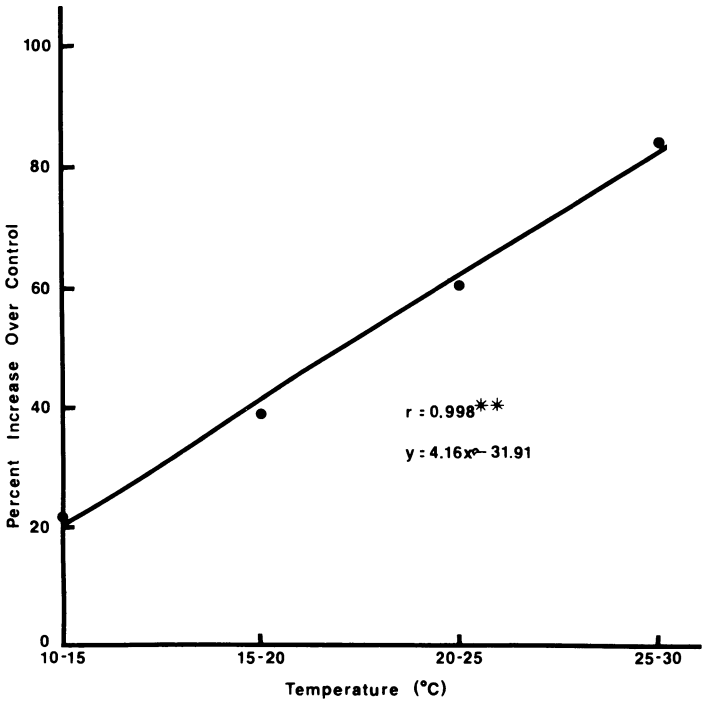

Fig. 1. The relationship between temp regimes and the response of carrot, tomato, and wheat to seed treatments with triacontanol dissolved in dichloromethane. The regression is expressed as the average % increase over the control of both concn of triacontanol with all 3 crops.

regression analysis indicated there was a positive correlation between temp and the growth response (Fig. 1). Even though the best crop growth occurred with the 20-25°C regime, the triacontanol response on a percentage basis was greatest with the 25-30° regime. Average increase for all 3 crops over the controls changed from about 20% at the lowest temp to 80% at the highest temp. This may explain the lack of response from the seed soak treatments with triacontanol which were planted during the relatively cool spring months.

Triacontanol stimulates the growth of plants quickly at rates as low as a few milligrams per hectare. All of the annual crop species tested in the greenhouse have responded indicating a lack of specificity. When plants respond to triacontanol in the greenhouse or field, it is usually over a very wide range of rates. Multiple applications do not seem to be beneficial, and our observations indicate that plants respond best when conditions for growth are optimal.

Foliar applications of triacontanol increased the yield of several crops in the field during this first year of testing, but much more research must be conducted before it can be used as another aid to increase agricultural crop production. Its efficacy must be proven under many different environmental conditions. The optimum method of triacontanol application in the field must be established, such as granular formulations, and also the rate and time of application to crops in the field.

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Apple Fruit β-galactosidase and Softening in Storage[1]

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Abstract. **Fruit softening of apple (*Malus domestica* Borkh.) and the activity of β-galactosidase in 0.5 M citrate extracts of fruit cortex were compared for 'Lodi', 'McIntosh', 'Golden Delicious', and 'York Imperial' during storage at 0° or 3.3°, 10.0°, and 18.3°C. Enzyme activity per g fresh weight increased as fruits softened, but specific activity did not change. Cell wall galactose content also decreased during softening. The decrease in wall galactose was least in 'Lodi' which contained the lowest β-galactosidase activity. 'York Imperial' which softened most slowly showed the highest β-galactosidase activity at harvest and throughout storage. Concentrated enzyme preparations did not release measurable amounts of reducing sugar, uronic acid, or neutral hexose from isolated cell walls.**

Enzymatic modifications of the cell wall are generally thought to be responsible for fruit softening. Knee (5) showed that the galactose content of apple cortex cell walls decreased as the fruits ripened. Although the significance of this change in terms of wall structure and fruit firmness is unknown, a relationship to polyuronide solubilization was suggested (6); the increase in soluble polyuronide was closely correlated with apple fruit softening. Polygalacturonase has not been detected in apples, but Bartley (1) showed that β-galactosidase activity increased during ripening. He felt that this enzyme hydrolyzed a cross-linking galactan and thereby caused the release of polyuronide from the wall. The studies cited above were conducted

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primarily with 'Cox's Orange Pippin'. We have examined the proposed relationship between β-galactosidase activity and fruit softening by comparing these parameters in several other apple cultivars.

Materials and Methods

'Lodi', 'McIntosh', 'Golden Delicious', and 'York Imperial' were harvested on July 12, Sept. 9, Sept. 30, and Oct. 13, respectively, and stored at 0° or 3.3°, 10.0° and 18.3°C, as indicated below. Fruit firmness was measured at intervals throughout the storage period with a Magness-Taylor pressure tester; 3 readings were made with each of the 16 fruits in a sample. After measuring firmness, the fruits were placed in plastic bags and frozen at –20°.

Tissue samples for enzyme extraction were obtained by removing 1.0 × 1.5 cm cylinders of cortex tissue from each of the 16 apples in a sample; all tissue handling operations were conducted at or near 0°C. For enzyme extraction, cortex tissue (26-30 g) was first homegenized in a Waring blender with 2 volumes of cold acetone for 2 min; the residue was separated by filtration through Miracloth, and washed on the filter with 8 volumes of 80% acetone. The residue from this extraction was washed with double-distilled water and then suspended in 2 volumes 0.5 M citrate buffer, (ph 4.6) for 30 min to solubilize cell wall enzymes. The residue was removed by filtration through Miracloth and the filtrate was dialyzed against 10 volumes of double-distilled water for 2 days. The water-wash fraction was assayed and found to contain β-galactosidase acitivty equal to 20 to 30% of that in the 0.5 M citrate extract. It is reasonable to assume that the enzyme solubilized by 0.5 M citrate was associated with the cell wall *in situ* (9). However, there was always β-galactosidase activity left on the citrate-extracted cell walls; the amounts of this activity throughout ripening were proportional to that found in the citrate extract. Bartley (1) also observed that a constant proportion of total enzyme remained associated with the insoluble cell wall material. The enzyme assay results reported in this paper are for the 0.5 M citrate extract as described above.

The activity of β-galactosidase was determined by measuring the hydrolysis of p-nitrophenyl-β-D-galactopyranoside (12). Protein was estimated by the method of Lowry et al. (7) with bovine serum albumin as a standard.

Cell walls were isolated from cortex tissue by sequential extraction with phosphate buffer, chloroform: methanol, and acetone as described previously (12). These were analyzed for neutral sugar content using methods previously described (11). Walls prepared from the cortex of unsoftened fruits were also used to test enzyme preparations for wall-degrading activity. For this purpose, walls were suspended (5 mg/ml) in enzyme which had been concentrated 10-fold using an Amicon ultrafiltration apparatus with a PM 10 membrane. After incubation for several hours at 30°C, cell walls were removed by filtration; the filtrate was analyzed for wall hydrolysis products using tests for reducing sugar (8), uronic acid (2), and neutral hexose (10).

Results and Discussion

During the storage of 'McIntosh' apples at 3.3°, 10.0°, and 18.3°C, changes in β-galactosidase activity (per g fresh wt) and softening appeared to be closely related (Fig. 1). Enzyme activity increased only slightly more rapidly at 18.3° than at 10.0°C and this temp difference affected softening in the same way. Fruit softening and β-galactosidase were both suppressed at 3.3°. These results are very similar to the data reported for 'Cox's Orange Pippin' (1). However, when enzyme activity was expressed per unit protein, there was no substantial change as apples softened (Fig. 1, inset). This was due to an increase in protein content of the enzyme extracts from progressively riper fruits. Total protein content is known to increase

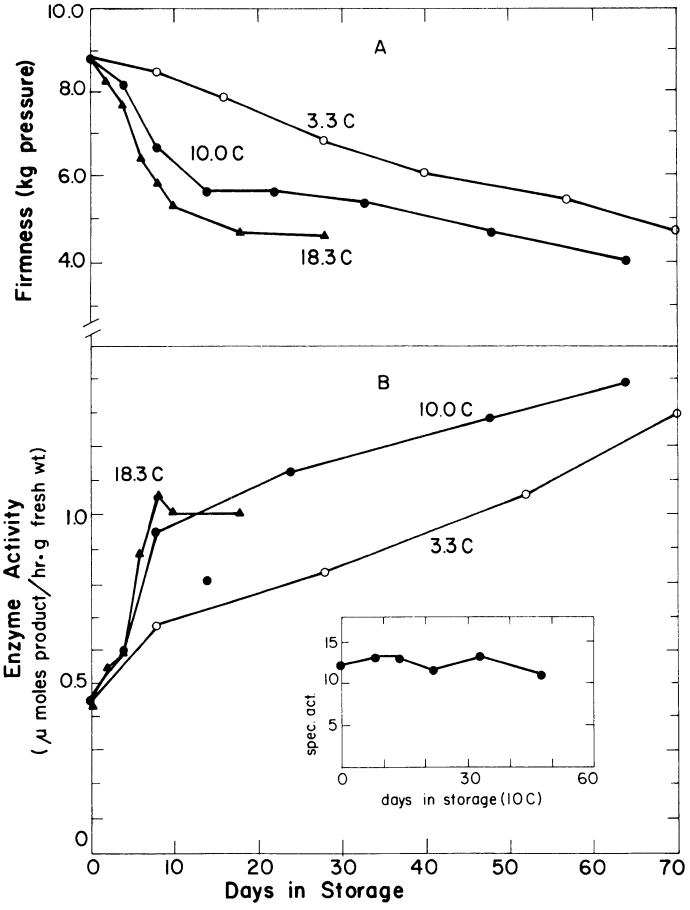

Fig. 1. Changes in 'McIntosh' fruit firmness (A) and β-galactosidase activity (B) during storage at 3.3°, 10.0°, and 18.3°C. Specific enzyme activity (inset) is expressed as μmoles product per hr per mg protein.

during apple ripening and may simply reflect enhanced overall metabolic activity (3).

In each of the 4 cultivars examined in this study, there was a substantial increase in β-galactosidase activity during storage at10.0°C (Fig. 2), as well as at the other storage temp (data not shown). Both at harvest and throughout storage, the detectable activity was highest in extracts of 'York Imperial'. Fruits of 'Lodi' and 'York Imperial' softened to 60% of at-harvest firmness in 15 and 57 days, respectively, yet the β-galactosidase activity of 'York Imperial' was many times greater than that of 'Lodi' (Table 1). The initial rate of softening was not related to β-galactosidase activity in a way which suggested that the amount of enzyme regulated the process. During the first 20 days of storage at 10.0°, 'Lodi', 'McIntosh', 'Golden Delicious', and 'York Imperial' fruits declined in firmness at rates of 0.19, 0.16, 0.14, and 0.03 kg per day, respectively; corresponding values for β-galactosidase activity at harvest were 0.03, 0.44, 1.37, and 1.92 μm per g fresh wt per hr. Although β-galactosidase activity and the decline in cell wall galactose content were both lowest in 'Lodi' fruits (Table 1), the cultivar comparisons do not show a clear correlation between these 2 parameters. However, Knee (4) reported that small amounts of galactose, arabinose, and glucose were released from acetone-extracted apple cell wall suspensions, suggesting that a wall hydrolase(s) was responsible. On the other hand, there is no direct evidence that β-galactosidase catalyzes the removal of wall galactose known to occur during the ripening of apples. We were unable to detect activity of β-galactosidase against isolated apple fruit cell walls. Tomato polygalacturonase

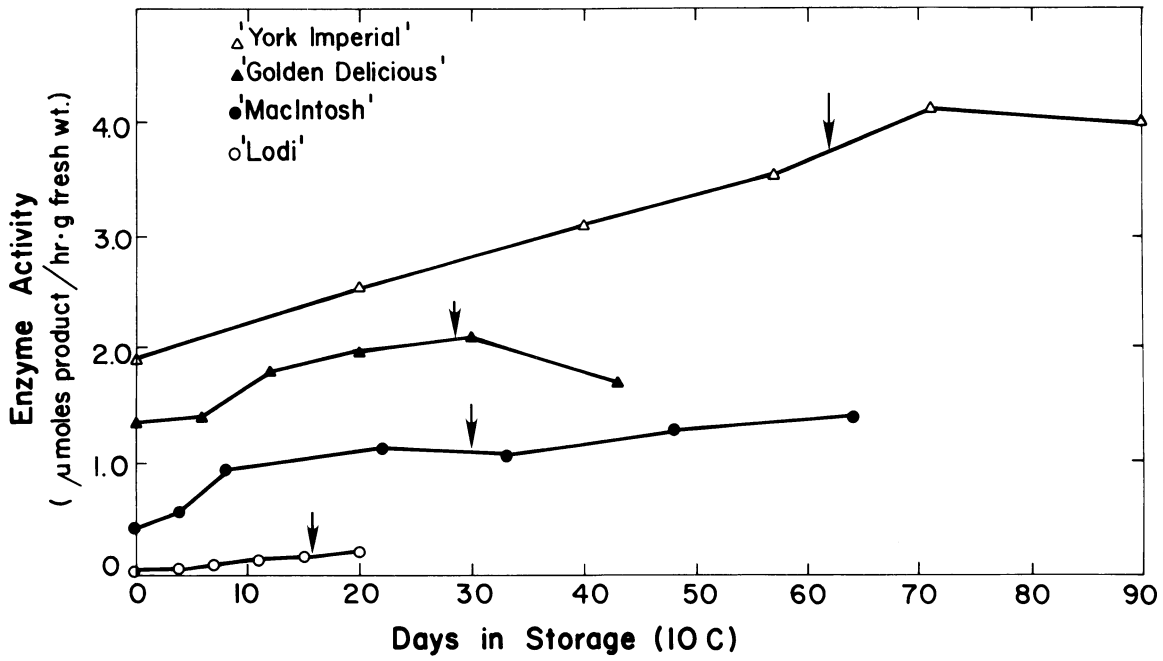

Fig. 2. Activity of β-galactosidase in extracts of 'Lodi,' 'McIntosh,' 'Golden Delicious,' and 'York Imperial' apples during storage at 10.0°C. Arrows indicate the times at which fruits had softened to 60% of at-harvest firmness.

Table 1. Initial firmness, rate of softening, β-galactosidase activity, and cell wall galactose content of cortex tissue from four apple cultivars; storage temp was 10.0°C.

Cultivar	Firmness at harvest (kg)	Rate of softening[z] (kg/day)	β-galactosidase activity[y]		Cell wall galactose	
			firm[x]	soft[x]	firm (mg/100mg wall)	soft (% of firm)
Lodi	8.2	0.19	0.03	0.13	10.2	14
McIntosh	8.8	0.16	0.44	0.06	8.1	50
Golden Delicious	8.1	0.14	1.37	2.10	9.0	35
York Imperial	10.4	0.03	1.92	3.56	11.7	28

[z]Avg over first 20 days storage.

[y]Enzyme activity expressed as μmoles product per hr per mg protein in extract.

[x]Firm = fruits on day of harvest; soft = fruits which softened to about 60% of initial firmness.

readily hydrolyzed fruit cell walls *in vitro* (11), but, in the present study, tests for the release of reducing groups, uronic acid, and neutral hexose from apple walls by a highly concentrated apple β-galactosidase were all negative. However, this does not rule out the possibility that β-galactosidase attacks cell walls *in situ*. Bartley (1) demonstrated that apple β-galactosidase preparations liberated reducing groups from galactose-rich potato pectin. It may be that some other agent (enzyme?) of ripening must first modify the cell wall to render it susceptible to the β-galactosidase.

The data reported here confirm the findings of Knee (5) and Bartley (1) that apple softening is accompanied by a decrease in wall galactose and an increase in β-galactosidase activity. In addition to these observations, the cultivar comparisons presented in this paper indicate that, if β-galactosidase is involved in the softening process, its activity in stored fruit is controlled by something other than enzyme synthesis. The amount of enzyme was clearly not the factor which restricted softening, especially in the case of 'York Imperial'. Other rate limiting factors to consider in such a situation include substrate (wall galactan) accessibility as well as enzyme inhibitors and activators.

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