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Histological and Physical Behavior of Tomato Skins Susceptible to Cracking[1]

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Abstract. **Cracking behavior of tomato skins (*Lycopersicon esculentum* Mill.) was investigated using failure and relaxation tests. Skin specimens were taken in 2 directions to represent concentric and radial cracks. Normal tissue and tissue subjected to mechanical forces were examined to determine the resulting histological distortions. The relaxation test gave more information than the failure test. No difference was observed for longitudinal or transverse skin strength for failure or the relaxation test indicating isotropic behavior. The shape of the cells and deposition of cuting appeared to affect cracking behavior. Generally, cells elongated and flattened during the failure test and failed between cells walls.**

Tomato cultivars differ in their ability to resist cracking and in the occurrence of concentric and/or radial cracks. The physical properties of the skins relate to the ability of tomatoes to resist mechanical damage (1, 5). The mechanical behavior of different tomato cultivars in relation to tomato skin cracking has been evaluated by Voisey et al. (5). A direct relationship existed between resistance to cracking, the ultimate stress or strength of the skins, and the percent elongation at failure, which is a measure of the ability of the skin to stretch. Substantiating the results of Voisey, Batal et al. (1) also reported a direct relationship between ultimate force, the percent elongation, and the resistance of tomato skins to cracking. In addition, from the slopes of the stress-strain curves, Batal determined the modulus of elasticity but could find no relationship between the elastic modulus and crack resistance.

In addition to the mechanical behavior of tomato skins, Voisey et al. (5) related skin strength to the histology of tomato skins and concluded that a greater cutinization of the epidermis and underlying cells may have contributed to crack resistance. However, the elongated shape of the epidermal cells did not appear to influence crack resistance. Cotner et al. (2) also could not relate the shape of the epidermal cells to tomato skin resistance to radial cracking but did notice the tomato cultivars resistant to concentric cracking possessed flattened epidermal cells.

The mechanical behavior and histology of tomato skins and their relation to crack resistance has been investigated. However, no study has been conducted to determine the histological distortions occurring in tomato skins as a result of force applications to observe the physical changes that take place in the cells undergoing various stresses. Therefore, this study was undertaken to determine the histological distortions and changes in physical structure brought about by mechanical forces in

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addition to the failure strength, relaxation behavior and normal histology. Furthermore, readings were taken for both a longitudinal and transverse direction in relation to the stem end to determine if a directional relationship existed for tomatoes susceptible to radial or concentric cracking or to both types of cracking.

Materials and Methods

Commercial cultivars of tomato were obtained from Dr. H. A. Mills, University of Georgia, Athens: 'Delicious', 'Sunripe', 'Burpee Early Hybrid', and 'Oxheart'. The tomatoes, cultivated in the same field under the same conditions, were handpicked when ripe according to color. At the time of harvesting, the tomatoes were assigned a "crack resistance score" as outlined by Reynard (3). The 5 numbered classes used for scoring ranged from 100 for fruits with no visible cracks, 80 for short cracks of little consequence, 60, 40, to 20 for progressively more severely cracked fruits. Separate scores were recorded for concentric and radial cracks. One person rated all the tomatoes to reduce variability in scoring. The 75-100 individual scores for each cultivar were averaged to yield a single crack resistance score for each tomato cultivar.

Mechanical tests. A failure test was performed using an Instron Universal Testing Machine, Table Model 1130, at a deformation of 0.508 cm/min until the specimen failed. A relaxation test was also conducted by applying a constant force and allowing the specimen to relax. For either test, a skin specimen was taken in 2 directions from the same tomato as close to the stem end as was practical. One section was longitudinal (with respect to the stem and blossom end) which, when subjected to stress, simulated the conditions considered to cause concentric cracking. The other was a transverse section which, when tested, simulated radial cracking. The flesh was scraped away carefully and, to prevent dehydration, the skin dipped in water prior to loading. The specimens were clamped between 2 rubber-faced jaws set 1 cm apart.

For the failure test, skin thickness was measured after the specimen had failed. To encourage breakage toward the center of the sample, a bell-shaped cutter 2.0 cm long with an end width of 1.0 cm reducing smoothly to a 0.5 cm minimum width at the midpoint was used. Ten longitudinal samples and 10 transverse specimens were taken for the failure test.

The shapes of the skin specimens were different for the 2 tests. For the relaxation test, a portion of skin about 1 cm wide and 2 cm long was used. The crosshead was stopped at a force reading of about 27 N, and the specimen allowed to relax for 5 min while relaxation behavior was recorded. Eight specimens were taken for each direction. A nondeformed section adjacent to the test specimen was used to determine skin thickness.

The stress relaxation in a 3-element model is given by the following formula.

$$\sigma = \sigma_e + (\sigma_o - \sigma_e) \exp \quad -t \frac{(E_o + E_2)}{\eta}$$

where σ is the stress at time t, σ_e is the equilibrium stress at $t = \infty$, σ_o is the stress at $t = 0$, E_o is the instantaneous modulus of elasticity, E_2 is the modulus of elasticity of the Kelvin element spring, and η is the viscous constant. The 3-element model is a Kelvin model with a spring in series. Relaxation data were analyzed using the modified Gauss-Newton method outlined by Wylie (6) for non-linear regression. The multiple regression model applied to the several relaxation data was significant at P = 1%. The model explained 90 to 98% of the variation in the data, indicating a very good fit.

Data from the failure test were used to calculate stress and modulus of elasticity at failure using the following formulas:

$$\sigma_f = F/A$$

where σ_f is the failure stress, F is the force failure, and A is cross sectional area at the site of failure measured after the specimen had failed.

$$\epsilon_f = d/L$$

where ϵ_f and d are strain and deformation at failure; L is the original length of the specimen.

$$E_f = \frac{\sigma_f}{\epsilon_f}$$

where E_f and σ_f are modulus of elasticity and stress at failure.

Data for both the failure test and for the relaxation test were analyzed by ANOVA to determine differences due to cultivars, and the Least Significant Difference test (LSD) was used to detect differences existing between cultivars.

Histology. For both the longitudinal and transverse tests, a strip about 1 mm wide was cut parallel to the region of specimen failure. This strip was cut into 1 × 3 mm tissue blocks, fixed, dehydrated, infiltrated with epoxy resin, flat embedded, and sectioned. The tissue sections were fixed in 2% glutaraldehyde on 0.1 M cocodylate buffer (pH 6.8) for 2 hr. After rinsing in cold 5% sucrose on 0.1 M cocodylate buffer, the specimens were postfixed 1.5 hr with osmium tetroxide in 0.1 M cocodylate buffer (pH 6.8), then rinsed in cold sucrose/cocodylate buffer. The tissues were left in the buffer overnight. The next day, the tissues were block stained with undiluted Paragon-1301 stain (4). The specimens were dehydrated slowly with a 10% graded series of acetone with 15 min changes in each concentration. They were aspirated during the 30, 40, and 50% changes of acetone to remove any trapped air bubbles. The tissue blocks were left for 1 hr in a final change of 100% acetone dried with the addition of sodium sulfate. A low viscosity epoxy resin, Spurr (Polysciences, Inc.), was used. A slow infiltration was accomplished by 15 hr changes in 3:1, 1:1, and 1:3 acetone:epoxy resin mixtures followed by 2 changes in pure resin for 30 hr. The tissues were flat embedded to facilitate orientation and incubated at 70°C for 8 hr. Sections 1 to 2 μm thick were placed on slides and stained with Paragon-1301. Photomicrographs of normal tissue and of tissue in the regions of failure were taken.

Results and Discussion

Physical tests. Reynard (3) suggested that a crack resistant score near 75 was the dividing line between tomatoes resistant and not resistant to cracking. Using this value, the tomatoes susceptible to radial cracking were 'Delicious' with a crack resistant score (CRS) of 66 and 'Oxheart' with a CRS of 63. 'Oxheart' was also susceptible to concentric cracking with a CRS of 70 as was 'Burpee Early Hybrid' with a CRS of 65. 'Sunripe' was resistant to both radial and concentric cracking (Table 1).

Data from the failure and relaxation tests were analyzed to determine if there was any interaction between cultivar and direction and none existed. In addition, no significant differences existed between the longitudinal or transverse direction for any of the cultivars for either the failure test or the relaxation test. The lack of a directional difference led to the conclusion that the properties governing tomato skin strength were the same in all directions indicating isotropic behavior. Hence the data from the longitudinal and transverse directions for each test were combined.

'Sunripe', the cultivar most resistant to cracking, had the shortest relaxation time (Table 1), although not significantly different from cultivars which exhibited predominately radial ('Delicious') or concentric ('Burpee Early Hybrid') cracking. 'Oxheart', which exhibited both radial and concentric cracking, had the longest relaxation time (P = 1%). The longer relaxation time for 'Oxheart' could be interpreted as indication that its skin relaxes and dissipates stress more slowly than the skin of other cultivars and therefore is more likely to crack.

'Oxheart, which cracked both concentrically and radially, had the smallest instantaneous modulus of elasticity, E_o, of

Table 1. Means and analysis of variance of individual crack resistant scores[z], failure test data[y], and the relaxation data[x], for the skins of the four tomato varieties.

Tomato cultivar	Crack score		E_f Modulus of elasticity at failure (Pa x 10^3)	τ Relaxation time (sec)	E_o (Pa x 10^3)	η Viscous constsnt (Pa S x 10^3)
	Radial	Concentric				
Delicious	66	85	17.00	39.27	24.67	2951.2
Sunripe	89	92	22.93	35.06	21.27	2395.3
Burpee Early Hybrid	86	65	20.58	36.58	19.42	2155.0
Oxheart	63	70	22.20	45.46	17.16	2747.5
F value	29.38	28.76	4.9296	8.12551	5.5422	2.8993
Prob F	0.001	0.001	0.0038	0.0003	0.0023	0.0414
LSD						
1%	9.50	8.80	4.45	6.06	5.07	785.7
5%	7.16	6.63	3.35	4.56	3.81	590.7

[z]Means of 75 to 100 samples.

[y]Means of 20 samples.

[x]Means of 16 samples.

all the cultivars (Table 1). 'Burpee Early Hybrid', which cracked concentrically, also had a low E_o. 'Sunripe', which was resistant to both types of cracking had a higher E_o. 'Delicious', which cracked predominately radially, had the largest E_o. The instantaneous modulus of elasticity appears to play a vital role in concentric cracking, with higher E_o values contributing to greater resistance to cracking. However, E_o does not seem to affect the radial cracking behavior in the cultivars investigated.

'Oxheart' and 'Delicious' which exhibited radial cracking, had the largest viscous constants (η). 'Sunripe', resistant to radical and concentric cracking had a low η. A high η seems to lead to radial cracks with no effect on concentric cracking.

The parallel modulus of elasticity (E_2) was not significantly different among the cultivars and was not a factor in cracking behavior. For all parameters, 'Sunripe', the cultivar most resistant to cracking, was intermediate in value and not significantly different from the other cultivars except that 'Oxheart', which cracked both radially and concentrically, differed in relaxation time and E_o.

In the failure test (Table 1), failure stress was not significantly different for any of the tomato cultivars. The modulus of elasticity at failure was significantly lower (P = 5%) from all other tomato cultivars for 'Delicious', a cultivar which exhibited predominately radial cracking.

Histology. In general, the basic anatomy of the skins from these tomato cultivars (Fig. 1) consisted of an epidermis with an outer layer of cutin which varied in its deposition between and/or below the epidermis and underlying cells. Underneath the epidermis were several layers of hypodermal cells which can be divided into 2 distinct regions. For the first 4 to 6 layers, the hypodermis was composed of flat, elongated cells which thereafter became progressively larger and more rounded.

Cotner et al. (2) reported that tomato cultivars possessing flattened epidermal cells were resistant to concentric cracking. However, he did not find any relationship between radial cracking and anatomical differences. In this study the cultivars resistant to concentric cracking, 'Delicious' and 'Sunripe', possessed flattened epidermal cells, while those of 'Oxheart' and 'Burpee Early Hybrid' had rounded epidermal cells, substantiating the findings of Cotner. In addition, the first few rows of hypodermal cells of 'Delicious' and 'Sunripe' were more flattened than those of 'Oxheart' and 'Burpee Early Hybrid.' Although Cotner did not find any difference between tomato skins that resisted radial cracking, some anatomical differences existed in this study. In the second division of the hypodermis of skins resistant to radial cracking, 'Sunripe' and 'Burpee Early Hybrid', the cells were much larger than those of cultivars susceptible to radial cracking, 'Delicious' and 'Oxheart'. The cutin in resistant cultivars also penetrated into the third layer of cells, while the cutin in susceptible cultivars only penetrated into the second layer of cells. 'Oxheart', the cultivar susceptible to both concentric and radial cracking, was quite different anatomically from the other 3 cultivars. It had rounded epidermal cells and the cells of the first few rows of the hypodermis were not flattened

Some relationships between the histology and the physical test results were also noted. 'Delicious' had the highest E_o and η in the relaxation test, (Table 1) and appeared to have less total cutin deposited in the first 2 or 3 layers of cells (Fig. 1). The other cultivars were similar in the amount of cutin deposited in their first 2 or 3 layers of cells. Furthermore, 'Delicious' had a significantly lower E_f (Table 1) than did the other cultivars in the failure test. Apparently cutin deposition is an important factor in the strength of tomato skins.

A relationship between relaxation time (τ) and cell anatomy was observed. As τ increased (Table 1), the cells became smaller but less flattened in the first 4 layers of the hypodermis (Fig. 1). The hypodermal cells of 'Sunripe' (τ = 35.06 sec) were very flat and elongate, the cells of 'Burpee Early Hybrid' and 'Delicious' (τ = 36.38 and 39.27 sec, respectively) were less flat, while those of Oxheart (τ = 45.46 sec) were more round.

Photomicrographs of the tomato skins in the region of failure can be seen in Fig. 2. As might be expected, the cells were flattened and elongated as a result of force application. The cutin was slightly distorted, encompassing some cells in places. The actual separation of the cells occurred primarily between the cell walls. In cases where failure was across cell walls, the cells were not as distorted or flattened as in cases where the specimen failed between cells. Apparently if the cell walls were weak, they broke before the cells had time to elongate. If the cell walls were strong, the cells flattened or elongated until the cells separated. No definite pattern of cell separation could be detected for the different cultivars.

The relaxation test gave more information than the failure test. The failure test required less time but the results were not an accurate indicator of cracking behavior. When screening new tomato cultivars for their susceptibility to cracking, the relaxation test would be more accurate. Resistant cultivars exhibited shorter relaxation times and higher E_o. The direction the sample was taken did not affect results since no directional difference was found for either the relaxation or the failure test.

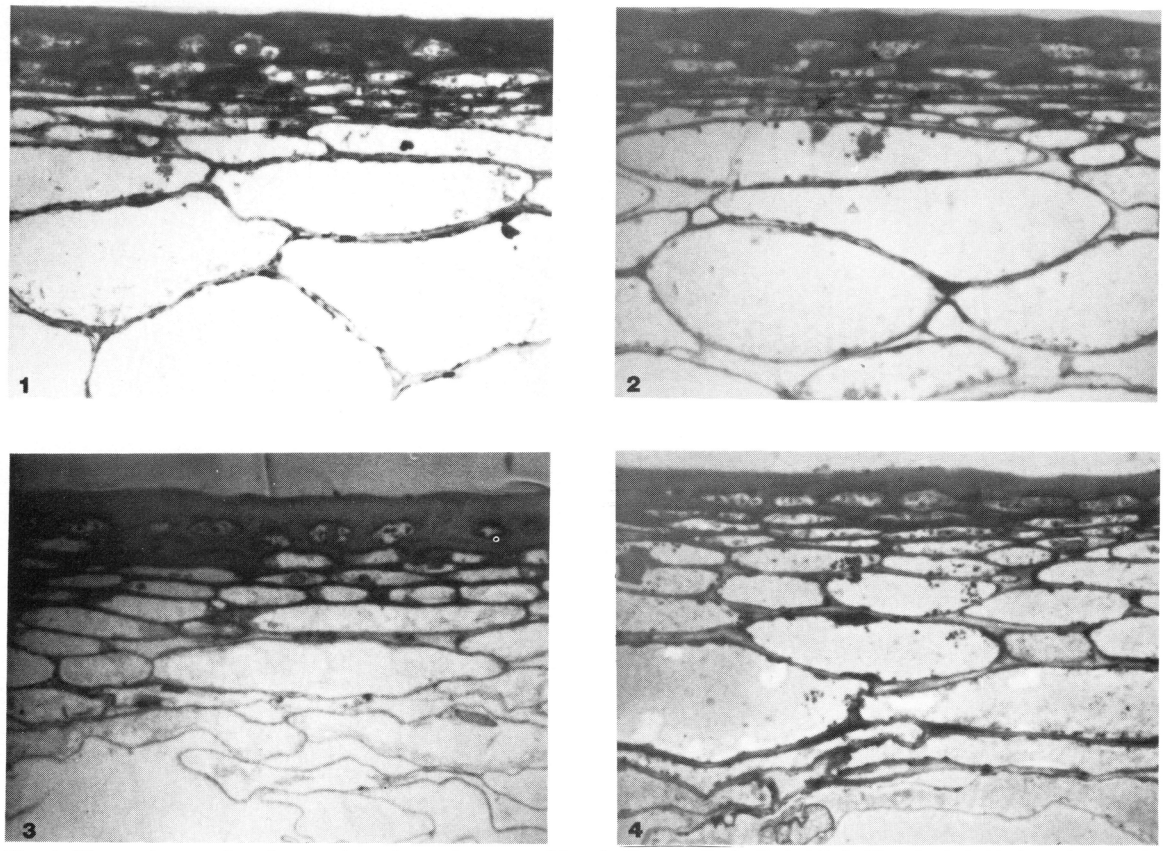

Fig. 1. Photomicrographs of normal sections of tomato skins. 1. 'Burpee Early Hybrid'; 2. 'Sunripe'; 3. 'Oxheart'; 4. 'Delicious' (150×).

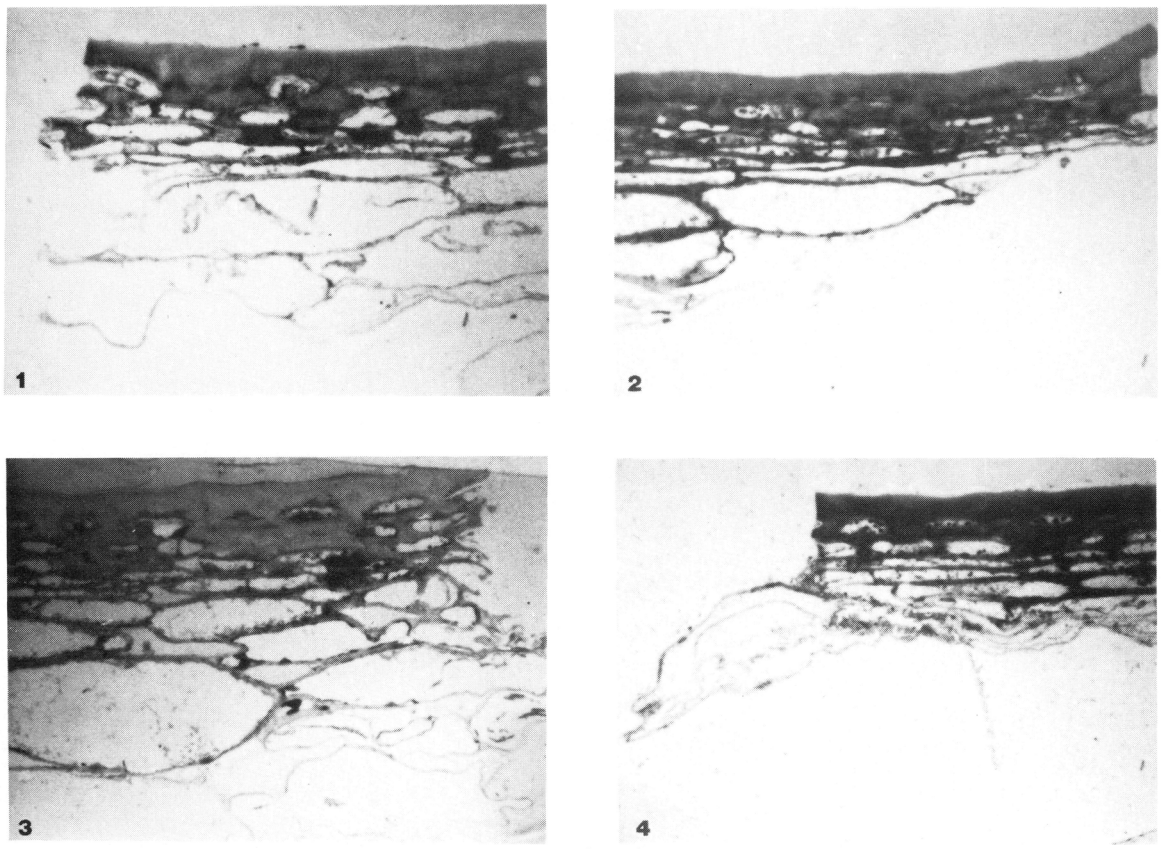

Fig. 2. Photomicrographs of tomato skin tissues that have been subjected to force & failed. 1. 'Burpee Early Hybrid'; 2. 'Sunripe'; 3. 'Oxheart'; 4. 'Delicious' (150×).

Histological analysis may be another rapid indication of the susceptibility of new tomato cultivars to cracking. The shape of the cells and deposition of cutin appear to play an important role in the cracking of tomato skins. Tomato cultivars resistant to concentric cracking possessed flattened epidermal and hypodermal cells for the first few rows. For tomato cultivars resistant to radial cracking, the cutin penetrated into the third layer of cells. Less total cutin resulted in a higher τ, η, and E_f. Flat cells in the first few rows of the hypodermis were associated with a reduced τ.

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Relationships and Interactions between Phenylalanine Ammonia-lyase, Phenylalanine Ammonia-lyase Inactivating System, and Anthocyanin in Apples[1]

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Additional index words. Malus domestica, light, temperature, antibiotics

Abstract: **Low temperature (6°C) in light stimulated the accumulation of phenylalanine ammonia-lyase (PAL) and anthocyanin and reduced the level of phenylalanine ammonia-lyase inactivating system (PALS-IS) in the skin of whole apples. Without light, anthocyanin synthesis did not take place in the skin of whole apples, and the level of PAL was found to be very low. In skin disks held in the dark, however, PAL activity increased but no anthocyanin was synthesized. In both the light and dark conditions, PAL-IS in the skin of whole apples was about 3.5 times higher than that in the apple skin disks after 44 hours of incubation at 18°. In the skin of whole apples, cycloheximide (10, 25, and 50 μM), chloramphenicol (50, 100, and 200 μM), and puromycin (10 and 25 μM) increased the accumulation of PAL and anthocyanin and reduced the level of PAL-IS. In the apple skin disks, cycloheximide (0.1, 1.0, 5.0, and 10.0 μM) and chloramphenicol (10.0 and 50.0 μM) inhibited the accumulation of PAL and anthocyanin to varying degrees. Puromycin had little effect on the accumulation of PAL but strongly inhibited the synthesis of anthocyanin. None of the antibiotics, at the concentrations tested, had any effect on the accumulation of PAL-IS in apple skin disks.**

The development of red color is one of the serious problems confronting the growers of red apples in New York State. Since Extra Fancy or Fancy grades are based on quantity of color, the red coloration is an important economic factor in the production and marketing of red apples.

The major red pigment of apple is a soluble anthocyanin called cyanidin-3-galactoside (24). Its synthesis is influenced by phenylalanine ammonia-lyase (PAL), first described by Koukol and Conn (20). The synthesis of anthocyanin in several plant tissues is associated with increased PAL activity (6, 7, 13, 14, 19). PAL activity is affected by light, temperature, growth regulators, inhibitors of RNA and protein synthesis, wounding (3, 11), and by mineral nutrition (21). The recent finding that a phenylalanine ammonia-lyase inactivating system (PAL-IS) capable of inactivating PAL *in vitro* has increased interest in the study of the possible role of this inactivating system as a regulator of PAL in the plant. PAL-IS has been demonstrated in the extracts of leaf disks of sunflower (8, 9, 10, 26), red cabbage seedlings (9), and sweet potato root (27). In addition, a macromolecule inhibitor of PAL has been reported in gherkin hypocotyls (1, 17). The reaction of PAL inhibitor is reversible (1) while the reaction of PAL-IS is not reversible (27). The main difference between the PAL-IS from sunflower leaf and sweet potato root tissue is in their pH optima. The assay pH optima of the sunflower PAL-IS is 9.5, and that of sweet potato is 6.0.

The objective of the present investigation was to study the relationships and the interactions between the activity of PAL and PAL-IS and the synthesis of anthocyanin in apples. This paper reports on the effect of light, low temperature, and antibiotics on the synthesis of anthocyanin and the accumulation of PAL and PAL-IS in the skin of whole apples and in apple skin disks under these varying conditions.

Materials and Methods

Experimental materials and conditions.

Green apples ('Red Spy') were harvested in mid-September from shaded positions of trees in the Cornell orchard, Ithaca, during the 1976 and 1977 growing season and stored in a cold room for about 1½ month (about 2°C) until used for the whole apple and apple skin disk experiments. When whole apples were

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