

# Comparison of Softening-related Changes during Storage of ‘Honeycrisp’ Apple, Its Parents, and ‘Delicious’

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**ABSTRACT.** Many studies of apple (*Malus ×domestica* Borkh.) softening have been done using cultivars that eventually become mealy. We wanted to determine whether observations in these studies would be seen in a cultivar that maintains its crispness. In this paper, we compared the texture, ultrastructure, and some physiological parameters of Honeycrisp, an apple cultivar introduced in 1991 by the Minnesota Agricultural Experiment Station, with its parents and Delicious. Sensory evaluations and instrumental texture measurements showed that ‘Honeycrisp’ maintained a crisp texture from harvest through 6 months of cold storage, whereas its parents, ‘Macoun’ and ‘Honeygold’, softened over the same time period. Turgor potential, cell wall composition, and ultrastructural comparisons of the fruit were made. Cell turgor potentials of ‘Honeycrisp’ and ‘Delicious’ were similar and greater than those of ‘Macoun’ and ‘Honeygold’, and clearly correlated with firmness. There were no differences in cell wall neutral sugar composition, except for arabinose, which was not highly correlated with crispness. ‘Honeycrisp’ fruit maintained cell wall integrity after 6 months of storage, while cell walls of ‘Macoun’ and ‘Honeygold’ deteriorated. These data show that it is important to compare more than one cultivar when studying crispness. Honeycrisp is a cultivar that maintains its crispness through long storage without controlled atmosphere conditions. After 6 months of storage, this crispness can be attributed to a maintenance of high turgor potential and cell wall integrity.

Fruit texture is a complicated quality (Jackman and Stanley, 1995). It has been studied at the whole fruit level, using sensory evaluations and physical measurements, to the cellular (turgor potential and ultrastructural examinations) and subcellular (biochemical determinations) levels.

For ripening of stored apples, changes in the firmness, cell wall chemistry, and ultrastructure of the tissue have been well documented. Most of the studies documenting these changes have been done with a single cultivar, such as Golden Delicious (Fischer et al., 1994; Glenn and Poovaiah, 1990; Gross and Sams, 1984; Roy et al., 1997; Siddiqui et al., 1996) or Cox’s Orange Pippin (Bartley, 1976; Knee, 1973, 1978; Stevens and Selvendran, 1984; Stow, 1993). A few studies have used other cultivars, but only one cultivar per study (Ben-Arie et al., 1979; Ben Shalom et al., 1996; Harker et al., 1996; Ross et al., 1994; Schoorl and Holt, 1983; Stow, 1989; Yoshioka et al., 1992). Using sensory evaluations or textural measurements, these cultivars have been shown to soften or turn mealy during ripening or storage.

To understand why these fruit soften, researchers have used microscopy and done biochemical studies of the cell wall. Ben-Arie et al. (1979) described the major ultrastructural change in softening

‘Calville de San Sauveur’ apples as “dissolution of the middle lamella,” although areas containing plasmodesmata retained their structure. In one of the few studies comparing several apple cultivars differing in their firmness, Lapsley et al. (1992) concluded that mechanical forces applied to firm apples causes structural failure through cells, while in mealy apples, the failure is between cells. The structure of firm apples used in this study was similar to that of ‘Golden Delicious’ apples treated with calcium (Glenn and Poovaiah, 1990). Harker and Hallett (1992) also showed that in mealy ‘Braeburn’ apples stored for 4 months, failure occurs at the middle lamella between cells. These studies suggest that dissolution of the middle lamella plays a large role in the loss of apple fruit texture.

Biochemical studies have focused on changes in the cell wall. Knee (1973) showed that levels of cell wall arabinose, galactose, glucose, and galacturonic acid decrease during storage of ‘Cox’s Orange Pippin’. Bartley (1976) reported less dramatic decreases in cell wall arabinose and galactose. An increase in soluble polyuronides in the same cultivar was correlated with a decrease in fruit firmness. However, in a comparison of preclimacteric and postclimacteric ‘Golden Delicious’ fruit, Gross and Sams (1984) found no change in cell wall arabinose, an increase in xylose, and decreases in glucose and galactose levels. On the other hand, Glenn and Poovaiah (1990) and Ben Shalom et al. (1993) reported decreases in arabinose and galactose levels in ‘Golden Delicious’ apples during 5 or 7 months of storage. Fischer and Amadò (1994) reported similar results, but did not find a decrease in galacturonic acid content for ‘Golden Delicious’ apples stored for 6 months. Neutral sugar analyses were performed by gas chromatography of alditol acetate or trimethylsilyl derivatives in all of these studies except in those by Knee (1973 and 1978), and the ranges of values were similar in all studies. When decreases were observed in arabinose and galactose levels, initial values were greater than when no or slight differences were detected. Siddiqui et al. (1996) found that levels of Na<sub>2</sub>CO<sub>3</sub>-soluble pectin and hemicellulose decrease during storage of ‘Golden Delicious’ apples, while levels of water-soluble pectins increases. Ben Shalom et al.

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(1996) obtained similar data for 'Anna' apples stored for 2 months, and found a decrease in cell wall galactose but not in arabinose during this time period. Hence the roles of cell wall neutral sugars and uronides in apple fruit texture are unclear.

Unlike the cultivars used in the above studies, Honeycrisp is a cultivar introduced by the Minnesota Agricultural Experiment Station in 1991, which remains crisp through 9 months of cold storage under normal atmospheric conditions (Luby and Bedford, 1992). The parents of 'Honeycrisp' are 'Macoun' and 'Honeygold', which soften noticeably within 6 months of cold storage. We wanted to determine whether changes in the studies described above would be observed in 'Honeycrisp' and its parents. We compared the textural, ultrastructural, and cell wall and turgor potential changes occurring during their storage. Such a comparison of a long-storing cultivar with short-storing parents would be useful in determining what characteristics may be important in breeding for crispness. Delicious was included in this study because it is a cultivar that is familiar to many people around the world.

## Materials and Methods

**PLANT MATERIAL.** Fruit from four cultivars (Honeycrisp, Delicious, Honeygold, and Macoun) were harvested from at least 10 trees and pooled at each of two sites. The sites were the Horticulture Research Center of the University of Minnesota in Chanhassan (northern site, lat. 45°52'N) and commercial orchards (southern site, lat. 43°10'N). Fruit were harvested when in the 3.0 to 4.0 range according to a starch test using 'Granny Smith' as a standard (1 = 100% starch, 6 = 0% starch). At least one fruit was harvested from each side (in the order north, east, west, south) of a tree, at a height of 90 to 150 cm, and within the first 30 cm from the periphery. Fruit were stored at 0 to 2 °C in open-topped cardboard boxes under ambient air conditions. Relative humidity varied between 92% to 97%. On each measurement date, fruit were randomized using a random number table and selected for each of the evaluations and analyses described below. Because fruit were harvested from many trees, pooled, then randomized, the experimental design was completely randomized with each fruit as an experimental unit. Cultivar, location, and storage time were considered as treatments.

Many 'Honeygold' fruit from the southern location were rotten by the sixth month of storage. Rotten fruit were discarded, and the remaining fruit were randomized for the sensory evaluations and turgor potential measurements. Because these measurements were done first, there were no fruit left for textural and ultrastructural studies.

**SENSORY EVALUATIONS.** Subjects were consumer panels of 72 students and staff from the University of Minnesota that liked apples and were available for the test. Many were employees or students of the Departments of Food Science and Nutrition or Horticultural Science at the University of Minnesota. They were paid for their participation. Some subjects participated in more than one of the storage tests.

Before testing, the apples were brought to room temperature, washed, cored and cut into eight wedges (except the 'Macoun' apples, which were small and were cut into six wedges). Immediately after cutting, the wedges were immersed into a mixture of 5 g Fruit Fresh, dissolved in 250 mL water, then placed into coded 120-mL cups and served. Slices were held no longer than 1 h between cutting and serving. Fruit Fresh has a taste base similar to that of apples, but its effects on the sensory evaluations were not documented.

Each judge received all nine samples at one time with nine separate scoresheets. Judges tasted the samples in random order and

rated their overall liking for the apple, their liking of the flavor and of the texture, and the crispness, firmness, mealiness, and juiciness. Ratings were made on line scales labeled at the ends. The hedonic scales were labeled dislike extremely at the left end and like extremely at the right end. Other scales were labeled none at the left and extreme at the right.

**ETHYLENE AND RESPIRATION MEASUREMENTS.** Ethylene and carbon dioxide production were measured from 10 fruit of each cultivar and location using an automated system (Watada and Massie, 1981) equipped with a gas chromatograph (model 5890; Hewlett Packard, Palo Alto, Calif.) with flame-ionization and thermal conductivity detectors and two 80/100 Porapak Q (182 × 318 mm) columns (Supelco, Bellefonte, Pa.).

**TEXTURE ANALYSES.** Fruit used in the ethylene and respiration measurements were used for texture analyses. Individual fruit were cut in half lengthwise. Half of a fruit was peeled, weighed, and frozen at -20 °C for later cell wall extractions. The other half of the fruit was used for texture analyses.

Texture analyses were done using a texture analyzer (TA.XT2; Texture Technologies Corp., Scarsdale, N.Y.). For fracture force measurements, 7-mm cylinders of apple cortex tissue were removed between the periphery and seed core from the equator of the fruit and placed on a stainless steel support with 2 anvils set ≈4 cm apart. Force was applied perpendicularly to the apple cylinder with a blunt-edged anvil descending at a speed of 0.5 mm·s<sup>-1</sup>. The force required to break the cylinder was recorded. Broken cylinders were put immediately into fixative for transmission electron microscopy.

Compression (firmness) was measured by taking a quarter of an apple, and cutting the skin off to form a flat surface. The quarter piece was laid skin side up on a flat platform. Force was applied to the piece using a round probe 1 cm in diameter descending at a speed of 0.5 mm·s<sup>-1</sup>. The force required to puncture the apple tissue to a depth of 5 mm was recorded.

**TURGOR MEASUREMENTS.** The turgor potential of cells located 150 to 1000 mm below the surface of an intact apple fruit were measured on a sample of three to four fruit from each cultivar, orchard location, and storage period unless stated otherwise. Fruit were removed from cold storage after 3 or 6 months and allowed to reach room temperature (≈20 °C) at least 2 h before turgor potential measurements. Due to instrument problems, measurements were not made for 1 month old apples. Turgor potential was measured with a micropressure probe (e.g., Steudle, 1993) using glass capillaries prepared as described by Shackel et al. (1987). The orientation of the tissue, probe, and microscope was similar to that described by Shackel et al. (1991), with the capillary parallel to the microscope plane of focus and penetrating the skin surface of the apple at right angles to a depth of 0.1 to 1 mm. To achieve this orientation with intact apples, a very long working distance (31 mm) microscope objective was used (20×, SL20, Mitutoyo, Yokohama, Japan), in combination with vertical illumination (BHMJ system, Olympus, Tokyo, Japan). The preparation of strong and sharply pointed glass capillaries presumably avoided the difficulties of tip breakage described by Steudle and Wieneke (1985) in apple, so that penetration through an intact apple skin was possible. The methodology used to measure the turgor of subepidermal cells was to press the probe tip into the tissue, sacrificing the epidermal and first few subepidermal cells in order to obtain an observable oil/cell sap boundary external to the tissue, as described in Cosgrove and Cleland (1983). It was usually possible to penetrate the tissue, obtain an observable oil/sap boundary, and subsequently measure 3 to 5 cells at progressively deeper positions below the surface. A motorized piezo-electric manipulator (Stoelting Co., Wood Dale, Ill.) was used to carry the pressure probe, impale the cells, and to measure the

## Results

depth of the cell below the tissue surface. At least two series of cells were measured at each of 2 equatorial positions on opposite sides of the fruit, and as found by Cosgrove and Cleland (1983), after meniscus establishment the measured turgor of sequentially penetrated subepidermal cells was quite uniform (typically  $\pm$  SE = 0.02 MPa).

For fruit stored for 6 months, puncture tests were used to estimate the firmness of each fruit following the measurement of fruit cell turgor. Two to four measurements of the maximum force required to press an 11.1-mm-diameter probe 5 mm into an intact fruit (skin removed) were made at a speed of 0.5 mm·s<sup>-1</sup>, using an Instron universal testing system (Instron Corp., Canton, Mass.).

**CELL WALL ANALYSES.** Cell wall material was extracted and fractionated from previously frozen tissue using the method of Gross and Sams (1984). Samples from fruit stored for 1 and 6 months were fractionated all at the same time. Neutral sugar analyses were performed according to the method of Blakeney et al. (1983) using an SP-2340 fused silica (30 m × 0.32 mm ID) capillary column (Supelco, Bellefonte, Pa.). Uronide determinations were done using the method of Ahmed and Labavitch (1977) except that cell walls were hydrolyzed in 2 mol·L<sup>-1</sup> trifluoroacetic acid, instead of sulfuric acid. The presence of neutral sugars was corrected for using sodium hydroxide in place of p-diphenylphenol.

**TRANSMISSION ELECTRON MICROSCOPY (TEM).** Apple cylinders from the fracture tests were used for TEM. Fractured cylinder pieces were oriented fracture side down in tubes containing cold fixative solution. Two sets of samples were fixed in 2.5% glutaraldehyde in 0.1 mol·L<sup>-1</sup> sodium cacodylate buffer, pH 7.2, at room temperature for at least 8 h (Roland and Vian, 1991). After 3 buffer washes, 1 mm cubes were cut from the fixed tissue and postfixed for 2 h in 1% osmium tetroxide prior to ethanol dehydration, infiltration, embedding, and polymerization in Spurr's resin. Sections (90 nm) were cut, poststained, and photographed in a Philips CM12 Transmission Electron Microscope. Sections were cut from three to five different samples for each apple type; ≈6 to 10 photos were taken of cell walls at magnifications from 3000 to 25,000×. Lower magnifications were used first to observe cell walls, followed by higher magnifications to see finer cell wall structures. Samples were compared for discontinuity of the plasma membranes, separation of plasma membrane from cell walls, and fraying of cell walls.

**STATISTICAL ANALYSES.** Statistical analyses of sensory data, ethylene production and respiration rates, texture measurements, turgor potential data, and cell wall neutral sugar composition were done using the GLM procedure of SAS (SAS Institute Inc., Cary, N.C.). Means were separated using the Student-Newman-Keuls test with  $P = 0.05$ , except for turgor potential data in which  $P = 0.01$ . Correlation analyses of arabinose and texture data were done using CoStat (CoHort Software, Minneapolis, Minn.). Except for ultrastructural determinations, only data significant at  $P = 0.05$  is discussed, unless stated otherwise.

For sensory evaluations, an analysis of variance (ANOVA) was used at each test time to determine whether the nine apple samples differed from each other on any of the attributes (model: sensory attribute = judge, apple). ANOVAs were also used to determine differences among the four cultivars and the two growing locations (model: attribute = judge, cultivar, location, cultivar × location). For turgor potential and cell wall analyses, individual fruit were used as replications for each combination of cultivar, location, and storage period (models: turgor potential = cultivar, location, cultivar × location; cell wall attribute = time, location, cultivar, time × location, location × cultivar, time × cultivar, time × location × cultivar). All mean squares are from type III (partial) sum of squares.

**SENSORY EVALUATIONS.** The best liked apple cultivar at 1 month was 'Honeygold', followed by 'Honeycrisp' and then 'Delicious' and 'Macoun' (Fig. 1A). The overall liking scores showed a similar pattern with the flavor liking scores and the sweetness scores (data not shown). At 1 month, subjects liked the texture of 'Honeycrisp' best (Fig. 2A). However, at this age the flavor, as opposed to the texture, appeared to have driven the liking of the apples (Fig. 1B).

'Honeycrisp' apples grown from the northern site were generally better liked and had better liked flavor and texture than the 'Honeycrisp' apples from the southern location. The other three apple cultivars had better flavor, texture, and overall liking when grown in the south. Apple cultivar was highly significant ( $p < 0.0001$  difference for all sensory attributes). Growing location was generally not significant, but most cultivar × growing location interactions were significant.

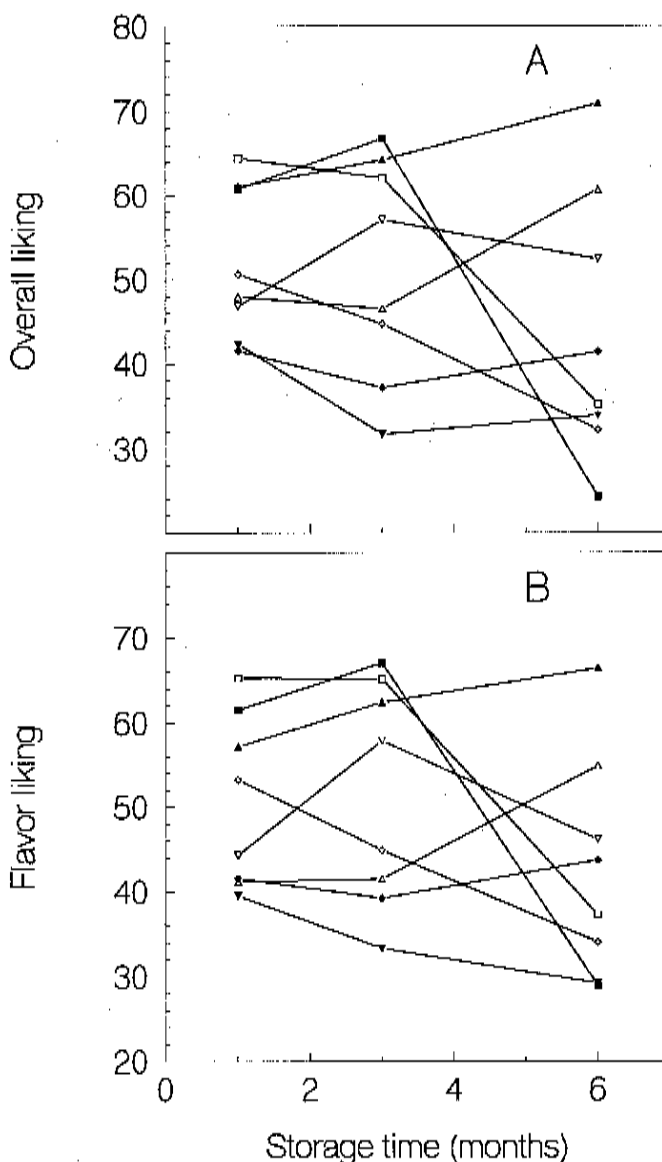


Fig. 1. Evaluations of overall liking (A) and flavor liking (B) by sensory panels of apple fruit stored for 1, 3, or 6 months. Different samples were tasted at each time point. Lines connecting time points show trends, not continuous changes, over time. ▲ = 'Honeycrisp', ▼ = 'Delicious', ■ = 'Honeygold', ◆ = 'Macoun'; closed symbols = northern samples, open symbols = southern samples.

'Honeycrisp' apples from the north were better liked throughout storage time and had better liked flavor (Fig. 1) and texture (Fig. 2) than 'Honeycrisp' apples grown in the south. 'Honeycrisp' and 'Honeygold' apples from both sites and the 'Delicious' apples grown in the southern area maintained their relatively higher liking with storage time, while the 'Delicious' apples from the north and all the 'Macoun' apples declined slightly in overall liking compared with the others. These declines in overall liking were matched by declines in flavor liking. Again, the flavor liking appeared to drive the overall liking of the apples.

'Honeycrisp' apples were clearly the best liked apples at 6 months. They had the best liked flavor (Fig. 1B) and texture (Fig. 2A). They were firmer and juicier (data not shown) at 6 months than were the other apples.

A dramatic drop in relative liking for 'Honeygold' apples was the most striking observation from the 3 to the 6 month data. This was accompanied by similar decreases in both flavor and texture liking (Figs. 1B and 2A). The crispness (Fig. 2B), firmness, and juiciness of these apples dropped considerably from 3 to 6 months.

'Macoun' and 'Delicious' apples were generally liked better, and had better texture and flavor when grown at the southern locations

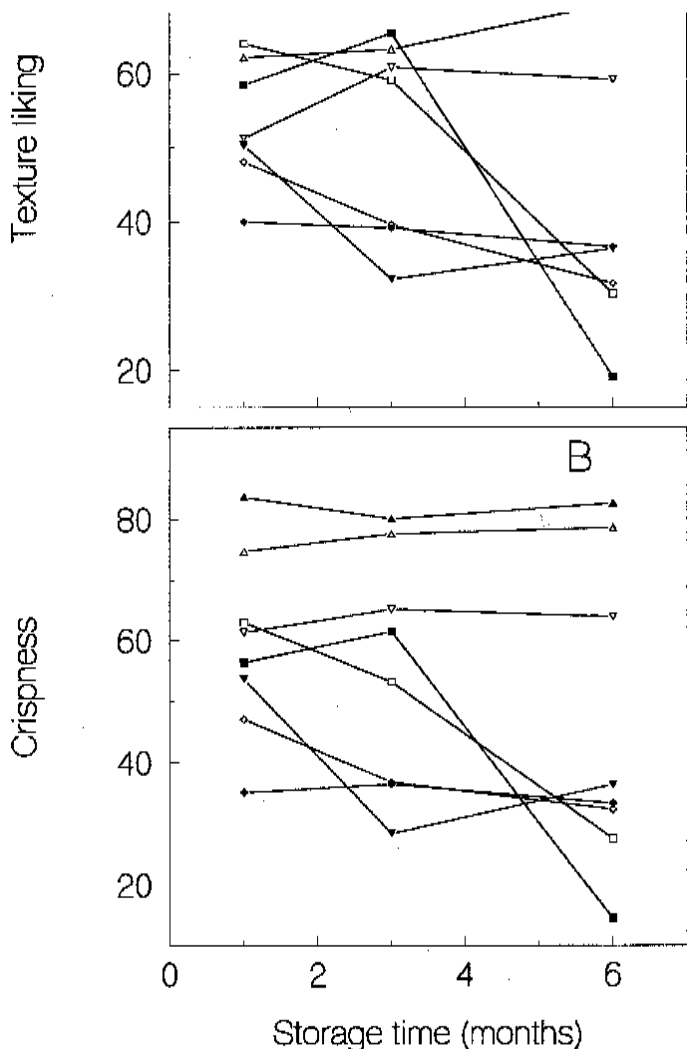


Fig. 2. Evaluations of texture liking (A) and crispness (B) by sensory panels of apple fruit stored for 1, 3, or 6 months. Different samples were tasted at each time point. Lines connecting time points show trends, not continuous changes, over time. ▲ = 'Honeycrisp', ▼ = 'Delicious', ■ = 'Honeygold', ◆ = 'Macoun'; closed symbols = northern samples, open symbols = southern samples.

(Figs. 1 and 2A) whereas 'Honeycrisp' apples were always liked better, and had better flavor and texture when grown at the northern location. Statistical interactions between growing location and cultivar were nearly always significant for all sensory attributes and time periods.

**ETHYLENE PRODUCTION AND RESPIRATION.** Overall, ethylene production and respiration rates were affected by cultivar or storage time but not orchard location (data not shown). There were interactions between cultivar and location or time, and between time, location, and cultivar. Variabilities in ethylene production and respiration rates between samples for each cultivar and location were large, so it is not possible to determine whether changes that occurred over time for individual cultivars were significant, suggesting that these fruit were climacteric or postclimacteric after 1 month of storage.

**TEXTURE ANALYSES.** In general, there was good agreement be-

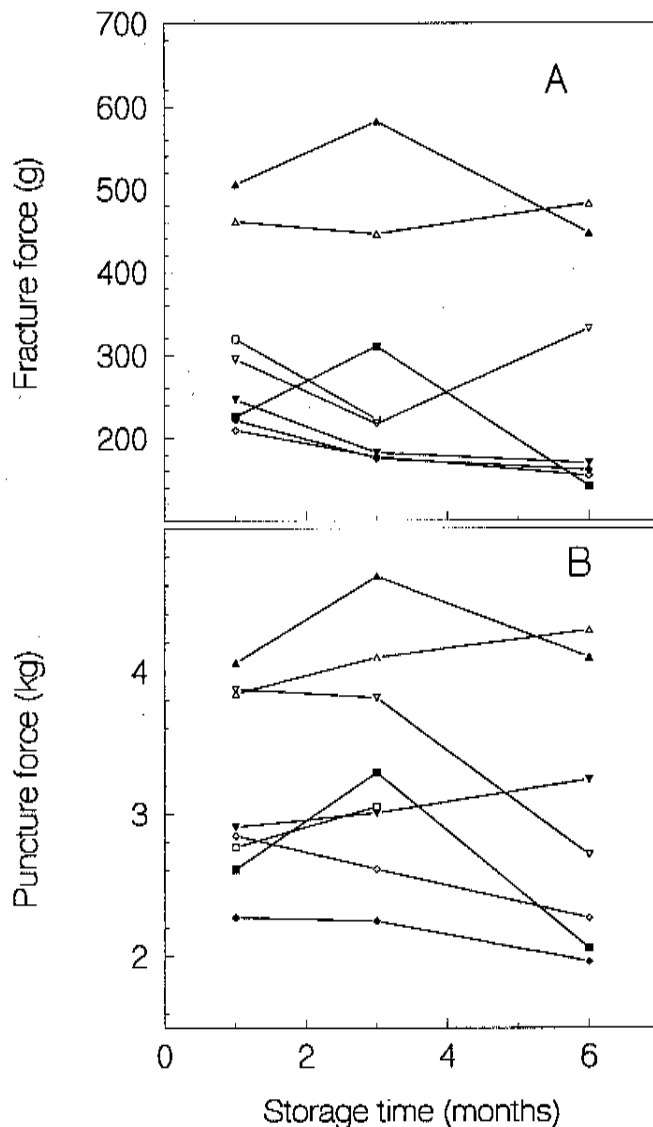


Fig. 3. Fracture (A) and puncture (B) force of apples stored for 1, 3, or 6 months. Different fruit were used at each time point, so lines connecting time points indicate trends, not continuous changes, over time. At 6 months, all 'Honeygold' apples from the southern location exhibited internal breakdown, so were not used for these measurements. Means with different letters are significantly different at  $P = 0.05$  with the Student-Newman-Keuls test. ▲ = 'Honeycrisp', ▼ = 'Delicious', ■ = 'Honeygold', ◆ = 'Macoun'; closed symbols = northern samples, open symbols = southern samples.

Table 1. Mean cell wall neutral sugar content, percentage, and uronide levels of the different apple varieties after 1 and 6 months of storage and abbreviated ANOVA. HC = 'Honeycrisp', D = 'Delicious', MC = 'Macoun', HG = 'Honeygold', rha = rhamnose, ara = arabinose, xyl = xylose, man = mannose, gal = galactose, glc = glucose, cw = cell wall. Units for the neutral sugars and uronide is mg·g<sup>-1</sup> (fresh weight basis). For each mean at one month, n = 56 and at 6 months, n = 65. In the ANOVA table, T = time, C = cultivar, L = location.

Time (month)	rha		ara		xyl		man		gal		glc		cw (%)		Uronide	
	1	6	1	6	1	6	1	6	1	6	1	6	1	6	1	6
HC	0.13	0.2	1.17	1.13	0.46	0.74	0.07	0.12	0.52	0.72	0.26	0.30	1.05	1.02	0.84	0.79
D	0.13	0.20	1.08	0.84	0.50	0.73	0.07	0.09	0.35	0.49	0.48	0.31	1.15	1.03	1.09	0.65
MC	0.14	0.22	1.38	0.76	0.61	0.82	0.08	0.10	0.78	0.80	0.30	0.33	1.03	0.92	0.66	0.49
HG	0.10	0.22	1.09	0.86	0.46	0.73	0.06	0.09	0.63	0.91	0.22	0.32	1.03	0.80	0.83	0.40
pooled SE	0.005	0.005	0.04	0.03	0.02	0.02	0.005	0.005	0.02	0.02	0.02	0.01	0.004	0.006	0.03	0.02
Mean squares																
Factor	df	rha		ara		xyl		man		gal		glc		cw (%)		Uronide
T	1	0.167		3.345		1.331		0.022		0.477		0.017		0.423		3.266
L	1	0.014		0.038		0.236		0.005		0.0007		0.120		0.005		0.001
C	3	0.003		0.401		0.119		0.002		0.827		0.134		0.411		1.130
T × L	1	0.002		1.685		0.234		0.0002		0.122		0.511		0.182		1.714
L × C	3	0.004		0.415		0.115		0.0003		0.118		0.061		0.488		0.408
T × C	3	0.002		0.577		0.013		0.002		0.042		0.076		0.303		1.207
T × C × L	2	0.001		0.505		0.022		0.004		0.020		0.084		0.005		0.286

tween fracture force and sensory crispness data (Figs. 3A and 2B). Fracture force analyses showed that the 'Honeycrisp' apples were harder to fracture than the other apple cultivars (Fig. 3A). Instrumental firmness measurements (Fig. 3B) showed that 'Honeycrisp' apples were also firmer than all other cultivars except for the southern 'Delicious' samples at 1 and 3 months.

When the southern 'Honeygold' apples were cut open after 6 months of storage, all of them exhibited internal breakdown, so were not used for texture, cell wall, and ultrastructural analyses.

**CELL WALL ANALYSES.** The only consistent difference in neutral sugars between 'Honeycrisp' and the other cultivars was in arabinose content (Table 1). Arabinose content increased between 1 and 6 months in the 'Honeycrisp' and northern 'Delicious' samples. The correlation coefficient (*r*) between the 6-month data for arabinose content and fracture force for all cultivars and locations was 0.58, while *r* = 0.51 for arabinose and firmness. The correlation coefficients for mean arabinose and mean fracture force or mean firmness were 0.86 and 0.78, respectively. However, these correlation coefficients were derived from only four genotypes.

The percentage of cell wall decreased for 'Macoun' and 'Delicious' apples over storage time, but remained constant for the other cultivars (Table 1). Uronide content decreased over storage time for all cultivars.

**TURGOR POTENTIAL MEASUREMENTS.** Cell turgor potential declined in all cultivars between 3 and 6 months of cold storage, but for both storage durations, consistent and highly significant cultivar differences in cell turgor were observed, with 'Honeycrisp' and 'Delicious' exhibiting the highest and 'Honeygold' and 'Macoun' exhibiting the lowest turgors (Table 2). Time and cultivar were both very highly significant (*p* < 0.001). For the fruit measured after 6 months of storage, there was a clear, positive correlation of puncture force to turgor potential (Fig. 4). Within each location and cultivar there was some fruit-to-fruit variability, but in particular, two 'Delicious' fruit exhibited much higher turgor and firmness than any of the other 'Delicious' fruit (shown separately in Fig. 4).

**ULTRASTRUCTURAL OBSERVATIONS.** Representative TEM photographs are shown in Figs. 5–8. In general, a decline in overall plasma membrane continuity with storage time was observed for all cultivars, with the exception of 'Honeycrisp' and 'Delicious' from the southern location. This was characterized by thinning, breaks, and

convolution of the plasma membrane, and its separation from the cell wall. Deterioration in cell wall integrity over storage time, characterized by separation of laminates and appearance of fenestration, was also observed, similar to that observed by Ben-Arie et al. (1979) and Roy et al. (1997). Depolymerization of the cell wall was characterized not only by the deterioration of the middle lamella (Roy et al., 1997), but also by deterioration of cell wall next to the plasma membrane. This was especially notable in the vicinity of the plasmodesmata, and was evident in all the cultivars studied. 'Honeycrisp' apples showed less of this deterioration after 6 months of storage.

'Macoun' apples from the northern location showed good middle lamella and cell wall continuity after 1 month of storage (Fig. 5A). Very fine lamellate laminates were visible everywhere in the cell walls. The plasma membrane was somewhat undulating and closely followed the cell wall. After 6 months, a breakdown in cell wall lamellae and plasma membrane continuity was observed (Figs. 5B and C). Plasma membranes not only became very convoluted, separating from the cell walls, but also dissociated, leaving very frayed lamellae at the surface of the cell wall (Fig. 5C). The middle lamellae appeared more diffuse. Lucent areas of cell corners were large and sometimes separated more with increasing age. Electron-dense deposits were numerous in cell walls, especially at cell

Table 2. Cell turgor potential (mean ± 1 SD) for each cultivar at 3 and 6 months of cold storage and abbreviated ANOVA with significance of F tests indicated by asterisks. Sample number for each mean is in parentheses, SS = type III sum of squares.

Cultivar	Cell turgor potential (MPa)			
	3 months		6 months	
Honeycrisp	0.63 ± 0.08 (5)		0.39 ± 0.06 (6)	
Delicious	0.59 ± 0.06 (6)		0.48 ± 0.20 (8)	
Honeygold	0.35 ± 0.04 (6)		0.22 ± 0.05 (8)	
Macoun	0.32 ± 0.08 (6)		0.19 ± 0.02 (8)	
Factor	df	SS	df	SS
Cultivar (C)	3	0.438***	3	0.465***
Location (L)	1	0.001 <sup>NS</sup>	1	0.008 <sup>NS</sup>
C × L	3	0.018 <sup>NS</sup>	3	0.056 <sup>NS</sup>
Error	15	0.0708	22	0.2521

<sup>NS,\*\*\*</sup> Nonsignificant or significant at *P* < 0.001, respectively

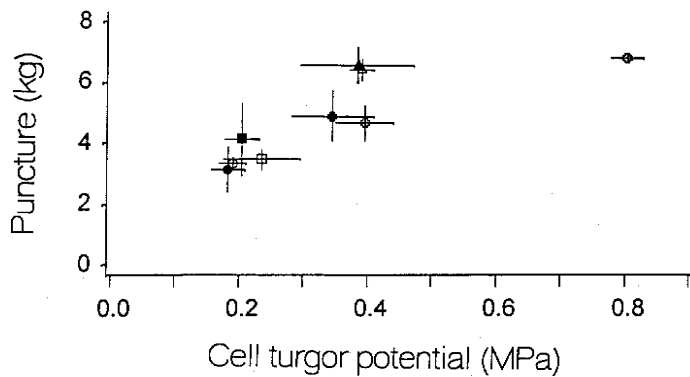


Fig. 4. (above) Relationship between tissue puncture force and cell turgor potential after 6 months of cold storage, measured on three to four fruit of each variety and orchard location (▲ = 'Honeycrisp', ◆ = 'Delicious', ■ = 'Honeygold', and ● = 'Macoun'; closed symbols = northern samples, open symbols = southern samples). Each point represents the mean value, with vertical and horizontal lines representing  $\pm 1$  SD. Two 'Delicious' fruit from the southern orchard location that had particularly high turgor potential values are plotted separately from the other 'Delicious' fruit.

corners. There was a reduction of fine laminates to broader light and dark bands (Fig. 5B).

'Macoun' apples from the southern location, like the northern apples, showed extensive degradation of cell walls after 6 months. Some degradation was already observable after 1 month. Little of the laminated structure of the cell wall was visible and the plasma membrane was loosely attached, elongated, and convoluted (Fig. 5D). Very large electron-dense deposits were often seen in the cell walls at this stage. In older samples, many of the electron-lucent regions of the cell corners were coming apart, always starting from the intercellular space (Fig. 5E). Light and dark banding was visible instead of the fine laminated lamellae in the cell walls of most cells in older samples, as was the fraying of surface layers (Fig. 5F).

Northern 'Honeygold' apples showed more severe cell wall degradation than the 'Macoun' fruit (Fig. 6). One-month-old samples showed much better cell wall continuity than older apples. Fine laminations of lamellae were visible after 1 month, but after 6 months, they tended to break down, especially at cell corners, and become disorganized. Fenestrations were sometimes observed, especially starting at cell corners and extending to plasmodesmata (Figs. 6B and C).

Cell walls of 'Delicious' apples from both locations changed over time also, but not as much degradation was observed as in 'Macoun' and 'Honeygold' fruit (Fig. 7). Fine lamellar laminations were sometimes still visible after 6 months of storage (Figs. 7B and C). Light and dark banding of the cell wall was also seen in the older samples (Fig. 7E). In some of the 6-month-old samples, the light and dark patterns appeared as blotches instead of bands (Fig. 7F). Electron-lucent corner regions remained intact. Some cell wall lamellar integrity was lost around intercellular spaces. Plasma membranes tended to stain more densely in 6-month samples.

'Honeycrisp' samples from both locations showed

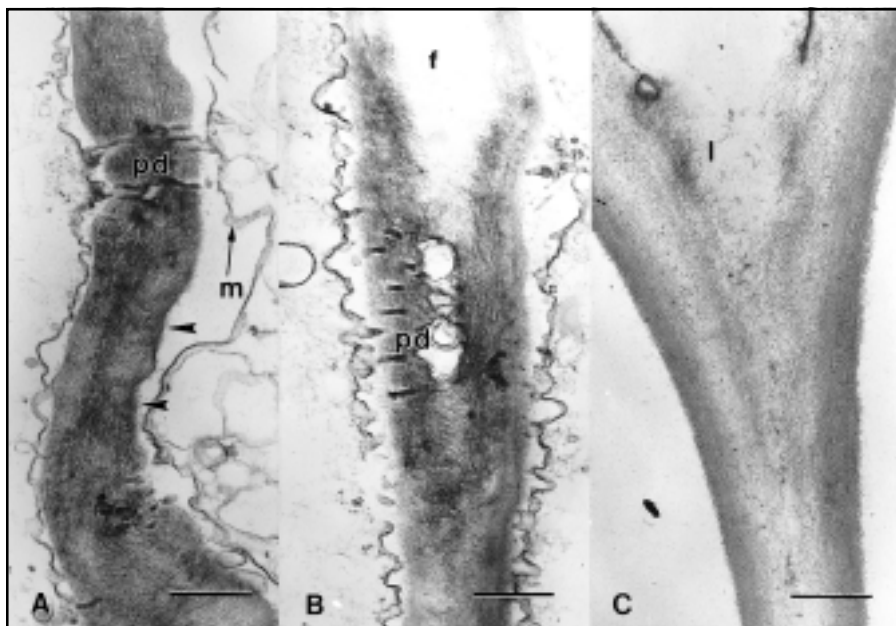
less plasma membrane and cell wall degradation than the other cultivars (Figs. 8A–F). Little or no depolymerization of cell wall lamellae was observed. Intercellular spaces also seemed to retain their integrity through 6 months (Figs. 8B and E). Most plasmodesmata looked as good in structural detail (note plasmodesmata pits and channels in Fig. 8C) at 6 months as at 1 month.

## Discussion

These data show that 'Honeycrisp' is an unusual apple that is more firm and crisp than its parents. It maintained its crispness and

Fig. 5. (below) Transmission electron micrographs of representative 'Macoun' fruit, in which A–C are from the northern location and D–F are from the southern location. (A) Cell wall after 1 month of storage. Note the middle lamella (arrowheads), the finely laminated structure of the cell wall (cw), and the bound and continuous plasma membrane as it cups and follows the contour of the cell wall (small arrows); plastid (p). (B) Cell wall corner after 6 months. Densely staining deposits (d) are present in the corners, as are lucent (l) regions. (C) Cell wall between two cells after 6 months showing disorientation of the middle lamella (arrowheads). Note the degradation of the plasma membrane (m). (D) Electron-dense deposits cover large areas of the cell wall in 1-month-old fruit. (E) Cell corner with intercellular space and plasmodesmata after 6 months. (F) Cell wall after 6 months. Bar = 1  $\mu$ m unless otherwise indicated.





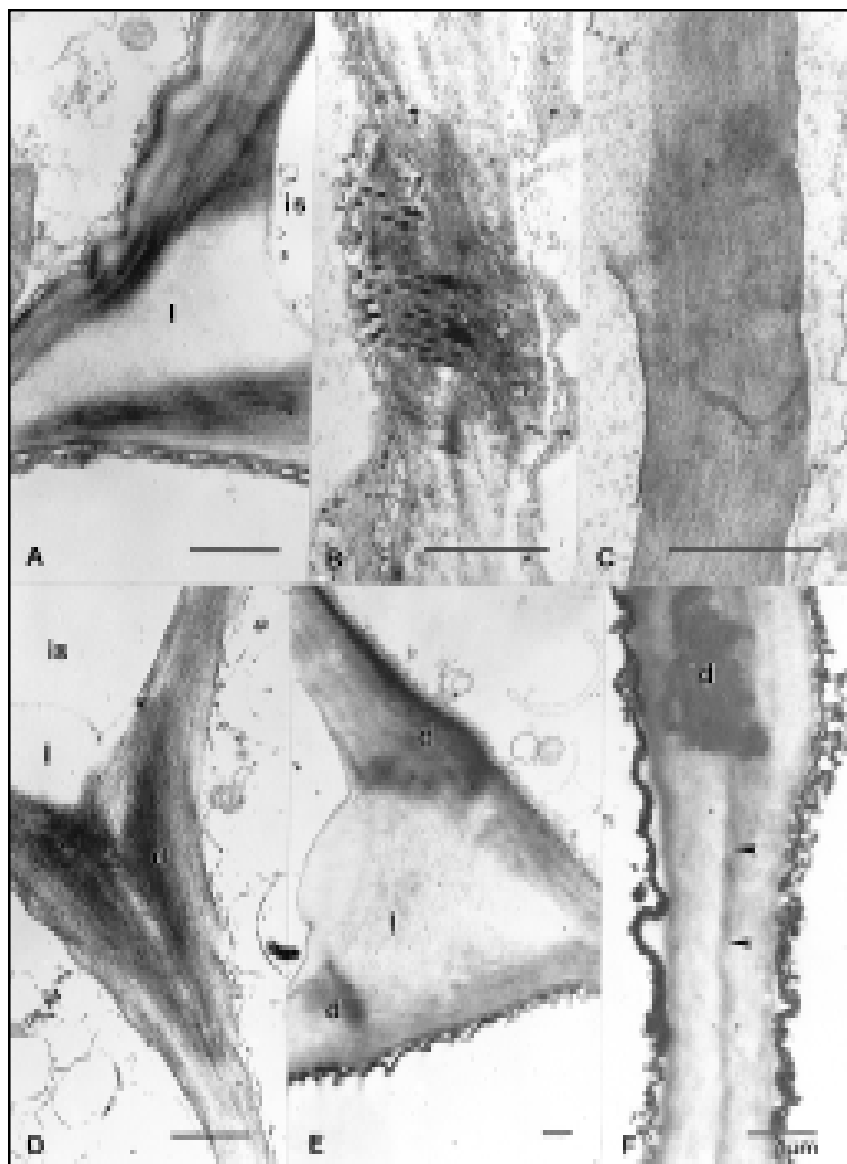
turgor is similar in all these cultivars, and that the two high turgor 'Delicious' fruit (Fig. 4) indicate that an upper limit of tissue firmness is reached for turgors above  $\approx 0.5$  MPa. In any case, the range of turgors measured in these intact apples was quite substantial (0.1 to 0.8 MPa), generally much higher than turgors previously measured in ripening intact tomato fruit (0.0 to 0.1 MPa, Shackel et al., 1991), but similar to those reported for excised tissue of 'Cox's Orange Pippin' apples that were incu-

Fig. 7. (**below**) Transmission electron micrographs of representative 'Delicious' fruit; **A–C** are of northern fruit and **D–F** are of southern fruit. (**A**) Cell corner with intercellular space (is) at 1 month. Note electron-lucent area (l). (**B–C**) Cell wall with plasmodesmata at 6 months. (**D–E**) Cell corners with electron-lucent (l) and electron-dense (d) areas at 1 and 6 months, respectively. (**F**) Cell wall with degraded lamellae and laminations at 6 months. Note electron-dense middle lamella (arrowheads) and dense cell wall deposits (d). Bar = 1  $\mu$ m.

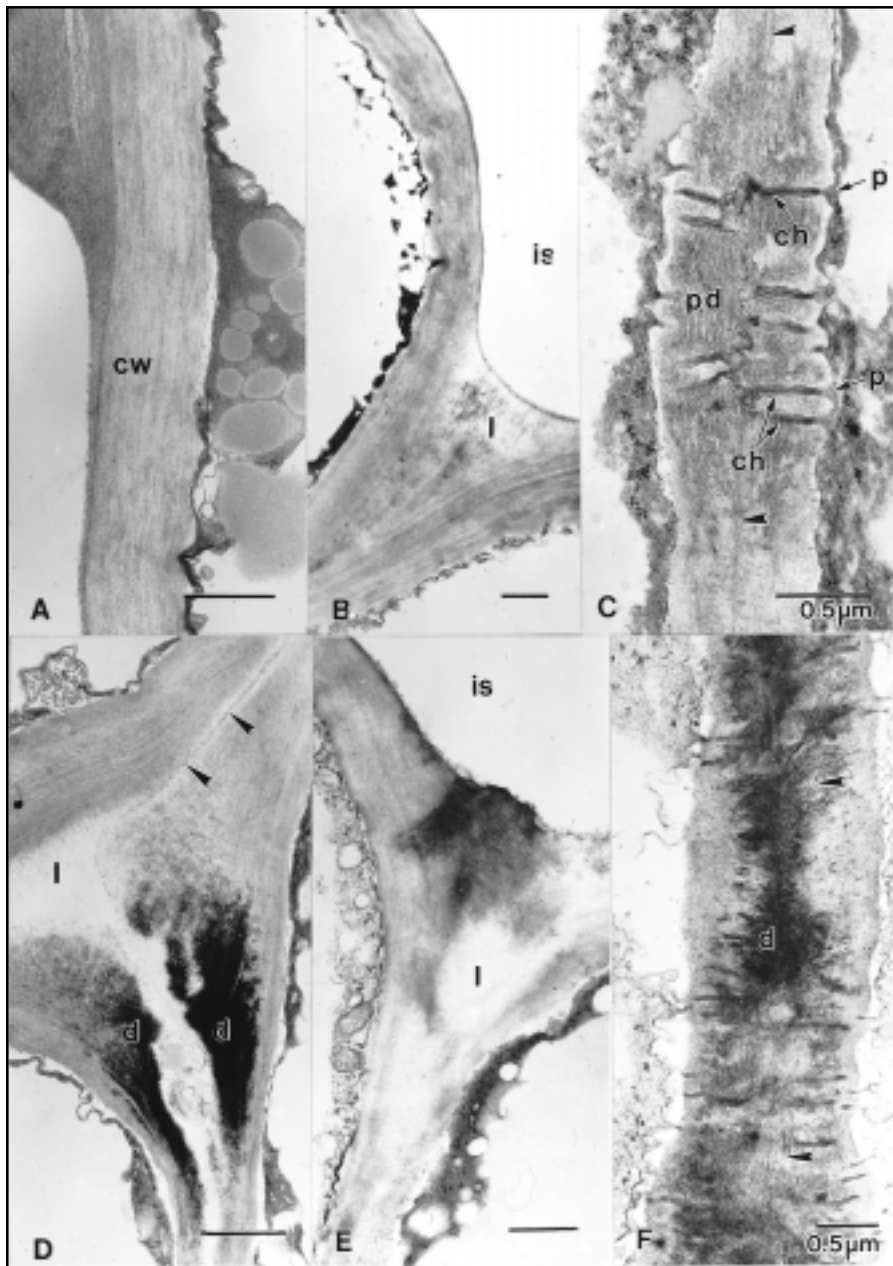
Fig. 6. (**above**) Transmission electron micrographs of representative 'Honeygold' fruit from the northern location. (**A**) Cell wall after 1 month showing plasmodesmata (pd). Note the separation of the plasma membrane (m) from the cell on the right, and frayed cell wall surface (arrowheads). (**B**) Cell wall after 6 months showing fenestration (f). (**C**) Cell wall junction after 6 months. Note the electron lucent (l) area. Bar = 1  $\mu$ m.

cell wall integrity through 6 months of cold storage without controlled atmosphere conditions. Two possible factors that may be involved in the maintenance of this crispness are high turgor potential and maintenance of cell wall integrity and arabinose content.

The positive relationship between cell turgor potential and tissue puncture firmness in these apple cultivars (Fig. 4) is consistent with the hypothesis that turgor may be generally an important component of the texture of plant tissues, as suggested for both carrot (*Daucus carota* L., Greve et al., 1994) and tomato (*Lycopersicon esculentum* Mill., Shackel et al., 1991) tissues. The fact that turgor potential generally declined over time in storage (Table 2) is also consistent with this hypothesis in that apple tissue firmness also generally declines over time in storage. With more extensive sampling it should be possible to determine whether each cultivar exhibits a unique relationship of firmness to turgor, or whether all cultivars are generally scattered around the same overall positive relation. If the two 'Delicious' fruit that had notably high firmness and turgor (Fig. 4) are indicative of a linear increase in firmness with turgor for the cultivars 'Macoun', 'Honeygold', and 'Delicious', then 'Honey-crisp' may be noteworthy in having a higher firmness for its level of turgor. This may indicate that both a turgor and a structural component of firmness may play a role in differentiating the postharvest behavior of 'Honeycrisp' from its parents and from 'Delicious'. On the other hand, it is possible that the relationship of firmness to cellular







the postharvest behavior of these tissues.

The depolymerization of cell walls and plasma membranes demonstrated with transmission electron microscopy after 6 months of storage was extensive in some cells. However, the percentage of cells showing membrane deterioration was not quantified. Although there were many cells in 6-month-old 'Macoun' and 'Honeygold' fruit showing membrane separation and deterioration, there must have been enough intact cells to account for measurable turgor potential. Deterioration of cell walls was also difficult to quantify; however, spaces observed between plasma membranes and cell walls indicate that such deterioration was extensive at each plasmodesma in most samples except 'Honeycrisp'. Similar observations were also made by Fuller (1976) with 'Cox's Orange Pippin' and Ben-Arie et al. (1979) with 'Calville de San Sauveur'. This membrane separation may be a result of a loss in turgor potential or digestion of cell wall components. Glenn and Poovaiah (1990) reported fenestrations in stored 'Golden Delicious' apples. 'Macoun' and 'Honeygold' showed minor fenestrations after 6 months of cold storage. However, no fenestrations were observed in 'Delicious' or 'Honeycrisp' samples, even after 6 months of storage.

The role of arabinose in fruit firmness or crispness is less clear than that of turgor potential or cell wall integrity. There were large correlations between cultivar mean arabinose values at 6 months and firmness or fracture force. These relationships should be studied over a larger sample of genotypes and environments to determine whether high arabinose content after storage may indeed be a useful trait for apple breeders to consider in selection. For individual genotypes at each location, the correlation coefficients for arabinose content and fracture force or firmness were insignificant, making it difficult to determine from these data whether arabinose content has a

Fig. 8. Transmission electron micrographs of representative 'Honeycrisp' fruit; **A–C** are of northern fruit and **D–F** are of southern fruit. (**A**) Cell wall of 1 month-old fruit, showing detailed laminations and closely appressed plasma membrane. (**B**) Cell wall with intercellular space (is) at 6 months with electron-lucent (l) area. (**C**) Plasmodesmata (pd) between cells at 6 months. Note middle lamella (arrowheads). (**D**) Juncture of 3 cells at 1 month, showing electron-lucent (l) and electron-dense (d) regions. Arrowheads point to the middle lamella. (**E**) Cell wall with electron-lucent area at 6 months. (**F**) Plasmodesmata showing electron-dense (d) deposits and some very fine laminations (arrowheads). Bar = 1 μm unless otherwise indicated.

bated in an osmotic nutrient solution (Steudle and Wieneke, 1985). It is well known that the elastic properties of plant cells is strongly dependent on cell turgor potential (Steudle and Wieneke, 1985), and hence it should be anticipated that fruit cell turgor, and any physiological factor which may influence turgor, such as the presence of apoplasmic solutes or the occurrence of water deficits in the fruit tissue, may have a strong influence on fruit physical characteristics such as firmness. Understanding the physiological basis for such differences in turgor may lead to a better general understanding of

physiological role in the maintenance of fruit firmness.

Although more locations are necessary to make generalizations regarding location effects on fruit quality, the anomalous responses of the 'Delicious' fruit from the southern site compared with 'Delicious' from the northern site and the other cultivars may be due to its lack of adaptation in the northern site. Although 'Delicious' is commonly grown in the southeastern corner of Minnesota, and in orchards around the southern site in Wisconsin, the northern site is well north of the range that is recommended for 'Delicious' (Hoover and Zins, 1998). In this northern site the length of growing season is typically marginal for properly maturing 'Delicious' fruit and the trees are often injured by cold winter temperatures. This may have been a critical factor as the winter minimum temperature at the northern site was  $-38^{\circ}\text{C}$  in early February, 1996. The trees survived this episode but may have suffered damage to their vascular system that affected fruit maturity and ripening.

Overall, our data suggest that a comparison of apple cultivars can be useful in determining what factors are important in fruit texture.



Although much of fruit firmness could be accounted for by turgor potential at 6 months of storage, 'Honeycrisp' and 'Delicious' fruit had similar turgor potentials but differed in firmness, suggesting that one or more factors in addition to turgor potential may be important in the maintenance of apple fruit firmness. Determining what these factors are is a challenge because not only is fruit tissue anisotropic and heterogeneous (Jackman and Stanley, 1995), but also because cell walls are heterogeneous (Roy et al., 1992).

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