JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Peroxidase Activity and Superficial Scald Development in Apple Fruit

J. Pablo Fernández-Trujillo,[†,#] Jacqueline F. Nock,[†] Eugene M. Kupferman,[‡] Susan K. Brown,[§] and Christopher B. Watkins*[,†]

Department of Horticulture, Cornell University, Ithaca, New York 14853; Tree Fruit Research Center, Washington State University, 1100 North Western Avenue, Wenatchee, Washington 98801; and Department of Horticultural Science, Cornell University, Geneva, New York 14456

The relationship between soluble peroxidase (EC 1.11.1.7; POX) activity and the development of a chilling-related disorder, superficial scald, was studied in three apple fruit (*Malus* × *domestica* Borkh.) systems: a White Angel × Rome Beauty population with progeny with different scald susceptibilities; Delicious from three harvests with progressively declining scald susceptibility; and the scald-resistant Idared and the scald-susceptible Law Rome. Differences in incidence and severity of scald in progeny from White Angel × Rome Beauty progeny tended to show relationships with POX activity at harvest, but, overall, associations were not consistent. However, greater scald incidence and lower POX activity were found in less mature Delicious fruit than in later harvested fruit. Also, the scald-resistant Idared had a much higher POX activity compared with the scald-susceptible Law Rome. A general hypothesis that POX activity is related to scald susceptibility was generally supported, but exceptions were observed.

KEYWORDS: Apple; antioxidant enzymes; cold storage; cultivars; crabapple; *Malus* × *domestica*; peroxidase; superficial scald

INTRODUCTION

Production of active oxygen species (AOS) such as O_2^- and H_2O_2 is a continuous process in all respiring cells. These species are relatively unreactive by themselves, but together they form the hydroxyl radical (OH^-) and singlet oxygen ($^1O_2$), the most reactive species in chemistry. Unless these products are metabolized, they can react indiscriminately to cause lipid peroxidation, protein denaturation, and DNA mutation (*1*, *2*). Under normal conditions AOS are maintained below damaging levels by the activity of antioxidant enzymes such as catalase (CAT; EC 1.11.1.6), peroxidase (POX; EC 1.11.1.7), ascorbate peroxidase (EC 1.11.1.11), superoxide dismutase (SOD; EC 1.15.1.1), and glutathione reductase (EC 1.6.4.2) (*3*).

The elevation of AOS in plant tissues subjected to stress, such as low temperatures (*4*, *5*), is thought to be involved in plant susceptibility to chilling injury and death. Most research on chilling has focused on AOS and related metabolism of growing plants (*4*, *6*, *7*), but increasing evidence suggests that AOS metabolism may be involved in the development of chilling injury in fruit during storage (*8*).

A serious chilling-related injury of many apple cultivars is superficial scald, a physiological disorder that develops in the skin during prolonged low-temperature storage (*9*). Damage appears as browning of the skin that results from damage to hypodermal cells (*10*). The browning may develop during cold storage but usually is expressed after fruit are warmed to ambient temperatures. Scald susceptibility is influenced greatly by cultivar: Gala, Empire, and Idared tend to be scald resistant, whereas others, Cortland, Delicious, McIntosh, Granny Smith, and Law Rome, tend to be scald susceptible, suggesting that scald may be genetically determined. Scald susceptibility was segregated in a seedling population from a cross between Rome Beauty, a dessert apple that is highly susceptible to scald, and White Angel, a crabapple that is resistant to scald (*11*). Maturity is another critical factor in scald susceptibility, with more mature fruit usually having lower scald susceptibility than less mature ones (*12*, *13*).

Although the precise oxidative events that occur during scald development are not understood well, the generally accepted hypothesis of scald development is that the sesquiterpene, α-farnesene, is oxidized in the fruit peel, producing several conjugated trienol (CT) species including the triene hydroperoxide radical (*12*, *14*−*16*). A close relationship between scald incidence and accumulation of CT species is often, but not always, found, suggesting that other factors may be involved (*17*, *18*). These include antioxidative factors that may inhibit α-farnesene oxidation. Scald may not occur if antioxidant

* Author to whom correspondence should be addressed [telephone (607) 255-1784; fax (607) 255-0599; e-mail cbw3@cornell.edu].
† Department of Horticulture, Cornell University.
# Present address: Department of Agricultural and Food Engineering, Technical University of Cartagena, Paseo Alfonso XIII 48, 30203 Cartagena (Murcia), Spain.
‡ Tree Fruit Research Center, Washington State University.
§ Department of Horticultural Science, Cornell University.

10.1021/jf034079d CCC: $25.00
Published on Web 10/25/2003

concentrations remain adequate to prevent, or sufficiently limit, the extent of α-farnesene oxidation (*19*). Du and Bramlage (*20*, *21*) were not able to demonstrate relationships between the activities of SOD, CAT, or POX and scald susceptibility. However, disorder development in the susceptible fruit was low, making comparisons difficult. In contrast, high activities of POX and CAT, but not SOD, were related to lower scald susceptibility in selections of a Rome Beauty × White Angel population (*17*). These results suggested that POX activity in the peel of fruit at harvest could be a predictor of scald susceptibility. POX is an oxidoreductase enzyme that decomposes H_2O_2 by oxidation of cosubstrates such as phenolic compounds and/or antioxidants (*22*). Increased POX activity, and/or enhancement or synthesis of its isoenzymes, has been shown to occur in response to stresses, such as wounding (*23*), ozone or ultraviolet-B light (3), and mechanical injury (*24*), or in relation to the development of internal browning in peaches (*25*). The objective of this study was to test the hypothesis that POX activity in the fruit peel was related to scald susceptibility.

MATERIALS AND METHODS

Plant Material. Three fruit systems were used in these experiments.

1. Progeny from a White Angel × Rome Beauty Population with different scald susceptibilities were grown at the New York State Agricultural Experimental Station, Geneva, NY (*11*). On a single harvest date, 200−300 fruits from the tree of each of 45 selections were harvested and transported to the postharvest laboratory in Ithaca, NY, for assessment of maturity. The remaining fruits were stored at 0.5 °C for 21 weeks. Eight red-skinned (no. 1, 3, 7, 16, 20, 22, 48, and 85) and six yellow-skinned (no. 26, 28, 38, 55, and 65) selections were also sampled after 7, 14, and 21 weeks of storage.

2. Delicious. Three hundred fruits of the Starkrimson strain were harvested from trees at a single orchard at three 2-week intervals during September and October 1997 in Washington state and air-freighted to Ithaca, NY. Fruits were stored at 0.5 °C for 20 weeks. Fruits were also sampled at 10 weeks and after 1 week at 20 °C after 20 weeks of storage.

3. Idared and Law Rome. Three hundred fruits of each cultivar were harvested from mature trees growing at the Cornell University orchard in Lansing, NY. Fruits were stored at 0.5 °C for 8, 12, 16, and 20 weeks.

A minimum of 30 fruits of each selection or cultivar were used at harvest or immediately after removal from storage and peeled using a hand-held fruit peeler, and the peels were flash frozen in liquid N_2. The samples were stored at −80 °C until used for analysis.

Internal Ethylene Concentrations (IEC). At harvest, IEC were measured on 0.5 mL (progeny) or 1 mL (Delicious, Idared, Law Rome) samples of internal gas from the core of 10 fruits as described by Alwan and Watkins (*26*) except that the gas chromatograph used was a Hewlett-Packard 5890, series II (Hewlett-Packard Co., Wilmington, DE).

Scald Assessment. Scald incidence and severity were assessed after removal from cold storage plus 7 days at 20 °C. Incidence was recorded as percent of fruit with scald, whereas severity was assessed using a subjective scale where 0 = 0%, 1 = 1−10%, 2 = 11−33%, 3 = 34−66%, and 4 = 67−100% of the surface area affected (*9*).

Tissue Extraction. Ten grams of frozen tissue was ground to a fine powder under liquid N_2 and homogenized with 20 mL of 200 mM potassium phosphate buffer (pH 7.8) containing 2 mM ethylenediaminetetraacetic acid (EDTA), 1% polyvinylpyrrolidone (PVP)-40, and 1 mM phenylmethanesulfonyl fluoride (PMSF). The homogenate was filtered through four layers of cheesecloth and centrifuged at 25000*g* (30 min at 0−4 °C). Each extraction was carried out in quadruplicate. All procedures were conducted under ice. The supernatants were stored in aliquots at −80 °C.

Protein Concentration. The protein content of each sample was determined in triplicate according to the method of Bradford (*27*) using bovine serum albumin (BSA) as a standard.

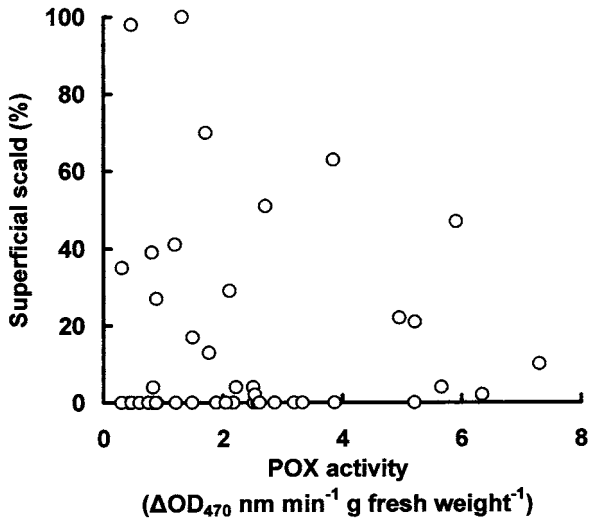

Figure 1. Percentage of fruit affected with superficial scald plotted against POX activity in apple peel from 45 White Angel × Rome Beauty selections.

Peroxidase Activity. POX activities were measured using guaiacol as a substrate following the method described by Rao et al. (*17*). The assay mixture (3 mL) contained 100 mM phosphate buffer (pH 6.5), 16 mM guaiacol, and 100 μL or less of protein extract at 22 °C, and the reaction was initiated by adding 10 μL of 30% H_2O_2. Changes in absorbance of guaiacol at 470 nm were recorded every 10 s for 5 min with a spectrophotometer (model DU 7600; Beckman Instruments, Fullerton, CA).

All enzyme activities were assayed using equal amounts of tissue protein (10 μg for Delicious, 20 μg for Idared and Law Rome), different protein concentrations being based on the reaction rates. In the selections, 500 μL of crude extract was used because of the lower protein concentrations. Each replicate was assayed at least in triplicate. Enzyme activity rates were proportional to protein concentrations, and appropriate controls were used throughout the experiments. Activities are expressed on a fresh weight basis.

Statistical Analysis. Data were subjected to analysis of variance using the general linear model procedure for calculation of least-squared means and least significant differences (LSDs; $P = 0.05$). Data from Delicious were transformed by the natural logarithms; storage time and harvest time were considered as factors. The relationship between scald percentage or score and POX activity was plotted in the selections, and Pearson multiple correlation coefficients and significance were calculated.

RESULTS

White Angel × Rome Beauty Progeny. Of the 45 selections harvested, 20 did not develop scald, 7 had fewer than 10% fruit with scald, and 15 were highly susceptible to scald. Scald percentage and scald severity were highly correlated ($R = 0.856$, $P < 0.0001$). A general trend between scald incidence and POX activity in the peel of selections at harvest was observed either on a fresh weight basis (**Figure 1**) or on a protein basis (data not shown). However, the overall correlation between the two factors was poor (−0.23, $P = 0.15$). The range of scald incidence was particularly large in fruit with the lowest POX activities. More mature fruit might be less susceptible to scald development, and therefore we considered the possibility that differences between scald incidences in some selections could be a function of maturity differences. However, even when fruit IEC was taken into account, we could not detect better relationships between scald incidences at a similar POX activity (data not shown).

The activities of POX in several red- and yellow-fruited selections during storage were measured (**Figure 2**). Two red-

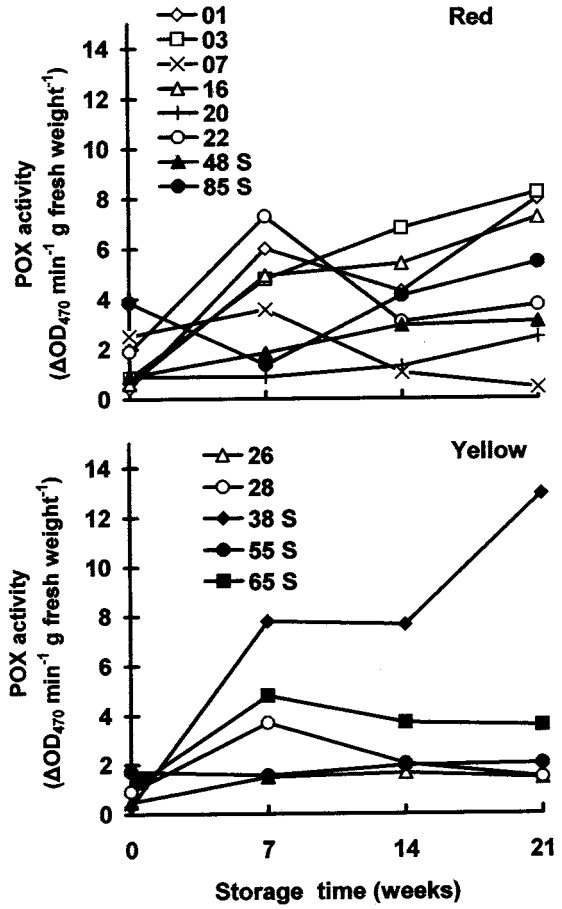

Figure 2. POX activity in apple peel of scald-susceptible (solid symbols) and scald-resistant (open symbols) of red and yellow White Angel × Rome Beauty selections stored for up to 21 weeks at 0.5 °C.

and three yellow-fruited selections developed scald during storage, with incidences of 27 and 63% in reds 48 and 85, respectively, and 35, 70, and 100% in yellows 38, 55, and 65, respectively.

In the red-fruited selections, POX activities showed no patterns of change consistent with scald resistance or scald susceptibility (**Figure 2**). On a protein basis, scald-resistant red selections generally had higher POX activities throughout the storage period than did scald-susceptible selections (data not shown). Increases in POX activity on a protein basis during storage were extremely high in selections 1 and 16 and were much greater than for the other apple systems described below. These increases were a function of the overall low extractable protein contents in these selections.

In the yellow selections, the highest POX activity was measured with a scald-susceptible selection (**Figure 2**), and no relationships between scald susceptibility and enzyme activity were evident. Changes in POX activity over time on a protein basis also were not related to scald susceptibility (data not shown).

Delicious. After 20 weeks of storage plus 1 week at 20 °C, fruits from harvests 1, 2, and 3 had 93, 57, and 16% scald, respectively. POX activity was lower in the least mature fruit (harvest 1) compared with the more mature fruit at harvests 2 and 3 (**Figure 3**). The lower activity in fruit from harvest 1 was maintained at 10 weeks of storage, but by 20 weeks there was no difference between fruits from harvests 1 and 2. POX activity of fruit from harvests 2 and 3 were similar except at 20 weeks, when activity in the harvest 3 samples increased. POX

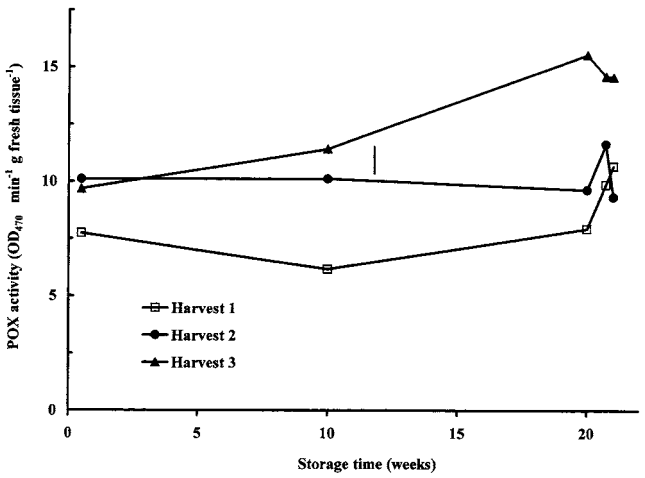

Figure 3. POX activity in apple peel of Delicious harvested at three different stages of maturity and stored for 20 weeks at 0.5 °C plus 1 week at 20 °C. Fruits were transferred to the warmer temperature at week 20. Bar represents LSD at $P = 0.05$ ($n = 3$) calculated for the significant harvest date effect using the natural logarithm of the POX activity data.

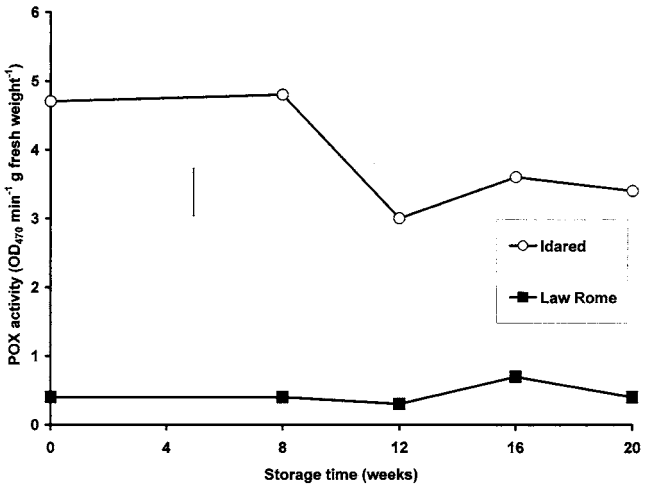

Figure 4. POX activity in apple peel of Idared and Law Rome cultivars stored for up to 20 weeks at 0.5 °C ($n = 3$). Bar represents LSD at $P = 0.05$ for the significant cultivar effect ($n = 3$).

activity during the 1-week period at 20 °C increased in fruit from harvest 3 but decreased in fruit from the other harvests.

Changes in POX activities on a protein basis were similar to those shown on a fresh weight basis except that activity did not continue to increase during storage in fruit from harvest 3 (data not shown). Also, during the 1-week period at 20 °C, POX activity on a protein basis showed changes opposite to those found on a fresh weight basis.

Law Rome and Idared. Law Rome had 98% scald, whereas Idared had no detectable scald after 20 weeks of storage plus 7 days at 20 °C. POX activities in Idared peel tissues were similar at harvest and after 8 weeks of storage, declined between 8 and 12 weeks of storage, and then increased slightly (**Figure 4**). Activity of POX did not change greatly during storage of Law Rome, but overall activity was much lower than that measured in Idared. Patterns of change for POX activities were similar when expressed on a protein basis.

DISCUSSION

The observation that scald-resistant selections from a White Angel × Rome Beauty cross had higher POX activities at

harvest and during storage (*17*) suggested that measurements of this enzyme might be a useful indicator of scald risk of harvested apples. Therefore, we tested this hypothesis using three systems.

In the first, the White Angel × Rome Beauty cross, the use of a much greater number of selections, 45 compared with 8 in the study of Rao et al. (*17*), did not strongly support a view that higher POX activities are closely related with lower scald development in fruit during storage. Although there was a tendency for the scald incidence of individual selections to decrease as POX activities increased, many anomalies between the two factors were observed. Scald incidences ranged from 0 to 100% in selections with the lowest POX activities. In the original study, selections were chosen on the basis of IECs to ensure similar fruit maturities. Ripeness may affect the activities of POX in apple (*28*, *29*) and other fruit (*30*, *31*), and, therefore, differences in fruit maturity at harvest could have influenced the relationships between the two factors.

In the second system, POX activities were measured in the scald-susceptible cultivar, Delicious, from sequential harvests. POX activity was lower in fruit from harvest 1 with the highest scald incidence, but while scald incidences declined from 57 to 16% between harvests 2 and 3, differences in POX activity were relatively small until late in storage. In contrast, the third system showed that the POX activity of a scald-resistant cultivar, Idared, was markedly higher than that of the scald-susceptible Law Rome. Although our results provide partial support for the hypothesis that POX activity is related to scald susceptibility, overall, our results, together with those in the literature (*21*), suggest that relationships can be inconsistent.

POX plays an important role in plant growth, development, and differentiation, catalyzing the oxidation of many substrates (e.g., phenolics, aromatic amines) using H_2O_2. Important physiological functions include those in lignification, removal of highly toxic reactive oxygen species, ethylene biosynthesis and pathogen defense, and polymer cross-linking. In apple, the soluble form of POX reached a peak of activity at about the midpoint of fruit softening, whereas the ionically bound forms decreased (*28*). The existence of POX isoenzymes has been demonstrated in apple fruit (*33*, *34*), and the occurrence of POX in several polymorphic forms and the high diversity of in vitro substrates make it difficult to postulate definitive physiological roles for POX. The activity of CAT also increases during ripening of apple fruit (*32*), but CAT metabolizes H_2O_2 to water and O_2 and does not need an additional hydrogen to donate substrate to dismutate H_2O_2.

In summary, although evidence of POX activity relationships to scald development has been provided by our study, these relationships were inconsistent among the different systems tested. The relationships are not strong enough to predict scald susceptibility or resistance in apple fruits. Research that has examined the interaction of other antioxidant enzymes with resistance of fruit to scald also has been inconclusive (*35*). A possible involvement of isoenzymes in this resistance, however, was indicated on that study, and further research into the possible role of antioxidant isoenzymes is continuing.

ABBREVIATIONS USED

POX, peroxidase; CAT, catalase; SOD, superoxide dismutase; AOS, active oxygen species; OH^-, hydroxyl radical; $^1O_2$, singlet oxygen; IEC, internal ethylene concentration; CT, conjugated trienol; EDTA, ethylenediaminetetraacetic; PVP, polyvinylpyrrolidone; PMSF, phenylmethanesulfonyl fluoride; BSA, bovine serum albumin; LSD, least significant difference.

ACKNOWLEDGMENT

We thank Dr. Mulpuri Rao for advice during this study.

LITERATURE CITED

(1) Elstner, E. F. Metabolism of activated oxygen species. In *Biochemistry of Metabolism: The Biochemistry of Plants*; Davies, D. D., Ed.; Academic Press: New York, 1987; Vol. 11, pp 253−315.

(2) Halliwell, B.; Gutteridge, J. M. C. *Free Radicals in Biology and Medicine*; Clarendon Press: Oxford, U.K., 1989.

(3) Rao, M. V.; Paliyath, G.; Ormrod, D. P. Ultraviolet-B- and ozone-induced biochemical changes in antioxidant enzymes of *Arabidopsis thaliana*. *Plant Physiol.* **1996**, *110*, 125−136.

(4) Anderson, M. D.; Prasad, T. K.; Stewart, C. R. Changes in isozymes profiles of catalase, peroxidase, and glutathione reductase during acclimation to chilling in mesocotyls of maize seedlings. *Plant Physiol.* **1995**, *109*, 1247−1258.

(5) Prasad, T. K.; Anderson, M. D.; Stewart, C. R. Acclimation, hydrogen peroxide, and abscisic acid protect mitochondria against irreversible chilling injury in maize seedlings. *Plant Physiol.* **1994**, *105*, 619−627.

(6) Horvath, E.; Janda, T.; Szalai, G.; Paldi, E. In vitro salicylic acid inhibition of catalase activity in maize: differences between the isozymes and a possible role in the induction of chilling tolerance. *Plant Sci.* **2002**, *163*, 1129−1135.

(7) Seppanen, M. M.; Fagerstedt, K. The role of superoxide dismutase activity in response to cold acclimation in potato. *Physiol. Plant.* **2000**, *108*, 279−285.

(8) Watkins, C. B.; Rao, M. V. Genetic variation of horticultural crops for resistance to oxidative stress induced by postharvest conditions, and prospects for genetic engineering. In *Postharvest Oxidative Stress in Horticultural Crops*; Hodges, M., Ed.; Hawthorn Press: Binghamton, NY, 2003; Chapter 10, pp 199−224.

(9) Watkins, C. B.; Bramlage, W. J.; Cregoe, B. A. Superficial scald of 'Granny Smith' apples is expressed as a typical chilling injury. *J. Am. Soc. Hortic. Sci.* **1995**, *120*, 88−94.

(10) Bain, J. M.; Mercer, F. J. The sub-microscopic cytology of superficial scald, a physiological disease of apples. *Aust. J. Biol. Sci.* **1963**, *16*, 442−449.

(11) Weeden, N. F. Genetic control of apple storage scald. *N. Y. Fruit Q.* **1993**, *Winter*, 12−13.

(12) Anet, E. F. L. J. Superficial scald, a functional disorder of stored apples. IX. Effect of maturity and ventilation. *J. Sci. Food Agric.* **1972**, *23*, 763−769.

(13) Bramlage, W. J.; Watkins, C. B. Influences of preharvest temperature and harvest maturity on susceptibility of New Zealand and North American apples to superficial scald. *N. Z. J. Crop Hortic. Sci.* **1994**, *22*, 69−79.

(14) Du, Z.; Bramlage, W. J. A modified hypothesis on the role of conjugated trienes in superficial scald development on stored apples. *J. Am. Soc. Hortic. Sci.* **1993**, *118*, 807−813.

(15) Huelin, F. E.; Coggiola, I. M. Superficial scald, a functional disorder of apples. VII. Effect of applied α-farnesene, temperature and diphenylamine on scald and the concentration and oxidation of α-farnesene in the fruit. *J. Sci. Food Agric.* **1970**, *21*, 584−589.

(16) Whitaker, B.; Solomos, T.; Harrison, D. J. Quantification of α-farnesene and its conjugated trienol oxidation products from apple peel by C18-HPLC with UV detection. *J. Agric. Food Chem.* **1997**, *45*, 760−765.

(17) Rao, M. V.; Watkins, C. B.; Brown, S. K.; Weeden, N. F. Active oxygen species metabolism in 'White Angel' × 'Rome Beauty' apple selections resistant and susceptible to superficial scald. *J. Am. Soc. Hortic. Sci.* **1998**, *123*, 299−304.

(18) Whitaker, B. D.; Nock, J. F.; Watkins, C. B. Peel tissue α-farnesene and conjugated trienol concentrations during storage of 'White Angel' × 'Rome Beauty' hybrid apple selections susceptible and resistant to superficial scald. *Postharvest Biol. Technol.* **2000**, *20*, 231−241.

(19) Anet, E. F. L. J. Superficial scald, a functional disorder of stored apples. XI. Apple antioxidants. *J. Sci. Food Agric.* **1974**, *25*, 299−304.
(20) Du, Z.; Bramlage, W. B. Superoxide dismutase activities in senescing apple fruit (*Malus domestica* Borkh.) *J. Food Sci.* **1995**, *59*, 581−584.
(21) Du, Z.; Bramlage, W. J. Peroxidative activity of apple peel in relation to development of poststorage disorders. *HortScience* **1995**, *30*, 205−209.
(22) Campa, A. Biological roles of plant peroxidases: Known and potential functions. In *Peroxidases in Chemistry and Biology*; Everse, J., Everse, K., Grisham, M. B., Eds.; CRC Press: Boca Raton, FL, 1991; Vol. II, pp 25−50.
(23) Ke, D.; Saltveit, M. E. Wound-induced ethylene production, phenolic metabolism and susceptibility to russet spotting in iceberg lettuce. *Physiol. Plant.* **1989**, *76*, 412−418.
(24) Miller, A. R.; Kelley, T. J. Mechanical stress stimulates peroxidase activity in cucumber fruit. *HortScience* **1989**, *24*, 650−652.
(25) Stutte, G. W. Quantification of net enzymatic activity in developing peach fruit using computer video image analysis. *HortScience* **1989**, *24*, 113−115.
(26) Alwan, T. F.; Watkins, C. B. Intermittent warming effects on superficial scald development of 'Cortland', 'Delicious' and 'Law Rome' apple fruit. *Postharvest Biol. Technol*. **1999**, *16*, 203−212.
(27) Bradford, M. M. A rapid assay for the quantification of microgram quantities of protein utilizing the principle of protein−dye binding. *Anal. Biochem.* **1976**, *72*, 248−254.
(28) Ingham, L. M.; Parker, M. L.; Waldron, K. W. Peroxidase: Changes in soluble and bound forms during maturation and ripening of apples. *Physiol. Plant.* **1998**, *102*, 93−100.
(29) Gorin, N.; Heidema, F. T. Peroxidase activity in Golden Delicious apples as a possible parameter for ripening and senescence. *J. Agric. Food Chem.* **1976**, *24*, 200−201.
(30) Haard, N. F. Upsurge of particular peroxidase in ripening banana fruit. *Phytochemistry* **1973**, *12*, 555−560.
(31) Marin, M. A.; Cano, M. P. Patterns of peroxidase in ripening mango *Mangifera indica* L. fruits. *J. Food Sci.* **1992**, *57*, 690−692.
(32) Masia, A. Superoxide dismutase and catalase activities in apple fruit during ripening and post-harvest and with special reference to ethylene. *Physiol. Plant.* **1998**, *104*, 668−672.
(33) Moulding, P. H.; Grant, H. F.; McLellan, K. M.; Robinson, D. S. Heat stability of soluble and ionically bound peroxidases extracted from apples. *Int. J. Food Sci. Technol.* **1987**, *23*, 391−397.
(34) Moulding, P. H.; Singleton, D. E.; McLellan, K. M.; Robinson, D. S. Purification and heat stability of Cox's apple pulp peroxidase isoezymes. *Int. J. Food Sci. Technol.* **1988**, *23*, 343−351.
(35) Kochhar, S.; Conklin, P. L.; Brown, S. K.; Watkins, C. B. A quantitative and qualitative analysis of antioxidant enzymes in relation to susceptibility of apple fruit to superficial scald. *J. Am. Soc. Hortic. Sci.* **2003**, *128*, 910−916.

Received for review January 27, 2003. Revised manuscript received September 18, 2003. Accepted September 18, 2003. This research was supported by Research Grant Award US-3165-99R from BARD, the New York Apple Research and Development Program, and federal formula funds, Regional Project NE103. J.P.F.-T. was supported by a postdoctoral grant (Séneca Foundation, Región de Murcia, Spain).

JF034079D