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Mortality of stored-grain insects in stored wheat (*Triticum* sp.) fumigated with ozone

FUJI JIAN¹, VELLAICHAMY CHELLADURAI², PAUL G FIELDS³, DIGVIR S JAYAS⁴, NOEL D G WHITE⁵

University of Manitoba, Winnipeg, MB, R3T 5V6 Canada

ABSTRACT

Ozone as a fumigant to control stored-product insects was tested inside a metal bin filled with 10 tonnes of 11.0 or 12.0% moisture content wheat (*Triticum* sp.). The tested insects were the egg and adult stages of rusty grain beetle, *Cryptolestes ferrugineus* (Stephens); red flour beetle, *Tribolium castaneum* (Herbst); rice weevil, *Sitophilus oryzae* (L.); and lesser grain borer, *Rhyzopertha dominica* (Fabricius). The insects were located at the top surface, 5 cm below the top of grain surface, and middle depth of the grain mass (about 1 m below grain surface). The wheat and the insects were continually fumigated for 72 h with an average ozone generation rate of 100 g/h. The generated ozone was injected into the test bin through a patent-pending distribution system with an air-flow rate of 27 L/min. Adults held at the surface of the grain mass had 100% mortality, but not all of introduced adults were killed inside the grain mass. Fewer eggs survived the ozone fumigation when the adult mortality was 100% than when the adult mortality was less than 100%. There was a correlation between wheat germination loss and high adult mortality. The germination of wheat located at 5 cm below the top of grain surface was reduced to 0%.

Key words: Fumigation, Mortality, Ozone, Stored wheat, Stored-product insects

Control of insect pests in stored grain requires alternative fumigants, due to the development of resistance of insects to pesticides, the cryptic behaviour of most major stored-product insects, the limited options of available fumigants, safety concerns, and the consumers' demand for residue-free food, and environmentally friendly treatment methods. Ozone (O₃) as a potential fumigant to control stored-grain insects has attracted attention recently owing to its advantages of easy generation at the treatment site and no chemical residues (Jian et al., 2013). Toxicity of O₃ on stored grain insects was evaluated in the laboratory (Levy et al., 1974; Isikber and Oztekin, 2009; Hansen et al., 2012) and in field studies (Kells et al., 2001; Hardin et al., 2010; Bonjour et al., 2011; Campabadal et al., 2013). The laboratory studies

showed the toxic effect of O₃ to both external and internal feeders of stored-grain insects with different combinations of ozone concentrations and exposure times (Jian et al., 2013).

Field studies showed that ozone did not achieve 100% mortality if the ozone was lower than the target concentration, 3600 ppm-h, inside grain mass (Kells et al., 2001; Bonjour et al., 2011; Campabadal et al., 2013). The reason for the lower ozone concentration inside the grain mass is that the ozone is consumed by grain and degraded to oxygen (Hardin et al., 2010; Mendez et al., 2003). To solve these issues, aeration in combination with ozone (Hardin et al., 2010) or point fumigation (Campabadal et al., 2013) have been suggested. Ozograin International Inc. (Ozograin) developed a technique which can help ozone penetrate into a grain mass (Falcon, 2015); hence the total fumigation can be completed in 3 days. The aim of this study was to assess ozone as a fumigant to control major stored-grain insects under Canadian conditions by using the new developed technique of Falcon (2015).

^{1,2,4}Department of Biosystems Engineering, ^{3,5}Agriculture and Agri-Food Canada, c/o Department of Biosystems Engineering, University of Manitoba, Winnipeg, MB, R3T 5V6 Canada

⁴Corresponding author e-mail: Digvir.Jayas@umanitoba.ca

MATERIALS AND METHODS

Wheat preparation

A total of 60 tonnes of hard red spring wheat (*Triticum* sp.) (No. 2 grade) was used and this wheat was purchased from two different sources. The first 30 tonnes wheat was delivered from an elevator and the second 30 tonnes was delivered from a producer. Both the elevator and the producer were located near Winnipeg, Manitoba, Canada. The physical properties of the two batches of wheat were slightly different. The test weights of the first and second batches were 777.5 ± 1.5 and 806.9 ± 2.4 kg/m³, respectively. The per cent foreign material of the first and second batches were $0.9 \pm 0.3\%$ and $0.4 \pm 0.1\%$ by weight, respectively. The germination of the both batches was similar ($90.0 \pm 0.6\%$). The temperatures of the grain were less than 10°C at delivery. After the grains were warmed to 14°C (by aeration in less than 6 h), the first 30 tonnes of wheat was used for the first three treatments and the second 30 tonnes of wheat was used for the last three treatments without further modifications. For each test, 10 tonnes wheat was filled in 2.8 m diameter welded steel bin with perforated flat bottom and 1.8m high plenum.

Insect preparation

The following four insect species were tested in this study: rusty grain beetle, *Cryptolestes ferrugineus* (Stephens); red flour beetle, *Tribolium castaneum* (Herbst); rice weevil, *Sitophilus oryzae* (L.); and lesser grain borer, *Rhyzopertha dominica* (Fabricius). The insect species were originally collected from farms located in western Canada and reared inside our laboratory for less than 2 years at $30 \pm 1^\circ\text{C}$ and $75 \pm 5\%$ r.h. The red flour beetle was reared on white flour with 5% brewers yeast, and the other insect species were reared on whole wheat kernels. The grain used for the rusty grain beetle was mixed with 5% wheat germ (w:w) and 5% cracked grain. To create similar aged adults; 200 adults were introduced into 4-L jars with approximately 1.5 kg of diet. Two days later, the 200 adults were sieved out and the jars were kept inside an environmental chamber at $30 \pm 1^\circ\text{C}$ and $75 \pm 5\%$ r.h. for 6 to 8 weeks.

Twenty-five adults were sieved from the feed and collected using an aspirator. These 25 adults were introduced into a nylon mesh bag. The holes on the nylon bags were about #30 to 40 mesh. The size of the bag was about 4 cm diameter with 8 cm high. Before the 25 adults were introduced into the bag, approximately 20 g wheat (the same wheat used in the fumigation test) was placed into the bag. After the

adults were introduced into the bag, the bag was closed by folding up the top of the bag and these folded bags were sealed by a paper clamp. Each nylon bag with 25 adults was kept inside a polyethylene tube. The size of the tube was 4 cm inner diameter and 10 cm long. There were 18 holes in the tube. The inner diameter of the holes was 1.5 cm. Both ends of the tube and 12 holes were covered by the same nylon mesh. The 6 holes which were located at the middle of the tube were not covered during fumigation. Before the tubes were introduced into the test bin, 4 tubes with different insect species were grouped together by using cable ties. Before the grouped tubes were introduced into the wheat bulk, the grouped tubes were kept at $25 \pm 1^\circ\text{C}$ and $50 \pm 5\%$ r.h. for 24 h and the adults laid eggs before the ozone fumigation.

Ozone generating and monitoring

Ozone was produced by an ozone generator (Absolute Ozone® Atlas 100 Industrial Ozone generator, Absolute System Inc., Edmonton, Canada). An oxygen generator (Pro-8, Site Gas System Inc, Newington, CT, USA) supplied the oxygen required by the ozone generator. The maximum and average ozone generation rates were 113 g/h and 100 g/h, respectively. About 27 L/min air-flow rate was used to inject the ozone into the bin through a patent-pending distribution system (Falcon, 2015). Ozone concentration inside the bin was measured approximately every 3 h at seven locations after the fumigation was started (Fig. 1) by using a Portasens-II (Analytical Technology Inc., PA, USA) equipped with either a 0 to 200 ppm (Model: 00-1037) or 0 to 1,000 ppm (Model: H10-14) sensor. The ozone concentration was not measured in the first treatment and the 0 to 1,000 ppm sensor was not used in the first four treatments due to the non-availability of the sensor at that time. The Portasens-II drew air from PVC tubes with ½ inch (1.3 cm) inner diameter. In the second and third treatments, the ozone concentrations were measured at the walls of the bin at 75, 125 and 175 cm height from the flat bottom of the test bin. In the last three treatments, the ends of the ½ inch tubes were located at 60 or 120 cm from the wall in the horizontal direction and 25, 75, 125 and 175 cm from the flat bottom of the test bin in the vertical direction (Fig. 1).

Test procedure

A perforated flat bottom metal bin with a hopper plenum was used to conduct the fumigation test (Fig.1). Before grain was loaded, the bin was sealed and could hold 500 Pa pressure when the air was supplied by a test fan. The air tightness of the bin did not meet the

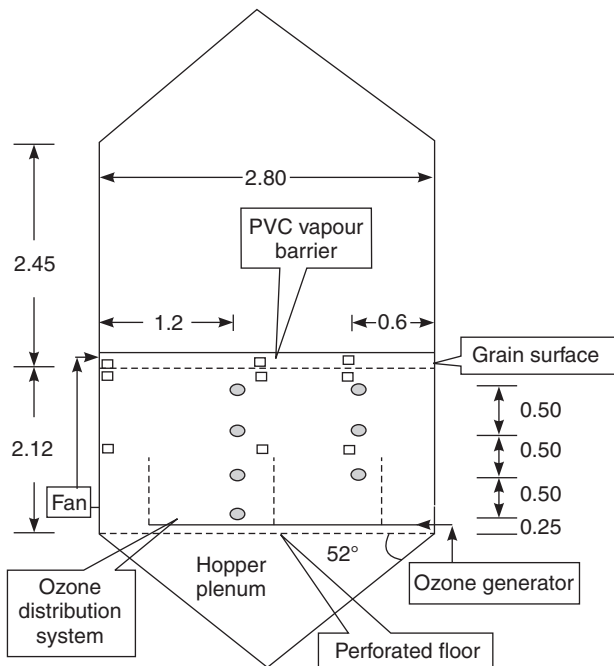


Fig. 1. Dimension of the test bin and locations of the introduced insects and ozone measurement (meter); the dots show the locations of the introduced insects, and empty cylinders show the locations of the ozone measurement

recommendation for phosphine fumigation and the half-loss time of the pressure was less than 1 min. Approximately 44 h after the insects were introduced into the small nylon bags and after 5 tonnes of the wheat was loaded into the test bin and the top of grain was leveled, the grouped tubes with insects were introduced at the locations of the wall, half radius, and center of the top of the 5 tonnes grain mass (referred to as Middle-Wall, Middle-Half, and Middle-Center, respectively; Fig. 1). At each location, about 6 kg wheat was collected. These collected grains were divided into two halves and loaded into two nylon cloth bags. One nylon bag was kept at the location where the wheat was sampled from, and another bag was kept at -10°C . To monitor the grain temperature, one HOBO[®] was introduced into the nylon bag which was close to the grouped tubes. After the insects and HOBO[®] were introduced, another 5 tonnes of wheat was loaded into the test bin. The total grain depth was 2.25 m (Fig. 1). After the top grain was leveled, the grouped tubes with insects were introduced at 5 cm deep or on the top of the grain mass by following the same procedure as that conducted after the first 5 tonnes grain were loaded. The insects introduced at the 5 cm deep or on the surface of the grain mass were referred to as Top-5 cm and Top-S respectively. There were three replicates at each location.

After the insects were introduced, the top of the

grain mass was leveled and covered by PVC sheeting (commercial vapor barrier). About 32 h after the grouped tubes were introduced into the test bin, the ozone was injected into the test bin through the ozone distribution system (Falcon, 2015) and continually fumigated for 72 h. The test insects stayed inside the small bags with 20 g wheat for 76 h before the fumigation was started, allowing adults to lay eggs. A Ring Compressor pump (Model PRB40-420, CE, Aurora, Ontario) with 4.7 L/s (10 CFM) volumetric flow rate was used to help the ozone penetrate into the grain mass. The Ring Compressor pump was continually run for about 6 h after the ozone generator was stopped, then the grain was aerated at about 1.5 L/s m³ airflow rate for about 2 h. Six hours later after the aeration, the grain was unloaded out of the test bin and the grouped tubes with insects and the large nylon bags were removed from the test bin. Germination and moisture content of the grain sampled from the nylon bags were determined. The removed tubes were kept at $30\pm 1^{\circ}\text{C}$ and 75% r.h. for 24 h, and then the dead and live adults were counted by sieving the insects from the wheat. The separated wheat was kept inside 0.5 L jars at $30\pm 1^{\circ}\text{C}$ and 75% r.h. for 35 d, and then the offspring of the fumigated adults were counted by following the same counting procedure.

Data analysis

The recovery rate of the introduced adults after fumigation was greater than 84% even though not all introduced adults were recovered in some treatments. Therefore, Schneider-Orelliequation was used to correct the adult mortality. To find the relationship between the grain temperature and insect mortality or between the grain germination and insect mortality, Pearson Product Moment Correlation was conducted. To find the difference of tolerance between different insect species to toxic ozone, paired *t* tests were conducted.

RESULTS AND DISCUSSION

Ozone concentration

Inside the test bin, the O₃ concentrations were different in different treatments. The reason for these different O₃ concentrations was not known. The O₃ concentrations between the first four treatments and the last two treatments were difficult to compare because different O₃ sensors with different measurement ranges were used. In the last two treatments, the following locations had consistently high concentrations of O₃: 75 cm high from bottom and 120 cm from the wall, and 60 cm and 120 cm from the wall at 175 cm high

from bottom. The O₃ concentration was consistently low at 120 cm from the wall and 125 cm high from bottom.

Temperature and moisture content of the grain

The grain temperature inside the grain mass was 14 to 26°C after the insects were introduced and removed from the wheat. The correlation coefficient between the average grain temperature and mortality of adults introduced at the middle of the grain mass was 0.268 ($P < 0.0001$). This correlation coefficient and p value indicated a weak positive relationship. In all of the treatments, about 0.9 and 0.5% point of m.c. of the wheat at the top and inside the wheat bulk were removed, respectively. This removed moisture was caused by the aeration during and after fumigation and the higher temperature at the surface of the grain bulk. This drying effect should not influence the adult mortality because the grain at the same depth had the same drying rate, but the insect mortality at the middle centre was significantly different from that at the middle-half radius (Paired *t* test; $t = 4.381$, $P < 0.001$), while all the adults were killed at the Top-S and Top-5cm (Table 1).

Grain germination

Grain germination was influenced by the O₃ fumigation. Almost all of the wheat kernels located at the surface of the grain bulk were killed. The correlation coefficient between the insect mortality and the wheat germination was -0.64 ($P < 0.0001$). This strong correlation indicated that the O₃ also killed the wheat kernel if insects were killed.

Insect mortality

The four species of the tested insects under

controlled condition ($25 \pm 1^\circ\text{C}$, $50 \pm 5\%$ r.h.) had less than 25% average mortality (Table 1). In a few replicates, the natural mortality of *C. ferrugineus*, *S. oryzae* and *R. dominica* adults was higher than 25%. This high mortality was possibly due to the low r.h. This low r.h. was the equilibrium r.h. corresponding to the tested grain m.c. This high natural mortality under the tested grain m.c. was similar with previous studies (Howe, 1965).

All of the introduced insects were killed on the top of the grain bulk. Almost all adults were killed at the locations of 5 cm under the surface of the grain mass. Not all of introduced adults were killed at the middle of the grain mass (Table 1). These results might indicate that there was lower O₃ concentration inside the grain mass than that at the surface of the grain mass. Though there was a big difference in O₃ concentration in different treatments, the insect mortality at the same location was similar (Table 1). Insect mortalities at the center were significantly different from that at the wall or half radius locations (Table 1). This significance was caused by the higher mortality at the Middle-Center location in five out of six treatments than that at wall and half radius locations. This might indicate that the ozone concentration at the middle of the grain bulk was not consistent at different locations and in different treatments. The low and inconsistent O₃ concentration resulted in less than 87% of overall mortality at the middle of the grain mass (Table 1).

The mortalities among insect species introduced at the middle of the grain bulk were significantly different. This might be caused by the uneven distribution of the introduced ozone (Kells et al., 2001) and different tolerances of the insect species to toxic ozone (Hansen et al., 2012).

Table 1 Mortality and offspring number of adults after fumigation with ozone

Location		<i>Cryptolestes ferrugineus</i>		<i>Tribolium castaneum</i>		<i>Sitophilus oryzae</i>		<i>Rhyzopertha dominica</i>	
		M ^a	O ^b	M ^a	O ^b	M ^a	O ^b	M ^a	O ^b
Top-S	wall	100±0	0.0±0.0	100±0	0.1±0.1	100±0	0.0±0.0	100±0	0.0±0.0
	half	100±0	0.1±0.1	100±0	0.1±0.1	100±0	0.1±0.1	100±0	0.1±0.1
	center	100±0	0.1±0.1	100±0	0.1±0.1	100±0	0.2±0.1	100±0	0.1±0.1
Top-5cm	wall	97±3	0.0±0.0	93±7	0.0±0.0	95±6	0.9±0.4	94±6	5.1±2.7
	half	100±0	0.1±0.1	100±0	0.1±0.1	100±0	0.3±0.3	100±0	1.2±0.8
	center	100±0	0.0±0.0	100±0	0.0±0.0	100±0	0.0±0.0	100±0	0.1±0.1
Middle	wall	50±11	3.7±1.7	19±11	0.8±0.4	56±11	23.6±6.6	33±9	43.4±7.7
	half	72±18	1.7±1.0	62±20	0.2±0.1	62±14	16.9±6.2	37±12	14.4±3.2
	center	50±14	0.5±0.3	29±11	0.2±0.1	87±11	7.4±3.3	74±17	12.9±4.9
Control		21±3	3.1±1.0	4±1	1.3±0.6	18±2	44.6±9.6	16±4	56.9±9.4

^a Mortality of adults (mean ±SE, %). n = 18; ^b offspring number of adults (mean ±SE). n = 18.

Number of the offspring

Different insect species had different offspring numbers (Table 1). The correlation coefficient between offspring number and adult mortality was -0.5 ($P < 0.001$). This result indicated that fewer eggs survived the ozone fumigation when the adult mortality was 100%. Therefore, ozone killed some eggs in this study.

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