August and September were 34.9 and 32.2°C, respectively, with average nighttime lows of 21.4 and 17.7°C, respectively.

All bougainvillea were tip-pruned (0.5 to 1 cm) on 3 Aug. Plants were then sprayed with dikegulac at 600 or 1200 ppm (Atrimmec; PBI/Gordon, Kansas City, Kan.) at the time of pruning or 2, 4, or 6 weeks later. Bougainvillea not sprayed at 4 weeks were pinched; these plants also were pruned for shaping when it was necessary. The experiment was set up as a completely randomized design with six replications per treatment. Plants were hand-watered as needed, usually every 2 to 3 days.

On 3 Oct., height, width, the number of structural branches (branches longer than 15.2 cm) with and without inflorescences, and the total number of inflorescences were recorded. The mean number of inflorescences per flowering structural branch was calculated. The overall aesthetic quality as a finished bougainvillea in the hanging baskets was rated on a scale where 1 = poor and 10 = excellent. Plants with an overall quality rating of 7 were considered marketable. Marketable plants were defined as well branched with many fully expanded bracts, and at least 15 cm growth beyond the pot perimeter. The rating values recorded were our consensus. Data were subjected to analysis of variance by general linear model (GLM) procedures (SAS Institute, Inc., 1985). Means were separated using Duncan's multiple range test at $P = 0.05$. Single-degree-of-freedom contrasts were conducted to determine the effect of timing of dikegulac treatment.

Marketable hanging baskets were produced 9 weeks after transplanting and pruning (WATP), but only when the bougainvillea were treated with 1200 ppm dikegulac 4 WATP. Nine weeks is the average production time for this climate during the summer (Hatten's Nursery, personal communication). The number of inflorescences per branch and overall aesthetic quality were higher for a single 1200 ppm dikegulac application 4 WATP than for the control (Table 1). Application of 600 ppm dikegulac at 4 and 6 WATP similarly enhanced flowering, but the overall quality of these bougainvillea was lower than for one application of 1200 ppm (Table 1), due to the proportion of bracts that had not yet fully expanded (no data collected). Therefore, dikegulac applied 4 WATP appeared to be the primary factor that promoted flowering during decreasing daylengths of late summer and early fall. Dikegulac at 1200 ppm did not appear to reduce bract size, although 1600 ppm appeared to reduce 'Barbara Karst' bougainvillea bract size $\approx 25\%$ to 50% as previously noted.

Height was unaffected by dikegulac (range 31.5–40.0 cm), but width tended to increase as time of application was delayed (Table 1). Bougainvillea treated with 600 ppm dikegulac 0 and 2 WATP, then pruned 4 WATP, generally had more pendulous growth habits, an observation not shown in the quantitative data. However, bougainvillea not pruned 4 WATP occasionally had upright shoots that

detracted from the overall aesthetic rating. Dikegulac had no effect on total number of structural branches per basket (13.1 ± 0.5).

In conclusion, marketable hanging baskets of 'Rainbow Gold' bougainvillea were produced from rooted liners in 9 weeks when treated with dikegulac under decreasing daylengths and high temperatures. However, one application of dikegulac did not reduce vegetative growth.

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HORTSCIENCE 27(1):36–39. 1992.

Calcium and Heat Treatments to Improve Storability of 'Anna' Apples

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Additional index words. *Malus domestica*, storage, cell walls

Abstract. Apples (*Malus domestica* Borkh. cv. Anna) were treated at harvest by a dip in 3% CaCl₂ solution, heated for 4 days at 38°C, or the two treatments combined, before being placed in OC storage. After removal of the apples from storage and holding them for 1 week at 20°C, the combined treatment maintained fruit quality best. The fruit remained firmer than with either treatment separately, and peel yellowing and decreased titratable acidity caused by the heat treatment were less pronounced. Heat treatment alone maintained fruit firmness, while CaCl₂ alone had no effect on fruit quality, although it raised the fruit calcium level more than the combined treatment in most experiments. Altering the temperature (0, 20, or 38°C) of the CaCl₂ dip did not change its efficacy. There was less soluble and more insoluble pectin in cell wall extracts of apples from the combined treatment than from other treatments. In addition, proportionally less Ca was present in the water-soluble pectin fraction of the combined treatment compared to other treatments, indicating different binding properties in the cell wall.

'Anna' is an early summer apple that stores very poorly. It ripens very quickly after harvest and softens even during cold storage. However, it has very low cold requirements for growth and for that reason is the main apple variety grown in many subtropical countries. It would, therefore, be beneficial

to enhance its storage properties and allow for a longer postharvest marketing period.

Calcium applications during the growing season or as postharvest dips are often used to enhance the storage life of apples (Poo-vaiah, 1986). Calcium has long been associated with regulation of fruit ripening. Specifically, maintenance of relatively high calcium concentrations in fruit tissues results in slowing of ripening, as seen in lower respiration rates, reduced ethylene production, and slower softening of the fruit flesh. There are also some specific fruit disorders, such as bitter pit, that can be prevented if sufficient calcium is present (Ferguson, 1984).

A postharvest heat treatment was also found to enhance the storability of 'Anna' and 'Granny Smith' apples (Klein and Lurie, 1990). The rate of softening of fruit after

Received for publication 31 Dec. 1990. This article is publication no. 3167-E, 1990 series, from the ARO, Bet Dagan, Israel. The research was funded in part by the United States-Israel Binational Agricultural Research and Development Foundation. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked advertisement solely to indicate this fact.

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Table 1. Ripeness characteristics of 'Anna' apples treated at harvest and then stored for 6 weeks at 0C and 90% relative humidity. Fruits were examined at removal from storage and following 7 days at 20C.

Treatment	Color ^a		Firmness (N)		Titratable acidity (%)	
	Removal	Holding	Removal	Holding	Removal	Holding
Control	4.8	5.2	60.8	52.1	0.60	0.55
3% CaCl ₂	4.9	5.0	61.7	55.9	0.61	0.54
38C, 4 days	5.6	5.9	65.7	59.7	0.55	0.44
38C, 4 days + 3% CaCl ₂	5.3	5.3	72.6	68.6	0.58	0.49
LSD _{0.05}	0.39		2.16		0.38	

^aColor was measured with a Techwest Apple Color Meter, where 1 = green and 10 = yellow.

Table 2. Calcium content and firmness of 'Anna' apple flesh heated 4 days at 38C, dipped in 3% CaCl₂, or both treatments together. Fruits were examined at harvest, following 7 days at 20C after harvest or heat treatment, and after storage for 1 month at 0C and 90% relative humidity followed by 7 days at 20C.

Time of examination and treatment	Ca (mg/100 g fresh wt)			Firmness (N)
	Soluble	Insoluble	Total	
Harvest	2.72	2.83	5.55	72.4
Holding after harvest				
Control	2.71	2.72	5.43	49.7
4 days 38C	2.78	2.32	5.50	57.5
Holding after storage				
Control	2.41	2.30	4.71	54.8
4 days 38C	2.33	2.61	4.71	61.6
3% CaCl ₂	5.04	4.68	9.72	56.8
4 days 38C + 3% CaCl ₂	3.56	3.46	7.02	70.7
LSD _{0.05}	0.52	0.57	0.75	3.14

Table 3. Calcium content of acetone-insoluble solids (AIS) prepared from 'Anna' apples at harvest or after 1 month of storage followed by 7 days at 20C.

Time of examination and treatment	Ca content (µg/100 mg AIS)	
	Water soluble	AIS
Harvest	30	222
Following storage and holding		
Control	46	236
4 days 38C	55	255
3% CaCl ₂	84	420
4 days 38C + 3% CaCl ₂	47	407
LSD _{0.05}	24.9	84.2

storage was greatly reduced by holding the apples at 38C for 4 days before storage. Ethylene production was also inhibited by the heat treatment, but recovered during shelf life. Respiration was depressed following the treatment (Klein and Lurie, 1990; Lurie and Klein, 1990).

A combination of these two treatments was examined to determine whether it was possible to achieve an additive effect on extension of storage and shelf life of 'Anna' apples. Dipping 'Anna' and 'Granny Smith' apples in Ca before the heat treatment did not raise the Ca level of the fruit, nor enhance storability over that conferred by heating alone (Klein et al., 1990). However, the Ca dip at the end of the heat treatment was found to be beneficial to the apples. The current report describes experiments to optimize the heat treatment and subsequent Ca dip. It ex-

amines the effect of treatments alone and together on fruit softening, cell wall pectic components, and ripeness characteristics after storage. In addition, the distribution of the Ca in different subcellular compartments is analyzed.

Apples were harvested during the commercial harvest period when the fruit background color was still green and firmness was around 70 N. The fruits were divided into three lots. One lot was placed immediately in cold storage (0C, 90% relative humidity), another was dipped for 5 min in 3% CaCl₂ solution and then placed in cold storage, while the third lot was placed in a heating chamber for 4 days. The Ca dip was normally performed at 20C, but in one experiment it was conducted at 0, 20, and 38C. The heating chamber was thermostatically controlled and had forced air circulation. The usual heat treatment was 4 days at 38C. Trays of water were placed on the chamber floor to maintain humidity, and each plastic box containing 50 apples was covered with a plastic bag to retard weight loss. Humidity within the unsealed plastic bag was > 97%. At the end of the heat treatment, the apples were either placed in 0C storage or dipped in 3% CaCl₂ for 5 min before storage.

At harvest, upon removal from storage and following holding for 1 week at 20C, four replicates of 10 fruits per treatment were analyzed for peel color, firmness, titratable acidity (TA), and soluble solids content (SSC). Color was measured with a Techwest Apple Color Meter (Techwest Enterprises, Vancouver, B.C., Canada) with a scale of 1 to 10, where 1 = green and 10 = yellow.

Pressure tests for firmness were made with a Hunter-Spring penetrometer (Hunter-Spring Corp., Hatfield, Pa.; 11-mm tip) on opposite pared sides of the apples. SSC and TA were measured on expressed juice from peeled sections of fruit. The former was determined with a hand refractometer and the latter by titrating the juice with 0.1 N NaOH to pH 8.2 and expressing the result as percent malic acid.

Calcium concentration of the fruit flesh was determined by atomic absorption. For soluble and insoluble Ca, four replicates of 50 g of fruit (10 g from each of five apples) of each treatment were ground in 40 ml of water. The slurry was centrifuged at 20,000 × g for 10 min and the supernatant taken for soluble Ca. The pellet was lyophilized, acid-digested, and taken as insoluble Ca. Total flesh Ca was also taken from four replicates of 50 g (10 g from each of five fruits) for each treatment. The samples were frozen, lyophilized, acid-digested, and the content of Ca determined.

Calcium and pectic fractions were also measured in acetone powders [acetone-insoluble solids (AIS)] prepared from apple flesh. Four replicates of each treatment were prepared, and aliquots of each powder (100 mg) were serially extracted in water, 0.5% EDTA, and 0.4 mg pectinase (Sigma, St. Louis)/liter. The galacturonic acid concentration in each filtrate was measured by the method of Blumenkrantz and Asboe-Hansen (1973) and expressed as water-soluble pectin, calcium pectate, and insoluble pectin.

Analyses of variance and statistical significance were calculated using SAS software (SAS Institute, 1985).

In the first season, 'Anna' apples were stored for 6 weeks and then held for 1 week at 20C. Their ripeness characteristics were determined at removal and at the end of the holding period (Table 1). The Ca dip alone minimally affected the ripeness characteristics; firmness was slightly enhanced by the Ca dip. Heating the apples before storage affected all the ripeness characteristics, except SSC, which varied from 11.2% to 13.0% (data not shown). Background color change of the peel from green to yellow was enhanced, TA was lowered, and flesh softening was decreased. These differences between heated and unheated apples were apparent at removal of the apples from storage and after holding them at 20C. The combination treatment of heating plus Ca dip mitigated both the peel color change and the loss of TA, while it enhanced retention of flesh firmness.

In a separate, but similar experiment, heat-treated and control apples had about the same Ca concentrations and pattern of Ca distribution, equally divided between soluble and insoluble fractions (Table 2). However, following holding at harvest and after storage, control apples softened much more than heated apples. The Ca dip alone raised the Ca level in the flesh more than heat treatment followed by Ca dip, although the distribution remained evenly divided between soluble and insoluble fractions. The Ca concentration of dipped fruit was double that of control fruit,

Table 4. Galacturonic acid residues in water-soluble, calcium pectate, and insoluble pectin fractions of acetone-insoluble solids from 'Anna' apples and their firmness at harvest and after 1 month of storage followed by 7 days holding at 20C (percentage of total).

Time of examination and treatment	Water-soluble pectin		Calcium pectate		Insoluble pectin		Firmness (N)
	(mg·g ⁻¹)	(%)	(mg·g ⁻¹)	(%)	(mg·g ⁻¹)	(%)	
Harvest	0.23	11.3	0.16	8.4	1.74	80.3	72.4
Following storage and holding							
Control	0.70	33.6	0.35	16.8	1.04	49.6	54.8
4 days 38C	0.58	26.8	0.39	17.6	1.22	55.6	61.6
3% CaCl ₂	0.59	30.1	0.33	16.8	1.04	53.0	56.8
4 days 38C + 3% CaCl ₂	0.47	21.7	0.47	19.3	1.34	59.0	70.7
LSD _{0.05}	0.15		0.12		0.37		3.1

and heated fruit dipped in Ca had 42% more Ca than heated fruit alone. However, the effect on fruit firmness was much more pronounced in the heated plus Ca-dipped fruit than in Ca-treated fruit alone (Table 2). It appeared that there was a qualitative aspect to Ca concentration that was not associated with the quantitative amount.

Calcium levels were raised 78% in the AIS of CA-dipped apples compared with control apples and 59% in heated and Ca-dipped apples compared with heat treatment alone (Table 3). The striking difference is that Ca in the combined treatment did not elute into the water-soluble fraction to the same extent as it did in the other treatments. Of the total AIS Ca, 11% was found in the water-soluble fraction in heated and Ca-dipped apples, whereas in the other treatments, it was ≈ 20%. The additional Ca in the fruit of the combined treatment was apparently bound in a different manner than that of fruit in the other treatments.

Heated apples contained less soluble pectin and more insoluble pectin than the controls (Table 4). At harvest, 80% of the galacturonic acid was in the insoluble pectin fraction, while after storage followed by holding at 20C for 7 days, it dropped to 50% in the control apples, 55% in heated apples, and 59% in the combined treatment (Table 4). An opposite pattern existed for galacturonic acid in the water-soluble and calcium pectate fractions of the apples from the various treatments. When the treatments were rated from the firmest fruit (heat and Ca) to

the softest (control), the water-soluble pectin fraction varied inversely with firmness. The largest amount of water-soluble pectin was present in control fruit and the least in those of the combined treatment. Calcium pectate and insoluble pectin levels were higher in the firm fruit than in the softer fruit. However, the amount of galacturonic acid in these fractions did not always significantly differ among the various treatments.

The Ca dips had very little effect on unheated apples, regardless of the dip temperature. The exception was the 38C dip, which decreased flesh softening and loss of TA during storage. After holding the apples for 1 week at 20C, apple firmness was similar to that of control apples, although the TA was still higher. None of the Ca dips affected fruit color.

Heating the apples for 4 days at 38C before applying Ca dips led to differences in ripening characteristics. Fruit yellowing, caused by heat treatment, was mitigated somewhat by Ca dips, but the differences were mostly nonsignificant. Apples dipped at 20 or 38C were softer at removal than those only heated, but the apples softened less during the week at 20C. Calcium dips reduced TA loss during storage, but by the end of 1 week at 20C, these differences were no longer apparent. SSC was not affected by treatment (data not shown).

The increase in Ca content due to the dips ranged from 20% to 56%, with the highest increase resulting from the 38C Ca dip for unheated apples and the dip at 20C for heated

apples (Table 5). Again, ripeness characteristics bore no connection to the amount of Ca in the tissue. In unheated apples, the 38C Ca dip enhanced the Ca level 56% with no significant effect on fruit firmness, while in heated apples, the 20C Ca dip raised Ca levels 53% without enhancing fruit firmness more than the heating alone.

The combined treatment of 4 days at 38C and then a dip in 3% CaCl₂ solution before storage was found to maintain fruit quality after storage better than with either treatment alone. Fruit remained firmer than in either treatment separately, and yellowing of the peel and decreased TA (both enhanced by heat treatment) were less pronounced.

The marked reduction in softening, which was the main benefit of heating, was observed by Liu (1978) and Porritt and Lidster (1978) in other cultivars. The reasons for the slower softening are not entirely clear, but may be due to inhibition of cell wall-degrading enzymes. This was indirectly determined from the polyuronide content (water-soluble, calcium pectate, and insoluble pectins) of pectic fractions prepared from cell wall extracts. During holding at 20C, the insoluble pectin fraction remained larger in heated apples, and the water-soluble fractions were smaller. Porritt and Lidster (1978) found lower levels of soluble pectin in juice of heat-treated 'Golden Delicious' apples than in control fruit, but no differences were found in 'Spartan' apples treated likewise.

Calcium treatments raised flesh Ca levels between 20% to 100% over that in untreated apples. However, the effect on retention of fruit firmness was not related to the amount of Ca in the fruit. The fruit was potentiated to use the Ca more effectively by a heat treatment before the Ca dip: Ca generally did not increase as much from a dip following heat treatment as from a dip of nonheated apples. Nonetheless, the ripeness characteristics of heated apples were more affected by Ca than were those of unheated apples. In addition, we found that dipping the apples before the heat treatment did not confer the benefits that were found when the treatments were reversed (Klein et al., 1990).

The Ca content of AIS was raised in both unheated and heated apples dipped in Ca. However, only half as much Ca eluted into

Table 5. Ripeness characteristics and Ca content of 'Anna' apples dipped in 3% CaCl₂ at 0, 20, or 38C at harvest, or heated at 38C for 4 days and then dipped in Ca. Following treatment, the apples were stored for 6 weeks at 0C and 90% relative humidity and then removed for 1 week at 20C.

Time of dipping and temp (°C) of Ca dip	Color ^a		Firmness (N)		Titratable acidity (%)		Ca content (mg·100 g ⁻¹ fresh wt)
	Removal	Holding	Removal	Holding	Removal	Holding	
At harvest							
No Ca	4.4	4.3	66	48	0.80	0.71	7.6
0	4.1	4.1	62	47	0.79	0.66	9.2
20	4.0	4.4	66	48	0.77	0.63	10.5
38	3.8	4.4	69	50	0.87	0.78	11.9
After heating							
No Ca	4.7	5.5	71	58	0.50	0.53	6.7
0	4.9	5.3	69	59	0.71	0.55	8.8
20	4.6	5.1	66	60	0.70	0.51	10.3
38	4.3	5.3	67	61	0.66	0.56	8.1
LSD _{0.05}	0.51		3.9		0.05		1.5

^aColor was measured with a Techwest Apple Color Meter, where 1 = green and 10 = yellow.

the water-soluble pectin fraction of heat- plus Ca-treated apples as in the other treatments. This result suggests that the increased Ca in the combined treatment may have different binding sites than Ca dips of unheated fruit and that this may contribute to the enhanced firmness measured in this treatment.

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HORTSCIENCE 27(1):39-41. 1992.

Controlled-atmosphere Storage of Sugar Peas

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Additional index words. weight loss, chlorophyll, soluble sugars, insoluble solids, soluble protein, subjective quality

Abstract. Sugar peas (*Pisum sativum* var. *saccharatum* cv. Manoa Sugar) were stored for 14 or 21 days under controlled atmospheres (CA) of 21% or 2.4% O₂, plus 0%, 2.6%, or 4.7% CO₂ at 10 or 1°C. Changes in appearance, weight, and in the concentrations of chlorophyll, total soluble sugars, insoluble solids, and soluble protein were evaluated before and after storage. After 14 days of storage at 10°C there were minor changes in all indicators of quality under the various storage conditions, but the appearance of sugar peas was better under CA than under 21% O₂. When quality was evaluated after 21 days, however, storage under CA at 10°C was not as beneficial as storage in 21% O₂, at 1°C. Holding peas in 2.4% O₂, for up to 3 weeks at 10°C, a higher than recommended storage temperature, maintained better quality than 21% O₂. Increasing the CO₂ concentration from 0% to 2.6% or 4.7% had no adverse effects on quality and had a beneficial effect in some treatments. Compared with storage in 21% O₂, the appearance of the peas was better, the concentrations of chlorophyll and soluble sugar were maintained at higher levels, and the insoluble solids were decreased in all atmospheres with 2.4% O₂. Appearance and concentrations of chlorophyll, soluble sugars, and proteins were maintained at 1°C regardless of treatments.

Very little information is available on the recommended CO₂ and O₂ levels for controlled atmosphere (CA) storage of the sugar or edible podded pea. Controlled-atmosphere research on unshelled peas showed that color and flavor were maintained under storage at 5°C in 5% CO₂ for 20 days (Tomkins, 1957). Chlorophyll levels of 'Harvester' green beans were retained at 7°C for

14 days in 2% to 3% O₂ + 5% to 10% CO₂ (Groeschel et al., 1966). Sugars were retained in peas (Miller and Brooks, 1932) and lima beans (Miller and Dowd, 1936) when stored in 42% CO₂ for 1 to 2 days at 15, 20, or 25°C. Oxygen at 1% was more beneficial than elevated CO₂ in maintaining the chlorophyll and sugar levels in the outer laminae of Chinese cabbage (Wang, 1983).

In this study we examined the use of CA for the storage of sugar peas at temperatures higher than the recommended 0°C Hardenburg et al., 1986). Changes in appearance, weight, and concentrations of chlorophyll, total sugars, insoluble solids, and soluble protein were monitored as measures of quality.

Sugar peas (cv. Manoa Sugar) were either hand harvested from local growers near Honolulu, Hawaii, or obtained from commercial sources in Honolulu within 1 day of harvest. Uniform pods free of visual defects were selected and randomly sorted into groups of 25 or 45 pods and placed in 0.4- or 1-liter jars. Three jars were used per atmosphere and each experiment was repeated between Mar. and May 1987. The sugar peas were weighed and rated for appearance before and after the storage treatments. There are no industry quality standards for sugar peas; therefore, a subjective index was devised similar to that for lettuce (Kader et al., 1973) with modifications: 9 = green calyx and pod, free from defects and firm; 7 = green calyx and pod, minor defects, slightly wilted; 5 = slightly brown calyx, green pod, obvious defects on pod, wilted pod; 3 = brown, shriveled calyx, obvious defects on pod, obvious wilt, slight infection from disease; and 1 = unsalable. Data are presented as percent of initial values because of the high degree of variability among the three experiments.

A flow-through system (Morris, 1969) with flow rates of 20 or 14 liter·(kg·hr)⁻¹ was used at 10 or 1°C, respectively. The flow rates were based on respiration rates of garden peas (Tewfik and Scott, 1954). The selected flow rates maintained the O₂ and CO₂ concentrations within ± 0.3% of the desired values. All gases at 1°C were humidified, while only N₂ was humidified at 10°C. Daily measurements of CO₂ and O₂ were made with an infrared CO₂ analyzer (Model IR 703, Infrared Industries, Santa Barbara, Calif.) and a paramagnetic oxygen analyzer (Servomex Model 570A, Sybron Boston, Mass.), respectively. Weight loss was calculated from the difference between initial and final weight and expressed as a percentage of the initial fresh weight. Compositional data were adjusted for weight loss. The jars were held at 10°C for 14 and 21 days or at 1°C for 21 days.

Sugars were extracted from pod tissue that had been chopped with a razor blade and mixed. A 2-g sample was placed in 20 ml of cold 95% ethanol and held at -16°C for 2 weeks (Pauli et al., 1984). The supernatant was diluted and soluble sugars assayed colorimetrically (Dubois et al., 1956). Glucose was used as the standard.

For further compositional analyses, three

Received for publication 22 Oct. 1990. Accepted for publication 6 Aug. 1991. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.