

## Role of Seed and Gibberellic Acid on Return Bloom in Olive (*Olea europaea* L. c.v. 'Tokhme Kabki')

M. Zare<sup>1</sup>, M. Rahemi<sup>1\*</sup>, and S. Eshghi<sup>1</sup>

### ABSTRACT

Alternate bearing is one of the most important problems in olive production around the world. This experiment was performed on 25-year-old olive trees of Tokhme Kabki cultivar in an olive orchard located in Shiraz, in 2018-2019. In this experiment, the role of normal fruits, shot berries, fruit removal, and Gibberellic Acid (GA<sub>3</sub>) application on the amount and type of return flower were determined. We demonstrated that seed has a significant role in flower induction in olive. Shot berry fruits actually induced return bloom and removing the fruit before pit hardening stimulates induction of flower bud in 'Tokhme Kabki' olive cultivar. GA<sub>3</sub> application before pit hardening significantly inhibited flower formation. Endogenous GA<sub>3</sub>-like substances was also determined in fruit flesh and seed tissues support the idea that, high concentration of GA<sub>3</sub>-like during pit hardening is responsible for the inhibition of flowering. According to the rapid increase in GA<sub>3</sub>-like substances in the fruit tissues, it appears that this compound may be transferred to the buds and then directed toward vegetative growth. Data suggest that GA<sub>3</sub>-like level in the fruit flesh and seed tissues is one of the main factors in alternate bearing of olive tree. Therefore, thinning the seeded fruit till 6 weeks after full bloom or before pit hardening would be effective in reducing the concentration of GA<sub>3</sub> in the olive tree and reducing the severity of alternate bearing.

**Keywords:** Alternate bearing, Full bloom, GA<sub>3</sub> application, Seeded fruit, Shotberry fruit.

### INTRODUCTION

Alternate bearing is a wide spread phenomenon in many fruit tree species, and yet it remains a problem with numerous fruit tree crops even today (Kour *et al.*, 2018). Alternate bearing is one of the most important horticultural problems in both deciduous and evergreen tree fruits, producing 'On' and 'Off' years, therefore, making yield estimation almost unpredictable. Alternate bearing can be observed among branches and shoots in a tree, trees in an orchard, and different areas in the same region (Prasad *et al.*, 2017). Olive tree has a strong alternate habit and sometimes even if flower development occurs after 'on' year, fruit set will be very

low because the majority of flowers will be male due to pistil abortion during the process of flower formation (Uriu, 1959). The sequence of flower development in the olive trees passes through induction, initiation in November (autumn), differentiation (winter) and, finally, maturation of flower (spring) before anthesis (Connor and Fereres, 2005).

Induction of flower buds is a quantitative change governed by hormonal balance or other cues such as nutritional status and C/N ratio. After stimulus has been received by the bud, the bud changes from vegetative to reproductive meristem. (Faust, 1989). Flower induction in olive buds occurs in July (7-8 weeks after full bloom) near the time of pit hardening of the

<sup>1</sup> Department of Horticultural Sciences, School of Agriculture, Shiraz University, Shiraz, Islamic Republic of Iran.

\*Corresponding author; e-mail: rahemi@shirazu.ac.ir



present season's fruit (Sanz-Cortés *et al.*, 2002). Some data suggest that floral induction in fruit trees is influenced by compounds released by the developing fruit and seeds that are translocated to the buds (Fabbri and Benelli, 2002; Rallo and Martin, 1991; Stutte and Martin, 1986). Endogenous plant growth hormone is one of the main factors that influence the alternate bearing of the olive trees (Monselise and Goldschmidt, 1982).

It is well documented that an application of Gibberellic Acid (GA<sub>3</sub>) during flower induction in fruit trees interrupts the floral process and reduces the intensity of flowering. Spraying olive trees with GA<sub>3</sub> before the expected 'on' year, decreased the number of flowers per inflorescence in the following year (Hassan, 1987). Floral initiation of olive is thought to be suppressed by Gibberellic Acids (GAs) released by the developing seeds (Fabbri and Benelli, 2000). External application of GA<sub>3</sub> and GA<sub>4</sub> both in May has been shown to prevent flower formation, and GA<sub>1</sub> and GA<sub>4</sub> have been endogenous inhibitors of flowering in Valencia orange (Mullins *et al.*, 1989). Fernandez- Escobar *et al.* (1992) found that injection of GA<sub>3</sub> to the scaffold of nonbearing "off" olive trees, inhibited flowering the following year. Also, they removed fruit and distracted seed and reported that they were effective in improving return bloom in 'Manzanillo' olives when done before pit hardening. Fruit removal after this period had no effect on return bloom in the following year.

Dag *et al.* (2010) found similar results and found that early de-fruited induced return bloom in the subsequent year, but poor or no bloom developed on late de-fruited olive trees. In another study, application of GA<sub>3</sub> succeeded in inhibiting olive trees flowering in the subsequent year (El-Sharkawy, 1999). Andreini *et al.* (2008) distinguished between 'on' and 'off' axillary buds in July, near to pit hardening. They observed accumulation of zeatin in meristem of 'off' axillary buds with strong RNA signal. Abu-Zahra and Al-

Dmoor (2013) reported that relative balance between GA<sub>3</sub> and ABA concentration in the tissue might play a key role in flower formation and alternate bearing in olive trees.

Kour *et al.* (2018) reported that gibberellins (GA<sub>s</sub>) are produced abundantly in the seed during its development and act as inhibitor in floral induction in olive. Also, pit hardening has a physiological importance in the floral induction. In loquat (*Eriobotrya japonica* Lindl.), GA<sub>3</sub> applied directly to the apex near the period of flower differentiation reduced the number of flower per panicle by 23-33%, but the morphological characteristics of the panicle did not change (Reig *et al.*, 2011). Gibberellic acid can change the pattern of flowering in plants. To study the patterns of walnut pistillate flowers, GA<sub>3</sub> at different concentrations were applied to walnut cultivar 'Chandler', 2 and 4 weeks after full bloom. The results of this study showed that GA<sub>3</sub> application increased the number of male flowers and male/female flowers ratio in the following year (Hassankhah *et al.*, 2018). Thus, it is well-known that flower induction process in olive starts in July, but the role of fruit type and the presence of seeds in fruits (seeded, shot berry or fruit removal) and endogenous GA<sub>3</sub> during pit hardening on flowering have not been well investigated.

The purposes of the present study were to determine the levels of endogenous GA-like compounds during fruit development and its application on return bloom and the number of perfect flowers in highly alternate bearer 'Tokhme Kabki' olive cultivar. Also, the potential role of seeded and parthenocarpic olive fruits (shot berry) on flowering in subsequent year was examined.

## MATERIALS AND METHODS

### Plant Material

The experiment was conducted using 25-year old olive trees cv. 'Tokhme Kabki',

which had been propagated by cutting, at a commercial orchard in Shiraz region, Iran (2018-2019). The trees were in an ON year. Tree spacing was 10×6 m. Trees received routine cultural practices for commercial fruit production including fertigation by drip irrigation system. The experiment was designed as a completely randomized block (RCBD) with three replicates. There were 4 trees in each block. Two branches per tree were selected in two directions (north and south) with uniform length, diameter, and flowers numbers. Two Weeks After Flowering (WAFB), with the identification of shot berry and normal fruits from each other, fruits were removed depending on the treatment. The treatments included control (intact fruits), only normal fruits (shot berry removal), only shot berry fruits (normal fruit removal), and defruited (removing all fruits). In the following year, the number of flowers on each treated branch was counted at balloon stage. The number of flowers per branch was determined according to the following formula:

$$\text{No of flowers per branch} = \text{No of cluster per branch} \times \text{Average no of flowers per cluster}$$

### Exogenous GA<sub>3</sub> Application

In the same orchard, exogenous application of GA<sub>3</sub> around the pit hardening period (around the first week of July (3/4/2018)) was conducted in factorial experiment in a randomized complete block design with four replications. Four 25-year-old uniform trees of 'Tokhme Kabki' cultivar were selected and each tree was used as a block; and on each tree four uniform branches were selected. Before foliar application of GA<sub>3</sub>, all flowers on the selected branches were removed immediately after Full Bloom (FB). The first factor included GA<sub>3</sub> (0, 25, 50 and 100 mg L<sup>-1</sup>) and the second factor was time of application, 2 weeks before and 2 weeks after pit hardening. Solutions were prepared

by dissolving GA<sub>3</sub> in small volumes of isopropyl alcohol. The final volume was made with double distilled water after adjusting the pH to 7.5-7.8, and applied to the branch units. In the following year, the number of flowers per branch and the percentage of staminate and perfect flowers were recorded.

### Endogenous GA<sub>3</sub>-Like Analysis

Two weeks after FB, endogenous GA<sub>3</sub>-like substances in lateral buds, fruit flesh, and randomly collected seeds were determined at 2-weeks interval until 4 Weeks After Pit Hardening (WAPH). The samples were transferred to the laboratory and stored at -80°C for further analysis. Changes of GA<sub>3</sub>-like in growing season were determined according to the following method.

### GA<sub>3</sub>-Like Analysis

Analyses of Gibberellin (GA<sub>3</sub>) were determined according to Topcuoglu and Unyayar (1995) and Ulger *et al.* (2004). Samples (1 g) were homogenized by a cold methanol: chloroform mixture (14: 6 v/v) at laboratory room temperature, then, were stored at -20°C for 1 week. The extracts were filtered through a Whatman No. 5 paper. The chloroform was separated from the methanol, by means of a funnel, and discarded. The pressurized methanol phase was separated from the aqueous phase by evaporation at 40°C. The pH was adjusted to 2.5 with 1N HCl and extracted three times with ethyl acetate. The resulting aqueous phase was adjusted to pH 7, using 1N NaOH, and extracted three times with ethyl acetate. Then, these free hormone extracts were dried under a vacuum at 40°C. The residue was dissolved in 1 mL methanol, and line-loaded on to thick silica gel 60 F254 TLC plate (Merck Plc, Darmstadt, Germany). Standard GA<sub>3</sub> was also spot-loaded at both edges of the plates in scored strips. Using isopropyl alcohol:



ammonia: water (84:8:8) as the solvent method, the plate was allowed to grow in the vertical direction for 15 cm. The positions of GA<sub>3</sub> were detected under UV light (254 nm wavelength) and marked after growth. A silica band corresponding to the RF values of the standards was scraped off, dissolved in an Eppendorf in 0.5 mL methanol, and then samples were screened before injection with a 0.45 µm filter. High-Pressure Liquid Chromatography (HPLC) analysis was performed with HPLC model AZURA (KNAUER, Germany), equipped with a Zorbax Eclipse XDB-C18 (4.6×250 mm, 5 µm), detector: at 254 nm, wave length: For GA<sub>3</sub> 254 nm, a flow of 1 mL min<sup>-1</sup>, and an injection amount of 30 µL, eluted with an isocratic mixture of 30% (v/v) HPLC- grade, water and 70% acetonitrile (including 0.05% acetic acid), at ambient temperature (Ulger *et al.*, 2004).

### Data Analysis

Data analyses were performed with SAS software package v. 9.01 (Institute Inc., Cary, NC), and subjected to Analysis Of Variance (ANOVA) with mean separation by Least Significant Difference (LSD) test ( $P < 0.05$ ) with standard errors of means. The charts were made using the Excel software (Version 2016, Microsoft Inc., and Redmond, WA, USA).

## RESULTS

### Effect of Seeded Fruit Removal and Shot berry Fruit on Return Bloom

In this study, we used ‘Tokhme Kabki’ cultivar, which produces shot berry fruits that are small, commonly seedless fruit expressing quantitative parthenocarpy. Seeded fruits removal before 2WAFB significantly induced return bloom. The complete removal of both, seeded and seedless (shot berry) fruits, strongly promoted return bloom in the subsequent

year compared to the control (Figure 1). The presence of both seeded and shot berry fruits decreased flowering. Shot berries alone allowed return bloom (Figure 1). The effect of shot berry fruits on total and perfect flowers in the following year was more than other treatments (Figure 1). Thus, shot berry fruit and early removal of seeded fruits before pit hardening allow flowering in the following year.

### Exogenous GA<sub>3</sub> Application on Return Bloom

GA<sub>3</sub> application significantly reduced return bloom, as compared to untreated branches in ‘Tokhme Kabki’ cultivar (Table 1). The magnitude of response depended on the concentration applied, although GA<sub>3</sub> treatments significantly reduced flowering (Table 1). Based on the results of this study, it seems that application of GA<sub>3</sub> altered the return flowering pattern in olive. GA<sub>3</sub> may have an effect on flower formation since results showed that the number of perfect and staminate flowers decreased by increasing GA<sub>3</sub> concentration (Table 1).

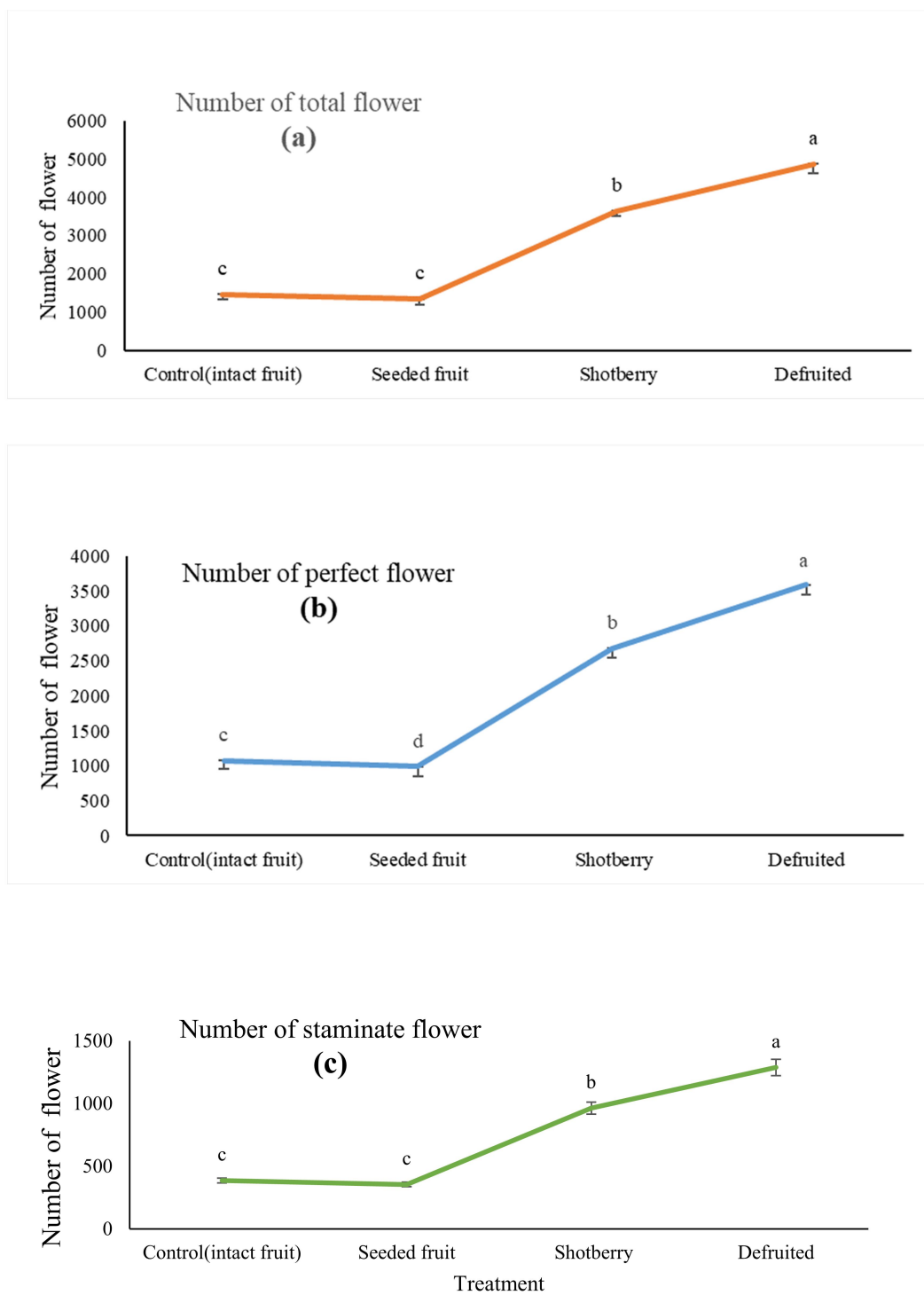
The application of GA<sub>3</sub> two Weeks Before Pit Hardening (2 WBPH) significantly reduced return bloom compared to its application after pit hardening (Table 2). Perfect and staminate flowers were significantly reduced when GA<sub>3</sub> was applied 2 WBPH (Table 2).

Interaction of GA<sub>3</sub> concentration and time of application showed that GA<sub>3</sub> at 50 and 100 mg L<sup>-1</sup> completely inhibited return bloom when applied 2WBPH (Table 3). GA<sub>3</sub> applied after pit hardening had no, or very mild, effect on inhibiting flower development in 2019.

### Effect of Endogenous GA<sub>3</sub> on Return Bloom

#### *GA<sub>3</sub>-Like Substances in Fruit Flesh Tissue*

The activity of GA<sub>3</sub>-like in fruit flesh tissue increased from 2 WAFB and reached the maximum level at pit hardening (6 WAFB)



**Figure 1.** Effect of seeded and shot berry fruits on flowering pattern of olive cv. 'Tokhme Kabki' in 2019. Data points represent mean±SE.

**Table 1.** Effect of foliar application of GA<sub>3</sub> on return bloom in olive cv. 'Tokhme Kabki' (2019).<sup>a</sup>

GA <sub>3</sub> (mg L <sup>-1</sup> )	No flower/ BCSA (cm <sup>2</sup> )	No total flower per each branch	No staminate flower/ BCSA (cm <sup>2</sup> )	No staminate flower	No perfect flower/ BCSA (cm <sup>2</sup> )	No perfect flower
Control	7.4019a	1300.2a	5.4474a	956.88a	1.9550a	343.33a
25	3.1095b	634.0b	2.2884b	466.56b	0.8213b	167.40b
50	0.9092c	187.8c	0.6691c	138.24c	0.2400c	49.60c
100	0.5909c	111.5c	0.4349c	82.08c	0.1563c	29.45c

<sup>a</sup> Means within column followed by the same letter are not significantly different by LSD at P ≤ 0.05 level. BCSA: Branch Cross Section Area (cm<sup>2</sup>).

**Table 2.** Effect of time of GA<sub>3</sub> application on the number of flowers of olive tree cv. 'Tokhme Kabki' in subsequent year (2019).<sup>a</sup>

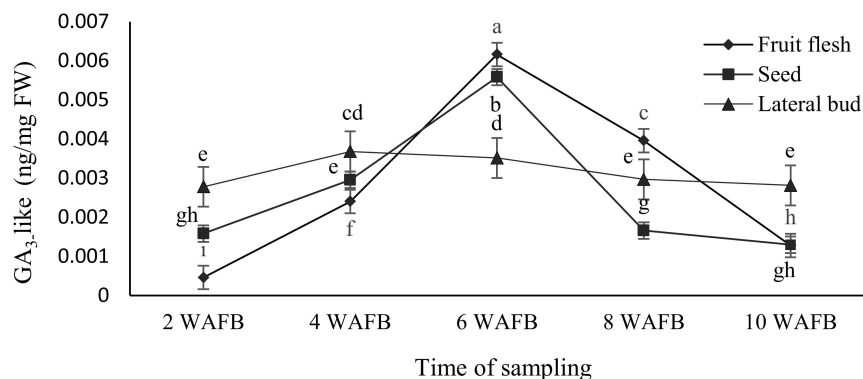
Spraying time	No flower/ BCSA (cm <sup>2</sup> )	No. flower	No staminate flower/ BCSA (cm <sup>2</sup> )	No staminate flower	No perfect flower/ BCSA (cm <sup>2</sup> )	No perfect flower
2 weeks before pit hardening (2WBPH)	1.2b	154.1b	0.85b	113.4b	0.30b	40.7b
2 weeks after pit hardening (2WAPH)	4.9a	962.7a	3.57a	708.5a	1.28a	254.2a

<sup>a</sup> Means within a column followed by the same letter are not significantly different by LSD at P ≤ 0.05 level. BCSA: Branch Cross Section Area (cm<sup>2</sup>).

**Table 3.** Interaction of GA<sub>3</sub> concentration and spraying time on the number of flowers of olive tree cv. 'Tokhme Kabki' in subsequent year (2019).

Treatment		Spraying time 2 WBPH				
GA <sub>3</sub> (mg L <sup>-1</sup> )	No flower/ BCSA (cm <sup>2</sup> )	No flower	No staminate flower/ BCSA (cm <sup>2</sup> )	No staminat e flower	No perfect flower/ BCSA (cm <sup>2</sup> )	No perfect flower
Control	3.15bc	440.3c	2.316bc	324.0c	0.833bc	116.25c
25	1.46cd	176.1cd	1.075cd	129.6cd	0.385cd	46.50cd
50	0	0	0	0	0	0
100	0	0	0	0	0	0
Treatment		Spraying time 2 WAPH				
GA <sub>3</sub> (mg L <sup>-1</sup> )	No. flower/ BCSA (cm <sup>2</sup> )	No. flower	No. staminate flower/ BCSA (cm <sup>2</sup> )	No. staminat e flower	No. perfect flower/ BCSA (cm <sup>2</sup> )	No. perfect flower No. perfect flower
Control	11.66a	2160.2a	3.078a	570.40a	8.579a	1589.8a
25	4.76b	1091.8b	1.258b	288.30b	3.502b	803.5b
50	1.82cd	375.7c	0.480cd	99.20c	1.338cd	276.5c
100	1.18cd	223.1cd	0.313cd	58.90cd	0.870cd	164.2cd

<sup>a</sup> Means within column followed by the same letter are not significantly different by LSD at P ≤ 0.05 level. BCSA: Branch Cross Section Area (cm<sup>2</sup>).



**Figure 2.** Concentration of GA<sub>3</sub>-like in olive tissues during growing season in 'Tokhme Kabki' (2019). Data points represent mean±SE.

and then sharply decreased to the lower level at 10 WAFB or 4 weeks after pit hardening (4 WAPH) (Figure 2).

#### *GA<sub>3</sub>-Like Substances in Seed*

The amount of GA<sub>3</sub>-like in the seeds was higher than that of fruit flesh until 4 WAFB and then it was lower at pit hardening (6 WAFB). After pit hardening, the level of GA<sub>3</sub>-like substances were always lower than fruit flesh tissue and reached the lowest level at 10 WAFB or 4 WAPH (Figure 2).

#### *GA<sub>3</sub>-Like Substances in Lateral Bud*

The concentration of GA<sub>3</sub>-like substances in lateral buds were higher than in fruit flesh and seed tissues until 4 WAFB and then slowly decreased until 10 WAFB. The GA<sub>3</sub>-like level in lateral buds was lower than in any other olive tissues at pit hardening, but higher at 10 WAFB (Figure 2).

## DISCUSSION

Alternate bearing involves a wide range of changes in activation and depression of endogenous metabolic pathway (Sharony, 2013). Alternate bearing is controlled by an

interaction between vegetative growth and fruit load (Hegazi *et al.*, 2011). Researcher reported that the seed produces a dominance signal (Bangerth, 1989). Dennis and Neilson (1999) and Huet (1973) reported that seedless apple fruits have less inhibitory effects on return bloom than seeded fruits. Lavee (2007) reported that flower induction in olive is influenced by signals produced by developing embryos. In this study, we found the same results. When we left shot berries (parthenocarpic fruits) on the branches, return bloom was significantly enhanced (Figure 1). This finding agrees with previous report, in which killing the seeds allowed the buds to differentiate into flowers in olive (Fernandez-Escobar *et al.*, 1992). This study suggests that seeded fruits, but not shot berries, inhibited flower induction in olive (Figure 1). Seeds are one of the sources of GAs relevant for flower induction in olive. Smith and Samach (2013) reported that, in perennial tree crops, labeled GAs appeared to move from seeds to neighboring shoots. Seeded fruits removal before pit hardening is a critical time to affect flowering in olive.

In this study, it appears that trees having seedless fruits actually had sufficient perfect flower in the following year, because of the greater availability of assimilates (Proietti and Tombesi, 1996). Seedless fruits not always induce flowering. In citrus, seedless fruit can cause alternate bearing too



(Verreyne and Lovatt, 2009). In olive, the early fruit removal has significant effects on return bloom (Lavee, 2007). In the present study, fruit removal 2 WAFB increased return bloom while any later time had only minor effects on flowering (Dag *et al.*, 2010).

The function of a plant depends on specific levels of natural endogenous hormones (Sharony, 2013). In this respect, it has been reported that the amount and rates of plant growth regulators in a plant affects the occurrence and severity of alternate bearing (Gunes *et al.*, 2010). The balance between gibberellic acid-like substances and abscisic acid concentrations of tissues appears to exhibit evidence of being a key regulator of floral development and alternate bearing (Baktir *et al.*, 2004). Foliar application of GA<sub>3</sub> can play an important role in the olive tree vegetative growth as well as in flower bud formation as reported by Abd El-Naby *et al.*, 2012)

Gibberellins (GAs), produced in great abundance by the seed during its development, inhibit floral induction (Kour *et al.*, 2018). Olive tree has a natural tendency to produce a high number of flowers and fruits, yet, the developing seeds produce molecular messengers (like the gibberellins) that inhibit floral induction, arresting the buds and directing them towards forming vegetative buds (Kour *et al.*, 2018).

The earlier seeded fruit removal, the lower GA<sub>3</sub> concentration transfer to the buds and more flower induction occurs at pit hardening in olive. A better understanding of GA<sub>3</sub> as floral inhibitors in olive trees begins with findings that seeded fruits, but not shot berries, inhibit flower induction in the subsequent year. Most of the studies carried out to investigate the effect of GA<sub>3</sub> on flower induction have been done by exogenous hormone application. In this study, GA<sub>3</sub> application significantly inhibited return bloom (Table 1). GA<sub>3</sub> has a high degree of hydroxylation, which is necessary for the movement of GAs in the bud tissue where flower induction occurs

(Kour *et al.*, 2018). The activity of GA<sub>3</sub> was dependent on time and concentration of hormone. Application of GA<sub>3</sub> at 50 and 100 mg L<sup>-1</sup> before pit hardening completely inhibited flower development for the next year. The results agree with report of Fernandez-Escobar *et al.* (1999) following GA<sub>3</sub> injection into the scaffold 2 WAFB reduced flower formation for the following year (Kafkas *et al.*, 2020). Single buds of orange shoots that were treated with 75 ng GA<sub>3</sub> showed a 75% reduction in flowering. In our experiment, application of GA<sub>3</sub> after pit hardening had low effects on return bloom. The result of this study was in accordance with previous reports where the injection of GA<sub>3</sub> in February before floral differentiation increased the length of inflorescence and it had no effect on return bloom, even though it has been stated that injections of GA<sub>3</sub> in November affect the growth of flower primordia. (Fernandez-Escobar, *et al.*, 1992). The olive has two flower types: perfect and staminate flowers. Application of GA<sub>3</sub> in this study changed the pattern of olive flowering. GA<sub>3</sub> at all concentrations reduced both types. GA<sub>3</sub> at 50 and 100 mg L<sup>-1</sup> significantly reduced the number of both types of flowers in 'Tokhme kabki' olive (Table 1). In walnut, GA<sub>3</sub> application at 100 mg L<sup>-1</sup> increased the number of staminate, and the total and the ratio of male/female flowers (Hassankhah *et al.*, 2018). The time of GA<sub>3</sub> application had an influence on flowering patterns (Table 2).

The results showed that the time of application of gibberellic acid affects the flower formation of the next year. GA<sub>3</sub> succeeded in inhibition of olive tree blooming in the following season. GA<sub>3</sub> has the potential control on growth and flowering process (Nafea and Abdulfatah, 2015). GA<sub>3</sub> succeeded in inhibition of olive tree blooming in the following season (El-Sharkawy, 1999). This finding suggested that GA<sub>3</sub> application before pit hardening has more influence on flower differentiation (Fernandez-Escobar *et al.*, 1992) than after



pit hardening. GA<sub>3</sub> controls growth and flowering. Spraying olive trees with Gibberellic acid (GA<sub>3</sub>) before an expected “on” year decreased flower buds per shoot and flowers per inflorescence the following year (Lavee and Haskal, 1994; Nafea and Abdulfatah, 2015; El-Iraqy, 2001).

Limited information is available on the endogenous GA<sub>3</sub>-like substances during the development of olive fruit. Badr *et al.* (1970) analyzed endogenous GAS and ABA-like inhibitors during floral induction and differentiation in vegetative (terminal) and potentially lateral reproductive buds. Two GA-like substances were found to accumulate during floral induction and quantitatively decrease during flower initiation. After fruit set (2 WAFB), GA<sub>3</sub>-like substances increased in agreement with previous reports that GA<sub>3</sub>-like substances level sharply increased in all tissues after fruit set (Abu-Zahra and Al-Dmoor, 2013). The highest GA<sub>3</sub>-like substances level in ‘On’ trees were found in fruit flesh and seeds in June and July (6 WAFB) during pit hardening (flower induction), and then gradually decreased until 10 WAFB (Figure 2) (Abu-Zahra and Al-Dmoor, 2013).

This finding was also in parallel with Shulman and Lavee (1980) who reported a gradual reduction in GAs content during fruit development, finding the lowest level at full maturation. Fruit flesh and seeds are not the only source of GA<sub>3</sub>-like substances relevant for flower induction in trees. Shoot tips are also rich in bioactive GA<sub>5</sub>, particularly GA<sub>1</sub>, and could be involved in floral inhibition of lateral buds of long shoots (Forshey and Elfving, 1989). Experimental studies proposed that auxin stimulates gibberellin biosynthesis in various shoot tissues as well as in seeds (Van Huizen *et al.*, 1997). Researcher hypothesized seeds produce the dominant signal (Bangerth, 1989).

Indeed, shot berry olive fruits have less inhibitory effect on floral induction than seeded fruits. A high positive correlation was shown between fruit load and level of chlorogenic acid (phenolic compound) in the

leaves of olive tree in mid-summer (during pit hardening). Chlorogenic acid accumulates in leaves of fruiting olive tree so that these trees enter the induction period with high level of this phenolic acid (Lavee *et al.*, 1986). GA<sub>3</sub> spraying during flower induction significantly inhibited the activity of PAL, PPO, POD enzymes in lignin biosynthesis in the leaves during flower induction, inhibiting flower bud initiation (Li *et al.*, 2003). It seems that endogenous GA<sub>3</sub>-like substances in fruit flesh and seed of olive send signals to the buds and inhibit floral induction, directing buds to a vegetative fate. These data suggest that high concentrations of GA<sub>3</sub>-like 6 WAFB (pit hardening) promoted vegetative bud growth.

## CONCLUSIONS

The presence of seeded fruits and the level of GA<sub>3</sub>-like in fruit flesh and seed tissues at a high-level during pit hardening seem to translocate signals to the lateral vegetative bud inhibiting flower induction, but, shot berry olive fruits do not inhibit flower induction for the subsequent year. This finding reveals that flowering in ‘Tokhme Kabki’ olive cultivar is controlled by endogenous as well as exogenous GA<sub>3</sub>. Application of GA<sub>3</sub> before pit hardening during ‘On’ year reduces flower induction and well-differentiated and developed flowers in the following year. Therefore, in this situation, high levels of GA<sub>3</sub> in fruits during the ‘On’ year increase the sink strength of the fruit and compete with the buds for carbohydrates and nitrogen compounds. Under such conditions, the growth of the branches of the current season will decrease and there will be less position for the formation of next year's flowers, thus, there will be fewer flowers next year. Also, due to the carbohydrate depletion of the buds, most of the induced flower buds do not fully develop and most of the flowers are male, and fruit formation will be less.



## ACKNOWLEDGEMENTS

We thank Shiraz University for performing relevant tests in the central laboratory of Shiraz University.

## REFERENCES

1. Abd El-Naby, S. K. M., El-Sonbaty, M. R., Hegazi, E. S., Samira, M. M. and El-Sharony, T.F. 2012. Effect of Gibberellic Acid Spraying on Alternate Bearing of Olive Trees. *J. Appl. Sci. Res.*, **8(10)**: 5114-5123.
2. Abu-Zahra, T. and Al-Dmoor, H. 2013. Seasonal Changes in Endogenous Plant Hormones and Alternate Bearing of Nabali Olive (*Olea europaea* L.) Trees. *Asian J. Plant Sci.*, **12(6/8)**: 241-246.
3. Andreini, L., Bartolini, S., Guivarc'h, A., Chriqui, D. and Vitagliano, C. 2008. Histological and Immunohistochemical Studies on Flower Induction in the Olive Tree (*Olea europaea* L.). *Plant Biol.*, **10(5)**: 588-595.
4. Badr, S. A., Hartmann, H. T. and Martin, G. C. 1970. Endogenous Gibberellins and Inhibitors in Relation to Flower Induction and Inflorescence Development in the Olive. *Plant Physiol.*, **46(5)**: 674-679.
5. Baktir, I., Ulger, S. and Kaynak, L. 2004. Relationship of Seasonal Changes in Endogenous Plant Hormones and Alternate Bearing of Olive Trees. *HortScience*, **39(5)**: 987-990.
6. Bangerth, F. 1989. Dominance among Fruits/Sinks and the Search for a Correlative Signal. *Plant Physiol.*, **76(4)**: 608-614.
7. Connor, D. J. and Fereres, E. 2005. The Physiology of Adaptation and Yield Expression in Olive. *Hortic. Rev.*, **31**: 155-229.
8. Dag, A., Bustan, A., Avni, A., Lzipori, I., Lavee, S. and Riov, J. 2010. Timing of Fruit Removal Affects Concurrent Vegetative Growth and Subsequent Return Bloom and Yield in Olive (*Olea europaea* L.). *Sci. Hortic.*, **123(4)**: 469-472.
9. Dennis, F. G. and Neilsen, J. C. 1999. Physiological Factors Affecting Biennial Bearing in Tree Fruit: The Role of Seeds in Apple. *HortTechnology*, **9(3)**: 317-322.
10. El-Sharkawy, S. M. 1999. Physiological Studies on Flowering and Fruiting of Olive Tree. PhD. Thesis, Fac. Agric. Moshtohor Zagazig Univ., Egypt, 168 PP.
11. Emongor, V. E. 2004. Effect of Gibberellic Acid on Postharvest Quality and Vase Life of Gerbera Cut Flowers (*Gerbera jamesonii*). *J. Agron.*, **3**: 191-195.
12. El-Iraqy, M. 2001. Physiological Studies on Alternate Bearing of Olive. PhD. Thesis, Fac. Agric., Zagazig University., Egypt, 148 PP.
13. Fabbri, A. and Benelli, C. 2000. Flower Bud Induction and Differentiation in Olive. *J. Hortic. Sci. Biotechnol.*, **75(2)**: 131-141.
14. Faust, M. 1989. *Physiology of Temperate Zone Fruit Trees*. John Wiley and Sons, Inc., New York, 338 PP.
15. Fernández-Escobar, R., Benlloch, M., Navarro, C. and Martin, G. C. 1992. The time of floral induction in the olive. *J. Am. Soc. Hortic. Sci.*, **117(2)**: 304-307.
16. Fernández-Escobar, R., Moreno, R. and García-Creus, M. 1999. Seasonal Changes of Mineral Nutrients in Olive Leaves during the Alternate-Bearing Cycle. *Sci. Hortic.*, **82(1-2)**: 25-45.
17. Forshey, C. G. and Elfving, D. C., 1989. The Relationship between Vegetative Growth and Fruiting in Apple Trees. *Hortic. Rev.*, **11**: 229-287.
18. Francis, D. and Sorrell, D. A. 2001. The Interface between the Cell Cycle and Plant Growth, Regulators: A Mini Review. *Plant Growth Regul.*, **33**: 1-12.
19. Gunes, N. T., Okay, O., Koksall, I. A. and Koroglu, M. 2010. The Effect of Nitrogen and Phosphorus Fertilization on Yield, Some Fruit Characteristics, Hormone Concentrations, and Alternate Bearing in Pistachio. *Turk. J. Agric. For.*, **34**: 33-43.
20. Hassan, L. H. O. 1987. Effect of Some Growth Substances on Flowering and Fruiting of Olive Trees. PhD. Thesis, Faculty of Agriculture, Ain Shams University, Egypt, 157 PP.

21. Hassankhah, A., Rahemi, M., Mozafari, M. R., and Vahdati, K. 2018. Flower Development in Walnut: Altering the Flowering Pattern by Gibberellic Acid Application. *Notulae Botanicae Horti Agrobotanici Cluj –Napoca*, **46(2)**: 700-706.
22. Hegazi, E. S., Mohamed, S. M., El-Sonbaty, M. R., Abd El-Naby, S. K. M. and El-Sharony, T. F. 2011. Effect of Potassium Nitrate on Vegetative Growth, Nutritional Status, Yield and Fruit Quality of Olive CV. "Picual". *J. Hortic. Sci. Ornament. Plants*, **3(3)**: 252-258.
23. Huet, J. 1973. Floral Initiation in Pear Trees. *Acta Hort.*, **34**: 193-198.
24. Kafkas, N. E., Gundesli, M. A., Guney, M. and Kafkas, S. 2020. Endogenous Gibberellin and Abscisic Acid Influence Alternate Bearing in Pistachio (*Pistacia vera* L.). *Pak. J. Bot.*, **52(4)**: 1235-1241.
25. Khan, A. S. and Chaudhry, N. Y. 2006. GA<sub>3</sub> Improves Flower Yield in Some Cucurbits Treated with Lead and Mercury. *Afr. J. Biotechnol.*, **5**: 149-153.
26. Kour, D., Bakshi, P., Wali, V. K., Sharma, N., Sharma, A. and Iqbal, M. 2018. Alternate Bearing in Olive: A Review. *Int. J. Curr. Microbiol. Appl. Sci.*, **7**: 2281-2297.
27. Lavee, S., Harshemesh, H. and Avidan, N. 1986. Phenolic Acids-Possible Involvement in Regulating Growth and Alternate Fruiting in Olive Trees. *V Int. Symp. Growth Regul. Fruit Prod.*, **179**: 317-328.
28. Lavee, S. and Haskal, A. 1994. Partial Fruiting Regulation of Olive Trees (*Olea europaea* L.) with Paclobutrazol and Gibberellic Acid in the Orchard. *Adv. Hortic. Sci.*, **7(2)**: 83-86.
29. Lavee, S. 2007. Biennial Bearing in Olive (*Olea europaea* L.). *Annales Ser. Hist. Nat.*, **17**: 101-112.
30. Li, X., Li, S. and Lin, J. 2003. Effect of GA<sub>3</sub> Spraying on Lignin and Auxin Contents and the Correlated Enzyme Activities in Bayberry (*Myrica rubra* Bieb.) during Flower-Bud Induction. *Plant Sci.*, **164(4)**: 549-556.
31. Monselise, S. P. and Goldschmidt, E. E. 1982. Alternate Bearing in Fruit Trees. *Hortic. Rev.*, **4(1)**: 128-173.
32. Mullins, M. G., Plummer, J. A. and Snowball, A. M. 1989. Flower Initiation: New Approaches to the Study of Flowering in Perennial Plants. In: "Manipulation of Fruiting", (Ed.): Wright C. J. Butterworth and Co Publishers Ltd. London. 65-77.
33. Nafea, S. M., and Abdelfattah, H. K. 2015. Effect of Foliar Application of GA<sub>3</sub> and NAA for Reducing Alternate Bearing of Olive Trees (*Olea europaea* L. cv. Ashrasie). *Zanco J. Pure Appl. Sci.*, **27**: 1-6.
34. Prasad, A., Sakai, K. and Hoshino, Y. 2017. Direct Coupling: A Possible Strategy to Control Fruit Production in Alternate Bearing. *Sci. Rep.*, **7(1)**: 1-12
35. Proietti, P. and Tombesi, A. 1996. Effects of Gibberellic Acid, Asparagine and Glutamine on Flower Bud Induction in Olive. *J. Hortic. Sci.*, **71(3)**: 383-388.
36. Rallo, L. and Martin, G. C. 1991. The Role of Chilling in Releasing Olive Floral Buds from Dormancy. *J. Am. Soc. Hortic. Sci.*, **116(6)**: 1058-1062.
37. Reig, C., and Farina, V., Volpe, G., Mesejo, C., Martínez-Fuentes, A., Barone, F. and Agusti, M. 2011. Gibberellic Acid and Flower Bud Development in Loquat (*Eriobotrya japonica* Lindl.). *Sci. Hortic.*, **129(1)**: 27-31.
38. Sanz-Cortés, F., Martínez-Calvo, J., Badenes, M.L., Bleiholder, H., Hack, H., Llacer, G. and Meier, U. 2002. Phenological Growth Stages of Olive Trees (*Olea europaea* L.). *Ann. Appl. Biol.*, **140(2)**: 151-157.
39. Sharony, T. F. 2013. Influence of Foliar Application of Gibberellic Acid on the Endogenous Phytohormones. *J. Appl. Sci. Res.*, **9(3)**: 2196-2204.
40. Shulman, Y. and Lavee, S. 1980. Gibberellin-Like Substances during Ripening of Olive Fruit. *Sci. Hortic.*, **12(2)**: 169-175.
41. Smith, H. M. and Samach, A. 2013. Constraints to Obtaining Consistent Annual Yields in Perennial Tree Crops. I: Heavy Fruit Load Dominates over Vegetative Growth. *Plant Sci.*, **207**: 158-167.



42. Stutte, G. W. and Martin, G. C. 1986. Effect of Killing the Seed on Return Bloom of Olive. *Sci. Hortic.*, **29(1-2)**: 107-113.
43. Topcuoglu, F. and Unyayar, S. 1995. Determination of Growth Substances (Auxin, Gibberellic Acid, Abscisic Acid and Cytokinin) Production and Biological Activity in White Rot Fungus *Phanerochate chrysosporium* ME 446. Arastırma Fonu Proje. No. 93-119, İnönü Univ.
44. Ulger, S., Sonmez, S., Karkacier, M. and Akdesir-Aksu, M. 2004. Determination of Endogenous Hormones, Sugars and Mineral Nutrition Levels during the Induction, Initiation and Differentiation Stage and Their Effects on Flower Formation in Olive. *Plant Growth Regul.*, **42(1)**: 89-95.
45. Uriu, K. 1959. Periods of Pistil Abort in Development of the Olive Flowers. *Proc. Am. Soc. Hortic. Sci.*, **73**: 194-202.
46. Van Huizen, R., Ozga, J. A. and Reinecke, D. M. 1997. Seed and Hormonal Regulation of Gibberellin 20-Oxidase Expression in Pea Pericarp. *Plant Physiol.*, **115(1)**: 123-128.
47. Verreynne, J. S. and Lovatt, C. J. 2009. The Effect of Crop Load on Budbreak Influences Return Bloom in Alternate Bearing 'Pixie' Mandarin. *J. Am. Soc. Hortic. Sci.*, **134(3)**: 299-307.

### نقش بذر و اسید جیبرلیک در شکوفایی بازگشت در زیتون (*Olea europaea* L. c.v. ) 'Tokhme Kabki'

م. زارع، م. راحمی، و س. عشقی

#### چکیده

باردهی متناوب یکی از مهمترین مشکلات تولید زیتون در سراسر جهان است. این آزمایش بر روی درختان زیتون ۲۵ ساله رقم تخم کبکی در باغ زیتون واقع در شیراز در سال ۹۸-۱۳۹۷ انجام شد. در این آزمایش نقش میوه‌های معمولی، میوه‌های ساچمه‌ای، حذف میوه و کاربرد اسید جیبرلیک بر میزان گلدهی سال بعد تعیین شد. در مطالعه ما نشان دادیم که دانه نقش مهمی در القای گل در زیتون دارد. میوه‌های ساچمه‌ای سبب ایجاد گلدهی بیشتر در سال بعد شده و حذف میوه قبل از سفت شدن مغز باعث تحریک القای جوانه گل در رقم زیتون تخم کبکی می‌شود. کاربرد اسید جیبرلیک قبل از سفت شدن مغز به طور قابل توجهی از تشکیل گل جلوگیری کرد. شبه اسید جیبرلیک درون‌زا نیز در بافت‌های گوشت و دانه میوه مشخص شد که از این ایده پشتیبانی می‌کند که غلظت بالای شبه اسید جیبرلیک در طول سفت شدن مغز مسئول مهار گلدهی است. با توجه به افزایش سریع مواد شبه اسید جیبرلیک در بافت میوه، به نظر می‌رسد که این ترکیب ممکن است به جوانه‌ها منتقل شده و سپس به سمت رشد رویشی هدایت شود. داده‌ها نشان می‌دهد که سطح ماده شبه‌اسید جیبرلیک در گوشت میوه و بافت دانه یکی از عوامل اصلی در باروری متناوب درخت زیتون است. بنابراین تک کردن میوه بذر تا شش هفته پس از شکوفه کامل یا قبل از سفت شدن مغز در کاهش غلظت اسید جیبرلیک در درخت زیتون و کاهش شدت باردهی متناوب موثر خواهد بود.