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Efficacy of Ozone Fumigation to Control Some Stored Product Insects

Lidia Limonta * , Massimiliano Stampini and Daria Patrizia Locatelli

Abstract: Ozone is a toxic gas with potential to replace methyl bromide for controlling stored product pests. The efficacy of an ozone fumigation treatment on beetles (*Sitophilus oryzae*, *Rhyzopertha dominica*, and *Tribolium confusum*), and moths (*Cadra cautella*, *Corcyra cephalonica*, *Ephestia kuehniella*, and *Plodia interpunctella*) was evaluated. Insects at various stages of development (eggs, larvae, pupae, and adults of *S. oryzae* and *R. dominica*; eggs, third instar larvae and adults of *T. confusum*; eggs, and third instar larvae of moths) were treated with ozone at 600 ppm v/v, 20 ± 2℃, for different time periods (from 30 min to 3 hours). Treatments were carried out in a column (10 cm dia. × 110 cm) containing 4 kg of rough rice (paddy). Mortality was assessed at 24 and 48 hours after treatment, and weekly thereafter.

The egg stage was the most tolerant to ozone fumigation treatment for all the species tested. Ozone fumigation for 30 min killed 100% of the third instar larvae of the four species of moths and of *S. oryzae* adults. More than 50% of the adults of *R. dominica* and of *T. confusum* and 30% of third instar larvae of *T. confusum* were alive 24 hour after a 30 minute treatment; but they were found dead after 48 hours. After a 1 hour treatment, some third instar larvae and adults of *T. confusum* were alive after 24 hours; but they were found dead after 48 hours. To obtain a 100% mortality of all the stages of the species tested, a 3 hour ozone fumigation treatment was required.

Key words: *Sitophilus oryzae*, *Rhyzopertha dominica*, *Tribolium confusum*, *Cadra cautella*, *Corcyra cephalonica*, *Ephestia kuehniella*, *Plodia interpunctella*, fumigation, ozone

Introduction

Ozone is a highly oxidising agent and widely used in food industry for disinfection of surfaces, for hygiene in processing plants and to treat some foods for controlling insects and fungi [1,2,3,4,5,6,7]. Ozone is highly reactive and damages cell membranes of organisms by causing oxidative stress[8], but does not affect intrinsic grain properties or seed germination [9].

In 1965, Beard[10] indicated that prolonged exposure to high levels of ozone was lethal to adult house flies and caused flies to lay fewer eggs per female. Levy et al. [11] observed that eggs and larvae of *Musca domestica* (L.) and *Stomoxys calcitrans* (L.) are less susceptible to ozone compared to pupae and adults.

Both laboratory tests[9,12,13] and field trials have been carried out to verify the efficacy of ozone fumigation on stored product insects with different concentrations and different exposure times. Kells et al. [14], treating 8.9 tonnes of maize with 50 ppm ozone for 3 days, obtained 92% – 100% mortality of some insects of stored products. Field experiments on strains of *Rhyzopertha dominica* (F.) with a high level resistance to phosphine showed susceptibility to ozone fumigation[15].

Although low pressures and CO_2 may improve efficacy of some fumigants, requiring shorter fumigation periods, the efficacy of high concentration ozone on *Ephestia kuehniella* (Zell.) is not enhanced under low pressure of 100 mmHg and 92% CO_2[16].

The efficacy of an ozone fumigation treatment on beetles and moths was evaluated at high concentrations of ozone with short exposures.

Materials and Methods

Tests were performed on *Cadra cautella* (Walk.), *Corcyra cephalonica* (Staint.), *Ephestia kuehniella* (Zell.), *Plodia interpunctella* (Hbn.), *Sitophilus oryzae* (L.), *Rhyzopertha dominica* (F.) and *Tribolium confusum* J. du Val. The procedure adopted was a) to place insects at different stages of development with paddy rice (rough rice) inside a column, and b) to treat with ozone and observe their survival.

Tests were carried out by SAPIO produzione idrogeno ossigeno srl., Via Malcontenta 49, Porto Marghera (VE) Italy.

Istituto di Entomologia agraria, Universitdegli Studi di Milano, Via Celoria 220133, *Milano, Italy.*
* *Corresponding author: lidia. limonta@ unimi. it*

Test insects were reared in thermostatically controlled incubators at 26 ± 1℃; 70 ± 5% r. h. at the Istituto di Entomologia agraria, Universitdegli Studi, Milan, Italy.

The moth species were reared on a diet used for mass rearing [17].

For the moth species, the eggs were collected from Petri dishes placed under a special plexiglass egg-laying cylinder, 15 cm dia. x 40 cm, with the base fitted with an 18 mesh metal net through which the eggs fall. Eggs were collected from 50 moths in each cylinder. Tests were carried out on eggs laid 30 – 48 hours previously, and held at the laying conditions.

T. confusum was reared on soft wheat flour, maize flour and bran (in equal parts). To obtain Eggs and larvae of *T. confusum* were obtained by sieving (60 mesh) a 50 g sample of sieved soft wheat flour, previously infested for a period of 5 days by 100 adults.

Eggs of *S. oryzae* and *R. dominica* were obtained by exposing rice samples (50 g) to 100 adults for 5 day. Larvae were obtained from 50 g samples of cereals containing eggs laid 8 – 11 days, 13 – 16 days and 18 – 23 days before the treatment. For pupae, the period prior to exposure was 26 – 30 days.

The fumigation system was made of an ozone generator connected to a polycarbonate cylinder (10 cm dia., 110 cm high). The generator produced ozone from purified oxygen (0.8 L/min). In the cylinder, ozone concentration was 600 ppm v/v. Ozone was introduced from the bottom of the cylinder through a perforated plate. The top of the cylinder had a lid with holes used to regulate the ozone exit. The exit gas stream was heated to 80℃ to decompose the ozone present to oxygen.

Insects were added to 4 kg of paddy rice in the column: groups of 20 larvae for each moth species and for *T. confusum*, while for the other beetles, 20 g of infested material for each tested stages or instars were added. Groups of a 100 eggs for each moth species and for *T. confusum*, were placed in gauze bags (70 mesh). In tests with adult beetles, groups of 20 individuals of *T. confusum* and 50 of *R. dominica* and of *S. oryzae* were introduced in the column at 20 ± 2℃ for different time periods (from 30 min to 3 hours). Each test was repeated 4 times, each with an untreated control batch.

The mortality adults of all the species and the third instar larvae of the moths and of *T. confusum* were assessed after 24 and 48 hours. In the other cases, the biological samples were maintained at a temperature of 26 ± 1℃ and 70 ± 5% r. h. and checked weekly until adult emergence. Mortality was corrected according to Abbott's formula [18].

Results

One hundred percent mortality of all the tested stages of moths and beetles was obtained with a 3 hours ozone fumigation.

Eggs of moths were the most tolerant stage to ozone fumigation. With 30 minutes fumigation, a corrected mortality of < 50% for eggs of *Cadra cautella*, *Corcyra cephalonica*, and *Ephestia kuehniella* was obtained (Table 1). *Plodia interpunctella* (79.7% corrected mortality) was the least tolerant species.

Table 1. Effect of ozone fumigation at 600 ppm on eggs of *Cadra cautella*, *Corcyra cephalonica*, *Ephestia kuehniella* and *Plodia in terpunctella* for different exposure times, expressed as percentage mortality (percentage values corrected according to Abbott's formula).

Species	Corrected mortality %			
	30 min	1 h	2 h	3 h
Cadra cautella	49.1	85.6	91.6	100
Corcyra cephalonica	31.6	67.5	80.6	100
Ephestia kuehniella	34.7	69.9	98.7	100
Plodia interpunctella	79.7	88.7	92.1	100

After a 1 hour fumigation, mortality of moth eggs was between 67.5 (*C. cephalonica*) and 88.7% (*P. interpunctella*). After 2 hours fumigation, mortality was > 90%, except for *C. cephalonica* (80.6%). Third instar larvae of moths were most susceptible to ozone fumigation: a 30 minutes fumigation was enough to obtain a 100% corrected mortality.

As far as the beetles were concerned, with a 2 hours fumigation, eggs of *T. confusum* were the least susceptible stage (Table 2). Mortality of eggs of *R. dominica* after 30 minutes and 1 hour were 87.7 and 97.5% mortality. For eggs of *S. oryzae* these values were 41.7 and 72.5%, respectively. With a 2 hours treatment, a mortality higher than 95% was obtained for eggs of both *R. dominica* and *S. oryzae*.

32.5% of third instar larvae and 63% of adults of *T. confusum*, and 64.5% of adults of *R. dominica* survived for > 24 hours after an ozone fumigation of 30 minutes (Table 3), but all were dead (100% mortality) after 48 hours. All adults of *S. oryzae* were dead after 24 hours succeeding the fumigation treatment. Larvae and pupae of *R. dominica* and of *S. oryzae* were susceptible to a 30 minutes fumigation. After 24 hours following a 1 hour fumigation, 2.5% of third instar larvae and 1% of adults of *T. confusum* were still alive, but all were dead after 48 hours.

Table 2. Effect of ozone fumigation at 600 ppm on eggs of *Sitophilus oryzae*, *Rhyzopertha dominica* and *Tribolium confusum* for different exposure times, expressed as percentage mortality (percentage values corrected according to Abbott's formula).

Species	Corrected mortality %			
	30 min	1 h	2 h	3 h
Rhyzopertha dominica	87.7	97.5	100	100
Sitophilus oryzae	76.0	77.4	97.2	100
Tribolium confusum	41.7	72.5	92.1	100

Table 3. Percentage of adults of *Rhyzopertha dominica* and larvae and adults of *Tribolium confusum* surviving a 30 min exposure at 600 ppm ozone at 24 hours and 48 hours after the fumigation.

Species	Survival %	
	24h	48h
Rhyzopertha dominica adults	64.5	0
Tribolium confusum Ⅲ *instar larvae*	32.5	0
Tribolium confusum adults	63.0	0

Adult insects, derived from eggs surviving the ozone fumigation, displayed the postembryonal development period observed to those untreated (controls).

Discussion

With 600 ppm ozone concentration, the egg was the most tolerant stage. To obtain a 100% mortality, 2 hours fumigation were required for *Rhyzopertha dominica* and 3 hours for *Sitophilus oryzae*, *Tribolium confusum*, *Cadra cautella*, *Corcyra cephalonica*, *Ephestia kuehniella* and *Plodia interpunctella*.

Ozone, unlike nitrogen and carbon dioxide, was lethal at short periods of fumigation to stages of insects that develop inside the kernels, such as *Rhyzopertha dominica* and *Sitophilus oryzae*. In this research, with a high concentration of ozone, a complete mortality was observed after a 30 minutes fumigation.

Erdman[19] fumigated insects at 300 ppm ozone and observed a complete mortality of adults of *T. castaneum* and *T. confusum* during few days after the treatment. Also in this research, with the 30 minutes fumigation, third instar larvae and adults of *T. confusum* and adults of *R. dominica* were alive after 24 hours and dead after 48 hours.

With high concentration of ozone the fumigation period is reduced. With low concentrations it was observed, in a laboratory study, that 5 ppm of ozone resulted in a 100% mortality of adult *Oryzaephilus surinamensis* (L.) and *T. confusum* after exposure times of 3 and 5 days respectively[20]. Laboratory studies have indicated that 50 ppm ozone for 3 days killed adult insects commonly found in stored grain[1]. Kells et al.[14] fumigated insects at 25 ppm and 50 ppm ozone for 3 days and observed that adults of *S. oryzae* are more susceptible than adults of *T. confusum*. In our tests, we observed the same response. Besides, Erdman[19] showed that *T. castaneum* was consistently more ozone – sensitive than *T. confusum*.

Ozone fumigation does not leave toxic residues and requires, at high concentrations, a short period of treatment. It is an interesting alternative to the use of fumigants and of physical methods that require a long period of application, such as nitrogen and carbon dioxide. An attractive aspect of ozone is that it decomposes rapidly (half life in air of 20 – 50 min) to molecular oxygen without residues, and ozone can be generated on – site, eliminating the need to store or dispose of chemical containers. A high content of dust in cereals can negatively affect the efficacy of CO_2 treatment[21,22] and the same result was observed also for ozone fumigation[23]. A disadvantage of ozone application is its corrosive properties towards most metals[9,12]. Nevertheless, the use of ozone fumigation is interesting in the disinfestation of stored products such as cereals stocked in concrete silos and in steel silos after coating with specific resins. It is noteworthy that inox steel (AISI 316) silos is not susceptible to ozone corrosion.

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