Physiology and Control of Apple Scald

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(ABSTRACT)

The effect of controlled atmosphere (CA) storage, and antioxidant treatment on polyphenoloxidase (EC 1.1418.1:PPO), peroxidase (EC1 1.11.1.7:POD), and superoxide dismutase (EC 1.15.1.1:SOD) activities and superficial scald and soft scald development in 'Virginiagold', 'Stayman', and 'Rome' apples (*Malus domestica* Borkh.) was investigated. 'Virginiagold' apples treated postharvest with an aqueous solution of diphenylamine (DPA) + 1,2, dihydro -6- ethoxy -2,2,4-trimethylquinoline (ethoxyquin) and stored in CA exhibited lower soft scald incidence and higher firmness than comparable fruits stored at 0°C in air storage. Fruit firmness and titratable acids (TA) decreased continuously, while ethylene increased in storage. Titratable acids and firmness were positively correlated, while TA and ethylene and firmness and ethylene were negatively correlated. 'Stayman' and 'Rome' apples analyzed during storage showed increase in levels of α-farnesene and its oxidation products conjugated trienes. During storage fruits showed an increase in the activities of polyphenoloxidase (PPO), peroxidase (POD), and superoxide dismutase (SOD), which reached a maximum when scald symptoms in 'Stayman' were observed. Our results indicate that 'Stayman' apples exhibited scald and 'Rome' did not, because 'Stayman' apples had a ten fold higher PPO activity than 'Rome'

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Table of Contents

List of Illustrations

List of Tables

Review of Literature

Introduction

Superficial scald, also known as storage scald or common scald, is one of the most serious and most extensively studied postharvest physiological disorders in apples (*Malus domestica* Borkh). Research on superficial scald of apples has been reviewed previously by Hardenburg (1965) and Ingle and D'Souza (1989). In this review attention will be given to factors affecting superficial scald development, and control of superficial scald.

Superficial scald (hereafter referred to as scald), causes considerable economic loss to growers, shippers, handlers, and consumers. It is estimated that 4-8% of stored apples develop some sort of scald. Scald is caused by the progressive internal browning of the hypodermal cells. Light brown coloring of the skin deepens with increasing severity of the disorder and browning extends throughout the outer five or six layers of the hypodermis. The epidermis, however, is not affected unless the disorder is very severe (Bain, 1956).

The cuticle may be directly involved in scald development by producing toxic substances that cause the disorder or it may be indirectly involved in scald development by preventing the dissipation of toxic substances from the fruit (Shutak and Christopher, 1960). Scald development has been associated with volatile substances produced in the waxy coating of the fruit (Brooks and Cooley, 1919; Meigh, 1967). Huelin and Murray (1966) identified α-farnesene, an unsaturated sesquiterpene, in the natural coating of apples. Later, Huelin and Coggiola (1968) presented evidence linking α-farnesene to scald development. Diphenylamine (DPA) and ethoxyquin (6 - ethoxy - 1,2 - dihydro - 2,2,4 - trimethylquinoline) controlled scald development in 'Rhode Island Greening', 'Cortland', and 'Rome' apples (Smock, 1957). Huelin and Coggiola (1968) reported that DPA delayed the oxidation of α-farnesene in pure solution and natural coating of the fruit. Therefore it was suggested that scald might be caused by the oxidation of α-farnesene and that DPA delays this oxidation process (Meigh and Filmer, 1969; Huelin and Coggiola, 1970).

Scald is associated with conjugated trienes that accumulate progressively in the apple peel rather than with α-farnesene (Huelin and Coggiola, 1970; Sal'Kova *et al.*,1975; Meir and Bramlage, 1988). Anet (1969) reported that α-farnesene autoxidised to form conjugated triene hydroperoxides in 'Granny Smith' apples. Low molecular-weight carbonyl compounds and unsaturated volatile ketones are also produced by α-farnesene oxidation but these compounds do not induce scald (Filmer and Meigh, 1971; Anet, 1972 a). In addition to α-farnesene, lipoxygenase may be involved in the induction of scald. Lipoxygenase is an enzyme involved in the formation of free radical species which enhance plant senescence. Feys *et al* (1980) reported a possible involvement of lipoxygenase in scald formation, in crude extracts of 'Schone Van Boskoop' apples.

Peel flavonols may also be involved in scald induction. Flavonols have been shown to have antioxidant properties and are found in the apple peel (Lea and Swaboda, 1956). Albrigo and Childers (1970) reported that, as the level of flavonols in 'Stayman' apples increased from 225 $ug.g^{-1}$, to 313 $ug.g^{-1}$ the level of scald decreased from 17.4 to 7.5%.

A positive corelation between α-farnesene or its oxidation products and scald has been found. Lack of natural antioxidants may lead to apples developing scald, (Anet and Coggiola, 1974). Anet (1974) detected 11 antioxidants in 'Granny Smith', 'Jonathan', 'Delicious', 'Rome', and 'Starkrimson Delicious' apples using thin layer chromatography. α-tocopherol and three other antioxidants were detected in all apple cultivars tested. In addition, there were other unidentified antioxidants in some of the cultivars tested. Scald does not occur in storage if the antioxidants remain adequate to prevent or limit α-farnesene oxidation. The amount of antioxidant required to prevent scald development depends on the quantity of α-farnesene produced by the apple.

Factors affecting scald development

Scald is influenced by apple cultivar, rootstock, maturity, preharvest air temperatures, orchard location, and storage conditions. Huelin and Murray (1966) and Huelin and Coggiola (1968) reported that in 'Granny Smith', a scald susceptible cultivar, α-farnesene concentration was 3 fold higher than in 'Crofton', a scald resistant cultivar. O'Loughlin and Jotic (1978) observed a lower incidence of scald in 'Delicious' on 'M.1' rootstock than on 'M.13', 'M.16', and 'M.25'. Maturity and har-

vest dates also influence the development of scald in storage. Immature 'Granny Smith', 'Stayman', 'Delicious', 'Cortland', 'Rhode Island Greening', and 'Starking Delicious' fruits scald more readily than mature fruits from the same cultivars (Christopher, 1941; Huelin and Murray, 1966; Albrigo and Childers, 1970; Anet, 1972 b; Zebbini *et al*, 1978; Chen *et al* 1985). The development of scald in immature fruits can be attributed to: accumulation of a smaller amount of of antioxidants in immature than mature fruits and/or lower resistance of immature fruits to the toxic effects of the oxidation products of α-farnesene (Anet, 1972 b).

Preharvest air temperature also affects scald incidence. Merritt *et al.*,(1961) reported a quantitative relationship between number of hours below 10°C and scald severity in 'Stayman' apples. A significant reduction in scald was observed when 150 hours or more of temperature below 10°C had accumulated. Orchard locality has also been reported to influence scald susceptibility. Little and Taylor (1981) reported that 'Granny Smith' apples harvested from a district with relatively warm temperatures had 50% more scald than fruits that were harvested from a relatively cool district.

Mineral content has been reported to influence scald development. 'McIntosh' apple peel with low calcium level (less than 125 ppm,dry wt.basis) developed 50% more scald than apples with high calcium levels (greater than 175 ppm, dry wt.basis) (Drake *et al.*, 1979; Bramlage *et al.*, 1985). Phosphorus concentrations in apples may also influence scald susceptibility. Yogaratnam and Sharples (1982) reported that a 1% phosphorus spray between mid-June and mid-July, reduced scald in 'Bramley's Seedling' in certain years. In addition, copper and cobolt increased scald, while barium and strontium reduced the disorder (Wills and Scott, 1974).

Controlled atmosphere (CA) has reduced scald development. Little *et al.*,(1982), reported that 1.5% O_2 and 1% CO_2 prevent scald development satisfactorily in 'Granny Smith' apples. Scald development is also influenced by ethylene in storage. Knee and Hatfield (1981) reported that when 'Bramley's Seedling' apples were stored in a flow-through system of 5% CO_2, 3% O_2, and 1100 $ul.l^{-1}$ ethylene scald was noticed after 200 days; in a storage atmosphere that did not have ethylene, no scald was noticed at 200 days. Apples stored in an atmosphere containing an ethylene absorbant showed only 21% scald, while atmospheres without an ethylene absorbant showed 85% scald.

Chemical control of scald

Preharvest

Preharvest chemical application is a convenient control method as scald inhibitors can be combined with routine sprays of fungicides and calcium. Daminozide (Alar) spray reduced scald and extended fruit shelf-life when 'Delicious' was sprayed with 700-1000 ppm daminozide (Blanpied *et al.*,1967; Shutak *et al.*, 1968; Williams *et al.*,1964; Couey and Williams, 1973; Campbell, 1977). A high concentration of daminozide (2500-5000 ppm) eliminated scald but also reduced fruit size (Shutak *et al.*, 1968). Preharvest sprays of ethephon have shown satisfactory control of scald. A single application of 250-1000 $mg.liter^{-1}$ ethephon caused a considerable scald reduction in 'Red Delicious', 'Golden Delicious', 'Granny Smith', and 'Starkrimson Delicious' (Greene

et al.,1977; Padfield, 1977; Watkins *et al.*,1982). However, in 'Red Delicious' ethephon increased brown-core incidence (Greene *et al.*,1977).

The effect of growth regulators on scald has been investigated on several apple cultivars. Gibberellins (GA_{4+7} at 100-250 mg.liter^{-1}) had no influence on scald in 'Delicious' but increased scald in 'Rome' (Ingle and D'Souza, 1989). In addition, napthaleneacetic acid, abscisic acid, and glutathione increased scald in 'Granny Smith' (Wills *et al.*,1977).

Although preharvest sprays are convenient, they are not used on a commercial scale. Insufficient control and variation among cultivars in response to preharvest chemicals has directed research efforts at postharvest control.

Postharvest

Wrapping apples with paper impregnated with mineral oil had been used commercially for many years to control scald. However, this treatment was labor intensive in that each apple had to be wrapped individually and it was not completely effective in controlling scald. Therefore, alternative methods to control scald became necessary. Smock (1957) reported that when DPA and ethoxyquin were applied as dip treatment at 500-2000 ppm they successfully controlled scald in 'Rhode Island Greening', 'Cortland', and 'Rome'.

In addition to DPA and ethoxyquin, several other chemicals have been tested for scald control. Butylated hydroxytoluene (BHT) at 10,000 ppm reduced scald in 'Granny Smith', 'Red Delicious', and 'Cortland' as effectively as 2000 ppm DPA (Wills and

Scott, 1977); however, BHT is not used on a commercial scale because of high residue on fruits. Application of monoterpenes controlled scald in 'Jonathan' and 'Granny Smith' apples (Wills and Scott, 1974; Wills *et al.*,1977).

Although hundreds of chemicals have been tried for scald control, the best results have been obtained when apple fruits were dipped in a combination of 2000 ppm DPA and 2700 ppm ethoxyquin, except in 'Golden Delicious' where DPA caused fruit injury (Pierson and Schomer, 1968).

Both DPA and ethoxyquin have been compatible with fungicides and bactericides. Benomyl and thiabendazole with 2700 ppm ethoxyquin and 2000 ppm DPA controlled gray mold in wounded 'Stayman' and 'Delicious' apples (Hardenburg and Spalding, 1972). Recently, Hardenburg and Anderson (1981) used a combination of DPA and ethoxyquin with 4% calcium chloride and reported excellent scald control in 'Stayman' apples.

Non-Chemical control of scald

Scald in 'Stayman' and 'Delicious' was controlled by dipping fruits in a 54°C water bath for 30-60 sec. This treatment caused very little injury to the fruits and was as effective as DPA or ethoxyquin (Hardenburg, 1967). However, in 'Rome' apples scald was not controlled by hot water treatments (Hardenburg and Anderson, 1965).

Controlled atmosphere (CA) storage has also controlled scald. Little *et al*, (1973) reported that 'Jonathan' apples stored at 3% CO_2 and 2.5% O_2 showed 7% scald, while

at 6% CO_2 and 2.5% O_2, only 1% showed scald. Low O_2 storage reduces scald incidence. 'Spartan' apples stored at 1% O_2 had less scald than fruits stored in 2.5% O_2 (Lau, 1983). In addition, fruits exposed to 0-0.5% O_2 for the first 9-14 days of storage showed less scald than fruits that were not exposed to this low oxygen treatment (Little *et al.*,1982). However, short term ultra-low O_2 treatment caused increased scald and low-oxygen injury in 'Grand Alexander', 'Granny Smith', and 'Delicious' apples (Chen *et al.*,1985).

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Control of scald in 'Virginiagold' apples

Abstract

The effect of controlled atmosphere (CA) storage and a combination of diphenylamine (DPA) and 1, 2, dihydro -6- ethoxy -2,2,4- trimethylquinoline (ethoxyquin) on scald control and fruit quality of 'Virginiagold' apples (*Malus domestica* Borkh.) was examined in two field experiments. In the first experiment, fruit was dipped in an aqueous mixture of 2000 ppm DPA + 2700 ppm ethoxyquin and stored in CA or in 0°C air storage. After 5 months in storage fruits were evaluated for scald, firmness, titratable acids (TA), soluble solids concentration (SSC) and ethylene. Another set of apples from the same treatments was placed at 21°C for 7 days and evaluated for the same factors. Fruit stored in CA did not show any scald symptoms, whereas, fruits stored in 0°C air had 10% scald. Fruit firmness was higher in CA than in 0°C air storage and declined after 7 days at 21°C. Ethylene increased in both CA and 0°C air stored fruit after 7 days at 21°C. In the second experiment, fruit was dipped in 2000 ppm DPA + 2700 ppm ethoxyquin and stored at 0°C in air storage. At monthly intervals fruits

were evaluated for firmness, TA, SSC, and ethylene. At the end of 4 months in storage, fruit was evaluated for scald development. Treated fruit had 25% less scald than control fruit and showed no visible injury. Firmness and TA declined linearly with days in storage (DS) with $R^2 = 0.80$ and $R^2 = 0.79$ respectively. Ethylene biosynthesis increased linearly with DS ($R^2 = 0.34$). There was a positive correlation between firmness and TA ($r = 0.86$), a negative correlation between firmness and ethylene, ($r = -0.68$), and TA and ethylene ($r = -0.63$). Soluble solids concentration was not correlated to any of the factors measured.

Introduction

'Virginiagold' is a cross between 'Golden Delicious' and 'Albemarle Pippin'. The fruit size is large, skin is golden yellow, and the flesh is crisp and juicy at optimum maturity. The cultivar performs well in areas where 'Golden Delicious' is grown.

'Virginiagold' fruit is susceptible to soft scald and superficial scald, two serious postharvest disorders in apple. Soft scald, also called ribbon scald, is a postharvest disorder characterized by ribbon-like areas of brown tissue on the surface of the apple and a sharp line of demarcation between the diseased and healthy tissue (Brooks and Harley, 1934). Browning on the surface of the fruits often extends into the flesh for 2 cm or more. This disorder is believed to be caused by abnormal respiratory conditions of the fruit and the severity of the disorder is increased by delayed cooling (Kidd and West, 1935; Haller and Lutz, 1936). Superficial scald, also refered to as common scald or scald is characterized by brown patches on the surface of the fruit. In severe cases,

the brown coloring of the skin extends through the outer five or six layers of the hypodermis (Bain, 1956). Superficial scald symptoms generally appear a few days after the fruit has been removed from storage and held at ambient temperature (Fisher and Cooley, 1947). In addition, maturity greatly influences the development of scald, with immature fruits being more susceptible than mature fruits (Albrigo and Childers, 1970; Anet, 1972; Chen *et al.*, 1985; Christopher, 1941; Huelin and Murray, 1966). The etiology of scald is not fully understood, but the oxidation products of the sesquiterpene α-farnesene have been implicated in its development (Anet, 1972; Huelin and Coggiola, 1968). In many apple cultivars scald is controlled commercially by dipping the fruit postharvest in an aqueous mixture of diphenylamine (DPA) and 1,2 - dihydro -6- ethoxy -2,2,4- trimethylquinoline (ethoxyquin) (Smock, 1957).

In addition to chemical treatment with DPA and/or DPA + ethoxyquin, controlled atmosphere (CA) storage has reduced scald development in 'Jonathan' apples held at 3% CO_2 and 2.5% O_2 and in 'Granny Smith' apples held at 1% CO_2 and 1.5% O_2 (Little *et al.*, 1973; 1982). The objectives of this study were to determine if DPA + ethoxyquin and CA storage could reduce scald development without causing fruit injury in 'Virginiagold' apples.

Materials and Methods

Storage treatment: The effect of controlled atmosphere (CA) and 0°C air storage on scald was examined. Forty fruits per tree were harvested on 28 October 1988 from each of six mature 'Virginiagold'/MM.111 trees grown at the Horticulture Farm at

Blacksburg, VA, using each tree as a replicate. Fruits were dipped for 30 sec in an aqueous solution of 2000 ppm DPA and 2700 ppm ethoxyquin. Twenty fruits per tree were stored in 0°C air and the remaining 20 fruits were stored in a commercial CA storage at 3% CO_2 and 3% 0_2. After 5 months in storage, 10 fruits per sample were evaluated immediately for soft scald, superficial scald, bitter-pit (BP), internal break-down (IB), fruit firmness, titratable acids (TA), soluble solids concentration (SSC), and ethylene production. The remaining 10 fruits were kept at 21°C for 7 days and then evaluated for the same factors.

Fruit firmness was measured with an Effegi tester using the 11 mm plunger tip. Titratable acids level was measured by titrating 0.01N NaOH into a 5 ml juice sample to a pH 8.2 using phenolpthalein as an indicator. Soluble solids concentration was measured with a hand refractometer. Ethylene was measured by sealing 5 fruits per replicate in a 4 liter jar for 1hr and taking a 1-ml gas sample which was injected into a Shimadzu GC-8A Gas Chromatograph ® (Shimadzu corporation, Japan) equipped with a Pora-Pac 80/100 mesh column and a flame ionization detector. The temperature of the injector and detector was 200°C and column was 100°C. Helium was the carrier gas.

DPA + ethoxyquin treatment: Two hundred fruits were harvested on 18 October 1988 from each of six mature (12 yr-old) 'Virginiagold'/MM.111 trees. Each tree represented one replicate. The 200 fruits were divided into two groups of 100 fruits each. The first group was dipped in an aqueous solution of 2000 ppm DPA + 2700 ppm ethoxyquin for 30 sec and stored in 0°C air. The second group was stored in 0°C air without treatment (control). During the 4 months of storage, ten fruits per treatment/replicate were tested monthly for firmness, SSC, TA, and ethylene.

After 4 months of storage 30 fruits per treatment/replicate were evaluated immediately for scald, BP, IB, firmness, SSC, TA, and ethylene; an equal number of fruits were evaluated for the same factors after 7 days at 21°C

Results and Discussion

Scald Control: Controlled atmosphere storage prevented soft scald in 'Virginiagold' apples more efficiently than 0°C air storage (Table 1.1). It has been reported that soft scald is induced by abnormal respiration of the fruit. Kidd and West, (1935) and Haller and Lutz, (1936) showed that the rate of respiration was directly propotional to soft scald incidence in 'Jonathan' apples. Kader, (1986) and Weichman, (1986) reported that the rate of respiration is lower in CA-stored fruits than 0°C air-stored fruit. 'Virginiagold' fruit stored in CA had limited O_2 and elevated CO_2 and hence lower rate of respiration. Our data indicate that fruits stored in CA showed lower level of scald, possibly due to lower rates of respiration. 'Virginiagold' fruits treated with DPA + ethoxyquin and stored in air at 0°C had less soft scald than untreated fruits (Table 1.3) Soft scald is known to be caused by high respiratory activities. It is possible that DPA + ethoxyquin treated fruits had lower soft scald because of lower respiratory rates. It has been shown that DPA treated fruits have lower rates of respiration than untreated fruits (Lurie *et al.*, 1989).

Incidence of superficial scald was very low ($< 0.5\%$) in all treatments (Table 1.3). Most apple cultivars exhibit superficial scald symptoms after approximately 15 weeks in 0°C air storage (Lurie *et al.*, 1989). However, no scald was observed in

'Virginiagold' apples even when fruit was stored for 16 weeks at 0°C in air. In addition, when fruits were evaluated after 7 days at 21°C following storage, no increase in superficial scald was noticed (Table 1.3). It is possible that cultivar is the overriding factor in scald development (Huelin and Coggiola, 1968). Diphenylamine and ethoxyquin control scald successfully in almost all apple cultivars. However, in 'Golden Delicious' a combination of DPA and ethoxyquin have been reported to cause skin injury, possibly due to DPA (Pierson and Schomer, 1968); hence only ethoxyquin has been used to control scald in this cultivar. Our results show that DPA + ethoxyquin did not cause fruit injury in 'Virginiagold' even though 'Golden Delicious' is a parent of ' Virginiagold'.

Internal breakdown and bitter-pit: Internal breakdown incidence was very low to non-existent in all treatments (Tables 1.1 and 1.3). This was probably because of a very small gap between harvest and storage and also because fruits were stored for a relatively short time. Porritt and Mehereiuk, (1973) showed that fruits stored without delayed cooling for a relatively short period (4-5 months) had very low IB as compared to fruits stored with delayed cooling for 8-9 months.

Incidence of bitter-pit was low (< 6%) in all treatments (Tables 1.1 and 1.3). This was probably because fruits were harvested at optimum maturity. Allen (1932) has reported that mature fruit is more susceptible to BP than immature or over mature fruit.

Fruit firmness:

'Virginiagold' fruit firmness was higher in fruits from CA storage than in 0°C air storage (Table 1.1). These data confirm earlier findings that CA storage retains firmness in 'Delicious' sports (Mehereiuk and Porritt, 1971). The relatively low decline in fruit firmness in CA could be due to a decrease in respiratory activity and/or de-

crease in the activity of fruit softening enzymes such as polygalacturonase (EC 3.2.1.15:PG) (Kader, 1986; Weichman, 1986).

When fruit held in 0°C air storage was evaluated at monthly intervals, a linear decline in fruit firmness with days in storage (DS) was observed (R^2 = 0.80; Fig. 1.1). The decrease in fruit firmness could also be due to an increase in ethylene biosynthesis, because a negative correlation between ethylene production and fruit firmness was seen (r = -0.46; Fig. 1.4). In addition, ethylene biosynthesis linearly increased with DS (R^2 = 0.34; Fig. 1.3). Fruit firmness decreased significantly (P = 0.01) after 7 days at 21°C following storage, while a concomitant increase (five fold) in ethylene biosynthesis was observed (Table 1.2). These results confirm earlier findings by Ingle and D'Souza (1989) of a negative correlation between fruit firmness and ethylene and a positive relationship between DS and ethylene.

Titratable acidity and soluble solids concentration: Titratable acids showed a linear decline with DS (R^2 = 0.79; Fig.1.2). Liu and Samelson (1986) showed that TA decreased continuosly during storage. Titratable acids and fruit firmness showed a positive correlation (r = 0.86; Fig. 1.5). Fruit analyzed after 7 days at 21°C following storage had significantly lower TA and higher ethylene production than fruit analyzed immediately after storage (Table 1.2). In addition, a negative correlation (r = -0.63) between ethylene and TA was observed (Fig. 1.6). Our results show that TA and firmness are not good indicators of scald as they did not correlate with the degree of scald incidence. However, these factors have been reported to be related to the ripening of the fruits (Lurie *et al.*, 1989).

In this study, soluble solids concentration did not change with the type of storage (Table 1.2) or storage duration. It has been reported that SSC changed while fruits

were still attached to the tree but no changes were observed after fruits were stored in 0°C air storage or CA storage (Ingle and D'Souza 1989; Mehereiuk and Porritt, 1971).

Table 1.1. **Postharvest disorders and fruit firmness following controlled atmosphere (CA) and 0°C air storage in 'Virginiagold' apples.**

Storage[y] duration (days)	Storage type	Disorder[z] (%) Soft scald	BP[x]	IB[x]	Firmness (Newtons)
150	CA (0°C)	0	2	0	58
150	0°C air	12	3	2	52
		**	NS[w]	NS	**
150 + 7[v]	CA (0°C)	0	3	0	54
150 + 7	0°C air	12	3	0	47
		**	NS		**

[z]Data transformed by arcsin square root prior to statistical analysis.

[y]Data are the means of six replicates of 10 fruits each.

[x]Bitterpit ; Internal breakdown.

[w]NS, ** Nonsignificant and significant at P = 0.05 and P = 0.01 respectively.

[v]Fruits evaluted after 7 days at 21°C.

Table 1.2. Fruit firmness, titratable acids (TA), soluble solids concentration (SSC), and ethylene as influenced by test date and type of storage in 'Virginiagold' apples.

Storage[z] duration (days)	Storage type	Firmness (Newtons)	TA (mg/ml)	SSC (%)	Ethylene (ul/kg/hr)
150	CA (0°C)	57.6	3.7	11.3	44
150 + 7[y]	CA (0°C)	54.5	3.0	11.2	199
		**	**	NS[x]	**
150	0°C air	52.0	3.6	11.3	39
150 + 7	0°C air	46.8	2.6	11.0	193
		**	*	NS	**

[z]Data are the means of six replicates of 10 fruits each.

[y]Fruit was evaluated after 7 days at 21°C.

[x]NS, * , ** Nonsignificant and significant at P = 0.05 and P = 0.01 respectively.

Table 1.3. Postharvest disorders as influenced by scald inhibitors and test day in 'Virginiagold' apples held at 0°C in air storage.

Storage[y] duration (days)	Treatment	Disorder[z] (%)			
		Soft scald	Superficial scald	BP[x]	IB[x]
120	Control	37	0.0	5	0
120	DPA + EQ[w]	9	0.4	1	0
		**	NS[v]	*	
120 + 7[u]	Control	37	0.0	6	2
120 + 7	DPA + EQ	10	0.4	1	0
		**	NS	**	NS

[z]Data transformed by arcsin square root prior to statistical analysis.

[y]Data are means of 6 replicates of 30 fruits each.

[x]Bitterpit ; Internal breakdown.

[w]Fruits dipped in 2000 ppm DPA + 2700 ppm ethoxyquin (EQ).

[v]NS, *, ** Nonsignificant and significant at P = 0.05 and P = 0.01 respectively.

[u]Fruits evaluated after 7 days at 21°C.

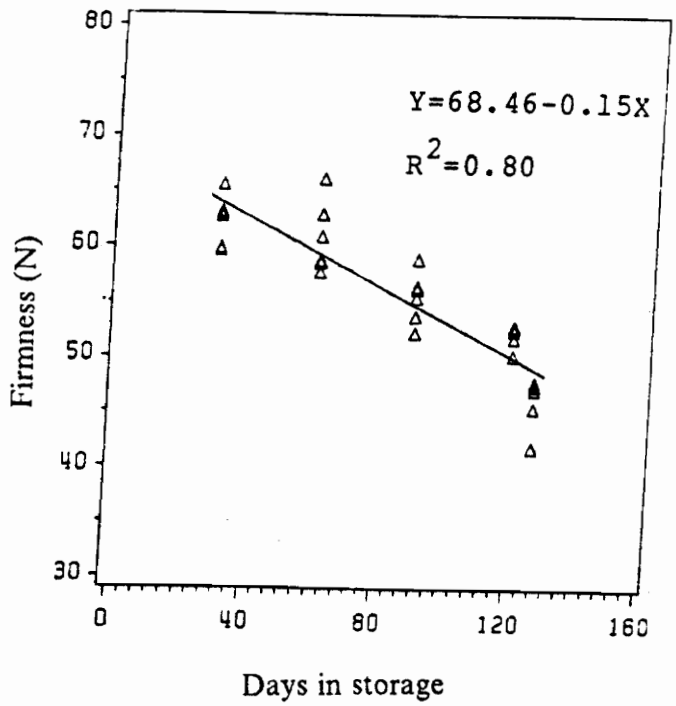

Figure 1.1. Fruit firmness (Newtons) as affected by storage (0°C air) duration in 'Virginiagold' apples.

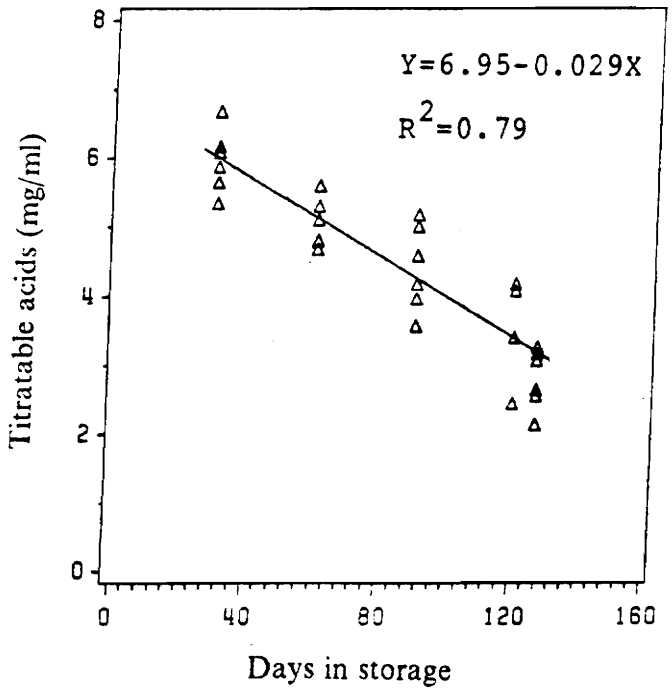

Figure 1.2. Titratable acids (mg/ml) as affected by storage (0°C air) duration in 'Virginiagold' apples.

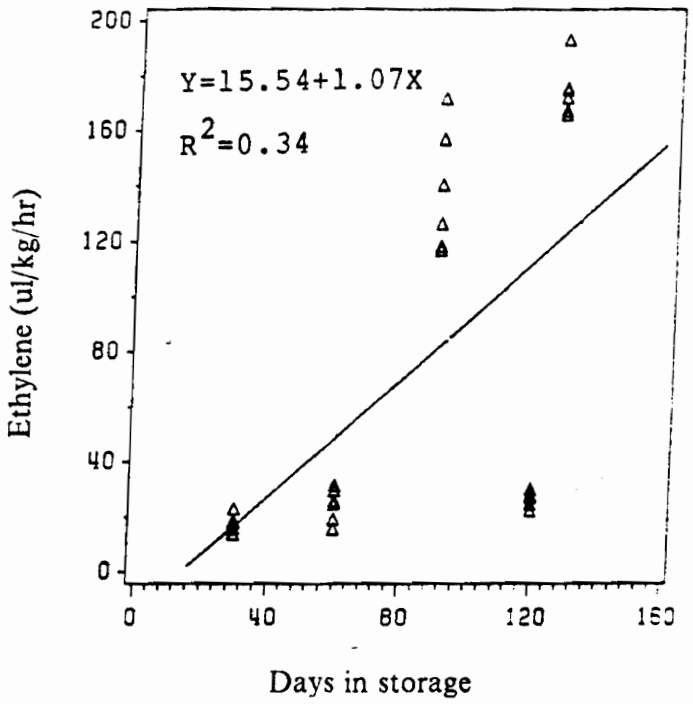

Figure 1.3. Ethylene production (ul/kg/hr) as affected by storage (0°C air) duration in 'Virginiagold' apples.

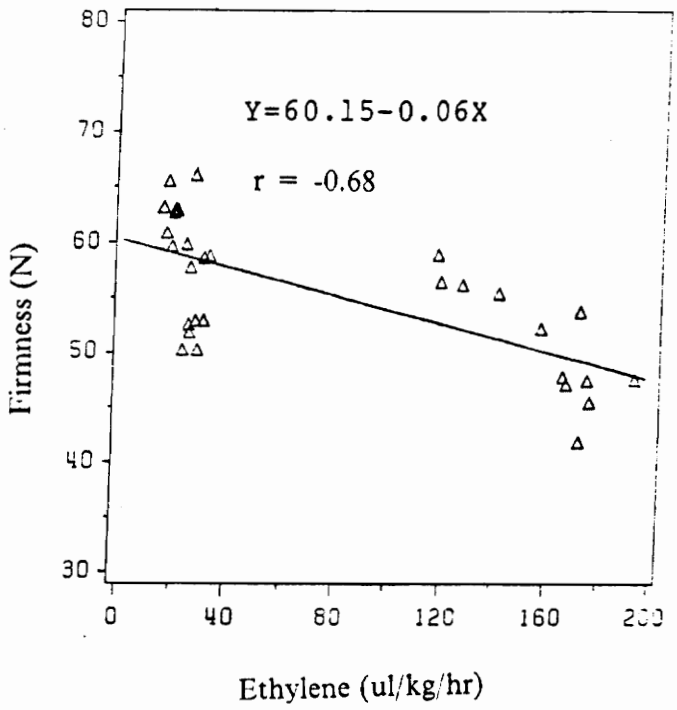

Figure 1.4. Relatioship between fruit firmness and ethylene: Decrease in fruit firmness (Newtons) with increasing ethylene production (ul/kg/hr) during 0°C air storage of 'Virginiagold' apples.

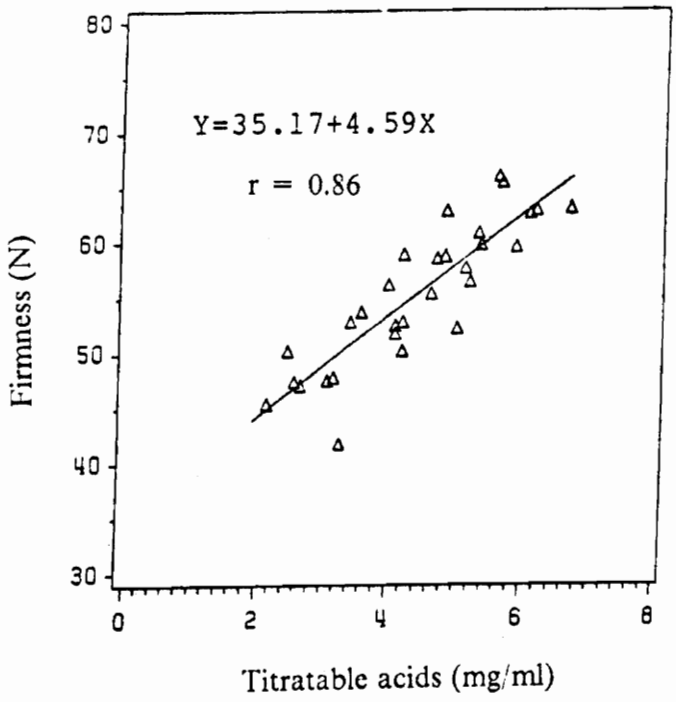

Figure 1.5. Relationship between fruit firmness and titratable acidity: Increase in fruit firmness (Newtons) with increasing titratable acids (mg/ml) in 0°C air stored 'Virginiagold' apples.

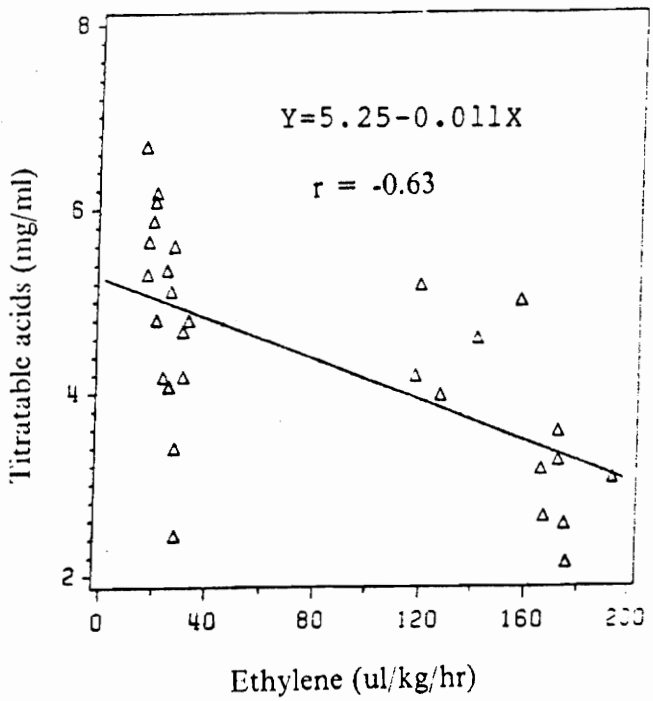

Figure 1.6. Relationship between titratable acidity and ethylene: Decrease in titratable acids (mg ml) with increase in ethylene (ul/kg/hr) in 0°C air stored 'Virginiagold' apples.

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Changes in polyphenoloxidase, peroxidase, and superoxide dismutase in apple fruit in relation to scald development

Abstract

'Rome ' and 'Stayman' apples (*Malus domestica* Borkh.) were analyzed for polyphenoloxidase (PPO), peroxidase (POD), and superoxide dismutase (SOD) activities and α-farnesene and conjugated trienes, 15 days prior to commercial harvest and at commercial harvest. At commercial harvest fruit was subjected to a postharvest dip of 2000 ppm diphenylamine (DPA) and 2700 ppm ethoxyquin for 30 sec and stored in air at 0°C. Following 30 and 75 days of storage fruit was held for 7 days at 21°C before analysis for α-farnesene and conjugated trienes. Polyphenoloxidase, POD, and SOD activities were determined immediately after 30 and 75 days of storage. α-farnesene and conjugated trienes increased with storage duration. When fruits were analyzed after

30 and 75 days of storage, conjugated trienes were lower in DPA + ethoxyquin treated than in untreated fruits. α-farnesene did not vary with treatment. When fruits were evaluated visually after 75 days of storage, treated 'Stayman' had half as much scald as untreated. No scald was observed in 'Rome'. Polyphenoloxidase, POD, and SOD activity in both cultivars increased in storage with the exception of PPO activity in untreated 'Stayman' which reached a peak at 30 days and then declined at 75 days. Enzyme activities were highest when scald symptoms were noticed in 'Stayman'. Our results suggest that 'Stayman' apples exhibited scald but 'Rome' did not, because 'Stayman' apples had a ten fold higher PPO activity than 'Rome'

Introduction

Superficial scald is one of the most serious storage disorders of apples. Scald symptoms appear a few days after the fruits have been removed from storage and held at ambient temperature. Maturity greatly influences the development of scald, with immature fruits being more susceptible than mature fruits (Albrigo and Childers, 1970; Anet, 1972; Chen *et al.*, 1985; Christopher, 1941; Huelin and Murray, 1966).

The etiology of scald is not fully understood, but conjugated trienes, the oxidation products of the sesquiterpene α-farnesene, have been implicated in scald develpoment (Anet, 1972; Huelin and Coggiola, 1968). Scald is controlled on a commercial scale by a postharvest dip in an aqueous mixture of 2000 ppm diphenylamine (DPA) and 2700 ppm 1,2 - dihydroxy -6- ethoxy -2,2,4- trimethylquinoline (ethoxyquin) (Smock, 1957).

However, the mode of action by which these chemicals control scald is not fully understood.

Scald is manifested as a progressive internal browning of the hypodermal cells. Okamoto (1959) suggested that the browning reaction is due to the action of polyphenoloxidase (PPO). Polyphenoloxidase is an enzyme mainly involved in the catalysis of natural phenolic compounds into quinones, which are polymerized to brown-red or black pigments (Mathew and Parpia, 1971). The activities of PPO, peroxidase (POD), and lipoxygenase (EC 1.13.11.12) were lower in the peel of DPA treated than in untreated 'Granny Smith' apples (Lurie *et al.*, 1989).

Peroxidase is involved in cell membrane disintegration and senescence (Sacher, 1973; Winkenbach and Matile, 1970). It has been generalized that senescence corresponds to tissue oxidation, which may be due to an increase in peroxides, lipoxygenase and POD activities (Bredemeijer, 1973; Brennan and Frenkel, 1977; Carfantan and Daussant, 1975; Mishra *et al.*, 1976).

Lipoxygenase is involved in the formation of free radicals which enhance plant senescence. Feys *et al.* (1980) reported a possible involvement of this enzyme in scald formation in apples. Free radical populations are decreased by free radical scavenging, a defense mechanism in tissues (Leshem *et al*, 1979). Scavengers are a class of compounds which react with superoxides to form molecular oxygen. The enzyme superoxide dismutase (SOD) is a free radical scavenger. It catalyzes the dismutation of the superoxide free radicals to innocuous molecular O_2 and H_2O_2. This enzymatic antioxidant has a protective action against oxidative reactions brought about by various physiological stresses (Monk *et al.*, 1989). Since superficial scald is primarily an autoxidation process, it is possible that SOD level is affected by scald induction. The

objective of this research was to investigate the changes in the activities of PPO, POD, and SOD and to determine the role of these enzymes in scald development.

Materials and methods

Fruits were harvested twice (Oct. 1 and Oct. 15, 1989) from 24-yr old 'Stayman' and 'Rome'/MM.111 trees grown at the Horticulture Farm at Blacksburg, VA. Five trees per cultivar were used with trees as replicates. At each harvest 5 fruits per replicate were analyzed for PPO, POD, and SOD activities and α-farnesene and conjugated trienes. In addition, at second harvest, 40 fruits per replicate were harvested and divided into two groups of 20 each. One group was dipped in 2000 ppm DPA + 2700 ppm ethoxyquin for 30 sec, the other group was a control. Ten fruits per group were stored for 30 days at 0°C and 10 fruits per group were stored for 75 days at the same temperature. At the end of each storage period, 5 fruits per group were analyzed for PPO, POD, and SOD activities, while an equal number were kept at 21°C for 7 days and analyzed for α-farnesene, conjugated trienes, and scald.

Determination of PPO and POD activities: The activities of PPO and POD were measured in crude enzyme extract from the outer 1 cm of the fruit surface. A 2 g sample from 5 fruits was ground in liquid nitrogen and suspended in 20 ml of 50 mM potassium phosphate buffer (pH 6.2), containing 0.5% (w/v) polyvinylpyrrolidone (PVP) and 0.3% (v/v) Triton x-100® (Rohm and Haas Company, U.S.A.). The mixture was homogenized with a polytron homogenizer® (KINEMATICA gmbh LITTAU, Switzerland) at low speed (3x) for 20 sec. and centrifuged at 18000 x g for

20 minutes. The supernatant was dialyzed over-night against the extraction buffer and the enzyme avtivity was determined as follows: The enzyme activity of PPO was assayed in a 6 ml reaction mixture which included 3 ml of 100 mM sodium citrate buffer (pH 5.0), 2 ml of 20 mM 4-methyl catechol, as the enzyme substrate, 0.5 ml extraction buffer, and 0.5 ml crude extract. Absorbance at 410 nm was measured in a Shimadzu UV-160 Spectrophotometer ® (Shimadzu Corporation, Japan). Polyphenoloxidase activity was measured as the change in optical density over a 3 min. period and expressed as $\Delta OD \cdot min \cdot^{-1} mg\ protein^{-1}$ (Sciancalepore, 1985).

The activity of POD was assayed in a total volume of 3 ml, which included 2.2 ml of 50 mM citrate-phosphate buffer (pH 5.5), 0.2 ml of 0.5% (v/v) guaiacol, 0.15% (v/v) hydrogen peroxide, 0.3 ml extraction buffer, and 0.3 ml crude extract. The absorbance was determined at 470 nm. Peroxidase activity was measured as a change in optical density over a 3 min period and expressed as $\Delta OD \cdot min \cdot^{-1} mg\ protein^{-1}$ (Sciancalepore, 1985). Protein concentration was determined according to Bradford (1976) using bovine serum albumin as a standard.

Determination of SOD activity: SOD activity was measured in the crude extract. A 2 g sample (from 5 fruits) from the outer 1 cm of the fruit surface was ground in liquid nitrogen and suspended in 20 ml of 50 mM potassium phosphate buffer (pH 7.0), containing 1% (w/v) PVP, 0.1 mM EDTA, and 0.3% (v/v) Triton x-100. The resulting mixture was homogenized with a polytron homogenizer at a low speed (3x) for 20 sec., centrifuged at 15000 x g for 10 min, and supernatant was dialyzed over night against the extraction buffer.

The activity of SOD was assayed by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT), using the methods of Beauchamp and

Fridovich (1971) and Dhindsa *et al.* (1981). The reaction mixture in a total volume of 3 ml of the extraction buffer, was placed in a 5 ml test tube which contained 13 mM methionine, 75 uM NBT, 2 uM riboflavin as the enzyme substrate, and 0-300 ul crude extract. The reaction was started by addition of riboflavin and placing the tubes 30 cm below a 15W fluorescent light for 10 min. and the absorbance was recorded at 560 nm. A non-irradiated mixture, without crude extract was used as the control (dark blank). An irradiated mixture lacking crude extract developed maximum color (light blank) and decreased with increasing volume of extract added. The volume of the extract that brought about 50% inhibition of the light blank was considered as one enzyme unit (U) (Beauchamp and Fridovich, 1971). SOD activity was expressed as $U \cdot g$ f. wt.$^{-1}$

Analysis of α-farnesene and conjugated trienes: α-farnesene and conjugated trienes were measured from extracted apple cuticle. Apples were dipped individually for 2 min, with occasional agitation, in 100 ml spectroscopic grade hexane. The solution was filtered through a Whatman No. 1 filter paper and OD measured at 232 nm for α-farnesene and 281-290 nm for conjugated trienes in a Shimadzu UV-160 Spectrophotometer. The amounts of α-farnesene and conjugated trienes in nmol.cm^{-2} of fruit surface was calculated by the modified method of Meir and Bramlage (1988) using the extinction coefficient, as follows:

- Extinction coefficient for α-farnesene = 27.7
- Extinction coefficient for conjugated trienes = 25.0
- ((OD/27.7) 10^5)/fruit surface area = α-farnesene in nmol.cm^{-2}

- ((OD/25.0) 10^5)/fruit surface area = Conjugated trienes in nmol.cm^{-2}

- Fruit surface area (cm^2) was calculated by measuring r, the radius of the fruit, and using the formula $4\Pi r^2$

- 10^5 was used to express the concentration in nmoles.

Results

α-farnesene and conjugated trienes: The levels of α-farnesene and conjugated trienes in 'Stayman' apples did not change with harvest date. When fruits were analyzed after 30 and 75 days in storage α-farnesene and conjugated trienes increased (Figs. 2.0 and 2.1). α-farnesene and conjugated trienes were lower in treated than in untreated fruits after 30 and 75 days storage. Visible scald symptoms were not seen at 30 days, but at 75 days treated fruits had only half as much scald as untreated fruits (Table 2.0).

'Rome' fruits analyzed at commercial harvest had higher α-farnesene and conjugated triene levels than fruits analyzed 15 days earlier. When fruits were analyzed after storage, α-farnesene exhibited an increasing trend (Fig. 2.2). α-farnesene level did not differ among treatments at 30 and 75 days storage. However, conjugated trienes were lower in treated than in untreated fruits at 30 and 75 days (Fig. 2.3). No visible scald symptoms were seen after 7 days at 21°C following 30 or 75 days in storage.

Polyphenoloxidase: Polyphenoloxidase activity in 'Stayman' apples did not change with fruit maturity (Fig. 2.4). Treated fruits analyzed after 30 and 75 days of storage ex-

hibited a gradual increase in activity. In untreated fruits, PPO activity reached a maximum at 30 days and declined at 75 days. Polyphenoloxidase activity in 'Rome' was 10 times lower than in 'Stayman' apples (Figs. 2.4 and 2.5). PPO activity did not change with fruit maturity (Fig. 2.5) but increased in storage. In general, treated fruit had consistently lower PPO activity than untreated fruits.

Peroxidase: Peroxidase activity in 'Stayman' apples did not change before storage (Fig. 2.6). Peroxidase activity of untreated fruits increased in storage, wheras, in treated fruits POD activity did not change up to 30 days but increased after 75 days in storage. Similar to PPO, POD activity in 'Rome' apples was 10 times lower than 'Stayman' (Figs 2.6 and 2.7). When treated fruits were analyzed after 30 days, no change in POD activity was observed (Fig. 2.7). However, after 75 days POD activity increased. In untreated fruits, POD activity increased up to 30 days and began to level off. Treated fruits had lower POD activity than untreated fruits at 30 days, but, at 75 days the difference was minimal.

Superoxide dismutase: Superoxide dismutase activity in 'Stayman' decreased with fruit maturity but increased in storage (Fig. 2.8). The enzyme activity in treated fruit was higher than untreated fruit after 75 days but not after 30 days in storage. Similar to 'Stayman', SOD activity in 'Rome' decreased with fruit maturity and increased in storage (Fig. 2.9). However, SOD activity did not differ among treatments after 30 or 75 days in storage.

Discussion

α-farnesene and conjugated trienes: Data from our experiments suggest that conjugated trienes are a good indicator of scald because in 'Stayman' apples they were higher in fruits that developed scald than fruits that did not develop scald (Table 2.0). A recent report by Lurie *et al.* (1989) suggests that α-farnesene is not a good indicator of scald, because 'Granny Smith' apples treated with DPA had higher α-farnesene level but lower level of scald than untreated fruits. Our data do not refute this observation.

Polyphenoloxidase: The increase in PPO activity in (Figs. 2.4 and 2.5) storage was opposite to what has previously been reported in 'Delicious' apples where the activity of PPO decreased (Klapp *et al.*, 1989). However, these authors also reported that 'Granny Smith' fruit exhibited no change in PPO activity with storage. Previous studies have provided no clear trend in PPO activity. Harel *et al.* (1966) and Macheix (1970) reported that total PPO activity dropped at the later stages of 'Grand Alexander' apple development. The reverse was found by Zocca and Ryugo (1975) in 'Golden Delicious'. It is possible that such differences may be due not only to the different cultivars studied but also due to the extraction procedure used.

Studies on 'Granny Smith' apples by Lurie *et al.* (1989) have shown that fruits treated with DPA had lower levels of scald and PPO activity than untreated fruits. Data from our experiments do not refute this claim. 'Stayman' apples exhibited scald while 'Rome' did not, probably because 'Stayman' apples had a ten fold higher PPO activity than 'Rome'. It has been hypothesized that DPA could prevent scald by maintaining a high level of reducing power and by inhibiting the activity of PPO Lurie *et al.* (1989).

Okamoto (1959) has suggested that the browning reaction in the apple skin during scald development is due to the action of PPO. Histological studies have shown that scald begins with an accumulation of oxidative browning products (Bain, 1956). In addition, electron micrographs have shown dense material accumulating on the inner side of the tonoplast of scald susceptible fruits (Bain and Mercer, 1963). These compounds may be condensed tannins which would lead to tonoplast breakdown and subsequent mixing of vacuolar and cytoplasmic contents (Bain and Mercer, 1963).

Peroxidase and superoxide dismutase: Peroxidase has been associated with cell disintegration, free radical formation, and senescence (Sacher, 1973; Winkenbach and Matile, 1970). Superoxide dismutase has been reported as a free radical scavenger and has been associated with anti-senescence (Droillard and Mascot, 1987; Leshem *et al.*, 1986; Dhindsa *et al.*, 1981). Our data indicate that POD and SOD activities increased simultaneously during storage. This is probably a facet of normal cell metabolism because it has been shown that POD and SOD activities increase with aging (Warzburger *et al.*, 1984; Gorin and Heidema 1976). Our data indicate a possible involvement of POD in scald induction because α-farnesene and conjugated trienes increased along with an increase in POD activity. In addition, POD activity was highest when scald symptoms were observed in 'Stayman'. Our study with SOD indicates that DPA and ethoxyquin enhanced the activity of this enzyme in 'Stayman' apples. Previous reports have shown that application of DPA could replace endogenous antioxidants, such as ascorbic acid and α-tocopherol, and increase the antioxidant pool in senescing tisssue (Kunert and Ederer, 1985). Diphenylamine + ethoxyquin may have controlled scald in 'Stayman' fruit by enhancing the activity of SOD. This observation was distinct in 'Stayman', where treated fruits had a higher SOD activity than untreated fruits, and fruits with higher SOD activity had 50% less scald than fruits with lower activity.

Table 2.0. Superficial scald, α-farnesene, and conjugated trienes in 'Stayman' and 'Rome' apples after 75 days at 0°C in air storage.

Cultivar	Treatment[z]	Scald[y] (%)	α-farnesene[x]	Conjugated trienes[x]
Stayman	Control	32.0	89.7	4.7
Stayman	DPA + EQ[v]	16.0	83.1	1.4
		NS[u]	NS	**
Rome	Control	0.0	77.9	3.1
Rome	DPA + EQ	0.0	78.3	0.8
			NS	**

[z]Fruits evaluated after 7 days at 21°C following 75 days in storage.

[y]Data transformed by arcsin square root prior to statistical analysis.

[x]nmoles.cm^{-2}

[v]Fruits dipped in 2000 ppm DPA + 2700 ppm ethoxyquin (EQ).

[u]NS , ** Nonsignificant and significant at P = 0.05 and P = 0.01 respectively.

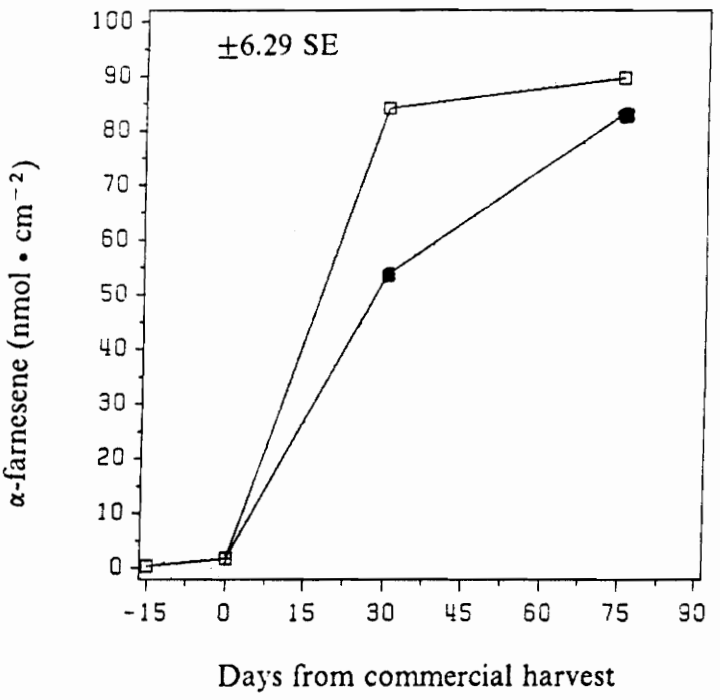

Figure 2.0. α-farnesene levels (nmol.cm^{-2}) in 'Stayman' apples following commercial harvest: in untreated (□) and treated (■) fruits held at 0°C in air storage. Treated fruits were dipped in 2000 ppm DPA and 2700 ppm ethoxyquin for 30 sec.

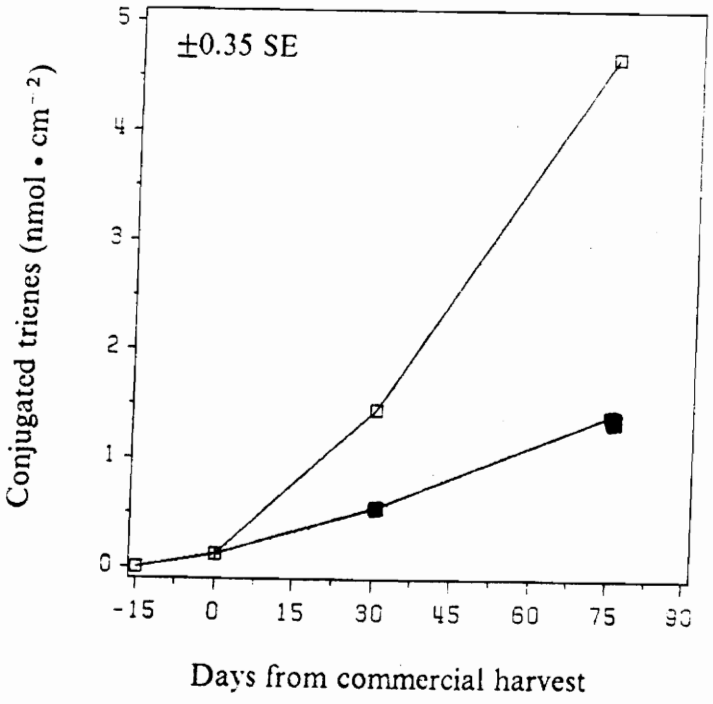

Figure 2.1. **Conjugated trienes levels (nmol.cm^{-2}) in 'Stayman' apples following commercial harvest:** in untreated (□) and treated (■) fruits held at 0°C in air storage. Treated fruits were dipped in 2000 ppm DPA and 2700 ppm ethoxyquin for 30 sec.

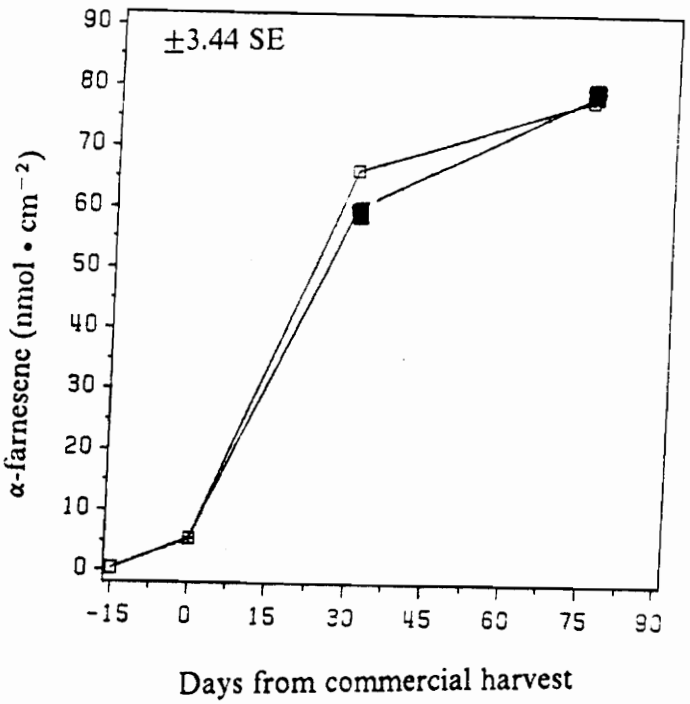

Figure 2.2. **α-farnesene levels (nmol.cm^{-2}) in 'Rome' apples following commercial harvest: in untreated (□) and treated (■) fruits held at 0°C in air storage. Treated fruits were dipped in 2000 ppm DPA and 2700 ppm ethoxyquin for 30 sec.**

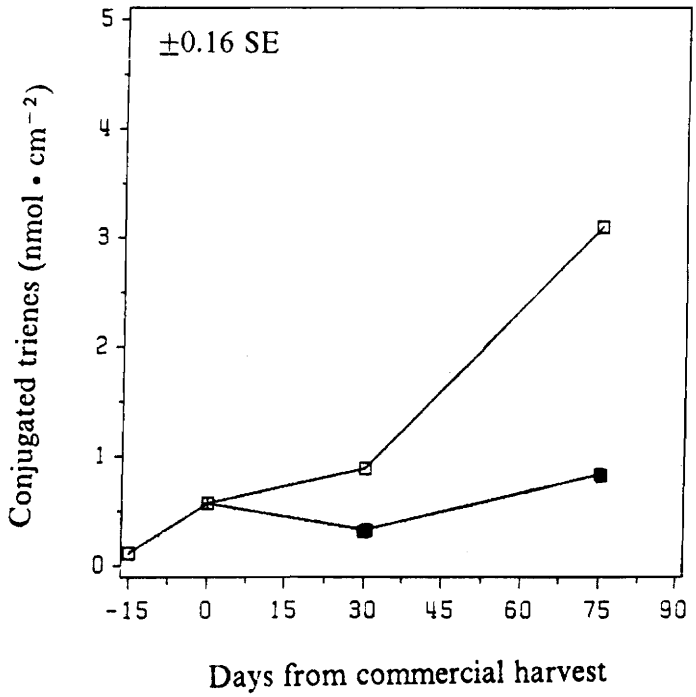

Figure 2.3. Conjugated trienes levels (nmol.cm^{-2}) in 'Rome' apples following commercial harvest: in untreated (□) and treated (■) fruits held at 0°C in air storage. Treated fruits were dipped in 2000 ppm DPA and 2700 ppm ethoxyquin for 30 sec.

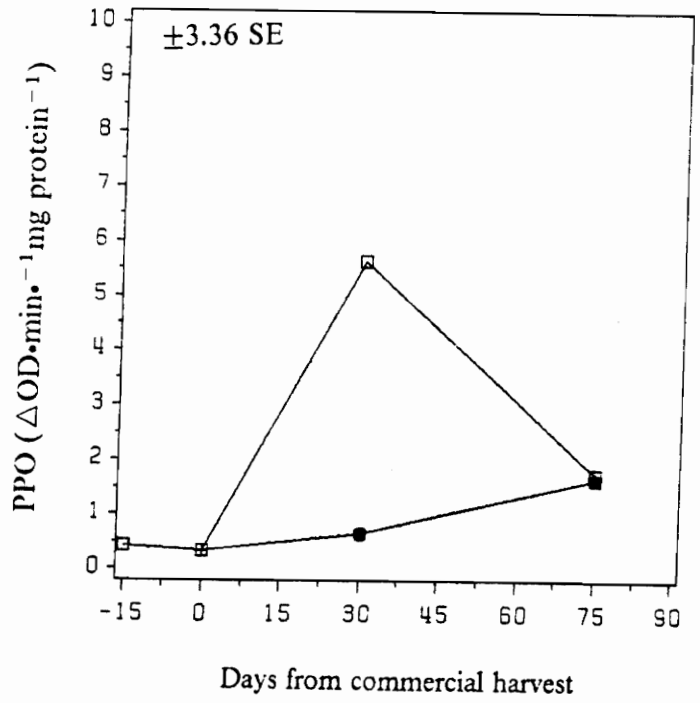

Figure 2.4. Polyphenoloxidase (PPO) activity (ΔOD•min•$^{-1}$mg protein^{-1}) in 'Stayman' apples following commercial harvest: in untreated (□) and treated (■) fruits held at 0°C in air storage. Treated fruits were dipped in 2000 ppm DPA and 2700 ppm ethoxyquin for 30 sec.

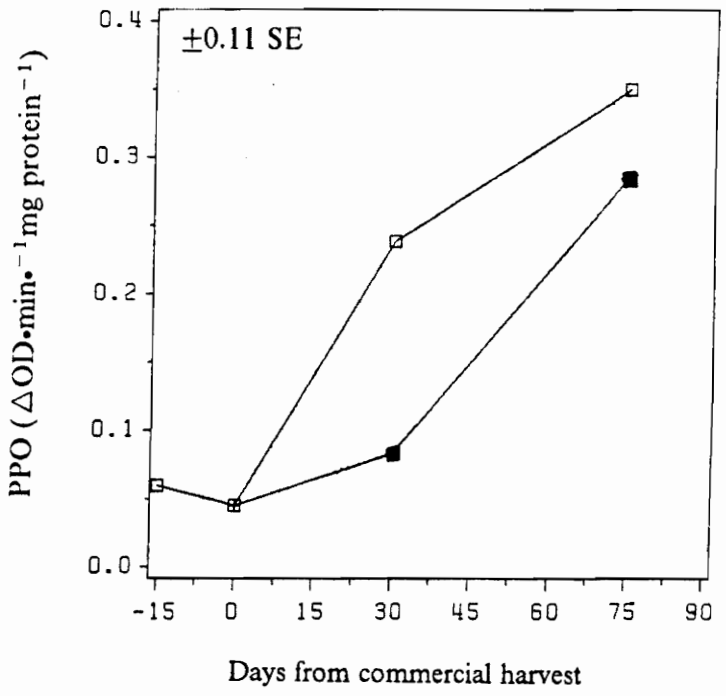

Figure 2.5. **Polyphenoloxidase (PPO) activity (ΔOD•min•$^{-1}$mg protein^{-1}) in 'Rome' apples following commercial harvest:** in untreated (□) and treated (■) fruits held at 0°C in air storage. Treated fruits were dipped in 2000 ppm DPA and 2700 ppm ethoxyquin for 30 sec.

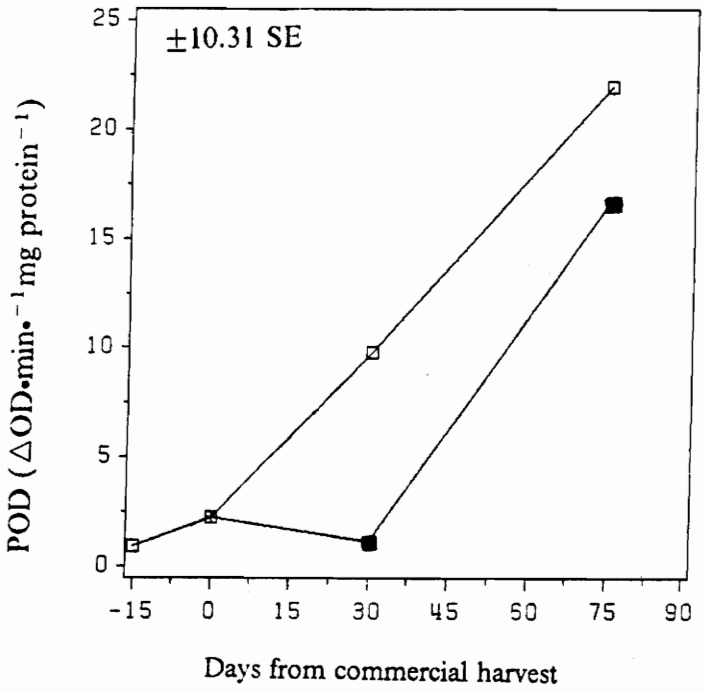

Figure 2.6. Peroxidase (POD) activity ($\Delta OD \cdot min \cdot^{-1} mg\ protein^{-1}$) in 'Stayman' apples following commercial harvest: in untreated (□) and treated (■) fruits held at 0°C in air storage. Treated fruits were dipped in 2000 ppm DPA and 2700 ppm ethoxyquin for 30 sec.

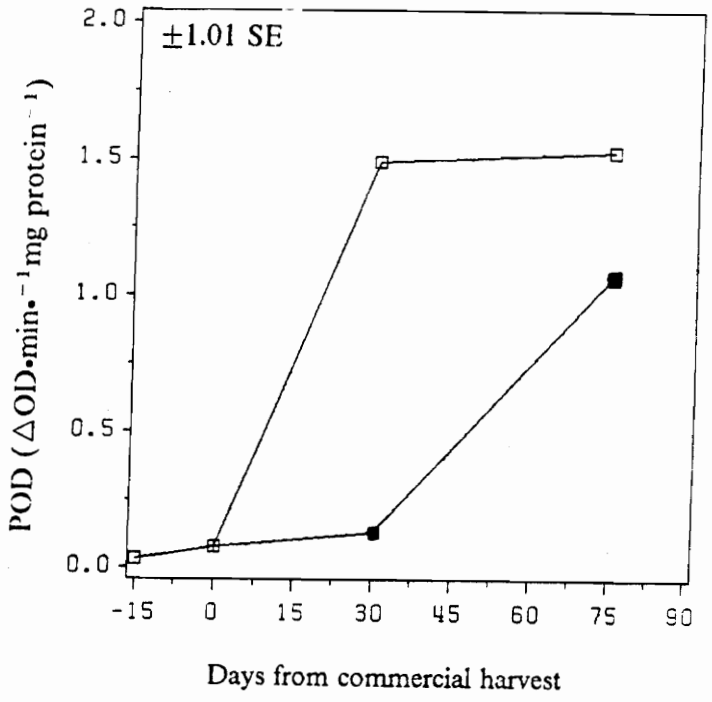

Figure 2.7. Peroxidase (POD) activity ($\Delta OD \cdot min \cdot^{-1} mg\ protein^{-1}$) in 'Rome' apples following commercial harvest: in untreated (□) and treated (■) fruits held at 0°C in air storage. Treated fruits were dipped in 2000 ppm DPA and 2700 ppm ethoxyquin for 30 sec.

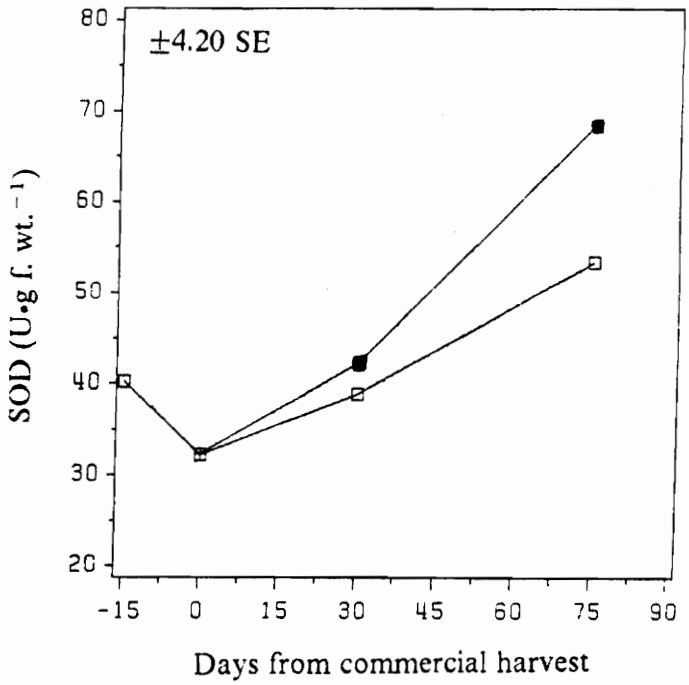

Figure 2.8. **Superoxide dismutase (SOD) activity (U•g f. wt.$^{-1}$) in 'Stayman' apples following commercial harvest:** in untreated (□) and treated (■) fruits held at 0°C in air storage. Treated fruits were dipped in 2000 ppm DPA and 2700 ppm ethoxyquin for 30 sec.

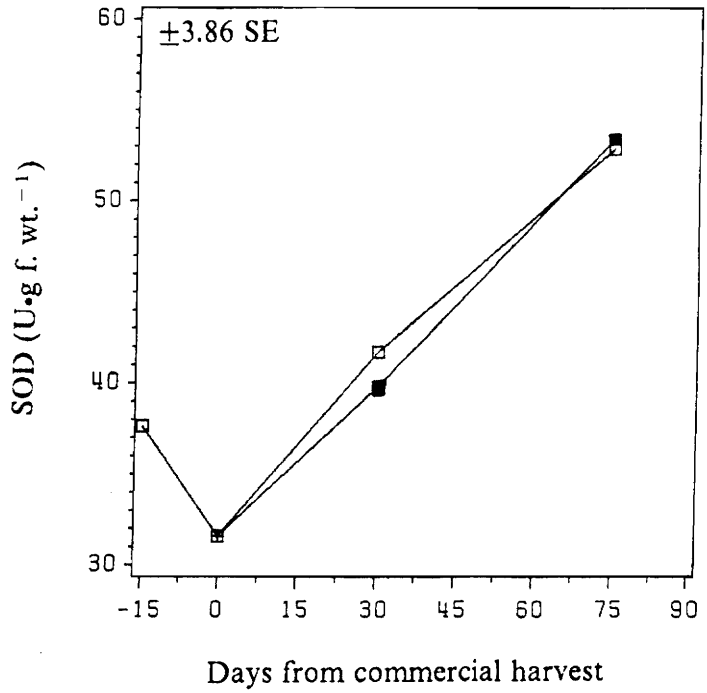

Figure 2.9. **Superoxide dismutase (SOD) activity (U•g f. wt.$^{-1}$) in 'Rome' apples following commercial harvest:** in untreated (□) and treated (■) fruits held at 0°C in air storage. Treated fruits were dipped in 2000 ppm DPA and 2700 ppm ethoxyquin for 30 sec.

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Appendix A. Sodium erythorbate is ineffective in controlling superficial scald in 'Stayman' apples

Abstract

The effectiveness of sodium erythorbate (SET) as a preharvest or postharvest treatment to reduce superficial scald and enhance fruit quality of 'Stayman' apples was investigated in two field experiments. In both treatments fruits were harvested twice, at commercial harvest and 15 days earlier. In the preharvest treatment, fruits were sprayed with 2000 ppm SET immediately prior to harvest and stored at 0°C. In the postharvest treatment, fruits were dipped in 2000 ppm SET immediately following harvest and stored at 0°C. After 135 days in storage fruits were kept at 21°C for 7 days and evaluated for scald, bitter-pit (BP), titratable acids (TA), and soluble solids concentration (SSC). Sodium erythorbate treated fruits showed no difference in scald, BP, or fruit quality from the untreated fruits. Fruits harvested early (harvest 1) had greater

scald incidence and firmness, and lower SSC than fruits harvested 15 days later (harvest 2) (Table 3.0).

Table 3.0. Postharvest disorders and fruit quality of 'Stayman' apples as affected by sodium erythorbate and harvest date.

Harvest	Scald[y] (%)		BP[x] (%)		TA[w] (mg/ml)		SSC[v] (%)		Firmness (Newtons)	
	C[u]	T[t]	C	T	C	T	C	T	C	T
PREHARVEST STUDY[z]										
1	95	93	18	5	5.7	5.1	13.5	13.7	40.8	38.3
2	48	52	0	3	5.5	4.6	14.2	14.6	37.2	36.9
	**	**	NS[s]	NS	NS	*	**	**	**	*
POSTHARVEST STUDY[z]										
1	93	90	10	8	6.5	5.7	13.6	13.8	43.8	44.4
2	52	25	5	3	5.7	5.5	14.5	14.8	43.1	41.6
	*	**	NS	NS	**	NS	*	**	NS	**

[z]Data are means of 6 replicates of 10 fruits each.

[y]Percent data transformed by arcsin square root prior to statistical analysis.

[x]Bitterpit ; [w]Titratable acids; [v]Soluble solids concentration

[u]Control ; [t]Fruits treated with 2000 ppm sodium erythorbate.

[s]NS , *, ** Nonsignificant and significant at P = 0.05 and P = 0.01 respectively.

Appendix B. Superficial scald cannot be reversed after its initiation

Abstract

'Stayman' apples with potential for scald development were treated with diphenylamine (DPA) and ethoxyquin to determine if scald could be reversed after it was manifest. 'Stayman' apples were harvested at the commercial harvest and stored at 0°C. Fruits were examined at 15 day intervals for scald, bitter-pit (BP), and internal breakdown (IB). After 102 days in storage fruits when fruits started to show slight scald symptoms, they were dipped in 2000 ppm DPA and 2700 ppm ethoxyquin for 30 sec and stored at 0°C. After an additional 60 days in storage, fruits were immediately evaluated for scald, BP, and IB and kept at 0°C for 7 days and evaluated again. 'Stayman' apples exhibited a dramatic increase in scald at the end of storage (Table 3.1). The disorder increased further after 7 days at 21°C. Thus superficial scald could not be reversed or

checked after it was initiated. Bitter-pit and IB incidence was very low or nonexistent at the end of storage, but increased after 7 days at 21°C.

Table 3.1. Postharvest disorders in 'Stayman' apples treated with DPA and ethoxyquin after scald was manifest.

Days in[y] storage	Postharvest disorder[z] (%)					
	Scald[x]		BP[w]		IB[v]	
	C[u]	T[t]	C	T	C	T
102	19 a	16 a	2 a	4 a	0 a	0 a
162	81 b	89 b	1 a	2 a	0 a	0 a
162 + 7[s]	93 c	93 b	2 a	4 a	9 b	9 b

[z]Data transformed by arcsin square root prior to statistical analysis.

[y]Data are means of 6 replicates of 10 fruits each.

[x]Mean separation within columns by Tukey's LSD, P = 0.05.

[w]Bitterpit ; [v]Internal breakdown.

[u]Control ; [t]Fruits treated with 2000 ppm DPA and 2700 ppm ethoxyquin.

[s]Fruits evaluated after 7 days at 21°C.

Vita

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