Can Ozone be a New Control Strategy for Pests of Stored Grain?

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Abstract Ozone (O_3) is a strong oxidant with a long history of safe use in many fields. Researches have been recently focused on the application of O_3 as a fumigant to control stored-grain insects and microorganisms and to reduce mycotoxins. This review found the following facts: (1) O_3 significantly suppressed insect populations at ≤ 50 ppm with 4 days treatment; (2) to eradicate insect infestation, >135 ppm with more than 8 days treatment would be required; (3) O_3 at 50 ppm with 3 days treatment reduced 63 % of stored fungi; (4) O_3 at 5–30 ppm could reduce mycotoxin contamination; however, high concentration and long treatment time were required to eliminate mycotoxins; (5) application of O_3 at doses that were sufficient for the effective disinfestation of grain might affect qualities of grain; and (6) O_3 at 47–106 ppm could noticeably damage equipment in 2 months by corrosion. Based on these facts, we recommended that ≤ 50 ppm O_3 should be used in the stored-grain industry and its potential method of application was also analyzed.

Keywords Stored grain · Ozone · Insects · Microorganisms · Mycotoxins · Decontamination · Disinfestation

Introduction

Ozone (O_3), or trioxygen, is much less stable than O_2 and breaks down with a half-life of 20–50 min at room temperature, to normal O_2 . Ozone is a strong oxidant with a long history of safe use in many fields and mostly used in the industries of pharmaceuticals, synthetic lubricants, and many other organic compounds. It is also used for bleaching substances, controlling microorganisms in water and air sources, eradicating water-borne parasites, and bleaching wood pulp and paper. For example, O_3 was first used to treat water as early as 1893 [12, 27]. At present, many municipal water treatment systems control bacteria with O_3 instead of chlorine [12, 15, 47]. Once it has

decayed, O_3 leaves no taste or odour in the treated materials [8]. It is also used to treat cold room air, eliminate odours, improve taste, and bleach colour [24].

Since 1930s, researches have been done on a wide variety of fruits and vegetables [46]. These researches used O_3 to prevent microorganism activity on food surfaces and extended the shelf life of fruits and vegetables, including apples, oranges, peaches, pears, grapes, cranberries, strawberry, broccoli, potatoes, and tomatoes. For decades, O_3 was successfully used as a disinfectant and sanitizer for the treatment of agriculture products and equipment such as vegetables and fruits, dairy and swine effluent, meat, gelatin, manufacturing equipment, and packaging materials [16], to control insects and microorganisms, and to remove pesticides, inorganic and organic compounds [27].

The advantage of O_3 application is that it is more environmentally friendly than traditional pesticides and fungicides. Ozone can be easily generated at the treatment site and only electricity and air are required. Therefore, O_3 provides several safety advantages over conventional pesticides and fungicides. There are no residues on products and no presence of toxic chemicals, no danger of chemical

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mixing hazards, or no issues about disposal of left over insecticides or containers [25].

Researches have been recently focused on the application of O_3 as a fumigant to control stored-grain insects and microorganisms and to reduce mycotoxins on grain. This attention is timely due to the development of insect resistance to pesticides, the phase out of methyl bromide in 2005 as a fumigant, increased demand for organic grains, and innovation to control insects and microorganisms. Looking at past literature (especially the last 2 years), we saw rapidly accumulated information on this powerful oxidizing agent. The purpose of this article was to present a general overview of published information related to O_3 as a fumigant to control insects and microorganisms and to reduce mycotoxins. Its potential application in the grain storage industry was also assessed.

Control of Insects of Stored Grain

Before 1980, the toxicity of O_3 on agriculture field insects was studied [5, 14, 29, 30] and little was known on stored grain insects [13]. In recent years, O_3 toxicity on stored-grain insects was evaluated in several laboratory and field studies. Those studies showed the toxic effect of O_3 to stored-grain insects. However, there were several inconsistencies in the published reports summarized below.

Susceptible Species and Insect Stages

Ozone was lethal to both external and internal feeders. The external feeders include beetles such as *Cryptolestes ferrugineus* (Stephens) [6], *Oryzaephilus surinamensis* (L.) [6, 18, 34, 52], *Tribolium castaneum* (Herbst) [6, 13, 18, 34], *T. confusum* (J. Duv.) [13, 18, 34, 52], and *Stegobium paniceum* (L.) [18]; moths such as *Ephestia kuehniella* (Zell) [18] and *Plodia interpunctella* (Hübner) [6, 18, 53]; and Psocids [6]. The internal feeders include weevils such as *Sitophilus* spp. [6, 18, 53]; lesser grain borer, *Rhyzopertha dominica* (F.) [6, 18, 52]; and Angoumois grain moth, *Sitotroga cerealella* (Oliv.) [18]. Studies showed that the most resistant stage of insects was the egg or O_3 had no effect on eggs [6, 18, 26]. The susceptible stage among larvae, pupae, and adults was different in different published studies. Bonjour et al. [6] and Hansen et al. [18] found the pupa or adult was the most susceptible stage for most tested species. Leesch [26] reported pupae of *P. interpunctella* were the most resistance stage after the egg. The dose and exposure times leading to 100 % mortality were not different for young, medium-aged and old larvae of eleven stored-product pests tested [18]. However, Erdman [13] found larval and pupa stages of *T. castaneum* were O_3 sensitive with sensitivity decreasing with age.

Leesch [26] found younger pupae of *P. interpunctella* were more susceptible whereas with larvae the opposite was true. Isikber and Oztekin [21] observed a higher susceptibility for larvae, pupae, and adult stages of *E. kuehniella* (90–100 %) compared to *T. confusum* (1.3–22.7 %) under similar experimental conditions. A higher susceptibility rate for insects was reported for *P. interpunctella* compared to the low mortality of *T. confusum* [26]. However, Strait [53] and Hansen et al. [18] reported a high mortality rate of *T. confusum* (Table 1). Ozone treatment on larvae of *P. interpunctella* inside a wheat bin was less effective than that on pupae [6]. Bonjour et al. [6] found *S. oryzae* adults were the most susceptible species with 100 % mortality reached after 2 days in all O_3 treatments (from 25 to 70 ppm). Hansen et al. [18] found *S. oryzae* adults were not the most susceptible species with 100 % mortality reached after 6 days with 21 ppm O_3 treatment.

Dose and Exposure Time

Lethal dose and exposure time were reported to range between 5 and 500 ppm with a few hours up to 8 days treatments. Leesch [26] used up to 500 ppm in a study to develop relationship of dose and mortality. The author found that 200–500 ppm with many hours was required to kill insects [26]. Hansen et al. [18] found “freely exposed stages (with a few exceptions) were controlled with 35 ppm of O_3 in 6 days, while full mortality of internal stages within kernels required exposure to 135 ppm for 8 days”. Inside grain bins, ≥ 35 ppm was required for the control of freely exposed stages (Table 1). Inside different media, the same dose and treatment time would not reach the same mortality (Table 1). This difference was caused by the different structures and chemical components on the surface of the materials. Different structures and chemical components would influence the reaction of ozone on the surface of the materials.

Correlation Between Insect Respiration Rate and Their Susceptibility to O_3

Ozone-caused tissue damage even at low concentrations [4, 7, 31]. If the insect respiratory system was the major entry route of O_3 into the insect body [31], increased respiration rate with an increase of temperature might result in more mortality because the increased gas exchange would increase the amount of O_3 inside insect bodies [41, 49]. Sousa et al. [51] and Pereira et al. [41] found the O_3 toxicity increased with the increase of the grain temperature and O_3 concentration. Carbon dioxide, a respiratory stimulus and synergising agent that acts by keeping insect spiracles open, did potentiate the toxicity of O_3 [26]; however, Sousa et al. [52] found there was no correlation

Table 1 Comparison of disinfestation results of adult insect pests under different treatment conditions

Insects	Media ^a	Dose (ppm)	Time ^b (days)	Mortality (%)	Source
<i>R. dominica</i>	Wheat bin	70	4	97	Bonjour et al. [6]
	Freely exposed	35	6	100	Hansen et al. [18]
	Inside corn	120	1.2	100 ^d	Qin et al. [44]
	Inside wheat	120	1.2	100 ^d	Qin et al. [44]
	Inside paddy	120	1.2	94	Qin et al. [44]
<i>Tribolium confusum</i>	Inside corn	50	3	100	Strait [53]
	Freely exposed	35	6	100	Hansen et al. [18]
<i>T. castaneum</i>	Corn bin	50	3	92	Kells et al. [23]
		25	5	77	Kells et al. [23]
	Wheat bin	50	4	100	Bonjour et al. [6]
	Freely exposed	35	6	100	Hansen et al. [18]
	Inside corn	50	3	50	Pereira et al. [41]
		120	1.2	93 ^d	Qin et al. [44]
	Inside wheat	120	1.2	90 ^d	Qin et al. [44]
	Inside paddy	120	1.2	88 ^d	Qin et al. [44]
	Freely exposed	5	3	100	Mason et al. [34]
	Wheat bin	70	4	67	Bonjour et al. [6]
<i>Sitophilus zeamais</i>	Corn bin	50	3	100	Kells et al. [23] and Strait [53]
		25	5	99	Kells et al. [23]
	Freely exposed	78	5	100	Hansen et al. [18]
	Inside corn	120	1.2	97 ^d	Qin et al. [44]
	Inside wheat	120	1.2	70 ^d	Qin et al. [44]
	Inside paddy	120	1.2	100 ^d	Qin et al. [44]
	Freely exposed	100	1	100	Qin et al. [44]

Table 1 continued

Insects	Media ^a	Dose (ppm)	Time ^b (days)	Mortality (%)	Source
<i>Sitophilus oryzae</i>	Wheat bin	50	4	100	Bonjour et al. [6]
	Freely exposed	21	5	100	Hansen et al. [18]
<i>P. interpunctella</i> ^c	Inside corn	50	3	100	Strait [53]
	Corn bin	25	5	91	Kells et al. [23]
	Freely exposed	36	6	100	Hansen et al. [18]

^a Insects are kept inside cages located in grain bin (corn or wheat bin), in small tubes with small amount grain (inside corn or wheat) or there is no or small amount grain (freely exposed) during treatment

^b Time exposed to O₃

^c Older larval

^d Phosphine-resistant strain

between insect respiration rate and their susceptibility to O₃. Therefore, the reasons causing insect death should be further studied.

Correlation Between the Resistance to Phosphine and Susceptible to O₃

There was no significant correlation between the resistance to phosphine and susceptibility to O₃, which clearly indicated the absence of cross-resistance to both fumigants [52]. However, Qin et al. [44] found phosphine resistant strains were more susceptible to O₃.

These discrepancies created the difficulty to correctly choose O₃ dose and treatment time in grain storage bins. Further testing inside storage bins showed that O₃ significantly suppresses insect populations [6, 23]; however, 100 % mortality inside storage bins could only be achieved when much higher O₃ concentration and longer treatment time were applied than that under laboratory conditions (Table 1). For example, to reach 100 % mortality inside commercial bins, higher than 70 ppm with more than 4 days treatment would be required to kill adults [6]. To control insect eggs, a second treatment after egg hatching was required [6]. The difference between laboratory and field studies might be caused by the highly reactive nature and short half-life time of O₃ that limit O₃ penetrating into a grain mass and grain kernels. Insects might not receive the supplied does of O₃ at the beginning of the fumigation under field conditions. It should not be different under laboratory and field conditions if insects receive the same

amount of O₃. Therefore, insects inside stored grain bins might receive sub-lethal doses. Insects receiving a sub-lethal dose or surviving after treatment were sluggish and uncoordinated; however, the survived insects were able to reproduce [53]. To eradicate insect infestation, therefore, ≥ 135 ppm with more than 8 days treatment would be required [18].

Control of Microorganisms and Reduction of Mycotoxins

The effect of O₃ on food microorganisms was reviewed by Rice et al. [46], Kim et al. [24], and Tiwari et al. [55]. Food microorganisms inherently varied in sensitivity to O₃ [24, 46]. The physical state (e.g., the stage of growth, inside or outside of the treated materials, and whether materials were treated before by O₃) and environmental factors (e.g., pH of the medium, temperature, and humidity) greatly affected the disinfection by O₃. Yeasts appeared more sensitive than molds to O₃ treatment [38, 45]. Ozone also reacted with other particles and compounds present in an environment such as food systems that were rich in organic matter. Therefore, O₃ was more effective on microorganisms when the organism was inside water and air or on the surface of treated materials.

Except for a few published papers in the 1960s and 1980s little was known about the effects of O₃ on stored grain and associated microorganisms [11, 32, 38–40, 48]. Ozone was effectively used to control stored grain and flour fungal growth; however, these effective results were only presented at certain conditions. Inside cereal grains, beans, and spices, counts of *Bacillus* and *Micrococcus* decreased 1–3 logs by <50 mg/L O₃ treatment [39]; however, with few exceptions, longer exposure time and lower temperature resulted in higher or the same microbial activity. Mason et al. [34] also found radial growth of *Aspergillus flavus* Link and *Fusarium moniliforme* J. Sheld in agar media was inhibited for the first 2 days, however after 3 days of O₃ exposure, growth of the microorganism was the same as the control. The “number of viable *A. parasiticus* Spore on the grain surface was reduced by 63 % when grain was exposed to 50 ppm O₃ for 3 days, whereas 25 ppm for 5 days failed to significantly reduce spore viability” [23]. Therefore, researchers concluded that O₃ was only effective on microorganisms on the surface of grain kernels and control required higher than 1,752 ppm [2, 23, 34, 35, 57].

Further pilot studies in grain storage bins showed that microorganisms were significantly decreased. Even though increasing treatment time and dose decreased the count of microorganisms, the maximum reduction rate was less than 80 % and only the microorganisms on the surface of the

grain kernels were reduced. To reach this maximum reduction rate, 1,752 ppm O₃ with 3 h treatment was required [23, 35]. Kells et al. [23] reported that 50 ppm O₃ with 3 days treatment reduced only 63 % of the contamination level of the fungus *A. parasiticus*. White et al. [57] concluded that O₃ treatment decreased dry matter loss compared to the control, but treatment at high rates and long treatment times would result in an unacceptable cost for the grain industry. In addition, $\geq 1,752$ ppm O₃ was corrosive [26]. Therefore, using higher concentration of O₃ to control microorganisms in stored grain might not be justified, while lower than 1,752 ppm could only suppress populations of microorganisms.

Ozone was reported to “be effective in the detoxification and degradation of commonly occurring mycotoxins such as patulin, cyclopiazonic acid, secalonic acid D, ochratoxin A, ZEN, aflatoxins, deoxynivalenol” [28, 36, 59]. Decontamination of mycotoxins depended upon several factors including exposure time, O₃ concentration, temperature, pH, and moisture content of the grain mass. Application of O₃ gas under dry conditions was reported to be less effective than that under moist conditions [59]. Young et al. [60] observed “a rapid degradation of trichothecene mycotoxins at a low pH (pH 4–6) compared to a higher pH (pH 7–8)”. Proctor et al. [42] reported “a greater degradation of aflatoxins in peanut kernels at high temperature”. Degradation was specific to the molecular structure of toxins. McKenzie et al. [36] observed greater resistance of aflatoxin B2 compared to B1 and G2. To reduce mycotoxin contamination, O₃ at about 5–30 ppm was required [55]; however, to eliminate mycotoxins from grain required a high concentration and long treatment time.

Effect on Stored-Grain Quality

Tiwari et al. [55] reviewed the effect of O₃ on grain quality and concluded that application of O₃ at doses that were sufficient for the decontamination of grains might affect grain quality. In the published literature, there were discrepancies in the effect of O₃ on quality of grain and derived products. These discrepancies might be mainly caused by the difference of dose and treatment time, material, and application method.

The most affected part of the grain kernels or food products was the surfaces of the treated materials [20]. Mechanisms of decomposition by O₃ were complex processes that depend on factors such as the surface structure of a grain kernel, its products, and packing density of materials. Ozone converted many nonbiodegradable organic materials into biodegradable forms [24]. Ozone was sparingly soluble in water. At 20 °C, the solubility of 100 % O₃ was only 570 mg/L. Ozone decomposed in solution in a stepwise fashion, producing in turn hydroperoxyl ($\cdot\text{HO}_2$), hydroxyl

($\bullet\text{OH}$), and superoxide ($\bullet\text{O}_2^-$) radicals [1, 17, 20]. Ozone might directly react with substrates with organic double bonds as a general oxidant [54]. When O_3 was broken down to dioxygen it gave rise to oxygen-free radicals, which were highly reactive and capable of damaging many organic molecules. These reactions were so rapid and the decomposition of O_3 was so fast that its disinfection action might take place mainly at the surface [20] until a saturation state of O_3 was reached. The surface reaction might explain the following experimental reports.

- 1) During O_3 treatment, surface oxidation happened and this oxidation might promote oxidative spoilage, discolouration, and produce undesirable odours [24]. However, the internal part of the treated grain and its products were not affected, and milling and baking quality of these discoloured products might not be influenced. For example, Naitoh et al. [39] found that oxidation of lipids in cereals, peas, beans, and spices rarely occurred at <5 ppm but was considerable at higher concentrations. The husk of the rice treated with O_3 at 50 ppm for 30 days was darker brown than that in the control and had a smell of vinegar and an acidic odour. This acidic odour decreased over time but was still detectable 4 months later. Both the acidic odour and brown discolouration could be removed during the milling process, and the baking and milling quality of the polished rice was not influenced [37]. Mendez et al. [37] also found that amino acid and fatty acid contents and the content of saturated or unsaturated fatty acids of hard and soft wheat, soybean, and maize did not alter. They suggested that the O_3 did not penetrate into the kernels. Dehulling of moistened wheat treated with O_3 was enhanced [9]. Desvignes et al. [10] observed a “significant reduction (10–20 %) in the required total energy for milling without significant changes in biochemical characters of the milling fractions”.
- 2) Ozone penetrated into the inside of the treated materials. Compared with grain kernels, processed products with low packing density might be easy to be penetrated by O_3 . Therefore, the baking quality of these processed products might be influenced. For example, O_3 treatment of rice starch was reported to “enhance swelling with a reduced retrogradation tendency” [3]. Ozone had a negative effect on the sensory quality of ground spices, milk powder, and fish cake due to lipid oxidation [40]. Thiamin content in wheat flour decreased after O_3 treatment [40]. This surface reaction also explained that O_3 treatment improved the sensory quality in beef and egg and it did not alter the sensory quality of some fruits and vegetables significantly [24].

- 3) Germination of treated grain might be influenced by the O_3 concentration and treatment time. Germination of corn treated with $20 \text{ g/m}^3 \text{ O}_3$ for 6.8–20.5 min increased [56]. However, O_3 at ground level with a maximum 11 ppm reduced the germination of Northern pine provenances [43]. Wu et al. [58] found germination of wheat was not affected in less than 15 min treatment while it was reduced after 20 min treatment with O_3 concentration at 0.98 mg/g wheat. Strait [53] found germination of corn is not influenced after ozone treatment.

Toxicity to Humans and Corrosion

When O_3 concentration was less than 0.05 ppm, O_3 had a pleasant odour, similar to the fresh air after a thunderstorm. When concentration was higher than this pleasant level, O_3 was a primary irritant and affected especially the eyes and respiratory systems. Therefore, O_3 was detectable by many people at concentrations of 10 ppm in air. Ozone-damaged respiratory tissues in humans above concentrations of 100 ppm. Ground level O_3 could harm lung function and irritate the respiratory system. Ozone pollution was linked to premature death, heart attack, bronchitis, asthma, and other cardiopulmonary problems. In the USA, O_3 in the work environment was limited to a maximum of 0.1 ppm on a basis of 8 h per day and 40 h per week. Risk of O_3 to humans could be kept at a minimum level if lower than 50 ppm was applied and treated facilities were sealed.

Ozone is a powerful oxidant, second only to the hydroxyl-free radical. Therefore, it was capable of oxidizing many inorganic and organic compounds in air and water. Ozone could oxidize most metals (except gold, platinum, and iridium) to oxides of the metals or in their highest oxidation state. Ozone attacked copper, silver, aluminium (at damp or wet condition), brass, steel, and iron [22, 26]. Ozone reacted directly with some hydrocarbons such as aldehydes, polymer, or double bonds within its chain structure such as in natural rubber, wool, protein, and paint. The rubber couplings used to connect PVC ducts to the distribution plenum was rapidly degraded during 2-month duration of the test with O_3 concentration from 47 to 106 ppm [19]. Corrosion by O_3 to materials used by the grain industry was a potential disadvantage for O_3 application and should be evaluated. One of the choices to reduce the corrosion might be that the grain storage and handling system was protected by ozone-resistant materials. Ozone-resistant materials included the austenitic (300 series) stainless steels, titanium, glass, ceramics, concrete, waxes, Teflon, Hypalon[®], polytetrafluorethylene, or polyvinylidene fluoride.

Ozone must be continually supplied and evenly distributed throughout bulk of grain. It might increase corrosion rates on metal components and degrade equipment such as rubber seals, and electrical equipment at unacceptable rates [19]. Therefore, to reduce the corrosion, O_3 should be used at low concentration and contact between O_3 and equipment should be minimized. These disadvantages required complex application techniques such as mixing with treated materials which make O_3 treatment much more complicated than conventional treatments. This might result in a high cost of equipment and operational costs.

Application of O_3

Because most microorganisms, mycotoxins, and insects might not be found in free suspension as discrete particles or on the surface of the treated materials, the effectiveness of O_3 depended on the amount of O_3 applied but more on the O_3 reacting with the microorganisms, mycotoxins, and insects after demands by the substrate organic matter had been met or the organic matter was saturated with O_3 . In the published studies, there were discrepancies on the effect of O_3 on insects, microorganisms, and mycotoxins. These discrepancies might be caused by the highly reactive nature and short half-life time of O_3 that limit O_3 penetrating into grain mass and grain kernels. Therefore, the most failures by various investigators might be their inability to distinguish between the concentration of applied O_3 and O_3 reacting with microorganisms, mycotoxins, and insects.

This review found the following facts: (1) O_3 significantly suppressed insect populations at 50 ppm with 4 days treatment; (2) to eradicate insect infestation, ≥ 135 ppm with more than 8 days treatment would be required; (3) O_3 at 50 ppm with 3 days treatment reduced 63 % of the contamination level of fungi and even >50 ppm with long-treatment time might not eliminate fungi; (4) to reduce mycotoxin contamination, O_3 at 5–30 ppm was required; however, to eliminate mycotoxins from grain required a high concentration and long treatment time; (5) application of O_3 at doses that were sufficient for the disinfestation of grain might affect grain qualities; and (6) O_3 at 47–106 ppm could seriously damage the rubber couplings in 2 months. Therefore, O_3 at 50 ppm might be the proper dose to be used to suppress insect and microorganism populations and reduce mycotoxins. To exterminate insects, microorganisms, and mycotoxin contamination, a much higher dose will be required but it might not be justified because the complex application technique and requirement of ozone-resistant materials would result in a cost challenge for the grain

storage industry. This recommended dose might be used in the fumigation of empty facilities and grain storage bins.

Empty Facility and Flour Mill Treatment

Empty facility and flour mill treatment with O_3 might be more effective and faster than that inside grain storage bins if corrosion was not an issue. Because empty facilities have less grain mass than inside grain bins, the total amount of O_3 applied to the empty facility would be much lower. Insects and microorganisms would also be directly exposed to the applied dose of O_3 . Ozone fumigation might be applied on a room to room basis if each room was airtight enough to stop O_3 leakage. This might avoid shutting down the entire facility. There were several reports on storage room treatments with O_3 [46].

Application in Stored Grain Bins

Even though the chemical components on the grain surface that react with O_3 were not well known, studies showed that O_3 movement through a grain mass was restricted [23, 37]. When O_3 reached the grain, most of the O_3 reacted with the grain and only a small amount reacted with pathogens or insects unless pathogens and insects were located on the surface of treated materials or the surface of the treated materials was saturated with O_3 . This surface reaction explained two distinct phases of O_3 movement in bulk grain [23, 53]. When grain was not saturated with O_3 (phase one), O_3 was reduced as it moved through a grain mass because interaction with grain rapidly degraded the O_3 . Once the grain was saturated with O_3 (phase two), O_3 could be moved through the grain mass with less degradation. For example, O_3 concentration at 2.7 m high in a bulk of stored corn could reach 50 ppm in 0.7 day when O_3 was pushed with an apparent velocity of 0.03 m/s and O_3 concentration supplied at the bottom of the bulk grain was 80 ppm (calculated from the equation published by Mendez et al. [37]). In both the USA and Canada, grain is usually stored inside silos higher than 6 m and larger than 6 m diameter. If 50 ppm was the lethal dose and the apparent air velocity was 0.03 m/s (about 5 (L/s)/m³) and coming from the bin bottom, insects at 6 m high would be exposed to this lethal dose after 1.8 days (calculated from the equation published by Mendez et al. [37]). To control adults of *T. castaneum*, continually supplying 50 ppm of O_3 for at least 4 days was required [6] because O_3 concentration inside the grain mass would decrease to less than 30 ppm in less than 7 min if the O_3 supply was stopped [50]. Less than 5 (L/s)/m³ is the airflow rate for aeration. Both temperature and O_3 fronts would pass the grain during the O_3 treatment period. Grain would

lose some moisture at this airflow rate in 5.8 days. This O₃ fumigation time was similar to the fumigation time using phosphine. Therefore, using O₃ to control insects and microorganisms and reduce mycotoxin contamination inside small farm bins was fiscally possible and could be an alternative control method. When grain was warm and wet, near-ambient drying or aeration combined with O₃ treatment might have advantages over phosphine fumigation or near-ambient drying because: (1) O₃ would suppress the populations of insects and microorganisms, reduce mycotoxins, and eliminate odours, improve taste, and bleach colour; (2) near-ambient drying would dry grain or aeration would cool the grain; and (3) low population of insects and microorganisms at dry and/or cold environment would not cause storage problems (depending on temperature and moisture). This treatment would at least extend the safe storage time. The O₃ could be supplied from the headspace of bins and the fan could move air downward. The advantage of this method was that the fan would be exposed to low concentration of O₃ and O₃ might help itself move down because O₃ is heavier than air. The fan could be connected with a closed-loop recirculation system to capture and reuse O₃ gas. The fan could be stopped at any time because the concentration of O₃ could return to previous levels in 30 min after O₃ flow was resumed [23]. For moderately tall structures, the O₃ introduction point could be any place of the grain mass and air flow could be downward or upward. There were several patents available to treat herbs, spices, fruits and vegetables by O₃ treatment in small storage facilities [24, 33]. There were several pilot studies in grain storage bins [6, 19, 23, 33]. This combination method should be evaluated and compared financially with conventional methods for treatment of grain in bins.

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