Table 2. Calcium content in the flavedo tissue of the abaxial and adaxial sides of 'Marsh' grapefruit.

Orientation	Ca (mg g^{-1} dry wt)[z]
Abaxial side	9.06 ± 1.97
Adaxial side	9.61 ± 1.76

[z]Mean ± SD. Means were not significantly different from each other at 5% level.

was rated after 21 and 35 days of storage on a scale of 0 to 5 (0 = no injury, and 5 = severe injury). The total score for the lot was divided by the maximum possible score (30 fruit × a score of 5 = 150) to give the chilling injury index. An index of 10 represents unmarketable fruit.

Calcium determinations were made only on flavedo tissue, since chilling injury symptoms are restricted primarily to the outer layers of the peel. The flavedo was removed in 5-mm strips from around the equatorial position of 6 fruit from each canopy position and harvest. The strips were cut into 5-mm lengths and dried overnight in a drying oven at 60°C. One gram samples of the dried tissue were placed in platinum crucibles and dry ashed in a muffle furnace at 500°. The resulting white fine powder was dissolved in 2 ml of concentrated HNO_3 and then diluted volumetrically to 100 ml with deionized glass distilled water. Calcium content was determined with a Perkin-Elmer 503 Atomic Absorption Spectrophotometer at a wavelength of 422.7 nm and compared to a Ca standard.

Exterior canopy fruit from one grove were marked for orientation prior to harvest. The peel from the sun-exposed (abaxial) and shaded (adaxial) sides of the fruit were analyzed for Ca separately.

Fruit harvested from interior canopy positions developed significantly less pitting after 21 and 35 days of storage at 5°C than fruit harvested from exterior canopy positions (Table 1). Calcium levels in the flavedo tissue of interior and exterior canopy fruit were not different. In addition, Ca levels in the flavedo tissue of the sun-exposed surface were not different from the shaded surface of the same fruit (Table 2). The sun-exposed surface previously was found to be more susceptible to chilling injury than the shaded surface of the same fruit (7).

The severity of pitting in fruit stored at 5°C for 4 weeks was not significantly correlated ($r = -0.239$, n = 12) with the Ca level in the flavedo tissue. Calcium content of fruit exhibiting no chilling injury symptoms was similar to the Ca content in the flavedo tissue of fruit which was severely pitted (data not shown). Calcium content of the tissue also did not change during storage at 5° for 5 weeks.

In contrast to chilling injury of avocados (1), no evidence was obtained in this study to indicate that chilling injury of grapefruit is related to the endogenous Ca content of the flavedo tissue. Nagy et al. (4) recently reported only minor variations in the endogenous Ca levels in grapefruit peel throughout the season. However, seasonal trends in susceptibility to chilling injury consistently have been observed (8, 9). Thus, the variation in susceptibility of grapefruit to chilling injury does not appear to be related to endogenous Ca levels of the flavedo tissue. However, a certain threshold level of Ca in the tissue may be necessary to maintain membrane integrity and thereby enable grapefruit to resist injury during storage at low temperatures.

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Chemical Control of Vegetative Growth in Citrus Trees by Paclobutrazol

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Additional index words. 'Minneola' (*Citrus paradisi* x *C. reticulata*) tangelo, growth regulation, morphactin

Abstract. **Paclobutrazol (PP333) [(2RS,3RS)-1-(4-chlorophenyl)-4, 4-dimethyl-2-(1,2,4-triazol-1-yl-)pentan-3-ol] sprays applied to 'Minneola' tangelo trees, at 500 or 1000 ppm, before the onset of summer flush, markedly reduced total growth, number of shoots, and internode length. Soil treatments (0.4 and 0.8 g per square meter) had only a small effect, probably because of the short time from application to flush inception. Sprays of Morphactin (an auxin transport inhibitor), at 250 and 500 ppm did not reduce growth, but rather enhanced it by increasing the number of emerging shoots. The spring flush of the same trees also showed effects of paclobutrazol soil treatments, whereas other treatments were not different from control. Paclobutrazol sprays on comparable 'Minneola' trees just before the spring flush also reduced this growth. Paclobutrazol may become a tool for the control of vegetative growth of mature citrus trees.**

The chemical control of vegetative growth in citrus trees has not yet been attained. Growth retardants, effective on many deciduous woody perennials, have almost no effect on vegetative growth rates of citrus and other evergreen trees (6). Some of those retardants, however, influence flowering and fruit dimensions (7, 4). Other growth regulators have effects too strong to be acceptable, such as those growth inhibitors which prevent regrowth of branches (2) but cannot be used to retard shoot elongation.

A reliable chemical which would reduce shoot elongation would therefore be of great practical interest. Although it seems logical to use an inhibitor of biosynthesis of naturally occurring gibberellins, those which have been tried until now have had little effect, especially when sprayed on leaves. (1) Paclobutrazol has been reported as very effective in reducing growth of different plants, including deciduous fruit trees (3, 9, 10). Tests of paclobutrazol on mature citrus trees have given some success, and these results

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are presented here. A morphactin (methylflurenol, EMD 7301W) believed to inhibit growth through the inhibition of auxin transport (11), was sprayed for comparison with paclobutrazol, at concentrations just under the threshold for damage (S.P. Monselise, A. Goren, and A. Shemi, unpublished data).

Summer flush growth, composed of relatively few but long branches, is considered as the main growth whereby citrus trees increase dimensions of their leafy crown, more than flushes occurring at other seasons. Moreover, since it neither occurs at the blooming season nor directly carries flowers, it is the ideal flush to control in order to prevent excessive vegetative growth of the tree. The 'Minneola' tangelo, a relatively new acquisition by the Israeli citrus industry, is well-known for its vigorous growth, especially in relatively young nucellar clones.

'Minneola' trees (11 years from the planting of original 'Marsh' seedless grapefruit trees on rough lemon stock, and 4 years from topworking 'Minneola' on its scaffold branches), growing on light soil, at Kevutzat Schiller on the coastal citrus belt of Israel, were used in this trial. There were 42 experimental trees, 4 m by 6 m apart, separated by guard trees in both directions, in a randomized block experiment, including 6 single tree replicates and 7 treatments. Treatments included: untreated control; a single spray of 250 or 500 ppm morphactin; a single spray of 500 or 1000 ppm paclobutrazol; soil treatment by either 0.4 or 0.8 g m^{-2} paclobutrazol. The dose was calculated on the basis of crown projection (16 m^2) rather than distances between trees (which would have required 25 m^2), as irrigation is by microjets which do not wet the whole orchard area. Soil treatments were dissolved in 10 liter H_2O applied in a shallow wide circular furrow at about 80 cm from the trunk, and washed down successively by 20 liters of H_2O. Soil treatment and sprays were applied on 1 June 1983, and measurements were taken on 15 July after the end of branch elongation. The length of all branches of the summer flush on each tree was categorized according to 3 lengths, averaging 10, 40, and 80 cm, respectively, and branches were counted separately for upper and lower portions of the tree ('upper' starting at 180 cm). The total length of shoots was calculated by multiplying average length by number of shoots for each tree portion. The average internode length was calculated from 3 shoots per tree by accurate measurement of length and number of nodes. Results are listed in Table 1.

Data (not presented) show that most flushes on these 'Minneola' trees were in the upper rather than lower portion of the crown (21.8 m new growth per tree in the upper vs. 13.8 in the bottom portion of the tree). Since trees were about 4 m high, this difference was due to increased growth, not to greater volume of upper portion of the trees. In general, treatments which caused long branches also caused long internodes. The morphactin treatments, however, increased rather than decreased total shoot length. This increase was due to about twice as many shoots, compared to control, plus a minor increase in internode length. However, the total increase in length was less than 2-times, because average shoot length was reduced by morphactin. Tree sprays of paclobutrazol caused a remarkable reduction of growth, evident in all parameters reported in Table 1. Apparently the low concentration was enough, although there was a slight but significant decrease in internode length by doubling the paclobutrazol concentration. A significant reduction in internode length and a slight reduction in shoot number and total length also was caused by soil treatments; however, the relatively limited effect was probably due to the shortness of time elapsed between treatment and flush inception.

The residual effect of treatments on trees was examined in May 1984, to study the reactions of spring flush. Due to the great number of shoots emerging during the spring flush, 3 branches in each of 6 replicate trees were evaluated for shoot number and total shoot length. Results, presented in Table 2, show that sprays applied in the summer have no residual effect on the following spring flush. Soil treatments, however, had a strong residual effect on total shoot length per branch, individual shoot length and internode length of the following spring flush, as found on deciduous trees (3).

An additional spray experiment was initiated in February of 1984, before the start of the spring flush, and measured in May. Data in Table 3 show no effects of the relatively low morphactin concentrations applied to minimize possible damage to bloom. All paclobutrazol concentrations, however, caused a significant reduction on total and average shoot length and internode length. These effects are smaller than those experienced with the summer flush, possibly because spring shoots are much shorter than summer growth and carry flowers.

No visible damage to leaves, bloom, or fruit could be detected on all paclobutrazol treated trees by inspections carried out during more than one year.

Morphactin is believed to affect auxin transport (11), but did not, in this test, yield satisfactory results. Paclobutrazol is considered an inhibitor of gibberellin biosynthesis (3), and a reduction of shoot elongation could be expected on the basis of gibberellin effects on citrus shoot elongation (5). Nevertheless, other retardants, with similar effects on gibberellin biosynthesis, either did not affect elongation or required repeated treatments and high concentrations (8, 12). These results require additional trials with other citrus cultivars; if satisfactory results occur regularly, paclobutrazol may become very useful in controlling vegetative growth in mature citrus trees.

Table 1. Effects of morphactin and paclobutrazol treatments on the growth of summer shoots in 'Minneola' trees.

Treatments	No. of shoots[z] (No.)	(%)[x]	Total length[z] (m)	(%)[x]	Internode length[y] (mm)	(%)[x]
Control	33.4 b[w]	100.0	37.1 b	100.0	20.89 b	100.0
Morphactin 250 ppm	73.8 a	220.9	68.8 a	185.4	23.38 a	111.9
Morphactin 500 ppm	68.9 a	206.3	64.1 a	172.8	23.12 a	110.7
Paclobutrazol 500 ppm	15.1 c	45.2	14.6 c	39.2	16.99 d	81.3
Paclobutrazol 1000 ppm	14.6 c	43.7	15.1 c	40.7	15.97 e	76.4
Paclobutrazol (soil) 0.4 g m^{-2}	25.7 bc	76.9	25.6 bc	69.1	18.67 c	89.4
Paclobutrazol (soil) 0.8 g m^{-2}	22.6 bc	67.7	21.8 bc	58.9	17.50 d	83.8

[z]Based on all summer flushes of 42 experimental trees.
[y]Based on averages of 3 branches per tree, 42 experimental trees.
[x]Percentage of control.
[w]Mean separation in columns by Duncan's multiple range test, 5% level.

Table 2. Effect of treatments on 3 June 1983, on spring flush of 'Minneola' tangelo trees measured on 23 May 1984.[z]

Treatments	Percentages of control[y] No. of shoots	Total length	Avg. shoot length	Avg. internode length
Control	100.0 a[x]	100.0 a	100.0 a	100.0 a
Morphactin 250 ppm	98.7 a	101.1 a	103.9 a	98.1 ab
Morphactin 500 ppm	95.3 a	107.5 a	112.6 a	100.6 a
Paclobutrazol 500 ppm	100.4 a	97.2 a	96.5 a	96.8 ab
Paclobutrazol 1000 ppm	96.6 a	98.7 a	101.3 a	93.6 b
Paclobutrazol (soil) 0.4 g m^{-2}	85.2 a	66.6 b	78.1 b	86.0 c
Paclobutrazol (soil) 0.8 g m^{-2}	86.0 a	65.4 b	75.6 b	84.1 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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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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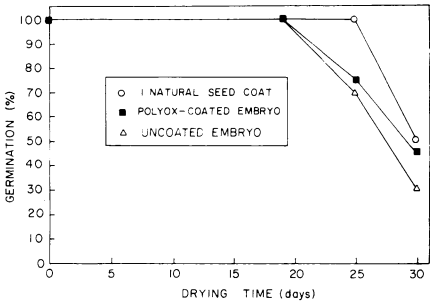

Fig. 1. Effect of drying time and seed coat treatment on germination of 'Temple' tangor seed. Each treatment consisted of 10 embryos.

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