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Toxic effects of ozone on selected stored product insects and germ quality of germinating seeds

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Abstract

The merchant grain beetle (MGB), *Oryzaephilus mercator* (Fauvel), the cigarette beetle (CB) *Lasioderma serricorne* (F.) and the rice weevil, *Sitophilus oryzae* (L.) cause significant damage to stored grain, grain-based products, and other durable commodities. Ozone, a highly oxidative toxic gas, has the potent to kill insects, meantime degrades rapidly to oxygen, making it a potential alternative to phosphine, a fumigant to which insects are developing resistance. The adults of MGB and CB were exposed to ozone concentrations of 100 - 400 ppm at 50 ppm increments for one hour and at 100 ppm for 1-6 h. Adults of rice weevil buried at 5, 15 or 25 cm depths within a wheat mass placed in 10 cm diameter 30 cm high PVC pipes were exposed to ozone concentration of 200 ppm for six hours and then at 12-h increments up to 60 h. Adult survival was recorded at 0, 24, and 48 h post-treatment. Significantly fewer MGB or CB adults survived when exposed to higher ozone concentrations or when exposed to ozone in the absence of food. RW adult mortality at 5 cm depth were significantly higher than that of 15 or 25 cm depths. This paper further discusses about mortality of MGB, CB and RW adults at different exposure periods at various ozone concentrations and effect of ozone on wheat germination.

Keywords: Fumigants, germination, ozone, stored product insects, wheat

Introduction

The ban of methyl bromide, the most effective fumigant for the control of many stored product insect pests, has necessitated the search for other potential alternative management methods. One of the potential alternatives is ozone (O_3), a highly oxidative, environmentally safe gas that degrade into molecular oxygen (O_2) within 20-50 minutes. Ozone is formed by the excitation of molecular oxygen, into atomic oxygen (O), and then combination of three atomic oxygen to form ozone. The use of ozone against stored product insect pests has gained tremendous attention over the past decade (Mahroof el al., 2018).

There have been few studies (Hasan et al., 2012) on the effect of ozone on the merchant grain beetle, *Oryzaephilus mercator* (Fauvel), the cigarette beetle, *Lasioderma serricorne* (F.) and the rice weevil, *Sitophilus oryzae* (L.). Published studies did not investigate the effect of ozone when externally feeding insects are treated in the presence or absence of food. Therefore, the study described here was conducted using adults of *O. mercator*, *L. serricorne* and *S. oryzae* exposed to different concentrations of ozone for different durations. The objectives of this study were to evaluate the relative susceptibility of adults to different ozone concentrations, determining concentration-mortality and time-mortality relationships and to determine germination quality of wheat treated using ozone.

Materials and Methods

A bench-top ozone generating equipment that produces ozone in the range of 0-8000 ppm was used in the experiment. Detailed descriptions of the Ozone generator were provided in Mahroof et al. (2018).

Exposure of *O. mercator* and *L. serricorne* to different concentrations of ozone

Adults of *O. mercator* and *L. serricorne* were exposed to ozone concentrations beginning 100 ppm and then at 50 ppm increments up to 400 ppm for one hour. Ten jars with 20 adults were used for

ozone treatment and 10 jars served as control. Five of the either treatment or control jars had 10 g diet mix and the other five were without food. Insects were treated either in the presence or absence of diet to determine the effect of ozone, if any. For each concentration tested, each treatment was replicated five times. Each experiment for any particular concentration was repeated for a total of three times. Adults were observed for mortality soon after treatment (0). Then, adults were placed in an incubator at 28 °C and 65% RH and observed again at 1 and 2 days after treatment (DAT).

Exposure of *S. oryzae* to 200 ppm ozone concentration for different durations

The effect of 200 ppm ozone concentration on adult *S. oryzae* adults at different depths within a column of wheat mass was tested using PVC pipes. A nylon pouch containing 20 adults was placed at the 5, 15 and 25 cm depths from the top of the PVC pipe filled with wheat. Three of the pipes were exposed to ozone by placing them in the ozone chamber set at 200 ppm for 12, 24, or 36 h. The remaining three pipes served as control. Adults were observed for mortality immediately after ozone exposure (0 DAT). Then, adults were provided with wheat kernels and placed in an incubator at 28 °C and 65% RH and observed again at 1 and 2 DAT.

Germination test

Wheat kernels exposed at 200-ppm of ozone for 12-60 h with an increment of 12 h were germinated in a Petri dish and germination percentage was compared with untreated wheat kernels. Seeds layered on a wet paper towel placed in the Petri dish were maintained in an environmental growth chamber at 28°C, 65% RH and 18:6 D: L photoperiodism for 10 days to record germination.

Data analyses

For experiments exposing the adults of *O. mercator* and *L. serricorne* to different concentrations of ozone, the corrected mortality data were fitted to a complementary log-log (CLL) regression model to estimate the concentration required to kill 50% (LC_{50}) and 99% (LC_{99}) of insects exposed to ozone (SAS Institute 2013). In the CLL model, the concentration was transformed to $\log_{10}$ scale. The goodness-of-fit of the model to the data was compared using a χ^2 statistic (SAS Institute 2013). For *S. oryzae*, we determined the differences between treated and corresponding control for each depth. The means were separated using Tukey's Honest Significant Difference (HSD). ANOVA was considered significant if $P < 0.05$ (PROC GLM, SAS Institute, 2013). Results from germination tests were summarized in a graph and represented as mean % germination at 5, 15 and 25 cm depths.

Results and discussion

Exposure of *O. mercator* and *L. serricorne* to different concentrations of ozone

The Probit estimates from the mortality response of adult *O. mercator* exposed to different ozone concentrations are summarized in Tab. 1. The results showed that susceptibility for adults varied to ozone when they were treated with or without diet. When adults were treated with diet, they were more tolerant to ozone than without diet. In *L. serricorne*, when diet were provided, similar to *O. mercator* adults were more tolerant than when diet was absent (Tab. 1). The χ^2 values were significant for the concentration-mortality regression models, an indication that the responses to ozone by the *O. mercator* and *L. serricorne* adults were heterogeneous whether they were treated with or without food. In height of heterogeneous response, the CLL model estimated a very high LC_{50} and LC_{99} values that did not yield any fiducial limits for O. Mercator adults, those were treated with food.

Tab. 1: Relative toxicity of ozone to adults of *O. mercator* and *L. serricorne* determined through concentration-mortality bioassays

Stage	Treatment type	Total # of insects	Intercept ± SE	Slope ± SE	LC_{50} (95% FL) (ppm) [a]	LC_{99} (95% FL) (ppm) [a]	χ^2 (df) [b]
***O. mercator* Adult**	With food	2100	-4.1 ± 1.5	0.7 ± 0.6	338432 [c]	212033356 [c]	157.5 (40) *
	Without food	2100	-13.5 ± 1.9	5.5 ± 0.8	239.0 (211.3–261.0)	525.7 (454.6 – 669.8)	2,207.7 (69) *
							739.0 (103) *
***L. serricorne* Adult**	With food	2100	-9.3 ± 1.4	2.8 ± 0.6	1442 (879–4620)	6692 (2628–62082)	
	Without food	2100	-9.0 ± 1.2	2.9 ± 0.5	854 (630–1549)	3769 (1931–14532)	955.0 (103) *

[a] FL, Fiducial Limits

[b] χ^2 values for goodness-of-fit of the CLL regression model to the observed mortality data

[c] Fiducial Limits were not calculated

Significant ($P < 0.05$)

Means within treatments followed by different letters are significantly different (Tukey's HSD test, $P < 0.05$). Upper case letters are for comparisons within 15 cm depth

* indicates a significant difference between a treatment and its corresponding control for a given day.

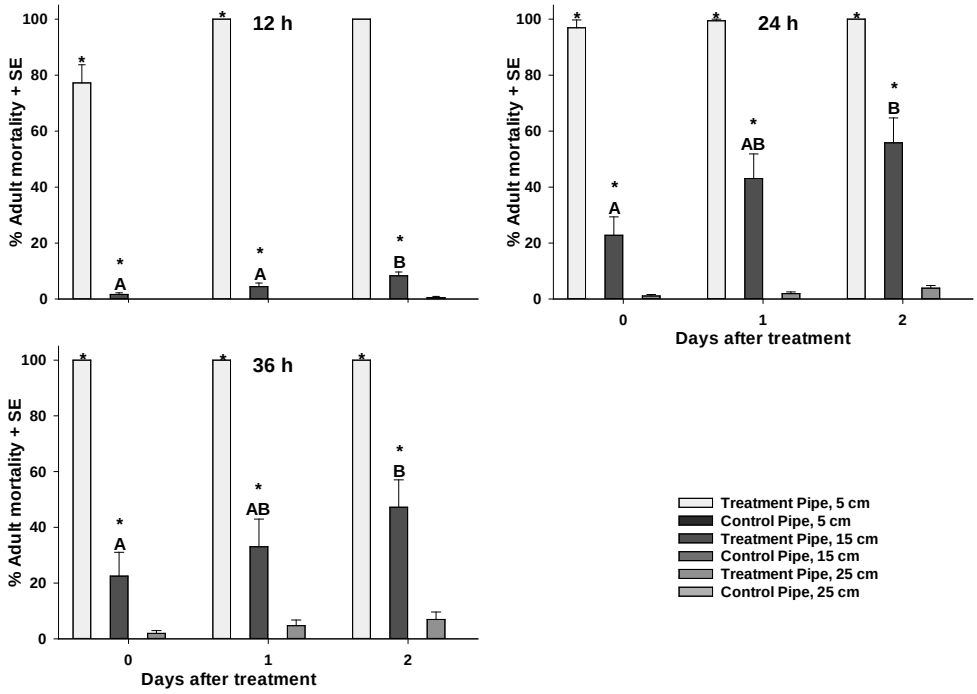

Fig. 1: Mean % mortality ± SE of adult *S. oryzae* exposed to 200 ppm of ozone for 12, 24 and 36 hours at 5, 15 and 25 cm depths in a wheat column.

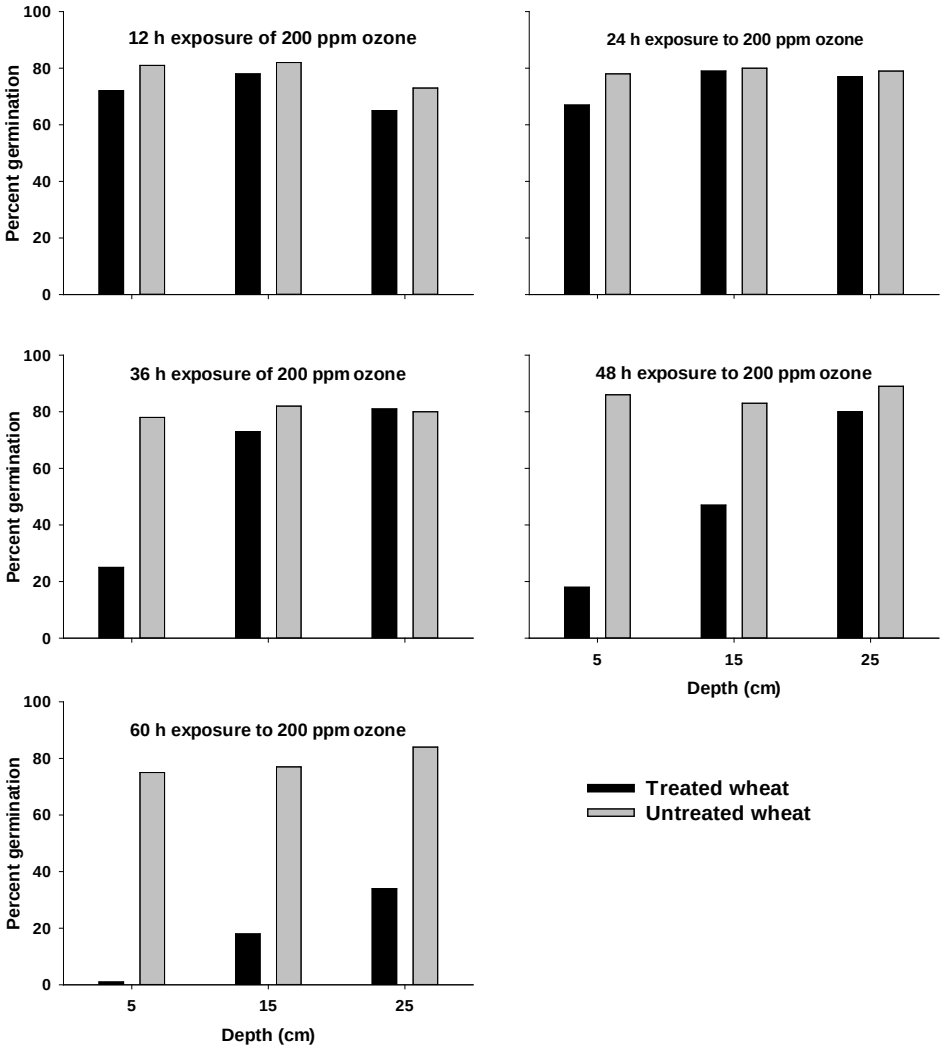

Fig. 2: Percentage germination of wheat seeds exposed to 200 ppm of ozone for 12-60 h at an increment of 12 h. Seeds were collected from 5, 15 and 25 cm depths in a wheat column.

Exposure of *S. oryzae* to 200 ppm ozone concentration for different durations

The percentage mortality of *S. oryzae* adults when exposed to 200 ppm for different durations, is presented in Fig. 1. Higher mortality was recorded at the 5 cm depths for each of the durations investigated. The longer the exposure period, the higher the mortality recorded. Exposure durations of 12 h or higher resulted in 100% mortality at the 5 cm depths by 2 DAT. At 24 h exposure duration, there were significant higher adult mortality at the 15 cm depth compared to control, soon after treatment, or at 1 and 2 DAT ($P < 0.001$). However, at the deepest depth, at 25 cm, there was no significant difference among days and between a treatment and its corresponding control for three durations tested ($P > 0.05$).

Based on our data, the highest concentration of ozone tested, 400 ppm, could not result in 100% mortality for both *O. mercator* and *L. serricorne*. Complete mortality in adult *L. serricorne* was achieved with 24 ppm after 48 h exposure, and shorter exposure periods required significantly

higher ozone concentrations to elicit similar effects (Hassan et al., 2012). Longer exposure durations and or higher concentrations will also be required to be able to control *S. oryzae* adults placed at the 25 cm depth of the PVC pipes. Ozone adsorption in grain layer depends on factors such as the ozone concentration supplied to the layer, ozone degradation rate, and the exposure duration to ozone (Tojanowska, 1991). Hence, longer exposure periods and or higher ozone concentrations may be required to control *S. oryzae* adults placed in deeper depths of a wheat column.

Germinations tests

Wheat germination was not adversely affected when seeds were exposed to 200 ppm up to 24 hours (Fig. 2). However, percentage germination was reduced, when compared to control germination at 5 cm depth, beyond the 36 hour-exposure to ozone at 200 ppm. Germination of wheat seeds considerably decreased when wheat was exposed to 60 hours at depths of 15 or 25 cm. Seeds collected and germinated from the 5 cm depth at 60 hours of exposure had germination closer to 0%. To our knowledge this is the first experiment to report effect of ozone on germination of wheat seeds in relation to insect mortality. It is clear with this study, that prolonged exposure of grain to ozone may adversely affect the germ quality. If grain is stored for seeding and cultivation purposes, or extracting germ for commercial uses, care must be taken if ozone treatment is an option for stored insect management.

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Update on ProFume® gas fumigant (sulfuryl fluoride) use for post-harvest pest control

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ProFume® gas fumigant (99.8% sulfuryl fluoride), first registered in 2003, is a broad spectrum, non-ozone depleting fumigant for the control of rodent, insect and other invertebrate pests. It is used to treat a wide range of stored products and structures which transport, store, and process commodities and is currently registered in 22 countries. Sulfuryl fluoride is not cross-resistant with phosphine and has been documented to effectively control quarantine pests, including the pinewood nematode and brown marmorated stinkbug.

Continued reduction of methyl bromide availability for non-quarantine fumigations coupled with the emergence of phosphine tolerant or resistant populations has led to increased interest in registration of sulfuryl fluoride in several tropical and sub-tropical countries. As part of the registration effort additional efficacy data has been developed to support the use in these countries. hile found in temperate zones, both Flat grain beetle (*Cryptolestes pusillus* (Schönherr)) and Maize Weevil (*Sitophilus zeamais* (Motschulsky)) are more problematic in tropical environments. As