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Dose-response of selected stored product insects to ozone treated on various surface materials

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Abstract

Since the phase out of methyl bromide, researchers are looking for alternatives to control stored insect pests and ozone has shown some promises as an effective control agent for stored insect pests. However, there exist many discrepancies on the effects of ozone on stored insect pests, which might be due to the differences on dose, exposure time, storage surface material, and method of application. The goal of this study was to determine the effects of ozone toxicity for different stored insect pests that could occur in different storage structures. The materials used commonly in storage structures included aluminum, cement, wood, glass, and vinyl. Preliminary experiments were done with eggs, larvae, pupae and adults of two stored insect pests, Indianmeal moth, *Plodia interpunctella* (Hubner), and red flour beetle, *Tribolium castaneum* (Herbst). Insect life stages were exposed to ozone gas on glass, aluminum, concrete, vinyl, and wood surfaces at 900 ppm. This study explored the time-concentration requirements of ozone gas to attain LT_{99} for the most tolerant life stage of *P. interpunctella* and *T. castaneum*.

Keywords: Fumigations, Grain storage, Ozone, Indianmeal moth, Insect management, Red flour beetle, Surface materials, Stored products

Introduction

The consequences of insect infestation in storage, milling, processing and warehouse facilities are far greater than the dollar costs of products (Arthur and Phillips, 2003). In general, stored product insects in bulk grain have been managed by fumigation using phosphine. Phosphine fumigation is an effective method to control stored-product insects, but its continuous and indiscriminate use has resulted in the evolution of resistant populations and control failures (Zettler and Keever, 1994; Shi et al., 2012).

More research on viable alternatives that can effectively control stored product insects in bulk storages deem necessary. Ozone is one such alternative, it can control insects and moulds associated with grain (Mahroof et al., 2017; 2018 a, b). Ozone is a toxic gas that can kill insects effectively in relatively short period. The most desirable aspect of ozone is that it decomposes rapidly to molecular oxygen without leaving a residue in treated food. Stored product insects such as Indianmeal moth, Plodia interpunctella (Hübner) (Lepidoptera: Pyralidae) and red flour beetle, Tribolium castaneum (Herbst) (Coleoptera: Tenebrionidae) are known to successfully feed, develop, and breed on a variety of durable commodities, causing significant losses to stored grains, pulses, dried fruits and nuts, and processed foods (Howe, 1957). Losses due to these insect infestations and damage can account for millions of dollars in the food industry. These insects are cosmopolitan in distribution and the most common indication of infestation is adult activity on the surface of the food. Plodia interpunctella commonly forms webbing or silken cocoons. Larval and adult tunnels are common in infested commodities as well as on dusty surfaces in food-processing facilities and grain elevators (Hagstrum and Subramanayam, 2009). In heavy infestations, the food material may be discolored, kernel structure may be disintegrated and dust and particles may be prevalent.

Ozone kills stored-grain insects such as Sitophilus oryzae (L.), Oryzaephilus surinamensis (L.), O. *mercator* (Fauvel), *Lasioderma serricorne* (F.), and *Ephestia elutella* (Hübner) and various fruit flies (Endman, 1979; Endman, 1980; Mahroof et al., 2017; 2018 a; Amoah and Mahroof, 2018). Endman (1980) who pioneered the effort of ozonation for killing stored product insects used low concentration gas with extended exposure time, up to 7 h. He reported that the younger larval and pupal stages of *T. confusum* were more ozone sensitive than similar older stages; 28-d-old pupae were the most ozone resistant of the stages tested. Other studies, including those by Mahroof et al. (2018 a, b) and Amoah and Mahroof (2018) have suggested that toxicity caused by ozone is usually delayed but dependent on the species, life stages, dose and exposure time.

Lack of data on dose-mortality response for various life stages of stored product insects alongside flow and penetration characteristic of ozone through various building materials or floor surfaces render uncertainly in application of this technique to various types of storage bins, food processing buildings or packaging. Therefore, there is a need to close the data gap that exist in the literature, in order to better popularize and utilize this pest control technique. The objective of this study was to evaluate the efficacy of ozone on the most tolerant life stages of *P. interpunctella* and *T. castaneum* and using the most tolerant stage to expose on surfaces, resembling flooring or construction of food processing plants or grain storage structures.

Methods

Ozone equipment, ozone generation, and application

A bench-top model of ozone generating equipment that produces ozone in the range of 0-8000 ppm with a continuous flow rate of 1-2 L/min using the corona discharge method, was custom built and obtained from Ozone Solutions Inc., Hull, IA. The equipment was made of five major components: the oxygen concentrator, a control box with ozone generator, the analyser, the ozone chamber, and the ozone destruction unit. The ambient air was taken up by the oxygen concentrator; O_2 in the air was concentrated and delivered between high voltage plates to simulate corona discharge. Oxygen was broken apart and recombined into ozone. The concentration of ozone was regulated by adjusting the push button selector switch on a touch screen panel. The accuracy of the concentration was further verified by the analyzer. The analyser was calibrated yearly according to NIST standards to maintain accuracy. Accuracy of the set concentrations varied by ± 5 ppm. The ozone chamber, where test specimens were placed, maintained user-defined ozone concentrations. Ozone was delivered to the chamber through three outlets. A circulation fan located inside the ozone chamber evenly distributed ozone throughout the chamber.

Establishing optimal lethal concentration
As a preliminary study, eggs, larvae, pupae, and adults of *P. interpunctella* and *T. castaneum* life stages were tested at 400, 500 and 600 ppm for 6 h exposure to select the most tolerant life stages. We did not report the results of the preliminary data herein. Based on the study, we selected eggs of *P. interpunctella* and *T. castaneum* for further studies. Twenty-four-hour old eggs transferred to Petri dishes either with 20 g of diet or without diet were exposed at 600 ppm concentration for 9 h. Lab artificial medium or 15 (whole wheat flour):1 (yeast) was used as the diet for *P. interpunctella* and *T. castaneum*, respectively. The control experiment was not to expose eggs to ozone gas, but rather to apply every other condition given to experimental unit including control treatments with or without diet. Post treatment, eggs were transferred to an environmental growth chamber at 28ºC and 60-65% RH. Egg hatch was observed daily until 10 d. The number of eggs that hatched both in treated and control experiments were recorded. If mortality exceeded >15% in control units, then treatment mortality was corrected based on the Abbott's Formula.

The experiment was a Randomized Complete Block Design (RCBD) with replication over time (block based on time). Experiments for *P. interpunctella* eggs were replicated six times and for *T. castaneum*, it was replicated nine times. At a given time for a give species, a total of 100 eggs were treated as 10 eggs/dish ($10 \times 10 = 100$). These 10 dishes were considered as pseudo replicates. Egg that did not hatch were expressed as percentage mortality and subjected to two-way ANOVA using PROC MIXED program. Means were separated using LS means procedure at $\alpha = 0.05$.

Evaluating the efficacy of ozone when exposed to various surfaces
The toxicity of ozone was evaluated for eggs of *P. interpunctella* by exposing them on various surfaces that resemble flooring or construction materials of food processing plants or grain storage structures. Glass, aluminum, concrete, vinyl, and wood were tested. Twenty-four-hour old eggs with or without diet were placed on the laboratory-fabricated arena. Eggs were exposed to ozone at 600 ppm for 9 h. A control experiment was maintained by introducing eggs to various surfaces with or without diet but not exposing them to ozone. Post treatment, eggs were transferred to an environmental growth chamber at 28ºC and 60-65% RH. Egg hatch was observed daily until 10 d. Number of eggs that hatched both in treated and control experiments were recorded. If mortality exceeded >15% in control units, then treatment mortality was corrected based on Abbott's Formula. The experiment was replicated over six times (block based on time). At a given time, a total of 100 eggs were treated as 50 eggs/dish ($50 \times 2 = 100$). These 2 dishes were considered as pseudo replicates. Mean percentage mortality in treated arenas and corresponding control arenas, whether with diet or without diet were estimated.

Results and discussion

Establishing optimal lethal concentration
Susceptibility of *P. interpunctella* and *T. castaneum* eggs was found when exposed to ozone at 600 ppm for 9 h. Egg mortality was not dependent upon whether presence or absence of diet in the treatment arena (Table 1). Ozone treated eggs with or without diet showed significantly lower hatch, resulting high egg mortality when compared to corresponding controls, for both species (*P. interpunctella:* $F = 337.41$, $df = 3,116$ $P < 0.0001$; *T. castaneum*: $F = 116.31$, $df = 3,176$ $P < 0.0001$). Egg mortality was consistently high for *P. interpunctella* eggs in control treatments. We presume this could be due to mechanical and physical damages caused during handling.

Comparing the two species, *P. interpunctella* eggs were more susceptible to chosen Ct (Concentration X time) resulting in closer to 100% mortality. However, *T. castaneum* eggs required a higher Ct value to attain 100% mortality. Moth eggs, in general, had more aerophyles and micropyles when compared to beetle eggs, allowing them to intake a relatively higher amount of gas during exposure, making ozone relatively more toxic to moth eggs. In *T. castaneum*, a higher percentage of egg mortality was encountered when eggs were treated without diet. Ozone is adsorbed by physical surfaces including diet and the gas had to penetrate through food by diffusion, resulting in a significant proportion of the gas loss. Therefore, it is suggested to use a higher Ct value in the presence of grain.

Table 1. Mean mortality (%) ± SE for *P. interpunctella* and *T. castaneum* eggs treated using 600 ppm ozone exposed to 9 h.

Species	Type of treatment	Mean %Mortality ± SE
Plodia interpunctella	Control with food	59.67 ± 8.32^{a}
	Control without food	56.00 ± 7.81^{a}
	Treatment with food	98.74 ± 1.69^{b}
	Treatment without food	99.54 ± 0.91^{b}
Tribolium castaneum	Control with food	23.00 ± 4.59^{a}
	Control without food	23.33 ± 3.91^{a}
	Treatment with food	68.33 ± 9.59^{b}
	Treatment without food	78.11 ± 8.45^{b}

**n* = 6 for *P. interpunctella* and *n* = 9 for *T. castaneum*

Data were analyzed separately for each species using ANOVA and the mean numbers with different letters for a given species are significantly different (LS means at $\alpha = 0.05$).

Evaluating the efficacy of ozone when exposed to various surfaces

Susceptibility of *P. interpunctella* eggs on five surfaces with or without diet was tested in laboratory-fabricated arenas. Studies clearly showed, regardless of whether diet present or absent in the testing arena, eggs exposed to ozone failed to hatch compared to corresponding controls (Fig. 1). Treated surfaces did not influence the toxicity of the ozone, mortality remained high and above 95% for all surfaces. Studies have demonstrated that the movement of ozone through a grain mass is restricted and most of the gas reacts with the grain or food surfaces. Results from this preliminary study show, construction materials made of glass, aluminum, cement, vinyl, or wood behave in a similar manner in reacting to ozone. These surfaces appear to be not absorbing a significant proportion of the gas, leaving the ozone to react with the insects.

Overall, our study has shown that variability in egg susceptibility to ozone gas depended on the dose, exposure time, species, and presence or absence of food during the treatment. The study also showed common construction materials used in grain bins, mill equipment, processing, and storage plants absorbed insignificant proposition of the ozone gas, allowing ozone to be used in these facilities. Detailed further studies are ongoing.

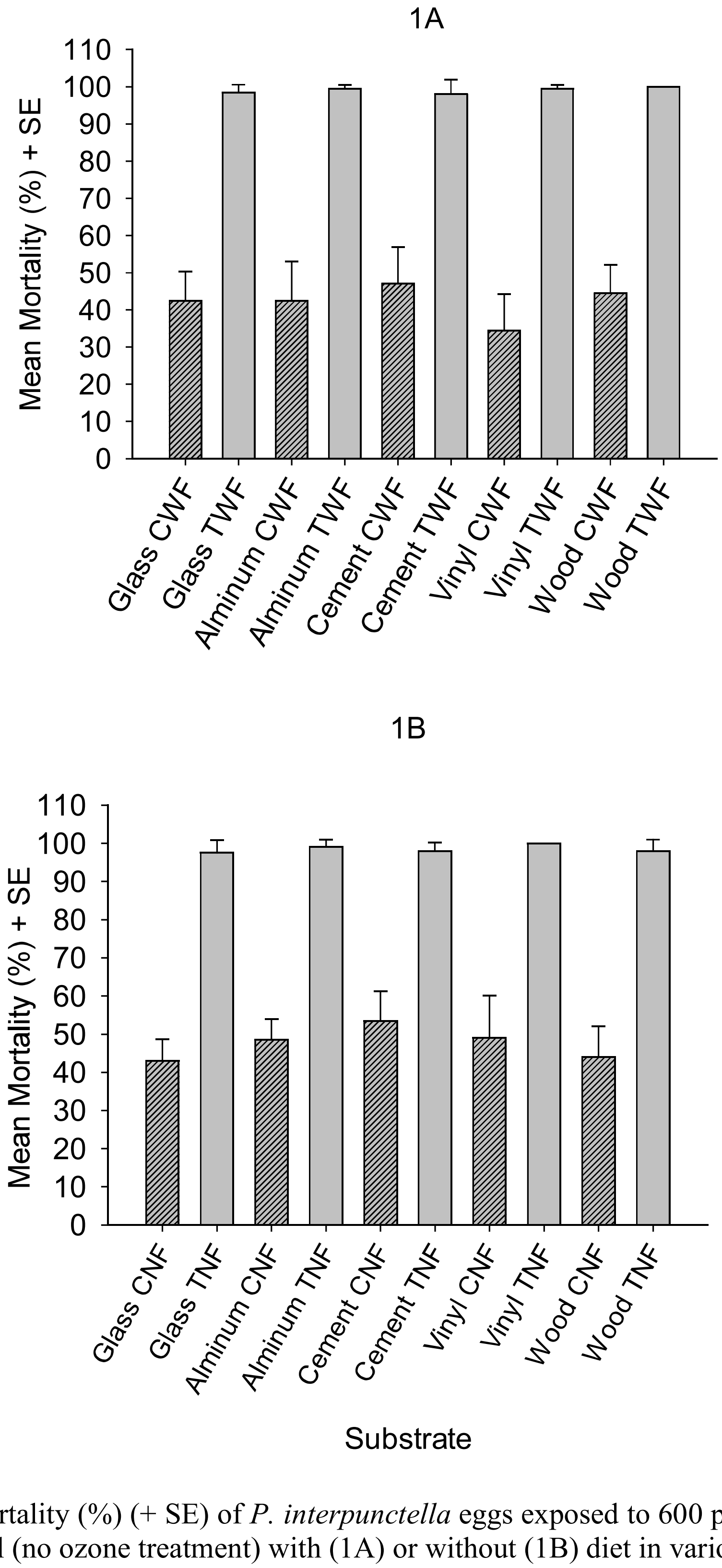

Fig. 1. Mean mortality (%) (+ SE) of *P. interpunctella* eggs exposed to 600 ppm of ozone for 9 h treated or control (no ozone treatment) with (1A) or without (1B) diet in various surfaces (*n*=6).

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