Morphological and Yield Characteristics of 'Washington' Navel Orange and 'Tahiti' Lime Trees Produced with Buds from "Floral" versus "Vegetative" Mother Shoots

Carol J. Lovatt[1,*] and Robert R. Krueger[2]
[1]Department of Botany and Plant Sciences, University of California, Riverside, CA, USA
[2]USDA-ARS National Clonal Germplasm Repository, Riverside, CA, USA
*Corresponding author: carol.lovatt@ucr.edu

Abstract

Citrus cultivars bear "floral" shoots that produce more inflorescences than vegetative shoots (411 inflorescences, 11 vegetative shoots). Each cultivar also bears "vegetative" shoots that produce fewer inflorescences (208) and more vegetative shoots (202) than floral shoots on a similar number of nodes. Canopy sections in which floral shoots develop contribute a greater percent of tree total yield than those dominated by vegetative shoots. We hypothesized that trees propagated from buds taken from floral shoots would retain the internal physiological status of a floral mother shoot to produce more floral daughter shoots than trees propagated from buds taken from vegetative mother shoots and thus be more productive. Buds from the two types of mother shoots collected from 'Tahiti' lime and 'Washington' navel orange were budded on 'Carrizo' citrange rootstocks. Forty trees were established in a field at UC-Riverside. At age 3 years, 'Tahiti' lime trees propagated from buds from floral mother shoots produced 28% more fruit than trees propagated from vegetative mother shoot buds. Similarly, 3-yr-old 'Washington' navel orange trees produced with buds from floral mother shoots yielded 26% more fruit than trees produced with buds from vegetative mother shoots. For 'Washington' navel orange trees propagated with buds from floral mother shoot, 4-year cumulative and 4-year average yield as number of fruit per tree was significantly greater than trees propagated with buds from vegetative mother shoots; the same relationship was true for 'Tahiti' lime, but the results were not significant at the 5% confidence level.

Keywords: *Citrus latifolia* (Yu Tanaka) Tanaka, *Citrus sinensis* L. Osbeck, epigenetics, inflorescence, thorns, topophysis

INTRODUCTION

Researchers investigating citrus floral development have found it useful to be able to distinguish in advance of bloom shoots that will produce predominantly floral shoots (inflorescences) (named "floral" mother shoots) from those that will produce significantly more vegetative than floral daughter shoots (named "vegetative" mother shoots). Using the criteria reported in Table 1, Lord and Eckard (1985) were able to select floral mother shoots of the 'Washington' navel orange several months before bloom with 90% accuracy; vegetative mother shoots were predicted with 70% accuracy. In California, in orchards with rows that run east to west, floral mother shoots are found in greater number in the southwest (SW) tree quadrant of citrus trees. In contrast, vegetative mother shoots are the majority in the northeast (NE) tree quadrant. Using the criteria of Lord and Eckard (1985), floral and vegetative mother shoots were tagged in December in the SW and NE quadrants, respectively, of 'Washington' navel orange trees planted in east to west rows. At spring bloom, both types of mother shoots produced approximately the same number of daughter shoots (Table 2). However, for floral mother shoots, a greater absolute number and percentage of the daughter shoots that developed at bloom were inflorescences (>95%) rather than vegetative shoots. In contrast, for vegetative mother shoots, the absolute number and percentage of the daughter shoots that were inflorescences was much lower (~50%). Floral mother shoots produced 9-fold more leafless (totally floral) inflorescences than vegetative mother shoots, whereas vegetative mother shoots produced 18-fold more vegetative daughter shoots than floral mother shoots. The inflorescences produced by vegetative mother shoots were predominantly leafy (flowers and leaves) inflorescences. The greater proportion of the tree's total fruit number was produced by areas of the tree with the greater proportion of floral mother shoots compared to sectors of the tree with numerous vegetative mother shoots (Verreynne and Lovatt, 2007).

Table 1. Characteristics used to distinguish 'Washington' navel orange floral mother shoots, which produce a greater proportion of floral to vegetative daughter shoots, from vegetative mother shoots, which produce fewer floral and more vegetative daughter shoots (Lord and Eckard, 1985).

Mother shoot charcateristics	Branching	Leaf area[1] (cm^2)	Nodes (No.)	Thorns (No.)
Floral	Frequent	17.59 ± 5.79	5-6	Few or absent
Vegetative	Infrequent	27.73 ± 7.34	>6	Numerous

[1] Data are the mean ± SE for 100 mother shoots of each type.

Table 2. Characteristics (mean ± SE) for 100 floral and vegetative mother shoots, respectively, (Part A) and the number and type of daughter shoots produced per 100 floral and vegetative mother shoots of the 'Washington' navel orange (Part B).

Part A mother shoot characteristics	Shoot length (mm)		Leaf area (cm^2)	Nodes (No.)	Thorns (No.)
Floral	77 ± 2.2		18 ± 0.5	7 ± 0.1	1 ± 0.2
Vegetative	158 ± 5.0		25 ± 0.8	10 ± 0.3	5 ± 0.4
Part B daughter shoots per 100 mother shoots	**Total (No.)**	**Floral shoots (No.)**	**Leafless inflorescences (No.)**	**Leafy inflorescences (No.)**	**Vegetative shoots (No.)**
Floral	422	411	343	68	11
Vegetative	410	208	38	170	202

Results derived from "mapping" 3-year-old container grown 'Limco Lisbon 8-A' lemon trees [*Citrus limon* (L.) Burm. f.] on *C. macrophylla* rootstock provided strong evidence that the shoot characteristics described above were passed from one flush of shoots to the next (Hake, 1995). Shoot internode length was highly consistent from shoot to shoot, decreasing slightly with each younger growth flush. Thorns per shoot were significantly positively related to branch length and to the number of vegetative daughter shoots produced. The correlation coefficient between thorns and the number of flowers per branch was negative and significant, consistent with the fact that thorns are flower homologues in citrus, i.e., an aborted axillary floral bud (Lord and Eckard, 1987). From one flush to the next, mother shoots that were long with many thorns tended to produce daughter shoots that had many thorns. Mother shoots with many flowers produced daughter shoots with many flowers.

Taken together, these results suggested two possibilities. Trees propagated from buds taken from shoots having the characteristics of floral mother shoots will retain the internal physiological status of the floral mother shoot and hence have a higher proportion of floral shoots than trees propagated with buds taken from vegetative mother shoots. The higher proportion of floral shoots would result in these trees being more productive. Topophysis, the effect of the position of the axillary bud used in propagation on the growth habit and flowering potential of the resulting progeny, is well known in the propagation of woody perennials. It is consistent with an epigenetic change in a bud that is passed from one shoot flush to the next and also retained by the grafted bud. Alternatively, the characteristics of a bud might change in response to its new environment, which includes a direct vascular connection to the juvenile rootstock. The objective of the research presented herein was to determine whether productivity could be improved by propagating trees with buds taken from floral mother shoots compared to trees propagated with buds from vegetative mother shoots or whether buds would change phenotype in response to budding or due to location with a specific quadrant of the tree. This research was undertaken using 'Washington' navel orange, the source of the original observations, and 'Tahiti' lime, which has a tree architecture that is different from sweet orange but similar to lemon. Demonstration that trees propagated with buds from floral mother shoots are more productive than trees propagated with buds from vegetative mother shoots would be of significant benefit to the citrus industry.

MATERIALS AND METHODS

Plant Material

Trees of 'Washington' navel orange (*Citrus sinensis* L. Osbeck) and 'Tahiti' lime (*Citrus latifolia* (Yu Tanaka) Tanaka) were budded on 'Carrizo' citrange (*C. sinensis* × *Poncirus trifoliata* L. Raf.) in 2005. Ten trees of each cultivar were propagated from buds obtained from each of the two shoot types (floral and vegetative) of mature field-grown 'Washington' navel trees and mature, potted (190 L), lathehouse-grown 'Tahiti' lime trees on the campus of the University of California, Riverside (UCR) (33°57' N, 117°23' W). Vegetative budsticks were selected from the southwest quadrant and floral budsticks from the northeast tree quadrant of the source trees, which were planted in rows running east to west. The trees propagated from these buds were established in the field at UCR in September 2006 ('Tahiti' lime) and June 2007 ('Washington' navel). The trees were spaced at 7.3 m between rows and 5.2 m within the row, with the rows running in a north-south direction. Trees were irrigated with mini-sprinklers and grown with the cultural practices standard in a commercial orchard in Riverside. Phenotypic characteristics of the young trees were determined in spring of 2009 and winter 2012. Crops produced by the spring bloom in 2009, 2010, 2011 and 2012 were harvested in December (lime) or January (navel). The entire crop on each tree was harvested, weighed and counted; average fruit weight was calculated as kilograms per tree divided by fruit number per tree. A sub-sample of 54 to 250 fruit per tree, representing 100 to 25% of the total crop was collected at harvest; the transverse diameter of each fruit was measured with calipers.

Statistical Analysis

For each cultivar, the experimental design, with 10 individual tree replications per treatment (type of mother shoot as the bud source), was a randomized complete block. Repeated measure analysis was used to test treatment effects on yield parameters with year as the repeated measure factor (Tables 4 and 6). This analysis was performed using General Linear Model procedures of the SAS statistical program (SAS Inst., Inc., Cary, N.C.). Analysis of variance was used to test for treatment effects on yield parameters for a specific year and for the 4-year cumulative yield (Tables 4 and 6). Means were separated using Fisher's protected LSD test at $P \leq 0.05$. Note that for 4-year cumulative yield and the 4-year average yield, a missing datum point for a tree in any year excluded all the data for that tree from the statistical analysis. Note that only one 'Tahiti' lime tree was removed from the data sets.

RESULTS

Floral mother shoots (budsticks) used as the source of buds to propagate 'Washington' navel orange trees in 2005 had greater branching (more lateral shoots) than the vegetative mother shoot budsticks ($P=0.0289$) (Table 3). Whereas node number was not significantly different between the two shoot types (Data not shown), floral mother shoots, which served as the source of the budsticks, were shorter due to shorter internode length (P=0.0455) and had smaller leaves (P=0.0255) and fewer thorns (P=0.0624) than vegetative mother shoots used as budsticks. At spring bloom 2009, approximately 2 years after the trees were planted in the field, there were no significant differences in the number of inflorescences or vegetative shoots produced by shoots on trees propagated with buds from floral mother shoots (FL trees) versus vegetative mother shoots (VG trees) (data not shown). By winter (Dec.) 2012, shoots in the SW quadrant of FL trees had significantly greater branching (more lateral shoots) than shoots in the NE quadrant of VG trees, branching on shoots in the NE quadrant of FL trees and SW quadrant of VG trees was intermediate and not significantly different (P=0.0655) (Table 4). There were no differences in any other shoot characteristics associated with floral or vegetative mother shoots. 'Washington' navel orange 4-year cumulative yield and 4-year average yield was significantly greater for FL trees as number, but not kg, of fruit per tree compared to VG trees (P=0.0163 and P=0.0145, respectively) (Table 5). At the end of 4 years, FL trees had produced a cumulative 21 kg (177 fruit) more fruit per tree than VG trees. For 'Washington' navel orange trees, yield increased significantly from 2010 through 2012 with the average weight of individual fruit inversely related to annual yield (P<0.0001). However, averaged across the 4 years of the experiment, there was no significant difference in the average weight of individual fruit for FL and VG trees. There was a significant interaction between mother shoot type and year on fruit number per tree and the weight of individual fruit because the difference in number of fruit per tree became greater each year for FL trees relative to VG trees (P=0.0164 and P=0.0322, respectively). By 2012, FL trees produced 7.1 kg (92 fruit) more than VG trees.

Table 3. Characteristics of 'Washington' navel orange floral and vegetative mother shoots used as the source of buds for tree propagation in 2005.

Mother shoot bud source	Lateral shoots (No.)	Internode length (mm)	Leaf length (mm)	Thorns (No.)
Floral	5.5 a[1]	14.8 b	64.6 b	0.0 a
Vegetative	1.7 b	17.3 a	79.9 a	0.7 a
P-value	0.0289	0.0455	0.0255	0.0624

[1]Means within a vertical column followed by different letters are significantly different at the *P*-value specified by Fisher's protected LSD test.

Table 4. Characteristics of shoots in the southwest (SW) and northeast (NE) quadrants of trees propagated with buds from floral and vegetative mother shoots of the 'Washington' navel orange by winter (Dec.) 2012. Trees were budded in 2005 and planted in the orchard in June 2007.

Mother shoot (bud source)	Quadrant	Lateral shoots (No.)	Shoot length (cm)	Leaf length (cm)	Thorns (No.)
Floral	SW	0.5 a[1]	11.7 a	7.8 a	3.0 a
Floral	NE	0.2 ab	11.4 a	7.9 a	1.9 a
Vegetative	SW	0.4 ab	13.6 a	8.3 a	2.9 a
Vegetative	NE	0.1 b	12.2 a	8.3 a	3.1 a
P-value		0.0655	0.5237	0.1003	0.2483

[1] Means within a vertical column followed by different letters are significantly different at the *P*-value specified by Fisher's Protected LSD Test.

Table 5. Four-year cumulative yield and 4-year average yield and average weight per fruit for trees propagated with buds from floral and vegetative mother shoots of the 'Washington' navel orange. Trees were budded in 2005 and planted in the orchard in June 2007.

Mother shoot bud source	4-year cumulative yield			4-year average yield		4-year average fruit wt.
	kg/tree	No./tree		kg/tree	No. /tree	(g/fruit)
Floral	147.3 a[1]	800 a		36.8 a	200 a	210.9 a
Vegetative	125.9 a	623 b		31.5 a	156 b	226.6 a
P-value	0.1571	0.0163				
			Year			
			2009	15.4 c	60 c	261.7 a
			2010	13.9 c	71 c	230.3 b
			2011	47.0 b	233 b	202.2 c
			2012	59.8 a	342 a	182.4 d
			P-value			
			Mother shoot	0.1611	0.0145	0.1179
			Year	<0.0001	<0.0001	<0.0001
			M x Y	0.2669	0.0164	0.0322

[1]Means within a vertical column followed by different letters are significantly different at the *P*-value specified by Fisher's protected LSD test.

Floral mother shoot budsticks used to propagate 'Tahiti' lime trees in 2005 had greater branching (more lateral shoots) than budsticks of vegetative mother shoots (*P*=0.0001) (Table 6). Node number was not significantly different between the two shoot types (Data not shown), but floral mother shoots used as the source of buds were shorter due to shorter internode length (*P*=0.0083) and had smaller leaves (*P*=0.0003) and fewer thorns (*P*=0.0014) than vegetative mother shoots used as budsticks. At spring bloom 2009, approximately 2.5 years after the trees were planted in the field, shoots on FL trees produced significantly more total inflorescences per shoot, as both leafless and leafy inflorescences, than shoots on VG trees. Compare 14 leafless inflorescences per shoot for FL trees to 6 for VG trees (*P*=0.0084), 30 leafy inflorescences per shoot for FL trees to 21 for VG trees (*P*=0.0820), and 44 total inflorescences per shoot for FL trees to 27 for VG trees (*P*=0.0042). There were no differences in the number of vegetative shoots per shoot between FL and VG trees. By winter (Dec) 2012, shoots in the SW quadrant of FL 'Tahiti' lime trees had significantly greater branching (more lateral shoots) than shoots in the NE quadrant of FL trees and SW quadrant of VG trees, whereas branching on shoots in the NE quadrant of VG trees was intermediate and not significantly different (*P*=0.0282) (Table 7). Surprisingly, shoots in the SW quadrant of FL trees were significantly longer (*P*=0.0026) due to having longer internodes (*P*=0.0304) and more nodes (*P*=0.0374) than shoots in the SW and NE quadrants of VG trees; shoots in the NE tree quadrant of FL trees were intermediate and not significantly different.

Table 6. Characteristics of 'Tahiti' lime floral and vegetative mother shoots used as the source of buds for tree propagation in 2005.

Mother shoot bud source	Lateral shoots (No.)	Internode length (mm)	Leaf length (mm)	Thorns (No.)
Floral	6.0 a[1]	14.7 b	66.5 b	1.8 b
Vegetative	0.0 b	27.1 a	115.6 a	9.3 a
P-value	0.0001	0.0083	0.0003	0.0014

[1] Means within a vertical column followed by different letters are significantly different at the *P*-value specified by Fisher's Protected LSD Test.

Table 7. Characteristics of shoots in the southwest (SW) and northeast (NE) quadrants of trees propagated with buds from floral and vegetative mother shoots of the 'Tahiti' lime by winter (Dec.) 2012. Trees were budded in 2005 and planted in the orchard in September 2006.

Mother shoot (bud source)	Quadrant	Lateral shoots (No.)	Shoot length (cm)	Leaf length (cm)	Thorns (No.)
Floral	SW	0.5 a[1]	18.1 a	7.8 a	6.6 a
Floral	NE	0.2 b	14.5 b	7.3 a	5.6 a
Vegetative	SW	0.0 b	11.7 b	7.9 a	5.2 a
Vegetative	NE	0.2 ab	12.9 b	7.8 a	5.4 a
P-value		0.0282	0.0026	0.2513	0.4362

[1] Means within a vertical column followed by different letters are significantly different at the *P*-value specified by Fisher's protected LSD test.

Annual, 4-year cumulative and 4-year average yields, including the average weight of individual fruit, were consistently greater for FL compared to VG 'Tahiti' lime trees, but the yield differences were not significant at the 5% confidence level due to large tree to tree variation in yield (Table 8). At the end of 4 years, FL trees had produced a cumulative 20 kg (>250 fruit) more fruit per tree than VG trees. Annual yield in kilograms per tree for the 'Tahiti' lime was the same in 2009 and 2010 and then increased dramatically in 2011 and remained at this level in 2012; fruit number per tree increased annually from 2009-2011 ($P<0.0001$). Individual fruit weighed the most when fruit number was the least in 2009 ($P<0.0001$).

Table 8. Four-year cumulative yield and 4-year average yield and average weight per fruit for trees propagated with buds from floral and vegetative mother shoots of 'Tahiti' lime. Trees were budded in 2005 and planted in the orchard in September 2006.

Mother shoot	4-year cumulative yield			4-year average yield		4-year average fruit wt.
bud source	kg/tree	No./tree		kg/tree	No./tree	(g/fruit)
Floral	181.6 a[1]	2660 a		45.4 a	665 a	82.6 a
Vegetative	161.5 a	2395 a		40.4 a	599 a	80.9 a
P-value	0.3514	0.2482				
			Year			
			2009	13.6 b	140 c	95.1 a
			2010	15.6 b	418 b	77.9 b
			2011	71.3 a	917 a	79.0 b
			2012	71.8 a	1061 a	75.3 b
			P-value			
			Mother	0.3514	0.2482	0.9119
			Year	<0.0001	<0.0001	<0.0001
			M × Y	0.9370	0.9320	0.7235

[1]Means within a vertical column followed by different letters are significantly different at the *P*-value specified by Fisher's Protected LSD test.

DISCUSSION

All shoots in *Citrus* are determinate since growth is sympodial. According to Lord and Eckard (1987), this system sets up a negative relationship between shoot length and flowering. Vegetative shoots, having a vegetative apical bud that continues the growth of the shoot, produce thorns instead of flowers in the axils of leaves. Thus, shoots that terminate with a floral apical bud are shorter and branch more frequently. The characteristics of floral and vegetative mother shoots selected as the bud source (budsticks) for propagation of FL and VG trees in 2005 were not evident in the progeny trees in 2009. Trees propagated with buds from floral mother shoots (FL trees) of 'Tahiti' lime produced more inflorescences, including more leafless inflorescences, than VG trees in spring 2009, but bud source had no effect on the floral intensity of 'Washington' navel orange trees. Quadrant effects on shoot characteristics were not evident in 2009. By the winter of 2012, greater frequency of branching (greater number of lateral shoots), a characteristic of the floral mother shoot bud source compared to the vegetative mother shoot bud source, was greater in the SW quadrant of both 'Washington' navel orange and 'Tahiti' lime FL trees compared to VG trees, suggesting an interaction between bud source and the location of shoots within the trees 7 years from budding. Greater branching would provide more nodes (sites) at which to bear lateral inflorescences. Trees propagated with buds from floral mother shoots from 'Washington' navel orange and 'Tahiti' lime tended to have greater annual yields. The 4-year cumulative and 4-year average number of fruit per tree, but not kilograms per tree, was significantly greater for 'Washington navel orange FL versus VG trees. For the 'Washington' navel orange trees in this experiment, the weight of individual fruits decreased as the number of fruits per tree increased. For 'Tahiti' lime, the number of fruits produced by FL trees was not significantly greater than VG trees. The tendency toward a greater number of fruits per FL tree versus VG tree observed for both cultivars is consistent with FL trees of each cultivar having more lateral shoots. Though inconclusive at this time, the results suggest that trees propagated with buds from floral mother shoots might be more productive on a cumulative basis, especially the 'Washington' navel orange, for which these characteristics were first developed. A modified set of characteristics may be required for cultivars with a tree architecture (growth habit) that is different from that of *C. sinensis*.

ACKNOWLEDGEMENTS

The authors thank Toan Khuong for statistical analysis of the data and Alec Weitzel, Gavin Meeks, Jose Aviles, Jacob Hernandez, Christopher Jordan and Dwight Williams for their technical support.

Literature Cited

Hake, K.D. 1995. Regulation of flowering in *Citrus limon* by water-deficit stress and nitrogen compounds. Ph.D. Dissertation, University of California, Riverside, CA.

Lord, E. and Eckard, K.J. 1985. Shoot development in *Citrus sinensis* L. (Washington navel orange). I. Floral and inflorescence ontogeny. Bot. Gaz. 146:320-326.

Lord, E.M. and Eckard, K.J. 1987. Shoot development in *Citrus sinensis* L. (Washington navel orange). II. Alteration of developmental fate of flowering shoots after GA_3 treatment. Bot. Gaz. 148:17-22.

Verreynne, J.S. and Lovatt, C.J. 2007. Citrus fruit reduce summer and fall vegetative shoot growth and return bloom. Proc. Int. Soc. Citricult. 2:520-524.