11) in the tissue may have caused this variability. Cooke (2) demonstrated that addition of pectin to synthetic apple juice depressed the ionic Ca to about the same level as that found in natural juice (15.7% of recovered total Ca.) Other soluble components of apple fruit tissue which could have interfered with analysis of Ca^{++} include oxalic acid (6), which may comprise 0.2 to 0.6 micromoles per grams of dry weight (15), phytic acid (11, 12) which occurs mainly in storage organs (6), and other organic acids occurring in the vacuole, such as malic and citric acids (8). Juice pH was consistently low during the early fruit growth period; additonal KOH was required to adjust the pH to 6.0 and may have affected the readings.

High fruit Ca at harvest is associated with improved flesh firmness and reduced bitter pit development during storage. However, preharvest measurement of ionic Ca in fruit does not seem to be a practical means for determining whether the fruit should be sold or stored for later sale.

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Effects of a Vacuum Infusion of a Partially Purified β-Galactosidase Inhibitor on Apple Quality

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Additional index words. *Malus domestica*, firmness

Abstract. **An ethyl acetate extract of ground apples (*Malus domestica* Borkh.) was concentrated and purified for a specific β-galactosidase inhibitory function. Vacuum infusion of either H_2O or the extract restricted initial C_2H_4 evolution from apples but had no effect on fruit soluble solids and titratable acid levels in apples held at 20°C. Infusion of the extract did not affect the mean CO_2 evolution from 'McIntosh' apples over a 5-day period but reduced the rate of CO_2 evolution over time. Vacuum infusion of the extract containing the β-galactosidase inhibitor resulted in retention of fruit firmness in 'McIntosh' and 'Gravenstein' apples held at 20°.**

Apple firmness, which depends upon cellular wall matrix integrity and cohesion, is disrupted by enzymatic (1, 2, 4) or possibly by chemical (5) processes during ripening and storage. Galactose residues account for the largest proportion of monomeric carbohydrates released from cell wall polysaccharides (4). β-galactosidase was found to degrade β-1, 4-galactan (1) and to release galactose residues from cellular preparations, although the release of galactose residues was not dependent upon increased β-galactosidase activity (2). Bartley (2) concluded however, that hydrolysis of galactan was responsible for the observed loss of fruit firmness.

A previous study (3) indicated the presence of an inhibitor fraction that could inhibit reversibly β-galactosidase activity of acetone powder preparations. This inhibitory fraction may retard in vivo the release of galactose residues implicated in apple softening (2). This study investigated the effect of a concentrated, partially purified apple extract with β-galactosidase inhibitory properties on apple softening at 20°C.

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Extract preparation and purification. A 400-kg lot of 'McIntosh' apples, held in CA storage (5% CO_2 + 3% O_2, 3.3°C) for 8 months plus another 2 months in air at 0°, were ground in an Apex 314 comminuting mill (Apex Construction Ltd., London, England) equipped with a 0.562 mm screen using a 1:1 ratio intermediate speed and the sharp side of the blades. Batches of 25-kg each of ground apples were extracted twice (10 min each, with continuous agitation) with equal volumes (1:1 v/v) of purified ethyl acetate (Anachemia Ltd., Mississauga, Ontario). The ethyl acetate supernatant was decanted, concentrated by evaporation (30°, 0.1 atmosphere) to a combined volume of about 1 liter, and filtered to remove insoluble material. The addition of 5 volumes of hexane (reagent grade) to the concentrate resulted in the separation of a semi-solid gummy precipitate. This precipitate was collected, resuspended in 5 liters of distilled water, and filtered. The aqueous solution was re-extracted with ethyl acetate, and this extract was evaporated to dryness. The residue was dissolved in 1 liter of distilled water. These preparations contained chlorogenic acid, catechins, and quercetin glycosides. Over 75% of the inhibitor activity of the initial ethyl acetate extract was contained in the final preparation.

Assay of β-galactosidase inhibition. Fresh solutions of β-galactosidase were prepared by extracting acetone powders of 'McIntosh' apples with sodium phosphate buffer (0.005 M, pH 7.0) (3). β-galactosidase activity in

Table 1. Effect of vacuum infusion on CO_2 and C_2H_4 evolution from 'McIntosh' apples held at 20°C (n = 4).

	CO_2 evolution (ml $CO_2 \cdot kg^{-1} \cdot hr^{-1}$)				C_2H_4 evolution (log_{10} $\mu l \cdot kg^{-1} \cdot hr^{-1}$)			
		Vacuum infusion				Vacuum infusion		
Days at 20°C	Control	H_2O	Extract	SE	Control	H_2O	Extract	SE
1	10.2	10.0	11.1	0.50	0.93	0.14	0.23	0.15
2	10.8	10.6	12.2	0.50	1.22	1.16	1.49	0.15
3	13.4	13.8	13.8	0.50	2.12	2.23	2.28	0.15
4	15.0	15.1	14.5	0.50	2.52	2.52	2.51	0.15
5	15.9	16.0	14.8	0.50	2.69	2.61	2.61	0.15
Mean	13.0	13.1	13.3	0.27	1.90	1.73	1.82	0.08
Response components								
Linear	15.7	16.3	9.6	1.05**[z]	4.82	6.30	5.78	0.713 NS
Quadratic	−0.32	−1.17	−2.73	1.438 NS	−0.76	−2.62	−2.87	0.202**

[z]Significant at the 1% level (**); not significant (NS) at the 5% level.

the filtered and centrifuged suspensions was determined colorimetrically at pH 4.0 using p-nitrophenyl-β-D-galactopyranoside as a substrate (3) or fluorometrically using 4-methylumbelliferyl β-D-galactoside (6, 8). The Kratos Fluorometer (model FS 950) was calibrated using standard solutions of 4-methylumbelliferone excited at 365 nm and fluorescence detected at 460 nm. Inhibitor concentration of the extract solutions was determined from standard curves relating inhibition to the amount of inhibitor added to the β-galactosidase reaction mixtures. One unit of inhibition (activity) was defined as that amount which caused 50% inhibition of β-galactosidase activity in the standard fluorometric assay.

Extract application. Preclimacteric 'Gravenstein' and 'McIntosh' apples [starch index 2-3 (7)] were harvested from each of 5, 15-yr-old trees taken at random from 2 commercial orchards. Five replicates, each of 75 fruit, were assigned randomly to each of 3 treatments: a nondip control, a vacuum infusion with H_2O (vacuum control), and a vacuum infusion with apple extract. The infiltration treatments consisted of immersing 15 fruit per batch in either H_2O or apple extract, reducing the pressure to 0.1 atmosphere for 2.5 min, releasing the vacuum, and allowing the fruit to equilibrate to atmospheric pressure in solution for a further 0.5 min.

Five samples of 10 'McIntosh' fruit per treatment were placed in airtight polyethylene jars with CO_2-and C_2H_4-free air passing over the fruit sample at 1.0 liters$\cdot hr^{-1}$ at 20°C. CO_2 and C_2H_4 evolution were determined from the effluent air by infrared and gas chromatographic techniques, respectively, every day for 5 days. All remaining fruit were placed in 80% relative humidity at 20°, and the 10-apple samples were withdrawn at 5-day intervals. Fruit firmness was determined on opposite pared sides of apples using a Ballauf penetrometer with an 11.1-mm tip. Titratable acids were determined on a 2-ml juice sample diluted with 25 ml distilled H_2O, by titration with 0.1 N NaOH to an endpoint of pH 8.1. Soluble solids were determined on a juice sample by a hand refractometer.

Polynomial regression methods were used to fit a response curve over time for each variate of the 15 treatment-replicates; individual polynomial components (mean, linear, quadratic, cubic) of the treatment response curves were compared in analyses of variance (9, 10).

Vacuum infusion of the extract did not affect ($P = 0.05$) the mean CO_2 evolution significantly over the 5-day period but did reduce ($P < 0.01$) the rate of increase in CO_2 evolution over time compared with that from H_2O-infused or nondipped apples. After 1 day the extract-infused apples exhibited greater CO_2 evolution than water-infused apples, whereas after 5 days the relationship was reversed (Table 1). The initial increase in CO_2 production in apples infused with extract, compared to those infused with H_2O, may be an injury response resulting from the slight superficial lenticular damage observed in the former fruit. Vacuum-infusion of H_2O or apple extract reduced C_2H_4 evolution from 'McIntosh' apples 1 day after treatment but not thereafter (Table 1). The initial reduction in apparent C_2H_4 production may have been due to reduced C_2H_4 diffusion from the fruit by the infusion of water into the intercellular spaces near the surface. Dispersion of the intercellular liquids may have occurred over a 1-day period, allowing C_2H_4 evolution to return to normal.

Infusion of H_2O or apple extract demonstrated no consistent effect on titratable acids or soluble solids ($P = 0.05$, data not presented) for either cultivar.

For fruit firmness, the response curves for each treatment within cultivars were quadratic, and the curves for the nondipped control and the H_2O-infused treatments were not significantly different ($P = 0.05$). Vacuum infusion of the apple extract, containing a component inhibitory to β-galactosidase activity, retained firmness ($P < 0.01$) in both 'Gravenstein' and 'McIntosh' apples (Table 2). The quadratic components for both controls were larger ($P < 0.01$) than for the extract infused apples, indicating earlier fruit softening. Shelf times for samples to reach 45 N were 12.0 and 18.5 days for control and inhibitor treatments, respectively. The maximum firmness retention resulting from the extract infusion occurred at about 14 days for either cultivar, after which the firmness benefit declined.

The observed retention of firmness was not associated with a persistent effect on C_2H_4 evolution or a consistent effect on 'McIntosh' apple respiration as determined by CO_2 evolution. The results suggest that the presence of the β-galactosidase inhibitor observed in a previous study (3) and contained within the apple extract may be responsible in part for the retention of firmness, although there is insufficient data to implicate a direct cause and effect relationship. Further work is necessary to identify these compounds, to isolate the substance in a pure state, and to record apple firmness in response to the addition of this entity.

Table 2. Fruit firmness (N) of 'Gravenstein' and 'McIntosh' apples at 20°C after vacuum infusion with water and apple extract (n = 4).

	Fruit firmness (N)							
	'Gravenstein'				'McIntosh'			
		Vacuum infusion[z]				Vacuum infusion[z]		
Days at 20°C	Control	H_2O	Extract	SE	Control	H_2O	Extract	SE
0	74.0	74.0	74.0	0.72	72.7	72.7	72.7	0.89
5	66.1	65.4	70.9	0.72	58.0	58.8	63.2	0.89
10	51.4	51.1	57.0	0.72	46.9	45.9	52.6	0.89
15	39.9	43.8	47.2	0.72	42.6	42.6	49.3	0.89
20	39.4	40.5	45.3	0.72	40.1	40.0	44.7	0.89
25					38.7	38.9	41.4	0.89
Response components								
Linear	−95.4	−88.6	−81.1	1.97**[y]	−227.7	−228.6	−215.2	4.71*
Quadratic	18.0	17.4	6.4	2.31**	100.7	104.8	54.6	4.14**

[z]Ten fruit were submersed in liquid and pressure reduced to 0.1 atmosphere for 2.5 min, then vacuum released; fruit were allowed to equilibrate to atmosphere in liquid for a further 0.5 min.
[y]*,**Significant at 5% and 1% levels, respectively.

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Pubescence as a Factor in Codling Moth, Oviposition, and Fruit Entry in Five Apple Selections

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Additional index words. *Malus domestica*, codling moth preference, *Laspyresia pomonella*

Abstract. **Significant differences ($P = 0.05$) in fruit infestation by codling moth larvae were found when fruit of 5 apple selections, with different levels of leaf pubescence, were evaluated. No differences in entry into fruit were found when larvae were placed on the relatively glabrous upper leaf surface. Selections having a pubescent lower leaf surface had significantly ($P < 0.05$) reduced numbers of entries. Females allowed to oviposit freely on fruit and leaves preferred to oviposit on the glabrous upper leaf surface. In all but one selection, more eggs were laid on the leaves than on the fruit. About 70% of larval entries were found in the midsection of the fruit, with 14% and 15% occurring at the calyx and stem ends, respectively. Larval entry was increased on the side of the fruit closest to the light source. Leaf pubescence seems to be a factor in 1st brood codling moth preference of apple cultivars.**

Production of commercially acceptable apple fruit in the United States is hampered by the presence of a complex of arthropod pests and diseases. The codling moth (CM) is a key pest of this complex, and the damage it causes has zero tolerance during fruit grading. Among other factors, morphology, specifically pubescence, has been reported to influence the behavior of this insect (3, 4, 6, 7). The literature also suggests different preferences for oviposition sites by CM on apple and pear (4, 7). This study quantifies leaf pubescence and investigates its effect on female oviposition and the patterns of apple fruit infestation by neonate larvae.

Five apple selections (1225-100, 673-20, 1500-100, 1569-100, and 1689-110) were chosen, based on reported (6) differences in their leaf hair densities, to give us a range of leaf pubescence. Newly hatched CM larvae were used in all tests, except Test 3 (leaf hairs), which were conducted in a growth chamber operating at 22.5° ± 1.5°C, 90% + 10% RH and 16 hr illumination with 24, 40 W cool-white fluorescent lights. Fruit bearing spurs (FS), removed from the tree 14 to 19 days after the first male CM was trapped with a pheromone baited trap (Zoecon Corp., Palo Alto, Calif.), were placed about 60 cm from the light source. All larvae were placed on a leaf of the FS 6.5 cm from a leaf axil. The twig was considered the main axis in both tests. Testing was timed to conform with the phenological events (1st cover) of apple tree development at Lafayette and emergence of the 1st brood of CM. Tests were terminated after 48 hr.

Test 1. Oxine-citrate-sugar solution (5) (hereafter called "sugar solution") has been reported to promote freshness in cut flowers (5), but its effect on apple plant parts and CM larvae is unknown. We therefore investigated its effect on our apple test system by comparing it with distilled water. FS's taken 14 days after the capture of the 1st CM male were cut to give a twig segment (2.5 cm long) bearing a single fruit (4 cm o.d.) and 3 leaves. This unit was immersed in a shell vial (21 × 70 mm) containing either the sugar solution or distilled water (Fig. 1). There were 4 treatments (water only, sugar solution only, larvae + water only, larvae + sugar solution only) which were replicated 4 times for each selection. We placed one larva on the midrib (generally the most densely pubescent area of a pubescent leaf) of the upper leaf surface of one leaf and another larva on the lower leaf surface of the opposite leaf of each replicate in each of 2 treatments, the other 2 treatments serving as controls.

A leaf disk (0.75 cm o.d.) was removed for leaf hair counts from one-half of the uninfested leaf of each spur at the beginning of the experiment and from the same location of the opposite half of the leaf at the end (2

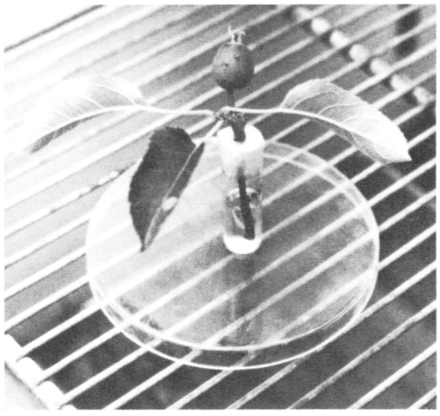

Fig. 1. A fruit spur exposed to codling moth larvae in Test 1, showing a larval entry(s) used in evaluating apple selections.

Table 1. Mean number of leaf hairs per disk[z] (Test 1) and mean number codling moth larval entries[y] per apple found when larvae were placed at the same point of the upper and lower surface of a leaf in a growth chamber study (Test 2).

Apple selection	Mean no. leaf hairs disk	Mean no. of entries/apple by CM larvae placed on	
		Upper leaf surface	Lower leaf surface[x]
673-20	12.8[w] a	1.75 a	2.38 a
1569-100	18.4 a	1.63 a	2.00 a
1225-100	19.4 a	0.63 b	1.00 b
1689-110	30.8 b	1.75 a	1.00 b
1500-100	35.9 b	1.75 a	0.63 b

[z]Leaf disks of 0.75 cm diameter were taken from opposite sides of the leaf midrib from the same location near the leaf tip.
[y]An entry was determined if fresh frass was present.
[x]Lower leaf surface more pubescent than upper leaf surface.
[w]Mean separation within a column by Duncan's multiple range test, 5% level.

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