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The effects of preharvest factors on the accumulation of antioxidants in fruit peel, and their relationships to superficial scald development after cold storage of apples (Malus domestica Borkh.).

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THE EFFECTS OF PREHARVEST FACTORS ON THE ACCUMULATION OF ANTIOXIDANTS IN FRUIT PEEL, AND THEIR RELATIONSHIPS TO SUPERFICIAL SCALD DEVELOPMENT AFTER COLD STORAGE OF APPLES (*MALUS DOMESTICA* BORKH.)

A Dissertation Presented

by

CYNTHIA L. BARDEN

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ABSTRACT

THE EFFECTS OF PREHARVEST FACTORS ON THE ACCUMULATION OF ANTIOXIDANTS IN FRUIT PEEL, AND THEIR RELATIONSHIPS TO SUPERFICIAL SCALD DEVELOPMENT AFTER COLD STORAGE OF APPLES (MALUS DOMESTICA BORKH.)

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Superficial scald, a physiological disorder of apples, develops during storage, and is believed to result from the oxidation of ∝farnesene to conjugated trienes. Antioxidants are believed to protect against this oxidation, thus providing scald resistance. The hypothesis examined in this study was that temperatures below 10°C before harvest facilitate the accumulation of antioxidants in the apple peel, and that this effect is enhanced by ripening and light.

Cortland and Delicious apples were harvested following exposure to increasing hours below 10°C during three years. Cortland apples were sprayed with ethephon to induce ripening at warm temperatures, and were bagged in late August to produce ripening at low light intensities, both during two years. Percent inhibition of oxidation and water-soluble reducing activities, as well as concentrations of ∝tocopherol, ascorbic acid, glutathione, ∝farnesene,

conjugated trienes and several pigments, were determined at harvest and following intervals of storage. Scald development was determined after 3 to 5 months of storage at 0°C.

Correlations and regressions demonstrated that increasing exposure to temperatures below 10°C was the primary factor in development of scald resistance. Less resistance developed when low light intensity existed during cool periods. Ripening alone was only a small factor in development of scald resistance.

Cool preharvest temperatures slightly increased the concentrations of ∝ tocopherol and carotenes, and slightly increased water-soluble reducing capacity and percent inhibition of oxidation. Ripening increased both percent inhibition of oxidation and total water-soluble reducing activities, and increased concentrations of ∝ tocopherol, carotenes and ascorbic acid. Reduced light intensity (bagging) decreased concentrations of ∝ tocopherol, carotenes, and ascorbic acid, as well as total water-soluble reducing capacity. However, percent inhibition of oxidation and glutathione were not significantly influenced. During storage, as conjugated trienes accumulated, the ∝ tocopherol and carotene concentrations increased, while total water-soluble reducing capacity, ascorbic acid, and glutathione decreased.

Although some support is given to the widely accepted model of scald development, it was concluded that while

antioxidants play a role in scald resistance, they are probably not the key factor.

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CHAPTER 1

INTRODUCTION

Superficial scald is a physiological disorder of apples that develops during storage. The disorder has been responsible for large losses of apples after storage and during marketing (Pierson et al., 1971). Symptoms include patches of brown, dead tissue which in mild cases involve only the fruit peel. In more severe cases, however, some of the underlying tissue may be affected (Pierson et al., 1971). The amount of surface affected can range from small patches surrounded by healthy tissue, up to large areas of dead tissue. These symptoms usually do not become evident during storage until after 4 to 5 months at -1 to 0°C, however, the damage may become obvious after only 2 to 3 months of storage if the fruit are removed and kept in warm air for several days (Pierson et al., 1971).

Although a definite cause of scald has not been established, several factors are known to influence scald susceptibility. These include cultivar, orchard locality, weather, harvest maturity, and storage conditions (Pierson et al., 1971; Fidler, 1957; Merritt et al. 1961; Meigh and Filmer, 1969; O'Loughlin and Jotic, 1978). The occurrence of scald is more frequent in hot, dry seasons than in cool, damp seasons (Fidler, 1957). Immature apples usually develop more scald than those harvested at maturity (Anet, 1972; Chen et al., 1985; Huelin and Murray,1966; Zebbini et

al., 1978), and the green portions of the surface are more susceptible than are the red areas (Albrigo and Childers, 1970; Shutak and Kitchin, 1966). Also, storage conditions such as temperature, ethylene level, ventilation, and atmosphere (O_2 and CO_2 concentrations) all may affect scald development (Little et al., 1982; Lau, 1983; Knee and Hatfield, 1981; Little et al., 1985; Huelin and Coggiola, 1970).

The accumulation of the volatile sesquiterpene ∝ farnesene and its subsequent oxidation to conjugated trienes have been related to scald development (Huelin and Murray, 1966; Huelin and Coggiola, 1968; Meigh and Filmer, 1969). Scald severity was proportional to maximum conjugated triene concentrations in apple peel (Huelin and Coggiola, 1970.; Anet and Coggiola, 1972). Huelin and Coggiola (1970) suggested that scald may develop from the following sequence of events:

- ∝ farnesene is secreted extracellularly by the epidermis and hypodermis;
- ∝ farnesene is oxidized to conjugated trienes (which is prevented perhaps by conditions in the intact living cell);
- oxidation products cause damage by entering cells or by polymerizing to form gas-impermeable films impairing gas exchange.

Evidence suggests that scald does not occur if sufficient endogenous antioxidant is present to prevent or limit the oxidation of ∝ farnesene (Anet, 1972). Anet (1972) reported that immature apples generally do not produce significantly

greater amounts of ∝ farnesene than more mature apples. Thus, their inability to prevent the oxidation of ∝ farnesene, possibly due to a less efficient antioxidant system, may be responsible for their higher susceptibility (Anet, 1972). In a study of sixteen apple cultivars, eleven different endogenous antioxidants were detected (Anet, 1974). Five of the antioxidants, including ∝ tocopherol, were found in all apple cultivars. The occurrence of the remaining six antioxidants varied with cultivar, maturity, and origin of sample. There was a tendency for all antioxidants to increase during the first 2 to 3 weeks of storage (Anet, 1974). Anet (1974) found that the concentration of antioxidant necessary to limit or prevent its autoxidation depended greatly on the amount of ∝ farnesene present in the fruit.

The most common currently used control for scald is postharvest application of diphenylamine and/or ethoxyquin (6-ethoxy-1,2-dihydro-2,2,4-trimethylquinoline) (Pierson et al., 1971). Both of these synthetic antioxidants inhibit the oxidation of ∝ farnesene to conjugated trienes (Huelin and Coggiola, 1970). However, the use of these chemicals has come under scrutiny by regulatory agencies in this country and is not permitted in some European countries (Anonymous, 1991). Ethoxyquin use on apples is being voluntarily withdrawn by its manufacturers (Joseph R. Solga, ATOCHEM North America, personal communication). In order to store apples successfully with reduced dependence on

chemicals, new and more environmentally acceptable control methods must be investigated.

The risks of fruit losses in many circumstances are so high that storage operators often apply maximum treatment levels even though fruit susceptibility, which is quite variable, is unknown. Consequently, antioxidant chemicals often are applied unnecessarily, or in dosages higher than needed. If a method were available to predict scald development accurately at harvest, use of antioxidants might be keyed to the actual risk involved. This could lead to application of less or even no antioxidants in many instances, since actual risk is often less than is feared (W. J. Bramlage, unpublished data).

Meir and Bramlage (1988) found a negative correlation between the absorbance at 200 nm of a hexane extract of apple surfaces and the scald susceptibility of Cortland apples. The total antioxidant activity of the apple peel correlated with the OD 200 values. There are many antioxidants which absorb in the vicinity of 200 nm (Anet, 1974). The authors proposed that OD 200 estimates antioxidant activity and may be a valuable method to predict susceptibility to scald (Meir and Bramlage, 1988).

Weather records present another possibility for predicting scald susceptibility. Fidler (1957) found that hot, dry weather increased scald when compared to cool, damp seasons in England. He concluded that water relations were the critical factor. Merritt et al. (1961) found that

increased hours below 10°C during the period shortly preceding harvest decreased scald in New Jersey. While these observations suggest that scald susceptibility may be predictable from meteorological data, such data must be quantified carefully and tested extensively before commercial recommendations can be justified. Such studies are occurring in conjunction with the investigation reported here.

This study was designed to examine the physiological basis for development of scald resistance in apples prior to harvest. Factors examined for effects on scald resistance were preharvest temperature (as hours below 10°C), ripening, and light. The hypothesis to be examined is that temperature below 10°C before harvest facilitates the accumulation of endogenous antioxidants in apple peel. It is further proposed that ripening and light enhance this effect of cool temperature by contributing to the accumulation of these endogenous antioxidants. It is assumed that endogenous antioxidants can inhibit the oxidation of α farnesene and thus block or impede the sequence of events suggested by Huelin and Coggiola (1970) and described above.

CHAPTER 2

REVIEW OF LITERATURE

Scald Development

Superficial scald is the result of browning and death of hypodermal cells. The cellular contents of the entire hypodermis turn brown in severe cases, and a sunken appearance develops due to radial collapse of the cells of the hypodermis and outer cortex (Bain, 1956; Bain and Mercer, 1963). Except when very severe scald develops, the epidermis cells are not affected.

Evidence suggests that the overall scald-promoting factor is the aerial oxidation of surface lipids (Meigh, 1967). The oxidation of the sesquiterpene ∝ farnesene to conjugated trienes has been implicated in the development of scald (Huelin and Coggiola, 1970; Anet and Coggiola, 1974). Alpha farnesene accumulates in the cuticle and adjacent cells during the initial 2 to 4 months of storage of fruit, and then declines (Huelin and Coggiola, 1968). The large increase in ∝ farnesene seems to correlate with the onset of the respiratory climacteric (Meigh and Filmer, 1969). The first products of the oxidation of ∝ farnesene are conjugated triene hydroperoxides (Anet, 1972). Huelin and Coggiola (1970) showed that the conjugated triene levels in Granny Smith apples reached a maximum after 17 weeks of storage at 1°C. The concentrations subsequently declined,

suggesting further reaction of oxidation products. Anet (1970) reported that in Granny Smith apples harvested on three dates, after an induction period there was a constant rate of conjugated triene production until a maximum was reached. The induction period for autoxidation of ∝ farnesene ranged from 3 weeks for the first harvest to 10 weeks for the last two harvests. While scald does not correlate well with levels of ∝ farnesene, there is a good correlation with conjugated triene levels (Huelin and Coggiola, 1968; Anet, 1970).

In the early stages of autoxidation of ∝ farnesene, 6-methyl-5-hepten-2-one is the major volatile produced (Anet, 1972). Several of the low molecular weight carbonyl compounds which are products of ∝ farnesene oxidation apparently have no effect on scald, but pyruvaldehyde, methyl vinyl ketone, and 6-methyl-5-hepten-2-one require further investigation (Filmer and Meigh, 1971). It appears that the scald-promoting factor is formed by an oxidative process and not by direct synthesis (Meigh, 1967). Feys et al. (1980) suggested that lipoxygenase (LOX) oxidation of polyunsaturated acids in the peel may be involved in induction of scald. They found the highest LOX activity to be in the core and peel. Lipoxygenase activity increased most markedly in the core and peel during storage.

Anet (1972) concluded that scald was induced by the products of ∝ farnesene oxidation and that the amount and time of appearance of the products determined the severity

of the disorder (see Figure 2.1). A free radical attack on ∝ farnesene can occur in two ways: either by hydrogen atom abstraction or by addition of the radical to one of the unsaturated systems. Upon loss of a hydrogen atom, ∝ farnesene becomes a free radical which quickly reacts with molecular oxygen forming a peroxyradical. If this peroxyradical then abstracts a hydrogen atom from either ∝ farnesene or an oxidation product of the conjugated triene, a hydroperoxide is formed. Alternatively, the peroxyradical may add to an olefinic group. If this takes place intramolecularly a free radical is formed. Again, this free radical can quickly react with molecular oxygen to form a peroxyradical which may form a hydroperoxide by abstraction of a hydrogen atom or may form a dimer by addition to an olefin. Such intermolecular addition of peroxyradicals to conjugated olefins forms dimers and polymers. Degradative chain reactions can take place during the oxidation of ∝ farnesene which can lead to the formation of carbonyl compounds. Bain (1956) and Bain and Mercer (1963) proposed a tannin-like effect causing a breakdown of the cytoplasm as the final step in scald development.

Scald Control

Commercial control for scald has been the subject of numerous research projects. It also has been known for several years that different cultivars have different

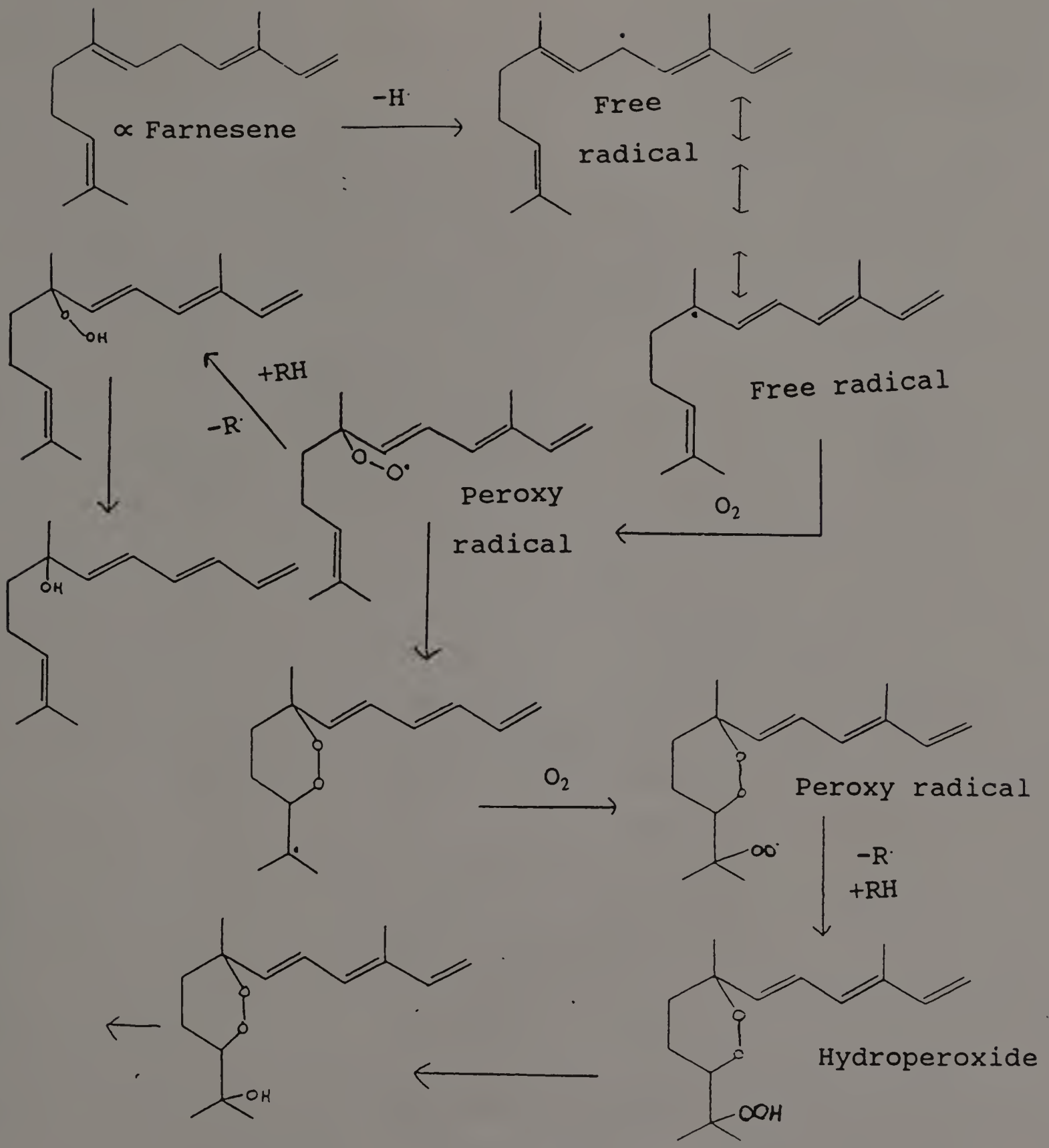


Figure 2.1 Oxidation of ∝ farnesene

susceptibilities to scald development and may respond differently to control measures (Meigh and Filmer, 1969; de Villiers, 1961; Dewey and Dilley, 1964; Southwick, 1963). Southwick (1963) concluded from four years of data that no generalizations could be made about the relationships between scald and maturity or storage conditions for different cultivars, as responses to treatments are variable. Brooks and Cooley (1917) reported that Grimes Golden and York Imperial apples in well-aerated experimental chambers stored at temperatures above 0°C did not develop scald. These authors concluded that scald was the result of abnormal respiratory conditions due to poor aeration. Brooks et al. (1919) confirmed the effects of aeration and reported that scald was always prevented or reduced to negligible amounts by constant air movements of 1/8 to 1/4 mile per hour. They found that air movement was more important than air renewal. Efficient aeration was most important during the first eight weeks of storage. Also in this study the authors tested various gas absorbants, both placed in the storage barrels and impregnated into paper wraps. Excelsior, sawdust, or animal charcoal in the barrel reduced scald significantly. Apple wrappers were prepared by dipping paper in specific hot oils or waxes, followed by draining and cooling. Wrappers with mixtures of olive oil, cocoa butter, vaseline, or beeswax decreased or prevented scald development in Newtown Pippins. The beneficial effects of oil-impregnated wrappers were reported again

later that year by the same authors (1919). The effectiveness of oiled wraps was confirmed many times (Brooks et al., 1923: Carrick and Oskamp, 1928; Anonymous, 1928; Smock and Southwick, 1945; Padfield, 1955). This was the first relatively consistent control for scald and for many years oil-impregnated wrappers were the commercial control procedure for scald (Smock, 1957; Smock, 1961).

While oiled wraps gave relatively good commercial scald control, it was not always successful and had some disadvantages, including cost, consumer objections to paper in the packages, absorption of odors by the oiled papers, and the often incomplete control (Smock and Southwick, 1945). Therefore, scientists continued research on control methods. In 1945, Smock and Southwick reported that air-conditioning agents were effective in decreasing scald, with the most promising being activated charcoal with added bromine. The same authors later reported consistently effective scald control with air purification using activated coconut-shell carbon, if a single cultivar was stored (Smock and Southwick, 1948). Effectiveness decreased if multiple cultivars were stored together; however, they found equal control to that of oiled wraps when mixed cultivars were stored.

In 1955, Smock reported the reduction of scald incidence by treatment with the antioxidant diphenylamine (DPA). Many subsequent reports have supported the effectiveness of DPA as a commercial scald control (e.g.,

Hardenburg and Anderson, 1959, 1960, 1962; Padfield, 1959; Padfield and Smock, 1960; Smock, 1957). In 1957, the antioxidant ethoxyquin (6 ethoxy-1,2,-dihydro-2,2,4-trimethyl quinoline) was reported also to have scald reducing capabilities (Smock, 1957). In 1959, Monsanto Chemical Company released ethoxyquin as 'Stop Scald'.

The effectiveness of diphenylamine (DPA) and ethoxyquin as commercial scald inhibitors is due to their antioxidant activity (Huelin and Coggiola, 1970). Huelin and Coggiola (1970) reported that the oxidation of ∝ farnesene to conjugated trienes was negligible after treatment with DPA. Lurie and Ben-Arie (1989) concluded from their results that DPA may act as an antioxidant influencing other processes as well. They reported effects of DPA on ethylene evolution and some enzyme activities (peroxidase, polyphenol oxidase, and lipoxygenase). Some researchers believe that DPA and ethoxyquin control scald by increasing the pentose phosphate pathway in respiration (Faust, 1967; Sims, 1962).

The best control of scald for some cultivars and conditions is a combination of DPA and ethoxyquin (Porritt and Meheriuk, 1968). The U.S. Environmental Protection Agency set tolerance levels for apple fruit residues at 10 and 3 $mg \cdot kg^{-1}$ for DPA and ethoxyquin, respectively. Residue tolerances in other countries vary from 0 to 5 and 0 to 3 $mg \cdot kg^{-1}$ for DPA and ethoxyquin, respectively (Anonymous, 1991). Since the introduction of DPA and ethoxyquin, use of these chemicals has been the most common commercial control

procedure for scald in the US. However, federal review of these chemicals is due in the next 5 years (Blanpied, 1990). Currently the only variable considered when determining treatment concentrations is varietal differences, when in reality several factors influence susceptibility. Quantities of DPA and ethoxyquin used might be reduced by forecasting of scald susceptibility (Blanpied, 1990) (see Scald Prediction section).

Other practices have been attempted to control scald with mixed success. Antioxidants other than DPA and ethoxyquin have been tested with some success. Ascorbic, gallic and tannic acids were reported to reduce scald (Padfield, 1960 as quoted by Smock, 1961). However, others found these same antioxidants to be ineffective as postharvest dips (Smock, 1961; Stevenson, 1962). Vitamin treatments (such as Vitamins C and E) were reported to give reasonable, short-term scald control (Little and Barrand, 1989). Butylated hydroxytoluene (BHT) was an effective scald inhibitor for Granny Smith when used as a postharvest dip or as BHT-saturated wraps (Wills and Scott, 1977). In order to get inhibition equivalent to that of ethoxyquin, much higher concentrations of BHT than ethoxyquin were required (Gough et al., 1973). Acceptable control of scald also was achieved with postharvest dips of $CaCl_2$ with lecithin (Sharples et al., 1979). The lecithin molecules may form a network bridged by multivalent ions, which might raise the CO_2 and lower the O_2 in the internal atmosphere of

the fruit by affecting the skin porosity. Hot water dips at 54°C for 30 to 60 seconds were effective in inhibiting scald in some cultivars (Hardenburg and Anderson, 1965). Ionizing radiation was reported to reduce scald (Faust et al., 1967). Lurie et al. (1991) reported adequate short-term control (3 months) of scald on Granny Smith apples with prestorage heat treatments of 38°C for four days.

Modified and controlled atmospheres also can influence the incidence of scald. In 1919, Brooks et al. found no consistent benefit to increased oxygen levels but reported a tendency for carbon dioxide concentrations of 1 to 6% to decrease scald. Roberts et al. (1963) reported that at an O_2 concentration of 2.5%, scald was reduced linearly with increasing CO_2 concentrations from 3 to 9%. The use of box liners (e.g., polyethylene), which modify the package atmosphere by allowing a relatively rapid decrease in O_2 and increase in CO_2, has been reported by several studies to reduce scald (Hardenburg and Siegelman, 1957; Hardenburg and Anderson, 1959; Ryall and Uota, 1955; Smock, 1957; Workman, 1957). Several researchers have reported that rapidly established controlled atmosphere with O_2 at 2 to 3% decreased scald (Blanpied and Dewey, 1960; Hardenburg and Anderson, 1959; Patterson, 1959, Smock and Southwick, 1945). Low oxygen storage (1.0-1.5% O_2) has been shown to control scald in Delicious and Newtown effectively (Chen et al., 1985; Chen et al., 1989). For Delicious grown in Oregon the best atmosphere for scald control is 1% O_2 with 1% CO_2 (Chen

et al., 1985). For Washington apples, scald was reduced or eliminated and dessert quality maintained at 1% O_2 with less than 0.03% CO_2 (Chen et al., 1985). However, apples often will not tolerate such low O_2 concentrations. Satisfactory control of scald in Granny Smith apples has been accomplished with initial oxygen stress (0% to 0.5% O_2) followed by ultra low O_2 storage (O_2 < 1.7% at 0°C, 92-96% RH) (Little et al., 1985). Lau (1989) reported better control in Delicious apples with 0.7% O_2 storage than with DPA in 1.5% O_2.

Ethylene reduction from 100 ppm down to less than 6 ppm reduced scald (Little and Barrand, 1989). Johnson et al. (1989) reported complete control of scald in Bramleys Seedling apples by a combination of ethylene removal and 1% O_2 storage. Data from several laboratories suggest that at greater than 100 ppm ethylene, scald is severe and that scald is controlled if ethylene is less than 1 ppm (Blanpied, 1990).

Climatic Relationships

In a controlled climate experiment, Uota (1952) found a linear relationship between increased temperatures during the three weeks prior to harvest and increased scald development. He also found an inverse relationship between anthocyanin development and the percent scald. Fidler (1957) studied many facets of weather as related to scald

development in England. He collected data on total rainfall, total sunshine, and mean temperature, each of which were plotted against scald incidence to determine if simple correlations existed. Extended periods of sunshine during fruit growth increased scald incidence, and scald essentially was absent when heavy rainfall occurred. It follows from this that in irrigated areas scald generally is not a problem. However, heavy irrigation can increase scald susceptibility (Anonymous, 1928; Brooks and Fisher, 1919). The water deficit, which takes into account rainfall and sunshine, correlated very well with scald development in Fidler's studies. Fidler (1957) concluded that water relations were the most important factor in this relationship, but that it would be preferable to consider several parameters since many weather factors are interrelated. Fidler was able to predict scald incidence in England for the years 1954 and 1955 using rainfall data from 1946 to 1953.

These experiments did not show any linear relationship between average temperature and scald; however, high temperatures (>62°F) led to high scald incidence. Smock also reported significant positive correlations between increased temperature and scald development in McIntosh and R.I. Greening but found no relationship with rainfall (Smock, 1953). There are data that suggest that in hot seasons, when there is minimal soil moisture stress, scald is likely to occur (Little and Barrand, 1989). Australian

Granny Smith apples from cooler districts are less scald susceptible than those from the warmer districts (Little and Taylor, 1981). Little and Barrand (1989) reported that in Australia the concentrations of DPA required to control scald were highest in the hottest areas (Manhjimup and Shepparton districts) and the lowest DPA was required in Tasmania, the coolest district.

Merritt et al. (1961) determined that the temperature best related to scald development on Stayman apples in New Jersey was 10°C (50°F). They measured accumulated hours below 10°C after the beginning of cool weather. When apples had experienced 150 hours below 10°C before harvest, scald development after storage was decreased greatly. After fruit had experienced 190 hours below 10°C, scald was almost, if not entirely, absent after storage. However, an unseasonably warm period (from Sept. 22 and Oct. 11, 1959) during the accumulation of hours below 10°C apparently nullified any previous cool hours. They suggested that fruit temperature, rather than air temperature, likely affects scald development. Therefore, they concluded that the relationship between air temperature and scald depends on three factors:

1. temperature of the apples when the air temperature begins to decline;
2. rate at which the air cools;
3. time period of cool temperature.

Morris (1964) used controlled temperature chambers to study the relationship among cool temperature, maturity, and scald development. He found that scald decrease was slightly better correlated with the accumulation of hours below 55°F (12.8°C) than below 50°F (10°C), as Meritt et al. (1961) reported. In a controlled temperature study, Morris (1964) found that apples experiencing many hours of cool temperatures early in the season still scalded to a large degree. The scald incidence in these apples did not decline with cool temperature accumulation unless the apples had matured as well. Morris (1964) consistently found that accumulated hours below 55°F (12.8°C), in combination with maturity (as measured by flesh color), gave the best correlation with scald development.

Maturity

Physiological maturity is defined as "the stage of development when a plant or plant part will continue ontogeny even if detached", and horticultural maturity is defined as "the stage of development when a plant or plant part possesses the prerequisites for utilization by consumers for a particular purpose" (Watada et al., 1984). Immature apples scald more than apples harvested at horticultural maturity (Brooks et al., 1923; Christopher, 1941; Comin and Ting, 1951; Smock and Southwick, 1945; Little and Taylor, 1981; Carrick and Oskamp, 1928; Padfield,

1955). Smock (1961) not only found a decrease in scald with maturity, but in many cases also found a sharp break in susceptibility with later harvests. Shutak and Kitchin (1966) reported that both immature and overmature apples tended to scald more than those harvested at a middle date. Anet (1972) reported that immature apples did not produce appreciably more ∝ farnesene than more mature apples. He attributed their increased susceptibility to a less efficient system of antioxidants and/or to a lower resistance to the toxicity of the products of autoxidation.

Ethylene is implicated as a fruit-ripening hormone and may coordinate several metabolic processes involved in ripening (Mattoo and Aharoni, 1988). Ripening can be delayed by spraying fruit with inhibitors of ethylene biosynthesis (Mattoo and Aharoni, 1988). On the other hand, applied ethylene induces a burst of respiration and hastens ripening.

Preharvest application of (2-chloroethyl)phosphonic acid (ethephon) to fruit stimulates ripening in blueberries (Eck, 1970), tomatoes (Iwahori and Lyons, 1970), pears (Edgerton and Blanpied, 1968), peaches (Sims et al., 1974), and apples (Couey and Williams, 1973; Greene et al., 1974; Hammett, 1976; Pollard, 1974; Unrath, 1972). Application to apples also increases soluble solids concentration (sugar), CO_2 and ethylene evolution, and the rate of flesh softening (Windus and Shutak, 1977).

Several researchers have studied the effects of ethephon on scald development; however, the results are mixed. Greene et al. (1977) found an increase in scald incidence following treatment of McIntosh apples with 150 or 250 ppm ethephon 2 weeks before storage. With Cortland apples, scald development increased following application of 300 or 1000 ppm ethephon 6 days prior to harvest (Windus and Shutak, 1977). However, scald incidence was less on Delicious apples treated with 500 ppm ethephon 3 weeks before harvest, or 250 ppm ethephon 4 weeks before harvest, or 500 to 1000 ppm ethephon 3 months prior to harvest (Couey and Williamns, 1973; Hammett, 1976; Greene et al., 1977). Lurie et al. (1989) reported that application of 500 ppm ethephon 1.5 or 2.5 months before harvest reduced scald development on Granny Smith apples. Padfield (1977) greatly reduced scald with 500 ppm ethephon treatment 12 days before harvest in Granny Smith apples. The variability in results indicates that effects of ethephon on scald incidence may vary with cultivar as well as with time and concentration of application.

Prediction of Scald Development

As discussed, many factors are known to influence scald susceptibility. Several researchers have proposed potential scald predictive methods. As mentioned in the climatic relationships section, Fidler (1957) was able to predict

scald successfully using rainfall data. He found a good correlation ($r=0.995$, $p<0.001$) between scald index and potential evaporation minus rainfall over the period July 23 to September 3. From these data, Fidler (1957) concluded that prediction of scald (as absent or slight, moderate, or severe) was possible measuring rainfall and sunshine.

Merritt et al. (1961) reported the potential to predict scald on the basis of accumulated hours below 10°C (50°F). They found substantial scald reduction in Stayman apples with the accumulation of 150 hours at less than 10°C and almost no scald after the accumulation of 190 hours. Morris (1964) determined that hours below 12.8°C (55°F) in combination with maturity data (determined by flesh color) provided a good basis for scald prediction.

A relationship between accumulated hours below 10°C and scald was reported for Delicious in Washington, with 101 to 200 hours below 10°C reducing scald (average 30% but varying from 2 to 80%), and more than 200 hours below 10°C almost controlling scald completely (Curry, 1990). Some relationships also were found with Julian date, days after full bloom, and maturity indices (Curry, 1990).

Meir and Bramlage (1988) found a negative correlation between the OD 200 of a hexane extract of apple surfaces at harvest and scald development after 4 to 5.5 months of storage in Cortland apples. They concluded that this relatively simple determination may be a practical method of predicting scald susceptibility.

In a cooperative study involving three Canadian and four eastern U.S. locations, scald on Starkrimson Delicious apples was controlled or greatly reduced by harvesting after the accumulation of 90 hours below 10°C or after a starch index (a qualitative evaluation of starch hydrolysis) of at least 5.3 was reached (Blanpied et al., 1991). While these data indicated that scald may be predictable, more data must be collected and analyzed before a predictive model can be developed and used commercially (Curry, 1990).

Effects of Light

Anthocyanin formation in the apple is light-dependent, and low temperatures promote anthocyanin formation (Siegelman and Hendricks, 1958; Uota, 1952). Brooks et al. (1919a, 1919b) found less scald on well-colored apples than on those with poor color. In 1928, the USDA Farmers Bulletin reported that highly colored apple surfaces were resistant to scald. Shutak and Kitchen (1966) enclosed Cortland and Grimes Golden apple fruit in white or black muslin bags in mid-August to study the effects of light on scald incidence. Bagged apples scalded more than those not enclosed in bags, with those in black scalding more severely than those enclosed in white bags. While temperature and humidity likely were affected by bagging, the authors believed that light intensity was the major factor. When they plotted percentages of apples with scald versus percent

red color on the surface, there was a positive correlation until September 18 and then a negative correlation at later harvests. Therefore, the authors concluded that scald was more related to time of picking than to red color. Olsen and Martin (1980) found that as preharvest temperatures decreased, the percentage of red surface increased and scald incidence decreased. Uota (1952) reported that the amount of pigment formed was inversely related to percent scald. Albrigo and Childers (1970) found that the side of the apple exposed to sunlight developed less scald than the shaded side.

Lipid Catabolism in Plants

Meigh (1967) suggested that the overall scald promoting factor is the aerial oxidation of surface lipids. As already discussed, scald is thought to be the result of the oxidation of ∝ farnesene to conjugated triene hydroperoxides and eventual membrane damage; therefore, a discussion of lipid oxidation is relevant here.

Lipid Composition

Acyl lipids are major constituents of plant tissues, and in seeds and fruit flesh are mostly triacylglycerols. In vegetative tissues, however, glyco- and phospholipids comprise the majority of the acyl lipids. The polyunsaturated fatty acids (PUFA) linoleate (18:2) and

linolenate (18:3) are present in varying amounts in both galacto- and phospholipids. These PUFA are very susceptible to oxidation and are substrates in formation of lipid hydroperoxides (LOOX) (Douce and Joyard, 1980). The lipid composition of apple peel is similar to that of leaves, since both contain chloroplasts (Douce and Joyard, 1980). The levels of galacto- and phopholipids decrease during senescence, with glycolipids decreasing prior to phospholipids (Sylvestre and Paulin, 1987, Galliard, 1968). With senescence there is a decrease in unsaturation (Thompson, 1988). Concurrent with this catabolism, there is an increase in products such as free fatty acids (FFA), hydroperoxides, and malondialdehyde.

LAH Activity

Fatty acids are removed from phospho- and galactolipids by polar lipid acyl hydrolase (LAH). These fatty acids either may accumulate or be oxidized. The activities of LAH on phospho- and galactolipids are summarized in Figure 2.2. The enzymic hydrolysis must occur in a lipoprotein phase, as both phospholipids and galactolipids are mainly membrane-bound. Disruption of the bilayer by free fatty acids, due to their detergent-like activity, exposes the acyl-ester bonds to enzymes, resulting in an autocatalytic effect.

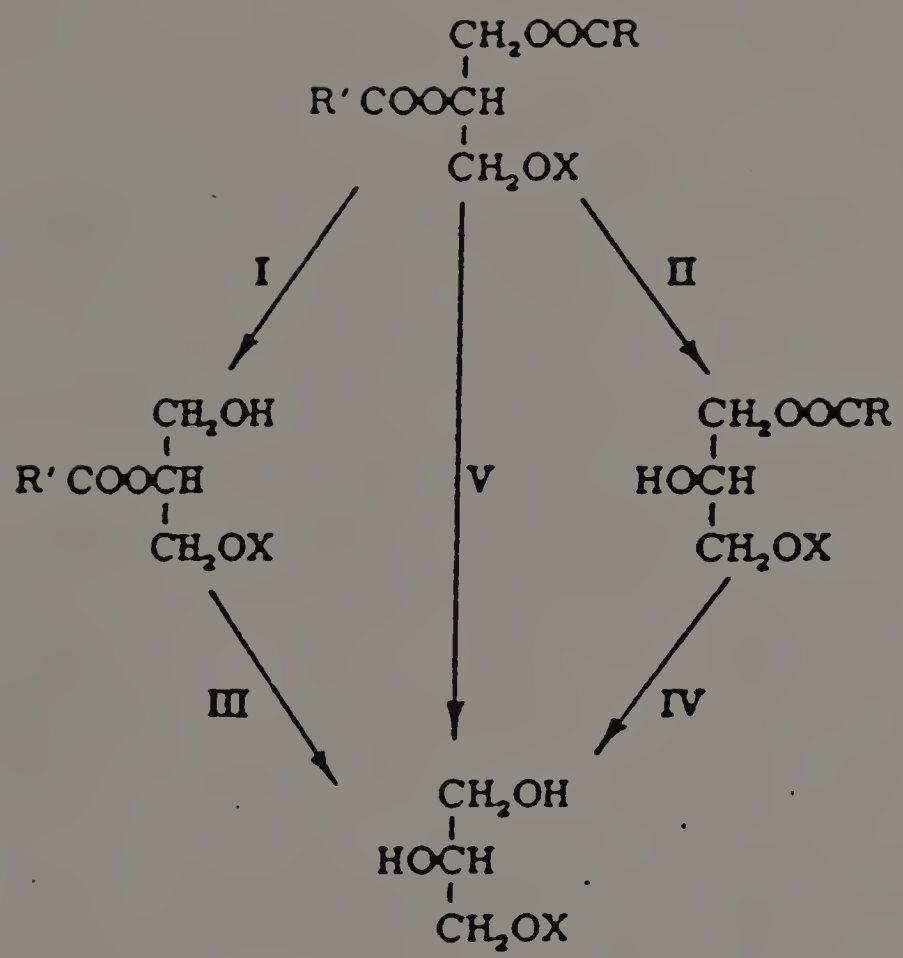


Figure 2.2. Summary of the activities of LAH on phospho- and galactolipids. I=phopholipase-1, II= phopholipase-2, III=phopholipase B, IV= lysophospholipase, V=phospholipase B (phospholipids) or galactolipid lipase (galactolipids).

Lipid Peroxidation

Lipid peroxidation may take place via enzymatic or non-enzymatic mechanisms. Due to its unpaired electrons, atmospheric oxygen easily reacts with organic free radicals in chain reactions. The initial product of lipid peroxidation is hydroperoxide (ROOH).

Initiation: $RH \rightarrow R\cdot$ (via initiator)

Propagation: $R\cdot + O_2 \rightarrow ROO\cdot$

$ROO\cdot + RH \rightarrow ROOH + R\cdot$

Termination: $R\cdot + R\cdot \rightarrow RR$

$R\cdot + ROO\cdot \rightarrow ROOR$

$ROO\cdot + ROO\cdot \rightarrow ROOR + O_2$

The unsaturated fatty acids oleate (18:1), linoleate (18:2), and linolenate (18:3) are the most important substrates for such chain reactions in plants. Their susceptibility increases with degree of unsaturation.

Lipoxygenase (LOX) is a group of enzymes which catalyze the formation of conjugated hydroperoxides in the above reaction sequence. The substrates for LOX are PUFA with a cis,cis-1,4- pentadiene structure. Kim and Grosch (1979) reported that linolenate (18:3) was the preferred substrate for LOX in apples. Evidence indicates that LOX activity is dependent on the release of PUFA by LAH (Leshem, 1987).

The reduction of oxygen in one-electron steps results in the formation of the superoxide anion radical ($O_{2\cdot}$), the

perhydroxyl radical ($HO_2\cdot$), hydrogen peroxide (H_2O_2), and the hydroxyl radical ($HO\cdot$). These species all may participate in lipid peroxidation. Due to its high positive redox potential, $HO\cdot$ is a strong oxidant which can oxidize most biomolecules. Although under biological conditions relatively large amounts of $O_2\cdot$ and H_2O_2 may be generated, neither of these is a strong oxidant, or has been shown to react directly with PUFA. However, the conjugate acid of $O_2\cdot$, the perhydroxyl radical ($HO_2\cdot$), is a much stronger oxidant and can initiate oxidation of linoleic acid. A one-electron reduction of H_2O_2 leads to formation of a hydroxyl anion and hydroxyl radical, which can initiate lipid peroxidation. Redox compounds generate transition metal ions (ferrous, cuprous) which are involved in generation of hydroxyl radicals and initiation of peroxidation. The following "iron-redox cycling" can catalyze lipid peroxidation.

$$Fe^{+3} + RC^- \rightarrow Fe^{+2} + RC\cdot$$

$$Fe^{+2} + O_2 \rightarrow Fe^{+3} + O_2\cdot$$

$$O_2 + O_2 \rightarrow H_2O_2 + O_2$$

$$Fe^{+2} + H_2O_2 \rightarrow Fe^{+3} + HO^- + HO\cdot$$

$$LH + HO\cdot \rightarrow L\cdot + HO^-$$

RC = reducing compounds LH = unsaturated FA

The hydroperoxides resulting from lipid peroxidation are unstable and may cleave to form alkoxyl and hydroxyl radicals, aldehyde esters and aldehydes. The formation of

radicals can initiate further lipid peroxidation chain reactions.

In plants, hydroperoxides may be broken down by a hydroperoxide lyase or a hydroperoxide dehydrase. One of the possible products from hydroperoxide dehydrase reactions is an intermediate in synthesis of jasmonic acid and methyl jasmonate (Vick and Zimmerman, 1987). Both jasmonate and methyl jasmonate promote senescence. Several secondary products, volatile and non-volatile, are formed from hydroperoxides. In apples, C-6 aldehydes were formed from linoleic acid, and volatile production was several times greater in the peel than in the pulp of the apples (Guadagni et al., 1971).

Control of Lipid Peroxidation

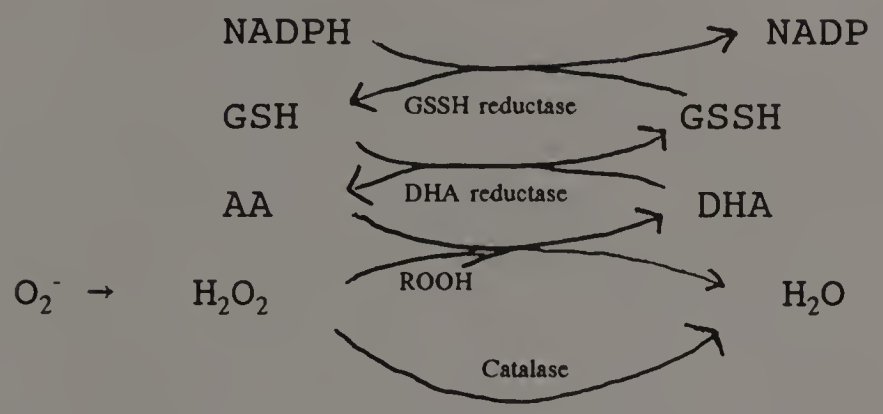
In order for lipid peroxidation to be avoided, plant metabolism must control oxygen, active oxygen species, catalysts, and free radicals. As discussed under lipid peroxidation, one-electron reductions of oxygen lead to formation of four oxidants: $O_2^{\cdot-}$, HO_2, H_2O_2 and $HO^{\cdot}$. These all can participate in lipid peroxidation. Unsaturated fatty acids are present in all cellular membranes. Linoleic acid and linolenic acid are the major unsaturated fatty acids in plant cell membranes and both are prone to oxidation by oxygen radicals. Many products, including short chain alcohols, aldehydes, and ketones, result from this oxidation

(Elstner, 1987). Such free radical attack on membranes may result in lysis, fatty acid deesterification, and decreased bulk lipid fluidity (Thompson, 1988). A decrease in membrane fluidity affects functions of membrane-associated enzymes and receptors. There is a correlation between lipid peroxidation and increased membrane permeability in senescing leaves (Thompson, 1988). The accumulation of peroxidized lipids also can contribute to membrane bilayer destabilization. The plant has many lines of defense to avoid such oxygen toxicity, which are discussed in more detail below.

Oxygen and Active-oxygen Species

Plants depend mostly on superoxide dismutase (SOD), catalase, and ascorbate peroxidase to control active-oxygen species (Rabinowitch and Fridovich, 1983). SOD catalyzes the conversion of superoxide to H_2O_2 and oxygen. There are three classes of superoxide dismutases present in different cellular compartments, which have different prosthetic groups but which all catalyze the same reaction (Fridovich, 1977). There are superoxide dismutases which contain copper and zinc, others which contain manganese, and still others which contain iron (Fridovich, 1977). The cuprozinc superoxide dismutases are found in the cytoplasm. The other superoxide dismutases are found in the mitochondria. In addition to converting superoxide, SOD protects plant tissue from "iron-redox cycling". The H_2O_2 generated by the SOD

reactions is converted to water and oxygen by catalase. Active oxygen species are controlled by the following general scheme:



AA = ascorbate DHA = dehydroascorbate GSH = reduced glutathione GSSH = oxidized disulfide glutathione

Older tobacco leaves showing signs of membrane damage have decreased levels of SOD and catalase. A positive correlation between the levels of these two enzymes and the degree of lipid peroxidation in tobacco leaves has been reported (Larson, 1988).

Lipoxygenase

Lipoxygenase catalyzes peroxidation of unsaturated fatty acids leading to the formation of various secondary lipid oxidation products (Hildebrand, 1989). This oxidation of membranes fatty acids can lead to increased permeability which may result in increased Ca^{+2} in the cells, stimulation of phospholipases, and release of fatty acids. LOX therefore accelerates deterioration of membranes and

metabolic functioning (Hildebrand, 1989). Several compounds can inhibit LOX activity. Catechols reduce the iron in the active center of the enzyme, thus inhibiting its action. ∝ Tocopherol is also an inhibitor of LOX. This inhibition appears to be via formation of a LOX-tocopherol complex rather than by free radical scavenging (.

Free Radicals

Free radicals are atoms or molecules with an unpaired electron such that they can accept (or donate) an electron from (or to) an adjacent molecule. Such free radicals can contribute to deesterification of membrane lipids, leading to substrates for LOX. Again, it is critical for plants to control such activity. ∝ Tocopherol and other antioxidants control lipid peroxidation by trapping radicals (i.e., $ROO^{\cdot}$, $RO^{\cdot}$) and effectively breaking the chain reactions. Most of the chain - breaking antioxidants (AH) are phenolics and stop the chain as follows:

$$ROO^{\cdot} + AH \rightarrow ROOH + A^{\cdot}$$

$$ROO^{\cdot} + A^{\cdot} \rightarrow ROOA$$

$$A^{\cdot} + A^{\cdot} \rightarrow AA$$

An effective antioxidant can be a donor of a hydrogen, electron, or radical and results in a stable compound that will not initiate a new chain reaction.

Endogenous Plant Antioxidants

Many naturally occurring substances in plants have antioxidant activity.

Vitamin E (∝ Tocopherol)

The tocopherols are a group of phenolic benzochroman derivitives having alkylated rings (Larson, 1988). These lipid-soluble molecules are found in the hydrophobic interior of membranes, especially chloroplast membranes. The major role of ∝ tocopherol (the most biologically active tocopherol) is activity as a lipid-soluble chain-breaking antioxidant. Tappel (1980) reported effective protection against peroxidation by vitamin E. Each molecule of ∝ tocopherol inactivates two equivalents of peroxy radicals, thus terminating two chain reactions. There is potential for regeneration of vitamin E via vitamin C (Niki et al., 1983). Ascorbic acid has the ability to reduce the ∝ tocopheryl radical back to ∝ tocopherol and this may occur in intact membranes. Actually, the reduction probably occurs near the surface of the membrane since vitamin C is polar and likely will not enter the hydrophobic membrane interior. Several researchers have reported a synergistic effect with vitamin E and vitamin C (Tappel 1962, Niki et al. 1984, Finckh and Kunert 1985). The proposed relationship is vitamin E as the primary antioxidant, and

reductive regeneration carried out by vitamin C. This synergism also has been reported in vitro (Packer, 1979).

Vitamin C (Ascorbic Acid and Dehydroascorbic Acid)

Two equivalents of $O_2^{\cdot}$ are reduced by ascorbate to form H_2O_2 and dehydroascorbic acid (DHA). DHA then can be converted back to AA by dehydroascorbate reductase (Englard and Seifte, 1986). Ascorbate may also react with $^1O_2$ and scavenge peroxy radicals, thus breaking a chain reaction. Evidence suggests that vitamin C regenerates vitamin E activity by donating a hydrogen atom to the vitamin E-derived phenolate (Niki et al., 1983). Kunert and Ederer (1985) found that the ratio of vitamin C to vitamin E was the important determination for antioxidant activity as related to age-related peroxidation in leaves. Others found a ratio of 10:1 to 15:1 (C:E) provided good protection against peroxidative damage (Finckh and Kunert, 1985). Kunert and Ederer (1985) concluded that the amount of peroxidation is directly related to the efficiency of the antioxidant system, e.g., the system involving vitamin C and vitamin E.

Apple peel has been reported to contain three to four times as much ascorbic acid as the apple pulp (Fellus et al., 1932; Fish, 1943; Murneek and Wittwer, 1948). The cultivar Stayman from one season contained 14.5 mg per 100 g fresh weight (Fish et al., 1944). Researchers have reported that the sun-exposed sides of the apples have more ascorbic

acid than the shaded (green) sides (Murneek and Wittwer, 1948; Albrigo, 1968). During the first two months of cold storage Stayman apples were reported to lose as much as 26% of their at-harvest ascorbic acid; however, little additional loss occurred over the next two months of storage (Fish, 1943).

Glutathione

Glutathione (GSH) is a tripeptide with a thiol group and is found in relatively high concentrations in many cells. Known functions of glutathione include herbicide detoxification, removal of toxic derivatives of oxygen in the ascorbate-glutathione cycle, enzyme induction and involvement in sulfur metabolism (Alscher, 1989; Rennenberg, 1982). Some have suggested that glutathione is involved in plant adaption to environmental stresses (Wise and Naylor, 1987; Esterbauer and Grill, 1978)

Glutathione reacts with oxidants such as H_2O_2 to form the oxidized form, a disulfide GSSG;

$$ROOH + 2\ GSH \rightarrow ROH + GSSG + H_2O$$

It also can react with other oxidants such as $^1O_2$, O_2^- and $HO\cdot$ (Larson, 1988). In leaves, glutathione is synthesized in the chloroplast and in the cytosol (Foyer, 1991). Data suggest that glutathione synthesis might be light dependent (Smith, 1985). Glutathione reductase maintains glutathione in the reduced form (GSH). Although located mostly in the chloroplast stroma, glutathione reductase also is found in

the mitochondria and cytosol (Edwards et al., 1990). The chloroplast envelope has the ability to transport ascorbate (Anderson et al., 1983), thus it is possible that the ascorbate-glutathione cycle functions in the chloroplast and cytosol of leaves through a redox link between these compartments (Foyer, 1991). This would provide protection against oxidative damage. Evidence suggests that glutathione reductase may be a critical enzyme in protection against environmental stress (Wise and Naylor, 1987).

Wise and Naylor (1987) implicated glutathione in a protective antioxidant role following a study of glutathione response to cold stress in chilling-sensitive and chilling-resistant plants. They found that in cucumber (chilling-sensitive), chilling caused photooxidation and lipid peroxidation, with a corresponding GSSG accumulation and decrease in total glutathione. In pea (chilling resistant), however, total glutathione decreased only slightly with chilling treatment and lipid peroxidation, and photooxidation did not occur. Total glutathione was shown to increase in hybrid poplar with ozone exposure (Alscher, 1989).

Flavonoids

The flavonoids, like the tocopherols, are phenolic compounds. One major class of flavonoids is the water-soluble anthocyanins, which impart the red and blue colors of many fruits. Anthocyanins are found for the most part in

the fruit peel, located in the vacuoles of the cells of the epidermis (Gross, 1987). The flavonols are another group of flavonoids. Several flavonoids, such as myricetin, robinetin, kaempferol, and quercetin, show antioxidant activity, and some may inhibit destruction of ascorbic acid (Larson, 1988). Flavonoid content is lower in plants grown in the shade (Larson, 1988). The antioxidant activity of flavonoids appears to be due to the ability to donate a hydrogen atom to the peroxy radical. Some flavonoids, such as luteolin and 3',4'-dihydroxyflavone, can inhibit LOX activity (Larson, 1988). Environmental conditions, including light, temperature, and water, influence the amount of anthocyanin formation in plants (Gross, 1987). Light is the most influential of these factors and may activate enzymes involved in the biosynthesis of anthocyanins. Phenylalanine ammonia lyase (PAL) directly influences anthocyanin synthesis and is influenced by all of the above-mentioned environmental factors (Gross, 1987). Most fruits which have anthocyanin develop more color when grown in regions which experience cooler temperatures during the growing season.

Other Phenolic Compounds

Several simple phenolic acids, including caffeic acid and chlorogenic acid, have shown antioxidant activity. Other phenols such as ubiquinone may also contribute to protection from oxidation (Larson, 1988).

Carotenoids

Carotenoids, or at least beta-carotene, are known antioxidants. Beta-carotene is an effective quencher of singlet oxygen (Larson, 1988). Free radicals react readily with beta-carotene under certain conditions. The rate constant for quenching by beta-carotene is fast enough so that relatively low concentrations can effectively protect membrane lipids from reaction of $^1O_2$ which leads to peroxidation. The highest concentration of carotenoid in fruit is most often in the rind or peel. In apples the carotenoid concentration is five times higher in the peel than in the pulp (Goodman and Goad, 1970). Several researchers have reported that carotenoid levels in fruits increase with maturation (Goodwin, 1952). As Golden Delicious apples matured, however, the carotene content of the peel and the pulp decreased (Workman, 1963). During ripening the level of carotenoids in banana peel is constant (Loesecke, 1929). This is also true in some apple cultivars (Valadon and Mummery, 1967). Based on studies with detached tomato fruit, total carotenoid levels are not affected by ethephon treatment and carotenoid biosynthesis is light-dependent (Paynter and Jen, 1976). In addition, others have reported that more carotenoid synthesis occurred in the dark than in the light in some tomatoes, peaches, nectarines and apricots (Smith and Smith, 1931); however, Raymundo et al. (1976) found evidence that carotenogenesis is dependent on light. They reported that the pattern of carotenoids was

the same in tomatoes ripened in either the dark or the light, and they suggested that light may be stimulatory for the process but may not be required for induction.

Nitrogen Compounds

Several nitrogen compounds have been implicated as antioxidants (Larson, 1988). A number of alkaloids are inhibitors of $^1O_2$. Superoxide radicals are scavenged by high concentrations of the polyamine, spermine. In the dark, chlorophyll reportedly can act as an antioxidant.

Antioxidants and Scald Development

Anet (1972) attributed the high scald susceptibility of immature apples to a less efficient antioxidant system. He reported an induction period of 3 weeks for the beginning of α farnesene autoxidation in early picked Granny Smith apples, while later picked apples showed autoxidation only after 10 weeks, due to a more effective antioxidant system. In later studies, Anet (1974) isolated 11 antioxidants from apple peel tissue and stated that scald did not occur if sufficient antioxidants were present to limit the α farnesene oxidation. Meir and Bramlage (1988) found that antioxidant activity of apple peel tissue at harvest in Cortland apples was negatively correlated with scald development.

CHAPTER 3

MATERIALS AND METHODS

Plant Materials

Apples for all experiments were harvested from trees at the University of Massachusetts Horticulture Research Center, Belchertown. Cortland, Delicious, and McIntosh apples were used (Table 3.1). Trees selected for experiments were as uniform as possible. The apples were stored in air at 0°C.

McIntosh apples were sampled in 1989 and 1990, but no scald occurred on the fruit. Thus, analysis of McIntosh tissue were discontinued, and data accumulated to that point are presented in Appendix D.

Preharvest Factors

Cool Temperatures

Temperature records were kept in the orchard, using a recording thermometer, during each season beginning August 1. The hours below 10°C were recorded for each harvest date. Apples were harvested three or four times each season in order to have a range of hours below 10°C. Depending on the year, the accumulated hours ranged from 62 to 119 for McIntosh; 21 to 187 for Cortland; and 62 to 365 for Delicious. Six replications were used for each cultivar,

Table 3.1. Characteristics of trees used in all experiments.

Experiment	Cultivar	Strain	Rootstock	Year Planted
1988 Hrs < 10°C	McIntosh	Geneva	M.7	1971
1988 Hrs < 10°C	Cortland	Standard	M.7	1963
1988 Hrs < 10°C	Delicious	Gardner	MM.106	1976
1989 Hr < 10°C	McIntosh	Summerland	M.7	1971
1989 Hrs < 10°C	Cortland	Standard	M.7	1963
1989 Hrs < 10°C	Delicious	Gardner	MM.106	1976
1989 Ripening	Cortland	Standard	M.7	1963
1989 Light	Cortland	Standard and Redcort	M.7a	1980
1990 Hrs < 10°C	Cortland	Standard	M.7	1963
1990 Hrs < 10°C	Delicious	Gardner	MM.106	1976
1990 Ripening	Cortland	Standard	MM.106;M.9/ MM.111	1970;1977
1990 Light	Cortland	Standard	M.7	1963

with one or two trees serving as a replication, depending on the crop load.

Light

Bagging of fruit was used to investigate the effects of light. In 1989, Cortland (Standard strain and Redcort), and in 1990 Cortland (Standard strain), were covered with Kraft paper bags in mid- to late August. Five replications were used and each replication included one tree with bagged fruit and one non-bagged control tree. The pairs were chosen randomly. In 1989 and 1990, 250 and 200 apples per replication were bagged, respectively. The apples were bagged by pulling bags over one or more apples, rolling down bag tops and stapling the bags closed. Apples were harvested twice in each year: September 18 and October 2, 1989 and October 1 and 9, 1990.

In 1991 thermocouples were placed near exposed apples and in bags with apples to determine if any temperature differences occurred. The average temperature inside the bags was 0.5°C lower than the average temperature of the air surrounding the apples. Although small, the difference was statistically significant. Plots of the temperatures on relatively warm and relatively cool days are presented in Figures 3.1 and 3.2, respectively.

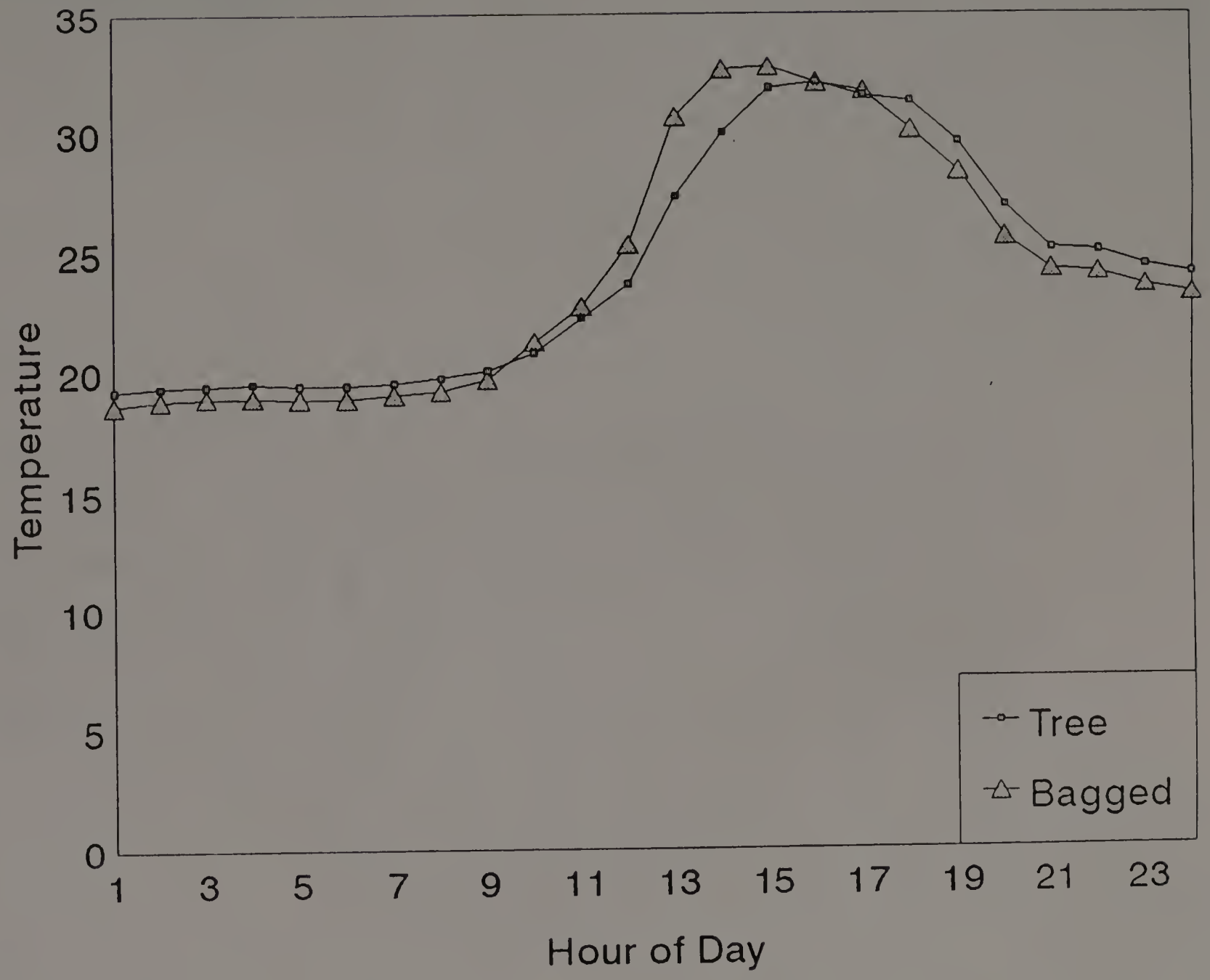


Figure 3.1. Temperatures measured during a 24-hour period both inside a bag enclosing a fruit, and near an unbagged fruit similarly positioned in the tree canopy. Relatively warm day, beginning 1 a.m. September 16, 1991.

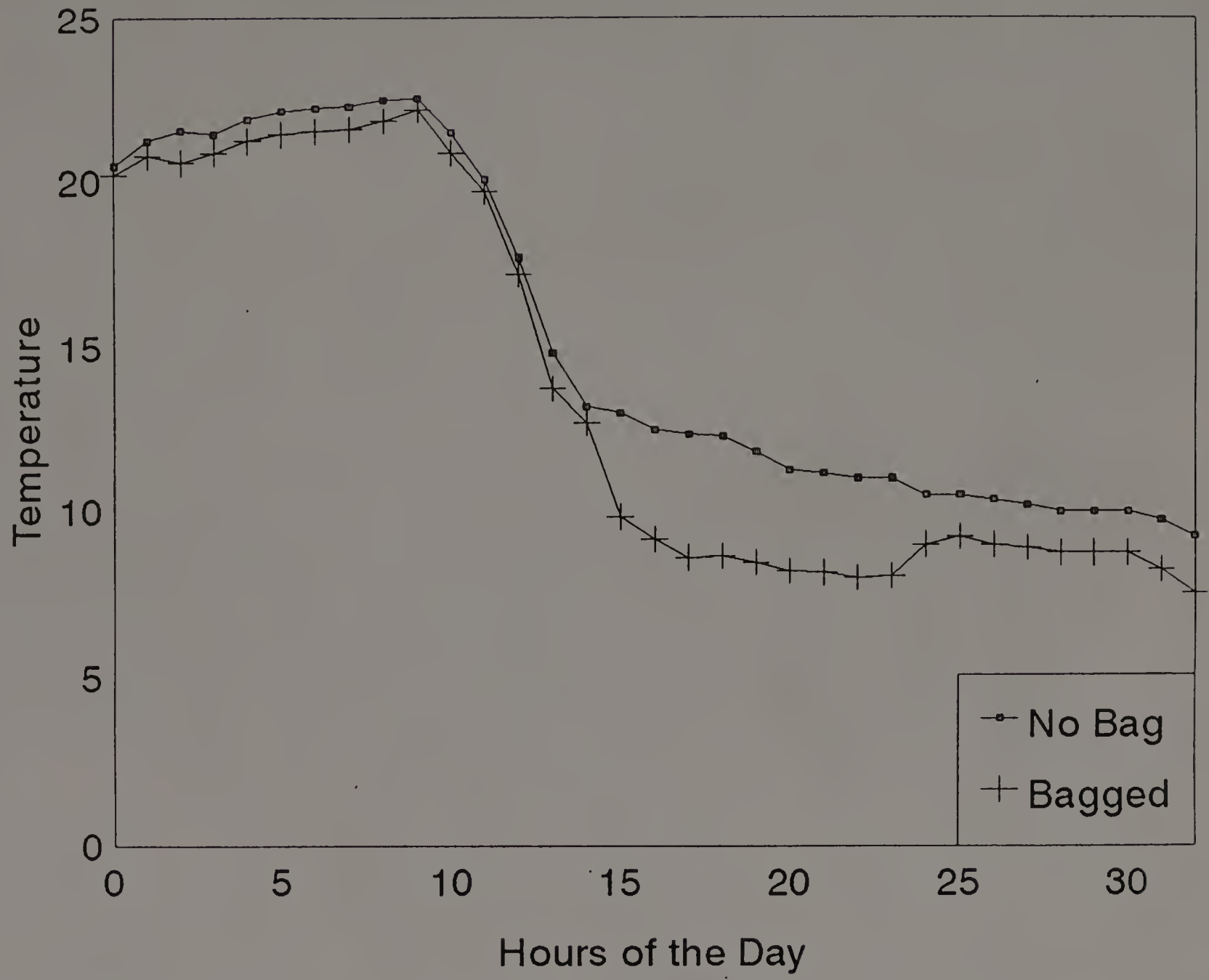


Figure 3.2. Temperatures measured during a 30-hour period both inside a bag enclosing a fruit, and near an unbagged fruit similarly positioned in the tree canopy. Relatively cool day, beginning 1 a.m. September 19, 1991.

Ripening

During ontogeny of fruit on the tree, maturation progresses as long as the fruit are attached, because fruit development continues. This "maturation" ceases at harvest, when the fruit becomes a detached organ. "Ripening" is a specific set of changes that occur in fruit at a certain stage of maturity. In a climacteric fruit such as the apple, ripening occurs simultaneously with the respiratory and ethylene climacterics.

Ripening can occur both preharvest and postharvest. When a fruit begins to ripen on the tree, it therefore can be said to be both "maturing" (since developmental changes continue) and "ripening". Thus, there is a confounding of terminology and of physiological changes when fruit are harvested on different dates, and ripening begins to occur during this harvesting period. Until ripening begins, differences among samples can be referred to as "maturity differences". Once ripening begins, however, differences among fruit at different sampling dates represent both maturity and ripening changes. In the study reported here, ripening will be induced while fruit are attached to the tree, and changes in fruit and differences among samples will be referred to as "ripening" differences and changes. It is recognized that some maturation may also have occurred, but the ripening changes should be of greater magnitude.

Ethephon at 500 ppm was applied on August 16, 1989. In 1990 ethephon was applied on August 20 at 250 or 500 ppm. In both years the ethephon was applied in combination with a surfactant and sprayed to the drip point. The ethephon was applied to induce ripening in order to have apples which were at different stages of ripeness but which had accumulated the same numbers of hours below 10°C. Five replications were used and each replication consisted of two trees for each rate of ethephon treatment (0, 250, or 500 ppm). The apples were harvested twice each season: September 6 and 13, 1989, and September 1 and 6, 1990.

Statistical Analysis

Replications in experiments consisted of one tree or a pair of trees, depending on crop load. Blocking was done when possible and in experiments involving treatments, randomly selected trees (replications) were treated.

Statistical analyses included analysis of variance and regression where appropriate. The SAS statistical package was used to analyze the data.

Harvesting and Storage Sampling

Harvesting

Apples were harvested during the morning of each harvest date. Sufficient numbers of apples were picked at

each harvest for the at-harvest analyses, storage samples, and scald evaluation as dictated by each experiment (see Tables 3.2, 3.3 and 3.4).

Ripening

The mean ripeness of ten apples per replication of each treatment was determined within a day of harvest, using three indices; internal ethylene concentration, starch pattern, and flesh firmness. Ethylene was measured using a Shimadzu GC-8A gas chromatograph with an 18 inch activated alumina column. The injector temperature was 110°C and the column was 40°C. A gas sample was withdrawn from the core cavity with a 4 ml syringe and 1 ml was injected into the gas chromatograph. A Shimadzu C-R3A Chromatopac integrater was used to assess ppm ethylene. Firmness on two sides of each apple was determined using a Magness-Taylor penetrometer. Starch was evaluated by dipping apples sliced at their equator into iodine solution, and comparing pattern development to a standard chart (Lau, 1985 through personal communication).

Hexane Extract

At each harvest and each storage sampling, ten apples from each replication were dipped one at a time into 100 ml of HPLC-grade hexane (Fisher Scientific Co.). Each apple remained in the hexane for three minutes with periodic turning. After all ten apples had been extracted, the

Table 3.2. Dates of harvest and intervals of storage at 0°C prior to storage sampling and scald analyses, 1988.

Experiment	Cultivar	Harvest date	Storage sampling	Scald evaluation
Hours<10°C	McIntosh	Sept. 13	7, 14, 21	19
		Sept. 20	"	18
		Sept. 27	"	17
Hours<10°C	Cortland	Sept. 15	"	19
		Sept. 22	"	18
		Sept. 29	"	17
		Oct. 6	"	16
Hours<10°C	Delicious	Oct. 1	"	25
		Oct. 8	"	24
		Oct. 15	"	23

Table 3.3. Treatment and harvest dates and weeks of storage at 0°C prior to storage sampling and scald analyses, 1989.

Experiment	Cultivar	Treatment dates	Harvest dates	Storage sampling	Scald evaluation
Hrs <10°C	McIntosh	N/A	Sept. 12	6,12,18	18
			Sept. 19	N/S	17
			Sept. 26	N/S	16
Hrs <10°C	Cortland	N/A	Sept. 15	"	18
			Sept. 22	"	17
			Oct. 4	"	16
Hrs <10°C	Delicious	N/A	Sept. 29	"	24
			Oct. 5	N/S	23
Ripening	Cortland	Aug. 16	Sept. 6	"	17
			Sept. 13	"	16
Light	Cortland	Aug. 21-25	Sept. 18	"	17
			Oct. 2	"	15

Table 3.4. Treatment and harvest dates and weeks at 0°C prior to storage sampling and scald analyses, 1990.

Experiment	Cultivar	Treatment dates	Harvest dates	Sampling dates	Scald evaluation
Hrs<10°C	Cortland	N/A	Sept. 17	8, 20	20
			Sept. 24	"	19
			Oct. 3	"	18
			Oct. 11	"	17
Hrs<10°C	Delicious	N/A	Sept. 21	"	27
			Sept. 26	"	26
			Oct.3	"	25
			Oct. 11	"	24
Ripening	Cortland	Aug. 20	Sept. 1	6,12,18	20
			Sept. 6	N/S	19
Light	Cortland	Aug. 13-15	Oct. 1	N/S	17
			Oct. 9	N/S	16

hexane was made up to 100 ml and stored in the dark at room temperature. Appropriate dilutions of the hexane extract were analyzed in a scanning u.v. spectrophotometer (Beckman Model 25), recording absorbance from 300 to 190 nm. A recorder was used until November 1989, after which time readings were taken manually from the digital readout. From the resulting peaks, the OD200 values and the ∝ farnesene and conjugated triene concentrations were calculated (see Appendix A). The OD200 value was calculated as the OD at 200 nm multiplied by 1000 and divided by the total peel area of the extracted apples (cm^2). Appropriate extinction coefficients and surface areas were used to calculate the concentrations of ∝ farnesene and conjugated trienes (ϵ_{232} = 27,700; $\epsilon_{281\text{-}290}$ = 25,000). A sample calculation is shown in Appendix A.

Sample Preparation for Antioxidant Assays

Freeze-dried apple peel was used for all antioxidant assays. Apples were peeled using a White Mountain Apple Peeler (White Mountain Freezer, Inc., Winchendon, MA). The peels from 10 apples per replication were composited and placed in plastic bags, frozen on dry ice, stored in a freezer at -30°C and subsequently freeze-dried in a freeze dryer (Repp Industries, Inc., Gardiner, N.Y.). The freeze-dried tissue was ground using a Wiley mill with a 20-mesh screen. The ground tissue was stored in a dessicator in a freezer at -30°C.

Storage Sampling

Samples of some of the experiments were removed following periods of storage (See Table 1). The storage samples were evaluated for the same parameters as the at-harvest samples, with the exception of the ripeness indices.

Scald Evaluation

After 5 to 6 months of cold storage, samples of 25 to 150 fruit from each replication from each harvest were evaluated for scald incidence and scald severity. The incidence was reported as the percent of fruit with scald. The scald severity was determined by assigning a score of 1 to 4, based on the percent of the surface exhibiting scald, as follows:

1 = 1-10%

2 = 11-33%

3 = 34-66%

4 = >66%

Scald scores for samples are the mean scores of only fruit that developed scald, not mean scores for all fruit in the sample.

Analytical Procedures

Percent Inhibition of Oxidation

A modification of the method of McKersie et al. (1982) was used to assay percent inhibition of oxidation in the apple peel. Samples of 0.5 g of freeze-dried apple peel were extracted in 10 ml of HPLC-grade hexane (Fisher Scientific) at room temperature in the dark, overnight. The extracts were filtered through Whatman No. 4 filter paper and 20 μl aliquots of the extract were dried in air in large test tubes for 30 minutes and then redissolved in 0.2 ml of 100% ethanol. Standards of α tocopherol were in ethanol with 0 to 5μl of α tocopherol (Sigma). To each tube 0.2 ml of 0.2 M linoleic acid (Sigma) was added. Three ml of 10 mM KH_2PO_4 (pH 6.8) was added to each emulsion. To initiate the reaction, 1.5 ml of 0.2 mM $FeSO_4$ were added and the tube was placed in a water bath at 37°C. Immediately after adding $FeSO_4$ and again following 3 hours of incubation, 0.5 ml aliquots were taken. To each aliquot 2 ml of 0.1 N NaOH were added to clear the emulsion. The absorbance at 232 nm was measured to determine linoleic acid oxidation. Results of the assay were reported as percent inhibition of linoleic acid oxidation. Absorbance at 232 nm measures conjugated dienes and is generally accepted as one indicator of oxidation. The assumption made by the author, which may or may not be completely valid, is that the changes in oxidation caused by the addition of samples to the system

were due to the presence of lipid-soluble antioxidants rather than a decrease in pro-oxidants. Therefore, care should be taken in interpretation of these results. The standard curve with ∝ tocopherol shows that a decrease in oxidation does occur in this system with increased abtioxidant concentrations.

∝ Tocopherol

∝ Tocopherol was determined by a modification of the method of Spychalla and Desborough (1990). Freeze-dried peel (1 g) was mixed by vortexing with 25 ml of 80% ethanol in water (v/v). This homogenate was then centrifuged for 5 minutes at 2500g and filtered through Whatman No. 1 filter paper. The filtrate was mixed with 10 ml of petroleum ether and centrifuged. The upper phase was partitioned, the solvent was removed at 50°C under vacuum, and the residue was dissolved in 0.7 ml HPLC-grade methanol. The sample was then passed through a 0.45μm filter and stored in a freezer at -30°C until analysis by High Performance Liquid Chromatography. The HPLC column used was a Bondclone (300 x 3.9 mm) 10μm C18 particle column in series with a 10μm C18 guard column (30 x 3.9) (Phenomenex, Torrance, CA). The mobile phase was 97% methanol at a flow rate of 2ml/min, and the sample size was 200 μl. Absorption was recorded at 295 nm.

Carotenoids

Total carotenoids were determined by extracting freeze-dried peel tissue (0.5g) in 20 ml of acetone. The acetone and samples were placed in vials and purged with nitrogen. The samples then were left in the dark at room temperature for 2 hours. Following the two hour incubation, the samples were centrifuged at 27000g for 15 minutes and the absorbance at 440nm was measured. Using the average extinction coefficient for carotenoids (2500), the concentrations were calculated.

Hydrocarbon carotenes were determined by extracting freeze-dried peel tissue (0.5g) in 10 ml of hexane following the procedure for total carotenoids. The samples were centrifuged at 39000g to remove all cloudiness. The absorbance at the absorption maximum was determined and the extinction coefficient (2500)was used to calculate the concentrations.

Total Water-soluble Reducing Capacity

Total water-soluble reducing capacity was determined by monitoring reduction of iron using an unpublished colorimetric method (Meir, private communication). Freeze-dried samples (0.05g) were homogenized in 10ml acetate buffer (pH 4.5) and centrifuged for 10 minutes at 1800g. Aliquots (0.1ml) of extract, 0.9ml buffer and 1ml $FeCl_3$ (made up as 24.3mg/100ml H_2O + 50μl H_2SO_4) were placed in tubes and allowed to incubate in the dark for 24 hours.

After the incubation, 0.8 ml of ammonium acetate (1.3M) and 0.2 ml ferrozine reagent (75mg ferrozine + 75mg Neocuproine) (Sigma) were added. Ferrozine is 3-(2-pyridyl)-5-6-bis(4-phenyl-sulfonic acid)1,2,4-triazine. The absorption at 562 nm was measured one hour after the addition of ferrozine. The total reducing capacity (units/g dry weight) was defined as the Fe^{+3}-reducing capacity of the tissue resulting in the absorbance of 1 OD at 562 nm (Meir, 1990, private communication).

Ascorbic Acid

Ascorbic acid was determined by a modification of the method of Arakawa et al. (1981). Samples (0.1 gm) were extracted in 10 ml of 5% TCA and centrifuged. Aliquots (0.5 ml) of suitably diluted samples were added to 10 ml volumetric flasks containing 1 ml of ethanol and 1 ml of 5% TCA. The following were then added to each flask in order: 0.5 ml of 0.4 mM H_3PO_4 in ethanol, 1 ml of 0.5% Bathophenanthroline, and 1 ml of 0.18mM $FeCl_3$ in ethanol. The samples were made up to 10 ml with ethanol. The absorbance at 534 nm was determined after one hour at room temperature and compared to a standard curve of 0 to 15 μg of ascorbic acid (Sigma).

Anthocyanin/Total Flavonol

The freeze-dried peel was analyzed for anthocyanin and total flavonol by a modification of the procedure of Lees

and Francis (1972). One-half-gram samples were mixed with 15 ml of 95% ethanol:1.5 N HCl (85:15 v/v) extracting solvent and refrigerated overnight at 4°C. Samples then were filtered through Whatman No. 1 filter paper using a Buchner funnel. The peel tissue then was remixed with 15 ml extracting solvent and filtered. The filtrate was transferred quantitatively to a 50 ml volumetric flask and made to volume with extracting solvent. The samples were left in the dark at room temperature for 2 hours. The pH was measured to ensure that it was 1.0±0.1. The absorbance of the samples was read at 535 nm for total anthocyanin, and at 374 nm for total flavonol. Calculations were done as follows:

Total anthocyanin (mg)=Abs_{535} x Dilution factor/98.2*

Total flavonol (mg quercetin)=Abs_{374} x Dilution factor/76.6**, with * being average E_{535} and ** being average E_{374} (Lees and Francis,1972).

Glutathione

Glutathione was determined using a modification of the method of Buwalda et al. (1988) as modified by Hariyadi and Parkin (1991) (clarified by personal communication). One-half gram samples of freeze-dried apple peel were extracted in 10 ml of 80 mM sulfosalicylic acid with 1mM EDTA. The sulfosalicylic acid and EDTA were dissolved separately and then mixed to avoid insolubility. The samples were centrifuged at 27000g for 15 minutes and filtered through a

0.45μm nylon filter. To 1 ml aliquots of the samples were added 2 ml of 0.5M Na-phosphate, pH 8.25 and 0.1 ml of 10mM DTNB (5,5'-dithio-bis(2-nitrobenzoic acid) pH 7.0) to derivatize the samples. The DTNB was dissolved at a slightly alkaline pH (8.0) and then brought to pH 7.0. The samples were derivitized for 5 minutes and then neutralized by the addition of 0.5 ml of 0.5M H_3PO_4. The prepared samples were stored frozen until analyzed by HPLC. The column was a C18 reverse phase 250mm X 4.0mm (Phenomenex, Torrance, CA) at 22°C. The mobile phase was 2% acetonitrile in 30mM Na-phosphate buffer, pH 7.0, and the flow rate was 1 ml/minute. The absorbance was determined at 280nm and compared to external standards. A 7-minute cleaning phase with 33% acetonitrile was run after each sample.

Chlorophyll

Freeze-dried peel was analyzed for chlorophyll using an acetone extraction (Holden, 1965). One-gram samples were mixed with 15 ml of cold acetone and filtered through Whatman No. 2 filter paper. The tissue was remixed with another 15 ml of cold acetone and filtered. The filtrate was transferred quantitatively to a 50 ml volumetric flask with 10 ml of water in it, and made to volume with acetone for a final concentration of 80% acetone. The samples were centrifuged for 15 minutes at 2500g to remove cloudy materials and the absorbance was read at 663 and 645 nm to

determine chlorophylls a and b. The calculations were as follows and reported as total chlorophyll (a + b).

$$\text{Chlorophyll a (mg/g)} = [12.3D_{663}-0.86D_{645}/d \text{ X } 1000 \text{ X } W]V$$

$$\text{Chlorophyll b (mg/g)} = [19.3D_{645}-3.6D_{663}/d \text{ X } 1000 \text{ X } W]V$$

CHAPTER 4

RESULTS

Changes Associated with Different Exposures of Apples to Low Temperature (as Hours Below 10°C) Before Harvest

Differences in Ripeness at Harvest

In each season (1988, 1989, and 1990), apples increased in ripeness as they experienced increasing amounts of low temperature before harvest (Tables 4.1 to 4.3). In 1988, Cortland apples were harvested four times, having accumulated between 73 and 187 hours below 10°C. During this time the firmness decreased by 2.5 lbs and the starch index (indicating starch hydrolysis) increased four-fold. The internal ethylene concentration increased, and the apples at the fourth harvest were in the climacteric rise ($\log C_2H_4 \geq 0$). All three of these factors (firmness, starch index, and ethylene) were significantly correlated with hours below 10°C (Table 4.4).

In 1989, the Cortland apples were harvested three times. At the first harvest, 62 hours below 10°C had been recorded, but no additional hours below 10°C were recorded between the first and second harvests. There was an increase in ripeness, however, between these two harvests. Between the second and third harvest, an additional 90 hours below 10°C had been recorded, and during this period starch indices and internal ethylene concentrations increased and firmness decreased. In 1990, the four Cortland harvests

Table 4.1. At-harvest ripeness measurements for Cortland and Delicious apples, versus scald development after storage at 0°C, 1988.

Cultivar	Harvest date	Hours < 10°C	Flesh firmness (N)	Starch index[z]	C_2H_4 conc. (log ppm)	Scald (%)	Scald score[y]
Cortland	Sept. 15	73	82	1.2	-1.1271	71	1.8
	Sept. 22	102	75	2.0	-1.0021	36	1.2
	Sept. 29	134	71	4.0	-0.6485	11	1.6
	Oct. 6	187	71	5.0	0.1481	4	1.3
Delicious	Oct. 1	160	92	1.3	-1.5726	12	1.1
	Oct. 8	232	92	1.6	-2.5168	2	1.0
	Oct. 13	365	87	1.9	-1.1595	2	1.0
Significance (ANOVA)							
Cortland							
HOURS			***	***	***	***	ns
Delicious							
HOURS			**	**	***	***	ns

[z] 1 to 3 = immature; 4 to 6 = mature; 7 to 9 = overmature

[y] Score reflects % of surface affected: 1 = 1 to 10%; 2 = 11 to 33%; 3 = 34 to 66%; 4 = ≥ 67%.

***,**,*,ns: Significant at 0.1%, 1%, or 5%, or not significant, respectively.

Table 4.2. At-harvest ripeness measurements for Cortland and Delicious apples, versus scald development after storage at 0°C, 1989.

Cultivar	Harvest date	Hours < 10°C	Flesh firmness (N)	Starch index[z]	C_2H_4 conc. (log ppm)	Scald (%)	Scald score[y]
Cortland	Sept. 15	62	82	1.0	-1.0631	99	2.9
	Sept. 22	62	78	1.7	-0.8564	99	2.7
	Oct. 4	152	74	4.7	0.0795	29	1.3
Delicious	Sept. 29	125	90	1.4	-0.7121	83	2.2
	Oct. 5	170	91	2.6	0.3213	72	2.0
Significance (ANOVA)							
Cortland							
HOURS			*	***	***	***	***
Delicious							
HOURS			ns	***	***	ns	*

[z] 1 to 3 = immature; 4 to 6 = mature; 7 to 9 = overmature.

[y] Score reflects % of surface affected: 1 = 1 to 10%; 2 = 11 to 33%; 3 = 34 to 66%; 4 = ≥ 67%.

***,**,*,ns: Significant at 0.1%, 1%, or 5% or not significannt, respectively.

Table 4.3. At-harvest ripeness measurements for Cortland and Delicious apples versus scald development after storage at 0°C, 1990.

Cultivar	Harvest date	Hours < 10°C	Starch index[z]	C_2H_4 conc. (log ppm)	Scald (%)	Scald score[y]
Cortland	Sept. 17	21	1.3	-0.9694	98	2.7
	Sept. 24	79	1.5	-1.1863	78	1.9
	Oct. 3	127	4.3	0.2288	46	1.6
	Oct. 11	150	6.8	2.0750	49	1.6
Delicious	Sept. 21	62	1.2	-1.5382	94	2.7
	Sept. 26	104	1.6	-0.9645	88	2.8
	Oct. 3	127	3.2	-0.2627	68	2.1
	Oct. 11	150	5.5	1.1818	51	1.7
Significance (ANOVA)						
Cortland						
HOURS			***	***	***	***
Delicious						
HOURS			***	***	***	***

[z] 1 to 3 = immature; 4 to 6 = mature; 7 to 9 = overmature.

[y] Score reflects % of surface affected: 1 = 1 to 10%; 2 = 11 to 33%; 3 = 34 to 66%; 4 = ≥ 67%.

***,**,*,ns: Significant at 0.1%, 1%, or 5%, or not significant, respectively.

Table 4.4. Correlation coefficients between hours below 10°C and ripeness measurements.

Cultivar	Year	Starch	C_2H_4	Firmness
Cortland	1988	0.92^{***}	0.84^{***}	-0.73^{***}
	1989	0.95^{***}	0.81^{***}	-0.58^{*}
	1990	0.89^{***}	0.81^{***}	-
Delicious	1988	0.74^{***}	0.37^{ns}	-0.73^{***}
	1989	0.94^{***}	0.89^{***}	0.12^{ns}
	1990	0.87^{***}	0.85^{***}	-

^{***,**,*,ns}: Significant at the 0.1%, 1%, or 5% level or not significant, respectively.

spanned a range of 21 to 150 hours below 10°C. The starch indices increased more than five-fold over this period, and the apples from the last two harvests were in the climacteric rise (log $C_2H_4 \geq 0$). In both 1989 and 1990, starch scores and hours below 10°C were highly correlated (Table 4.4).

Similar results were recorded for Delicious apples. In 1988, they were harvested three times, with 160 to 365 hours below 10°C having been recorded at these harvest dates. The apples ripened between harvests but not as rapidly as did the Cortlands. The apples from all harvests in 1988 were non-climacteric (log $C_2H_4 < 0$). Firmness losses and starch indices were highly correlated with the hours below 10°C; however, internal ethylene concentrations were not correlated with the accumulated hours below 10°C (Table 4.4). In 1989, Delicious apples were harvested twice, with 125 and 170 hours below 10°C, respectively, having been recorded. Fruit firmness did not change between harvests; however, starch indices almost doubled and internal ethylene concentrations increased (Table 4.2). Apples from the second harvest were in the climacteric rise, based on ethylene concentrations. Both starch indices and internal ethylene concentrations were highly correlated with hours below 10°C (Table 4.4). In 1990, Delicious were harvested four times, with 62 to 150 hours below 10°C having accumulated. The starch indices increased more than four-fold between the first and fourth harvests. Internal

ethylene concentrations increased steadily with later harvest, and apples from the final harvest were in the climacteric rise (Table 4.3).

In summary, accumulated hours below 10°C were highly correlated with the ripeness measurements (internal ethylene concentration, starch index and firmness) (Table 4.4). In general, fruit firmness decreased and starch hydrolysis (starch scores) and internal ethylene concentrations increased during the season. Based on starch scores and internal ethylene concentrations, Cortlands were ripe enough for commercial harvest on September 29, 1988, October 4, 1989, and October 3, 1990. Delicious were ripe enough for harvest on September 29, 1989 and October 3, 1990, but they were still unripe at all harvests in 1988.

Scald Development After Storage

Percent incidences of scald and scald intensities (scald scores) are shown in Tables 4.1 to 4.3 for references to changes in hours below 10°C and ripeness. Scald in most cases was highly correlated with ripeness indices and with accumulated hours below 10°C (Table 4.5). For Cortland apples, scald incidence was highly negatively correlated with hours below 10°C, starch indices, and internal ethylene concentrations in all years. Scald was negatively correlated with firmness in 1988 and 1989; firmness was not measured in 1990. The correlation coefficients for hours below 10°C versus scald ranged from -0.84 to -0.99, and for

Table 4.5. Correlation coefficients between percent scald incidence and hours below 10°C or ripeness indices.

Cultivar	Year	Hours < 10°C	Starch score	C_2H_4	Firmness
Cortland	1988	-0.85^{***}	-0.85^{**}	-0.64^{***}	0.78^{***}
	1989	-0.99^{***}	-0.92^{***}	-0.74^{***}	0.54^{*}
	1990	-0.88^{***}	-0.76^{***}	-0.66^{***}	-
Delicious	1988	-0.67^{**}	-0.60^{**}	-0.08^{ns}	0.40^{ns}
	1989	-0.39^{ns}	-0.45^{ns}	-0.34^{ns}	-0.35^{ns}
	1990	-0.78^{***}	-0.65^{***}	-0.77^{**}	-

^{***,**,*,ns}: Significant at the 0.1%, 1%, or 5% level or not significant, respectively.

starch index versus scald they ranged from -0.76 to -0.92. These were all significant at the 0.1% level. Regression, as % of model sums of squares due to the non-rep effects, showed that hours below 10°C versus scald had significant regression values ranging from 82 to 98% (Table 4.6). The relationship in 1988 was linear and quadratic; however, in 1989 and 1990, only significant linear components existed. Starch scores versus scald incidences had regression values ranging from 65 to 85% and all were highly significant.

In Delicious apples in 1988 and 1990, hours below 10°C before harvest and scald development after storage were highly correlated (1% level), with correlation coefficients of -0.67 and -0.78, respectively (Table 4.5). In 1989, however, scald and hours below 10°C were not correlated significantly. In 1989, there were only two harvests of Delicious, and the difference in hours below 10°C was not very large. Regression analyses showed significant regressions for scald versus hours below 10°C of 85 and 79% for 1988 and 1990, respectively. In 1988, the relationship was linear and quadratic, but it was only linear in 1990. In 1989, as with the correlation, the regression was not significant.

Starch hydrolysis, as an index of ripening, was related significantly to scald in 1988 and 1990, with regression values of 65% (5% level) and 71%, respectively, and the relationships were linear. Internal ethylene concentrations

Table 4.6. Regression (as % of non-rep sums of squares with the linear and/or quadratic portions of the model) for scald, hours below 10°C and ripening indices.

	Cortland			Delicious		
	1988	1989	1990	1988	1989	1990
Hours vs Scald	93	98	82	85	39	79
Linear	***	***	***	***	ns	***
Quadratic	***	ns	ns	***	ns	*
Starch vs Scald	85	85	78	65	47	71
Linear	***	***	***	**	ns	***
Quadratic	**	ns	**	*	ns	ns
Ethylene vs Scald	49	58	53	20	44	57
Linear	***	**	***	ns	ns	***
Firmness vs Scald	74	43	-	22	43	-
Linear	***	*	-	ns	ns	-

***,**,*,ns Significant at the 0.1%, 1%, or 5% level or not significant, respectively

were correlated with scald only in 1990, and firmness measurements were not correlated with scald in any year.

The relationships between preharvest temperatures (hours below 10°C) and scald development over the three seasons 1988 to 1990 for Cortland and Delicious apples are shown in Figures 4.1 and 4.2. The regression value for the regression with Cortland was 91% (with the replication sums of squares removed) and was highly significant. The linear component accounted for 89% of the regression. The regression value of 67% (with replication sums of squares removed) for Delicious was highly significant and had significant linear and quadratic components.

These results show that there was a clear, largely linear, negative relationship between increasing hours below 10°C before harvest and scald development after storage. Since ripening occurred between harvests, however, it too was significantly correlated with scald development, though correlations and regressions between ripening indices and scald were lower than those between hours below 10°C and scald.

General Estimates of Antioxidant Activity in Apple Peel at Harvest

The OD200 values (1988-1990), total lipid-soluble antioxidant concentrations (1989-1990), and total water-soluble reducing capacities (1990) are shown in Tables 4.7 to 4.9. OD200 values and total lipid-soluble antioxidant

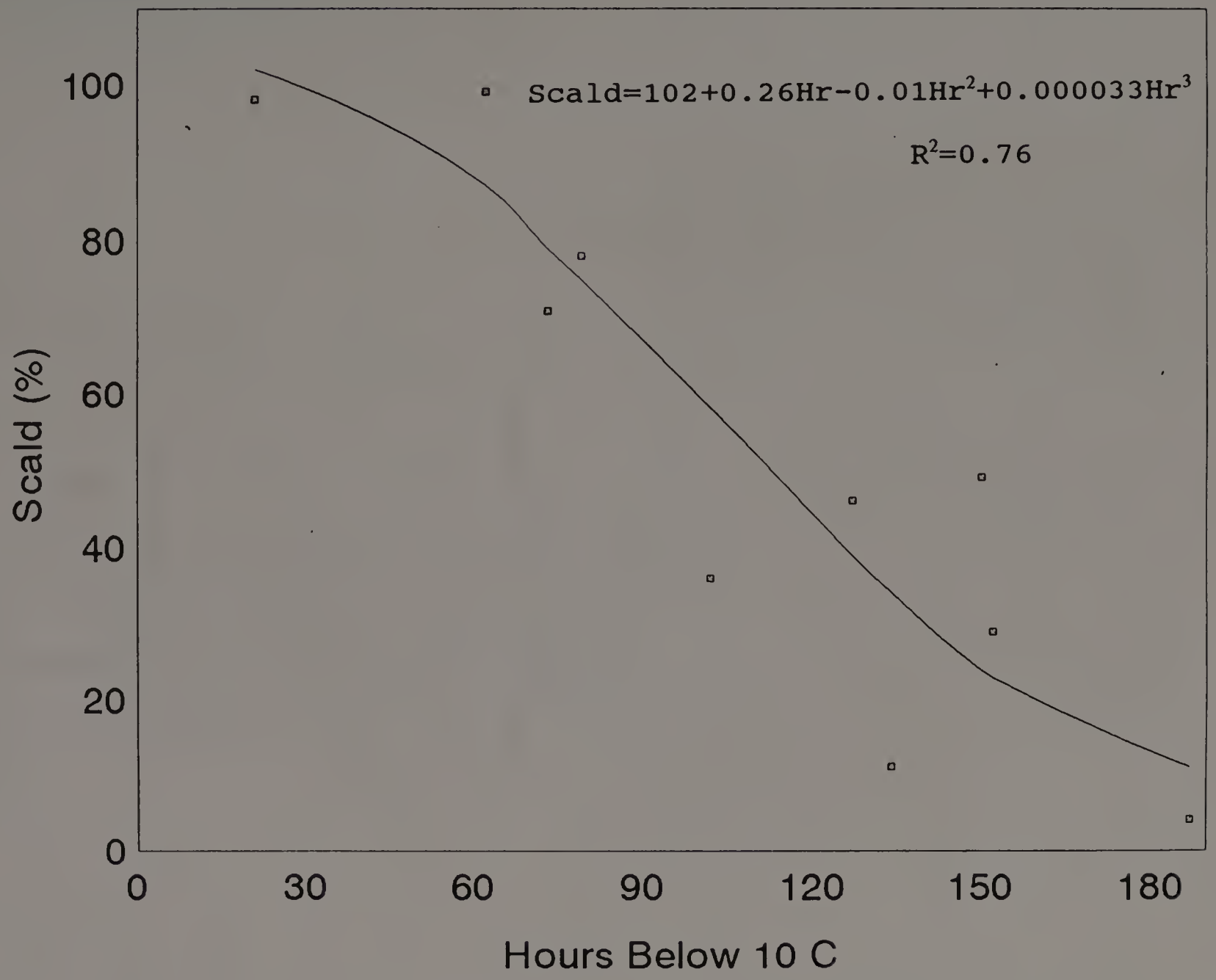


Figure 4.1. Regression of scald development with preharvest hours below 10°C for Cortland apples harvested in three seasons (1988 to 1990). The R^2 value increased to 0.94 when the variation due to replication was included in the model.

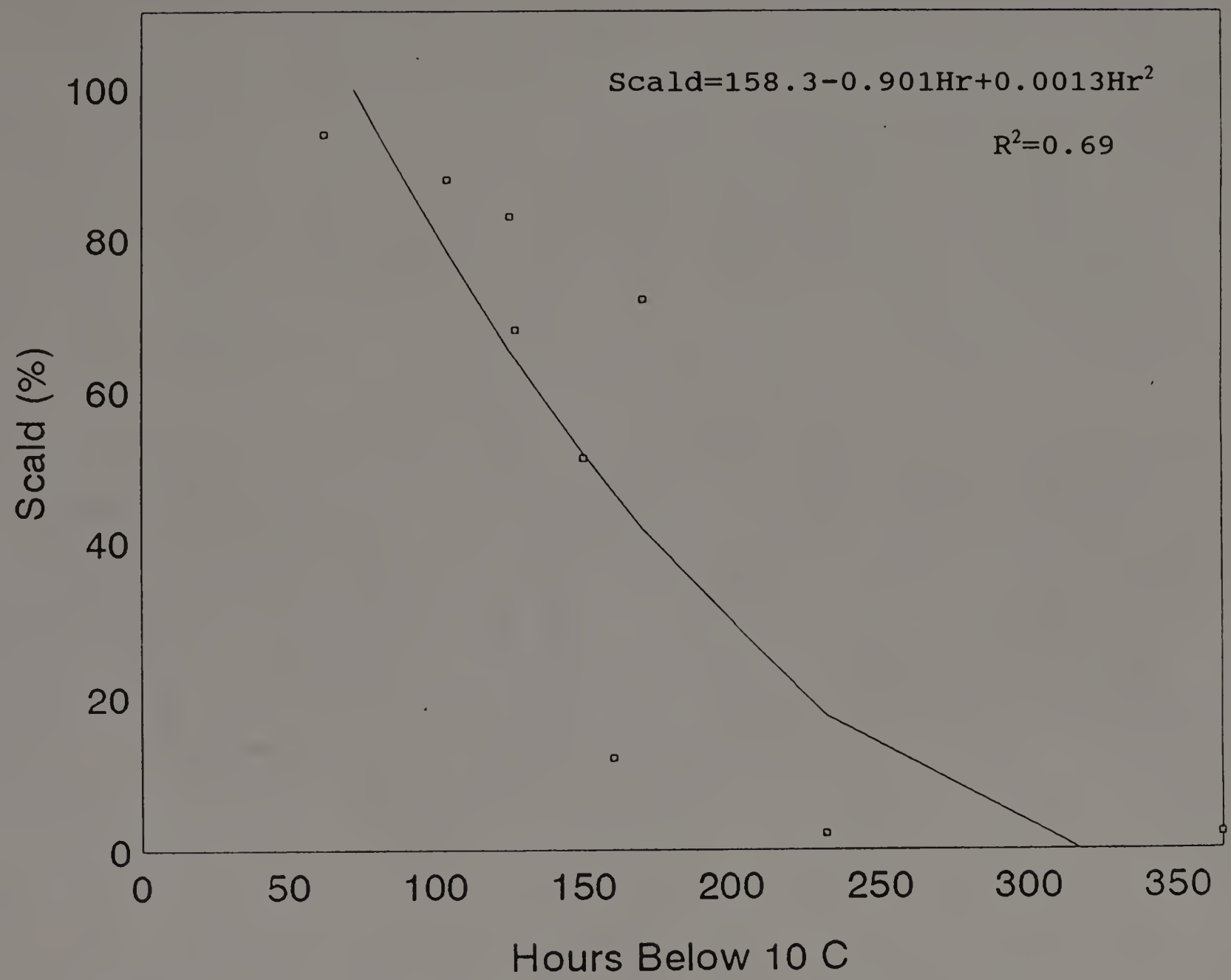


Figure 4.2. Regression of scald development with preharvest hours below 10°C for Delicious apples harvested in three seasons (1988 to 1990). The R^2 value increased to 0.96 when the variation due to replication was included in the model.

Table 4.7. OD200 values of at-harvest hexane extracts of fruit surfaces from Cortland and Delicious apples, and scald development after storage at 0°C, 1988.

Cultivar	Harvest date	Hours < 10°C	OD200 (ODX1000/cm^2)	Scald (%)	Scald score[z]
Cortland	Sept. 15	73	4.2	71	1.8
	Sept. 22	102	10.9	36	1.2
	Sept. 29	134	13.2	11	1.6
	Oct. 6	187	15.4	4	1.3
Delicious	Oct.10	160	10.9	12	1.1
	Oct. 8	232	12.5	2	1.0
	Oct. 13	365	13.2	2	1.0
SIGNIFICANCE (ANOVA)					
Cortland					
HOURS			***	***	ns
Delicious					
HOURS			ns	***	ns

[z] Score reflects % of surface affected: 1 = 1 to 10%; 2 = 11 to 33%; 3 = 34 to 66%; 4 = ≥ 67%.

***,**,*,ns: Significant at 0.1%, 1%, or 5% level, or not significant, respectively.

Table 4.8. Antioxidant and % oxidation inhibition estimates of at-harvest hexane extracts from fruit surfaces of Cortland and Delicious apples, and scald development after storage at 0°C, 1989.

Cultivar	Harvest date	Hours < 10°C	OD 200 (ODX1000/cm^2)	Oxidation[z] (% inhibition)	Scald (%)	Scald score[y]
Cortland	Sept. 15	62	5.3	55	99	2.9
	Sept. 22	62	8.8	60	99	2.7
	Oct. 4	152	14.8	65	29	1.3
Delicious	Sept. 29	125	7.1	12	83	2.2
	Oct. 5	170	10.9	16	72	2.0
SIGNIFICANCE (ANOVA)						
Cortland						
HOURS		***	ns	***	***	
Delicious						
HOURS		**	ns	ns	*	

[z] % Inhibition if linoleic acid oxidation as indicated by conjugated dienes.

[y] Score reflects % of surface affected: 1 = 1 to 10%; 2 = 11 to 33%; 3 = 34 to 66%; 4 = ≥ 67%.

***,**,*,ns: Significant at 0.1%. 1%, or 5% level or not significant, respectively.

Table 4.9. At-harvest antioxidant and % oxidation inhibition estimates of peel from Cortland and Delicious apples, and scald development after storage at 0°C, 1990.

Cultivar	Harvest date	Hours <10°C	OD200 (ODX1000/cm^2)	Oxidation[z] (% inhibition)	TWRC[y] (units/gdw)	Scald (%)	Scald score[x]
Cortland	Sept. 17	21	5.5	40	11.2	98	2.7
	Sept. 24	79	7.7	60	11.3	78	1.9
	Oct. 3	127	17.9	76	12.0	46	1.6
	Oct. 11	150	23.5	87	13.8	49	1.6
Delicious	Sept. 21	62	2.4	22	13.9	94	2.7
	Sept. 26	104	5.5	34	14.1	88	2.8
	Oct. 3	127	10.9	44	14.9	68	2.1
	Oct. 11	150	18.1	55	15.8	51	1.7
Significance (ANOVA)							
Cortland							
HOURS			***	***	***	***	***
Delicious							
HOURS			***	***	**	***	***

[z] % Inhibition of linoleic acid oxidation as estimated by conjugated dienes.

[y] TWRC=Total water-soluble reducing capacity

[x] Score reflects % of surface affected: 1 = 1 to 10%; 2 = 11 to 33%; 3 = 34 to 66%; 4 = ≥ 67%.

***,**,*,ns: Significant at 0.1%, 1%, or 5% level or not significant, respectively.

activities of apple peels increased with each harvest of both cultivars in each year that they were measured. Scald data are shown in these tables for reference. In Cortland apples, from all three seasons, preharvest temperature (hours below 10°C) was correlated highly with OD200 values, the correlation coefficients ranging from 0.86 to 0.93 (Table 4.10). The OD200 values for all years were highly correlated with the subsequent scald development (Table 4.11). The regression, removing replication sums of squares, yielded highly significant regression values of 78 to 90% for Cortlands (Table 4.12). For Delicious, similar correlations between hours below 10°C and OD200 values existed in 1989 and 1990, but not in 1988 (Table 4.10). However, OD200 and scald development were correlated in all three years: at the 5% level in 1988 and 1989 and at the 0.1% level in 1990 (Table 4.11). The regression between OD200 values and scald in Delicious yielded regression values ranging from 41 to 76%, with only the 1988 and 1990 values being significant (Table 4.12).

The percent inhibition of linoleic acid oxidation in Cortland apples was not correlated with scald development but was correlated with hours below 10°C (5% level) in 1989 (Table 4.11). However, in 1990 % inhibition of oxidation was highly correlated with both hours below 10°C and scald development. The regression between % inhibition of oxidation and scald was not significant in 1989, but very

Table 4.10. Correlation coefficients between hours below 10°C and OD200, inhibition of oxidation and TWRC values for extracts of at-harvest apple peel.

Cultivar	Year	OD 200	% Inhibition	TWRC
Cortland	1988	0.86^{***}	-	-
	1989	0.86^{***}	0.50^{*}	-
	1990	0.93^{***}	0.92^{***}	0.61^{**}
Delicious	1988	0.37^{ns}	-	-
	1989	0.79^{**}	0.46^{ns}	-
	1990	0.93^{***}	0.85^{***}	0.69^{***}

^{***,**,*,ns}: Significant at the 0.1%, 1%, or 5% level or not significant, respectively.

Table 4.11. Correlation coefficients between % scald and OD200, % inhibition, or TWRC values of at-harvest peel extracts of Cortland and Delicious apples.

Cultivar	Year	OD 200	% inhibition	TWRC
Cortland	1988	-0.90^{***}	-	-
	1989	-0.84^{***}	-0.47^{ns}	-
	1990	-0.84^{***}	-0.79^{***}	-0.58^{**}
Delicious	1988	-0.48^{*}	-	-
	1989	-0.47^{*}	-0.08^{ns}	-
	1990	-0.87^{***}	-0.70^{**}	-0.51^{*}

^{***,**,*,ns}: Significant at the 0.1%, 1%, or 5% level or not significant, respectively.

Table 4.12. Regressions (as % of non-rep sums of squares with the linear and/or quadratic portions of the model) among scald incidence, hours below 10°C before harvest, at-harvest % inhibition of oxidation and at-harvest TWRC values for Cortland and Delicious apples.

	Cortland			Delicious		
	1988	1989	1990	1988	1989	1990
Hours vs OD200	89	76	97	27	87	98
Linear	***	***	***	ns	**	***
Quadratic	***	-	***	ns	-	***
Scald vs OD200	86	90	78	50	41	76
Linear	***	***	***	*	ns	***
Quadratic	ns	**	ns	ns	ns	ns
Scald vs % Inhib[z]	-	22	71	-	49	68
Linear		ns	**		ns	**
Scald vs TWRC	-	-	45	-	-	60
Linear			**			**
Quadratic			ns			ns

***,**,*,ns: Significant at 0.1%, 1%, or 5% level or not significant, respectively.

[z] % Inhibition of linoleic acid oxidation as estimated by conjugated dienes.

significant in 1990 (71%). The same results were recorded for Delicious as for Cortland.

The total water-soluble reducing capacities (TWRC) also were correlated significantly with hours below 10°C and scald in both Cortland and Delicious apples; however, the correlation coefficients were lower than those for % inhibition of oxidation. The regressions between TWRC and scald also were significant (Table 4.12).

These results show that while there was a clear trend toward increased estimates of both % inhibition of oxidation and water-soluble antioxidant activities in apple peel with later harvests of apples, the relationships of these estimates to either hours below 10°C before harvest or scald development after storage were not completely consistent. However, the frequency with which significant correlations occurred suggests more than casual associations among preharvest hours below 10°C, antioxidant activity in the apple peel at harvest, and scald development after storage.

Measurements of Specific Antioxidant and Pigment Concentrations in Apple Peel at Harvest

In 1990, the specific antioxidants ∝ tocopherol, ascorbic acid, and glutathione were measured in the peel of Cortland and Delicious apples at harvest after different periods of temperature below 10°C (Table 4.13). In both cultivars ∝ tocopherol was highest in peel at the latest harvests. ∝ Tocopherol was correlated with low temperature

Table 4.13. Antioxidant concentrations in apple peel at harvest of Cortland and Delicious apples, 1990.

Cultivar	Harvest date	Hours < 10°C	∝ Tocopherol (µg/gdw)	Ascorbic acid (µg/gdw)	Glutathione (µg/gdw)	Scald (%)	Scald Score[z]
Cortland	Sept. 17	21	103	380	91	98	2.7
	Sept. 24	79	90	376	106	78	1.9
	Oct. 3	127	100	365	139	46	1.6
	Oct. 11	150	145	411	81	49	1.6
Delicious	Sept. 21	62	72	624	39	94	2.7
	Sept. 26	104	69	600	39	88	2.8
	Oct. 3	127	78	592	55	68	2.1
	Oct. 11	150	78	603	35	51	1.7
Significance (ANOVA)							
Cortland							
HOURS			***	ns	*	***	***
Delicious							
HOURS			*	ns	ns	***	***

[z] Score reflects % of surface affected: 1 = 1 to 10%; 2 = 11 to 33%; 3 = 34 to 66%; 4 = ≥ 67%.

***,**,*,ns: Significant at 0.1%, 1%, or 5% level or not significant, respectively.

for both cultivars, and with scald development in Delicious (Table 4.14); however, ascorbic acid did not change substantially during the harvest period. Ascorbic acid was not correlated with either hours below 10°C or scald development (Table 4.14). Glutathione tended to increase for the first three harvests and then decline in both Cortland and Delicious but was only significant in Cortland (Table 4.13). There were no relationships between glutathione and hours below 10°C or scald (Table 4.14).

Changes in concentrations of chlorophyll, carotenoids, anthocyanin, and total flavonols in apple peel at harvest are shown in Table 4.15. Chlorophyll decreased and anthocyanin increased in both cultivars as the season progressed. Total flavonols were highest at the last harvest of Cortland, but did not change during harvests of Delicious.

Chlorophyll was significantly correlated with scald development, with correlation coefficients of 0.84 and 0.62 in Cortland and Delicious, respectively (Table 4.16). There were also significant linear regressions between chlorophyll and scald, with higher significance in Cortland than in Delicious (Table 4.17).

Anthocyanins were correlated significantly with both accumulated hours below 10°C and scald (Table 4.12). In Cortland the regression between scald and anthocyanins was both linear and quadratic, but in Delicious it was only linear. The regression values were 73 and 75% for Cortland

Table 4.14. Correlation coefficients and regressions (as % of non-rep sums of squares with the linear and/or quadratic portions of the model) between hours below 10°C before harvest or percent scald development after storage, and concentrations of ∝ tocopherol, ascorbic acid, or glutathione in peel at harvest of Cortland and Delicious apples, 1990.

	Correlation coefficients					
	Cortland			Delicious		
	∝ Tocopherol	Ascorbic acid	Glutathione	∝ Tocopherol	Ascorbic acid	Glutathione
Hours <10°C	0.52^{**}	0.16^{ns}	0.13^{ns}	0.46^{*}	-0.27^{ns}	0.05^{ns}
Scald	-0.40^{ns}	-0.35^{ns}	-0.32^{ns}	-0.50^{*}	0.08^{ns}	0.09^{ns}
	Regressions					
	Cortland			Delicious		
	∝ Tocopherol	Ascorbic acid	Glutathione	∝ Tocopherol	Ascorbic acid	Glutathione
Hours <10°C	76	17	19	35	20	15
Linear	***	ns	ns	*	ns	ns
Quadratic	***	ns	ns	ns	ns	ns
Scald	16	5	12	36	5	0.4
Linear	ns	ns	ns	ns	ns	ns

***,**,*,ns: Significant at 0.1%, 1%, or 5% level or not significant, respectively.

Table 4.15. Concentrations of pigments in peel at harvest of Cortland and Delicious apples, 1990.

Cultivar	Hours < 10°C	Chlorophyll (μg/gdw)	Carotenoids (μg/gdw)	Carotenes (μg/gdw)	Anthocyanin (μg/gdw)	Flavonols (μg/gdw)
Cortland	21	203	105	22	261	1194
	79	163	109	21	383	1072
	127	114	100	23	594	1167
	150	92	83	33	1028	1550
Delicious	62	120	76	18	433	1600
	104	100	74	15	483	1356
	127	107	74	17	639	1344
	150	72	68	20	917	1506
Significance (ANOVA)						
Cortland						
HOURS		***	***	**	***	**
Delicious						
HOURS		***	**	ns	***	ns

***,**,*,ns: Significant at 0.1%, 1%, or 5% level or not significant, respectively.

Table 4.16. Correlations between hours below 10°C or percent scald and pigment concentrations in peel at harvest of Cortland and Delicious apples, 1990.

	Cortland		Delicious	
	Hours < 10°C	Scald	Hours < 10°C	Scald
Chlorophyll	-0.92^{***}	0.84^{***}	-0.76^{***}	0.62^{**}
Carotenoid	-0.61^{**}	0.49^{*}	-0.59^{**}	0.50^{*}
Carotenes	0.53^{**}	-0.34^{ns}	0.17^{ns}	-0.26^{ns}
Anthocyanin	0.84^{***}	-0.69^{***}	0.84^{***}	-0.71^{***}
Flavonols	0.38^{ns}	-0.30^{ns}	-0.23^{ns}	-0.07^{ns}

^{***,**,*,ns}: Significant at 0.1%, 1%, or 5% level or not significant, respectively.

Table 4.17. Regressions (as % of non-rep sum of squares with the linear and/or quadratic portions of the model) between hours below 10°C or scald and pigment concentrations in peel at harvest of Cortland and Delicious apples, 1990.

	Cortland		Delicious	
Constituent	Scald	Hours < 10°C	Scald	Hours < 10°C
Chlorophyll	78	87	49	64
Linear	***	***	**	***
Quadratic	ns	ns	ns	ns
Carotenoid	28	84	38	53
Linear	*	***	*	**
Quadratic	ns	***	ns	ns
Carotenes	24	65	62	25
Linear	ns	**	*	ns
Quadratic	ns	**	**	ns
Anthocyanin	73	87	75	95
Linear	***	***	***	***
Quadratic	**	***	ns	***
Flavonol	19	47	4	37
Linear	ns	*	ns	ns
Quadratic	ns	**	ns	*

***,**,*,ns: Significant at 0.1%, 1%, or 5% level, or not significant, respectively.

and Delicious apples, respectively. The total flavonol concentrations showed no relationship with either scald or hours below 10°C in either cultivar.

These results show no relationship between either ascorbic acid or total flavonol concentrations to either preharvest temperature, ripeness index, or scald development. ∝ Tocopherol, however, increased with increasing hours below 10°C (and accompanying fruit ripening), and was negatively correlated with scald development. Anthocyanin concentration also increased with later harvest and was negatively correlated with scald development, while chlorophyll concentration decreased with later harvest and was positively correlated with scald development after storage.

Storage measurements

Except for the ripeness indices, all of the constituents reported above were measured following intervals of storage at 0°C.

The OD200 values for Cortland and Delicious apples at harvest and at selected storage dates are presented in Tables 4.18 to 4.22. As mentioned previously, the at-harvest OD200 values were strongly related to the number of hours below 10°C that were experienced by apples before harvest. There were highly significant effects of hours below 10°C and storage periods on OD200 values in all three seasons for Cortland but only in 1990 for Delicious

Table 4.18. OD200, ∝ farnesene, and conjugated triene values of hexane extracts of Cortland apple surfaces at harvest and after 7, 14, and 21 weeks of stQrage at 0°C, 1988.

Harv. date	Hrs< 10°C	OD200 ([ODx1000]/cm²)				∝ Farnesene (nmoles/cm²)				Conjugated trienes (nmoles/cm²)			
		Weeks at 0°C				Weeks at 0°C				Weeks at 0°C			
		0	7	14	21	0	7	14	21	0	7	14	21
9-15	73	4	32	36	33	8	136	91	65	0	10	15	4
9-22	102	11	35	32	31	19	122	76	62	0	7	7	4
9-29	134	13	38	34	33	20	123	78	49	0	7	6	4
10-6	187	15	36	35	33	21	102	75	53	0	5	5	4
Significance (Orthogonal polynomial comparisons within storage period)													
Linear		**	**	ns	ns	*	***	*	***	ns	***	***	ns
Quadratic		***	ns	*	ns	ns	ns	ns	***	ns	ns	***	ns
Cubic		ns	ns	ns	ns	ns	ns	ns	**	ns	ns	*	ns

***,**,*,ns: Significant at 0.1%, 1%, or 5% level or not significant, respectively.

Table 4.19. OD200, ∝ farnesene, and conjugated triene values of hexane extracts of Cortland apple surfaces at harvest and after 6, 12, and 18 weeks of storage at 0°C, 1989.

Harv. date	Hours < 10°C	OD200 (ODX1000/cm²)				∝ Farnesene (nmoles/cm²)				Conjugated trienes (nmoles/cm²)			
		Weeks at 0°C				Weeks at 0°C				Weeks at 0°C			
		0	6	12	18	0	6	12	18	0	7	14	21
9-15	62	5	26	26	24	3	124	112	86	0	9	20	22
9-22	62	9	33	29	27	9	155	106	101	0	8	19	23
10-4	152	15	33	33	27	22	121	98	68	0	6	11	10
Significance (Orthogonal polynomial comparisons within storage period)													
Linear		***	ns	***	ns	**	ns	*	***	ns	**	***	***

***,**,*,ns: Significant at 0.1%, 1%, or 5% level or not significant, respectively.

Table 4.20. OD200, ∝ farnesene, and conjugated triene values of hexane extracts of Cortland apple surfaces at harvest and after 8 and 20 weeks of storage at 0°C, 1990.

Harv. date	Hours < 10°C	OD200 (ODX1000/cm²)			∝ Farnesene (nmoles/cm²)			Conjugated trienes (nmoles/cm²)		
		Weeks at 0°C			Weeks at 0°C			Weeks at 0°C		
		0	8	20	0	8	20	0	8	20
9-17	21	6	29	27	5	129	67	0	11	17
9-24	79	8	31	27	11	130	66	1	8	12
10-3	127	18	32	29	21	97	51	2	6	6
10-11	150	24	31	27	39	86	44	3	5	4
Significance (Orthogonal polynomial comparisons within storage period)										
Linear		***	*	ns	***	***	***	***	ns	***
Quadratic		***	ns	ns	**	**	*	ns	***	ns
Cubic		ns	ns	ns	ns	ns	ns	ns	ns	ns

***,**,*,ns: Significant at 0.1%, 1%, or 5% level or not significant, respectively.

Table 4.21. OD200, ∝ farnesene, and conjugated triene values of hexane extracts of Delicious apple surfaces at harvest and after 7, 14, and 21 weeks of storage at 0°C, 1988.

Harv. date	Hours < 10°C	OD200 (ODX1000/cm²)				∝ Farnesene (nmoles/cm²)				Conjugated trienes (nmoles/cm²)			
		Weeks at 0°C				Weeks at 0°C				Weeks at 0°			
		0	7	14	21	0	7	14	21	0	7	14	21
10-1	160	11	33	28	27	0	110	74	53	0	4	5	5
10-8	232	13	34	31	25	0	116	76	48	0	3	5	4
10-15	365	13	38	33	23	0	109	62	41	0	3	4	2
Significance (Orthogonal polynomial comparisons within storage period)													
Linear		ns	*	***	*	ns	ns	*	***	ns	ns	***	***
Quadratic		ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	*

***,**,*,ns: Significant at 0.1%, 1%, or 5% level or not significant, respectively.

Table 4.22. OD200, ∝ farnesene, and conjugated triene values of hexane extracts of Delicious apple surfaces at harvest and after 8 and 20 weeks of storage at 0°C, 1990.

Harv. date	Hours < 10°C	OD200 (ODX1000/cm²)			∝ Farnesene (nmoles/cm²)			Conjugated trienes (nmoles/cm²)		
		Weeks at 0°C			Weeks at 0°C			Weeks at 0°C		
		0	8	20	0	8	20	0	8	20
9-21	62	2	33	25	1	136	64	0.13	5	13
9-26	104	6	37	27	1	142	55	0.11	5	9
10-3	127	11	35	31	4	94	41	0.26	4	6
10-11	150	18	37	32	7	80	38	0.39	3	4
Significance (Orthogonal polynomial comparisons within storage period)										
Linear		***	**	***	***	***	*	***	***	***
Quadratic		***	ns	ns	ns	***	ns	*	**	ns
Cubic		ns	ns	*	*	***	ns	ns	ns	ns

***,**,*,ns: Significant at 0.1%, 1%, or 5% level or not significant, respectively.

(Appendix Table B.1). During the initial 6 to 8 weeks of storage at 0°C, the OD200 values of apples from all harvests increased greatly, and then remained constant or declined slightly during the remaining storage time. After 5 or more weeks of storage, differences that existed at harvest had diminished greatly or disappeared.

The ∝ farnesene concentrations in Cortland and Delicious apples over storage are shown in Tables 4.18 to 4.22. Generally, ∝ farnesene increased to a maximum during the first 10 weeks of storage at 0°C and then declined. There were significant effects of harvest date (hours < 10°C) and storage time on ∝ farnesene concentrations in all years of both cultivars. In most cases the earlier harvested apples reached a higher maximum than those harvested later, with more hours below 10°C (to be presented in later paragraph). In Cortland apples harvested later in the season, ∝ farnesene had already begun to accumulate by harvest time. In Delicious, at harvest the ∝ farnesene was either absent or was present at much lower concentrations than in Cortland; however, the patterns of change in storage were similar to those seen in Cortlands.

The patterns of changes of conjugated triene concentrations in peel of apples during storage are shown in Tables 4.18 to 4.22. At harvest little or no conjugated trienes were present in extracts of apple peel, but during 0°C storage they increased in all samples of both cultivars. Conjugated trienes in some samples increased in

concentration until the end of storage, but in other samples they reached maximum concentrations at about 14 weeks and then declined. The apples harvested earlier in the season, having experienced fewer hours below 10°C before harvest and also being less ripe, reached higher conjugated triene concentrations than those harvested later in the season (to be presented in later paragraph). Harvest date (hours below 10°C) significantly influenced the conjugated triene concentrations in all samples except the 1988 Delicious, where many hours below 10°C had accumulated at all three harvests. Storage time significantly influenced the conjugated triene accumulation in all samples.

The maximum concentrations of ∝ farnesene and conjugated trienes measured during storage for each year and cultivar are shown in Tables 4.23 and 4.24. Scald development also is shown in these tables for reference. In the Cortland apples harvested in 1988 and 1989, the maximum ∝ farnesene did not correlate with either the hours below 10°C or with scald development (Table 4.25). The maximum conjugated triene concentrations, however, showed strong correlations and regressions with hours below 10°C and with scald development. The regression values for maximum conjugated trienes and hours below 10°C were 79% and 89% for 1988 and 1989, respectively, and were linear. For the maximum conjugated triene concentrations versus scald, the regression values were 72% and 92% and also were linear. In 1990 both the maximum ∝ farnesene and the maximum conjugated

Table 4.23. Maximum ∝ farnesene and conjugated triene concentrations in hexane extracts of fruit surfaces of Cortland apples after storage at 10°C, and scald development after 20 weeks of storage.

Year	Harvest date	Hours < 10°C	Max ∝ farn (nmoles/cm^2)	Max CT (nmoles/cm^2)	Scald (%)	Scald score[z]
1988	Sept. 15	73	136	14	71	1.8
	Sept. 22	102	122	7	36	1.2
	Sept. 29	134	123	7	11	1.6
	Oct. 6	187	102	5	4	1.3
1989	Sept. 15	62	124	22	99	2.9
	Sept. 22	62	155	23	99	2.7
	Oct. 4	152	121	10	29	1.3
1990	Sept. 17	21	129	17	98	2.7
	Sept. 24	79	130	12	78	1.9
	Oct. 3	127	97	6	46	1.6
	Oct. 11	150	86	5	49	1.6

[z] Score reflects % of surface affected: 1 = 1 to 10%; 2 = 11 to 33%; 3 = 34 to 66%; 4 = ≥ 67%.

Table 4.24. Maximum ∝ farnesene and conjugated triene concentrations in hexane extracts of fruit surfaces of Delicious apples after storage at 0°C, and scald after 20 weeks of storage, 1988 and 1990.

Year	Harvest date	Hours < 10°C	Max ∝ farn (nmoles/cm^2)	Max CT (nmoles/cm^2)	Scald (%)	Scald score[z]
1988	Oct. 1	160	110	5	12	1.1
	Oct. 8	232	116	5	2	1.0
	Oct. 15	365	109	4	2	1.0
1990	Sept. 21	62	136	13	94	2.7
	Sept. 26	104	142	9	88	2.8
	Oct. 3	127	94	6	68	2.1
	Oct. 11	150	80	4	51	1.7

[z] Score reflects % of surface affected: 1 = 1 to 10%; 2 = 11 to 33%; 3 = 34 to 66%; 4 = ≥ 67%.

Table 4.25. Correlation coefficients between maximum ∝ farnesene or conjugated trienes and hours below 10°C or scald development on Cortland and Delicious apples.

	1988				1989		1990			
	Cortland		Delicious		Cortland		Cortland		Delicious	
	Hours	Scald	Hours	Scald	Hours	Scald	Hours	Scald	Hours	Scald
Max ∝ farn	-0.20^{ns}	0.23^{ns}	-0.14^{ns}	0.35^{ns}	-0.35^{ns}	0.35^{ns}	-0.82^{***}	0.73^{***}	-0.81^{***}	0.80^{***}
Max CT	-0.88^{***}	0.80^{***}	-0.11^{ns}	0.13^{ns}	-0.90^{***}	0.89^{***}	-0.96^{***}	0.86^{***}	-0.93^{***}	0.75^{***}

^{***,**,*,ns}: Significant at 0.1%, 1%, or 5% level or not significant, respectively.

triene concentrations were strongly correlated with hours below 10°C and with scald. The regression values ranged from 60 to 90% and were significant.

In Delicious apples in 1988, there were no significant correlations between maximum ∝ farnesene or maximum conjugated triene concentrations, and either hours below 10°C or scald development. In 1990, however, both maximum ∝ farnesene and maximum conjugated triene concentrations correlated strongly with hours below 10°C and with scald. The regression value for maximum ∝ farnesene versus scald was 72%, and for maximum conjugated trienes versus scald was 72%; both relationships were linear.

The % inhibition of oxidation and total water-soluble reducing activities of apple peel were measured over the course of fruit storage time in the 1990 Cortland and Delicious apples (Tables 4.26 and 4.27). In both cultivars the at-harvest % inhibition of oxidation was linearly related to hours below 10°C. The activities changed little during fruit storage, and the at-harvest differences between harvest dates were maintained. At harvest, TWRC increased with later harvest (greater numbers of hours below 10°C), but these values declined rapidly during storage and after 8 weeks or more at 0°C, there were no differences among harvest dates.

In 1990, the individual antioxidants that were measured at-harvest were also measured during storage in Cortland and Delicious apples and are presented in Tables 4.28 and 4.29.

Table 4.26. Antioxidant and % oxidation inhibition estimates for peel of Cortland apples at harvest and after 8 and 20 weeks of storage at 0°C, 1990.

Harvest date	Hours < 10°C	Oxidation[z] (% inhibition)			TWPC (units/gdw)		
		Weeks at 0°C			Weeks at 0°C		
		0	8	20	0	8	20
Sept. 17	21	40	32	33	11.2	9.5	10.7
Sept. 24	79	60	54	58	11.1	9.7	9.3
Oct. 3	127	76	69	69	12.0	8.7	11.8
Oct. 11	150	87	81	80	13.8	9.3	11.0
Significance (Orthogonal polynomial comparisons within storage period)							
Linear		***	***	***	***	ns	ns
Quadratic		ns	ns	ns	*	ns	ns
Cubic		ns	ns	ns	ns	ns	ns

***,**,*,ns: Significant at 0.1%, 1%, or 5% level or not significant, respectively.

[z] % Inhibition of linoleic oxidation as estimated by conjugated dienes.

Table 4.27. Antioxidant and % oxidation inhibition estimates for peel of Delicious apples at harvest and after 8 and 20 weeks of storage at 0°C, 1990.

Harvest date	Hours < 10°C	Oxidation[z] (% inhibition)			TWRC (units/gdw)		
		Weeks at 0°C			Weeks at 0°C		
		0	8	20	0	8	20
Sept. 21	62	22	23	45	13.9	16.4	12.8
Sept. 26	104	34	47	56	14.1	15.9	14.4
Oct. 3	127	44	61	67	14.9	16.3	12.5
Oct. 11	150	55	69	77	15.8	12.8	12.3
Significance (Orthogonal polynomial comparisons within storage period)							
Linear		***	***	***	***	***	ns
Quadratic		ns	ns	ns	ns	**	ns
Cubic		ns	ns	ns	ns	**	*

***,**,*,ns: Significant at 0.1%, 1%, or 5% level or not significant, respectively.

[z] % Inhibition of linoleic acid oxidation as estimated by conjugated dienes.

Table 4.28. Antioxidant concentrations in peel of Cortland apples at harvest and after 8 and 20 weeks of storage at 0°C, 1990.

Harv. date	Hours < 10°C	∝ Tocopherol (μg/gdw)			Ascorbic acid (μg/gdw)			Glutathione (μg/gdw)		
		Weeks at 0°C			Weeks at 0°C			Weeks at 0°C		
		0	8	20	0	8	20	0	8	20
9-17	21	103	94	132	380	362	365	91	77	61
9-24	79	91	100	111	376	364	295	106	91	43
10-3	127	100	116	118	365	349	354	139	86	58
10-11	150	146	112	128	411	327	357	81	40	74
Significance (Orthogonal polynomial comparisons within storage period)										
Linear		***	**	ns	ns	ns	ns	ns	*	ns
Quadratic		***	ns	**	ns	ns	**	*	**	ns
Cubic		**	ns	ns	ns	ns	ns	*	*	ns

***,**,*,ns: Significant at 0.1%, 1%, or 5% level or not significant, respectively.

Table 4.29. Antioxidant concentrations in peel of Delicious apples at harvest and after 8 and 20 weeks of storage at 0°C, 1990.

Harv. date	Hours < 10°C	∝ Tocopherol (μg/gdw)			Ascorbic acid (μg/gdw)			Glutathione (μg/gdw)		
		Weeks at 0°C			Weeks at 0°C			Weeks at 0°C		
		0	8	20	0	8	20	0	8	20
9-21	62	72	59	87	624	571	568	39	19	29
9-26	104	70	69	-	601	590	623	39	45	34
10-3	127	78	62	74	593	560	564	55	8	15
10-11	150	79	63	90	604	499	555	35	12	15
Significance (Orthogonal polynomial comparisons within storage period)										
Linear		*	*	-	ns	ns	*	ns	ns	ns
Quadratic			*	-	ns	*	*	ns	**	ns
Cubic			ns	-	ns	ns	ns	*	***	ns

***,**,*,ns: Significant at 0.1%, 1%, or 5% level or not significant, respectively.

The ∝ tocopherol concentrations did not change greatly during the storage period but differences among harvest dates at harvest time slowly disappeared during storage. The ascorbic acid concentrations also changed little in apple peel throughout the time of fruit storage, at no sampling time were there differences among harvest dates. The glutathione concentrations decreased during storage in both cultivars (Tables 4.28 and 2.29).

The pigments also were measured at intervals during storage at 0°C (Tables 4.30 and 4.31) (Storage ANOVA tables in Appendix B). All of the measured pigments changed during cold storage. Chlorophyll and anthocyanin decreased significantly in both cultivars, but differences among harvest dates that existed at harvest generally persisted throughout the storage period. With the exception of total carotenoids in Delicious, both the total carotenoids and the hydrocarbon carotenes generally increased during storage. Harvest time differences generally persisted during storage of Cortlands but not of Delicious. Delicious contained lower amounts of both carotenoids and carotenes than did Cortland.

Table 4.30. Pigment concentrations in peel of Cortland apples at harvest and after 8 and 20 weeks of storage at 0°C, 1990.

Hours < 10°C	Chlorophyll (μg/gdw)			Carotenoids (μg/gdw)			Carotenes (μg/gdw)			Anthocyanin (μg/gdw)		
	Weeks at 0°C			Weeks at 0°C			Weeks at 0°C			Weeks at 0°C		
	0	8	20	0	8	20	0	8	20	0	8	20
21	209	149	157	105	102	113	22	24	37	261	213	226
79	163	132	111	109	99	112	21	26	41	383	293	106
127	114	77	109	100	65	92	23	22	39	594	241	606
150	92	44	72	83	61	86	33	25	42	1028	784	667
Significance (Orthogonal polynomial comparisons within storage period)												
Linear	***	***	***	***	***	***	**	ns	*	***	***	***
Quadratic	ns	***	ns	***	**	*	*	ns	ns	***	***	ns
Cubic	ns	ns	***	ns	**	ns	ns	**	ns	*	***	ns

***,**,*,ns: Significant at 0.1%, 1%, or 5% level or not significant, respectively.

Table 4.31. Pigment concentrations in peel of Delicious apples at harvest and after 8 and 20 weeks of storage at 0°C, 1990.

Hours < 10°C	Chlorophyll (μg/gdw)			Carotenoids (μg/gdw)			Carotene (μg/gdw)			Anthocyanin (μg/gdw)		
	Weeks at 0°C			Weeks at 0°C			Weeks at 0°C			Weeks at 0°C		
	0	8	20	0	8	20	0	8	20	0	8	20
62	120	56	27	76	58	59	18	22	26	433	552	353
104	100	78	52	74	73	72	15	25	35	483	562	486
127	107	51	35	74	53	57	17	22	24	639	621	427
150	72	33	29	68	45	55	20	23	31	917	668	587
Significance (Orthogonal polynomial comparisons within storage period)												
Linear	***	ns	*	**	***	ns	ns	ns	ns	***	***	***
Quadratic	ns	***	***	ns	***	**	ns	ns	ns	ns	ns	ns
Cubic	**	ns	***	ns	***	**	ns	ns	**	ns	ns	ns

***,**,*,ns: Significant at 0.1%, 1%, or 5% level or not significant, respectively.

Changes Associated with Fruit Ripening Independent of Differences in Low Temperature Exposure

Effects of Ethephon Application on Fruit Ripening

Cortland apples were used for the ethephon experiments in 1989 and 1990. Ethephon treatments were applied as single applications in mid-August of both seasons. In 1989, apples were harvested twice, with 52 and 62 hours below 10°C accumulated, respectively. In 1990, the apples were also harvested twice, but there were no hours below 10°C at either harvest, which provided an opportunity to study the effects of ethephon-induced ripening in the absence of confounding effects of temperatures below 10°C.

Ethephon significantly hastened ripening in both years as shown by firmness loss, starch hydrolysis, and ethylene production (Table 4.32). In 1989, ethephon significantly decreased the flesh firmness, and in both years the starch indices (hydrolysis) increased greatly as a result of ethephon treatment. In both seasons, only those apples that were treated with ethephon were in the climacteric rise at harvest (log $C_2H_4 \geq 1$). The two rates of ethephon used in 1990 affected the fruit ripening to about the same extent. The correlations between treatment and ripening indices were very strong (Table 4.33).

Since both control and ethephon-treated fruit experienced the same environmental conditions by harvest, differences in composition at harvest and changes after

Table 4.32. At-harvest ripeness indices in the 1989 and 1990 ethephon experiments. Ethephon was applied on August 16, 1989 and on August 20, 1990.

Year	Harvest date	Hours < 10°C	Ethephon (ppm)	Firmness (N)	Starch index[z]	C_2H_4 (log ppm)	Scald (%)	Scald score[y]
1989	Sept. 6	52	0	96	1.2	-1.0500	87	2.2
		52	500	71	7.4	1.9987	81	2.8
	Sept. 13	62	0	84	1.3	-0.6418	96	2.5
		62	500	45	8.4	2.1314	67	2.0
1990	Sept.1	0	0	-	1.0	-2.3498	97	2.4
		0	250	-	4.8	1.8596	90	2.8
		0	500	-	5.9	2.0795	92	2.8
	Sept. 6	0	0	-	1.3	-1.8181	99	2.4
		0	250	-	7.3	2.1180	91	3.2
		0	500	-	6.9	1.9520	96	3.2
Significance (ANOVA)								
1989	TREATMENT		*	***	***	**	ns	
	HOURS		**	ns	ns	ns	ns	
1990	TREATMENT		-	***	***	**	***	

***,**,*,ns: Significant at 0.1%, 1%, or 5% level or not significant, respectively.

[z] Scores of 1 to 9, with 1 to 3 indicating immature, 4 to 6 mature (climacteric) and 7 to 9 overmature.

[y] Score reflects % of surface affected: 1 = 1 to 10%; 2 = 11 to 33%; 3 = 34 to 66%; 4 = ≥ 67%.

Table 4.33. Correlation coefficients between ethephon treatment, scald development after storage, and fruit ripening indices, antioxidant and % oxidation inhibition estimates and maximum ∝ farnesene and conjugated triene concentrations in the 1989 and 1990 ethephon experiments.

	1989			1990		
	Treatment	Scald (%)	Scald Score	Treatment	Scald (%)	Scald score
Treatment	-	-0.71^{***}	-0.04^{ns}	-	-0.31^{ns}	0.65^{***}
Firmness	-0.82^{***}	0.47^{*}	-0.04^{ns}	-	-	-
Starch index	0.98^{***}	-0.71^{***}	-0.11^{ns}	0.82^{***}	-0.33^{ns}	0.86^{***}
Ethylene	0.98^{***}	-0.67^{**}	-0.18^{ns}	0.85^{***}	-0.46^{*}	0.72^{***}
OD200	0.76^{***}	-0.76^{***}	-0.30^{ns}	0.89^{***}	-0.49^{**}	0.74^{***}
% Inhib.[z]	0.94^{***}	-0.59^{**}	0.03^{ns}	0.85^{***}	-0.50^{**}	0.74^{***}
TWRC	-	-	-	0.47^{**}	-0.18^{ns}	0.54^{**}
Max. ∝ farnesene	-0.89^{***}	0.70^{***}	0.21^{ns}	0.61^{*}	-0.12^{ns}	0.63^{*}
Max. conj. trienes	-0.51^{*}	0.58^{**}	0.21^{ns}	0.01^{ns}	0.51^{ns}	-0.05^{ns}

[z] % Inhibition of linoleic acid oxidation as estimated by conjugated dienes.

Total water-soluble reducing capacity

^{***,**,*,ns}: Significant at 0.1%, 1%, or 5% level or not significant, respectively.

harvest should represent only the effects of differences in ripeness at harvest.

Effects on Concentrations of ∝ Farnesene and Conjugated Trienes in Apple Peel

As mentioned previously, ∝ farnesene usually is relatively low at harvest and increases to a maximum in storage after about 6 weeks, and then decreases. This pattern was recorded for the control apples in the ethephon experiments in both 1989 and 1990 (Tables 4.34 and 4.35). Apples treated with ethephon, however, generally had very elevated concentrations of ∝ farnesene at harvest, and these levels did not increase during storage (Tables 4.34 and 4.35). The at-harvest concentrations of conjugated trienes also were very high in ethephon-treated fruit, and changed little if any during storage, whereas in control fruit conjugated triene concentrations were low at harvest and increased during storage (Tables 4.34 and 4.35).

Effects on Estimates of Antioxidant Activities and Percent Inhibition of Oxidation in Apple Peel

The at-harvest OD200 values were much higher after ethephon treatment, as were the % inhibitions of oxidation (Table 4.36). The total water-soluble reducing capacities (TWRC) also were increased by treatment, but to a lesser extent. Ethephon treatments were correlated highly (0.1% level) with at-harvest OD200 values and % inhibition of

Table 4.34. OD200, ∝ farnesene and conjugated triene values of hexane extracts of Cortland apple surfaces in the ethephon experiment at harvest and after 6, 12, and 18 weeks of storage at 0°C, 1989.

Harv. date	Ethephon (ppm)	OD200 ([ODx1000]/cm²)				∝ Farnesene (nmoles/cm²)				Conjugated trienes (nmoles/cm²)			
		Weeks at 0°C				Weeks at 0°C				Weeks at 0°C			
		0	6	12	18	0	6	12	18	0	6	12	18
9-1	0	3	33	26	24	0	141	106	75	0	10	15	12
	500	14	42	38	35	97	95	90	76	12	12	11	10
9-6	0	8	29	20	25	8	124	84	70	1	9	11	17
	500	38	37	34	35	78	70	73	59	8	9	9	13
Significance (Orthogonal polynomial comparisons within storage period)													
TREATMENT		**	**	***	***	***	***	ns	ns	***	ns	ns	ns
HOURS		ns	ns	ns	ns	ns	ns	*	*	ns	ns	*	*

***,**,*,ns: Significant at 0.1%, 1%, or 5% level or not significant, respectively.

Table 4.35. OD200, ∝ farnesene, and conjugated triene values of hexane extracts of Cortland apple surfaces in the ethephon experiment at harvest and after 6, 12, and 18 weeks of storage at 0°C, 1990.

Harv. date	Ethephon (ppm)	OD200 ([ODx1000]/cm^2)				∝ Farnesene (nmoles/cm^2)				Conjugated trienes (nmoles/cm^2)			
		Weeks at 0°C				Weeks at 0°C				Weeks at 0°C			
		0	6	12	18	0	6	12	18	0	6	12	18
9-1	0	6	24	30	27	10	110	109	88	1	5	12	12
	250	30	35	36	36	91	129	89	58	10	9	10	9
	500	34	35	36	33	108	129	89	61	13	11	11	11
Significance (Orthogonal polynomial comparisons within storage period)													
Linear		***	***	***	**	***	*	*	*	***	*	ns	ns
Quadratic		**	***	**	ns	*	ns	ns	ns	ns	ns	ns	*

***,**,*,ns: Significant at 0.1%, 1%, or 5% level or not significant, respectively.

Table 4.36. Effects of ethephon applications on estimates of antioxidant and % oxidation inhibition activities in peel of Cortland apples at harvest, 1989 and 1990.

Year	Harvest date	Ethephon (ppm)	OD 200 (ODX1000/cm^2)	Oxidation[z] (% inhibition)	TWRC (units/gdw)	Scald (%)	Scald score[y]
1989	Sept. 6	0	3.1	56	-	87	2.2
		500	14.0	93	-	81	2.8
	Sept.13	0	7.8	61	-	96	2.5
		500	38.0	93	-	67	2.0
1990	Sept. 1	0	4.2	61	9.6	97	2.4
		250	28.8	89	11.2	90	2.8
		500	32.1	88	11.6	92	2.8
	Sept. 6	0	6.0	62	11.3	99	2.4
		250	29.5	90	13.9	91	3.2
		500	33.9	91	14.2	96	3.2
Significance (ANOVA)							
1989	TREATMENT		***	***	-	**	ns
	HOURS		***	ns	-	ns	ns
1990	TREATMENT		***	***	*	**	***

[z] % Inhibition of linoleic acid oxidation as estimated by conjugated dienes.

TWRC=Total water-soluble reducing capacity

[y] Score reflects % of surface affected: 1=1 to 10%; 2=11 to 33%; 3=34 to 66%; 4= ≥ 67%.

***,**,*,ns: Significant at 0.1%, 1%, or 5% level or not significant, respectively.

oxidation, and were correlated at the 1% level with at-harvest TWRC (Table 4.33). In both 1989 and 1990, the % inhibition of oxidation were correlated significantly with scald development, although the correlation coefficients were only -0.59 and -0.50, respectively (Table 4.33). In 1990, the correlation between % inhibition of oxidation and scald score was higher (0.74) than in 1989. The TWRC was not correlated with scald incidence but was related to scald score (intensity) (Table 4.33). These results indicate that ethephon-induced ripening may increase antioxidant activity in apple peel, with greater effect on % inhibition of oxidation which was presumably due to lipid-soluble antioxidants than on water-soluble antioxidants.

Effects on Activities of Specific Antioxidants in Apple Peel

After measuring the estimates of antioxidant activities and % inhibition of oxidation in 1990, three individual antioxidants were quantified. The concentrations of ∝ tocopherol, ascorbic acid and glutathione in the apple peel are shown in Table 4.37. Both ∝ tocopherol and ascorbic acid were correlated significantly with ethephon treatment, having been increased by treatment, and the regressions were linear (Tables 4.28, 4.29, and 4.38). These data, along with the antioxidant estimates and % inhibition of oxidation (Tables 4.26, 2.27, and 4.36), indicate that both lipid-soluble and water-soluble antioxidants increase during fruit ripening.

Table 4.37. Effects of ethephon treatment on concentrations of specific antioxidants in Cortland apple peel at harvest, 1990.

Harvest date	Ethephon (ppm)	∝ Tocopherol (µg/gdw)	Ascorbic acid (µg/gdw)	Glutathione (µg/gdw)
Sept. 1	0	79	423	87
	250	120	493	98
	500	116	511	83
Sept. 6	0	72	383	87
	250	149	479	88
	500	154	481	93
Significance (ANOVA)				
TREATMENT		***	***	ns

***,**,*,ns: Significant at 0.1%, 1%, or 5% level or not significant, respectively.

Table 4.38. Correlations between ethephon treatment, scald development, antioxidant concentrations, and pigment concentrations, 1990.

Constituent	Ethephon treatment	Scald (%)	Scald score[z]
∝ Tocopherol	0.69***	-.034ns	0.63***
Ascorbic Acid	0.66***	-0.44*	0.43*
Glutathione	0.02ns	-0.01ns	0.00ns
Chlorophyll	-0.86***	0.36*	-0.71***
Carotenoids	-0.69***	0.40*	-0.61***
Carotenes	0.55**	-0.21ns	0.54**
Anthocyanin	0.71***	-0.40*	.067***
Flavonols	0.00ns	-0.25ns	-0.44ns

[z] Score reflects % surface affected: 1=1 to 10%; 2=11 to 33%; 3=34 to 66%; 4= ≥ 67%.

***,**,*,ns: Significant at 0.1%, 1%, or 5% level or not significant, respectively.

Effects on Antioxidant Activities During Storage at 0°C

The OD200 values and antioxidant concentrations also were measured during storage at 0°C in 1990 (OD200 also in 1989). As seen in previous experiments with Cortland apples, the OD200 values of control fruit greatly increased during storage (Tables 4.34 and 4.35). This pattern also was true for apples treated with 500 ppm ethephon and harvested September 6, 1989. In all other ethephon-treated apples in 1989 and 1990, however, the OD200 values were high at time of harvest and changed little during storage. The treated apples had higher OD200 values throughout the storage time than did the control apples (Tables 4.34 and 4.35).

The % inhibition of oxidation after different intervals of storage at 0°C for apples harvested September 1, 1990 are shown in Table 4.39. As shown previously, the % inhibition of oxidation at harvest was increased by ethephon treatment. These differences were maintained throughout eighteen weeks of storage, the patterns being similar to those seen in Cortlands exposed to increasing hours below 10°C prior to harvest (Table 4.26). The % inhibition of oxidation did not change over harvest as seen in Cortland in the sequential harvests experiments. The TWRC also increased during storage, but then they decreased after prolonged storage and treatment differences had disappeared after 18 weeks at 0°C (Table 4.39).

Table 4.39. Effects of ethephon treatment on % oxidation inhibition and total water-soluble reducing capacities (TWRC) in Cortland apple peel at harvest and after storage at 0°C, 1990.

Ethephon application (ppm)	Oxidation[z] (% inhibition)			TWRC (units/gdw)		
	Weeks at 0°C			Weeks at 0°C		
	0	12	18	0	12	18
0	61	62	58	10	12	12
250	89	94	96	11	14	12
500	88	95	95	12	14	11
Significance (Orthogonal polynomial comparisons)						
Linear	***	***	***	*	*	ns
Quadratic	***	*	***	ns	ns	ns

***,**,*,ns: Significant at 0.1%, 1%, or 5% level or not significant, respectively.

[z] % Inhibition of linoleic acid oxidation as estimated by conjugated dienes.

The concentrations of ∝ tocopherol in apple peel during storage increased but the at-harvest differences were maintained throughout storage (Table 4.40).

The ascorbic acid concentrations declined during storage (Table 4.40). The differences between ethephon-treated and control apples that existed at harvest were not significant after either 12 or 18 weeks at 0°C, although there continued to be a trend toward greater concentrations in treated fruit.

Effects on Pigment Concentrations in Apple Peel

Chlorophyll concentrations at harvest were decreased greatly by ethephon treatment (Table 4.41) Chlorophyll decreased in all samples during storage, but the treatment differences persisted throughout storage at 0°C. The at-harvest anthocyanin concentrations were increased significantly by ethephon treatment (Table 4.41). These at-harvest differences were maintained during the 18-week period of storage, as the concentrations did not change. There were no treatment differences in total flavonol concentrations and these concentrations at harvest did not change during cold storage (storage data not shown).

Total carotenoids decreased and carotenes increased at harvest with ethephon treatment. Concentrations of both increased during storage but the carotenes increased more dramatically than the total carotenoids (Table 4.41).

Table 4.40. Effects of ethephon treatment on antioxidant concentrations in apple peel at harvest and after storage at 0°C for Cortland apples harvested on September 1, 1990.

Ethephon (ppm)	∝ Tocopherol (μg/gdw)			Ascorbic acid (μg/gdw)			Glutathione (μg/gdw)		
	Weeks at 0°C			Weeks at 0°C			Weeks at 0°C		
	0	12	18	0	12	18	0	12	18
0	79	111	122	423	436	364	87	68	44
250	120	179	172	493	473	388	98	70	60
500	116	184	154	511	522	381	83	55	35
Significance (Orthogonal polynomial comparisons within storage period)									
Linear	*	***	ns	*	ns	ns	ns	ns	ns
Quadratic	ns	*	*	ns	ns	ns	ns	ns	ns

***,**,*,ns: Significant at 0.1%, 1%, or 5% level or not significant, respectively.

Table 4.41. Effects of ethephon treatment on pigment concentrations at harvest and after intervals of storage at 0°C for Cortland apples harvested September 1, 1990.

Ethephon (ppmy y)	Chlorophyll (μg/gdw)			Carotenoids (μg/gdw)			Carotenes (μg/gdw)			Anthocyanin (μg/gdw)		
	Weeks at 0°C			Weeks at 0°C			Weeks at 0°C			Weeks at 0°C		
	0	12	18	0	12	18	0	12	18	0	12	18
0	209	218	182	130	74	151	25	38	87	178	156	169
250	151	131	103	111	65	124	31	47	102	506	517	461
500	114	104	74	109	63	121	27	54	102	456	517	400
Significance (Orthogonal polynomial comparisons within storage period)												
Linear	***	***	***	**	*	**	ns	***	***	***	***	**
Quadratic	ns	**	*	ns	ns	ns	*	ns	*	***	**	**

***,**,*,ns: Significant at 0.1%, 1%, or 5% level or not significant, respectively.

Effects on Scald Development After Storage at 0°C

Incidences and intensities of scald development after storage are shown for reference in Tables 4.32, 4.34, and 4.42. Ethephon treatments reduced scald development significantly in both years; however, differences were substantial only after the second harvest in 1989 (Table 4.32). In 1989, ethephon treatment and scald incidence were correlated strongly (Table 4.33). There was no relationship, however, between scald score (intensity) and ethephon treatment. In 1989, 52 and 62 hours below 10°C, had been recorded at the two harvests, respectively. In 1990, when there were no hours below 10°C at either harvest, there was no correlation between ethephon treatment and scald development (Table 4.33). However, there was a highly significant positive correlation between ethephon treatment and scald score, indicating that ethephon may in fact negatively influence resistance to scald development, or at least scald intensity, under certain conditions, such as in the absence of cool temperatures during ripening.

As mentioned above, in both 1989 and 1990, ethephon greatly advanced ripening, and in 1989, there was a concomitant decrease in scald development. In 1990, with substantial increase in ripening and increased antioxidant levels but no accumulated hours below 10°C, there was no correlation between scald and ethephon treatment. The analysis of variance showed a significant (at 1% level) decrease in scald incidence with treatment, but in all

Table 4.42. Maximum ∝ farnesene and conjugated triene concentrations measured during storage at 0°C for the 1989 and 1990 ethephon experiments.

Year	Harvest date	Ethephon (ppm)	Max. ∝ farn. (nmoles/cm^2)	Max. CT (nmoles/cm^2)	Scald (%)	Scald score[z]
1989	Sept. 6	0	141	15	87	2.2
		500	95	11	81	2.8
	Sept. 13	0	124	11	96	2.5
		500	73	9	67	2.0
1990	Sept.1	0	110	12	97	2.4
		250	129	10	90	2.8
		500	129	11	92	2.8
Significance (ANOVA)						
1989 TREATMENT			***	*	***	ns
HOURS			*	ns	ns	ns
1990 TREATMENT			ns	*	**	***

[z] Score reflects % of surface affected: 1=1 to 10%; 2=11 to 33%; 3=34 to 66%; 4=≥ 67%.

***,**,*,ns: Significant at 0.1%, 1%, 5% level or not significant, respectively

samples the percent scald was at least 90%. Therefore, in practical terms, there was no substantial decrease in percent scald development.

Maximum ∝ farnesene and maximum conjugated triene concentrations measured during storage are reported in Table 4.42. Ethephon did not consistently affect maximum ∝ farnesene but decreased maximum conjugated triene levels in both years. In 1989, the maximum ∝ farnesene and maximum conjugated trienes measured during storage were correlated with scald incidence but not with scald score (Table 4.33). In 1990, however, there were no correlations between either maximum ∝ farnesene or maximum conjugated triene concentrations and scald, and only a slight correlation between maximum ∝ farnesene concentrations and scald score (at 5% level).

In summary, ethephon induced fruit ripening, and the ripe and unripe (control) fruits all had experienced the same exposures to low temperatures before harvest. Thus, differences between treated and control fruit represent effects of ripening independent of effects of low temperature.

Ripening (ethephon-induced) increased OD200 values, % inhibition of oxidation, and TWRC, and increased both ∝ tocopherol and ascorbic acid concentrations in apple peel. It also increased anthocyanin concentrations and decreased chlorophyll concentrations in peel.

Ethephon-induced ripening resulted in high concentrations of ∝ farnesene and conjugated trienes in apple peel at harvest. High incidences of scald development occurred on fruit in this experiment, and while ripening significantly reduced this incidence, the reductions were not of large magnitudes.

Changes Associated with Reduction of Light Intensity During Fruit Development as a Result of Bagging the Fruit

The at-harvest ripening indices for fruit in this experiment are shown in Table 4.43. Bagging had no effect on ripening as measured by flesh firmness, starch index, or ethylene concentration. Both control and bagged apples, however, did ripen between harvests.

Effects on Concentrations of ∝ Farnesene and Conjugated Trienes in Apple Peel

The ∝ farnesene and conjugated triene levels measured during storage at 0°C for the 1989 experiment are shown in Table 4.44. The patterns of change in both are similar to those seen in previous experiments. ∝ Farnesene concentrations increased to maxima early in storage then declined, and the conjugated trienes steadily increased throughout storage. The maximum ∝ farnesene and conjugated triene concentrations during storage were increased by bagging fruit during their maturation period in the orchard

Table 4.43. At-harvest ripeness measurements for 1989 and 1990 bagging experiments.

Year	Harvest date	Treatment	Hours < 10°C	Flesh firmness (N)	Starch index[z]	C_2H_4 (log ppm)	Scald (%)	Scald score[y]
1989	Sept. 18	Control	62	82	1.3	-1.1841	95	2.6
		Bagged	62	82	1.6	-1.2713	100	3.2
	Oct. 2	Control	147	75	5.1	-0.2060	27	1.4
		Bagged	147	75	4.4	0.0224	90	2.1
1990	Oct. 1	Control	107	-	3.2	-0.0741	31	1.4
		Bagged	107	-	3.4	0.2441	62	1.4
	Oct. 9	Control	150	-	6.4	1.2076	13	1.4
		Bagged	150	-	6.0	1.4105	42	1.2
Significance (ANOVA)								
1989	TREATMENT			ns	ns	ns	*	**
	HOURS			*	***	ns	**	*
	TREATMENT X HOURS			ns	**	ns	*	ns
1990	TREATMENT			-	ns	ns	*	ns
	HOURS			-	***	**	ns	ns
	TREATMENT X HOURS			-	ns	ns	ns	ns

[z] 1 to 3= immature; 4 to 6= mature; 7 to 9= overmature.

[y] Score reflects % of surface affected: 1=1 to 10%; 2=11 to 33%; 3=34 to 66%; 4 = ≥67%.

***,**,*,ns: Significant at 0.1%, 1%, 5% level or not significant, respectively

Table 4.44. OD200, ∝ farnesene and conjugated triene values for hexane extracts of Cortland apple surfaces in the bagging experiment at harvest and after 6, 12, and 18 weeks of storage at 0°C, 1989.

Harv. date	Treatment	OD200 ([ODx1000]/cm²)				∝ Farnesene (nmoles/cm²)				Conjugated trienes (nmoles/cm²)			
		Weeks at 0°C				Weeks at 0°C				Weeks at 0°C			
		0	6	12	18	0	6	12	18	0	6	12	18
9-18	Control	7	26	28	22	3	141	102	72	0	8	15	15
	Bagged	7	26	28	25	6	149	118	85	0	10	22	24
10-2	Control	15	34	26	27	16	122	80	75	0	7	9	13
	Bagged	14	35	28	28	20	154	103	86	0	9	15	22
Significance (Orthogonal polynomial comparisons within storage period)													
TREATMENT		ns	ns	*	ns	ns	**	*	ns	ns	*	*	***
HOURS		**	***	ns	*	ns	ns	ns	ns	ns	ns	ns	ns

***,**,*,ns: Significant at 0.1%, 1%, or 5% level or not significant, respectively.

(Table 4.45). Bagged fruit from the later harvest accumulated higher concentrations of both ∝ farnesene and conjugated trienes than unbagged fruit from the earlier harvest, demonstrating that light exclusion had a greater effect than harvest time on formation of these substances.

Effects on Antioxidant Activities of Apple Peel

The antioxidant estimates, specifically OD200 values, % inhibition of oxidation, and total water-soluble reducing capacities (TWRC), are shown in Table 4.46. Bagging had no significant effect on OD200 values or % inhibition of oxidation in either year although these estimates tended to be lower for the bagged fruit. Bagging did significantly decrease TWRC. In 1989, the OD 200 values increased as the hours below 10°C increased between harvests, but the % inhibition of oxidation did not change significantly between harvests. In 1990, both the OD200 values and % inhibition of oxidation increased with hours below 10°C. Interactions between bagging and hours below 10°C were small or not significant. As seen in previous experiments, the OD200 values increased early during storage and then leveled off, the final values being the same regardless of treatment or harvest date (Table 4.44).

In 1990 at-harvest levels of the antioxidants ∝ tocopherol, ascorbic acid, and glutathione were measured in the apple peel (Table 4.47). Bagging significantly decreased ∝ tocopherol and ascorbic acid concentrations in

Table 4.45. Maximum ∝ farnesene and conjugated triene concentrations measured during storage at 0°C for the 1989 bagging experiment.

Harvest date	Treatment	Hours < 10°C	Max. ∝ farn (nmoles/cm^2)	Max CT (nmoles/cm^2)	Scald (%)	Scald score[z]
Sept. 18	Control	62	142	16	95	2.6
	Bagged	62	153	26	99	3.2
Oct. 2	Control	147	127	13	27	1.4
	Bagged	147	160	23	90	2.1
Significance (ANOVA)						
TREATMENT			**	***	*	**

[z] Score reflects % of surface affected: 1=1 to 10%; 2=11 to 33%; 3=34 to 66%; 4= ≥ 67%.

***,**,*,ns: Significant at 0.1%, 1%, 5% level or not significant, respectively.

Table 4.46. At-harvest antioxidant and % oxidation inhibition estimates for the 1989 and 1990 bagging experiments.

Year	Harvest date	Treatment	Hours < 10°C	OD 200 (ODX1000/cm^2)	Oxidation[z] (% inhibition)	TWRC (units/gdw)	Scald (%)	Scald score[y]
1989	Sept. 18	Control	62	7.0	56	-	95	2.6
		Bagged	62	7.0	51	-	99	3.2
	Oct. 2	Control	147	14.5	61	-	27	1.4
		Bagged	147	13.4	58	-	90	2.1
1990	Oct. 1	Control	107	20.5	37	10	31	1.4
		Bagged	107	19.3	40	8	62	1.4
	Oct. 9	Control	150	26.3	67	10	13	1.4
		Bagged	150	26.3	49	7	42	1.2
Significance (ANOVA)								
1989	TREATMENT			ns	ns	-	*	**
	HOURS			*	ns	-	**	*
	TREATMENT X HOURS			ns	ns	-	*	ns
1990	TREATMENT			ns	ns	***	*	ns
	HOURS			*	**	ns	ns	ns
	TREATMENT X HOURS			ns	*	ns	ns	ns

[z] % Inhibition of linoleic acid oxidation as estimated by conjugated dienes.
TWRC=Total water-soluble antioxidant activity
[y] Score reflects % of surface affected: 1=1 to 10%; 2=11 to 33%; 3=34 to 66%; 4= ≤67%.
***,**,*,ns: Significant at 0.1%. 1%, or 5% level or not significant, respectively

Table 4.47. At-harvest antioxidant concentrations in apple peel, 1990 bagging experiment.

Harvest date	Treatment	∝ Tocopherol (μg/gdw)	Ascorbate acid (μg/gdw)	Glutathione (μg/gdw)
Oct.1	Control	106	399	102
	Bagged	91	324	117
Oct. 9	Control	122	384	103
	Bagged	93	297	84
Significance (ANOVA)				
TREATMENT		*	***	ns
HOURS		**	ns	ns
TREATMENT X HOURS		*	ns	ns

***,**,*,ns: Significant at 0.1%, 1%, or 5% or not significant, respectively.

the peel. The ∝ tocopherol increased between the two harvests (i.e., with increased hours below 10°C). The ascorbic acid levels, however, were the same at both harvests. Glutathione concentrations were not affected by bagging at either harvest (Table 4.47).

Effects on Pigment Concentrations in Apple Peel

The at-harvest values for the measured pigments are shown in Table 4.48. Chlorophyll levels were not influenced by bagging in either season, or by the accumulated hours below 10°C between harvests in 1990. Since chlorophyll declines with senescence, this indicated that bagging did not influence the rate of senescence in the tissue, which is consistent with the lack of any effect on the rate of ripening (Table 4.43). Chlorophyll concentration decreased between harvests in 1989, but not in 1990.

Bagging decreased anthocyanin levels in the peel by approximately five-fold in 1989 and by more than ten-fold in 1990 (Table 4.48). Flavonol levels were not altered by bagging in 1989 but were significantly decreased by bagging in 1990. Harvest date (hours below 10°C) did not affect the results significantly for either anthocyanin or flavonols. There were many correlations between the measured components within the apple peel, indicating potential interactions within the system in the peel tissue (Appendix C).

Table 4.48. At-harvest pigment concentrations in apple peel, 1990 bagging experiment.

Year	Treatment	Hours ≤ 10°C	Chlorophyll (µg/gdw)	Carotenoid (µg/gdw)	Carotenes (µg/gdw)	Anthocyanin (µg/gdw)	Flavonol (µg/gdw)
1989[z]	Control	62	171	-	-	622	1156
	Bagged	62	171	-	-	133	1200
	Control	147	121	-	-	1089	961
	Bagged	147	141	-	-	172	894
1990	Control	107	115	96	23	550	1172
	Bagged	107	131	85	18	39	744
	Control	150	116	97	30	678	1178
	Bagged	150	121	79	17	39	589
Significance (ANOVA)							
1989	TREATMENT		ns	-	-	**	ns
	HOURS		**	-	-	ns	ns
	TREATMENT X HOURS		ns	-	-	ns	ns
1990	TREATMENT		ns	ns	*	**	*
	HOURS		ns	ns	ns	ns	ns
	TREATMENT X HOURS		ns	ns	ns	ns	ns

***,**,*,ns: Significant at 0.1%, 1%, or 5% level or not significant, respectively.

[z] In 1989, two replicates were standard Cortland and two replicates were Redcort.

Bagging had no effect on the concentrations of total carotenoids; however, the carotenes were significantly decreased by bagging (Table 4.48).

Effects on Scald Development

In both 1989 and 1990, scald incidence was increased significantly by bagging (Table 4.43). In all but the first harvest in 1989, where almost all apples scalded, the bagged fruit had at least twice as much scald as the control fruit. Scald severity also was increased in 1989; however, in 1990 the scald scores (severity) were not different for bagged fruit compared to controls. The OD 200 values, % inhibition of oxidation, TWRC, α tocopherol, and ascorbic acid all were correlated significantly with scald incidence (Appendix Table C.20). The at harvest concentrations of anthocyanin, flavonol, total carotenoids, and carotenes were all negatively correlated with scald development (Appendix Table C21).

These results show that bagging (reducing light intensity) during fruit development greatly increased scald in both years. It had no effect on fruit ripening, however, and any effect it had on fruit temperature would favor the development of scald resistance. Thus, the bagging effects should represent the independent effects of light intensity on antioxidant accumulation and scald susceptibility. Data in Tables 4.46 and 4.48 indicate that low light intensity reduced the accumulation of antioxidants in apple peel, and

these effects all were correlated negatively with scald development (Appendix Table C.19).

CHAPTER 5

DISCUSSION

General Relationships to Scald Development

The commonly held explanation of scald development is that the disorder results from the accumulation of ∝ farnesene in fruit peel, its subsequent oxidation to conjugated trienes, and then cellular disruption as a result of conjugated triene action. The discoloration of tissue presumably results from phenolic oxidations in the disrupted cells. This model is based on correlations between ∝ farnesene concentrations, and especially conjugated triene concentrations, and scald development. From this model it follows that apples which accumulated more ∝ farnesene, or more critically, more conjugated trienes, likely would develop more scald, and such evidence has been reported (Huelin and Coggiola, 1970; Anet and Coggiola, 1974). Anet (1974) reported a case in which one harvest of Granny Smith apples had a high concentration of ∝ farnesene and also high conjugated triene formation, but no scald occurred. He concluded that the autoxidation had begun late enough in the storage period that it was not an effective scald inducer.

The data reported here showed consistencies with this model. The changes in ∝ farnesene and conjugated triene concentrations during storage followed previously reported patterns. Furthermore, in the sequential-harvests

experiments, both cultivars of apples exhibited significant correlations between maximum conjugated triene concentrations measured during storage and scald development following storage (Table 4.25), except for Delicious in 1988, when all three harvests took place after the accumulation of at least 160 hours below 10°C and very little scald developed. Maximum ∝ farnesene did not correlate with scald in 1988 or 1989, but did correlate in 1990. Both maximum ∝ farnesene and conjugated trienes correlated with scald in the 1989 ethephon and bagging experiments (Appendix Tables C.5 and C.6). Bagging increased conjugated triene concentrations to a greater extent than ethephon decreased them. In the 1990 ethephon experiment, there was no correlation between conjugated triene concentration and scald development, but there was not enough scald variance to expect significant correlations.

In the model described above, antioxidants are believed to protect against the oxidation of ∝ farnesene to conjugated trienes, thus controlling the development of scald. From this, it follows that apples with lower antioxidant concentrations would have higher conjugated triene concentrations, and that the rise in conjugated trienes that occurs during storage might correspond to a fall in antioxidant concentrations. Anet (1974) found that in apples that were susceptible to scald there was a decrease in antioxidants during storage. In apples that did

not scald, however, the antioxidant levels were maintained or even increased in some cases during storage. The at-harvest data in the sequential-harvests experiments give support to this model, in that there were significant negative correlations between antioxidant estimates at harvest and the maximum conjugated triene concentrations measured during storage (Appendix Tables C.3, C.4, C.8, and C.12). In experiments in which ethephon was applied or fruit were bagged, however, there were almost no relationships between the antioxidant estimates and maximum conjugated triene concentrations (Appendix Tables C.5, C.6, and C.16). Contrary to what might be expected from the model, during the time that conjugated triene concentrations were increasing, the OD 200 values (purported estimates of lipid-soluble antioxidants) increased in both Cortland and Delicious, and the % inhibition of oxidation increased in Delicious (Tables 4.18 through 4.22 and 4.27). The estimates of water-soluble antioxidant activity (TWRC) declined slightly in both cultivars, however, and % inhibition of oxidation decreased in Cortland as conjugated trienes increased (Tables 4.18 through 4.22, 4.26 and 4.27). Thus, some of the data reported here support the model, but others do not. The bulk of the data, however, support the involvement of endogenous antioxidant activity to some extent in suppression of conjugated triene accumulation and scald development.

Scald susceptibility is influenced by the preharvest environment, as reported many times before (e.g. Fidler, 1957; Merritt et al., 1961). Morris (1964) reported that in Stayman apples grown in New Jersey, accumulated hours below 55°F were more important in determining scald susceptibility than was maturity; however, prediction of scald was more accurate if hours below 55°F and maturity were used in combination. In Rome Beauty apples, while it was still best to use both parameters, maturity was the more important factor. Little and Taylor (1981) reported that in Australia, apples grown in the cooler districts scalded less than those from warmer districts. Shutak and Kitchin (1966) found that bagging fruit with black or white muslin increased scald development, with black having the greater effect.

In the experiments reported here, both scald incidence (% scald) and scald intensity (scald score) were negatively correlated with accumulated hours below 10°C before harvest. Preharvest ripening (due to ethephon application) slightly suppressed scald development. Low light intensities (bagging) increased scald development.

Under normal orchard conditions in the northeastern U.S., hours below 10°C, ripening, and light intensity all are confounded. The sequential harvests, therefore, represent the combined effects of all three factors. This is evidenced by the strong correlations between the ripeness indices and the hours below 10°C for both Cortland and

Delicious apples in the sequential-harvests experiments (Table 4.4). Post-storage development of scald decreased as fruit were harvested progressively later in the season, and scald generally was correlated significantly with increases in both hours below 10°C and ripeness (Table 4.5). Although scald was related to both hours below 10°C and to ripeness, the correlations and regressions were generally higher for hours below 10°C versus scald than for ripeness indices versus scald (Tables 4.5 and 4.6). This result is similar to what Merritt et al. (1961) reported for Stayman. In addition, scald incidence began to decrease when fruit were harvested at times when little or no ripening had occurred yet, and often the greatest reductions in scald occurred when fruit were harvested while they were still preclimacteric. In the 1989 Cortlands, there were no additional hours below 10°C recorded between the first and second harvest but there was a substantial increase in ripening (Table 4.2); however, there was no reduction in scald incidence from the first to the second harvest. Furthermore, the apples generally did not enter the climacteric rise until the last harvest, but scald decreased substantially at earlier harvests. Perhaps most tellingly, in 1988, the Delicious apples had not entered the climacteric rise at any of the three harvests, but had accumulated 160 to 365 hours below 10°C, and scald incidence on these apples was minimal (Table 4.7).

In order to study the effects of ripening with minimal influence of hours below 10°C, ethephon application was used to advance ripening during warm weather. At each harvest in these experiments (1989 and 1990), all apples had been subjected to the same environmental conditions, and therefore any differences in scald development should have been the result solely of differences in ripeness at harvest. Furthermore, in 1990 there were no hours below 10°C recorded at either harvest, providing an opportunity to study the effects of ripening independent of temperature (hours below 10°C).

The ethephon treatments statistically decreased scald development (Table 4.32); however, at least 90% of the fruit developed scald in all treatments from both harvests in 1990, when ripening occurred in the absence of any low temperature. (The ethephon treatment actually significantly increased the scald intensity on the fruit that scalded in 1990.) Therefore, in practical terms there was no substantial decrease in scald development despite substantial ripening of the fruit. Ripening in itself was not a major factor in development of resistance to scald.

In 1989, when 62 hours below 10°C accumulated before harvest, the ethephon treatment reduced the percent of fruit that developed scald by about 30% (96% vs. 67%) (Table 4.32). The differences in response between 1990, when ripening occurred in the absence of cool temperatures, and 1989 when it occurred during cool temperature accumulation,

again suggest that ripening alone provided little scald resistance, but also that there may have been an interaction between ripening and temperature: the 62 hours were not enough to give resistance (Figure 4.1), but when combined with ripening, some resistance occurred. Since these results of the ethephon experiments indicate that ripening alone is a small factor in the development of scald resistance, the majority of the effects observed in the sequential-harvests experiment must be due to the cool temperatures that occurred during the harvest period.

In the bagging experiment, thermocouples placed in the bags showed that bagging did not affect temperature significantly (Figures 3.1 and 3.2). Likewise, ripeness indices showed that bagging had no effect on ripening (Table 4.43). Therefore, differences between bagged and control fruit should be the result of decreased light intensity, essentially independent of temperature and ripening. Bagging fruit significantly increased the percent of fruit that developed scald in both seasons, and also increased scald intensity in 1989 (Table 4.43). These data indicate that light significantly enhanced the development of scald resistance in the presence of cool temperatures. Apples ripening under low light intensity retained scald susceptibility, even though they experienced sufficient cool temperature to become highly resistant.

In summary, in the sequential-harvests experiments (measuring hours below 10°C), apples experienced

progressively more preharvest cool temperatures, and ripening occurred primarily at the latest harvests. Scald development decreased with progressively later harvests, most of this decrease occurring prior to ripening. Results of the ethephon treatments indicated that ripening alone contributed little to this development of scald resistance. Therefore, the decrease in scald development was primarily due to the cool temperatures experienced during the harvest period. The data from the bagging experiment indicated that light intensity was a factor in this development of scald resistance, which can account for the commonly observed greater scald development on poorly colored fruit, on fruit from the interior of the tree canopy, and on the green area of an otherwise red fruit.

These results generally supported the conventional model for the mechanism of scald development, and for the likely involvement of endogenous antioxidants in this mechanism. Most importantly, however, they showed that cool temperatures probably were essential for development of scald resistance, that low light intensity inhibited that development, and that ripening was only marginally involved.

Relationships of Antioxidant Estimates to Scald Development

Three purported estimates of antioxidant activity (OD 200 values, % inhibition of oxidation, and total water-

soluble reducing capacity (TWRC)) were measured in the apple peel. The OD 200 values proposed by Meir and Bramlage (1988) as crude estimates of antioxidant activity were highly correlated with % inhibition of oxidation in most cases (Appendix Tables C.3 through C.6, C.8, C.12, C.16, and C.20), suggesting that OD 200 indeed may estimate the activity of lipid-soluble antioxidants. In both the sequential-harvests experiments and the ethephon experiments, the % inhibition of oxidation and TWRC were correlated (Appendix Tables C.9, C.13, C.17), indicating an integration of both lipid-soluble and water-soluble antioxidant systems in the fruit peel. There have been several reports of interactions involving ascorbic acid, glutathione, and ∝ tocopherol. Niki et al. (1983) reported results in line with others that vitamin C can regenerate vitamin E. They concluded that the combination of vitamin C and vitamin E is an effective antioxidant system since lipid peroxy radicals would be preferentially trapped by vitamin E and then the vitamin E would be regenerated by vitamin C. Glutathione also has been linked to the regeneration of ∝ tocopherol (Tappel, 1962).

The OD 200 values increased linearly with hours below 10°C except in the 1988 Delicious (Tables 4.18 to 4.20), and were increased greatly by ethephon treatment (Table 4.34 and 4.35). Meir and Bramlage (1988) found similar increases with later harvests. Ethephon increased the OD 200 values to substantially more than those that were measured in the

sequential harvests: 38 vs 15 in 1989 and 34 vs 24 in 1990 for final harvests in the ethephon-treated and sequentially-harvested Cortland experiments, respectively (Tables 4.19, 4.20, 4.34 and 4.35). This difference was greater in 1989, when the difference in ripeness between the fruit from the final Cortland harvest and the ethephon-treated apples was greater. Bagging the fruit did not influence OD 200 values (Table 4.46). From these data it appears that OD 200 is influenced greatly by ripening, much less by hours below 10°C, and little if at all by light intensity. The OD 200 values showed strong negative correlations with scald development in the sequential-harvests experiments. This also was reported by Meir and Bramlage (1988). While OD 200 was correlated with scald development in the ethephon and bagging experiments as well (Appendix Tables C.5, C.6,C.15, and C.19), the correlation coefficients were only about 0.50, indicating somewhat weak relationships. It appears, then ,that OD 200 values are not reliable indicators of scald potential, since they were related most closely to ripening, a factor that was not associated closely with development of scald resistance.

The % inhibition of oxidation, presumably due to lipid-soluble antioxidants, like the OD 200 values, increased with hours below 10°C (Tables 4.8 and 4.9). At this point the reader again should be cautioned that assumptions have been made about the measured % inhibition of oxidation (see page 51). These values also were increased greatly by ripening

(ethephon treatment) (Table 4.36) and were not significantly affected by bagging although they tended to decrease with bagging (Table 4.46). The final sequential harvest of Cortlands in 1990 had starch indices similar to those of the 500 ppm ethephon-treated apples, and these two lots of fruit exhibited the same % inhibition of oxidation. These data indicate that ripening influenced % inhibition of oxidation, while cool temperatures had little or no influence on it. In the sequential-harvests experiments, % inhibitions were significantly correlated with scald development in 1990 (Table 4.11). Also, in the ethephon and bagging experiments, % inhibition of oxidation and scald were correlated negatively (Appendix Tables C.5, C.15, and C.19), but the correlation coefficients were much lower than those in the sequential-harvests experiments. Therefore, some environmental factors did influence % inhibition of oxidation; however, relationships of this inhibition to scald development were not consistent. Thus, it appears that there is a linkage between scald and preharvest environmental factors, via effects of the environment on accumulation of lipid-soluble antioxidants, but this linkage accounts only partially for the relationship between preharvest environment and scald development.

The TWRC increased during the season as hours below 10°C accumulated (Table 4.9). The increase between the first and last harvest was almost 20% in Cortlands and somewhat less than 20% in Delicious. The TWRC correlated

positively with hours below 10°C, and correlated negatively with scald development (Tables 4.10 and 4.11). In the ethephon experiment the TWRC also increased approximately 20% with ethephon treatment (Table 4.36) and correlated with scald intensity (score), but not with scald incidence (percent) (Appendix Table C.15). Therefore, it appears that TWRC increased substantially with ripening and was influenced only weakly by cool temperatures. Bagging decreased TWRC approximately 20% (Table 4.46). There also was a significant negative correlation ($p=0.05$) between TWRC and scald in the bagging experiment (Appendix Table C.19). These data suggest that TWRC were not primary contributors to endogenous scald resistance, but that they may have been secondary contributors.

In summary, lipid-soluble antioxidants (as indicated by inhibition of oxidation) appeared to be strongly associated with ripening but more weakly associated with cool temperatures and light. The water-soluble antioxidants appeared to be strongly associated with ripening and light and weakly associated with cool temperatures. When relationships are compared, lipid-soluble antioxidants appeared to be greater contributors to scald resistance than were water-soluble antioxidants.

Relationships of Specific Compounds to Scald Development

Anet (1974) found evidence for eleven different lipid-soluble antioxidants in apple cuticle (and probably some underlying cells). Of the eleven antioxidants, only alpha-, gamma-, and delta- tocopherol were identified. Which of the antioxidants that were present varied with cultivar, maturity, and origin of the sample. Most of the antioxidants had a tendency to increase during the initial 2 to 3 weeks of storage while at least one, when present, increased for 20 weeks of storage. Anet's (1974) data indicated very low concentrations of the tocopherols.

∝ Tocopherol is a presumed contributor to inhibition of ∝ farnesene oxidation (Anet, 1974; Meir and Bramlage, 1988; Gallerani et al., 1990). The ∝ tocopherol concentrations in apple peel increased significantly with sequential harvests of both Cortland and Delicious (Table 4.13). The concentrations of ∝ tocopherol measured in the peel were similar to those reported in the literature (as cited by Gallerani et al., 1990). The correlations and regressions between ∝ tocopherol and hours below 10°C were significant, but generally had coefficients of only about 0.50 (Table 4.14). Starch indices (ripening) also were correlated significantly with ∝ tocopherol (coefficient = 0.74 in Cortland) (Appendix Tables C.9 and C.13), and most of the increase in ∝ tocopherol came at the final harvest when fruit were ripening (Table 4.13). The fact that the

increase occurred only between the third and final harvest, when ripening was occurring, would explain the lower correlation coefficients between hours below 10°C and ∝ tocopherol than between starch and ∝ tocopherol (Appendix Tables C.9 and C.13) . Kunert and Ederer (1985) found an increase in ∝ tocopherol concentrations during aging of both beech leaves and fir needles. In 1990, ethephon treatments increased ∝ tocopherol (Table 4.37), and the apples treated with ethephon and harvested September 6 had slightly more ∝ tocopherol than did Cortlands harvested October 11 in the sequential-harvests experiment. These data suggest that ripening was the primary factor influencing changes in the ∝ tocopherol concentrations. The results of the bagging experiment (Table 4.47) indicated that light was involved in accumulation of ∝ tocopherol, since bagging decreased its concentrations significantly. Scald was correlated with ∝ tocopherol only in the bagging experiment (0.1% level) (Appendix Table C.20) and in the sequential harvests of Delicious in 1990 (5% level) (Appendix Table C.13). It appeared that ∝ tocopherol alone did not play a large role in the development of scald resistance at low temperatures, as has been proposed (Anet, 1974; Meir and Bramlage, 1988).

Ascorbic acid and glutathione are components of the TWRC. The concentrations of ascorbic acid did not change during the sequential harvests of either Cortland or Delicious in 1990 (Table 4.13). Kunert and Ederer (1985) found some increase in ascorbic acid in beech leaves and fir

needles but much less change than that of ∝ tocopherol. Ethephon treatments in 1990 (ripening in the absence of cool temperature), however, increased ascorbic acid concentrations to levels higher than those in Cortlands harvested October 11 (ripening in the presence of cool temperature) (Table 4.37). Bagging fruit decreased ascorbic acid concentrations to levels slightly below those of similar harvest dates in the sequential harvests (Table 4.47). These data indicated that ripening can increase ascorbic acid concentrations in apple peel, and light can enhance this increase; however, it appeared that cool temperatures did not independently influence ascorbic acid concentrations to a significant extent. Not suprisingly, then, scald was not related to ascorbic acid concentrations in either Cortland or Delicious apple peel in the sequential-harvests experiment (Table 4.14). In both the ethephon (Appendix Table C.15) and bagging experiments (Appendix Table C.19), however, there were significant negative correlations between scald and ascorbic acid concentrations. Albrigo (1968) found that the sun-exposed red side of the apples had higher levels of ascorbic acid. Scald is known to occur more frequently on the green side of the fruit. Ascorbic acid may play a role in scald resistance, but it does not appear to be an important factor in the development of scald resistance at low temperatures.

Glutathione concentrations were not influenced consistently by cool temperatures (Table 4.13). Neither

ethephon treatment nor bagging fruit affected concentrations of glutathione (Tables 4.37 and 4.47). There were no relationships between glutathione and hours below 10°C, scald, or any of the other parameters that were measured (Appendix Tables C.9, C.13, C.17, and C.20). Therefore, it appears that glutathione does not play a direct role in scald resistance. Total flavonols also were determined in the peel tissue. Flavonols have been reported to protect against ascorbic acid degradation (Larson, 1988). Flavonols correlated with TWRC and ascorbic acid in all cases except in the sequential harvests of Delicious (Appendix Tables C.10, C.14, C.18, and C.21). Flavonols were increased in Cortland by cool temperature and decreased by bagging (Tables 4.15 and 4.48); however, they were not influenced by ethephon in Cortland (Table 4.38), and did not change significantly in Delicious (Table 4.15). Apparently, flavonols are not involved substantially in development of scald resistance.

Another lipid-soluble component of the extract was the carotenes, which are reported to have antioxidant activity (Larson, 1988). The carotenes increased with hours below 10°C (Table 4.15) and with ethephon treatment (Table 4.41). The increase in both cases was about the same. Bagging decreased the carotenes by 20 and 40% at the first and second harvest, respectively (Table 4.48). Raymundo et al. (1976) suggested that carotenogenesis may be stimulated by light but does not require light for induction. This would

account for the lower concentrations of carotenes in the bagged fruit. The data indicated that carotenes were influenced by ripening and light but not by cool temperatures. In the sequential harvests and ethephon experiments, carotenes were not correlated with scald (Appendix Tables C.7, C.11, and C.15); however, in the bagging experiment carotenes were negatively correlated with scald (Appendix Table C.19). Both the carotenes (Table 4.15) and the % inhibition of oxidation (Table 4.9) increased with sequential harvests; however, the % inhibition of oxidation increased by approximately 54% over the season while the carotenes increased by only 33%. The carotenes are only small contributors to the inhibition of oxidation, and apparently are not a major factor in development of scald resistance.

Total carotenoids, anthocyanin, and chlorophyll also were measured in the peel tissue. Total carotenoids decreased with increasing hours below 10°C (Table 4.15). Ethephon also significantly decreased the total carotenoids (Table 4.41). The decrease with hours below 10°C was similar to that due to ripening (ethephon). Bagging of fruit did not affect the carotenoid concentrations significantly, but the concentrations tended to decline with bagging (Table 4.48). It appears that total carotenoids are influenced strongly by ripening, weakly by light, and little at all by temperature. In the sequential harvests (Table 4.16) scald was positively correlated with total carotenoids

and this was true for the ethephon (Appendix Table C.15) experiment as well. In the bagging experiment, scald was negatively correlated with carotenoid concentrations. The data suggest that carotenoids are not involved in the development of scald resistance.

Light, temperature, and ethephon are factors known to influence anthocyanin accumulation (Gross, 1987). Anthocyanin concentrations increased with sequential harvests (Table 4.15) as well as with ethephon treatment (Table 4.41), but bagging decreased anthocyanin concentrations greatly (Table 4.48). Scald development correlated with anthocyanin in all experiments (Appendix Tables C.10, C.14, C.18, and C.21). The procedure for TWRC includes an acidic extraction and the extract was red, so anthocyanins were present in this extract. It is unlikely, however, that anthocyanins influenced scald to any great degree. This was evident in the 1989 bagging experiment when both standard Cortland and Redcort, a red strain of Cortland, were used. Although Redcorts had approximately three times as much anthocyanin as the standard Cortlands (27.6 vs. 8.5 μg/gdw for Redcort and standard Cortland, respectively), scald incidence (78% vs. 77%, Redcort and standard Cortland, respectively) and scald score (2.4 vs. 2.3, Redcort and standard Cortland, respectively) were the same for both strains.

Chlorophyll decreased with sequential harvests (Table 4.15) and ethephon treatment (Table 4.41); however, bagging

did not affect chlorophyll concentrations (Table 4.48). Since chlorophyll loss is commonly used as an indicator of senescence, these changes were expected because senescence occurs during ripening. Scald was correlated significantly with chlorophyll except in the bagging experiment (Appendix Tables C.10, C.14, C.18, and C.21), with both chlorophyll and scald decreasing with later harvests and ripening. Despite these correlations, however, it should not be concluded that senescence imparts scald resistance, since scald does not occur until substantial senescence develops in the fruit during and following prolonged periods of storage. Kunert and Ederer (1985) reported that as beech leaves and fir needles aged and senescence occurred, the ratio of Vitamin C to Vitamin E decreased. This decrease was correlated with an increase in the formation of TBA reactants. Therefore, one might expect the same to be true in the peel of senescing apples if this is indeed a reflection of senescence-related changes. Indeed, both in later harvested apples of the sequential-harvests experiments and in the ethephon-treated apples, the ratios of Vitamin C: Vitamin E decreased. The ratios dropped from 4:1 to 3:1, 9:1 to 7:1, and 5:1 to 3:1 for sequentially harvested Cortland and Delicious, and ethephon-treated Cortland, respectively. In addition bagging, which did not influence ripening, did not affect the ratio (4:1 at the first harvest and 3:1 at the second, for both control and bagged fruit). During storage the ratio declined in all

cases. Therefore, the observed changes in antioxidant concentrations and ratios during storage may be representative of the progress of senescence.

In summary, these data suggest that ripening substantially influenced concentrations of the specific antioxidants that were measured. Hours below 10°C also had some effects on concentrations of antioxidants, but it appears that the effects were relatively small. From the results of the bagging and ethephon experiments, it appears that effects of light and ripening on scald resistance may have been related to their effects on ∝ tocopherol, ascorbic acid, and the carotenes. Low preharvest temperature, however, which imparted the greatest amount of resistance to scald development, did not substantially affect any of the specific antioxidant compounds that were measured.

Changes Occurring During Storage

Scald development requires continuous storage at low temperatures for 12 to 25 weeks. During storage, ∝ farnesene first increases and then decreases after about 6 to 10 weeks, while conjugated trienes progressively increase over time. If oxidation of ∝ farnesene to conjugated trienes is regulated by antioxidant concentrations, differences in conjugated triene accumulation could be associated with either initial antioxidant concentrations at

harvest, or to changes in them that occur during low temperature storage.

The % inhibition of oxidation (which was increased by cool preharvest temperatures) decreased slightly during storage at 0°C in Cortland but increased in Delicious (Tables 4.26 and 4.27). The at-harvest differences among samples, however, were maintained through 20 weeks of storage in both Cortland and Delicious apples. The at-harvest differences in % inhibition of oxidation in apples in the ethephon experiments also were maintained during storage, as these values remained relatively constant during storage (Table 4.39).

The TWRC declined during storage at 0°C, and the at-harvest differences disappeared (Tables 4.26 and 4.27). The at-harvest TWRC and % inhibition of oxidation were correlated with scald development (Table 4.11). Since at-harvest differences in TWRC disappeared during storage, while different amounts of conjugated trienes accumulated and different amounts of scald occurred (Tables 4.23, 4.24, 4.26, and 4.27), it follows that % inhibition of oxidation, which potentially is due to lipid-soluble antioxidants, would be more associated with scald resistance than are TWRC. It is puzzling, however, to observe progressive increases in % inhibition of oxidation in Delicious accompanying progressive increases in conjugated triene concentrations and occurrences of scald (Tables 4.27, and 4.22).

During storage the concentrations of ∝ tocopherol tended to increase (Tables 4.28, 4.29, and 4.40). Spychalla and Desborough (1990) reported an even larger increase in ∝ tocopherol concentration in potato tubers during storage at both 3°C and 9°C. Hariyadi and Parkins (1991) reported that the concentration of ∝ tocopherol remained constant during storage of cucumbers unless subjected to temperatures causing chilling injury, in which case the ∝ tocopherol concentrations declined. The carotenes increased during storage and the at-harvest differences were maintained (Tables 4.30 and 4.41). Total carotenoids also increased during storage (Tables 4.30 and 4.41).

Ascorbic acid concentrations tended to decline during storage, (Tables 4.29, 4.30, and 4.40) as was observed for the TWRC (Tables 4.26, 4.27, and 4.39). The magnitudes of change were remarkably similar for ascorbate and TWRC, and changes in ascorbate and TWRC among fruit from different harvests followed very similar patterns: levels in fruit from the first and third harvests of Cortland changed little (2 to 4%), while those in fruit from the second and fourth harvests decreased by 13 to 22%. Glutathione concentrations decreased as much as 60 to 73% during storage in Cortland and Delicious, respectively. Hariyadi and Parkins (1991) found that chilled cucumbers lost ascorbic acid during storage while in unchilled fruit the ascorbic acid content remained about constant. Lieberman et al. (1959) found loss of ascorbic acid in sweet potatoes stored at chilling

temperatures. Hariyadi and Parkins (1991) reported a 67% decrease in glutathione within 7 days of chilling in cucumber peel. The unchilled fruit also lost 43% of their glutathione but the loss was over a 21 day period. Apple scald has been assumed to be a chilling injury (Bramlage and Meir, 1990), so the declines in ascorbic acid and glutathione observed here are consistent with that assumption.

Conformity of Results to the Scald Development Model

While some of the data collected and reported here lend support to the widely accepted model for scald development, other data apparently conflict with the model.

Strongly supporting the model are the high correlations between scald and maximum conjugated triene concentrations measured during storage in the sequential harvests experiments (Table 4.25), which often had coefficients greater than 0.80. There were also significant negative correlations between antioxidant estimates and maximum conjugated triene concentrations (Appendix Tables C.8 and C.12) and some negative correlations between antioxidants and scald development (Appendix Tables C.9 and C.13). Correlations such as these are the types of evidence on which the model initially was built. Probably the strongest evidence in this study to support the involvement of antioxidants in scald resistance was that obtained in the

bagging experiments. Decreasing light intensity by bagging the fruit substantially decreased antioxidants (Tables 4.46 and 4.47), increased the maximum conjugated triene concentrations during storage (Table 4.45), and increased scald development following storage.

In contrast, much of the other data raise serious questions as to the accuracy of the model. Scald develops after 2 to 5 months of continuous storage at 0°C, never has been reported to occur without cold storage, and has been associated with the accumulation of ∝ farnesene and its autoxidation to conjugated trienes during cold storage. However, both ∝ farnesene and conjugated trienes can accumulate in the peel tissue while the apples are still on the tree (Tables 4.20, 4.21, 4.34, and 4.35). Obviously, accumulation of these compounds does not require cold temperatures. In addition, those apples which had large quantities of ∝ farnesene and conjugated trienes at harvest (Tables 4.20, 4.21, 4.34, and 4.35) also had higher concentrations of antioxidants (Tables 4.9, 4.13, 4.36, and 4.37) than any of the other apples in these experiments. In the 1990 ethephon experiment, there was no significant correlation between scald and maximum conjugated triene concentrations(Appendix Table C.15), indicating that this relationship does not always hold true. There also were data sets where antioxidants were not correlated with maximum conjugated triene concentrations. For example, in 1990, ∝ tocopherol was doubled and ascorbic acid increased

by 20% by ethephon treatment (Table 4.37), but there was only a slight decrease in maximum conjugated triene concentrations measured during storage (Table 4.42).

Antioxidants often were not related closely to scald. In 1990, ethephon treatment substantially increased antioxidants, even doubling ∝ tocopherol by the second harvest, yet had essentially no effect on scald development (Table 4.36). On the other hand, accumulated hours below 10°C had a large impact on scald with minimal effect on antioxidants. For example, in the 1990 sequentially harvested Cortlands, ∝ tocopherol did not increase until the final harvest when apples had entered their ethylene climacteric rise, but scald decreased substantially on fruit harvested before this increase occurred. Ripening, which had minimal effect on scald development, apparently had a stronger influence on antioxidants than hours below 10°C, which had the greatest effect on scald susceptibility. Some changes occurring in storage, such as the increase in ∝ tocopherol as conjugated trienes accumulated, are hard to reconcile with a scheme in which antioxidants protect against autoxidation of ∝ farnesene.

Conclusions

The purpose of this research was to examine the effects of preharvest temperature (as hours below 10°C), ripening, and light on the development of scald resistance.

Specifically studied was the hypothesis that temperatures below 10°C before harvest facilitate the accumulation of antioxidants in the apple peel, and that ripening and light enhance this effect by contributing to antioxidant accumulation. These three environmental factors were successfully separated using sequential harvests, ethephon application, and bagging of fruit. Scald development was greatly decreased with increased numbers of preharvest hours below 10°C. Preharvest ripening (due to ethephon application) only slightly suppressed scald incidence, and scald development was increased by low light intensity (bagging).

The data showed that antioxidants were influenced significantly by both ripening and light. Ethephon greatly increased antioxidants and bagging decreased them. Therefore, the influences that these factors have on scald development may involve their influence on antioxidants. However, hours below 10°C, which imparted the greatest resistance to scald, had much less effect on concentrations of those antioxidants measured. Alpha-tocopherol, ascorbic acid, and carotenes all appeared to be associated with the effects of light and ripening on development of scald resistance, but none of these antioxidants was strongly associated with the effects of cool temperatures. Glutathione did not appear to be a significant factor in scald resistance.

There were some data collected in this study which fit well into the widely accepted model in which conjugated trienes contribute to scald development, and antioxidants protect against conjugated triene formation. However, there was at least as much evidence, if not more, reported here that raised questions about the model. From the results it is concluded that while antioxidants play a role in the development of scald resistance, they probably are not the key factor in endogenous protection against scald.

CHAPTER 6

SUMMARY

The results of these experiments are summarized briefly as follows:

1. Experiments successfully separated the independent effects of preharvest cool temperature, ripening, and light intensity on the development of scald resistance in the peel of Cortland and Delicious apples. The experiments demonstrated that increasing exposure to low temperature (as hours below 10°C) was the primary factor in development of this resistance. Resistance developed more slowly when low light intensity existed during the cool periods. Ripening alone had only small effects on development of this resistance.

2. Some of the results generally supported the commonly held model of scald development. ∝ Farnesene accumulated early in storage, and then declined. Conjugated trienes generally accumulated throughout the storage period. In most experiments, occurrence of scald among samples was highly correlated with maximum conjugated triene concentrations in the peel tissue, and in some experiments it was also correlated with maximum ∝ farnesene concentrations.

3. Endogenous antioxidant concentrations appeared to be involved in scald resistance. Lipid-soluble antioxidants were correlated more strongly with development of scald resistance than were water-soluble antioxidants.

4. Increasing exposures to cool preharvest temperatures were accompanied by small increases in concentrations of ∝ tocopherol and carotenes. Cool temperature had considerably less effect on water-soluble reducing activity. Although concentrations of ascorbic acid increased somewhat at these temperatures, glutathione was not consistently affected. Total flavonol and anthocyanins increased, while total carotenoids and chlorophyll decreased with increasing amounts of cool temperature.

5. Ripening increased both percent inhibition of oxidation (assumed to be due to lipid-soluble antioxidants) and total water-soluble reducing activity of the fruit peel. Concentrations of ∝ tocopherol, carotenes, ascorbic acid, and anthocyanins increased with ripening, while chlorophyll concentrations declined and glutathione concentrations were not affected.

6. Reducing light intensity by bagging fruit decreased their concentrations of ∝ tocopherol, carotenes, total

water-soluble reducing activity, ascorbic acid, anthocyanins, and total flavonols. However, percent inhibition of oxidation was not affected significantly, and the concentrations of total carotenoids, glutathione and chlorophyll were not altered significantly.

7. During storage at 0°C, as conjugated trienes accumulated and scald was initiated, percent inhibition of oxidation increased in some samples and declined in others, and total water-soluble reducing activities decreased. Correspondingly, concentrations of ∝ tocopherol, carotenes, and total carotenoids increased, while concentrations of ascorbic acid and glutathione decreased. Anthocyanins and chlorophyll also decreased.

8. From the data reported here, it was apparent that cool temperature greatly decreased scald development. Low light intensity increased scald, and preharvest ripening slightly reduced scald. Antioxidants appeared to be strongly related to the effects of light and ripening, but only weakly to those of cool temperature. From the results it is concluded that while antioxidants play a role in the development of scald resistance, they probably are not the key factor in the development of endogenous protection against scald.

APPENDIX A

SAMPLE CALCULATIONS

Appendix A. Sample Calculation of ∝ farnesene and conjugated triene concentrations.

I. Information needed and formulas used in calculations.

A. Weight of 10 apples (or number of apples extracted if other than 10).

B. Volume = Weight X Factor*

*Factor (Factor = Weight/Volume)

McIntosh = 1.259	Red Rome = 1.254
Jonathan = 1.245	Red York = 1.213
Empire = 1.201	Golden Delicious = 1.266
Spartan = 1.257	Red Delicious = 1.144
Cortland = 1.280	Spencer = 1.254

C. Volume of 1 apple = Volume/10

D. $(4/3)\pi r^3$ = Volume

From this, calculate r, using the volume of 1 fruit.

E. Surface area = $4\pi r^2$

This gives the surface area of 1 fruit. Multiply by the number of fruit extracted. Multiply by 1.02 to account for the variation from a perfect sphere.

F. OD/ϵ = molar concentration

Multiply the OD by dilution factor (dil. of the extract)
ϵ_{232} = 27,700
$\epsilon_{281\text{-}290}$ = 25,000

G. Molar conc. X 0.1 liter X 10^9 nmoles/mole = nmoles

Divide by surface area calculated in step E.

II. Sample calculation

OD_{232} = 0.604

$OD_{281\text{-}290}$ = 0.136

Wt. of 10 apples = 1498 g

Sample diluted 1:50

Volume = 1498g X 1.280 = 1917.44

Volume of 1 apple = 191.744

$(4/3)\pi r^3 = 191.744$

$r = 3.58$

Surface area $= 4\pi r_2$
$= 4\pi(3.58)^2$
$= 161.056$

Surface area of 10 apples = 1610.56

1610.56 X 1.02 = 1642.8 cm^2

$(OD_{232}$ X dil.$)/\epsilon_{232} = (0.604$ X 50$)/27,700$

$= 1.09 \text{ X } 10^{-3}$ M

$(1.09 \text{ X } 10^{-3}$ M$)$(0.1 liter$)(10^9$ nmoles/mole$)$

$= 1.09\text{X}10^5$ nmoles

$1.09\text{X}10^5$ nmoles/1642.8 cm^2

$= 66.35$ nmoles/cm^2 $\propto$ farnesene

$(OD_{281\text{-}290}$ X dil.$)/\epsilon_{281\text{-}290} = (0.136$ X 50$)/25,000$

$= 2.72 \text{ X } 10^{-4}$ M

$(2.72 \text{ X } 10^{-4}$ M$)$(0.1 liter$)(10^9$ nmoles/mole$)$

$= 2.72 \text{ X } 10^4$ nmoles

$2.72\text{X}10^4$ nmoles/1642.8 cm^2

= 16.56 nmoles/cm^2 conjugated trienes

APPENDIX B

ANOVA TABLES

Table B.1. ANOVA table for storage OD 200, ∝ farnesene, and conjugated triene values for hexane extracts of fruit surfaces of Cortland and Delicious apples after storage, 1990.

	OD 200			∝ Farnesene			Conjugated trienes		
Source	1988	1989	1990	1988	1989	1990	1988	1989	1990
Cortland									
REP	ns	ns	ns	ns	ns	ns	ns	*	ns
HARVEST	***	***	***	***	**	***	***	***	***
STORAGE	***	***	***	***	***	***	***	***	***
H X S	***	***	***	***	***	***	***	***	***
Delicious									
REP	**	-	*	ns	-	ns	ns	-	ns
HARVEST	ns	-	***	**	-	***	ns	-	***
STORAGE	***	-	***	***	-	***	*	-	***
H X S	***	-	***	*	-	***	ns	-	***

***,**,*,ns: Significant at 0.1%, 1%, or 5% level or not significant, respectively.

Table B.2. ANOVA table for storage OD 200 values, ∝ farnesene, and conjugated triene concentrations in a hexane extract of surfaces of Cortland apples in the ethephon experiment, 1989.

Source	OD 200	∝ Farnesene	Conjugated trienes
REP	*	ns	*
TREATMENT	***	ns	ns
HARVEST	ns	**	ns
STORAGE	***	***	***
TRT X HARV	**	ns	ns
TRT X STOR	***	***	***
HARV X STOR	***	*	***

***,**,*,ns: Significant at 0.1%, 1%, or 5% level or not significant, respectively.

Table B.3. ANOVA table for OD 200, ∝ farnesene, and conjugated triene concentrations in a hexane extract of apple surfaces of Cortland apples in the ethephon experiment, 1990.

Source	OD 200	∝ Farnesene	Conjugated trienes
REP	*	*	ns
TREATMENT	***	**	*
STORAGE	***	**	***
TRT X STOR	***	***	***

***,**,*,ns: Significant at 0.1%, 1%, or 5% level or not significant, respectively.

Table B.4. ANOVA table for OD 200 values, ∝ farnesene, and conjugated triene concentrations in a hexane extract of surfaces of Cortland apples in the bagging experiment, 1989.

Source	OD 200	∝ Farnesene	Conjugated trienes
REP	ns	*	*
TREATMENT	ns	**	**
HARVEST	**	ns	**
STORAGE	***	***	***
TRT X HARV	ns	ns	ns
TRT X STOR	ns	ns	**
HARV X STOR	***	*	**

***,**,*,ns: Significant at 0.1%, 1%, or 5% level or not significant, respectively.

Table B.5. ANOVA table for antioxidants and oxidation inhibition during storage for Cortland and Delicious apples, 1990.

Source	Oxidation (% inhibition)	TWRC	∝ Tocopherol	Ascorbic acid	Glutathione
Cortland					
Rep	***	ns	**	*	ns
Hours	**	*	***	ns	*
Storage	*	***	***	*	***
H X S	ns	*	***	*	**
Delicious					
Rep	*	ns	ns	ns	ns
Hours	***	ns	ns	**	*
Storage	***	**	**	**	**
H X S	ns	*	*	**	*

***,**,*,ns: Significant at 0.1%, 1%, or 5% level or not significant, respectively.

Table B.6. ANOVA table for antioxidant concentrations and oxidation inhibition in the peel of Cortland apples in the ethephon experiment, 1990.

Source	Oxidation (% inhibition)	TWRC	∝ Tocopherol	Ascorbic acid	Glutathione
REP	ns	ns	*	ns	ns
TRT	***	ns	**	ns	***
STOR	ns	***	***	***	ns
TRT X STOR	ns	ns	**	ns	ns

***,**,*,ns: Significant at 0.1%, 1%, or 5% level or not significant, respectively.

Table B.7. ANOVA table for pigments during storage for Cortland and Delicious apples, 1990.

Source	Chlorophyll	Anthocyanin	Flavonols	Carotenoids	Carotenes
Cortland					
Rep	ns	*	*	ns	ns
Harvest	***	***	**	**	***
Storage	***	***	*	***	***
H X S	**	***	ns	**	***
Delicious					
Rep	ns	*	**	*	ns
Harvest	***	***	*	***	***
Storage	***	**	ns	***	***
H X S	***	**	ns	***	***

***,**,*,ns: Significant at 0.1%, 1%, or 5% level or not significant, respectively.

Table B.8. ANOVA table for pigment concentrations in Cortland apple peel at harvest and during storage in the ethephon experiment, 1990.

Source	Chlorophyll	Anthocyanin	Flavonols	Carotenoids	Carotenes
REP	*	*	ns	ns	**
TREATMENT	***	***	ns	ns	**
STORAGE	***	ns	ns	**	***
TRT X STOR	ns	ns	ns	ns	ns

***,**,*,ns: Significant at 0.1%, 1%, or 5% level or not significant, respectively.

APPENDIX C

CORRELATION TABLES

Table C.1. Simple correlations between constituents of Cortland apple peel, 1988.

	Hours	Scald	Score	Starch	C_2H_4	Firmness	OD 200	Max ∝ Max CT Farn
Hours	-							
Scald	-0.85^{***}	-						
Score	-0.20^{ns}	0.23^{ns}	-					
Starch	0.92^{***}	-0.83^{***}	-0.25^{ns}	-				
C_2H_4	0.84^{***}	-0.64^{***}	-0.32^{ns}	0.87^{***}	-			
Firm.	-0.73^{***}	0.78^{***}	0.36^{ns}	-0.83^{***}	-0.64^{***}	-		
OD 200	0.86^{***}	-0.90^{***}	-0.24^{ns}	0.78^{***}	0.65^{***}	-0.79^{***}	-	
Max ∝ F	-0.20^{ns}	0.23^{ns}	0.21^{ns}	-0.25^{ns}	-0.32^{ns}	0.36^{ns}	-0.24^{ns}	-
Max CT	-0.83^{***}	0.80^{***}	0.39^{ns}	-0.82^{***}	-0.75^{***}	0.63^{**}	-0.77^{***}	0.21^{ns} -

^{***,**,*,ns}:Significant at 0.1%, 1%, or 5% level or not significant, respectively.

Table C.2. Simple correlations between constituents of Delicious apple peel, 1988.

	Hours	Scald	Score	Starch	C_2H_4	Firmness	OD 200	Max ∝ Farn	Max CT
Hours	-								
Scald	-0.67^{**}	-							
Score	-0.14^{ns}	0.35^{ns}	-						
Starch	0.74^{***}	-0.59^{**}	-0.36^{ns}	-					
C_2H_4	0.37^{ns}	0.07^{ns}	0.00^{ns}	0.27^{ns}	-				
Firm.	-0.73^{***}	0.41^{ns}	0.44^{ns}	-0.54^{*}	-0.51^{*}	-			
OD 200	0.37^{ns}	-0.47^{*}	-0.40^{ns}	0.45^{ns}	0.09^{ns}	-0.18^{ns}	-		
Max ∝ F	-0.14^{ns}	0.35^{ns}	-0.12^{ns}	-0.36^{ns}	0.00^{ns}	0.44^{ns}	-0.40^{ns}	-	
Max CT	-0.11^{ns}	0.13^{ns}	-0.05^{ns}	-0.05^{ns}	-0.575^{*}	0.24^{ns}	0.16^{ns}	-0.12^{ns}	-

^{***,**,*,ns}: Significant at 0.1%, 1%, or 5% level or not significant, respectively.

Table C.3. Simple correlations between constituents of Cortland apple peel, 1989.

	Scald	Score	Starch	C_2H_4	Firm	OD 200	Max ∝F	Max CT	% Inhib.[z]
Scald	-								
Score	0.93***	-							
Starch	-0.92***	-0.93***	-						
C_2H_4	-0.75***	-0.76***	0.87***	-					
Firm	0.53*	0.64**	-0.68**	-0.60**	-				
OD 200	-0.84***	-0.83***	0.87***	0.84***	-0.60**	-			
Max ∝F	0.35ns	0.23ns	-0.19ns	-0.24ns	0.08ns	-0.05ns	-		
Max CT	0.89***	0.80***	-0.87***	-0.76***	0.53*	-0.76***	0.43ns	-	
% Inhib.[z]	-0.47ns	-0.39*	0.51*	0.62**	-0.42ns	0.58*	-0.05ns	-0.47*	-

***,**,*,ns: Significant at 0.1%, 1%, or 5% level or not significant, respectively.
[z] % Inhibition of linoleic acid oxidation.

Table C.4. Simple correlations between constituents of Delicious apple peel, 1989.

	Scald	Score	Starch	C_2H_4	Firm	OD 200	% Inhib.[z]
Scald	-						
Score	0.55^{ns}	-					
Starch	-0.44^{ns}	-0.35^{ns}	-				
C_2H_4	-0.34^{ns}	-0.13^{ns}	0.83^{***}	-			
Firm	-0.35^{ns}	0.04^{ns}	0.00^{ns}	0.29^{ns}	-		
OD 200	-0.64^{*}	-0.58^{*}	0.08^{ns}	0.60^{*}	0.07^{ns}	-	
% Inhib.[z]	-0.08^{ns}	-0.02^{ns}	0.33^{ns}	0.60^{*}	0.28^{ns}	0.04^{ns}	-

^{***,**,*,ns}: Significant at 0.1%, 1%, or 5% or not significant, respectively.

Table C.5. Simple correlations between constituents of Cortland apple peel in the ethephon experiment, 1989.

	Scald	Score	Starch	C_2H_4	Firm	Max∝F	Max CT	OD	% Inhib.[z]
Scald	-								
Score	0.53*	-							
Starch	-0.71***	-0.11ns	-						
C_2H_4	-0.67**	-0.18ns	0.99***	-					
Firm	0.47*	-0.04ns	-0.87***	-0.88***	-				
Max∝F	0.70***	0.21ns	-0.89***	-0.90***	0.77***	-			
Max CT	0.58**	0.29ns	-0.45*	-0.40ns	0.09ns	0.55*	-		
OD	-0.73***	-0.30ns	0.82***	0.81***	-0.78***	-0.85***	-0.28ns	-	
% Inhib.[z]	-0.59**	0.03ns	0.92***	0.92***	-0.77***	-0.87***	-0.49*	0.73***	-

***,**,*,ns: Significant at 0.1%, 1%, or 5% level, or not significant, respectively.

[z] % Inhibition of linoleic acid oxidation

Table C.6. Simple correlations between constituents of Cortland apple peel in the bagging experiment, 1989.

	Scald	Score	Starch	C_2H_4	Firm	Max∝F	Max CT	OD	% Inhib.[z]
Scald	-								
Score	0.83^{***}	-							
Starch	-0.73^{**}	-0.83^{***}	-						
C_2H_4	-0.46^{ns}	-0.66^{**}	0.74^{**}	-					
Firm	0.57^{*}	0.74^{**}	-0.82^{***}	-0.71^{**}	-				
Max∝F	0.63^{**}	0.41^{ns}	-0.24^{ns}	-0.05^{ns}	0.05^{ns}	-			
Max CT	0.60^{*}	0.65^{**}	-0.20^{ns}	-0.24^{ns}	0.14^{ns}	0.55^{*}	-		
OD	-0.59^{*}	-0.77^{***}	0.79^{***}	0.77^{***}	-0.75^{***}	-0.26^{ns}	-0.31^{ns}	-	
% Inhib.[z]	-0.23^{ns}	-0.35^{ns}	0.39^{ns}	0.31^{ns}	-0.05^{ns}	0.05^{ns}	-0.30^{ns}	0.28^{ns}	-

^{***,**,*,ns}: Significant at 0.1%, 1%, or 5% level, or not significant, respectively.

[z] % Inhibition of linoleic acid oxidation.

Table C.7. Simple correlations between constituents of Cortland apple peel in the sequential-harvests experiment, 1990-1.

	Hours	Scald	Score	Starch	C_2H_4
Hours	-				
Scald	-0.88^{***}	-			
Score	-0.72^{***}	0.70^{***}	-		
Starch	0.89^{***}	-0.76^{***}	-0.54^{**}	-	
C_2H_4	0.80^{***}	-0.66^{***}	-0.49^{*}	0.97^{***}	-
OD200	0.93^{***}	-0.84^{***}	-0.61^{**}	0.95^{***}	0.90^{***}
Max∝F	-0.82^{***}	0.73^{***}	0.41^{*}	-0.85^{***}	-0.78^{***}
Max CT	-0.96^{***}	0.86^{***}	-0.59^{**}	-0.84^{***}	-0.78^{***}
% Inhib[z]	0.92^{***}	-0.79^{***}	-0.71^{***}	0.84^{***}	0.78^{***}
TWRC	0.61^{**}	-0.58^{**}	-0.51^{*}	0.67^{***}	0.72^{***}
∝ Toc	0.52^{**}	-0.40^{ns}	-0.21^{ns}	0.74^{***}	0.80^{***}
Ascorbate	0.16^{ns}	-0.03^{ns}	-0.20^{ns}	0.27^{ns}	0.32^{ns}
Glut	0.14^{ns}	-0.31^{ns}	-0.26^{ns}	-0.15^{ns}	-0.21^{ns}
Chlor	-0.92^{***}	0.84^{***}	0.73^{***}	-0.84^{***}	-0.78^{***}
Antho	0.84^{***}	-0.69^{***}	-0.61^{**}	0.90^{***}	0.92^{***}
Flav	0.38^{ns}	-0.30^{ns}	-0.27^{ns}	0.54^{**}	0.63^{***}
Carot	-0.61^{**}	-0.49^{*}	0.33^{ns}	-0.79^{***}	-0.83^{***}
Carotene	0.53^{**}	-0.34^{ns}	-0.39^{ns}	0.65^{***}	0.67^{***}

^{***,**,*,ns}: Significant at 0.1%, 1%, or 5% or not significant, respectively.

[z] % Inhibition of linoleic acid oxidation

Table C.8. Simple correlations between constituents of Cortland apple peel in the sequential-harvests experiment, 1990-2.

	OD 200	Max. ∝ Farn.	Max. CT
Hours	0.93^{***}	-0.82^{***}	-0.96^{***}
Scald	-0.84^{***}	0.73^{***}	0.86^{***}
Score	-0.61^{**}	0.41^{ns}	0.59^{**}
Starch	0.95^{***}	-0.85^{***}	-0.84^{***}
C_2H_4	0.90^{***}	-0.78^{***}	-0.78^{***}
OD 200	-	-	-
Max ∝Farn	-0.90^{***}	-	-
Max CT	-0.89^{***}	0.79^{***}	-
% Inhib.[z]	0.90^{***}	-0.76^{***}	-0.86^{***}
TWRC	0.69^{***}	-0.53^{**}	-0.56^{**}
∝ Toc	0.68^{***}	-0.58^{**}	-0.52^{**}
Ascorbate	0.21^{ns}	-0.06^{ns}	-0.14^{ns}
Glutathione	0.07^{ns}	-0.06^{ns}	-0.21^{ns}
Chlorophyll	0.89^{***}	0.78^{***}	0.87^{***}
Anthocyanin	0.88^{***}	-0.71^{***}	-0.81^{***}
Flavonoids	0.50^{*}	-0.31^{ns}	-0.42^{*}
Carotenoids	-0.75^{***}	0.67^{***}	0.61^{**}
Carotenes	0.62^{**}	-0.53^{**}	-0.56^{**}

^{***,**,*,ns}: Significant at 0.1%, 1%, or 5% level or not significant, respectivey.

[z] % Inhibition of linoleic acid oxidation.

Table C.9. Simple correlations between constituents of Cortland apple peel in the sequential-harvests experiment, 1990-3.

	% Inhib[z]	TWRC	∝ Toc	Ascorbate	Glut.
Hours	0.92***	0.61**	0.52**	0.16ns	0.14ns
Scald	-0.80***	-0.58**	-0.40ns	-0.03ns	-0.32ns
Score	-0.71***	-0.51*	-0.21ns	-0.20ns	-0.26ns
Starch	0.84***	0.67***	0.74***	0.27ns	-0.15ns
C_2H_4	0.78***	0.72***	0.80***	0.32ns	-0.21ns
OD	0.90***	0.69***	0.68***	0.21ns	0.07ns
Max ∝F	-0.76***	-0.53**	-0.58**	-0.06ns	-0.06ns
Max CT	-0.86***	-0.56**	-0.52**	-0.14ns	-0.21ns
% Inhib[z]	-				
TWRC	0.55**	-			
∝ Toc	0.53**	0.59**	-		
Ascorbate	0.13ns	0.54**	0.39ns	-	
Glut	0.13ns	-0.20ns	-0.21ns	-0.39ns	-
Chlor	-0.83***	-0.64***	-0.55**	-0.21ns	-0.07ns
Antho	0.78***	0.83***	0.77***	0.50*	-0.10ns
Flav	0.33ns	0.67***	0.79***	0.62**	-0.07ns
Carot	-0.58**	-0.72***	-0.72***	-0.50*	0.35ns
Carotene	0.53**	0.63**	0.60**	0.36ns	-0.29ns

***,**,*,ns: Significant at 0.1%, 1%, or 5% level or not significant, respectively.

[z] % Inhibition of linoleic acid oxidation.

Table C.10. Simple correlations between constituents of Cortland apple peel at harvest in the sequential-harvests experiment, 1990-4.

	Chlor	Antho	Flav	Carotenoid	Carotene
Hours	-0.92^{***}	0.84^{***}	0.38^{ns}	-0.61^{**}	0.53^{**}
Scald	0.84^{***}	-0.69^{***}	-0.30^{ns}	0.49^{*}	-0.34^{ns}
Score	0.73^{***}	-0.61^{**}	-0.27^{ns}	0.33^{ns}	-0.39^{ns}
Starch	-0.84^{***}	0.90^{***}	0.54^{**}	-0.79^{***}	0.65^{***}
C_2H_4	-0.78^{***}	0.92^{***}	0.63^{***}	-0.83^{***}	0.67^{***}
OD200	-0.89^{***}	0.88^{***}	0.50^{*}	-0.75^{***}	0.62^{**}
Max ∝F	0.78^{***}	-0.71^{***}	-0.31^{ns}	0.67^{***}	-0.58^{**}
Max CT	0.87^{***}	-0.81^{***}	-0.42^{*}	0.61^{**}	-0.44^{*}
% Inhib[z]	-0.83^{***}	0.78^{***}	0.33^{ns}	-0.58^{**}	0.53^{**}
TWRC	-0.64^{***}	0.83^{***}	0.67^{***}	-0.72^{***}	0.63^{**}
∝ Toc	-0.55^{**}	0.77^{***}	0.79^{***}	-0.72^{***}	0.60^{**}
Ascorbate	-0.21^{ns}	0.49^{*}	0.62^{**}	-0.50^{*}	0.36^{ns}
Glut	-0.07^{ns}	-0.10^{ns}	-0.07^{ns}	0.35^{ns}	-0.29^{ns}
Chlor	-	-	-	-	-
Antho	-0.81^{***}	-	-	-	-
Flav	-0.44^{*}	0.76^{***}	-	-	-
Carotenoids	0.62^{**}	-0.79^{***}	-0.53^{*}	-	-
Carotenes	-0.58^{**}	0.67^{***}	0.38^{ns}	-0.69^{***}	-

^{***,**,*,ns}: Significant at 0.1%, 1%, or 5% level or not significant, respectively.

[z] % Inhibition of linoleic oxidation.

Table C.11. Simple correlations between constituents of Delicious apple peel at harvest in the sequential-harvests experiment, 1990-1.

	Hours	Scald	Score	Starch	C_2H_4
Hours	-				
Scald	-0.78^{***}	-			
Score	-0.72^{***}	0.56^{**}	-		
Starch	0.87^{***}	-0.77^{***}	-0.74^{***}	-	
C_2H_4	0.84^{***}	-0.64^{***}	-0.71^{***}	0.92^{***}	-
OD	0.93^{***}	-0.83^{***}	-0.79^{***}	0.94^{***}	0.88^{***}
Max ∝F	-0.81^{***}	0.80^{***}	0.74^{***}	-0.90^{***}	-0.78^{***}
Max CT	-0.93^{***}	0.75^{***}	0.72^{***}	-0.78^{***}	-0.75^{***}
% Inhib[z]	0.85^{***}	-0.70^{***}	-0.68^{***}	0.77^{***}	0.79^{***}
TWRC	0.69^{***}	-0.51^{*}	-0.65^{***}	0.72^{***}	0.73^{***}
∝ Toc	0.46^{*}	-0.50^{*}	-0.56^{**}	0.47^{*}	0.40^{ns}
Ascorbate	-0.27^{ns}	0.08^{ns}	0.10^{ns}	-0.12^{ns}	-0.15^{ns}
Glut	0.05^{ns}	0.09^{ns}	-0.05^{ns}	-0.05^{ns}	-0.04^{ns}
Chlor	-0.76^{***}	0.62^{**}	0.61^{**}	-0.71^{***}	-0.73^{***}
Antho	0.84^{***}	-0.71^{***}	-0.88^{***}	0.89^{***}	0.88^{***}
Flav	-0.23^{ns}	-0.07^{ns}	-0.15^{ns}	-0.12^{ns}	-0.08^{ns}
Carot(tot)	-0.59^{**}	0.50^{*}	0.43^{*}	-0.61^{**}	-0.55^{**}
Carotene	0.17^{ns}	-0.26^{ns}	-0.21^{ns}	0.28^{ns}	0.31^{ns}

^{***,**,*,ns}: Significant at 0.1%, 1%, or 5% level or not significant, respectively.

[z] % Inhibition of linoleic acid oxidation.

Table C.12. Simple correlations between constituents of Delicious apple peel in the sequential-harvests experiment, 1990-2.

	OD 200	Max ∝ Farn.	Max CT
Hours	0.93^{***}	-0.81^{***}	-0.93^{***}
Scald	-0.83^{***}	0.80^{***}	0.75^{***}
Score	-0.79^{***}	0.74^{***}	0.72^{***}
Starch	0.94^{***}	-0.90^{***}	-0.78^{***}
C_2H_4	0.88^{***}	-0.78^{***}	-0.75^{***}
OD	-		
Max ∝F	-0.87^{***}	-	
Max CT	-0.87^{***}	0.82^{***}	-
% Inhib.[z]	0.81^{***}	-0.70^{***}	-0.76^{***}
TWRC	0.70^{***}	-0.63^{***}	-0.63^{**}
∝ Toc	0.51^{*}	-0.50^{*}	-0.52^{**}
Ascorbate	-0.11^{ns}	0.19^{ns}	0.30^{ns}
Glutathione	-0.2^{ns}	-0.07^{ns}	-0.04^{ns}
Chlorophyll	-0.77^{***}	0.59^{**}	0.63^{***}
Anthocyanin	0.91^{***}	-0.81^{***}	-0.76^{***}
Flavonoids	-0.07^{ns}	0.06^{ns}	0.12^{ns}
Carotenoids	-0.59^{**}	0.54^{**}	0.44^{*}
Carotenes	0.28^{ns}	-0.34^{ns}	-0.15^{ns}

^{***,**,*,ns}: Significant at 0.1%, 1%, or 5% level or not significant, respectively.
[z] % Inhibition of linoleic acid oxidation.

Table C.13. Simple correlations between constituents of Delicious apple peel in the sequential-harvests experiment, 1990-3.

	% Inhib[z]	TWRC	∝ Toc	Ascorbate	Glut
Hours	0.85***	0.69***	0.46*	-0.27ns	0.05ns
Scald	-0.70***	-0.51*	-0.50*	0.08ns	0.09ns
Score	-0.68***	-0.65***	-0.56**	0.10ns	-0.05ns
Starch	0.77***	0.72***	0.47*	-0.12ns	-0.05ns
C_2H_4	0.79***	0.73***	0.40ns	-0.15ns	-0.04ns
OD	0.81***	0.70***	0.51*	-0.11ns	0.02ns
Max ∝F	-0.70***	-0.63***	-0.50*	0.19ns	-0.07ns
Max CT	-0.76***	-0.63**	-0.52**	0.30ns	-0.04ns
% Inhib[z]	-				
TWRC	0.62**	-			
∝ Toc	0.53**	0.47*	-		
Ascorbate	-0.13ns	0.26ns	0.22ns	-	
Glut	-0.03ns	0.06ns	-0.05ns	-0.23ns	-
Chlor	-0.66***	-0.43*	-0.21ns	0.34ns	0.24ns
Antho	0.85***	0.73***	0.53**	-0.12ns	-0.05ns
Flav	0.08ns	0.07ns	0.30ns	0.35ns	-0.19ns
Carot(tot)	-0.53**	-0.19ns	-0.05ns	0.56**	-0.09ns
Carotenes	0.28ns	0.25ns	-0.02ns	-0.12ns	0.15ns

***,**,*,ns: Significant at 0.1%, 1% or 5% level or not significant, respectively.
[z] % Inhibition of linoleic acid oxidation.

Table C.14. Simple correlations between constituents of Delicious apple peel at harvest in the sequential-harvests experiment, 1990-4.

	Chlor	Antho	Flav	Carotenoid	Carotene
Hours	-0.76^{***}	0.84^{***}	0.23^{ns}	-0.59^{**}	0.17^{ns}
Scald	0.62^{**}	-0.71^{***}	-0.07^{ns}	0.50^{*}	-0.26^{ns}
Score	0.61^{**}	-0.88^{***}	-0.15^{ns}	0.43^{*}	-0.21^{ns}
Starch	-0.71^{***}	0.89^{***}	-0.12^{ns}	-0.61^{**}	-0.28^{ns}
C_2H_4	-0.73^{***}	0.88^{***}	-0.08^{ns}	-0.55^{**}	0.31^{ns}
OD200	-0.77^{***}	0.91^{***}	-0.07^{ns}	-0.59^{**}	0.28^{ns}
Max ∝F	0.59^{**}	-0.81^{***}	0.06^{ns}	0.54^{**}	-0.35^{ns}
Max CT	0.63^{***}	-0.76^{***}	0.12^{ns}	0.44^{*}	-0.15^{ns}
% Inhib[z]	-0.66^{***}	0.85^{***}	0.08^{ns}	-0.53^{**}	0.28^{ns}
TWRC	-0.43^{*}	0.73^{***}	0.07^{ns}	-0.19^{ns}	0.25^{ns}
∝ Toc	-0.21^{ns}	0.53^{**}	0.27^{ns}	-0.05^{ns}	-0.02^{ns}
Ascorbate	0.34^{ns}	-0.12^{ns}	0.35^{ns}	0.56^{**}	-0.11^{ns}
Glut	0.24^{ns}	-0.05^{ns}	-0.19^{ns}	-0.09^{ns}	0.15^{ns}
Chlor	-	-	-	-	-
Antho	-0.78^{***}	-	-	-	-
Flav	0.06^{ns}	0.19^{ns}	-	-	-
Carotenoids	0.74^{***}	-0.61^{**}	0.12^{ns}	-	-
Carotenes	-0.16^{ns}	0.34^{ns}	0.24^{ns}	-0.42^{*}	-

^{***,**,*,ns}: Significant at 0.1%, 1%, or 5% level or not significant, respectively.

[z] % Inhibition of linoleic acid oxidation.

Table C.15. Simple correlations between constituents in Cortland apple peel in the ethephon experiment, 1990-1.

	Treatment	Scald	Score	Starch	C_2H_4
Trt	-				
Scald	-0.31^{ns}	-			
Score	0.65^{***}	-0.15^{ns}	-		
Starch	0.82^{***}	-0.33^{ns}	0.86^{***}	-	
C_2H_4	0.85^{***}	-0.50^{*}	0.72^{***}	0.91^{***}	-
OD	0.89^{***}	-0.49^{**}	0.74^{***}	0.91^{***}	0.95^{***}
Max $\propto$F	0.61^{*}	-0.12^{ns}	0.63^{*}	0.73^{**}	0.66^{**}
Max CT	0.01^{ns}	0.51^{ns}	-0.05^{ns}	-0.05^{ns}	-0.31^{ns}
% Inhib[z]	0.85^{***}	-0.49^{**}	0.74^{***}	0.90^{***}	0.94^{***}
TWRC	0.50^{**}	-0.18^{ns}	0.54^{**}	0.61^{***}	0.57^{**}
$\propto$ Toc	0.69^{***}	-0.34^{ns}	0.63^{***}	0.79^{***}	0.80^{***}
Ascorbate	0.66^{***}	-0.44^{*}	0.43^{*}	0.61^{***}	0.74^{***}
Glut	0.02^{ns}	-0.01^{ns}	-0.00^{ns}	-0.02^{ns}	-0.08^{ns}
Chlor	-0.86^{***}	0.36^{*}	-0.71^{***}	-0.85^{***}	-0.84^{***}
Antho	0.71^{***}	-0.40^{*}	0.68^{***}	0.83^{***}	0.82^{***}
Flav	0.00^{ns}	-0.25^{ns}	-0.15^{ns}	0.04^{ns}	0.10^{ns}
Carotenoids	-0.69^{***}	0.40^{*}	-0.61^{***}	-0.72^{***}	-0.78^{***}
Carotene	0.55^{**}	-0.21^{ns}	0.54^{**}	0.71^{***}	0.63^{***}

^{***,**,*,ns}: Significant at 0.1%, 1%, or 5% level or not significant, respectively.
[z] % Inhibition of linoleic acid oxidation.

Table C.16. Simple correlations between constituents in Cortland apple peel in the ethephon experiment, 1990-2.

	OD 200	Max ∝ Farn	Max CT
Trt	0.89^{***}	0.61^{*}	0.01^{ns}
Scald	-0.49^{**}	-0.12^{ns}	0.51^{ns}
Score	0.74^{***}	0.63^{*}	-0.05^{ns}
Starch	0.91^{***}	0.73^{**}	-0.05^{ns}
C_2H_4	0.95^{***}	0.66^{**}	-0.31^{ns}
OD	-		
Max ∝F	0.70^{**}	-	
Max CT	-0.17^{ns}	0.11^{ns}	-
% Inhib[z]	0.95^{***}	0.67^{**}	-0.33^{ns}
TWRC	0.59^{***}	0.47^{ns}	-0.19^{ns}
∝ Toc	0.81^{***}	0.36^{ns}	-0.25^{ns}
Ascorbate	0.74^{***}	0.60^{*}	-0.16^{ns}
Glut	0.06^{ns}	0.15^{ns}	-0.29^{ns}
Chlor	-0.89^{***}	-0.64^{**}	0.05^{ns}
Antho	0.84^{***}	0.59^{*}	-0.40^{ns}
Flav	0.08^{ns}	0.14^{ns}	-0.11^{ns}
Carotenoids	-0.81^{***}	-0.60^{*}	0.24^{ns}
Carotene	0.63^{***}	0.03^{ns}	-0.61^{*}

^{***,**,*,ns}: Significant at 0.1%, 1%, or 5% level or not significant, respectively.
[z] % Inhibition of linoleic acid oxidation.

Table C.17. Simple correlations between constituents of Cortland apple peel in the ethephon experiment, 1990-3.

	% Inhib[z]	TWRC	∝ Toc	Ascorbate	Glut
Trt	0.85***	0.47**	0.69***	0.66***	0.02ns
Scald	-0.50**	-0.18ns	-0.34ns	-0.44*	-0.01ns
Score	0.74***	0.54**	0.63***	0.43*	-0.00ns
Starch	0.90***	0.61***	0.79***	0.61***	-0.02ns
C_2H_4	0.95***	0.57**	0.80***	0.74***	0.08ns
OD	0.95***	0.59***	0.81***	0.74***	0.06ns
Max ∝F	0.67**	0.47ns	0.36ns	0.60*	0.15ns
Max CT	-0.33ns	-0.19ns	-0.25ns	-0.16ns	-0.29ns
TLSA	-				
% Inhib[z]	0.54**	-			
∝ Toc	0.80***	0.80***	-		
Ascorbate	0.69***	0.58***	0.72***	-	
Glut	0.14ns	0.26ns	0.28ns	0.26ns	-
Chlor	-0.83***	-0.69***	-0.80***	-0.74***	0.03ns
Antho	0.82***	0.82***	0.92***	0.72***	0.17ns
Flav	0.08ns	0.43*	0.35ns	0.51**	0.41*
Carotenoids	-0.78***	-0.69***	-0.82***	-0.71***	-0.16ns
Carotene	0.63**	0.52**	0.69***	0.40*	-0.05ns

***,**,*,ns: Significant at 0.1%, 1%, or 5% level or not significant, respectively.
[z] % Inhibition of linoleic acid oxidation.

Table C.18. Simple correlations between constituents of Cortland apple peel in the ethephon experiment, 1990-4.

	Chlor	Antho	Flav	Cartenoid	Carotene
Trt	-0.86^{***}	0.71^{***}	0.00^{ns}	-0.69^{***}	0.55^{**}
Scald	0.36^{*}	-0.40^{*}	-0.25^{ns}	0.40^{*}	-0.21^{ns}
Score	-0.71^{***}	0.68^{***}	-0.015^{ns}	-0.61^{***}	0.54^{**}
Starch	-0.85^{***}	0.83^{***}	0.04^{ns}	-0.72^{***}	0.71^{***}
C_2H_4	-0.84^{***}	0.82^{***}	0.10^{ns}	-0.78^{***}	0.63^{***}
OD	-0.89^{***}	0.84^{***}	0.08^{ns}	-0.81^{***}	0.63^{***}
Max ∝F	-0.64^{**}	0.59^{*}	0.14^{ns}	-0.60^{*}	0.03^{ns}
Max CT	0.05^{ns}	-0.40^{ns}	-0.11^{ns}	0.24^{ns}	-0.61^{*}
% Inhib[z]	-0.83^{***}	0.82^{***}	0.08^{ns}	-0.78^{***}	0.63^{**}
TWRC	-0.69^{***}	0.82^{***}	0.44^{*}	-0.69^{***}	0.52^{**}
∝ Toc	-0.80^{***}	0.92^{***}	0.35^{ns}	-0.82^{***}	0.69^{***}
Ascorbate	-0.74^{***}	0.72^{***}	0.51^{**}	-0.71^{***}	0.40^{*}
Glut	0.03^{ns}	0.17^{ns}	0.41^{*}	-0.16^{ns}	-0.05^{ns}
Chlor	-				
Antho	-0.83^{***}	-			
Flav	-0.15^{ns}	0.38^{*}	-		
Carotenoids	0.85^{***}	-0.75^{***}	-0.20^{ns}	-	
Carotene	-0.59^{**}	0.80^{***}	0.22^{ns}	-0.44^{*}	-

^{***,**,*,ns}: Significant at 0.1%, 1%, or 5% level or not significant, respectively.
[z] % Inhibition of linoleic acid oxidation.

Table C.19. Simple correlations between constituents of Cortland apple peel in bagging experiment, 1990-1.

	Scald	Score	Starch	C_2H_4
Scald	-			
Score	0.14^{ns}	-		
Starch	-0.44^{ns}	-0.07^{ns}	-	
C_2H_4	-0.29^{ns}	-0.25^{ns}	0.84^{***}	-
OD	-0.44^{*}	-0.29^{ns}	0.69^{***}	0.65^{**}
% Inhib[z]	-0.49^{*}	-0.15^{ns}	0.65^{**}	0.66^{**}
TWRC	-0.54^{*}	0.22^{ns}	-0.13^{ns}	-0.35^{ns}
∝ Toc	-0.69^{***}	-0.08^{ns}	-0.32^{ns}	0.28^{ns}
Ascorbate	-0.54^{*}	0.07^{ns}	-0.22^{ns}	-0.25^{ns}
Glut	0.11^{ns}	-0.27^{ns}	-0.21^{ns}	-0.28^{ns}
Chlor	0.13^{ns}	-0.11^{ns}	-0.20^{ns}	-0.18^{ns}
Antho	-0.69^{***}	0.04^{ns}	0.08^{ns}	-0.03^{ns}
Flav	-0.47^{*}	-0.02^{ns}	-0.17^{ns}	-0.15^{ns}
Carotenoids	-0.52^{*}	-0.01^{ns}	-0.07^{ns}	-0.09^{ns}
Carotene	$-0.65^{[1]**}$	-0.15^{ns}	0.20^{ns}	0.26^{ns}

^{***,**,*,ns}: Significant at 0.1%, 1%, or 5% level or not significant, respectively.

[z] % Inhibition of linoleic acid oxidation.

Table C.20. Simple correlations between constituents of Cortland apple peel in the bagging experiment, 1990-2.

	OD	% Inhib[z]	TWRC	∝ Toc	Ascorbate	Glut
Scald	-0.44^{*}	-0.49^{*}	-0.54^{*}	-0.69^{***}	-0.54^{*}	0.11^{ns}
Score	-0.29^{ns}	-0.15^{ns}	0.22^{ns}	-0.08^{ns}	-0.07^{ns}	-0.27^{ns}
Starch	0.69^{***}	0.65^{**}	-0.13^{ns}	0.32^{ns}	-0.22^{ns}	-0.21^{ns}
C_2H_4	0.65^{**}	0.66^{**}	-0.35^{ns}	0.28^{ns}	-0.25^{ns}	-0.28^{ns}
OD	-					
% Inhib[z]	0.67^{**}	-				
TWRC	-0.10^{ns}	0.11^{ns}	-			
∝ Toc	0.38^{ns}	0.77^{***}	0.57^{**}	-		
Ascorbate	-0.11^{ns}	0.14^{ns}	0.89^{***}	0.61^{**}	-	
Glut	-0.07^{ns}	0.18^{ns}	0.13^{ns}	-0.06^{ns}	0.15^{ns}	-
Chlor	-0.14^{ns}	-0.43^{ns}	-0.25^{ns}	-0.55^{*}	-0.21^{ns}	0.08^{ns}
Antho	0.13^{ns}	0.38^{ns}	0.82^{***}	0.81^{***}	0.90^{***}	0.02^{ns}
Flav	-0.04^{ns}	0.23^{ns}	0.77^{***}	0.67^{**}	0.94^{***}	0.13^{ns}
Carenoids	0.00^{ns}	0.05^{ns}	0.58^{*}	0.48^{*}	0.70^{**}	0.02^{ns}
Carotene	0.18^{ns}	0.59^{**}	0.60^{**}	0.90^{***}	0.74^{***}	0.04^{ns}

^{***,**,*,ns}: Significant at 0.1%, 1%, or 5% level or not significant, respectively.
[z] % Inhibition of linoleic acid oxidation.

Table C.21. Simple correlations between constituents of Cortland apple peel in the bagging experiment, 1990-3.

	Chlor	Antho	Flav	Carotenoid	Carotene
Scald	0.13^{ns}	-0.69^{***}	-0.47^{*}	-0.52^{*}	-0.65^{**}
Score	-0.11^{ns}	0.04^{ns}	-0.02^{ns}	-0.01^{ns}	-0.15^{ns}
Starch	-0.20^{ns}	0.08^{ns}	-0.17^{ns}	-0.07^{ns}	0.20^{ns}
C_2H_4	-0.18^{ns}	-0.03^{ns}	-0.15^{ns}	-0.09^{ns}	0.26^{ns}
OD	-0.14^{ns}	0.13^{ns}	-0.04^{ns}	0.00^{ns}	0.18^{ns}
% Inhib[z]	-0.43^{ns}	0.38^{ns}	0.23^{ns}	0.05^{ns}	0.59^{**}
TWRC	-0.25^{ns}	0.82^{***}	0.77^{***}	0.58^{*}	0.60^{**}
∝ Toc	-0.55^{*}	0.81^{***}	0.67^{**}	0.48^{*}	0.90^{***}
Ascorbate	-0.21^{ns}	0.90^{***}	0.94^{***}	0.70^{**}	0.74^{***}
Glut	0.08^{ns}	0.02^{ns}	0.13^{ns}	0.02^{ns}	0.04^{ns}
Chlor	-				
Antho	-0.40^{ns}	-			
Flav	-0.30^{ns}	0.92^{***}	-		
Carotenoids	0.22^{ns}	0.67^{**}	0.66^{**}	-	
Carotene	-0.35^{ns}	0.88^{***}	0.81^{***}	0.63^{**}	-

^{***,**,*,ns}: Significant at 0.1%, 1%, or 5% level or not significant, respectively.
[z] % Inhibition of linoleic acid oxidation.

APPENDIX D

McINTOSH DATA

Table D.1. OD200, ∝ farnesene and conjugated triene values of hexane extracts of McIntosh apple surfaces at harvest and after 7, 14, and 21 weeks of storage at 0°C, 1988.

Harv. date	Hours < 10°C	OD200 (ODX1000/cm²)				∝ Farnesene (nmoles/cm²)				Conjugated trienes (nmoles/cm²)			
		Weeks at 0°C				Weeks at 0°C				Weeks at 0°			
		0	7	14	21	0	7	14	21	0	7	14	21
9-13	68	1	15	20	23	0	38	48	43	0	1.3	5	2
9-20	102	5	15	16	21	0	34	43	41	0	0.9	2	2
9-27	119	5	15	14	18	0	37	38	35	0	1.0	2	3
Significance (Orthogonal polynomial comparisons within storage period)													
Linear		***	ns	***	***	ns	ns	***	***	ns	**	***	ns
Quadratic		ns	ns	ns	ns	ns	*	ns	***	ns	*	***	**

***,**,*,ns: Significant at 0.1%, 1%, or 5% level or not significant, respectively.

Table D.2. ANOVA table for the OD 200 values, ∝ farnesene, and conjugated triene concentrations in a hexane extract of surfaces of McIntosh apples, 1988.

Source	OD 200	∝ Farnesene	Conjugated trienes
REP	*	ns	ns
HARVEST	*	***	***
STORAGE	***	***	***
HARV X STOR	***	***	***

***,**,*,ns: Significant at the 0.1%, 1%, or 5% level or not significant, respectively.

Table D.3. OD200, ∝ farnesene, and conjugated triene values of hexane extracts of McIntosh apple surfaces at harvest and after 6, 12, and 18 weeks of storage at 0°C, 1989.

Harv. date	Hours < 10°C	OD200 (ODX1000/cm²)				∝ Farnesene (nmoles/cm²)				Conjugated trienes (nmoles/cm²)			
		Weeks at 0°C				Weeks at 0°C				Weeks at 0°			
		0	7	14	21	0	7	14	21	0	7	14	21
9-12	62	4	7	14	11	0	23	39	32	0	0.3	4	5
9-19	62	4	-	-	-	0	-	-	-	0	-	-	-
9-26	95	4	-	-	-	0	-	-	-	0	-	-	-

Table D.4. At-harvest ripeness measurements for 1988 and 1989 McIntosh.

Year	Harvest date	Hours < 10°	Starch Index[z]	Firmness (N)	C_2H_4 (log ppm)
1988	Sept. 13	68	3.7	76	-1.2421
	Sept. 20	102	5.5	75	-0.1041
	Sept. 27	119	6.7	72	0.6215
1989	Sept. 12	62	3.3	77	-1.4111
	Sept. 19	62	4.7	81	-1.0218
	Sept. 26	95	6.2	73	-

[z] 1 to 3 = immature; 4 to 6 = mature; 7 to 9 = overmature.

There was no scald development on the McIntosh apples in either 1988 or 1989. Therefore, the data collection on this variety was terminated.

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