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Efficacy of ozone gas against laboratory and field strains of four stored-product insect species

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ABSTRACT

The efficacy of ozone, as an alternative fumigant to phosphine, was tested against four economically important stored-product insect species at 27.2°C and 20.4% r.h. Adults of phosphine susceptible laboratory strains and phosphine resistant field strains of the red flour beetle, *Tribolium castaneum* (Herbst); sawtoothed grain beetle, *Oryzaephilus surinamensis* (L.); maize weevil, *Sitophilus zeamais* Motschulsky; and rice weevil, *Sitophilus oryzae* (L.), were exposed to an ozone concentration of 0.42 g/m^3 (200 ppm) for 1, 2, 3, 5, 6, 8 and 10 h in the presence (10 g) or absence (0 g) of wheat (*Triticum* sp.). Mortality after the ozone exposure was assessed 5 days later. Complete mortality of *Sitophilus* spp. required a 3 to 4 h exposure to ozone, whereas that of *O. surinamensis* required 6 to 10 h. Adults of *T. castaneum* were the least susceptible to ozone, and after a 10 h exposure, the highest mortality recorded was 82 to 95%. The LT_{50} and LT_{99} values were estimated by probit analysis based on 5 d corrected mortality data. The LT_{99} values for *Sitophilus* spp., *O. surinamensis*, and *T. castaneum* were 2.00 to 5.56, 4.33 to 11.18, and 14.35 to 29.89 h respectively.

Key words: Efficacy assessment, Fumigation, Ozone, Phosphine resistant strains, Stored-product insects

Ozone is a highly reactive gas, and has been used as a disinfectant in water treatment as well as in the food-processing industry (Khadre et al., 2001, Tiwari et al., 2010). The decomposition of ozone leads to formation of free radicals including superoxide radical ion (O_2^-) which can oxidize sulfhydryl groups found in enzymes, double bonds of polyunsaturated fatty acids, including DNA (Khadre et al., 2001; Tiwari et al., 2010). Ozone have been reported to not only successfully deactivate microflora and its related mycotoxins but also effectively kill coleopterous and lepidopterous stored-product insect pests (Sousa et al., 2008, Isikber and Oztekin, 2009; Tiwari et al., 2010; McDonough et al., 2011; White et al., 2013; Isikber and Athanassiou, 2015). Due to its highly reactive nature, the rate of ozone penetration through the grain is related to the surface characteristic of grain kernels (Tiwari et al., 2010; Isikber and Athanassiou, 2015). The ozonation process has been categorized into phase I and phase II. During phase I, ozone reacts with the active sites on the kernel surface, and once all sites are saturated, ozone concentration gradually increases (phase II) to levels lethal for target insect pests (Kells et al., 2001; Campabadal et al., 2013). The length of these two phases are affected by the amount of grain, ozone flow rate, initial concentration of ozone, and as well as temperature and grain moisture content. In this study, both phosphine susceptible laboratory strains and phosphine resistant field strains of four stored-product insect species were exposed to ozone to investigate its effectiveness against insect pests.

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MATERIALS AND METHODS

Insect cultures

Cultures of the red flour beetle, *Tribolium castaneum* (Herbst),were reared on organic wheat (*Triticum* sp.) flour (Heartland Mills, Marienthal, Kansas, USA) fortified with 5% (by wt) brewer's yeast. The rice weevil, *Sitophilus oryzae* (L.), was reared on organic hard red winter wheat (Heartland Mills, Marienthal, Kansas, USA). The maize weevil, *Sitophilus zeamais* Motschulsky, was reared on organic yellow corn (*Zea* sp.) (Heartland Mills, Marienthal, Kansas, USA). The sawtoothed grain

Table 1 Sites and years of collection of field strains of fourstored-product insect species

Species	County, state	Commodity	Strain	Collection year	Survival (%)
Tribolium castaneum	Russell, Kansas	Wheat	PD	2011	45.0
	Washington, Kanas	Wheat	CF	2011	15.0
Oryzaephilus surinamensis	Abilene, Kansas	Wheat	AB2	2011	1.3
Sitophilus zeamais	Texas[a]	Corn	TX	2011	6.7
Sitophilus oryzae	Texas[a]	Corn	TX	2011	9.3

[a]County unknown.

beetle, *Oryzaephilus surinamensis* (L.), was reared on organic rolled oats (*Avena* sp.) (Heartland Mills, Marienthal, Kansas, USA) plus 5% by wt brewer's yeast diet. All cultures were held 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 cultures through an 841-µm opening square-holed sieve.

Phosphine resistant strains of *T. castaneum* and *O. surinamensis* were collected from farm-stored grain in Kansas, USA, whereas phosphine resistant strains of *S. zeamais* and *S. oryzae* were collected from farm-stored grain in Texas, USA. Laboratory strains of the four species served as the phosphine susceptible strains, and phosphine resistance of all species and strains (three replications and total 150 individuals for each strain) was verified following a discriminating dose test (Champ and Dyte, 1976). Phosphine concentrations used during the test for *Sitophilus* spp., *T. castaneum,* and *O. surinamensis* were 0.042, 0.042, and 0.052 g/m^3(30, 30, and 37.5 ppm) respectively. Fumigation lasted for 20 h, and mortality was assessed 14 d after the fumigation (Table 1).

Table 2 Time required for 100% mortality of adults of four stored-product insect species exposed to 0.42 g/m^3 of ozone in vials with and without wheat. Mortality was assessed 5 d after exposure

Species	Strain	With wheat (hour)	Without Wheat (hour)
Tribolium castaneum	Lab[a]	8	8
	CF[b]	8	10
	PD[c]	10	10
Oryzaephilus surinamensis	Lab	6	8
	AB2	10	8
Sitophilus zeamais	Lab	4	4
	TX	3	4
Sitophilus oryzae	Lab	4	3
	TX	3	3

[a]The mortality of *T. castaneum* Lab strain with and without wheat after 8 h of exposure was 96.0 ± 2.4 and 98.0 ± 2.0%, respectively, [b]the mortality of *T. castaneum* CF strain with and without wheat after 10 h of exposure was 97.0 ± 2.0 and 99.1 ± 0.9%, respectively, [c]the mortality of *T. castaneum* PD strain with and without wheat after 10 h of exposure was 95.1 ± 1.5 and 83.0 ± 4.6% respectively

Bioassays

Bioassays were carried out in snap cap vials (23 mm in diameter and 55 mm in height) that had mesh bottoms (250 µm opening) and mesh caps with similar size openings to ensure diffusion of ozone through the vials, and also to prevent insect escape. The ozone treatment was conducted in an air-tight polymethyl methacrylate (PMMA) chamber (0.50 m × 0.35 m × 0.35 m). Ozone was generated by a custom-built corona discharge ozone generator (O_3Co, Idaho Falls, Idaho, USA) with a capacity of 2.5 g/h, and monitored by a gas analyzer (IN2000-L2-LC, IN USA, Norwood, Massachusetts, USA). The ozone concentration in the testing chamber was recorded every minute and acquired by a program written in LABVIEW (National Instruments, Austin, Texas, USA). The ozone generator was housed inside a trailer that was parked outdoors on the premises of Department of Grain Science and Industry, North Campus, Kansas State University, Manhattan, Kansas, USA. The temperature and relative humidity of the chamber were monitored by HOBO© data loggers (Model: U10-003, Onset Computer Corp, Massachusetts, USA). The mean ± SE temperature during tests was 27.2 ± 0.08°C with the minimum and maximum temperatures being 22.9 and 32.5°C. The mean ± SE relative humidity for the test was 20.4 ± 0.9%, and during tests the humidity levels ranged from 15.0 to 30.6%. Each vial held 20 adults of a specific species and strain with no wheat (0 g) or 10 g of wheat. The feeding air flow rate was 0.02 m^3/minute. Samples were exposed to 0.42 g/m^3 (200 ppm) of ozone for 1, 2, 3, 4, 5, 6, 8, and 10 h. After the intended exposure to ozone, vials were brought back to the laboratory, and kept in environmental growth

Table 3 Probit regression estimates for adults of four stored-product insect species exposed to 0.42 g/m^3 of ozone in the presence of wheat

Species	Strain	N[a]	Mean ± SE		Lethal time (h, 95% CL)		χ^2 (df)[b]
			Intercept	Slope	LT_{50}	LT_{99}	
Tribolium castaneum	Lab	700	–3.56 ± 1.22	5.18 ± 1.66	4.86 (3.17-6.98)	13.64 (8.64-92.79)	4189.55 (33)
	CF	700	–1.42 ± 0.20	3.11 ± 0.33	2.85 (2.42-3.29)	15.91 (11.79-24.91)	447.45 (33)
	PD	700	–2.28 ± 0.40	3.26 ± 0.55	4.99 (4.05-6.23)	25.81 (16.19-64.99)	1019.84 (33)
Oryzaephilus surinamensis	Lab	180	–0.19 ± 0.16	2.97 ± 0.58	1.16 (0.83-1.43)	7.07 (4.24-25.46)	72.79 (9)
	AB2	760	–1.21 ± 0.14	3.37 ± 0.25	2.28 (2.03-2.51)	11.18 (9.33-14.16)	195.84 (36)
Sitophilus zeamais	Lab	600	–1.42 ± 0.20	5.03 ± 0.49	1.92 (1.72-2.11)	5.56 (4.65-7.20)	250.33 (28)
	TX	560	–1.68 ± 0.22	6.91 ± 0.65	1.75 (1.61-1.88)	3.80 (3.37-4.50)	135.55 (26)
Sitophilus oryzae	Lab	580	–1.34 ± 0.20	7.45 ± 0.69	1.51 (1.39-1.63)	3.11 (2.76-3.65)	149.12 (27)
	TX	580	–1.55 ± 0.10	12.85 ± 0.64	1.32 (1.28-1.36)	2.00 (1.90-2.13)	29.78 (27)

[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

chambers at 28°C and 65% r.h. Prior to incubation in the growth chamber, vials without wheat received 10 g of wheat as food for insects. Mortality was assessed 5 d after ozone exposure. Control vials were kept in the trailer where the ozone treatment was conducted, and were handled similarly as vials that were exposed to ozone. Treatment and controls had five replicates.

Data analysis

Mortality was expressed as a percentage based on number of dead insects out of the total exposed. Mortality of exposed insects was corrected for control mortality (Abbott, 1925). The 5 d corrected mortality data were subjected to probit analysis (SAS Institute, 2008) to determine the exposure times resulting in 50% (LT_{50}) and 99% (LT_{99}) mortality of insects.

RESULTS AND DISCUSSION

Exposure to ozone for 3 to 10 h resulted in 100% mortality of *O. surinamensis*, *S. zeamais*, and *S. oryzae* adults, whereas mortality of *T. castaneum* strains were 82 to 100% (Table 2). *Sitophilus* spp. generally were more susceptible to ozone than *O. surinamensis* and *T. castaneum*. Adults of *T. castaneum* were the least

Table 4 Probit regression estimates for adults of four stored-product insect species exposed to 0.42 g/m^3 of ozone in the absence of wheat

Species	Strain	N[a]	Mean ± SE		Lethal time (h, 95% CL)		χ^2 (df)[b]
			Intercept	Slope	LT_{50}	LT_{99}	
Tribolium castaneum	Lab	480	–4.54 ± 1.15	6.69 ± 1.52	4.78 (3.77-5.59)	10.65 (8.31-19.90)	868.37 (22)
	CF	620	–2.30 ± 0.29	4.00 ± 0.42	3.76 (3.31-4.21)	14.35 (11.37-20.30)	341.71 (29)
	PD	660	–1.94 ± 0.20	2.89 ± 0.28	4.70 (4.18-5.27)	29.89 (21.74-47.59)	250.42 (31)
Oryzaephilus surinamensis	Lab	800	0.03 ± 0.13	3.61 ± 0.41	0.98 (0.80-1.14)	4.33 (3.49-5.98)	233.45 (38)
	AB2	800	–1.18 ± 0.13	3.67 ± 0.25	2.10 (1.90-2.30)	9.05 (7.64-11.25)	208.33 (38)
Sitophilus zeamais	Lab	600	–0.55 ± 0.13	4.62 ± 0.42	1.32 (1.18-1.44)	4.20 (3.56-5.29)	147.16 (28)
	TX	600	0.38 ± 0.14	3.92 ± 0.57	0.80 (0.61-0.95)	3.14 (2.52-4.59)	162.42 (28)
Sitophilus oryzae	Lab	600	–0.29 ± 0.12	7.14 ± 0.78	1.10 (1.02-1.17)	2.32 (2.02-2.87)	116.64 (28)
	TX	160	0.16 ± 0.13	4.67 ± 0.99	0.92 (0.68-1.07)	2.91 (2.07-8.17)	32.13 (6)

[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

susceptible to ozone compared to the other species, because complete mortality was not observed after a 8 or 10 h exposure. Other studies also reported that *Sitophilus* spp. to be more susceptible to ozone fumigation than *T. castaneum*. Kells et al. (2001) exposed adults of *T. castaneum* and *S. zeamais* to an ozone concentration of 0.10 g/m^3 (50 ppm) for 3 d, and mortality of these two species was 92.2 and 100% respectively. Hansen et al. (2012) exposed adults of *T. castaneum*, *O. surinamensis*, *S. oryzae*, and *S. zeamais* at low concentrations of ozone for several days, and complete mortality was observed at different dosages based on the insect species. A dosage of 0.074 g/m^3 (35 ppm) for 6 d resulted in complete mortality of *T. castaneum* adults, whereas for *O. surinamensis*, *S. oryzae*, and *S. zeamais* the dosages for complete mortality were 0.042 g/m^3 (20 ppm) for 5 d, 0.044 g/m^3 (21 ppm) for 5 d, and 0.164 g/m^3 (78 ppm) for 5 d respectively. Adults of *S. zeamais* were the least susceptible to ozone, followed by *T. castaneum, O. surinamensis,* and *S. oryzae* (Hansen et al., 2012). McDonough et al. (2011) applied 3.78 g/m^3 (1 800 ppm) of ozone to corn infested with adults of *T. castaneum*, *S. oryzae*, and *S. zeamais*, and complete mortality was obtained after 120, 60, and 120 h respectively.

The probit estimates for lethal times (LT_{50} and LT_{99}) are shown in Tables 3 and 4. The Chi-square (χ^2) values for goodness-of-fit test were significant for all probit regressions, indicating poor fit of model to data. Heterogeneity can be the major contributor to the poor fit (Mahroof et al., 2003; Subramanyam et al., 2014). Insects used in tests were unsexed adults of mixed ages, and differences in sex and age may have contributed to the heterogeneity in responses observed. In addition, ozone concentrations and contact durations in vials located at different spots in the testing chamber may vary and contribute to the heterogeneity observed, as does the effect of insects exposed with and without wheat. In insecticide bioassays, heterogeneous responses in insects are not uncommon (Mahroof et al., 2003; Subramanyam et al., 2014). Based on 5 d mortality, in the presence of 10 g wheat, LT_{50} values for *T. castaneum, O. surinamensis, S. zeamais,* and *S. oryzae* were 2.85 to 4.99, 1.16 to 2.28, 1.75 to 1.92, and 1.32 to 1.51 h respectively; corresponding LT_{99} values were13.64 to 25.81, 7.07 to 11.18, 3.80 to 5.56, and 2.00 to 3.11 h respectively (Table 3). *Sitophilus* spp. was more susceptible to ozone, because the lethal times were less than those of *O. surinamensis* and *T. castaneum* by at least 2- to 5-fold. Bonjour et al. (2011) exposed adults of *T. castaneum* and *S. oryzae* to 0.10 g/m^3 (50 ppm) of ozone for 1 d inside a steel grain bin which contained 13.6 MT of hard red winter wheat. Adults of each species were confined in a cotton muslin tea bag with 50 g of wheat. The survival ratio of *T. castaneum* and *S. oryzae* was 100 and 35%, respectively, indicating more tolerance of *T. castaneum* to ozone than *S. oryzae*, which is comparable to current findings.

When insects were exposed to ozone without wheat, LT_{50} values for *T. castaneum, O. surinamensis, S. zeamais,* and *S. oryzae* were 3.76 to 4.78, 0.98 to 2.10, 0.80 to 1.32, and 0.92 to 1.10 h respectively.The corresponding LT_{99} values were 10.65 to 29.89, 4.33 to 9.05, 3.14 to 4.20, and 2.32 to 2.91 h respectively. As expected *Sitophilus* spp. more susceptible to ozone than the other species. Sousa et al. (2008) exposed 20 unsexed adults of *T. castaneum* and *O. surinamensis* collected from 23 locations in Brazil with 0.32g/m^3 (150 ppm) of ozone in the absence of food, and LT_{99} values (h) based on day 8 mortality were 22.17 to 37.90 h, and 11.03 to 18.72 h, respectively, which are comparable to our results.The LT values of *O. surinamensis*, *S. zeamais*, and *S. oryzae* strains in the vials with wheat were higher than in vials without wheat, which indicated that wheat kernels may protect these three species by adsorbing or chemically reacting with ozone (Kells et al., 2001). However, LT values of *T. castaneum* did not show this trend.

In conclusion, ozone at a concentration of 0.42 g/m^3 (200 ppm) can effectively kill adults of phosphine susceptible and resistant strains of *T. castanesum*, *O. surinamensis*, *S. oryzae*, and *S. zeamais*. In addition, no significant cross-resistance between ozone and phosphine was observed among the tested species and strains.*T. castaneum* was less susceptible to ozone than the other three species.

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REFERENCES

Abbott WS (1925). A method for computing the effectiveness of an insecticide. Economic Entomology **18**: 265–267.

Bonjour EL, Opit GP, Hardin J, Jones CL, Payton, ME, Beeby RL (2011). Efficacy of ozone fumigation against the major grain pests in stored wheat. Economic Entomology **104**: 308–316.

Campabadal CA, Maier DE, Mason LJ (2013) Efficacy

of fixed bed ozonation treatment to control insects in stored bulk grain. Applied Engineering in Agriculture **29**: 693–704.

Champ BR, Dyte CE (1976) Report of the FAO global survey of pesticide susceptibility of stored grain pests FAO Plant Production & Protect Series No. 5. Food and Agricultural Organisation of the United Nations, Rome.

Hansen LS, Hansen P, Jensen KMV (2012) Lethal doses of ozone for control of all stages of internal and external feeders in stored products. Pest Management Science **68**: 1,311–1,316.

Isikber AA, Athanassiou CG (2015) The use of ozone gas for the control of insects and micro-organisms in stored products. Journal of Stored Products Research **64**: 139–145.

Isikber AA, Oztekin S (2009) Comparison of susceptibility of two stored-product insects, *Ephestia kuehniella* Zeller and *Tribolium confusum* du Val to gaseous ozone. Journal of Stored Products Research **45**: 159–164.

Khadre MA, Yousef AE, Kim JG (2001) Microbiological aspects of ozone applications in food: a review. Food Science **66**: 1,242–1,252.

Kells SA, Mason LJ, Maier DE, Woloshuk CP (2001) Efficacy and fumigation characteristics of ozone in stored maize. Journal of Stored Products Research **37**: 371–382.

Mahroof R, Subramanyam Bh, Throne JE, Menon A (2003). Time-mortality relationships for *Tribolium castaneum* (Coleoptera: Tenebrionidae) life stages exposed to elevated temperatures. Journal of Economic Entomology **96**: 1 345–1 351.

McDonough MX, Mason LJ, Woloshuk CP (2011) Susceptibility of stored product insects to high concentrations of ozone at different exposure intervals. Journal of Stored Products Research **47**: 306–310.

SAS Institute (2008) SAS/STAT 9.2 User's Guide. SAS Institute, Cary, North Carolina, USA

Sousa AH, Faroni LRD, Guedes RNC, Totola MR, Urruchi WI (2008) Ozone as a management alternative against phosphine-resistant insect pests of stored products. Journal of Stored Products Research **44**: 379–385.

Subramanyam Bh, Boina DR, Sehgal B, and Lazzari F (2014) Efficacy of partial treatment of wheat with spinosad against *Rhyzopertha dominica* (F.) adults. Journal of Stored Products Research **59**: 197–203.

Tiwari BK, Brennan CS, Curran T, Gallagher E, Cullen PJ, O'Donnell CP (2010) Application of ozone in grain processing. Journal of Cereal Science **51**: 248–255.

White SD, Murphy PT, Leandro LF, Bern CJ, Beattie SE (2013). Journal of Stored Products Research **55**: 84–89.