**Effects of chlorine dioxide and ozone gases against immature stages of stored-grain insects**

by

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DOCTOR OF PHILOSOPHY

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# Abstract

Utilization of ozone and chlorine dioxide has been well documented in a variety of applications, such as for treatment of drinking water, microbial inactivation in food processing settings, and for odor control. The two gases have been evaluated for managing immature stages of phosphine resistant field populations of stored-product insects. Laboratory studies were conducted to determine the effectiveness of chlorine dioxide gas against various life stages of common stored-product insect species, namely the red flour beetle, *Tribolium castaneum* (Herbst); lesser grain borer, *Rhyzopertha dominica* (Fabricius); rice weevil, *Sitophilus oryzae* (Linnaeus); maize weevil, *Sitophilus zeamais* Motschulsky; and sawtoothed grain beetle, *Oryzaephilus surinamensis* (Linnaeus). Several phosphine resistant field populations of these insect species were exposed to chlorine dioxide gas including phosphine susceptible laboratory populations. Target life stages were eggs (0-day-old), young larvae (14-day-old), old larvae (21-day-old), and pupae (3-day-old). Life stages were exposed to a chlorine dioxide gas concentration of 0.95 $g/m^3$ (350 parts per million, ppm) for 2, 4, 6, 8, and 10 hours. Both phosphine resistant and phosphine susceptible populations were equally susceptible to chlorine dioxide. In general, for all species and strains mortality increased with increasing exposure time. Eggs and 14-d-old larvae were easier to kill, even at the lowest gas dosage (concentration × time product, *ct* product) of 1.90 $g\text{-}h/m^3$. Although less susceptibility was observed in 21-d-old larvae and 3-d-old pupae, increased gas concentrations and/or exposure time were able to produce higher mortalities in these two stages. Progeny production of treated pupae was severely suppressed as compared to the control, indicating adverse effects of chlorine dioxide on reproductive organs. Chlorine dioxide gas can be a very effective alternative to phosphine in controlling stored-product insects associated with raw commodities.

Immature stages of a phosphine susceptible laboratory strain and four phosphine resistant field strains of *T. castaneum*, were used for this study. Eggs, young larvae, old larvae, and pupae of *T. castaneum* were exposed in vials with 10 g of wheat to chlorine dioxide gas at 2.02 g/$m^3$ (750 ppm) concentration for 2, 4, 6, and 8 h. Mortality was determined by subtracting the number of adults that emerged from the immature insects out of the total insects exposed and expressed as a percentage. Adult progeny production was determined 8 weeks after adult emergence. Complete mortality was achieved in eggs and young larvae. The highest mortality of old larvae and pupae was 86.8-95.2% and 81.1-93.8%, respectively, after an 8 h exposure. The order of susceptibility of immature stages to chlorine dioxide was: young larvae > eggs > old larvae > pupae. Mortality of immature stages increased with an increase in exposure time. At longer exposure times there was a significant reduction in progeny production, perhaps a result of higher mortality of immature stages. Adults that emerged from treated pupae revealed less progeny production. In addition, all strains of phosphine resistant insects were equally susceptible to chlorine dioxide when compared with susceptible strains. These results suggest that chlorine dioxide gas is highly effective in controlling immature life stages of laboratory and field strains of *T. castaneum*.

Ozone was investigated as a potential alternative to control phosphine resistant strains of the lesser grain borer, *Rhyzopertha dominica* (F.). The efficacy of ozone against one phosphine-susceptible laboratory and two phosphine resistant field strains of *R. dominica* was evaluated at two concentrations (0.21 and 0.42 g/$m^3$). Vials holding 20 adults with 0 and 10 g of wheat were exposed to each ozone concentration for up to 24 h to estimate lethal doses required for 50 ($LD_{50}$) and 99% ($LD_{99}$) mortality. After ozone exposure, mortality was assessed 5 d later. After exposure to 0.21 g/$m^3$ of ozone for 24 h, the 5-d mortality was 77-86% with wheat and 96-97%

without wheat. After exposure to 0.42 g/m$^3$ of ozone for 16 h, the 5-d mortality with and without wheat was 100%. There were no significant differences between $LD_{50}$ values of the samples treated at 0.21 and 0.42 g/m$^3$, regardless of strains and presence or absence of wheat. The small amount of wheat (10 g) affected efficacy at 0.21 g/m$^3$, but showed a non-significant effect at 0.42 g/m$^3$. Ozone tends to react with active sites on the surface of wheat kernels prior to reaching an effective lethal concentration for insects. High ozone concentration in the supply air reduced the time to saturate all active sites and ensured that lethal levels of free ozone were available to kill insects. Emergence of adults from eggs, young larvae, and old larvae were reduced by 96-100% relative to the emergence in the control treatment after a 72 h exposure to an ozone concentration of 0.42 g/m$^3$. At the same concentration of ozone, pupae exposed for 2 to 10 h, had a 33 to 97% reduction in adult emergence relative to control treatment.

Exposure to chlorine dioxide at concentration of 0.95 g/m$^3$ for 3 h reduced both production and hatchability of eggs of *T. castaneum* and egg production of the Indian meal moth, *Plodia interpunctella* (Hübner). Severe effects of chlorine dioxide in both *T. castaneum* and *P. interpunctella* reproductive performance are important in terms of increase in population of these insect species after a fumigation. Population of these stored product insects is highly related to the reproductive performance of surviving insects. Sanitation of storage facilities prior to fumigation plays a critical role in the effectiveness in controlling insects due to bonding property of chlorine dioxide with organic matter.

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# Abstract

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Immature stages of a phosphine susceptible laboratory strain and four phosphine resistant field strains of the red flour beetle, *T. castaneum*, were used for this study. Eggs, young larvae, old larvae, and pupae of *T. castaneum* were exposed in vials with 10 g of wheat to chlorine dioxide gas at 2.02 $g/m^3$ (750 ppm) concentration for 2, 4, 6, and 8 h. Mortality was determined by subtracting the number of adults that emerged from the immature insects out of the total insects exposed and expressed as a percentage. Adult progeny production was determined 8 weeks after adult emergence. Complete mortality was achieved in eggs and young larvae. The highest mortality of old larvae and pupae was 86.8-95.2% and 81.1-93.8%, respectively, after an 8 h exposure. The order of susceptibility of immature stages to chlorine dioxide was: young larvae > eggs > old larvae > pupae. Mortality of immature stages increased with an increase in exposure time. At longer exposure times there was a significant reduction in progeny production, perhaps a result of higher mortality of immature stages. Adults that emerged from treated pupae revealed less progeny production. In addition, all strains of phosphine resistant insects were equally susceptible to chlorine dioxide when compared with susceptible strains. These results suggest that chlorine dioxide gas is highly effective in controlling immature life stages of laboratory and field strains of *T. castaneum*.

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Exposure to chlorine dioxide at concentration of 0.95 $g/m^3$ for 3 h reduced both production and hatchability of eggs of *T. castaneum* and egg production of the Indian meal moth, *Plodia interpunctella* (Hübner). Severe effects of chlorine dioxide in both *T. castaneum* and *P. interpunctella* reproductive performance are important in terms of increase in population of these insect species after a fumigation. Population of these stored product insects is highly related to the reproductive performance of surviving insects. Sanitation of storage facilities prior to fumigation plays a critical role in the effectiveness in controlling insects due to bonding property of chlorine dioxide with organic matter.

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# Dedication

**To my amazing parents,**

**Fengjiang Li and Chenxia Wang**

# Chapter 1 - Introduction

## 1.1. Phosphine

Phosphine hydrogen phosphide ($PH_3$) is a colorless gas with fish-like odor, and slightly heavier (density = 1.38 g/L) than air (1.28 g/L). Phosphine, has molecular weight of 34, melting point of -132.8 °C and boiling point of -87.7 °C. Phosphine is one of the two US EPA registered (besides sulfuryl fluoride) fumigants, and has been extensively applied to stored commodities, oil seeds, and other dried commodities for controlling insect pests (Phillips 2012). Numerous studies were conducted to understand the mode of action of the phosphine in the past several decades due to development of resistance among many species of stored-product insects. Nath et al. (2011) reviewed phosphine toxicity, and the mechanisms of action can be summarized to three possible aspects: neural, metabolic, and redox aspects that may also be interrelated, contribute to phosphine toxicity. Regarding neural aspects of phosphine toxicity, studies performed on vertebrates (such as rats) showed phosphine increases acetylcholine neurotransmission through suppressing the enzyme activity of acetylcholinesterase (AChE) (Al-Azzawi et al., 1990; Potter et al., 1993; Mittra et al., 2001). Such enzyme suppression can cause an attenuation of acetylcholine signaling, and result a hyperactivity. Exposed to phosphine can lead to a reduction of acetylcholine esterase activity in insect, both *in vitro* and *in vivo.* Research conducted to investigate the inhibitory action of phosphine on acetylcholinesterases (AChEs) using *Ephestia cautella* by Al-Hakkak et al. (1989), indicating that adults emerged from treated pupae showed much lower enzyme activities (0.433 ± 0.11 to 0.650 ± 0.13 µmol/mL/min) compare to controls (0.815 ± 0.18 µmol/mL/min). Result was confirmed later with *in vitro* experiments and showed inhibitory effects of phosphine on AChEs. When used as a grain fumigant, acetylcholine-mediated inhibitory action is due to atropine inhibiting G-protein coupled acetylcholine

signaling, rather than acute neural excitotoxicity which associated with ligand gated ion channel receptors.

Inhibition of respiration found in insect mitochondria and nematodes when treated with phosphine. Because the inner membrane of mitochondria is highly impermeable, it inhibits phosphine uptake unless this membrane is physically disrupted or activated by reactions, such as ATP synthesis. *In vitro* studies demonstrated that inhibition of respiration took place via directly altering electron transport chain function following phosphine exposure, whereas such effect found reduced or absent when examined the isolated mitochondria from treated live insects. Furthermore, modified function of electron transport chain seemed unrelated with the resistance mechanism as *in vitro* studies demonstrated that both phosphine-resistant and -susceptible strains of insects expressed similar inhibition regarding respiration in mitochondria (isolated from phosphine-treated insects) (Price 1980; Schlipalius et al., 2006).

## 1.2. Potential fumigants

### 1.2.1. Chlorine dioxide

Chlorine dioxide is a US EPA proved disinfectant, sanitizer, and sterilizer which functions "to destroy or eliminate all forms of microbial life including fungi, viruses, and all forms of bacteria and their spores". In addition, there is no evidence showed any organisms that are resistant to chlorine dioxide. At atmospheric pressure, chlorine dioxide gas has a yellow to green-yellowish color under room temperature (O'Neil et al., 2001; NIOSH, 2003) and relative dense in water, approximately 1.6 (water = 1). Chlorine dioxide has been successfully utilized as a disinfectant against many microorganisms, including bacteria, fungi, and viruses, due to its potent oxidizing property.

***Antibacterial activity.*** Chlorine dioxide acts as a free radical which possesses 19 electrons and can react with substances that give off electrons. Regarding the oxidation potentail, chlorine dioxide (0.95 V) is relatively weak compared to ozone (2.07 V). The oxidation capacity of chlorine dioxide is 5 $e^-$ which chlorine dioxide can accept 5 electrons as reduced to chloride. Numerous studies have been undertaken regarding the mode of action of chlorine dioxide on various microorganisms in the past several decades. Chlorine dioxide reacts with selected substances such as amino acids, sulpfuric substances, and reactive organic substances. Benarde et al. (1968) pinpointed the mechanism of chlorine dioxide (liquid) disinfection. They reported that chlorine dioxide did not react with amino acids to modify their functional structures, but disrupt *Escherichia coli* via interfering its protein synthesis. Roller et al. (1980) suggested that there was no significant alteration of dehydrogenase, nor protein synthesis may not be the direct cause in bacterial lethality. They reported that low dose of chlorine dioxide (2 mg/L) had no effect on characteristic structure of DNA when stripped DNA exposed to chlorine dioxide, whereas an initial dose 20mg/L would slightly inhibit DNA transforming activity after 10 min.

Berg et al. (1986) reported that outer membrane integrity is a major factor regarding cell sensitivity to chlorine dioxide. Potassium efflux is the primary indication of membrane injury in bacterial cells as treated with membrane-active antimicrobials, such as chlorine and phenol (Lambert and Hammond, 1973; Kroll and Anagnostopoulos, 1981; Haas and Englebrecht, 1980, 1981). The “lethality index” indicated the rate of potassium efflux was correlated to the doses of antimicrobials (such as phenol), whereas losses of potassium was too rapid to develop such index at any given doses of chlorine dioxide. Studies conducted by Mogoa et al. (2011) reported that exposure of *Acanthamoeba castellanii* to chlorine dioxide led to a decrease in size and modify the cell permeability related to control, even with the lowest dose (0.2 mg·min/L). Moreover,

under transmission electron microscopy, chlorine dioxide-induced cells appeared to be highly vacuolated while cytoplasm remained dense.

Injury of inner cell membrane with oxidizing agents has been documented as a key factor for killing some pathogenic microorganisms. Cortezzo et al. (2004) reported that the rate of methylamine uptake was much faster in oxidizing agent treated spores compared to untreated spores. The treated spores unable to retain methylamine were damaged the most. Spores pretreated with oxidizing agents obtained severe injury in their inner membrane. Spore damage/death after treated with oxidizing agents are not due oxidation of unsaturated fatty acids in the spore inner membrane, rather the targets are some membrane proteins, of which facilitate with spore germination, dormancy, and metabolism (Stelow et al., 2003; Cortezzo et al., 2004). Oxidative damage may undergo by attacking iron-sulphur centers and amino acids of the proteins or more readily conformational changes which may increase the permeability of inner membrane (Imlay, 2002). Spores killed by ozone germinate much quicker compared to those killed by chlorine dioxide which were noted to germinate normally (Cortezzo et al., 2004; Young and Setlow, 2003). Further investigation on germinated chlorine dioxide killed spores showedd significant reduction level of metabolism since they germinated normally. In addition, bacterial viability analysis indicated that there was approximately 35% of the spores which were germinated from chlorine dioxide-killed spores (Young and Setlow, 2003). Some other factors found in resistant spores showed that chlorine dioxide may be associated with relatively low permeability of inner membrane to small hydrophilic molecules (Gerhart et al., 1972; Swerdlow et al., 1981).

It has been reported that ozone may affect cell membrane through interferences with proteins, respiratory enzymes, and unsaturated fatty acids. Cho et al. (2010) illustrated that ozone

primarily reacted with various components on the cell wall. Protein release, lipid peroxidation and changes in cell permeability were observed during *E. coli* inactivation with ozone treatment (*ct* = 0.0021 mg/L min). Effect of ozone on bacteria indicated that ozone interacted with cell envelopes, such as peptidoglycans, and cytoplasm, including enzymes, nucleic acids, and led to bacterial inactivation. Degradation of nucleic acids after *E. coli* exposed to ozone showed guanine base of RNA and guanine and thymine bases of DNA were susceptible to ozone (Shirnriki et al., 1981, 1983; Minura et al., 1984a; Ishizaki et al., 1984; Shinriki et al., 1984).

***Antiviral activity.*** US Occupational Safety and Health Administration permits that chlorine dioxide gas with concentration of 0.1 ppm can be applied in human workplaces for disinfection purposes (US Department of Labor, Occupational Safety and Health Administration, 2006: http://www.osha.gov/SLTC/healthguidelines/ chlorinedioxide/recognition.html). Ogata and Shibata (2008) proposed that fumigation with chlorine dioxide gas using a low concentration (0.03 ppm) could be carried out against viruses that are associated with respiratory infection. Particularly it can be used for managing influenza A virus, in public places such as hotels, airports, schools, and offices, with the presence of humans (WHO, 2003, Influenza: Report by the Secretariat to the Fifty-Sixth World Health Assembly (WHO, Geneva), A56/23, 17 March 2003: http://www.who.int/gb/ebwha/pdf_files/WHA56/ea5623.pdf). Two major glycoproteins on virus envelope, haemagglutinin (HA) and neuraminidase (NA), were denatured upon chlorine dioxide gas treatment in *in vitro* which led to effective inhibition ofviral infection. (Tsuchita et al., 2001; Wagner et al., 2002; Benz and Mittal, 2003; Solorano et al., 2000; Gong et al., 2007). Specifically, denaturation of HA and NA proteins by chlorine dioxide was through altering amino acid residues, primarily tryptophan and tyrosine. Other proteins may also get involved in the infectivity and denatured by chlorine dioxide. For instance, M2 is a proton channel protein

located in viral envelope, of which tryptophan residue ($Trp^{41}$) inside of the proton channel which is attributed to a 'gate' responsible for proton exchange (Tang et al., 2002). Therefore, there may be a possibility that tryptophan residue ($Trp^{41}$) could be modified by chlorine dioxide in M2, leading to its malfunction, and which in turn caused inactivation of influenza A virus (Ogata, 2007; Ogata and Shibata 2008). Ogata (2012) confirmed the previous findings and identified the specific residue (tryptophan residue W153) in HA peptide which get modified by chlorine dioxide.

***Antifungal activity.*** Some fungal species produce mycotoxins which are secondary metabolites and highly toxic to humans and other animals. The action level and tolerances of mycotoxins were established by FDA in human food and animal feed (Guidance for industry, FDA, 2017: https://www.fda.gov/Food/GuidanceRegulation/ucm077969.htm). One of the antifungal mechanisms about chlorine dioxide is its oxidation and degradation of sugars and polysaccharides leading to a major loss of fungal invasion. Research found that more than 99% of *C. albicans* cells were inactivated following treatment with 15 mmol/L of chlorine dioxide, however, cells remained their shape and integrity, also there was no visible damages observed on the plasm membrane, such as plasmolysis, holes, or gaps. Although the cell membrane was still intact, the inner membrane of the cell wall appeared to be pushed on the plasm membrane and present many irregular invaginations on the innermost layer. Rapid and gross potassium leakage was observed as both time- and dose-dependent increases prior 99% cell death. Maximum potassium efflux was about 23% even with the highest dose of chlorine dioxide (200 mmol/L), which was significant low compared to that of *E. coil* (Berg et al., 1986). Membrane composition of these microorganisms might be responsible for such dissimilarity. ATP leakage can also be considered as an indicator for cell membrane damage (Anséhn, and Nilsson, 1984;

Kulakovskaya, et al., 2003; Higgins et al., 2005). Wei et al. (2008) pinpointed that substantial ATP leakages were observed from both time- and dose-dependent experiments, however, loss of ATP was quite low as compared to that of potassium and there was no severe destruction in the integrity of the plasm membrane. Therefore, the ATP leakage did not appear to be critical in relation to cell inactivation (Wei et al., 2008). Young et al. (2003) proposed that one of possible antifungal mechanisms of chlorine dioxide could be disrupting sulfur metabolism in *C. albicans*. Protein disulfide reductase is an enzyme which converts C-S-S-C linkage of proteins to the sulfhydryl form (-SH) by introducing hydrogen. Such reaction is known to be critical during cell division. Chlorine dioxide might keep sulfur-containing groups in oxidized state with its potent oxidizing ability; this restrained *C. albicans* cells from cell division and replication. Furthermore, an "alternate pathway" in Candida respiration was proposed regarding the mechanism of chlorine dioxide as an anti-fungal agent. Although the extract biochemical mechanism for respiratory pathway remains unknown, research showed in Young et al. (2008) indicated alternate pathway occurs in mitochondria.

***Insecticidal activity.*** Insecticidal activities of chlorine dioxide against chironomids, *Tribolium castaneum*, *Plodia interpunctella, Cimex lectularius, Citnex hemipterus*, *Oryzaephilus surinamensis, Rhyzopertha dominica, Sitophilus zeamais*, and *Sitophilus oryzae* have been reported by several researchers (Sun et al., 2007; Gibbs et a., 2012; Kim et al., 2015; Kumar et al., 2015; Han et al., 2017; E et al., 2017). Chlorine dioxide increases the oxidative stress in animals such as rat and chicken, and enzymes (i.e. catalase) play key role to overcome the oxidative stress (Couri and Abdel-Rabman, 1979). Kim et al. (2015) demonstrated that enzymes, superoxide dismutase (Tc-SOD) and thioredoxin-peroxidase (Tc-Tpx*)*, as main antioxidants to overcome the oxidative stress induced by chlorine dioxide. Experiments carried out using larvae

and adults of *T. castaneum* were killed at chlorine dioxide concentration of 200 ppm in 24 h. Kim et al. (2015) reported that chlorine dioxide gas may impair the physiological system of *T. castaneum* and weaken its ability to repair the injured tissues. Insecticidal activity of chlorine dioxide was significantly magnified as *Tc-SOD* and *Tc-Tpx* were silenced by introducing dsRNAs. Accordingly to larvae, and this was evident that Tc-SOD and Tc-Tpx were the major enzymes to defense against oxidative stress induced by chlorine dioxide. Additionally, to further confirm whether the oxidative stress was produced due to chlorine dioxide treatment, the reactive oxygen species were quantified in fat body of treated larvae.

### 1.2.2. Ozone

Ozone gas is colorless or pale blue gas with a pungent odor. Ozone is a triatomic molecule ($O_3$) and has molecular weight of 48, melting point of -192.7°C, and boiling point of -111.9°C under atmospheric pressure (The Merck Index, 2013). Gaseous ozone is denser (2.14 g/L) than air (1.28 g/L) (Wojtowicz, 1996), and possesses a high oxidizing potential of 2.07 V (Manley and Niegowski 1967; Brady and Humiston, 1978; Pehkonen 2001). Gaseous ozone is more stable than aqueous ozone regarding to the half-life, of which half-life of gaseous ozone is 12 h, and aqueous ozone varies from 2 to 65 min at 20 °C (Koike et al., 1998; Weavers and Wickramanayake, 2001; Wynn et al., 1973; Wickramanayake, 1984; Graham, 1997). Ozone is Generally Recognized as Safe (GRAS) in the United States as a disinfectant in bottled water (FDA1995). Other successful applications of ozone approved by US FDA included decontamination of packaging materials, sanitation of processing equipment, disinfection of drinking water (Mahapatra et al., 2005). Ozone is highly unstable and can easily decompose to hydroxyl ($HO^{\circ}$), hydroperoxyl ($HO_2$), and superoxide radicals ($O_2^{\ -}$) which attribute to potent oxidizing power of ozone (Manousaridis et al., 2005). The inactivation mechanisms of ozone

have been well studied on numerous microorganisms, mainly bacteria and viruses, including Gram-positive (*Listeria monocytogenes, Staphylococcus aureus, Bacillus cereus, B. subtilis, B. meaaterium*, and *Enterococcus faecalis*), Gram-negative bacteria (*Pseudomonas aeruginosa* and *Yersinia enterocolitica, yeasts;* and *Candida albicans, Zygosaccharomyces bacilli* and spores of *Aspergillus niger*), and other organisms ((Ishizaki et al., 1986; Restaino et al., 1995). Research showed that ozone is more effective compared to chlorine dioxide regarding microorganism inactivation (Botzenhart et al., 1993).

***Antiviral activity.*** Several studies on the mechanism of inactivation by ozone revealed that ozone can directly react with proteins and inactivate protein with susceptible amino acids such as cysteine, tryptophan and methionine (Mudd et al., 1969). Ozone inactivated bacteriophages f2 and T4 via attacking the protein capsid to liberate nucleic acids and then inactivated (Kim et al., 1980; Sproul et al., 1982). Ward (1958) demonstrated the oxidation of polio virus by ozone, and suggested that RNA strand was broke to many short segments following ozone exposure and viruses lost their infectivity in 6 sec. De Mik and De Groot (1977) investigated the mechanism of inactivation of bacteriophage φX174 by ozone gas and found that the inactivation of the phages was mainly because of the protein damage; ozone oxidized the capsid protein to subunits led to loss of integrity, and then release the DNA. Researchers found that a mixture of four nucleotides of polio virus RNA exposed to ozone, only guanine bases were degraded. When its transfer RNA was exposed to ozone, guanine was the first nucleotide to be degraded (Ward 1958). Tobacco mosaic virus (TMV) treated with ozone resulted a rapid inactivation in 20 min due to the preferential degradation of guanine (Shinriki et al., 1988, 2014). Menzel (1971) reported that ozone may react with DNA, and Prat et al. (1969) also showed that ozone might modify pyrimidine in *E. coli* nucleic acids. De Mik and De Groot (1977)

demonstrated that DNA extracted from ozone-induced phage did not show significant difference from the DNA extracted from the control phage when compared their biological activity. Kim et al. (1980) examined inactivation of bacteriophage f2 using ozone and elucidated that ozone broke the capsid of the phage and disrupted adsorption to the host pili and reduced biological activity of the RNA. Damage of RNA by ozone was also implied to be the main cause for poliovirus type 1 inactivation (Roy et al., 1981).

***Antibacterial activity.*** Botzenhart et al. (1993) conducted experiments on inactivation of *B. subtilus* spores with ozone in water treatment and found that ozone is a more effective sterilant compare to chlorine dioxide. Lack of enzyme (SleB) in *B. atrophaeus* mutant spores main causes for rapid inactivation by ozone (Khadre and Yousef 2001; Young and Setlow 2004). Reduced activities were observed among various enzymes in *E. coli* for different degrees (Takamoto et al., 1992). Kim et al. (2003) demonstrated the inactivity mechanism of *B. subtilis* spores following ozone treatment using TEM, and it was revealed that the lethality was caused by damage to both inner and outer layers of the spore coats which comprise approximately 80% of spore proteins. Cortezzo et al. (2004) reported that ozone can penetrate and disrupt the key proteins located on the inner membrane which caused increased penetration of methylamine entry to the core. Moreover, loss of this permeability barriers may liberate the spore core contents and lead to lethal injury (Khadre and Yousef 2001; Young and Setlow 2004).

***Insecticidal activity.***

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 and poultry (FDA, 2001). Due to its rapid decomposition, ozone is

commonly produced onsite to ensure a continuous supply. The potential of ozone as a fumigant to control stored-product insects was explored more than three decades ago (Erdman, 1980). A comprehensive review by Iskber and Athanasiou (2015) adequately addressed the use of ozone gas for the control of insects and microorganisms in stored products. Erdman (1980) applied 0.0963 g/m3 (1 g/m3 ¼ 476.19 ppm at 25 °C) ozone for 1–6 h to kill larvae, pupae, and adults of the confused flour beetle, *Tribolium confusum* Jacquelin du Val, and red flour beetle, *Tribolium castaneum* (Herbst). He found immature stages (larvae and pupae) were more sensitive to ozone than adults for both species. The adults of *T. confusum* were more tolerant to ozone than adults of *T. castaneum*. All exposed adults of *T. confusum* died after a 6.5 h of exposure to ozone, whereas 5 h was necessary to kill all *T. castaneum* adults. Mason et al. (1998) exposed *T. confusum*, *T. castaneum*; the maize weevil, *Sitophilus zeamais* Motschulsky; and Indian meal moth, *Plodia interpunctella* (Hübner), to low concentrations of ozone (0.0214 or 0.107 g/m$^3$). The mortality of adults of *T. confusum*, *T. castaneum*, and *S. zeamais*, and fifth instars of *P. interpunctella* was 100% after exposure to an ozone concentration of 0.0214 g/m$^3$ for 4 d or to a concentration of 0.107 g/m$^3$ for 1 d. However, the highest mortality was not observed until 3–12 d after the treatment, which indicated delayed toxic effects of ozone. Hansen et al. (2012) used ozone to control eggs, young and medium-aged larvae, pupae, and adults of 11 stored product insect species. They found internally developing insect species to require higher ozone concentrations than externally developing insect species. Unlike Erdman (1980), for all species tested, adults in general tended to be more susceptible to lower ozone concentrations than other stages tested (Hansen et al., 2012).

The lethal dosages for complete adult mortality of the insect species tested ranged from 5.14 to 20.04 g-h/m3. The immature stages of all insect species were found to be more tolerant to

ozone and required a dose of 9.55 - 56.04 g-h/m3 for 100% mortality. Holmstrup et al. (2011) also reported that the larvae of *T. castaneum* required a higher lethal dose for complete mortality than adults (4.109 vs. 3.082 g-h/$m^3$). The mechanism of lethal effect of ozone against insects has not yet been widely studied, but we hypothesize that the insects succumb to ozone because of oxidative stresses at the physiological level. Chlorine dioxide, another highly oxidative gas, and one that has a lower oxidation potential than ozone (Simpson, 2005) has been pointed out to cause insect mortality by oxidative stresses (Kim et al., 2015). In addition, chlorine dioxide can oxidize fatty acids and thiol groups (-SH) in proteins and enzymes located on the cell membrane, which then lead to degradation and increased permeability of cell membrane, and eventually inducing a lethal effect on microorganisms (Gómez-López et al., 2009; Finnegan et al., 2010).

## 1.3. Research objectives

Fumigation has been an effective control measure for postharvest pest management for many centuries (Fumigants and Pheromones, 1989, https://www.fumigationzone.com/assets/docs/Issue%2019.pdf; Phillips et al., 2012). Phosphine has been used extensively for stored-commodities against a variety of insect pests, and it became the primary fumigant for grain storage especially during the period of phaseout of methyl bromide (Price and Mills, 1988; Bell, 2000; Zeller and Arthur, 2000; Rajendran, 2001; Daglish, 2004; Collins et al., 2005). Application of a single fumigant for long period of time, inadequate fumigation and leakages in the storage structure resulted in increase of selection pressure for phosphine resistance (Chaudhry, 1997; Bengston et al., 1999; Chaudhry, 2000; Collins et al., 2002; Benhalima et al., 2004; Daglish, 2004; Pimentel et al., 2008). Opit et al. (2012) collected several insect populations from various locations in Oklahoma between years of 2010-1011 and

were evaluated for phosphine resistance. Of which eight out of nine *T. castaneum* populations were resulted to be resistant, and the highest survival was 94% in one population. All five populations of *R. dominica* exhibited be resistant to phosphine, and the highest survival rate was 97% (Opit et al., 2012). Survey carried out to characterize the phosphine-resistance status in Brazil by Pimentel et al. (2010) and three insect species of total thirty one populations were assayed for phosphine resistance, including *R. dominica, T. castaneum,* and *O. surinamensis*. Results found that none of these populations revealed greater than 90% of mortality using the corresponding discriminating concentration recommended by FAO (0.03 mg/l for *R. dominica*, 0.04 mg/l for *T. castaneum* and 0.05 mg/l for *O. surinamensis*) (FAO 1975). In Australia, research documented that several insect species developed high level of phosphine resistance, including *R. dominica, T. castaneum* and *Cryptolestes ferrugineus* (Collins et al., 2002; Nayak et al., 2013). Development of phosphine resistance by insect species was also documented in Bangladesh (Mills, 1983; Tyler et al., 1983), China (Ren et al., 1994), Philippines (Acda and Daglish, 2000), and India (Rajendran and Narasimhan, 1994). There has been an urgent demand for environmentally friendly and effective fumigants in replacing phosphine for stored-product insect control. Chlorine dioxide gas was proposed and evaluated as an alternative to phosphine for control of stored-product insects.

Several studies have investigated the effectiveness of chlorine dioxide gas in control of urban and stored-product insect pests. Gibbs et al. (2012) reported the application of chlorine dioxide gas for control of bedbugs (*Cimex lectularius* and *Cimex hemipterus*). Exposure carried out with two higher concentrations 1.95 g/m3 (724 ppm) and 2.93 g/m3 (1086 ppm) for 94 and 167 min, respectively, and complete mortality was obtained immediate after fumigation under laboratory setting. Complete mortality was observed using lower concentration of 0.98 $g/m^3$ (362

ppm), after 6 h when exposed for 176 min and after 18 h when exposed for 89 min, respectively. Channaiah et al. (2012) evaluated the efficacy of chlorine dioxide against life stages of *T. castaneum* and *T. confusum.* The highest mortality was achieved when insects exposed to the highest dosage (834.4 g-h/$m^3$) without food. For *T. castaneum*, 9.3, 100, 18.8, and 100% of mortality was observed for eggs, young larvae, old larvae and adults, respectively. For *T. confusum*, the mortality under highest dosage was 11.1, 100, 31.3, and 100% for eggs, young larvae, old larvae, and pupae, respectively. Phosphine susceptible and resistant populations of five major store-product adult insects were assayed for the efficacy of chlorine dioxide with gas concentration 0.54 g/$m^3$ (200 ppm) for various exposure times (3-34 h) (E et al., 2017). With food, $LD_{99}$ reported for phosphine susceptible and resistant populations of these five insect species, including *T. castaneum, R. dominica, S. zeamais, O. surinamensis,* and *S. oryzae*, were 14.79-22.57, 15.79-21.60, 10.66-14.53, 8.20-8.41, and 7.67-12.20 g-h/$m^3$, respectively. However, the efficacy of chlorine dioxide in controlling immature stages is not known. Therefore, the research objectives listed below were conducted using immature stages of several phosphine-resistant strains and one susceptible strain of *T. castaneum* (Herbst), *Rhyzopertha dominica* (Fabricius), *Sitophilus zeamais* (Motschulsky), *Oryzaephilus surinamensis* (Linnaeus), and *Plodia interpunctella* (Hübner).

- To evaluate the effect of insecticidal properties of chlorine dioxide gas against immature stages of varies field- and laboratory populations (chapter 2),
- To responses of immature stages of *Tribolium castaneum* to chlorine dioxide gas (chapter 3),
- To determine the effect of reproductive performance in *Tribolium castaneum* pupae and *Plodia interpunctella* larvae to sublethal chlorine dioxide exposure (chapter 4),

- To determine the efficacy of ozone at two concentrations against adults of phosphine susceptible and resistant strains of *Rhyzopertha dominica* and other insect species (chapter 5),

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# Chapter 2 - Efficacy of chlorine dioxide gas against immature stages of three stored-product insect species

## 2.1. Abstract

Laboratory-scale study was to focus on the effectiveness of chlorine dioxide gas against various life stages of common stored-product insect species, including *Rhyzopertha dominica* (Fabricius), *Sitophilus zeamais* (Motschulsky), and *Oryzaephilus surinamensis* (Linnaeus). Phosphine-resistant populations of these insect species were exposed to chlorine dioxide gas, and laboratory (susceptible) populations were served as control. Life stages, including eggs (0-day-old), young larvae (14-day-old), old larvae (21-day-old), and pupae (3-day-old), were exposed to chlorine dioxide gas at concentrations of 0.95 g/m$^3$ (350 ppm) and 2.70 g/m$^3$ (1000 ppm) for 2, 4, 6, 8, and 10 hours, respectively. Results showed that phosphine-resistant field populations of each species were observed similar susceptibility in relation of its corresponding phosphine-susceptible laboratory population. Eggs were the most susceptible to chlorine dioxide for all insect species and the lowest dosage of chlorine dioxide (1.90 g/m$^3$) was about to produce 88.3 – 100 % mortality. Young larvae of *S. zeamais* and *O. surinamensis* was observed 91.5-100% mortality after exposure to chlorine dioxide at concentration of 0.95 g/m$^3$.whereas 8.3-55.0% mortality was obtained when *R. dominica* young larvae were treated with gas concentration of 0.95 g/m$^3$ for 2-10 h. Although less susceptibility was observed in 21-d-old larvae and 3-d-old pupae for all species, higher gas concentrations and longer exposure times were able to achieve higher mortality. Progeny production of all three species was severely suppressed when treatments conducted on both egg and young larval stages. Number of adult progeny found in treated eggs after 8 weeks were ranged from 0-9 and 0-4 adult progeny per container in *R. dominica* and *S. zeamais,* respectively. Progeny production observed in treated larvae ranged

from 0-27 adult progeny per container in *S. zeamais*, whereas progeny reduction of progeny did not show significance until insects were tested with longer exposure time. no progeny production of *O. surinamensis* was observed in treated eggs and young larvae due to 100% mortality after exposure to either concentrations (0.95 g/m$^3$ and 2.70 g/m$^3$) at any given time. All insect species were observed significant difference between control and treated groups for 6-10 h exposure time relative to progeny production when exposed as old larvae and pupae. Progeny production of all tested species was found only in control insects while using chlorine dioxide concentration of 2.70 g/m$^3$ (1000 ppm) at any given exposure time. In conclusion, chlorine dioxide gas can be a very effective phosphine alternative in control of stored-product insects in raw commodities.

## 2.2. Introduction

Since early twentieth century, when Taylor et al. (1940) found the potent bleaching characteristics of sodium chlorite, meanwhile these researchers considered to separate gaseous chlorine dioxide produced by a chemical reaction between sodium chlorite and chlorine. Chlorine dioxide gas became commercially available for drinking water treatment, and the first published application was in a water treatment plant located in Niagara Falls, New York in 1944. As the commercial availability was highlighted, the application of chlorine dioxide was expanded to other areas such as bleaching, taste and odor control, microorganism inactivation (Solomon et al., 1996; Kolar et al., 1983; Steynberg, 1996; Huang et al., 1997; del Rıo et al., 1998; Han et al., 1999; Plummer and Edzwald, 2002; Singh et al., 2002; Lee et al., 2004; Mahovic et al., 2007; Vandekinderen et al., 2009; Jonnalagadda and Nadupalli, 2014). In 1967, chlorine dioxide solution approved as a disinfectant by United States Environmental Protection Agency (US-EPA) under the supervision of Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), and chlorine dioxide solution has been used for sanitation of food processing equipment, storage facilities, fruit and vegetables, and animal farms (Liu and Du, 2011). In 1988, gas form of chlorine dioxide became an EPA registered sterilant and has been used to sanitize laboratory equipment, medical devices, food processing surfaces, and clean rooms (Gómez-López et al., 2009; Lerouge 2012; Trinetta et al., 2013; Prodduk et al., 2014). However, its utilization as a stored-product fumigant remains uncertain. Meanwhile, majority of stored-product insects have developed resistance to phosphine, the most commonly used fumigant on stored grains, which has restrained the effectiveness of phosphine (Saxena and Bhatia, 1980; Zettler et al., 1989and1990; Rajendran and Narasimhan, 1994; Schlipalius et al., 2008; Pimentel et al., 2009; Opit et al., 2012; Benhalima et al., 2004). Consequently, alternative fumigants for

stored commodities becomes critical. Before considering chlorine dioxide as a potential fumigant on stored products, an insecticidal demand study ought to be carried out.

Previous laboratory studies found that chlorine dioxide gas has the potential to control adults of insect species in both wheat and flour. Channaiah et al. (2012) conducted experiments to evaluate the efficacy of chlorine dioxide gas using *Tribolium castaneum* (Herbst) and *Tribolium confusum* Jacquelin du Val on life stages of eggs, larvae and adults. These insects were fumigated with gas dosages (concentration × time, *ct*) of 380.1, 685.6, 745.0, and 834.4 g-h/m$^3$ and concluded that the presence of food has a significant impact on the mortality of larvae, which *Tribolium* spp. mortality was ranged from 40.1-100% without food, and 6.8-38.8% with present of food, respectively. Adults treated adults from both species showed complete mortality after exposed to dosages of 745.0 and 834.4 g-h/m$^3$. Kumar et al. (2015) investigated the toxicity at chlorine dioxide to *Plodia interpuntella* (Hübner) larvae by applying gas concentration at 0.54 g/m$^3$ for several exposure times, and results indicated that mortality is proportionally related to chlorine dioxide dosage (*ct*) products and increases significantly with incubation time after exposure. Han et al. (2016) tested all life stages of *P. interpuntella* (eggs, larvae, pupae and adults) with concentrations of 0.27 and 0.54 g/m$^3$ for 48 h and 24 h, respectively, and complete mortality was reported in all stages with both exposure treatments. Furthermore, molting failure occurred when treated larvae underwent pupation, and treated pupae died in the process of eclosion, which indicated that chlorine dioxide disrupts insect development.

The most recent study by E et al. (2017) reported that the dosages of chlorine dioxide required to achieve 100 % mortality was tested on five species of stored-product insect adults, including phosphine-susceptible and –resistant populations, using 0.54 g/m$^3$ for varies exposure times. Results illustrated that insect field populations of each species had the similar

susceptibility to chlorine dioxide as compared to corresponding laboratory populations. Dosages to achieve 100 % lethality required extended exposure time for all five insect species and populations as compared the presence of food during exposure with that of absence of food. To obtain complete mortality with presence of wheat, the exposure time increased by 17-41, 38-61, 42, 61-67, and 81% for *R. dominica*, *S. zeamais T. castaneum*, *S. oryzae,* and *O. surinamensis* respectively, when compared to those of treated without wheat. Furthermore, adult progeny production was completely suppressed.in *T. castaneum O. surinamensis*, and *Sitophilus* spp. E et al. (2018) revealed that temperature is an important factor relative to the effectiveness of chlorine dioxide gas. Adults of five insect species (both phosphine-susceptible and –resistant populations), *R. dominica*, *S. zeamais T. castaneum*, *S. oryzae*, and *O. surinamensis* were tested with gas concentration of 1.4 g/m$^3$ (520 ppm) during months of July (32.8 ± 0.5 ℃) and October (24.8 ± 0.6 ℃). Complete mortality was observed across all insect species and populations after 4- or 8-h exposure with the presence of food wheat experiments was conducted in July; whereas under cooler weather of October, longer exposure time was needed to achieve 100% mortality. To continue such breakthrough in investigating the insecticidal characteristic of chlorine dioxide gas against stored-product insects, this current study was designed to study the efficacy of chlorine dioxide against immature stages (eggs, young larvae, old larvae, and pupae) of these five different insect species, including lesser grain borer, *Rhyzopertha dominica* (Fabricius), maize weevil, *Sitophilus zeamais* (Motschulsky), and sawtoothed grain beetle, *Oryzaephilus surinamensis* (Linnaeus). Of which phosphine-resistant population were tested for each insect species and phosphine-susceptible (laboratory) population was used as control.

## 2.3. Materials and methods

### 2.3.1. Insects

All insect cultures were reared in environmental chambers (Model I-36VL; Percival Scientific, Perry, Iowa) set at temperature of 28°C, relative humidity (r. h.) of 65% r.h., and a photoperiod of 14h:10h (light: dark). For each species, insects were kept in 0.95-L Mason jars with stainless steel mesh screens and filter papers, and approximately 250 g of diet. *T. castaneum* was reared on organic whole wheat flour (Heartland Mills, Mariental, Kansas, USA) blended with 5% Brewer's yeast (by wt, Lesaffre Yeast Corporation, Milwaukee, Wisconsin, USA); *Rhyzopertha dominica* were reared on organic hard red winter wheat (Heartland Mills); *Sitophilus zeamais* was kept on organic yellow corn (Heartland Mills); and *Oryzaephilus surinamensis* was reared on organic rolled oats (Heartland Mills) mixed with 5% brewer's yeast (by wt). All laboratory populations, without any insecticide exposure, have been used as control groups and in rearing since 1999 in the postharvest protection laboratory in Department of Grain science and Industry at Kansas State University. Field populations included in this study were collected from various locations showed in Table 2.1. (Sehgal et al., 2014).

### 2.3.2. Life stage identification

Unsexed insects were used for egg collections for all insect species in this study. Organic whole wheat flour which was pre-sifted with 177-µm mesh sieve (Seedburo Equipment Company, Chicago, IL, USA) was used in collecting eggs of *T. castaneum*, *R. dominica*, and *O. surinamensis*. Adults were removed after the 72-h incubation and eggs were obtained by sifting the flour through a 250- µm mesh sieve, and these eggs were treated as 0-d-old eggs. To obtain 14-d-old young larvae for each insect species, a separate batch of eggs was collected and kept in the rearing chamber with respect diet for 19 d, included 5 d for eggs to hatch. The same method

was used to collect 21-d-old larvae and 3-d-old pupae. Immature insects were reared on their corresponding standard diet right after hatching till the treatments to avoid starvation.

The same developmental stages of *S. zeamais* were collected according to the laboratory protocol demonstrated by Khamis et al. (2010). Twenty unsexed *S. zeamais* adults were put in a customized snap-cap vial (described below) filled with 10 g organic hard red winter wheat and adults removed after 72-h incubation and such heat sample then contained eggs and were considered as 0-d-old eggs.

### 2.3.3. Bioassays

Bioassays conducted using snap-cap vials (diameter × height, 23 mm × 55 mm) with stainless steel mesh covered bottoms and caps (250 µm openings), which ensured chlorine dioxide gas to diffuse through the vails and also prevent insects from escape. Sample preparation for *R. dominica*, and *O. surinamensis* was to have a total of 20 insects placed into a snap-cap vial filled with 10 g organic hard red winter wheat for each life stage of the population of each individual insect species, and this was treated as one replicate of total three replicates. In case of *S. zeamais,* an individual vial collected for various life stages considered as a replicate of total three replicates.

Gaseous chlorine dioxide ($ClO_2$; CAS# 10049-04-4) was generated by a HP-10E Pure$ClO_2$ ™ electrochemical system (PureLine Treatment System, LLC, Bensenville, Illinois, USA). Approximately 99.5% pure chlorine dioxide gas was produced utilizing a liquid precursor PureCide®E solution (active ingredient: sodium chlorite, 31.0%) and passed through an electrochemical exchange membrane with chemical reaction: $2NaClO_2 + 2H_2O \rightarrow 2ClO_2 + 2NaOH + H_2$. Chlorine dioxide gas was extracted from the stripping column by a negative pressure created by compressed air (344.7 cubic feet per meter), and was channeled to an air-

tight polymethyl methacrylate (PMMA) test chamber (dimension: 0.6 m × 0.6 m × 1.0 m). Chlorine dioxide gas was then mixed with ambient air to achieve the desired concentrations, monitored by a chlorine dioxide analyzer (Control 4000, Optek®; Germantown, Wisconsin), and channeled into PMMA test chamber where bioassay was conducted. HOBO© data logger (Model: U 10-003, Onset Computer Corp., Bourne, Massachusetts, USA) was utilized to record temperature and humidity inside of the chamber every minute during exposures.

Concentrations of chlorine dioxide gas were 0.95 $g/m^3$ (350 parts per million, ppm), 1.35 $g/m^3$ (500 ppm), and 2.70 $g/m^3$ (1000 ppm). Vials that were served as controls held in laboratory growth chambers (28°C and 65% r.h.) while the treatment vials were exposed to chlorine dioxide in the PMMA test chamber. Chlorine dioxide-induced vials were taken out from the test chamber, and transferred back to the laboratory growth chamber (28°C and 65% r.h.) with their corresponding rearing diet. Assessment of mortality was carried out on the days when adult eclosion takes place for each immature stage. The assessment took approximately a week for each replicate to avoid miscounting due to delayed toxicity effects caused by chlorine dioxide (Subramanyam and E, 2015). All insects, including the dead ones, and diet were placed back in the vials after mortality assessment, and maintained in the growth chamber for 8 weeks to analyze progeny production.

### 2.3.4. Mortality assessment and statistical analysis

To obtain insect mortality for both control and chlorine dioxide treated samples, the number of adults emerged from immature stages, starting around the day 30 for *R. dominica*, *S. zeamaize* and *O. surinamensis* (Howe, 1956 and 1960), and mortality was determined using the total number of treated insects divided by the number of dead insects. For *S. zeamais*, because insects developed inside the kernels, mortality observation started on day 42 (Khamis et al.,

2015) and the effectiveness of chlorine dioxide gas can be ascertained via monitoring the number of emerged insects after exposure to chlorine dioxide relative to emerged adults from control group. Mortality of the treatments was corrected based on the corresponding controls according to Abbott formula (Abbott, 1925). Analysis of Variance (ANOVA) (SAS Institute, 2008) was used for data analysis on insect mortality and to determine the main effects relative to effectiveness of chlorine dioxide, including developmental stages, exposure durations, gas concentrations, susceptibility of insect population to chlorine dioxide, and interactions within these effects. Adult progeny of each insect species was calculated as the average number of adults per container (mean ± SE) and means were separated using Ryan-Einot-Gabriel-Welsh Q (REGWQ) test at significant level $\alpha = 0.05$.

## 2.4. Results and Discussion

### 2.4.1. Chlorine dioxide impact on *R. dominica*

***Efficacy of chlorine dioxide against immature stages of R. dominica***. Mortality from control groups were 6.7 – 20.0, 3.3 – 13.3, 6.7 – 10.0, and 5.0 – 11.7% for eggs, young larvae, old larvae, and pupae, respectively (Table 2.1). Susceptibility was found similar as compared laboratory population with field populations. Four field strains of *R. dominica* showed no significant differences as compared the mortality data from that of susceptible laboratory strain at any given exposure time and gas concentration of 0.95 g/m$^3$. Egg was the most susceptible stage followed by young larval and old larval stages, and pupal stage showed the least susceptibility among all developmental stages. Increased mortality was observed when exposure time increased. Three-way ANOVA revealed that corrected mortality was significantly different among insect stages ($F = 1403.31$; df = 3, 99; $P < 0.0001$) and exposure time ($F = 84.38$; df = 3,99; $P < 0.0001$). Interaction between stage and exposure was also significantly different when

compared to other interactions ($F$ = 6.69; df = 12, 99; $P$ < 0.0001). One-way ANOVA reported significant differences in young larvae, old larvae and pupae among exposure times when compared within the same strain, respectively (young larvae: $F_{range}$ = 7.80 – 12.89; df = 4, 10; $P_{range}$ = 0.0006 – 0.0040; old larvae: $F_{range}$ = 4.95 – 6.00; df = 4, 10; $P_{range}$ = 0.0100 – 0.0184; and pupae: $F_{range}$ = 3.74 – 18.85; df = 4, 10; $P_{range}$ = 0.0001 – 0.0411), whereas no significant difference observed in corrected egg mortality of all strains and young larvae of CF population. Significant differences among life stages were observed from mortality of each population of *R. dominica* at all exposure times (laboratory: $F_{range}$ = 44.57 – 124.72; df = 3, 8; $P$ < 0.0001; CF: $F_{range}$ = 31.39 – 103.49; df = 3, 8; $P$ < 0.0001; CS: $F_{range}$ = 25.20 – 92.84; $P_{range}$ = < 0.0001 – 0.0002; PD: $F_{range}$ = 32.25 – 99.98; df = 3, 8; $P_{range}$ < 0.0001; and RL: $F_{range}$ = 21.64 – 450.67) (Table 2.2).

***Suppression of progeny production***. Adult progeny production from control groups of all *R. dominica* populations was 119 to 180 insects per container (Table 2.4.1-2.4.4). Three-way ANOVA revealed that there were significant differences among stages ($F$ = 1018.57; df = 3, 240; $P$ < 0.0001) and exposure times ($F$ = 111.78; df = 5, 240; $P$ < 0.0001) in the mean of adult progeny production. The only significant interaction observed was between stage and exposure time ($F$ = 41.46; df = 15, 240; $P$ < 0.0001), whereas interactions of stage × strain ($F$ = 1.29; df = 12, 240; $P$ = 0.2251), strain × exposure ($F$ = 0.64; df = 20, 240; $P$ = 0.8831) and stage × strain × exposure time ($F$ = 0.24; df = 60, 240; $P$ = 0.9998) were not significant. One-way ANOVA analysis showed that progeny production was significantly reduced when eggs were exposed to chlorine dioxide gas due to few numbers of insects emerged after treatments (Table 2.4.1) and significant differences were found among exposure times within each insect population. Similarly, it was significant regarding the progeny produced by adults emerged from treated

young larvae, old larvae and pupae and showed in Table 2.4.2-2.4.4, except the adult progeny produced by old larvae of the field populations which showed no significant difference (CF: $F$ = 1.64; df = 5, 12; $P$ = 0.2228; CS: $F$ = 2.63; df = 5, 12; $P$ = 0.0788; PD: $F$ = 2.22; df = 5, 12; $P$ = 0.1195; and RL: $F$ = 3.07; df = 5, 12; $P$ = 0.2228). Both laboratory and field populations were highly susceptible to chlorine dioxide gas at concentration of 2.70 $g/m^3$ (1000 ppm) at any given exposure times, and only adult progeny was only observed in controls (Table 2.18).

### 2.4.2. Chlorine dioxide impact on *S. zeamais*

***Efficacy of chlorine dioxide against immature stages of S. zeamais.*** *S. zeamais* was less susceptible to chlorine dioxide gas and the number of emerged adults obtained from control groups of both laboratory and field populations ranging from 71 to 144. Field population revealed similar susceptibility as of mortality values ranged from 98.7 to 100.0 % for eggs, 94.7 to 98.8 % for larvae, 42.0 to 65.5 % for old larvae, and 23.9 to 69.6 for pupae when compared to laboratory population 99.4 to 100.0 % for eggs, 91.5 to 99.5 % for young larvae, 31.3 to 58.7 % for old larvae, and 5.9 to 68.2 for pupae (Table 2.5). Three-way ANOVA reported that significant differences were found among stages ($F$ = 286.42; df = 3, 80; $P$ < 0.0001), populations ($F$ = 5.51; df = 1, 80; $P$ = 0.0214), and exposure times ($F$ = 15.76; df = 4, 80; $P$ < 0.0001) regarding the mean of mortality. Interaction between stage and exposure was statistically different ($F$ = 7.68; df = 12, 80; $P$ < 0.0001), whereas interactions of strain × exposure ($F$ = 1.41; df = 4, 80; $P$ = 0.2383) and stage × strain × exposure time ($F$ = 0.88; df = 12, 80; $P$ = 0.5742) were not significant. Mortality assessment in one-way ANOVA showed that stages were significantly different within each exposure time (Table 2.5). In addition, exposure times was found significant in chlorine dioxide treated young larvae (LAB: $F$ = 4.26; df = 4, 10; $P$ = 0.0287; and TX: $F$ = 3.31; df = 4, 10; $P$ = 0.0567) and pupae (LAB: $F$ = 7.33; df = 4, 10; $P$ = 0.0050 and TX: $F$ = 5.11; df = 4, 10; $P$ = 0.0167), however, not significant in eggs and old larvae (P > 0.05).

***Suppression of progeny production***. Adult progeny produced by insects emerged after exposure to chlorine dioxide revealed that there were significant differences among stages ($F$ = 437.16; df = 3, 96; $P$ < 0.0001), populations ($F$ = 8.56; df = 1, 96; $P$ = 0.0043), and exposure times ($F$ = 111.05; df = 5, 96; $P$ < 0.0001). Furthermore, there was no significant difference observed in interactions of stage × population ($F$ = 1.32; df = 3, 96; $P$ = 0.2730), population × exposure time ($F$ = 0.59; df = 15, 96; $P$ = 0.7106), and stage × population × exposure time ($F$ = 1.01; df = 15, 96; $P$ = 0.4519), except the interaction of stage × exposure time which was significant ($F$ = 20.59; df = 15, 96; $P$ < 0.0001). One-way ANOVA showed that treatments among exposure times resulted significant differences for both laboratory and field populations (Laboratory: $F$ = 10152.2; df = 5, 12; $P$ < 0.0001 for eggs; $F$ = 17.73; df = 5, 12; $P$ < 0.0001 for young larvae; $F$ = 4.28; df = 5, 12; $P$ = 0.0181 for old larvae; and $F$ = 9.06; df = 5, 12; $P$ = 0.0009 for pupae; TX: $F$ = 49.29; df = 5, 12; $P$ < 0.0001 for eggs; $F$ = 9.43; df = 5, 12; $P$ = 0.0008 for young larvae; $F$ = 14.59; df = 5, 12; $P$ < 0.0001 for old larvae; and $F$ = 4.35; df = 5, 12; $P$ = 0.0173 for pupae). Additionally, it was statistically different in adult progeny when insects were exposure as different stages (Table 2.6.1-2.6.4). Exposure carried out with chlorine dioxide gas concentration of 2.70 g/$m^3$ for various durations revealed complete mortality in both laboratory and field populations and adult progeny was only found in the controls (Table 2.19).

### 2.4.3. Chlorine dioxide impact on *O. surinamensis*

***Efficacy of chlorine dioxide against immature stages of O. surinamensis.*** Mortality (%, mean ± SE) in control groups of *O. surinamensis* were 1.3 % for eggs, 0 to 1.3 % for young larvae, and 2.7 to 4.0 % for both old larvae and pupae to 4.0 % for laboratory population and 1.3 to 4.0 % for field population (Table 2.7.1). Corrected mortality data showed that eggs and young larvae were highly susceptible to chlorine dioxide followed by old larvae, and pupal stage was least susceptible; and the susceptibility were similar when compared laboratory population to

field population. Although no differences were found between eggs and young larvae or between old larvae and pupae, there were statistically differences found as compare eggs or young larvae to old larvae or pupae. All eggs and young larvae were completely killed at any given exposure time for both laboratory and field populations. Old larvae and pupae obtained less susceptible to chlorine dioxide with corrected mortality of old larvae at any given exposure times ranging from 16.0 to 65.0 % for AB2 population and 14.7 to 45.3 % for laboratory population, and corrected pupae mortality 5.6 to 43.1 % for AB2 population and 14.7 to 45.3 % for laboratory population. Increased mortality was observed as exposure time increased, moreover, there were significantly differences found in old larval and pupal stages of AB2 field population as well as pupal stage of laboratory population (Table 2.7.2).

***Suppression of progeny production***. Adult reproduction in controls were 171 to 262 insects per container in laboratory population and 174 to 270 insects per container for AB2 strain (Table 2.8.1-2.8.3). Three-way ANOVA showed that stages ($F$ = 6526.12; df = 3, 96; $P <$ 0.0001) and exposure times ($F$ = 968.48; df = 5, 96; $P <$ 0.0001) were significant, additionally, interaction between stage and exposure time ($F$ = 231.89; df = 15, 96; $P <$ 0.0001) was the only interaction revealed important impact on the progeny results. Significant differences were found in regard to life stages within the same exposure time (AB2: eggs, $F$ = 4694.57; df = 5, 12; $P <$ 0.0001; young larvae, $F$ = 8557.43; df = 5, 12; $P <$ 0.0001; old larvae, $F$ = 5.93; df = 5, 12; $P =$ 0.0055; pupae, $F$ = 11.76; df = 5, 12; $P$ = 0.0003; laboratory: eggs $F$ = 1229.65; df = 5, 12; $P <$ 0.0001; young larvae, $F$ = 1701.12; df = 5, 12; $P <$ 0.0001; old larvae, $F$ = 5.27; df = 5, 12; $P =$ 0.0086; pupae, $F$ = 4.32; df = 5, 12; $P$ = 0.0176). Similarly, adult progeny was significantly different related to exposure times for both laboratory and field populations (AB2: $F_{range}$ = 197.29 - 2388.46; df = 3, 8; $P <$ 0.0001; laboratory, $F_{range}$ = 177.24 - 2924.15; df = 3, 8; $P <$ 0.0001). Chlorine dioxide gas at concentration of 2.70 g/m$^3$ were able to kill all immature stages of insects with various exposure times, and the only progeny observed was from controls (Table 2.20).

### 2.4.2. Discussion

Phosphine-resistant populations of *R. dominica*, *S. zeamais*, and *O. surinamensis* revealed similar susceptibility to chlorine dioxide gas as compared to the corresponding laboratory populations. Findings in this study is consistent with those tested on adults of stored product insect species reported by E et al. 2017 and 2018. Experiments were performed on adults of *T. castaneum, O. surinamensis, R. dominica, S. zeamais,* and *S. oryzae* using chlorine dioxide gas concentrations, 0.54, 1.35, 2.02, and 2.70 g/m$^3$, for various exposure times. Mortality was assessed every day for 5 days after exposure and progeny production was observed after 8 weeks. Results suggested that no strong relationship found between phosphine-resistant and -susceptible populations (E et al., 2017). Similar results were documented in another chlorine dioxide study on adults of phosphine susceptible and resistant populations using these five insect species when tested with gas concentration of 1.41 g/m$^3$ for various exposure times (E et al., 2018). Kumar et al. (2015) assessed the toxicity and insecticidal mechanism of chlorine dioxide gas in larvae of *P. interpunctella*, of which all larvae were killed with 200 ppm of chlorine dioxide within 24 h, reactive oxygen species (ROS) was introduced by chlorine dioxide causing oxidative stress, genes of *P. interpunctella* superoxide dismutase (Pi-SOD) and thioredoxinperoxidase (Pi-Tpx) was identified with antioxidant function, and RNA interference (RNAi) confirmed such association. Another study performed on both larvae (6-7$^{th}$ instar) and adults (unsexed, less than 1 wk old) of *T. castaneum* GA-1 strain and results were very similar to that of *P. interpunctella* (Kim et al., 2015).

Increased mortality was observed as dosage (*ct*) of chlorine dioxide gas increased. Each life stage of *T. castaneum* and *T. confusum* was exposed to gas concentration of 380.1, 685.6, 745.0, and 834.4 gh/m$^3$ and these dosages were achieved in 1.53-2.07 h under room temperature

(25-30 °C). Results indicated an increase of mortality as dosage increased, in addition, chlorine dioxide was more effective when *Tribolium* spp. exposed to the same concentration over 24 h as compared to 1.53-2.07 h (Channaiah et al., 2012). Laboratory settings was conducted to determine the efficacy of chlorine dioxide gas in discriminating of mix-aged bedbugs (*Cimex lectularius* and *Cimex hemipterus*). Mortality assessment indicated that 84.3 % insects was killed after exposure to gas concentrations of 362 ppm for 176 min, and 100% mortality was obtained by 6-h postexposure. The same concentration was applied for 519 min, only 59.8% mortality was achieved immediately after the exposure, and insects were all killed by 18-h postexposure (Gibbs et al., 2012). In the same study, although the similar dosages (1132 vs. 1029) were used, initial concentration (724 ppm) with relatively shorter exposure time (94 min) achieved higher mortality (100%) immediately after the treatment, whereas later dosage with initial concentration of 362 ppm for 176 min resulted 84.3 % mortality after fumigation completed and 100% mortality was obtained by 6-h postexposure.

Developmental stages and insect species had strong impact regarding their susceptibility to chlorine dioxide. Egg stage of all tested insect species was highly susceptible to chlorine dioxide gas as well as young larvae of *S. zeamais* and *O. surinamensis*, whereas there was low susceptibility found in that of *R. dominica* young larvae. Old larvae and pupae were the least susceptible to chlorine dioxide. Pimental et al. (2007) reported that degrees of phosphine resistance may relate to the physiological differences, and adults of *T. castaneum*, *R. dominica,* and *O. surinamensis* field populations collected from in Brazil were evaluated for such objective. Populations possessed higher phosphine resistance had lower rate of carbon dioxide production (Pimental et al., 2007). Eggs and pupae were very less susceptible to chlorine dioxide than that of larval stages and this corresponds to the respiration rate of these immature stages reported by

Emekci et al. (1998). In addition, metabolism rate varies among different insect species and this may have impact on insects' susceptibility to chlorine dioxide. E et al. (2017) exposed adults to chlorine dioxide at concentration of 0.54 g/m$^3$ with present of food, and complete mortality required were 30, 26, 21, 16.5 and 16 h for *R. dominica, T. castaneum, S. zeamais*, *S. oryzae,* and *O. surinamensis,* respectively. Heat production reported in adult insects of *R. dominica, T. castaneum* and *S. oryzae* were 35.3-32.8, 39.7-38.1, and 56.4-55.3 µW/insect (Cofie-Agblor et al., 1995). These two studies demonstrated that insect species possessed higher metabolism rate (higher heat production) tended to be more susceptible to chlorine dioxide, which corresponded to the corrected mortality in this study. (E et al., 2017).

## 2.5. Conclusion

In conclusion, chlorine dioxide at gas concentrations of 0.95 and 2.70 g/m$^3$ was effective in control of immature stages (eggs, young larvae, old larvae, and pupae) of phosphine-resistant and -susceptible populations of three major stored-product insects, namely *R. dominica, S. zeamais*, and *O. surinamensis*. Complete mortality was observed in egg and young larval stage of all species, except larvae of *R. dominica* (55 %) which needed longer exposure time with gas concentration of 0.95 g/m$^3$. Old larval and pupal stages required longer exposure time since the highest mortality were 45 – 65 % among the species. There was no progeny or very few progenies produced when eggs and young larvae were exposed to chlorine dioxide due to few number of adult survival, except young larvae of *R. dominica*. Field studies are needed to confirm the effectiveness of chlorine dioxide in control of major stored-product insects in grain storage and grain silos.

## 2.6. References


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Table 2.1**.** Information of field strains of five stored-product insect species.

| Species | Population | County, State | Commodity | Time of collection |
|---|---|---|---|---|
| ***T. castaneum*** | AB1 | Dickinson, KS | Wheat | 2011 |
| | AB2 | Dickinson, KS | Wheat | 2011 |
| | CF | Washington, KS | Wheat | 2011 |
| | MN | Ottawa, KS | Wheat | 2011 |
| | PD | Russell, KS | Wheat | 2011 |
| ***R. dominica*** | CF | Washington, KS | Wheat | 2011 |
| | CS | Chase, KS | Wheat | 2011 |
| | PD | Russell, KS | Wheat | 2011 |
| | RL | Riley, KS | Flour mill | 2007 |
| ***S. zeamais*** | TX | Texas | Corn | 2011 |
| ***O.surinamensis*** | AB2 | Abilene, KS | Wheat | 2011 |

[a] Phosphine resistance levels were verified by conducting the discriminating dose tests (Champ and Dyte, 1976) with phosphine concentrations of 0.042, 0.028, 0.042, and 0.052g/m$^3$ for *T. castaneum*, *R. dominica*, *Sitophilus* spp., and *O. surinamensis*, respectively. All laboratory populations showed 0% survival indicating complete susceptibility to phosphine.

Table 2.2. Control mortality (%, mean ± SE) of immature stages of *R. dominica* laboratory and field strains without exposing to chlorine dioxide[a].

| Strain | Stage | | | |
|---|---|---|---|---|
| | Egg | Young larva | Old larva | Pupa |
| LAB | 6.7 ± 4.4 | 13.3 ± 4.4 | 6.7 ± 1.7 | 8.3 ± 3.3 |
| CF | 20.0 ± 2.9 | 8.3 ± 6.0 | 10.0 ± 5.0 | 5.0 ± 2.9 |
| CS | 8.3 ± 4.4 | 10.0 ± 2.9 | 6.7 ± 1.7 | 11.7 ± 4.4 |
| PD | 13.3 ± 4.4 | 13.3 ± 7.3 | 8.3 ± 4.4 | 10.0 ± 5.0 |
| RL | 11.7 ± 6.7 | 3.3 ± 1.7 | 6.7 ± 4.4 | 6.7 ± 1.7 |

[a]Each mean is based on $n = 3$.

Table 2.3**.** Corrected mortality (%, mean ± SE) of *R. dominica* laboratory and field strains exposed as eggs, young larvae, old larvae, and pupae to chlorine dioxide concentration of 0.95 $g/m^3$ for various durations.

| Strain | Exposure time (h) | Mortality (%, mean ± SE)* | | | |
|---|---|---|---|---|---|
| | | Eggs[a] | Young larvae[b] | Old larvae | Pupae |
| LAB | 2 | 91.7 ± 8.3 A | 15.0 ± 2.9 cB | 10.0 ± 2.9 cB | 8.3 ± 1.7 cB |
| | 4 | 100.0 ± 0.0 A | 23.3 ± 1.7 bcB | 13.3 ± 4.4 bcB | 15.0 ± 5.8 bcB |
| | 6 | 100.0 ± 0.0 A | 40.0 ± 5.8 abB | 20.0 ± 5.8 abcB | 20.0 ± 5.8 abcB |
| | 8 | 100.0 ± 0.0 A | 40.0 ± 0.0 abB | 33.3 ± 6.7 abB | 31.7 ± 6.0 abB |
| | 10 | 100.0 ± 0.0 A | 50.0 ± 5.8 aB | 40.0 ± 5.8 aB | 36.7 ± 3.3 aB |
| CF | 2 | 91.7 ± 4.4 A | 20.0 ± 10.0 B | 13.3 ± 3.3 bB | 13.3 ± 3.3 bB |
| | 4 | 96.7 ± 3.3 A | 23.3 ± 4.4 B | 23.3 ± 4.4 abB | 21.7 ± 1.7 bB |
| | 6 | 100.0 ± 0.0 A | 30.0 ± 7.6 B | 23.3 ± 1.7 abB | 23.3 ± 1.7 bB |
| | 8 | 98.3 ± 1.7 A | 35.0 ± 7.6 B | 35.0 ± 7.6 aB | 35.0 ± 2.9 aB |
| | 10 | 100.0 ± 0.0 A | 51.7 ± 6.0 B | 38.3 ± 1.7 aC | 38.3 ± 1.7 aC |
| CS | 2 | 88.3 ± 6.0 A | 16.7 ± 6.0 bB | 16.7 ± 6.0 bB | 18.3 ± 3.3 bB |
| | 4 | 91.7 ± 8.3 A | 21.7 ± 1.7 bB | 21.6 ± 1.7 bB | 23.3 ± 1.7 abB |
| | 6 | 96.7 ± 3.3 A | 26.7 ± 7.3 bB | 26.7 ± 3.3 abB | 26.7 ± 8.2 abB |
| | 8 | 96.7 ± 3.3 A | 26.7 ± 3.3 bB | 26.7 ± 7.3 abB | 26.7 ± 4.4 abB |
| | 10 | 98.3 ± 1.7 A | 55.0 ± 2.9 aB | 45.0 ± 2.9 aB | 45.0 ± 5.0 aB |
| PD | 2 | 93.3 ± 6.7 A | 11.7 ± 6.0 bB | 11.7 ± 6.0 bB | 13.3 ± 6.0 bB |
| | 4 | 95.0 ± 5.0 A | 16.7 ± 4.4 bB | 17.7 ± 4.4 bB | 20.0 ± 2.9 bB |
| | 6 | 96.7 ± 3.3 A | 25.0 ± 2.9 bB | 18.3± 4.4 abB | 18.3 ± 4.4 bB |
| | 8 | 98.3 ± 1.7 A | 33.3 ± 4.4 bB | 30.0 ± 5.0 abB | 30.0 ± 5.0 abB |
| | 10 | 100.0 ± 0.0 A | 55.0 ± 7.6 aB | 40.0 ± 5.8 aB | 40.0 ± 2.9 aB |
| RL | 2 | 95.0 ± 2.9 A | 8.3 ± 1.7 cB | 8.3 ± 1.7 bB | 8.3 ± 1.7 cB |
| | 4 | 100.0 ± 0.0 A | 21.7 ± 4.4 bcB | 16.7 ± 1.7 bB | 15.0 ± 2.9 bcB |
| | 6 | 100.0 ± 0.0 A | 23.3 ± 8.8 abcB | 23.3 ± 8.8 abB | 20.0 ± 2.9 bcB |
| | 8 | 100.0 ± 0.0 A | 48.3 ± 4.4 abB | 28.3 ± 6.0 abC | 28.3 ± 6.0 abC |
| | 10 | 95.0 ± 5.0 A | 51.7 ± 10.1 aB | 38.3 ± 1.7 aB | 38.3 ± 1.7 aB |

*Means followed by different upper case letters are significantly different among life stages within one exposure time and one strain, and means followed by different lower case letters are significantly different for pupae among different exposure times within one strain ($P < 0.05$, by Ryan-Einot-Gabriel-Welsh Q tests).

[a] No significant differences observed from corrected egg mortality of all strains (LAB: $F = 1.00$; df = 4,10; $P = 0.4516$; CF: $F = 1.79$; df = 4,10; $P = 0.2071$; CS: $F = 0.67$; df = 4,10; $P = 0.6273$; PD: $F = 0.42$; df = 4,10; $P = 0.7931$; and RL: $F = 1.13$; df = 4,10; $P = 0.3981$).

Table 2.4. Adult progeny (mean ± SE) of *R. dominica* laboratory and field strains produced by adults developed from eggs exposed to chlorine dioxide at concentration of 0.95 g/m$^3$ for five durations.

| Strain | Exposure time (h) | No. live adult / container (mean ± SE)[a] | No. adult progeny / container (mean ± SE)[b] |
|---|---|---|---|
| LAB | 0 | 18.0 ± 0.9 | 162.0 ± 14.2a |
| | 2 | 1.7 ± 1.7 | 5.3 ± 5.3b |
| | 4 | 0 | 0b |
| | 6 | 0 | 0b |
| | 8 | 0 | 0b |
| | 10 | 0 | 0b |
| CF | 0 | 16.0 ± 0.6 | 118.7 ± 19.4a |
| | 2 | 1.7 ± 0.9 | 8.0 ± 4.6b |
| | 4 | 0.7 ± 0.7 | 3.7 ± 3.7b |
| | 6 | 0 | 0b |
| | 8 | 0.3 ± 0.3 | 0.3 ± 0.3b |
| | 10 | 0 | 0b |
| CS | 0 | 18.3 ± 0.9 | 157.7 ± 8.1a |
| | 2 | 2.3 ± 1.2 | 8.7 ± 8.7b |
| | 4 | 1.7 ± 1.7 | 8.3 ± 8.3b |
| | 6 | 0.7 ± 0.7 | 3.3 ± 3.3b |
| | 8 | 0.7 ± 0.7 | 0b |
| | 10 | 0 | 0b |
| PD | 0 | 17.3 ± 0.9 | 157.7 ± 8.1a |
| | 2 | 1.3 ± 1.3 | 8.7 ± 8.7b |
| | 4 | 1.0 ± 1.0 | 8.3 ± 8.3b |
| | 6 | 0.7 ± 0.7 | 3.3 ± 3.3b |
| | 8 | 0.3 ± 0.3 | 0b |
| | 10 | 0 | 0b |
| RL | 0 | 17.7 ± 1.3 | 141.0 ± 10.0a |
| | 2 | 1.0 ± 0.6 | 5.0 ± 4.5b |
| | 4 | 0 | 0b |
| | 6 | 0 | 0b |
| | 8 | 0 | 0b |
| | 10 | 1.0 ± 1.0 | 1.3 ± 1.3b |

[a]Number of live insects for progeny production.

[b]Means followed by different lower case letters are significantly different among five durations within one strain. Number of adult progeny per container was significantly different after exposed to chlorine dioxide for various durations within each strain of LAB ($F$ = 37.63, df = 5, 12; $P$ < 0.0001), CF ($F$ = 13.46; df = 5, 12; $P$ = 0.0001), CS ($F$ = 7.08; df = 5, 12; $P$ = 0.0027), PD ($F$ = 10.71; df = 5, 12; $P$ = 0.0004) and RL ($F$ = 23.65; df = 5, 12; $P$ <0.0001).

Table 2.5. Adult progeny (mean ± SE) of *R. dominica* laboratory and field strains produced by adults developed from young larvae exposed to chlorine dioxide at concentration of 0.95 g/$m^3$ for five durations.

| Strain | Exposure time (h) | No. live adult / container (mean ± SE)[a] | No. adult progeny / container (mean ± SE)[b] |
|---|---|---|---|
| LAB | 0 | 17.3 ± 0.9 | 155.0 ± 10.7a |
| | 2 | 17.0 ± 0.6 | 135.3 ± 13.3ab |
| | 4 | 15.3 ± 0.3 | 99.0 ± 10.1bc |
| | 6 | 12.0 ± 1.2 | 84.7 ± 10.9cd |
| | 8 | 12.0 ± 0.0 | 78.3 ± 1.8cd |
| | 10 | 10.0 ± 1.2 | 64.0 ± 1.0d |
| CF | 0 | 18.3 ± 1.2 | 119.7 ± 19.2 |
| | 2 | 16.0 ± 2.0 | 80.7 ± 11.6 |
| | 4 | 15.3 ± 0.9 | 77.0 ± 6.1 |
| | 6 | 14.0 ± 1.5 | 64.7 ± 24.4 |
| | 8 | 13.0 ± 1.5 | 59.3 ± 11.7 |
| | 10 | 9.7 ± 1.2 | 43.3 ± 5.2 |
| CS | 0 | 18.0 ± 0.6 | 146.0 ± 16.7a |
| | 2 | 16.7 ± 1.2 | 114.0 ± 4.4ab |
| | 4 | 15.7 ± 0.3 | 91.0 ± 6.0b |
| | 6 | 14.7 ± 1.5 | 82.7 ± 7.0b |
| | 8 | 14.7 ± 0.7 | 80.7 ± 5.6b |
| | 10 | 9.0 ± 0.6 | 32.7 ± 5.6c |
| PD | 0 | 17.3 ± 1.5 | 150.0 ± 19.6a |
| | 2 | 17.7 ± 1.2 | 125.7 ± 16.2ab |
| | 4 | 16.7 ± 0.9 | 118.0 ± 6.1ab |
| | 6 | 15.0 ± 0.6 | 83.7 ± 6.7ab |
| | 8 | 13.3 ± 0.9 | 76.7 ± 9.0b |
| | 10 | 9.0 ± 1.5 | 39.0 ± 9.9c |
| RL | 0 | 19.3 ± 0.3 | 179.3 ± 11.8a |
| | 2 | 18.3 ± 0.3 | 91.0 ± 13.8b |
| | 4 | 15.7 ± 0.9 | 88.3 ± 2.3b |
| | 6 | 15.3 ± 1.8 | 84.0 ± 5.5b |
| | 8 | 10.3 ± 0.9 | 53.0 ± 11.7bc |
| | 10 | 9.7 ± 2.0 | 38.7 ± 7.8c |

[a]Number of live insects for progeny production.

[b]Means followed by different lower case letters are significantly different among five durations within one strain. Number of adult progeny per container was significantly different after exposed to chlorine dioxide for various durations within each strain of LAB ($F$ = 14.82, df = 5, 12; $P$ < 0.0001), CF ($F$ = 1.82; df = 5, 12; $P$ = 0.1827), CS ($F$ = 23.60; df = 5, 12; $P$ < 0.0001), PD ($F$ = 12.36; df = 5, 12; $P$ = 0.0002) and RL ($F$ = 13.75; df = 5, 12; $P$ = 0.0001).

Table 2.6. Adult progeny (mean ± SE) of *R. dominica* laboratory and field strains produced by adults developed from old larvae exposed to chlorine dioxide at concentration of 0.95 g/m$^3$ for five durations.

| Strain | Exposure time (h) | No. live adult / container (mean ± SE)[a] | No. adult progeny / container (mean ± SE)[b] |
|---|---|---|---|
| LAB | 0 | 18.7 ± 0.3 | 172.3 ± 13.4a |
| | 2 | 18.0 ± 0.6 | 140.3 ± 12.3ab |
| | 4 | 17.3 ± 0.9 | 88.3 ± 15.0bc |
| | 6 | 16.0 ± 1.2 | 76.7 ± 4.3c |
| | 8 | 13.3 ± 1.3 | 61.0 ± 11.1c |
| | 10 | 12.0 ± 1.2 | 61.0 ± 9.5c |
| CF | 0 | 18.0 ± 1.0 | 119.7 ± 19.2 |
| | 2 | 17.3 ± 0.7 | 102.7 ± 10.8 |
| | 4 | 15.3 ± 0.9 | 99.0 ± 14.5 |
| | 6 | 15.3 ± 0.3 | 93.0 ± 4.6 |
| | 8 | 13.0 ± 1.5 | 84.0 ± 6.6 |
| | 10 | 12.3 ± 0.3 | 77.7 ± 9.3 |
| CS | 0 | 18.7 ± 0.3 | 146.0 ± 16.7 |
| | 2 | 16.7 ± 1.2 | 122.3 ± 8.4 |
| | 4 | 15.7 ± 0.3 | 114.7 ± 8.6 |
| | 6 | 14.7 ± 1.5 | 109.7 ± 10.6 |
| | 8 | 14.7 ± 0.6 | 108.0 ± 16.7 |
| | 10 | 11.0 ± 0.6 | 87.3 ± 2.3 |
| PD | 0 | 18.3 ± 1.9 | 150.0 ± 19.5 |
| | 2 | 17.7 ± 1.2 | 125.7 ± 16.2 |
| | 4 | 16.7 ± 0.9 | 118.0 ± 6.2 |
| | 6 | 16.3 ± 0.9 | 102.3 ± 20.9 |
| | 8 | 14.0 ± 1.0 | 96.0 ± 8.9 |
| | 10 | 12.0 ± 1.2 | 92.7 ± 9.9 |
| RL | 0 | 18.7 ± 0.9 | 179.3 ± 12.0 |
| | 2 | 18.3 ± 0.3 | 113.3 ± 25.0 |
| | 4 | 16.7 ± 0.3 | 105.7 ± 11.5 |
| | 6 | 15.3 ± 1.8 | 95.3 ± 9.8 |
| | 8 | 14.3 ± 1.2 | 83.0 ± 16.9 |
| | 10 | 12.3 ± 0.3 | 82.7 ± 17.0 |

[a]Number of live insects for progeny production.

[b]Means followed by different lower case letters are significantly different among five durations within one strain. Number of adult progeny per container was significantly different after exposed to chlorine dioxide for various durations within each strain of LAB ($F$ = 10.21, df = 5, 12; $P$ = 0.0005), CF ($F$ = 1.64; df = 5, 12; $P$ = 0.2228), CS ($F$ = 2.63; df = 5, 12; $P$ = 0.0788), PD ($F$ = 2.22; df = 5, 12; $P$ = 0.1195) and RL ($F$ = 3.07; df = 5, 12; $P$ = 0.0517).

Table 2.7**.** Adult progeny (mean ± SE) of *R. dominica* laboratory and field strains produced by adults developed from pupae exposed to chlorine dioxide at concentration of 0.95 $g/m^3$ for five durations.

| Strain | Exposure time (h) | No. live adult / container (mean ± SE)[a] | No. adult progeny / container (mean ± SE)[b] |
|---|---|---|---|
| LAB | 0 | 18.3 ± 0.7 | 172.0 ± 10.1a |
| | 2 | 18.3 ± 0.3 | 135.3 ± 13.3ab |
| | 4 | 17.0 ± 1.2 | 120.0 ± 11.1ab |
| | 6 | 16.0 ± 1.2 | 103.7 ± 10.4bc |
| | 8 | 13.7 ± 1.2 | 90.0 ± 13.1bc |
| | 10 | 12.7 ± 0.7 | 71.7 ± 7.7c |
| CF | 0 | 19.0 ± 0.6 | 123.3 ± 22.7 |
| | 2 | 17.3 ± 0.7 | 105.0 ± 8.5 |
| | 4 | 15.7 ± 0.3 | 103.7 ± 10.3 |
| | 6 | 15.3 ± 0.3 | 93.7 ± 4.3 |
| | 8 | 13.0 ± 0.6 | 87.3 ± 2.7 |
| | 10 | 12.3 ± 0.3 | 77.3 ± 10.8 |
| CS | 0 | 17.7 ± 0.9 | 145.7 ± 16.0a |
| | 2 | 16.3 ± 0.7 | 122.3 ± 6.8ab |
| | 4 | 15.3 ± 0.3 | 114.7 ± 6.8ab |
| | 6 | 14.7 ± 1.8 | 110.3 ± 10.3ab |
| | 8 | 14.7 ± 0.9 | 100.0 ± 16.3ab |
| | 10 | 11.0 ± 1.0 | 87.3 ± 2.3b |
| PD | 0 | 18.0 ± 1.0 | 159.3 ± 19.4a |
| | 2 | 17.3 ± 1.2 | 129.7 ± 14.8ab |
| | 4 | 16.0 ± 0.6 | 111.0 ± 3.2ab |
| | 6 | 16.3 ± 0.9 | 104.3 ± 19.9ab |
| | 8 | 14.0 ± 0.9 | 96.3 ± 8.3b |
| | 10 | 12.0 ± 0.6 | 93.7 ± 10.4b |
| RL | 0 | 18.7 ± 0.3 | 179.0 ± 8.7a |
| | 2 | 18.3 ± 0.3 | 84.0 ± 8.7b |
| | 4 | 17.0 ± 0.6 | 69.3 ± 4.5bc |
| | 6 | 16.0 ± 0.6 | 61.7 ± 4.9cd |
| | 8 | 14.3 ± 1.2 | 48.0 ± 2.1de |
| | 10 | 12.3 ± 0.3 | 37.0 ± 1.2e |

[a]Number of live insects for progeny production.

[b]Means followed by different lower case letters are significantly different among five durations within one strain. Number of adult progeny per container was significantly different after exposed to chlorine dioxide for various durations within each strain of LAB ($F$ = 10.13, df = 5, 12; $P$ = 0.0006), CF ($F$ = 2.12; df = 5, 12; $P$ = 0.1322), CS ($F$ = 3.36; df = 5, 12; $P$ = 0.0397), PD ($F$ = 3.15; df = 5, 12; $P$ = 0.0482) and RL ($F$ = 65.57; df = 5, 12; $P$ < 0.0001).

Table 2.8. Corrected mortality (%, mean ± SE) of *S. zeamais* laboratory and field strains exposed as eggs, young larvae, old larvae, and pupae to chlorine dioxide concentration of 0.95 $g/m^3$ for various durations.

| Strain | Exposure time (h) | Mortality (%, mean ± SE)* | | | |
|---|---|---|---|---|---|
| | | Eggs[a] | Young larvae | Old larvae | Pupae |
| LAB | 2 | 100.0 ± 0.0A | 91.5 ± 2.8Ab | 48.4 ± 6.4B | 2.4 ± 15.7Cb |
| | 4 | 99.4 ± 0.6A | 98.6 ± 1.4Aa | 31.4 ± 4.2B | 5.9 ± 9.2Cb |
| | 6 | 99.4 ± 0.3A | 98.1 ± 1.2Aa | 31.3 ± 2.9B | 33.3 ± 13.0Bab |
| | 8 | 100.0 ± 0.0A | 99.5 ± 0.5Aa | 58.3 ± 14.7B | 58.8 ± 10.3Ba |
| | 10 | 99.0 ± 0.6A | 98.6 ± 0.8Aa | 58.7 ± 6.4B | 68.2 ± 2.4Ba |
| TX | 2 | 100.0 ± 0.0A | 94.7 ± 1.0Ab | 53.9 ± 8.1B | 23.9 ± 8.9Cb |
| | 4 | 100.0 ± 0.0A | 97.9 ± 0.6Aab | 42.0 ± 1.7B | 32.5 ± 8.4Bb |
| | 6 | 98.7 ± 1.3A | 97.4 ± 1.3Aab | 42.3 ± 1.7B | 47.8 ± 7.6Bab |
| | 8 | 100.0 ± 0.0A | 98.2 ± 0.5Aab | 60.5 ± 8.9B | 45.0 ± 8.7Bab |
| | 10 | 100.0 ± 0.0A | 98.8 ± 0.8Aa | 65.5 ± 3.3B | 69.6 ± 3.3Ba |

*Means followed by different upper case letters are significantly different among life stages within one exposure time and one strain, and means followed by different lower case letters are significantly different for pupae among different exposure times within one strain ($P < 0.05$, by Ryan-Einot-Gabriel-Welsh Q tests).

** Significant differences observed among stages from corrected mortality in both laboratory and field strains as exposure time of 2 h (LAB: $F = 27.43$; df = 3, 8; $P = 0.0001$ and TX: $F = 35.35$; df = 3, 8; $P < 0.0001$), 4 h (LAB: $F = 86.56$; df = 3, 8; $P < 0.0001$ and TX: $F = 69.23$; df = 3, 8; $P < 0.0001$), 6 h (LAB: $F = 32.89$; df = 3, 8; $P < 0.0001$ and TX: $F = 58.55$; df = 3, 8; $P < 0.0001$), 8 h (LAB: $F = 7.03$; df = 3, 8; $P = 0.0124$ and TX: $F = 19.43$; df = 3, 8; $P = 0.0005$), and 10 h (LAB: $F = 36.11$; df = 3, 8; $P < 0.0001$ and TX: $F = 61.12$; df = 3, 8; $P < 0.0001$).

***No significant differences found among exposure times when treated as eggs (LAB: $F = 1.12$; df = 4, 10; $P = 0.3981$ and TX: $F = 1.00$; df = 4, 10; $P =0.4516$), young larvae TX strain ($F = 3.31$; df = 4, 10; $P = 0.0567$) and old larvae (LAB: $F = 2.89$; df = 4, 10; $P = 0.0787$ and TX: $F = 3.45$; df = 4, 10; $P = 0.0510$); whereas significant difference was observed when insects were treated as young larvae (LAB: $F = 4.26$; df = 4, 10; $P = 0.0287$) and pupae (LAB: $F = 7.33$; df = 4, 10; $P = 0.0050$ and TX: $F$ 5.11; df = 4, 10; $P = 0.0167$).

Table 2.9**.** Adult progeny (mean ± SE) of *S. zeamais* laboratory and field strain produced by adults developed from eggs exposed to chlorine dioxide at concentration of 0.95 $g/m^3$ for five durations.

| Strain | Exposure time (h) | No. live adult / container (mean ± SE)[a] | No. adult progeny / container (mean ± SE)[b] |
|---|---|---|---|
| LAB | 0 | 103.3 ± 8.7 | 437.0 ± 26.9a |
| | 2 | 0 | 0b |
| | 4 | 0.7 ± 0.7 | 0b |
| | 6 | 0.7 ± 0.3 | 0b |
| | 8 | 0 | 0b |
| | 10 | 1.0 ± 0.8 | 0b |
| TX | 0 | 129.7 ± 12.5 | 454.3 ± 16.4a |
| | 2 | 0 | 0b |
| | 4 | 0 | 0b |
| | 6 | 4.0 ± 4.0 | 4.0 ± 4.0b |
| | 8 | 0 | 0b |
| | 10 | 0 | 0b |

[a]Number of live insects for progeny production.

[b]Means followed by different lower case letters are significantly different among five durations within one strain. Number of adult progeny per container was significantly different after exposed to chlorine dioxide for various durations within each strain of LAB ($F$ = 4.28; df = 5, 12; $P$ = 0.0181) and TX ($F$ = 49.29, df = 5, 12; $P$ < 0.0001).

Table 2.10**.** Adult progeny (mean ± SE) of *S. zeamais* laboratory and field strain produced by adults developed from young larvae exposed to chlorine dioxide at concentration of 0.95 $g/m^3$ for five durations.

| Strain | Exposure time (h) | No. live adult / container (mean ± SE)[a] | No. adult progeny / container (mean ± SE)[b] |
|---|---|---|---|
| LAB | 0 | 71.0 ± 11.6 | 267.3 ± 36.4a |
| | 2 | 6.0 ± 2.0 | 26.3 ± 14.3b |
| | 4 | 1.0 ± 1.0 | 7.0 ± 7.0bc |
| | 6 | 1.3 ± 0.9 | 0c |
| | 8 | 0.3 ± 0.3 | 0c |
| | 10 | 1.0 ± 0.6 | 1.0 ± 1.0c |
| TX | 0 | 113.0 ± 9.3 | 322.0 ± 15.4a |
| | 2 | 6.0 ± 1.2 | 18.0 ± 9.0b |
| | 4 | 2.3 ± 0.7 | 8.3 ± 4.6b |
| | 6 | 2.7 ± 1.5 | 5.0 ± 3.6b |
| | 8 | 2.0 ± 0.6 | 2.0 ± 2.0b |
| | 10 | 1.3 ± 0.9 | 1.3 ± 1.3b |

[a]Number of live insects for progeny production.

[b]Means followed by different lower case letters are significantly different among five durations within one strain. Number of adult progeny per container was significantly different after exposed to chlorine dioxide for various durations within each strain of LAB ($F$ = 17.73; df = 5, 12; $P < 0.0001$) and TX ($F$ = 9.43, df = 5, 12; $P$ = 0.0008).

Table 2.11**.** Adult progeny (mean ± SE) of *S. zeamais* laboratory and field strain produced by adults developed from old larvae exposed to chlorine dioxide at concentration of 0.95 $g/m^3$ for five durations.

| Strain | Exposure time (h) | No. live adult / container (mean ± SE)[a] | No. adult progeny / container (mean ± SE)[b] |
|---|---|---|---|
| LAB | 0 | 84.0 ± 19.1 | 288.7 ± 79.6a |
| | 2 | 43.3 ± 5.4 | 119.3 ± 31.9a |
| | 4 | 57.7 ± 3.5 | 223.7 ± 15.7ab |
| | 6 | 57.7 ± 2.4 | 145.3 ± 13.4ab |
| | 8 | 35.0 ± 12.3 | 114.7 ± 24.9ab |
| | 10 | 34.7 ± 5.4 | 76.3 ± 25.0b |
| TX | 0 | 106.3 ± 12.9 | 466.3 ± 21.2a |
| | 2 | 49.0 ± 8.6 | 148.7 ± 15.1b |
| | 4 | 61.7 ± 1.8 | 207.7 ± 14.3bc |
| | 6 | 61.3 ± 1.9 | 101.7 ± 19.6c |
| | 8 | 42.0 ± 9.5 | 96.3 ± 36.3c |
| | 10 | 36.7 ± 3.5 | 87.0 ± 5.7c |

[a]Number of live insects for progeny production.

[b]Means followed by different lower case letters are significantly different among five durations within one strain. Number of adult progeny per container was significantly different after exposed to chlorine dioxide for various durations within each strain of LAB ($F$ = 4.28; df = 5, 12; $P$ = 0.0181) and TX ($F$ = 14.59, df = 5, 12; $P$ < 0.0001).

Table 2.12**.** Adult progeny (mean ± SE) of *S. zeamais* laboratory and field strain produced by adults developed from pupae exposed to chlorine dioxide at concentration of 0.95 $g/m^3$ for five durations.

| Strain | Exposure time (h) | No. live adult / container (mean ± SE)[a] | No. adult progeny / container (mean ± SE)[b] |
|---|---|---|---|
| LAB | 0 | 85.0 ± 8.7 | 348.7 ± 71.1a |
| | 2 | 83.0 ± 13.3 | 296.0 ± 96.4a |
| | 4 | 80.0 ± 7.8 | 354.7 ± 103.0ab |
| | 6 | 56.7 ± 11.1 | 171.0 ± 45.9ab |
| | 8 | 35.0 ± 8.7 | 83.7 ± 18.3bc |
| | 10 | 27.0 ± 2.1 | 45.7 ± 6.4c |
| TX | 0 | 143.7 ± 14.2 | 551.3 ± 37.2a |
| | 2 | 109.3 ± 12.8 | 357.0 ± 57.5ab |
| | 4 | 97.0 ± 12.1 | 280.3 ± 67.0ab |
| | 6 | 75.0 ± 11.0 | 245.0 ± 27.2ab |
| | 8 | 79.0 ± 12.5 | 341.7 ± 66.7ab |
| | 10 | 43.7 ± 4.7 | 148.3 ± 63.2b |

[a]Number of live insects for progeny production.

[b]Means followed by different lower case letters are significantly different among five durations within one strain. Number of adult progeny per container was significantly different after exposed to chlorine dioxide for various durations within each strain of LAB ($F$ = 9.06; df = 5, 12; $P$ = 0.0009) and TX ($F$ = 4.35, df = 5, 12; $P$ = 0.0173).

Table 2.13. Control mortality (%, mean ± SE) of immature stages of *O. surinamensis* laboratory and field strains without exposing to chlorine dioxide[a].

| Strain | Stage | | | |
|---|---|---|---|---|
| | Egg | Young larva | Old larva | Pupa |
| LAB | 1.3 ± 1.3 | 0 | 4.0 ± 4.0 | 2.7 ± 2.7 |
| AB2 | 1.3 ± 1.3 | 1.3 ± 1.3 | 2.7 ± 1.3 | 4.0 ± 2.3 |

[a]Each mean is based on $n$ = 3.

Table 2.14**.** Corrected mortality (%, mean ± SE) of *O. surinamensis* laboratory and field strain exposed as eggs, young larvae, old larvae, and pupae to chlorine dioxide concentration of 0.95 g/m$^3$ for various durations.

| Strain | Exposure time (h) | Mortality (%, mean ± SE) | | | |
|---|---|---|---|---|---|
| | | Eggs | Young larvae | Old larvae | Pupae |
| LAB | 2 | 100.0 ± 0.0A | 100.0 ± 0.0A | 14.7 ± 1.3B | 12.0 ± 6.1B |
| | 4 | 100.0 ± 0.0A | 100.0 ± 0.0A | 17.3 ± 8.7B | 21.3 ± 11.4B |
| | 6 | 100.0 ± 0.0A | 100.0 ± 0.0A | 21.3 ± 10.9B | 29.3 ± 5.8B |
| | 8 | 100.0 ± 0.0A | 100.0 ± 0.0A | 29.3 ± 9.6B | 32.0 ± 2.3B |
| | 10 | 100.0 ± 0.0A | 100.0 ± 0.0A | 45.3 ± 4.8B | 33.3 ± 7.4B |
| AB2 | 2 | 100.0 ± 0.0A | 100.0 ± 0.0A | 16.0 ± 8.0Bb | 5.6 ± 5.0Bb |
| | 4 | 100.0 ± 0.0A | 100.0 ± 0.0A | 18.7 ± 2.7Bb | 12.5 ± 2.4Bb |
| | 6 | 100.0 ± 0.0A | 100.0 ± 0.0A | 20.0 ± 4.6Bb | 15.3 ± 5.0Bb |
| | 8 | 100.0 ± 0.0A | 100.0 ± 0.0A | 24.0 ± 10.1Bb | 20.8 ± 2.4Bb |
| | 10 | 100.0 ± 0.0A | 100.0 ± 0.0A | 65.3 ± 4.8Ba | 43.1 ± 6.1Ca |

*Means followed by different upper case letters are significantly different among life stages within one exposure time and one strain, and means followed by different lower case letters are significantly different for pupae among different exposure times within one strain ($P < 0.05$, by Ryan-Einot-Gabriel-Welsh Q tests).

**Significant differences observed from corrected mortality in all stages ($P < 0.05$).

***Significant differences observed from corrected mortality in AB2 strain were old larvae ($F = 9.81$; df = 4,10; $P = 0.0017$) and pupae ($F = 10.39$; df = 4, 10; $P = 0.0014$).

Table 2.15**.** Adult progeny (mean ± SE) of *O. surinamensis* laboratory and field strain produced by adults developed from eggs and young larvae in control groups.

| Strain | Stage | No. live adult / container (mean ± SE)[a] | No. adult progeny / container (mean ± SE) |
|---|---|---|---|
| LAB | Egg | 24.7 ± 0.3 | 171.0 ± 23.2 |
| AB2 | Egg | 24.7 ± 0.3 | 174.3 ± 12.7 |
| LAB | Young larvae | 25.0 ± 0.0 | 201.3 ± 25.7 |
| AB2 | Young larvae | 24.7 ± 0.3 | 210.7 ± 12.1 |

[a]Number of live insects for progeny production.

Table 2.16**.** Adult progeny (mean ± SE) of *O. surinamensis* laboratory and field strain produced by adults developed from old larvae exposed to chlorine dioxide at concentration of 0.95 $g/m^3$ for five durations.

| Strain | Exposure time (h) | No. live adult / container (mean ± SE)[a] | No. adult progeny / container (mean ± SE)[b] |
|---|---|---|---|
| LAB | 0 | 24.0 ± 1.0 | 262.0 ± 41.7a |
| | 2 | 21.3 ± 0.3 | 227.7 ± 23.8a |
| | 4 | 20.7 ± 2.2 | 206.0 ± 13.6a |
| | 6 | 19.7 ± 2.7 | 196.0 ± 6.7a |
| | 8 | 17.7 ± 2.4 | 189.7 ± 32.4a |
| | 10 | 13.7 ± 1.2 | 110.0 ± 16.8b |
| AB2 | 0 | 24.3 ± 0.3 | 239.3 ± 40.3a |
| | 2 | 21.0 ± 2.0 | 230.7 ± 11.7a |
| | 4 | 20.3 ± 0.7 | 179.3 ± 13.2a |
| | 6 | 20.0 ± 1.2 | 168.0 ± 17.4a |
| | 8 | 19.0 ± 2.5 | 158.7 ± 17.7a |
| | 10 | 8.7 ± 1.2 | 94.7 ± 22.9b |

[a]Number of live insects for progeny production.

[b]Means followed by different lower case letters are significantly different among five durations within one strain. Number of adult progeny per container was significantly different after exposed to chlorine dioxide for various durations within each strain of LAB ($F$ = 5.27; df = 5, 12; $P$ = 0.0086) and AB2 ($F$ = 5.93, df = 5, 12; $P$ = 0.0055).

Table 2.17**.** Adult progeny (mean ± SE) of *O. surinamensis* laboratory and field strain produced by adults developed from pupae exposed to chlorine dioxide at concentration of 0.95 $g/m^3$ for five durations.

| Strain | Exposure time (h) | No. live adult / container (mean ± SE)[a] | No. adult progeny / container (mean ± SE)[b] |
|---|---|---|---|
| LAB | 0 | 24.3 ± 0.7 | 256.0 ± 14.6a |
| | 2 | 22.0 ± 1.5 | 230.3 ± 13.4a |
| | 4 | 19.7 ± 2.8 | 199.3 ± 24.4ab |
| | 6 | 17.7 ± 1.5 | 141.3 ± 12.0ab |
| | 8 | 17.0 ± 0.6 | 135.7 ± 5.0ab |
| | 10 | 16.7 ± 1.9 | 112.7 ± 41.7b |
| AB2 | 0 | 24.0 ± 0.6 | 270.3 ± 22.2a |
| | 2 | 22.7 ± 1.2 | 238.7 ± 27.0a |
| | 4 | 21.0 ± 0.6 | 191.0 ± 8.1ab |
| | 6 | 20.3 ± 1.2 | 122.7 ± 3.8ab |
| | 8 | 19.0 ± 0.6 | 172.0 ± 11.1bc |
| | 10 | 13.7 ± 1.5 | 83.7 ± 20.2c |

[a]Number of live insects for progeny production.

[b]Means followed by different lower case letters are significantly different among five durations within one strain. Number of adult progeny per container was significantly different after exposed to chlorine dioxide for various durations within each strain of LAB ($F$ = 4.32; df = 5, 12; $P$ = 0.0176) and AB2 ($F$ = 11.76, df = 5, 12; $P$ = 0.0003).

Table 2.18**.** Adult progeny (mean ± SE) of *R. dominica* laboratory and field strain produced by adults developed from immature stages in control groups.

| Strain | Stage | No. live adult / container (mean ± SE)[a] | No. adult progeny / container (mean ± SE) |
|---|---|---|---|
| LAB | Egg | 17.0 ± 0.7 | 165.3 ± 15.6 |
| CF | Egg | 18.3 ± 1.9 | 147.3 ± 13.3 |
| CS | Egg | 16.7 ± 0.7 | 154.0 ± 18.0 |
| PD | Egg | 15.3 ± 0.3 | 167.7 ± 12.1 |
| RL | Egg | 16.0 ± 1.3 | 129.0 ± 32.2 |
| LAB | Young larvae | 17.3 ± 0.6 | 164.0 ± 20.7 |
| CF | Young larvae | 15.0 ± 0.9 | 167.0 ± 10.8 |
| CS | Young larvae | 19.0 ± 0.6 | 163.7 ± 5.7 |
| PD | Young larvae | 16.0 ± 0.6 | 167.7 ± 22.0 |
| RL | Young larvae | 18.0 ± 0.3 | 146.7 ±30.0 |
| LAB | Old larvae | 16.3 ± 0.7 | 164.3 ±27.3 |
| CF | Old larvae | 17.0 ±0.6 | 176.0 ± 26.1 |
| CS | Old larvae | 17.7 ± 1.2 | 146.3 ± 23.1 |
| PD | Old larvae | 14.7 ± 1.5 | 164.7 ±35.9 |
| RL | Old larvae | 18.0± 1.0 | 133.7 ± 39.3 |
| LAB | Pupae | 18.3 ± 0.7 | 160.3 ± 10.3 |
| CF | Pupae | 18.3 ± 0.3 | 192.7 ± 23.6 |
| CS | Pupae | 19.0 ± 0.6 | 174.3 ± 31.8 |
| PD | Pupae | 16.7 ± 1.3 | 152.3 ± 41.7 |
| RL | Pupae | 17.3 ± 0.9 | 122.3 ± 47.9 |

[a]Number of live insects for progeny production.

Table 2.19**.** Adult progeny (mean ± SE) of *S. zeamais* laboratory and field strain produced by adults developed from immature stages in control groups.

| Strain | Stage | No. live adult / container (mean ± SE)[a] | No. adult progeny / container (mean ± SE) |
|---|---|---|---|
| LAB | Egg | 115.0 ± 9.8 | 437.0 ± 17.0 |
| TX | Egg | 126.3 ± 6.1 | 435.3 ± 37.4 |
| LAB | Young larvae | 76.7 ± 3.9 | 477.7 ± 32.8 |
| TX | Young larvae | 128.3 ± 11.1 | 474.7± 8.8 |
| LAB | Old larvae | 102.3 ± 10.0 | 382.7 ± 43.2 |
| TX | Old larvae | 121.3 ± 15.1 | 449.7 ± 15.5 |
| LAB | Pupae | 92.0 ± 8.7 | 415.7 ± 44.9 |
| TX | Pupae | 158.0 ± 13.5 | 464.3 ± 80.9 |

[a]Number of live insects for progeny production.

Table 2.20 . Adult progeny (mean ± SE) of *O. surinamensis* laboratory and field strain produced by adults developed from immature stages in control groups.

| Strain | Stage | No. live adult / container (mean ± SE)[a] | No. adult progeny / container (mean ± SE) |
|---|---|---|---|
| LAB | Egg | 24.0 ± 1.0 | 177.7 ± 10.4 |
| AB2 | Egg | 24.7 ± 0.3 | 177.3 ± 21.7 |
| LAB | Young larvae | 25.0 ± 0.0 | 203.7 ± 22.6 |
| AB2 | Young larvae | 24.7 ± 0.3 | 204.0 ± 12.7 |
| LAB | Old larvae | 25.0 ± 0.0 | 231.7 ± 8.4 |
| AB2 | Old larvae | 24.7 ± 0.3 | 230.3 ± 18.8 |
| LAB | Pupae | 25.0 ± 0.0 | 264.7 ± 19.2 |
| AB2 | Pupae | 25.0 ± 0.0 | 262.7 ± 12.0 |

[a]Number of live insects for progeny production

# Chapter 3 - Responses of immature stages of *Tribolium castaneum* to chlorine dioxide gas

## 3.1. Abstract

Chlorine dioxide gas was evaluated as an alternative to phosphine for the management of postharvest insects. Immature stages of a phosphine susceptible laboratory strain and four phosphine resistant field strains of the red flour beetle, *Tribolium castaneum* (Herbst), were used for this study. Eggs, young larvae, old larvae, and pupae of *T. castaneum* were exposed in vials with 10 g of wheat to chlorine dioxide gas at concentration of 2.02 g/$m^3$ (750 ppm) concentration for 2, 4, 6, and 8 h. Mortality was determined by subtracting the number of adults that emerged from the immature insects out of the total insects exposed, and expressed as percentage. Adult progeny production was determined 8 weeks after adult emergence. Complete mortality was achieved in eggs and young larvae after a 4-h and 2-h exposure, respectively. The highest mortality of old larvae and pupae of all five strains was 86.8-95.2% and 81.1-93.8%, respectively, after an 8-h exposure. The order of susceptibility of immature stages to chlorine dioxide was: young larvae > eggs > old larvae > pupae. Mortality of immature stages increased with increase of exposure time. Exposure time is an important influence on the progeny production when adults emerged from dioxide-induced immature insects. Adults that emerged from treated pupae produced very less progeny although the survival rate was relatively high among other stages. In addition, all strains of phosphine resistant insects showed similar susceptible to chlorine dioxide as compared to the phosphine susceptible strain. These results suggest that chlorine dioxide gas is highly effective in controlling immature stages of laboratory and field strains of *T. castaneum*.

## 3.2. Introduction

Chlorine dioxide is yellow to green-yellowish gas at room temperature (O'Neil et al, 2001; NIOSH, 2003), which has been successfully utilized across a broad-spectrum of areas. It performs as bleaching agent in paper-pulp, disinfectant and deodorizer in water and perishable foods, aging accelerator in flour processing, and sanitizer in food and beverage plants (O'Neil et al., 2001; Aieta et al., 1979; Synan et al., 1944; Lewis 2001; Tomás-Callejas et al., 2012; Clarke and Clarke, 1975).

Evaluated the efficacy of chlorine dioxide in killing larvae of *Chironomid, Chironomus riparius* in raw water. Chlorine dioxide was effective against larvae of *Chironomid, Chironomus riparius* with 100% mortality when tested in raw water at *ct* value of 45 mg-min/L, and temperature and pH of 23.2–25.7 °C and 6.7–7.1, respectively (Sun et al., 2007). Similarly, 100% mortality of bedbugs (*Cimex lectularius* and *Citnex hemipterus*) was achieved when fumigated by chlorine dioxide at 1086 ppm (2.93 g/$m^3$) and 724 ppm (1.95 g/$m^3$) immediately after treatment, and 362 ppm (0.98 g/$m^3$) after 6 h exposure (Gibbs et al., 2012). The same study on eggs showed no hatched larvae after treated with chlorine dioxide concentrations of 724 ppm exposure for 6 h under temperature of 20-21 °C.

However, there is limited data on the efficacy of chlorine dioxide against stored-product insect pests. Eggs and old larvae of red four beetle, *T. castaneum* (Herbst) and confused flour beetle, *T. confusum* (Jacquelin du Val) were least susceptible to chlorine dioxide gas as compared with other life stages when exposed to 248.4, 331.2, 413.9, and 496.6 g/$m^3$ concentrations for 1.53, 2.07, 1.80, and 1.68 h, with and without wheat flour under laboratory condition (Channaiah, et al., 2012). The same study also showed complete mortality of young larvae and adults when exposed to a chlorine dioxide concentration of 834.4 gh/$m^3$

concentration. In addition to this, a study by Kim et al. (2015) on *T. castaneum* showed 100% mortality in both larvae and adults when exposed to chlorine dioxide at concentration of 0.54 $g/m^3$ (200 ppm) for 24 h without food. A recent study by Han et al. (2016) on all developmental stages (eggs, larvae, pupae, and adults) of Indian Meal Moth, *Plodia interpunctella* (Hübner) showed 100% mortality of all life stages after exposed to clorine dioxide concentration of 0.27 $g/m^3$ (100 ppm) and 0.54 $g/m^3$ (200 ppm) concentrations of chlorine dioxide for 48 h and 24 h, respectively. The study also indicated that eggs were the most susceptible life stage and pupae were least susceptible to chlorine dioxide gas. Subramanyam and E (2015) studied the efficacy of chlorine dioxide gas against adults of laboratory (phosphine-susceptible) and field (phosphine-resistant) strains of five stored-products insect species, *T. castaneum* (red flour beetle), *Rhyzopertha dominica* (Fabricius) (lesser grain borer), *Sitophilus oryzae* (Linnaeus) (rice weevil), *Sitophilus zeamais* (Motschulsky) (maize weevil), and *Oryzaephilus surinamensis* (Linnaeus) (sawtoothed grain beetle) and obtained 100% mortality of *T. castaneum*, *R. dominica*, and *O. surinamensis* when exposed to chlorine dioxide concentration of 2.70 $g/m^3$ (1000 ppm) for 7 h. However, only 57 and 50% mortalities were obtained for *S. oryzae* and *S. zeamais*, respectively, with the same concentration and exposure time.

Apart from the availability of fumigants, resistance development is a major concern to implement effective stored product insect pest management. Previous studies suggest that chlorine dioxide can be a potential fumigant in controlling certain life stages of stored product insect pests, but data on the efficacy of chlorine dioxide on immature stages (eggs, young larvae, old larvae, and pupae) of phosphine-resistant insect species on stored-commodities is still limited. Therefore, this study was aimed at determining the efficacy of chlorine dioxide gas

against immature stages of one laboratory strain (phosphine susceptible) and four field strains of *T. castaneum* with various phosphine resistance.

## 3.3. Materials and methods

### 3.3.1. Chlorine dioxide gas generation

Chlorine dioxide gas (ClO2; CAS# 10049-04-4) was generated by A HP-10E $PureClO_2^{TM}$ electrochemical generator (PureLine Treatment System, LLC, Bensenville, Illinois) and supplied to an air-tight polymethyl methacrylate (PMMA) test chamber (0.6 m × 0.6 m × 1.0 m). Compressed air (344.7 kPa) was used to create a negative pressure to pull the gas out from the generator. Chlorine dioxide gas concentration was monitored and recovered using chlorine dioxide analyzer (Control 4000, Optek®; Germantown, Wisconsin). HOBO® data logger (Model: U 10-003, Onset Computer Corp., Bourne, Massachusetts, USA) was set to record temperature and relative humidity of the chamber every minute during exposure times.

### 3.3.2. Insects rearing

Adults of *T. castaneum* were collected from farm bins in Kansas during 2011, and phosphine resistance was evaluated according to the discrimination dose test by Food and Agriculture Organization of the United Nations (FAO) (Sehgal, 2013; E et al., 2017). Laboratory strain *T. castaneum* has been reared in stored product entomology laboratory at the department of Grain Science and Industry, Kansas State University since 1999 without insecticide exposure. This strain showed 0% survival after phosphine exposure. The resistance of field strains all weak except MN (strong) (Table 3.1). Organic whole wheat flour (Heartland Mills, Marienthal, Kansas) with 5% (by weight) brewer's yeast was used for rearing all *T. castaneum* strains in an environmental chamber (Model I-36VL; Percival Scientific, Perry, Iowa) set at 28°C and 65% relative humidity (r.h.).

### 3.3.3. Life stage identification

Eggs and young larvae were collected and determined according to Beeman et al. (2009). Organic whole wheat flour was sifted with 177-µm mesh sieve (Seedburo Equipment Company, Chicago, IL, USA) and used for egg collection. One hundred unsexed *T. castaneum* adults (1-week old) were placed on 100 g sifted flour as described above for egg laying. Adults were removed after 72 h. Eggs were collected from flour after 72h incubation by passing through a 297-µm mesh sieve. For the collection of the young larvae, eggs were collected first followed by the protocol described above, and these eggs were placed on organic hard red winter wheat flour (Heartland Mills, Marienthal, Kansas) with 5% (weight by weight) brewer's yeast. Young larvae were collected from the flour after a 19-d incubation (including egg hatching period). Old larvae (21-22 days old) and pupae (2-3days old) were collected directly from the culture jars.

### 3.3.4. Chlorine dioxide treatment

Fifty insects from each life stage (eggs, young larvae, old larvae and pupae) were placed in separated cylindrical plastic vial (55 × 23 mm, height × inner diameter) contained 10 g wheat kernels covered with stainless steel mesh (250-µm opening) at the top and bottom. Vials were exposed to a chlorine dioxide concentration of 2.02 g/m$^3$ (750 ppm) in the test chamber. Control vials were kept in the growth chamber with no gas exposure at 28°C and 65% (r.h.). The gas concentration in the test chamber reached to 2.02 g/m3 within 5 minutes. Treated vials were taken out from the test chamber after 2, 4, 6, and 8-h exposures and brought back to the environmental chamber for incubation prior to mortality assessment. Mortality was determined based on adult emergence. Adult emergence from eggs, young larvae, old larvae, and pupae was checked after 42, 28, 20, and 5d of incubation respectively in the environmental chamber. After checking the adult emergence, dead adults were removed from the sample, and live adults along

with treated wheat were transferred to 50 g of whole wheat flour into 100 ml plastic jar to facilitate reproduction. The adult progeny production was assessed after 8 weeks of incubation in the environmental chamber at 28 C and 65 % r.h. Each experiment was replicated three times.

### 3.3.5. Data analysis

Mortality was determined by subtracting the number of adults emerged from the total numer of exposed immature insects and expressed as percentage. Mortality data on chlorine dioxide-treated insects was corrected for control mortality (Abbott, 1925). Corrected mortality was tested with Levene's test for equality of variances and the population variances were found to be homoscedastic. Corrected mortality data was subjected to three-way Analysis of Variance (ANOVA) (SAS Institute, 2008) to observe the main effects and interactions in the treatments. One-way ANOVA was used to determine significant differences among exposure times and strains in relation to each life stage, and means were separated with Ryan-Einot-Gabriel-Welsh Q (REGWQ) test at $\alpha = 0.05$ (SAS Institute, 2008). Adult progeny data was subjected to three-way ANOVA. Then, progeny productions from eggs, old larvae and pupae were transforomed to $\log_{10}(x+1)$ then subjected one-way ANOVA to test the significant differences between control and exposure times for each strain and stages, and means were separated using Ryan-Einot-Gabriel-Welsh Q (REGWQ)test at significant level $\alpha = 0.05$. Progeny production was interpreated as the number of live adults per container. There was no progeny from young larvae due to complete mortality.

## 3.4. Results

Control mortality data was presented as mean ± standard error based on three independent replicates for all strains, ranged from 11.3 ± 5.6 to 17.3 ± 6.8, 0.0 ± 0.0 to 6.7 ± 3.3, 0.0 ± 0.0 to 17.0 ± 8.6 and 0.0 ± 0.0 to 10.0 ± 10.0 for egg, young larvae, old larvae and pupae

respectively (Table 3.2). Mortality of *T. castanem* from controls ranged from 11.3-14.0, 0-6.7, 0-17.0, and 0-10.0% for eggs, young larvae, old larvae, and pupa, respectively (Table 3.2). A 100% mortality of young larvae and eggs was obtained after 2-h and 4-h exposures, respectively (Table 3.3). Young larvae of all strains were the most susceptible life stage of all strains to chlorine dioxide gas followed by eggs, old larvae, and pupae. The mortality of egg, old larvae and pupae increased as exposure time increased. Three-way ANOVA showed that corrected mortality showed significant differences among all life stages ($F$ = 50.54; df = 3,150; $P$ < 0.0001) and exposure time ($F$ = 21.18; df = 3,150; $P$ < 0.0001). Stages and exposure time were the only significant interaction ($F$ = 7.17; df = 9,150; $P$ < 0.0001). One-way ANOVA showed pupal mortality was significantly different among exposure time within the same strain ($F_{range}$ = 4.38 - 17.77; df = 3,8; $P_{range}$ = 0.0007 - 0.0421), whereas no significant difference was observed on other life stages. Mortality of *T. castanem* laboratory strain showed significant differences among life stages except 2 h exposure ($F_{range}$ = 4.11 – 9.44; df = 3,8 for 4 h and 3, 6 for 8 h; $P_{range}$ = 0.0077 – 0.0489). Significant difference was only observed from 4-h exposures among life stages of AB1 strain ($F$ = 4.53, df = 3,8; $P$ = 0.0388). For CF strain, all exposure times showed significant differences among life stages ($F_{range}$ = 4.79 – 8.65; df = 3,8 for 2,4 and 6 h, and 3, 6 for 8 h; $P_{range}$ = 0.0068 – 0.0469). significant differences were showed in exposure of 2, and 4 h among life stages of MN strain ($F$ = 6.45 and 7.85; df= 3,8; $P$ = 0.0091 and 0.0157). AB2 showed no significant differences among stages ($F_{range}$ = 1.42 – 2.98; df = 3, 8 for 2, 4 and 6 h, and 3, 6 for 8 h; $P_{range}$ = 0.0966 – 0. 3075) (Table 3.3). Mortality differences among exposure times, life stages were detected only in pupae ($P$ < 0.05), and no differences observed from eggs, young larvae, and old larvae. Four phosphine resistant strains of *T. castanem* were as susceptible to chlorine dioxide gas as laboratory strain at any given exposure time.

Progeny production was obtained from the controls ranging from 323 to 865 adults per container (Table 3.4-3.6). Insects that were exposed to chlorine dioxide gas as young larvae showed no progeny production (Table 3.4). Drametice suppression in prgeny production was observed when *T. castaneum* old larvae were exposed to chlorine dioxide and mostly because there were very few numbers of old larvae survived after the exposures. AB2 strain of adults failed to produce offspring after exposed to chlorine dioxide gas for any given time (treatments vs. control, 0 vs. 501.0, $F = 234.15$, df = 4,9, $P < 0.0001$). For lab train, there were 15.0 adults per container after a 2-h exposure as compared to control (547.7 adults per container) ($F = 9.76$, df = 4,9, $P = 0.0025$). No progeny was observed after 4 h or longer exposure duration for lab strain. Similarly, strains of AB1, CF, and MN had no progeny production after a 4-h, 6-h, or 8-h exposure, respectively, whereas it was not statistically different after a 2-h exposure in progeny productions of these three strains (2-h exposure vs. control, 112.3 vs. 323.7, $F = 3.21$, df = 4,9, $P = 0.0676$ for AB1 strain; 266.3 vs. 588.0, $F = 4.54$, df = 4,9, $P = 0.0279$ for CF strain; 206.0 vs. 625.3, $F = 2.48$, df = 4,9, $P = 0.1189$ for MN strain) (Table 3.5). Chlorine dioxide treated *T. castaneum* pupae had very low numbers of offspring showed in Table 3.6. Progeny production was completely suppressed by chlorine dioxide gas for AB1 strain even though at a 2-h exposure. Significant differences were found in any given exposure time as compared to its corresponding control group (2-h exposure vs. control, 33.3 vs. 592.7, $F = 26.89$, df = 4,10, $P < 0.0001$ for Lab strain; 0 vs. 683.3, $F = 397.70$, df = 4,10, $P < 0.0001$ for AB1 strain; 6.3 vs. 583.0, $F = 161.13$, df = 4,10, $P < 0.0001$ for AB2 strain; 13.7 vs. 469.0, $F = 55.43$, df = 4,10, $P < 0.0001$ for CF strain; 79.0 vs. 642.0, $F = 10.96$, df = 4,10, $P = 0.0011$ for MN strain) (Table 3.6).

## 3.5. Discussion

Fumigation is a common practice used for stored product insect pest management in post-harvest chain. Phosphine has been the most commonly used grain fumigant for past several decades, however, there are many insect species have developed various level of resistance to over the past five decades (Benhalima et al., 2004; Hori and Kasaishi 2005; Lorini et al., 2007; Song, et al., 2011; Jagadeesan et al., 2012; Opit et al., 2012; Kaur et al., 2015; Gautam et al., 2017; Cato et al., 2017). Eggs and pupae are more tolerant to fumigants than the rest life stages of insect pests (Bell, 2000; Rajendran et al., 2008). Failure to kill these immatures leads survived insects to start breeding during post-fumigation storage, due to fumigants leave minimal or no residues after treatment.

In this study, the susceptibility of all immature stages (eggs, young larvae, old larvae, and pupae) of *T. castaneum* was found similar when compared the phosphine-resistant strains to laboratory (phosphine-susceptible) strain. E et al. (2017) reported that no strong relationship found between phosphine-resistant and -susceptible populations when exposed adults of *T. castaneum*, *O. surinamensis*, *R. dominica*, and *Sitophilus* spp. to chlorine dioxide gas at concentration of 0.54 g/m$^3$ for different exposure periods. Another study documented similar finding using adults of the same insect species when tested with gas concentration of 1.41 g/m$^3$ for various exposure times (E et al., 2018). Molecular studies revealed the mode of action of chlorine dioxide on microorganisms was to alter the enzymes on cell membrane, however, caused no damage on DNA or mutations (Roller et al., 1980; Young and Setlow, 2003; Finnegan et al., 2010). Chlorine dioxide was defined the ability of increasing oxidative stress in rat and chicken (Couri and Abdel-Rabman, 1979). *P. interpunctella* larvae were used to investage the insecticidal mechanism of chlorine dioxide. Fumigation with chlorine dioxide induced reactive

oxygen species (ROS) which led oxidative stress (Kumar et al., 2015). Similar results were reported by conducting experiments using *T. castaneum* (Kim, et al., 2015). Enzymes, superoxide dismutase (SOD) and thioredoxin-peroxidase (Tpx*)*, were the major antioxidants in cell defense against oxidative stress induced by chlorine dioxide in *P. interpunctella and T. castaneum* (Kumar et al., 2015; Kim et al., 2015).

Furthermore, Bond (1961) reported that the effectiveness of fumigants was associated with their mode of entry. Fumigants either entered in to the tracheal system via insect's spiracles or diffused through integument (Yu, 2014) and poison insects which caused death. Susceptibility among immature stages vary due to different morphological characteristics. Cuticular layer deposition and its relative thickness may play critical roles in response to fumigants. Our study suggested that eggs of *T. castaneum* are more susceptible to chlorine dioxide than old larvae and pupae. Chlorine dioxide enters eggs by diffusion through the shell (chorion layers) or by respiratory channels. Gautam et al. (2014, 2015) hypothesized two factors that can affect the gas uptake by eggs: number of respiratory and reproductive openings (aeropyle and micropyle), and the surface-to-volume ratio of the eggs. These features can provide entry sites for chlorine dioxide gas (Beament, 1949; Gautam et al., 2014, 2015). Scanning electron micrographs provided detailed images of the ultra-structures of *T. castaneum* eggs. Neither aeropyle nor micropyle is present on the surface of *T. castaneum* eggs (Gautam et al., 2015). Therefore, it is most likely that diffusion through the eggshell is the only way for chlorine dioxide entrance to *T. castaneum* eggs. In relation to life stages of larvae and pupae, fumigants may be taken via insect trachea and distributed systemically according to Yu (2014), however, research is required to provide evidence. Apart from the entrance of fumigants, respiration rate within different life stages determines the fumigant uptake rate (Emekci et al., 2002). Emekci et al. (1998 and 2002)

studied respiration rates of all life stages of *T. castaneum* at various modified atmospheres containing 1, 2, 3, 5, 10 or 15% oxygen mixed with nitrogen at 30 °C and 70% r.h. Respiration rate in each developmental stage of insects was measured based on the carbon dioxide production (µL CO2/insect/h). Profile of respiration rate at oxygen concentration (1%-21% oxygen) was approximated according to the figures in Emekci et al. (2002): 0.0025 - 0.0012 µL $CO_2$/h/egg, 0 - 9.25 µL $CO_2$/h/young larva, 4 - 8.45 µL $CO_2$/h/old larva, and 0.83 - 1.45 µL $CO_2$/h/pupa (data was adapted from figures). More $CO_2$ was produced by *T. castaneum*, and adults under 3% and 5% oxygen with a production rate of 4.77 and 4.98 µL $CO_2$/h/adult, respectively. Moreover, general trend of respiration rate in *T. castaneum* control group under normal atmospheric oxygen level (21% oxygen) was reported as (fast to slow): young larvae > mature larvae > adults > pupae > eggs (Emekci et al., 1998).

Chlorine dioxide is a highly oxidizing agent that reacts with tyrosine, tryptophan, and cysteine, but does not cause DNA mutation (Roller et al., 1980; Noss et al., 1986; Young and Setlow, 2003; Knapp and Battisti, 2001). Oxidative stress was suspected to be the cause of the mortality in chlorine dioxide-exposed insects (Kim et al., 2015; Kumar et al., 2015). Unlike chlorine dioxide, phosphine is a strong reducing agent and can inhibit aerobic respiration in insects and other animals (Bolter and Chefurka, 1990). It is believed that resistance to phosphine could be a combination of many factors, including inhibition of cytochrome-*c* oxidase (Kashi and Chefurka, 1976), catalase and peroxidase (Fridovich, 1983; Bolter and Chefurka, 1990), up-regulation of dihydrolipoamide dehydrogenase (DLD) (Schlipalius et al., 2012), and alteration of transcripts encoding phosphine resistance genes (i.e. increase of gene-encoding CYPs and decrease of anti-diruetic peptides) (Oppert et al., 2015).

Manivannan (2015) assessed the toxicity of phosphine against immature stages of *T. castaneum* at different exposure times (24, 48, 72, 96, 120, 144, 168 h) under laboratory condition (25 °C and 75 % r.h.), and treated samples were transferred to insect culture chamber (30 °C and 75 % r.h.) till mortality assessment. After a 24-h exposure, the $LC_{50}$ values of eggs, $2^{nd}$ instars, $4^{th}$ instars, $6^{th}$ h instars, and pupae were 1.571, 0.081, 0.212, 0.336, and 1.184 mg/L, indicating that eggs and pupae were less susceptible to phosphine than larval stages. This corresponds to the respiration rate of these immature stages reported by Emekci et al. (1998). It suggested that uptake of phosphine is facilitated with the higher respiration rate. Our dose-mortality data revealed a different pattern as compared to Manivannan's study (2015). In our study, the most susceptible stages are eggs and young larvae, followed by old larvae and pupae. The efficacy of chlorine dioxide cannot be solely explained by respiration rates of different immature stages. This may imply that chlorine dioxide performs lethal effect through other routes as well which needs further investigation.

Chlorine dioxide has significant impact on adult progeny. Progeny production from chlorine dioxide-induced immature stages, especially pupae, was suppressed. Park (1934) pointed out that in *Tribolium*, the only reliable external feature for any juvenile stage for sex determination is pupal stage. This may be due to the different sensitivity between two sexes of *T. castaneum*. If one sex is more susceptible than the other, there would be no progeny or very less progeny produced after treatment. This suggested that chlorine dioxide directly disrupts insect mating behavior. Or it may impair the reproductive organs of insects, and insects lose the ability to reproduce.

## 3.6. Conclusion

Chlorine dioxide can be a potential alternative to phosphine in controlling insects in stored-grains. Our study showed the effectiveness of chlorine dioxide gas against all immature stages of *T. castaneum*. Eggs of *T. castaneum* were completely suppressed from development to the next stage after exposed to chlorine dioxide, which indicated an advantage compared to other traditional fumigants. Fumigation with chlorine dioxide significantly suppressed progeny production, indicating that chlorine dioxide may be a good alternative to phosphine for controlling store product pests.

## 3.7. References


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Table 3.1. Collection of adults *T. castaneum* field strains from various locations and time periods in Kansas, 2011[a].

| Strain | County, State | Location | Commodity | Collection date | % Survival[b] | Phosphine resistance |
|---|---|---|---|---|---|---|
| AB1 | Dickinson, KS | Abilene | Wheat | Aug. 3, 10 | 43 | Weak |
| AB2 | Dickinson, KS | Abilene | Wheat | Aug. 24, Sept. 12, Oct. 4 | 52 | Weak |
| CF | Washington, KS | Clifton | Wheat | Aug. 3, 24 | 15 | Weak |
| MN | Ottawa, KS | Minneapolis | Wheat | Aug. 10 | 98 | Strong |

[a]Reference: Sehgal, (2013) and Subramanyam and E, (2016).

[b]Survival rate was based on FAO discrimination dosage tests. Laboratory (LAB) strain was susceptible to phosphine due to 0% survival and was not shown here.

Table 3.2. Control mortality (%, mean ± SE) of immature stages of *T. castaneum* laboratory and field strains without exposing to chlorine dioxide[a].

| Strain | Stage | | | |
|---|---|---|---|---|
| | Egg | Young larva | Old larva | Pupa |
| LAB | 14.0 ± 6.9 | 0 | 17.0 ± 8.6 | 3.5 ± 3.5 |
| AB1 | 13.3 ± 4.7 | 6.7 ± 3.3 | 0 | 2.6 ± 2.6 |
| AB2 | 17.3 ± 6.8 | 0 | 0 | 0 |
| CF | 11.3 ± 5.7 | 0 | 10.0 ± 10.0 | 10.0 ± 10.0 |
| MN | 13.3 ± 4.4 | 3.3 ± 3.3 | 1.8 ± 1.8 | 1.8 ± 1.8 |

[a]Each mean is based on $n = 3$.

Table 3.3. Corrected mortality (%, mean ± SE) of *T. castaneum* laboratory and field strains exposed as *eggs*, young larvae, old larvae, and pupae to chlorine dioxide concentration of 2.02 $g/m^3$ for various durations.

| Strain | Exposure time (h) | Mortality (%, mean ± SE)* | | | |
|---|---|---|---|---|---|
| | | Eggs[a] | Young larvae[b] | Old larvae[c] | Pupae |
| LAB | 2 | 100.0 ± 0.0 | 100.0 ± 0.0 | 69.4 ± 27.3 | 43.3 ± 15.9b |
| | 4 | 100.0 ± 0.0A | 100.0 ± 0.0A | 86.7 ± 13.3AB | 69.6 ± 4.9abB |
| | 6 | 100.0 ± 0.0A | 100.0 ± 0.0A | 95.0 ± 5.0AB | 84.7 ± 0.3aB |
| | 8 | 100.0 ± 0.0[c]A | 100.0 ± 0.0A | 92.5 ± 7.5[c]AB | 81.1 ± 2.2aB |
| AB1 | 2[e] | 96.0 ± 4.0 | 100.0 ± 0.0 | 62.6 ± 29.1 | 33.4 ± 19.2b |
| | 4 | 100.0 ± 0.0A | 100.0 ± 0.0A | 84.2 ± 12.3AB | 70.0 ± 5.8abB |
| | 6 | 100.0 ± 0.0 | 100.0 ± 0.0 | 90.0 ± 10.0 | 77.9 ± 5.1ab |
| | 8[e] | 100.0 ± 0.0[c] | 100.0 ± 0.0 | 92.5 ± 7.5[c] | 88.3 ± 6.0a |
| AB2[e] | 2 | 94.7 ± 5.3 | 100.0 ± 0.0 | 70.0 ± 25.2 | 44.1 ± 14.9 |
| | 4 | 100.0 ± 0.0 | 100.0 ± 0.0 | 91.7 ± 8.3 | 86.7 ± 7.3 |
| | 6 | 100.0 ± 0.0 | 100.0 ± 0.0 | 89.5 ± 10.5 | 81.5 ± 7.7 |
| | 8 | 100.0 ± 0.0[c] | 100.0 ± 0.0 | 86.8 ± 13.2[c] | 84.6 ± 6.2 |
| CF[f] | 2 | 100.0 ± 0.0A | 100.0 ± 0.0A | 44.4 ± 23.8B | 33.3 ± 13.0bB |
| | 4 | 100.0 ± 0.0A | 100.0 ± 0.0A | 90.7 ± 9.3AB | 74.3 ± 6.1aB |
| | 6 | 100.0 ± 0.0A | 100.0 ± 0.0A | 95.0 ± 5.0A | 82.3 ± 2.7aB |
| | 8 | 100.0 ± 0.0[c]A | 100.0 ± 0.0A | 92.5 ± 7.5[c]AB | 84.1 ± 3.7aB |
| MN | 2 | 99.3 ± 0.7A | 100.0 ± 0.0A | 72.8 ± 19.8AB | 43.1 ± 8.5cB |
| | 4 | 100.0 ± 0.0A | 100.0 ± 0.0A | 89.6 ± 8.1AB | 72.2 ± 5.0bB |
| | 6 | 100.0 ± 0.0 | 100.0 ± 0.0 | 95.0 ± 5.0 | 88.3 ± 3.3ab |
| | 8[i] | 100.0 ± 0.0[c] | 100.0 ± 0.0 | 95.2 ± 4.8[c] | 93.9 ± 3.0a |

*Means followed by different upper case letters are significantly different among life stages within one exposure time and one strain, and means followed by different lower case letters are significantly different for pupae among different exposure times within one strain ($P < 0.05$, by Bonferroni *t*-tests).

[a]Corrected egg mortality in LAB and CF strains was 100% after exposure to chlorine dioxide at all four durations, so no ANOVA was conducted. There were no significant differences in

corrected egg mortality from strains of AB1, AB2, and MN strains ($F$ = 0.85; df = 3, 7; $P$ = 0.5099).

[b]Corrected young larva mortality in each strain reached 100% at exposure to all four durations, so ANOVA was not run on data.

[c]Means were based on $n$ = 2.

[d]There were no significant differences observed in the mortality of AB1 strain among four stages after exposed to chlorine dioxide for 2 and 8 h, respectively ($F$ = 3.81 or 1.65; df = 3, 8 or 3, 6; P = 0.0578 or 0.2755).

[e]There were no significant differences observed in the mortality of AB2 strain among five exposure durations within one stage ($F$ = 0.32-3.41; df (eggs and old larvae) = 3, 7 and df (young larvae and pupae) = 3, 8; P = 0.0735-0.8078). In addition, there were no significant differences observed in the mortality of AB2 strain among four stages within each exposure duration (F =1.61-3.64; df = 3, 8, except df (8-h) = 3, 6; P = 0.0638-0.2624).

[f]Corrected mortality of eggs and young larvae was 100% among exposure time. Therefore, data were not subjected to one-way ANOVA.

Table 3.4. Control adult progeny (mean ± SE) of *T. castaneum* laboratory and field strains produced by adults developed from young larvae of control samples[a].

| | Strains | | | | |
|---|---|---|---|---|---|
| | LAB | AB1 | AB2 | CF | MN |
| No. live adults / container (mean ± SE)[b] | 20.0 ± 0.0 | 18.7 ± 0.7 | 20.0 ± 0.0 | 20.0 ± 0.0 | 19.3 ± 0.7 |
| No. adult progeny / container (mean ± SE) | 367.3 ± 28.7 | 865.3 ± 8.3 | 590.0 ± 0.0 | 742.0 ± 0.0 | 498.0 ± 0.0 |

[b]Number of live insects for progeny production.

Table 3.5. Adult progeny (mean ± SE) of *T. castaneum* laboratory and field strains produced by adults developed from old larvae exposed to chlorine dioxide at concentration of 2.02 g/$m^3$ for five durations.

| Strain | Exposure time (h) | No. live adult / container (mean ± SE)[a] | No. adult progeny / container (mean ± SE)[b] |
|---|---|---|---|
| LAB | 0 | 20.7 ± 4.6 | 547.7 ± 150.7a |
| | 2 | 6.7 ± 5.2 | 15.0 ± 15.0b |
| | 4 | 2.7 ± 2.7 | 0b |
| | 6 | 1.0 ± 1.0 | 0b |
| | 8 | 1.5 ± 1.5 | 0b |
| AB1 | 0 | 26.3 ± 6.8 | 323.7 ± 87.5a |
| | 2 | 8.3 ± 5.2 | 112.3 ± 112.3ab |
| | 4 | 3.7 ± 2.3 | 0b |
| | 6 | 2.0 ± 2.0 | 0b |
| | 8 | 1.5 ± 1.5 | 0b |
| AB2[c] | 0 | 27.0 ± 6.5 | 501.0 ± 24.1 |
| | 2 | 6.7 ± 4.8 | 0 |
| | 4 | 1.7 ± 1.7 | 0 |
| | 6 | 2.0 ± 2.0 | 0 |
| | 8 | 2.5 ± 2.5 | 0 |
| CF | 0 | 20.0 ± 3.2 | 588.0 ± 95.2a |
| | 2 | 10.0 ± 4.6 | 266.3 ± 266.3ab |
| | 4 | 1.7 ± 1.7 | 0b |
| | 6 | 1.0 ± 1.0 | 0b |
| | 8 | 1.5 ± 1.5 | 0b |
| MN | 0 | 28.3 ± 6.4 | 625.3 ± 62.1a |
| | 2 | 5.3 ± 3.3 | 206.0 ± 206.0ab |
| | 4 | 2.3 ± 1.5 | 0b |
| | 6 | 1.0 ± 1.0 | 0b |
| | 8 | 1.0 ± 1.0 | 0b |

[a]Number of live insects for progeny production.

[b]Means followed by different lower case letters are significantly different among five durations within one strain. Number of adult progeny per container was significantly different after exposed to chlorine dioxide for various durations within each strain of LAB ($F$ = 18.5; df = 4, 9; $P$ = 0.002), AB1 ($F$ = 6.99; df = 4, 9; $P$ = 0.0076), CF ($F$ = 6.66; df = 4, 9; $P$ = 0.0089), and MN ($F$ = 7.38; df = 4, 9; $P$ = 0.0064). AB2 strain old larvae had no progeny produced after exposed to chlorine dioxide for any duration.

[c]No ANOVA was conducted.

Table 3.6. Progeny (mean ± SE) of *T. castaneum* laboratory and field strains exposed as pupae to chlorine dioxide at concentration of 2.02 g/m$^3$ for various durations.

| Strain | Exposure time (h) | No. live adult /container (mean ± SE)[a] | No. adult progeny /container (mean ± SE)[b] |
|---|---|---|---|
| LAB | 0 | 19.0 ± 1.0 | 592.7 ± 70.9a |
| | 2 | 11.3 ± 3.2 | 33.3 ± 33.3b |
| | 4 | 6.3 ± 0.9 | 0b |
| | 6 | 3.3 ± 0.3 | 0b |
| | 8 | 4.0 ± 0.6 | 0b |
| AB1[c] | 0 | 21.3 ± 1.5 | 683.3 ± 74.8 |
| | 2 | 13.0 ± 3.6 | 0 |
| | 4 | 6.0 ± 1.2 | 0 |
| | 6 | 4.7 ± 0.9 | 0 |
| | 8 | 2.3 ± 1.2 | 0 |
| AB2 | 0 | 20.3 ± 0.7 | 583.0 ± 5.7a |
| | 2 | 12.7 ± 3.3 | 6.3 ± 6.3b |
| | 4 | 2.7 ± 1.5 | 0b |
| | 6 | 3.7 ± 1.5 | 0b |
| | 8 | 3.0 ± 1.2 | 0b |
| CF | 0 | 18.3 ± 2.2 | 469.0 ± 87.0a |
| | 2 | 13.3 ± 2.6 | 13.7 ± 13.7b |
| | 4 | 5.0 ± 1.2 | 0b |
| | 6 | 4.0 ± 1.0 | 0b |
| | 8 | 3.3 ± 0.9 | 0b |
| MN | 0 | 20.0 ± 1.2 | 642.0 ± 113.0a |
| | 2 | 12.0 ± 2.3 | 79.0 ± 79.0b |
| | 4 | 6.3 ± 1.9 | 0b |
| | 6 | 2.3 ± 0.7 | 0b |
| | 8 | 1.3 ± 0.7 | 0b |

[a]Number of live insects for progeny reduction.

[b]Means followed by different lower case letters are significantly different among five durations within one strain. Number of adult progeny per container was significantly different among exposure durations within each strain of LAB ($F = 15.99$; df = 4, 10; $P = 0.0002$), AB2 ($F = 38.50$; df = 4, 10; $P < 0.0001$), CF ($F = 22.25$; df = 4, 10; $P < 0.0001$), and MN ($F = 11.60$; df = 4, 10; $P = 0.0009$). AB1 strain pupae had no progeny produced after exposed to chlorine dioxide for any duration.

[c]No ANOVA was conducted.

# Chapter 4 - Sublethal chlorine dioxide exposure leads to reproductive disruption in *Tribolium castaneum* (Coleoptera: Tenebrionidae) pupae and *Plodia interpunctella* (Lepidoptera: Pyralidae) larvae

## 4.1. Abstract

Fumigation is an effective control treatment implemented in managing stored-product insects for many centuries. Due to increase of phosphine resistance among stored-product insects, chlorine dioxide gas has been proposed as a replacement to phosphine. This study addressed the subleathal concentration of chlorine dioxide gas affected the reproductive performance. Research demonstrated when exposed 3-d pupae of *Tribolium. castaneum* and 5$^{th}$ larval instars of *Plodia interpuntella* at concentration of 0.95 g/m$^3$ (350 ppm) for 3 h. A dramatic reduction was observed in fecundity and egg hatchability when crossed treated females with either treated or untreated male insects for both species. Our finding underlined that fumigation with chlorine dioxide gas using sublethal concentration for short exposure time had significant impact on the reproductive performance of *T. castaneum* and *P. interpunctella* and to restrict the insect population.

## 4.2. Introduction

Sublethal dosage has known to be highly associated with development of resistance (Whiting et al., 1999), however, studies shown it dose may be effective in interfering reproductive performance. Jacob and Qamar (2013) evaluated the lethal effects of fifteen combinations of six essential oils on rice moth, *Corcyra cephalonica*. Fecundity of treated adult females of *C. cephalonica* showed complete suppression at concentration of 1.75% or greater

with any given essential oil combinations (Cedarwood + Eucalyptus, Cedarwood + Peppermint, Cedarwood + Camphor, Cedarwood + Lemongrass, and Cedarwood + Bitterorange). Harburguer et al. (2014) investigated the sublethal effect of pyriproxyfen, an insect growth regulator, on adults of *Aedes aegypti* under laboratory condition. Pyriproxyfen, 2- [1-methyl-2-(4-phenoxyphenoxy) ethoxy] pyridine, was discharged from a fumigant formula of which dosages resulting 20 to 40% emergence inhibition (% EI). Larvae of *Ae. aegypti* were exposed to dosages required to achieve for EI of 20-25% or 40-45%, respectively. Sublethal effects of pyriproxyfen conducted on survived *Ae. aegypti* larvae which later completed adult eclosion. After exposed to EI of 40% ($EI_{40}$), number of eggs produced by each *Ae. aegypti* female (33.7 ± 5.5) showed significant reduction as compared to the control (77.4 ± 1.4) and the egg hatchability in treated group was different from the control by 23.4 ± 2.2 % (Harburguer et al., 2014).

Although several studies have documented the direct impact of chlorine dioxide gas (Channaiah et al., 2012; Kim et al., 2015; Kumar et al., 2015; E et al., 2017 and 2018), limited information was known relative to the sublethal effect of chlorine dioxide gas. Thus, it would be provocative if sublethal doses have impacts on the reproductive performance on stored-product insects. If sublethal doses suppress insect reproduction, then reduction in population could be achieved. The objective of this study was to investigate the impacts of sublethal dose of chlorine dioxide on fecundity and fertility of *T. castaneum* and *P. interpunctella*.

## 4.3. Materials and Methods

### 4.3.1. Insect rearing

*T. castaneum* and *P. interpunctella* were reared in an environmental chamber (Model I-36VL; Percival Scientific, Perry, Iowa) set at 28°C and 65% relative humidity (RH) with a photoperiod of 14:10 (L:D) h. A diet consisting of whole wheat flour (Heartland Mills, Marienthal, Kansas) fortified with 5% (weight by weight) brewer's yeast was used for rearing *T. castaneum* laboratory population. Culture of *P. interpunctella* laboratory population was reared in 0.95-liter Mason jars on a chicken-mash based diet (Subramanyam and Cutkomp 1987).

Pupae of *T. castaneum* (Coleoptera: Tenebrionidae) and larvae of *P. interpunctella* (Lepidoptera: Pyralidae) were used for the experiments tested with chlorine dioxide gas concentration at 350 ppm (0.95 $g/m^3$), and laboratory populations of these two species have never been exposed to insecticides previously. Pupae of *T. castaneum* used in the experiments were from the insect culture reared in stored product entomology laboratory at department of Grain Science and Industry of Kansas State University since 1999. One-day-old pupae were selected for testing because the sexual maturity of *T. castaneum* begins at the early pupal stage according to Sokoloff 1974. Twenty – five-day-old *P. interpunctella* larvae were used in the tests, because the sexual maturity of *P. interpunctella* can be distinguished during larval stage.

### 4.3.2. Sexing *T. castaneum* pupae and *P. interpunctella* larvae

Prepupae of *T. castaneum* were collected from insect cultures and then transferred to petri culture dishes (Pyrex ® 3160, 100 mm diameter × 15 mm height), and kept in the rearing chamber described previously. Prepupae were monitored hourly for pupation, and sexing took place within 8 h as *T. castanuem* pupae by examining the external genitalia using a stereo microscope (Nikon® SMZ 654) (Mahroof et al., 2005). Male and female pupae were placed in

petri dishes and kept in the rearing chamber prior for testing. Ten insects were tested for each replicate with total 3 replicates for each sex, respectively.

To obtain 25-d-old *P. interpunctella* larvae, eggs were collected every 12 h from unsexed adults, which were placed in 0.95-liter glass jars with screen lid and inverted on Petri dishes. Eggs can drop through the screen onto the Petri dish and were transferred into clean 0.95-liter glass jars containing fresh *P. interpunctella* diet and placed in the rearing chamber. *P. interpunctella* eggs hatching took approximately 5 days in general, which was considered as day 0 in this experiment, and 25-d-old larvae were collected accordingly. *P. interpunctella* larvae were obtained from culture jars and segregated by sex, and placed in snap cap vials (23 mm in diameter and 55 mm in height) with caps and bottoms customized with 250-μm-opening stainless steel mesh screen. Male and female larvae were placed in snap cap vials separately, rearing in the rearing chamber till fumigation. Each vial contained 10 insects and 3 replicates total for each sex. The control samples were prepared followed the same procedure and insects were maintained in the growth chamber all the time.

**4.3.3. Insect exposure and reciprocal crosses**

Reproductive effect of chlorine dioxide was assayed on *T. castaneum* and *P. interpunctella*, and the exposure was conducted using gas concentration 0.95 g/m$^3$ for a 2-h duration. A 2-h exposure time used for the test was because such condition was given to provide minimal *ct* product (concentration × time). Experiment was carried out inside of an air-tight polymethyl methacrylate (PMMA) test chamber (0.6 m × 0.6 m × 1.0 m) at 26 °C.

One-day-old male and female pupae were transferred from petri dishes to snap cap vials (diameter × height, 55 × 23 mm) with mesh top and bottom. Each vial held 10 male or female pupae, respectively, and such vials for each sex were exposed to 0.95 g/m$^3$ chlorine dioxide gas

for 2 h at 26 °C. Vials were brought back to the laboratory, and placed on the bench for 30 min to allow chlorine dioxide residues to decompose, and transferred to the rearing chamber set at 28 °C, 65 % RH with a photoperiod of 14:10 (L: D) h. Insects from the control treatment were remained in the rearing chamber (28 °C, 65 % RH) without gas exposure. Mortality was examined 3 d after the gas exposure, and pupae which become dark drown were considered dead and were disposed. The same gas concentration was used for *P. interpunctella* for 30 min of exposure time due to high susceptibility of larvae to chlorine dioxide. Ten 5$^{th}$ instar larvae, male or female, were held in a snap cap vial described above, and 6 such vials were treated with chlorine dioxide gas at 26 °C. Vials that exposed to chlorine dioxide were taken back and placed on the laboratory bench for 30 min to allow decomposition of chlorine dioxide residues, and male and female larvae were transferred into 100-ml plastic jars with 20 g poultry-mash diet, respectively, rearing in the growth chamber.

Reciprocal crosses conducted in *T. castaneum* were using adults emerging from chlorine dioxide-induced (0.95 g/m$^3$) pupae and control groups (without exposure to gas). All possible combinations included: a control male crossed with a control female, a control male crossed with a chlorine dioxide-treated female, a chlorine dioxide-treated male crossed with a control male, and a chlorine dioxide-treated male crossed with a chlorine dioxide-treated female. Each crossed pair was introduced into a snap cap vial (diameter × height, 55 × 23 mm) then covered with a cap that customized with 250-µm-opening stainless steel mesh screen, containing 5 g of 177-µm-opening sifter sieved whole wheat flour. All snap cap vials were maintained at 28 °C, 65 % RH with a photoperiod of 14:10 (L: D). Thirty subsamples were used for each of reciprocal crosses, and experiment was repeated three times. The crosses of *P. interpunctella* utilized adults emerged from chlorine dioxide treated larvae and control larvae, and all possible combinations

included: 20 control males with 20 control females, 20 control males with 20 chlorine dioxide-treated female, 20 chlorine dioxide-treated male with 20 control, and 20 chlorine dioxide-treated male with 20 chlorine dioxide-treated female. Each combination was introduced in to a 0.45-liter glass jar and reared in insect growth chamber.

**4.3.4. Measuring fecundity and hatchability of the eggs**

To determine the effects of chlorine dioxide treatment on the reproductive performance of *T. castaneum* pupae and *P. interpunctella* larvae*,* the number of eggs produced by each mated *T. castaneum* female was counted every 3 d for total 9 d; eggs laid by mated *P. interpunctella* females from each crossed combination were counted daily till no adult moths found alive. When collecting eggs from each pair of *T. castaneum,* the adults were removed from the vials, flour was sieved with a 250-µm-opening sieve to obtain the eggs and these eggs were later transferred on to a glass petri dish and counted under a stereomicroscope (Nikon SMZ 654). After collecting the eggs, each pair of the adults were introduced to a new snap cap vial held 5 g new sieved whole wheat flour (using 177-µm-opening sieve) and maintained at 28 °C, 65 % RH with a photoperiod of 14:10 (L: D) for next egg collection in 3 d. At day 9, the average number of eggs (mean ± SE) was determined across 30 subsamples within one replicate. To obtain *P. interpunctella* eggs, each combination (20 pairs) of adult moths was maintained in a 0.45-liter glass jar closed with a stainless steel sprouting lid, was placed upside-down vertically on a Pyrex ® glass petri dish (diameter × height, 100 × 15 mm). A clean glass petri dish was placed at the lid to gather another batch of eggs the next day. Eggs were harvested daily, counted under a stereomicroscope, and incubated in the growth chamber. When it was no adult moth alive, average number of eggs laid was calculated across total 20 subsamples in one replicate.

Furthermore, the egg hatchability of both insect species was examined after 7 d for each combination by using the number of eggs hatched divided by total number of eggs.

## 4.4. Statistical analysis

The impact of chlorine dioxide gas on number of eggs produced and adult progeny was analyzed by transforming the data to log (x + 1) scale (Zar 1984). Data was subjected to PROC GLM of SAS (SAS Institute 1999) for determination of the differences between stages and treatments in eggs laying and the hatchability. Furthermore, analysis of variance (ANOVA) was used to evaluate any significant differences within four reciprocal crossings in fecundity and hatchability, then means were separated at $\alpha = 0.05$ using REGQW.

## 4.5. Results

Production of offspring was severely affected by chlorine dioxide fumigation of parental *T. castanuem* and there were significant differences found in the means of eggs production among reciprocal crosses in *T. castaneum* ($F = 12.38$; df = 3, 11; $P = 0.0022$) (Figure 1). The range of eggs laid by *T. castaneum* adults that exposed to chlorine dioxide as pupae was 0 to 66 eggs per female per 9 days as the mean of eggs in control group was 102 eggs per female per 9 days. As pupae were induced by chlorine dioxide, mean number of eggs laid by a treated female crossed with an untreated male was approximately 67 % fewer on average as compared to a control female paired with a control male, whereas a 36 % decrease on average number of eggs produced by an untreated female which was crossed with a treated male (Figure. 1). There was no egg laid when a female which exposed to chlorine dioxide paired with a treated male. Statistical differences were found in treated female × control male and treated female × treated male while compared to control female × control male ($P < 0.05$), respectively, it was not significantly different, however, in treated male × control female when compared to mean of

eggs laid by female and male paired from control group ($P > 0.05$). Likewise, regarding number of eggs laid in combinations in 9 days among four combinations of crossing in treatments on *P. interpunctella*, significant differences observed in treated females paired with either treated or untreated males, whereas no statistical difference found in treated males when crossed with untreated females. Number of eggs laid in the control group (20 control females x 20 control males) was 2497 ± 79 on average. Egg numbers (2091 ± 130) were relatively similar laid by 20 untreated females after mating with 20 males that exposed to chlorine dioxide gas as compared to the control group (described above), which did not show a statistical difference as compared to the control group. A great reduction found in mean number of eggs laid when 20 treated females paired with 20 untreated males, which was 258 ± 40. Only 101 ± 13 eggs were collected from 20 treated females that mated with 20 treated males. (Figure 2).

Experiments with *T. castaneum* revealed significant reduction in egg hatchability in which treated pupae completed adult eclosion and produced eggs, compared to the hatchability obtained from the control group that both females and males were not exposed to chlorine dioxide. Significant differences in the mean hatchability rate obtained in all treatments after one week from each batch of eggs produced among the four reciprocal crossings ($F = 277.36$; df = 3, 296; $P < 0.0001$). All reciprocal crossings were statistically different from each other ($P < 0.05$) (Figure 3). The highest hatchability in eggs obtained in control treatment (untreated female x untreated male) was 87 %. Experiment with treated male x untreated female resulted approximately 47% of hatchability on average, and only 38% eggs hatched in treated female x control male treatment. There was no egg hatched due to zero production of eggs in reciprocal crossing of treated female x treated male.

### 4.6. Discussion

The current study in exposure of *T. castaneum* and *P. interpunctella* to 0.95 $g/m^3$ for 3 h disclosed that as a fumigant, chlorine dioxide gas has significant impact on reproductive output and egg hatchability when insects were exposed during their late larval or pupal stage. Exposure to 0.95 $g/m^3$ chlorine dioxide gas revealed adverse effect in *T. castaneum* pupae which was probably because, ovaries and spermatheca reach sexual maturation during early pupal stage (Sokoloff 1974). Smith et al. (1964) suggested that chemicals (chemosterilants) enable to causing sterility in insects may act via one of the following ways: first, chemosterliants may lead insects to fail to produce sperms or ovum; secondly, they may lead inviable of sperms or ovum after having been produced by insects; finally, because of the radiomimetic property, chemosterilant generate dominant lethal mutation or injurious genetic material in sperms and ovum. Sperms and ovum stay alive, however, fertilized ovum does not complete their development.

Chlorine dioxide affects the function of the reproductive system on both sex insects however, in our study females revealed more vulnerability in pupae of *T. castaneum*. Greater impact observed when females were exposed to chlorine dioxide at gas concentration of 0.95 $g/m^3$ as compared to males. Oviposition studies reported that there was no indication of sterility when control female crossed with male that was not exposed to chlorine dioxide. Treated females resulted severe reduction in number of offspring production and egg hatchability when mated with control males, indicating partial sterility. Complete sterility was obtained when reciprocal crossing occurred between treated females treated males. Mahroof et al. (2005) found greater adverse effects in early fecundity, egg-to-adult survival rate, and progeny production when female pupae or adults of *T. castaneum* were exposed to 50 °C. Similar research conducted

in *Cadra cautella* showed that sterility was more pronounced in exposed female pupae than males, using high temperature (45 °C) for 2 h (Arbogast, 1981).

There was significant reduction when *P. interpunctella* old larvae were subjected to chlorine dioxide at concentration of 0.95 g/m$^3$ for 3 h. Research in treating immature stages of some insect species might increase the possibility to induce sterility. Weidhaas (1962) showed that partial or complete sterility was obtained in *Aedes aegypti* in treatment using rearing medium with 10 ppm of chemosterilants, namely tepa or apholate. Fertility was significantly reduced when larvae of screw-worm flies were fed using apholate (25 to 50 ppm) containing medium (Chamberlain, 1962). Furthermore, more that 40 % of sterility produced in screw-worm adults via immersing pupae of different ages in apholate solution (Chamberlain, 1962). Reproductive performance in *T. castaneum* reported by Mahroof (2005) was magnitude of effects in fecundity, egg survival and progeny which they were greater when exposure carried out on pupae than on adults. *T. castaneum* pupae and *P. interpunctella* larvae revealed similar susceptibility to heat treatments. This perhaps contributed to reduced egg production and hatchability although low gas concentration was used in the treatments.

## 4.7. Conclusions

Exposure to chlorine dioxide at concentration of 0.95 g/m$^3$ for 3 h reduced both production and hatchability of eggs of *T. castaneum* and egg production of *P. interpunctella*. Severe effects of chlorine dioxide in both *T. castaneum* and *P. interpunctella* reproductive performance are important in term of increase in population of these insect species after fumigation. Population of these stored product insects is highly related to the reproductive performance of survived insects. Sanitation of storage facilities prior fumigation plays a critical

role in the effectiveness in controlling insects due to bonding property of chlorine dioxide. Thus, the adverse effects might be more prominent as insects survive during fumigation.

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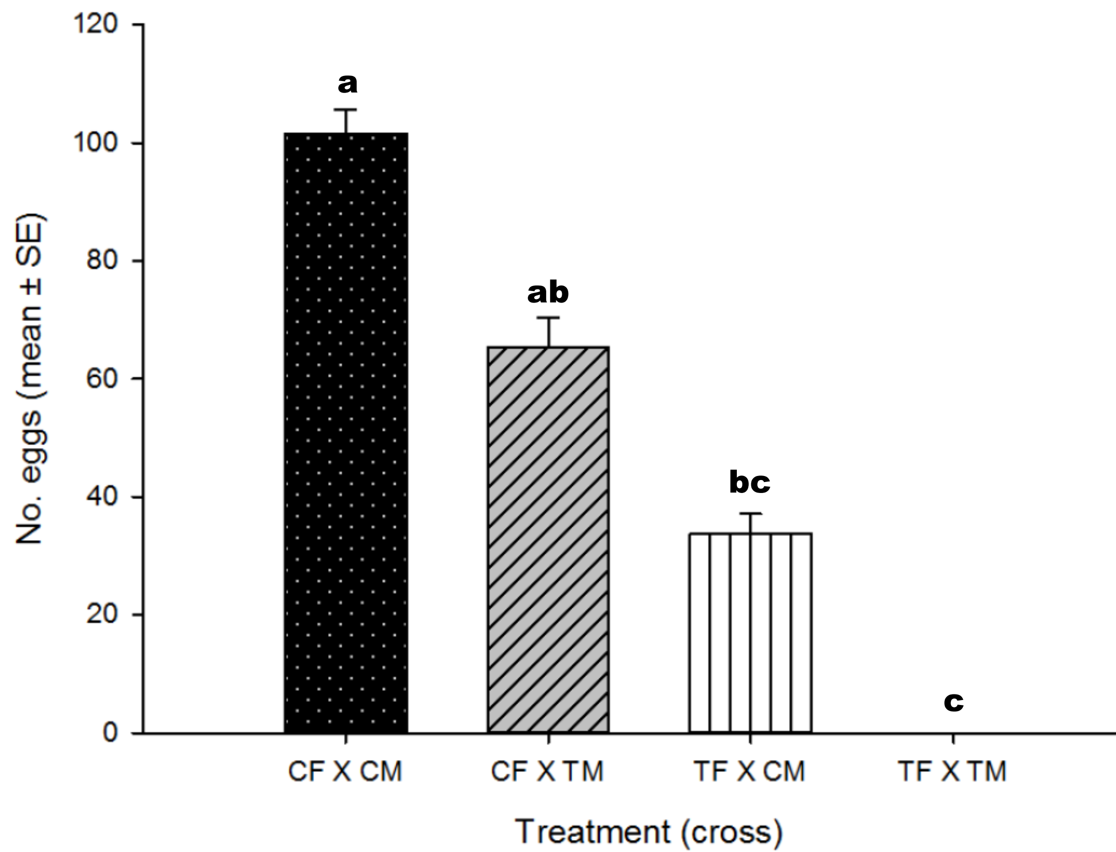


Figure 4.1. Number (mean ± SE) of eggs produced by *T. castaneum* treated with 0.95 g/m$^3$ chlorine dioxide as pupae. Control group was exposed to 28 ºC. CF × CM, control female mated with control male; CF × TM, control female mated with treated male; TF × CM, treated female mated with control male; TF × TM, treated female mated with treated male.

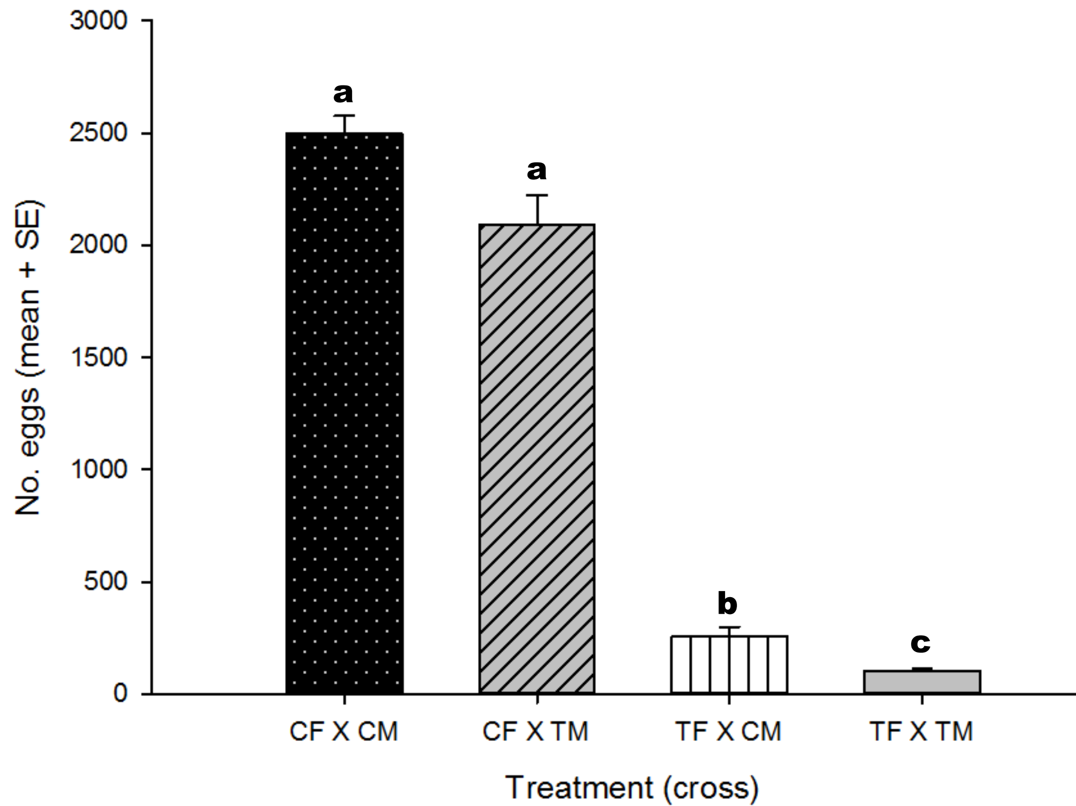


Figure 4.2. Number (mean ± SE) of eggs produced by *P. interpuntellas* treated with 0.95 g/m$^3$ as 5$^{th}$ instars. Control group was exposed to 28 ºC. CF × CM, control female mated with control male; CF × TM, control female mated with treated male; TF × CM, treated female mated with control male; TF × TM, treated female mated with treated male.

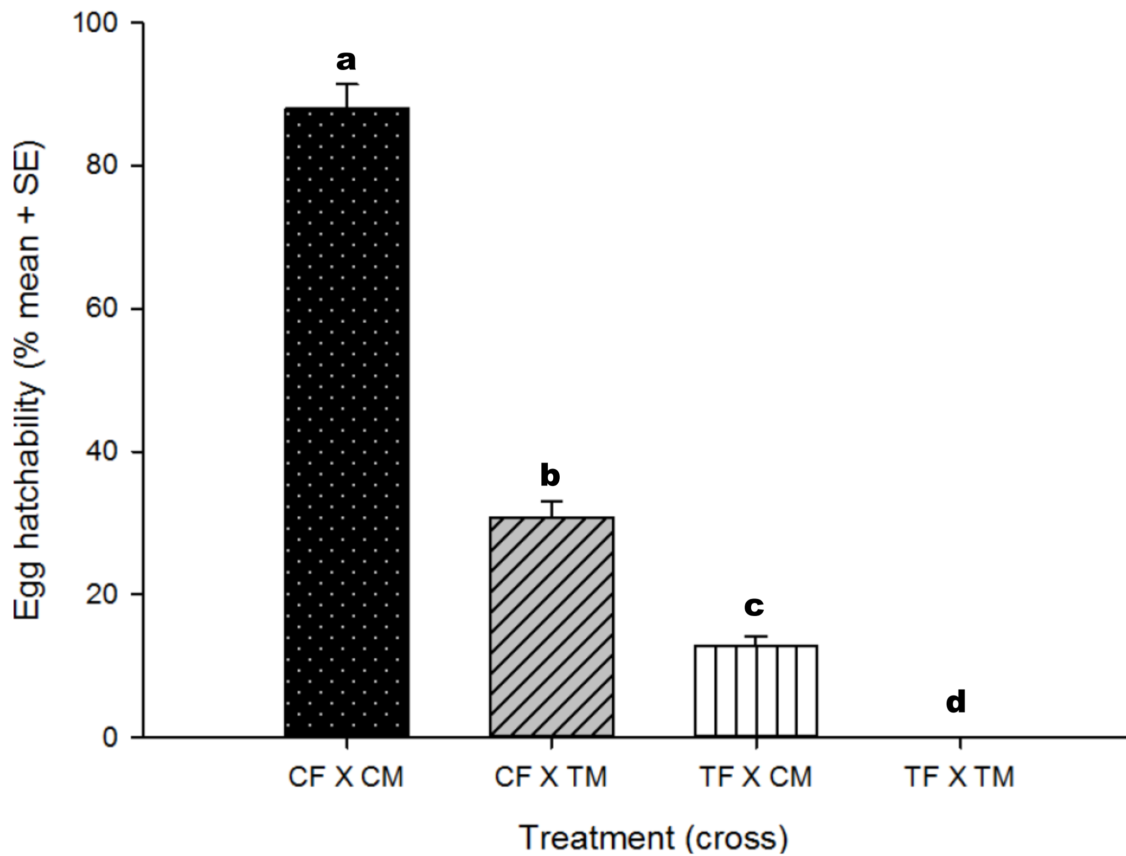


Figure 4.3. Percentage (mean ± SE) of insects which emerged from eggs laid within 9 d by *T. castaneum* treated with 0.95 g/m$^3$ chlorine dioxide as pupae. Control group was exposed to 28 ºC. CF × CM, control female mated with control male; CF × TM, control female mated with treated male; TF × CM, treated female mated with control male; TF × TM, treated female mated with treated male.

# Chapter 5 - Efficacy of ozone at two concentrations against adults of phosphine susceptible and resistant strains of *Rhyzopertha dominica*

## 5.1. Abstract

Ozone was investigated as a potential alternative to control phosphine resistant strains of the lesser grain borer, *Rhyzopertha dominica* (F.). The efficacy of 0.21 and 0.42 g/m$^3$ concentrations of ozone against one phosphine-susceptible laboratory and two phosphine-resistant field strains of *R. dominica* was evaluated. Vials holding 20 adults with 0 and 10 g of wheat were exposed to each ozone concentration for up to 24 h to estimate lethal doses required for 50 ($LD_{50}$) and 99% ($LD_{99}$) mortality. After ozone exposure, mortality was assessed 1 and 5 d later. After exposure to 0.21 g/m$^3$ of ozone for 24 h, the 5-d mortality was 77-86% with wheat and 96-97% without wheat. After exposure to 0.42 g/m$^3$ of ozone for 16 h, the 5-d mortality with and without wheat was 100%. There were no significant differences between $LD_{50}$ values of the samples treated at 0.21 and 0.42 g/m$^3$ regardless of strains and the presence/absence of wheat. The small amount of wheat (10 g) affected efficacy at 0.21 g/m$^3$, but showed no significant effect at 0.42 g/m$^3$. Ozone tends to react with active sites on the surface of wheat kernels prior to reaching an effective concentration for insects. High ozone concentration in the supply air reduced the time to saturate all active sites and ensured that lethal levels of free ozone were available to kill insects. In addition, ozone effectively killed eggs, young larvae, and old larvae of *R. dominica* strains after exposure to a concentration of 0.42 g/m$^3$ for 10 to 34 h.

## 5.2. Introduction

Ozone is a highly oxidative gas, and can inactivate various bacteria and fungi (Khadre et al., 2001, Raila et al., 2006, Wu et al., 2006, Isikber and Athanassiou, 2015). In a confined space, ozone decays into oxygen within minutes to hours, and its half-life time is governed by temperature, gas flow rate, relative humidity, and the availability of reactive sites (Hardin et al., 2010, 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 and poultry (FDA 2001). In addition, ozone has the ability to penetrate a large grain mass, and leaves no residues (Khadre et al., 2001, Raila et al., 2006, Isikber and Athanassiou, 2015). Grain quality, including nutrient profiles, milling and baking performance were affected marginally after exposure to ozone (Mendez et al., 2003). These merits make ozone a potential candidate as an alternative to phosphine, a fumigate that has been used for decades to manage pest in stored-products.

The lesser grain borer, *Rhyzopertha dominica* (F.), is a common stored-product insect pest found in many countries of the world (Hagstrum and Subramanyam, 2009). This pest has been found in natural habitats, but it can easily migrate to agricultural areas due to its ability to fly (Heaps 2005, Hagstrum and Subramanyam 2009, Edde, 2012). As an internal feeder, both larvae and adults feed on germ and endosperm of grain kernels causing severe kernel damage (Chanbang et al., 2008, Edde, 2012). Phosphine has been used as a predominant fumigant to control *R. dominica* in raw agricultural commodities. As a result, resistance in *R. dominica* has been detected in many regions of the world (Benhalima et al., 2004, Song et al., 2011, Opit et al., 2012, Kaur et al., 2015).

Ozone has been used to control *R. dominica* in a few laboratory and pilot scale studies (Athanassiou et al., 2008, Sousa et al., 2008, Bonjour et al., 2011, Hansen et al., 2012, Silva et al., 2016). *R. dominica* adults were placed in columns of 70-cm high and 8-cm diameter along with maize or wheat to test the efficacy of ozone at two concentrations, 0.12 and 0.24 g/m$^3$ (Athanassiou et al., 2008). Complete mortality was achieved after a 6-h exposure to 0.24 g/m$^3$ of ozone throughout the column of wheat or maize. However, at a concentration of 0.12 g/m$^3$, less than 30% of mortality was observed after an 8-h exposure. Sousa et al. (2008) reported that the time to kill 95% of *R. dominica* adults ($LT_{95}$) exposed to 0.32 g/m$^3$ of ozone ranged from 21.85 to 37.32 h. In another study, complete mortality of *R. dominica* adults was observed after exposure to an ozone concentration of 0.05 g/m$^3$ for 4 d (Bonjour et al., 2011). Hansen et al. (2012) reported complete mortality of eggs, young larvae, medium-aged larvae, pupae, and adults after exposure to an ozone concentration of 0.065 g/m$^3$ for 6 d, 0.281 g/m$^3$ for 8 d, 0.281 g/m$^3$ for 8 d, 0.074 g/m$^3$ for 6 d, and 0.074 g/m$^3$ for 6 d, respectively. In wheat, Silva et al. (2016) reported zero instantaneous growth rate of *R. dominica* after exposed to 1.61 g/m$^3$ of ozone for 17 h.

In this study, the efficacy of ozone at concentrations of 0.21 and 0.42 g/m$^3$ against *R. dominica* adults was investigated. Both phosphine susceptible and resistant strains were exposed to ozone for various durations with and without wheat. In addition, eggs, young larvae, old larvae, and pupae of *R. dominica* were exposed to 0.42 g/m$^3$ of ozone for different durations to test its efficacy against these pre-adult stages. Progeny production from both adults and pre-adult stages was assessed to evaluate the effect of ozone on progeny suppression.

## 5.3. Material and methods

### 5.3.1. Insect Cultures

Culture of *R. dominica* was reared on organic hard red winter wheat (Heartland Mills, Marienthal, Kansas, USA) at 28°C and 65% RH in an environmental growth chamber (Model I-36 VL; Percival Scientific, Perry, IA, USA). Unsexed adults of mixed ages were collected directly from culture jars after sifting the culture through a 707-µm opening square-holed sieve (Seedburo Equipment Company, Chicago, IL, USA). Phosphine resistance of two field strains was verified following a discriminating dose test (Champ and Dyte, 1976). Insects were exposed to a phosphine concentration of 0.027 g/m$^3$ for 20 h, and mortality was assessed after 14 d to score insects as either susceptible or resistant to phosphine. The survival rates of the laboratory strain (LAB), and the field strains (CS and FL) were 0, 64.4, and 84.0%, respectively. This indicated that the laboratory strain was susceptible to phosphine while the two field strains were resistant to phosphine (Opit et al., 2012).

### 5.3.2. Ozone Generation

Ozone was generated by a custom-built corona discharge ozone generator (O3Co, Idaho Falls, ID, USA) with a capacity of 2.5 g/h, and monitored by a gas analyzer (IN2000-L2-LC, IN USA, Norwood, MA, USA). The ozone concentration in the testing chamber was recorded every minute and acquired by a program written in LABVIEW (National Instruments, Austin, TX, USA). The temperature and relative humidity of the chamber was monitored by microprocessor-based temperature sensors (HOBO® data logger Model U10-003, Onset Computer Corp, MA, USA). The average temperature for the test was 26.9 ± 0.2°C, and the minimum and maximum temperatures observed during the test period ranged from 20.9 to 33.3°C. The average humidity during tests was 23.3 ± 0.4%, and the minimum and maximum humidity levels observed ranged

from 15.0 to 36.8%. The air flow rate was 0.02 $m^3$/min. The two ozone concentrations tested were 0.21 (100 ppm) and 0.42 g/$m^3$ (200 ppm).

### 5.3.3. Bioassays with Adults

Ozone exposure was conducted in an air-tight polymethyl methacrylate (PMMA) chamber (0.50 m × 0.35 m × 0.35 m). Bioassays were carried out in snap cap vials (23 mm in diameter and 55 mm in height), which have mesh bottoms and caps (250 µm opening) to ensure good penetration of ozone through vials and prevent insects escape. Each vial had 20 unsexed adults of mixed-ages along with 0 and 10 g of wheat during ozone exposure. Vials were exposed to each ozone concentration for 4, 6, 8, 10, 12, 14, 16, 18, and 24 h. After exposure, vials were brought back to the laboratory, and kept in an environmental growth chamber at the rearing condition. Vials without wheat received 10 g of wheat prior to incubation in the growth chamber. Mortality was assessed 1 and 5 d after ozone exposure. In previous tests (Subramanyam et al., 2017), we observed delayed toxic effects of ozone, and mortality tended to become stable on day 5. Therefore, the end-point mortality was determined 5 d after incubation. Control vials were handled similarly with no ozone exposure. Live and dead adults were counted by gently prodding adults with a camel's hair brush. Insects that failed to move were considered dead. Mortality was expressed as a percentage based on number of dead adults out of the total exposed. After mortality assessment, live and dead insects were placed in vials which were then held at rearing conditions for 42 d to count number of adult progeny produced. There were five replicates for each combination of strain, ozone concentration, and exposure time.

The vials with same dimensions as those used in adult bioassay were used to hold eggs, young larvae, old larvae, and pupae of *R. dominica*. Each vial contained 10 g of organic wheat and infested with 20 unsexed adults of mixed ages of each *R. dominica* strain. Infested vails were

kept under rearing conditions for 3 d, after which all adults were removed from vials. The infested vials with eggs were treated with ozone immediately (day 0); vials containing young larvae, old larvae, and pupae were treated 14, 21, and 28 d after day 0, respectively. We followed the procedure reported by Khamis et al. (2010) that documented which life stages were present based on X-ray examination of infested kernels. Eggs were exposed to an ozone concentration of 0.42 $g/m^3$ for 24, 48, and 72 h; young larvae were exposed for 10, 24, and 34 h; old larvae were exposed for 10, 24, and 34 h, and pupae were exposed for 2, 6, and 10 h. Control treatment consisted of vials not exposed to ozone (0 h treatment). Vials after exposure were held in a growth chamber at rearing condition until emergence of adults from each life stage. Adult emergence in ozone-exposed and unexposed vials of eggs, young larvae, old larvae, and pupae was examined after 35, 21, 14, and 7 d, respectively. Each strain, stage, and treatment time combination was replicated five times.

## 5.4. Data Analysis

Mortality of *R. dominica* adults was expressed as a percentage, and mortality of treated insects was corrected based on control mortality (Abbott, 1925). The corrected 5 d mortality was subjected to probit regression (SAS Institute 2008) to determine the time-concentration product (dosage) resulting 50% ($LD_{50}$) and 99% ($LD_{99}$) mortality. The $LD_{50}$ values were compared by ratio tests to determine the effects of phosphine resistance, food availability, and ozone concentrations on the efficacy of ozone exposure (Robertson and Preisler, 1992). If the 95% confidence interval (CI) for the ratio included one, the difference between the pairs being compared was not significant ($P > 0.05$) (Robertson and Preisler, 1992).

The percent reduction in adult *R. dominica* progeny production relative to production in control was calculated as: [1-(progeny in treated samples/progeny in control samples)] × 100.

The percentage reduction was subjected to probit regression analysis to determine the dosages for 50% ($ED_{50}$) and 99% ($ED_{99}$) reduction in adult progeny production.

Percent reduction in adult emergence from each pre-adult stage exposed to ozone relative to the control was calculated as: [1 – (number of adults that emerged in ozone exposed vials/number of adults that emerged in control vials)] × 100. The percent reduction data were transformed to angular values and data by stage were subjected to two-way analysis of variance to determine significant main and interactive effects among strains and exposure times (SAS Institute, 2008). In addition, one-way analysis of variance was used to determine significant difference in the percentage of adult emergence reduction among different strains at each exposure duration for each stage, and means were separated using Bonferroni *t*-tests at $\alpha=0.05$ (SAS Institute, 2008).

## 5.5. Results

The mortality of adults of three *R. dominica* strains is shown in Fig. 5.1. In most cases, control mortality for all three strains ranged from 0 to 8.9 ± 2.4%, irrespective of presence or absence of wheat. However, the mean ± SE control mortality of CS and FL strains was 11.9 ± 3.6% and 23.7 ± 1.1%, respectively, for the ozone treatment at 0.21 $g/m^3$. For treated samples, more insects died as the ozone exposure time increased. Mortality of LAB, CS, and FL strains was less than 30% in the vials without wheat after exposure to 0.21$g/m^3$ ozone for 4 h, and the corresponding mortality after a 24-h exposure ranged from 96.0 ± 2.4 to 97.0 ± 3.0% (Fig. 5.1A). When 10 g of wheat was present during the 24-h ozone exposure (Fig. 5.1C), the mortality of three strains ranged from 77.4 ± 2.2 to 86.3 ± 3.7%, indicating a negative effect of wheat on the mortality. Results of the exposure to 0.42 $g/m^3$ of ozone showed a similar trend, but required less time to reach the complete mortality (Fig. 5.1B and 5.1D). For example, in vials exposed to

ozone for 16 h without wheat, adult mortality of LAB, CS, and FL strains was 100%. In vials with 10 g of wheat, to obtain 100% mortality, a 24-h ozone exposure was required.

Probit analysis to estimate the lethal doses (*ct*) to kill 50 and 99% of insects was shown in Tables 1 and 2. After exposure to 0.21 g/m$^3$ of ozone with 0 and 10 g of wheat, the range of $LD_{99}$ values of three strains were 5.77-8.45, and 12.05-40.43 g-h/m$^3$, respectively. The corresponding values when exposed to 0.42 g/m$^3$ of ozone were 6.65-9.86, and 7.82-9.48 g-h/m$^3$, respectively.

The efficacy of two ozone concentrations is shown in Table 5.3, where $LD_{50}$ values obtained by exposure to 0.21 and 0.42 g/m$^3$ of ozone were compared. In the presence of wheat, $LD_{50}$ of three strains showed no significant difference at two ozone concentrations, which indicated that the dosage (*ct*, the product of concentration × time) to kill 50% of insects was consistent at ozone concentration of 0.21 and 0.42 g/m$^3$. The range of $LD_{50}$ values in the presence of wheat at two concentrations was 3.28 to 4.59 g-h/m$^3$. When exposed to ozone without wheat, $LD_{50}$ generated by exposure to 0.42 g/m$^3$ of ozone showed slightly higher values than those exposed to 0.21 g/m$^3$ of ozone. In the absence of wheat, $LD_{50}$ values ranged from 1.82 to 3.38 g-h/m$^3$, which was comparable to the values reported by Sousa et al. (2008) (2.92-3.86 g-h/m$^3$) when they exposed 20 unsexed adults without food to 0.32 g/m$^3$ of ozone.

The effect of wheat on the efficacy of ozone is summarized in Table 5.4. Comparisons of $LD_{50}$ values between with and without wheat for all three strains at both ozone concentrations were significant based on the ratio tests. Fewer insects died in the presence of wheat compared to those without wheat at both ozone concentrations.

The response of phosphine susceptible and resistant strains to ozone was compared by ratio tests (Table 5.5). In most cases, LAB, the susceptible strain, showed either higher or similar $LD_{50}$ values as compared to the resistant CS and FL strains. Except at 0.21 g/m$^3$ of ozone, the CS

strain had significantly higher $LD_{50}$ value than the LAB strain with and without wheat, indicating that the CS strain was less susceptible to ozone. The LAB and FL strains showed similar responses to ozone. These results suggest that phosphine resistant strains of *R. dominica* are susceptible to ozone.

After exposure to an ozone concentration of 0.21 g/m$^3$ for 24 h with wheat, progeny reduction for LAB, CS, and FL strains was 43.5, 75.4, and 82.9%, respectively (Table 5.6). The low progeny reduction may attribute to the low mortality when assessments were made 1 d after exposure (40.2, 42.5, and 55.0% for LAB, CS, and FL strains, respectively). By contrast, after exposure to an ozone concentration of 0.42 g/m$^3$, the 1-d mortality was relatively high (greater than 92.2%), which led to more significant progeny reduction (73.1 to 100%). Interestingly, the 1-d mortality for CS strain after exposure to 0.42 g/m$^3$ of ozone for 24 h was 100%, however the progeny reduction was only 73.1 ± 9.5%. In vials with wheat, $ED_{50}$ values of LAB, CS, and FL strains of *R. dominica* after exposure to the ozone concentration of 0.21 g/m$^3$ were 47.98, 3.68, and 2.36 g-h/m$^3$, respectively (Table 5.7). When wheat was absent during ozone exposure, lower $ED_{50}$ values were required for the three strains (0.96-1.72 g-h/m$^3$). $ED_{50}$ values were lower when insects were exposed to an ozone concentration of 0.42 g/m$^3$ (Table 5.8). For example, in the presence of wheat, $ED_{50}$ values for LAB, CS, and FL strains were 3.20, 0.21, and 1.31 g-h/m$^3$, respectively. In the absence of wheat, corresponding values were 0.81, 1.06, and 0.98 g-h/m$^3$.

Eggs, young larvae (14-d old), old larvae (21-d old), and pupae (28-d old) of laboratory and field strains of *R. dominica* were exposed to 0.42 g/m$^3$ of ozone for 2 to 72 hours (Table 5.9). Young larvae were more susceptible to ozone exposure than the other stages, and the reduction in adult emergence among strains and exposure durations was 98 to 100%. Two-way ANOVA results indicated that the reduction in adult emergence from eggs exposed to ozone was

similar among strains ($F$ = 0.51, df = 2, 36, $P$ = 0.6068), but was significantly different among exposure durations ($F$ = 43.98, df = 2, 36, $P < 0.0001$). The interaction of strain and exposure duration also showed significant effect on the reduction of adult emergence from eggs ($F$ = 2.87, df = 4, 36, $P$ = 0.0366). Reduction in adult emergence from young larvae was not significantly different among strains ($F$ = 1.97; df = 2, 36; $P$ = 0.1536), exposure durations ($F$ = 1.21; df = 2, 36; $P$ = 0.3101), and the interaction of strains and exposure durations was not significant ($F$ = 0.85; df = 4, 36; $P$ = 0.5027). In the case of old larvae, significant differences were observed among strains, exposure durations, and the interaction of strain and exposure duration ($F_{range}$ = 10.44 – 69.07; df = 2, 36 for strain and exposure duration, and 4, 36 for interaction; $P \leqslant 0.0003$). Differences in adult emergence from pupae were significant among strains, exposure durations, and strains and exposure durations interaction ($F_{range}$ = 3.49 – 30.19; df = 2, 36 for strain and exposure duration and 4, 36 for the interaction; $P_{range}$ = 0.0197 - <0.0001). Complete reduction of adult emergence was observed for eggs, young larvae, and old larvae of all three strains after exposure to 0.42 g/m$^3$ of ozone for 72, 34, and 34 h, respectively. For pupae, the maximum reduction was observed after a 10-h exposure for the LAB (32.8%), CS (54.4%), and FL (96.6%) strains.

## 5.6. Discussion

Ozone is an excellent oxidizing agent, and reacts with microorganisms, dust, organic compounds on the surface of wheat kernels (Kells et al., 2001, Hardin et al., 2010). It is plausible that insects in vials with 10 g of wheat were not exposed to 0.21 g/m$^3$ of ozone immediately, instead the ozone concentration around them gradually increased to 0.21 g/m$^3$ after the active sites on kernels were saturated. However, when higher ozone concentration was used during the exposure, a quicker kill was observed regardless of the presence or absence of wheat. Under

current experimental setup, complete mortality can be achieved for all three strains after a 24-h exposure to ozone at a concentration of 0.42 g/m$^3$.

Chi-squares ($\chi^2$) for goodness-of-fit tests were significant for all probit regressions, indicating poor fit of model to data. Heterogeneity can be the major contributor to the poor fit (Mahroof et al., 2003; Subramanyam et al., 2014). Insects used in tests were unsexed adults of mixed-age, and differences in sex and age may have contributed to the heterogeneity in responses observed here. In addition, ozone concentrations and exposure durations in vials located at different spots in the testing chamber varies and contributes to the heterogeneity. In these cases, variances and covariances were adjusted by a heterogeneity factor (the ratio of Chi-square and the degree of freedom), and a critical value from the *t* distribution was used to compute the confidence limits (Subramanyam et al., 2014).

$LD_{50}$ of LAB and CS strains exposed to 0.21 g/m$^3$ of ozone without wheat was significantly lower than those exposed to the ozone concentration of 0.42 g/m$^3$ (1.82 vs. 3.15 and 2.08 vs. 2.45 g-h/m$^3$), indicating that these two strains were more susceptible to the combination of low ozone concentration and longer exposure duration compared to the combination of a shorter exposure duration and a high ozone concentration. However, with only two concentrations tested, this conclusion may not be extrapolated to other concentration regimes. LD values for some fumigants may stay same within a certain concentration regime, but can be different at other concentrations. Winks (1984) studied the relation between phosphine concentrations and LD values. $LD_{50}$ value decreased from 0.35 to 0.179 mg-h/L when phosphine concentration increased from 0.0016 to 0.0038 mg/L; however, it stayed in the range of 0.171 to 0.197 mg-h/L when the concentration increased from 0.0038 to 0.01 mg/L.

As ozone enters vials, it reacts with active sites on the surface of wheat kernels, resulting its simultaneous decomposition (Kells et al., 2011). Once most sites were saturated, free ozone started to accumulate and reach to a concentration lethal for insects. Athanassiou et al. (2008) exposed 70-cm tall columns filled with or without wheat at an ozone concentration of 0.24 g/m$^3$. The mortality of *R. dominica* adults without wheat reached 100% at all sampling points in the column after a 6-h exposure. However, when filled with wheat, after a 6-h exposure, sampling points away from the ozone entering opening (at the bottom of the column) only had 89-96% mortality, compared to the sampling points close to the bottom of the column where 100% mortality was observed. Wheat kernels appeared to restrict the penetration of ozone and lead to the decrease of insect mortality.

Adults surviving ozone exposure were able to lay eggs during incubation in the growth chamber. Even though more adults died when mortality assessments were made on day 5, eggs laid during days 1 through 5 by adults, survived to adulthood. Therefore, the reductions in adult progeny production after exposure to ozone at two concentrations for various hours, respectively, were not 100%, especially to the ozone concentration of 0.21 g/m$^3$ with a few exceptions. Another reason for eggs to successfully develop to adults could be attributed to ozone not having any residual effect (FDA 2001) when vials were incubated after ozone exposure.

Since mortality was checked 24 h after the exposure, it is plausible that there were survivors who laid eggs before the 24 h mortality checking point, and eggs developed to adults later on. Additionally, as Table 6 shown, the adult emergence reduction from eggs for CS was only 64.4 ± 3.6% after exposure to ozone at 0.42 g/m$^3$ for 24 h, which indicated that eggs laid in vials prior to the ozone exposure can also develop into adults later on. In addition, progeny reduction results indicated that a lower dosage was required to reduce progeny production by

50% ($ED_{50}$) compared to dosage require for 50% of mortality ($LD_{50}$), especially when exposed to 0.42 g/m$^3$ vs. 0.21 g/m$^3$ of ozone. Sehgal et al. (2013) using spinosad on wheat showed that the $ED_{50}$ values for one laboratory and two field strains of *O. surinamensis* and *R. dominica* were lower than corresponding $LD_{50}$ values. The $ED_{50}$ for laboratory and field strains of *O. surinamensis* were 0.56-0.85 mg (AI)/kg of grain whereas the $LD_{50}$ for these strains ranged from 0.64-2.86 mg (AI)/kg. Similarly, for *R. dominica* $ED_{50}$ values ranged from 0.007 to 0.009 mg (AI)/kg and the $LD_{50}$ values for one laboratory and two field strains was 0.011 mg (AI)/kg.

Young larvae were most susceptible to ozone exposure, and complete reduction of adult emergence (100%) was observed after the 10-h exposure, followed by old larvae and eggs which required a 24- and 72-h exposure, respectively, to achieve 100% reduction. Pupae of these three strains were only exposed to ozone for up to 10 h, and adult emergence reduction observed among strains ranged from 32.8 to 96.6%. Differences in responses of life stages to ozone could be related to their respiration rates. Pimentel et al. (2007) found that lower respiration rates were related to higher phosphine resistance in adults of *T. castaneum*, *R. dominica*, and *O. surinamensis*. It seems that at low respiration rates, the up-take of ozone or phosphine by insects was less, which induced less susceptibility to the gas. At normal atmosphere, the respiration rate of eggs, young and old larvae, and pupae of *R. dominica* was 0.0029, 0.41, 2.52, and 0.82 µl $CO_2$/insect/h, respectively (Emekci et al. 2004). The adult emergence results here indicated that eggs were the most tolerant stage to ozone fumigation, followed by pupae and old larvae, which generally corresponded to their respiration rates as observed by Emekci et al. (2004).

## 5.7. Conclusions

Ozone exposure can result complete the mortality of *R. dominica* within 24 h with or without wheat. $LD_{50}$ values remain constant between two concentrations (0.21 and 0.42 $g/m^3$). Therefore, in situations where time is a constraint, 0.42 $g/m^3$ of ozone is recommended for use, since the treatment time to obtain 50% mortality can be reduced by half compared to 0.21 $g/m^3$ of ozone. However, when treating larger quantity of wheat, and higher ozone concentration is difficult to maintain, and in such situation use of 0.21 $g/m^3$ of ozone is more practical, but the fumigation time has to be extended by one fold. Pre-adult stages of *R. dominica* can be controlled by exposure to ozone, and the ozone dosage required was significantly higher than those required for controlling the adults. The survivors after ozone exposure were able to lay eggs which subsequently developed into viable adults, since ozone has short half-life time and does not leave any residues on the grain surface. To ensure zero infestation in grain after the treatment, ozone exposure should be carried out until complete mortality is achieved.

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Table 5.1. Probit regression estimates and dosages required for 50 and 99% mortality for laboratory and field strains of *R. dominica* exposed to 0.21 g/m$^3$ of ozone with 0 and 10 g of wheat.

| Strain | Wheat (g) | $N^a$ | Mean ± SE | | Lethal Dose (g-h/m$^3$, 95% CI) | | $\chi^2$ (df)$^b$ |
|---|---|---|---|---|---|---|---|
| | | | Intercept | Slope | $LD_{50}$ | $LD_{99}$ | |
| LAB | 0 | 800 | -0.91 ± 0.13 | 3.50 ± 0.30 | 1.82 (1.65-1.99) | 8.45 (6.87-11.28) | 276.75 (38) |
| CS | 0 | 800 | -1.67 ± 0.19 | 5.26 ± 0.45 | 2.08 (1.93-2.22) | 5.77 (5.02-6.98) | 307.81 (38) |
| FL | 0 | 800 | -4.18 ± 0.33 | 8.25 ± 0.66 | 3.21 (3.08-3.34) | 6.14 (5.55-7.04) | 256.77 (38) |
| LAB | 10 | 800 | -2.18 ± 0.34 | 4.17 ± 0.68 | 3.33 (2.95-3.89) | 12.05 (8.33-24.79) | 866.86 (38) |
| CS | 10 | 800 | -1.13 ± 0.18 | 2.15 ± 0.38 | 3.34 (2.81-4.28) | 40.43 (19.35-188.54) | 542.58 (38) |
| FL | 10 | 800 | -1.85 ± 0.23 | 2.99 ± 0.46 | 4.59 (3.91-5.97) | 25.05 (14.78-67.74) | 512.58 (38) |

$^a$*N* = total number of insects used in generating the probit regression estimates.

$^b\chi^2$ values for goodness-of-fit of model to data were significant ($P < 0.0001$), indicating poor fit of model to data.

Table 5.2. Probit regression estimates and dosages required for 50 and 99% mortality for lab and field strains of *R. dominica* exposed to 0.42 g/$m^3$ of ozone with 0 and 10 g of wheat.

| Strain | Wheat (g) | $N$[a] | Mean ± SE | | Lethal Dose (g-h/$m^3$, 95% CI) | | $\chi^2$ (df)[b] |
|---|---|---|---|---|---|---|---|
| | | | Intercept | Slope | $LD_{50}$ | $LD_{99}$ | |
| LAB | 0 | 800 | -3.04 ± 0.25 | 6.10 ± 0.44 | 3.15 (2.96-3.33) | 7.57 (6.79-8.70) | 267.12 (38) |
| CS | 0 | 800 | -2.08 ± 0.26 | 5.36 ± 0.49 | 2.45 (2.22-2.65) | 6.65 (5.8-8.05) | 346.24 (38) |
| FL | 0 | 800 | -2.65 ± 0.28 | 5.01 ± 0.55 | 3.38 (3.14-3.67) | 9.86 (7.9-13.95) | 132.58 (38) |
| LAB | 10 | 800 | -3.53 ± 0.30 | 6.40 ± 0.46 | 3.56 (3.34-3.76) | 8.20 (7.46-9.26) | 214.47 (38) |
| CS | 10 | 800 | -3.19 ± 0.33 | 6.18 ± 0.52 | 3.28 (3.04-3.52) | 7.82 (6.98-9.12) | 307.87 (38) |
| FL | 10 | 800 | -3.71 ± 0.35 | 6.17 ± 0.52 | 3.98 (3.72-4.22) | 9.48 (8.44-11.12) | 303.74 (38) |

[a]$N$=total number of insects used in generating the probit regression estimates.

[b]$\chi^2$ values for goodness-of-fit of model to data were significant ($P < 0.0001$), indicating poor fit of model to data.

Table 5.3. Comparison of $LD_{50}$ values for *R. dominica* adults between 0.21 and 0.42 g/m$^3$ of ozone.

| Strain | Wheat (g) | $LD_{50}$ (g-h/m$^3$, 95% CI)[a] | | Ratio (95% CI) |
|---|---|---|---|---|
| | | 0.21 g/m$^3$ ozone | 0.42 g/m$^3$ ozone | |
| LAB | 10 | 3.33 (2.95-3.89) | **3.56** (3.34-3.76) | 1.07 (0.96-1.19) |
| CS | 10 | **3.34** (2.81-4.28) | 3.28 (3.04-3.52) | 1.00 (0.83-1.20) |
| FL | 10 | **4.59** (3.91-5.97) | 3.98 (3.72-4.22) | 1.05 (0.87-1.26) |
| LAB | 0 | 1.82 (1.65-1.99) | **3.15** (2.96-3.33) | 1.74 (1.53-1.98)* |
| CS | 0 | 2.08 (1.93-2.22) | **2.45** (2.22-2.65) | 1.17 (1.04-1.33)* |
| FL | 0 | 3.21 (3.08-3.34) | **3.38** (3.14-3.67) | 1.05 (0.96-1.14) |

[a]$LD_{50}$ values in bold are greater in the pair being compared.

*Significant ($P < 0.05$).

Table 5.4. Comparison between $LD_{50}$ values of *R. dominica* adults with 0 and 10 g of wheat when exposed to 0.21 or 0.42 $g/m^3$ of ozone.

| Strain | Ozone ($g/m^3$) | Ratio (95% CI)[a,*] |
|---|---|---|
| LAB | 0.21 | 1.82 (1.56-2.13) |
| CS | 0.21 | 1.58 (1.30-1.93) |
| FL | 0.21 | 1.29 (1.08-1.53) |
| LAB | 0.42 | 1.12 (1.03-1.22) |
| CS | 0.42 | 1.35 (1.21-1.51) |
| FL | 0.42 | 1.17 (1.07-1.30) |

[a]$LD_{50}$ values were greater when there was wheat present during ozone exposure.

*Significant ($P < 0.05$).

Table 5.5. Comparison of $LD_{50}$ values between phosphine susceptible and resistant strains of *R. dominica* adults exposed to 0.21 or 0.42 g/m$^3$ of ozone with 0 and 10 g of wheat.

| Strain[a] | Ozone (g/m$^3$) | Wheat (g) | Ratio (95% CI) |
|---|---|---|---|
| CS vs. LAB | 0.21 | 10 | 1.26 (1.05-1.51)* |
| FL vs. LAB | 0.21 | 10 | 1.00 (0.82-1.23) |
| CS vs. LAB | 0.21 | 0 | 1.78 (1.54-2.05)* |
| FL vs. LAB | 0.21 | 0 | 1.15 (0.98-1.35) |
| Lab vs. CS | 0.42 | 10 | 1.12 (1.03-1.23)* |
| FL vs. LAB | 0.42 | 10 | 1.07 (0.96-1.19) |
| FL vs. LAB | 0.42 | 0 | 1.07 (0.98-1.17) |
| LAB vs. CS | 0.42 | 0 | 1.29 (1.18-1.41)* |

[a]Strain mentioned first has a higher $LD_{50}$ value.

*Significant ($P < 0.05$).

Table 5.6. Mean ± SE mortality (%) and adult progeny reduction (%) of three strains of *R. dominica* adults after 24 h of exposure to ozone at 0.21 and 0.42 $g/m^3$.

| Ozone Concentration ($g/m^3$) | Strain | Wheat (g) | Mean ± SE mortality (%) | | Mean ± SE progeny reduction (%) |
|---|---|---|---|---|---|
| | | | Day 1 | Day 5 | |
| 0.21[a,b] | LAB | 10 | 40.2 ± 7.2 | 78.1 ± 2.9 | 43.5 ± 7.4 |
| | | 0 | 83.9 ± 4.0 | 97.0 ± 2.0 | 96.8 ± 1.5 |
| | CS | 10 | 42.5 ± 2.7 | 86.3 ± 3.7 | 75.4 ± 5.7 |
| | | 0 | 75.6 ± 4.7 | 97.0 ± 3.0 | 93.6 ± 2.3 |
| | FL | 10 | 55.0 ± 2.2 | 77.4 ± 2.2 | 82.9 ± 5.4 |
| | | 0 | 81.2 ± 1.7 | 96.0 ± 2.4 | 96.6 ± 1.5 |
| 0.42[c,d] | LAB | 10 | 92.2 ± 2.9 | 100.0 ± 0 | 88.2 ± 6.3 |
| | | 0 | 98.9 ± 1.1 | 100.0 ± 0 | 97.3 ± 0.9 |
| | CS | 10 | 100.0 ± 0 | 100.0 ± 0 | 73.1 ± 9.5 |
| | | 0 | 100.0 ± 0 | 100.0 ± 0 | 100.0 ± 0 |
| | FL | 10 | 99.1 ± 0.9 | 100.0 ± 0 | 89.6 ± 2.4 |
| | | 0 | 100.0 ± 0 | 100.0 ± 0 | 100.0 ± 0 |

[a]Mean ± SE mortality (%) on day 1 and day 5 in the control treatment for LAB, CS, and FL strains, with or without wheat, ranged from 0 to 7.4 ± 3.9% and 0 to 23.7 ± 1.0%, respectively.

[b]Mean ± SE progeny production in the control treatment for LAB, CS, and FL strains, with and without wheat, ranged from 69.2 ± 6.9 to 127.4 ± 15.0 adults and 75.6 ± 16.3 to 180.6 ± 18.8 adults, respectively.

[c]Mean ± SE mortality (%) on day 1 and day 5 in the control treatment for LAB, CS, and FL strains, with or without wheat, ranged from 0 to 2.9 ± 2.9% and 0 to 10.6 ± 4.3%, respectively.

[d]Mean ± SE progeny production in the control treatment for LAB, CS, and FL strains, with and without wheat, ranged from 120.0 ± 31.3 to 157.4 ± 28.7 adults and 111.8 ± 10.9 to 251.6 ± 31.1 adults, respectively.

Table 5.7. Effective dose (ED) estimates for adult progeny reduction in *R. dominica* after exposure to 0.21 g/m$^3$ of ozone.

| Strain | Wheat (g) | $N$[a] | Mean ± SE | | Effective dose (g-h/m$^3$, 95% CI) | | $\chi^2$ (df)[b] |
|---|---|---|---|---|---|---|---|
| | | | Intercept | Slope | $ED_{50}$ | $ED_{99}$ | |
| LAB | 10 | 800 | -0.99 ± 0.28 | 0.59 ± 0.60 | 47.98 (---)[c] | 425535 (---)[c] | 1414.2 (38) |
| CS | 10 | 800 | -0.95 ± 0.19 | 1.68 ± 0.41 | 3.68 (2.87-5.9) | 88.21 (27.04-2783) | 679.90 (38) |
| FL | 10 | 800 | -1.06 ± 0.22 | 2.83 ± 0.49 | 2.36 (1.96-2.79) | 15.63 (9.55-42.91) | 821.98 (38) |
| LAB | 0 | 780 | -0.81 ± 0.26 | 3.42 ± 0.62 | 1.72 (1.32-2.05) | 8.25 (5.67-17.97) | 1072.95 (37) |
| CS | 0 | 800 | -0.33 ± 0.17 | 2.40 ± 0.42 | 1.37 (0.97-1.69) | 12.75 (7.93-33.38) | 652.31 (38) |
| FL | 0 | 740 | 0.26 ± 0.12 | 2.18 ± 0.32 | 0.96 (0.49-0.99) | 8.84 (6.11-17.09) | 278.79 (35) |

[a]$N$ = total number of insects used in generating probit regression estimates.

[b]All $\chi^2$ values for goodness-of-fit of model to data were significant ($P < 0.05$), indicating poor fit of model to data.

[c]Confidence intervals were not generated due to the large $\chi^2$ value.

Table 5.8. Effective dose (ED) estimates for adult progeny reduction in *R. dominica* after exposure to $0.42 g/m^3$ of ozone.

| Strain | Wheat (g) | $N^a$ | Mean ± SE | | Effective dose ($g\text{-}h/m^3$, 95% CI) | | $\chi^2$ (df)[b] |
|---|---|---|---|---|---|---|---|
| | | | Intercept | Slope | $ED_{50}$ | $ED_{99}$ | |
| LAB | 10 | 800 | -1.01 ± 0.25 | 2.00 ± 0.35 | 3.20 (2.34-3.89) | 46.58 (25.00-170.21) | 514.81 (38) |
| CS | 10 | 800 | 0.40 ± 0.13 | 0.60 ± 0.19 | 0.21 (0-0.73) | 1562 (165.11- ----)[c] | 137.69 (38) |
| FL | 10 | 800 | -0.18 ± 0.12 | 1.57 ± 0.17 | 1.31 (0.90-1.68) | 39.92 (26.50-75.21) | 111.85 (38) |
| LAB | 0 | 780 | 0.22 ± 0.13 | 2.42 ± 0.25 | 0.81 (0.56-1.04) | 7.41 (6.20-9.57) | 74.19 (33) |
| CS | 0 | 800 | -0.06 ± 0.18 | 2.45 ± 0.32 | 1.06 (0.69-1.39) | 9.45 (7.30-14.33) | 129.94 (28) |
| FL | 0 | 740 | 0.03 ± 0.20 | 2.59 ± 0.36 | 0.98 (0.60-1.31) | 7.73 (6.04-11.67) | 152.06 (30) |

[a]*N* = total number of insects used in generating probit regression estimates.

[b]All $\chi^2$ values for goodness-of-fit of model to data were significant ($P < 0.05$), indicating poor fit of model to data.

[c]Confidence intervals were not generated due to the large $\chi^2$ value.

Table 5.9. Percent reduction (mean ± SE) in adult emergence after exposure to 0.42 g/m$^3$ of ozone for various durations[a]

| Stage | Exposure duration | Percent reduction (mean ± SE) in adult emergence after ozone exposure[b] | | |
|---|---|---|---|---|
| | | Lab | CS | FL |
| Eggs | 24 | 73.3 ± 3.9 | 64.4 ± 3.6 | 47.7 ± 12.4 |
| | 48 | 85.4 ± 3.9 | 85.3 ± 4.5 | 92.4 ± 4.8 |
| | 72 | 99.7 ± 0.3 | 96.3 ± 2.0 | 100.0 ± 0 |
| Young larvae | 10 | 100.0 ± 0 | 100.0 ± 0 | 99.0 ± 1.0 |
| | 24 | 100.0 ± 0 | 99.2 ± 0.5 | 97.1 ± 2.0 |
| | 34 | 99.7 ± 0.3 | 98.4 ± 0.7 | 99.0 ± 1.0 |
| Old larvae | 10 | 53.3 ± 3.9b | 64.6 ± 6.7b | 93.3 ± 4.3a |
| | 24 | 99.1 ± 0.4 | 98.8 ± 1.2 | 98.3 ± 1.0 |
| | 34 | 99.6 ± 0.3 | 98.8 ± 0.5 | 99.2 ± 0.8 |
| Pupae | 2 | 12.4 ± 8.9 | 12.1 ± 11.6 | 19.6 ± 17.7 |
| | 6 | 16.4 ± 13.8b | 35.2 ± 6.0b | 76.5 ± 7.0a |
| | 10 | 32.8 ± 8.6b | 54.4 ± 6.9b | 96.6 ± 3.4a |

[a]Each mean is based on $n = 5$.

[b]Mean ± SE adults emerged in control treatment for eggs of Lab, CS, and FL strains were 69.8 ± 4.4, 38.2 ± 2.8, and 26.4 ± 2.3, respectively. Corresponding values for young larvae were 62.0 ± 17.5, 50.4 ± 5.1, and 20.4 ± 5.5; for old larvae were 92.0 ± 12.9, 52.0 ± 2.1, 24.0 ± 4.0; and for pupae were 95.2 ± 11.2, 56.2 ± 7.1, 35.8 ± 2.8. Means by stages and hours followed by different letters are significant different among strains (Bonferroni *t*-tests, $\alpha$=0.05).

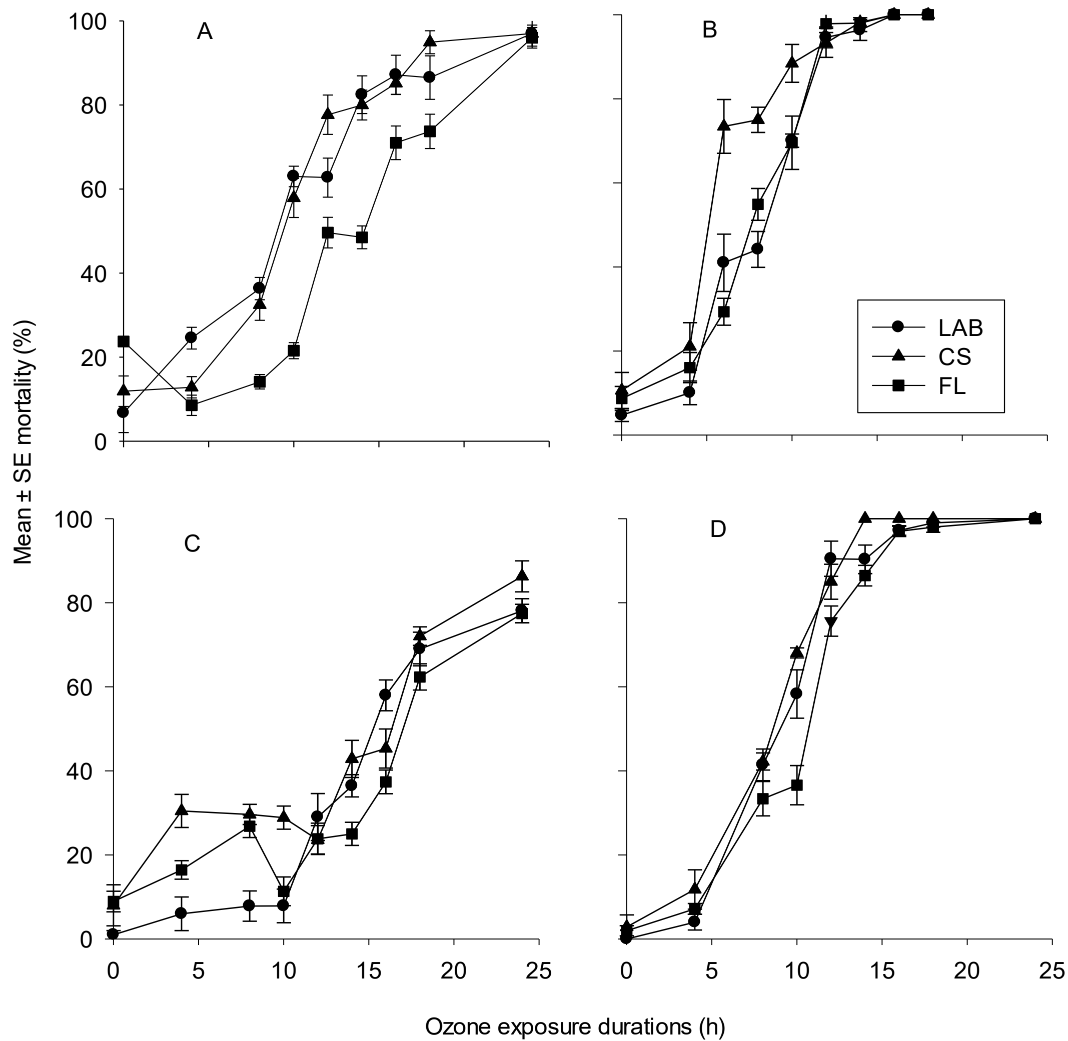


Figure 5.1. Mortality assessed on day 5 of *R. dominica* exposed with 0 and 10 g of wheat to 0.21 or 0.42 g/m$^3$ of ozone. (A) 0 g of wheat and 0.21 g/m$^3$ of ozone; (B) 0 g of wheat and 0.42 g/m$^3$ of ozone; (C) 10 g of wheat and 0.21 g/m$^3$ of ozone; and (D) 10 g of wheat and 0.42 g/m$^3$ of ozone.

# Chapter 6 - Overall Conclusions

## 6.1. Insecticidal property of chlorine dioxide gas against life stages of major stored-product insect species

Exposure to 0.95 g/m$^3$ for various time periods, *Tribolium castaneum* (Herbst), and *Oryzaephilus surinamensis* (Linnaeus) were very susceptible to chlorine dioxide gas, whereas *Rhyzopertha dominica* (Fabricius), *Sitophilus oryzae* (Linnaeus), and *Sitophilus zeamais* (Motschulsky) varied in mortality data depending on the immature stages which exposure was conducted. With exposure at 2.7 g/m$^3$, complete mortality was achieved at any given time all for species.

## 6.2. Responses of immature stages of *Tribolium castaneum* to chlorine dioxide gas

Based on the laboratory study, current results showed the effectiveness of chlorine dioxide gas against immature stages of *T. castaneum,* and it may be used as phosphine alternative to manage stored product insects. Additional, a fumigation with chlorine dioxide gas may be conducted within much less time than that of phosphine.

## 6.3. Efficacy of ozone at two concentrations against adults of phosphine susceptible and resistant strains of *Rhyzopertha dominica*

According to investigation on ozone conducted under laboratory condition, immature stages of *R. dominica* exposed to 0.21 g/m$^3$ of ozone for 24 h revealed that 5-d mortality was 77-86% with food and 96-97% without food. Exposure with 0.42 g/m$^3$ of ozone for 16 h, complete mortality was achieved on the 5$^{th}$ d. Lethal dose of ozone is highly related to ozone concentration

in the supply air due to its binding property. In addition, ozone can be used in control of eggs, young larvae, and old larvae of *R. dominica* as exposed to ozone at 0.42 $g/m^3$ for 10 to 34 h.

### 6.4. Sublethal chlorine dioxide exposure leads to reproductive disruption in *Tribolium castaneum* (Coleoptera: Tenebrionidae) pupae and *Plodia interpunctella* (Lepidoptera: Pyralidae) larvae

Studies reported that female pupae of *T. castaneum* are more vulnerable than males to chlorine dioxide gas at 0.95 $g/m^3$ for 3 h treatment. Production of eggs was adversely reduced when females exposed to chlorine dioxide as compared to the control. Egg hatchability was severely reduced after the treatment. Results from chlorine dioxide induced *P. interpunctella* old larvae showed very little production in number of offspring. Chlorine dioxide acts as a chemosterilant in reproductive performance.