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Efficacy of Ozone against Insect Pests in Wheat Stored in Steel Grain Bins

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Abstract: Field experiments were conducted in steel bins containing 13.6 metric tons of hard red winter wheat. One bin was treated with ozone and the second bin served as a control. Stored grain insects were placed in bins for 1, 2, 3, and 4 days exposure in sampling tubes at three ozone levels: low, medium, and high concentrations. Ozone treatments on eggs, larvae, and pupae of *Plodia interpunctella* were not effective except for pupae at 3 and 4 days exposure at the highest ozone level. *Sitophilus oryzae* adults were the most susceptible species with 100% mortality reached after 2 days exposure at the medium and high ozone levels and after 4 days exposure at the low ozone level. However, some progeny were produced at all treatment levels for all exposure periods. *Tribolium castaneum* adults had 100% mortality only after 4 days exposure at the medium and high ozone levels. No *T. castaneum* progeny were produced at the high ozone treatment after 2 – 4 days exposure. For *Rhyzopertha dominica*, *Cryptolestes ferrugineus*, and *Oryzaephilus surinamensis*, 100% mortality was never achieved and progeny were produced at all ozone levels.

Key words: ozone, stored wheat, insect control

Introduction

Alternative control measures to eliminate pests from stored products are being continually investigated because of concerns of pesticide residues in foods from grain protectants. Fumigants, which leave no pesticide residues on stored products, are effective in controlling pests of stored products but there are concerns about transporting, handling, storing, and applying these products and of insects developing resistance to them.

Electrical generation of ozone is an attractive alternative for controlling pests as it eliminates some of the concerns of using traditional post-harvest pesticides. However, there have been few published studies on its effectiveness as an insecticide. Erdman[1] observed the toxicity of ozone on two *Tribolium*spp. in laboratory trials where the insect life stages were exposed to a continuous flow of air containing 45 ppm ozone and 100% mortality was obtained in 6.5 hours or less. In a laboratory study on corn[2], 100% mortality was achieved after three days exposure of ozone at 50 ppm for adults of *Tribolium confusum* Jacquelin du Val, *T. castaneum* (Herbst), and *Sitophilus zeamais* Motschulsky, and after six days for late instar *Plodia interpunctella* (Hübner). Kells et al. [3] demonstrated that 8.9 metric tons of maize treated with 50 ppm ozone for 3 days resulted in 92% – 100% mortality of adult *T. castaneum*, adult *S. zeamais*, and larval *P. interpuntella*.

In Oklahoma, wheat is the major stored grain commodity. To our knowledge, no field experiments using ozone for insect control in stored wheat have been conducted in the United States. The objective of this study was to determine the effectiveness of ozone fumigation on various stored product pests in a grain mass of wheat under field conditions during the month of October, which is the traditional time during storage when grain is fumigated in Oklahoma.

Materials and Methods

Experiments were conducted in October 2007 in central Oklahoma in two steel grain bins each containing 13.6 metric tons of hard red winter wheat (*Triticum aestivum* L.). One bin was treated with ozone and the second bin served as a control. Grain quality was poor because of the extreme wet conditions at the end of the 2007 growing season. Grain in the ozone bin was grade 4 with a test weight of 55.1 pounds per bushel, moisture content of 11.3%, and total defects (dockage, foreign material, and shrunken and broken kernels) of 3.8%. Grain in the control bin was also grade 4 with a test

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weight of 55.3 pounds per bushel, moisture content of 10.3%, and total defects of 5.6%.

Ozone was produced by an OZAT Model CFS – 3A generator (Ozonia International, France) with a rated capacity of 120 g/hr on dry air but the best output we could achieve under our environmental conditions was 60 g/hr. For our study, the generator was operated at about 30 g/hr to produce the three targeted ozone concentrations of 25, 50, and 70 ppm within the bin. The ozone was introduced into the bin through a 6.4 mm inside diameter polytetrafluoroethylene tube from the generator just upstream of the fan into a 10.2 cm diameter poly-vinyl chloride pipe connected to the aeration fan transition into the plenum in the bottom of the bin. The axial fan moved 0.21 cubic meters/sec of air/ozone gas through the 2.8 m diameter by 3.4 m depth grain mass. As the ozone traveled through the grain mass, its concentration rapidly decreased due to natural decay and reaction with materials it contacted.

For testing purposes, 10.2 cm diameter poly-vinyl chloride insect sampling tubes were placed in the bins so that test insects could be easily hung in bags within the tubes and retrieved. The tubes were inserted into the grain mass to depths corresponding to the targeted ozone levels of 25, 50, and 70 ppm. Ozone rising through the grain mass entered the bottom of the insect sampling tubes and continued to flow up through the tubes thereby coming in contact in the suspended bags containing insects. A recirculation system was used to recover the residual air/ozone gas exiting the top of the grain mass and then injected it back into the bottom of the bin through a closed loop system. This minimized ozone leakage to the environment and reduced the load on the ozone generator. Ozone concentrations in the insect sampling tubes were monitored approximately every 8 hours through 6.4 mm polytetrafluoroethylene sampling lines in each of the insect sampling tubes using a Series IN – 2000 5 – channel Ozone Analyzer (IN USA, Inc., Norwood, MA, USA) that has a monitoring range of 0 – 200 ppm.

Because of limited space within the tubes, *Sitophilus oryzae* (L.), *T. castaneum*, and *Rhyzopertha dominica* (F.) were tested during week one and *Cryptolestes ferrugineus* (Stephens), *Oryzaephilus surinamensis* (L.), and *P. interpunctella* were tested during week two.

For *S. oryzae* and *R. dominica*, 25 adults of each species were placed in 7.0 × 10.2 cm cotton muslin tea bags with a drawstring (Mountain Rose Herbs, Eugene, OR, USA) containing 50 g whole wheat kernels and 20 g infested wheat kernels of the species. For *T. castaneum*, 25 adults and 20 middle-late instars were placed on 50 g whole wheat kernels, 15 g ground wheat kernels, and 2.5 g infested flour which contained eggs. For bags holding *O. surinamensis*, 25 adults were placed on 15 g ground wheat kernels and 8 g of infested rolled oats containing eggs and larvae. For *C. ferrugineus*, 25 adults were placed on 15 g ground wheat kernels, 8 g infested oats containing eggs and larvae, and 2.5 g infested flour containing eggs. For *P. interpunctella*, five pupae and five fifth instars were placed in 15 g ground wheat. Also in the bags for *P. interpunctella*, 25 eggs less than 21 hr old were adhered to double-stick tape on black filter paper strips placed in a small folded copper wire envelope to protect them from being crushed.

Stored grain insects in the bags were placed in bins for 1, 2, 3, and 4 days exposure by suspending the bags on ropes in insect sampling tubes corresponding to the targeted ozone exposure values of 25, 50, and 70 ppm. At the end of each time period, bags were removed from the sampling tubes, taken to the laboratory, and adult beetles and *P. interpunctella* larvae were removed and assessed whether alive or dead. All grain particles and dust from the bags were placed in 226.8 g glass jars fitted with lids of wire screen sandwiched between filter paper disks and held in a growth chamber at 28 C. Beetle progeny were counted at 14 and 28 days. For *P. interpunctella*, eggs were evaluated after one week to determine egg hatch and pupae were evaluated after two weeks for adult emergence.

Data were analyzed as a completely randomized design. Arcsine transformations of proportionate insect mortality values were used for percentage values before analysis to normalize data and make variances homogeneous. Statistical inferences were made after subjecting data to general linear models procedure using SAS software[4]. Treatment means were separated using least significant difference at the 0.05 level. Untransformed means are presented in figures.

Results and Discussion

The mean grain temperature in the ozone treated bin was 25.3℃ during week one and 22.3 ℃ during week two. In the control bin, the mean grain temperature during week one was

26.9 ℃ and during week two was 25.6 ℃. The temperature in the ozone treated bin may have been slightly lower than the control bin due to the movement of air/ozone through the bin during the study. Air/ozone in the external closed loop tubes would have been cooled by the ambient temperature at night resulting in cooler air/ozone being circulated through the bin.

The mean ozone levels during week one were 27.3, 3.3 ppm for the low level, 52.3, 3.5 ppm for the medium level, and 67.7, 3.5 ppm for the high level. During week two, the mean ozone levels were 27.4, 3.7, 53.0, 3.2, and 68.9, 2.2 ppm for the low, medium, and high levels, respectively.

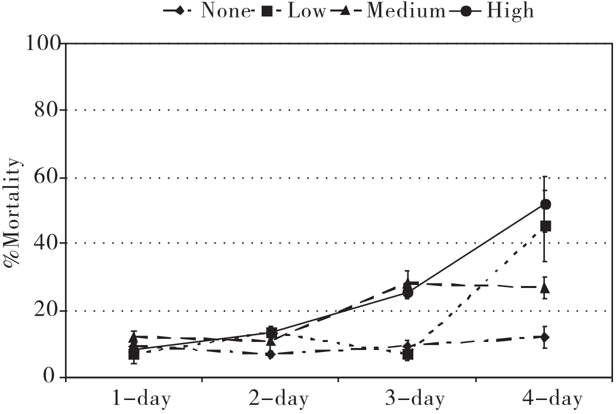

Fig. 1 Percent mortality *P. interpunctella* eggs

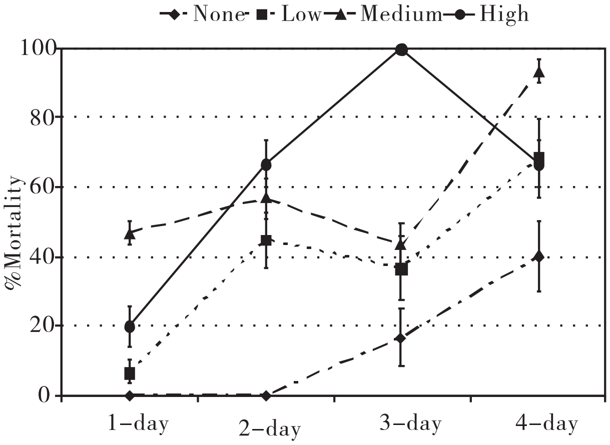

Fig. 2 Percent mortality *P. interpunctella* larvae

P. interpunctella egg mortality only reached 52% after four days under treatment at the highest ozone level (Fig. 1). Larval mortality was mixed with 100% mortality being reached after 3 days but then the mortality dropped to 66.7% after 4 days in the highest ozone treatment (Fig. 2). 100% control was never reached at the medium ozone level. Surprisingly, pupae seemed to be the most sensitive to ozone treatments. At the medium ozone level, 100% mortality was reached after 2 and 3 days but then fell to 86.7% after 4 days (Fig. 3). At the high ozone level, 100% mortality was reached after 3 days exposure.

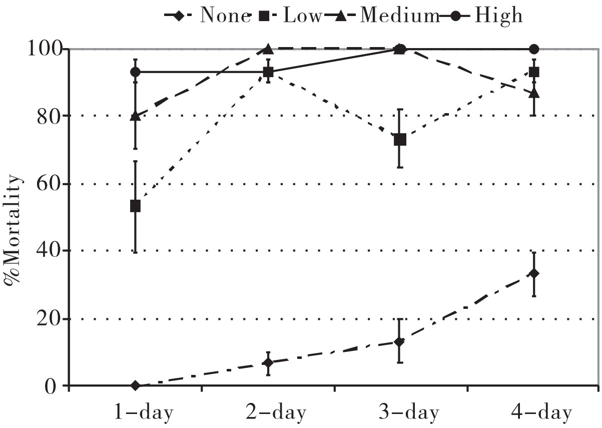

Fig. 3 Percent mortality *P. interpunctella* pupae

Mortality for *R. dominica* did not reach 100% even after 4 days exposure to the highest ozone level (Fig. 4). Progeny were produced at all ozone levels with the fewest being produced at the medium and high ozone levels (Fig. 5).

S. oryzae adults seemed to be the most sensitive to ozone treatments of the beetles tested. After 2 days exposure at the medium and high ozone levels, 100% mortality was achieved (Fig. 6). At the low level, 100% mortality was reached after 4 days exposure. However, at all ozone treatment levels, progeny were produced (Fig. 7). Evidently, some larvae and pupae inside the wheat kernels were able to survive even the high ozone treatment.

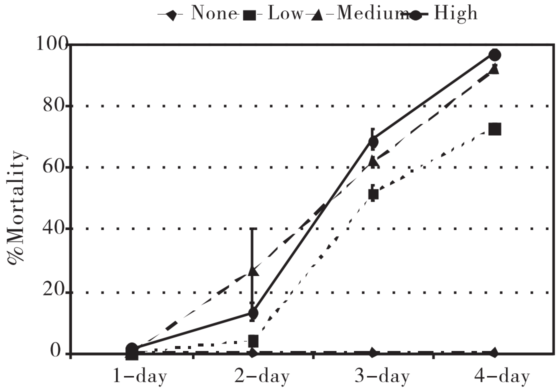

Fig. 4 Percent mortality *R. dominica* adults

No *T. castaneum* adults succumbed at the low or medium ozone levels until after 2 days exposure (Fig. 8) and the mortality was still very slight at the low ozone level. 100% mortality of adults was reached at the medium and high ozone levels after 4 days exposure. No progeny were produced at the high ozone level at 2 – 4 days exposure and only 0 – 1 progeny

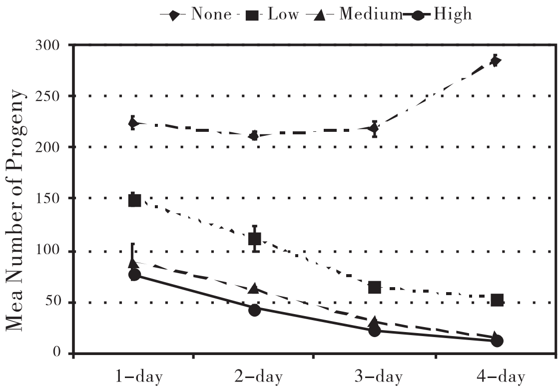

Fig. 5 Mean number *R. dominica* progeny

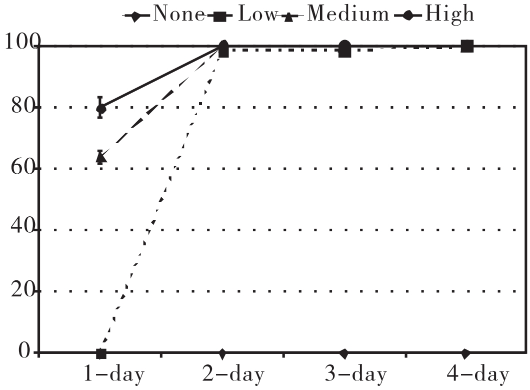

Fig. 6 Percent mortality *S. oryzae* adults

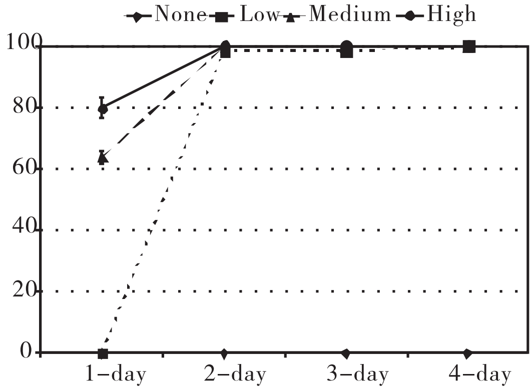

Fig. 7 Mean number *S. oryzae* progeny

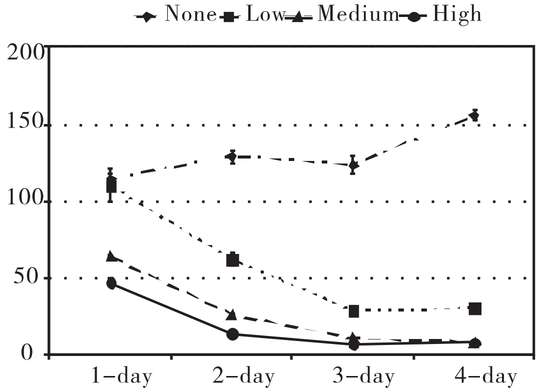

Fig. 8 Percent mortality *T. castaneum* adults

were produced at the medium ozone level during this same time period of exposure (Fig. 9).

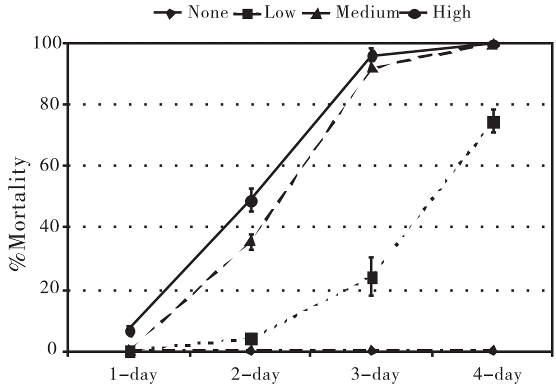

Fig. 9 Mean number *T. castaneum* progeny

C. ferrugineus adults seemed to be the toughest beetle to kill at the ozone levels tested. No adults died after 1 day exposure at any ozone levels (Fig. 10). No adults were killed at the low ozone level after 2 days exposure and there was only 1.3% mortality after 3 days at this level. Mortality only reached 88% after 4 days exposure at the high ozone level. Progeny were produced at all ozone levels with the fewest being produced at the medium and high ozone levels after four days exposure (Fig. 11).

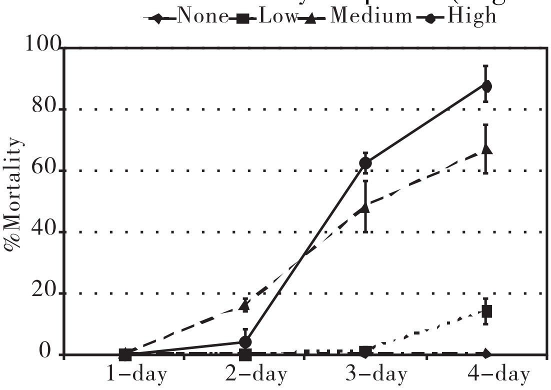

Fig. 10 Percent mortality *C. ferrugineus* adults

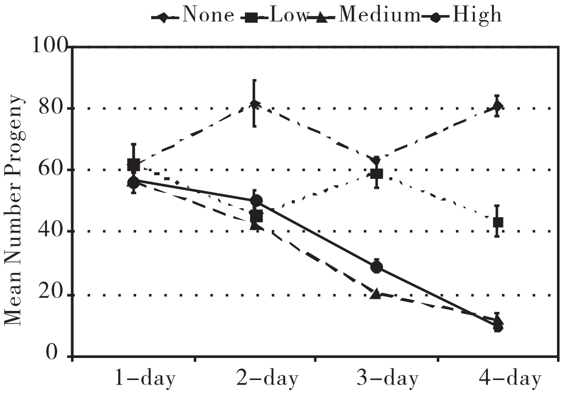

Fig. 11 Mean number *C. ferrugineus* progeny

Mortality of *O. surinamensis* adults was significantly higher at the medium and high ozone levels than at the low and no ozone levels after 2 – 4 days exposure (Fig. 12). However, 100%

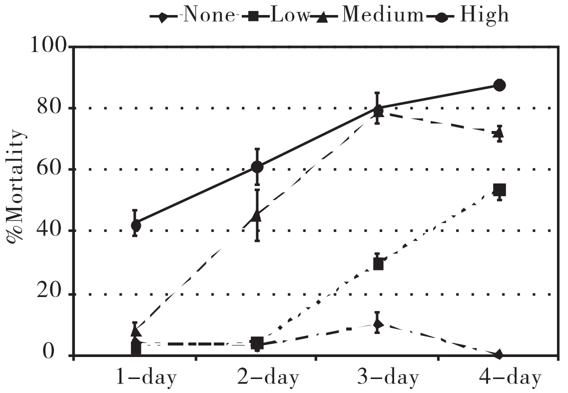

Fig. 12 **Percent mortality *O. surinamensis* adults**

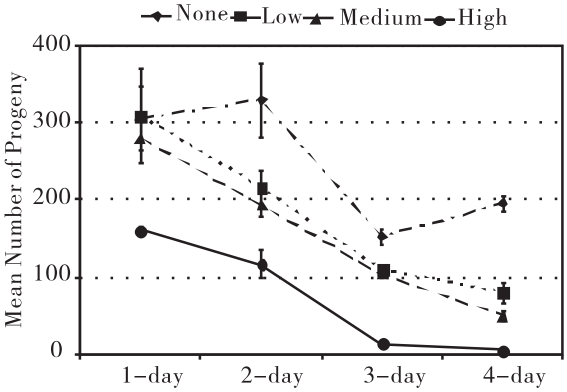

Fig. 13 **Mean no. *O. surinamensis* progeny**

mortality was never reached, even at the high ozone level. Progeny production decreased as exposure time increased but there was production at every ozone level (Fig. 13).

Overall, adult beetle mortality increased with increasing time of exposure although 100% mortality was only realized for *S. oryzae* and *T. castaneum*. Total suppression of progeny production was only seen for *T. castaneum* at the high ozone level.

To obtain total control of beetle populations, either the level of ozone concentration should be increased or the time of exposure at the tested levels should be increased. An economic analysis should be conducted to determine if ozone treatments can compare in cost to traditional methods of fumigation with products such as aluminum or magnesium phosphide.

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