

Repeating cycles of ON and OFF yields in alternate bearing olive, pistachio and citrus trees – different mechanisms, common solutions

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Abstract

Alternating high yield ON-crop years and low yield OFF-crop years (alternate bearing) is a common problem of significant economic consequence to the commercial production of woody perennial fruit and nut crops. Not only does alternate bearing complicate orchard management and reduce grower income, it also negatively impacts packinghouse operation, marketing, and the stability of commodity-based industries. Alternate bearing is typically initiated by an adverse climate event that results in an OFF crop, which is followed by an intense bloom that sets an ON crop, with little to no flowering the year following the ON crop. There are four mechanisms, by which crop load (fruit number tree⁻¹) influences return bloom, listed here as the effect of the ON crop: i) reduced summer vegetative shoot growth, with fewer, shorter vegetative shoots resulting in fewer nodes to bear inflorescences the following spring; ii) abscission of floral buds; iii) repression of key genes required for normal floral development; and iv) inhibition of spring bud break. All four mechanisms perpetuate alternate bearing in olive (*Olea europaea*), three in citrus (*Citrus reticulata*), with only one, presently demonstrated in pistachio (*Pistacia vera*). For each species, the effects of the ON crop are greater for bearing shoots than non-bearing shoots, which by default are the major source of inflorescences the following spring. Thus, ON-crop trees need to be managed to create more non-bearing shoots with properly timed fruit thinning by hand, chemical treatment, or pruning or potentially by using foliar-applied plant growth regulators to overcome the effects of the ON crop and stimulate summer vegetative shoot growth, restore floral gene transcription, prevent floral bud abscission, and promote spring bud break to increase return bloom and yield.

Keywords: vegetative shoot growth, floral bud abscission, *FLOWERING LOCUS T*, spring bud break, non-bearing shoots, cytokinins

INTRODUCTION

Woody perennial fruit and nut trees frequently bear in repeating biennial (2-year) cycles, in which a high yield ON-crop year alternates with a low yield OFF-crop year. The alternating high and low yields cause significant economic problems. In the ON-crop year, the large number of fruit set tree⁻¹ reduces fruit and nut size and the percentage of marketable crop. Although fruit and nut size is good in the OFF-crop year, there is too little crop to provide the grower an adequate income. In addition, the lack of fruit in the OFF-crop year has a negative economic impact on every step in the production chain from farm to consumer, including orchard management, harvesting, packinghouse operation, manufacture of value-added products, and marketing, which jeopardizes the stability and sustainability of tree-crop commodity-based industries. Alternate bearing is typically initiated by an adverse climate event (low or high temperature, limited or excess water, etc.) during stages of floral development, bloom or fruit set that reduces floral intensity, pollination, fertilization and fruit set, resulting in an OFF crop, which is followed in one to two years, depending on how long it takes the trees to recover, by an ON crop. Conversely, alternate bearing can be initiated when all conditions are optimal during reproductive development, flowering and fruit set, such that



natural fruit thinning fails to occur. In this case, the trees set an ON crop that is followed the next year by an OFF crop. Once initiated, the pattern of ON and OFF crops that characterizes alternate bearing is perpetuated by the influence of crop load (number of fruit tree⁻¹) on the intensity of the return bloom the following spring, i.e., low yield OFF-crop trees flower profusely the following year, whereas high yield ON-crop trees produce few to no inflorescences at return bloom.

Our results and those published in the literature provide evidence that there are four mechanisms, by which the ON crop of fruit reduces return bloom: i) reduced summer vegetative shoot growth results in fewer nodes to bear inflorescences the following spring (Fichtner and Lovatt, 2018; Sibbett, 2000; Verreyne and Lovatt, 2009); ii) abscission of floral buds (Chao, 2014; Crane and Nelson, 1971; Lovatt et al., 2006); iii) repression of key genes required for normal floral development (Muñoz-Fambuena et al., 2012; Shalom et al., 2012; Tang, 2017); and iv) inhibition of spring bud break (Fichtner and Lovatt, 2018; Tang, 2017; Verreyne and Lovatt, 2009). In this research, the four mechanisms were compared in three alternate bearing tree crops of commercial importance to Iran and California, USA, olive (*Olea europaea*), citrus (*Citrus reticulata*), and pistachio (*Pistacia vera*).

MATERIALS AND METHODS

Plant material

The research was conducted in two commercially bearing 'Manzanillo' olive orchards located in Exeter, CA (latitude 36.3°N; longitude 119.2°W; elevation 106 m a.s.l.): 1) 26-year-old trees on their own roots planted at 156 trees ha⁻¹ with 'Sevillano' pollenizer trees at a ratio of 1 to 7 (Table 1); and 2) 17-year-old trees on their own roots planted at 357 trees ha⁻¹ with 'Barouni' pollenizer trees at a ratio of 1 to 10 (Tables 3 and 5). In year 1, OFF- and ON-crop control trees were selected based on floral intensity. Ten non-bearing shoots (no inflorescences) and ten bearing shoots (many inflorescences) were tagged at a height of 1.5 m above ground around the control trees and additional ON-crop trees. In July or the following February, scaffold branches of ON-crop trees were injected with plant growth regulators (PGRs) to increase summer vegetative shoot growth, spring bud break and return bloom: i) 6-benzyladenine (BA), a synthetic cytokinin; ii) BA + tri-iodobenzoic acid (TIBA), an auxin-transport inhibitor; iii) adenosine (ADO), a natural precursor of purines and cytokinins (Lovatt et al., 2014); and iv) Ado + TIBA. Each treatment was injected at 1 g active ingredient in 50 mL of appropriate solvent divided in two syringes per compound per one main scaffold branch per tree. A randomized complete block design (RCBD) with seven individual tree replications per treatment was used. Research was also conducted in two commercially bearing mandarin orchards each planted on citrange rootstock (*C. sinensis* × *Poncirus trifoliata*) at 625 trees ha⁻¹ with no pollenizer trees: 1) 'Pixie' mandarin trees located in Ojai, CA (latitude 34.3°N; longitude 119.1°W; elevation 227 m a.s.l.); and 2) 'Nules Clementine' mandarin trees in Fillmore, CA (latitude 34.2°N; longitude 118.6°W; elevation 139 m a.s.l.). Twenty non-bearing and 20 bearing shoots without and with inflorescences, respectively, were tagged on OFF- and ON-crop control trees as described above. Additional ON-crop 'Nules Clementine' mandarin trees were selected at bloom. In July and July plus the following January, the trunks were injected as described above with: i) BA; ii) BA+TIBA; iii) adenosine (ADO); iv) Ado+TIBA; and v) gibberellic acid (GA₃), which is known to stimulate bud break and vegetative or floral shoot growth (Salazar-García and Lovatt, 1998). A RCBD with 14 individual tree replications per treatment was used. Two separate experiments were conducted with 17-year-old 'Kerman' pistachio trees on 'Pioneer Gold' rootstock (*P. integerrima*) planted at 292 trees ha⁻¹ with 'Peters' pollenizer trees in a ratio of 8 to 1 in a commercial orchard in Madera, CA (latitude 36.6°N; longitude 120.0°W; elevation 83 m a.s.l.). One bearing shoot (>70 fruit shoot⁻¹) per ON-crop tree was tagged and the trees were left untreated or sprayed at the initiation of embryo growth (mid-June) and start of exponential embryo growth (mid-July) with the following: 1) BA at 70 g ha⁻¹; 2) low-biuret urea (0.49% N, 0.25% biuret) at 7 kg ha⁻¹; and 3) BA (70 g ha⁻¹) + urea (7 kg ha⁻¹) in water at 378.5 (pH 5.5) L ha⁻¹ using a handgun sprayer at 2.76 MPa. A RCBD with 16 individual tree replications per treatment was used. The

fate of the buds was quantified at each pair of opposing nodes for olive and each node for citrus and pistachio.

Statistical analysis

Analysis of variance (ANOVA) was used to test for treatment effects on the number of nodes produced, inflorescence number at bloom, and percent bud break at spring bloom averaged across non-bearing and bearing shoots, respectively, per individual tree replication. When ANOVA output indicated significant differences, post-hoc comparisons were run using Fisher's least significant difference (LSD) test with a family error rate of $\alpha \leq 0.05$.

RESULTS

Effect of fruit on summer vegetative shoot growth and return bloom

In alternate bearing tree crops, there is both a localized effect of fruit set on a bearing shoot and a whole tree effect of the total fruit produced by the tree (crop load). For 'Manzanillo' olive trees, evidence of the localized effect of the fruit was that bearing shoots (8.6 fruit shoot⁻¹) of OFF-crop trees produced 54% fewer node pairs than non-bearing shoots (0 fruit shoot⁻¹) of OFF-crop trees ($P < 0.0001$) (Table 1). No effect of crop load (kg tree⁻¹) was observed for non-bearing shoots of ON-crop trees versus OFF-crop trees (Table 1). The localized effect of fruit borne on a shoot combined with the effect of crop load resulted in a 67% reduction in summer vegetative shoot growth (node pairs) for bearing shoots of ON-crop trees compared to non-bearing shoots of OFF-crop trees ($P < 0.0001$). A localized effect of fruit set on a shoot on the number of inflorescences produced by summer vegetative shoots at return bloom was also observed. Bearing shoots of OFF-crop trees produced 63% fewer inflorescences per summer shoot than non-bearing shoots of OFF-crop trees. Bearing shoots of ON-crop trees also produced 68% fewer inflorescences per summer shoot than non-bearing shoots of ON-crop trees ($P < 0.0001$) (Table 1). The effect of crop load combined with the localized effect of fruit borne on a shoot further reduced the number of inflorescences produced by summer shoots on bearing shoots of ON-crop trees by 68% compared to summer shoots on bearing shoots of OFF-crop trees. For bearing shoots of ON-crop trees, summer vegetative shoot growth and the number of inflorescences produced by summer flush vegetative shoots at spring bloom were reduced 66 and 88%, respectively, relative to non-bearing shoots of OFF-crop trees. The greater reduction in inflorescence number than summer vegetative shoot growth (nodes pairs shoot⁻¹) for ON-crop 'Manzanillo' olive trees suggests that additional factors play a role in reducing floral intensity at return bloom, consistent with the demonstration of floral bud abscission and inhibition of floral gene transcription (Chao, 2014). For 'Manzanillo' olive, fruit exerted a similar effect on the contribution of spring versus summer shoots to return bloom, with summer shoots contributing more than 55% of the inflorescences the following spring (Table 1).

Table 1. Effect of non-bearing and bearing shoots of OFF- and ON-crop 'Manzanillo' olive trees on summer vegetative shoot growth measured in September and the number of inflorescences produced by summer vegetative shoots the following spring.

Tree status	Year 1		Year 2		
	Yield (kg tree ⁻¹)	Shoot status	Summer vegetative shoot growth (no. of node pairs shoot ⁻¹)	Inflorescences (no. per summer shoot ⁻¹)	Inflorescences Produced by summer shoots (% total)
OFF	49.6 b	Non-bearing	6.7 a	11.2 a	57
		Bearing	3.1 b	4.1 b	59
ON	151.7 a	Non-bearing	7.2 a	3.4 b	58
		Bearing	2.3 c	1.3 c	65
<i>P</i> -value	<0.0001		<0.0001	<0.0001	

Means in a vertical column followed by different letters are significantly different at specified *P*-values by Fisher's LSD test.

Similar results were observed for citrus. Summer vegetative shoot growth was reduced 58% on bearing shoots compared to non-bearing shoots of OFF-crop 'Pixie' mandarin trees ($P < 0.0001$) (Table 2). However, for 'Pixie' mandarin the effect of crop load was stronger than that observed for 'Manzanilla' olive. Thus, summer vegetative shoot growth was reduced 73% on non-bearing shoots of ON-crop 'Pixie' mandarin trees compared to non-bearing shoots of OFF-crop trees ($P < 0.0001$). The combined localized and crop load effects of fruit reduced summer vegetative shoot growth in 'Pixie' mandarin 89%; compare 1.1 node per bearing shoot of ON-crop trees to 10 nodes per non-bearing shoot of OFF-crop trees ($P < 0.0001$). The localized and crop load effects of fruit on the number of inflorescences produced at return bloom by summer vegetative shoots followed a similar pattern. Non-bearing shoots of OFF-crop trees produced the greatest number of inflorescences per summer vegetative shoot ($P < 0.0001$) (Table 2). The presence of fruit on bearing shoots of OFF-crop trees (localized effect) reduced the number of inflorescences produced by summer shoots 70% at return bloom ($P < 0.0001$). Comparison of the number of inflorescences produced by summer shoots of non-bearing shoots of OFF- and ON-crop trees demonstrated that the ON crop of fruit (crop load effect) reduced the number of inflorescences produced by summer shoots 99% ($P < 0.0001$). The strength of the crop load effect on flowering was also demonstrated by the 95% reduction in inflorescence number for summer shoots on bearing shoots of ON-crop trees versus bearing shoots of OFF-crop trees ($P < 0.0001$). For 'Pixie' mandarin, the ON crop reduced the number of inflorescences produced by summer shoots on both non-bearing and bearing shoots 99.9% compared to non-bearing shoots of OFF-crop trees. 'Pixie' mandarin fruit reduced the contribution of the summer shoots to return bloom more than the contribution of the spring shoots (Table 2).

Table 2. Effect of non-bearing and bearing shoots of OFF- and ON-crop 'Pixie' mandarin trees on summer vegetative shoot growth and the number of inflorescences produced by summer vegetative shoots the following spring.

Tree status	Yield (kg tree ⁻¹)	Year 1		Year 2	
		Shoot status	Summer vegetative shoot growth (no. of nodes shoot ⁻¹)	Inflorescences (no. per summer shoot ⁻¹)	Inflorescences produced by summer shoots (% total)
OFF	51	Non-bearing	10.0 a	7.0 a	42
		Bearing	4.2 bc	2.1 bc	27
ON	177	Non-bearing	2.7 cd	0.8 c	24
		Bearing	1.1 d	0.1 c	33
P-value			<0.0001	<0.0001	

Means in a vertical column followed by different letters are significantly different at specified P -values by Fisher's LSD test.

Effect of fruit on floral bud abscission

For pistachio, excessive abscission of floral buds beginning in June (initiation of embryo growth) and intensifying at the time of embryo and seed growth (nut fill) in July during the heavy ON-crop year results in the following year's light OFF bloom and OFF crop. In two separate experiments, 89 and 73% of the floral buds on bearing shoots of ON-crop 'Kerman' pistachio trees abscised by harvest in September (data not shown). Until recently, the abscission of floral buds during the ON-crop year had not been documented for any other tree crop. Chao (2014) provided the first evidence demonstrating that 75% of the floral buds per bearing shoot of ON-crop 'Manzanillo' olive trees abscised by September (data not shown). Increased floral bud abscission during the ON-crop year does not occur in *C. reticulata* (Lovatt, unpublished results; Tang, 2017; Verreyne and Lovatt, 2009).

Effect of fruit on floral gene transcription

Chao (2014) also was the first to document that expression of the floral timing gene *FLOWERING LOCUS T (FT)* was continuous in buds of non-bearing shoots of OFF-crop 'Manzanillo' olive trees from June through the following March, one month before bloom (data

not shown). For buds of bearing shoots of ON-crop ‘Manzanillo’ olive trees, *FT* expression, which was equal to that of non-bearing shoots of OFF-crop trees from June through August, was reduced below the limit of detection from September through March (data not shown). Buds on non-bearing shoots of OFF-crop ‘Manzanillo’ olive trees expressed downstream floral organ identity genes and flowered at return bloom (Chao, 2014). In contrast, floral organ identity genes were never expressed in buds on bearing shoots of ON-crop trees and no inflorescences developed at spring bloom.

Similarly, Tang (2017) was the first to provide evidence that the ON-crop of ‘Pixie’ mandarin fruit reduced the expression of *FT* below the limit of detection from October through March (one month before bloom) in buds on bearing shoots of ON-crop trees, whereas *FT* was continuously expressed during this period in buds of non-bearing shoots of OFF-crop ‘Pixie’ mandarin trees (data not shown). Buds on non-bearing shoots of OFF-crop trees subsequently expressed downstream floral organ identity genes in March, the month before spring bloom, and flowered profusely. For buds on bearing shoots of ON-crop ‘Pixie’ mandarin trees, expression of the floral organ identity genes was significantly lower, i.e., at the limit of detection or below it, compared to buds of non-bearing shoots of OFF-crop trees and no inflorescences developed the following spring. The similarities in the negative effects of the ON crop of ‘Manzanillo’ olive and ‘Pixie’ mandarin fruit on the activities of the same floral genes suggest a common fruit-derived factor is produced, at least among, subtropical evergreen woody perennial tree crops, that downregulates flowering through the same underlying genetic mechanism.

Effect of fruit on spring bud break

Although the harvest period for the ‘Manzanillo’ olive trees for the “ripe” or “table” olive market is from late September through early October in California, USA, the ON crop of fruit significantly reduced spring bud break on both spring and summer shoots borne on non-bearing and bearing shoots of ON-crop trees compared to non-bearing shoots of OFF-crop trees ($P < 0.0001$) (Table 3). Spring bud break was reduced 60% for both spring and summer vegetative shoots on non-bearing shoots as a result of the whole tree effect of the ON crop compared to the OFF crop. The whole tree effect of the ON crop combined with the localized effect of fruit set on bearing shoots reduced spring bud break on spring shoots by 88% and on summer shoots 99% relative to non-bearing shoots of OFF-crop trees, respectively. The combined localized and whole tree effects of fruit had a more negative effect on spring bud break on summer shoots than spring shoots ($P < 0.0001$).

Table 3. Percent spring bud break in year 2 for spring and summer vegetative shoots produced in year 1 by non-bearing and bearing shoots of OFF- and ON-crop ‘Manzanillo’ olive trees.

Year 1		Year 2 spring bud break (%)	
Tree status	Shoot status	Spring shoots	Summer shoots
OFF	Non-bearing	49.5 a	54.8 a
	Bearing	-	-
ON	Non-bearing	19.8 b	20.9 b
	Bearing	5.7 c	0.8 c
<i>P</i> -value		<0.0001	<0.0001

Means in a vertical column followed by different letters are significantly different at specified *P*-values by Fisher’s LSD test.

Similar results were obtained with ‘Pixie’ mandarin. Comparison of spring bud break for spring and summer vegetative shoots on non-bearing shoots of OFF- and ON-crop trees provided evidence that the ON crop of fruit more strongly inhibited spring bud break on summer shoots than spring shoots ($P < 0.0001$) (Table 4). For ON-crop ‘Pixie’ mandarin trees, bud break of spring shoots on non-bearing shoots was reduced 52%, but spring bud break for summer shoots on non-bearing shoots was reduced 85% relative to non-bearing shoots of

OFF-crop trees, respectively. The localized effect of the fruit set on a shoot also reduced the bud break of summer shoots to a greater degree than spring shoots. There was a 66% reduction in spring bud break for spring shoots versus a 75% reduction for summer shoots on bearing shoots of OFF-crop trees relative to non-bearing shoots of OFF-crop trees ($P<0.0001$). The localized effect of fruit combined with the whole tree effect of the ON crop reduced spring bud break of spring and summer shoots of bearing shoots of ON-crop trees 94 and 97%, respectively, compared to non-bearing shoots of OFF-crop 'Pixie' mandarin trees ($P<0.0001$) (Table 4).

Table 4. Percent spring bud break in year 2 for spring and summer vegetative shoots produced in year 1 by non-bearing and bearing shoots of OFF- and ON-crop 'Pixie' mandarin trees.

Year 1		Year 2 spring bud break (%)	
Tree status	Shoot status	Spring shoots	Summer shoots
OFF	Non-bearing	31.4 a	33.0 a
	Bearing	10.8 b	8.4 b
ON	Non-bearing	15.5 b	5.0 b
	Bearing	2.1 c	1.0 c
<i>P</i> -value		<0.0001	<0.0001

Means in a vertical column followed by different letters are significantly different at specified *P*-values by Fisher's LSD test.

Efficacy of cytokinin compounds alone or combined with other materials in mitigating the negative effects of the ON crop of fruit

For 'Manzanillo' olive, injection of BA or ADO (alone or combined with TIBA) into scaffold branches of ON-crop 'Manzanillo' olive trees in July significantly increased summer vegetative shoot growth for non-bearing shoots of ON-crop trees to a value equal to that of non-bearing shoots of OFF-crop (untreated) trees and significantly greater than that of bearing shoots of ON-crop (untreated) trees ($P<0.0001$) (Table 5). However, only ADO (alone or combined with TIBA) significantly increased summer vegetative shoot growth on bearing shoots of ON-crop olive trees to a value equal to that of non-bearing shoots of OFF-crop (untreated) trees ($P<0.0001$). For 'Manzanillo' olive, combining ADO with TIBA provided no benefit over using ADO alone to increase summer vegetative shoot growth. Injecting these same compounds into the scaffold branches of a second set of ON-crop 'Manzanillo' olive trees in February demonstrated that both BA and ADO had a positive effect on spring bud break and floral intensity at return bloom. As a result, BA and ADO increased the number of inflorescences produced by non-bearing shoots of ON-crop trees to values significantly greater than those of non-bearing shoots on both OFF- and ON-crop (untreated) trees at return bloom ($P<0.0001$) (Table 5). Supplying TIBA with ADO reduced the benefit of ADO alone. All three treatments increased inflorescence number at return bloom for bearing shoots of ON-crop 'Manzanillo' olive trees relative to bearing shoots of ON-crop (untreated) trees, but not to the level of non-bearing shoots of either ON- or OFF-crop trees ($P<0.0001$) (Table 5).

For 'Nules Clementine' mandarin, ADO combined with TIBA injected in both July and January increased bud break and the number of inflorescences produced by spring shoots on bearing shoots of ON-crop trees to a value greater than that of non-bearing shoots of OFF-crop (untreated) trees and bearing shoots of ON-crop (untreated) trees and ON-crop trees in all other treatments at return bloom ($P=0.0067$) (Table 6). For summer shoots, BA, ADO and ADO with TIBA injected in July and ADO and ADO with TIBA injected in July plus January all increased bud break and the number of inflorescences produced by summer shoots on bearing shoots of ON-crop 'Nules Clementine' mandarin trees at return bloom, with the combination of ADO with TIBA in July and January producing the best results ($P=0.0033$) (Table 6). The greater increase in the inflorescence number of summer shoots in response to the PGR treatments is likely due to an increase in summer vegetative shoot growth in summer

in addition to a positive effect on spring bud break. The negative effect of GA₃ when injected in July or July and January on the number of inflorescences produced by spring and summer shoots at return bloom is consistent with the known inhibitory effect of GA₃ on floral development. However, this is the first report of the foliar-application of GA₃ as early as July followed by the quantification of floral intensity at spring bloom (Table 6).

Table 5. Effects of 6-benzyladenine (BA), adenosine (Ado), and ADO plus tri-iodobenzoic acid (TIBA) injected into scaffolding branches of ON-crop trees in July on summer vegetative shoot growth or in February on the number of inflorescences at spring bloom for non-bearing and bearing shoots of ON-crop 'Manzanillo' olive trees.

Tree status	Year 1		Year 2
	Shoot status	Summer shoot growth (no. of node pairs shoot ⁻¹)	Inflorescences (no. shoot ⁻¹)
OFF	Non-bearing	3.3 a	15.4 b
ON	Non-bearing	0.7 cd	13.3 b
+ BA	Non-bearing	2.6 ab	22.0 a
+ Ado	Non-bearing	3.5 a	22.2 a
+ Ado + TIBA	Non-bearing	3.6 a	15.8 b
ON	Bearing	0.6 d	0.8 d
+ BA	Bearing	1.9 bc	4.1 c
+ Ado	Bearing	2.6 ab	5.1 c
+ Ado + TIBA	Bearing	2.4 ab	4.9 c
<i>P</i> -value		<0.0001	<0.0001

Means in a vertical column followed by different letters are significantly different at specified *P*-values by Fisher's LSD test.

Table 6. Effects of 6-benzyladenine (BA), adenosine (Ado) and Ado plus tri-iodobenzoic acid (TIBA) injected into the trunks of ON-crop trees in July and July plus January on the number of inflorescences produced by spring and summer shoots of bearing shoots of ON-crop 'Nules Clementine' mandarin trees.

Tree status	Year 1		Year 2	
	Shoot status	Inflorescences (no. spring shoot ⁻¹)	Inflorescences (no. summer shoot ⁻¹)	
OFF	Non-bearing	14 b	28 ab	
ON	Bearing	3 bc	10 cd	
July GA ₃	Bearing	0 c	4 d	
July BA	Bearing	3 bc	17 bc	
July Ado	Bearing	2 bc	14 bc	
July Ado + TIBA	Bearing	4 bc	22 abc	
July + Jan GA ₃	Bearing	0 c	2 d	
July + Jan BA	Bearing	2 bc	9 c	
July + Jan Ado	Bearing	5 bc	16 bc	
July + Jan Ado + TIBA	Bearing	33 a	33 a	
<i>P</i> -value		0.0067	0.0033	

Means in a vertical column followed by different letters are significantly different at specified *P*-values by Fisher's LSD test.

For 'Kerman' pistachio, the results of two separate experiments demonstrated that foliar-applied BA combined with urea increased bud retention on bearing shoots of ON-crop trees when applied in both June and July. The increase in bud retention was 3-fold and 1.6-fold for the two experiments, respectively ($P \leq 0.05$) (Table 7). The treatment increased bud retention 39 and 43% in the two experiments, respectively ($P \leq 0.05$) (Table 7).

Table 7. Effects of foliar-applied 6-benzyladenine (BA), urea and BA with urea in June and July on floral bud retention on bearing shoots of ON-crop 'Kerman' pistachio trees.

Tree status	Shoot status	Floral buds (no. shoot ⁻¹)	Floral bud retention (% total)
Experiment 1			
ON	Bearing	0.97 b	11 b
June + July BA	Bearing	1.30 b	15 b
June + July Urea	Bearing	1.86 b	24 b
June + July BA + Urea	Bearing	3.07 a	39 a
<i>P</i> -value		≤0.05	≤0.05
Experiment 2			
ON	Bearing	2.4 b	27 b
June + July BA + Urea	Bearing	4.0 a	43 a
<i>P</i> -value		≤0.05	≤0.05

Means in a vertical column followed by different letters are significantly different at specified *P*-values by Fisher's LSD Test.

DISCUSSION

The localized effect of fruit set on a shoot and the whole tree effect of the total fruit produced (crop load) per tree on summer vegetative shoot growth were documented herein for both 'Manzanillo' olive and 'Pixie' mandarin, along with their corresponding effects on the number of inflorescences contributed by both spring and summer shoots at return bloom. For olive, summer shoots contributed more inflorescences (≥57%) than spring shoots at return bloom the following spring, with the contribution of the summer shoots largely independent of crop load. The results are consistent with earlier reports (Fichtner and Lovatt, 2018; Sibbett, 2000). In contrast, for 'Pixie' mandarin, the summer shoots contributed a smaller proportion of the inflorescences (≤42%) at return bloom, with the contribution of the summer shoots decreasing in response to both the localized and whole tree effect of the ON crop of fruit, consistent with the earlier of the report of Verreynne and Lovatt (2009). The results suggest that summer vegetative shoot growth is a more important factor in alternate bearing in *C. reticulata* than *O. europaea*.

For 'Manzanillo' olive, at return bloom, the localized effect and whole tree effect of the fruit on bud break were similar for spring and summer shoots, with the exception that the combined effects dramatically reduced spring bud break on summer shoots compared to spring shoots. In contrast, for 'Pixie' mandarin, the whole tree effect of the ON crop of fruit reduced spring bud break to a greater degree on summer shoots than spring shoots. Thus, for 'Pixie' mandarin the negative effect of the ON-crop of fruit on both the growth of summer vegetative shoots and the percent bud break on these shoots at return bloom are critical factors in the number of inflorescences that develop at spring bloom following the ON-crop year as first demonstrated for citrus by Verreynne and Lovatt (2009). Taken together, the results further support the suggestion that that summer vegetative shoot growth is a more important factor in alternate bearing in *C. reticulata* than *O. europaea*.

However, 'Manzanilla' olive is also impacted during the ON-crop year by the abscission of floral buds not observed in citrus and previously only reported to occur in pistachio (Chao, 2014). Inhibition of floral development at the level of gene transcription for the floral timing gene *FT* and downstream floral organ identity genes were documented for both *O. europaea* (Chao, 2014) and *C. reticulata* (Tang, 2017) in response to the ON crop of fruit, suggesting a fruit-produced signal that directly or indirectly regulates floral gene transcription common to the two species. Mitigating the negative effect of the ON crop of fruit on floral gene transcription is a new factor to be addressed when developing strategies for increasing yield in alternate bearing orchards.

Verreynne (2005) was the first to demonstrate the role of the ON-crop of fruit in changing the hormone balance (homeostasis) of buds of an alternate-bearing tree crop. In

contrast to OFF-crop trees, the ON crop of 'Pixie' mandarin fruit increased the concentration of auxin, indole-3-acetic acid (IAA), and reduced the concentration of cytokinin, isopentenyladenine (IPA), in buds during the summer and by spring, along with an increase in bud abscisic acid (ABA) concentration in spring. These imbalances were reversed in trees with the ON crop of fruit removed in July or January. Consistent with these results, treatment of ON-crop trees with BA and ADO to artificially increase bud cytokinin concentrations to counter-balance the increased concentrations of IAA and ABA, without or with TIBA to reduce the accumulation of IAA in buds, mitigated the negative effects of the ON crop of 'Manzanillo' olive fruit and 'Nules Clementine' mandarin fruit on summer vegetative shoot growth, percent spring bud break and floral intensity at return bloom. Further, BA combined with urea increased floral bud retention on bearing shoots of ON-crop 'Kerman' pistachio trees by correcting the 40% reduction in the concentrations of two cytokinins, IPA and zeatinriboside (ZR), that occurred in buds on bearing shoots of ON-crop pistachio trees between June and July (Lovatt et al., 2006). In experiment 2 reported herein, BA with urea applied in June and July increased percent floral bud retention 1.6-fold on bearing shoots of treated ON-crop trees compared to bearing shoots on untreated ON-crop trees. This resulted in a 1.8-fold increase in kg fruit (fresh weight) per tree ($P=0.0005$) and a 1.6-fold increase in kg split nuts (dry weight) per tree ($P=0.0018$) as 2-year cumulative yield compared to untreated trees (Lovatt et al., 2006).

Additional research is required to optimize the effects of the injected PGR treatments as foliar sprays to increase yield of *O. europaea* and *C. reticulata*. For 'Manzanillo' olive, the capacity of cytokinins to mitigate the negative effects of the ON crop of fruit on summer vegetative shoot growth when injected in July to one set of trees and increase spring bud break and floral intensity at return bloom when injected in January to the second set of trees, suggests that combining the two treatments could have additive benefits. Whether the increase in auxin relative to cytokinin in buds of ON-crop trees, which reduces summer vegetative shoot growth, also reduces *FT* expression remains to be determined. Thus, it is not known at this time whether PGR treatments that reverse the ratio of auxin > cytokinin to cytokinin > auxin in buds of ON-crop trees in July also restore *FT* transcription and subsequently the activity of the downstream floral organ identity genes. For both 'Manzanillo' olive and 'Nules Clementine' mandarin the negative effects of the ON crop were greater for bearing shoots than non-bearing shoots. Moreover, the efficacy of PGR treatments was greater for non-bearing than bearing shoots of ON-crop trees, suggesting that creating more non-bearing shoots on ON-crop trees by properly timing fruit thinning (by hand, chemical treatment, or pruning) would increase the efficacy of the PGR treatments and their capacity to mitigate the negative effects of the ON crop on the floral intensity at return bloom and increase return yield.

ACKNOWLEDGEMENTS

The authors thank the University of California (UC)-Riverside, NIFA, UC Cooperative Extension-Tulare County, UC Division of Agriculture and Natural Resources Thelma Hansen Fund, California Olive Committee, and California Pistachio Commission for funding; E. Jorgensen and M. Jorgensen for PGR injections; L. Summers for foliar PGR applications; R. Sandejas, J. Hernandez and A. Weitzel for determination of the fate of buds; G. Klein and T. Khuong for data analysis; and B. Faber for critically reviewing the manuscript.

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