Efficacy of ozone against stored grain insect species in wheat: laboratory and field observations

Subramanyam, Bh.*#[1], E, X.[1], Savoldelli, S.[2], Sehgal, B.[1], Maier, D.E.[1], Ren, Y.L.[3]
[1]Department of Grain Science and Industry, Kansas State University, Manhattan, Kansas 66506, USA
[2]DiPSA, University of Milan, Milano, Italy
[3]School of Biological Sciences and Biotechnology, Murdoch University, Western Australia

*Corresponding author, Email: sbhadrir@k-state.edu
#Presenting author, Email: sbhadrir@k-state.edu

DOI: xx.xxxx/xxx.2014.xxx.xxx.xxx

Abstract

Adults of the lesser grain borer, *Rhyzopertha dominica* (F.), were exposed to an ozone concentration of 0.43 or 0.86 g/m^{3}for 15-36 h or 4-30 h to estimate lethal time (LT) and lethal dose (LD, or concentration x time (*Ct*) product) to kill 99% of the adults at 28°C and 65% r.h. After ozone exposure adult mortality was counted daily for 5 d. At 0.43 and 0.86 g/m^3, the LT_{99} values for adult mortality on day 1 were about 67 and 42 h, respectively. Corresponding LD_{99} values for adult mortality were 28 and 36 g-h/m^3, respectively. On day 5, the LT_{99} and LD_{99} values decreased by 52 to 54% of day 1 values. Doubling the ozone concentration did not reduce the LT_{99} values by half. In general, the LD_{99} values on days 1 through 5 at an ozone concentration of 0.86 g/m^3 were significantly greater than similar values at an ozone concentration of 0.43 g/m^3. This suggested that *R. dominica* adults tend to be more susceptible when exposed for long time period to a low ozone concentration. In a bin holding 125 MT of hard red winter wheat, ozone was flushed through the grain mass for 5 d. Ozone concentrations at the plenum showed an increase from 0 to 0.107 g/m^3. All adults of *R. dominica*; the rusty grain beetle, *Cryptolestes ferrugineus* (Stephens), *T. castaneum*, and eggs of the Indian meal moth, *Plodia interpunctella* (Hübner), in bioassays succumbed to ozonation. A laboratory strain and four phosphine-resistant field strains of the red flour beetle, *Tribolium castaneum* (Herbst), and a laboratory strain and two phosphine-resistant field strains of *R. dominica* were exposed to 0.43 and 0.86 g/m^3 of ozone for 24 h. After one day of incubation following the 24h exposure, the mortality of the laboratory and phosphine-resistant field strains of both species was 100%.

Keywords: ozonation, wheat, stored-grain insects, efficacy assessment

1. Introduction

Ozone is a highly oxidative gas, and is lethal to a wide range of microorganisms (Wu et al., 2006) without leaving any residues. Moreover, it effectively degrades mycotoxins (Tiwari et al., 2010). Ozone decomposes into oxygen within hours under regular conditions (Baba et al., 2002; McClurkin et al., 2013). Ozone is Generally Recognized as Safe (GRAS) by the United States Food and Drug Administration, and it is approved for use as an antimicrobial agent on processed food, including meat (Federal Register, 2001). Due to its rapid decomposition, ozone is commonly produced onsite to ensure a continuous supply. Via UV radiation or corona discharge, oxygen is converted to ozone. The amount of ozone produced is limited by the flow rate of oxygen fed to the generator.

The potential of ozone to manage stored-grain insets has been explored more than a decade ago. Kells et al. (2001) used ozone to disinfest stored maize in pilot scale bins (8.9 tonnes) and a laboratory scale bin (208 liters). When applying ozone in the storage bins (8.9 tonnes each), 100% mortality of maize weevil adults, *Sitophilus zeamais* Motschulsky, was achieved

by using 0.107 g/m^3 of ozone for 3 d. At the same ozone treatment, the highest mortalities of larvae of the Indian meal moth, *Plodia interpunctella* (Hübner), and adults of red flour beetle, *Tribolium castaneum* (Herbst), were 94.5 and 92.2%, respectively. Ozone was introduced from the top and pushed down to the plenum of the bins. The ozone penetration was closely related to the air flow rate, and higher flow rate facilitated faster penetration. Kells et al. (2001) reported two distinct phases of ozonation, the initial phase and the stabilizing phase. As a strong oxidant, ozone firstly reacts with the active sites on the surface of the grains, which accelerates ozone degradation. Once ozone reacts with active sites, the concentration is stabilized and disinfestation is achieved. Hansen et al. (2012) evaluated ozone against eggs, young and medium-aged larvae, pupae, and adults of 11 stored-product insect species. They found the internally-developing insect species to require higher ozone concentrations than externally-developing insect species. Campabadal et al. (2013) reported 100% mortality of *S. zeamais* and *T. castaneum* in bins at 22.4°C and 73.8% r.h. when exposed to a minimum ozone concentration x time product (*Ct*) of 7.704 g-h/m^3.

Ozone has adverse effects on grain microflora. Ozone degrades the outer layer of the spores and makes them inactive (Tiwari et al., 2010). McDonough et al. (2011) applied ozone in a screw conveyor to control fungal contamination in corn. Ozone was shown to effectively reduce the mold propagules (cfu/g) by 2 logs after three passes through the conveyor. Humidified ozone was more reactive and showed higher disinfection potential.

In the present study, ozone was evaluated at two concentrations and different exposure times under laboratory conditions against adults of the lesser grain borer, *Rhyzopertha dominica* (F.). The objectives of this work were to determine the ozone concentrations needed over time and ozone dosages (concentration x time products [Ct]) for 99% mortality of exposed adults. Additionally, postexposure mortality of ozone-exposed adults was evaluated daily for 5 d to determine any delayed toxic effects. Field tests were conducted in one untreated bin and in one ozone-treated bin at a farm site to determine effects on insect species within the grain mass and on the mold infection and mold propagules, and on selected insect species confined in bioassay containers. Finally, the effect of ozone in controlling laboratory reared and field collected phosphine-resistant strains of *T. castaneum* and *R. dominica* was evaluated through laboratory experiments.

2. Materials and Methods

2.1. Insect rearing

Laboratory strains of *R. dominica*, the rice weevil, *Sitophilus oryzae* (L.); rusty grain beetle, *Cryptolestes ferrugineus* (Stephens), *T. castaneum*, and *P. interpunctella* were used in laboratory and field tests. Cultures of *R. dominica* and *S. oryzae* were reared on organic hard red winter wheat (Heartland Mills, Marienthal, KS, USA). Cultures of *T. castaneum* were reared on white wheat flour plus 5% (w/w) brewer's yeast. Cultures of *C. ferrugineus* were reared on rolled oats with 5% brewer's yeast. Cultures of *P. interpunctella* were reared on a poultry mash diet (Subramanyam and Cutkomp, 1987), which consisted of poultry mash from a local feed mill (1,000 g), glycerol (150 ml), honey (150 ml), and distilled water (75 ml). All cultures were kept at 28°C and 65% r.h. in the Department of Grain Science and Industry, Kansas State University, Manhattan, KS, USA.

2.2. Ozone generation and exposure of R. dominica adults in the laboratory

Ozone was generated by a custom-built corona discharge ozone generator (O3Co, Idaho Falls, ID, USA) with a capacity of 2.5 g/h. The ozone produced was introduced into a stainless steel cylindrical chamber of 20 cm internal diameter and 24 cm height from the bottom, and ozone

was vented out from the top of the cylinder to maintain a constant ozone concentration inside the chamber. The bottom of the chamber above the ozone inlet had a perforated floor. The vented ozone passed through a gas analyzer (IN2000-L2-LC, INUSA, Norwood, MA, USA) to monitor the ozone concentration in the chamber. It took approximately five minutes to increase the ozone concentration from 0 to 0.86 g/m^3 in the chamber. A program written in LABVIEW (National Instruments, Austin, TX, USA) was used for ozone data acquisition.

Prior to bioassays with *R. dominica* adults, the organic hard red winter wheat was cleaned manually by sieving it over a 2 mm round-holed aluminum sieve (Seedburo Equipment Company, Des Plaines, IL, USA) to remove dockage and broken kernels. Cleaned wheat was frozen for 1 week at -13ºC to kill any live insects present. The moisture content of wheat was equilibrated to 12±1% in an environmental growth chamber maintained at 28ºC and 65% r.h. Wheat (277 g) was placed in 500-ml volume paper cups (top diameter, 8.5 cm; bottom diameter 6.5 cm; height 12 cm). The bottom of the cup was cut out and a wire-mesh screened lid (160 µm openings) was taped at the bottom to facilitate ozone diffusion through wheat in the cup. Unsexed adults of mixed ages of *R. dominica* were exposed for 15-36 h at an ozone concentration of 0.43 g/m^3 or for 4-30 h at an ozone concentration of 0.86 g/m^3. At the lower ozone concentration the wheat was infested with 50 adults and at the higher concentration with 100 adults. At each concentration-time combination there were three replications. Wheat with 50 or 100 adults of *R. dominica* unexposed to ozone, and handled similarly at 28°C and 65% r.h. served as the control treatment.

After the intended period of exposure at each concentration, adults and wheat were incubated at 28°C and 65% r.h. in 0.45-L glass jars covered with lids fitted with wire-mesh screens (160 µm opening). The contents of control and ozone-exposed wheat were examined daily for 5 d to determine mortality. After 5 d, the live and dead insects in unexposed and ozone exposed wheat were counted to determine mortality.

2.3. Farm-bin tests with ozone

Two identical round metal bins of 125 MT capacity were selected for conducting the field trials. Both bins had wheat filled to the eaves. One bin was used as a control bin while the other was ozonated. Grain samples were collected before and after the ozonation. The collection points were near the grain surface at the top center and the side of the bins. A 1.52 m long grain probe was used to draw 500 g of grain samples twice at each location. These samples were bagged and labeled and analyzed within 24 h of collection in the laboratory to determine types and numbers of live insects present and for mycological analyses.

The percentage of mold infection of wheat grains was determined by using tergitol plates with malt salt agar (MSAT). Twenty grains were taken from the bins before and after treatment, and were transferred on the plate by a flame sterilized forceps. The plates were incubated at 37 ºC for 7 d. The percentage of mold infection was calculated as:

$$100 \times \frac{moldy \quad grains}{total \quad grains}$$

The procedure for isolating mold propagules from wheat kernels was described by Samson et al. (1996). Wheat sample (25 g) was soaked in 250 ml of sterile peptone (0.1%) water for 30 minutes before stomaching for 2 minutes. One milliliter of the sample was serially diluted in 9 ml of sterile peptone water and a 100µl sample from serial dilutions was drop-plated on Dichloron Glycerol-18 (DG-18) agar medium (Oxoid Chemicals, Hampshire, UK) and incubated at 35°C for 4-5 d in an upright position. After incubation the colony forming units were recorded to determine mold concentration per gram of wheat (cfu/g).

Ozone was generated by a four-chamber generator (97D4) made by O3Cowith a capacity of 250 g/h of ozone. The feeding gas was dried and pressurized with air to ensure a constant flow. The ozonation was conducted using a recirculation system as mentioned in a previous paper (Campabadal et al., 2003). Ozone entered the bin from the top through a roof cover using 0.025 m diameter Teflon® hose, and was forced downward by a fan (0.25 kW with velocity of 0.06 meters/second). The ozone as it passed through the grain mass exited from the bottom of the bin through the plenum. The ozone leaving the plenum was drawn by the fan and flowed back to the top of the bin through a recirculation duct. The ozone concentration at the bin bottom was monitored by an ozone analyzer (IN-2000), and data were acquired by Hydra logger 2620A (Fluke Corporation, Everett, WA, USA). The target ozone concentration to be attained was 0.107 g/m^3. Two identical bins were located on a farm in Abilene, KS, USA. Prior to tests, producers cleaned the bins to remove old grain, debris and insects.

Insect bioassays were carried out in 150-ml round plastic containers with perforated lids (1 cm diameter) covered with a nylon mesh (841 µm opening). Each container with 50 g of organic hard red winter wheat was infested with 50 adults each (except *P. interpunctella*) of *R. dominica*, *T. castaneum*, or the rusty grain beetle, *Cryptolestes ferrugineus* (Stephens). In the case of *P. interpunctella*, 50 eggs were added to 50 g of wheat. The containers were placed 0.6 m below grain surface (top) or near the side door of the bins and at the bottom near the plenum area. There were three containers below the grain surface and three near the plenum for each species. After 5 d of ozonation, the containers from control and ozone-treated bins were removed, and number of live and dead adults was recorded. For *P. interpunctella* the containers were held at 28°C and 65% r.h. and examined after 21 d to count the number of live larvae present.

2.4. Susceptibility of phosphine-resistant strains of T. castaneum and R. dominica to ozone

Phosphine resistant strains of *T. castaneum* (RFB-CF, RFB-MN, RFB-AB1, and RFB-AB2) and *R. dominica* (LGB-CF and LGB-RL) were collected from farm bins at cooperating farm sites between July and November 2011 by inserting five perforated probe traps below the stored-grain surface (Sega et al., 2013). After capture, they were reared in the laboratory on standard diets and kept in the environmental chamber at 28℃ and 65 % r.h. Bioassays with laboratory reared and phosphine-resistant field strains of *T. castaneum* or *R. dominica* were conducted by placing 20 unsexed adults of mixed ages in plastic vials of 23 mm diameter and 55 mm height. The plastic bottom was removed and a wire mesh bottom (231 µm) cut to size was glued to the vial bottom. The vial lid was cut and a wire mesh (231 µm) was glued to cover the lid opening. In vials with *T. castaneum* 10 g of flour was used and for *R. dominica* 10 g of organic hard red winter wheat was used. Infested vials with food were exposed to ozone concentrations of 0.43 or 0.86 g/m3 generated by the laboratory ozone generator for 24 h. Vials were placed in a polymethylmethacrylate chamber of 0.5 x 0.35 m x 0.35 m for exposure. There were three vials for each species and strain. Vials prepared similarly but unexposed to ozone served as the control treatment. After 24 h, all vials were incubated at 28°C and 65% r.h. and examined for mortality after an additional 24 h.

2.5. Data analysis

Mortality was calculated as a percentage from the number of insects that died out of the total exposed. Mortality of ozonated insects was corrected for the corresponding control mortality (Abbott, 1925). The corrected time-mortality data by day (1-5) and concentration x time (*Ct*)-mortality data means by day were subjected to probit regression analysis (SAS Institute, 2008) to determine lethal time (LT) or lethal dose (LD) resulting in 99% mortality of insects. Table Curve 2D® (v5.01, SYSTAT 2014) was used to fit a polynomial equation to describe

LT_{99} or LD_{99} as a function of postexposure time in days, and the graphs were plotted using Sigma Plot® (12.5, SYSTAT 2013).

The field tests were done using one control and one ozonated bin. Therefore, data collected from multiple samples taken were totaled (for live insects only) or averaged and tabulated. Similarly, mortality data from the vial bioassays using phosphine susceptible and resistant strains of *T. castaneum* and *R. dominica* exposed to ozone were averaged and tabulated.

3. Results and Discussion

3.1. Ozone toxicity against adults of R. dominica in the laboratory

The lethal time (LT) in hours required to kill 99% of exposed *R. dominica* adults at an ozone concentration of 0.43 g/m^3 was 66.65 hours based on observations after 1 d. There was a decrease in LT_{99} values with successive observations times. On days 2, 3, 4, and 5 the LT_{99} values were 38.03, 36.95, 32.27, and 30.72 h, respectively. The LT_{99} values after 1, 2, 3, 4, and 5 d following exposure of *R. dominica* adults to an ozone concentration of 0.86 g/m^3 were 41.81, 29.83, 20.97, 27.38, and 20.18 h, respectively. The LT_{99} values on days one through five at 0.86 g/m^3 when compared with 0.46 g/m^{3}were reduced by only 15-37%. Doubling the ozone concentration did not reduce the time in hours required for LT_{99} by half. Therefore, the lethal dose (LD) values for 99% mortality of exposed *R. dominica* adults at 0.86 g/m^3 were higher than those exposed to 0.46 g/m^3. For example, at 0.86 g/m^3, the LD_{99} values on 1, 2, 3, 4, and 5 d were 35.79, 25.53, 17.95, 23.44, and 17.28 g-h/m^3, respectively. Corresponding LD_{99} values at 0.43 g/m^3 were 28.10, 16.28, 15.82, 13.81, and 13.15 g-h/m^3, respectively. The LT_{99} (Fig. 1A) or LD_{99} (Fig. 1B) values as a function of postexposure observation time in days was satisfactorily described (r^2=0.861-0.988) by a simple polynomial regression, $y=a+b/x^2$. These results suggest that adults of *R. dominica* were more susceptible to low concentration of ozone when exposed for longer duration. A decrease in LT_{99} with time showed that ozone exhibited delayed toxicity effects against *R. dominica* adults. Delayed toxicity with ozone against adults of *S. zeamais*, *T. castaneum*, and the confused flour beetle, *Tribolium confusum* (Jacquelin du Val); and larvae of *P. interpunctella* were reported by Mason et al. (1998). Mason et al. (1998) reported that for all four inset species mortality after 2 d of exposure to an ozone dose of 2.054 g-h/m^3 was less than 50%. Complete mortality of *S. zeamais* adults and *P. interpunctella* larvae occurred 3 d after exposure, whereas for adults of *Tribolium* spp. complete mortality occurred after 6-12 d.

3.2. Farm-bin tests with ozone

Ozone level at the plenum reached 0.107 g/m^3 after 96 h. On the fifth and last day of treatment, the ozone concentration was 0.113 g/m^3. Ozone is highly oxidative and quickly degrades to diatomic oxygen. Once entering the grain bin, ozone first reacts with the surface of kernels (Kells et al., 2001; Campabadal et al., 2013). After all active sites were eliminated, ozone concentration started to stabilize. This explains the slow rise in ozone concentration measured at the bin plenum. The total *Ct* product (dosage) over 5 d of ozonation was 326.94 g-h/m^3.

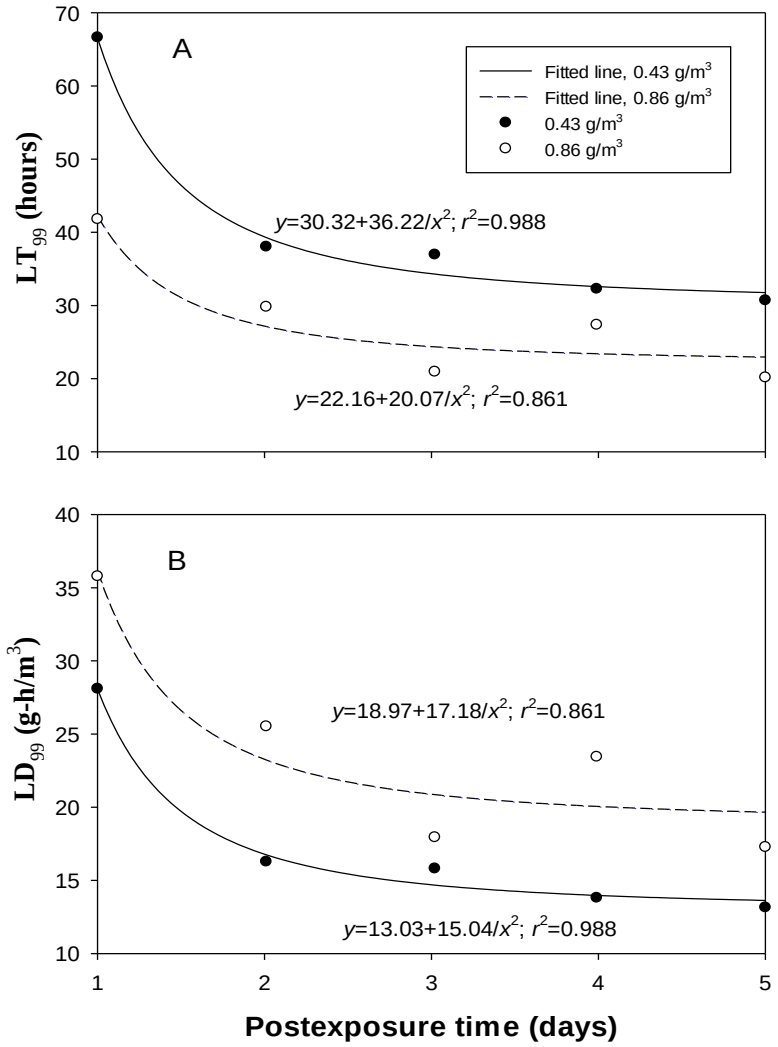

Figure 1 Observed and fitted data at two ozone concentrations showing a decrease in LT_{99} (A) and LD_{99} (B) as a function of postexposure time.

Mold infection in the control bin was 84.0% prior to ozonation and after 5 d it was 80.3%. In the ozonated bin prior to ozone treatment mold; infection was 21.3% and after treatment it was 19.3%. The mold propagules (cfu/g) in the control bin before ozone treatment was 2300 and after 5 d it increased to 17175. In the ozone treated bin, prior to ozone treatment there were 575 cfu/g and after the ozone treatment, there were 325 cfu/g. The *Ct* product used in this study was 13.59 g-h/m^3. These data were collected from four samples from each bin before and after ozonation, but no firm conclusions can be reached. The control and ozone treated bins did not have similar mold infection rates or mold concentration at the beginning of the experiments. The low mold infection prior to ozonation in the ozone-treated bin is unclear. Several researchers have reported ozone to be effective against fungal microorganisms. Kells et al. (2001) tested the ozone efficacy against *Aspergillum sparasiticus*, and found that the number of viable conidia on the surface of maize kernels was reduced by 63% when exposed to 0.107 g/m^3 of ozone after 3 d (*Ct*=7.70 g-h/m^3). White et al. (2013) tested the effect of ozone on maize kernel infection with fungi at various moisture

contents (18, 22 or 26%, wet basis). Without ozone treatment the kernel infection was 98-100%. After 1 h of exposure to an ozone concentration of 2.14 g/m^3, the infection percentage dropped to 50. Increasing the ozone concentration to 3.21 g/m^3 while keeping the exposure time the same resulted in reducing the infection percentage to 20% at 18% moisture. These results indicated that the moisture content of grain influences the effectiveness of ozone against fungal microorganisms. The effectiveness of ozone in reducing fungal infection varies with grain temperature, moisture, and the type of fungal species (McDonough et al., 2011).

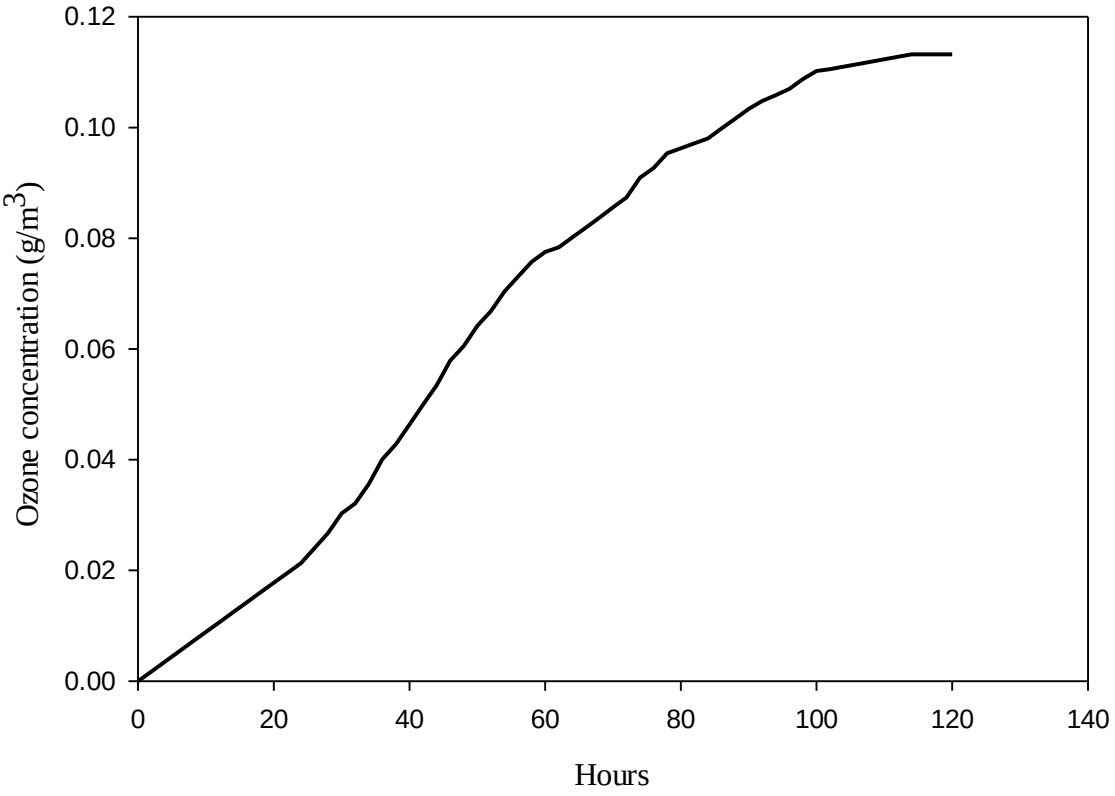

Figure 2 Ozone concentration profile measured at the plenum of the farm bin. The target concentration of 0.107 g/m^3 was reached at the plenum after 96 h.

No firm conclusions can be reached regarding the effect of ozone based on the total number of live adults of different insect species found in wheat samples collected from the control and ozone-treated bins before and after the treatment (Table 1). There were more insects of five different species after ozonation than before the treatment. Similarly, more adults of five species were found in the control bin after 5 d than before the start of the experiments.

The three bioassay containers placed below the grain surface and in the plenum area in the ozonated bin showed 100% mortality of *R. dominica* and *C. ferrugineus* adults (Table 2). Mortality of these two species in the control bin ranged from 2.7 to 40.7%. The mortality of *T. castaneum* adults in the control bin was 0-5.7%, but in the ozonated bin, the top samples showed 100% mortality of adults while the bottom samples showed only 88% mortality. It is plausible that the samples near the top were exposed to high concentrations of ozone as ozone was introduced from the top of the bin. No live larvae of *P. interpunctella* were found in samples from the ozonated bin; in the control bin samples had 6.7-17.3 larvae when examined after 21 d. These results suggested ozone to be effective against the four insect species tested.

Table 1 The number of live adults of insect species observed in grain samples from control and ozone treated farm bins. Data were based on total number of insects found in four subsamples per bin taken before and after ozonation.

	Insect species	Before	After
Control bin	*C. ferrugineus*	17	447
	R. dominica	1	0
	T. castaneum	0	2
	O. surinamensis	0	55
	T. stercorea	0	7
	S. oryzae	0	1
	P. interpunctella larvae	1	0
Ozonated bin	*C. ferrugineus*	38	173
	R. dominica	9	26
	T. castaneum	3	21
	O. surinamensis	2	14
	T. stercorea	0	2
	S. oryzae	0	0
	P. interpunctella larvae	1	0

Table 2 The mortality of adults of three species in control and ozone treated bins.

Bin	Sample location	Mean mortality (%) of:		
		R. dominica	*C. ferrugineus*	*T. castaneum*
Control	Top	2.7	40.7	0
	Bottom	6.2	22	5.7
Ozonated	Top	100	100	100
	Bottom	100	100	88

3.3. Susceptibility of phosphine-resistant strains of T. castaneum and R. dominica to ozone

Adults of the laboratory and phosphine-resistant field strains of *T. castaneum* and *R. dominica* succumbed to ozone concentrations of 0.43 and 0.86 g/m^3 within 24 h (Table 3). Sousa et al. (2008) tested ozone on phosphine-resistant insects in the laboratory, and found them to be susceptible to ozone at a concentration of 0.321 g/m^3.

Table 3 The mean mortality of laboratory and phosphine-resistant field strains of *R. dominica* and *T. castaneum* after 24 h of exposure to ozone concentrations of 0.43 and 0.86 g/m^3. Each mean is based on three samples.

Strains	Phosphine resistance	0.43 g/m^3		0.86 g/m^3	
		Control	Ozone	Control	Ozone
RFB-Lab	Susceptible	0	100	0	100
RFB-CF	Strong	1.7	100	0	100
RFB-MN	Strong	0	100	0	100
RFB-AB1	Weak	0	100	0	100
RFB-AB2	Weak	0	100	0	100
LGB-Lab	Susceptible	0	100	0	100
LGB-CF	Weak	0	100	3.3	100
LGB-RL	Weak	1.7	100	3.3	100

RFB = Red flour beetle, *T. castaneum*; LGB = Lesser grain borer, *R. dominica*.

4. Conclusions

In the laboratory study, adults of *R. dominica* was more susceptible when exposed to a low concentration of ozone for longer time period as opposed to high concentration of ozone for shorter time period. The toxicity of ozone to *R. dominica* adults was delayed with greater mortalities occurring five days after exposure compared to one day after exposure. The farm-bin tests did not show any effect on live insects in grain and on the grain microflora. However, ozone did kill 88-100% of four insect species exposed in 50 g of wheat in plastic containers. Phosphine-resistant adults of *T. castaneum* and *R. dominica* were highly susceptible to ozone concentrations of 0.43 or 0.86 g/m^{3}after a 24 h exposure.

Acknowledgement

The authors acknowledge the support of Plant Biosecurity Cooperative Research Centre (Project No: PBCRC3038) established and supported under the Australian Government's Cooperative Research Centres Program.

References

Abbott, W.S., 1925. A method of computing the effectiveness of an insecticide. Journal of Economic Entomology 18, 265-267.

Baba, S., Satoh, S., Yamabe, C., 2002. Development of measurement equipment of half life of ozone. Vacuum 65, 489-495.

Campabadal, C.A., Maier, D.E., Mason, L.J., 2013.Efficacy of fixed bed ozonation treatment to control insects in stored bulk grain. Applied Engineering in Agriculture 29, 693-704.

Federal Register, 2001. Secondary direct food additives permitted in food for human consumption. Federal Register 66, 33829-33830.

Hansen, L.S., Hansen, P., Jensen, K.M.V., 2012. Lethal doses of ozone for control of all stages of internal and external feeders in stored products. Pest Management Science 68, 1311-1316.

Kells, S.A., Mason, L.J., Maier, D.E., Woloshuk, C.P., 2001. Efficacy and fumigation characteristics of ozone in stored maize. Journal of Stored Products Research 37, 371-382.

Mason L.J., Strait, C.A., Woloshuk, C.P., Maier, D.E., 1998. Controlling stored grain insects with ozone fumigation, pp. 536-547. In Jin, Z.X., Liang, Q., Liang, Y.S., Tan, X.C., Guan, L.H. (Eds.), Proceedings of the 7th International Working Conference on Stored-Product Protection, October 14-19, 1998, Beijing, China. Sichuan Publishing House of Science and Technology, Sichuan, China.

McClurkin, J.D., Maier, D.E., Ileleji, K.E., 2013. Half-life time of ozone as a function of air movement and conditions in a sealed container. Journal of Stored Products Research 55, 41-47.

McDonough, M.X., Campabadal, C.A., Mason, L.J., Maier, D.E., Denvir, A., Woloshuk, C.P., 2011. Ozone application in a modified screw conveyor to treat grain for insect pests, fungal contaminants, and mycotoxins. Journal of Stored Products Research 47, 249-254.

Samson, R.A., Hoekstra, E.S., Frisvad, J.C., Filtenborg. O., 1996. Isolation of fungi from food samples, pp. 156-168. In Samson, R.A., Hoekstra, E.S., Frisvad, J.C., Filtenborg, O. (Eds.), Introduction to Food-borne Fungi, 5th edition. Centraal bureauvoor Schimmelcultures, Baarn, The Netherlands.

SAS Institute. 2008. SAS/STAT 9.2 user's guide. SAS Institute, Cary, NC, USA.

Sehgal, B., Subramanyam, Bh., Arthur, F.H., Gill, B.S., 2013. Variation in susceptibility of field strains of three stored-grain insect species to spinosad and chlorpyrifos-methyl plus deltamethrin on hard red winter wheat. Journal of Economic Entomology 106, 1911-1999.

Sousa, A.H., Faroni, L.R., Guedes, R.N., Totola, M.R., Urruchi, W.I., 2008. Ozone as a management alternative against phosphine-resistant insect pests of stored products. Journal of Stored Products Research 44, 379-385.

Tiwari, B.K., Brennan, C.S., Curran, T., Gallagher, E., Cullen, P.J., Donnell, C.P., 2010. Application of ozone in grain processing. Journal of Cereal Science 51, 248-255.

White, S.D., Murphy, P.T., Leandro, L.F., Bern, C.J., Beattie, S.E., van Leeuwen, J., 2013. Mycoflora of high-moisture maize treated with ozone. Journal of Stored Products Research 55, 84-89.

Wu, J.N., Doan, H., Cuenca, M.A., 2006. Investigation of gaseous ozone as an anti-fungal fumigant for stored wheat. Journal of Chemical Technology and Biotechnology 81, 1288-1293.