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Citrus Shoot Age Requirements to Fulfill Flowering Potential

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Three hurricanes from late summer through the fall of 2004 caused severe leaf loss, sometimes over the same citrus production areas in Florida. A vegetative flush occurred after each hurricane, and by December, new shoots were 3 to 12 weeks old prior to trees accumulating over half the cool temperatures required for good flower bud induction. Two experiments were conducted to evaluate the potential for young flushes to develop buds that could be induced to flower. Flushes on potted trees in a greenhouse were stimulated and shoots were allowed to develop 4 to 10 weeks before moving the trees to cool, flower-inducing conditions for 6 weeks in a growth chamber (15/10 °C, day/night). Plants were subsequently returned to the greenhouse, which was at 20 °C or higher (ambient), until buds sprouted. In both experiments, less than 2% of sprouting buds flowered on shoots that had developed for only 4 weeks. Sprouting buds flowered on 25% and 76% of the shoots that had developed for 6 weeks in the first and second experiments, respectively. After 8 to 10 weeks of shoot development, sprouted buds that flowered increased to 54% in Experiment 1 and were 50% in Experiment 2. Consequently, more than 4 weeks of shoot development were necessary for citrus shoots and/or their leaves to reach their maximum flowering level in these experiments. In the second experiment, conducted during the following year, fewer buds sprouted, possibly due to low carbohydrate levels in new leaves after repeated cycles of forced flushing and root restriction in the greenhouse.

Under Florida conditions, flower bud induction usually occurs from late October until January in response to cool temperatures (Valiente and Albrigo, 2004). Most of the flower buds are produced on spring and summer flush shoots from the previous growing season. Buds on these shoots are more likely to flush the next spring than buds on older shoots (Guardiola, 1981). In Spain, fall flush in September was observed to flower during the following spring (personal observations). About half of the sprouting buds on all of last year's shoots will be flower buds if adequate induction occurs during the winter (Moss, 1969). Drought conditions can also lead to flower bud induction in citrus and differentiation and expansion of the flower buds to full bloom requires warm temperatures and soil moisture for growth (Cassin et al., 1969). Any disturbance of vegetative growth and inductive conditions can be expected to affect the timing and success of this normal process.

In 2004, Florida citrus production areas were hit by two or three hurricanes depending on their location resulting in significant leaf losses to all cultivars, which stimulated successive fall flushes after each hurricane, often from the same flushes on the same trees (Albrigo et al., 2005; Salvatore et al., 2005). After Hurricane Charley (13 Aug., Ft. Myers through Polk and Orange counties), the subsequent flush, which took about 2 weeks to initiate, developed 8 to 12 weeks until November or December when cool temperatures stopped maturation and initiated flower bud induction. After Hurricane Frances (5 Sept., Ft. Pierce through central Florida to Brooksville), a new flush was initiated after another severe leaf loss event, and these shoots had 5 to 9 weeks to develop until November or December, respectively. After Hurricane Jeanne (25 Sept., Ft. Pierce through central Florida to Brooksville), the newly initiated flush from this leaf loss event had 3 to 7 weeks to develop before sufficient cold induction for flowering occurred in late 2004. In central Florida, the two east-to-west hurricanes plus the earlier Hurricane Charley resulted in up to three fall flushes after leaf loss events where these hurricanes crossed paths . These latter two hurricanes caused damage and new fall flush in the Indian River District as their landfall and paths were almost identical (Albrigo et al., 2005).

These atypical flushing episodes that developed from spring and summer flush buds resulted in fewer spring and summer shoot buds for flower bud induction during the winter period, and each new flush had less time to mature before cool weather started flower bud induction. However, these new flushes produced more total buds than were present on the original spring and summer flush. Each new shoot produced multiple node (bud) shoots from each forced bud. Therefore the new flushing events could have produced more flower buds as long as no maturation requirement existed for the buds and associated leaves to meet before responding to inductive conditions.

During the 2004–2005 flower bud induction period, sustained cool weather started the first week of November and continued until 25 Dec. when a warm period initiated bud differentiation as determined by the on-line "Citrus Flowering Monitor" system (Albrigo et al., 2006; http://disc.ifas.ufl.edu/bloom). Therefore, shoot and bud maturation was able to progress until November or perhaps December, but only half of the necessary inductive temperatures accumulated after 1 Dec. (http://disc.ifas.ufl.edu/bloom). The assumption of how long the shoots and buds had to mature are based on this flower monitoring information. An earlier report, not available when the first of these experiments was started, substantiated that fewer flowers were produced on defoliated limbs in the Ft. Pierce area after the two hurricanes impacting that area (Salvatore et al., 2005).

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To understand the likely impact of the hurricanes—resulting in excessive fall flushes that might or might not be able to produce flower buds—greenhouse and growth chamber experiments were carried out in late 2004–2005 (simultaneously with the field fall growth events after the hurricanes) and in 2005–2006. The major hypothesis for this work was that shoots (buds and associated leaves) must mature to a certain stage (age) before they are able to be induced to become flower buds under inductive conditions. We also wanted to characterize carbohydrate levels in leaves that might impact the buds' ability to flower (Goldschmidt and Golomb, 1982)

Materials and Methods

Greenhouse and growth chamber studies were initiated in the fall of 2004 using 2-year-old 'Valencia' orange trees in 15-gal containers. These trees were producing a strong fall vegetative flush (about 40 new shoots from 100 to 160 available buds) after weak cool temperature induction levels were applied in August–September (2 weeks at 24/19 °C). Four trees per shoot development time were randomized in the greenhouse into four replicates, one per bench, and allowed to develop for 4, 6, 8 or 10 weeks (treatments) before being moved to a cool growth chamber (15/10 °C, day/night) for 6 weeks to induce buds to flower. The experimental design was a completely randomized block. The growth chamber had lighting of 600–800 $\mu mol \cdot m^{-2} \cdot s^{-1}$ during the 15 °C period of 12 h each day. The buds on these plants were then forced to grow in a greenhouse with a minimum setting of 20 °C. Flushing and flowering on the various aged shoots was recorded. For field data, fall flushes stimulated on grapefruit trees by two hurricanes in the Indian River District were monitored for flowering in the spring of 2005 and data was previously reported. (Salvatore et al., 2005).

In summer 2005, the 16 potted trees used in the 2004 study plus 16 additional trees from the same cultivar tree lot and maintained in the greenhouse were randomly divided into two groups, one of which was defoliated of 75% of the older, mature leaves prior to flushing in an attempt to create lower carbohydrate levels. The trees were forced to flush in the summer by applying a short drought stress by withholding irrigation for 2 weeks after tip pruning about 10 shoots per plant. Plants were randomized on greenhouse benches after four trees from both the defoliated and non-defoliated plants were randomly chosen for flush development times of 4, 6, 8, or 10 weeks. The plants were arranged on benches for shoot development in a randomized block design.

After 4 weeks of new shoot development, six new flush leaves were sampled from each of six normal and six defoliated plants selected at random. Total sucrose and available carbohydrates were determined by the methods of Van Handel (1968) and Baroja-Fernández et al. (2004) to see if defoliation led to reduced carbohydrates that might affect flowering levels. All plants were allowed to develop their new shoots in the greenhouse and, at the end of their assigned flush develop time, plant groups were moved to a growth chamber for 6 weeks of 15/10 °C, day/night, induction. Buds were forced in the greenhouse at 20 °C, after which bud sprouting and flowering were recorded.

Results and Discussion

In the first experiment, bud break for new shoots was higher on 6- and 8-week-old than 4-week-old shoots, but they were not different from each other (Fig. 1). Bud break was highest for shoots allowed to develop 10 weeks. Percentage of sprouted buds of 8- and 10-week-old shoots was similar to that reported by Moss (1969) for buds induced for 6 weeks on mature shoots that were several months old. Lower bud sprouting and little flowering occurred on shoots that were only allowed 4 weeks to develop (Fig. 1). The percentage of flowering buds increased from 2% in 4-week-old shoots to nearly 25% at 6 weeks and increased approximate two times to 57% in 8-week-old shoots compared to 6-week-old shoots with no apparent change from 8 to 10 weeks (average of 54% for these two time periods). The results from this test suggested that new shoots need at least 6 weeks of development before many of the sprouting buds could be induced to differentiate into flower buds by cool temperature induction, and the response was better if the shoots were allowed 8 weeks to develop and mature before they were subjected to cool temperature induction conditions. The 6 weeks of flower bud inducing conditions in a growth chamber were adequate for a flowering response as report by Moss (1969) and 6 weeks of varying cool temperatures provided adequate flowering in the field (Valiente and Albrigo, 2004).

Fall shoots on grapefruit trees in the Indian River area that were initiated after the last hurricane (Jeanne) in 2004 did not flower abundantly (Salvatore et al., 2005). Flowering levels for buds on shoots that sprouted after this hurricane had less flowering than earlier fall flushes. These shoots (buds and leaves) had 3 to 4 weeks to develop before the cool induction temperatures began to occur and less than 7 weeks before half of the total cool induction temperatures had accumulated. The poor flowering response of these buds on late developing shoots in the field is in agreement with the first greenhouse experiment reported here, indicating that 3 to 7 weeks of shoot development and maturation was not adequate time for the shoot leaves or buds to be normally responsive to cool temperature induction of flower buds.

In the following year, the experiment was repeated, but included a 75% defoliation treatment to simulate leaf loss similar to a hurricane that might reduce available carbohydrates for bud development and maturation. Potted plants from the same lot were reused after inducing some shoot growth using tip pruning of 10 longer shoots/plant and a short drought period by withholding irrigation for 2 weeks in the summer. Bud sprouting did

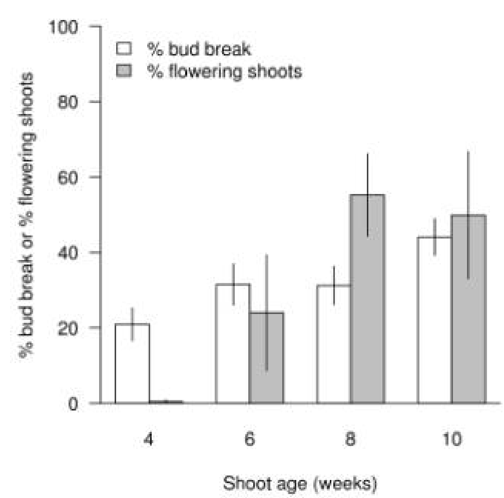

Fig. 1. Percentage of bud break and flowering shoots as a factor of shoot (bud) age in potted 'Valencia' trees. Shoots were developed in a greenhouse and flower bud induction was provided by 6 weeks of 15/10 °C after the specified shoot maturation period. Vertical bars indicate the standard error of the mean.

not increase in non-defoliated plants as weeks of development increased, averaging about 10 sprouts per plant (Fig. 2A). There was a significant increase in sprouted buds for defoliated plants over non-defoliated plants for all development times except 6 weeks, with 8- and 10-week-old defoliated shoots producing numerically more sprouts than shoots having less development times (Fig. 2A). Flowering bud counts were higher from 6- to 10-week-old compared to 4-week-old shoots (Fig. 2B). Flowering bud counts were higher for defoliated than normal shoots at 8 weeks, but not at 6 or 10 weeks. The percentage flowering for these three later development times were larger (Fig. 2C) than for shoots matured 4 weeks. Values were greatest at 6 weeks, which was significantly higher than for 8- or 10-week-old shoots. Only at 10 weeks did defoliated shoots have a significantly lower percentage of flowering versus normal shoots, but usually twice as many buds sprouted and produced only numerically more flowers on defoliated than non-defoliated shoots so that the per cent flowering buds was usually slightly less for defoliated plant shoots. Overall, the number of buds sprouting and numbers of flowering buds/plant were much lower than in the first experiment for all treatments.

The trees used in the second experiment were pot bound and had been manipulated before, which might have contributed to their being somewhat non- responsive to induction, resulting in the relatively low flushing and flowering. One factor presumed to cause a low flowering response is low carbohydrate levels. Defoliation should have reduced available carbohydrates and supposedly affected flowering response (Goldschmidt and Golomb, 1982). The carbohydrate data did show uniformly reduced carbohydrates in the new leaves on defoliated shoots; however, some of the new shoot growth leaves on non-defoliated trees also had similarly low carbohydrate levels (Fig. 3). Furthermore, defoliated plants had slightly more sprouting than non-defoliated plants and as much flowering as non-defoliated plants with significantly more in 8-week-old shoots (Fig. 2 A and B). Evidently, factors other than carbohydrates accounted for the low flowering in the second experiment.

In spite of this growth difference in Experiment 2, the lack of flowering for 4-week-old shoots followed the same pattern as in Experiment 1, in which more than 4 weeks of shoot development and maturation were required before significant flowering could be induced. The declining flowering after 6 weeks in the second experiment (Fig. 2 B and C) was not observed in the first experiment. Since the flowering responses in the second experiment were weak, the decrease in flowering after 6 weeks may not be an important effect, particularly as flowering was still two or more fold larger than flowering of 4-week-old shoots.

The data obtained from the experiments presented here indicate that more than 4 weeks are needed for a 'Valencia' orange shoot and its buds and/or associated leaves to develop sufficiently for a flower bud induction response similar to normal spring or summer shoot growth, induction, and spring flowering (Valiente and Albrigo, 2004). Flowering response after 8 weeks of growth was significantly greater than after 6 weeks in the first experiment, but 6 weeks appeared to be sufficient in Experiment 2. Six to 8 weeks is also about the time required for leaves on a new shoot to fully develop. A similar time may be needed for shoot parts to be able to react to flower induction conditions. Work on flowering signals and flowering gene up-regulation implicate leaves as a source of a flowering signal (Endo et al., 2005; Kobayashi et al., 1999; Nishikawa et al., 2007). The bud itself may also produce

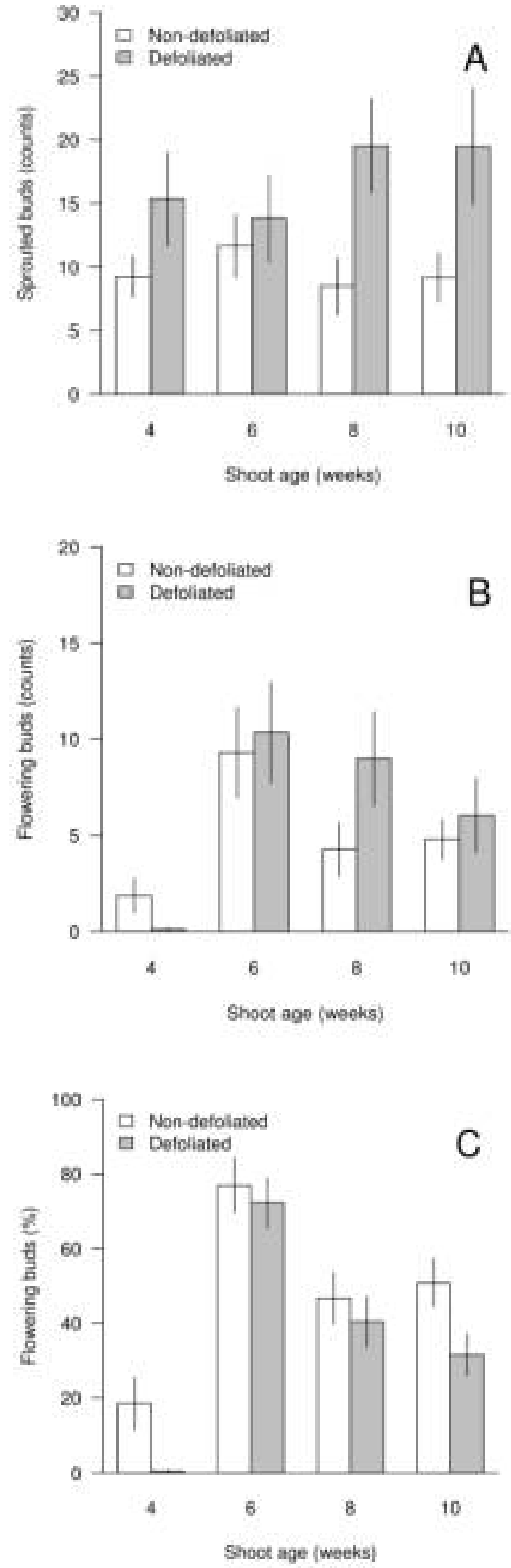

Fig. 2. The effect of weeks of shoot development and defoliation of potted 'Valencia' trees on bud sprouting (**A**), buds that flowered per plant (**B**), and percentage of sprouting buds that flowered (**C**). Shoots were developed and matured in a greenhouse and flower bud induction was provided by 6 weeks of 15/10 °C after the specified shoot growth period. Vertical bars indicate the standard error of the mean.

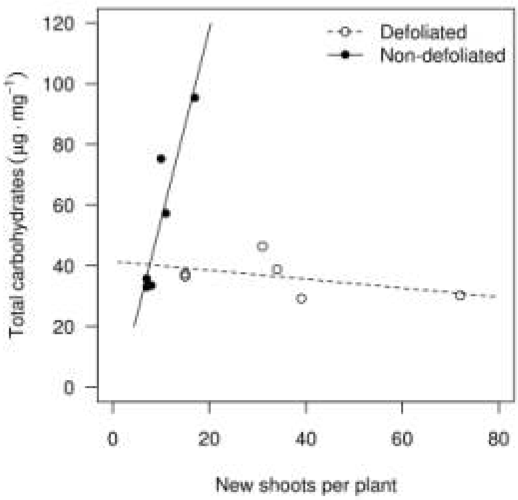

Fig. 3. Total available carbohydrate levels of leaves of 4-week-old shoots on six defoliated and six non-defoliated trees with corresponding new flush numbers for each plant. Regression lines were fitted to the defoliated and non-defoliated plant data with the non-defoliated regression being significant ($r^2 = 0.79$).

a signal and must be able to up-regulate important floral identity genes (Abe et al., 2005; Weigel and Meyerowitz, 1994). Therefore, both buds and leaves could require development and maturation until they can respond to these flowering gene up-regulation events and apparently this maturation period exceeds 4 weeks.

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