Active Oxygen Species Metabolism in 'White Angel' x 'Rome Beauty' Apple Selections Resistant and Susceptible to Superficial Scald

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ABSTRACT. Fruit from seedlings selected from a population obtained by crossing superficial scald-resistant 'White Angel' and superficial scald-susceptible 'Rome Beauty' apple (*Malus ×domestica* Borkh.) were used to investigate the role of oxidative processes in the development of superficial scald. Selections were identified for study based on fruit coloration and scald susceptibility. Plant material had one of the following three physiognomies: 1) red-skinned fruit resistant to scald; 2) red-skinned fruit susceptible to scald; and 3) yellow-skinned fruit susceptible to scald. The concentrations of α -farnesene, conjugated triene (CT) species, hydrogen peroxide (H_2O_2), thiobarbituric acid-reactive substances, carbonyl groups, and the activities of superoxide dismutase, guaiacol-peroxidase, and catalase were measured at harvest and during 0.5 °C storage. Relationships were poor between scald susceptibility and α -farnesene and its oxidation products, CT258, CT281, and the CT258/CT281 ratio. Tissue concentrations of H_2O_2 were lower in scald-resistant than in scald-susceptible fruit at harvest and after storage, and these lower concentrations were associated with less damage to cellular membranes (lipid peroxidation) and proteins (carbonyl group content). Higher activities of the H_2O_2 -degrading enzymes, guaiacol-peroxidases, and catalases, were related to lower H_2O_2 content and lower scald susceptibility. Activities of superoxide dismutase, a H_2O_2 -generating enzyme, were not related to scald susceptibility or storage period. We hypothesize that fruit susceptibility to scald is strongly influenced by cellular efficiency in metabolizing active oxygen species such as H_2O_2 .

Superficial scald is a physiological disorder associated with long-term storage of certain apple and pear cultivars (Emongor et al., 1994; Ingle and D'Souza, 1989). The damage due to scald is confined to a zone a few cells thick, just below the skin, and manifested as skin browning (Bain and Mercer, 1963). Although scald has been recognized as a problem by the apple industry for many years, research interest has been limited because of the availability and effectiveness of diphenylamine (DPA) as a post-harvest antioxidant drench to prevent the disorder. The generally accepted hypothesis of scald development is that the sesquiterpene α -farnesene is oxidized in the fruit peel, producing several conjugated triene (CT) species that cause disruption, discoloration, and death of surface cells (Anet, 1972, 1974; Huelin and Coggiola, 1970a). However, the physiology and biochemistry of scald development still is not well understood.

Production of active oxygen species (AOS) such as O_2^- and H_2O_2 in all respiring cells is a continuous process, and accumulation of AOS occurs during senescence or is influenced by physiological stress factors such as low temperature (Halliwell and Gutteridge, 1989; Prasad et al., 1994; Purvis and Shewfelt, 1993). By themselves, O_2^- and H_2O_2 are relatively unreactive, but together they can form hydroxyl radicals ($OH^\cdot$) and singlet oxygen (1O_2), the most reactive species in chemistry (Halliwell and Gutteridge, 1989; Bowler et al., 1992). Unless metabolized, these reactive

oxygen species react indiscriminately to cause lipid peroxidation, protein denaturation, and DNA mutation (Halliwell and Gutteridge, 1989). Plant cells are equipped with several enzymic- and nonenzymic-based antioxidant defense systems that keep these deleterious reactions to a minimum. Superoxide dismutase (SOD; EC 1.15.1.1) localized in chloroplast, cytosol, and mitochondria dismutates O_2^- to produce H_2O_2 (Van Camp et al., 1994), which is metabolized to H_2O by a wide array of antioxidant enzymes such as catalase (CAT; EC 1.11.1.6), peroxidase (POX; EC 1.11.1.7), ascorbate peroxidase (APX; EC 1.11.1.11), and glutathione reductase (GR; EC 1.6.4.2) (Criessen et al., 1994; Rao et al., 1996).

Minimization of damage induced by AOS may be associated with tolerance of apples to scald development. While studies by Du and Bramlage (1995a, 1995b) provided little evidence that marked changes in peroxidative activity were related to scald development, the studies compared different commercial cultivars whose genetic makeup varied and therefore had possible confounding effects of maturity and ripeness and environmental conditions. As scald susceptibility is believed to be influenced by climatic factors, nutrient availability, fruit size, and maturity (Anet, 1972; Emongor et al., 1994), conclusive evidence on the role of antioxidant-based defense systems and scald susceptibility has not yet been obtained.

Since scald susceptibility may be associated with enhanced production of free radicals and peroxidative events, we investigated the role of AOS and their metabolism in relation to scald. Selections from a population derived from a cross between 'White Angel', a crab apple highly resistant to scald, and 'Rome Beauty', a commercial apple cultivar highly susceptible to scald, were used for study. These selections are hybrids of the same age and were segregated for scald resistance under similar environmental conditions, thereby minimizing possible influences of environmental factors and management practices on scald susceptibility. Detailed

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information on the genetic map (Hemmat et al., 1994; Lawson et al., 1995) and genetic markers for scald resistance (Weeden, 1993) are available. The main objective of our study was to investigate whether or not the resistance to scald in this population is related to the ability to maintain small pool sizes of AOS, such as H_2O_2 . α -Farnesene and CT concentrations in hexane extracts of scald-resistant and scald-susceptible fruit were also measured spectrophotometrically to examine this relationship.

Materials and Methods

PLANT MATERIAL AND FRUIT SOURCE. A family derived from the cross between 'White Angel' and 'Rome Beauty' growing at the New York State Agricultural Experimental Station, Geneva, N.Y., was used for our studies. Details of the cross and the plantings have been described in detail (Hemmat et al., 1994; Lawson et al., 1995). The seedlings derived from this cross segregate for red- and yellow-skinned fruit (Cheng et al., 1996) with varying susceptibility to scald (Weeden, 1993).

SAMPLING AND ESTIMATING INTERNAL ETHYLENE CONCENTRATION (IEC) AND CHROMATICITY VALUES. Based on 2 years of scald data (N.F. Weeden, unpublished data), 19 selections were initially chosen for our experiments. Fruit (160 to 200) were harvested on 14 Oct. 1996 from each selection and brought to the laboratory at Ithaca, N.Y. IEC and skin color at harvest were measured on 20 fruit per selection. The remaining fruit were stored at 0.5 °C. Internal gas samples (0.5 mL) were drawn into a syringe through a hypodermic needle inserted into the core cavity of each fruit, and ethylene concentrations were measured by gas chromatography (model 3700; Varian, Walnut Creek, Calif.). Skin color was assessed using a color reflectance meter (chroma meter II; Minolta, Tokyo) under CIE illuminant D65 conditions as described by Watkins et al. (1995). The L^* variable is a lightness value that measures black (0) to white (100). The a^* scale measures the degree of red (+ a^*) or green (− a^*) color, and the b^* scale measures the degree of yellow (+ a^*) or blue (− b^*) color.

For further analysis, only fruit with similar IECs were used (Table 1). Red or yellow fruit types were designated based on their skin color as determined by their chromaticity values (Table 1). Eight selections were chosen for further study: three different trees each that produced red-skinned fruit either resistant (selection no. 3, 16, and 17; red-R) or susceptible (selection no. 44, 47, and 48; red-S) to scald, and two different trees each that produced yellow-skinned fruit (selection no. 38 and 65; yellow-S) susceptible to scald. Each tree was treated as a replication; therefore, red-S and red-R had three replicates and yellow-S had two replicates.

Twenty-fruit samples were taken from each tree replicate at harvest and after 4 or 16 weeks of storage. Stored fruit were held at 20 °C for 24 h before sampling. The outer portions (0 to 3 cm) of fruit were peeled, frozen under liquid nitrogen, and stored at −80 °C until used for biochemical analyses. Another set of 10 fruit was used to monitor changes in α -farnesene and CTs. After 16 weeks of storage plus 7 d at 20 °C, 100 fruit were scored for scald symptoms visually, and the remaining fruit scored for scald after 20 weeks plus 7 d at 20 °C.

α -FARNESENE AND CTs. Fruit from each replication were immersed in 90 mL of hexane [high-performance liquid chromatography (HPLC)-grade] for 2 min and rinsed with fresh hexane. The solution plus wash was adjusted to 100 mL, and appropriately diluted aliquots were used to measure UV-absorbance at 232 nm for estimating α -farnesene and at 258, 281, and 290 nm for estimating CTs. Concentrations of α -farnesene in hexane extracts were calculated from OD232 with a extinction coefficient of E_{232}

= 27,700 (Huelin and Coggiola, 1968). Concentrations of all CTs were calculated from OD258-290 and OD281-290, with an extinction coefficient of 25,000 (Anet, 1972), and are referred to as CT258 and CT281, respectively.

PEROXIDE CONTENT. Five grams of frozen tissue was homogenized with 10 mL of ice cold acetone. The homogenate was centrifuged at 20,000 g_n (15 min at 4 °C) and the supernatant was stored at −80 °C until further analysis. Peroxide levels were assessed by reacting the acetone extract with 20% titanous tetrachloride (w/v) for 15 min at 20 °C following the method of Brennan and Frenkel (1977). The concentration of the peroxide in the extracts was determined by comparing the absorbancy with a standard curve representing the titanium- H_2O_2 complex from 0.1 to 1 mM. Although the hydroperoxides extracted by the above procedure represents H_2O_2 and lipid hydroperoxides, the latter represent only 5% to 10% of the total peroxides in the fruit (Brennan and Frenkel, 1977).

LIPID PEROXIDATION AND OXIDATIVE DAMAGE TO PROTEINS. Lipid peroxidation was monitored by assaying the thiobarbituric acid-reactive substances using the method of Du and Bramlage (1995a). Oxidative damage to proteins was assessed by measuring protein carbonyl groups. Five grams of frozen tissue was ground in a mortar and pestle with 10 mL of 200 mM potassium phosphate buffer (pH 7.5) containing 2 mM EDTA, 5% PVP-40, and 1 mM PMSF. The homogenate was filtered through four layers of cheesecloth and centrifuged at 20,000 g_n (25 min at 4 °C). Carbonyl contents were monitored by reacting the protein extract (20 mg) with 2,4-dinitrophenyl hydrazine following the method of Rao et al. (1996).

ENZYME EXTRACTION. Ten grams of frozen tissue was ground to a fine powder with a mortar and pestle under liquid nitrogen. Frozen powder was homogenized with 20 mL of 200 mM potassium phosphate buffer (pH 7.8) containing 2 mM EDTA, 1% PVP-40, and 1 mM PMSF. The homogenate was filtered through four layers of cheesecloth and centrifuged at 25,000 g_n (30 min at 4 °C). The supernatant was stored in aliquots at −80 °C for enzyme activity assay. Protein content was determined according to Bradford (1976). All enzyme activities were assayed with equal amounts of tissue protein (20 μ g).

SOD ACTIVITY. SOD activity was estimated by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) following the method of Dhindsa et al. (1981). The assay mixture (3 mL) contained 100 mM phosphate buffer (pH 7.8), 15 mM methionine, 100 mM NBT, 2 mM riboflavin, and 0.1 mM EDTA with 20 μ g of tissue protein. The reaction was initiated by illuminating the mixed assay mixture with fluorescent lamps. A similar assay mixture covered with aluminum foil served as a

Table 1. Chromaticity values and internal ethylene concentrations (μ L·L^{−1}) of red and yellow fruit collected from scald resistant (R) and scald susceptible (S) selections from a 'White Angel' × 'Rome Beauty' apple population at harvest.

Parameter	Fruit type ²		
	Red-R	Red-S	Yellow-S
L^*	45.7 a	45.6 a	75.8 b
a^*	24.4 a	25.7 a	−8.3 b
b^*	19.6 a	21.4 a	45.8 b
a^*/b^*	1.25 a	1.20 a	−0.2 b
IEC (mL·L ^{−1})	12.2 a	16.4 a	11.2 a

²Values are averages of means (n = 3 for red and n = 2 for yellow) for each fruit type, and those followed by same letter within a row are not significantly different ($p > 0.05$).

Table 2. Superficial scald incidence (%) after 16 weeks storage at 0.5 °C and the changes in α -farnesene, CT258, CT 281 (fresh mass basis) and CT258/CT281 ratio in hexane extracts of scald resistant (R) and scald susceptible (S) selections derived from a population of 'White Angel' x 'Rome Beauty'.

Fruit type	Scald (%)	α -Farnesene (nmol·g ⁻¹)			CT258 (nmol·g ⁻¹)			CT281 (nmol·g ⁻¹)			258/281 Ratio		
		Storage period (weeks)											
		0	4	16	0	4	16	0	4	16	0	4	16
Red-R	0	13.5 a ^z	53.7 b	73.5 c	1.16 a	2.99 b	4.42 c	0.26 a	0.43 b	1.09 c	4.54 a	7.12 b	4.10 a
Red-S	70	28.3 a	42.4 b	69.4 b	2.46 a	3.39 b	3.88 b	0.49 a	0.58 a	0.91 b	4.96 a	5.89 a	4.36 a
Yellow-S	100	33.8 a	52.5 b	57.0 b	2.97 a	3.37 a	3.68 b	0.67 a	0.76 a	0.90 b	4.55 a	4.40 a	4.10 a

²Mean followed by same letter within a row are not significantly different ($p > 0.05$).

control. The reaction duration was 20 min and was stopped by removing the light source. The absorbance of the assay mixture was read at 560 nm. One unit of SOD activity is defined as the amount of enzyme that inhibits the NBT photoreduction by 50% under the conditions of the assay.

POX ACTIVITY. Total POX activities were measured using guaiacol as a substrate following the method described by Rao et al. (1996). The assay mixture (3 mL) contained 100 mM phosphate buffer (pH 6.5), 16 mM guaiacol, and 20 μ g protein at 22 °C, and the reaction was initiated by adding 10 μ L of 30% H₂O₂. Changes in absorbance at 470 nm were recorded for 5 min with a spectrophotometer (model DU-60; Beckman Instruments, Fullerton, Calif.).

CAT ACTIVITY. CAT activity was assessed by measuring the decomposition of H₂O₂ (Du and Bramlage, 1995a). The assay mixture (1 mL) contained 100 mM phosphate buffer (pH 6.5), 5 mM H₂O₂, and 20 μ g protein. The reaction was allowed to continue for 20 min at 22 °C and stopped by adding 100 mL of 20% titanate tetrachloride. The absorbance of the assay cocktail was used to calculate H₂O₂ content as described previously.

STATISTICAL ANALYSES. Mean values were subjected to ANOVA using the General Linear Model (Minitab Inc., State College, Pa.).

Results

Trees were selected for presumed scald resistance and susceptibility based on preliminary storage trials (N.F. Weeden, unpublished data). Fruit maturity can influence scald susceptibility (Anet, 1972; Emongor et al., 1994; Huelin and Coggiola, 1968); therefore, any differences in the physiological and biochemical aspects of fruit from the selections could be due to the differences in their maturity. IEC can be used as a physiological determinant of apple maturity (Knee et al., 1983). To reduce variation in fruit maturity as a confounding factor in these experiments, only fruit selections with uniform IECs (10 to 20 μ L·L⁻¹) were used (Table 1).

After 16 weeks of storage, the maximum time for all chemical analyses, red-R fruit did not develop scald symptoms, whereas red-S and yellow-S susceptible fruit had a scald incidence of 70% and 100%, respectively (Table 2). After 20 weeks of storage, 100% of red-S and yellow-S fruit exhibited severe scald symptoms (70% to 80% of the total surface area). At this time, two replications of the red-R fruit were still free of scald. However, 12% of the red-R selection no. 17 developed scald symptoms, although the severity of these symptoms was slight (<30% of the total surface area).

α -FARNESENE AND CTs. At harvest, the levels of α -farnesene and CTs (CT258 and CT281) of red-R fruit were significantly lower ($p < 0.001$) compared to red-S and yellow-S fruit (Table 2). During storage, α -farnesene and CTs increased in resistant and susceptible populations ($p < 0.001$), but the increases were much greater in resistant fruit. After 16 weeks of storage, α -farnesene, CT258,

and CT281 concentrations of red-R fruit increased by 444%, 281%, and 319%, respectively, compared with those at harvest (Table 2). During the 16 weeks of storage, α -farnesene concentrations in red-S and yellow-S fruit increased 145% and 68%,

Fig. 1. Peroxide levels (nmol·g⁻¹) (fresh mass basis) at harvest and during storage at 0.5 °C for 16 weeks of different apples from selections derived from a cross between 'White Angel' x 'Rome Beauty'. Error bars indicate SE. Effects of fruit type and storage time and the interaction of fruit type x storage time were significant at $p < 0.001$, 0.001, or 0.006, respectively.

Fig. 2. Lipid peroxidation (nmol·g⁻¹ TBARS, fresh mass basis) at harvest and during storage at 0.5 °C for 16 weeks of different apples from selections derived from a cross between 'White Angel' x 'Rome Beauty'. Error bars indicate SE. Effects of fruit type and storage time and the interaction of fruit type x storage time were each significant at $p < 0.001$.

respectively, while CT258 species increased 58% and 24%, respectively (Table 2). Similarly, CT281 species increased by 86% and 34%, respectively, in red-S and yellow-S fruit during storage. CT258/CT281 ratios were similar in all the fruit types (Table 2).

PEROXIDE CONTENT, LIPID PEROXIDATION, AND OXIDATIVE DAMAGE TO PROTEINS. Peroxide levels and lipid peroxidation were monitored at harvest and during storage to assess changes in production of AOS and subsequent damage to cell membranes. Even at harvest, H_2O_2 levels and lipid peroxidation of both scald-susceptible groups were significantly higher ($p < 0.001$) than the scald-resistant group (Figs. 1 and 2). Peroxide levels and lipid peroxidation in resistant fruit did not increase significantly over the 16-week storage period. In contrast, H_2O_2 levels for red-S and yellow-S fruit stored for 16 weeks increased 65% and 84%, respectively, relative to harvest levels (Fig. 1). Similarly, lipid peroxidation in red-S and yellow-S fruit increased by 69% and

92%, respectively, compared with harvest values (Fig. 2).

At harvest, carbonyl groups were similar in all fruit types (Fig. 3). Carbonyl group content in red-S and yellow-S increased during storage, although the increase was only 28% and 39%, respectively, compared with harvest values. No major changes were observed in the carbonyl group content of red-R fruit during storage (Fig. 3).

ENZYME ACTIVITY. SOD activity was similar in all three fruit types at harvest, and during storage increased between 36% and 39% over activities at harvest (Fig. 4).

At harvest, POX and CAT activities of red-R fruit were markedly higher than that of red-S and yellow-S fruit (Figs. 5 and 6). Although POX activity declined during storage, the activities of red-R fruit were 400% and 1800% higher than red-S and green-S fruit, respectively (Fig. 5). CAT activity declined significantly during storage only in scald-susceptible fruit (Fig. 6). By the end of the 16-week storage period, CAT activity of red-R fruit was 500% and 1500% higher than that of red-S and yellow-S fruit, respectively (Fig. 6).

Discussion

Scald development is hypothesized to result from the action of CT species produced by oxidation of α -farnesene on cellular membranes, which ultimately causes cell death (Anet, 1972; Huelin and Coggiola, 1970a, 1970b). The hypothesis is based on the determination of a close relationship between the accumulation of CTs, particularly CT281, and the occurrence of scald (e.g., Anet and Coggiola, 1974; Huelin and Coggiola, 1970b; Meir and Bramlage, 1988). Du and Bramlage (1993, 1994) found that CT281 concentrations were correlated positively with scald development, while those of CT258 were negatively correlated. Moreover, high and low ratios of CT258/CT281 were, respectively, negatively and positively associated with scald development. Therefore, if CT production during apple storage is associated with scald susceptibility, then low CT258, high CT281, and low CT258/CT281 ratios would be expected in scald-susceptible fruit. However, in the selections investigated, CT258 and CT281 species were significantly enhanced, and the CT258/CT281 ratio remained the same in all fruit types (Table 2). Further, there was little relationship between CT258 ($r = 0.69$), CT281 ($r = 0.40$), or the CT258/CT281 ratio ($r = 0.00$) with scald incidence. In addition, in separate experiments we found that the relationship between the CT258/CT281 ratio and scald incidence is convincing for 'Cortland' but not for 'Delicious' or 'Law Rome' (T.F. Alwan and C.B. Watkins, unpublished data), indicating that cultivar effects on such relationships may be significant.

Recently, Rowan et al. (1995) and Whitaker et al. (1997) have demonstrated that the bulk of conjugated trienes produced in vivo are two isomers of 2,6,10-trimethyldodeca-2,7,9,11-tetraen-6-ol. Limitations of the use of CTs from crude hexane extracts to understand the processes underlying the development of scald are apparent because of the possible presence of interfering UV compounds. Nevertheless, we believe data on CT281 and CT258 are still relevant to scald physiology, as they appear to indicate the changes in other metabolic processes during disorder development. In this light, it is interesting that CT258 may be associated with an antioxidant phenolic in apple peel (B.D. Whitaker, personal communication).

Plant cells exposed to low temperatures often have enhanced production of AOS such as O_2^- and H_2O_2 (Pinhero et al., 1997; Prasad et al., 1994; Purvis and Shewfelt, 1993). It is believed that these AOS, either directly or indirectly by generating other highly

Fig. 3. Carbonyl groups (nmol·g⁻¹) (protein basis) at harvest and during storage at 0.5 °C for 16 weeks of different apples from selections derived from a cross between 'White Angel' x 'Rome Beauty'. Error bars indicate SE. Effects of fruit type and storage time and the interaction of fruit type x storage time were significant at $p < 0.001$, 0.001, or 0.009, respectively.

Fig. 4. Superoxide dismutase (SOD) activities (U/mg) (protein basis) at harvest and during storage at 0.5 °C for 16 weeks of different apples from selections derived from a cross between 'White Angel' x 'Rome Beauty'. Error bars indicate SE. Effects of fruit type and storage time were each significant at $p < 0.001$, while the interaction of fruit type x storage time was not significant.

reactive AOS such as $\text{OH}^\cdot$, disrupt cellular organization and, thereby, metabolism. Apples are generally stored at low temperature, and scald development exhibits many characteristics similar to chilling injury (Watkins et al., 1995). Du and Bramlage (1995a) hypothesized that low-temperature-induced generation of AOS is responsible for the development of physiological disorders such as scald.

The ability of plant tissues to maintain low pool levels of H_2O_2 may be related to preventing lipid peroxidation (Prasad et al., 1994) and carbonyl group formation (Levine et al., 1990; Rao et al., 1996). Interestingly, in our study, lipid peroxidation was significantly higher in all fruit types when compared with protein damage, regardless of scald susceptibility, suggesting that membranes may be a primary site of damage during senescence and ripening. In several other systems, plant membranes have been shown to be highly sensitive to oxidative stress compared with plant proteins (Levine et al., 1990; Prasad et al., 1994; Rao et al.,

1996). Fruit resistant to scald did not accumulate H_2O_2 (Fig. 1), but the CT258 and CT281 species were significantly higher in these fruit than in susceptible fruit (Table 2), which argues against the suggestion that increased production of CTs perturb cellular membranes (Du and Bramlage, 1994). These results suggest that resistant fruit may have acquired additional mechanisms to tolerate the increased CT production if CTs are involved in damaging events related to scald development.

Peroxide levels in plant cells are regulated by coordination between the enzymes such as SOD, which generate H_2O_2 , and enzymes such as POX and CAT, which degrade H_2O_2 (Creissen et al., 1994; Rao et al., 1996; Van Camp et al., 1994). Increased accumulation could be due to enhanced generation of H_2O_2 or decreased degradation of H_2O_2 (Allen, 1995; Foyer et al., 1994). Since significant accumulation of H_2O_2 occurred in scald susceptible fruit, the activities of different enzymes that generate and degrade H_2O_2 were assessed.

Changes in SOD activities of scald-resistant and scald-susceptible fruit during storage were almost identical (Fig. 4). Thus, storage of apples at low temperature may enhance the production of $\text{O}_2^\cdot$ species, which, in turn, are dismutated to H_2O_2 by SOD. In spite of the significant increase in SOD activity, however, H_2O_2 levels remained unaltered in red-R fruit (Fig. 1), indicating that H_2O_2 generated by SOD was perhaps more efficiently removed by H_2O_2 -degrading enzymes than it was in susceptible fruit. Supporting this view, the activities of POX and CAT of red-R fruit were significantly higher at harvest and during subsequent storage compared with those of red-S and yellow-S fruit (Figs. 5 and 6). A constitutive presence of higher POX and CAT activities may be associated with a greater ability of scald-resistant fruit to metabolize H_2O_2 and thereby to minimize damage to cellular membranes and proteins. Thus, higher POX and CAT activities could be one mechanism involved in resistance of fruit to scald.

Scald-resistant fruit accumulated CTs during storage, while scald-susceptible fruit had already accumulated significant amounts of CTs and peroxides by the harvest stage (Table 2, Fig. 1). This observation raises some interesting possibilities. The greater ability of red-R fruit to minimize the production of AOS may develop only during long-term storage of fruit under low temperature. On the other hand, damaging events may have already been initiated in susceptible fruit by the harvest stage with increased production of AOS coupled with a lesser capacity to metabolize them. Storage under low temperature may have enhanced these damaging events further, resulting in scald symptoms.

By using a sibling population segregating for resistance and susceptibility to scald, we have demonstrated that increased efficiency to metabolize AOS may enhance resistance to scald. We believe that this is the first report to show clearly the relative importance of antioxidant-based defense systems in conferring resistance to a physiological disorder such as scald. Collectively, the data indicate that scald is genetically determined, and resistance to scald might be enhanced by modifying the ability of apples to metabolize AOS.

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Fig. 5. Guaiacol-peroxidase (POX) activities ($\mu\text{mol} \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$) (protein basis) at harvest and during storage at 0.5°C for 16 weeks of different apples from selections derived from a cross between 'White Angel' $\times$ 'Rome Beauty'. Error bars indicate SE. Effects of fruit type and storage time were each significant at $p < 0.002$, while the interaction of fruit type $\times$ storage time was not significant.

Fig. 6. Catalase (CAT) activities ($\mu\text{mol} \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$) (protein basis) at harvest and during storage at 0.5°C for 16 weeks of different apples from selections derived from a cross between 'White Angel' $\times$ 'Rome Beauty'. Error bars indicate SE. Effects of fruit type and storage time and the interaction of fruit type $\times$ storage time were significant at $p < 0.001$.

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