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Comparison of susceptibility of two stored-product insects, *Ephestia kuehniella* Zeller and *Tribolium confusum* du Val to gaseous ozone

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ABSTRACT

In this study, the susceptibility of two stored-product insects, *Ephestia kuehniella* and *Tribolium confusum*, to gaseous ozone was investigated. Two ozone fumigation methods were used, an empty space fumigation with only one flush of ozone treatment held for 2 h, and a reflush ozone treatment at 30-min intervals for 5 h in the presence of 2 kg wheat, with an initial ozone concentration of 13.9 mg/L. Toxicity data for empty space ozone treatments indicated a remarkable difference in susceptibility between the life stages of *E. kuehniella* and *T. confusum*. For *E. kuehniella*, empty space ozone treatment resulted in complete mortality of adults, pupae and larvae, while only 62.5% of the eggs were killed. For *T. confusum*, ozone treatment resulted in very low mortality of adults, pupae and eggs, ranging from 4.2 to 14.1% while only larvae had a high mortality (74%). Generally *T. confusum* was more tolerant to ozone treatment than *E. kuehniella*. Ozone flush treatment at 30-min intervals for 5 h resulted in almost complete mortality of all life stages of *E. kuehniella* placed in the top position of 2 kg wheat, whereas eggs of *E. kuehniella* placed in the bottom position of 2 kg wheat were hard to kill. For *T. confusum*, larvae placed in the bottom position of 2 kg wheat were easily killed, whereas eggs, pupae and adults survived.

1. Introduction

Ozone is a triatomic form of oxygen (O_3) and is referred to as activated oxygen, or allotropic oxygen. It is an unstable gas with a half-life of about 20 min, depending on the temperature. Thus it does not persist and therefore accumulate substantially without continual generation (Peleg, 1976; Miller et al., 1978). Ozone has a pungent, characteristic odor described as similar to "fresh air after a thunderstorm" (Coke, 1993). It has a longer half-life in the gaseous state than in aqueous solution (Rice, 1986). Ozone in pure water rather quickly degrades to oxygen, and even more rapidly if impurities are present (Hill and Rice, 1982). Ozone is a blue gas at ordinary temperatures but at concentrations at which it is normally produced the colour is not noticeable. Ozone can be generated by electrical charges in air and is currently used in the medical industry as a disinfection technique against microorganisms and viruses, as a means of reducing odor, and for removing taste, colour, and environmental pollutants in industrial applications (Kim et al., 1999).

In 1997, ozone was recognized as being generally safe (GRAS) for food contact applications in the United States (Graham et al., 1997; U.S. Food and Drug Administration, 1997). Since that time, interest in developing ozone applications in the food industry has increased, although some regulatory issues regarding ozone use for this purpose have not been resolved. Electrical generation of ozone eliminates the handling, storage, and disposal problems of conventionally used post-harvest pesticides. An attractive aspect of ozone is that it decomposes rapidly (within about 50 min) to molecular oxygen without leaving a residue. These attributes make ozone an attractive candidate for controlling insects and fungi in stored products. At low concentrations ozone protects clean surfaces from subsequent fungal contamination and growth, although higher doses are required to kill fungi on contaminated surfaces (Rice et al., 1982). Five ppm ozone inhibited surface growth, sporulation, and mycotoxin production by cultures of *Aspergillus flavus* Link and *Fusarium moniliforme* Sheldon (Mason et al., 1997).

Ozone in its gaseous form has also been shown to have potential to kill insect pests in commodities (Erdman, 1980; Mason et al., 1997; Kells et al., 2001). High mortality was achieved for adults of the maize weevil, *Sitophilus zeamais* Motschulsky, and the confused flour beetle *Tribolium confusum* du Val, and the larval stage of the Indian meal moth, *Plodia interpunctella* (Hübner) exposed to low ozone concentrations ranging from 5 to 45 ppm (Erdman, 1980; Kells et al., 2001). Erdman (1980) also observed mortality of larvae

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of *T. confusum* and the red flour beetle, *Tribolium castaneum* (Herbst) when exposed to a 45 ppm ozone environment. Leesch (2003) tested ozone as a toxicant to stored-product insects in the hope of killing insects at low dosages in short periods of time. In his study, even high concentrations of 200–500 ppm (v/v) required many hours to kill the insects exposed. Other than these studies, little has been done to determine susceptibility of stored-product insects to ozone treatments. Our study was therefore designed to test the susceptibility of life stages of two stored-product insects, the Mediterranean flour moth *Ephestia kuehniella* Zeller and *T. confusum* to generated gaseous ozone.

2. Materials and methods

2.1. Test insects

Tests were carried out on all life stages (eggs, larvae, pupae and adults) of *E. kuehniella* and *T. confusum.* All life stages of *T. confusum* were obtained from cultures reared at 26 ± 1 °C and 65 ± 5% relative humidity (r.h.) on a diet of wheat flour mixed with yeast (17:1, w/w) using standard culture techniques (Donahaye, 1990). *Ephestia kuehniella* were reared on a 10:2:1 mixture of wheat flour, wheat germ and dried brewer's yeast at the same environmental conditions as *T. confusum* (Rahman et al., 2007). Eggs aged 1–2 days, in 9 cm Petri dishes, were placed in 3-L jars for exposure to the treatments. Larvae were removed from culture jars and exposed to the treatments 21 days after oviposition. Pupae were obtained by daily separation from culture jars and were exposed to the treatments at age two days. Newly-emerged (0–1 day) adults were exposed to the treatments in empty exposure jars.

2.2. Commodity

The Seri-82 variety of hard winter wheat (*Triticum* sp.) with moisture content (m.c.) of $10.8\% \pm 0.2\%$ was used in the tests. In order to minimize the reaction of microbial loads in the commodity with ozone, the wheat used in the tests was sterilized with steam under pressure.

2.3. Experimental setup

The experimental set up consisted of an ozone generator, oxygen cylinder, check valve, mass flow-meter, vacuum pump, vacuum digital gauge and 3-L exposure jars (9 cm ID $\times$ 24 cm length), each of the latter capped with a metal stopper equipped with entry and exit tubing. A magnetic stirrer placed in the bottom beneath a wire-mesh disc served to mix the air with the ozone. Two pieces of rubber tubing, 5 cm long, 6.2 mm ID, were attached to the tubing and sealed with pinch-clamps. Entry and exit tubing were connected to ozone generator and vacuum pump respectively. Pressure in each jar was measured using a $0–800 \pm 3$ mm Hg vacuum digital gauge (Celesco-model SE-2000, U.S.A.). The low pressure measure is referred to herein as absolute pressure, with 760 mm Hg considered as atmospheric pressure.

Gaseous ozone was generated using a laboratory corona discharge ozone generator (Model OZO-1VTT provided by the company Ozomax Inc. (http://www.ozomax.com), Canada) with 5 g per h of output from purified extra dry oxygen (O_2) feed gas. The generator is capable of producing 13.88 mg/L ozone at an O_2 flow rate of 6 L/min at room temperature. Gaseous ozone was introduced into the fumigation chamber from above. The exposure jars were sealed with silicone vacuum grease. A schematic view of the 3-L fumigation chamber and the position of the insect cages in 2 kg of wheat are presented in Fig. 1. Wire-mesh insect cages (2.5 cm ID $\times$ 5 cm length) were placed vertically into top and bottom positions in the 2 kg wheat loaded into each fumigation chamber. The upper opening of insect cages in the top position was level with the surface of the 2 kg wheat. The lower insect cages rested on the bottom of the fumigation chamber (Fig. 1).

2.4. Ozone fumigation procedures

Prior to each test, 20 larvae, pupae or adults were confined, separately, inside 3 cm diameter by 8 cm long wire-mesh cages. For eggs, fifty were placed in open Petri dishes for fumigation.

For empty space ozone fumigation, 100 eggs and 25 each of pupae, larvae and adults of *E. kuehniella* and *T. confusum* were exposed to only one flush of ozone at a generated concentration of 13.88 mg/L for 2 h. The insects were first placed in exposure jars which were then briefly evacuated to 3–5 mm Hg. The check valve of the O_2 cylinder was opened and the flow rate of O_2 gas was adjusted. Oxygen entered at the bottom of the ozone generator and an ozone-containing atmosphere was produced which was flushed into the exposure jar until it reached atmospheric pressure, whereupon the insects were exposed to ozone for 2 h.

For the ozone flush treatment at 30-min intervals for 5 h in the presence of the commodity, 100 eggs and 25 each of pupae, adults and larvae confined inside the wire-mesh cages (2.5 cm ID $\times$ 5 cm length) were placed into the top and bottom positions in the 2 kg wheat loaded into each fumigation chamber (Fig. 1). Chambers were then briefly evacuated to 3–5 mm Hg and gaseous ozone at 13.88 mg/L was flushed into the system until atmospheric pressure was restored, and this was repeated every 30 min for a total of 10 flushes. An extra batch of larvae of *T. confusum* was included in this experiment to follow survival through to the adult stage. Untreated control insects were exposed to atmospheric conditions.

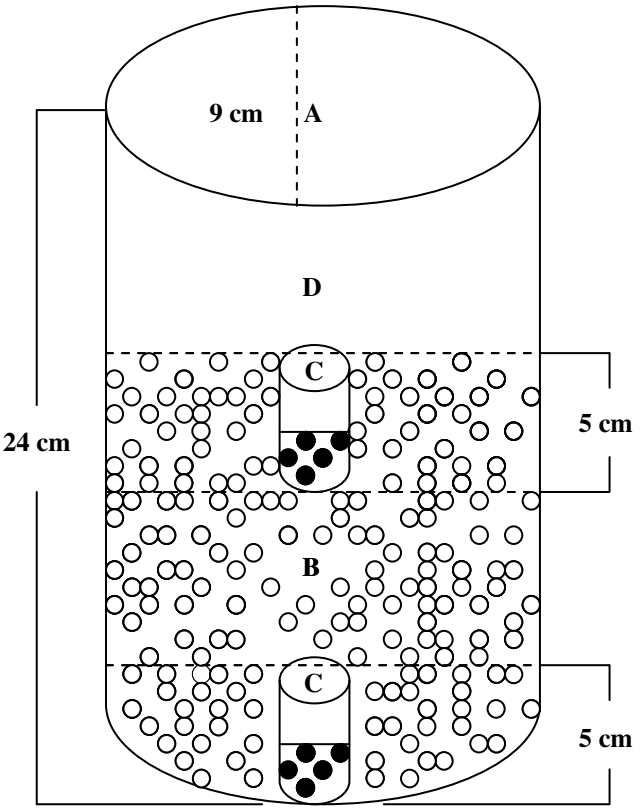

Fig. 1. Schematic view of 3-L fumigation chamber and the position of insect cages in the presence of 2 kg of wheat (A) 3-L fumigation chamber, (B) 2 kg of wheat in fumigation chamber, (C) insect cages, and (D) head space in fumigation chamber.