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Winter-injury Following Horticultural Treatments to Overcome Juvenility in Citrus Seedlings

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Citrus seedling juvenility delays new hybrid evaluation, slows cultivar release, and slows introgression of new traits. A horticultural program reported to overcome citrus juvenility was tested at the Florida Citrus Research Foundation Whitmore Farm (Lake County), using replicated Hirado Buntan × Clementine seedlings and standard cultivars all propagated onto US-812 rootstock. Due to cold-winters, influence of treatments on cold-injury was evaluated and damage was too great to assess juvenility reduction uncompromised by this injury. Treatments compared on each genotype were: 1) non-trained control, 2) training to a single upright shoot (TSUS), 3) TSUS with trunk-scoring in December, 4) TSUS with soil paclobutrazol in December, 5) TSUS with weekly thorn removal, and 6) TSUS with weekly thorn removal and scoring in December (complete juvenility reduction program). Trunk diameter increased faster on non-trained vs. TSUS trees. In Jan 2010, there were 23 hours < –4.4 °C and 1 hour < –6.7 °C. Trees were assessed for cold damage: 26% of non-trained trees vs. 100% of TSUS trees displayed damage ranging from nearly complete defoliation to death. In control trees 11% were killed. TSUS trees of treatments 2, 4, and 5 had mortality of 9% to 17%, while trunk-scored trees (treatments 3 and 6) had higher mortality at 23% to 34%. Even though a cold protection tarp system was installed and used on surviving trees during the period of coldest weather in early December, further damage occurred following freezing conditions in Dec. 2010 with 17 hours < –4.4 °C and 1 hour < –6.7 °C. TSUS trees again showed much more damage than non-trained trees with a cumulative 66% to 83% mortality vs. only 17% for non-trained controls. These techniques increase risk of serious winter-injury, and require more elaborate cold protection to be implemented in colder areas such as Lake County.

Juvenility in citrus seedlings is a major impediment to rapid evaluation and selection of improved citrus cultivars. Many field grown citrus seedlings will not produce significant amounts of fruit until they are 6+ years of age, making it necessary for breeding programs to maintain large numbers of plants for many years before they can be evaluated. In an active breeding program with progeny at all stages of assessment and development, and low per unit cost of maintaining plants, there is little value in accelerating flowering for routine material in the breeding program. However, for new material that is expected to include urgently needed properties such as resistance to a priority disease or utilizes a new parent with novel traits, techniques that would permit more rapid flowering and fruiting may be invaluable.

Seedlings of perennial plants often display an extended juvenility period, with juvenility defined as an inability to induce flowering using any means (Zimmerman, 1972). "Phase change" occurs, permitting flowering and fruiting, after plants transition into maturity. Transition to flowering in many plants is correlated with morphological/physiological "aging" rather than mere passage of time (e.g., Lamoreaux et al., 1978). Shoots near the base of trees often retain juvenile characteristics and when such wood is used for propagation the resulting plants display juvenile traits even though more apical shoots and the plants propagated from them are mature. For example, Zimmerman (1971) showed that growing *Malus hupehensis* as a single shoot resulted in flowering at 1.8–2.0 m in height and at the 75th to 80th node. Grafting with buds from the upper portion of the tree produced plants with rapid onset of flowering, while use of lower buds for propagation resulted in plants with delayed flowering until further nodes were developed. Similarly, initial flowering of some citrus genotype seedlings was earlier and/or more profuse when apical buds from seedlings were used in propagations compared to more basal buds (Furr, 1961). Snowball et al. (1994a) reported that removal of lateral branches and maintaining optimal growth to maximize node number on a single shoot resulted in flowering within 4 years of seed germination in 13 families of citrus seedlings. In that trial, lateral shoots were permitted to grow after 24 months of single shoot growth, and shoots near the plant apex produced most of the observed flowers.

A number of studies have shown a correlation between earlier seedling flowering and greater tree size in non-trained fruit trees (e.g., Visser et al., 1976). In citrus, Mitani et al. (2008) reported that non-trained grafted seedlings of diverse hybrids displayed flowering of one-third of the trees within 2.5 years of grafting. Greater tree size (height, diameter, and number of branches) was associated with greater early flowering, but substantial differences were seen between seedlings of different parentage. Early flowering was attributed to influence of grafting, though no own-rooted seedlings were compared.

In most woody plants, including citrus, mild rejuvenation occurs even in young plants produced through vegetative propagation. A

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number of cultural practices have been routinely used to accelerate transition from juvenility in orchard trees propagated from mature buds. Most notable among these is the use of clonally propagated rootstocks that are associated with earlier flowering and cropping. In Florida citrus production a good example of this effect is the earlier bearing of orchards on Swingle vs. Cleopatra rootstocks (Stover and Castle, 2002). Bending branches to the horizontal is routinely used to enhance early cropping in apples (Childers et al., 1995; MacDaniels and Heinicke, 1925).

Some citrus breeding programs have attempted to accelerate maturation by collecting budwood from the upper shoots of seedlings and grafting it onto mature rootstocks. Japanese researchers were reported to use this technique in citrus, and combined it with bending of scions to the horizontal to significantly shorten time to flowering (Soost, 1987). However, as recently as 1996, leading citrus breeders reported that "efforts to reduce this period (of juvenility in citrus seedlings) … have been largely unsuccessful" (Soost and Roose, 1996).

Another practice that sometimes enhances earlier bearing is girdling or scoring (a single thin cut around the trunk or branch circumference) before the period of flower induction to alter distribution of growth regulators, carbohydrates etc., between the root system and canopy, and enhance both flowering and fruit set (reviewed in Goren et al., 2003). In citrus, such a scoring treatment was reported to have little effect on 4-year-old seedlings, but greater effect on trees at 7 years after germination (Furr et al., 1947) and greatly increased flowering in some mature citrus trees (Goldschmidt et al., 1985). Combining elements from numerous established practices and extrapolating from research results and including more speculative observations, a combination of practices is reportedly used in some breeding programs to markedly accelerate transition to cropping in citrus seedlings. The reported set of practices includes: budding onto rootstocks providing early productivity, maintaining rapid growth from a single shoot on a rigid support (bending over rather than cutting when exceeding the support height), complete removal of thorns from the segregating progeny scions (seemingly because juvenility in citrus is associated with thorniness), and trunk-scoring each scion in the fall. This method is anecdotally reported to result in cropping at 3 years after germination rather than 6+ years (P. Chaires, personal communication). While extremely laborious, the time savings may justify this procedure where rapid advancement is a priority. This procedure has not been published, but its adoption in several regions appears to be a testimonial to its success.

While branch bending, use of rootstocks, and girdling/scoring likely influence gibberellins indirectly, an additional group of practices to reduce juvenility is application of chemicals that directly inhibit production of gibberellins within the plant. While many aspects of plant metabolism are altered in the progression from juvenile to adult trees, gibberellins play a central role: high levels of gibberellins inhibit flowering and enhance cell and stem elongation while lower levels of gibberellins promote flowering and decrease vegetative growth. Use of gibberellin biosynthesis inhibitors has been demonstrated to enhance early flowering in a wide array of plant species, including citrus (e.g., Acosta et al., 1998; Snowball et al., 1994b). Aggressive application of several different gibberellin biosynthesis inhibitors has been shown to enhance flowering in citrus seedlings. Lime and kumquat seedlings treated with paclobutrazol flowered within 11 months of germination (Snowball et al., 1994b), whereas untreated plants did not flower until 14 months. Related compounds have also been shown to be effective as gibberellin inhibitors in a wide array of citrus genotypes (Stover et al., 2004).

The purpose of this project was to implement and refine methods for accelerating flowering and fruiting in citrus to permit earlier evaluation of fruit quality and enhance both efficiency and rapidity of developing improved citrus varieties. Unfortunately, low winter temperatures severely damaged many trees, and instead we documented the effects of juvenility-reduction treatments on resulting winter-injury.

Materials and Methods

The experimental planting was established at the Florida Citrus Research Foundation Whitmore Farm in Lake County, FL. Plants used were 30 seedling hybrids of Hirado Buntan pummelo × Clementine, from seedlings grown out in 2005 and grafted onto US-812 in 2006. Also included were plants propagated from same age seedlings of Sun Chu Sha mandarin, Duncan grapefruit and Ridge Pineapple sweet orange, and mature budwood-sourced Sun Chu Sha and Hirado Buntan pummelo, all grafted onto US-812.

A trellis was constructed with two horizontal wires at 50 cm (20 inches) and 200 cm (80 inches) and a vertical wire for tree training every 30 cm (12 inches). Six plants of each budwood source (hybrid seedlings and standards for comparison) were planted in Apr. 2009 using adjoining spaces except that empty spaces were left on either side of the non-trained control tree. Individual treatment assignments were randomized within each group and one plant of each was subjected to the following treatments:

1) Control, not trained, left with empty space on either side to minimize crowding;

2) Trained to single upright shoot (TSUS) by removing lateral branches, attaching to vertical wire with a Max-Tapener (Max Co, Limited, Tokyo, Japan) and bending over shoot and Max-tapening to trellis when height exceeded ≈250 cm (10 ft);

3) TSUS, scored in first week of Dec. 2009 and 2010 using a tool with three circular blades that fit around trunk. Scoring was conducted at 5–12.5 cm (2–5 inches) above soil where trunk diameter was suitable for using the scoring tool. Tool was rinsed with 95% ethanol between trees;

4) TSUS, treated with paclobutrazol at 50 mg (0.0018 oz) per plant per application (in the formulation Bonzi, Syngenta Crop Protection, Inc., Greensboro, NC) applied in 500 mL (16.9 fl oz) per plant to a circle of soil 15 cm (5.9 inches) in diameter centering on tree trunk in first week of Dec. 2009 and 2010;

5) TSUS, all thorns removed each week;

6) TSUS, all thorns removed each week, scored in Dec. 2009 and 2010.

A removable cold protection system was built over the trellis in Nov. 2010, using continuous polyethylene tarp taped at the seams. It was pulled into place over the treatment block on 7 Dec., sealed at the edges by covering with soil, and kept over the trees through 15 Dec. 2010. Microsprinklers were run when temperatures were below freezing. Based on an initial forecast of less severe cold than was actually experienced, and reports of minimal value in providing protection, it was not in use on 27 Dec.–30 Dec.

Tree trunk circumference 2.5 cm (1 inch) above the graft union, tree height, and canopy width along and across the row were measured quarterly. Temperature data were obtained from the Okahumpka site of the Florida Agricultural Weather Network, which is within 400 m of the trees examined in this study. Following freezing temperatures, defoliation was assessed within 6 weeks while death and dieback were assessed at 12 weeks. Trees were scored as follows: 1 = death; 2 = terminal dieback; 3 = complete defoliation with no dieback; 3.5 = defoliated except for terminal leaves; 4 = slight defoliation; 5 = no damage. Analysis of variance was conducted using SAS (Cary, NC). Correlation between tree circumference and freeze damage was conducted using Excel Analysis Tools (Microsoft Corp., Redmond, Wash.).

Results and Discussion

Results 2009–2010

Although all trees grew well, the non-trained control trees grew more quickly than the trees subjected to lateral shoot removal. By Dec. 2009, the non-trained trees averaged 50% greater trunk diameter but 65% of the height of TSUS trees (Table 1). As severity of pruning is increased in fruit trees, reduced growth is a common response (e.g., Greene and Lord, 1983; Mika et al., 2003).

Unfortunately, extreme cold weather (for central Florida) was experienced at the experimental site on 7 Jan. through 12 Jan. 2010 with 23 h in which average temperature was below –4.4°C (24 °F) and temperatures below –6.7 °C (20 °F) were recorded on 11 Jan. for more than an hour (Table 2). Considerable damage occurred to many citrus trees throughout the farm. Trees trained to the upright single shoot showed much more damage than the "control" non-trained trees on the trellis. Defoliation was fairly widespread and some tree death occurred. Virtually no damage occurred to trees of the sister Hirado Buntan × Clementine hybrid populations, which were growing nearby with conventional training and spacing, even though many were planted in lower areas that historically have shown a higher frequency of winter-injury (data not shown). Defoliation and dieback recorded on 8 May 2010 (Table 3), displayed much greater damage on trees trained to a single upright shoot. In non-trained trees, 74% were only minimally damaged and 11% died, while in trained trees all suffered significant damage (ranging from almost complete defoliation to death) and an average of 20% of trees were killed. There was also evidence that in trained trees, scoring trunks increased damage (pdiff = 0.012 in contrast analysis of treatments 2 and 5, vs. 3 and 6), and that application of paclobutrazol decreased mortality (pdiff = 0.051 in contrast analysis of treatment 2 vs. 4).

Since the larger non-trained trees showed much less damage, it was reasonable to ask whether greater bulk of tree trunks may have helped maintain a greater core temperature to enhance tree winter-hardiness. Hume (1949) stated that "as a rule, the (freeze) resistant power of a branch becomes less and less as it decreases in size, or in other words, varies directly with the diameter." This same relationship may extend to trunks, as younger and smaller trees are often more damaged by freezing temperatures (Young and Peynado, 1963). Regressions shown in Table 4 do indicate a negative relationship when winter injury was correlated with trunk diameter across all experimental trees, but since non-trained trees uniformly had much greater trunk diameter, this relationship was confounded by treatment. To eliminate this confounding effect, only all trained trees were compared, and there was a weakly significant positive relationship, however this was confounded by genotype, since the 'Sun Chu Sha' and 'Pineapple Sweet Orange' scion trees had smaller diameters and among the lowest levels of winter-injury. Looking exclusively at Hirado Buntan × Clementine scions, there was no significant relationship between trunk diameter and winter injury when either only non-trained or only trained trees were evaluated (Table 4). Wheaton et al. (1986) conducted a trial with 'Hamlin' and 'Valencia' at four tree densities ranging from 371 to 890 trees/ha (150–360 trees/acre) and a severe freeze was observed at 5 years after planting. Trees at the higher plant densities had substantially less winter-injury even

Table 1. Growth of Hirado Buntan pummelo × Clementine seedlings grafted onto US-812 as influenced by training treatments intended to accelerate passage through juvenility. Trees were planted on Apr. 2009. Measurements were made in. Dec 2009 and indicate tree size when exposed to freezing.

Training treatments	Trunk diam (cm)	Ht (cm)	Width (cm)
1) Non-trained controls	33.1 a[z]	211 c	130 a
2) Trained to single upright shoot (TSUS)	19.9 b	291 b	24 b
3) TSUS, scored in Dec.	19.6 b	306 ab	24 b
4) TSUS, paclobutrazol in Dec.	20.5 b	317 a	24 b
5) TSUS, dethorned weekly	20.3 b	303 ab	24 b
6) TSUS dethorned and scored	19.6 b	299 b	24 b
P value treatment	<0.0001[y]	<0.0001[y]	<0.0001[y]
P value genotype	<0.0001	0.2428	0.059

[z]Means followed by the same letter within a column are not different at $P = 0.05$ as determined by Duncan's New Multiple Range Test.
[y]*P* values within this column were determined for main effects by analysis of variance.

Table 2. Cold weather at the Whitmore research farm in winters 2009–10 and 2010–11. The data presented are the cumulative hours below the indicated temperature at 60 cm (Okahumpka Weather Station from FAWN) on the indicated dates.

Dates	< 0 °C (32 °F)	< –2.2 °C (28 °F)	< –4.4 °C (24 °F)	< –6.7 °C (20 °F)
1–13 Jan. 2010	92	59	23	1
8–29 Dec. 2010	90	47	17	1

Table 3. Winter-injury, following damaging cold in 2009-2010 growing season, of Hirado Buntan pummelo × Clementine seedlings grafted onto US-812. Data are compared for different training treatments intended to overcome seedling juvenility, and winter-injury was assessed in May 2010. Initial planting was Apr. 2009. Trees were scored as follows: 1 = death; 2 = terminal dieback; 3 = complete defoliation with no dieback; 3.5 = defoliated except for terminal leaves; 4 = slight defoliation; 5 = no damage. Minimally damaged was defined as those trees scoring ≤2.

Training treatments	Winter	Dead (%)	Minimally damaged (%)
1) Non-trained controls	3.7 a[z]	11	74
2) Trained to single upright shoot (TSUS)	2.2 bcd	17	0
3) TSUS, scored in Dec.	2.1 cd	23	0
4) TSUS, paclobutrazol in Dec.	2.6 b	9	0
5) TSUS, dethorned weekly	2.5 bc	17	0
6) TSUS dethorned and scored	1.9 d	34	0
P value treatment	<0.0001[y]	0.0847[x]	<0.0001[x]
P value genotype	<0.0001	<0.0001	1.0000

[z]Means followed by the same letter within a column are not different at $P = 0.05$ as determined by Duncan's New Multiple Range Test.
[y]*P* values within this column were determined for main effects by analysis of variance.
[x]*P* values within this column were determined for main effects by Kruskal Wallis nonparametric analysis.

Table 4. Hirado Buntan pummelo × Clementine seedlings grafted onto US-812 and subjected to training treatments intended to overcome seedling juvenility were exposed to damaging cold in Jan. 2010. Regressions were run between trunk diameter at the time of freeze exposure and winter-injury observed the following Spring 2010. These regressions test the hypothesis that greater trunk diameter results in greater temperature buffering and thus reduced winter-injury. All genotypes all treatments (comparison 1) includes non-trained controls, which averaged 50% greater trunk diameter, and were markedly lower in winter-injury in all analyses.

Comparison	*P*-value regression	r^2	Nature of correlation
1) All genotypes all treatments	<0.00001	0.11	negative
2) All genotypes no non-trained controls	0.079	0.018	positive
3) H × C data no non-trained controls	0.56	0.002	none
4) All genotypes non-trained controls	0.312	0.03	none
5) H × C non-trained controls	0.127	0.08	none

though they displayed smaller trunk circumferences. Reduced winter-injury may have been the result of greater interception and retention of radiant heat given off from the soil (Jackson, 2006) by having more of the orchard floor covered by tree canopies, and this may also have played a crucial role in reducing winter-injury in non-trained control trees in our study.

Results 2010–2011

Trees that were trained to an upright shoot the previous year were almost completely defoliated in the winter of 2009–10 and following the established protocol were maintained with the same training and not allowed to create new lateral shoots. Sufficient trees survived to continue the juvenility reduction experiment as planned. A cold protection structure was installed over the rows of trees with trained trees prior to the early Dec freeze and micro-sprinklers were run during the hours below freezing. Unfortunately, extreme cold weather (for central Florida) was again experienced, producing new records for early season low temperatures. On 8 Dec. through 29 Dec. 2010 there were 17 h in which average temperature was below –4.4 °C (24 °F) and temperatures below –6.7 °C (20 °F) were recorded on 29 Dec. 2010 for 1 h (Table 2). Even trees under the cold protection structure were damaged, and all upright trained trees were completely defoliated. Most young trees maintained using conventional practices, including controls on the trellis, also experienced considerable defoliation but maintained a core of surviving foliage.

Again, considerable damage occurred to many citrus trees throughout the farm, and trees trained to the upright single shoot on the trellis showed much more damage than the control non-trained trees on the trellis. By May 2011, 66% to 77% of trees trained to a single upright shoot had been killed vs. 17% of the non-trained trees (Table 5). The great majority of tree death resulted from the Dec 2010 freezes. Low temperatures and hours of freezing accumulated in 2010–2011 were similar to those in 2009–2010 (Table 2), but freezing in Dec. 2010 occurred very early with little preceding cold weather for acclimation (Fig. 1). It is well established that cold hardening occurs in citrus that has been exposed to cool but not freezing conditions preceding a freeze event (reviewed in Yelenosky, 1985), and this may be the primary reason for much greater damage following 2010–2011 freezes. However, it is also possible that winter-injury from the previous year made trees more susceptible to further freeze damage (Young and Peynado, 1963).

During this 2-year trial, only two trees flowered, the non-trained trees from mature budwood of 'Sun Chu Sha' and 'Hirado Buntan' standards. It is noteworthy, that the trained trees using these sources of budwood did not flower or crop, but it is inappropriate to conclude that the training methods would not have accelerated flowering and cropping of seedlings if trees were unaffected by cold-damage.

Conclusions:

To our knowledge the components of this juvenility system, which represents substantial commitments of hand labor and cost, have not been tested. We established this experiment to provide such a test and permit us to determine what components merit implementation in our breeding program. Unfortunately, winter-injury compromised comparison of treatment affects on juvenility of healthy trees, but did alert us to the enhanced danger of cold-damage from using these procedures. Dedicated use of these juvenility reduction techniques in areas subject to sporadic

Table 5. Winter-injury, following damaging cold in 2009–2010 and 2010–2011 growing seasons, of Hirado Buntan pummelo × Clementine seedlings grafted onto US-812. Data are compared for different training treatments intended to overcome seedling juvenility and winter-injury was assessed in May 2011. Initial planting was Apr. 2009. Trees were scored as follows: 1 = death; 2 = terminal dieback; 3 = complete defoliation with no dieback; 3.5 = defoliated except for terminal leaves; 4 = slight defoliation; 5 = no damage.

Training treatments	Cumulative winter-injury on May 2011	Data excluding dead from May 2010, winter-injury on May 2011	Cumulative dead (%)	Newly dead in 2011 (%)
1) Non-trained controls	3.0 a[z]	3.3 a	17	6
2) Trained to single upright shoot (TSUS)	1.3 b	1.4 b	66	49
3) TSUS, scored in Dec.	1.2 b	1.3 b	77	54
4) TSUS, paclobutrazol in Dec.	1.2 b	1.3 b	77	69
5) TSUS, dethorned weekly	1.2 b	1.3 b	77	60
6) TSUS dethorned and scored	1.2 b	1.3 b	83	49
P value treatment	<0.0001[y]	<0.0001[y]	<0.0001[x]	<0.0001[x]
P value genotype	<0.0001	0.0059	0.0039	0.0245

[z]Means followed by the same letter within a column are not different at $P = 0.05$ as determined by Duncan's New Multiple Range Test.
[y]*P* values within this column were determined for main effects by analysis of variance.
[x]*P* values within this column were determined for main effects by Kruskal Wallis nonparametric analysis.

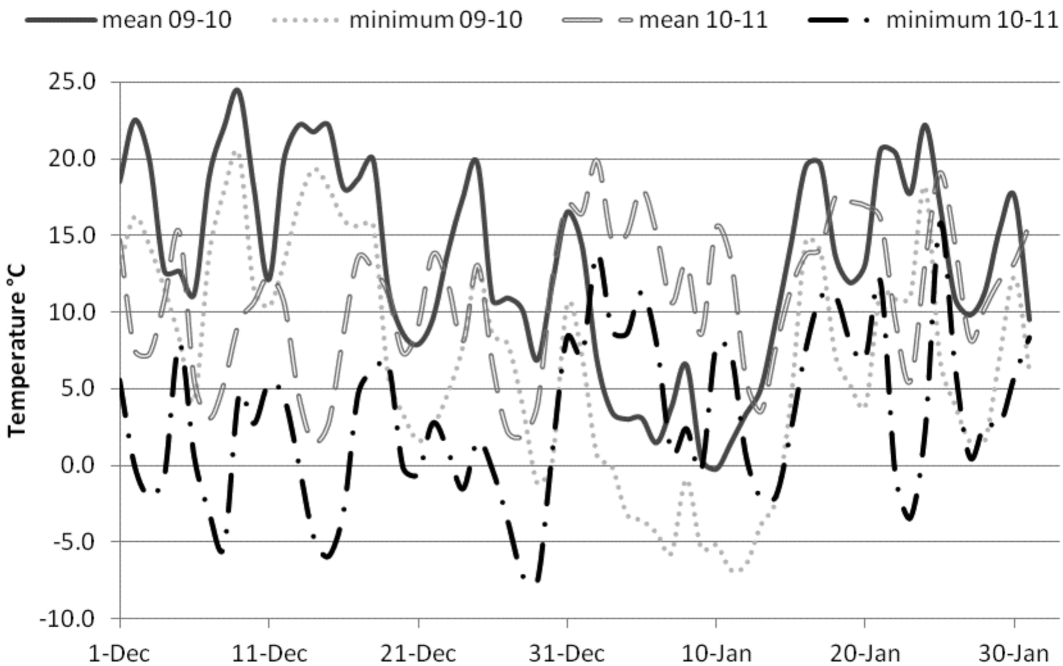

Fig. 1. Temperature data from Okahumpka FAWN station near experiment. Data are mean and minimum daily temperatures at a height of 60 cm, for the periods 1 Dec.–31 Jan. in the winters of 2009–10 and 2010–11.

cold winters, such as Lake County, Florida appears to be inadvisable, unless substantial cold protection is available. It is fortunate that we entered into use of the juvenility-reduction system with a focus on testing these practices with a modest number of plants. Winter-damage from a large scale effort to use this system would have set back our breeding efforts substantially.

Further experiments are needed to test these reported juvenility reduction practices in trees uncompromised by winter-injury. Firstly, effectiveness in accelerating flowering needs to be demonstrated to merit this substantial additional effort, and secondly value of individual management components needs to be established. The concept that removal of thorns will enhance transition to maturity may require special scrutiny. If thorn removal provides little or no contribution to maturation, then elimination of this step would permit inclusion of many more seedlings in the rapid maturation process. Certainly it is well established that thorniness declines in citrus as plants become mature, but thorns have generally been considered a symptom of citrus juvenility rather than a cause. Thorns are considered determinate axillary branches in citrus (Tan and Swain, 2006) suggesting no obvious physiological benefit to phase change through their removal. A more recent suggestion that thorns may be derived from flowers that failed to complete development (Tan and Swain, 2006) also provides little support for the notion that their removal may enhance subsequent flowering.

In an era of ongoing threat of haunglongbing infection in field-planted citrus, and possibly a frequent recurrence of citrus-damaging cold, it may be advisable to use protected cultivation of new hybrids for their initial evaluation at the Whitmore Farm and other areas with significant damaging cold. If these practices are verified to accelerate seedling maturation, the high-density juvenility-reduction trellis, within a screenhouse with cold protection, may offer a practical solution for continued generation and testing of new hybrids in cold sites.

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