Table 1. Effect of different chemical treatments on time of 50% fruit drop and germination percentage of embryos of 'Fla. 3–1' and 'Flordaking' and seed of 'Fla. 7–3N', 'Fla. 7–4N', 'Earligrande', and 'Fla. M6–6N' (1981 and 1982).

		Control		Daminozide (2000 ppm)		Maleic hydrazide (1000 ppm)		Thiourea (2000 ppm)	
Cultivar	FDP (days)	50% fruit drop (days)	Germination (%)	50% fruit drop (days)	Germination (%)	50% fruit drop (days)	Germination (%)	50% fruit drop (days)	Germination (%)
Fla. 3–1[z]	50	68	8	70	35*	72*	20	71*	25*
Flordaking[z]	65	77	28	79	60*	81*	34	79	60*
Fla. 7–3N	70	86	26	90*	19	90*	35	93*	59*
Fla. 7–4N	70	85	18	88*	55*	90*	36*	88*	63*
Earligrande	75	83	0	89*	53*	88*	81*	88*	25*
Fla. M6–6N	80	96	66	---[y]	---	---[y]	---	98	100*
Mean	68	82	24	83	44*	84	41*	86*	55*

[z]Only 1982 embryo culture data
[y]No treatment applied
*Significant at 5% from control

The objectives of using chemical treatments in this study were to enhance seed germination either by 1) delaying fruit drop to allow more time for embryo maturation or 2) speeding up embryo development in relation to fruit maturity.

In controls, 50% fruit drop occurred from 8 to 18 days (average of 1981 and 1982) after normal commercial harvest stage (Table 1). This variability was probably due to cultivar differences in physiological maturity rates of firmness. The softer cultivar at ground color break would have been more mature physiologically. Daminozide, maleic hydrazide, and thiourea delayed fruit drop from one to 4 days (Table 1), but generally had the least effect on the earliest-maturing cultivars.

Daminozide, maleic hydrazide, and thiourea generally delayed fruit drop, but this delay did not appear to account for all of the increase in seed germination. From our experiences in seed germination, an increase of 1–4 days in FDP of any early-ripening cultivars would not be expected to increase germination in the range of 30%. Daminozide, maleic hydrazide, and thiourea increased average germination in all cultivars 28%, 25%, and 31%, respectively, over the unsprayed control. The increase was present in embryos cultured both *in vitro* and in seed planted in perlite.

The chemical treatments caused no statistical differences in length of immature embryos of 'Fla. 3–1' and 'Flordaking' (data not shown). However, different germination rates were obtained from each treatment even though embryos were equal in size. This is not in agreement with Toledo et al. (5) where germination rate was affected by embryo size. The other 4 cultivars had embryos that filled so that no endosperm was evident; thus, embryo lengths were not measured.

Daminozide (1000 ppm), maleic hydrazide (2000 ppm), and thiourea (1000 ppm) with single and double spray applications (data not shown) were equally effective in delaying fruit drop and in increasing embryo germination. A second application of these 3 chemicals was made in 1982 one week after the first application but had little additional effect.

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In Vitro Shoot-tip Grafting to Eliminate Citrus Viruses and Virus-like Pathogens Produces Uniform Budlines

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Abstract. **Individual shoot-tip-grafted budlines of 'Willowleaf' mandarin (*Citrus reticulata* Blanco) and 'Temple' tangor [*C. reticulata* x *C. sinensis* (L.) Osbeck] were grown to fruiting for evaluation. Fruits of both cultivars were highly uniform among shoot-tip cultures, indicating that this technique for producing disease-free citrus germplasm is reliable and does not increase the production of variant budlines.**

Prior to development of shoot-tip grafting (5, 6, 7) and thermotherapy (1, 8) techniques, growing nucellar seedlings was the only method available for producing disease-free citrus cultivars from clones infected with virus or other graft-transmissible pathogens.

The primary disadvantage of producing citrus budlines through nucellar embryony is the phenomenon of juvenility (2). Young nucellar seedlings exhibit excessive thorniness, vigorous and upright habit of growth, slowness to fruit, alternate bearing in early years, and physical differences in fruit characteristics which are often detrimental in marketing the fruit. These characteristics may persist for many years and over many budded gen-

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Table 1. Range of values, percentage of deviation from the mean, and significance of variation among fruit samples from 15 'Temple' and 15 'Willowleaf' shoot-tip-graft budlines and from 15 samples of 'Willowleaf' from the same tree.

Variable	Temple (15 budlines)			Willowleaf (15 budlines)			Willowleaf (15 samples—same tree)		
	Range	% dev.	Sig.[z]	Range	% dev.	Sig.	Range	% dev.	Sig.
Fruit weight (g)	159 –208	16.4	**	74 –97	13.6	**	79 –86	5.4	*
Length/width ratio	0.88– 0.96	4.1	**	0.75– 0.85	6.6	**	0.76– 0.83	4.4	**
Rind texture (rating)	3.9 – 4.9	12.7	NS	3.3 – 3.7	6.8	NS	3.2 – 3.7	9.7	NS
Rind thickness (mm)	3.3 – 4.5	17.6	**	3.2 – 3.8	7.7	NS	2.9 – 3.7	13.3	**
Seed count	6.1 – 9.9	24.3	**	9.3 –11.6	14.8	NS	7.3 –10.0	16.5	**
Percentage of juice	44.6 – 52.1	9.3		27.4 –31.9	11.6		22.2 –27.2	12.5	
Solids/acids ratio	7.7 – 8.9	7.8		11.9 –14.3	10.2		13.5 –15.3	6.6	

[z]Significant at 5% (*) and 1% (**) levels.

erations. Nucellar budlines usually produce higher yields of fruit than their parental clones over a period of 8–10 years or more (2, 3). The portion of this higher yield that can be attributed to elimination of virus and virus-like pathogens in the parental budline has not been determined. Variations among citrus nucellar budlines and differences, other than juvenility, from the parental budline have been reported (4) in numbers indicating that genetic variants may occur more often during production of nucellar budlines than occur during standard nursery trees production by bud propagation.

Shoot-tip grafting currently appears to be the most useful method for producing pathogen-free citrus propagative stock. While thermotherapy is highly effective for eliminating most citrus viruses, it is ineffective in eliminating exocortis and cachexia viroids. As noted, nucellar embryony has the disadvantages of juvenility and possible variability.

In 1974 and 1975, several shoot-tip grafts of 'Willowleaf' mandarin and 'Temple' tangor were made at Riverside (6). The 'Willowleaf' source was infected with tristeza virus and exocortis viroid and the 'Temple' with exocortis and cachexia viroids. Following shoot-tip grafting and subsequent growth of the grafts, the respective pathogens were no longer present based on standard index testing (7). These individual shoot-tip graft budlines were then budded on 'Troyer' citrange [*Poncirus trifoliata* (L.) Raf. x *C. sinensis*] rootstock and planted in the field in June 1976 for evaluation for uniformity and trueness to type.

In February 1982, fruit samples were collected from 26 'Willowleaf' and 15 'Temple' trees, each representing an individual shoot-tip graft. This fruit, 10 per sample picked at random, was brought to the laboratory for analysis. No apparent abnormal variations were detected during visual observations of the trees and fruit of these cultivars in the field.

Samples of whole fruit and cut halves were visually observed in the laboratory. Except for size variation, which was expected and was probably due to crop load on the trees, there were no readily observable differences among samples within each cultivar, indicating that there were no obvious variants present.

Fruit analysis measurements included: 1) fruit weight; 2) length/width ratio; 3) rind texture rating, by visual comparison with a photographic texture chart depicting 8 gradations from smooth to very rough and pebbly; 4) rind thickness; 5) seed number; 6) percentage of juice by weight; 7) percentage of soluble solids and acid in the juice; and 8) juice solids–acids ratio. The first 5 measurements and ratings were done on an individual fruit basis so that differences among samples could be analyzed using Duncan's multiple range test.

As expected, fruit-weight differences among samples of both cultivars were statistically significant (Table 1). Also, unexpectedly, significant differences were found among 'Temple' samples for length/width ratio, number of seeds, and rind thickness, and among 'Willowleaf' samples for length/width ratio.

To ascertain whether these differences represented true differences that might be genetic in nature, normal variation, or an error in sampling, fruit quality analysis, and/or statistical analysis methods, another set of samples was collected from the field. One 'Willowleaf' mandarin tree of shoot-tip graft origin with a medium-heavy crop load was chosen, and 15 samples were collected from this tree by walking around it 15 times and collecting the samples in the same random manner as before. There were not enough fruit to do this with 'Temple' tangor.

These 'Willowleaf' samples were then analyzed as above. Statistically significant differences among samples from this one tree occurred for fruit weight, length/width ratio, rind thickness, and seed count (Table 1). The data in Table 1 are for the first 15 trees of 'Willowleaf' in the row rather than for all 26 trees; thus, the comparison is for 15 samples in all 3 cases. Since twice as many fruit characteristics measured (4 vs. 2) were significantly different among 'Willowleaf' samples from the same tree as among samples from different trees representing individual shoot-tip-grafted budlines, we conclude that differences in fruit characteristics among individual shoot-tip-grafted budlines did not represent genetic change but were due to normal variation.

It would appear, based on the production of 41 uniform budlines of 2 cultivars, that there need be no hesitancy in the use of the shoot-tip-grafting method for the production of pathogen-free citrus clones. Shoot-tip progeny of 'Temple' tangor, 'Washington' navel orange [*Citrus sinensis* (L.) Osbeck], 'Clementine' mandarin (*C. reticulata* Blanco), and 'Bearss' lime [*C. aurantifolia* (Christm.) Swing.] grown in test locations at Riverside and Lindcove, Calif., have also been consistent in yielding uniform and apparently true-to-type fruit without exception. Additional shoot-tip-cultured citrus cultivars just now coming into bearing also appear normal to date.

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