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Influence of Ethylene Action, Storage Atmosphere, and Storage Duration on Diphenylamine and Diphenylamine Derivative Content of Granny Smith Apple Peel

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The application of diphenylamine (DPA) to prevent the apple peel disorder superficial scald can result in accumulation of a number of DPA derivatives resulting from *C*-nitration, *C*-hydroxylation, *O*-methylation, and *N*-nitrosation during fruit storage. As the presence of these compounds may be indicative of metabolic processes leading to superficial scald development, the contents of DPA and DPA derivatives were determined in fruits treated at harvest with DPA or DPA plus the ethylene action inhibitor 1-methylcyclopropene (1-MCP), which also prevents scald development. Influences of fruit maturity, storage environment, storage duration, and a 14 day poststorage ripening period on accumulation of DPA metabolites were also assessed. Poststorage ripening, 1-MCP treatment, and controlled atmosphere storage had varied effects on DPA derivative contents suggesting that reactive oxygen and nitrogen species, such as •OH, •NO, and •NO_2, or enzyme-catalyzed reactions may be present during certain ripening and senescence-related physiological processes. Definitive correlations between superficial scald incidence and contents of specific derivatives were not observed.

KEYWORDS: ***Malus sylvestris* var. *domestica* (Borkh.) Mansf; apple; superficial scald; diphenylamine; LC-MS; antioxidant**

INTRODUCTION

Diphenylamine (DPA) is a diarylamine antioxidant used in a variety of applications, including control of a physiological storage disorder of apple fruits called superficial scald or "scald" (*1*). For this purpose, 1−2 g L^{-1} DPA is typically applied to fruits as an aqueous emulsion prior to cold storage.

Apples that are susceptible to scald can develop brown patches under the peel surface resulting from the death of the first 5−6 cell layers of the hypodermis after 2−4 months of cold storage or following removal from storage (*2*, *3*). Scalded peels can attain a sunken appearance as the severity of the disorder increases (*2*). Scald incidence and severity are affected by cultivar susceptibility, fruit maturity, preharvest environment, fruit mineral content, light exposure, storage conditions, and ethylene levels in storage (*4*). Oxidation of the sesquiterpene α-farnesene, forming various conjugated trienes and 6-methyl-5-hepten-2-one (*5*), purportedly plays a role in scald development; therefore, the prevention of α-farnesene oxidation using antioxidants may directly lead to scald control (*6*, *7*). Treatment with ethylene synthesis inhibitors, such as aminoethoxyvinylglycine (*8*), or ethylene action inhibitors including diazocyclopentadiene (*9*) and 1-methylcyclopropene (1-MCP) (*10*) greatly reduces scald incidence and the content of α-farnesene and conjugated trienes. Controlled atmosphere (CA) storage also reduces peel α-farnesene contents as well as scald incidence (*11*).

DPA may halt oxidation cascades that lead to conjugated triene and 6-methyl-5-hepten-2-one formation by providing its amino hydrogen for abstraction as described by Boozer and Hammond (*12*). A similar mechanism for DPA-mediated control of lipid oxidation occurs in red blood cells (*13*). In contrast, DPA-mediated smokeless gun powder and propellant stabilization are purportedly consequences of direct interactions with •NO and •NO_2 (*14*). These interactions form various *N*-nitrosated and *C*-nitrated DPA derivatives, including *N*-nitrosodiphenylamine (NODPA), 2-nitrodiphenylamine (2NO2DPA), and 4-nitrodiphenylamine (*14*, *15*).

DPA treatment of apple fruits followed by cold storage also results in DPA derivative production, including 4-hydroxydiphenylamine (4OHDPA), 3-hydroxydiphenylamine (3OHDPA), 2-hydroxydiphenylamine (2OHDPA), 2,4-dihydroxydiphenylamine, indophenol (*16*), NODPA (*17*), 4-methoxydiphenylamine (4MeODPA), and 3-methoxydiphenylamine (3MeODPA) (*18*). *C*-hydroxylated derivatives can undergo glycosidic conjugation (*16*). A similar metabolism of DPA has been reported in mammals (*19*). The mechanism of *C*-hydroxylated species production has not been characterized in apple fruits but may result from interaction with •OH (*20*, *21*) or possibly catalysis by native,

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nonspecific oxygenases similar to those in other biological systems (*22*), although no unmodified cycloogenase has been shown to catalyze this reaction with DPA. Methoxylated derivatives result from *O*-methylation of hydroxylated DPA derivatives by way of an unresolved mechanism (*23*). The mechanisms for NODPA may be similar to interactions with $^{\bullet}NO$ and $^{\bullet}NO_2$ reported in smokeless powder.

Whether DPA confers scald control through interactions leading to DPA derivative production is unknown. The presence of $^{\bullet}OH$, a destructive reactive oxygen species (ROS), and $^{\bullet}NO$, a reactive nitrogen species associated with signal mediation, have been well-documented in plants (*24*, *25*). Hydroxyl radicals, which can react with DPA to form 4OHDPA, are widely believed to be a major initiator of lipid radical cascades in many biological systems. However, the main DPA derivative formed during apple storage, 4OHDPA, and DPA provide similar levels of scald control suggesting that the hydroxylation reaction and the presence of a functional group in the para position are not related to the mechanism by which DPA ameliorates scald (*23*). Conversely, occupation of the meta, ortho, and amino positions inhibits scald protection, emphasizing the relevance of amino hydrogen donation and subsequent resonance stabilization of the diphenylamidogen radical in this process (*23*).

Characterization of derivative formation over the storage and poststorage ripening period as well as following various treatments known to affect scald incidence may provide additional mechanistic insight into the scald development process. DPA metabolism and scald incidence in Granny Smith apple peel as affected by pre- and postharvest fruit maturity, storage environment, storage duration, and ethylene responsiveness were evaluated for this purpose.

MATERIALS AND METHODS

Apple Fruit Treatments. Granny Smith apples [*Malus sylvestris* var. *domestica* (Borkh.) Mansf] were harvested September 7, October 18 (commercial harvest), and November 1, 2002 in a commercial orchard near Wenatchee, WA. The internal ethylene concentration (IEC) was measured on a subsample of 18 fruits on the day of harvest. Ethylene analysis was performed by withdrawing 1 cm^3 gas from the core of each fruit and injecting the sample into a Hewlett-Packard 5880 gas chromatograph (Agilent, Palo Alto, CA) equipped with a 46 cm (length) × 0.32 cm (diameter) glass column packed with Porapack PQ and a flame ionization detector. Flow rates for N_2 carrier, H_2, and compressed air makeup gas were maintained at 30, 10, and 100 mL min^{-1}, respectively. Oven, injector, and detector temperatures were 60, 100, and 200 °C, respectively. Treatments were as follows: control, 1 μL L^{-1} 1-MCP, 2 g L^{-1} DPA, and 1 μL L^{-1} 1-MCP plus 2 g L^{-1} DPA. 1-MCP [0.14% (w/v), EthylBloc formulation, Floralife, Inc., Walterboro, SC] was applied as outlined by Fan and Mattheis (*26*). Fruits were submerged in a commercially formulated DPA emulsion (Shield 31% DPA, Pace International, Seattle, WA) for 1 min. Fruits exposed to 1-MCP for 12 h immediately following harvest were treated with DPA the following day. The scald incidence was evaluated, and peel samples were collected after 0, 7, and 14 days at 22 °C following harvest or removal from storage. All fruits were stored at 1 °C in air for 1, 2, 4, and 6 months or a 1 kPa O_2/1 kPa CO_2 CA for 6 months. Fruit peels were collected using a potato peeler to compile three samples (six fruits/sample) for each treatment. Peel samples were frozen using N_2(l) and stored at −80 °C until extraction and analysis of DPA derivatives.

Storage chamber atmospheres were established within 60 h after harvest and monitored at 90 min intervals (Techni-Systems, Chelan, WA.). Semistatic chamber atmospheres (purged only when the atmosphere composition was adjusted) were maintained with N_2 generated from a membrane system (Permea, St. Louis, MO), compressed air, and CO_2.

Extraction of DPA and DPA Derivatives from Frozen Apple Peel. DPA and DPA derivatives were extracted by adding 50 mL of methanol and 200 μL of 0.282 mM *p*-isobutylhydratropic acid in methanol (internal standard) to approximately 25 g of frozen peel tissue and immediately homogenizing the mixture. After 20 min, the homogenate was vacuum filtered through Whatman #2 paper and the macerate was washed twice with 50 mL of 80:20 methanol/dH_2O. The methanol was evaporated from the filtrate using a rotary evaporation apparatus in a water bath at 34 °C, acidified with acetic acid to approximately 0.4% (v/v), and partitioned three times with 50 mL of chloroform. The epiphase was discarded, the chloroform fraction was evaporated using rotary evaporation, and the residue was dissolved in 8 mL of methanol. When fully dissolved, 2 mL of deionized water was added. Relatively nonpolar constituents were removed by passing this solution through a C_{18} cartridge (Sep-pak, Waters, Milford, MA). Following the addition of 70 mL of deionized water to the eluate, the extract was again acidified by adding acetic acid to 0.4% (v/v) and the analyte was collected on another C_{18} cartridge. The analyte was eluted from the cartridge using 5 mL of 2-propanol, dried using rotary evaporation, and dissolved in 200 μL of methanol.

Reversed Phase High-Performance Liquid Chromatography (HPLC)−Ultraviolet/Visible (UV/Vis)−Atmospheric Pressure Chemical Ionization Mass Spectrometry (APCI-MS) Conditions. Samples were analyzed by injecting 0.5−10 μL into a Series 1100 HPLC system (Agilent Technologies) controlled by Chemstation software (A.09.03) and equipped with a 5 μm Agilent Hypersil ODS (4.0 mm × 125 mm) reverse phase column, a G1315B diode array detector (DAD), and a G1946D single quadrupole mass selective detector (MSD) using an APCI source. Elution solvents used for a linear gradient were (A) 50:49.8:0.2 and (B) 99.8:0:0.2 methanol/deionized water/formic acid. The column temperature and mobile phase flow rate were 20 °C and 0.5 mL min^{-1}, respectively. Immediately following injection, the mobile phase was comprised entirely of solvent A for 2 min, followed by a linear gradient of solvent A plus B (starting with A) until reaching 100% B at 25 min, and then remaining entirely solvent B until 30 min. The eluate was first analyzed by the DAD and then the MSD. The DAD was adjusted to continuously monitor and record spectra (230−700 nm) for the entire analysis.

The APCI spray chamber conditions were as follows: N_2 drying gas flow, 5 L min^{-1}; drying gas temperature, 350 °C; nebulizer pressure, 414 kPa; vaporizer temperature, 425 °C; and coronal discharge, 4 μA. The fragmentor and capillary potentials were maintained at 130 and 4000 V, respectively. The MSD was adjusted to monitor positive ions in the scanning mode, continuously monitoring and recording entire mass spectra within a 100−1000 *m*/*z* range, or selective ion monitoring mode when compounds were identified and increased sensitivity was required.

Positive identification was achieved through comparison of extract constituents with the UV−vis and/or mass spectra of authentic standards as well as column retention comparisons. Quantification of constituents was performed by comparison with known amounts of authentic standards added to the same volume (as added to each sample) of *p*-isobutylhydratropic acid, the internal standard. Areas from the 4OHDPA and the smaller *N*-phenyl-4-quinoneimine peaks were compiled for quantification (*18*).

Analytical Standards. DPA, 3MeODPA, 2NO2DPA, and *p*-isobutylhydratropic acid were purchased from Sigma-Aldrich (St. Louis, MO). NODPA, 4OHDPA, and 3OHDPA were purchased from TCI (Portland, OR). 4MeODPA was prepared as outlined previously (*18*).

Statistical Analyses. Day 0 values for individual compounds from all storage durations as well as poststorage ripening periods were regressed to evaluate significance ($p \leq 0.05$) of linear and/or polynomial fits using the general linear model (GLM, Version 9.0, SAS Institute, Cary, NC). Analysis of variance, also using GLM, was performed on 4OHDPA/DPA values. Mean separation was determined using Fisher's least significant difference analysis. Significant differences in scald incidence between harvest dates for control (untreated) fruits were determined using *z*-statistics.

Table 1. Concentration of DPA and DPA Derivatives Recovered from the Peel of Granny Smith Apples Harvested on September 7, 2002, Treated with 1 μL L^{-1} 1-MCP and/or 2 g L^{-1} DPA, Stored for up to 6 Months at 1 °C in Air or 1 kPa O_2/1 kPa CO_2, and Ripened for up to 14 Days at 22 °C

	compound (ng g^{-1} fresh weight)							
	4-hydroxyDPA		4-methoxyDPA		DPA		*N*-nitrosoDPA	
sample treatment	check	1-MCP	check	1-MCP	check	1-MCP	check	1-MCP
initial day 0	7.134	11.36	0.3870	0.5714	7336	4752	2.140	1.184
initial day 7	16.16	13.99	1.133	1.595	3408	1932	0.8916	0.1888
initial day 14	13.81	32.75	1.936	3.886	1609	1763	0.3473	0.2886
significance ($n = 3$, $p \leq 0.05$)[a]	NS[b]	NS	*	NS	*	*,**	*	*,**
1 month air day 0	22.60	55.85	1.739	3.094	2673	3038	0.4362	0.1884
1 month air day 7	39.49	60.34	7.721	5.732	876.6	1347	0.5496	0.1039
1 month air day 14	49.05	145.6	41.44	8.126	462.8	1143	1.072	0.6280
significance ($n = 3$, $p \leq 0.05$)	*,**	*	*,**	*	*,**	*	NS	NS
2 month air day 0	186.3	217.7	13.48	26.77	5491	3778	1.158	1.252
2 month air day 7	150.3	414.3	130.1	57.02	2588	4373	5.256	0.6979
2 month air day 14	485.5	99.40	487.4	18.97	3668	1270	15.07	0.9110
significance ($n = 3$, $p \leq 0.05$)	NS	NS	*,**	NS	*,**	NS	*	NS
4 month air day 0	755.6	956.5	93.52	93.24	4882	2970	1.292	0.3295
4 month air day 7	509.4	880.0	258.7	115.3	2698	2082	1.718	0.4082
4 month air day 14	1476	572.7	775.9	72.46	2484	1064	1.328	0.2175
significance ($n = 3$, $p \leq 0.05$)	NS	NS	*,**	NS	*	*	NS	NS
6 month air day 0	906.7	1077	265.5	87.49	5185	2540	1.822	1.011
6 month air day 7	898.0	313.5	400.6	67.94	3938	904.1	2.313	0.5965
6 month air day 14	1640	171.4	1306	52.00	2716	463.8	1.656	0.3673
significance ($n = 3$, $p \leq 0.05$)	NS	*	*,**	NS	*	*	**	*
significance at day 0, all storage durations ($n = 3$, $p \leq 0.05$)	*	*	*,**	*	NS	*	**	NS
6 month CA day 0	977.6	227.9	120.7	227.6	3193	1723	0.4519	0.2601
6 month CA day 7	1721	666.2	196.0	521.0	3550	2014	0.3608	0.0726
6 month CA day 14	1529	667.8	534.5	582.6	2216	1368	0.4214	ND
significance ($n = 3$, $p \leq 0.05$)	NS	NS	*	NS	NS	NS	NS	*

[a] Significant linear (*) or polynomial (**) trend. [b] ND, not detected; NS, not significant.

RESULTS AND DISCUSSION

Fruit Maturity, Storage Duration, and DPA Derivative Content. Mean IEC values for harvest 1, 2, and 3 fruits were 0, 0.144, and 0.0944, respectively. 4OHDPA was the major DPA metabolite recovered in peels as in all previous studies of DPA metabolism in apple fruit (**Tables 1−3**) (*16*, *18*, *23*). These results also corroborate a previous report (*23*) indicating that 4MeODPA was the second most abundant DPA derivative. Minute quantities of NODPA and 2NO2DPA were also detected. Trace amounts of 3OHDPA were detected but not reported as the pattern of 3OHDPA accumulation was similar to that of 4OHDPA. 2OHDPA was detected in minute quantities, but its identity was not confirmed with an authentic standard (*18*).

Significant changes in DPA content were only noted in apples from harvests 1 and 3 where peel DPA contents of 1-MCP-treated fruits significantly decreased with storage duration. The DPA content typically decreased during poststorage ripening. The DPA content of harvest 1 peels was generally lower than the other two harvests suggesting either decreased uptake and DPA volatilization from the surface, increased absorption into interior tissues, or metabolism so that less DPA was present in the peel. These same phenomena may have been factors contributing to the lack of treatment differences for DPA. A previous report indicating that DPA loss is less in fruits stored in CA as compared to air suggests that DPA content dynamics are affected by storage environment (*16*). Another report indicates that DPA metabolism is reduced in Delicious apples treated with 1-MCP suggesting that ethylene action contributes to DPA metabolism (*27*). In the current study using Granny Smith apples, DPA metabolism was altered following 1-MCP treatment only in harvest 1 fruits stored in air for 6 months where the DPA content was lowest in 1-MCP-treated fruit. The difference in results between the two studies may be due to the use of different cultivars, harvest maturity, storage conditions, and/or storage duration. Decreases in DPA content with poststorage ripening may indicate increased absorption or metabolism producing 4OHDPA and smaller amounts of other metabolites.

4OHDPA accumulated during ripening at 22 °C immediately following harvests 1 and 3 and with increasing storage duration regardless of the harvest date. The impact of poststorage ripening on 4OHDPA content was inconsistent. The formation of 4OHDPA may be related to a phenomenon by which 4OHDPA production and metabolism are regulated in part by fruit maturity. For instance, decreased 4OHDPA may reflect its glycosydic conjugation, glycosides being major DPA metabolites in apple fruits (*16*), and formation of DPA glycosides could be governed by fruit maturity. Glycosidic conjugates of 4OHDPA were not quantified in this study.

Harvest maturity affected 4OHDPA content throughout the evaluation period. This may relate to differential DPA contents in fruits from different harvests. However, the 4OHDPA/DPA ratio reveals that 4OHDPA production in harvest 1 fruits was lower than production of harvest 2 or harvest 3 fruits indicating that the lower DPA presence was not the only factor promoting this characteristic (**Table 4**). 4OHDPA/DPA in harvest 2 fruits was higher than harvest 3 fruits following 4 months of air storage.

CA storage had little effect on 4OHDPA contents indicating that the low O_2 concentration during storage was sufficient for 4OHDPA production. Hypoxia can stimulate ROS production

Table 2. Concentration of DPA and DPA Derivatives Recovered from the Peel of Granny Smith Apples Harvested on October 18, 2002, Treated with 1 μL L^{-1} 1-MCP and/or 2 g L^{-1} DPA, Stored for up to 6 Months at 1 °C in Air or 1 kPa O_2/1 kPa CO_2, and Ripened for up to 14 Days at 22 °C

	compound (ng g^{-1} fresh weight)							
	4-hydroxyDPA		4-methoxyDPA		DPA		*N*-nitrosoDPA	
sample treatment	check	1-MCP	check	1-MCP	check	1-MCP	check	1-MCP
initial day 0	4.290	4.820	0.2152	0.2374	3676	3634	1.873	1.042
initial day 7	332.7	121.8	2.801	2.849	9339	6514	2.817	1.263
initial day 14	132.0	190.7	3.457	8.039	2439	3408	1.175	0.7361
significance ($n = 3$, $p \leq 0.05$)[a]	*	*	*	*	**	NS[b]	**	NS
1 month air day 0	1722	1153	3.425	7.677	13110	13973	3.638	2.242
1 month air day 7	628.7	392.7	62.60	17.10	5715	3911	3.085	0.8126
1 month air day 14	1173	475.8	486.8	24.48	3130	2384	2.485	0.6350
significance ($n = 3$, $p \leq 0.05$)	*,**	*,**	*,**	NS	*	*,**	*	*
2 month air day 0	2776	1324	17.24	28.91	7107	6120	4.133	1.915
2 month air day 7	916.3	783.6	57.22	29.83	2462	2005	1.142	0.4427
2 month air day 14	1498	418.7	508.0	23.42	1561	831.2	1.217	0.2973
significance ($n = 3$, $p \leq 0.05$)	NS	NS	*	NS	*,**	*	*,**	*
4 month air day 0	4657	3723	74.02	77.84	6568	5660	2.686	0.5606
4 month air day 7	3169	2198	256.0	74.94	3397	1880	1.623	0.3850
4 month air day 14	1643	2109	551.6	66.17	1501	1367	0.8386	0.2468
significance ($n = 3$, $p \leq 0.05$)	NS	NS	*	NS	*	*,**	NS	*
6 month air day 0	7796	3917	443.6	266.8	9285	6695	8.120	0.7895
6 month air day 7	5154	5850	634.8	335.8	5116	3868	2.405	0.4173
6 month air day 14	3944	3495	947.1	382.2	2856	2350	1.182	0.2909
significance ($n = 3$, $p \leq 0.05$)	*	*	*	NS	*	*	*,**	*
significance at day 0, all storage durations ($n = 3$, $p \leq 0.05$)	*	*	*,**	*,**	NS	NS	*,**	*
6 month CA day 0	5021	7415	64.96	140.6	6465	10355	1.091	0.7618
6 month CA day 7	5784	2315	82.18	135.3	6855	2407	0.8239	0.4019
6 month CA day 14	5294	6079	544.2	223.6	4284	3901	0.7650	0.3421
significance ($n = 3$, $p \leq 0.05$)	NS	NS	*,**	NS	NS	*,**	NS	*

[a] Significant linear (*) or polynomial (**) trend. [b] ND, not detected; NS, not significant.

in plants (*28*) where reduction of O_2 to form $O_2^{-\bullet}$, H_2O_2, and $^{\bullet}OH$ can occur (*23*, *29*, *30*). As previously indicated, $^{\bullet}OH$ can hydroxylate benzene (*31*) as well as substituted aryl compounds (*21*). This reaction typically occurs primarily in the ortho and para positions although largely in the para position when steric hinderence in the ortho position is present (*21*). As the aryl structure(s) of DPA is substituted by aniline, para hydroxylation of DPA appears to be favored in apple peels. 4OHDPA accumulates more than 3OHDPA and 2OHDPA (*16*), implying that such a mechanism may occur in this medium. Additionally, enzyme-catalyzed hydroxylation reactions are also aerobic (*22*) indicating that enzymatic hydroxylation of DPA can occur at 1.0 kPa O_2 as used in this study or is the result of processes where existing oxygenated substrates are available for this reaction during storage.

The reduced content of 4OHDPA in the peel during poststorage ripening was not related to increased 4MeODPA production (*23*). In the current study, a decrease in 4OHDPA that coincided with increased 4MeODPA during poststorage ripening was not typically observed. Synthesis of 4MeODPA by air-stored fruit from harvests 1 and 2 appears to be related to ethylene action as the 4MeODPA content was lowest in fruits treated with 1-MCP and the 4MeODPA content increased more during poststorage ripening in fruits not treated with 1-MCP. Storage duration was associated with increases in 4MeODPA for all harvests. 1-MCP treatment reduced the total amount of 4MeODPA in the peels of harvest 1 and harvest 2 fruits, albeit to a lesser extent than harvest 1, but not harvest 3 fruits where the control fruit contained less 4MeODPA. This result may be indicative of a maturity-dependent process regulated by ethylene as the inhibition provided by 1-MCP diminishes as fruit maturation and ripening progress. While the exact *O*-methylation mechanism is unknown, a wide variety of enzymes catalyze *O*-methylation of many hydroxylated aryl compounds, including phenylpropanoids, in plants (*32*).

NODPA production was also influenced by harvest maturity and inhibition of ethylene action. Harvest 1 fruits exhibited a pronounced burst of NODPA during poststorage ripening following 2 months of air storage. Harvest 3 fruits demonstrated a similar capacity during ripening immediately after harvest and following 1 month of air storage (**Table 3**). However, harvest 2 fruits did not exhibit patterns similar to the other two harvests. NODPA was relatively stable in harvest 2 fruits except immediately after 6 months of air storage where the NODPA content decreased with poststorage ripening. CA storage reduced or did not impact NODPA levels. 1-MCP treatment prevented the increase in NODPA content associated with poststorage ripening, and in fact, 1-MCP-treated fruits had the lowest NODPA contents regardless of harvest date. 2NO2DPA was only detected in harvest 3 fruits during the postharvest ripening period and coincided with a similar increase in NODPA. 1-MCP treatment also inhibited the formation of this compound.

As already mentioned, NODPA contents may reflect interactions between $^{\bullet}NO$ or $^{\bullet}NO_2$ and DPA. Likewise, the formation of 2NO2DPA may result from the reaction of DPA and $^{\bullet}NO_2$ as in smokeless gun powder (*14*, *15*). If this is the case, increased NODPA and 2NO2DPA contents in the peel may indicate increased amounts of $^{\bullet}NO$ or $^{\bullet}NO_2$, the product of its reaction with O_2. As $^{\bullet}NO$ has been implicated as an inhibitor of ethylene synthesis and senescence in fruit and other plant organs (*33*), the changes in NODPA and 2NO2DPA content may reveal a

Table 3. Concentration of DPA and DPA Derivatives Recovered from the Peel of Granny Smith Apples Harvested on November 1, 2002, Treated with 1 μL L^{-1} 1-MCP and/or 2 g L^{-1} DPA, Stored for up to 6 Months at 1 °C in Air or 1 kPa O_2/1 kPa CO_2, and Ripened for up to 14 Days at 22 °C

	compound (ng g^{-1} fresh weight)									
	4-hydroxyDPA		4-methoxyDPA		DPA		*N*-nitrosoDPA		2-nitroDPA	
sample treatment	check	1-MCP	check	1-MCP	check	1-MCP	check	1-MCP	check	1-MCP
initial day 0	2.641	9.085	ND[b]	ND	6676	13180	1.072	1.046	1.030	ND
initial day 7	132.5	186.3	8.847	63.17	7376	9308	15.42	3.904	1.971	ND
initial day 14	778.8	999.0	174.8	121.6	5446	9796	24.38	2.570	3.092	ND
significance ($n = 3$, $p \leq 0.05$)[a]	*,**	*	*,*	*	NS	*	*	**	*	NS
1 month air day 0	1950	1602	8.218	127.3	12820	10790	3.852	1.236	ND	ND
1 month air day 7	2152	890.3	136.1	277.6	5081	5093	11.15	3.474	ND	ND
1 month air day 14	1040	2139	270.0	534.2	2323	4234	11.96	2.432	ND	ND
significance ($n = 3$, $p \leq 0.05$)	NS	**	*	NS	*,**	*	*,**	**	NS	NS
2 month air day 0	3887	2751	39.68	822.7	8550	5884	2.314	0.7329	ND	ND
2 month air day 7	2446	1847	316.4	427.2	5395	4328	2.558	0.6870	ND	ND
2 month air day 14	2445	2123	843.8	673.1	3563	4007	3.034	1.118	ND	ND
significance ($n = 3$, $p \leq 0.05$)	NS	NS	*,**	NS	*	NS	NS	NS	NS	NS
4 month air day 0	2066	3378	106.4	916.2	6835	8685	1.923	0.7265	ND	ND
4 month air day 7	3599	2126	368.4	896.7	6308	4980	2.320	0.5505	ND	ND
4 month air day 14	4416	1274	1136	690.8	4964	3619	1.579	0.6197	ND	ND
significance ($n = 3$, $p \leq 0.05$)	*	*	*,**	NS	NS	*	NS	NS	NS	NS
6 month air day 0	3338	5612	589.6	1983	10720	13350	4.627	1.425	ND	ND
6 month air day 7	3088	3983	1206	2585	8704	7575	2.465	0.7218	ND	ND
6 month air day 14	2402	2983	1983	1887	6422	4554	1.780	0.5185	ND	ND
significance ($n = 3$, $p \leq 0.05$)	NS	*	*	NS	NS	*	*	*	NS	NS
significance at day 0, all storage durations ($n = 3$, $p \leq 0.05$)	*	*	*,**	*	NS	**	*	**	NS	NS
6 month CA day 0	4705	4940	289.3	813.6	13920	15300	3.504	2.382	ND	ND

[a] Significant linear (*) or polynomial (**) trend. [b] ND, not detected; NS, not significant.

Table 4. Ratio of 4OHDPA and DPA Collected from Granny Smith Apple Peel Harvested and Treated with 2 g L^{-1} DPA on September 7, 2002 (Harvest 1), October 18, 2002 (Harvest 2), and November 1, 2002 (Harvest 3), Stored for up to 6 Months at 1 °C in Air or 1 kPa O_2/1 kPa CO_2, and Ripened for up to 14 Days at 22 °C

	4OHDPA/DPA (%)[a]			
sample	harvest 1	harvest 2	harvest 3	LSD[b]
initial day 0	0.180	0.131	0.0546	0.0797
initial day 7	0.593	2.72	1.89	0.975
initial day 14	1.39	5.39	12.1	2.76
1 month air day 0	1.33	11.2	15.1	3.64
1 month air day 7	4.45	10.3	28.6	11.1
1 month air day 14	11.9	27.8	46.9	12.3
2 months air day 0	4.18	29.7	47.3	12.6
2 months air day 7	7.56	33.6	42.2	19.7
2 months air day 14	10.7	73.6	60.0	29.0
4 months air day 0	23.5	72.2	34.2	31.8
4 months air day 7	31.0	103	48.3	36.4
4 months air day 14	58.2	140	59.7	46.3
6 months air day 0	29.5	71.3	37.0	19.6
6 months air day 7	27.6	129	43.9	39.8
6 months air day 14	49.2	142	52.3	35.3
6 months CA day 0	20.2	72.6	33.4	5.62
6 months CA day 7	39.0	88.8	*c*	33.0
6 months CA day 14	57.7	137	*c*	41.6

[a] Values from DPA and DPA + 1-MCP treatments for each evaluation were averaged to obtain this value. [b] LSD, least significant difference calculated using Fisher's least significant difference analysis ($n = 6$; $p \leq 0.05$). [c] No data.

mechanism by which apple fruits can reduce ethylene production at particular points during ripening and senescence. In particular, increased NODPA and 2NO2DPA occurring before storage of harvest 3 fruits and after 2 months of storage in the less mature fruits from harvest 1 would seem to suggest such a mechanism in apples. Likewise, the reduction of NODPA by 1-MCP treatment supports this hypothesis as 1-MCP treatment reduces the ripening rate via inhibition of ethylene action. The lack of an increase in the content of these compounds with ripening at any point of examination in this study for harvest 2 fruits may support a developmental stage specific characteristic for this mechanism. Furthermore, because NODPA is metabolized into DPA during storage (*23*), fluctuations in NODPA contents during storage may be masked or otherwise altered.

DPA Derivative Content and Scald Incidence. Apples stored after treatment with DPA, 1-MCP, or stored in CA did not develop scald (data not shown). Control apples from harvests 1 and 3 began to develop scald between 2 and 4 months of air storage and after 4 months plus 7 days of ripening in the case of harvest 2 apples (**Figure 1**). Overall, harvest 1 apples had the highest scald incidence followed by apples from harvest 3 and then harvest 2. This pattern is unusual as scald incidence typically declines as apples mature (*4*). This result indicates that these apples may have developed a scald control mechanism with increasing maturity but then lost it due to some unknown circumstance. In addition to a higher 4OHDPA/DPA ratio, harvest 2 fruits did not exhibit the NODPA increase detected in apples from the other harvest dates. Furthermore, fruits treated with 1-MCP had less NODPA content and scald incidence as compared to untreated controls. These observations may imply a role for •NO in scald formation, although beyond these interpretations, no conclusions about associations between DPA derivative content and scald incidence can be deduced from these data.

Conclusions. The peel DPA content in DPA-treated Granny Smith apples was affected by harvest maturity and generally decreased over the storage and poststorage ripening period. Likewise, the DPA derivative content increased during this period and the individual derivative content was affected

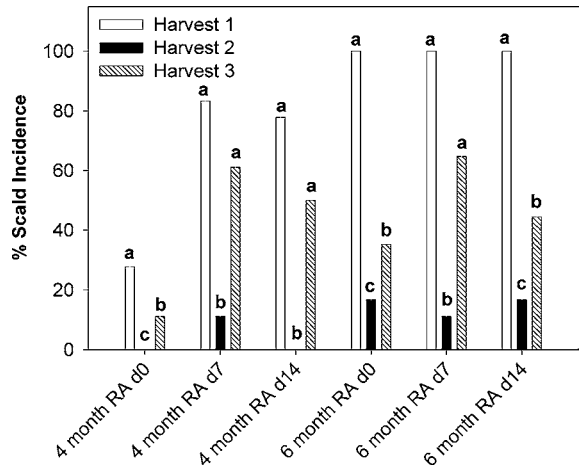

Figure 1. Scald incidence of untreated Granny Smith apples harvested on September 7, 2002 (harvest 1), October 18, 2002 (harvest 2), and November 1, 2002 (harvest 3), stored for up to 6 months at 1 °C in air, and ripened for up to 14 days at 22 °C. No scald symptoms were observed prior to 4 months of air storage or in CA-stored fruit. Different lower case letters denote significant differences between means according to *z*-statistics ($n = 18$; $\alpha = 0.05$).

differently by the various treatments employed in this study. 4OHDPA was affected by harvest maturity, storage duration, and storage environment while NODPA and 2NO2DPA contents were lower in fruits treated with 1-MCP and/or stored in CA. Harvest maturity and 1-MCP treatment reduced 4MeODPA content in fruits from harvests 1 and 2, but 1-MCP had no effect on harvest 3 fruits indicating that harvest maturity may influence the mechanism for *O*-methylation in this process. CA storage had no effect on 4MeODPA content.

The evidence shows that DPA uptake and derivatization are affected by fruit maturity at harvest, storage duration, and storage conditions. While 4OHDPA content appears to be unaffected by 1-MCP treatment and, surprisingly, CA storage, 4OHDPA accumulation is affected by harvest maturity indicating that hydroxylation capacity may develop while apples are still attached to the tree. The rate of glycosylation of hydroxylated DPA derivatives may also affect 4OHDPA levels. NODPA and 2NO2DPA contents may denote $^{\bullet}NO$ and $^{\bullet}NO_2$ generation at crucial points in postharvest development. Likewise, increased 4MeODPA contents may be indicative of other processes regulated by maturation such as *O*-methylation of phenylpropanoid species. Definitive correlations between scald incidence and derivative production are wanting; therefore, much of the evidence provided in these studies (*23*) supports the amino hydrogen abstraction model for scald control rather than the radical trapping model characterized in smokeless powder. Further studies revealing the donation of the amino hydrogen to various species during apple storage would be useful for further clarification of the chemical species influencing scald and their interactions with DPA.

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