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Efficacy of chlorine dioxide gas against laboratory and field strains of five stored-product insect species

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ABSTRACT

The toxicity of chlorine dioxide gas at a concentration of 0.54 g/m^{-3} (200 ppm) was evaluated against five stored-product insect species at 24.8°C and 29.4% r.h. Adults of phosphine-susceptible laboratory strains and phosphine-resistant field strains were exposed in vials to chlorine dioxide for up to 34 h in the presence (10 g) or absence (0 g) of wheat (*Triticum* sp.). Fumigation times required to cause complete mortality of adults of the red flour beetle, *Tribolium castaneum* (Herbst); saw toothed grain beetle, *Oryzaephilus surinamensis* (L.); lesser grain borer, *Rhyzopertha dominica* (Fabricius); maize weevil, *Sitophilus zeamais* (Motschulsky); and rice weevil, *Sitophilus oryzae* (L.), in vials with 10 g of wheat was 26, 16, 34, 24, and 18 h respectively. In the absence of wheat, corresponding times were 15, 3, 20, 15, and 7 h respectively. The longer exposures needed in the presence of wheat could be due to reaction of chlorine dioxide gas with active sites on wheat kernel surfaces. No progeny was observed after 8 weeks in either control or treatment samples for *T. castaneum* (Herbst) and *O. surinamensis.* No *Sitophilus* spp. progeny were produced in chlorine dioxide-treated samples. Progeny was observed in *R. dominica* samples, and progeny reduction was100% compared to the control vials after a 24 or 20 h exposure to chlorine dioxide with or without wheat respectively. This laboratory scale study indicated that chlorine dioxide gas is effective in killing adults of five stored-product insect species and suppressing adult progeny production.

Key words: Chlorine dioxide gas, Efficacy assessment, Fumigation, Phosphine resistant strains, Stored-product insects

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, people started using chlorine dioxide for drinking water treatment to inactivate microorganisms and remove off-odors. In 1990s, researchers gained interest in using chlorine dioxide for disinfection of food products and to improve their shelf-life. Gómez-López et al. (2009) summarized several studies where gaseous chlorine dioxide was used to inactivate pathogenic microorganisms (*Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella*, and *Allicyclobacillusacidoterrestris*) from the surface of fruits and vegetables. Both low and high concentrations of chlorine dioxide (1 to 18 mg/L or 372 to 6,671 ppm) have been tested, and the exposure times varied between 6 and 120 min. The highest pathogenic log reduction was 8.04 for *E. coli* O157:H7 when chlorine dioxide gas was applied at 1.6 mg/L (600 ppm) for 30 min. A few entomologists in stored-product pest management have investigated the possibility of using chlorine dioxide as a fumigant to control insects (Channaiah et al., 2012; Kim et al., 2015; Kumar et al., 2015). One paper reported the study on 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 (Channaiah et al., 2012). Channaiah et al. (2012) exposed life stages of *T. castaneum* and *T. confusum* without food and with 5 g wheat flour in vials to chlorine dioxide dosages 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 the greatest

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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
Tribolium castanesum	Dickinson, Kansas	Wheat	AB1	2011
	Minneapolis, Kansas	Wheat	MN	2011
Rhyzopertha dominica	Chase, Kansas	Wheat	CS	2011
	Riley, Kansas	Flour	RL	2007
Oryzaephilus surinamensis	Abilene, Kansas	Wheat	AB2	2011
Sitophilus zeamais	Texas[a]	Corn	TX	2011
Sitophilus oryzae	Texas[a]	Corn	TX	2011

[a]County unknown

at the highest dosage. The mortality of eggs, young larvae, old larvae, or adults of *T. castaneum* was 9.3, 100, 18.8, or 100% after exposure to 834.4 g-h/m^3 chlorine dioxide without any food. Similarly, for *T. confusum*, the mortality of the four life stages was 11.1, 100, 31.3, or 100% when exposed to 834.4 g-h/m^3 chlorine dioxide without any food. Longer than 2 h exposures may be needed for effective control of all life stages. In the present investigation, five common stored-product insectspecies were treated with gaseous chlorine dioxide at 0.54 g/m^3(200 ppm) for different durations. The efficacy of chlorine dioxide was evaluated in the presence or absence of wheat. The effect of exposure to chlorine dioxide on adult progeny production was also studied.

MATERIALS AND METHODS

Cultures of *T. castaneum* were reared on organic wheat flour (Heartland Mills, Marienthal, Kansas, USA) fortified with 5% (by w) brewer's yeast (Lesaffre Yeast Corporation, Milwaukee, Wisconsin, USA). Cultures of the lesser grain borer, *Rhyzopertha dominica* (Fabricius), and rice weevil, *Stiophilus 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). The sawtoothed grain beetle, *Oryzaephilus surinamensis* (L.), was reared on organic rolled oats (Heartland Mills) plus 5% brewer's yeast diet. Field strains of *T. castaneum*, *R. dominica* and *O. surinamensis* were collected 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). Laboratory strains of the five species served as the phosphine susceptible strains, and phosphine resistancein laboratory and field strains (three replications and total 150 individuals for each strain) was verified following discriminating dose tests (Champ and Dyte, 1976). In the discriminating dose tests, phosphine concentrations 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. Insects were exposed to phosphine for 20 h, and mortality was assessed after 14 d to score insects as either susceptible or resistant to phosphine (Table 2). All cultures were reared in the laboratory at 28°C and 65% r.h. in environmental growth chambers. Unsexed adults of mixed ages were collected directly from culture jars after sifting the diet and insects through an 841-µm opening round-holed sieve (Fisher Scientific Company, Hampton, New Hampshire,USA).

Table 2 Survival (%) of laboratory and field strains of the five stored-product insect species exposed to discriminating doses of phosphine

Species	Strain	Survival (%)	Resistance status
Tribolium castaneum	Lab	0	Susceptible
	AB1	43.0	Weak
	MN	98.0	Strong
Rhizopertha dominica	Lab	0	Susceptible
	CS	64.4	Weak
	RL	27.8	Weak
Oryzaephilus surinamensis	Lab	0	Susceptible
	AB2	1.3	Weak
Sitophilus zeamais	Lab	0	Susceptible
	TX	6.7	Weak
Sitophilus oryzae	Lab	0	Susceptible
	TX	9.3	Weak

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 mesh caps with similar size openings to ensure diffusion of chlorine dioxide through the vials, and also to prevent insect escape. The chlorine dioxide treatment was conducted in an air-tight polymethyl methacrylate (PMMA) chamber (dimensions: 0.6 m × 0.6 m × 1.0 m). Chlorine dioxide (ClO_2) gas was produced by a custom-built chlorine dioxide generator by Pure Line Treatment Systems, LLC (Chicago, Illinois, USA), housed inside

a trailer. The trailer was located on the north campus next to the O.H. Kruse Feed Technology Innovation Center, onthe Kansas State University North Campus, Manhattan, Kansas, USA. Chlorine dioxide gas was produced from sodium chlorite (31% solution) via two electrochemical reactions:

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

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

These reactions produced 99% pure chlorine dioxide gas, which was then admixed with ambient air prior to entering the PMMA chamber where bioassay vials 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. The mean ± SE humidity was 29.4 ± 1.2% (range, 21.1 to 52.3%).

About 10 g organic hard red winter wheat of 11–12% moisture (wet basis) was placed in individual vials along with 20 unsexed adults of mixed ages for each species and strain. Vials were placed horizontally to ensure maximum gas diffusion through the vials. 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. However, the mean concentration during the fumigation, despite these fluctuations, was 201 ppm (range, 147 – 255 ppm). Insects were exposed to chlorine dioxide for various time periods (Table 3). Vials with and without wheat, but infested with insects, were held at 28°C and 65% r.h. and sampled at the same time intervals as chlorine dioxide exposed insects, served as the control treatment. Prior to beginning the tests, all samples were placed in the testing chamber, and were collected after the intended exposure. Each species, strain, and chlorine dioxide exposure treatment combination was replicated three times.

After exposure to chlorine dioxide, the vials were brought back to the laboratory and kept in environmental chambers maintained at 28°C and 65% r.h. For samples fumigated or not fumigated without wheat, 10 g wheat were added prior to incubation in the environmental growth chambers. Mortality of insects in control and chlorine dioxide exposed vials was checked 1 and 5 d after exposure, to determine any delayed toxicity effects of chlorine dioxide. After mortality assessments, insects (live and dead) were placed back into the vials and all vails were held in environmental growth chambers for 8 weeks to determine adult progeny production.

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

Species	Exposure time (h) for vials with wheat
Tribolium castanesum	10, 12, 15, 18, 22, 26
Oryzaephilus surinamensis	3, 5, 7, 10, 16
Rhyzopertha dominica	10, 14, 16, 20, 24, 26, 28, 30, 34
Sitophilus zeamais	10, 15, 18, 20, 22, 24, 26, 28, 30
Sitophilus oryzae	5, 7, 10, 15, 18, 20, 22, 24, 26, 28, 30
	Exposure time (h) for vials without wheat
Tribolium castanesum	5, 7, 10, 15
Oryzaephilus surinamensis	1, 3, 5, 7
Rhyzopertha dominica	7, 10, 14, 18, 20, 24
Sitophilus zeamais	5, 7, 10, 15, 20, 24, 28
Sitophilus oryzae	5, 7, 10, 15, 20, 24, 28

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 control mortality across all species and strains ranged between 0 and 20%. Progeny was transformed to $\log_{10}(x+1)$, and subjected to one-way analysis of variance (ANOVA), and means were separated by Bonferroni *t*-tests (SAS Institute, 2008). The actual adult progeny produced was determined by subtracting the original number of adults added to vials. Progeny reduction was calculated as (1-mean number of progeny production in treatment/ mean number of progeny production in control)×100.

RESULTS AND DISCUSSION

The 1 d mortality of *Sitophilus oryzae*, *S. zeamais*, *T. castaneum* and *R. dominica* strains was not 100% even after the longest exposure (30, 30, 26, and 26 h respectively). Complete mortality of *O. surinamensis* strains was observed after a 16h exposure. In the presence of wheat, the fumigation time to obtain 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 15–24, 3, 18–20, 7–15, and 5–7 h, respectively

Table 4 Exposure times required for complete mortality after 5 d for adults of five stored-product insect species exposed to a chlorine dioxide concentration of 0.54 g/m^3

Species[a,b]	Strain	Exposure time (h)	
		With wheat	Without wheat
Tribolium castanesum	Lab	26	15
	AB1	26	15
	MN	26[c]	15
Oryzaephilus surinamensis	Lab	16	3
	AB2	16	3
Rhyzopertha dominica	Lab	24	20
	CS	34	18
	RL	34	20
Sitophilus zeamais	Lab	24	15
	TX	18	7
Sitophilus oryzae	Lab	15	5
	TX	18	7

[a]Mean ± SE control mortality was less than 10%, except for *O. surinamensis* AB2 strain (11.2 ± 2.3%) and *R. dominica* CS strain (14.7 ± 3.0%) in the presence of wheat. In the absence of wheat mean ± SE control mortality of *O. surinamensis* AB2 strain, *R. dominica* CS strain, *S. zeamais* TX strain, and *S. oryzae* Lab strain was 12.6 ± 0.9, 16.6 ± 1.4, 26.7 ± 1.9, and 11.7 ± 4.7% respectively.

[b]Mortality assessments made 1 d after exposure to chlorine dioxide did not produce 100% mortality in adults of *S. oryzae*, *S.zeamais*, *T. castaneum*and *R. dominica* when exposed to 30, 30, 26, and 34 h respectively. Only 100% mortality in *O. surinamensis* was observed at 16 h.

[c]Less than 100% mortality was obtained after 26 h of exposure to chlorine dioxide. The mean ± SE mortality for *T. castaneum* MN strain was 93.3 ± 6.7 %

(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 such as respiration and metabolic rates. Respiration rates of insects have been linked to phosphine resistance (Pimental 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 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 had a lower chlorine dioxide tolerance. Chlorine dioxide, like other fumigants, enters insect's respiratory system, and a lower carbon dioxide production indicates lower respiratory rate and consequently less chlorine dioxide up-take. On the other hand, 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* 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. Species with higher heat production were more susceptible to chlorine dioxide.

Table 5 Mean ± SE number of progeny produced (% reduction) of *Rhyzopertha dominica* strains after exposure to 0.54 g/m^3 of chlorine dioxide at various exposure times in vials with wheat

Hour	Lab [a]	CS [a]	RL[a]
0	282.0±53.9a	107.0±11.5a	105.0±40.7a
10	162.0± 43.0ab (42.6%)	55.0±16.3ab(48.6%)	22.7±8.7ab(78.4%)
14	52.7±12.7ab(81.3%)	29.7±15.2ab(72.3%)	13.7±0.3ab(87.0%)
16	58.7±40.2ab(79.2%)	6.7±3.4b(93.8%)	11.3±5.7ab (89.2%)
20	31.7±16.8ab(88.8%)	17.3±17.3ab(83.8%)	3.0±3.0b(97.1%)
24	0±0b(100%)	0±0b(100%)	0±0b(100%)
F	10.53	6.96	5.49
df	5, 12	5, 12	5, 12
P	0.0005	0.0029	0.0009

[a]Means by strain followed by different letters are significantly different ($P< 0.05$, by Bonferroni *t*-tests)

Table 6 Mean ± SE number of progeny produced (% reduction) of *Rhyzopertha dominica* strains after exposure to 0.54 g/m^{3}ofchlorine dioxide for various time periods in vials without wheat

Hour (h)	Lab[a]	CS[a]	RL[a]
0	211.0±51.2a	92.3±12.5a	119.3±36.7a
5	63.3 ± 35.3ab (70%)	21.7 ± 11.1ab (76.5%)	88.3 ± 26.5ab (26%)
7	0 ± 0b (100%)	10.7 ± 2.9ab (88.4%)	14.3 ± 2.9c (88%)
10	17.7 ± 7.3ab (91.6%)	13.7 ± 7.7ab (85.2%)	20.7 ± 3.7bc (82.7%)
14	8.0 ± 8.0b (96.2%)	8.3 ± 4.4ab (91%)	18.0 ± 3.2bc (84.9%)
16	4.3 ± 4.3b (97.9%)	2.7 ± 2.7b (97.1%)	39.3 ± 15.4abc (67%)
20	0 ± 0b (100%)	0 ± 0b (100%)	0 ± 0d (100%)
F	5.93	4.89	25.30
df	6, 14	6, 14	6, 14
P	0.0029	0.0068	<0.0001

[a]Means by strain followed by different letters are significantly different ($P< 0.05$, by Bonferroni *t*-tests)

Adults of *T. castaneum* and *O. surinamensis* exposed to chlorine dioxide and incubated with wheat 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 by surviving adults of these species to inability of larvae to infest and survive on whole wheat kernels, as these two species are secondary feeders and require grain dust or dockage (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 adult progeny 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 adult progeny respectively. Adult progeny were not found in any chlorine dioxide exposed insects, regardless of the presence or absence of wheat. There were notable numbers of progeny produced by all *R. dominica*strains in chlorine dioxide exposed vials regardless of the presence of wheat (Tables 5 and 6). After exposure to chlorine dioxide for 24 h, the progeny reduction of *R. dominica* Lab, CS, and RL strains was100% in vials with wheat; in vials without wheat, no progeny were produced after a 20h exposure. The progeny production decreased significantly at higher chlorine dioxide exposure times.

CONCLUSION

Chlorine dioxide at 0.54 g/m^3 was effective in killing adults of phosphine susceptible and phosphine resistant strains of five 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 presence or absence of wheat. Progeny production was only observed in *R. dominica* vials, and the progeny reduction compared to the control reached to 100% after 24 or 20 h chlorine dioxide exposure with or without wheat. The laboratory findings should be confirmed by tests under practical field conditions such as grain bins and silos.

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