Responses of phosphine susceptible and resistant strains of five stored-product insect species to chlorine dioxide

Xinyi E, Bhadriraju Subramanyam*, Beibei Li

Department of Grain Science and Industry, Kansas State University, Manhattan, Kansas 66506, USA

*Corresponding author.

E-mail address: sbhadrir@k-state.edu (B. Subramanyam).

Abstract

Adults of phosphine susceptible laboratory strains and phosphine resistant field strains of five stored-product insect species were exposed for different time periods to a chlorine dioxide gas concentration of 0.54 g/m^3 (200 ppm) in the presence (10 g) and absence (0 g) of wheat. After exposure, mortality of adults was determined 5 d later at 28°C and 65% r.h. The 5-d mortality was 100% in laboratory and field strains of the red flour beetle, *Tribolium castaneum* (Herbst); sawtoothed grain beetle, *Oryzaephilus surinamensis* (L.); lesser grain borer, *Rhyzopertha dominica* (F.); maize weevil, *Sitophilus zeamais* Motschulsky; and rice weevil, *Sitophilus oryzae* (L.) that were exposed in vials with 10 g of wheat to chlorine dioxide for 26, 16, 24-34, 18-24, and 15-18 h, respectively. Corresponding exposure durations for these species and strains in vials without wheat were 15, 3, 18-20, 7-15, and 5-7 h, respectively. The dosages of chlorine dioxide producing 99% mortality (LD_{99}) of *T. castaneum*, *O. surinamensis*, *R. dominica*, *S. zeamais*, and *S. oryzae* strains in the presence of wheat ranged from 14.79-22.57, 8.20-8.41, 15.79-21.60, 10.66-14.53, and 7.67-12.20 g-h/m^3, respectively. In the absence of wheat, corresponding LD_{99} values for *T. castaneum*, *R. dominica*, and *S. zeamais* strains were 6.51-8.66, 11.46-23.17, and 5.79-10.26 g-h/m^3, respectively. LD_{99} values for *O. surinamensis* and *S. oryzae* could not be computed, because of 100% mortality after a 3-5 h exposure to chlorine dioxide. Both phosphine susceptible and resistant strains of all five species were equally susceptible to chlorine dioxide. No adult progeny production of *T. castaneum* and *O. surinamensis* was observed after 8 weeks in control and chlorine dioxide-exposed samples. Adult progeny production of *Sitophilus* spp. was found only in the control samples. The dosage for 99% adult progeny reduction relative to control for *R. dominica* strains ranged from 10.07-18.11 g-

h/m^3. Chlorine dioxide gas is effective in killing adults of five stored-product insect species and suppressing adult progeny production of three of the five species.

Keywords: Chlorine dioxide; Stored-product insects; Phosphine resistance; Fumigation; Efficacy assessment

1. Introduction

Chlorine dioxide gas was discovered in early 1800s, and was initially used as a bleaching agent in the paper industry for pulp bleaching (Simpson, 2005). In 1950s, chlorine dioxide was used for treating drinking water to remove microorganisms and off-odors. Chlorine dioxide gas easily dissolves in water and stays as dissolved gas instead of being hydrolyzed. Once it separates from the solution, the gas tends to decompose into chlorine and oxygen when exposed to sunlight, high temperatures, electric sparks, or high pressure (Simpson, 2005).

A few researchers investigated the possibility of using chlorine dioxide as fumigant to control stored-product insect species. The efficacy of chlorine dioxide against four life stages of the red flour beetle, *Tribolium castaneum* (Herbst), and confused flour beetle, *Tribolium confusum* Jacquelin du Val, was reported by Channaiah et al. (2012). Channaiah et al. (2012) exposed eggs, young larvae, old larvae, and adults of *T. castaneum* and *T. confusum* without food in vials to chlorine dioxide concentrations of 380.1, 685.6, 745.0, and 834.4 g-h/m^3. The exposure times varied only from 1.53 to 2.07 h. Mortality was greatest at the highest dosage. The mortality of eggs, young larvae, old larvae, and adults of *T. castaneum* was 9.3, 100, 18.8, and 100%, respectively, after exposure to 834.4 g-h/m^3 chlorine dioxide. Similarly for *T. confusum*, the mortality of the four life stages was 11.1, 100, 31.3, and 100%, respectively, when exposed to a chlorine dioxide concentration of 834.4 g-h/m^3. In the presence of 5 g of flour in vials, only the mortality of adults of both species was 100% at the highest dosage, whereas mortality of eggs, young larvae, and old larvae ranged from 4 to 37%. Channaiah et al. (2012) hypothesized that longer than 2 h exposures may be needed for effective control of all life stages. Kumar et al. (2015) exposed late instars of the Indian meal moth, *Plodia interpunctella* (Hübner), to a chlorine dioxide concentration of 0.54 g/m^3 for various time periods, and complete mortality was

observed after 24 h. Kim et al. (2015) reported 100% mortality of both larvae (6-7th instars) and adults of *T. castaneum* after exposure to 0.54 g/m^3 concentration of chlorine dioxide for 24 h. They also investigated the mode of action of chlorine dioxide against insects by tracking changes in the quantity of reactive oxygen species and levels of two antioxidant enzymes (superoxide dismutase and thioredoxin-peroxidase) in the larvae of *T. castaneum* before and after chlorine dioxide exposure. After exposure to chlorine dioxide the production of the two antioxidant enzymes failed to keep up with the production of reactive oxygen species, and the authors inferred that this oxidative stress may have likely led to cellular damage and mortality of larvae (Kim et al., 2015).

Chlorine dioxide gas can be produced chemically or electrochemically. Most studies using chlorine dioxide for food applications followed the chemical method, which included using sachets containing sodium chlorite and an acid or an acid precursor (ferric chloride), or occurred in cartridges packed with sodium chlorite fed with chlorine gas (Sy et al., 2005; Trinetta et al., 2013; Prodduk et al., 2014). Another patented method to generate chlorine-free chlorine dioxide is to run sodium chlorite solution through a set of electrolytic cells where chlorite ion is electrochemically oxidized to chlorine dioxide gas (Cawlfield and Kaczur, 1990). In this study, the latter means of chlorine dioxide production was used.

In the present investigation, responses of phosphine susceptible and resistant strains of five common stored-product insects were evaluated by exposing them to a chlorine dioxide concentration of 0.54 g/m^3 for different durations. The efficacy of chlorine dioxide was evaluated in the presence and absence of wheat. The effect of chlorine dioxide on adult progeny production was also studied.

2. Materials and methods

2.1. Insects

Cultures of *T. castaneum* were reared on organic wheat flour (Heartland Mills, Marienthal, Kansas, USA) fortified with 5% (by wt) brewer's yeast (Lesaffre Yeast Corporation, Milwaukee, Wisconsin, USA). The sawtoothed grain beetle, *Oryzaephilus surinamensis* (L.), was reared on organic rolled oats (Heartland Mills) plus 5% brewer's yeast diet. Cultures of the lesser grain borer, *Rhyzopertha dominica* (F.), and rice weevil, *Sitophilus oryzae* (L.), were reared on organic hard red winter wheat (Heartland Mills). The maize weevil, *Sitophilus zeamais* Motschulsky, was reared on organic corn (Heartland Mills). Laboratory strains of all species have been in rearing since 1999. Field strains of *T. castaneum*, *R. dominica*, and *O. surinamensis* were collected during 2011 from farm-stored grain in Kansas, USA, whereas field strains of the *S. zeamais* and *S. oryzae* were collected from farm-stored grain in Texas, USA (Table 1). Unsexed adults used in all bioassays were 1- to 4-week old.

Phosphine susceptibility or resistance in laboratory and field strains of five insect species was verified following discriminating dose tests (Champ and Dyte, 1976). In the discriminating dose tests, phosphine concentrations used for *T. castaneum*, *O. surinamensis*, *R. dominica*, and *Sitophilus* spp., were, 0.042, 0.052, 0.028, and 0.042g/m^3 (30.0, 37.5, 20.0, and 30.0 ppm), respectively. Fifty unsexed adults of each strain were exposed to phosphine in triplicate. Adults of each strain were exposed to phosphine for 20 h, and mortality was assessed after 14 d to score insects as susceptible, weakly resistant, or strongly resistant to phosphine. All laboratory strains of the five species were susceptible to phosphine whereas the field strains of the species were resistant to phosphine (Table 2).

Cultures of all insect species were reared in 0.95-L glass jars with approximately 250 g of diet at 28°C and 65% r.h. in environmental growth chambers (model I-36 VL; Percival Scientific, Perry, Iowa, USA). All jars had metal lids fitted with wire mesh screens and filter papers. Adults for use in bioassays were collected directly from culture jars after sifting the culture through an 841-µm opening round-holed sieve (Fisher Scientific Company, Hampton, New Hampshire, USA).

2.2. Bioassays

Bioassays were carried out in snap cap vials (23 mm in diameter and 55 mm in height) that had mesh bottoms (250 µm openings) and perforated plastic caps covered with 250 µm opening sieve to ensure diffusion of chlorine dioxide through the vials, and also to prevent insects from escaping. Chlorine dioxide treatments were conducted in an air-tight polymethyl methacrylate (PMMA) chamber (0.6 m × 0.6 m × 1.0 m). Chlorine dioxide gas was produced by a customized chlorine dioxide generator donated by PureLine Treatment Systems, LLC (Bensenville, Illinois, USA), housed inside a trailer. The trailer was located on the north campus next to the O.H. Kruse Feed Technology Innovation Center, Department of Grain Science and Industry, Kansas State University, Manhattan, Kansas, USA. Chlorine dioxide gas was produced from sodium chlorite (31% solution) via two electrochemical reactions:

Anode (oxidation): $ClO_2^- \rightarrow ClO_2 + e^-$

3. Cathode (reduction): $2H_2O + 2e^- \rightarrow H_2 + 2OH^-$

Chlorine dioxide gas was then admixed with ambient air prior to entering the PMMA chamber where bioassays were held. Chlorine dioxide concentrations were adjusted by mixing different amounts of ambient air, and gas concentrations were monitored by an optical sensor converter (Control 4000, Optek, Germantown, Wisconsin, USA). Temperature and humidity

inside the testing chamber were monitored by HOBO© data loggers (Model: U10-003, Onset Computer Corp., Bourne, Massachusetts, USA). The mean ± SE temperature was 24.8 ± 0.6 °C (range, 17.6 to 28.8 °C) during tests, and the mean ± SE humidity was 29.4 ± 1.2% (range, 21.1 to 52.3%).

A total of 10 g of organic hard red winter wheat of 11-12% moisture (wet basis) was placed in a snap-cap vial along with 20 adults of each species and strain. Vials without wheat had just 20 insects per vial. Inside the PMMA chamber vials were placed horizontally to ensure proper gas penetration. The target concentration of chlorine dioxide was 0.54 g/m^3 (200 ppm). Maintaining a steady chlorine dioxide gas concentration was difficult and there were minor fluctuations in gas concentrations. The mean concentration during the fumigation was 201 ppm (range, 147 - 255 ppm). Insects were exposed to chlorine dioxide for different time periods (Table 3). Vials, with and without 10 g of wheat, held at 28 °C and 65% r.h. and sampled at the same time intervals corresponding chlorine dioxide-exposed vials served as the control treatment. Prior to starting the tests, all vials were placed in the testing chamber, and were collected after the intended exposure time. Each species, strain, and chlorine dioxide exposure treatment combination was replicated three times. After exposure to chlorine dioxide, vials were brought back to the laboratory and kept in environmental chambers maintained at 28 °C and 65% r.h. For samples exposed to chlorine dioxide without wheat, 10 g of wheat was added after the treatment to see if any adults would recover when given access to food. Mortality of adults in control and chlorine dioxide exposed vials was checked 5 d after exposure to chlorine dioxide. In our previous research (Subramanyam and E, 2015) adult mortality of laboratory and field strains of these same insect species exposed to 0.54, 1.35, 2.02, and 2.70 g/m^3 of chlorine dioxide gas for various time periods steadily increased when mortality assessments were made during days 1

through 5, indicating delayed toxic effects of chlorine dioxide. The mortality was stable on day 5, and therefore in the present study, end-point mortality was determined 5 days after chlorine dioxide exposure. After mortality assessments, insect adults (live and dead) and wheat were placed back into the vials and all vials were held at 28°C and 65% r.h. for 8 weeks to count adult progeny production.

2.3 Data analysis

Mortality was calculated as a percentage based on the number of dead insects out of the total exposed. Mortality in treatments was corrected for control mortality (Abbott, 1925). The dosage of chlorine dioxide was determined by multiplying the mean concentration (*C*) with the exposure time (*t)*, to provide a *Ct* product (dosage). The corrected 5 d mortality data as a function of dosage were subjected to probit analysis (SAS Institute, 2008) to generate probit regression estimates and lethal dosages (LD) producing 50 and 99% mortality. LD_{99} value of each field strain of a species was compared to the corresponding laboratory strain using the ratio test (Robertson and Preisler, 1992). Difference between any two LD_{99} values was considered significant ($P \leq 0.05$) if the 95% confidence interval (CI) for the ratio did not include 1. The adult progeny produced was counted and the original number of adults added to vials (20) was subtracted prior to data analysis. The percent reduction in adult progeny production relative to production in the control treatment was calculated as: (1-treatment progeny/control progeny) × 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 (SAS Institute, 2008).

3. Results and discussion

In the presence of wheat, the time required for complete mortality after 5 d for adults of *T.castaneum, O. surinamensis, R. dominica, S. zeamais,* and *S. oryzae* strains was 26, 16, 24-34, 18-24, and 15-18 h, respectively (Table 4). In the absence of wheat, the time to obtain complete mortality after 5 d was 15, 3, 18-20, 7-15, and 5-7 h, respectively (Table 4). Adults of *O. surinamensis* were most susceptible to chlorine dioxide followed by *S. zeamais* and *S. oryzae*. Adults of *T. castaneum* and *R. dominica* were least susceptible to chlorine dioxide. Susceptibility differences among species to chlorine dioxide may be due to their physiological differences. Respiration rates of insects have been linked to phosphine resistance (Pimentel et al., 2007). Pimental et al. (2007) collected field strains of *T. castaneum*, *R. dominica*, and *O. surinamensis* from 17 locations in Brazil and tested them for phosphine resistance and examined respiration and other fitness parameters. They found that strains with lower carbon dioxide production rate had higher phosphine resistance levels. Lu et al. (2009) reported carbon dioxide production in adults of *T. castaneum, R. dominica,* and *S. oryzae* after 2 h of incubation was 3.8, 4.2, 6.0 mL/g of an insect, respectively, which corresponded to the order of chlorine dioxide tolerance in these three species observed in our study. Species that had a higher carbon dioxide production showed a lower chlorine dioxide tolerance. Lower carbon dioxide production indicates lower respiratory rate and consequently less chlorine dioxide up-take.

The rate of metabolism of different insect species may also affect their susceptibility to chlorine dioxide. Cofie-Agblor et al. (1995) reported that at 30°C and 14.5 % r.h., the heat production of *S. oryzae, T. castaneum,* and *R. dominica* adults was 56.4-55.3, 39.7-38.1, and 35.3-32.8 µW/individual, which also corresponded to the order of chlorine dioxide tolerance

shown in Table 4. Adults of species (*S. oryzae*) with higher heat production (higher metabolism rate) were more susceptible to chlorine dioxide based on our study (Cofie-Agblor et al., 1995).

The presence of small quantities of wheat increased the exposure time to achieve 100% mortality of all five species and strains (Table 4). The presence of wheat increased the time to 100% mortality of *T. castaneum* adults by 42% when compared to those exposed without wheat for both laboratory and field strains. Similarly, the increase in time to 100% mortality of *O. surinamensis*, *R. dominica*, *S. zeamais*, and *S. oryzae* strains was 81, 17-41, 38-61, and 61-67%, respectively, when compared to strains exposed to chlorine dioxide without wheat. Chlorine dioxide is a highly reactive gas, and is able to not only react with organic matter but also adsorb onto plastic surfaces (Sampson, 2005; Gómez-López et al., 2007; Nam et al., 2014). Although the penetration of chlorine dioxide in grain mass has not been characterized, the mechanism is speculated to be similar to ozone, which has a higher oxidation potential than chlorine dioxide (Simpson, 2005), and both gases affect aerobic respiration in insects (Lu et al., 2009; Kim et al., 2015; Kumar et al., 2015). Raila et al. (2006) concluded that the penetration of ozone in grain mass was governed by gas diffusion, ozone velocity, and adsorption by the grain surface. Even with a continuous ozone flow, the gas concentration in grain gradually increased over time; depending on the quantity of the grain, it can take from hours to days to reach a constant concentration (Kells et al., 2001; Mendez et al., 2003; Campabadal et al., 2013; Subramanyam et al., 2014a). In the grain mass, ozone is prone to react with inherent sites on the grain surface first, and once all sites became saturated, ozone accumulation starts to increase (Kells et al., 2001). This phenomenon occurs because the degradation of ozone decreases after reaction with all of the active sites on kernels (Kim et al., 1999; Kells et al., 2001; Campabadal et al., 2013), and the un-degraded ozone is then available to slowly accumulate to levels lethal for insects. Chlorine

dioxide in the grain mass may behave like ozone by binding to active sites on the surface of kernels. Therefore, longer exposures were needed to completely kill insects in the presence of wheat when compared to time required for similar effect in the absence of wheat.

Probit analysis results for tests conducted with wheat are presented in Table 5, and results for tests without wheat are presented in Table 6. All χ^2 values for goodness-of-fit of the model to data were significant ($P < 0.05$), indicating poor fit of model to data, which may be due to heterogeneous adults responses to chlorine dioxide. Fitting logit and complementary log-log models to data (Robertson and Priesler, 1992) also yielded similar results, suggesting that the responses of adults were truly heterogeneous. In cases where the *P*-value for the test is small or significant, variances and covariances are adjusted by a heterogeneity factor (χ^2 value divided by the degrees of freedom (df)), and a critical value from the *t* distribution is used to compute the confidence intervals (SAS Institute, 2008). Heterogeneous responses of adults could be due to age-related or sex-related differences in susceptibility as unsexed adults of mixed ages were used in the experiments. Heterogeneous responses in insects exposed to high temperatures (Mahroof et al., 2003) and insecticides (Sehgal et al., 2013; Subramanyam et al., 2014b) are not uncommon. In the presence of wheat, LD_{99} values of *T. castaneum*, *R. dominica*, *O. surinamensis*, *S. zeamais*, and *S. oryzae* after 5 d were 14.79-17.16, 15.79-21.60, 8.20-8.41, 10.66-14.53, and 7.67-12.20 g-h/m^3 respectively. In the absence of wheat, LD_{99} values of *T. castaneum*, *R. dominica*, and *S. zeamais* after 5 d were 6.51-8.66, 11.46-23.17, and 5.79-10.26 g-h/m^3 respectively. The 5 d LD_{99} values for *O. surinamensis* and *S. oryzae* were not estimated since the mortality was at or near 100% at all exposure times.

In the presence of wheat, there was no significant difference between LD_{99} values of phosphine susceptible (laboratory) strain and phosphine resistant (field) strains of *T. castaneum*,

R. dominica, and *O. surinamensis* based on ratio tests (Table 7). The *S. zeamais* Lab strain had a significantly higher LD_{99} value than the TX strain (ratio [95% CI] =1.35 [1.17-1.55]). The *S. oryzae* TX strain had a significantly higher LD_{99} value than the Lab strain (ratio [95% CI] =1.62 [1.24-2.12]). In the absence of wheat (Table 8), there was no significant difference between LD_{99} values of phosphine susceptible and phosphine resistant strains for all species, except for *S. zeamais*, where phosphine resistant strain had a higher LD_{99} value than the susceptible strain (ratio [95% CI] =1.78 [1.03-3.07]).

The current data did not indicate a strong relationship between phosphine resistance and susceptibility to chlorine dioxide. Chlorine dioxide mode of action on microorganisms involves inactivation and irreversible damage to the enzymes on the cell membrane by oxidizing the thiol groups, but it does not cause DNA damage or mutations (Roller et al., 1980; Young and Setlow, 2003; Finnegan et al., 2010). Additionally, oxidative stress was shown to be another mechanism for the lethal effect of chlorine dioxide in insects (Kim et al., 2015; Kumar et al., 2015). Phosphine is known to inhibit aerobic respiration in several different organisms (Chefurka et al., 1976; Dua and Gill, 2004; Jian et al., 2000; Singh et al., 2006; Zuryn et al., 2008). Phosphine inhibits cytochrome *c* oxidase of the mitochondrial electron transport chain (Chefurka et al., 1976; Nakakita, 1976). Other proposed mode of action for phosphine includes chemical reaction with hydrogen peroxide to form hydroxyl radical, which can cause oxidative damage (Quistad et al., 2000). This theory was supported by *in vivo* and *in vitro* studies using mammalian cell lines exposed to phosphine (Hsu et al., 1998, 2002a,b; Quistad et al., 2000). Phosphine was shown to inhibit the antioxidant enzymes catalase and peroxidase (Chaudhry, 1997). Liu et al. (2015) using the larvae of the common fruit fly, *Drosophila melanogaster* Meigen, have shown that phosphine reduced the aerobic respiration rate with an increase in hydrogen peroxide and lipid

peroxidation. Additionally, phosphine down-regulated catalase encoding gene and the expression of catalase. These studies show that the modes of action of chlorine dioxide and phosphine may be essentially similar, although insects resistant to phosphine in our study were susceptible to chlorine dioxide. Given that both compounds act similarly in insects, there is a likelihood that insects may develop resistance to chlorine dioxide. However, more work is needed through laboratory selection experiments, or through commercial use of chlorine dioxide to control insects in stored grain and in grain-processing facilities, to confirm this hypothesis.

Adults of *T. castaneum* and *O. surinamensis* exposed to chlorine dioxide and cultured with wheat kernels for 8 weeks failed to produce adult progeny. Adults of these two species in the control treatment also failed to produce adult progeny. We attribute lack of adult progeny production in these two species due to the inability of larvae to effectively infest and survive on wheat kernels, as these two species are secondary feeders and require grain dust or dockage to survive (Sinha and Watters, 1985). The laboratory and field strains of *S. zeamais* in the control treatment produced a mean ± SE of 385.3 ± 30.5 and 231.7 ± 21.2 adults, respectively. In the control treatments laboratory and field strains of *S. oryzae* produced a mean ± SE of 344.0 ± 37.6 and 402.0 ± 9.6 adults, respectively. No adult progeny was found in any chlorine dioxide exposed *Sitophilus* spp., regardless of the presence or absence of wheat. Exposure to a chlorine dioxide concentration of 0.54 g/m^3 for only 5 and 10 h can effectively control adult progeny production of *S. zeamais* and *S. oryzae*, respectively.

In both control and chlorine dioxide treatments, *R. dominica* produced adult progeny (Table 9). The dosage of chlorine dioxide required for 50% reduction of adult progeny production was less than that required for 50% adult mortality. For example, in the presence of wheat, ED_{50} based on adult progeny and LD_{50} based on 5 d mortality for *R. dominica* were 3.57-

5.76 and 7.17-9.47 g-h/m^3, respectively. In the absence of wheat, ED_{50} and LD_{50} were 1.17-2.55 and 3.61-3.84 g-h/m^3, respectively. Sehgal et al. (2013), using spinosad on wheat, showed that the ED_{50} values for a 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 to 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} value for the laboratory and two field strains was 0.011 mg(AI)/kg (Sehgal et al., 2013).

In conclusion, a chlorine dioxide concentration of 0.54 g/m^3 was effective in killing adults of phosphine susceptible and resistant strains of five economically important stored-product insect species. Chlorine dioxide completely suppressed progeny of *Sitophilus* spp. indicating that the adults died before the females had a chance to lay eggs underneath the kernel pericarp. Adults of *R. dominica* needed longer exposure times (18-34 h) compared to other insect species tested for 100% mortality, regardless of the presence of wheat. This explains the presence of adult progeny production in *R. dominica* observed in this study. The laboratory findings should be confirmed by tests under practical field conditions such as grain bins and grain-processing facilities.

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Table 1. Sites and years of collection of field strains of five stored-product insect species.

Species	County, state	Commodity	Strain	Collection year
T. castaneum	Dickinson, Kansas	Wheat	AB1	2011
	Minneapolis, Kansas	Wheat	MN	2011
O. surinamensis	Abilene, Kansas	Wheat	AB2	2011
R. dominica	Chase, Kansas	Wheat	CS	2011
	Riley, Kansas	Flour	RL	2007
S. zeamais	Texas[a]	Corn	TX	2011
S. oryzae	Texas[a]	Corn	TX	2011

[a]County unknown.

Table 2. Survival of laboratory and field strains of five stored-product insect species exposed to discriminating doses of phosphine.

Species	Strain	% Survival (mean ± SE)	Resistance classification
T. castaneum[a]	Lab	0	Susceptible
	AB1	43.0	Weak
	MN	98.0	Strong
O. surinamensis	Lab	0	Susceptible
	AB2	1.3 ± 1.3	Weak
R. dominica	Lab	0	Susceptible
	CS	64.4 ± 2.9	Weak
	RL	27.8 ± 1.8	Weak
S. zeamais	Lab	0	Susceptible
	TX	6.7 ± 1.8	Weak
S. oryzae	Lab	0	Susceptible
	TX	9.3 ± 2.4	Weak

[a]Each mean is based on *n*=3. The test was conducted by a scientist in the Department of Entomology, Kansas State University, and the original values were not supplied to compute a SE.

Table 3. Exposure times chosen to test the efficacy of chlorine dioxide (0.54 g/m^3) against five stored-product insect species in vials with and without wheat.

Species	Exposure time (h) for vials with wheat
T. castaneum	10, 12, 15, 18, 22, 26
O. surinamensis	3, 5, 7, 10, 16
R. dominica	10, 14, 16, 20, 24, 26, 28, 30, 34
S. zeamais	10, 15, 18, 20, 22, 24, 26, 28, 30
S. oryzae	5, 7, 10, 15, 18, 20, 22, 24, 26, 28, 30
	Without wheat
T. castaneum	5, 7, 10, 15
O. surinamensis	1, 3, 5, 7
R. dominica	7, 10, 14, 18, 20, 24
S. zeamais	5, 7, 10, 15, 20, 24, 28
S. oryzae	5, 7, 10, 15, 20, 24, 28

Table 4. Time required for complete mortality of adults of five stored-product insect species exposed to a chlorine dioxide concentration of 0.54 g/m^3 based on mortality assessments made 5 d after exposure[a].

Species	Strain	Exposure time (h)	
		with wheat	without wheat
T. castaneum	Lab	26	15
	AB1	26	15
	MN	26[b]	15
O. surinamensis	Lab	16	3
	AB2	16	3
R. dominica	Lab	24	20
	CS	34	18
	RL	34	20
S. zeamais	Lab	24	15
	TX	18	7
S. oryzae	Lab	15	5
	TX	18	7

[a]Mean ± SE control mortality after 5 d for *T. castaneum, O. surinamensis, R. dominica, S. zeamais,* and *S. oryzae* strains was 0, 8.8 ± 2.4 to 11.2 ± 1.2, 5.0 ± 0.0 to 14.7 ± 2.6, 6.4 ± 1.4 to 10.0 ± 2.9, 0 to 5.0 ± 2.9%, respectively in the presence of wheat. In the absence of wheat mean ± SE control mortality after 5 d for *T. castaneum, O. surinamensis, R. dominica, S. zeamais,* and *S. oryzae* strains was 0, 11.0 ± 6.4 to 12.6 ± 4.7, 3.4 ± 1.7 to 16.6 ± 1.2, 9.2 ± 2.6 to 13.3 ± 3.3, 8.0 ± 4.4 to 11.7 ± 4.4%, respectively.

[b]The mean ± SE mortality for *T. castaneum* MN strain at 26 h was 93.3 ± 6.7 %.

Table 5. Probit regression estimates and dosages required for 50 and 99% mortality for laboratory and field strains of adults of five insect species exposed to 0.54 g/m^3 of chlorine dioxide in the presence of wheat based on mortality assessment made 5 d after exposure.

			Mean ± SE		LD (95% CI) (g-h/m^3)		
Species	Strain	*N*[a]	Intercept	Slope	LD_{50}	LD_{99}	χ^2 (df)[b]
T. castaneum	Lab	360	-5.93 ± 0.85	7.06 ± 0.96	6.92 (6.30-7.49)	14.79 (12.55-19.64)	151.92 (16)
	AB1	360	-5.13 ± 0.72	6.22 ± 0.80	9.70 (6.04-7.25)	15.86 (13.41-21.03)	121.48 (16)
	MN	360	-6.53 ± 1.22	7.17 ± 1.31	8.13 (7.15-9.16)	17.16 (13.72-27.70)	353.23 (16)
O. surinamensis	Lab	300	-0.36 ± 0.24	2.91 ± 0.51	1.33 (0.82-1.71)	8.41 (5.92-17.60)	72.08 (13)
	AB2	300	-1.59 ± 0.27	4.29 ± 0.57	2.35 (2.01-2.66)	8.20 (23.18-34.15)	57.89 (13)
R. dominica	Lab	540	-10.25 ± 1.24	10.50 ± 1.23	9.47 (8.84-10.07)	15.79 (14.23-18.49)	311.07 (25)
	CS	540	-7.08 ± 0.95	7.40 ± 0.97	9.03 (8.30-9.79)	18.62 (15.74-24.82)	153.49 (25)
	RL	540	-4.16 ± 0.36	4.86 ± 0.38	7.17 (6.70-7.60)	21.60 (18.69-26.38)	31.98 (25)
S. zeamais	Lab	540	-6.58 ± 0.68	7.67 ± 0.71	7.22 (6.67-7.7)	14.53 (13.23-16.56)	151.99 (25)
	TX	540	-6.91 ± 0.62	8.99 ± 0.74	5.87 (5.54-6.17)	10.66 (9.88-11.78)	67.37 (25)
S. oryzae	Lab	540	-3.85 ± 0.69	6.98 ± 1.15	3.56 (3.13-3.97)	7.67 (6.29-11.19)	290.61 (25)

TX	540	-7.27 ± 0.82	8.84 ± 0.90	6.66 (6.14-7.10)	12.20 (11.10-13.98)	161.55 (25)

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

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

Table 6. Probit estimates and dosages required for 50 and 99% mortality for laboratory and field strains of adults of five insect species exposed to 0.54 g/m^3 of chlorine dioxide in the absence of wheat based on mortality assessment made 5 d after exposure.

Species	Strain	*N*[a]	Mean ± SE		LD (95% CI) (g-h/m^3)		χ^2 (df)[b]
			Intercept	Slope	LD_{50}	LD_{99}	
T. castaneum	Lab	240	-2.61 ± 0.60	6.07 ± 1.11	2.69 (2.18-3.03)	6.51 (5.29-10.36)	63.42 (10)
	AB1	240	-3.09 ± 0.33	6.01 ± 0.57	3.27 (3.03-3.48)	7.96 (6.89-9.88)	25.98 (10)
	MN	360	-4.72 ± 0.69	7.52 ± 1.08	4.25 (3.84-4.70)	8.66 (7.13-12.39)	88.99 (16)
R.dominica	Lab	360	-2.13 ± 0.54	3.70 ± 0.67	3.77 (2.62-4.57)	16.02 (11.94-29.77)	143.48 (16)
	CS	360	-2.58 ± 0.43	4.64 ± 0.57	3.61 (3.00-4.08)	11.46 (9.62-15.21)	65.86 (16)
	RL	360	-1.74 ± 0.56	2.98 ± 0.67	3.84 (2.18-4.90)	23.17 (15.02-73.93)	182.23 (16)
S. zeamais	Lab	420	-1.21 ± 0.47	3.49 ± 0.72	2.21 (1.29-2.85)	10.26 (7.44-21.56)	190.01 (19)
	TX	420	-3.96 ± 1.29	8.25 ± 2.39	3.02 (2.27-3.53)	5.79 (4.56-13.69)	456.95 (19)

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

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

Table 7. Comparison of 5 d LD_{99} values of phosphine susceptible (Lab) and phosphine resistant adults of five stored-product insect species exposed to chlorine dioxide in the presence of wheat.

Species	Strain[a]	Ratio (95% CI)
T. castaneum	AB1 vs. Lab	1.07 (0.82 - 1.40)
	MN vs. Lab	1.15 (0.83 - 1.59)
O. surinamensis	Lab vs. AB2	1.02 (0.60 - 1.75)
R. dominica	CS vs. Lab	1.17 (0.91 - 1.51)
	RL vs. Lab	1.35 (1.11 - 1.64)*
S. zeamais	Lab vs. TX	1.35 (1.17 - 1.55)*
S. oryzae	TX vs. Lab	1.62 (1.24 - 2.12)*

[a]The strain mentioned first has a higher LD_{99} value in the pair being compared.

*Significant ($P < 0.05$).

Table 8. Comparison of 5 d LD_{99} values of phosphine susceptible (Lab) and phosphine resistant adults of five stored-product insect species exposed to chlorine dioxide in the absence of wheat.

Species	Strain[a]	Ratio (95% CI)
T. castaneum	AB1 vs. Lab	0.81 (0.60 - 1.10)
	MN vs. Lab	1.10 (0.84 - 1.43)
R. dominica	Lab vs. CS	1.38 (0.88 - 2.17)
	RL vs. Lab	1.45 (0.71 - 2.93)
S. zeamais	TX vs. Lab	1.78 (1.03 - 3.07)*

[a]The strain mentioned first has a higher LD_{99} value in the pair being compared.

*Significant ($P < 0.05$).

Table 9. Effective dose estimates for adult progeny reduction of *R. dominica* after exposure to chlorine dioxide[a].

Species	Wheat (g)	N[b]	Mean ± SE		ED (95% CI) (g-h/m^3)		χ^2 (df)[c]
			Intercept	Slope	ED_{50}	ED_{99}	
LGB-lab	10	300	-4.15 ± 1.22	5.46 ± 1.37	5.76 (3.86-6.78)	15.36 (11.69-36.00)	213.21 (13)
LGB-CS	10	300	-3.64 ± 1.70	4.94 ± 1.91	5.46 (0.90-6.99)	16.13 (11.03-674.78)	433.94 (13)
LGB-RL	10	300	-2.09 ± 0.84	3.78 ± 0.96	3.57 (1.44-4.76)	14.71 (11.21-34.51)	76.43 (13)
LGB-lab	0	360	-0.41 ± 0.63	2.73 ± 0.96	1.42 (0.06-2.41)	10.07 (6.38-137.46)	235.21 (16)
LGB-CS	0	360	-0.14 ± 0.47	1.96 ± 0.66	1.17 (0.03-2.23)	18.11 (9.8-507.37)	169.85 (16)
LGB-RL	0	360	-0.83 ± 0.65	2.05 ± 0.86	2.55 (0.01-4.18)	34.84 (13.64- -)	487.19 (16)

[a]Adult progeny were not observed in the control and chlorine dioxide treatments for both *T. castaneum* and *O. surinamensis* strains.

No adult progeny were observed only chlorine dioxide treatments for both *S. zeamais* and *S. oryzae* strains.

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

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