



Stimulating Flowering in Basal Buds of Sweet Orange Summer Shoots by Removal of Terminal Buds Early in the Flower Bud Induction Period

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Under Florida conditions, most sweet orange flowers ($\approx 80\%$) are formed in the four most apical buds of 1-year-old or younger shoots. Experiments were conducted to determine whether tip removal by clipping of the four most apical buds of 1-year-old sweet orange shoots before floral induction stimulated flowering in the remaining buds (more basal buds that do not usually flower). Clipped shoots had fewer buds starting growth in the spring and had fewer inflorescences than intact shoots. However, more buds started growth and more inflorescences were formed in clipped shoots than in buds at homologous positions in intact shoots (i.e., buds at position five or more below the original apex). The stimulation of basal buds to flower after clipping was stronger in 'Valencia' than in 'Hamlin' trees and greater when shoots were clipped in November (just before the onset of floral-inductive temperatures) than when shoots were clipped earlier in October or later in January. Clipped shoots produced more new vegetative shoots and formed inflorescences with a greater leaf: flower ratio than buds at homologous positions in intact shoots. These results indicate that removal of apical buds just before the onset of floral induction stimulates flowering at more basal positions of sweet orange shoots. Results are discussed in relation to the potential application of this knowledge to minimize the impact of routine hedging and topping on flowering in Florida.

In subtropical climates, citrus trees are induced to flower by exposure to temperatures typical of the fall–winter in these regions (5 to 20 °C). Exposure to floral-inductive temperature for as little as 2 weeks was enough to induce the initiation of flowering buds in potted trees, and more inflorescences were initiated after low temperature exposure for >2 weeks (Moss, 1969). Thus, the intensity of flowering was proportional to the length of the exposure to floral-inductive temperatures.

Like many other tropical and subtropical species, buds of citrus trees do not show visible external signs of floral commitment until the initiation of a new growth flush (Brundell, 1975; Cheng et al., 2005; Mustard and Lynch, 1946; Qiu et al., 2001; Reece, 1942). In citrus, new growth is not initiated unless warmer growth-promoting temperatures occur and water supply is non-limiting, so floral differentiation of buds must occur right after exposure to warmer growth-promoting temperatures.

Commercial citrus trees in Florida are usually mechanically hedged and topped regularly so that fruit productivity is maintained with a manageable tree size. Hedging and topping non-selectively removes terminal portions of branches, which promotes the growth of lateral buds (Davies and Albrigo, 1994). The nature and vigor of the promoted flush varies depending on the time of the year, cultivar, and severity of pruning (Bacon and Bevington, 1980). Less severe cutting typical for annual hedging results in less vigorous re-growth than more intense, less frequent, hedging and is desirable for maintaining better yield while controlling tree size and architecture (Bacon, 1981). In Australia (a

Mediterranean-like climate), hedging early in the fall reduced yield in the year of pruning, but hedging in spring just prior to anthesis had the least impact on yields (Bacon, 1981; Bacon and Bevington, 1980). Under Florida conditions, there were no consistent differences in yield between trees hedged in the fall or in the spring (Whitney et al., 2003). However, for most citrus cultivars, hedging and topping will invariably remove some of the fruit that make the current or next crop and buds for next year's crop regardless of when hedging and topping occur. This is most evident in late-season 'Valencia', because young-developing and/or mature fruit are always present on the tree.

In Florida, most of the inflorescences ($\approx 80\%$) initiated after floral induction in the winter are formed in the four most apical nodes of shoots formed during the previous summer and spring (Valiente and Albrigo, 2004). Buds at more basal positions are still capable of forming inflorescences since they can flower in years of high induction, suggesting that floral-induction of basal buds requires more induction or is possibly suppressed by buds at more apical positions. In this study, we hypothesized that removing the four most apical nodes of shoots formed in the previous spring and summer just before the onset of floral-inductive temperatures in the late-fall (November) would stimulate the induction of floral buds in basal buds that do not normally flower. Results are discussed in relation to their potential application to determining hedging times with least impact on floral induction and flowering.

Materials and Methods

Field experiments were conducted at the Citrus Research and Education Center in Lake Alfred, Florida (28°5'N, 81°43'W) using mature 'Valencia' and 'Hamlin' sweet orange trees [*Citrus*

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sinensis (L.) Osbeck] on ‘Carrizo’ [*C. sinensis* x *Poncirus trifoliata* (L.) Raf.] or ‘Swingle’ (*C. paradisi* Macf. x *P. trifoliata*) rootstocks. Trees of both cultivars were growing in Candler sand (hyperthermic, uncoated typic Quartzipsamments) in adjacent plots with similar characteristics. The experiment was conducted over three seasons (2005–06, 2006–07 and 2009–10) at this same experimental site.

In seasons 2005–06 and 2006–07, between 40 and 96 shoots (seven to nine nodes long) distributed among six to 10 uniform trees were selected and tagged in early October in each season for each cultivar. The selected shoots were all formed during the previous summer, had never flowered and had not lost any leaves (i.e., each node was associated with one leaf). The four most apical nodes of half of the selected shoots on each tree were clipped (shoot tipping) between early and mid-November using hand pruning shears. After clipping, shoots were three to five nodes long. The number of inductive cool hours (<20 °C) that accumulated from October 1 until the date of tipping were 322 h for 2005–2006 and 155 h for 2006–2007. During the experiments, trees received standard horticultural care and irrigation as in neighboring commercial groves.

In season 2009–2010, 49 similar shoots as in previous seasons were selected on six ‘Valencia’ trees in the same plot as before. The 49 shoots were divided into seven groups of seven shoots each, to which treatments were applied. Each tree-replicate contained at least seven shoot sub-replicates from which the tree mean was calculated. Treatments consisted of either (1) clipping the four most apical nodes as in previous seasons or (2) removing only the four most apical buds, but leaving their associated leaves intact. Buds were carefully removed with a razor blade, avoiding any damage to the stem or leaf petiole. These treatments were ap-

plied at three different dates (early-October, mid-November and late-February). The remaining group of seven paired shoots per plot were left intact as the control.

For all the experiments, the spring flush on the treatment and control shoots was described before petal fall as total new growth, new inflorescences, and inflorescence type and position. For each of the variables evaluated, differences between: (a) intact shoots and clipped shoots (shoots that had their four most apical buds removed), (b) the four most apical positions of intact shoots and new four apical buds of clipped/bud-removed shoots and (c) the buds at positions five to eight of intact shoots and formally positions five to eight of clipped/bud-removed shoots were analyzed. Inflorescences were divided into three types: leaf-abundant inflorescences (La; inflorescences with a leaf to flower ratio ≥ 1), leaf-deficient inflorescences (Ld; inflorescences with a leaf to flower ratio < 1) (Lovatt et al., 1987), and solitary flowers. Data analysis was conducted in R (R Development Core Team, 2011) using analysis of variance. All differences reported are significant ($P \leq 0.05$) unless noted otherwise.

Results and Discussion

Overall, fewer flowers and inflorescences formed in shoots that were clipped or had their buds removed in November than in intact shoots (Figs. 1 and 2). On average, clipping/bud removal in November reduced the number of inflorescences by two. Of the three different types of inflorescences, the number of leaf-deficient inflorescences was reduced the most by clipping/bud-removal in November, whereas the number of leaf-abundant inflorescences was in most cases unaffected by the treatments. More vegetative shoots tended to form on shoots that were clipped or had their

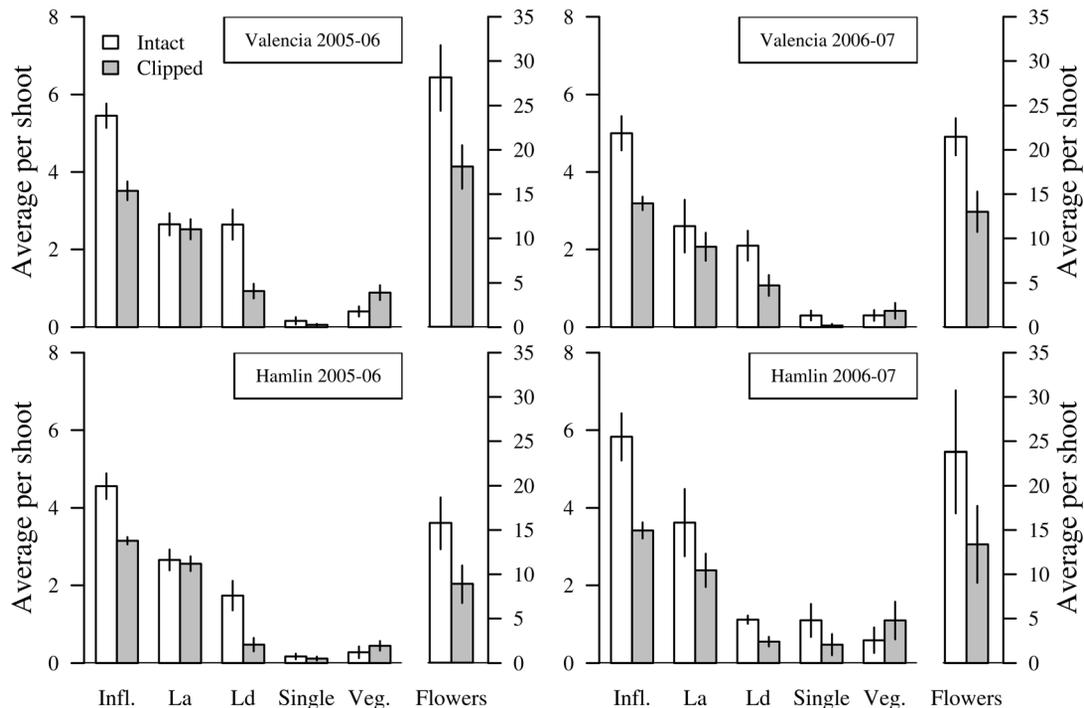


Fig. 1. Characteristics of the spring flush originating from ‘Valencia’ and ‘Hamlin’ shoots clipped in the fall. The four most apical nodes of seven to nine nodes-long shoots were clipped early in November in 2005 and 2006. A matching set of shoots from the same trees were tagged and left intact as paired controls with each treated shoot. The total number of inflorescences (Infl.), leaf-abundant inflorescences (La, leaf to flower ratio > 1), leaf-deficient inflorescences (Ld, leaf to flower ratio ≤ 1), single flowers, and new vegetative shoots (Veg.) were counted in the following spring before petal fall. Data are means of six-to-10-tree replicates. Each tree-replicate contained at least seven shoot-sub-replicates, from which the tree mean was calculated. Error bars represent standard errors of the mean.

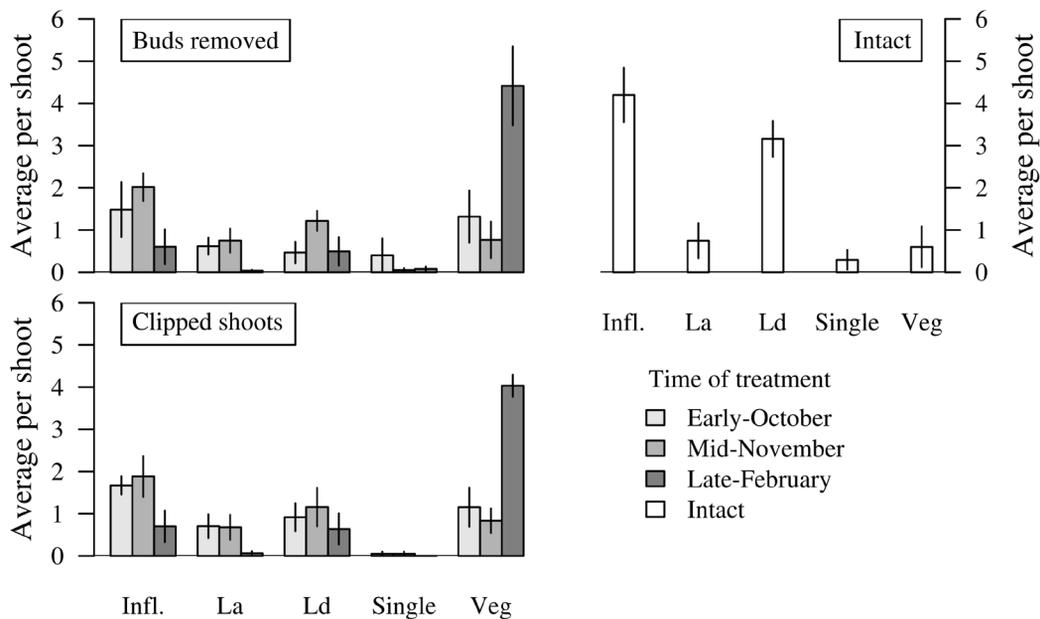


Fig. 2. Characteristics of the spring flush originating from 'Valencia' that were clipped or had their buds removed on three dates during the 2009-2010 fall/winter. Two sets of seven to nine nodes-long shoots either had their four most apical nodes clipped using hand pruner shears or had their four most apical buds removed with a razor-blade in early October, mid-November or late February during the 2009-2010 fall/winter. A matching set of shoots from the same trees were tagged and left intact as paired controls with each treated shoot. The total number of inflorescences (Infl.), leaf-abundant inflorescences (La, leaf to flower ratio >1), leaf-deficient inflorescences (Ld, leaf to flower ratio ≤ 1), single flowers, and new vegetative shoots (Veg.) were counted in the following spring before petal fall. Data are means of five single tree replications. Each tree-replicate contained seven shoot-sub-replicates, from which the tree mean was calculated. Error bars represent standard errors of the mean.

buds removed than in intact shoots. However, differences between the number of vegetative shoots on treated and intact trees were only significant in the experiments conducted in the fall/winter of 2005-06 (Fig. 1).

Even though fewer inflorescences were formed in clipped shoots than in intact shoots in the 2005-2006 and 2006-2007 experiments, flowering was stimulated in the buds that remained after clipping (Fig. 3). The number of inflorescences formed in new positions one to four of clipped shoots (formerly positions five to eight before clipping) was equivalent to the number of inflorescences formed in positions one to four of intact shoots and higher than the number of inflorescences in positions five to eight of intact shoots. However, in season 2009-2010, fewer inflorescences formed in positions one to four of shoots that were clipped or had their buds removed in November than in positions one to four of intact shoots (Fig 4.). Nonetheless, more inflorescences were formed on shoots that were clipped or had their buds removed in Oct. and Nov. 2009 than in positions five to eight of intact shoots, indicating that even though stimulation of flowering in basal buds after clipping/bud-removal was not high enough to equal flowering at apical positions of intact shoots, clipping/bud removal did stimulate flowering above levels at formerly equivalent positions of intact shoots.

In the experiments of 2005-2006 and 2007-2008, shoots were clipped on 4 and 14 Nov., respectively; by this time 322 h and 155 h at floral-inductive temperatures had occurred in the area where the experiments were conducted, and it was hypothesized that clipping earlier than November would increase the level of induction on the remaining buds. This hypothesis was tested in season 2009-2010 when shoots were clipped early in October after only 15 h of inductive temperature had accumulated. Shoots

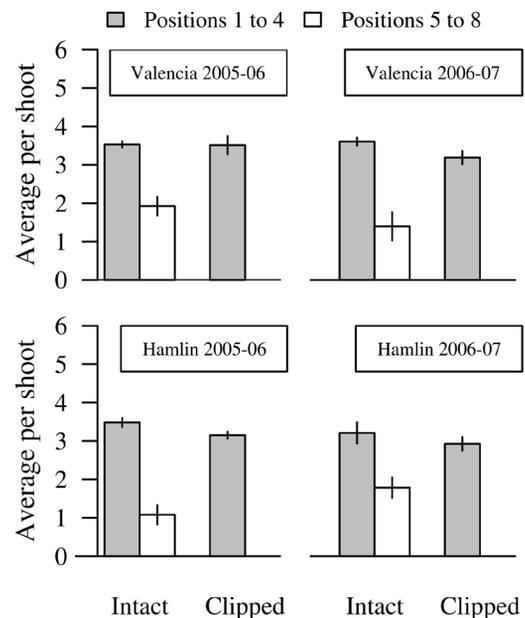


Fig. 3. Number of inflorescences originating in positions one to four and five to eight of 'Valencia' and 'Hamlin' shoots clipped in the fall. The four most apical nodes of seven to nine nodes-long shoots were clipped early in November in 2005 and 2006. A matching set of shoots from the same trees were tagged and left intact as paired controls with each treated shoot. Total inflorescences were counted in the following spring before petal fall. Positions one to four in clipped shoots were formerly positions five to eight before clipping. Figures are means of six-to-10-tree replicates. Each tree-replicate contained at least seven shoot-sub-replicates from which the tree mean was calculated. Error bars represent standard errors of the mean.

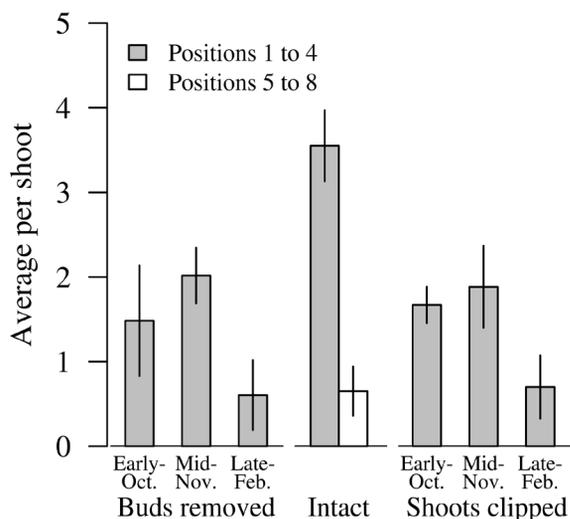


Fig. 4. Number of inflorescences originating in positions one to four and five to eight of 'Valencia' that were clipped or had their buds removed on three dates during the 2009–2010 fall/winter. Two sets of seven to nine nodes-long shoots either had their four most apical nodes clipped using hand pruner scissors or had their four most apical buds removed with a razor-blade in early October, mid-November or late February during the 2009–2010 fall/winter. A matching set of shoots from the same trees were tagged and left intact as paired controls with each treated shoot. Total inflorescences were counted in the following spring before petal fall. Positions one to four in clipped shoots or shoots that had their four most apical buds removed were formerly positions five to eight before clipping or bud removal. Data are means of five single tree replications. Each tree-replicate contained seven shoot-sub-replicates, from which the tree mean was calculated. Error bars represent standard errors of the mean.

were also clipped in mid-November (259 h of induction) and late-February after 1264 h of induction counted until 14 Jan. when differentiation was predicted. Shoots clipped in early-October had slightly fewer inflorescences than shoots clipped in mid-November, but differences were not statistically significant (Fig. 2). On the other hand, shoots clipped in late February had fewer inflorescences than shoots clipped at earlier dates. Again, the number of leaf-deficient inflorescences was most affected by clipping whereas the number of leaf-abundant was unaffected in shoots clipped in early-October and mid-November. All types of inflorescences were affected in shoots clipped in late-February. Shoots clipped in late-February produced mostly a vegetative flush, whereas the number of new vegetative shoots formed in shoots clipped in early October and mid-November was higher (but not statistically significant) than the number of new vegetative shoots in intact shoots.

In addition to clipping the four most apical buds as was done in seasons 2005–2006 and 2006–2007, in the experiment of season 2009–2010 the effect of removing only the buds at positions one to four of shoots formed during the previous years was also evaluated to test the hypothesis that the level of floral-induction in basal buds could be further increased by increasing the leaf area to bud ratio in shoots. Furr and Armstrong (1956) reported that grapefruit shoots girdled and defoliated at different times during the fall/winter produced fewer numbers of inflorescences the earlier the date on which the treatment was applied, supporting the hypothesis that flowering is induced by some factor produced in the leaves and transported to the meristems as has been reported for other species (Blázquez, 2005). In our trial, shoots clipped at any time in the fall/winter and shoots that had their four most

apical buds removed on the same dates produced an equivalent response for all the variables evaluated (Figs. 2 and 4). Thus, increasing the leaf area to bud ratio at the whole-shoot level did not increase the level of floral induction under the conditions evaluated in this experiment.

These results show that removing the buds at apical positions in shoots formed during the previous year stimulates flowering in more basal buds that do not normally flower under natural conditions in Florida (Valiente and Albrigo, 2004). Thus, basal buds of sweet orange shoots are florally-competent, but are apparently kept from responding to floral-induction by a flowering gradient established in buds at more apical positions that respond more readily to floral-inductive signals. Since the proportion of leaf-deficient inflorescences relative to the total number of inflorescences formed in clipped shoots was smaller than in the same proportion of intact shoots, clipped shoots were not induced to flower as intensely as positions one to four of intact shoots, which were considered equivalent in this study. Moss (1969) reported that inflorescence types equivalent to leaf-abundant inflorescences in this study formed in higher number in trees exposed to sub-optimal floral-inductive temperatures (19 to 22 °C), whereas more leaf-deficient inflorescences were formed in trees exposed to optimal floral-inductive temperature (10 to 18 °C). Thus, a higher proportion of leaf-abundant inflorescence indicates a lower, but sufficient, level of floral induction. From a practical standpoint, that the number of leaf-abundant inflorescences was not significantly reduced by clipping in mid-November could be important because leaf-abundant inflorescences set fruit better than leaf-deficient inflorescences (Moss, 1970; Sauer, 1954).

Increasing the level of induction of basal buds was unsuccessfully attempted during the 2009–2010 season by clipping the shoots in early-October to expose basal buds to more hours of floral-inductive temperatures and by increasing the leaf area to bud ratio in the shoot to increase the concentration of a hypothetical flowering-promoter factor(s) generated in leaves. Even though no statistically significant differences were detected, shoots that were clipped or had their buds removed, leaving the leaves intact in early October, showed a consistent trend to produce fewer inflorescences and more new vegetative shoots than shoots that were clipped or had their buds removed in mid November. This trend was consistent with reports in which pruning trees in the early-fall resulted in induction of a late-fall vegetative flush and a consequent reduction in flowering (Moss, 1973). In this study, no late-fall vegetative flush was visibly detected. However, clipping in early-October, when temperatures were still above the floral-induction threshold (≈ 20 °C), could have stimulated the molecular/microscopic determination of some of the basal buds to develop into vegetative shoots. This could have resulted in these buds becoming insensitive to floral-induction during the rest of the fall/winter. Hypothetically, to maximize the level of induction in basal buds, shoots need to be clipped as soon as ambient temperatures drop consistently below the floral-induction threshold (≈ 20 °C). However, since the time of the onset of consistent cooler weather in the fall in Florida varies markedly from year to year from early October to late November; precisely determining whether the lower level of induction in basal buds was due to reduced exposure to hours at floral-inductive temperatures would be difficult to determine under field conditions. On the other hand, it is also possible that basal buds, in spite of being florally-competent, are less sensitive to floral-inductive stimulus than buds at more apical positions. Still, some degree of sensitivity of basal buds to increasing accumulation of hours at

floral-inductive temperatures is apparent from the sharp reduction in the number of inflorescences and the increase in the number of new vegetative shoots when shoots were clipped in late February, compared with shoots clipped in mid November.

The results of this study show that buds in basal positions in sweet orange shoots can respond to natural floral induction during the fall/winter in Florida and that a gradient was established in buds at more apical locations keeping basal buds from responding to floral induction and initiating growth in the following spring. In these experiments, removal of the four most apical buds of shoots resulted in a net loss of only about two inflorescences. Furthermore, whereas clipped shoots produced roughly two inflorescences less than intact shoots, the number of leaf-abundant inflorescences was equivalent in both groups. Consequently, we hypothesized that fruit set could be equivalent in both clipped and intact shoots. Our results may be useful in determining hedging times that cause minimal impacts on flowering and ultimately yields. It is not known whether hedging whole trees would produce results similar to those reported here. The treatments applied in this experiment were considered to be comparable to a light hedging at the shoot-level, but since treatments were applied to only a subset of all the shoots in the trees, the impact on the physiology of the whole tree was probably minimal. Hedging, on the other hand, would be expected to induce major physiological changes at the whole-tree level because it is conducted non-selectively on all of the extended shoots on the sides of the tree. Still, it has been reported that the yield of trees hedged during the fall/winter in central Florida was equivalent to the yield of non-hedged trees (Spann et al., 2009). The results reported here provide useful information for future tests to determine the timing of hedging of sweet orange trees in Florida.

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