Ozone technology in the post-harvest storage environment- a comparison of efficacy of high doses of ozone to insects treated under laboratory conditions and field conditions

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DOI: 10.5073/jka.2010.425.195

Abstract

Efficacy experiments were conducted to determine the ozone concentration (C) and treatment time (T) needed to effectively kill adult stages of red flour beetle (*Tribolium castaneum*) and maize weevil (*Sitophilus zeamais*). Under laboratory conditions, insects were exposed to concentrations of ozone ranging from 50 to 1800 ppm for 30 and 60 min. After treatment, insect mortality was scored, and if 100% mortality was not reached, more treatment-time was added in 30 min intervals at 1800 ppm. For both species, 100% mortality was reached after treatment at 1800 ppm for 120 min, which equates to a concentration*time (CT) product of 216,000 ppm.min. A similar CT product was attained under field conditions with a prototype auger designed to treat moving streams of grain with ozone during transfer. In field tests, 100% mortality was achieved for both insect species after a treatment of 47,000 ppm ozone for 6 min (CT = 282,000 ppm.min). The results indicate that CT values obtained in laboratory experiments correlated well with those from field experiments. Based on these results, we now can calculate ozone concentration and auger length needed to treat grain in a fast-moving stream.

Keywords: Ozone, Maize weevil, *Sitophilus zeamais*, Red flour beetle, *Tribolium castaneum*

1. Introduction

There is a need for an alternative treatment strategy for insect control in stored grains due to issues such as: loss of registered control strategies, interest in biologically safe alternatives to chemical control methods, lack of control options for organic producers, and even resistance to some traditional fumigants such as phosphine (Zettler et al. 1989; Zettler and Cuperusi 1990; Zettler 1991). Ozone technology is one alternative for treating stored grains to manage insect pests. Ozone is an excellent alternative to currently available products, especially for on-farm use and grain already in bulk storage. It is a strong oxidizer and very unstable, breaking down into atmospheric oxygen very quickly. There is no need to store or dispose of potentially hazardous chemicals. Ozone has the ability to sanitize, disinfect, and is "Generally Recognized As Safe" (GRAS) for food processing in the United States (Sopher et al., 2002). Ozone can be used to deodorize kill microbes, and is effective against insects (Kells et al., 2001). Grains can be treated with ozone for extended periods of time without affecting its quality. Mendez et al. (2003) treated grain for 30 d at 50 ppm and found no affect on grain quality.

Insects are a major problem in stored-grain ecosystems. The weevils, and in particular, maize weevil, *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae), are internal feeders and are very destructive to grain. Adults bore holes into grain where eggs are laid and the entire life cycle takes place (Cotton, 1963). The red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), an external feeder that feeds on cracked kernels and dust (Mason and Obermeyer, 2006). Both species have a worldwide distribution and a fast reproductive ability. The objective of this study was to compare the efficacy of higher dosages of ozone on *S. zeamais* and *T. castaneum* treated in the laboratory and in the field to determine the concentration-time parameters necessary in constructing a commercial grain-auger ozone-treatment system.

2. Materials and methods

2.1. Experimental setup-laboratory

The laboratory ozone generator creates ozone using a corona discharge (O3Co Aberdeen, ID). Concentrations of ozone were measured using an ozone analyzer (INUSA, L2-LC Model 040977) located at the terminal end of the flow through system, consisting of three identical sealed plastic sandwich containers (101.6 mm x 101.6 mm x 25.4 mm) connected in series via 12.7 mm x 2.38 mm (diameter) Tygon® tubing.

2.2. Experimental setup-field

A modified screw conveyer, made of stainless steel, (Lynntech, Inc., College Station, TX), was used for the continuous ozonation treatments of the grain. The conveyor had a length of 6.3 m and a diameter of 0.102 m. A hopper (22.7 kg capacity) was placed on one side to load the grain into the system. A manifold was also attached to the system to introduce ozone at different points of the conveyor. Ozone was produced by corona discharge using an Ozoblast system (O_3Co, Aberdeen, ID. USA).

2.3. Insects/Treatments

2.3.1. Laboratory experiment.

Adult red flour beetle (*T. castaneum*) and adult maize weevil (*S. zeamais*) were taken from colonies maintained in environmental chambers (Percival Scientific Inc., Perry, IA, USA) (30°C and 60% r.h.) at Purdue University, West Lafayette, IN, USA. Insects were placed into Petri dishes (10/dish) and treated with ozone. Insects were exposed to concentrations of ozone ranging from 50 to 1800 ppm for 30 and 60 min. After treatment, insect mortality was scored, and if 100% mortality was not achieved, more replications were included by adding treatment-time in 30 min intervals at 1800 ppm. Each concentration/time combination (CT) was repeated three times with a total of 30 insects per CT combination. After treatment, insects were held at colony maintenance conditions for 24 h and then scored as dead or alive. These data were compared to controls which were untreated and held in dishes for 24 h at colony maintenance conditions.

2.3.2. Field experiment

Bioassay bags were constructed with fine nylon mesh (104 x 104 openings/in^2; 94 µm thread diameter; 150 µm opening size) by folding a 3.81 cm x 4.45 cm piece in half and hot gluing the sides together, leaving the top open for insertion of insects, after which the top was sealed. The size of the completed bags was 3.81 cm x 2.22 cm. Different sized bags were tested before the final size was chosen. The criteria used to decide which bag was chosen were a low crush rate in the auger and the bag had to make it all the way through the auger intact. Insects (10 per bag, randomly chosen from colony jars) were placed in bioassay bags 24 h prior to the experiment start. On the day of the experiment the bioassay bags were placed in buckets of maize (each bucket held 15.876 kg maize; 5 bioassay bags per bucket). These buckets were then subsequently dumped into a hopper which opened into the auger. The maize and bioassay bags moved through the auger and then were deposited down a PVC pipe into another bucket. Random bioassay bags were chosen after each run through the auger and insects within the bags were examined for insect alertness. One run constituted one time through the auger, which equaled two minutes residence (treatment) time with ozone. Treatments included one, two and three-runs through the auger. Between each "run", the ozone concentration was allowed to build back up in the auger (~15 m). Each 'run' was replicated three times. The concentration of ozone for each treatment was 47,820 ppm. Controls were run the same way through the conveyor, but without ozone flowing.

2.4. Analysis of data

To determine differences between control and corresponding treatments, a two-tailed t-test was run. After differences were found, treatment mortality was normalized to account for control mortality using the correction for Abbott's formula determined by Rosenheim and Hoy (1989). Data for comparison of the treatments was analyzed for differences ($\alpha = 0.05$) using analysis of variance (ANOVA). A Fisher's least significant difference (LSD) test using general linear model statistics was also performed (PROC GLM) (SAS Institute, 2001).

3. Results and discussion

In the laboratory, for both *S. zeamais* and *T. castaneum*, controls exhibited no mortality. A t-test revealed that controls and corresponding treatments were significantly different ($P < 0.05$). For both species, 100% mortality was only reached after an ozone treatment of 1800 ppm for 120 min. This equates to a concentration*time (CT) product of 216,000 ppm.min. The 90 min treatment for both insect species was not significantly different than the 120 min treatment. Although short treatment times are desirable for moving grain, the dosage rate of 1800 ppm (currently the highest achievable dose in our laboratory) does not achieve the desired mortality rates. Thus, if 100% control is desired, then the 120 min treatment time must be considered and/or a target CT of 216,000 must be achieved. Similar CTs have been reported by Kells et al. (2001) who achieved 100% mortality of *T. castaneum* adults with a treatment of 3 d at 50 ppm (CT = 216,000 ppm.min).

A CT product similar to the laboratory was attained under field conditions. For both *S. zeamais* and *T. castaneum*, 100% mortality was achieved after a treatment of 47,820 ppm ozone for 6 min (three runs)(CT = 286,920 ppm.min). The four-minute treatment (two-runs) at 47,820 ppm was not a long enough treatment to achieve the desired 100% mortality (CT = 191,280 ppm.min). For undetermined reasons, the two-run treatment mortality of red flour beetles was lower than the one-and three-run treatment. This was not the case for *S. zeamais* where there was an increase in mortality as treatment time increased.

A residence time of 6 min at 47,820 ppm in the treatment auger, or a CT of 286,920 ppm.min, must be applied for both species to achieve 100% mortality. All other treatment combinations tested did not achieve 100% mortality. Based on the CT products, if a treatment time of 5 min could have been tested, to achieve 100% mortality CT of 239,100 ppm.min would be necessary. This was not feasible based on the design of the auger we were testing, but when designing future augers this could certainly be accommodated.

Laboratory determined CT products necessary for 100% mortality (216,000 ppm.min) were lower, but close to the CT products achieved in the field experiments (286,920 ppm.min). Based on these results, ozone concentration and auger length needed to treat grain in a fast moving stream can now be determined and used to engineer an effective commercial grain-auger ozone-treatment system.

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