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Toxicity of chlorine dioxide gas to phosphine susceptible and resistant adults of five stored-product insect species: Influence of temperature and food during gas exposure

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Abstract

Adults of phosphine susceptible and resistant strains of the red flour beetle, *Tribolium castaneum* (Herbst); lesser grain borer, *Rhyzopertha dominica* (F.); sawtoothed grain beetle, *Oryzaephilus surinamensis* (L.); maize weevil, *Sitophilus zeamais* Motschulsky; and rice weevil, *Sitophilus oryzae* (L.), were exposed for 2-12 h to a chlorine dioxide gas concentration of 1.40 g/m^3 (520 ppm) in an outdoor trailer during July and October of 2015. The mean ± SE temperatures in July and October were 32.8 ± 0.5 °C and 24.8 ± 0.6 °C, respectively. In July, complete mortality after 5 d was achieved for all species and strains in vials with wheat after a 4- or 8-h exposure; in October a longer exposure time was needed for complete mortality of insects in vials with wheat. Chlorine dioxide was more toxic to all insect species and strains at warmer than cooler temperature and in vials without wheat than those with wheat. Both phosphine resistant and susceptible strains were equally susceptible to chlorine dioxide. The presence of wheat resulted in delayed mortality of insects because of reaction of chlorine dioxide with active sites on kernels. Progeny production 8 wk after chlorine dioxide exposure showed a significant reduction (72-100%), compared with that in control vials for strains of *R. dominica*, *S. zeamais*, and *S. oryzae*. There was no progeny production in control and treatment vials for *T. castaneum* and *O. surinamensis*, as these species require dockage. Chlorine dioxide is a potential fumigant to control phosphine resistant strains of the five stored-product insect species.

Keywords: Chlorine dioxide, Fumigant, Stored-product insects, Phosphine resistance

Fumigation is an integrated pest management tactic for stored-product protection (Phillips et al. 2012). Phosphine gas has been a predominant fumigant globally since the 1930s (Chaudhry 1997). However, weak and strong phosphine resistance in several species of stored-product insects has emerged over the decades in many parts of the world (Benhalima et al. 2004, Hori and Kasaishi 2005, Song et al. 2011, Opit et al. 2012, Jagadeesan et al. 2012, Kaur et al. 2015, Gautam et al. 2017). One solution to overcome phosphine resistance is to find suitable alternatives. Chlorine dioxide is recognized as a biocide, and its oxidative stress to organisms can cause massive cellular damage and lead to apoptotic and necrotic cell death (Finnegan et al. 2010). It exists almost completely as a monomeric free radical, which gives it a remarkable oxidizing ability (Simpson 2005, Gómez-López et al. 2009). Chlorine dioxide gas can easily dissolve in water and stay as dissolved gas instead of being hydrolyzed. Once it separates from the solution, the gas tends to quickly decompose into chlorine, chlorate, and chloride when exposed to sunlight, high temperature, electric sparks, or high pressure (Simpson 2005, Smith et al. 2014). Therefore, the only safe way to store chlorine dioxide gas is in the solution form. If gaseous form is required, a chlorine dioxide gas generator has to be equipped to produce the gas on-site. Chlorine dioxide is approved by the Food and Drug Administration for use as an antimicrobial agent in water to disinfect meats, fruits, and vegetables (FDA 2001). Unlike other chlorine disinfectants, chlorine dioxide does not form trihalomethane, which is an advantage for its use in disinfecting food products (Simpson 2005).

Limited studies investigated the use of chlorine dioxide gas for control of stored-product and urban insect pests. Channaiah et al. (2012) reported 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. The mortality of eggs, young larvae, old larvae, and adults

of *T. castaneum* was 9.3, 100, 18.8, and 100%, respectively, after 1.68 h exposure in vials without food (flour) to a chlorine dioxide concentration of 496.6 g/m^3 (184,203 ppm). Corresponding mortalities of the four stages of *T. confusum* were 11.1, 100, 31.3, and 100%, respectively. Gibbs et al. (2012) investigated the use of chlorine dioxide to control bed bugs (*Cimex lectularius* L. and *Cimex hemipterus* F.). Complete mortality of both species was observed immediately after exposure to 1.95 g/m^3 (723 ppm) of chlorine dioxide gas for 94 min. However, after exposure to 0.98 g/m^3 (363 ppm) of chlorine dioxide for 89 min, complete mortality of these two species was observed 18 h after the treatment, indicating delayed mortality effects. In our recent study (E et al. 2017), adults of five stored-product insect species were exposed to a chlorine dioxide concentration of 0.54 g/m^3 (200 ppm), and complete mortality was achieved 5 d after a 3-34 h exposure, depending on the species and the presence of wheat during the treatment. The presence of wheat delayed mortality of insects exposed to chlorine dioxide. In the absence of wheat, the LD_{99} values for phosphine susceptible and resistant strains of *T. castaneum*; lesser grain borer, *Rhyzopertha dominica* (F.); and maize weevil, *Sitophilus zeamais* Motschulsky, were 6.51-8.66, 11.46-23.17, and 5.79-10.26 g-h/m^3, respectively. In the presence of wheat, the LD_{99} values for all strains of *T. castaneum*, *R. dominica*, *S. zeamais*; the sawtoothed grain beetle, *Oryzaephilus surinamensis* L.; and rice weevil, *Sitophilus oryzae* (L.), 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 (E et al. 2017). In insect cells, chlorine dioxide triggers the production of reactive oxygen species (ROS), and the imbalance of ROS and antioxidant enzymes leads to oxidative stress, which causes cellular damage and subsequent mortality of insects (Kumar et al. 2015, Kim et al. 2015).

Temperature has a significant influence on the efficacy of fumigants (Reed 2006, Phillips et al. 2012). Insects are cold-blooded organisms, and their metabolic rates are affected by

temperature. At lower temperature, insect respiration decreases resulting in less fumigant uptake (Phillips et al. 2012). Aulicky et al. (2015) reported that low temperatures contributed to poor ovicidal effect of phosphine against *T. confusum* eggs during a structural mill fumigation. In this study, the average temperatures during the fumigation for mill floors 0 (ground floor), 4, and 5 were 20.1, 47.7, and 44.3 °C, respectively. When 5-cm flour was present, the number of larvae of laboratory and field strains of *T. confusum* that emerged from eggs on floor 0 was 8.4 and 7.2, respectively. However, no larval emergence was observed on floors 4 and 5. The authors noted larval emerge from eggs but did not indicate how many eggs were present in the flour. In general, fumigation is not recommended at temperatures below 15°C (Reed 2006). Higher temperatures improve the effectiveness of fumigation by accelerating gas distribution and fumigant uptake by insects.

In the present investigation, chlorine dioxide gas was evaluated against adults of five common and economically-important stored-product insect species that were susceptible and resistant to phosphine (E et al. 2017). Temperature is a critical factor for fumigation, therefore in this study, insects were exposed to chlorine dioxide during summer and fall to investigate the influence of temperature on efficacy against insects. The effects of food availability (wheat) and temperature on chlorine dioxide efficacy were evaluated by estimating lethal doses that produce 50 and 99% mortality of adults. The adult progeny production was determined at 42 d following exposure to chlorine dioxide.

Material and Methods

Insect Cultures

Cultures of *T. castaneum* were reared on organic wheat flour (Heartland Mills, Marienthal, KS) fortified with 5% (by wt) brewer's yeast. *R. dominica* and *S. oryzae* were reared on organic hard red winter wheat (Heartland Mills), and *S. zeamais* was reared on organic yellow corn (Heartland Mills). Cultures of *O. surinamensis* were reared on organic rolled oats (Heartland Mills). Phosphine resistant strains of *T. castaneum* (AB1 and CF strains), *R. dominica* (CS and RL strains), and *O. surinamensis* (AB2 strain) were collected from farm-stored grains in Kansas, USA, whereas the field strains of *S. zeamais* (TX strain) and *S. oryzae* (TX strain) were collected from farm-stored grains in Texas, USA (E et al. 2017). All cultures were reared at 28°C and 65% RH in environmental growth chambers (Model I-36 VL; Percival Scientific, Perry, IA). Unsexed adults of mixed ages were collected directly from culture jars after sifting the culture through an 841-µm opening square-holed sieve (Seedburo Equipment Company, Chicago, IL).

The number of field strains of *T. castaneum*, *R. dominica*, *O. surinamensis*, *S. zeamais* and *S. oryzae* used in this study was 2, 2, 1, 1, and 1, respectively. Laboratory (LAB) strains of the same species that have been in rearing in the Department of Grain Science and Industry, Kansas State University, for 16 years were assumed to be susceptible to phosphine. Phosphine susceptibility or resistant status of LAB and field strains was verified following a discriminating dose test (Champ and Dyte 1976). Fifty unsexed of mixed-age adults of each species and strain were exposed to phosphine for 20 h with three replications. Phosphine concentrations used during the test for *Sitophilus* spp., *T. castaneum, R. dominica,* and *O. surinamensis* were 0.042, 0.042, 0.028, and 0.052 g/m^3 (30, 30, 20, and 37.5 ppm), respectively. The phosphine resistance status of all field strains tested here was reported in our previous work (E et al. 2017), except for

T. castaneum CF, which showed a survival rate of 15% after the discriminating dose test. All LAB strains of the five species were susceptible to phosphine since the survival rate was 0%. All field strains of the five species were resistant to phosphine with survival rates varying from 1.3 to 64.4% (E et al. 2017).

Chlorine Dioxide Generation

Chlorine dioxide gas was produced by a custom-built chlorine dioxide generator (PureLine Systems, Bensenville, IL), 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, KS. Chlorine dioxide gas was generated from sodium chlorite (31% purity) solution via two electrochemical reactions:

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

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

The highly pure chlorine dioxide gas (99%) was extracted out from the liquid base system by a stripping column. Chlorine dioxide concentrations were obtained by mixing different amounts of ambient air with pure chlorine dioxide gas, and measured by an optical sensor (Control 4000, Optek, Germantown, WI).

Exposure of Insects to Chlorine Dioxide

Bioassays were carried out in snap-cap vials (23 mm in diameter and 55 mm in height) that had mesh bottoms and caps (250 µm openings) to ensure penetration of chlorine dioxide through the vials, and also to prevent insects from escaping. Chlorine dioxide exposure was conducted in an air-tight polymethyl methacrylate (PMMA) chamber (0.6 m × 0.6 m × 1.0 m). Temperature and

relative humidity (RH) inside the testing chamber were recorded at 1-min intervals over 12 h (*n* = 720) by HOBO® data loggers (Model: U10-003, Onset Computer Corp., Bourne, MA). The mean ± SE temperature during bioassays done in July 2015 was 32.8 ± 0.5 °C, and the minimum and maximum temperatures were 27.6 and 35.5 °C. During October 2015, the mean ± SE temperature was 24.8 ± 0.6 °C (range, 17.6 to 28.8 °C). The mean ± SE (range) humidity during July and October bioassays were 50.9 ± 1.4% (35.4 to 64.1%), and 29.4 ± 1.2% (21.1 to 52.3%), respectively.

A total of 10 g of organic hard red winter wheat (11-12% moisture, wet basis) were placed in vials along with 20 unsexed adults of mixed ages of each species and strain. Vials were placed horizontally in the PMMA chamber to ensure maximum gas penetration. The target concentration of chlorine dioxide was 1.35 g/m^3 (500 ppm). However, fluctuations occurred during exposure, and the mean ± SE concentration measured was 1.40 ± 0.02 g/m^3 (519 ± 6 ppm) for bioassays done in July 2015, and 1.41 ± 0.03 g/m^3 (522 ± 12 ppm) for the bioassays done in October 2015. In July bioassays, 10 g of wheat was introduced into the vials along with insects, whereas, in October bioassays, vials with and without wheat were used. The exposure times were 2, 3, 4, 5, 6, 7, and 8 h for the July tests, and 4, 5, 6, 7, 8, 10, and 12 h for the October tests. Exposure times for *O. surinamensis* adults, in vials without wheat, were shorter due to their greater susceptibility to chlorine dioxide based on our previous work (E et al. 2017). Exposure times for *O. surinamensis* were 0.5, 1, 1.5, 2, 2.5, 3, and 4 h. Each species, strain, and exposure time combination was replicated three times. Control vials were kept in an environmental chamber at 28°C and 65% RH, and checked at times corresponding to vials exposed to chlorine dioxide.

After exposure to chlorine dioxide, vials were brought back to the laboratory and kept in environmental growth chambers at 28°C and 65% RH before making mortality assessments. In vials without wheat (October bioassays), 10 g of wheat was added prior to incubation in an environmental growth chamber to see if any adults recovered on food. Mortality was checked 1 and 5 d after the exposure to determine delayed toxicity effects (E et al. 2017). Vials from October bioassays were kept in the growth chambers for 8 wk for adult progeny production. Vials from July were discarded after mortality assessments, and data on adult progeny production were therefore not collected.

Data Analysis

Mortality was calculated as a percentage based on the number of dead insects out of the total exposed. Mortality of chlorine dioxide exposed insects was corrected for control mortality (Abbott 1925). Corrected mortality data at the maximum exposure times (4, 8 or 12 h depending on species and the month of exposure) were transformed to angular values (Zar 1984), and data by species were subjected to one-way analysis of variance (ANOVA) to determine significant differences among strains, and means were separated using Bonferroni *t*-tests at α=0.05 (SAS Institute 2008). One-way ANOVA and Bonferroni *t*-tests were used separately for 1- and 5-d mortality data, because the same set of vials were examined over time following exposure to chlorine dioxide.

The dosage of chlorine dioxide that insects were exposed to was determined by multiplying the mean concentration (*C*) with the exposure time (*t*), to provide a *Ct* product (dosage). The corrected 5-d mortality and *Ct* product data were subjected to probit analysis (SAS Institute 2008) to estimate lethal dosages resulting in 50% mortality (LD_{50}) and associated statistics. The LD_{50} value of each LAB strain of a species was compared with LD_{50} value of each

field strain using a ratio test (Robertson and Preisler 1992) to determine differences in susceptibility between LAB and field strains to chlorine dioxide. The effects of temperature and food availability were also assessed similarly using ratio tests. The 5-d mortality data were used for probit analyses and ratio tests, because in our previous work, 1-d mortality of adults of the same five species after exposure to chlorine dioxide continued to increase and stabilized after 5 d (Subramanyam and E 2015, E et al. 2017). Progeny production by species and strains for different exposure durations was transformed to $\log_{10}(x+1)$, and subjected to one-way ANOVA, and means were separated using Bonferroni *t*-tests at α=0.05 (SAS Institute 2008). The percentage adult progeny reduction for each species and strain was calculated as: [1- (adult progeny produced in treated samples ÷ adult progeny produced in control samples)] ×100.

Results

Chlorine Dioxide Exposure During July

One-way ANOVA of July data showed no significant differences in the corrected 1-d mortality among LAB and field strains of each species (Table 1) that were exposed to chlorine dioxide for the maximum exposure duration of 4 or 8 h in vials with wheat ($F_{\text{range among species and strains}}$ = 0.02-4.13; df = 1, 4 [for *O. surinamensis*, and *Sitophilus* spp.] and 2, 6 [for *R. dominica* and *T. castaneum*]; P_{range} = 0.1099-0.8843). A significant difference between LAB and field strain was observed in the case of *S. oryzae* (F = 36.58; df = 1, 4; P = 0.0038), with the LAB strain being more susceptible than the field strain. Complete mortality was observed for all species and strains after 5 d, suggesting delayed toxicity of chlorine dioxide. Adults of *O. surinamensis* were most susceptible to chlorine dioxide based on complete mortality at 5 d after only a 4 h exposure to chlorine dioxide compared to 8 h for the other four species.

Probit regression estimates based on 5-d mortality in July bioassays are shown in Table 2. *O. surinamensis* LAB strain had the lowest LD_{50} (1.16 g-h/m^3) among all strains, making it the most susceptible insect species to chlorine dioxide. *R. dominica* LAB strain showed the highest LD_{50} value (3.26 g-h/m^3). The 5-d mortality of *S. zeamais* TX strain and *S. oryzae* LAB strain was 100% after an 8 h exposure to chlorine dioxide, respectively. Therefore, the probit regression models failed to generate the 95% confidence intervals for lethal dose estimates

Chlorine Dioxide Exposure During October

There was no significant difference in the corrected 1- or 5-d mortality between phosphine susceptible laboratory and resistant field strains of *O. surinamensis* exposed to chlorine dioxide for 4 h (maximum exposure time) in vials with wheat (Table 3). Similarly, differences in the corrected 1- or 5-d mortality between phosphine susceptible and resistant strains of *S. zeamais* were not significant after exposure to chlorine dioxide for 12 h in vials with wheat. The 1-d mortality of *T. castaneum* adults was different among strains ($F = 6.83$; df = 2, 6; $P = 0.0284$), and the mortality of AB1 strain was significantly lower than the CF and LAB strains. The 5-d mortality showed no differences among the three strains ($F = 1.82$; df = 2, 6; $P = 0.2408$). Based on 1-d mortality, the LAB strain of *S. oryzae* was significantly more susceptible to chlorine dioxide than the TX strain ($F = 19.60$; df = 1, 4; $P = 0.0114$), but no differences were observed in mortality between these two strains after 5 d ($F = 1.14$; df = 1, 4; $P = 0.3456$). In the case of *R. dominica*, differences among strains were significant based on 1-d mortality ($F = 23.06$; df = 2, 6; $P = 0.0015$) and 5-d mortality ($F = 38.91$; df = 2, 6; $P = 0.0004$). The CS strain had significantly lower 1- and 5-d mortality than the LAB and RL strains. The latter two strains were equally susceptible to chlorine dioxide. *O. surinamensis* was most susceptible to chlorine dioxide

among the insect species tested, because of 91-100% mortality only after a 4-h exposure. Other species, notably *T. castaneum* LAB strain, had 100% mortality after a 12-h exposure to chlorine dioxide.

No significant differences were observed in the 1- or 5-d mortality of laboratory and field strains of each species exposed in vials without wheat in October ($F_{\text{range among species}}$ = 0.07-3.88; df = 1, 4 [for *O. surinamensis*, *Sitophilus* spp.] 2, 6 [for *R. dominica* and *T. castaneum*]; P_{range} = 0.0831-0.8004), except for the 5-d mortality of *S. zeamais* (F = 39.22, df = 1, 4, P = 0.0033), where the TX strain was more susceptible to chlorine dioxide than the LAB strain (Table 4).

The probit regression estimates for bioassays done in October with wheat are summarized in Table 5. LD_{50} values for *T. castaneum*, *R. dominica*, *O. surinamensis*, *S. zeamais*, and *S. oryzae* strains ranged from 4.97-5.50, 10.80-20.02, 2.50-3.09, 7.87-8.60, and 4.39-6.33 g-h/m^3, respectively. Tests done in October with wheat showed that *O. surinamensis* LAB strain had the lowest LD_{50} value (2.50 g-h/m^3), and *R. dominica* CS strain had the highest LD_{50} value (20.02 g-h/m^3). LD_{50} values of *T. castaneum*, *R. dominica*, *O. surinamensis*, *S. zeamais*, and *S. oryzae* strains for tests done in October without wheat ranged from 2.56-2.93, 2.46-4.03, 0.94-1.57, 4.00-4.34, and 0.68-3.59 g-h/m^3, respectively (Table 6). The lowest LD_{50} value among all strains was observed with *S. oryzae* LAB strain (0.68 g-h/m^3), and *S. zeamais* LAB strain and *R. dominica* CS strain had the highest LD_{50} values (4.34 and 4.03 g-h/m^3, respectively).

Comparison of LD_{50s} Using Ratio Tests

Based on bioassays done in July, there were no significant differences in LD_{50} values between the phosphine susceptible LAB strain and resistant field strains of *T. castaneum* and *S. zeamais*, since their 95% confidence interval (CI) for the ratios included 1 (Table 7). Ratio tests showed

that the LAB strain of *R. dominica* had a significantly higher LD_{50} value than the field strains, which indicated that the former was less susceptible to chlorine dioxide. Mortality of *S. oryzae* LAB and TX strains was 100% after a 4-h exposure to chlorine dioxide. In the case of *O. surinamensis*, the field strain was less susceptible to chlorine dioxide than the laboratory strain. Tests done in October showed a similar trend. In the presence of wheat (Table 8), *O. surinamensis* and *S. oryzae* field strains had a significantly higher LD_{50} than the LAB strain; for the other three species, the field strains had either a similar or lower LD_{50} value than the corresponding LAB strains. Tests done without wheat (Table 9) showed the same exact pattern as the ones exposed to chlorine dioxide with wheat.

Temperature during July tests ranged from 28-35 °C, and for 7 h out of the total 8 h exposure period, and temperatures were above 30 °C. By contrast, the temperature during tests done in October varied between 15 and 27 °C, and for 8 h out of the total 12-h exposure period, temperatures were slightly above 25 °C. Except for differences in temperatures during exposure to chlorine dioxide in July and October tests, all vials from these tests were held in a growth chamber at 28 °C and 65% RH for mortality assessments and adult progeny production. Therefore, any differences in mortality could be attributed to the influence of temperature on chlorine dioxide efficacy. When exposed to the same chlorine dioxide concentration, bioassays done in July had lower LD_{50} values compared to values observed in October. Ratio tests (Table 10) suggested that all species and strains had a higher LD_{50} value in October than in July. The warmer temperature in July compared to October promoted chlorine dioxide efficacy against insects, as reflected in the lower lethal dosages needed for 50% mortality for all five species and strains.

The presence of wheat during exposure reduced the efficacy of chlorine dioxide against all species and strains (Table 11). Ratio tests indicated significantly higher LD_{50} values for each species and strain in bioassays when wheat was added to vials compared to LD_{50} values from bioassays without wheat.

Effect of Chlorine Dioxide on Progeny Production

T. castaneum and *O. surinamensis* adults failed to produce any progeny, including in the controls. Progeny was observed for all strains of *R. dominica* and *Sitophilus* spp. Progeny production of all species and strains when insects were exposed to chlorine dioxide in vials with wheat was significantly different among exposure times, except for *R. dominica* RL strain (Table 12). The TX strain of *S. zeamais* did not produce any progeny at the maximum exposure time of 8 h, but the LAB strain of *S. zeamais* produced progeny. Both LAB and TX strains of *S. oryzae* did not show any progeny production after a 5-h or longer exposure to chlorine dioxide. For each species and strain, there was a reduction in progeny production as the exposure time increased. A 50% progeny reduction was achieved only after a 2-h exposure to chlorine dioxide for all strains of these three species. The RL strain of *R. dominica* had a 72% suppression in progeny production relative to production in the control treatment at the maximum exposure time of 8 h, but all other species and strains showed a 95-100% reduction in progeny production.

In the absence of wheat, except for *R. dominica* LAB strain, the progeny production among exposure times showed significant differences (Table 13). In the case of *R. dominica* CS and RL strains after a 1-h exposure, progeny production in chlorine dioxide exposed insects was greater than production in the control treatment. Except for these anomalous results, progeny production decreased with increasing exposure time. Both *Sitophilus* spp. showed a greater

suppression of progeny production compared to other species and strains. The approximate chlorine dioxide dosage for 50% reduction in progeny production relative to the control treatment among species and strains ranged from 1.41 to 2.82 g-h/m^3. LD_{50} values for 50% mortality of the same species and strains, except for *S. oryzae* LAB strain (0.68 g-h/m^3), was between 3.59 to 4.34 g-h/m^3. In general, the dosage required for approximately 50% progeny reduction of the species and strains tested was lower than dosage required for 50% mortality.

In the absence of wheat, after exposure to chlorine dioxide for 2 h, 1-d mortality of *R. dominica* LAB, CS, and RL strains was 8.1 ± 4.2%, 3.3 ± 1.6%, and 19.1 ± 5.3%, respectively; and the corresponding progeny reduction compared to control was 89.0, -53.0 (lower progeny production in control compared to chlorine dioxide exposed samples), and 54.2%, respectively. After exposure to chlorine dioxide for 2 h, surviving adults of *R. dominica* were able to lay eggs outside the wheat kernels, and larvae hatching from eggs may have entered wheat kernels and completed development to adulthood. By comparison, after exposure to chlorine dioxide for 2 h, 1-d mortality of *S. zeamais* and *S. oryzae* strains ranged from 5 to 16%, which was comparable to *R. dominica* strains. However, the corresponding progeny reduction in *Sitophilus* spp. was much higher than *R. dominica* strains, and ranged from 93 to 99%. The low 1-d mortality of *Sitophilus* spp. fails to explain the lower progeny production of exposed insects. The reproductive activities of *S. zeamais* and *S. oryzae* may have been affected by chlorine dioxide exposure more severely than *R. dominica*. Additional studies are needed to confirm the adverse effects of chlorine dioxide on the reproductive organs of different species.

Discussion

The Chi-square (χ^2) values for the goodness-of-fit were significant for all probit regressions, except for *S. zeamais* LAB, indicating poor fit of model to data. This suggested that responses to chlorine dioxide were heterogeneous. Unsexed adults of mixed ages were used in all bioassays, and this may have resulted in heterogeneous response to chlorine dioxide. In addition, chlorine dioxide concentrations in vials placed at different locations in the testing chamber may vary and possibly contributed to the heterogeneity observed. In cases where heterogeneity occurrs, variances and covariances of the of the priobit regression analysis were adjusted by a heterogeneity factor (χ^2 divided by the degrees of freedom (df)), and a critical value from the *t* distribution is used to calculate the 95% CI (SAS Institute 2008, Subramanyam et al. 2014). Heterogeneous responses of stored-product insects were also observed in another chlorine dioxide study (E et al. 2017).

Strains of *T. castaneum*, *R. dominica*, and *S. zeamais* that were phosphine resistant did not have higher LD_{50} values compared to the corresponding phosphine susceptible LAB strains. Field strains of *O. surinamensis* and *S. oryzae* had slightly higher LD_{50} values than their corresponding LAB strains. Mortality of *T. castaneum* larvae after exposure to chlorine dioxide was attributed to oxidative stress (Kim et al. 2015). Chlorine dioxide was reported to enhance the reactive oxygen species (ROS) production in larvae of *T. castaneum*. Although more antioxidant enzymes (superoxide dismutase and thioredoxin-peroxidase) were produced after chlorine dioxide exposure to cope with the overflow of ROS, the imbalance of synthesis and degradation of ROS imposed a potent oxidative stress to the insects inducing a lethal effect. Another study suggested that chlorine dioxide causes the reduction of hemocytes and the impaired hemocyte-spreading behavior in mature larvae of the Indian meal moth, *Plodia interpunctella* (Hübner).

The impared hemocyte-spreading behavior adversely damages the immune system causing larval vulnerability to bacterial infection (Kumar et al. 2015).

Mortality increased over time after exposure to chlorine dioxide indicating delayed toxic effects. A similar pattern was reported in two recently published studies. After exposure to 0.54 g/m^3 (200 ppm) of chlorine dioxide only for 8 h, the mortality of mature larvae of *P. interpunctella* at 25 °C steadily increased from 20% immediately after the 8-h exposure to close to 50% at 96 h, indicating delayed toxic effect of chlorine dioxide (Kumar et al. 2015). Under the same concentration, exposure time, and temperature conditions mentioned above, the mortality of adults of *T. castaneum* steadily increased from about 15%, immediately after an 8-h exposure to about 42% at 96 h (Kim et al. 2015).

Temperature enhanced chlorine dioxide toxicity to insects. Since most fumigants enter the insect respiratory system through spiracles, high respiration rate can potentially promote fumigation efficacy (Bond 1969, Reed 2006, Phillips et al. 2012). At the normal fumigation temperature range between 15 and 35 °C, the rise of temperature can reduce the fumigant dosage required to kill same number of insects because of increased respiration rates. In addition, adsorption of gas onto the surfaces of any objects, including grains, during fumigation is affected by temperature (Bond 1969, Reed 2006). The lower the temperature, the more gas molecules are adsorbed to surfaces of grains, and less free gas molecules are available to act on insects. Our results support this hypothesis. All tested species and strains had a significantly lower LD_{50} value when tested at a higher temperature. To achieve complete mortality for *T. castaneum* adults, the minimum exposure time in July (32.8 °C) and October (24.8 °C) was 8 and 12 h, respectively. In the case of *R. dominica*, *S. zeamais*, and *S. oryzae* adults complete mortality was achieved after exposure to 1.40 g/m^3 of chlorine dioxide (500 ppm) for 8 h in July, but fewer number of insects

died after exposure to same concentration of chlorine dioxide for 12 h in October. Estes (1965) estimated the lethal dose (LD_{50} and LD_{95}) of methyl bromide against adults of *T. confusum*, and the granary weevil, *Sitophilus granarius* (L.), over a wide range of temperatures (4 to 32 °C). Both species showed a similar trend, where lower temperatures required a higher lethal dose. For example, when fumigation was carried out at 32 °C for 12 h, the LD_{95} values for *T. confusum* and *S. granarius* adults were 45.6 and 24.0 g-h/m^3, respectively. However, when temperature dropped to 4 °C, after 12 h exposure to methyl bromide, the LD_{95} values for these two species were 181.2 and 45.6 0 g-h/m^3, respectively, a 2.97 and 0.9 fold increase. The toxicity of methyl bromide against the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) and oriental fruit fly, *Bactrocera dorsalis* (Hendel), was also reported to be affected by fumigation temperatures (Armstrong and Whitehand 2005). The LT_{95} values for eggs, first instars, and third instars of *C. capitata* after exposure to 64 g/m^3 (16,448 ppm) of methyl bromide at 15 °C were 76, 51, and 33 min, respectively; the corresponding LT_{95} values at 30 °C for the three stages were 31, 23, and 30 min, respectively. Similarly, for *B. dorsalis*, the LT_{95} values for eggs, first instars, and third instars at 15 °C were 66, 50, and 61 min, respectively; and the corresponding LT_{95} values at 30 °C were 27, 16, and 19 min, respectively. The strongly phosphine-resistant psocid *Liposcelis bostrychophila* Badonnel was more tolerant to phosphine fumigation at a low temperature compared to a high temperature (Nayak and Collins 2008). Time to population extinction of *L. bostrychophila* after exposure to 1 g/m^3 of phosphine at 70% RH, and at 25 and 35 °C was 9 and 2 d, respectively.

Food availability during chlorine dioxide exposure influenced toxicity against all species and strains. The LD_{50} values were significantly greater when 10 g of wheat was present in vials during chlorine dioxide exposure compared to exposures 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 (Simpson 2005, Nam et al. 2014). Although the penetration of chlorine dioxide in grain mass has not been characterized, the mechanism is speculated to be very similar to ozone, a gas which has a higher oxidation potential than chlorine dioxide (Simpson 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, this process can take from hours to days to reach a constant concentration (Kells et al. 2001, Mendez et al. 2003, Campabadal et al. 2013). In the grain mass, ozone is prone to react with inherent sites on the grain surface first, and once all sites became saturated, ozone begins to accumulate in the treated commodity/space (Kells et al. 2001). If the concentration of chlorine dioxide in the grain mass was governed by the similar mechanism as that of ozone, insects with wheat may be exposed to less number of gas molecules compared to those without wheat in the initial phases of exposure. As most active sites on the surface of kernels were oxidized by chlorine dioxide, the free gas molecules start accumulating eventually reaching a lethal dose in the grain mass to kill insects.

Adults of *T. castaneum* and *O. surinamensis* exposed to chlorine dioxide and incubated with wheat kernels for 8 wk failed to produce adult progeny. Adults of these two species in the control treatment also failed to produce adult progeny. The lack of adult progeny production of these two species on wheat can be attributed to the inability of larvae to survive and develop, as these two species are secondary feeders and require broken kernels, dockage, or flour (Sinha and Watters 1985). A similar finding with these two species was reported in our previous study with chlorine dioxide (E et al. 2017). The progeny production of *R. dominica* and *Sitophilus* spp. was

inversely related to mortality of adults, which increased with increasing exposure time to chlorine dioxide.

In conclusion, our study indicated that chlorine dioxide is effective in controlling adults of phosphine resistant and susceptible strains of *T. castaneum*, *R. dominica*, *O. surinamensis*, *S. oryzae*, and *S. zeamais*. *O. surinamensis* was the most susceptible species with or without wheat. *S. zeamais* and *R. dominica* were the least susceptible species to chlorine dioxide with or without wheat. Significant adult progeny reduction was observed in strains of *R. dominica* and *Sitophilus* spp. Warmer temperature improved the efficacy of chlorine dioxide against all insect species and strains. The presence of wheat reduced chlorine dioxide efficacy. In order to replace phosphine with chlorine dioxide to control stored product insects, two key issues need to be addressed, namely the effect of chlorine dioxide on grain quality, and the cost of the fumigation.

Acknowledgements

We thank PureLine Systems, Inc. (Bensenville, Illinois, USA) for donating the trailer with the capability of generating chlorine dioxide gas, and for help in ensuring that the unit worked properly. The authors acknowledge the support of Plant Biosecurity Cooperative Research Centre (Project No: PBCRC3038) established and supported under the Australian Government's Cooperative Research Centre Program. Mention of a proprietary product name does not constitute an endorsement for its use by Kansas State University. This paper is contribution number 18-232-J of the Kansas State University Agricultural Experiment Station.

References

Abbott, W. S. 1925. A method for computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265-267.

Armstrong, J. W., and L. C. Whitehand. 2005. Effects of methyl bromide concentration, fumigation time, and fumigation temperature on Mediterranean and oriental fruit fly (*Diptera: Tephritidae*) egg and larval survival. J. Econ. Entomol. 98: 1116-1125.

Aulicky, R., V. Stejskal, B. Frydova, and C. G. Athanassiou. 2015. Susceptibility of two strains of the confused flour beetle (Coleoptera: Tenebrionidae) following phosphine structural mill fumigation: effects of concentration, temperature, and flour deposits. J. Stored Prod. Res. 108: 2823-2830.

Benhalima, H., M. Q. Chaudhry, K. A. Mills, and N. R. Price. 2004. Phosphine resistance in stored-product insects collected from various grain storage facilities in Morocco. J. Stored Prod. Res. 40: 241-249.

Bond, E. J. 1969. 2. Principles of fumigation: Toxicity of fumigation to insects. Manual of fumigation for insect control, FAO, Rome, Italy.

Campabadal, C. A., D. E. Maier, and L. J. Mason. 2013. Efficacy of fixed bed zonation treatment to control insects in stored bulk grain. Appl. Eng. Agric. 29: 693-704.

Champ, B. R. and C. E. Dyte. 1976. Report of the FAO global survey of pesticide susceptibility of stored grain pests. Rome FAO Plant Production & Protect Series No. 5. Food and Agricultural Organisation of the United Nations, Rome, ix+297 pp.

Channaiah, L., C. Wright, Bh. Subramanyam, and D. E. Maier. 2012. Evaluation of chlorine dioxide gas against eggs, larvae, and adults of *Tribolium castaneum* and *Tribolium*

confusum. pp. 403-407. *In* Proceedings, 9th International Conference on Controlled Atmosphere and Fumigation in Stored Products, 15-19 October 2012, Antalya, Turkey.

Chaudhry, M. Q. 1997. A review of the mechanisms involved in the action of phosphine as an insecticide and phosphine resistance in stored-product insects. Pesticide Sci. 49: 213-228.

E, X., Bh. Subramanyam, and B. Li. 2017. Responses of phosphine susceptible and resistant strains of five stored-product insect species to chlorine dioxide. J. Stored Prod. Res. 72: 21-27.

Estes, P. M. 1965. Effects of time and temperature on methyl bromide fumigation of adults of *Sitophilus granarius* and *Tribolium confusum*. J. Econ. Entomol. 58: 611-613.

Food and Drug Administration (FDA). 2001. Code of Federal Regulations Title 2, Section 173.300 Chlorine dioxide. http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=173.300

Finnegan, M., E. Linley, S. P. Denyer, G. McDonnell, C. Simons, and J. Y. Maillard. 2010. Mode of action of hydrogen peroxide and other oxidizing agents: differences between liquid and gas forms. J. Antimicrob. Chemother. 65: 2108-2115.

Gautam, S. G., G. P. Opit, and E. Hosoda. 2017. Phosphine resistance in adult and immature life stages of *Tribolium castaneum* (Coleoptera: Tenebrionidae) and *Plodia interpunctella* (Lepidoptera: Pyralidae) populations in California. J. Econ. Entomol. 110: 795-804.

Gibbs, S. G., J. J. Lowe, P. W. Smith, A. L. Hewlett. 2012. Gaseous chlorine dioxide as an alternative for bedbug control. Infect. Control Hosp. Epidemiol. 33: 495-498.

Gómez-López, V. M., A. Rajkovic, P. Ragaert, N. Smigic, and F. Devlieghere. 2009. Chlorine dioxide for minimally processed produce preservation: a review. Trends Food Sci. Technol. 20: 17-26.

Pimentel, M. A. G., L. R. D. Faroni, M. R. Totola, R. N. C. Guedes. 2007. Phosphine resistance, respiration rate and fitness consequences in stored-product insects. Pest Manag. Sci. 63: 876-887.

Phillips, T. W., E. M. Thoms, J. DeMark, and S. Walse. 2012. Fumigation, pp. 157-178. In Hagstrum, D. W., T. W. Phillips, and G. Cuperus (eds.), Stored Product Protection. Kansas State University. Manhattan, KS.

Hori, M. and Y. Kasaishi. 2005. Estimation of the phosphine resistance level of the cigarette beetle, *Lasioderma serricorne* (Fabricius) (Coleoptera: Anobiidae), by the knockdown time of adult. Appl. Entomol. Zool. 40: 557-561.

Jagadeesan, R., **P. J. Collins, G. J. Daglish, and P. R. Ebert. 2012.** Phosphine resistance in the rust red flour beetle, *Tribolium castaneum* (Coleoptera: Tenebrionidae): inheritance, gene interactions and fitness costs. PLoS ONE 7(2): e31582.

Kaur, R., M. Subbarayalu, R. Jagadeesan, G. J. Daglish, M. K. Nayak, H. R. Naik, S. Ramasamy, C. Subramanian, P. R. Ebert, and D. I. Schlipalius. 2015. Phosphine resistance in India is characterized by a dihydrolipoamide dehydrogenase variant that is otherwise unobserved in eukaryotes. Heredity 115: 188-194.

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

Kim, Y., J. Park, S. Kumar, H. Kwon, and J. Na. 2015. Insecticidal activity of chlorine dioxide gas by inducing an oxidative stress to the red flour beetle, *Tribolium castaneum*. J. Stored Prod. Res. 64: 88-96.

Kumar, S., J. Park, E. Kime, J. Na, Y. S. Chun, H. Kwon, W. Kim, and Y. Kim. 2015. Oxidative stress induced by chlorine dioxide as an insecticidal factor to the Indian meal moth, *Plodia interpunctella*. Pest. Biochem. Physiol. 124: 48-59.

Mendez, F., D. E. Maier, L. J. Mason, and C. P. Woloshuk. 2003. Penetration of ozone into columns of stored grains and effects on chemical composition and processing performance. J. Stored Prod. Res. 39: 33-44.

Nam, H., Seo, H. S., Bang, J., Kim, H., Beuchat, L. R., Ryu, J. H. 2014. Efficacy of gaseous chlorine dioxide in inactivating *Bacillus cereus* spores attached to and in a biofilm on stainless steel. Int. J. Food Microbiol. 188: 122-127.

Nayak, M. K., and P. J. Collins. 2008. Influence of concentration, temperature and humidity on the toxicity of phosphine to the strongly phosphine-resistant psocid *Liposcelis bostrychophila* Badonnel (Psocoptera : Liposcelididae). Pest Manag. Sci. 64: 971-976.

Opit, G. P., T. W. Phillips, M. J. Aikins, and M. M. Hasan. 2012. Phosphine resistance in *Tribolium castaneum* and *Rhyzopertha dominica* from stored wheat in Oklahoma. J. Econ. Entomol. 105: 1107-1114.

Raila, A., A. Lugauskas, D. Steponavicius, M. Railiene, A. Steponaviciene, and E. Zvicevicius. 2006. Application of ozone for reduction of mycological infection in wheat grain. Ann. Agric. and Environ. Med. 13: 287-294.

Reed, C. R. 2006. Grain fumigation, pp. 203-228. In C. R. Reed (eds.), Managing Stored Grain to Preserve Quality and Value. AACC International, St. Paul, MN.

Robertson, J. L., and H. K. Preisler. 1992. Pesticide bioassays with arthropods. Pesticide bioassays with arthropods.: xii + 127 pp.

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

Schlipalius, D., N. Valmas, A. G. Tuck, R. Jagadeesan, L. Ma, R. Kaur, Y. S. Mau, Q. Cheng, P. J. Collins, M. K. Nayak, H. J. Schirra, M. A. Hilliard, and P. R. Ebert. 2012. A core metabolic enzyme mediates resistance to phosphine gas. Science. 338: 807-810.

Simpson, G. D. 2005. Practical Chlorine Dioxide. Volume 1, Foundations. ISBN: 0-9771985-0-2.

Sinha, R. N.and F. L. Watters. 1985. Insect pests of flour mills, grain elevators, and feed mills and their control. Publication No. 1776E. Research Branch, Agriculture Canada.

Smith, D. J. 2014. Distribution and chemical fate of ^{36}Cl-chlorine dioxide gas during the fumigation of tomatoes and cantaloupe. J. Agric. Food Chem. 62: 11756-11766.

Song, X. H., P. Wang, and H. Y. Zhang. 2011. Phosphine resistance in *Rhyzopertha dominica* (Fabricius) (Coleoptera: Bostrichidae) from different geographical populations in China. Afr. J. Biotechnol. 10: 16367-16373.

Subramanyam Bh., Boina D. R., Sehgal B., and Lazzari F. 2014. Efficacy of partial treatment of wheat with spinosad against *Rhyzopertha dominica* (F.) adults. J. Stored Prod. Res. 59: 197-203.

Subramanyam Bh. and E X. 2015. Efficacy of chlorine dioxide gas against five stored-product insect speces. IOBC-WPRS Bulletin Vol. 111, pp. 159-168.

Yosep, S. M., P. J. Collins, G. J. Daglish, M. K. Nayak, H. Pavic, P. R. Ebert. 2012. The rph1 gene is a common contributor to the evolution of phosphine resistance in independent field isolates of *Rhyzopertha Dominica*. PLos One 7: 2, e31541.

Zuryn, S., J. Kuang, and P. R. Ebert. 2008. Mitochondrial modulation of phosphine toxicity and resistance in *Caenorhabditis elegans*. Toxicol. Sci. 102: 179-186.

Table 1. Corrected 1 and 5 d mortality (% mean ± SE) of adults of five insect species exposed to 1.40 g/m^3 of chlorine dioxide for 4 or 8 h with wheat in July 2015[a,b].

Species	Strain	Exposure time (h)	Mean ± SE mortality (%)	
			1 d after exposure	5 d after exposure
T. castaneum[c]	LAB	8	98.3 ± 1.7	100.0 ± 0.0
	AB1	8	100.0 ± 0.0	100.0 ± 0.0
	CF	8	100.0 ± 0.0	100.0 ± 0.0
O. surinamensis[d]	LAB	4	94.9 ± 5.1	100.0 ± 0.0
	AB2	4	92.3 ± 7.7	100.0 ± 0.0
R. dominica[e]	LAB	8	94.9 ± 3.0	100.0 ± 0.0
	CS	8	96.8 ± 3.2	100.0 ± 0.0
	RL	8	96.2 ± 3.8	100.0 ± 0.0
S. zeamais[f]	LAB	8	84.6 ± 2.3	100.0 ± 0.0
	TX	8	89.3 ± 0.3	100.0 ± 0.0
S. oryzae[g]	LAB	8	100.0 ± 0.0	100.0 ± 0.0
	TX	8	77.8 ± 6.4	100.0 ± 0.0

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

[b]The control mortality for *T. castaneum*, *O. surinamensis*. *R. dominica*, *S. zeamais*, and *S. oryzae* strains was 0 to 1.7 ± 1.7%, 7.5 ± 3.8 to 8.3 ± 3.3%, 4.9 ± 2.5 to 18.3 ± 1.7%, 6.7 ± 1.7 to 15.0 ± 0.0%, and 14.8 ± 3.0 to 15.3 ± 5.8%, respectively.

[c]There were no significant differences in 1-d mortality among strains (F = 1.00; df = 2, 6; P = 0.4219, by one-way ANOVA).

[d]There were no significant differences in 1-d mortality between strains (F = 0.02; df = 1, 4; P = 0.8843, by one-way ANOVA).

[e]There were no significant differences in 1-d mortality among strains (F = 0.17; df = 2, 6; P = 0.8508, by one-way ANOVA).

[f]There were no significant differences in 1-d mortality between strains (F = 4.19; df = 1, 4; P = 0.1099, by one-way ANOVA).

[g]There was a significant difference in 1-d mortality between the strains (F = 36.58; df = 1, 4; P = 0.0038).

Table 2. Probit regression estimates based on 5-d corrected adult mortality data for phosphine susceptible LAB and resistant field strains of five insect species exposed to 1.40 g/m^3 of chlorine dioxide in vials with wheat during July, 2015.

Species	Strain	*N*[a]	Mean ± SE		LD_{50} (g-h/m^3, 95% CI)	χ^2 (df)[b]	*P*
			Intercept	Slope			
T. castaneum	LAB	480	-0.71 ± 0.56	5.18 ± 1.32	1.85 (0.90-2.36)	159.05 (22)	<.0001
	AB1	480	-1.45 ± 0.42	7.54 ± 1.14	2.10 (1.74-2.33)	44.96 (22)	0.0027
	CF	480	-0.05 ± 0.61	3.66 ± 1.30	1.39 (0.08-2.21)	249.86 (22)	<.0000
R. dominica	LAB	420	0.29 ± 0.15	4.45 ± 0.58	3.26 (2.69-3.71)	148.77 (19)	<.0001
	CS	420	-1.03 ± 0.15	2.84 ± 0.58	1.98 (0.97-2.7)	154.56 (19)	<.0001
	RL	420	-1.70 ± 0.33	2.42 ± 0.42	1.78 (1.01-2.34)	33.04 (19)	0.001
O. surinamensis	LAB	180	-0.47 ± 0.34	4.48 ± 0.88	1.16 (0.8-1.39)	30.59 (7)	<.0001
	AB2	180	-0.29 ± 0.22	7.53 ± 0.72	1.85 (1.7-2.0)	19.89 (7)	0.0058
S. zeamais	LAB	240	-0.91 ± 0.20	4.10 ± 0.43	2.25 (1.87-2.55)	17.38 (10)	0.0663
	TX[c]	240	-5.16 ± 36275	20.49±120505	2.41 (-)	75.00 (10)	<.0001
S. oryzae	LAB[c]	300	-5.21 ± 17797	20.50 ±59121	2.42 (-)	31.36 (13)	0.003
	TX[d]	-	-	-	-	-	-

[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.

[c]Confidence intervals were not generated by SAS.

[d]The mortality was close to 100% for all tested hours based on 5-d mortality. Therefore, there were insufficient data points to fit a probit regression model to data.

Table 3. Corrected 1- and 5-d mortality (% mean ± SE) of adults of five insect species exposed to 1.41 g/m^3 of chlorine dioxide for 4 or 12 h with wheat in October 2015[a,b].

Species	Strain	Exposure time (h)	Mean ± SE mortality (%)	
			1 d after exposure	5 d after exposure
T. castaneum[c]	LAB	12	76.1 ± 4.8ab	100.0 ± 0.0
	AB1	12	61.7 ± 4.4b	90.0 ± 5.8
	CF	12	81.9 ± 1.9a	94.3 ± 3.8
O. surinamensis[d]	LAB	4	96.6 ± 1.7	100.0 ± 0.0
	AB2	4	91.1 ± 6.6	100.0 ± 0.0
R. dominica	LAB	12	65.6 ± 2.9a	84.1 ± 3.2a
	CS	12	29.8 ± 4.9b	38.4 ± 4.4b
	RL	12	66.2 ± 4.5a	83.1 ± 3.2a
S. zeamais[e]	LAB	12	47.1 ± 5.5	86.6 ± 4.0
	TX	12	38.7 ± 2.1	78.5 ± 3.9
S. oryzae[f]	LAB	12	74.1 ± 1.6	98.1 ± 1.9
	TX	12	59.3 ± 3.0	92.5 ± 3.7

[a]Each mean is based on *n*=3. Means by species followed by different letters are significantly different within the species ($P < 0.05$; by Bonferroni *t*-tests).

[b]The control mortality for *T. castaneum*, *O. surinamensis*. *R. dominica*, *S. zeamais*, and *S. oryzae* strains was 0%, 10.6 ± 1.1 to 16.9 ± 4.6%, 5.0 ± 0.0 to 14.7 ± 2.6%, 6.4 ± 1.4 to 10.0 ± 2.9%, and 0 to 8.3 ± 6.0%, respectively.

[c]There were no significant difference in 5-d mortality among strains (F = 1.82; df = 2, 6; P = 0.2408, by one-way ANOVA).

[d]There were no significant difference in 1-d mortality between strains (F = 0.29; df = 1, 4; P = 0.6196, by one-way ANOVA).

[e]There were no significant difference in 1-d or 5-d mortality between strains (F = 2.11 or 2.05; df = 1, 4; P = 0.2202 or 0.2253, by one-way ANOVA).

[f]There were no significant difference in 5-d mortality between strains (F = 1.14; df = 1, 4; P = 0.3456, by one-way ANOVA).

Table 4. Corrected 1- and 5-d mortality (% mean ± SE) of adults of five insect species exposed to 1.41 g/m^3 of chlorine dioxide for 4 or 8 h without wheat in October 2015[a,b].

Species	Strain	Exposure time (h)	Mean ± SE mortality (%)	
			1 d after exposure	5 d after exposure
T. castaneum[c]	LAB	8	96.7 ± 1.7	100.0 ± 0.0
	AB1	8	100.0 ± 0.0	100.0 ± 0.0
	CF	8	96.6 ± 1.7	100.0 ± 0.0
O. surinamensis[d]	LAB	4	100.0 ± 0.0	100.0 ± 0.0
	AB2	4	98.0 ± 2.0	97.8 ± 2.2
R. dominica[e]	LAB	8	93.3 ± 4.4	98.3 ± 1.7
	CS	8	79.4 ± 2.4	92.5 ± 2.5
	RL	8	84.9 ± 0.5	93.7 ± 1.9
S. zeamais[f]	LAB	8	47.2 ± 4.9	70.2 ± 1.7
	TX	8	48.8 ± 3.8	88.0 ± 2.1
S. oryzae[g]	LAB	8	53.5 ± 2.0	100.0 ± 0.0
	TX	8	43.0 ± 5.6	95.1 ± 4.9

[a]Each mean is based on n=3. Means by species followed by different letters are significantly different within the species ($P < 0.05$; by Bonferroni t-tests).

[b]The control mortality for strains of *T. castaneum*, *O. surinamensis*. *R. dominica*, *S. zeamais*, and *S. oryzae* was 1.7 ± 1.7% to 11.9 ± 3.5, 17.8 ± 2.2% to 21.4 ± 3.1%, 0%, 6.7 ± 1.7% to 9.2 ± 2.6%, and 8.0 ± 4.4% to 11.7 ± 4.4%.

[c]There were no significant difference in 1-d mortality among strains (F = 2.00; df = 2, 6; P = 0.2162, by one-way ANOVA).

[d]There were no significant difference in 1-d or 5-d mortality between strains (F = 1.00 ; df = 1, 4; P = 0.3739, by one-way ANOVA).

[e]There were no significant difference in 1-d or 5-d mortality among strains (F = 3.88 or 3.87; df = 2, 6; P = 0.0831 or 0.0868, by one-way ANOVA).

[f]There were no significant difference in 1-d mortality between strains (F = 0.07; df = 1, 4; P = 0.8004, by one-way ANOVA).

[g]There were no significant difference in 1-d or 5-d mortality between strains (F = 3.08 or 1.00; df = 1, 4; P = 0.1542 or 0.3739, by one-way ANOVA).

Table 5. Probit regression estimates based on 5-d corrected adult mortality data for phosphine susceptible LAB and resistant field strains of five insect species exposed to 1.41 g/m^3 of chlorine dioxide in vials with wheat during October, 2015.

Species	Strain	*N*[a]	Mean ± SE		LD_{50} (g-h/m^3, 95% CI)	χ^2 (df)[b]
			Intercept	Slope		
T. castaneum	LAB	480	-2.31 ± 0.4	3.25 ± 0.45	5.15 (4.19-5.90)	160.64 (22)
	AB1	480	-2.08 ± 0.31	2.82 ± 0.33	5.50 (4.67-6.19)	102.76 (22)
	CF	480	-1.97 ± 0.32	2.84 ± 0.35	4.97 (4.10-5.66)	105.11 (22)
R. dominica	LAB	480	-5.31 ± 0.65	4.92 ± 0.64	12.01 (10.95-13.55)	200.30 (22)
	CS	480	-5.84 ± 1.00	4.49 ± 0.93	20.02 (16.51-30.89)	191.55 (22)
	RL	480	-7.30 ± 0.67	7.06 ± 0.66	10.80 (10.21-11.48)	138.41 (22)
O. surinamensis	LAB	420	-2.03 ± 0.37	5.10 ± 0.77	2.50 (2.14-2.84)	302.16 (19)
	AB2	420	-4.30 ± 0.50	8.78 ± 0.95	3.09 (2.89-3.28)	154.15 (19)
S. zeamais	LAB	480	-2.78 ± 0.36	3.10 ± 0.38	7.87 (7.01-8.76)	142.01 (22)
	TX	480	-2.27 ± 0.29	2.43 ± 0.31	8.60 (7.67-9.65)	98.69 (22)
S. oryzae	LAB	480	-2.86 ± 0.46	4.44 ± 0.56	4.39 (3.68-4.95)	140.82 (22)
	TX	480	-1.83 ± 0.39	2.28 ± 0.42	6.33 (4.94-7.46)	180.01 (22)

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

[b]All 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 regression estimates based on 5-d corrected adult mortality data for phosphine susceptible LAB and resistant field strains of five insect species exposed to 1.41 g/m^3 of chlorine dioxide in vials without wheat during October, 2015.

Species	Strain	*N*[a]	Mean ± SE		LD_{50} (g-h/m^3, 95% CI)	χ^2 (df)[b]
			Intercept	Slope		
T. castaneum	LAB	360	-1.21 ± 0.27	2.58 ± 0.49	2.93 (2.25-3.68)	98.88 (16)
	AB1	360	-1.46 ± 0.16	3.58 ± 0.28	2.56 (2.28-2.82)	53.32 (16)
	CF	360	-2.00 ± 0.20	4.28 ± 0.34	2.93 (2.65-3.21)	61.01 (16)
R. dominica	LAB	480	-1.55 ± 0.26	2.91 ± 0.35	3.40 (2.73-4.05)	179.98 (22)
	CS	480	-2.36 ± 0.24	3.90 ± 0.31	4.03 (3.59-4.45)	89.49 (22)
	RL	480	-0.96 ± 0.20	2.46 ± 0.28	2.46 (1.90-2.97)	122.13 (22)
O. surinamensis	LAB	420	0.10 ± 0.12	3.42 ± 0.39	0.94 (0.75-1.10)	144.04 (19)
	AB2	420	-1.01 ± 0.24	5.13 ± 0.67	1.57 (1.34-1.78)	224.86 (19)
S. zeamais	LAB	480	-1.35 ± 0.28	2.12 ± 0.34	4.34 (3.32-5.17)	148.41 (22)
	TX	480	-1.78 ± 0.22	2.96 ± 0.28	4.00 (3.47-4.45)	82.63 (22)
S. oryzae	LAB	480	0.41 ± 0.38	2.45 ± 0.59	0.68 (0.11-1.26)	52.40 (22)
	TX	480	-1.95 ± 0.30	3.51 ± 0.38	3.59 (3.02-4.07)	129.41 (22)

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

[b]All 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. Comparisons of LD_{50} values based on 5-d corrected mortality data for phosphine susceptible LAB and resistant adults of five insect species after exposure to 1.40 g/m^3 of chlorine dioxide in vials with wheat during July, 2015.

Species	Strains[a]	Ratio (95% CI)
T. castaneum	AB1 vs. LAB	1.12 (0.77 - 1.63)
	LAB vs. CF	1.35 (0.59 - 3.10)
O. surinamensis	AB2 vs. LAB	1.62 (1.33 - 1.98)*
R. dominica	LAB vs. CS	1.62 (1.05 - 2.51)*
	LAB vs. RL	1.88 (1.22 - 2.70)*
S. zeamais[b]	TX vs. LAB	1.07 (0 - -)
S. oryzae[c]	LAB vs. TX	-

[a]The strain mentioned first has a higher LD_{50} value.

[b]The upper limit for the ratio was not calculated, because the LD_{50} for TX could not be estimated, since the mortality at all exposure times between 4 and 8 h was 100%.

[c]The ratio was not calculated, because the LD_{50} for TX could not be estimated, since the mortality at all exposure times between 4 and 8 h was 100%.

*Significant ($P < 0.05$).

Table 8. Comparison of LD_{50} values based on 5-d corrected mortality data for phosphine susceptible LAB and resistant adults of five insect species after exposure to 1.41 g/m^3 of chlorine dioxide in vials with wheat during October, 2015.

Species	Strains[a]	Ratio (95% CI)
T. castaneum	AB1 vs. LAB	1.07 (0.94 - 1.22)
	LAB vs. CF	1.02 (0.88 - 1.19)
O. surinamensis	AB2 vs. LAB	1.23 (1.07 - 1.41)*
R. dominica	CS vs. LAB	1.12 (0.99 - 1.27)
	LAB vs. RL	1.88 (1.22 - 2.70)*
S. zeamais	TX vs. LAB	1.07 (0.90 - 1.28)
S. oryzae	TX vs. LAB	1.44 (1.18 - 1.77)*

[a]The strain mentioned first has a higher LD_{50} value in the pair being compared.

*Significant ($P < 0.05$).

Table 9. Comparison of LD_{50} values based on 5-d corrected mortality data for phosphine susceptible LAB and resistant adults of five insect species after exposure to 1.41 g/m^3 of chlorine dioxide in vials without wheat during October, 2015.

Species	Strains[a]	Ratio (95% CI)[b]
T. castaneum	LAB vs. AB1	1.15 (0.92 – 1.44)
	LAB vs. CF	1.00 (0.81 – 1.23)
O. surinamensis	AB2 vs. LAB	1.70 (1.34 – 2.15)*
R. dominica	CS vs. LAB	1.20 (0.93 - 1.55)
	LAB vs. RL	1.38 (1.02 - 1.87)*
S. zeamais	LAB vs. TX	1.10 (0.81 - 1.49)
S. oryzae	TX vs. LAB	5.37 (2.19 - 13.16)*

[a]The strain mentioned first has a higher LD_{50} value in the pair being compared.

*Significant ($P < 0.05$).

Table 10. Comparison of LD_{50} values based on 5-d mortality of five species after exposure to chlorine dioxide during July (warmer temperature) and October (cooler temperature), 2015.

Species	Strains[a]	Ratio (95% CI)[*]
T. castaneum	LAB	2.75 (1.92-3.95)
	AB1	2.63 (2.25-3.08)
	CF	3.63 (1.69-7.8)
O. surinamensis	LAB	2.19 (1.76-2.73)
	AB2	1.66 (1.51-1.83)
R. dominica	LAB	3.72 (3.07-4.5)
	CS	10.00 (6.2-16.14)
	RL	6.03 (4.17-8.71)
S. zeamais	LAB	3.55 (3.15-4.00)
	TX[b]	3.55 (0 - -)
S. oryzae	LAB[b]	1.82 (0 - -)
	TX[c]	-

[a]The test done in October had a higher LD_{50} value than the one done in July.

[b]The upper limit for the ratio was not calculated, because the LD_{50} could not be estimated. The mortality at all exposure times between 4 and 8 h was 100%.

[c]The ratio was not calculated, because the LD_{50} could not be estimated. The mortality at all exposure times between 4 and 8 h was 100%.

[*]Significant ($P < 0.05$).

Table 11. Comparison of LD_{50} values based on 5-d mortality of five insect species after exposure to chlorine dioxide during with or without wheat during October, 2015.

Species	Strains[a]	Ratio (95% CI)[*]
T. castaneum	LAB	1.74 (1.41-2.14)
	AB1	2.14 (1.83-2.49)
	CF	1.70 (1.45-1.98)
O. surinamensis	LAB	2.69 (2.13-3.40)
	AB2	1.95 (1.68-2.26)
R. dominica	LAB	3.55 (2.79-4.52)
	CS	4.90 (3.68-6.51)
	RL	4.37 (3.49-5.47)
S. zeamais	LAB	1.82 (1.47-2.26)
	TX	2.14 (1.62-2.83)
S. oryzae	LAB	6.46 (2.66-15.65)
	TX	1.20 (1.02-1.42)

[a]The tests done with wheat had a higher LD_{50} value than those without wheat.

[*]Significant ($P < 0.05$).

Table 12. Progeny production (mean ± SE) and percentage reduction (in parenthesis) of LAB and field strains of five insect species after exposure to 1.41 g/m^3 of chlorine dioxide for different time periods in vials with wheat during October, 2015.

Exposure	*Ct*	*R. dominica*[a]			*S. zeamais*[a]		*S. oryzae*[a]	
time (h)	g-h/m^3	LAB	CS	RL	LAB	TX	LAB	TX
0	0	111.7±11.9a	41.7±10.7a	41±13.3	230±28.4a	209±23.9a	286.3±12.4a	263.7±39.6a
1	1.41	109.7±22.7a (1.8%)	32.3±7.0a (22.5%)	39±18.5 (4.9%)	119.7±28a (48.0%)	98.3±22.8ab (53.0%)	169.7±21.8a (40.7%)	185.7±23.5ab (29.6%)
2	2.82	41.3±8.7ab (63.0%)	21±5.2ab (49.6%)	21.0±6.4 (48.8%)	89.0±8.5a (61.3%)	11.0±1.0abc (94.7%)	138.7±15.8ab (51.6%)	65.7±23.4b (75.1%)
3	4.23	40.7±23.4ab (63.6%)	20.3±1.3ab (51.3%)	7.3±3.7 (82.2%)	112.3±19.4a (51.2%)	16.7±9.3abc (92%)	73.7±3.0ab (74.3%)	0.7±0.3c (99.7%)
4	5.64	37.7±14.9ab (66.2%)	13±6.1ab (68.8%)	48.3±11.2 (-17.8%)	4.0±3.5b (98.3%)	8.3±5.8abc (96%)	25.0±16.8b (91.3%)	2.0±0.6c (99.2%)
5	7.05	11±2.9ab (90.2%)	7.7±1.9ab (81.5%)	15.0±14.5 (63.4%)	27.3±12.5ab (88.1%)	1.0±0.6c (99.5%)	_[b]	_[b]
6	8.46	19±6.4ab (83.0%)	4.7±2.3ab (88.7%)	12.7±4.5 (69.0%)	12.3±10.3b (94.7%)	0.7±0.7c (99.7%)	_[b]	_[b]
7	9.87	13±6.4ab (88.4%)	5±2.1ab (88.0%)	8.0±4.6 (80.5%)	2.7±1.8b (98.8%)	24.3±24.3bc (88.4%)	_[b]	_[b]
8	11.28	3.3±2.0b (97.0%)	2.0±2.0b (95.2%)	11.7±9.7 (71.5%)	1.3±0.9b (99.4%)	0±0c (100%)	_[b]	_[b]
	F	4.59	4.93	1.47	15.55	7.71	5.53	79.40
	df	8, 18	8, 18	8, 18	8, 18	8, 18	4, 10	4, 10
	P	0.0035	0.0024	0.2342	<.0001	0.0002	0.013	<.0001

[a]Means by species and strain followed by different letters are significantly different ($P < 0.05$, by Bonferroni *t*-tests).
[b]Missing data.

Table 13. Progeny production (mean ± SE) and percentage reduction (in the parenthesis) of LAB and field strains of five insect species after exposure to 1.41 g/m^3 of chlorine dioxide for various time periods in vials without wheat during October, 2015.

Exposure time (h)	*Ct* g-h/m^3	*R. dominica*[a]			*S. zeamais*[a]		*S. oryzae*[a]	
		LAB	CS	RL	LAB	TX	LAB	TX
0	0	154.3±17.4	10.0±5.8	20.3±1.8a	147.7±16.2a	136.7±16a	158.0±23.6a	175.7±13.1a
1	1.41	49.7±25.2 (67.8%)	36.0±6.1 (-260.0%)	25.3±3.8a (-24.6%)	41.7±21.2ab (71.8%)	14.3±13.3b (89.5%)	143.7±13.2a (9.1%)	143±14.7a (18.6%)
2	2.82	17.0±11.5 (89.0%)	15.3±3.4 (-53.0%)	9.3±4.7ab (54.2%)	0.7±0.3b (99.5%)	10.3±10.3b (92.5%)	6.7±6.7b (95.8%)	2.7±2.7b (98.5%)
3	4.23	16.7±8.4 (89.2%)	3.3±1.7 (67.0%)	0±0b (100%)	14.7±14.7b (90.0%)	1.0±0.6b (99.3%)	0.3±0.3b (99.8%)	0±0b (100%)
4	5.64	11.0±4.9 (92.9%)	4.7±2.9 (53.0%)	3.7±1.5ab (81.8%)	0.3±0.3b (99.8%)	1.7±0.9b (98.8%)	0±0b (100%)	0.3±0.3b (99.8%)
5	7.05	16.7±7.3 (89.2%)	2.0±1.0 (80.0%)	3.7±0.7ab (81.8%)	0 ± 0b (100%)	0.3 ± 0.3b (99.8%)	_[b]	_[b]
6	8.46	_[b]	_[b]	_[b]	0 ± 0b (100%)	0.7 ± 0.7b (99.5%)	_[b]	_[b]
7	9.87	_[b]	_[b]	_[b]	0 ± 0b (100%)	0.7 ± 0.3b (99.5%)	_[b]	_[b]
8	11.28	_[b]	_[b]	_[b]	0 ± 0b (100%)	0.7 ± 0.7b (99.5%)	_[b]	_[b]
	F	3.10	3.20	8.50	7.39	6.02	28.93	56.38
	df	5, 12	5, 12	5, 12	8, 18	8, 18	4, 10	4, 10
	P	0.0502	0.0456	0.0012	0.0002	0.0008	<.0001	<.0001

[a]Means by species and strain followed by different letters are significantly different ($P < 0.05$, by Bonferroni *t*-tests).
[b]Missing data.