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Diphenylamine Residues in Apples Caused by Contamination in Fruit Storage Facilities

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Supporting Information

ABSTRACT: The potential of fruit storage facilities that are contaminated with the widely used chemical antioxidant diphenylamine to cross-contaminate untreated apples (*Malus* × *domestica* Borkh.) was studied. A new sample preparation method identified the storage room paint, contaminated from past treatments, as the major source of cross-contamination in the analyzed facilities. Diphenylamine amounts of up to 917 g were found in a single storage room and were shown to correlate with the extent of cross-contamination on stored apples. Our data support a diffusion-based mechanism where the wall paint releases the antioxidant to the storage room atmosphere even years after the last treatment. Given the extent of cross-contamination found in our model experiments and under commercial storage conditions, we deduce a significant risk of exceeding the potentially upcoming maximum residue level of 0.01 mg kg^{-1} on stored fruit in contaminated rooms even years after the last diphenylamine treatment.

KEYWORDS: *Malus* × *domestica Borkh., apple, diphenylamine, contamination, fruit storage, food safety*

INTRODUCTION

Diphenylamine (DPA) is an antioxidant[1,2] widely used in postharvest treatment to control the superficial scald during storage. Scald occurs through oxidation of α-farnesene, a naturally occurring terpene in apples, leading to damage to the outer cell layers, cell death, and browning of the apple skin.[3,4] DPA treatment is usually conducted as a drench at a concentration of 1000−2000 mg kg^{-1} in water immediately before storage or, alternatively, by nebulizing DPA directly into the storage chambers filled with apples (thermonebulization method: 600−1800 mg kg^{-1}). After treatment, typical DPA residues in apples ranged from 1 to 5 mg kg^{-1} independently of the chosen method (ref 2; own unpublished data).

In 2009, the European Union determined the withdrawal of authorizations for plant protection products containing DPA.[5] The current maximum residue level (MRL) of DPA on apples is 5 mg kg^{-1};[6] however, the MRL will likely be lowered to 0.01 or 0.05 mg kg^{-1}.[7] Efforts to substitute postharvest treatments with DPA have led to the development of new storage technologies, using different types of controlled atmosphere (CA)[8−12] such as dynamic controlled atmosphere (DCA), ultra low oxygen (ULO) with initial low oxygen stress (ILOS), or treatment of apples with 1-methylcyclopropene (1-MCP)[11−16] prior to storage.

These technological advances have removed the need for DPA treatment in commercial apple storage; however, DPA residues (0.01−0.2 mg kg^{-1}) have also been detected in untreated apples in South Tyrol as well as in other regions.[17−19] This suggests that the storage facilities themselves (storage bins, storage room walls, CO_2 scrubbers, ventilation system) are contaminated with DPA, causing cross-contamination of apples during commercial storage. Earlier studies,[17−19] carried out when DPA treatments were still conducted, tried to identify the contamination sources, with a focus on wooden storage bins that have been abandoned by most producers in South Tyrol. However, sample numbers were low and results not always conclusive. In particular, the amount of DPA in different parts of the facilities did not correlate clearly with the degree of cross-contamination in fruit. One study[17] examined the DPA content in the atmosphere of storage rooms filled either with untreated or DPA-treated apples and found considerable differences.

The aim of the present study was to clearly identify the sources of DPA cross-contamination in commercial storage facilities, which occurs even years after the last DPA treatments, and to quantify the amount of DPA in the major contamination sources using reliable extraction methods.

MATERIALS AND METHODS

Reagents and Equipment. Acetone, dichloromethane, toluene, acetonitrile, sodium chloride, sodium sulfate anhydrous, and diphenylamine (Pestanal) were purchased from Sigma-Aldrich (Milano, Italy). Water was purified using a Milli Rx 20 system (Millipore, Milano, Italy).

GC/MS Analysis. Gas chromatographic analysis was performed on an Agilent 6890 Series GC System (Agilent Technologies, Milano, Italy) equipped with a HP 5973 mass selective detector (Agilent Technologies, Milano, Italy). Separations of 1 μL samples were carried out on a 30 m × 0.25 mm i.d. 0.25 μm fused-silica capillary (Rxi-5Sil MS, Superchrom, Milano, Italy). The autosampler (Agilent 7683 Series Autosampler, Milano, Italy) was operated in splitless mode, and the temperature of the inlet was set to 250 °C. Helium was used as carrier gas with a constant flow rate of 1.5 mL min^{-1}. The temperature program of the oven was 50 °C (0 min), programmed with 16 °C min^{-1} up to 270 °C (8 min). Mass spectra were obtained after electron

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impact (EI) ionization at 70 eV using the selective ion monitoring (SIM) mode involving one target ion (m/z 168) and three qualifier ions (m/z 167, m/z 169, m/z 170). The interface heater, mass selective detector source, and quadrupole temperatures were 280, 230, and 150 °C, respectively. Quantitation was carried out with the target ion and an external standard using MSD ChemStation D.01.02.16 software (Agilent Technologies, Milano, Italy). Experiments were performed in commercial storage facilities in South Tyrol, Italy.

DPA Extraction from Apples. For each analysis, 10 apples cv. Golden Delicious of medium size (70−85 mm) and free of visual defects were pooled, immediately transported to the laboratory, and cut into small pieces using a cutter (Hobart model 84181D, Milano, Italy). 100 g of the fruit sample was homogenized and extracted with acetone (200 mL) using an Ultra-Turrax T25 digital mixer (IKA, Milano, Italy) for 2 min at room temperature.[20] The extract was filtered, and an aliquot (50% of the extract) was diluted with brine (40 mL) and extracted twice with dichloromethane (70 mL each). The combined organic layer was dried with anhydrous sodium sulfate, and the solvent was evaporated in vacuo after filtration. The dry residue was dissolved in acetone (2 mL) and analyzed by GC−MS.

DPA Extraction from Activated Carbon. Two grams of activated carbon was removed from CO_2 scrubbers and sonicated for 40 min in acetonitrile/toluene (100 mL, 1:1 v/v) at room temperature.[21,22] After filtration, the activated carbon was sonicated again for 15 min in acetonitrile/toluene (50 mL, 1:1 v/v). The combined filtrates were concentrated to dryness in vacuo. The residue was reconstituted in acetone (2 mL) and analyzed by GC−MS.

DPA Extraction from Storage Cell Wall Paint. As swab analyses delivered unreliable DPA quantification,[17] we have developed a novel method for determining the total DPA amount in cell wall paint (ISOLCOAT resin, Isolcell, Laives, Italy): A sample of approximately 2 cm × 2 cm cell wall paint (coating thickness: 0.25 mm for one layer and 0.50 mm for two layers) was chiselled off the wall, cut into small pieces (approximately 2 mm × 2 mm each), and sonicated for 1 h in acetone (100 mL) at room temperature. After filtration, the wall paint pieces were sonicated for another 30 min in acetone (50 mL) and filtered. The combined filtrates were concentrated to dryness in vacuo, and the residue was reconstituted in acetone (50 mL) and analyzed by GC−MS.

DPA Extraction from Air. Three consecutive silica cartridges (Orbo 52 small, Sigma-Aldrich) were installed on the air outlet of a pump (Schego model M2K3, air flow rate: 1.6 L min^{-1}) and placed into a contaminated storage cell. The cell atmosphere was aspirated for 24 h.[23] The adsorbent of each cartridge was extracted separately, sonicated for 40 min in acetone (100 mL) at room temperature, filtered, sonicated for another 15 min in acetone (50 mL), and filtered again. The combined filtrates were evaporated in vacuo, and the dry residue was reconstituted in acetone (2 mL) and analyzed by GC−MS. The total amount of DPA in air was calculated from the amount of DPA present in the three cartridges, divided by the total volume of air passed through the cartridges. For some experiments, one activated carbon cartridge (Orbo 32 small, Sigma-Aldrich) was used instead of three consecutive silica cartridges. In this case, DPA was extracted with acetonitrile/toluene (1:1, v/v), following the same extraction procedure. Recovery from activated carbon was determined to be 60%, whereas for silica, a quantitative recovery was assumed on the basis of the experience in our laboratory and the known recovery of close chemical analogues[23] such as phenylamine, *N*,*N*-dimethylaniline, or *N*,*N*-dimethyl-*p*-toluidine.

A device aimed to absorb DPA from the cell atmosphere into a sulfuric acid solution (10%; 22 L) was constructed using a pump with an operating performance of 30 m^3 air per hour. The device was operated in a contaminated storage room continuously for 13 days at room temperature, and sulfuric acid samples (5−10 mL) were taken at regular intervals and analyzed by GC−MS for their DPA content.

RESULTS

DPA Residues in Commercially Stored Apples. In a first step, existing results from our laboratory database containing analyses for DPA residues from apple samples (various cultivars) were grouped according to the degree of DPA content. The data came from commercial apple samples with unknown treatment status, analyzed between January 2006 and April 2010. As shown in Figure 1, samples with DPA amounts

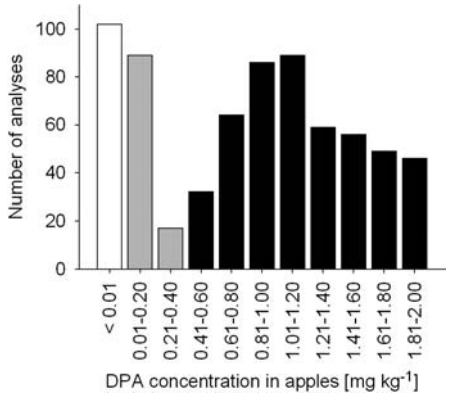

Figure 1. Apple samples (n = 689) analyzed for DPA residues between January 2006 and April 2010, grouped according to DPA concentration levels. The white bar indicates results below detection limit (<0.01 mg kg^{-1}), gray bars show presumptive cross contamination (0.01−0.40 mg kg^{-1}), and black bars represent residues presumably caused by postharvest treatment with DPA (≥0.41 mg kg^{-1}).

of up to 2.00 mg kg^{-1} (689 samples) cluster into three groups: The first group (102 out of 689 samples) consists of samples with a DPA content below the detection limit of 0.01 mg kg^{-1}. The second group contains 106 samples with residues ranging between 0.01 and 0.40 mg kg^{-1}, while the third group (481 samples) comprises residues between 0.41 and 2.00 mg kg^{-1}. Typical residues after DPA postharvest treatment are in the range of those occurring in the third group.[2] Even though the treatment history of the samples was not known, we hypothesized that residues below 0.40 mg kg^{-1} (second group) are probably caused by cross-contamination during storage. According to this assumption, as much as 50% of analyzed samples from untreated apples would be cross-contaminated during commercial storage.

Storage Room Wall Paint Was the Major Source of DPA Contamination. To identify the source of the above-mentioned cross-contamination in the storage facilities, three possible contamination sources were evaluated: storage bins, CO_2 scrubbers, and storage rooms.

Existing data from our laboratory database (see above) were used to assess the role of storage bins as potential source of DPA contamination. The bins, having been used for several years to store DPA-treated apples, are also typically used to store untreated apples and are not sorted into treated and untreated groups. Therefore, a random distribution of DPA residues across all storage rooms would be expected if bins were the major contamination source. However, analysis of the database clearly shows that DPA contamination clusters according to storage in contaminated and noncontaminated storage rooms, respectively. As illustrated in Table 1, untreated apples stored for several months in eight different storage rooms, which had been used previously for DPA treatment by nebulization and drenching, were contaminated with DPA (0.01−0.07 mg kg^{-1}). However, apples stored in DPA-free storage rooms lacked detectable DPA residues (<0.01 mg

Table 1. DPA Residues in Untreated Apples Stored in Contaminated Commercial Plastic Bins for Several Months in Storage Rooms Used for DPA Treatments and in DPA-Free Storage Rooms (SD = Standard Deviation)

room no.	DPA treatment history	cultivar	storage time (months)	DPA concentration ± SD [mg kg^{-1}][a]
2	4 years	Fuji	5	0.07 ± 0.05
19	4 years	Granny Smith	6	0.02 ± 0.02
1	4 years	Granny Smith	7	0.06 ± 0.04
18	4 years	Granny Smith	7	0.05 ± 0.03
7	7 years	Red Delicious	7	0.02 ± 0.01
11	>15 years	Red Delicious	6	0.04 ± 0.01
12	>15 years	Fuji	7	0.02 ± 0.01
10	>15 years	CIV G 198	7	0.01 ± 0.01
21	no treatment	Golden Delicious	2	<0.01
22	no treatment	Fuji	2	<0.01
3	no treatment	Fuji	7	<0.01
4	no treatment	Red Delicious	8	<0.01

[a]Three or more replicates consisting of 10 apples.

kg^{-1}), despite having been stored in used bins. We estimate that the probability that a 5-year-old bin has been in contact with DPA-treated apples is approximately 80%, based on the fact that around 30% of apple varieties produced in South Tyrol were routinely treated with DPA. This data analysis strongly suggests that storage rooms but not storage bins represent a significant source of DPA contamination in the samples present in the database.

Three different CO_2 scrubbers, which had been used for DPA-treated apple storage for at least 5 years, each filtrating the atmosphere of 4−6 heavily contaminated storage rooms, were analyzed for the presence of DPA by GC−MS. The extractable concentration of DPA residues was below the detection limit of 0.05 mg kg^{-1} in all cases (Supporting Information Table S1), thus excluding CO_2 scrubbers as a major contamination source.

These findings turned our attention to the storage room itself: Five storage bins filled to one-third with untreated apples cv. Golden Delicious were evenly distributed throughout a commercial storage room (room no. 4) and stored for 28 days at room temperature under air. During this period, apple samples were taken regularly and analyzed for the presence of DPA residues. DPA concentration increased over time, reaching a maximum of 1.62 mg kg^{-1} after 28 days (Figure 2).

DPA Quantification in Storage Room Wall Paint. Commercial storage room walls are usually made of prefabricated sandwich insulation panels covered on the room-facing side with a galvanized sheet metal coated with an insulating paint. As a first step, we tested the commonly used quantification method for DPA on room walls ("swab analysis")[17] and compared it to a novel method developed in our laboratory, designed to quantify the total amount of DPA present in the paint layer (see Materials and Methods). Swab analyses[17] on storage room walls were performed with different solvents, such as acetone, ethyl acetate, and water, to test their effectiveness in removing DPA from the room walls (data not shown). Acetone provided the best results; however, a direct

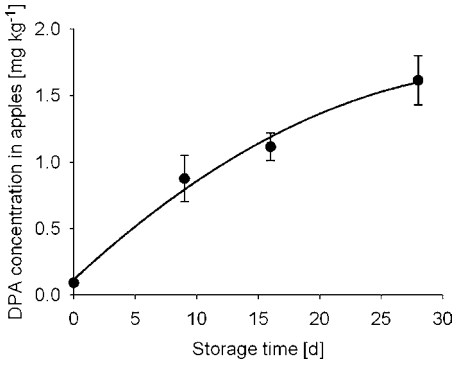

Figure 2. Temporal progression of DPA adsorption on apples during 28 days of storage in a contaminated storage room (DPA 1910 mg m^{-2}) at room temperature under air. Data points consist of five measurements on pools of 10 apples, taken from five storage bins that were evenly distributed throughout the storage room.

comparison of the two methods revealed that swab analysis was able to detect only 0.6% of the total DPA content present in the wall paint from a heavily contaminated storage room (1910 mg m^{-2}) (Supporting Information Table S2), presumably because it removes only the DPA present on the surface of the wall paint.

Using the novel DPA extraction procedure, we observed DPA amounts exceeding 1000 mg m^{-2} DPA in wall paint from storage rooms that had been nebulized with DPA for 3 years (Table 2). In contrast, walls of storage rooms in which

Table 2. DPA Amounts Detected in Samples of 2 cm × 2 cm Wall Paint from Storage Rooms[a]

	room history			
room no.	years of nebulization	years of drenching	last year of treatment	DPA concentration [mg m^{-2}][b]
5	3		2006	1210
6	1		2004	325
7		>7	2006	287
8		<2	2009	145
9				21.0
10[c]		>9	2009	1050
11[c]		>9	2009	1120
12[c]		>9	2009	517
13[d]		>5	1998	0.6

[a]Storage rooms had been used for storing DPA-treated apples and carrying out DPA nebulization treatments for the indicated time periods. [b]One sample; coefficient of variation (CV) for this method is 10%. [c]Room with two paint layers. [d]Room made of fiberglass instead of sheet metal coated with insulating resin.

drenched apples had been stored were contaminated with 150−300 mg m^{-2} DPA. If more layers of paint were applied, higher levels of DPA were detected, even if only drenched apples had been stored. Analyses of wall paint from a storage room that had never been used for storage of DPA-treated apples yielded DPA residues of 21.0 mg m^{-2}. Notably, a storage room wall made of fibreglass (outdated building type, last DPA treatment in 1998) revealed DPA amounts of less than 1 mg m^{-2}.

Furthermore, the effect of a fresh wall paint layer in a contaminated storage room was investigated. After the wall was repainted with ISOLCOAT, no reduction of the DPA concentration on apples under genuine storage conditions (1

°C, 8 months) was observed (0.03–0.11 mg kg^{-1} DPA residues; data not shown).

DPA Contamination of Apples Correlates with the DPA Amount Present in the Storage Room Wall Paint. To examine the amount of DPA adsorption in fruit, apples cv. Golden Delicious were placed in four storage bins filled up to 20% of their capacity and stored in four differently contaminated storage rooms at room temperature under air for 7 days. GC–MS analysis of apples after 0, 1, and 7 days revealed a considerable increase (up to 0.25 mg kg^{-1}) of DPA residues in apples stored in storage rooms with very high DPA levels (1330 mg m^{-2} DPA in the room wall paint). Storage of apples in storage rooms with high DPA levels (1210 mg m^{-2}) leads to residues of up to 0.20 mg kg^{-1}, whereas low DPA concentrations (325 mg m^{-2}) lead to the accumulation of up to 0.05 mg kg^{-1} DPA. Apples stored in storage rooms that had never come into contact with DPA (25.0 mg m^{-2}) showed no detectable accumulation of DPA (Figure 3).

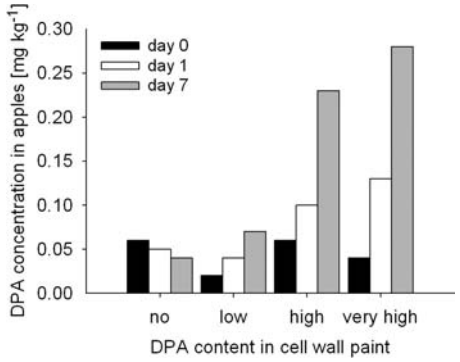

Figure 3. Temporal progression of DPA adsorption on apples stored for up to 7 days in differently contaminated storage rooms (no DPA, 25.0 mg m^{-2}; low DPA, 325 mg m^{-2}; high DPA, 1210 mg m^{-2}; very high DPA, 1330 mg m^{-2}) at room temperature under air. Data points consist of a pool of 10 apples. Available apple samples had an initial DPA contamination (day 0) ranging from 0.02 to 0.06 mg kg^{-1}.

To investigate the temperature dependence of DPA accumulation during apple storage in contaminated storage rooms, a storage bin filled up to 20% with apples cv. Golden Delicious was stored at 1 °C (typical commercial storage temperature) or 22 °C in a heavily contaminated storage room (1210 mg m^{-2}), respectively. As expected, the accumulation of DPA at 1 °C was slower than that at 22 °C, but still sufficient to contaminate the apples with approximately 0.15 mg kg^{-1} DPA in less than 10 days (Supporting Information Figure S1).

Unexpectedly High DPA Desorption Rate from the Storage Room Wall. Analyses of the DPA concentration in the air at different locations in three commercial storage facilities were carried out during summer (empty storage rooms with a temperature between 10 and 20 °C) and autumn (empty storage rooms or partially filled with apples, 1 °C). The DPA concentration measured in the air of storage rooms ranged from 0.9 to 7.3 μg m^{-3} and showed strong temperature dependence, with the highest values measured at 20 °C and the lowest at 1 °C. Considerable DPA concentrations were detected even in storage rooms that had never been used to store DPA-treated apples as well as in the aisles and in the weighing area of the storage facilities (Supporting Information Figure S2).

To investigate the rate of DPA desorption from the storage room walls, a centrifugal fan (Plastifer, Monte Cremasco, Italy), powerful enough to continuously replace the room atmosphere twice a day with fresh, DPA-free air, was operated in a heavily contaminated storage room (1260 mg m^{-2} DPA) at room temperature, and the DPA accumulation rate on apples cv. Golden Delicious was followed over 11 days. Continuous operation of the fan was not sufficient to reduce the contamination of the stored apples, reaching 0.60 mg kg^{-1} after 11 days, as compared to a maximum of 0.53 mg kg^{-1} after 11 days without fan operation (Figure 4). In addition, we

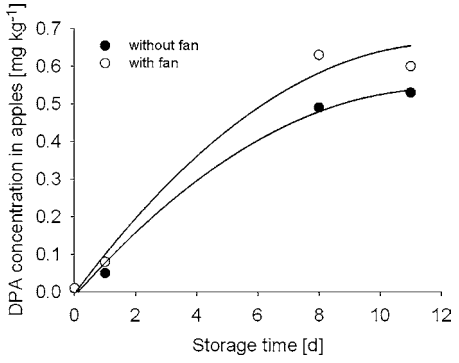

Figure 4. Temporal progression of DPA accumulation in apples during storage at room temperature under air in a heavily contaminated storage room (1260 mg m^{-2}), with and without continuous air replacement using a centrifugal fan.

constructed a device that pumped air from a contaminated storage room through 10% sulfuric acid (22 L), to absorb DPA from the room atmosphere. During a 13 day-period of continuous operation (720 m^3 air d^{-1}) in a contaminated storage room (784 mg m^{-2}, storage room size 1400 m^3) at room temperature, sulfuric acid samples (5–10 mL) were regularly taken and analyzed by GC–MS for their DPA content. The amount of absorbed DPA increased almost linearly over the first 13 days, reaching saturation at approximately 50 mg after 13 days due to the limited amount of sulfuric acid available (22 L) (Supporting Information Table S3).

High Percentage of Apples with DPA Residues after Storage in Contaminated Commercial Storage Rooms. To assess the probability of detectable DPA residues (≥0.01 mg kg^{-1}) occurring in untreated apples under commercial storage conditions, 42 apple samples were taken from seven fully loaded storage rooms with different DPA contamination levels (between 208 and 1330 mg m^{-2}) after 6–7 months of storage. Under these conditions, 35 samples (83%) tested positive for DPA residues. Depending on the DPA amount present in the storage room wall, the contamination risk was 33–100% (Figure 5). DPA residues ranged from 0.01 to 0.09 mg kg^{-1} (Supporting Information Table S4). On the basis of the observed average contamination, the amount of DPA present in the most contaminated storage room (1330 mg m^{-2}, total room wall surface 480 m^2) would be sufficient to cross-contaminate apples over 72 storage seasons (400 000 kg apples per storage season).

DISCUSSION

The results of this study demonstrate that fruit storage facilities contaminated with DPA through accumulation of this

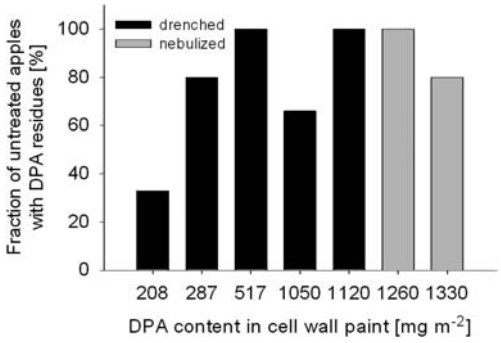

Figure 5. Fraction of untreated apples with detectable DPA residues after storage (6−7 months) in differently contaminated fully loaded storage rooms under commercial storage conditions.

postharvest antioxidant over the years have an alarming potential to contaminate untreated fruit with DPA. The extent of this contamination is surprisingly high, reaching more than 1.5 mg kg^{-1} DPA per apple in the course of 1 month in our model experiments. Here, we show for the first time that the storage room wall paint is the major source of this contamination. Using a newly devised extraction procedure that includes the whole paint layer, we were able to quantify the total DPA amount in a storage room that may total up to 1000 g. Previously used swab analyses[14] were limited to detecting only surface DPA, resulting in much lower values (12 mg m^{-2}). These results also explain why attempts to decontaminate storage facilities with adequate solvents or reagents have been unsuccessful: Apparently, DPA is only removed from the topmost paint surface, but not from the whole paint layer.

Our data show that the amount of DPA in the storage room wall clearly correlates with the degree of contamination of the fruit. As expected, the antioxidant contamination is highest in storage rooms where DPA nebulization treatments had been carried out repeatedly. However, high DPA amounts also occurred in storage rooms where DPA-drenched apples have been stored over several years, in particular if multiple layers of paint had been applied.

Our results are best explained by a diffusion-based mechanism where the wall paint of the storage room acts like a sponge adsorbing high amounts of DPA during years of treatment and releasing the antioxidant to the storage room atmosphere over time. The higher DPA concentration in double-coated walls points toward a significant mobility of the antioxidant in the paint layer and explains why attempts to seal the underlying DPA contamination with an additional layer of paint have been unsuccessful; rather, the latter increases the potential to absorb additional DPA from the air. Air analyses have detected DPA in different areas (storage rooms, aisles, weighing area) of various storage facilities, with DPA concentrations depending on the temperature (1−2 $\mu g\ m^{-3}$ at 1 °C; 3−13 $\mu g\ m^{-3}$ at 20 °C). Interestingly, the concentration variation in the atmosphere is much smaller than that in the wall paint (values between 16.0 and 1910 mg m^{-2}). DPA was also detected in the atmosphere throughout the investigated storage facility, even in newly built storage rooms that had never been exposed to DPA or DPA-treated fruit, amounting to 21.0 mg m^{-2} DPA in the wall paint, which, however, is not enough to cross-contaminate stored fruit. Previous studies on the contamination with DPA[17−19] in different parts of fruit storage facilities confirm our results, although, to our knowledge, this report is the first to show that even several years after the last DPA treatment detectable DPA concentrations are present in the storage room atmosphere, which can cross-contaminate fruit.

These findings support our model of a constant release of DPA into the air. Our attempts to remove DPA from air by replacing the atmosphere twice a day with DPA-free air or by absorbing it at a rate of 3.8 mg d^{-1} on average did not significantly decrease the DPA concentration in air and, in consequence, DPA residues in apples. We conclude that DPA is readily resupplied to the gas phase by desorption from the storage room wall, where it is not strongly bound, until an equilibrium is reached. In contrast, DPA appears to be strongly adsorbed to organic polymers[24,25] as well as to activated carbon, from which it cannot be recovered quantitatively even when using the strong eluent toluene. This supports our conclusion that plastic bins (and the charcoal in the CO_2 scrubbers) are not the main contamination sources, even though DPA has been found in wooden and plastic bins.[18] The antioxidant is bound too strongly to organic polymer materials (i.e., HD-PE) to be released in significant amounts.[18] The observed contamination patterns and the low contamination of a fiberglass storage room wall further support our conclusion, even though some caution is advised in the latter finding, as the last treatment was in 1998. According to our model, DPA is desorbed from the wall paint into the storage room atmosphere and then adsorbed onto the stored fruit. Our storage experiments show increasing concentrations of DPA on apples over time, with the expected temperature dependence. After 30 days of storage in a heavily contaminated storage room, the apple surface is not yet saturated, revealing that the apple peel has a very high adsorption capacity and a much higher affinity to DPA than the storage room wall. However, concentrations detected in our model are 4−15 times higher than those detected on commercially stored apples. Extrapolating from the 28 day experiment shown in Figure 3 to an entire storage season (6−12 months), it is surprising that DPA-free apples are found at all after storage in highly contaminated storage rooms. Two significant differences to commercial storage practices can explain this apparent contradiction: First, the amount of apples (60−500 kg) used in our experiments was much smaller than the total storage room capacity (400 000 kg). Therefore, desorbed DPA would disperse to 40 000 m^2 of total apple peel surface under commercial storage conditions, thus reducing the overall amount of DPA available per apple. In addition, it is likely that a DPA gradient is formed in a fully loaded storage room, causing apples in the middle of bins or in the middle of the storage room to have less contact with DPA. Second, GC−MS analysis of DPA-contaminated apples after 7 weeks of storage in a DPA-free storage room showed a reduction of DPA residues by 33%, suggesting a partial degradation during storage.[26] We rule out that DPA is lost by evaporation, as DPA desorption from the apple peel is negligible.[27,28]

Our results indicate that the risk of a DPA contamination of stored apples depends on the amount of DPA present in the storage room wall paint. At the moment, it is not possible to predict the exact extent of apple contamination after one storage season at a given contamination of the storage room. However, even if DPA diffusion is not fast enough to saturate all apples in a full storage room during one storage season and some of the DPA is degraded in the apples, it will be challenging to comply with a possible MRL of 0.01 mg kg^{-1}. Indeed, under commercial storage conditions in contaminated storage rooms, only 17% of the apple samples tested were free

of DPA residues after 6−7 months. Even a storage room with low contamination levels (200 mg m^{-2}) caused DPA residues ($\geq$0.01 mg kg^{-1}) in 33% of the samples. High contamination levels increased the risk significantly. Even though the level of DPA residues caused by cross-contamination under common practices is lower (0.01−0.20 mg kg^{-1}) than the one detected after postharvest DPA treatment (1−2 mg kg^{-1}), it exceeds the potential MRL of 0.01 mg kg^{-1}. In addition, the extent of contamination of the whole storage room infrastructure is unknown, and further investigation is needed to reliably predict the potential contamination of fruit after a storage period.

In summary, we have shown that DPA contamination of fruit storage infrastructures causes a cross-contamination of apples during storage. The storage room wall paint is the major source of DPA. Despite significant efforts, an effective decontamination strategy has not been reported. This work shows that the complete paint layer has to be decontaminated, not only its surface. Even waiving DPA treatments in the past cannot guarantee DPA-free apples, as we detected cross-contamination in storage rooms that had not been in contact with DPA for the last 3 years. Under the given circumstances, it will be challenging to avoid DPA residues for producers that have used DPA postharvest treatments in the past, and more research is needed to develop an effective and feasible decontamination method.

■ ASSOCIATED CONTENT

Supporting Information

Figures and tables giving additional test data. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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