

Effect of continuous 0.3 $\mu\text{L/L}$ gaseous ozone exposure on fungicide residues on table grape berries

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ABSTRACT

The persistence of residues of some fungicides, commonly applied in table grape vineyards to reduce bunch rot, was investigated during the cold storage of 'Thompson Seedless' table grape stemmed berries in atmospheres of air or 0.3 $\mu\text{L/L}$ ozone enriched air. Grape berries were sprayed with a mixture of boscalid, iprodione, fenhexamid, cyprodinil, and pyrimethanil solutions, dried in air for 24 h, and packed in plastic clamshell containers in expanded polystyrene boxes. The boxes were stored either in ozone or in ambient air atmosphere (2 °C, 95% RH) for 36 d. Residue analyses were done initially and at 12-d intervals using gas chromatography–mass spectrometry. Residues of boscalid, iprodione, fenhexamid, and pyrimethanil declined during storage in air, but cyprodinil residues did not change significantly during 36-d storage. Storage in the ozone atmosphere markedly accelerated the rates of decline of fenhexamid, cyprodinil, and pyrimethanil, but not those of boscalid or iprodione. At the end of storage, degradation of fenhexamid, cyprodinil, or pyrimethanil was 1.6-, 2.8-, or 3.6-fold higher, respectively, in the ozone atmosphere compared that in air. Despite their structural similarity, pyrimethanil declined more rapidly in an ozone atmosphere than cyprodinil. Fenhexamid declined in both air and ozone more rapidly than the other fungicides; at the end of storage period, only 59.2% or 35.5% of the initial residue remained after air or ozone storage, respectively. Our results have shown that gaseous ozone treatment during storage has a great potential for degrading contemporary fungicides related to table grape production.

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1. Introduction

In the production of table grapes, fungicides are applied in vineyards to reduce fungal disease losses before harvest, primarily the diseases powdery mildew and botrytis bunch rot, caused by *Erysiphe necator* and *Botrytis cinerea*, respectively. Dicarboximides (*i.e.* iprodione), the most popular class of specific fungicides against *B. cinerea*, have been used for years. Recently, new compounds with specific actions against this mold, such as the anilinopyrimidines (including cyprodinil and pyrimethanil), the hydroxyanilide fenhexamid, and the carboxamide boscalid, have been introduced into the market (Gabriolotto *et al.*, 2009). All are approved for use on table grapes and commonly used in vineyards (Smilanick *et al.*, 2010). The chemical structures of these fungicides are shown in Fig. 1. After harvest, sulfur dioxide gas is the primary means of controlling fungal decay, most of which is gray mold, also caused by *B. cinerea* (Lichter *et al.*, 2006). However, ozone has recently been evaluated for this purpose and it can be used in the cold storage atmosphere to prolong grape storage life after harvest (Smilanick

et al., 2010). Since it has been shown that ozone can oxidize many pesticides, the influence of ozone gas on residues on fruit is of interest, since the residues levels are important for regulatory and marketing purposes.

Agricultural products with lower pesticide residues are demanded by buyers in international trade due to increasing public concerns about health risks associated to residues on foods. Public perception and subsequent regulation surrounding non-target residue exposures, regardless of whether it is supported by acute or chronic toxicology, necessitates the development of safe and effective methods for residue reduction. Recently, many processes have been tested for degrading pesticides on various agricultural products.

In the field, degradation of pesticides on a product can occur by biologically (*i.e.* microbial activity) or chemically (*i.e.* oxidation, reduction, hydrolysis, photolysis). Industry also uses one of these chemical processes, oxidation, to effectively degrade pesticides. Oxidation of a compound is based on either a direct attack of an oxidative molecule, such as ozone, or the reaction of free radicals with the compound. Combined use of oxidants (*i.e.* ozone + hydrogen peroxide) or oxidant with ultraviolet radiation (*i.e.* ozone + UV) is referred to as advanced oxidation processes. Free radicals such as hydroxyl, hydroperoxyl and superoxide anion are

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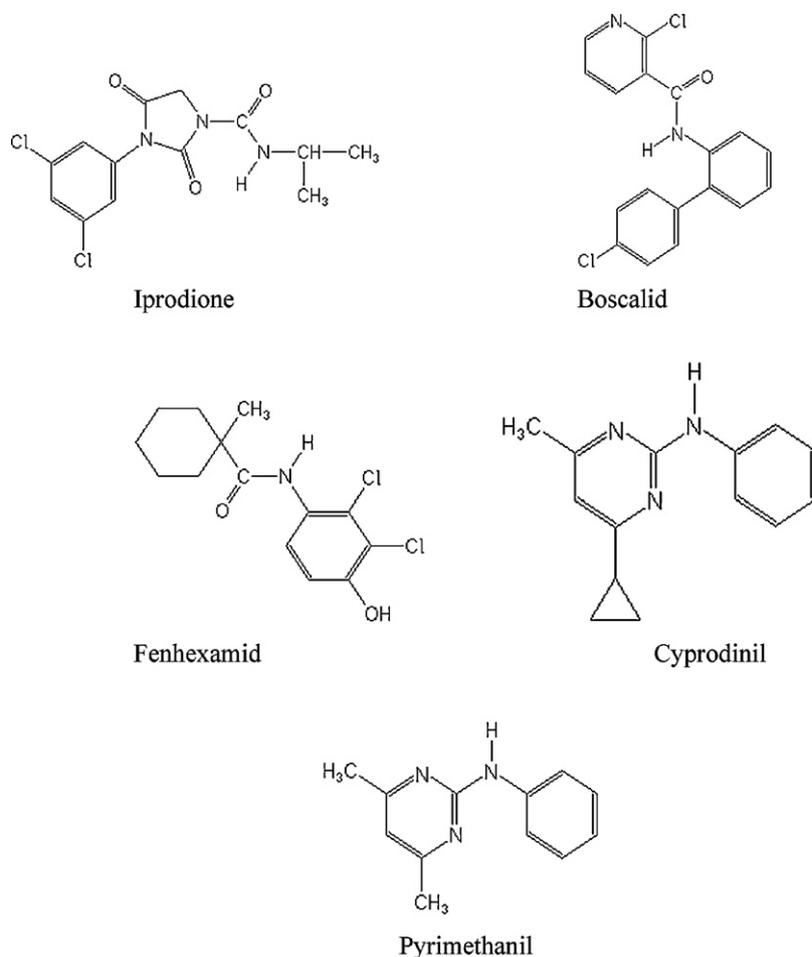


Fig. 1. Chemical structures of some of the common fungicides used in grapes.

involved in these reactions. Hydroxyl radical, a non-selective free intermediate, reacts strongly with the most of the organic species by hydrogen abstraction or electrophilic addition to double bonds. Free radicals further react with molecular oxygen, resulting in a peroxy radical that initiates a sequence of oxidative degradation reactions (Aguera and Fernandez-Alba, 1998).

Ozone has been evaluated for the removal of pesticides from different products. Ong et al. (1996) reported that dipping apples into ozonated water reduced levels of azinphos-methyl, captan and formetanate hydrochloric acid on the apple surface by 75%, 72% and 46%, respectively. Hwang et al. (2001) observed that mancozeb residues decreased by 56–97% and ethylene-thiourea completely removed after ozonated water treatment of apples. Even low levels of ozone (1.4–2.0 mg/L) in water reduced the insecticides diazinon, parathion, methyl-parathion and cypermethrin on Pak Choi (*Brassica rapa*) (Wu et al., 2007). Compared to aqueous ozonation studies, very little work has been done to examine the effectiveness of gaseous ozone to degrade pesticides. After storing waxed Navel oranges for 35 d in an atmosphere containing 180–200 nL/L ozone, Metzger et al. (2007) observed much lower levels of imazalil, malathion and chlorpyrifos on fruit stored in ozone than air. A brief study in our laboratory, the persistence of boscalid, iprodione, fenhexamid, cyprodinil, and pyrimethanil was determined on 'Ruby Seedless' grapes after a single exposure to 10,000 $\mu\text{L/L}$ ozone under a pulsing vacuum for 1 h at 5 °C (Gabler et al., 2010). This treatment reduced the residues of some of these fungicides, but this method of ozone application has not entered commercial use, since it is costly and some injuries to the grape cluster rachis can occur (Shimizu et al., 1982; Gabler et al., 2010). Moreover, some other

detrimental effects of ozone on various products have also been reported (Schomer and McColloch, 1948; Smilanick, 2011). In spite of considerable interest in the investigation of efficiency and possibility of ozone use in different food applications (Palou et al., 2002), the use of low concentration ozone atmospheres during cold storage of table grapes is only in limited commercial use.

Ozone was declared generally recognized as safe in the USA in 2001 (US FDA, 2001), and since that time it is being widely investigated and introduced into some commercial applications in food industry such as table grapes storage. It would be very useful if gaseous ozone shows effective results in reducing pesticide residues on the produce, and, if so, if the degradation products are characterized and safe. Fungicide degradation by any means can also have a negative effect on decay control during storage of conventional commodities. This work is the first step in determining if ozone influences degradation of these compounds. In subsequent work, we will examine their degradation products. The objective of this study was to investigate the persistence of the residues of some common vineyard fungicides during the cold storage of 'Thompson Seedless' table grapes in atmospheres of air or 0.3 $\mu\text{L/L}$ ozone.

2. Materials and methods

2.1. Chemicals

The analytical standards of boscalid (Riedel-de Haen), cyprodinil (Riedel-de Haen), fenhexamid (Fluka), iprodione (Fluka) with more than 99% purities were obtained from Sigma–Aldrich Chemie GmbH (Deisenhofen, Germany). Technical grade pyrimethanil

(>99% purity) was obtained from Janssen Pharmaceutica (Beerse, Belgium). Stock solutions of each fungicide (1000 mg/L) were prepared by dissolving 25 mg of standards in a mixture of 2-propanol and acetonitrile (50:50, v/v, 25 mL) and stored in a deep-freezer (-20°C).

Commercial fungicides used in the study were Pristine WG (BASF, Florham Park, NJ, 25.2% boscalid), Rovral (BASF, Mount Olive, NJ, 41.6% iprodione), Elevate 50WDG (Arysta LifeScience, Cary, NC, 50% fenhexamid), Vanguard 75WG (Syngenta Corp. Wilmington, DE, 75% cyprodinil) and Scala SC (Bayer CropScience, Research Triangle Park, NC, 54.6% pyrimethanil).

Acetonitrile (99.93% purity) and 2-propanol (99.5% purity) were obtained from Sigma–Aldrich. HPLC grade methyl tert-butyl ether (MTBE) was obtained from EMD Chemicals (Gibbstown, NJ, USA). An internal standard, *m*-bromoanisole, was obtained from Sigma–Aldrich and used to correct the variabilities in injection and detection response in gas chromatography (GC) and mass spectrometry (MS), respectively.

2.2. Fungicide treatments of grapes

Freshly harvested Thompson Seedless grapes were obtained from the vineyards of the USDA-ARS facility in Parlier (CA), and were not previously treated with any fungicides before harvest. After being taken to the laboratory, the grapes were stemmed manually and berries were used for the experiments.

About 90 kg of grape berries were sprayed in the laboratory to run-off with a fungicide solution that contained 0.27 g/L of cyprodinil (Vanguard 75WG), 37.1 g/L of pyrimethanil (Scala SC), 0.11 g/L of boscalid (Pristine WG), 0.29 g/L of fenhexamid (Elevate 50WDG), and 0.5 mL/L of iprodione (Rovral). Fungicide rates were calculated based on their maximum recommended label rate and a water volume of 1700 L per hectare. After fungicide application, the berries were dried in air for 24 h.

Approximately 1.8 kg of fungicide-treated grapes were packed in clamshell containers, then four clamshell containers were placed into an expanded polystyrene box. Clamshell containers and polystyrene boxes were perforated to provide openings to permit free circulation of air through the container interior. The boxes were stored either in ozone or in ambient air atmosphere (at 2°C , 95% RH) for 36 d. Residue analyses were done in the beginning and at 12-d time intervals. Berries from three replicate boxes were analyzed at each interval.

2.3. Storage in air and ozone atmosphere

Ozone was produced by a corona-discharge ozone generator (OG-4000, Purfresh Inc., Fremont, CA, USA) that employed an air drier and oxygen concentrator. The production capacity of the generator was 10.5 mg s^{-1} of ozone. Ozone gas was released into a cold room (27 m^3) with stainless steel surfaces through a perforated PVC tube anchored to the ceiling in front of the cooler unit fans. The ozone concentration in the room was controlled and adjusted to $0.300\text{ }\mu\text{L/L}$. It was continuously monitored by circulation of air from the room through an electro-chemical ozone analyzer (OSA-1000, Purfresh Inc., Fremont, CA, USA) with a minimum detection limit of $0.010\text{ }\mu\text{L/L}$. In order to have a comparative control room with an ambient air atmosphere, the same environmental conditions of temperature and RH were set and continuously monitored in another similar cold storage room.

2.4. Extraction of fungicides from grapes

About 0.6 kg of grape berries in a sample was macerated in a Waring Blender for 2 min at high speed. A sample 5 g of the homogenate was weighed into a polypropylene tube and 15 mL

of methyl tert-butyl ester (MTBE) with the internal standard was added. The tube content was thoroughly mixed using a vortex mixer and centrifuged at $2016\times g$ for 10 min (R5C5, Sorvall Instruments, Norwalk, CT, USA). The organic phase taken by a syringe was filtered through a polytetrafluoroethylene (PTFE) filter with $0.45\text{ }\mu\text{m}$ pore size (6874-2504, Whatman) and injected to the GC–MS.

2.5. Analyses of fungicides with GC–MS

The concentrations of fungicides in the samples were measured by GC–MS. Since filtered grape extracts showed no interference, they were directly injected to GC. A gas chromatograph (Agilent 6890) and an ion trap mass spectrometer (Agilent 5973) operated with 70 eV electron impact (EI) ionization were used. Full-scan spectra (m/z 50–550) were acquired at 0.34 s per scan. Cool on-column injection ($1\text{ }\mu\text{L}$) was at 40°C with He carrier gas ($23.3\text{ }\mu\text{L s}^{-1}$). Transfer-line and manifold temperatures were 240 and 220°C , respectively. The oven temperature was: isothermal at 100°C for 2 min, heated at 0.167°C/s to 200°C , isothermal for 12 min, heated at 0.167°C/s to 250°C , then isothermal for 25 min. The analytical column used was a J&W DB-5ms (L 30 m, i.d. 0.25 mm , d.f. $0.25\text{ }\mu\text{m}$).

2.6. Calibration curves

Standard solutions were prepared with the pesticides stock solutions in MTBE containing internal standards. A 7-point calibration curve was constructed for each fungicide covering a range of 0.01–10.0 mg/L. The resulting correlation coefficients were higher than 0.993 in all cases. Quantifications of pesticides based on peak area data were conducted using these curves.

2.7. Recovery studies and limit of detection

Homogenized grape samples (5 g) were spiked with appropriate volumes of stock solutions to concentrations of 1, 5, and 10 mg/L of each fungicide. After letting the samples settle for 20 min, they were extracted according to the procedure described previously. Three replicates were prepared for each concentration of five fungicides. The limits of detection were determined by analyzing fungicide mixtures in the grape extracts. These limits were 0.02 mg/L for boscalid, 0.08 mg/L for iprodione, 0.02 mg/L for fenhexamid, 0.01 mg/L for cyprodinil, and 0.01 mg/L for pyrimethanil.

2.8. Statistical analysis

The data were subjected to analysis of variance to determine the significant differences between means using Minitab statistical package (v. 13, MINITAB Inc., State College, PA, USA). Values were reported as mean fungicide level \pm standard deviation. Duncan's multiple range test, at a significance level of $P=0.05$, was conducted for the separation of means using MSTAT-C statistical software (MSTAT 1991, Michigan State University, MI, USA).

3. Results

Main fragments and retention times obtained from GC–MS are given in Table 1. Results of recovery tests are shown in Table 2. Average recovery values obtained for boscalid, iprodione, fenhexamid, cyprodinil, and pyrimethanil were 89.8–99.4%, 84.4–97.1%, 82.6–96.0%, 87.2–97.6%, and 88.7–96.5%, respectively. Boscalid, iprodione, fenhexamid, cyprodinil, and pyrimethanil residues during cold storage at 2°C of 'Thompson Seedless' table grapes in air or $0.3\text{ }\mu\text{L/L}$ ozone can be seen in Table 3. Storage of grape berries for 36 d in air resulted in 34.6%, 22.4%, 40.8%, 12.2% and

Table 1
Main fragments and retention times of the fungicides studied obtained from GC–MS.

Fungicide	Molecular weight	Retention time (min)	Main fragments <i>m/z</i> (% relative intensity)
Boscalid	343.21	31.44 ± 0.01	155(53.1), 342(32.0), 112(14.8)
Iprodione	330.17	21.40 ± 0.01	314(67.9), 187(20.1), 245(12.0)
Fenhexamid	302.20	20.10 ± 0.02	97(50.8), 177(44.3), 55(14.3), 301(12.0)
Cyprodinil	225.29	16.55 ± 0.01	224(87.3), 210(8.3), 77(4.4)
Pyrimethanil	199.25	14.03 ± 0.01	198(94.9), 77(5.0)

Table 2
Average recoveries and relative standard deviations (*n* = 3) of boscalid, iprodione, fenhexamid, cyprodinil and pyrimethanil from grape samples with different spiking levels.

Spiking level (mg/L)	(Recovery ± relative standard deviation) %				
	Boscalid	Iprodione	Fenhexamid	Cyprodinil	Pyrimethanil
1	89.8 ± 1.4	84.4 ± 3.8	82.6 ± 4.1	87.2 ± 8.2	88.7 ± 5.9
5	95.7 ± 2.8	97.1 ± 6.4	92.9 ± 5.9	95.5 ± 9.1	94.1 ± 7.2
10	99.4 ± 5.2	95.4 ± 4.7	96.0 ± 3.4	97.6 ± 8.5	96.5 ± 4.4

14.5% reductions in boscalid, iprodione, fenhexamid, cyprodinil, and pyrimethanil residues, respectively. These rates were 46.2%, 23.9%, 64.5%, 34.7%, and 51.6% in berries stored in ozone atmosphere

4. Discussion

Our results have shown both natural declines in the residues of fungicides related to table grape production and their susceptibility to degradation by ozone (Table 3). For a comparison purposes, Table 3 also shows the results of an earlier study (Gabler et al., 2010) conducted in our laboratory, where the persistence of these fungicides on 'Ruby Seedless' grapes after a single exposure to 10,000 µL/L ozone fumigation under pulsing vacuum for 1 h at 5 °C was determined. In general, the susceptibility of each fungicide to degradation by ozone was evident if the ozone was applied as a brief, very high concentration under pulsing vacuum as in the prior study or if it was constantly present during storage at 0.3 µL/L as in the present work.

Statistical analysis revealed that storage time in both the air and the ozone atmosphere in this study and the ozone treatment in the previous study (Gabler et al., 2010) significantly affected reduction of residues of all fungicides tested ($P < 0.05$). Moreover, these factors showed a significant interaction for fenhexamid, cyprodinil, and pyrimethanil ($P < 0.05$), but not for boscalid or iprodione ($P > 0.05$).

Storage for 36 d in air and ozone atmosphere resulted in 34.6% and 46.2% reductions in boscalid, and 22.4% and 23.9% reductions in iprodione residues, respectively. Residues of these fungicides were significantly lower on ozone-treated samples on the twelfth day of storage. However, this effect was transient and was not observed at the end of storage.

In our opinion, the resistance of boscalid to ozone is most likely due to the stability of aromatic and heteroaromatic rings in the structure of this compound. On the other hand, Lohmann et al. (2009) reported that boscalid could be oxidized electrochemically. They observed the formation of degradation products such as hydroxylated species and isomeric covalent glutathione adducts. Although the metabolism of boscalid in plants, hydroxylation in the biphenyl and pyrimidine rings, and cleavage reactions in both rings were observed, the unchanged parent was still the major part of the residue (Tomlin, 2003). Boscalid was reported to be stable to hydrolysis and aqueous photolysis. Moreover, it was not degraded during the simulation of pasteurization nor during simulated baking, boiling, brewing or during sterilization (Anon, 2006). Chen et al. (2007) studied boscalid residues in or on the cucumbers and observed that the residues declined quickly with time. They reported only 5–17% of the initial deposits were found in or on the cucumbers 6 d after the last application of this fungicide in the field. Gabriolotto et al. (2009) also reported low boscalid residues in harvested grapes after 65–90 d from the treatment.

Table 3
Persistence of residues (mg/kg) of fungicides iprodione, boscalid, fenhexamid, cyprodinil, or pyrimethanil during the cold storage at 2 °C of 'Thompson Seedless' table grape berries in air or 0.3 µL/L ozone, or after a single exposure to 10,000 µL/L ozone fumigation for 1 h at 5 °C 'Ruby Seedless'. The grapes were treated with the fungicides before ozone exposure. Each value is the mean of three replicates.^{a,b}

Fungicide	Constant low concentration ozone ^c	High concentration ozone ^d							
		Initial	12	24	36	Decline (%)	Pre-exposure	Post-exposure	Decline (%)
Boscalid	Air	2.6Aa	2.4Aa	1.8Ab	1.7Ab	34.6	2.6	2.5	5
	Ozone	2.6Aa	1.6Bb	1.3Bb	1.4Ab	46.2			
Iprodione	Air	6.7Aa	6.9Aa	6.0Aab	5.2Ab	22.4	0.5	0.4	15
	Ozone	6.7Aa	5.0Bb	4.5Bb	5.1Ab	23.9			
Fenhexamid	Air	7.6Aa	7.8Aa	5.7Ab	4.5Ac	40.8	1.1	0.4	68.5
	Ozone	7.6Aa	4.9Bb	3.0Bc	2.7Bc	64.5			
Cyprodinil	Air	4.9Aa	5.0Aa	4.9Aa	4.3Aa	12.2	5.1	1.2	75.4
	Ozone	4.9Aa	3.4Bb	3.1Bb	3.2Bb	34.7			
Pyrimethanil	Air	6.2Aa	6.1Aa	6.3Aa	5.3Ab	14.5	4.7	0.8	83.7
	Ozone	6.2Aa	4.4Bb	3.6Bbc	3.0Bc	51.6			

^a Different letters shown with upper case indicate significant differences between air and ozone storage ($P < 0.05$).

^b Different letters shown with lower case indicate significant differences among storage days in air or ozone atmosphere ($P < 0.05$).

^c Values indicate days in storage.

^d Residues after 1 h ozone exposure from Gabler et al. (2010).

Our results showed that the oxidation-sensitive regions of iprodione such as phenyl ring, alkyl chain and double bonds were somehow protected from oxidation by ozone. In a model system study, Hu et al. (2000) observed slow degradation (rate constant = $233 \text{ M}^{-1} \text{ s}^{-1}$) of iprodione by gaseous (2.4 mg/L) and aqueous (0.32 mg/L) ozone treatments. Information on the resistance of iprodione to some other chemical treatments is quite controversial. It has been reported to be degraded photochemically (Schwack et al., 1995) whereas according to the US Environmental Protection Agency it is quite stable to photolysis and hydrolysis (Anon, 1998). The residues of iprodione in produce widely varied depending on the application conditions. Some studies showed high persistency of this fungicide on treated fruits such as apricots (Cabras et al., 1998) and strawberries (Stensvand and Christiansen, 2000). Omirou et al. (2009) reported that iprodione residues in greenhouse tomato fruit were always low even when a double dose had been applied. Nevertheless, they observed significantly higher residues in fruit during postharvest storage compared to fresh harvested ones. According to the authors, this case was probably related to the photodegradation of iprodione, since tomato fruit were stored in dark chambers after harvesting, simulating the marketing procedure.

In our study, when stored in air for 36 d, 40.8%, 12.2%, and 14.5% of natural decline was observed in the residues of fenhexamid, cyprodinil, and pyrimethanil, respectively. Ozone treatments markedly accelerated the decomposition of these fungicides. From day 12 on, residues of all these fungicides were significantly lower on ozone-treated samples and this case lasted throughout storage. Reduction rates in fenhexamid, cyprodinil, and pyrimethanil levels were 64.5, 34.7%, and 51.6%, respectively, at the end of storage.

Among the fungicides we assessed, pyrimethanil was the most affected by ozone storage atmosphere. At the end of the storage period, its degradation was about 3.6-fold higher in the ozone atmosphere than in air. Likewise, addition of an oxidizer such as hydrogen peroxide to the treatment medium was reported to affect photocatalytic degradation of pyrimethanil (Arana et al., 2008). The authors also indicated that pyrimethanil degradation was notably faster in the presence of oxygen than in its absence. In addition, Vanni et al. (2006) reported that oxygen was the most important factor on the rate of degradation of pyrimethanil in soil. On ripening tomato plants, pyrimethanil residues dropped by half and reached detection limit level in 5.7 and 13.7 d after pesticide application in the field, respectively (Sadlo, 2002). Since pyrimethanil is also used on mature fruits, the disappearance of pyrimethanil could not be related to the dilution effect caused by fruit growth (Angioni et al., 2006).

Cyprodinil and pyrimethanil are from the same chemical class (anilinopyrimidines) and have similar chemical structures (Fig. 1). Despite this similarity, quite different degradation behaviors of these two substances could be observed. In contrast with our findings, cyprodinil was regarded as more sensitive to degradation reactions than pyrimethanil (Cabras and Angioni, 2000). The decay rate of cyprodinil in grapes was high, with a half-life of 12 d, whereas pyrimethanil disappeared slowly with a half-life of 57 d (Cabras et al., 1997). Anfossi et al. (2006) investigated the photo-induced degradation of 3 anilinopyrimidine fungicides (cyprodinil, pyrimethanil, and mepanipyrim). Photoproducts were very similar for the three fungicides and showed that three sites of the molecules were primarily attacked by hydroxyl radicals: the nitrogen bridge between the two rings, the phenyl ring and the primidyl ring.

In our study, maximum reduction rates, obtained at the end of storage period in both air and ozone atmosphere, were observed in fenhexamid levels. Fenhexamid has been shown to breakdown rapidly in the environment and to be ecologically benign and non-volatile (Hengel et al., 2003). Its relatively rapid disappearances

were reported in grapes (Cabras et al., 2001; Gabriolotto et al., 2009) and strawberries (Rabolle et al., 2006). Metabolization behavior of fenhexamid under aerobic conditions was investigated in soil samples (Brumhard and Bornatsch, 1996). Fenhexamid is characterized by rapid breakdown, conversion and mineralization in the soil. Half of the active ingredient disappeared in less than 1 d in each soil.

Compared to high-dose and short-time gaseous ozone treatments, low-dose and long-time treatments are more useful in an important way. For instance, Gabler et al. (2010) treated grapes with 10,000 $\mu\text{L/L}$ ozone soon after the fungicides were applied, so they did not have time to distribute into the grape tissue, while in the present study, the fungicide residues were characterized over many weeks. Another important issue with the fungicides is their 'systemic' activity. Some fungicides (systemic ones) penetrate the tissue and move through the flesh, while some others (non-systemic ones) are topical and reside on the berry surface. Three of the fungicides we evaluated, namely boscalid, cyprodinil, and pyrimethanil have systemic activity while iprodione and fenhexamid are locally systemic. They could be transported to the inner parts of the crop and therefore were limitedly subjected to the degradation process by ozone. In addition, all of them may dissolve into the waxy cuticle on the surface of the berry.

Toxicity of the degradation products from the parent compounds is probably the key factor for deciding whether a process is appropriate for a certain application (e.g. ozone treatment for removing pesticides). However, identification and toxicity determination of degradation products are quite difficult since many breakdown products (with no commercially available standards) are formed after exposure to various technological processes. For instance, up to 22 compounds are reported to be formed as degradation intermediates from pyrimethanil after photocatalytic degradation process (Aguera et al., 2000). Pugliese et al. (2004) evaluated aqueous solutions of various agents including some oxidizers such as hydrogen peroxide and sodium hypochlorite for pesticide reduction in nectarines. Ethanol, glycerol and sodium laurylsulfate removed approximately 50% of the residues while the other solutions were not found more effective than tap water. The authors mentioned the possible formation of toxic by-products from organophosphorus pesticides although none of them were identified in the extracts of the washed samples under the experimental conditions studied (Pugliese et al., 2004). Pesticides and other toxic compounds in this kind of produce, together with table grapes, constitute a more serious health risk than in the other agricultural products since they are consumed directly (without any processing, such as peeling). Lipophilic pesticides can remain on the skin of the produce and cannot be removed by washing treatments. In addition, commercial pesticide products, used for treating grapes before ozonation, contain not only active ingredients but also other ingredients such as solvents, surfactants, carriers, and intensifiers. These ingredients usually account for more than 50% of the commercial fungicide formulations and could also react with ozone during treatments. While degradation of a pesticide via any treatment is being studied, the fates of these ingredients should also be taken into consideration.

5. Conclusions

This study has shown the natural decline occurred in some contemporary fungicides related to table grape production. It is an important piece of information for the development of a process to manage over-tolerance of some residues. Residues of three of five popular fungicides evaluated in this study declined more rapidly in an ozone atmosphere than in air. Most probably, the reactivity of compounds with ozone varies largely due to their diverse structural features. Iprodione and boscalid resisted ozone while

the rates of decline of fenhexamid, cyprodinil, and pyrimethanil were markedly accelerated when grapes were stored in the ozone atmosphere. Among the recently introduced fungicides used in this study, boscalid is the only one that was not affected by ozone treatment. Ozonation employed to prolong the storage life of the grapes by controlling decay fungi may have a secondary benefit of reducing fungicide residues on table grapes. However, toxicology studies should be carried out to determine the possible human health risks of the breakdown products of fungicides after gaseous ozone treatment.

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