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Relationship of Soluble Solids, Alcohol-insoluble Solids, Fruit Calcium, and Pectin Levels to Firmness and Surface Pitting in 'Lambert' and 'Bing' Sweet Cherry Fruit[1]

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Additional index words. Prunus avium, crop load

Abstract. **Firmer 'Lambert' and 'Bing' sweet cherry fruit (*Prunus avium* L.) was associated with higher soluble solids (SS), higher concentration of alcohol-insoluble solids (AIS), and pectinase-soluble pectic substances and less crop, surface pitting, and lower concentration of water-soluble pectic substances. Fruit Ca and ethylene diamine-tetraacetic acid (EDTA) soluble pectic substances were not associated with fruit firmness. Total pectic substances were lower in AIS extracted with 70% ethanol held at 70°C for 1 hour as compared with AIS extracted with 70% ethanol at room temperature plus 80% ethanol in 5% HCl (v/v) for an additional hour. The major differences between the 2 methods were less EDTA and more pectinase pectins in 70°C ethanol-extracted AIS.**

Soft fruit is a major problem in marketing fresh sweet cherries. Softening can result from improper handling and fertilization (14, 16), excessive crop load (5), high temperature (7), or possibly preharvest rain (14). Surface pitting, a disorder caused by bruising (5, 10), is directly related to fruit firmness.

Changes in pectic substances are related to softening and maturation in some fruit species (16). Van Buren (19) reported that as 'Windsor' cherries ripened, water and calgon-soluble pectin fractions increased whereas the protopectin fraction decreased. Increases in total AIS and total pectic substances per cherry were found along with changes in intrinsic viscosity. Treatment with gibberellic acid (GA_3) has been shown to increase fruit AIS levels (4, 13) and fruit firmness (4, 5, 13, 18) and possibly to reduce water-soluble pectins (4).

Fruit Ca levels are also involved in firmness. Ca applications have been shown to increase sweet cherry firmness and to reduce surface pitting (2, 9, 12). The objectives of this study were to examine the relationships of fruit Ca, AIS, and water EDTA, and pectinase-soluble pectins on sweet cherry fruit firmness and surface pitting.

Materials and Methods

1979. Fruits were taken from 4 trees in each of 4 'Lambert' and 8 'Bing' sweet cherry orchards in the Mid-Columbia area at commercial maturity according to accepted color standards. Crop load, weight per fruit, and SS were determined as previously described (5), and fruit was stored for 2 weeks at 1°C. Fruit firmness was measured on the side opposite the suture on nonpeeled fruit with a Hunter mechanical force gauge (1.6 mm tip, 0–500 g). After storage, fruit were scored for surface pitting (5) and frozen at −23°C. Two orchards of each cultivar with maximum and minimum amounts of fruit surface pitting and firmness were selected for further examination. Two trees were then selected from each of the 4 orchards for determination of fruit AIS, Ca, and pectin levels.

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Fruit flesh was ground for 5 min in a Waring blender in sufficient amount of 95% ethanol to provide a final concentration of 70%. The residue was filtered on Whatman #1 filter paper, washed with 70% ETOH, and then resuspended in either 2 vol of 70% ethanol or 2 vol of a 5% solution of HCl (v/v) in 80% ethanol. The AIS were held at 70°C in the 70% ethanol for 1 hr (70°C ethanol method) and at room temperature for 1 hr in the HCl 80% ethanol (acidic ethanol method). The AIS from each method were then removed by suction using Whatman #1 filter paper and washed with 70% ethanol until free of chlorides. The filter cake (acidic ethanol method) was washed with acetone. AIS from both procedures were air dried at 65°C, then ground in a Wiley mill to pass a 40-mesh screen. Four, 10-fruit samples were extracted using each method. No differences were found in AIS or pectin levels using either method on fresh or frozen fruit. AIS levels from each orchard-cultivar-tree samples were measured in a similar manner, but 4 single fruit replicates were analyzed. Initially, only the 70°C ethanol method was used. Fruit samples the following year were extracted with acidic ethanol for titration of the free carboxyl groups (6). When differences in pectin fraction levels were found between the 2 methods, acidic ethanol extractions were then done on the original (1979) fruit. Water, EDTA, and pectinase-soluble pectin substances were determined in the AIS extracted by both methods. About 100 mg AIS was extracted in plastic centrifuge tubes (27 × 100 mm) 3 times with 25 ml distilled water (CO_2 free) after shaking the first water extraction for 1 hr. Soluble pectin substances were decanted off and brought to 100 ml after centrifugation at 3500 G. EDTA-soluble pectic substances were separated in the same manner. For the pectinase-soluble fraction, the AIS were resuspended in 50 ml distilled water, adjusted to pH 5.0, and shaken for 1 hr with the addition of 25 mg pectinase (ICN Pharmaceuticals, Inc.). All 3 fractions were filtered through Whatman #1 filter paper and 5 ml was saponified with 0.25 ml 1N NaOH at 25–30°C for 30 min prior to the determination of the anhydrouronic acid (AUA) contents (14). Ca determinations were made on the flesh from single fruits with 4 replicates for each orchard-cultivar-tree combination. Each sample was ashed at 600°C for 4 hr, dissolved in 20 ml 0.1 N HCl, brought to 50 ml with distilled water, and analyzed for Ca by atomic absorption spectroscopy.

1980. All data taken in 1979 (crop load, fruit weight, firmness, SS, and surface pitting) were repeated in 1980. One hundred fruit samples were harvested at commercial maturity according to accepted color standards from 5 trees in each of 5 'Lambert' and 4 'Bing' sweet cherry orchards. AIS were extracted from a composited fruit sample from each orchard using only the acidic ETOH method on three, 10-fruit samples from each orchard. Total fruit Ca was analyzed as in 1979 on three, 5-fruit samples from each orchard.

Firmness, SS, crop rating, fruit weight, and surface pitting were compared by *t* test. AIS, Ca and pectins were compared by *t* test in 1979 and by SE in 1980. Differences in pectin fractions were compared by SE.

Results

Orchards selected at maximum and minimum surface pitting and firmness in 1979 were significantly different from one another in those 2 variables (Table 1). These variables were related in a similar manner as previously reported (5) in that firmness was associated with larger and higher SS fruit, lower crop, and reduced surface pitting. Orchards selected at random in 1980 also exhibited these patterns. AIS concentration was greater in fruit from the 1979 'Lambert' orchard 2 than 1 (10% significance level) and also higher in the 1979 'Bing' orchard 2 than 1 (under 10% significance level) (Table 1). Higher AIS concentration, thus, was associated with firmer, higher SS, and larger fruit with less surface pitting. Fruit sampled in 1980 (Table 2) had the same pattern, but there was considerable variation, suggesting that more than AIS is involved in pitting and firmness. 'Bing' fruit had higher levels of AIS and less pitting than 'Lambert' in 1979 (Table 1) without being larger, firmer, or having more SS. 1980 'Bing' fruit also had higher levels of AIS than 'Lambert' but similar firmness, SS, and pitting (Table 2).

Table 1. Comparison of alchohol-insoluble substances, soluble solids, fruit weight, frimness, crop rating, Ca levels, and surface pitting between 2 'Lambert and 2 'Bing' orchards.

	Lambert orchard			Bing orchard		
Variable[z]	1	2	*t* prob.	1	2	*t* prob.
Firmness (g)[y]	262	363	0.99	254	345	0.99
SS (%)[x]	14.6	20.0	0.97	15.9	18.7	0.96
Surface pitted (%)[w]	25.5	0.2	0.99	8.0	0.5	0.97
Crop rating[v]	6.0	1.5	0.98	6.5	4.0	0.88
Fruit weight (g)	8.1	10.4	0.97	8.2	10.7	0.96
AIS (g/100 g fresh wt)	1.05	1.20	0.94	1.52	2.13	0.88
Ca (ppm g dry wt)	501	401	0.84	480	460	0.34
Ca (mg/fruit)	0.68	0.81	0.74	0.57	0.76	0.80

[z]Firmness, SS, surface pitting, crop rating, and fruit weight were compared by analysis of variance using 4 'Lambert' orchards (4 trees per orchard) and 8 'Bing' orchards (4 trees per orchard). AIS and Ca data were compared in 2 orchards and 2 trees of each cultivar by a *t* test.
[y]Fruit firmness was measured with a Hunter mechanical force gauge, 0-500 g (1.6 mm tip).
[x]SS were measured by a hand refractometer.
[w]Fruit were bruised by passing 8 times over a small, 2-level, conveyor belt system.
[v]Crop was rated visually, 0-10, 7 full crop.

'Lambert' orchards 1 and 2 were the only orchards sampled both years and response was similar. Fruit from orchard 1 was softer, smaller, had lower concentrations of AIS and SS, and more surface pitting both years (Tables 1 and 2). These 2 orchards have been sampled yearly since 1973 and have always exhibited similar differences in SS and pitting.

Pectin fraction patterns were complicated by the different levels found between the 2 extraction methods. In general, however, EDTA and total pectin levels were not associated with firmness in either cultivar either year (Tables 2 and 3). Firmer fruit of either cultivar tended to have less water soluble and greater amounts of pectinase-soluble pectins per g AIS (Tables 2 and 3), especially where fruit were sampled at extremes of firmness (Table 3).

Differences were found in the amounts of total pectic substances per g AIS between the 2 methods of AIS extraction (Table 2). Proportionally, the water-soluble fraction did not change. The EDTA fraction decreased from 30–35% to 19% of the total in the acidic and 70°C ethanol AIS extraction methods, respectively, while the pectinase-soluble fraction increased from 35–40% to 50–55% of the total. These results were consistent with both cultivars. Apparently the acid treatment made more pectins available for action by EDTA, probably from the pectinase-soluble fraction.

Discussion

Increased firmness of both 'Lambert' and 'Bing' sweet cherry fruit was associated with higher levels of SS, AIS, larger fruit, and reduced crop load. Reduced water-soluble pectins and higher levels of pectinase-soluble pectins were also found in the firmest

Table 2. Factors affecting firmness and surface pitting in sweet cherry fruit.

Orchard	wt (g)[z]	Firmness (g)[y]	SS (%)	Surface pitted (%)	AIS (g/100 g fresh wt)	Ca (ppm dry wt)	Ca (mg/fruit)	Pectin fraction (AUA mg/g AIS) Water soluble	EDTA soluble	Pectinase soluble	Total	Crop rating
					Lambert							
1	8.0 a[w]	231 a	14.6 a	62 a	1.34 ± 0.034[v]	82 ± 26	0.69	149 ± 4	92 ± 2	55 ± 8	296	7.5a
2	10.2 b	320 c	17.5 c	16 bc	1.49 ± 0.02	488 ± 31	0.77	92 ± 4	82 ± 3	95 ± 8	269	4.8 c
3	9.9 b	287 b	16.8 b	15 bc	1.34 ± 0.02	277 ± 18	0.35	69 ± 5	94 ± 3	91 ± 9	25	6.2ab
4	8.1a	287 b	17.0 bc	9 c	1.42 ± 0.03	329 ± 22	0.45	61 ± 2	84 ± 3	98 ± 10	243	5.4 b
5	8.2a	290 b	16.9 b	24 b	1.43 ± 0.02	584 ± 34	0.44	100 ± 6	87 ± 2	75 ± 7	262	5.6 b
					Bing							
1	8.9a	334a	18.2a	7a	1.65 ± 0.02	462 ± 27	0.62	103 ± 3	79 ± 3	76 ± 7	258	5.5a
2	7.9 b	284 c	16.6 b	15 b	1.58 ± 0.03	455 ± 28	0.62	178 ± 8	55 ± 4	55 ± 4	290	8.2 b
3	8.1ab	306 b	17.1 b	7a	1.55 ± 0.02	489 ± 21	0.53	84 ± 4	79 ± 3	98 ± 6	261	5.2a
4	7.6 b	286 c	16.1 b	51 c	1.33 ± 0.02	327 ± 19	0.43	132 ± 7	76 ± 4	62 ± 8	270	7.7 b

[z]Fruit firmness was measured with a Hunter mechanical force gauge, 0-500 g (1.6 mm tip).
[y]Soluble solids measured with a hand refractometer.
[x]Crop was rated visually, 0-10, 7 full crop.
[w]Means in column within cultivars separated by LSD, 5% level.
[v]Standard error of the means. AIS and pectin fractions based on 3 replicates.

fruit both years. Other studies have shown that increased firmness was related to increased size, SS, and reduced crop load (5). GA_3-firmed fruit have higher levels of AIS (4, 13) and possibly SS (4, 5). Firmness, thus, appears to be related in part to intercellular and cell wall components, plus some contribution of turgor pressure caused by the varying levels of SS. Even though fruit Ca has been shown to increase fruit firmness (2, 9, 12), endogenous Ca was not related to firmness in these studies. Endogenous Ca has been positively correlated with firmness, but those data are not conclusive (4). Lidster et al. (8) suggested that Ca levels increased with maturation, strengthening the middle lamella. However, their 1977 crop data shows no differences in Ca at various maturities and the 1978 crop data shows a decrease in fruit Ca during stage III of fruit growth with possibly a slight increase at the end of the sampling period. 'Lambert' sweet cherries, sampled during stage III of fruit growth, showed a decrease in Ca concentration and no changes in Ca on a per fruit basis (4). However, Ca has been shown to reduce surface pitting (3, 12). It is obvious that there is confusion as to the function of Ca in affecting cherry surface pitting and fruit firmness.

Table 3. Water, EDTA, and pectinase-soluble pectin fractions in 'Lambert and 'Bing' sweet cherry fruit where AIS were extracted by 2 methods.

Fraction	Extraction[z] method	Pectin (AUA mg/g AIS) Lambert orchard 1	Lambert orchard 2	Bing orchard 1	Bing orchard 2
Water soluble	Acidic ethanol	75±4[y]	59±3	92±3	66±4
EDTA soluble	"	91±7	104±6	85±2	89±3
Pectinase	"	98±6	120±8	106±4	114±6
Total	"	264	283	284	269
Water soluble	70°C ethanol	60±3	53±3	91±3	72±5
EDTA soluble	"	37±2	42±2	42±1	45±2
Pectinase	"	108±6	128±8	108±6	121±8
Total	"	205	224	242	238
Firmness (g)		262 a[x]	363 b	254 a	345 b

[z]AIS were extracted with either 70% ethanol for 1 hr plus 80% ethanol in 5% HCL (v/v) for an additional hour or 70% ethanol at 70 ° C for 1 hr.
[y]SE.
[x]Means within cultivars separated by *t* test, 5% level.

Firmer fruit may not necessarily reduce surface pitting. Firmness at harvest has been negatively correlated with surface pitting (5). GA_3-firmed fruit has reduced surface pitting (4, 5, 13). Fruit firmed by low temperatures, however, have increased surface pitting (2, 11) and bruising (1). Increasing firmness, *per se*, does not necessarily reduce pitting. Different methods used to measure firmness may not give comparable results and this may contribute to variable conclusions. However, in the data presented in this paper, 'Bing' and 'Lambert' cherries had comparable firmness values, using the same method, but different pitting levels.

Even though AIS concentration was related to fruit firmness and surface pitting, absolute AIS levels were not exact indications of either factor. AIS levels were generally higher in 'Bing' than in 'Lambert' fruit, but firmness was similar. 'Bing' fruit exhibited less pitting than 'Lambert' at similar firmness, AIS concentration, and SS levels. Obviously, other factors are involved in differences in pitting and firmness, one of which may be cell wall structure.

Probably 2 factors affecting firmness and surface pitting the most are crop load and tree vigor (3, 5). Within cultivars, the larger cropped trees had less SS, AIS, pectinase-soluble pectins, smaller fruit, and more surface pitting and water-soluble pectins. There are, of course, many other unknown factors, such as year-to-year environmental differences, that must play roles in firmness of sweet cherry fruit.

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Diphenyl Absorption and Decay in 'Dancy' and 'Sunburst' Tangerine Fruit[1]

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Additional index words. Citrus reticulata, postharvest physiology

Abstract. **Effects of storage time, temperature, fruit preparation, time of harvest and number of diphenyl pads per carton with 'Dancy' (*Citrus reticulata* Blanco) and 'Sunburst' a seedling from 'Robinson' x 'Osceola', each *C. reticulata* x (*C. paradisi* Macf. x *C. reticulata*) tangerines were studied to determine the amount of diphenyl absorbed and extent of decay. Storage of 'Dancy' and 'Sunburst' at 4°C for up to 4 weeks with 1 or 2 diphenyl pads resulted in diphenyl residues less than the U.S. legal tolerance of 110 ppm. However, storage of both cultivars for 2 weeks at 21°C with 2 diphenyl pads resulted in residues exceeding this tolerance limit. Decay and diphenyl residues both tended to be higher for 'Dancy' than for 'Sunburst'. 'Dancy' tangerines stored for 4 weeks at 21°C all decayed. Statistical examination of 2 harvests of 'Sunburst' showed that early harvested fruit were less susceptible to decay but prone to absorb higher amounts of diphenyl.**

Diphenyl is a vapor-phase fungistat that must be present in the free vapor form to be effective (2, 14). Growth and spore development of some fungi are inhibited so long as an atmosphere of diphenyl vapor persists. Diphenyl absorbed by citrus fruit will not control decay, mycelial growth or sporulation of the common fungi inhabiting the surface of the peel (7).

Factors affecting the amount of diphenyl absorbed by citrus fruit have been identified as storage temperature (3, 9, 10), diphenyl dosage (9, 11), packaging methods (5, 15), placement of diphenyl sheets in the cartons (12), cultivar (4, 10), maturity (17) and physiological state of the fruit (8, 10, 17). One of the more important constraints for marketing fresh fruit, however, is the residue limit of the pesticide. The legal diphenyl residue tolerance for citrus fruit marketed within the United States, Canada and Sweden, is 110 ppm, whereas 70 ppm is the legal tolerance for citrus fruit sold in Japan and countries belonging to the European Economic Community. Substantial monetary losses have occurred with exported citrus fruit because the legal tolerance limit of diphenyl was exceeded (17).

Previous investigations (3, 4) have shown that under similar storage conditions tangerines absorb more diphenyl than oranges, grapefruit, lemons or limes. Because of the commercial importance of tangerines, a comparative study was conducted with 'Dancy' and 'Sunburst' to evaluate the effects of temperature,

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