides are one of the main classes of polysaccharides detected in olive pulp cell walls and galacturonic acid residues (GalA) can bind to calcium ions allowing the pectic polysaccharide chains to assemble by calcium bridges [Ferreira et al. 2006]. The  $\text{Ca}^{2+}$ -GalA complexes are commonly found in the middle lamella, playing an important role in cell-cell adhesion and cohesion [Ferreira et al. 2006]. Highest values were measured in summer (June–August) probably because a lower mobility of Ca at that time (reduced xylem functionality); in general, a high concentration of Ca is measured in green olives (stage I) and lower values in ripening and black olives [Ferreira et al. 2006].

#### **NET photosynthetic rate**

Photosynthesis values were higher in ‘off’ year than in ‘on’ one, when the trees are more prone to the vegetative activity. The reduction of net photosynthetic activity in trees in ‘on’ year could be also due to the energy dissipated for reproductive structures.

The lowest photosynthesis values were reported in two periods, winter and summer, because of limited

metabolic processes reinforced by ‘low’ temperature values ( $< 12^{\circ}\text{C}$ ) and by the stomata closure for high temperatures (accentuated by climatic conditions and lower water content in olive leaves). These results are in accordance with Tombesi et al. [1986] and Chartzoulakis et al. [1999] for Leccino and Koroneiki cultivars. In both ‘off’ and ‘on’ year, a significant decrease of Pn activity was reported in November, in agreement with a previous study [Chartzoulakis et al. 1999]. The reduction of Pn was more evident in summer in the ‘on’ year with respect to the ‘off’ year.

#### **CONCLUSION**

On the basis of our results, in the ‘on’ year N fertilization can be accomplished in February–March (50–60%), another application at the beginning of spring (10–30%) and possibly after harvest (November–December) in a limited amount (20–30%) to be used at budburst in the successive season. During an ‘off’ year, an autumn and/or spring application of N would be appropriate to sustain a good vegetative growth. Our data confirmed that flowering, fruit-set and fruit growth (‘on’ year) require more P than vegetative activity, thus indicating a supply of P at autumn–winter and partially in spring–summer in particular in an ‘on’ year. A supply of K in January–February for trees in ‘on’ and ‘off’ year can be necessary for supporting the root system activity and the successive shoot growth (‘off’ year) and fruit development (‘on’ year). Another application of K in spring would be necessary for trees in ‘on’ year. Our data of the 4-year trial could be very useful to reduce alternate bearing in olive trees by means of an appropriate fertilization management. Nutrients would be applied to olive trees when required, taking into account if trees are in ‘on’ or ‘off’ year.

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