



# Characterization of biochemical factors affecting crop load in three olive cultivars

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## Summary

The dynamics of crop load due to alternate bearing in olive are poorly known, as many environmental conditions and endogenous factors can have a key role in this physiological phenomenon. The aim of this study was to explore the alternate bearing in three olive cultivars by following and determining some biochemical factors likely involved in this physiological phenomenon. This study was carried out in an olive orchard (*Olea europaea* L., cultivars Chemlali, Koroneiki and Arbosana) located in Sfax, Tunisia, during two consecutive years (2014–2015). Crop load alternation provoked good fruit and oil productions in the ON year (2014), followed by low crops (meanly -57% for fruits and -61% for oil) in the OFF year (2015). For all the three cultivars, leaves and fruits of olive plants in the OFF year generally showed reduced pigments, decreased enzymatic and non-enzymatic antioxidant defences, and their abscisic acid (ABA) homeostasis was disrupted. All the studied factors can interact in determining the alternation of high and low crop load in olive tree and its effects on fruit/oil yield and plant physiological status.

## Keywords

*Olea europaea*, antioxidant enzymes, phenols, pigments, phytohormones

## Introduction

The olive (*Olea europaea* L.) is an evergreen tree that has been cultivated from ancient times. Presently, there are more than eight million hectares producing olives in the world, most of them located in the Mediterranean Basin (Condelli et al., 2015). The productivity of an olive tree is a result of interaction among a number of components, including genotypes, foliar nutrient status and availability of nutrients in the soil (Jasrotia et al., 1999). In Tunisia, olive is one of the most important fruit trees. Its cultivation occupies about 79% of the total area planted with fruit trees (Bahloul et al., 2015). Moreover, olive production in Tunisia is erratic and varies from year to year.

This variation is partly due to alternate bearing phenomenon, an autonomous oscillation between two stages, one of pronounced vegetative and another one of pronounced generative growth. Alternate bearing in olive is caused by several factors, such as planted cultivars, environmental conditions,

## Significance of this study

*What is already known on this subject?*

- Crop load in olive tree is largely determined by flowering load, environmental factors during winter, and plant hormonal and mineral balance.

*What are the new findings?*

- The levels of enzymatic and non-enzymatic antioxidants, and those of abscisic acid (ABA) in olive leaf and fruits can interact in determining high and low crop load in olive tree, while auxin (IAA) seems to be not involved in this physiological phenomenon.

*What is the expected impact on horticulture?*

- Considering that olive oil is an important food product worldwide, this knowledge could help to understand how to reduce or mitigate alternate bearing in olive, a way for increasing fruit and oil yield and quality.

nutritional status of the trees, and endogenous plant hormonal balance, cultural practices and pests, that interact to influence tree physiology (Fernández-Escobar et al., 2004; Lavee, 2007; Yanik et al., 2013). The terms 'alternate' or 'biennial' bearing are used by horticulturists to designate the production of a heavy fruit crop one year followed by a light crop during the following year (Crane and Nelson, 1972). This phenomenon can be limited to an individual olive tree in an orchard or even to individual parts of a single tree (Davis, 1957). Alternate bearing is known to be a feature to a greater or lesser extent of many fruit tree species/cultivars. Monselise and Goldschmidt (1982) pointed out that irregular fruiting in fruit trees is the normal behavior of trees in their natural environment. Goldschmidt (2005) proposed that in a broad evolutionary sense, alternate bearing should perhaps be understood as a phenomenon of homeostasis.

The mechanism of alternate bearing is poorly known, but environmental conditions and endogenous factors appear to affect flower induction (Monselise and Goldschmidt, 1982; Bernier, 1988). In olive, alternate bearing is largely determined by crop load in the previous year (Cuevas et al., 1994). The early researches on alternate bearing in olive indicated that environmental factors during winter have a role in flower induction and subsequent initiation (Badr and Hartmann, 1971; Hartmann and Whisler, 1975). Determining the time of flower bud induction (changes in gene expression responsible for floral initiation) is important for determining

possible causes of alternate bearing and for developing management practices to correct this phenomenon (Yanik et al., 2013).

Besides flower bud induction, two other mechanisms are clearly involved in the maintenance of the alternate bearing condition in fruits: a) plant hormonal balance, and b) plant mineral control (Batkir et al., 2004; Fernández-Escobar et al., 2004; Ulger et al., 2004; Goldschmidt, 2005; Lavee, 2007; Yanik et al., 2013; Abaza et al., 2015). The synergistic and complementary effects of all these regulatory mechanisms are well demonstrated by the consequences of fruit thinning (Fernández-Escobar et al., 1992). Furthermore, the cause-and-effect relationships among these mechanisms with respect to alternate bearing are still not well defined. In addition, the activities of enzymes of antioxidant metabolism and the levels of antioxidant compounds, particularly abundant in olive tissues (Sofo et al., 2004, 2005; Bacelar et al., 2007; Abaza et al., 2015; Romero-García et al., 2016), could have a key role for their direct and/or indirect role as intra- and extra-cellular signal messengers. The characterization of oil quality could also be an important aspects in conjunction with the above-cited parameters (Hrncirik and Fritsche, 2005; Condelli et al., 2015).

On this basis, the objective of this study is to explore the alternation of high and low crop load in three olive cultivars ('Chemlali', 'Koroneiki' and 'Arbosana') by following and determining 1) enzymes of antioxidant metabolism and antioxidant compounds in olive organs at different vegetative growth phases; 2) changes in endogenous plant hormones in leaf and fruit samples. This knowledge could help to understand how to reduce or mitigate alternate bearing in olive, a way for increasing fruit and oil production.

## Materials and methods

### Plant material, experimental design and oil extraction

The experiments were carried out in an olive orchard (*Olea europaea* L., cultivars Chemlali, Koroneiki and Arbosana) located in the experimental station 'Taous' at the Olive Tree Institute of Sfax in the South of Tunisia (34°43'N; 10°41'E) in two consecutive years (2014–2015). Trees were 15-year-old, drip-irrigated, spaced at 7 m × 7 m (196 trees ha<sup>-1</sup>). Plants were fertigated (200 kg ha<sup>-1</sup> a<sup>-1</sup> NH<sub>4</sub>NO<sub>3</sub>, 40 kg ha<sup>-1</sup> a<sup>-1</sup> P<sub>2</sub>, and 140 kg ha<sup>-1</sup> a<sup>-1</sup> K<sub>2</sub>O) and 10 t ha<sup>-1</sup> a<sup>-1</sup> of organic matter were added. The irrigation water (3,000 m<sup>3</sup> ha<sup>-1</sup> a<sup>-1</sup>, equivalent to 100% ETc) was distributed from May to October by drip irrigation (four self-compensating drippers per plant delivering 8 L h<sup>-1</sup>).

Plant production and biochemical responses were compared between 2014, a good fruiting year (ON year), and 2015, a low fruiting year (OFF year). In 2014, for each cultivar, ten trees from two adjacent rows (total 20 trees per treatment) were selected to be similar in potential yield and canopy architecture. Five composite leaf samples (each from five randomly selected trees) were collected, at 08:00–09:00 h, in selected days of February, May, August and November 2014 and 2015. Fully expanded leaves with similar light exposition and position in the canopy were sampled from each plant along the median segment of new-growth shoots. Five composite samples (each from the same five randomly selected trees described above) of well-developed fruits were harvested in November 2014 and 2015, and chosen inside the canopy using the same method adopted for leaves. Fruit production per hectare was determined during the two experimental years for all the three cultivars. No fruit thinning

was carried out during the whole experimental period. In 2015 (OFF year), the selected 'Koroneiki' olive trees (the same ones of 2014) did not produce any fruits, so no results on fruits were available. All the leaf samples were transported to the laboratory in closed polyethylene bags, immediately frozen in liquid nitrogen and then stored at -80°C.

In all the three studied cultivars, no oil from the selected trees was produced in 2015 (OFF year). Oil extraction in the ON year was carried out in November 2014, using an Abencor system within 24 h after fruit sampling. Olives were crushed with a hammer mill and were slowly mixed for 30 min at 25°C. Then, the obtained paste was centrifuged at 3,500 × *g* over 3 min. The oil was then separated by decanting, transferred into dark glass bottles and stored in the dark at 4°C. Oil production per hectare was determined during the two experimental years for all the three cultivars.

### Chlorophyll and carotenoids

The extraction was carried out at 4°C in dark conditions. Samples of leaves (25 mg) and fruits (250 mg) were homogenized in a mortar and pestle with 1.5 mL of 80% acetone (v/v). The extracts were centrifuged in sealed tubes at 15,000 × *g* for 5 min. The supernatant was collected and the absorbance was read at 663 and 647 nm for chlorophyll *a* and chlorophyll *b*, respectively, and at 470 nm for carotenoid content. The concentrations for total chlorophyll (chlorophyll *a* + chlorophyll *b*), and the total carotenoids (xanthophylls and carotenes) were calculated according to the equations of Lichtenthaler and Buschmann (2001).

### Anthocyanins and phenols

Aliquots of leaves and fruits (2.5 g) were extracted in 15 mL of acidified methanol (1% HCl) for 2 h at room temperature in the dark, and then centrifuged at 1,000 × *g* for 15 min.

Anthocyanin levels in the methanolic extracts were calculated according to Gould et al. (2000) using a Jasco V-530 UV-vis spectrophotometer (Jasco Corp., Tokyo, Japan). Total phenolic content was determined by the Folin-Ciocalteu colorimetric method using chlorogenic acid as a standard. Total phenolic content was expressed as mg chlorogenic equivalents (CAE) per gram of fresh weight of tissue.

### Activities of the enzymes of antioxidant metabolism

For the enzymatic assays, 500 mg (fresh weight) of tissue (leaves and fruits) were homogenized with a pestle in an ice-cold mortar in 3 mL of an ice-cold 50 mM sodium phosphate buffer (pH 7.0) containing 0.2 mM EDTA and 1% (w/v) polyvinylpyrrolidone. The homogenates were filtered through four layers of cheesecloth, then centrifuged at 4°C for 20 min at 15,000 × *g*. The supernatant was collected and used for the assays of enzymatic activities.

Total superoxide dismutase (SOD; EC 1.15.1.1) activity was assayed in terms of its ability to inhibit the photochemical reduction of nitro blue tetrazolium (NBT) according to the method of Sofo et al. (2004). One unit of SOD activity was defined as the amount of the enzyme required to cause 50% inhibition of the rate of NBT reduction. The activity of catalase (CAT; EC 1.11.1.6) was measured in terms of H<sub>2</sub>O<sub>2</sub> decomposition rate, according to the method of Sofo et al. (2004). One unit of CAT activity was defined as the amount of the enzyme required to decompose 1 μmol of H<sub>2</sub>O<sub>2</sub> in 1 min. Total peroxidase (POX; EC 1.11.1.17) activity was determined measuring the oxidation of guaiacol to tetraguaiacol, which results in an increase in absorbance at 470 nm, according to the method

of Chance and Maehly (1955). One unit of POX activity was defined as the amount of the enzyme required to produce 1  $\mu\text{mol}$  of tetraguaiacol in 1 min. Ascorbate peroxidase (APX; EC 1.11.1.11) activity was assayed according to the method of Francini et al. (2006). One unit of APX activity was defined as the amount of enzyme required to oxidize 1  $\mu\text{mol}$  of AsA in 1 min. All the enzymatic activities were expressed as units on mg of proteins. Protein content was measured according to Bradford (1976).

### Phytohormones

The extraction of IAA and ABA was carried out according to Sofu et al. (2012). The quantitative determinations of IAA (indole-3-acetic acid) and ABA (abscisic acid) were carried out by a competitive enzyme-linked immunosorbent assay (ELISA) using the following kits, respectively: Phytodetek® t-ZR Test Kit, Phytodetek® DHZR Test Kit, and Phytodetek® IAA Test Kit (Agridia Biofords, Evry, France).

### Statistical analysis

The number of replicates ( $n$ ) for each measured parameter is specified in the Table and Figure captions. Statistical analysis of data was carried out using the software Sigmastat 3.1 SPSS software (SPSS Inc., IL, USA). Two-factorial (cultivars, years) analysis of variance (ANOVA) for all the analysed parameters was performed. Means were statistically analysed by Fisher's LSD test at  $P < 0.05$ .

## Results and discussion

### Productive parameters

Both fruit and oil yield at the orchard-level were higher in the ON year (2014), whereas no strong differences among the three cultivars were detected, excepting for 'Koroneiki' fruit production in 2015, that was markedly low than in 'Chemlali' and 'Arbosana' (Table 1). A similar trend was observed in the 20 selected plants for each treatment, with the absence of fruits for the cultivar Koroneiki in 2015 (Table 1).

In alternate bearing, the trees with a large fruit population were not expected to flower the next year (Cuevas et al., 1994; Moriana et al., 2003; Lavee, 2007). Particularly, olive trees under stress often show alternate bearing, shifting their resources to vegetative growth and structure for a year or two, thus providing the time necessary for fruiting in the long term. According to this, the productive data here obtained indicate that in the ON year both fruit and oil yield were higher. This alternation of large and small crops was likely caused by the competition between the crop in the ON season and the flower buds in the OFF year (Lavee, 2007; Yanik et al., 2013).

### Pigment analysis

The trends of total chlorophyll and total carotenoids in leaves and fruits of the three studied olive cultivars were similar (Table 2; Figure 1A, B). The values of leaf chlorophyll and carotenoids in ON were generally higher than those found in OFF, and this difference was more marked for chlorophyll (Figure 1A). In the ON year, 'Arbosana' had values of leaf chlorophyll lower than 'Chemlali' and 'Koroneiki' in May, while 'Koroneiki' showed significantly higher values in November (Figure 1A). Furthermore, 'Koroneiki' had significantly higher values of carotenoids in May and August of the ON year (Figure 1B). The differences among cultivars for the OFF year were less marked, with 'Koroneiki' leaves showing generally high levels of chlorophyll (August and November) and 'Chemlali' of carotenoids (February and November) (Figure 1B). Fruit chlorophyll and carotenoids in the ON year were significantly higher than in the OFF year (Table 2). In the ON year, 'Chemlali' fruits showed significantly higher carotenoids and lower chlorophyll, compared to 'Arbosana' (Table 2). Significant differences of anthocyanin levels were found between ON and OFF in November for all the cultivars, with 'Chemlali' significantly higher than all the other treatment also in August (Figure 1C). Regarding the differences in anthocyanin levels in fruits, as for chlorophyll and carotenoids, the values of the ON year were significantly higher than those of OFF one (Table 2).

The highest values of total chlorophyll and anthocyanin contents in the ON year were found in November of both the years (Figure 1A, C), in correspondence with plant vegetative maturity and fruit production, while the highest carotenoid contents were revealed in August, when the need of photo-protectant pigments against adverse arid climatic conditions is higher (Figure 1B). The observed values of pigments (Table 2; Figure 1) suggest that plant leaves and fruits during the OFF year did not manage to maintain an optimal physiological status, with a consequent decline of photosynthetic (chlorophyll), photo-protectant and free-radical scavenging pigments (carotenoids and anthocyanins), as pigments synthesis did not keep pace with their own degradation (Sofu et al., 2004, 2005; Romero-García et al., 2016).

### Antioxidant compounds and enzymes of antioxidant metabolism

Total phenol content in ON leaves resulted to be significantly higher in August and November (and also in May for 'Arbosana') compared to OFF leaves of all the three cultivars (Figure 1D). In fruits of the ON year, total phenols were significantly higher than in the OFF year, with 'Arbosana' ON having the highest value (Table 3).

**TABLE 1.** Fruit and oil production in 'Chemlali', 'Koroneiki' and 'Arbosana' olive cultivars in the whole orchard (196 plants) and in the selected trees (20 plants) in the two experimental years.

Cultivar	Year	Orchard		Selected trees	
		Fruit production (kg ha <sup>-1</sup> )	Oil production	Fruit production (kg plant <sup>-1</sup> )	Oil production
Chemlali	2014	5508	1266.8	29.1	6.2
	2015	3258	651.6	16.2	2.9
Koroneiki	2014	6628	1391.9	30.8	6.8
	2015	1152	207.4	-	-
Arbosana	2014	6732	1279.1	35.3	6.9
	2015	3500	630.0	18.9	3.8

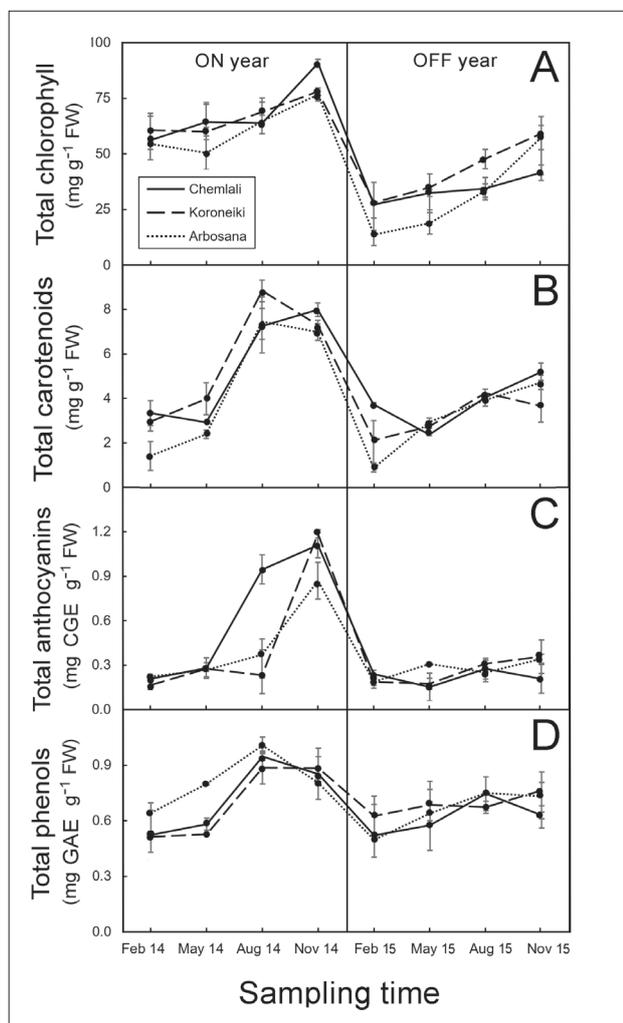
**TABLE 2.** Total chlorophyll, total carotenoids, total anthocyanins and total phenols in fruits of ‘Chemlali’ and ‘Arbosana’ olive cultivars. The values represent means ( $n = 5$ )  $\pm$  SD. Values followed by different letters are statistically different ( $P < 0.05$ ) within columns. CGE = cyaniding 3-glucoside equivalents; FW = fresh weight; GAE = gallic acid equivalents.

Cultivar	Year	Total chlorophyll	Total carotenoids	Total anthocyanins	Total phenols
		(mg g <sup>-1</sup> FW)		(mg CGE g <sup>-1</sup> FW)	(mg GAE g <sup>-1</sup> FW)
Chemlali	2014	27.16 $\pm$ 2.21 b	3.00 $\pm$ 0.38 a	0.17 $\pm$ 0.02 a	0.47 $\pm$ 0.01 a
	2015	16.56 $\pm$ 3.99 c	0.97 $\pm$ 0.26 c	0.06 $\pm$ 0.03 b	0.30 $\pm$ 0.05 b
Arbosana	2014	35.49 $\pm$ 5.59 ab	1.71 $\pm$ 0.28 b	0.12 $\pm$ 0.05 a	0.53 $\pm$ 0.02 a
	2015	16.61 $\pm$ 3.87 c	0.56 $\pm$ 0.03 c	0.06 $\pm$ 0.01 b	0.21 $\pm$ 0.04 b

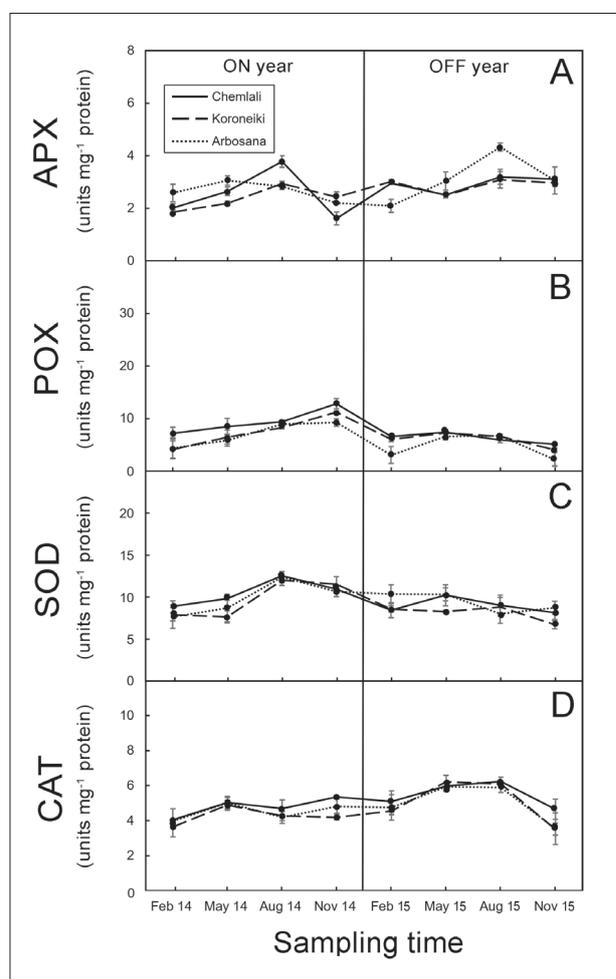
The trends of APX and POX activity in leaves were quite divergent, with ON treatments significantly higher in August and November compared to OFF ones for POX, and significantly lower in November for APX (Figure 2A, B). ‘Chemlali’ ON showed generally high values of APX compared to the other treatments (Figure 2A).

It was demonstrated that olive tissues have high amounts of phenols with free radical-scavenging properties, and that some antioxidant enzymes (such as SOD, CAT, POX, APX, IAA

oxidase, polyphenol oxidase) regulate their activity in response to abiotic stresses (Romero et al., 2002; Sofo et al., 2004; Bacelar et al., 2007; Lopez-Huertas and del Río, 2014; Abaza et al., 2015). While POX is less specific and can use a broad range of substrates as electron donors, preferably some phenolic compounds, APX is more specific and use ascorbate as electron donor (Sofo et al., 2005; Lopez-Huertas and del Río, 2014). Moreover, SOD and CAT, together with APX and POX, constitute the major defence system against the reactive oxygen species produced by the electron trans-



**FIGURE 1.** (A) Total chlorophyll, (B) total carotenoids, (C) total anthocyanins, and (D) total phenols in leaves of ‘Chemlali’ (continuous line), ‘Koroneiki’ (dashed line) and ‘Arbosana’ (dotted line) olive cultivars. The values represent means ( $n = 5$ )  $\pm$  SD. Where present, statistical differences ( $P < 0.05$ ) between cultivars and years are commented in the text. CGE = cyanidin 3-glucoside equivalents; FW = fresh weight; GAE = gallic acid equivalents.



**FIGURE 2.** Activities of (A) ascorbate peroxidase (APX), (B) total peroxidase (POX), (C) superoxide dismutase (SOD) and (D) catalase (CAT) in leaves (2014 and 2015) of ‘Chemlali’ (continuous line), ‘Koroneiki’ (dashed line) and ‘Arbosana’ (dotted line) olive cultivars. The values represent means ( $n = 5$ )  $\pm$  SD. Where present, statistical differences ( $P < 0.05$ ) between cultivars and years are commented in the text. FW = fresh weight.

**TABLE 3.** Activities of ascorbate peroxidase (APX), total peroxidase (POX), superoxide dismutase (SOD) and catalase (CTA) in fruits of 'Chemlali' and 'Arbosana' olive cultivars. The values represent means ( $n = 5$ )  $\pm$  SD. Values followed by different letters are statistically different ( $P < 0.05$ ) within columns. FW = fresh weight.

Cultivar	Year	APX	POX	SOD	CAT
		(units mg g <sup>-1</sup> protein)			
Chemlali	2014	2.24 $\pm$ 0.21 a	5.87 $\pm$ 0.20 a	8.66 $\pm$ 0.71 a	5.11 $\pm$ 0.56 b
	2015	0.36 $\pm$ 0.11 c	2.56 $\pm$ 0.32 b	4.95 $\pm$ 0.78 b	9.09 $\pm$ 0.54 a
Arbosana	2014	1.94 $\pm$ 0.17 b	6.68 $\pm$ 0.02 a	11.08 $\pm$ 1.98 a	5.54 $\pm$ 0.74 b
	2015	0.27 $\pm$ 0.08 c	0.93 $\pm$ 0.36 c	2.34 $\pm$ 0.57 c	8.46 $\pm$ 2.56 a

port chain located in chloroplast (Sofo et al., 2005). Leaf and fruit SOD activity showed a trend similar to that of POX, with ON treatments showing significantly higher values than OFF ones (Table 3; Figure 2C). Regarding leaf and fruit CAT activity, excepting in November, the situation seems to be reversed, with cultivars in the OFF year having significantly higher values than the ON ones (Table 3; Figure 2D).

Based on the results on phenol content (Table 2; Figure 1D) and antioxidant enzymes (Table 3; Figure 2), olive leaves and fruits of the ON year generally showed a better enzymatic and non-enzymatic antioxidant capacity. Sofo et al. (2005) observed an increase in some antioxidant enzymes in plants experiencing drought, with a kind of compensatory response that allowed plants to recover their physiological status after rewatering. In this case, the multi-factor stress experienced by olive plants during the OFF year resulted to be detrimental for plant, so the antioxidant capacity of olive tissue was depressed.

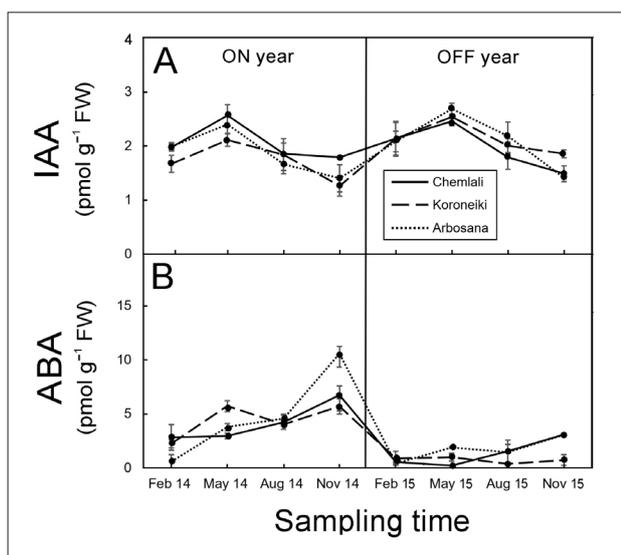
### Hormonal balance

The values of IAA foliar levels did not show a clear separation between ON and OFF years, but interestingly showed a common trend with peaks in May and declines until November (Figure 3A). In fruits, differences in ABA content, significantly higher in the ON year, were particularly evident for 'Arbosana' (Table 4). The values of foliar levels of ABA, ex-

cepting for 'Arbosana' in February, were significantly higher in the ON year for all the sampling dates (Figure 3B). Regarding fruits, differences in ABA content between cultivars and experimental years were not significant (Table 4).

It is known that the activation of many physiological responses is mediated through the synthesis of molecules with signal function, such as phytohormones. Many authors (Fernández-Escobar et al., 1992, Baktir et al., 2004, Ulger et al., 2004) reported that some endogenous plant growth hormones have an important role on regulating flowering and/or alternate bearing in olive. From a molecular point of view, Yanik et al. (2013) used an interesting approach by the identification of alternate bearing-associated microRNAs (miRNAs) in olive. They found that the nutritional and hormonal controls, together with the ones for the flowering processes, have noteworthy impacts on the alternate bearing in olive. In this experiment, we studied the trends of IAA and ABA concentrations in both leaves (Figure 3) and fruits (Table 4) in both ON and OFF years, in order to gain more information about the physiology of alternate bearing in olive tree. Particularly, IAA, the most abundant auxin in plants, stimulates cell division, expansion and differentiation in a majority of plants, including tree species (Sofo et al., 2012). On the other hand, ABA acts as a general inhibitor of growth and metabolism, and negatively affects the synthesis of proteins and nucleic acids (Kobashi et al., 2001, Srivastava, 2002). Its synthesis is enhanced in plants experiencing various types of abiotic stresses and it can be accumulated in leaves, where it is able to reduce stomatal conductance (Davies et al., 2000).

In most of the plants, IAA and ABA, together with other plant growth regulators, affect almost every event of plant growth and development, particularly stem and root growth, through cell elongation, and increased expression of several genes (Hedden and Thomas, 2006; Peleg and Blumwald, 2011). Furthermore, IAA and ABA appear to play a key but opposite role in the changes of growth processes, including fruit production (Maciejewska and Kopcewicz, 2002; Maksymiec, 2011). Unlike deciduous fruits having a short induction-to-initiation cycle, flower induction in olive may take up

**FIGURE 3.** Concentrations of (A) indole-3-acetic acid (IAA) and (B) abscisic acid (ABA) in leaves (2014 and 2015) of 'Chemlali' (continuous line), 'Koroneiki' (dashed line) and 'Arbosana' (dotted line) olive cultivars. The values represent means ( $n = 5$ )  $\pm$  SD. Where present, statistical differences ( $P < 0.05$ ) between cultivars and years are commented in the text. FW = fresh weight.**TABLE 4.** Concentrations of indole-3-acetic acid (IAA) and abscisic acid (ABA) in fruits of 'Chemlali' and 'Arbosana' olive cultivars. The values represent means ( $n = 5$ )  $\pm$  SD. Values followed by different letters are statistically different ( $P < 0.05$ ) within columns. FW = fresh weight.

Cultivar	Year	IAA	ABA
		(pmol g <sup>-1</sup> FW)	
Chemlali	2014	4.23 $\pm$ 0.10 b	1.30 $\pm$ 0.11 a
	2015	1.25 $\pm$ 0.72 d	1.64 $\pm$ 0.33 a
Arbosana	2014	7.62 $\pm$ 0.88 a	1.39 $\pm$ 0.08 a
	2015	2.02 $\pm$ 0.02 c	1.000 $\pm$ 0.49 a

to eight months, starting as early as July of the OFF year until about six weeks before full bloom. Although floral initiation occurs in November of the OFF year, the process of development of visible flower parts does not start until March. Indeed, differentiation takes place in late February and bloom in May, when the formation of each flower part occurs in the inflorescence. In this experiment, leaf IAA trend, with peaks in May and August, respectively, and declines during senescence (Figure 3A), was mainly synchronized with the vegetative growth of olive plants but not to their reproductive cycle. On the other side, the higher values of foliar ABA in the ON year (Table 4; Figure 3B), when fruits are abundant and well-developed, likely caused a depression of plant fruit production for the next OFF year, acting as a kind of biological clock for fruit induction.

## Conclusions

Crop load alternation provoked good fruit productions in the ON year, followed by low crops (OFF year). For all the three cultivars, the olive plants of the OFF year (2015) generally showed reduced pigments, decreased enzymatic and non-enzymatic antioxidant defences, and their ABA homeostasis was disrupted. All these studied factors can interact in determining the alternation of high and low crop load in olive tree. It is also true that differences in crop load, here monitored in a two-year and one-location experiment, could be not inevitably caused by an autonomous (internal) regulation but they may be affected by (one-time) external factors. Therefore, longer experiments are needed to have a complete picture of alternate bearing in olive tree. This notwithstanding, considering that olive oil is an important food product worldwide, this work could contribute to clarify the processes and factors regarding the effects of this phenomenon on fruit yield.

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