UNIVERSITY OF CALIFORNIA
RIVERSIDE

# Alternate Bearing in Olive (*Olea europaea* L.)

A Thesis submitted in partial satisfaction
of the requirements for the degree of

Master of Science

in

Plant Biology

by

Yi-Yun Chao

March 2015

Thesis Committee:
Dr. Carol J. Lovatt, Chairperson
Dr. Darleen A. DeMason
Dr. Peggy A. Mauk

The Thesis of Yi-Yun Chao is approved:

_____

_____

_____

Committee Chairperson

University of California, Riverside

**Acknowledgements**

I would like to thank my major professor, Dr. Carol Lovatt, for her training, encouragement, and support. I owe my deepest gratitude to her. She has given me a lot of advice and always been patient with me. I have really learned many things to improve myself under her guidance.

I would like to thank my Thesis Committee, Dr. Darleen DeMason and Dr. Peggy Mauk for their help in keeping me on the right track with my research.

I would like to thank people in Dr. Elizabeth Fichtner's lab, Katie Wilson and Walter Martinez, for their help in collecting olive shoots in the field.

I would like to thank Dr. Rui Li and Lisa Tang for helping me solve the problems I encountered in the gene expression experiments and process the samples every time I arrived back from the field and Toan Khuong, who helped me with the statistical analysis, making my figures and tables better, and the field work. I would like to thank Chris Jordan, Dwight Williams, Alec Weitzel, Jacob Hernandez, Peter Braun and Ryan Sendejas for helping me with the shoot data collection and grinding samples for me.

I would like to thank the Department of Botany and Plant Sciences at the University of California, Riverside, for providing me with the opportunity to learn professional knowledge here.

I would like to thank my new friends I made here, John Chater and Andrew Semotiuk, for their support and help.

I would like to thank all Taiwanese students here, Lisa Tang, Ariel Kuan, David Chen, Chia-Li Chen, Hsuan-chieh Peng, Chien-Yu Huang, Wei-Chi Wu, Jerry Tan, Kobe

Yang, Jimmy Liang, Kidd Tseng, and Carrie Cheng, who helped my life here and with whom I shared many experiences.

I would like to thank my parents and my family for their support, especially my wife who always stands by me and supports me with everything. Without their support, I would not have a chance to learn and improve myself.

Finally, I acknowledge the financial support from the California Olive Committee in the form of grants to support the research.

ABSTRACT OF THE THESIS

Alternate Bearing in Olive (*Olea europaea* L.)

by

Yi-Yun Chao

Master of Science, Graduate Program in Plant Biology
University of California, Riverside, March 2015
Dr. Carol J. Lovatt, Chairperson

In alternate bearing olive trees, high yield on-crop years alternate with low yield off-crop years. Research was undertaken in support of the California olive industry to determine how fruit number one year impacts floral intensity and yield the following year. Removing the on-crop of fruit before endocarp sclerification (mid-July) restored summer vegetative shoot growth and inflorescence number the following spring. Delaying fruit removal to 75 days after endocarp sclerification (September) significantly reduced spring bud break and floral intensity. Quantification of the fate of buds on nonbearing and bearing shoots of off- and on-crop trees provided striking results. The majority of buds on nonbearing shoots of off-crop trees produced inflorescences, whereas most buds on nonbearing shoots of on-crop trees remained inactive through spring bloom. In sharp contrast, abscission of 74% of buds on bearing shoots of on-crop trees occurred prior to spring bloom. A putative *O. europaea FLOWERING LOCUS T (FT)* gene, known to upregulate flowering in woody perennials, was expressed in buds on

nonbearing shoots of off-crop trees from June through March, but was repressed in buds of bearing and nonbearing shoots of on-crop trees starting in September and December, respectively. Lack of *OeFT* expression in buds on bearing shoots of on-crop trees may be due to the abscission of 51% of the buds by September. However, this result taken together with repression of *OeFT* in buds on nonbearing shoots of on-crop trees, for which bud abscission was not a factor, suggests the possibility that the on crop may inhibit determination of the floral meristem. Buds on nonbearing shoots of off-crop trees subsequently expressed two putative floral organ identity genes. The gene related to carpel development was expressed weakly in buds on nonbearing shoots of on-crop trees, which produced a few inflorescences at bloom; bearing shoots did not flower. This research is the first to provide evidence that several mechanisms in addition to inhibition of summer vegetative shoot growth, including inhibition of spring bud break, inhibition of floral development at the level of gene expression, and abscission of potential floral buds, contribute to the perpetuation of alternate bearing in olive.

## Table of Contents

## List of Tables

**Appendix A**

## List of Figures



## Appendix A

## 1. **Introduction**

Olive (*Olea europaea* L.) is an evergreen woody perennial native to the Mediterranean basin. Olive fruit are commercially important throughout the world for table consumption and their oil, which is known for its human health benefits. Over the past decade, the greatest olive production has been in Europe, especially in Spain, Italy and Greece, which are also the world's top three major producers of olive oil (FAOSTAT, 2014). In the United States, olive trees were brought to California in the late 18$^{th}$ century; a thriving industry had developed by the mid-19$^{th}$ century (Connell, 2005). Currently, California is the only state in the United States to commercially produce olives. Mazanillo and Sevillano are the two main cultivars grown on approximately 40,000 acres in California (Vilsack and Clark, 2013). In 2013, the 'Manzanillo' crop totaled 85,000 tons valued at US$ 70 million and the 'Sevillano' crop was 11,000 tons worth US$ 9 million (Vilsack and Clark, 2013). In California, Tulare County is the major producer of olives, based on crop value, followed by Glenn, Tehama, Fresno and Butte counties (County statistical data, 2012).

Olive yields are of economic importance to California's table and oil olive growers and olive processing industries. However, olive production has been highly variable over the past several years, largely due to alternate bearing (Tolomeo et al., 2013). Alternate bearing, also called biennial bearing or irregular bearing, is a widespread phenomenon in many deciduous and evergreen fruit and nut tree species in addition to olive, such as apple (*Malus domesticus*), pistachio (*Pistacia vera* L.), citrus (*Citrus sinensis*, *C. reticulata* Blanco), and avocado (*Persea americana* Mill). The term

describes the tendency of a fruit tree to produce a high or heavy yield, called an on crop (on-crop year), followed by a low or light yield the next year, called an off crop (off-crop year) (Monselise and Goldschmidt, 1982). The scale of alternate bearing can be a localized effect restricted to parts of a tree to a phenomenon affecting a large geographical area (Monselise and Goldschmidt, 1982). In an on-crop year, a fruit tree produces a large number of fruit of small sizes and low market value, whereas in an off-crop year, it produces a reduced number of fruit of larger sizes (Table 1). In the off-crop year, the large fruit size does not provide growers with a good income because there are simply too few fruit. Compared to the 2010 on-crop year in California, the 2011 yields of the two major varieties were reduced almost 80% (Tolomeo et al., 2013). In Tulare County, the loss in olive production between the 2010 on crop and the 2011 off crop was worth nearly US$51 million (Kinoshita, 2011). Obviously, alternate bearing causes dramatically severe economic problems for the olive industry in California. Thus, methods to mitigate the impact of alternate bearing on the olive industry and to keep production stable are important.

Climatic events that cause the failure of floral development or excessive abscission of reproductive structures during bloom or fruit set are the main cause of an off crop that is followed by an on crop the next year or, in some cases, two years later, depending on how long the trees need to recover. All unfavorable climatic conditions, such as high or low temperatures, water deficiency, heavy rain and wind, can cause the loss of reproductive structures and initiate alternate bearing. In addition, commercial ‘Manzanillo’ olive groves in California include ‘Sevillano’ as a pollenizer. Climatic

conditions that are unfavorable for bee activity can dramatically reduce pollination, fertilization, fruit set and yield and initiate alternate bearing. On the other hand, failure of natural flower and fruit thinning due to favorable climatic conditions during bloom or fruit set results in an on crop that is followed by an off crop. Once alternate bearing is initiated, it is perpetuated by the effects of the total number of fruit (crop load) on endogenous tree factors that ultimately impact floral intensity (inflorescence and flower number) the following year. Therefore, the on crop reduces return bloom the next spring, whereas the off crop increases return bloom. The endogenous physiological factors by which crop load one year influences floral intensity the next year are unknown in olive.

Known mechanisms demonstrated to function in the perpetuation of alternate bearing in different fruit and nut tree crops are discussed below. To evaluate whether any are potentially applicable to 'Manzanillo' olive production in California, it is important to understand the phenology of the tree under California growing conditions. The phenology presented here is specific to 'Manzanillo' olive grown in Tulare County for the table olive industry (Fig. 1). For this growing area, spring vegetative shoot growth occurs from late February through May and summer vegetative shoot growth is from June through September. Growth during the winter is negligible. According to Fernandez-Escorbar et al. (1992), annual phase transition from vegetative shoot development to reproductive development likely occurs just prior to endocarp sclerification (pit hardening) in early July. The floral meristems appear to be determined (committed to floral development) by October to November, at which time floral buds have increased in size with no accompanying visible changes in the floral primordia (Pinney and Polito, 1990; Cuevas et

al., 1999). Initiation of floral organ development starts with the formation of sepal primordia, which was observed to occur between mid-February and mid-March for 'Manzanillo' olive trees growing in California (Hartmann, 1951; Cuevas et al., 1999). Hence, flower formation begins approximately mid-February to mid-March followed by full bloom between April and May (Hartmann, 1951). Consistent with this description of olive floral development, Rallo and Martin (1991) demonstrated that low temperatures in winter were not required for floral initiation, but rather winter chilling temperatures were necessary for breaking bud dormancy in spring of floral buds initiated the previous summer. Olive fruit development is characterized by a double sigmoid growth curve like other stone fruit, such as peach (*Prunus persica* L.) and cherry (*Prunus avium* L.). There are three stages. Stage I, from May to June, is characterized by a rapid increase in fruit volume due largely to cell division in the ovary and ovule. Stage II, from June to July, is the period of endosperm and embryo growth and ends with lignification and hardening of the endocarp (pit hardening) (Stutte and Martin, 1986; Fernandez-Escorbar et al., 1992). In Stage III, from July to harvest (October-November) (Hartmann, 1949), the ovary resumes growing, but predominantly by cell enlargement. Additionally, olive is a non-climacteric fruit. The physiological significance is that olive fruit do not undergo a climacteric increase in respiration rate and do not ripen; they mature on the tree and continue to accumulate oil.

## 1.1 Inhibition of bud break

### 1.1.1 Summer vegetative shoot growth

According to Sibbett (2000), summer vegetative shoot growth of olive is very

important to floral intensity at spring bloom the following year. Shoot extension growth and lateral shoot development increase the number of nodes, which bear floral buds. During the summer of the on-crop year, shoot extension growth of olive is inhibited, reducing the number of nodes and floral buds that develop, resulting in an off bloom that sets the off crop. For many fruit tree species, one-year-old spring and summer vegetative shoots generally produce the majority of the inflorescences at spring bloom the next year. Moneselise and Goldschmidt (1982) found that crop load had a negative effect on summer vegetative shoot growth in citrus. Verreynne and Lovatt (2009) removed the entire on crop of 'Pixie' mandarin (*C. reticulata* Blanco) fruit monthly from fruit set to harvest to identify the inhibitory effect of the young developing fruit of the on crop on summer vegetative shoot growth and reduced inflorescence number the following spring. If fruit were removed before July, the number and length of summer vegetative shoots was increased, with a corresponding increase in inflorescence number at full bloom. In avocado (*Persea americana* Mill), the amount of summer vegetative shoot growth is also crucial to floral intensity because the summer shoots contribute 60% to 75% of the floral shoots to bloom the following spring (Lovatt, 2010). Similarly, crop load has a negative effect on vegetative shoot growth in avocado (Schaffer et al., 1991; Lovatt, 2010). These results indicate that the negative effect of crop load on summer vegetative shoot growth, which indirectly reduces inflorescence number at spring bloom by reducing the number of nodes that can bear inflorescences, is a mechanism perpetuating alternate bearing in a number of tree crops, including olive (Schaffer et al., 1991; Sibbett, 2000; Verreynne and Lovatt, 2009; Lovatt, 2010).

It is possible that in these tree crops fruit number reduces summer vegetative shoot growth by influencing the availability of carbohydrate energy reserves to support growth or by altering hormone homeostasis. Information from other tree crops suggests that carbohydrate availability is not a factor limiting summer vegetative shoot growth. For avocado, differences in accumulated starch concentrations in off- and on-crop trees were only detected in autumn through winter (Whiley and Wolstenholme, 1990). Further evidence provided by Lovatt (2006) showed that glucose concentrations were not significantly different between buds from nonbearing shoots of off-crop trees and buds from bearing shoots of on-crop trees from August through February. In citrus, Verreynne (2005) provided evidence that carbohydrate availability is not a factor inhibiting summer vegetative shoot growth. Removing all the fruit from on-crop trees in July increased the number of summer vegetative shoots but failed to increase glucose and starch concentrations above those of on-crop trees. Similarly, Ü lger et al. (2004) provided evidence that sucrose and glucose concentrations were not significantly different in leaves and nodes between off- and on-crop trees in summer in olive. These results support the possibility that other factors play a role in reducing summer vegetative shoot growth.

Research with 'Pixie' mandarin provided evidence that inhibition of summer vegetative shoot growth was due to correlative inhibition (Verreynne, 2005). Young developing fruit of on-crop trees caused a change in hormone homeostasis resulting in a high ratio of indole-3-acetic acid (IAA) to isopentenyladenine (IPA), a cytokinin, in the apical bud of bearing shoots. In August, apical bud IAA concentrations for bearing shoots

of on-crop trees were two-fold greater than those of nonbearing shoots of off-crop trees and bud IPA concentrations were 1.7-fold lower for on-crop trees than those of off-crop trees. Removal of the on-crop in July restored the IAA and IPA concentrations and summer vegetative shoot development to values equal to off-crop trees. Verreynne (2005) reported a strong positive correlation between apical bud IPA concentrations and the number of summer vegetative shoots that developed and a strong negative correlation between apical bud IAA concentrations and summer vegetative shoot development. It is possible that inhibition of summer vegetative shoot growth in 'Manzanillo' olive is due to correlative inhibition. Injecting the cytokinin 6-benzayladenine (6-BA) and the auxin transport inhibitor tri-iodobenzoic (TIBA) alone or in combination into branches of on-crop 'Manzanillo' olive trees in July significantly increased summer vegetative shoot growth and return bloom (Fichtner and Lovatt, 2013).

### 1.1.2 Spring bud break

There is evidence that the on crop of fruit delays spring bud break in citrus (Jones et al., 1976, 1977). For many citrus cultivars, the maturing fruit are not harvested until after spring bloom. Even for cultivars that mature early, fruit are frequently held on the tree to increase fruit size or to obtain a better price in the market. By removing the young on crop of fruit monthly and quantifying the total number of buds per shoot and the number of inflorescences, vegetative shoots and inactive buds per shoot at spring bloom, Verreynne and Lovatt (2009) were able to document that the on crop of fruit inhibited spring bud break on bearing shoots of 'Pixie' mandarin trees by 75% compared to nonbearing shoots of off-crop trees.

Verreynne (2005) reported a significant positive correlation between spring bud break and bud glucose and starch concentrations in January. Compared to off-crop trees, concentrations of starch and glucose were lower in on-crop trees in January. They also found that the concentration of both glucose and starch increased to the same level as off-crop trees when the entire on crop of fruit was removed in December and this had a positive effect on bud break and floral intensity at bloom.

Abscisic acid (ABA) has a well-known role in bud dormancy. Greater concentrations of ABA, or its isomer t-ABA, were found in on-crop citrus trees compared to off-crop trees (Jones et al., 1976; Goldschmidt, 1984; Shalom et al., 2014). Additionally, Garcia-Luis et al. (1986) found that bud break in citrus was delayed by exogenously applied ABA. Similarly, Ü lger et al. (1999) reported that floral bud break of olive was inhibited by ABA. Based on these results, the possibility that ABA might inhibit floral bud break in olive the spring following the on-crop year cannot be ruled out and merits further investigation.

### 1.2 Floral bud abscission

In pistachio (*Pistacia vera* L., cv. Kerman), the on crop of fruit causes the abscission of over 90% of potential floral buds for next year's crop (Crane and Nelson, 1971). Floral bud abscission begins with the initiation of embryo development in the seed (nut fill) in June and continues through the period of rapid embryo development in July (Crane and Nelson, 1971). This phenomenon is the major reason for the reduced number of inflorescences and an off bloom the spring following the on-crop year. In order to prevent a huge loss of floral buds, fruit removal from large branches or a cluster of small

branches with heavy fruit set has been shown to be a one practical method to reduce floral bud abscission- (James and Ferguson, 1990). James and Ferguson (1990) found that as long as fruit removal was completed before the period of nut growth, inflorescence bud abscission was prevented. Also, they found that the percentage of bud retention by nonbearing shoots on on-crop trees was equal to that of nonbearing shoots on off-crop trees.

A large accumulation of soluble sugars and starch were found in leaves of on-crop pistachio trees during the first 60 days after full bloom, but the concentration of starch subsequently decreased in on-crop trees, whereas the concentration of starch continuously increased in off-crop trees (Nzima et al., 1997). Rosecrance et al. (1998) reported that reserved starch content was reduced in canopy branches of on-crop trees compared to off-crop trees during the dormant season. The concentrations of sucrose and starch in floral buds on nonbearing branches of off-crop pistachio trees were significantly greater than those on bearing branches of on-crop trees (Vemmos, 1999). Furthermore, Baninasab and Rahemi (2006) provided evidence that concentrations of sucrose and starch in roots of on-crop pistachio trees were significantly lower than those in roots of off-crop trees during the period of floral bud abscission.

Cytokinins also may play an important role in floral bud retention. In pistachio, abscission of potential floral buds occurs with the initiation of embryo growth in June and increases during the period of rapid embryo growth in July. Coincidently, a 40% decrease in the concentrations of two cytokinins, IPA and zeatinriboside (ZR), was found during the initiation of embryo growth in June through the stage of rapid embryo growth in early

July, while bud concentrations of ABA increased 25% between June and July (Lovatt and Ferguson, 1994; Lovatt et al., 2005). The resulting change to a greater ABA to cytokinin ratio may be the underlying cause of the abscission of the floral buds for the next year's crop. Moreover, a positive correlation between abscission of floral buds and amount of ABA in the buds has been reported (Okay et al., 2011) and foliar application of the cytokinin 6-BA (with urea) to on-crop trees in early June and again in early July increased floral bud retention and yield the following year (Lovatt and Ferguson, 1994; Lovatt et al., 2005).

Floral bud abscission may contribute to the perpetuation of alternate bearing in olive since the phenology of olive is similar to that of pistachio. In olive, phase transition occurs between late June to early July, which coincides with embryo development, which typically starts in early June and ends in July with endocarp hardening (Stutte and Martin, 1986; Fernandez-Escorbar et al., 1992). Floral buds develop on new shoots with young developing leaves that might be poor sources of cytokinins for the developing buds. The developing fruit proximal to the floral buds would be strong sinks for cytokinins being translocated from the roots or mature leaves subtending the fruit. Failure of cytokinins to accumulate to a sufficient concentration relative to the concentration of ABA in the floral buds might result in abscission. Thus, abscission of floral buds might be a mechanism perpetuating alternate bearing in olive.

### 1.3 Inhibition of phase transition and subsequent stages of floral development

Floral development is regulated by four groups of genes. The floral timing genes, *FLOWERING LOCUS C* (*FLC*) and *TERMINAL FLOWER* (*TFL*), are involved in

transition from the juvenile phase to adult phase in the life history of plants and the annual transition from vegetative growth to reproductive development in woody perennials (Alvarez et al., 1992; Michaels and Amasino, 1999). Floral integrator genes, *FLOWERING LOCUS T* (*FT*), *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1* (*SOC1*), and *LEAFY* (*LFY*), are involved in integrating signals from different floral transduction pathways to upregulate the floral meristem identity genes, *LFY* and *APETALA 1* (*AP1*), which result in the meristem becoming committed to floral development (determined) (Weigel et al., 1992; Bowman et al., 1993; Lee et al., 2006; Hsu et al., 2011). Floral organ identity genes are involved in the formation of the organs of the flower. In the ABC model of floral organ specification, group A includes *AP1* and *APETALA 2* (*AP2*), group B includes *APETALA 3* (*AP3*) and *PISTILLATA* (*PI*) and group C includes *AGAMOUS 1* (*AG1*). Development of sepals is regulated by group A genes, development of petals is regulated by group A and B genes, development of stamens is regulated by group B and C genes and development of carpels is regulated by group C gene (Bowman et al., 1991). At the present time, it is not known whether olive flower formation follows the classic ABC model or another more recently reported model, nor is it known whether the on crop inhibits floral development at the level of gene expression in olive.

The mechanism underlying inhibition of floral development during alternate bearing has been more extensively investigated in apple than other fruit tree crops and gibberellens (GA) have been proposed to inhibit floral induction in apple. During the on-crop year, flower induction for next year's bloom, which occurs in early summer, is

inhibited. Gibberellins were identified as a key factor inhibiting flower induction. Findings of Chan and Cain (1967) pointed out that developing seeds are probably the source of GA activity. Luckwill (1970) suggested that gibberellin-like substances produced in seeds depressed or even fully inhibited flower induction. High GA activity has been found in diffusates from fruitlets of alternate bearing cultivars compared to diffusates from more regular bearing apple cultivars (Hoad, 1978). Li et al. (1995) also provided evidence that exogenous application of GA during flower induction inhibited apple flowering. In other fruit trees, several studies provided evidence that exogenous application of GA no later than the floral induction stage inhibited flowering, reducing return bloom the following spring (Kachru et al., 1971; Goldschmidt and Golomb, 1970; Lord and Eckard, 1987). In mango (*Mangifera indica* L.), Nakagawa et al. (2012) provided evidence that GA treatment inhibited *FT* expression and flowering. GA treatment resulted in a five-fold reduction in the expression of an *FT-like* gene in citrus (Goldberg-Moeller et al., 2013). Additionally, Shalom et al. (2012) and Muñoz-Fambuena et al. (2012) provided evidence that the expression of *FT* was reduced in bearing shoots of on-crop citrus trees. Moreover, in addition to *FT*, the on-crop of fruit inhibited the expression of *LFY* and *AP1* (Muñoz-Fambuena et al., 2012; Shalom et al., 2012; Goldberg-Moeller et al., 2013). Similarly, in avocado, the expression of these three genes was only detected in off-crop trees (Ziv et al., 2014).

GA may play a role in inhibiting phase transition and flowering in olive. Monthly seed destruction was used to demonstrate that phase transition occurred before endocarp hardening in early July (Stutte and Martin, 1986). Baktir et al. (2004) observed that the

ratio between GA and ABA is important for the transition from vegetative to reproductive growth. If bud ABA concentrations were greater than GA concentrations, a floral bud formed. Conversely, if GA concentrations were greater than ABA concentrations, then the bud remained vegetative. GA produced by developing olive seeds could cause such a result. Recently, Yanik et al. (2013) provided evidence that flower development may be inhibited by the on crop of fruit through microRNA regulation of target genes involved in carbohydrate metabolism, hormone signaling and organogenesis in olive.

To date, the basic mechanisms perpetuating alternate bearing in 'Manzanillo' olive under California growing conditions have not been identified. Knowledge of these mechanisms and when they occur would be of significant value to growers for improving existing cultural practices, such as pruning, girdling, fruit thinning, and exogenous application of plant growth regulators, and for developing new and better strategies for mitigating alternate bearing in olive (Hartmann, 1950; Lavee et al., 1983; Dag et al., 2010; Fichtner and Lovatt, 2012). Therefore, the objectives of the research presented here were: (1) to identify when the on crop of fruit exerts a negative effect on summer vegetative shoot growth; (2) to determine effect of the fruit number on spring bud break; (3) to quantify the effect of the on crop on floral bud abscission; (4) to quantify the effect of the on crop on the expression of key genes regulating floral development.

To meet these objectives shoot growth was quantified for nonbearing and bearing shoots of off- and on-crop control trees, and on-crop trees with the entire on crop of fruit removed monthly from June through October to determine when fruit inhibit summer

vegetative shoot growth and to quantify the relationship between the amount of summer vegetative shoot growth and the number of inflorescences produced the following spring. One month before full bloom, when floral buds were clearly visible, the fate of each bud on nonbearing and bearing shoots of off- and on-crop control trees was determined and used to calculate percent bud break and floral bud abscission. In a subsequent experiment, the fate of buds on nonbearing and bearing shoots of off- and on-crop control trees was assessed monthly from June through harvest in October to identify the major period of floral bud abscission. In addition, the expression of putative homologues of *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1* (*SOC1*) and *FLOWERING LOCUS T* (*FT*), two integrator genes whose expression signifies successful phase transition, and *PISTILLATA* (*PI*) and *AGAMOUS 1* (*AG1*), floral organ identity genes whose expression indicates flower formation, was quantified in olive buds from June through March the following year to determine if floral development is inhibited during the on-crop year and when. The results of this research provided evidence that several of the mechanisms reviewed above contribute to the perpetuation of alternating bearing in ‘Manzanillo’ olive trees in California.

## 2. Materials and Methods

### 2.1. Plant materials

The research utilized 26-year-old ‘Manzanillo’ olive trees (*Olea europaea* L.) planted on their own roots at a spacing of 7.62 m × 7.62 m in a commercially-producing orchard located at the University of California Lindcove Research and Extension Center, Exeter, California, USA (lat. 36˚21' N, long. 119˚3' W). Visually healthy trees of uniform canopy size were selected for the experiments, which were initiated in the spring of an on-crop year. On-crop trees were created by removing all setting fruit in June the year prior to the experiment. In each experiment, the trees were commercially harvested in October to obtain the total yield as kg fresh weight per tree.

### 2.2. Effect of off- or on-crop tree status and monthly removal of the on crop on summer vegetative shoot growth, spring bud break and return bloom.

The experimental design was a randomized complete block with six treatments replicated on five individual trees. The treatments included: (1) off-crop control trees created by removing all fruit from on-crop trees at the beginning of June; (2) on-crop control trees with no fruit removed; and (3-6) on-crop trees with the entire crop removed on the 15 of June, July, August and September, respectively. Five nonbearing shoots that did not set fruit were tagged on off- and on-crop control trees and on-crop trees in the fruit removal treatments. In addition, five bearing shoots that set fruit in spring were tagged on on-crop control trees and on-crop trees from which fruit were removed monthly. For tagged shoots, ribbons were placed five node pairs proximal to the new spring shoot growth in order to quantify additional new vegetative shoot growth and

determine the contribution of the buds on the current year's and previous year's shoot growth to spring bloom the following year. Shoot growth, as the number of new node pairs that developed distal to the ribbon, was determined monthly from July through October, and in December and February. At full bloom, the effects of fruit number and time of fruit removal on bud break and inflorescence number were quantified by counting the number of inflorescences and inactive buds per node pair on the new growth, the existing five node pairs distal to the ribbon, and 20 node pairs proximal to the ribbon.

**2.3. The fate of buds on nonbearing and bearing shoots of off- and on-crop trees.**

After fruit set in July, visually healthy trees of uniform canopy size producing on and off crops were selected. The following treatments were replicated on five individual trees in a randomized complete block design: (1) nonbearing shoots on off-crop trees; (2) nonbearing shoots on on-crop trees; and (3) bearing shoots on on-crop trees. At the time of tree selection, ten nonbearing and ten bearing shoots were selected on each tree. Approximately 1 month before full bloom, the fate of each bud at each node pair on each shoot was determined for the new spring vegetative shoot growth that developed since February of the current year (new growth), the eight node pairs proximal to the new shoot growth (distal 8 node pairs), the next eight node pairs (middle 8 node pairs), and a third set of eight node pairs (proximal 8 nodes; note that there were not always eight node pairs present at the proximal end of shoot where it intercepted a subtending branch) (Fig. 2). Node pairs were counted and the fate of the lateral buds at each node pair was evaluated. Node pairs typically bear one bud in the axil of each opposing leaf but can produce up to two buds in each leaf axil. The fate of each bud was assigned to one of the following four

categories: (1) inflorescence; (2) lateral vegetative shoot; (3) inactive bud; and (4) abscised bud. The results of this experiment provided evidence of significant bud abscission by spring bloom. Thus, subsequent research was conducted to quantify the rate of bud abscission over time from June through harvest in October. In this experiment, the following treatments were replicated on 15 individual trees in a randomized complete block design: (1) nonbearing shoots on off-crop trees; (2) bearing shoots on off-crop trees; (3) nonbearing shoots on on-crop trees; and (4) bearing shoots on on-crop trees. At the time of tree selection in June, ten nonbearing and ten bearing shoots were selected on each tree. For tagged shoots, a ribbon was placed 5 nodes proximal of the start of the current spring growth in order to distinguish the spring and summer growth from the previous year's growth. The fate of each bud at each node pair along the shoot was determined from June through harvest in October as described above.

### 2.4. Floral development in buds on nonbearing and bearing shoots of off- and on-crop trees.

The following treatments were replicated on five individual trees in a randomized complete block design: (1) nonbearing shoots on off-crop trees; (2) nonbearing shoots on on-crop trees; and (3) bearing shoots on on-crop trees. For each treatment, the distal eight nodes proximal to the new spring vegetative shoot growth were collected from 12 shoots monthly from June through harvest in October, and in December and March the following spring. Leaves were removed from the shoots. All samples for floral gene expression analysis were packaged with paper towels and stored at 4 ˚C in a cooler box for transportation from the field to the lab. In the lab, all samples were packaged in

aluminum foil, immediately frozen in liquid nitrogen and stored at −80 ˚C until they were removed and ground in liquid nitrogen to a fine powder using a mortar and pestle. All ground samples were stored in 15 ml plastic tubes at −80 ˚C prior to quantitative real-time PCR (qPCR) analysis.

### 2.4.1. Total RNA extraction

Total RNA was extracted from 50 mg of finely ground olive shoot tissue using Trizol reagent (Invitrogen, Carlsbad, CA) in final volume of 35µl. RNA quantity and quality was determined using NanoDrop 2000c spectrophotometer (Thermo Scientific, Wilmington, DE) and Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA). RNA with a ratio of 260/280 between 1.8 and 2.0 and ratio of 260/230 between 2.0 and 2.2 was considered good quality for cDNA synthesis.

### 2.4.2. Reverse transcriptase reactions

One µg of total RNA was treated with RQ1 RNase-free DNase (Promega, Madison, WI) and used as the template in the first strand synthesis. Synthesis of cDNA was conducted according Tetro cDNA Synthesis kit manual (Bioline, Taunton, MA) with oligo (dT) and reverse transcriptase in final reaction volume of 30 µl.

### 2.4.3. Primer Design

All primers used in qPCR are shown in Appendix A (Table A.1). For the reference genes, *alpha-tubulin* and *beta-actin* and the floral genes *FLOWERING LOCUS T* (*FT*) and *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1* (*SOC1*), primer pairs were designed based on nucleotide sequences from NCBI nucleotide database (http://www.ncbi.nlm.nih.gov/nuccore) using the PrimerQuest program of Integrated DNA

Technology (http://www.idtdna.com/primerquest/Home/Index). The qPCR products were designed to be 80 bp to 200 bp in length. Since sequence information for olive *PISTILLATA* (*PI*) and *AGAMOUS 1* (*AG1*) was not available, the primer pairs were designed based on the conserved regions of these two genes in *Arabidopsis thaliana*, *Malus domestica*, *Medicago truncatula*, *Vitis vinifera* and *Populus tomentosa* which were acquired by sequence alignment using the Clustal W alignment tool (http://www.genome.jp/tools/clustalw/). Subsequently, the conserved regions of *VvPI* from *Vitis vinifera* and *AtAG1* from *Arabidopsis thaliana* were used in the successful design of olive *PI* and *AG1* primer pairs, respectively.

### 2.4.4. Quantitative Real-Time PCR

Quantitative real-time PCR was performed using a CFX96 Touch™ real-time PCR detection system with a C1000 Touch™ thermal cycler (Bio-rad Laboratories, Hercules, California) and SensiMix™ SYBR & Fluorescein (2X) (Bioline, Taunton, MA). Each reaction (15 µl) contained 7.5 µl of SYBR & Fluorescein (2X), 2.0 µl of template cDNA, 0.6/0.9 µl of each gene-specific forward and reverse primer and 4.9/4.6 Ultra-Pure water (Invitrogen, Carlsbad, CA). The thermal cycling conditions were: one cycle at 95˚C for 10 min, followed by 40 cycles at 95 ˚C for 10 sec, and 1 min at the annealing temperature for each gene. The reaction was performed in three replicates for each sample. The expression levels of the target genes in floral buds were calculated using the Pfaffl method (Pfaffl, 2001). In the calculation, *alpha-tubulin* and *beta-actin* served as the reference genes and expression levels of the target genes in flowers served as the controls.

## 2.5. Statistics analysis

All data were statistically analyzed using the General Linear Model procedures of the SAS statistical program (version 9.2) (SAS Inst. Inc., Cary, N.C.). Analysis of variance (ANOVA) was used to test for treatment effects on vegetative shoot growth, bud break, and the number of inflorescences, lateral vegetative shoots, inactive buds and abscised buds, and gene expression, respectively. Means were separated using Fisher's LSD Test at $P \leq 0.05$.

## 3. Results

**3.1 Effect of fruit number on summer vegetative shoot growth, spring bud break and return bloom.** For off-crop control trees, which were created by removing all fruit in early June (0 kg fruit per tree at harvest), nonbearing vegetative shoots grew by 3.3 node pairs between mid-June and mid-September (Table 2). Growth slowed dramatically between mid-September and mid-December. The following spring, nonbearing vegetative shoots of off-crop trees grew by only 1.5 node pairs between February and May. In contrast, for on-crop control trees bearing 121 kg of fruit per tree, both nonbearing and bearing vegetative shoots barely grew between mid-June and mid-September (Table 2), confirming the inhibitory effect of the on crop of olive fruit on summer vegetative shoot growth first reported by Sibbett (2001). However, the following spring from February to May, nonbearing vegetative shoots of on-crop trees grew by 1.5 node pairs (42%), but bearing shoots grew by 2.4 node pairs (400%). Thus, summer vegetative shoot growth was greater than spring vegetative growth for nonbearing shoots of off-crop trees, whereas spring vegetative shoot growth was greater than summer vegetative growth for bearing shoots of on-crop trees. This greater spring vegetative shoot growth of previously on-crop trees followed by the greater summer vegetative shoot growth of the now off-crop trees resulted in significantly more nodes that produced floral buds at spring bloom the following year, and an on bloom compared to the reduced spring shoot growth of the previously off-crop trees that became on-crop trees, producing little summer vegetative shoot growth with few nodes and floral buds, which resulted in an off bloom the

following spring. Thus, this mechanism clearly contributes to the perpetuation of alternating on and off yields.

The length of the time the on crop remained on the tree prior to removal had an increasingly negative effect on summer vegetative shoot growth. For example, vegetative shoot growth for nonbearing shoots of on-crop trees with fruit removed in mid-June was not significantly different from that of nonbearing shoots of off-crop control trees from July through May (Table 2). When fruit were removed in mid-July, summer vegetative shoot growth was significantly reduced on bearing shoots only in August. Thereafter, there was no significant difference in the number of nodes on nonbearing and bearing shoots of on-crop trees with the fruit removed in July and the number of nodes on nonbearing shoots of off-crop control trees. However, when the on crop of fruit was removed in August, no summer vegetative shoot growth occurred between June and July (Table 2). As a result both nonbearing and bearing shoots had significantly fewer nodes than nonbearing shoots on off-crop control trees through October. Nonbearing shoots from on-crop trees with fruit removed in August continued to have significantly fewer nodes than nonbearing shoots of off-crop control trees in December, February and May, but not April, whereas the number of nodes on bearing shoots of on-crop trees with all fruit removed in August increased from December through May to a number equal to that of nonbearing shoots of off-crop control trees. There was no summer vegetative shoot growth from July to August for on-crop trees with their fruit removed in September (Table 2). Summer vegetative shoot growth (as node pairs) for both types of shoots on on-crop trees with fruit removed in September was not significantly different from on-

crop control trees and significantly less than nonbearing shoots of off-crop control trees for the entire period from October to May, with the exception of April. There was a significant negative correlation between the length of time the fruit remained on the tree during the period from June through October and the number of node pairs that developed on summer vegetative shoots during that period ($r = -0.66$; $P < 0.0001$).

In addition to determining the effect of crop load and fruit removal on summer vegetative shoot growth, the effects on the total number of inflorescences and percentage of bud break were studied at spring bloom. Nonbearing shoots of off-crop control trees produced the greatest number of inflorescences at 9.3 per shoot (Table 3). In contrast, bearing shoots of on-crop control trees produced 15.5-fold fewer inflorescences at just 0.6 per shoot ($P < 0.0001$). Nonbearing shoots of on-crop control trees produced 2.8 inflorescences per shoot, 4.7-fold more inflorescences than bearing shoots but still more than 3-fold less than nonbearing shoots of off-crop control trees ($P < 0.0001$) (Table 3). It is clear that following an on-crop year, only nonbearing shoots contribute significantly to return bloom. The monthly delay in removing the on-crop of fruit from June to September progressively reduced the total number of inflorescences on bearing shoots (Table 3). A delay of just one or two months from June to July or August reduced inflorescence number on bearing shoots by 38% and 42%, respectively. The number of inflorescences borne on nonbearing shoots was significantly reduced by the presence of the on crop when fruit removal was delayed until mid-September (Table 3). For bearing shoots, the results provided evidence that the number of inflorescences at return bloom was reduced by the on crop if the fruit remained on the tree just to mid-July (Table 3).

There was a negative correlation between the length of time fruit remained on the shoots from June through harvest in October and the total number of inflorescences on nonbearing and bearing shoots of on-crop trees (r = -0.75; $P < 0.0001$ and r = -0.73; $P = 0.0004$, respectively).

Additionally, at full bloom, the effect of off- and on-crop tree status and the time of fruit removal on bud break was determined for nonbearing and bearing shoots divided into five-node pair sections starting at the position immediately proximal to the current spring vegetative shoot growth (node pairs 1-5, 6-10, 11-15, 16-20, and 21-25) to determine which section of shoot contributed the majority of inflorescences to return bloom and which sections were affected by the on crop. For nonbearing shoots of off-crop control trees, bud break was lower than expected, only 55% and 50% for distal node pairs 1 to 5 and 6 to 10, respectively, and significantly less for the more proximal node pairs 11 through 20 (Table 3). It was also unexpected that 42% and 50% of the buds for node pairs 1 to 5 and 6 to 10, respectively, for nonbearing shoots of off-crop trees were inactive at spring bloom. Node pairs 1 to 5 consisted of the previous year's spring and summer vegetative shoot growth on off-crop trees, whereas node pairs 6 to 10 consisted predominantly of 2-year-old spring and summer vegetative shoot growth that developed on on-crop trees. In contrast, fruit of the on-crop control trees significantly reduce bud break for node pairs 1 through 5 to 20.9% and 5.7% on the nonbearing and bearing shoots, respectively. In light of the results of subsequent experiments (reported below) documenting that the on crop caused a high rate of floral bud abscission from bearing shoots, the low percentage of bud break on these shoots is likely due to the low number

of remaining buds. In contrast, nonbearing shoots of on-crop trees retained a large number of inactive buds at bloom (~80% of the node pairs), consistent with a strong inhibition of bud break. It is of interest that regardless of when the entire on crop was removed, for node pairs 1 through 5, the percentage of bud break on bearing shoots was significantly lower than that of nonbearing shoots, which was not significantly different from that of nonbearing shoots of the off-crop control trees even for trees with fruit removed in September (Table 3). Percent bud break for node pairs 6 through 10 on nonbearing and bearing shoots was not affected by the time the on crop of fruit was removed until September. In this case, percent bud break was significantly reduced on both nonbearing and bearing shoots compared to the off-crop control trees (Table 3). The percent bud break for node pairs 11 to 15 was variable, but documentation that inflorescences developed on this section of olive shoots was of interest because of the older age of this shoot section (Table 3).

**3.2 The fate of buds on nonbearing and bearing shoots of off- and on-crop trees.**

One month before full bloom, the fate of every bud was determined for new growth and the subtending three eight-node pair sections of nonbearing and bearing shoots of off- and on-crop trees (Fig. 2). The off-crop trees yielded 55 kg per tree, whereas the on-crop trees in this experiment produced 166 kg of fruit per tree ($P$ = 0.0022). It is important to note that there were no inflorescences produced on the section of new spring vegetative shoot growth one month before full bloom because this section of the shoot developed after the time of floral induction the previous summer. The results showed that nonbearing shoots of off- and on-crop trees produced more node pairs with a

commensurately significantly greater number of inactive buds than bearing shoots on on-crop trees (Table 4). No bud abscission occurred on the new spring growth for nonbearing or bearing shoots of off- and on-crop trees.

The distal eight node pairs on nonbearing shoots of off-crop trees produced the most inflorescences at 8.3 per 16 nodes (Table 4). Nonbearing shoots of on-crop trees produced 27.7-fold fewer inflorescences on this section of the shoot, whereas bearing shoots produced no inflorescences on this section (Table 4). Crop load and the presence or absence of fruit set on a shoot had no significant effect on the number of vegetative shoots that developed on this section of the shoot (Table 4). For nonbearing shoots of on-crop trees one month before full bloom, 14.3 of the distal 16 nodes were inactive buds (89%, the majority of the buds). This was significantly greater than the number of inactive buds on bearing shoots on on-crop trees and nonbearing shoots on off-crop trees ($P < 0.0004$) (Table 4). Strikingly, a great number of buds (75%) abscised from the distal eight node pairs of bearing shoots of on-crop trees compared to nonbearing shoots of off- and on-crop trees (Table 4). This is the first report that the on-crop causes floral bud abscission in 'Manzanillo' olive.

For the middle eight node pairs, only nonbearing shoots of off-crop trees produced a significant number of inflorescences, but only at 35% of the nodes (Table 4). Vegetative shoots were produced on nonbearing and bearing shoots of on-crop trees by 14% and 20% of the nodes, respectively (Table 4). A great number of inactive buds were observed on nonbearing shoots of off- and on-crop trees, 51% and 69% of the nodes had inactive buds, respectively (Table 4). Similar to the distal eight node pairs of bearing shoots of

on-crop trees, a great number of buds abscised from the middle eight node pairs (76%), significantly more than from nonbearing shoots of off- and on-crop trees (Table 4).

The section of proximal node pairs usually contained less than eight node pairs. Inflorescences were only produced on nonbearing shoots of off-crop trees, though the number for this shoot section was extremely low (7% of the nodes bore inflorescences) (Table 4). The number of lateral vegetative shoots developing on bearing shoots of on-crop trees was significantly greater than that of nonbearing shoots of off-crop trees (Table 4). The number of lateral vegetative shoots produced by nonbearing shoots of on-crop trees was intermediate to and not significantly different from that of nonbearing shoots of off-crop trees and bearing shoots of on-crop trees. The number of inactive buds for the proximal node pairs on nonbearing shoots of off-crop trees was significantly greater than that of nonbearing shoots on on-crop trees and both had significantly more inactive buds than bearing shoots of on-crop trees (Table 4). The number of buds that abscised from the proximal nodes of nonbearing shoots on off- and on-crop trees was 2.9 and 2.0, respectively, and not significantly different (Table 4). In contrast, 7.2 buds abscised from 71% of the proximal nodes of bearing shoots of on-crop trees, which was significantly greater than for the other two types of shoots (Table 4).

Taken together, most of the 24 node pairs proximal to the new spring growth on nonbearing shoots of off-crop trees produced either inactive (50%) or floral (35%) buds. For nonbearing shoots of on-crop trees, 76% of the nodes produced inactive buds and approximately 10% developed lateral vegetative shoots, whereas the buds abscised from 74% of nodes of bearing shoots of on-crop trees, with 16% producing lateral vegetative

shoots. The high percentage of inactive buds on nonbearing shoots of both off- and on-crop trees observed in two separate experiments in this research has not been reported previously for olive. It suggests the possibility that bud break and inflorescence number could potentially be increased through the foliar application of dormancy-breaking plant growth regulators (PGRs). The significant proportion of abscising buds from bearing shoots of on-crop olive trees also has not been reported previously. Similarly, it suggests the use of foliar-applied PGRs to prevent bud abscission as in the case of pistachio (Lovatt and Ferguson, 1994). Thus, these discoveries might prove useful in the development of new strategies to mitigate alternate bearing in olive. To this end, diagnosing when potential floral buds abscise is important. Thus, additional research was undertaken to determine the fate of all buds on the new vegetative shoot growth in spring and summer, and the subtending node pairs on nonbearing and bearing shoots of off- and on-crop trees from June through harvest in October. As part of this research, bearing shoots of off-crop trees were analyzed for the first time to determine the localized effect of fruit set on a bearing shoot independent from the whole tree effect of the on crop of fruit.

In this experiment, the off-crop trees produced 50 kg of fruit per tree compared to 152 kg per tree for the on-crop trees ($P < 0.0001$). The total numbers of node pairs on nonbearing shoots of off- and on-crop trees was greater than that of bearing shoots for both types of trees by October (Table 5). The number of lateral vegetative shoots on nonbearing shoots was greater than that of bearing shoots, with a significant number of lateral vegetative shoots developing on nonbearing shoots of on-crop trees between

September and October (Table 5). Additionally, most of the buds on this section remained inactive on both nonbearing and bearing shoots, but the number of inactive buds on nonbearing shoots was greater than that of bearing shoots due to the fact that there were more node pairs (Table 5). From June through October, there was insignificant bud abscission from the new spring and summer vegetative shoot growth of nonbearing or bearing shoots of off- and on-crop trees.

For the distal eight node pairs, the number of lateral vegetative shoots on nonbearing and bearing shoots of both off- and on-crop trees increased significantly from June to July. For nonbearing shoots of off-crop trees, the number of lateral vegetative shoots was significantly greater than that of bearing shoots of off- and on-crop trees in September and October and July, September and October, respectively, but lateral shoot number of nonbearing shoots of on-crop trees was significantly greater than that of bearing shoots of off- and on-crop trees from July to October (Table 6). Bud abscission for this section of bearing shoots of on-crop trees was significantly greater than bud abscission from all other shoots from June through October, with the exception of bearing shoots of off-crop trees by October (Table 6). This result suggests that some buds had already abscised from bearing shoots of on-crop trees between May and June. The lowest bud abscission was from nonbearing shoots of off- and on-crop trees, with bearing shoots of off-crop trees having an intermediate level of bud abscission, except in October (Table 6). The number of inactive buds was consistently greater for the distal eight node pairs of nonbearing shoots of off- and on-crop trees relative to bearing shoots on these trees from June through September (Table 6). By October, only nonbearing shoots of off-crop trees had

significantly more inactive buds in this section of the shoot. The distal eight node pairs of bearing shoots had more fruit than nonbearing shoots, but bearing shoots of off-crop trees set more fruit per shoot than bearing shoots of on-crop trees (Table 6).

For the middle eight node pairs, the number of lateral vegetative shoots increased significantly between June and July, except for bearing shoots on off-crop trees (Table 7). There was a significant increase in the number of lateral vegetative shoots between September and October for nonbearing and bearing shoots of on-crop trees. By October, there were significantly more lateral shoots on nonbearing versus bearing shoots of off- and on-crop trees, respectively. A significant increase in the number of abscised buds was observed on nonbearing and bearing shoots of off-crop trees between June and July, at 1.2 and 2.7, respectively (Table 7). However, the number of abscised buds for the middle eight nodes was consistently significantly greater for bearing shoots on both off- and on-crop trees than nonbearing shoots of trees with off or on crops from June through October (Table 7). The converse was true for the number of inactive buds, with nonbearing shoots of off- and on-crop trees having significantly more inactive buds from June through October than the bearing shoots of these trees (Table 7). For bearing shoots, the number of nodes bearing fruit on on-crop trees was greater than that of off-crop trees, and thus, the number of fruit on on-crop trees was greater than that of off-crop trees for this shoot section (Table 7).

For the proximal section of the shoot, there were less than eight node pairs (Table 8). Bearing shoots of off-crop trees had fewer node pairs than bearing shoots of on-crop trees. The presence or absence of fruit set on a shoot and crop load had no significant

effect on the number of lateral vegetative shoots that developed until October, at which time nonbearing shoots of on-crop trees had a greater number of lateral shoots than nonbearing and bearing shoots of off-crop trees (Table 8). Interestingly, nonbearing shoots of on-crop trees had the lowest number of abscised buds from June through October compared to all other shoots, which had equal levels of bud abscission from June through September (Table 8). In general, the number of inactive buds significantly decreased for both types of shoots on both off- and on-crop trees between June and July (Table 8). The proximal nodes of bearing shoots of off-crop trees had consistently significantly fewer inactive buds relative to all other shoot types from June through October. With the exceptions of the months of June and October, the proximal section on nonbearing shoots of on-crop trees had more inactive buds than all other shoot types. The number of inactive buds on the proximal section of nonbearing shoots of off-crop trees and bearing shoots of on-crop was intermediate. The proximal nodes of bearing shoots set very few fruit; only bearing shoots of on-crop trees had any remaining fruit by September (Table 8).

These results provided additional confirmation of the negative effect of fruit set on a bearing shoot in reducing summer vegetative shoot growth (as number of node pairs) and demonstrated a local effect of the fruit regardless of off- or on-crop tree status. With the exception of the proximal nodes, the number of lateral vegetative shoots was also reduced by the fruit set on bearing shoots independent of the total fruit number (crop load) on the tree. For all shoot sections, the number of inactive buds was significantly greater for nonbearing shoots and significantly reduced by the presence of fruit on bearing shoots

independent of crop load. This was because fruit caused bud abscission on bearing shoots, even on off-crop trees. Since there were no fruit set on the new apical vegetative shoot section of bearing shoots, there was minimal bud abscission. The major period of bud abscission occurred between September and October on bearing shoots. Bud abscission for the new apical spring and summer vegetative shoot growth of a bearing shoot increased from 2.8% in September to 3.4% by October, but from 50% to 71% for the distal and middle eight node pairs and 53% to 61% for the proximal node pairs. Moving basipetally along the shoot, the distal and middle eight node pairs represent approximately the previous year's spring and summer vegetative shoot growth and 2-year-old spring and summer shoot growth, respectively.

**3.3 Floral development in buds on nonbearing and bearing shoots of off- and on-crop trees.** Before doing floral gene analysis, the quality of all primers for the genes of interest was evaluated based on melting peak of the product. Primers were considered specific to the gene of interest if there was no non-specific peak or primer dimers observed in the preliminary qPCR tests and melting temperature was consistent for all three replicates. Thereafter, the sequences of qPCR products were checked against the NCBI database to search for homologous genes in other plant species to confirm that the primers were valid for the gene of interest. At least two to four target genes with a high degree of homology with the putative olive genes were found (Appendix A, Table A.2). The floral genes of interest were all expressed in flowers collected at full bloom (Appendix A, Fig. A.1.). In addition, *OeSOC1*, *OeFT*, and *OeAG1* were expressed in the mature leaves of 'Manzanillo' olive, but at a significantly lower level than in flowers ($P <$

0.001), consistent with reports that *SOC1* is expressed in leaves of apricot (*Prunus armeniaca*) (Trainin et al., 2013) and *Arabidopsis thaliana* (Samach et al., 2000), *FT* in leaves of citrus (Muñoz-Fambuena et al., 2012) and avocado (Ziv et al., 2014) and *AG1* in leaves of poplar (*Populus trichocarpa*) (Brunner et al., 2000) and peach (*Prunus persica*) (Tani et al., 2009).

Expression *OeSOC1* was continuous for nonbearing shoots of off-crop trees for all sampling dates from June through March (Table 9). For nonbearing shoots of on-crop trees, expression levels were equal to those of nonbearing shoots of off-crop trees from June through December, with no expression in March. The expression of *OeSCO1* in bearing shoots of on-crop trees was significantly greater in June and July than nonbearing shoots of off- and on-crop trees but not significantly different thereafter, including September and October, when no expression was detected. Thus, in March, *OeSOC1* expression was undetectable in buds of both nonbearing and bearing shoots of on-crop trees. The expression pattern of *OeSOC1* suggests that phase transition is likely not affected by the on crop in olive. Olive *FT* was expressed from June to March in nonbearing shoots of off-crop trees, whereas the expression of *OeFT* was repressed in nonbearing and bearing shoots of on-crop trees starting in December and September, respectively. For bearing shoots of on-crop trees, this corresponds with the abscission of 51% of the buds by September. However, for nonbearing shoots on on-crop trees, bud abscission was minimal (9%). The majority of buds developed into lateral vegetative shoots (61%), with 29% remaining inactive. Taken together, these results suggest the possibility that the on crop of fruit may inhibit the determination of the floral meristem.

For the putative floral organ identity gene *OePI*, expression was low and occurred only in March for nonbearing shoots of off-crop trees (Table 10). Expression of *OeAG1* was detected only for nonbearing shoots collected from off- and on-crop trees in March (Table 10). Expression was significantly greater for nonbearing shoots of off-crop trees than on-crop trees and the gene was not expressed in bearing shoots of on-crop trees (Table 10). The low level of *OeAG1* expression in nonbearing shoots of on-crop trees is consistent with the low number of inflorescences produced by these shoots by March, 2% of the distal eight node pairs, and suggests that a small pool of buds on nonbearing shoots of on-crop trees complete floral development.

## 4. Discussion

The results of this research provided strong evidence that four mechanisms contribute to the perpetuation of alternate bearing in 'Manzanillo' olive in California. Off- and on-crop tree status had statistically significantly different effects on summer vegetative shoot growth, including lateral vegetative shoot development, spring bud break and the number of inactive buds at bloom, abscission of potential floral buds, and expression of key genes regulating floral development. The heavy on crop of olive fruit was confirmed to reduce summer vegetative shoot growth, reducing the number of node pairs and potential floral buds that developed and floral intensity the following spring. The length of time the on crop of fruit remained on the tree from June to harvest in October was significantly negatively correlated with summer vegetative shoot growth (as node pairs) ($r = -0.66$; $P < 0.0001$) as well as the number of inflorescences that developed on nonbearing ($r = -0.75$; $P < 0.0001$ and bearing ($r = -0.73$; $P = 0.0004$) shoots the following spring. By far the more negative effects of the on crop of olive fruit documented for the first time by the results of this research was the strong inhibition of spring bud break, 76% of the buds on nonbearing shoots of on-crop trees were inactive at spring bloom, and the high rate of abscission of potential floral buds, 74% of the buds on bearing shoots had abscised by spring bloom. The results of this research also provided the first evidence suggesting that the on crop of olive fruit inhibited floral development at the level of gene expression. Identification of these mechanisms in olive provides a basic understanding of why the on crop year is followed by an off bloom and characterizes the factors that contribute to an on bloom and high yield following the off-crop year. This knowledge has the potential to contribute to improved

cultural practices for mitigating alternate bearing specifically but also for increasing bloom and yield in olive in general. The four mechanisms contributing to the perpetuation of on and off yield cycles in olive have been reported previously for other tree crops. However, this is the first demonstration that all four mechanisms are functioning in a single fruit tree crop.

Inhibition of summer vegetative shoot growth by the on crop of olive fruit and its negative impact on return bloom in olive was first proposed by Sibbett (2000). The results of this research demonstrated that the on crop exerted its negative effect on shoot growth between June and August. If fruit removal was delayed until August, shoot growth (as new node pairs) on both nonbearing and bearing shoots was significantly less than that of nonbearing shoots of off-crop trees through October. Delaying fruit removal to September, reduced shoot growth on both nonbearing and bearing shoots through February compared to nonbearing shoots of off-crop control trees. Despite an equal reduction in the number of node pairs on nonbearing and bearing shoots of on-crop trees resulting from delaying fruit removal until August or September, the number of inflorescences at return bloom was significantly reduced to a greater degree on bearing shoots than nonbearing shoots, suggesting that olive fruit set on a bearing shoot imposed additional local effects beyond the effect of total fruit number (crop load) on the whole tree. A new finding in this research was the relatively greater amount of vegetative shoot growth of nonbearing and bearing shoots in the spring following the on-crop year than following the off-crop year. Interestingly, nonbearing shoots of off-crop control trees, nonbearing and bearing shoots of on-crop trees with all fruit removed in July and nonbearing shoots of on-crop control trees with all fruit

removed in August produced between 1.0 and 1.8 node pairs from February through May versus bearing shoots of on-crop trees with fruit removed in August and nonbearing and bearing shoots of on-crop trees with fruit removed in September and on-crop control trees, which produced from 2.0 to 2.8 node pairs during the same period. This spring shoot growth combined with the greater summer vegetative shoot growth of the now off-crop trees increases the number of nodes and floral buds resulting in the on bloom the spring following the off-crop year.

However, it appears that the additional negative effects of the on crop identified in this research (bud abscission, inhibition of floral development at the level of gene expression and bud dormancy) reduce floral intensity at return bloom more dramatically than the 25% to 50% fewer node pairs at bloom in April or May might cause. Delaying removal of the on crop of fruit until September (75 days after endocarp scelrification), a critical time also identified by Dag et al. (2010) for olives in Israel, reduced inflorescence number 38% for nonbearing shoots and 58% for bearing shoots compared to nonbearing shoots of off-crop control trees. Removal of the on crop at harvest in October reduced floral intensity at return bloom by 70% for nonbearing shoots and 94% for bearing shoots relative nonbearing shoots of off-crop control trees. Buds abscission occurred at 50% of the 24 node pairs along a bearing shoot of an on-crop tree by September and at 71% of the first 16 node pairs and 61% of the proximal node pairs by October. This intense period of bud abscission from September to October for bearing shoots of on-crop trees was coincident with the repression of *OeSOC1* expression, although this gene was expressed again in December, and the complete cessation of *OeFT* expression in the buds of these shoots. How these changes in

gene expression are related to bud abscission is a matter of speculation. It is tempting to propose that the repression of *OeFT*, a gene involved with meristem determinacy, was the cause of bud abscission, but it cannot be ruled out that the lack *OeFT* expression was simply due to the lack of floral buds along the shoot since total RNA was extracted from the entire shoot due to the small size of the buds.

The latter interpretation is the more likely. Bud abscission during this period was low for nonbearing shoots of on-crop trees, less than 1%, 9%, 13% and 15% of the buds abscised from the nodes of the new apical vegetative shoot growth, the distal eight node pairs behind this growth, the middle eight node pairs and the proximal node pairs, respectively. However, expression of *OeFT* in these buds was repressed three months later (December) than the buds on bearing shoots of on-crop trees. Note that the expression of *OeSOC1* for buds on nonbearing shoots of on-crop trees was not significantly different than that of buds on nonbearing shoots of off-crop trees until one month before bloom in March, when expression was completely repressed. The results suggest that the on crop of fruit inhibits determinacy of the meristem of lateral buds of nonbearing shoots. Consistent with this interpretation, 44%, 61%, 53%, and 35% of the buds of the new apical vegetative shoot growth, the distal eight nodes behind this growth, the middle eight node pairs and the proximal node pairs, respectively, produced lateral vegetative shoots by October, which was significantly greater than lateral vegetative shoot development for any other shoot type. The subsequent expression of *OeAG1* in March is consistent with the low percentage of inflorescences that developed one month before bloom (2% and 1% of the node for the distal and middle eight node pairs) by nonbearing shoots of on-crop trees and suggests that a

small pool of buds completes floral development on these shoots. Reduced flowering following the on-crop year and repression of *FT* in on-crop fruit trees relative to off-crop trees has previously been reported for alternating bearing mango, citrus and avocado trees (Muñoz-Fambuena et al., 2012; Nakagawa et al., 2012; Shalom et al., 2012; Goldberg-Moeller et al., 2013; Ziv et al., 2014). However, in these other studies only nonbearing shoots of off-crop trees and bearing shoots of on-crop trees were compared.

Analysis of the fate of the buds on bearing shoots of off-crop trees in the final experiment of this research provided evidence for olive of a strong localized effect due to the fruit set on a shoot that was independent of the whole-tree effect from the number of fruit (crop load) on the tree. The localized effect of fruit set on a shoot significantly reduced the number of lateral vegetative shoots and inactive buds on the new apical vegetative shoot growth of nonbearing shoots by October independent of the off- or on-crop status of the tree. For both the distal eight and middle eight node pairs of bearing shoots of off- or on-crop trees, fruit set on the shoot significantly reduce lateral shoot development and inactive bud number with a concomitant increase in the number of abscised buds by October compared to these sections of nonbearing shoots of trees with off- or on-crops. Interestingly, this final experiment revealed that many of these differences were statistically significant as early as June. Thus, alternate bearing in 'Manzanillo' olive trees occurs predominantly at the level of the shoot, with on-crop trees having a greater ratio of bearing to nonbearing shoots and off-crop trees having the reverse. This is not the case for citrus or avocado. For these tree crops, there are clear localized and whole tree effects of the fruit on the shoots with the effects being additive for bearing shoots of on-crop trees (Verreynne, 2005; Verreynne and Lovatt,

2009; Lovatt, 2010). The situation in olive suggests that increasing the number of nonbearing shoots by flower or fruit thinning sufficiently early (June or July) would mitigate alternate bearing. However, the final interpretation of the results of this last experiment must await the determination of the final fate of the buds, especially the number of buds that produce inflorescences, on bearing versus nonbearing shoots of off- and on-crop trees at spring bloom.

Based on the information resulting from this research, increasing summer vegetative shoot growth, increasing spring bud break, reducing the rate of abscission of potential floral buds and preventing inhibition of floral development are ways that growers might be able to increase floral intensity and yield following the on-crop year in olive. Moreover, given the negative localized effect of fruit set on a shoot, some of these approaches might prove valuable for increasing yield of olive in the absence of alternate bearing. Results obtained by removing the on-crop monthly showed that the fruit reduced summer vegetative shoot growth when left on the tree after July. Quantification of the fate of buds from June to October indicated that many of the localized effects of the fruit, including bud abscission, began after bloom between May and June, but intensified between September and October. Thus fruit thinning and PGR applications probably need to be completed sometime after bloom and before July. Fichtner and Lovatt (2013) provided evidence that 6-BA and TIBA alone or in combination applied in July successfully increased vegetative shoot growth on both nonbearing and bearing shoots of on-crop trees and that cytokinin treatments in February increased bud break and inflorescence number 60% for nonbearing shoots of on-crop trees, with a positive, but nonsignificant, effect on bud

break and floral intensity for bearing shoots of on-crop trees, compared to nonbearing and bearing shoots, respectively, of the untreated off-crop control trees. Foliar-applications of 6-BA (with urea) have proven efficacious in preventing bud abscission in pistachio and might give a similar result for olive if properly timed (Lovatt and Ferguson, 1994; Lovatt et al., 2005). Application of cytokinin might have an additional beneficial effect on floral development in olive. Andreini et al. (2006) demonstrated that the cytokinin zeatin riboside (ZR) accumulated in the meristem of buds at the time of phase transition, which coincided with endocarp hardening (July). Zeatin riboside accumulated only in the buds of nonbearing shoots of off-crop trees, which flowered the following spring, and not in the buds of bearing shoots of on-crop trees, which produced no return bloom.

## 5. Conclusion

The results of this research are the first to provide evidence that four mechanisms contribute to perpetuating alternate bearing in olive under California growing conditions. The results documented the following effects of olive fruit that contribute to the reduction of floral intensity at spring bloom following the on-crop year: (1) inhibition of summer vegetative shoot extension growth and lateral vegetative shoot development, thereby reducing the number of nodes that can produce inflorescences the following spring; (2) inhibition of spring bud break, resulting in many inactive buds on nonbearing shoots; (3) abscission of buds on bearing shoots, which began after bloom but intensified between September and October, which reduces the number of lateral vegetative shoots and inflorescences; and (4) inhibition of floral development at the level of gene expression of putative *OeFT*, preventing the meristems of lateral buds from becoming determined in all but a small pool of buds on nonbearing shoots of on-crop trees, resulting in few inflorescences, an increased number of lateral vegetative shoots, and significant number of inactive buds. As a result of this research, four mechanisms known to individually perpetuate repeating cycles of off and on yields in multiple tree crops have for the first time been demonstrated to function in one tree crop, 'Manzanillo' olive. The information derived from this research suggest strategies for mitigating alternate bearing in olive and identifies the optimal time for implementing various cultural practices for 'Manzanillo' olive under California growing conditions.

## References


Alvarez, J., Guli, C.L., Yu, X.H., Smyth, D.R., 1992. Terminal flower: a gene affecting inflorescence development in *Arabidopsis thaliana*. The Plant J. 2(1), 103-116.

Andreini, L., Bartolini, S., Guivarćh, A., Chriqui, D., Vitagliano, C., 2006. Histological and immunohistochemical studies on flower induction in the olive tree (*Olea europaea* L.). Plant Biol. 10:588–595.

Baktir, I., Ü lger, S., Kaynak, L., Himelrick, D.G., 2004. Relationship of seasonal changes in endogenous plant hormones and alternate bearing of olive trees. HortScience. 39(5), 987-990.

Baninasab, B., Rahemi, M., 2006. Possible role of non-structural carbohydrates in alternate bearing of pistachio. Eur. J. Hortic. Sci. 71, 277-282.

Bowman, J.L., Alvarez, J., Weigel, D., Meyerowitz, E.M., Smyth, D.R. 1993. Control of flower development in *Arabidopsis thaliana* by *APETALA1* and interacting genes. Development, 119, 721–743.

Bowman, J.L., Smyth, D.R., Meyerowitz, E.M. 1991. Genetic interactions among floral homeotic genes of *Arabidopsis*. Development. 112(1), 1-20.

Brunner, A.M., Rottmann, W.H., Sheppard, L.A., Krutovskii, K., DiFazio, S.P., Leonardi, S., Strauss, S.H., 2000. Structure and expression of duplicate *AGAMOUS* orthologues in poplar. Plant Mol. Biol. 44, 619-634.

Chan, B.C., Cain, J.C., 1967. The effect of seed formation on subsequent flowering in apple. Proc. Am. Soc. Hortic. Sci. 91, 63-97.

Connell, J.H., 2005. History and scope of the olive industry. pp. 1-10. In: Sibbett, G.S., Ferguson, L. (Eds.). Olive Production Manual. UCANR Publications.

County statistical data, 2012. California agricultural statistics review 2011-2012. http://www.cdfa.ca.gov/statistics/PDFs/2013/CountyStatisticalData.pdf (accessed and verified 12 Dec 2014).

Crane, J.C., Nelson, M.M., 1971. The unusual mechanism of alternate bearing in the pistachio. HortScience. 6, 489–490.

Cuevas, J., Pinney, K., Polito, V.S., 1999. Flower differentiation, pistil development and pistil abortion in olive (*Olea europaea* L. 'Manzanillo'). Acta Hortic. 474, 293-296.

Dag, A., Bustan, A., Avni, A., Tzipori, I., Lavee, S., 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, 469–472.

FAOSTAT, 2014. Food and Agricultural Organization of the United Nations Statistical Division.
http://faostat3.fao.org/download/Q/QC/E (accessed and verified 1 Dec 2014).

Fernandez-Escorbar, R., Benlloch, M., Navarro, C., Martin, G.C., 1992. The time of floral induction in the olive. J. Am. Soc. Hortic. Sci. 117(2), 304-307.

Fichtner, E., Lovatt, C.J., 2012. Research advances on mitigation of alternate bearing in olive. Topics in Subtropics Newsletter.10, 5-7.

Fichtner, E., Lovatt, C.J., 2013. Advances in mitigation of alternate bearing of olive: vegetative growth response to plant growth regulators. Topics in Subtropics Newsletter. 11(4), 3-6.

Garcia-Luis, A., Almela, V., Monerri, C., Agusti, M., Guardiola, J.L., 1986. Inhibition of flowering in vivo by existing fruits and applied growth regulators in *Citrus unshiu*. Physiol. Plant. 66, 515-520.

Goldberg-Moeller, R., Shalom, L., Shlizerman, L., Samuels, S., Zur, N., Ophir, R., Blumwald, E., Sadka, A., 2013. Effect of gibberellin treatment during flowering induction period on global gene expression and the transcription of flowering-control genes in *Citrus* buds. Plant Sci. 198, 46-57.

Goldschmidt, E.E., 1984. Endogenous abscisic acid and 2-*trans*-abscisic acid in alternate bearing 'Wilking' mandarin trees. Plant Growth Regul. 2, 9-13.

Goldschmidt, E.E., Golomb, A., 1970. Hormonal control of flowering in citrus and some other woody perennials. pp. 758-765. In: Carr, J.D. (Eds.), Plant Growth Substances. Springer, Berlin.

Hartmann, H.T., 1949. Growth of the olive fruit. Proc. Am. Soc. Hortic. Sci. 54, 86-94.

Hartmann, H.T., 1950. The effect of girdling on flower type, fruit set and yield in the olive. Proc. Am. Soc. Hortic. Sci. 56, 217-226.

Hartmann, H.T., 1951. Time for floral differentiation of the olive in California. Bot. Gazette. 112, 323-3277.

Hoad, G.V., 1978. The role of seed derived hormones in the control of flowering in apple. Acta Hortic. 80, 93-103.

Hsu, C.Y., Adams, J.P., Kim, H., No, K., Ma, C., Strauss, S.H., Drnevich, J., Vandervelde, L., Ellis, J.D., Rice, B.M., Wickett, N., Gunter, L.E., Tuskan, G.A., Brunner, A.M., Page, G.P., Barakat, A., Carlson, J.E., dePamphilis, C.W., Luthe, D.S., Yuceer, C., 2011. *FLOWERING LOCUS T* duplication coordinates reproductive and vegetative growth in perennial poplar. Proc. Natl. Acad. Sci. U. S. A. 108(26), 10756-10761.

James, A.W., Ferguson, L., 1990. Inflorescence bud retention in 'Kerman' pistachio: Effect of defruiting date and branch size. HortScience. 25(8), 919-921.

Jones, W.W., Coggins, C.W., Jr., Embleton, T.W., 1976. Endogenous abscisic acid in relation to bud growth in alternate bearing 'Valencia' orange. Plant Physiol. 58, 681-682.

Jones, W.W., Coggins, C.W., Jr., Embleton, T.W., 1977. Growth regulators and alternate bearing. Proc. Intl. Soc. Citricult. 2, 657-660.

Kachru, R.B., Singh, R.M., Chacko, E.K., 1971. Inhibition of flowering in mango (*Mangifera indica* L.) by gibberellic acid. HortScience. 6, 140-141.

Kinoshita, M., 2011. Tulare County agricultural crop and livestock report. pp. 4. http://www.tularecounty.ca.gov/county/assets/File/2011%20CR%20web%20v3.pdf (accessed and verified 1 Dec 2014).

Lavee, S., Haskal, A., Ben-Tal, Y., 1983. Girdling olive trees, a partial solution to biennial bearing. I. Methods, timing and direct tree response. J. Am. Soc. Hortic. Sci. 58, 209-218.

Lee, J.H., Hong, S.M., Yoo, S.J., Park, O.K., Lee, J.S., Ahn, J.H. 2006. Integration of floral inductive signals by *FLOWERING LOCUS T* and *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1*. Physiol. Plant. 126(4), 475-483.

Li, S.H., Meng, Z.Q., Li, T.H., Liu, H.Z., Tu, Y.C., 1995. Critical period of flower bud induction in 'Red Fuji' and 'Ralls Janet' apple trees. Gartenbauwissenschaft. 60(5), 240-245.

Lord, E.M., Eckard, K.J., 1987. Shoot development in *Citrus sinensis* L. (Washington Navel orange). II. Alternation of developmental fate of flowering shoots after GA3 treatment. Bot. Gaz. 148, 17-22.

Lovatt, C.J., Ferguson, L., 1994. Using a foliar application of urea combined with 6-benzyladenine to decrease pistachio floral bud abscission in an on-year to increase yield the next year. California Pistachio Ind. Ann. Rep. p. 155-158.

Lovatt, C.J., Daoudi, H., Ferguson, L., 2005. Foliar-applied cytokinins and nitrogen reduce alternate bearing and increase cumulative yield of pistachio. Acta Hortic. 727, 353-364.

Lovatt, C.J., 2006. Eliminating alternate bearing of the 'Hass' avocado. http://www.californiaavocadogrowers.com/sites/default/files/documents/Eliminate-alternate-bearing-in-avocados-2006.pdf (accessed and verified 1 Dec 2014).

Lovatt, C.J., 2010. Alternate bearing of 'Hass' avocado. Calif. Avocado Soc. Yrbk. 93, 125-140.

Luckwill, L.C., 1970. The control of growth and fruitfulness of apple trees. pp. 237-253. In: Luckwill, L.C., Cutting, C.V. (Eds.), Physiology of Tree Crops. Academic Press,

Michaels, S.D., Amasino, R.M., 1999. *FLOWERING LOCUS C* encodes a novel MADS domain protein that acts as a repressor of flowering. Plant Cell. 11, 949-956.

Monselise, S.P., Goldschmidt, E.E., 1982. Alternate bearing in fruit trees. Hortic. Rev. 4, 128-173.

Muñoz-Fambuena, N., Mesejo, C., González-Mas, M.C., Primo-Millo, E., Agustí, M., Iglesias, D.J., 2012. Fruit load modulates flowering-related gene expression in buds of alternate-bearing 'Moncada' mandarin. Ann. Bot. 110(6), 1109-1118.

Nakagawa, M., Honsho, C., Kanzaki, S., Shimizu, K., Utsunomiya, N., 2012. Isolation and expression analysis of *FLOWERING LOCUS T*-like and gibberellin metabolism genes in biennial-bearing mango trees. Sci. Hortic. 139, 108-117.

Nzima, M.D.S., Martin, G.C., Nishijima, C., 1997. Seasonal changes in total nonstructural carbohydrates within branches and roots of naturally "off" and "on" 'Kerman' pistachio trees. J. Am. Soc. Hortic. Sci. 122, 856–862.

Okay, Y., Günes, N.T., Köksal, A.I., 2011. Free endogenous growth regulators in Pistachio (*Pistacia vera* L.). Afr. J. Agric. Res. 6, 1161–1169.

Pfaffl, M.W., 2001. A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res. 29, e45.

Pinney, K., Polito, V.S., 1990. Flower initiation in 'Manzanillo' olive. Acta Hortic. 286, 203-205.

Rallo, L., Martin, G.C., 1991. The role of chilling in releasing olive floral buds from dormancy. J. Am. Soc. Hortic. Sci. 116(6), 1058-1062.

Rosecrance, R.C., Weinbaum, S.A., Brown, P.H., 1998. Alternate bearing affects nitrogen, phosphorus, potassium and starch storage pools in mature pistachio trees. Ann. Bot. 82, 463-470.

Samach, A., Onouchi, H., Gold, S.E., Ditta, G.S., Schwarz-Sommer, Z., Yanofsky, M.F., Coupland, G., 2000. Distinct roles of *CONSTANS* target genes in reproductive development of Arabidopsis. Science. 288, 1613–1616.

Schaffer, B., Whiley, A.W., Kohli, R.R., 1991. Effect of leaf age on gas exchange characteristics of avocado. Sci. Hortic. 48, 21-28.

Shalom, L., Samuels, S., Zur, N., Shlizerman, L., Doron-Faigenboim, A., Blumwald, E., and Sadka, A., 2014. Fruit load induces changes in global gene expression and in abscisic acid (ABA) and indole acetic acid (IAA) homeostasis in citrus buds. J. Expt. Bot. 65(12), 3029-3044.

Shalom, L., Samuels, S., Zur, N., Shlizerman, L., Zemah, H., Weissberg, M., Ophir, R., Blumwald, E., Sadka, A., 2012. Alternate bearing in citrus: changes in the expression of flowering control genes and in global gene expression in on-versus off-crop trees. Plos One. 7(10), e46930.

Sibbett, S., 2000. Alternate bearing in olive trees. California Olive Oil News. 3(12). http://www.oliveoilsource.com/olivenews/olivenews3-12.htm (accessed and verified 3 Dec 2014).

Stutte, G.W., Martin, G.C., 1986. Effect of killing seeds on return bloom of olive. Sci. Hortic. 29, 107-113.

Tani, E., Polidoros, A.N., Flemetakis, E., Stedel, C., Kalloniati, C., Demetriou, K., Katinakis, P., Tsaftaris, A.S., 2009. Characterization and expression analysis of *AGAMOUS*-like, *SEEDSTICK*-like, and *SEPALLATA*-like MADS-box genes in peach (*Prunus persica*) fruit. Plant Physiol. Biochem. 47, 690-700.

Tolomeo, V., Krug, K., Hoffman, S., Flohr, D., Cosgrove, A., 2013. California olive probability survey report. http://www.nass.usda.gov/Statistics_by_State/California/Publications/Fruits_and_Nuts/201308olvpb.pdf (accessed and verified 3 Dec 2014).

Trainin, T., Bar-Ya'akov, I., Holland, D., 2013. *ParSOC1*, a MADS-box gene closely related to *Arabidopsis AGL20*/*SOC1*, is expressed in apricot leaves in a diurnal manner and is linked with chilling requirements for dormancy break. Tree Genet. Genomes. 9, 753-766.

Ülger, S., Baktir, İ., Kaynak L., 1999. Determination of the effects of endogenous plant hormones on alternate bearing and flower bud formation in olives. Turk. J. Agr. For. 23(3), 619-623.

Ü lger, S., Sonmez, S., Karkacier, M., Ertoy, N., Akdesir, O., 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.

Vemmos, S.N., 1999. Carbohydrate content of inflorescent buds of defruited and fruiting pistachio (*Pistacia vera* L.) branches in relation to biennial bearing. J. Hortic. Sci. Biotechnol. 74, 94-100.

Verreynne, J.S., 2005. The mechanism and underlying physiology perpetuating alternate bearing in 'Pixie' mandarin (*Citrus reticulata* Blanco). PhD dissertation, University of California, Riverside.

Verreynne, J.S., Lovatt, C.J., 2009. The effect of crop load on bud break influences return bloom in alternate bearing 'Pixie' Mandarin. J. Am. Soc. Hortic. Sci. 134(3), 299-307.

Vilsack, T., Clark, C.Z.F., 2013. United States summary and state data. Census of Agriculture. Vol.1.

Weigel, D., Alvarez, J., Smyth, D.R., Yanofsky, M.F., Meyerowitz, E.M. 1992. *LEAFY* controls floral meristem identity in Arabidopsis. Cell. 69, 843–859.

Whiley, A.W., Wolstenholme, B.N., 1990. Carbohydrate management in avocado trees for increased production. South Afr. Avocado Growers' Assn. Yrbk. 13, 25-27.

Yanik, H., Turktas, M., Dundar, E., Hernandez, P., Dorado, G., Unver, T., 2013. Genome-wide identification of alternate bearing-associated microRNAs (miRNAs) in olive (*Olea europaea* L.). BMC Plant Biol. 13(1), 10.

Ziv, D., Zviran, T., Zezak, O., Samach, A., Irihimovitch, V., 2014. Expression profiling of *FLOWERING LOCUS T-Like* gene in alternate bearing 'Hass' avocado trees suggests a role for PaFT in avocado flower induction. Plos One. 9(10), e110613.

Table 1. Width, length and weight of 'Manzanillo' olive fruit during development in off- and on-crop years.

| Tree Status | | Jun | Jul | Aug | Sep | Oct | *P*-value |
|---|---|---|---|---|---|---|---|
| | | Endocarp hardening | | | | Harvest | |
| Off crop | |  | | | | |  |
| On crop | |  | | | | | |
| Width (mm) | Off crop | 12.3 $a^{Dz}$ | 14.8 $a^{C}$ | 17.0 $a^{B}$ | 20.6 $a^{A}$ | 20.0 $a^{A}$ | <0.0001 |
| | On crop | 11.1 $b^{C}$ | 12.3 $b^{C}$ | 14.4 $a^{B}$ | 17.3 $b^{A}$ | 16.2 $b^{A}$ | <0.0001 |
| | *P*-value | 0.0243 | 0.0130 | 0.0619 | 0.0029 | 0.0009 | |
| Length (mm) | Off crop | 17.6 $a^{C}$ | 19.2 $a^{C}$ | 22.1 $a^{B}$ | 24.7 $a^{A}$ | 24.6 $a^{A}$ | <0.0001 |
| | On crop | 16.6 $a^{D}$ | 17.2 $a^{CD}$ | 18.6 $a^{BC}$ | 20.8 $b^{A}$ | 19.9 $b^{AB}$ | <0.0001 |
| | *P*-value | 0.0908 | 0.0817 | 0.0756 | 0.0070 | 0.0050 | |
| Weight (g) | Off crop | 1.7 $a^{D}$ | 2.7 $a^{C}$ | 4.6 $a^{B}$ | 6.1 $a^{A}$ | 5.9 $a^{A}$ | <0.0001 |
| | On crop | 1.3 $b^{C}$ | 1.7 $b^{C}$ | 2.6 $a^{B}$ | 3.7 $b^{A}$ | 3.2 $b^{A}$ | <0.0001 |
| | *P*-value | 0.0142 | 0.0239 | 0.0532 | 0.0020 | 0.0013 | |

[z] Values in a vertical column followed by different lower case letters or in a horizontal row followed by different uppercase superscript letters are significantly different at the *P*-value specified by Fisher's Protected LSD Test.

Table 2. Effect of removing the entire crop from on-crop 'Manzanillo' olive trees at progressively later times on vegetative shoot growth (node pairs) from June through May the following year. Each node pair has the potential to contribute typically two, but up to four, inflorescences at return bloom the following spring.

| Treatment | Total kg/tree | Shoot status | Jul | Aug | Sep | Oct | Dec | Feb | Apr | May |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | *monthly total no. of node pairs* | | | | | | | |
| Off-crop control | 0 | -fruit | 2.2 a[z] | 2.9 ab | 3.3 ab | 3.3 ab | 3.4 ab | 3.6 a | 5.0 a | 5.1 a |
| On-crop fruit removed[y] | | | | | | | | | | |
| Jun | | -fruit | 2.8 a | 3.3 a | 3.6 a | 3.6 a | 3.6 a | 3.4 a | 4.8 a | 5.2 a |
| Jul | | -fruit | -- | 1.8 bc | 2.3 bc | 2.4 abc | 2.5 abcd | 2.7 ab | 3.6 a | 3.7 ab |
| | | +fruit | -- | 1.6 cd | 2.3 bc | 2.3 bc | 2.7 abc | 2.9 a | 4.0 a | 4.2 ab |
| Aug | | -fruit | 0.0 b | -- | 1.0 d | 1.1 cd | 1.3 cde | 1.4 bcd | 2.4 a | 2.5 b |
| | | +fruit | 0.0 b | -- | 1.2 cd | 1.3 cd | 2.1 bcde | 2.3 abc | 3.6 a | 3.8 ab |
| Sep | | -fruit | -- | 0.0 e | -- | 0.8 d | 1.2 de | 1.4 bcd | 3.0 a | 2.8 b |
| | | +fruit | -- | 0.0 e | -- | 0.2 d | 0.8 e | 1.0 cd | 2.5 a | 3.0 b |
| On-crop control | 121 | -fruit | 0.6 b | 0.7 cde | 0.7 d | 0.7 d | 0.8 e | 1.0 cd | 2.7 a | 2.9 b |
| | | +fruit | 0.2 b | 0.5 de | 0.6 d | 0.6 d | 0.7 e | 0.8 d | 3.3 a | 3.2 ab |
| *P*-value | | | <0.0001 | <0.0001 | <0.0001 | <0.0001 | 0.0002 | 0.0004 | 0.1801 | 0.0795 |

[z] Values in a vertical column followed by different letters are significantly different at the specified *P*-value by Fisher's LSD Test.

[y] Fruit removal was done on approximately the 15th of each month. Harvest of the off- and on-crop control trees was at the end of October.

Table 3. Effect of removing the entire crop from on-crop 'Manzanillo' olive trees at progressively later times on total number of inflorescences and percentage of spring bud break at return bloom the following spring.

| Treatment | Shoot status | Total no. of inflorescences per shoot (25 node pairs) | Bud break (%) | | | | |
|---|---|---|---|---|---|---|---|
| | | | Node pairs 1-5 | Node pairs 6-10 | Node pairs 11-15 | Node pairs 16-20 | Node pairs 21-25 |
| Off-crop | -fruit | 9.3 a[z] | 54.8 a | 49.5 abc | 12.1 bc | 25.0 | |
| On-crop fruit removed[y] | | | | | | | |
| Jun | -fruit | 10.6 a | 57.4 a | 68.7 a | 28.1 abc | 0.0 | 0.0 |
| Jul | -fruit | 9.4 a | 56.4 a | 60.6 abc | 67.5 ab | 100.0 | |
| Jul | +fruit | 5.8 cd | 32.9 b | 40.0 bcd | 5.6 c | | |
| Aug | -fruit | 8.5 ab | 58.7 a | 65.8 ab | 83.3 a | | |
| Aug | +fruit | 5.4 cd | 26.4 bc | 42.3 abcd | 25.0 abc | | |
| Sep | -fruit | 6.7 bc | 52.8 a | 37.4 cd | 0.0 c | 0.0 | |
| Sep | +fruit | 3.9 de | 28.8 b | 18.5 de | 20.8 bc | | |
| On-crop | -fruit | 2.8 ef | 20.9 bc | 19.8 de | 0.0 c | | |
| On-crop | +fruit | 0.6 f | 5.7 c | 0.8 e | 0.0 c | | |
| *P*-value | | <0.0001 | <0.0001 | 0.0001 | 0.0332 | | |

[z] Values in a vertical column followed by different letters are significantly different at the specified *P*-value by Fisher's LSD Test.

[y] Fruit removal was done on approximately the 15$^{th}$ of each month. Harvest of the off- and on-crop control trees was at the end of October.

Table 4. Effect of fruit number (crop load) on the number of node pairs and the fate of buds at each node pair on the new spring apical vegetative shoot growth, distal eight node pairs, middle eight node pairs, and proximal eight node pairs of 'Manzanillo' olive trees one month before spring bloom.

| Tree status | Shoot status | Node pairs | Inflorescences | Lateral vegetative shoots | Inactive buds | Abscised buds |
|---|---|---|---|---|---|---|
| | | | New spring apical vegetative growth | | | |
| Off | Nonbearing | 2.3 a[z] | 0.0 a | 0.1 a | 4.4 a | 0.0 a |
| On | Nonbearing | 3.0 a | 0.0 a | 0.2 a | 5.8 a | 0.0 a |
| On | Bearing | 1.0 b | 0.0 a | 0.0 a | 2.0 b | 0.0 a |
| *P*-value | | 0.0039 | 0.4096 | 0.3667 | 0.0026 | -- |
| | | | Distal 8 node pairs | | | |
| Off | Nonbearing | 8.0 a | 8.3 a | 0.5 a | 6.8 b | 0.7 b |
| On | Nonbearing | 8.0 a | 0.3 b | 0.6 a | 14.3 a | 0.9 b |
| On | Bearing | 8.0 a | 0.0 b | 1.1 a | 3.2 b | 12.0 a |
| *P*-value | | -- | 0.0007 | 0.2294 | 0.0004 | <0.0001 |
| | | | Middle 8 node pairs | | | |
| Off | Nonbearing | 8.0 a | 5.6 a | 0.4 b | 8.2 a | 1.7 b |
| On | Nonbearing | 7.8 a | 0.1 b | 2.2 a | 10.7 a | 2.6 b |
| On | Bearing | 8.0 a | 0.0 b | 3.2 a | 1.1 b | 12.2 a |
| *P*-value | | 0.1083 | 0.0060 | 0.0007 | 0.0008 | 0.0002 |
| | | | Proxiaml 8 node pairs | | | |
| Off | Nonbearing | 5.1 a | 0.7 a | 0.5 b | 6.0 a | 2.9 b |
| On | Nonbearing | 3.2 a | 0.0 b | 1.0 ab | 4.0 b | 2.0 b |
| On | Bearing | 5.1 a | 0.0 b | 2.3 a | 1.0 c | 7.2 a |
| *P*-value | | <0.0001 | <0.0001 | <0.0001 | <0.0001 | 0.0111 |

[z] Values in a vertical column followed by different letters are significantly different at the specified *P*-value by Fisher's LSD Test.

Table 5. Effect of fruit number (crop load) on the number of node pairs and the fate of buds at each node pair on the new apical vegetative shoot growth of 'Manzanillo' olive trees from June to October.

| Tree status | Shoot status | Jun | Jul | Aug | Sep | Oct | *P*-value |
|---|---|---|---|---|---|---|---|
| | | | | Number of Node Pairs | | | |
| Off | Nonbearing | 5.8 $a^{Cz}$ | 6.3 $a^{B}$ | 6.6 $a^{AB}$ | 6.7 $a^{AB}$ | 6.9 $a^{A}$ | <0.0001 |
| On | Nonbearing | 6.5 $a^{C}$ | 6.8 $a^{B}$ | 7.1 $a^{A}$ | 7.2 $a^{A}$ | 7.3 $a^{A}$ | <0.0001 |
| Off | Bearing | 3.0 $b^{A}$ | 3.2 $b^{A}$ | 3.1 $b^{A}$ | 3.1 $b^{A}$ | 2.9 $b^{A}$ | 0.3156 |
| On | Bearing | 2.4 $b^{B}$ | 2.4 $b^{B}$ | 2.4 $b^{B}$ | 2.3 $b^{B}$ | 3.0 $b^{A}$ | 0.0012 |
| *P*-value | | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | |
| | | | | Number of Lateral Vegetative Shoots | | | |
| Off | Nonbearing | 0.5 $ab^{B}$ | 1.7 $a^{A}$ | 1.2 $b^{AB}$ | 1.0 $b^{AB}$ | 1.8 $b^{A}$ | 0.0812 |
| On | Nonbearing | 1.0 $a^{C}$ | 2.3 $a^{BC}$ | 2.7 $a^{B}$ | 3.0 $a^{B}$ | 6.4 $a^{A}$ | <0.0001 |
| Off | Bearing | 0.0 $b^{A}$ | 0.2 $b^{A}$ | 0.0 $c^{A}$ | 0.0 $b^{A}$ | 0.1 $c^{A}$ | 0.1559 |
| On | Bearing | 0.0 $b^{B}$ | 0.1 $b^{B}$ | 0.2 $c^{B}$ | 0.0 $b^{B}$ | 0.5 $bc^{A}$ | 0.0042 |
| *P*-value | | 0.0457 | 0.0007 | <0.0001 | <0.0001 | <0.0001 | |
| | | | | Number of Buds that Abscised | | | |
| Off | Nonbearing | 0.1 $b^{A}$ | 0.1 $a^{A}$ | 0.1 $a^{A}$ | 0.2 $a^{A}$ | 0.1 $a^{A}$ | 0.7483 |
| On | Nonbearing | 0.0 $b^{A}$ | 0.7 $a^{A}$ | 0.1 $a^{A}$ | 0.0 $a^{A}$ | 0.1 $a^{A}$ | 0.1543 |
| Off | Bearing | 0.0 $b^{A}$ | 0.0 $a^{A}$ | 0.1 $a^{A}$ | 0.0 $a^{A}$ | 0.1 $a^{A}$ | 0.3593 |
| On | Bearing | 0.4 $a^{A}$ | 0.2 $a^{A}$ | 0.2 $a^{A}$ | 0.1 $a^{A}$ | 0.2 $a^{A}$ | 0.3717 |
| *P*-value | | 0.0326 | 0.2623 | 0.4752 | 0.4023 | 0.4100 | |
| | | | | Number of Inactive Buds | | | |
| Off | Nonbearing | 10.7 $a^{C}$ | 10.8 $a^{BC}$ | 12.0 $a^{AB}$ | 12.3 $a^{A}$ | 11.9 $a^{AB}$ | 0.0281 |
| On | Nonbearing | 11.9 $a^{A}$ | 10.6 $a^{A}$ | 11.4 $a^{A}$ | 11.4 $a^{A}$ | 8.2 $b^{B}$ | <0.0001 |
| Off | Bearing | 5.9 $b^{A}$ | 6.3 $b^{A}$ | 6.1 $b^{A}$ | 6.2 $b^{A}$ | 5.7 $c^{A}$ | 0.3243 |
| On | Bearing | 4.4 $b^{B}$ | 4.4 $c^{B}$ | 4.3 $c^{B}$ | 4.5 $b^{B}$ | 5.2 $c^{A}$ | 0.0416 |
| *P*-value | | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | |
| | | | | Number of Nodes Bearing Fruit | | | |
| Off | Nonbearing | 0.0 $a^{A}$ | 0.0 $a^{A}$ | 0.0 $a^{A}$ | 0.0 $a^{A}$ | -- | -- |
| On | Nonbearing | 0.0 $a^{A}$ | 0.0 $a^{A}$ | 0.0 $a^{A}$ | 0.0 $a^{A}$ | -- | -- |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Off | Bearing | 0.0 a$^{A}$ | 0.0 a$^{A}$ | 0.0 a$^{A}$ | 0.0 a$^{A}$ | -- | 0.4023 |
| On | Bearing | 0.1 a$^{A}$ | 0.0 a$^{A}$ | 0.1 a$^{A}$ | 0.0 a$^{A}$ | -- | 0.5708 |
| *P*-value | | 0.4023 | -- | 0.4023 | 0.4023 | -- | |
| | | | | Number of Fruit | | | |
| Off | Nonbearing | -- | -- | 0.0 a$^{A}$ | 0.0 a$^{A}$ | -- | -- |
| On | Nonbearing | -- | -- | 0.0 a$^{A}$ | 0.0 a$^{A}$ | -- | -- |
| Off | Bearing | -- | -- | 0.0 a$^{A}$ | 0.0 a$^{A}$ | -- | 0.3343 |
| On | Bearing | -- | -- | 0.1 a$^{A}$ | 0.0 a$^{A}$ | -- | 0.3343 |
| *P*-value | | -- | -- | 0.4023 | 0.4023 | -- | |

[z] Values in a vertical column followed by different lower case letters or in a horizontal row followed by different uppercase superscript letters are significantly different at the *P*-value specified by Fisher's Protected LSD Test.

Table 6. Effect of fruit number (crop load) on the fate of buds at each of the distal eight node pairs of the vegetative shoot just proximal to the new vegetative shoot growth of 'Manzanillo' olive trees from June to October.

| Tree status | Shoot status | Jun | Jul | Aug | Sep | Oct | *P*-value |
|---|---|---|---|---|---|---|---|
| | | | | Number of Node Pairs | | | |
| Off | Nonbearing | 8.0 $a^{Az}$ | 8.0 $a^{A}$ | 8.0 $a^{A}$ | 8.0 $a^{A}$ | 8.0 $a^{A}$ | -- |
| On | Nonbearing | 8.0 $a^{A}$ | 8.0 $a^{A}$ | 8.0 $a^{A}$ | 8.0 $a^{A}$ | 8.0 $a^{A}$ | -- |
| Off | Bearing | 8.0 $a^{A}$ | 8.0 $a^{A}$ | 8.0 $a^{A}$ | 8.0 $a^{A}$ | 8.0 $a^{A}$ | -- |
| On | Bearing | 8.0 $a^{A}$ | 8.0 $a^{A}$ | 8.0 $a^{A}$ | 8.0 $a^{A}$ | 8.0 $a^{A}$ | -- |
| *P*-value | | -- | -- | -- | -- | -- | |
| | | | | Number of Lateral Vegetative Shoots | | | |
| Off | Nonbearing | 1.4 $a^{C}$ | 4.2 $ab^{B}$ | 4.2 $ab^{B}$ | 2.9 $b^{B}$ | 6.0 $b^{A}$ | <0.0001 |
| On | Nonbearing | 1.3 $a^{C}$ | 5.8 $a^{B}$ | 5.3 $a^{B}$ | 5.5 $a^{B}$ | 9.8 $a^{A}$ | <0.0001 |
| Off | Bearing | 1.2 $a^{B}$ | 2.6 $bc^{A}$ | 2.4 $b^{A}$ | 1.3 $c^{B}$ | 1.4 $c^{B}$ | 0.0016 |
| On | Bearing | 0.5 $a^{B}$ | 2.2 $c^{A}$ | 1.1 $b^{B}$ | 1.0 $c^{B}$ | 2.5 $c^{A}$ | <0.0001 |
| *P*-value | | 0.1352 | 0.0009 | <0.0001 | <0.0001 | <0.0001 | |
| | | | | Number of Buds that Abscised | | | |
| Off | Nonbearing | 2.1 $bc^{B}$ | 2.1 $c^{B}$ | 2.9 $c^{AB}$ | 2.7 $c^{AB}$ | 3.2 $b^{A}$ | 0.0686 |
| On | Nonbearing | 1.2 $c^{A}$ | 0.9 $c^{A}$ | 1.2 $c^{A}$ | 1.2 $c^{A}$ | 1.5 $b^{A}$ | 0.2660 |
| Off | Bearing | 3.1 $b^{D}$ | 4.2 $b^{C}$ | 5.3 $b^{B}$ | 6.1 $b^{B}$ | 10.7 $a^{A}$ | <0.0001 |
| On | Bearing | 7.3 $a^{C}$ | 7.6 $a^{C}$ | 8.8 $a^{B}$ | 8.1 $a^{BC}$ | 11.4 $a^{A}$ | <0.0001 |
| *P*-value | | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | |
| | | | | Number of Inactive Buds | | | |
| Off | Nonbearing | 12.5 $a^{A}$ | 9.6 $a^{B}$ | 8.9 $a^{B}$ | 10.4 $a^{B}$ | 6.8 $a^{C}$ | <0.0001 |
| On | Nonbearing | 13.5 $a^{A}$ | 9.3 $a^{B}$ | 9.5 $a^{B}$ | 9.3 $a^{B}$ | 4.6 $b^{C}$ | <0.0001 |
| Off | Bearing | 9.3 $b^{A}$ | 6.6 $b^{B}$ | 5.2 $b^{C}$ | 5.7 $b^{BC}$ | 3.8 $bc^{D}$ | <0.0001 |
| On | Bearing | 6.0 $c^{A}$ | 3.5 $c^{B}$ | 3.3 $c^{BC}$ | 4.0 $b^{B}$ | 2.2 $c^{C}$ | <0.0001 |
| *P*-value | | <0.0001 | <0.0001 | <0.0001 | <0.0001 | 0.0001 | |
| | | | | Number of Nodes Bearing Fruit | | | |
| Off | Nonbearing | 0.0 $c^{A}$ | 0.0 $c^{A}$ | 0.0 $c^{A}$ | 0.0 $c^{A}$ | -- | -- |
| On | Nonbearing | 0.0 $c^{A}$ | 0.0 $c^{A}$ | 0.0 $c^{A}$ | 0.0 $c^{A}$ | -- | -- |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Off | Bearing | 2.4 $a^{C}$ | 2.6 $a^{BC}$ | 3.1 $a^{A}$ | 3.0 $a^{AB}$ | -- | 0.0040 |
| On | Bearing | 2.1 $ab^{B}$ | 2.8 $a^{A}$ | 2.8 $ab^{A}$ | 2.9 $a^{A}$ | -- | 0.0012 |
| *P*-value | | <0.0001 | <0.0001 | <0.0001 | <0.0001 | -- | |
| | | | | Number of Fruit | | | |
| Off | Nonbearing | -- | -- | 0.0 $c^{A}$ | 0.0 $c^{A}$ | -- | -- |
| On | Nonbearing | -- | -- | 0.0 $c^{A}$ | 0.0 $c^{A}$ | -- | -- |
| Off | Bearing | -- | -- | 6.6 $a^{A}$ | 5.9 $a^{B}$ | -- | 0.0190 |
| On | Bearing | -- | -- | 4.2 $b^{A}$ | 4.2 $b^{A}$ | -- | 0.8916 |
| *P*-value | | -- | -- | <0.0001 | <0.0001 | -- | |

[z] Values in a vertical column followed by different lower case letters or in a horizontal row followed by different uppercase superscript letters are significantly different at the *P*-value specified by Fisher's Protected LSD Test.

Table 7. Effect of fruit number (crop load) on the fate of buds at each of the middle eight node pairs along a shoot of 'Manzanillo' olive trees from June to October.

| Tree status | Shoot status | Jun | Jul | Aug | Sep | Oct | *P*-value |
|---|---|---|---|---|---|---|---|
| | | | | Number of Node Pairs | | | |
| Off | Nonbearing | 7.9 $a^{Az}$ | 7.9 $a^{A}$ | 7.8 $a^{A}$ | 7.9 $a^{A}$ | 7.8 $a^{A}$ | 0.3544 |
| On | Nonbearing | 7.8 $a^{A}$ | 7.9 $a^{A}$ | 7.8 $a^{A}$ | 7.8 $a^{A}$ | 7.8 $a^{A}$ | 0.3912 |
| Off | Bearing | 7.7 $a^{AB}$ | 7.9 $a^{A}$ | 7.7 $a^{B}$ | 7.7 $a^{B}$ | 7.6 $a^{B}$ | 0.0192 |
| On | Bearing | 7.8 $a^{A}$ | 7.8 $a^{A}$ | 7.8 $a^{A}$ | 7.8 $a^{A}$ | 7.8 $a^{A}$ | 0.9943 |
| *P*-value | | 0.9202 | 0.7542 | 0.7834 | 0.5531 | 0.7122 | |
| | | | | Number of Lateral Vegetative Shoots | | | |
| Off | Nonbearing | 3.4 $a^{B}$ | 4.6 $ab^{A}$ | 4.8 $ab^{A}$ | 3.9 $ab^{AB}$ | 4.8 $b^{A}$ | 0.0271 |
| On | Nonbearing | 2.1 $b^{C}$ | 5.7 $a^{B}$ | 5.2 $a^{B}$ | 4.9 $a^{B}$ | 8.3 $a^{A}$ | <0.0001 |
| Off | Bearing | 3.2 $a^{AB}$ | 4.0 $bc^{A}$ | 3.6 $b^{AB}$ | 3.0 $bc^{B}$ | 3.0 $c^{B}$ | 0.0721 |
| On | Bearing | 1.8 $b^{C}$ | 2.8 $c^{AB}$ | 2.1 $c^{BC}$ | 1.9 $c^{C}$ | 3.2 $bc^{A}$ | 0.0022 |
| *P*-value | | 0.0126 | 0.0086 | <0.0001 | 0.0002 | <0.0001 | |
| | | | | Number of Buds that Abscised | | | |
| Off | Nonbearing | 3.2 $b^{B}$ | 4.4 $b^{A}$ | 4.8 $b^{A}$ | 4.9 $c^{A}$ | 5.2 $b^{A}$ | 0.0005 |
| On | Nonbearing | 1.6 $b^{A}$ | 1.4 $c^{A}$ | 1.6 $c^{A}$ | 1.4 $d^{A}$ | 2.0 $c^{A}$ | 0.1679 |
| Off | Bearing | 5.9 $a^{D}$ | 8.6 $a^{C}$ | 8.6 $a^{C}$ | 9.9 $a^{B}$ | 11.5 $a^{A}$ | <0.0001 |
| On | Bearing | 6.0 $a^{C}$ | 6.9 $a^{BC}$ | 7.8 $a^{B}$ | 7.8 $b^{B}$ | 11.0 $a^{A}$ | <0.0001 |
| *P*-value | | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | |
| | | | | Number of Inactive Buds | | | |
| Off | Nonbearing | 9.1 $b^{A}$ | 6.9 $a^{BC}$ | 6.1 $b^{BC}$ | 7.0 $b^{B}$ | 5.5 $a^{C}$ | <0.0001 |
| On | Nonbearing | 12.0 $a^{A}$ | 8.6 $a^{B}$ | 8.8 $a^{B}$ | 9.3 $a^{B}$ | 5.2 $a^{C}$ | <0.0001 |
| Off | Bearing | 5.0 $c^{A}$ | 1.8 $b^{BC}$ | 2.2 $c^{B}$ | 1.6 $c^{BC}$ | 0.7 $b^{C}$ | <0.0001 |
| On | Bearing | 3.7 $c^{A}$ | 2.6 $b^{B}$ | 2.5 $c^{B}$ | 2.5 $c^{B}$ | 1.4 $b^{C}$ | 0.0009 |
| *P*-value | | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | |
| | | | | Number of Nodes Bearing Fruit | | | |
| Off | Nonbearing | 0.0 $c^{A}$ | 0.0 $c^{A}$ | 0.0 $c^{A}$ | 0.0 $c^{A}$ | -- | -- |
| On | Nonbearing | 0.0 $c^{A}$ | 0.0 $c^{A}$ | 0.0 $c^{A}$ | 0.0 $c^{A}$ | -- | -- |
| Off | Bearing | 1.4 $b^{A}$ | 1.4 $b^{A}$ | 0.9 $b^{B}$ | 0.9 $b^{B}$ | -- | 0.0079 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| On | Bearing | 4.1 $a^{A}$ | 3.5 $a^{B}$ | 3.3 $a^{B}$ | 3.4 $a^{B}$ | -- | 0.0003 |
| *P*-value | | <0.0001 | <0.0001 | <0.0001 | <0.0001 | -- | |
| | | | | Number of Fruit | | | |
| Off | Nonbearing | -- | -- | 0.0 $c^{A}$ | 0.0 $c^{A}$ | -- | -- |
| On | Nonbearing | -- | -- | 0.0 $c^{A}$ | 0.0 $c^{A}$ | -- | -- |
| Off | Bearing | -- | -- | 1.9 $b^{A}$ | 1.9 $b^{A}$ | -- | 0.9614 |
| On | Bearing | -- | -- | 5.1 $a^{A}$ | 5.2 $a^{A}$ | -- | 0.4159 |
| *P*-value | | -- | -- | <0.0001 | <0.0001 | -- | |

[z] Values in a vertical column followed by different lower case letters and horizontal row followed by different uppercase superscript letters are significantly different at *P*-value specified by Fisher's Protected LSD Test.

Table 8. Effect of fruit number (crop load) on the fate of buds at each of the proximal eight node pairs along a shoot of 'Manzanillo' olive trees from June to October.

| Tree status | Shoot status | Jun | Jul | Aug | Sep | Oct | *P*-value |
|---|---|---|---|---|---|---|---|
| | | | | Number of Node Pairs | | | |
| Off | Nonbearing | 6.5 $a^{Az}$ | 5.3 $ab^{B}$ | 5.2 $a^{B}$ | 5.1 $a^{B}$ | 5.0 $a^{B}$ | <0.0001 |
| On | Nonbearing | 5.8 $ab^{A}$ | 5.7 $a^{A}$ | 4.9 $ab^{B}$ | 4.8 $ab^{B}$ | 4.6 $a^{B}$ | 0.0002 |
| Off | Bearing | 5.0 $b^{A}$ | 4.2 $b^{B}$ | 3.7 $b^{BC}$ | 3.6 $b^{BC}$ | 3.2 $b^{C}$ | <0.0001 |
| On | Bearing | 6.4 $a^{A}$ | 5.5 $a^{B}$ | 5.1 $a^{B}$ | 5.2 $a^{B}$ | 5.5 $a^{B}$ | 0.0016 |
| *P*-value | | 0.0916 | 0.0830 | 0.0738 | 0.0385 | 0.0045 | |
| | | | | Number of Lateral Vegetative Shoots | | | |
| Off | Nonbearing | 1.8 $a^{A}$ | 2.0 $a^{A}$ | 1.7 $a^{A}$ | 1.6 $a^{A}$ | 2.0 $b^{A}$ | 0.3362 |
| On | Nonbearing | 1.2 $a^{C}$ | 2.1 $a^{B}$ | 2.2 $a^{B}$ | 2.1 $a^{B}$ | 3.2 $a^{A}$ | 0.0002 |
| Off | Bearing | 1.8 $a^{A}$ | 1.7 $a^{A}$ | 1.6 $a^{A}$ | 1.8 $a^{A}$ | 1.4 $b^{A}$ | 0.3805 |
| On | Bearing | 1.8 $a^{ABC}$ | 2.1 $a^{AB}$ | 1.6 $a^{BC}$ | 1.3 $a^{C}$ | 2.4 $ab^{A}$ | 0.0246 |
| *P*-value | | 0.2435 | 0.8840 | 0.5641 | 0.3374 | 0.0116 | |
| | | | | Number of Buds that Abscised | | | |
| Off | Nonbearing | 4.3 $a^{A}$ | 4.3 $a^{A}$ | 4.9 $a^{A}$ | 4.7 $a^{A}$ | 5.0 $b^{A}$ | 0.4922 |
| On | Nonbearing | 2.0 $b^{A}$ | 1.9 $b^{A}$ | 1.8 $b^{A}$ | 1.4 $b^{A}$ | 1.7 $c^{A}$ | 0.7123 |
| Off | Bearing | 5.8 $a^{A}$ | 5.6 $a^{A}$ | 4.7 $a^{A}$ | 5.0 $a^{A}$ | 4.9 $b^{A}$ | 0.2714 |
| On | Bearing | 4.8 $a^{B}$ | 5.1 $a^{B}$ | 4.8 $a^{B}$ | 5.2 $a^{AB}$ | 6.5 $a^{A}$ | 0.0932 |
| *P*-value | | 0.0037 | 0.0002 | 0.0002 | <0.0001 | <0.0001 | |
| | | | | Number of Inactive Buds | | | |
| Off | Nonbearing | 7.1 $ab^{A}$ | 4.2 $b^{B}$ | 3.8 $ab^{B}$ | 3.9 $b^{B}$ | 3.2 $ab^{B}$ | <0.0001 |
| On | Nonbearing | 8.4 $a^{A}$ | 7.4 $a^{AB}$ | 5.7 $a^{CD}$ | 6.0 $a^{BC}$ | 4.3 $a^{D}$ | <0.0001 |
| Off | Bearing | 2.2 $c^{A}$ | 1.0 $c^{B}$ | 1.1 $c^{B}$ | 0.4 $c^{BC}$ | 0.2 $c^{C}$ | <0.0001 |
| On | Bearing | 5.3 $b^{A}$ | 3.1 $b^{B}$ | 3.4 $b^{B}$ | 3.5 $b^{B}$ | 2.1 $b^{C}$ | <0.0001 |
| *P*-value | | <0.0001 | <0.0001 | 0.0011 | <0.0001 | 0.0001 | |
| | | | | Number of Nodes Bearing Fruit | | | |
| Off | Nonbearing | 0.0 $b^{A}$ | 0.0 $b^{A}$ | 0.0 $b^{A}$ | 0.0 $b^{A}$ | -- | -- |
| On | Nonbearing | 0.0 $b^{A}$ | 0.0 $b^{A}$ | 0.0 $b^{A}$ | 0.0 $b^{A}$ | -- | -- |
| Off | Bearing | 0.2 $b^{A}$ | 0.1 $b^{AB}$ | 0.1 $b^{B}$ | 0.0 $b^{B}$ | -- | -- |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| On | Bearing | 0.9 $a^{A}$ | 0.7 $a^{AB}$ | 0.5 $a^{B}$ | 0.5 $a^{B}$ | -- | -- |
| *P*-value | | <0.0001 | <0.0001 | 0.0004 | 0.0001 | -- | |
| | | | | Number of Fruit | | | |
| Off | Nonbearing | -- | -- | 0.0 $b^{A}$ | 0.0 $b^{A}$ | -- | -- |
| On | Nonbearing | -- | -- | 0.0 $b^{A}$ | 0.0 $b^{A}$ | -- | -- |
| Off | Bearing | -- | -- | 0.1 $b^{A}$ | 0.0 $b^{A}$ | -- | -- |
| On | Bearing | -- | -- | 0.7 $a^{A}$ | 0.7 $a^{A}$ | -- | -- |
| *P*-value | | -- | -- | 0.0036 | 0.0002 | -- | |

[z] Values in a vertical column followed by different lower case letters and horizontal row followed by different uppercase superscript letters are significantly different at *P*-value specified by Fisher's Protected LSD Test.

Table 9. Olive *SOC1* and *FT* gene expression in buds of the distal eight node pairs from nonbearing shoots of off-crop trees (off-) and nonbearing (on-) and bearing (on+) shoots of on-crop trees from June to the following March.

| Month | | *SOC1/Tubulin* Off(-) | On(-) | On(+) | *P*-value |
|---|---|---|---|---|---|
| Jun | 2013 | 0.23 $b^{Bz}$ | 0.21 $ab^{B}$ | 0.84 $a^{A}$ | 0.0440 |
| Jul | 2013 | 0.15 $b^{B}$ | 0.32 $ab^{B}$ | 0.91 $a^{A}$ | 0.0112 |
| Aug | 2013 | 0.58 $b^{A}$ | 0.27 $ab^{A}$ | 0.55 $a^{A}$ | 0.2289 |
| Sep | 2013 | 0.34 $b^{A}$ | 0.19 $ab^{A}$ | 0.00 $a^{A}$ | 0.1281 |
| Oct | 2013 | 2.32 $b^{A}$ | 1.92 $a^{A}$ | 0.00 $a^{A}$ | 0.2913 |
| Dec | 2013 | 1.49 $b^{A}$ | 2.06 $a^{A}$ | 2.46 $a^{A}$ | 0.4332 |
| Mar | 2014 | 15.85 $a^{A}$ | 0.00 $b^{B}$ | 0.00 $a^{B}$ | 0.0148 |
| *P*-value | | <0.0001 | 0.1062 | 0.2238 | |

| Month | | *FT/Tubulin* Off(-) | On(-) | On(+) | *P*-value |
|---|---|---|---|---|---|
| Jun | 2013 | 0.05 $c^{A}$ | 0.03 $a^{A}$ | 0.12 $ab^{A}$ | 0.0668 |
| Jul | 2013 | 0.02 $c^{A}$ | 0.10 $a^{A}$ | 0.26 $a^{A}$ | 0.3997 |
| Aug | 2013 | 0.10 $c^{A}$ | 0.05 $a^{A}$ | 0.07 $b^{A}$ | 0.5521 |
| Sep | 2013 | 0.47 $ab^{A}$ | 0.08 $a^{B}$ | 0.00 $b^{B}$ | 0.0060 |
| Oct | 2013 | 0.25 $bc^{A}$ | 0.06 $a^{A}$ | 0.00 $b^{A}$ | 0.1157 |
| Dec | 2013 | 0.02 $c^{A}$ | 0.00 $a^{B}$ | 0.00 $b^{B}$ | 0.0168 |
| Mar | 2014 | 0.69 $a^{A}$ | 0.00 $a^{A}$ | 0.00 $b^{A}$ | 0.0544 |
| *P*-value | | 0.0005 | 0.5621 | 0.0729 | |

| Month | | *SOC1/Actin* Off(-) | On(-) | On(+) | *P*-value |
|---|---|---|---|---|---|
| Jun | 2013 | 0.18 $b^{Bz}$ | 0.20 $c^{B}$ | 0.77 $a^{A}$ | 0.0416 |
| Jul | 2013 | 0.15 $b^{B}$ | 0.20 $c^{B}$ | 0.44 $a^{A}$ | 0.0749 |
| Aug | 2013 | 0.24 $b^{A}$ | 0.13 $c^{A}$ | 0.25 $a^{A}$ | 0.4665 |
| Sep | 2013 | 0.18 $b^{A}$ | 0.16 $c^{A}$ | 0.00 $a^{A}$ | 0.2107 |
| Oct | 2013 | 0.68 $b^{A}$ | 0.18 $c^{B}$ | 0.00 $a^{B}$ | 0.0267 |
| Dec | 2013 | 0.99 $b^{A}$ | 2.10 $ab^{A}$ | 2.11 $a^{A}$ | 0.2934 |
| Mar | 2014 | 53.28 $a^{A}$ | 0.00 $c^{A}$ | 0.00 $a^{A}$ | 0.1129 |
| *P*-value | | 0.0047 | 0.0621 | 0.2183 | |

| Month | | *FT/Actin* Off(-) | On(-) | On(+) | *P*-value |
|---|---|---|---|---|---|
| Jun | 2013 | 0.04 $c^{B}$ | 0.03 $b^{B}$ | 0.14 $bc^{A}$ | 0.0046 |
| Jul | 2013 | 0.01 $c^{A}$ | 0.05 $b^{A}$ | 0.11 $bc^{A}$ | 0.4963 |
| Aug | 2013 | 0.04 $c^{A}$ | 0.02 $b^{A}$ | 0.03 $bc^{A}$ | 0.5526 |
| Sep | 2013 | 0.21 $c^{A}$ | 0.08 $b^{B}$ | 0.00 $c^{B}$ | 0.0074 |
| Oct | 2013 | 0.07 $c^{A}$ | 0.02 $b^{A}$ | 0.00 $c^{A}$ | 0.0722 |
| Dec | 2013 | 0.01 $c^{A}$ | 0.00 $b^{B}$ | 0.00 $c^{B}$ | 0.0168 |
| Mar | 2014 | 0.83 $b^{A}$ | 0.00 $b^{B}$ | 0.00 $c^{B}$ | 0.0001 |
| *P*-value | | <0.0001 | 0.3445 | 0.0080 | |

[z] Values in a vertical column followed by different lower case letters or in a horizontal row followed by different uppercase superscript letters are significantly different at the specified *P*-value by Fisher's Protected LSD Test.

Table 10. Olive *PI* and *AG1* gene expression in buds of the distal eight node pairs from nonbearing shoots of off-crop trees (off-) and nonbearing (on-) and bearing (on+) shoots of on-crop trees from June to the following March.

| Month | | *PI/Tubulin* | | | | Month | | *AG1/Tubulin* | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Off(-) | On(-) | On(+) | *P*-value | | | Off(-) | On(-) | On(+) | *P*-value |
| Jun | 2013 | 0.000 $b^{Az}$ | 0.00 $a^{A}$ | 0.00 $a^{A}$ | -- | Jun | 2013 | 0.01 $b^{A}$ | 0.00 $b^{A}$ | 0.00 $a^{A}$ | 0.3946 |
| Jul | 2013 | 0.000 $b^{A}$ | 0.00 $a^{A}$ | 0.00 $a^{A}$ | -- | Jul | 2013 | 0.01 $b^{A}$ | 0.00 $b^{A}$ | 0.00 $a^{A}$ | 0.4096 |
| Aug | 2013 | 0.000 $b^{A}$ | 0.00 $a^{A}$ | 0.00 $a^{A}$ | -- | Aug | 2013 | 0.03 $b^{A}$ | 0.00 $b^{A}$ | 0.00 $a^{A}$ | 0.3874 |
| Sep | 2013 | 0.000 $b^{A}$ | 0.00 $a^{A}$ | 0.00 $a^{A}$ | -- | Sep | 2013 | 0.00 $b^{A}$ | 0.00 $b^{B}$ | 0.00 $a^{B}$ | 0.0074 |
| Oct | 2013 | 0.000 $b^{A}$ | 0.00 $a^{A}$ | 0.00 $a^{A}$ | -- | Oct | 2013 | 0.00 $b^{A}$ | 0.00 $b^{B}$ | 0.00 $a^{B}$ | 0.0078 |
| Dec | 2013 | 0.000 $b^{A}$ | 0.00 $a^{A}$ | 0.00 $a^{A}$ | -- | Dec | 2013 | 0.00 $b^{A}$ | 0.00 $b^{B}$ | 0.00 $a^{B}$ | 0.0529 |
| Mar | 2014 | 0.004 $a^{A}$ | 0.00 $a^{A}$ | 0.00 $a^{A}$ | 0.1921 | Mar | 2014 | 0.36 $a^{A}$ | 0.05 $a^{B}$ | 0.00 $a^{B}$ | 0.0011 |
| *P*-value | | 0.0990 | -- | -- | | *P*-value | | <0.0001 | 0.1835 | 0.5256 | |

| Month | | *PI/Actin* | | | | Month | | *AG1/Actin* | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Off(-) | On(-) | On(+) | *P*-value | | | Off(-) | On(-) | On(+) | *P*-value |
| Jun | 2013 | 0.000 $b^{Az}$ | 0.00 $a^{A}$ | 0.00 $a^{A}$ | -- | Jun | 2013 | 0.01 $b^{A}$ | 0.00 $b^{A}$ | 0.02 $a^{A}$ | 0.4154 |
| Jul | 2013 | 0.000 $b^{A}$ | 0.00 $a^{A}$ | 0.00 $a^{A}$ | -- | Jul | 2013 | 0.01 $b^{A}$ | 0.00 $b^{A}$ | 0.00 $a^{A}$ | 0.4096 |
| Aug | 2013 | 0.000 $b^{A}$ | 0.00 $a^{A}$ | 0.00 $a^{A}$ | -- | Aug | 2013 | 0.01 $b^{A}$ | 0.00 $b^{A}$ | 0.00 $a^{A}$ | 0.3846 |
| Sep | 2013 | 0.000 $b^{A}$ | 0.00 $a^{A}$ | 0.00 $a^{A}$ | -- | Sep | 2013 | 0.00 $b^{A}$ | 0.00 $b^{B}$ | 0.00 $a^{B}$ | 0.0057 |
| Oct | 2013 | 0.000 $b^{A}$ | 0.00 $a^{A}$ | 0.00 $a^{A}$ | -- | Oct | 2013 | 0.00 $b^{A}$ | 0.00 $b^{A}$ | 0.00 $a^{A}$ | 0.4096 |
| Dec | 2013 | 0.000 $b^{A}$ | 0.00 $a^{A}$ | 0.00 $a^{A}$ | -- | Dec | 2013 | 0.00 $b^{A}$ | 0.00 $b^{B}$ | 0.00 $a^{B}$ | 0.0078 |
| Mar | 2014 | 0.009 $a^{A}$ | 0.00 $a^{A}$ | 0.00 $a^{A}$ | 0.2648 | Mar | 2014 | 1.44 $a^{A}$ | 0.21 $a^{B}$ | 0.00 $a^{B}$ | 0.0042 |
| *P*-value | | 0.1973 | -- | -- | -- | *P*-value | | <0.0001 | 0.0187 | 0.4936 | |

[z] Values in a vertical column followed by different lower case letters or in a horizontal row followed by different uppercase superscript letters are significantly different at the specified *P*-value by Fisher's Protected LSD Test.

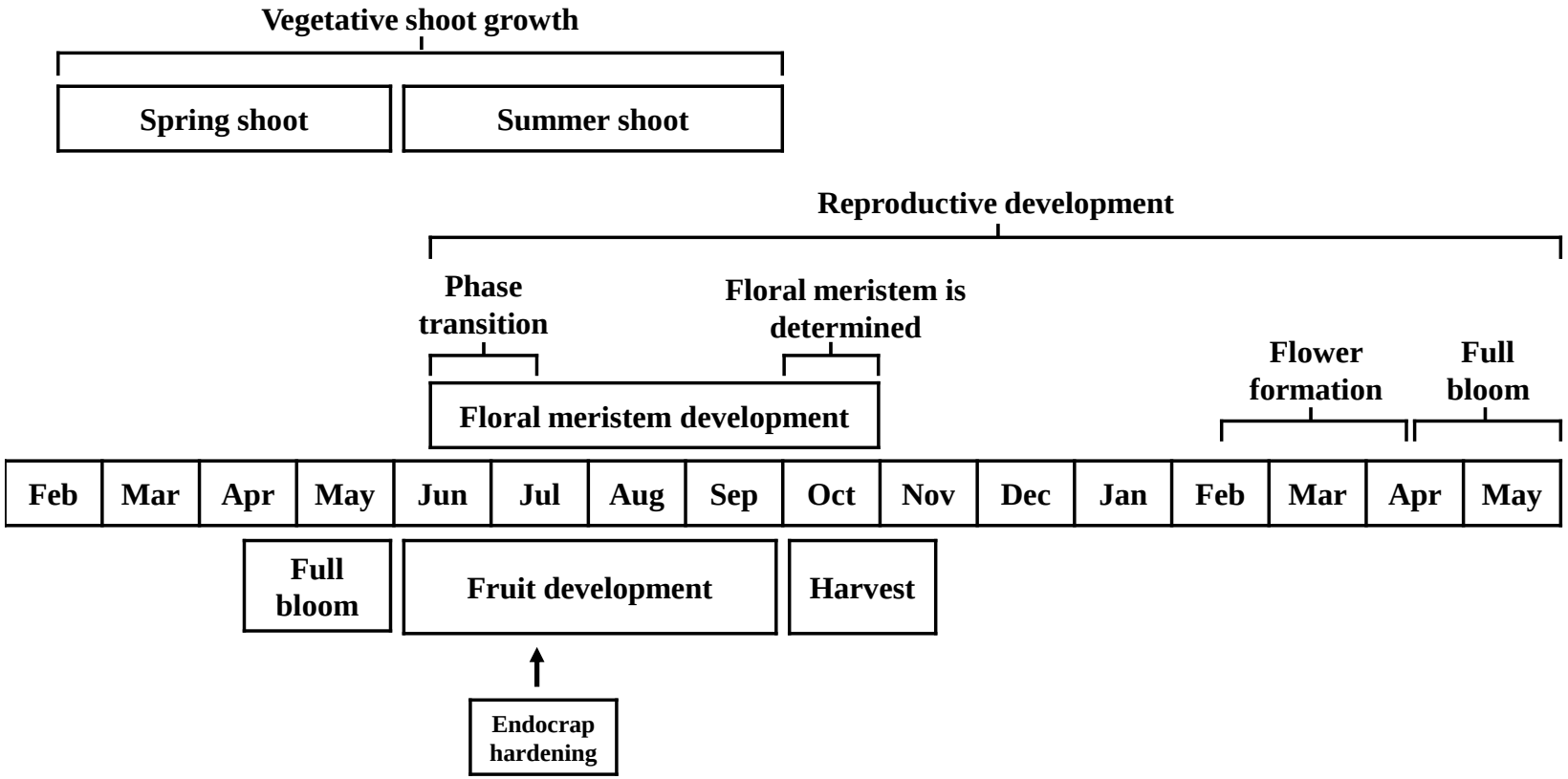


Fig. 1. Phenology of 'Manzanillo' olive trees in Tulare County, California. Vegetative shoot growth occurs in spring and summer and is minimal in fall and winter. Phase transition occurs in early July, 2 to 3 months after full bloom, followed by the meristem being committed to floral development (determined) with no apparent change in structure by October. Floral organ development occurs in late February to full bloom in April to May. Fruit development starts during bloom with fruit set and continues to commercial harvest in October.

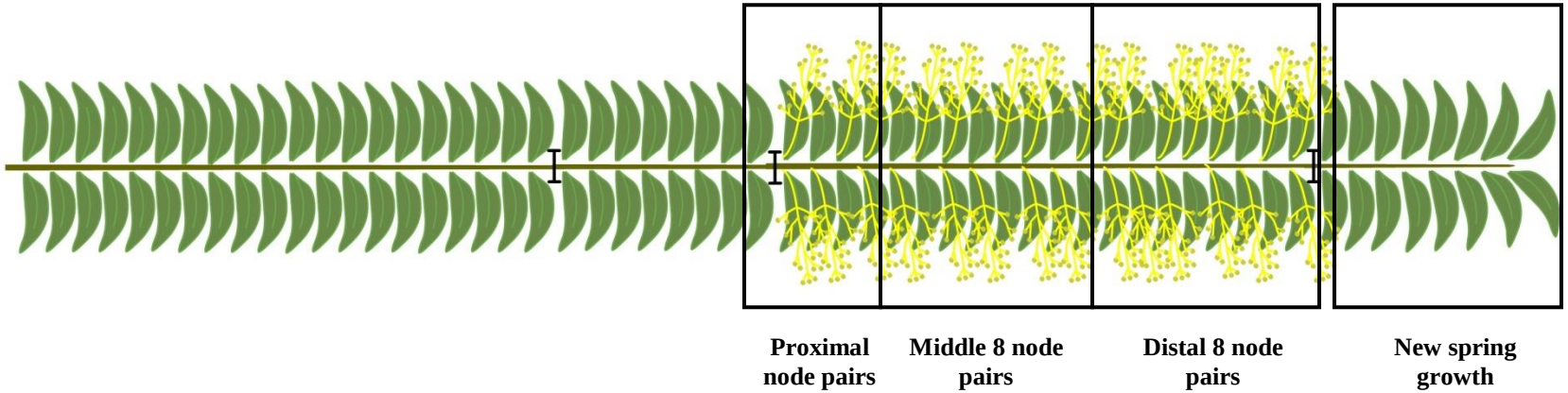


Fig. 2. Model of a ‘Manzanillo’ olive shoot at spring bloom. The shoot was divided into four sections, new spring growth, distal 8 node pairs, middle 8 node pairs, and proximal 8 node pairs, for quantification of the fate of buds. Every node pair has potential to produce two to four buds. Note that p node pairs do not have 8 node pairs in some cases.