Table 3. Effects of treatments on spring flush of 'Minneola' tangelo trees, sprayed on 22 Feb. measured on 22 May 1984.[z]

Treatments	Percentages of control[y]			
	No. of shoots	Total length	Avg. shoot length	Avg. intern. length
Control	100.0 a[x]	100.0 a	100.0 a	100.0 a
Morphactin 50 ppm	102.3 a	100.2 a	97.6 a	99.4 a
Morphactin 100 ppm	93.4 a	91.5 a	97.8 a	98.2 a
Morphactin 200 ppm	101.4 a	93.6 a	92.2 a	100.0 a
Paclobutrazol 500 ppm	96.3 a	71.5 b	74.2 b	90.3 b
Paclobutrazol 1000 ppm	91.1 a	60.2 c	66.1 c	78.8 c
Paclobutrazol 1500 ppm	93.0 a	60.9 c	65.5 c	80.6 c

[z]Average of 3 branches on each of 6 replicate trees per treatment.
[y]Results are presented as percentages of control, but statistics have been calculated on original data.
[x]Mean separation in columns by Duncan's multiple range test, 5% level.

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A Citrus Embryo Assay to Screen Water-soluble Resins as Synthetic Seed Coats

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Additional index words. *Citrus sinensis* x *C. reticulata, C. sinensis* x *Poncirus trifoliata*

Abstract. **An assay using in vivo-produced embryos, with and without tegmen, of monoembryonic 'Temple' tangor [*Citrus sinensis* (L.) Osbeck x *C. reticulata* Blanco) and polyembryonic 'Troyer' citrange (*C. sinensis* x *Poncirus trifoliata* Raf.) was developed to screen compounds as synthetic seed coats for in vitro-produced, asexual embryos. Of 8 compounds evaluated, Polyox WSR-N 750 proved to be the most suitable as a synthetic seed coat based on its film-forming ability, its ability to redissolve in water, and its nondeleterious effects on citrus embryos.**

The production of asexual embryos and their subsequent development suggests that in vitro-produced embryos of exalbuminous seed (those lacking endosperm at maturity) could be transformed into seeds if encapsulated with a synthetic seed coat. The objective of this research was to develop a system, using in vivo-produced embryos, to evaluate synthetic coating compounds. The concept was to replace natural coats with a synthetic coat and compare their protective properties.

Citrus seeds were chosen to screen synthetic coating compounds because of their reputed sensitivity to drying (1, 2, 6, 7), relatively large size, and ease of handling. Citrus seed has 2 easily removable coats, a thick, outer testa and a paper-thin inner tegmen. The assumption was made that citrus embryos with 1 natural seed coat (tegmen), when stressed by drying, would have a viablity advantage over embryos lacking a coat, and that this feature could be exploited to screen coating compounds applied to embryos as synthetic seed coats.

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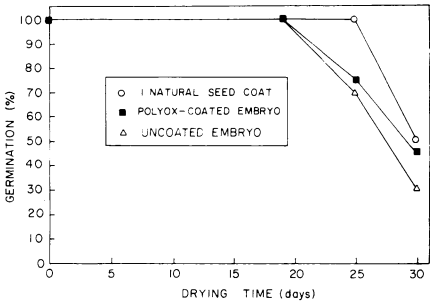

Fig. 1. Effect of drying time and seed coat treatment on germination of 'Temple' tangor seed. Each treatment consisted of 10 embryos.

Registered seeds of monoembryonic 'Temple' tangor and polyembryonic 'Troyer' citrange were disinfested in 0.5% NaClO (10% Clorox) plus 0.01% Tween 20 for 10 min, rinsed 3 times in sterile distilled water, and either one or both seed coats were removed. In a preliminary trial, embryos of 'Temple' tangor either uncoated, containing 1 natural coat, or a synthetic coat of 1% (w/v) Polyox WSR-N 750 (Table 1) were dried up to 30 days at room temperature and ambient humidity prior to germination for 12 days at 26°C in petri dishes (60 × 15 mm) containing moistened, Whatman No. 1 filter paper. Uncoated embryos had lower germination than embryos with 1 natural or synthetic coat after 25 days of drying, suggesting that 1 natural or synthetic coat gave embryos a viability advantage (Fig. 1).

A quicker and more reproducible drying environment was achieved by using a desiccator containing anhydrous $CaSO_4$ (Drierite) as a desiccant. Radicle length of tegmen-coated and uncoated embryos declined after a drying period in a desiccator up to 8 days (Fig. 2), although constant weight was

Table 1. Chemical compounds screened for synthetic coating properties.

Product	Chemical makeup	Source
Polyox WSR-N 750	Polyethylene oxide homopolymer	Union Carbide Corp., New York, N.Y.
Viscalex HV-30	Acrylic copolymer containing carboxyl groups	Allied Colloids, Inc., Fairfield, N.J.
Viterra II	Potassium propenoate-propenamide copolymers	Nepera Chem. Co., Inc. Harriman, N.Y.
Perfectamyl CMA-ZK	Carboxymethylized, cold-soluble swelling starch	Tunnel Avebe Starches Ltd., Gillingham, Kent, U.K.
Laponite XLS	Synthetic trioctahedral smectite	Laporte Industries, Ltd., Hackensack, N.J.
Laponite 508	Synthetic sodium magnesium lithium silicates	Laporte Industries,Ltd., Hackensack, N.J.
SGP 104	Starch plus synthetic polymer of acrylamide and sodium acrylate	Henkel Corp., Minneapolis, Minn.
Percol 511	Anionic flocculant	Allied Colloids, Inc., Fairfield, N.J.

Table 2. The effect of 8 coating compounds on vigor of 'Troyer' citrange embryos in response to desiccation stress.[z]

Embryo treatment		Radicle length (mm)	No. germinating embryos per seed	Contamination (%)
No. natural coats	Synthetic coat[y]			
1 (control)[x]		11.9 a[w]	5.0 a	1.0 a
0		9.3 ab	3.2 ab	1.0 a
0 +	Polyox WSR-N 750	9.2 ab	2.8 ab	6.6 ab
0 +	Viscalex HV-30	9.0 ab	3.2 ab	14.2 bc
0 +	Laponite XLS	7.8 ab	2.8 ab	4.8 ab
0 +	Viterra II	7.3 b	2.8 ab	6.6 ab
0 +	Perfectamyl CMA-ZK	6.2 b	1.8 ab	21.6 c
0 +	Laponite 508	5.8 bc	3.4 ab	10.4 ab
0 +	SGP 104	5.3 bc	0.6 b	35.6 d
0 +	Percol 511	1.6 c	0.5 b	76.2 e

Comparison	Correlation coefficient (*r*)
Radicle length vs. no. germinating embryos	+0.86**
Radicle length vs. contamination	−0.86**
No. germinating embryos vs. contamination	−0.81**

[z]Fifty embryos per treatment mean (5 petri dishes, 10 embryos per dish); 2 days in desiccator.
[y]Synthetic coats, 1%.
[x]Tegmen nicked after desiccation stress.
[w]Mean separation within columns by Duncan's multiple range test, 1% level.
**Significant at 1% level.

achieved after 2 days (Fig. 3). Embryos with 1 natural coat germinated more slowly than did uncoated embryos (Fig. 2), however, because the tegmen seemed to present a physical barrier to germination. This factor was eliminated by nicking the micropylar end of the inner coat after the desiccation stress.

A citrus desiccation (CD) assay was developed based on these and other studies (3) to screen potential synthetic coating compounds for asexual embryos. The protocol was as follows: Embryos of 'Temple' tangor or 'Troyer' citrange with 0 or 1 natural coats were used as controls. The synthetic coating compounds were mixed with sterile, distilled water into 1% solutions and applied to uncoated embryos. The coated embryos then were air dried for 3 hr. Controls and synthetically-coated embryos were dried in a desiccator for 2 days and then placed in petri dishes containing moistened filter paper for germination tests. The tegmen was nicked lightly before placing control embryos in petri dishes. Vigor of germinating embryos was determined after 6 days by measuring radicle length, contamination, and number of germinating embryos per polyembryonic seed in the case of 'Troyer' citrange. Seed was considered germinated if the radicle emerged at least 1 mm and regarded contaminated if visible bacterial or fungal growth was observed.

Eight synthetic coating compounds (Table 1) were screened using 'Troyer' citrange with the CD assay (Table 2). Radicle length was inversely correlated with extent of contamination ($r = -0.86$**), and directly correlated with number of germinating embryos per seed ($r = 0.86$**), indicating that all 3 variables were measures of vigor. None of the coating compounds improved vigor over that of uncoated embryos. In comparison to the control embryos (nicked tegmen), however, Percol 511 coating significantly increased contamination, and SGP 104 or Percol 511 coating significantly reduced the number of germinating embryos per seed. Radicle length of embryos coated with Polyox WSR-N 750, Viscalex HV-30, or Laponite XLS was not significantly lower than control embryos.

Use of Viscalex HV-30, a liquid, was discontinued because stock solutions became contaminated with fungal growth. Laponite XLS was unsuitable because it did not redissolve readily. Polyox WSR-N 750 was retained for further experiments because it dried to form a thin film (Fig. 4), did not support growth of contaminants, readily redissolved in water, and was nontoxic to embryos.

The viability of 'Temple' tangor and 'Troyer' citrange seeds after drying, as demonstrated in these studies, supports current findings that lemon, lime, and sour orange seeds remained viable after drying, although vigor decreased (4, 5). It was this reduction in vigor upon drying that was exploited in the CD assay to screen synthetic coating compounds. On the basis of this study, Po-

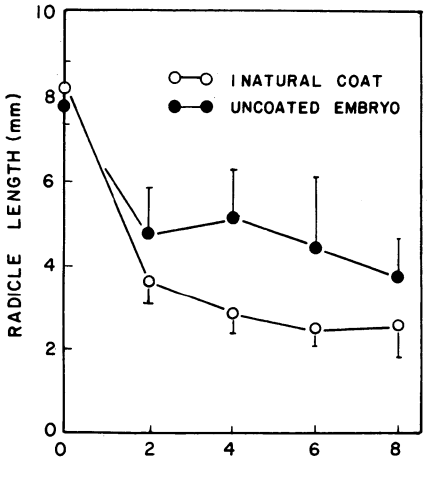

Fig. 2. Effect of desiccation time on radicle length of germinated 'Temple' tangor embryos. Each point is based on 6 embryos.

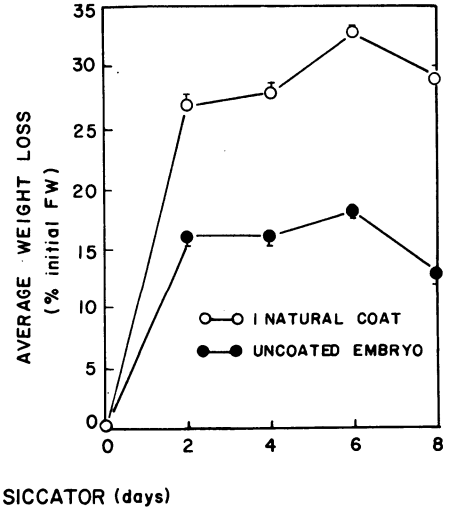

Fig. 3. Average weight loss of 'Temple' tangor embryos dried up to 8 days. Each point is based on 6 embryos.

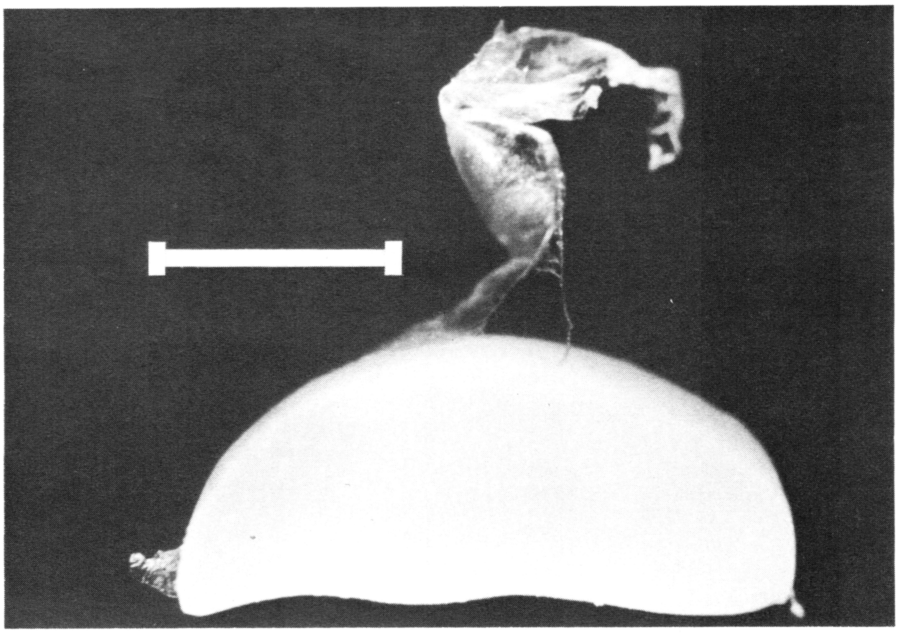

Fig. 4. A single cotyledon of a 'Temple' tangor coated with Polyox, partially removed to show its film forming ability (bar = 0.5 cm).

lyox WSR-N 750 was chosen for further consideration as a synthetic seed coat for in vitro-produced asexual embryos.

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Improving 'Manzanillo' Olive Seed Germination

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Additional index words. *Olea europaea*, stratification, chemical scarification

Abstract. **'Manzanillo' olive (*Olea europaea* L.) seeds were subjected to chemical scarification with NaOH and H_2SO_4 for various periods of time to determine the most appropriate treatment for improving the germination of the seeds. A critical balance of concentration and time was necessary to achieve high germination percentages without loss of viability of the seeds. H_2SO_4 was more effective than NaOH in increasing germination percentages. Germination percentages as high as 98% were obtained on stratified seed using H_2SO_4, compared to 0% without chemical scarification.**

Although several cultivars are used in olive production, none have the less pendulous form suitable for mechanical harvesting, resistance to *Verticillium*, or dwarf stature. These qualitites in a scion or rootstock would increase productivity and reduce production costs significantly. Yet, in spite of these needs, little breeding for cultivar and rootstock improvement is being conducted on olives. One reason for the lack of effort is the low germination percentage of olive seeds (1, 2, 6, 8, 10), often as low as 5% to 10%, especially in the highly desirable, large-fruited varieties grown in California (7).

Olive seeds can reach high germination percentages if the endocarp is removed and internal dormancy is eliminated (4, 5, 9). Crisosto (3) determined that the endocarp prevented olive seed germination by mechanical resistance of embryo expansion. Lagarda (7) reported that 'Manzanillo' embryos require 800–1000 hr of stratification at 15°C to obtain high germination percentages, but he worked only on seeds which had the endocarp removed.

Practical considerations preclude excising the embryo when many seeds are to be planted, as in a breeding program. Thus, it is imperative that a means for overcoming the restrictive role of the endocarp be found which would result in improving germination of 'Manzanillo' seeds. This paper describes the development of methods to improve the germination of 'Manzanillo' olive seeds planted intact with endocarp.

'Manzanillo' olive fruit were collected from a block of 5 trees in the Univ. of California, Davis, Pomology Experimental Orchard in 1981 and 1982, and the exocarp was removed with a seed cleaner. The heavy, well-developed seeds were separated from light, empty seeds by using an aqueous solution of common salt (30%, w:v). Seeds that floated in the solution had no embryo and were discarded.

Concentrated sulfuric acid (H_2SO_4) and 3% sodium hydroxide (NaOH) were used for scarification. Four samples of 60 seeds each were scarified with either H_2SO_4 or NaOH for various time periods. After scarification, the seeds were rinsed in cold running water for 15 min.

After scarification, seeds were mixed with moist vermiculite and placed in polyethylene bags for stratification at 15°C for 30 days. The seeds then were germinated for 30 days at 20° to 25°C under a 16-hr photoperiod. Seeds were evaluated for germination immediately after removal from stratification and after the 30-day germination period. Germination was determined as the total number of seeds with a radicle protruding, and of seeds that were split and showing a swollen, slightly elongated radicle.

Table 1. Effect of scarification with NaOH prior to stratification at 15°C on germination of 'Manzanillo' olive seeds harvested in 1981.

Scarification treatment (hr)	Germination 30 days after stratification[z] (%)
0	0
1	21
3	18
6	24
12	26
24	79
36	73
48	89
72	57
96	15
LSD	12.8

[z]Data analyzed after arc sine transformation.

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