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Efficacy and fumigation characteristics of ozone in stored maize

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Abstract

This study evaluated the efficacy of ozone as a fumigant to disinfest stored maize. Treatment of 8.9 tonnes (350 bu) of maize with 50 ppm ozone for 3 d resulted in 92–100% mortality of adult red flour beetle, *Tribolium castaneum* (Herbst), adult maize weevil, *Sitophilus zeamais* (Motsch.), and larval Indian meal moth, *Plodia interpunctella* (Hübner) and reduced by 63% the contamination level of the fungus *Aspergillus parasiticus* Speare on the kernel surface. Ozone fumigation of maize had two distinct phases. Phase 1 was characterized by rapid degradation of the ozone and slow movement through the grain. In Phase 2, the ozone flowed freely through the grain with little degradation and occurred once the molecular sites responsible for ozone degradation became saturated. The rate of saturation depended on the velocity of the ozone/air stream. The optimum apparent velocity for deep penetration of ozone into the grain mass was 0.03 m/s, a velocity that is achievable in typical storage structures with current fans and motors. At this velocity 85% of the ozone penetrated 2.7 m into the column of grain in 0.8 d during Phase 1 and within 5 d a stable degradation rate of 1 ppm/0.3 m was achieved. Optimum velocity for Phase 2 was 0.02 m/s. At this velocity, 90% of the ozone dose penetrated 2.7 m in less than 0.5 d. These data demonstrate the potential usefulness of using ozone in managing stored maize and possibly other grains.

Keywords: Ozone; Indian meal moth; Red flour beetle; Maize weevil; Post-harvest IPM; *Aspergillus*; Pest management

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1. Introduction

More than 400 million tonnes (15 billion bushels) of grain are stored every year in the United States. Insects and fungi create serious quality problems in stored grains and annual storage losses are estimated at more than $500 million (Scott, 1991). The only way to eliminate pests completely from a food grain without leaving pesticide residues is fumigation. Currently there are only two registered fumigants for stored food, methyl bromide and phosphine. Because of environmental concerns, the US government has mandated that methyl bromide will be completely eliminated from use by the year 2005 with partial reduction starting in 1999. Phosphine is currently undergoing re-registration by the Environmental Protection Agency (EPA, 1993). Assuming phosphine makes it through the re-registration process, it would be the only licensed fumigant for stored food grains. With only one fumigant remaining, insect resistance becomes a greater risk. Unfortunately some stored product insects already exhibit some levels of phosphine resistance and some show resistance to methyl bromide (Zettler et al., 1989; Zettler and Cuperus, 1990). Loss of fumigants, resistance to remaining fumigants and a trend by consumers to move away from residual chemicals, necessitates the development of additional control strategies.

Ozone (O_3) can be generated by electrical discharges in air and is currently used in the medical industry to disinfect against microorganisms and viruses, as a means of reducing odor, and for removing taste, color, and environmental pollutants in industrial applications (Kim et al., 1999). The attractive aspect of ozone is that it decomposes rapidly (half-life of 20–50 min) to molecular oxygen without leaving a residue. In 1982, the US Food and Drug Administration (FDA) classified ozone for treating bottled water as "generally recognized as safe" (GRAS) (FDA, 1982). An extension of this GRAS declaration to other agricultural uses was recently filed with the FDA and is currently awaiting affirmation (Graham, 1997).

Electrical generation of ozone eliminates the handling, storage, and disposal problems of conventionally used post-harvest pesticides. This attribute makes ozone an attractive candidate for controlling insects and fungi in stored grain; however, few studies have been published on its efficacy as an insecticide. Erdman (1980) observed mortality of larvae of red flour beetle, *Tribolium castaneum* (Herbst), and confused flour beetle, *Tribolium confusum* (du Val), when exposed to a 45 ppm ozone environment. In a laboratory study, 5 ppm of ozone resulted in 100% mortality of adult saw-toothed grain beetle, *Oryzaephilus surinamensis* (L.), and confused flour beetle after exposure times of 3 and 5 d, respectively (Mason et al., 1997).

Ozone is a strong oxidizing agent that has been effectively used to control fungal growth and reduce mycotoxin contamination (Kim et al., 1999). At low concentrations ozone protected clean surfaces from subsequent fungal contamination and growth, although higher doses were required to kill fungi on contaminated surfaces (Rice et al., 1982). Ozone (29 ppm) was effective in reducing or eliminating aflatoxin from cottonseed and peanut meal (Dollear et al., 1968; Dwarakanath et al., 1968). Ozone treatment also reduced the toxic effect of aflatoxin-contaminated maize fed to turkey pouts (McKenzie et al., 1998). Five ppm ozone inhibited surface growth, sporulation, and mycotoxin production by cultures of *Aspergillus flavus* Link: Fr and *Fusarium moniliforme* Sheldon (Mason et al., 1997).

Previously, Strait (1998) established that ozone, following fumigation of small-scale grain storage bins (18 kg) containing yellow maize, dispersed throughout the grain mass and was toxic to insects within that mass. A concentration of 50 ppm for 3 d resulted in 100% mortality of adult

confused flour beetles and maize weevils, *Sitophilus zeamais* (Motsch.), and greatly reduced emergence of larval Indian meal moths, *Plodia interpunctella* (Hübner). Initial movement of ozone through the grain was impeded by a phenomenon described as the ozone demand of the medium (Kim et al., 1999), which in this case was the maize. The chemical components on the grain surface that react with the ozone are not known. However, once the grain has been exposed to ozone, subsequent ozone movement through the grain was rapid with very little impedance. Because many factors influence fumigation, including equipment performance and environmental conditions, defining the abilities and limitations of ozone penetration of grain and insect efficacy are important for scale-up fumigation of commercial facilities. The objectives of this study were to quantify the efficacy of ozone fumigation in a grain mass (up to 8.9 tonnes) under field conditions, and to determine the movement characteristics of ozone through grain depths up to 3 m in both laboratory and field environments.

2. Materials and methods

2.1. Ozone generator

Ozone was produced electrostatically by a prototype ozone generator built by Heating Recovery Systems (Carmel, IN). The unit had two generator heads, each constructed of layered steel plates (30 cm × 12 cm) comprising five chambers. Application of 8000 V across the plates created a series of continuous electrical arcs in each chamber. Two rheostats (one for each generator head) that regulated the amount of arcing controlled the amount of ozone produced. At maximum generator voltage, the generator produced ozone concentrations between 20 ppm (0.054 m^3/s) and 50 ppm (0.019 m^3/s). Air was forced through the generating heads by 23–92 W (1/32–1/8 hp) centrifugal fans. Apparent velocity (m/s) through grain is defined as total airflow rate (m^3/s) divided by the cross-sectional floor area of the storage structure (m^2). Air velocity was measured at the generator outlet with either a 5.1 cm vane anemometer (DA-4, Davis Instruments, Hayward, CA) or a hot wire anemometer (Testo # 415, Davis Instruments, Hayward, CA).

2.2. Ozone penetration of grain mass: field study

The ozone generator was ducted to the top of a 12.7 tonne capacity galvanized steel grain bin via galvanized 113 liter steel ductwork (15.2 cm dia.). All seams in the ductwork were sealed with silicone and duct tape, with the transition pipe joints from the generator sealed with duct tape only. Ozone was forced downward through the grain to exit the plenum. Ozone was measured with an ozone analyzer (Model 1008-HC, Diasibi Environmental, Glendale, CA) having a detection range of 0.001–70.0 ppm at a sampling rate of 2 l/min. Teflon tubes (0.64 cm o.d. × 6.1 m) ducted air samples to a Teflon sampling manifold having 11 stainless-steel valves for multiple connections to the ozone analyzer. Teflon tubing provided sampling locations in the head space and plenum, as well as in the center of the grain mass at 0.30, 0.81, 1.5, and 1.8 m from the grain surface. Measurements at the wall were taken at 1.5 and 1.8 m from the grain surface. Ozone concentrations in the grain bin were measured at least every 24 h of operation. The field

study was conducted during July, August, and September at which time testing on insects and fungi was conducted.

2.3. Ozone penetration of grain mass: laboratory study

Columns (0.57 m dia. × 3 m) were constructed of four steel barrels (each 208 liters) bolted end-to-end with the lids and bottoms removed. A perforated steel floor set on four steel rods (1.27 cm dia.) was welded 15 cm from the bottom of the column to form a plenum. A lid was fastened to the top of the column with a 2.5 cm "L"-shaped aluminum strap that was bolted on the sides of the column. A gasket of closed-cell foam tape sealed the lid with the column after it was filled with food grade yellow maize.

The ozone generator was connected to the top of the column (through the lid) with 15 cm dia. galvanized duct (113 liters). All seams in the ductwork and the column were sealed with silicone and/or duct tape. Similar to the field experiments, ozone monitoring in the columns was achieved by Teflon tubing placed in the head space and plenum, as well as 0.3 m from the column wall at the following grain depths: 0.30, 0.9, 1.5, 2.1, and 2.7 m. This tubing was then connected to the sampling manifold, and ozone was measured by the ozone analyzer (Model 1008-HC, Diasibi Environmental, Glendale, CA).

The ozone generator ran continuously until readings for that column were completed. Two separate columns (each a replicate) were used per velocity. Ozone concentrations were measured 1–4 times per day.

2.4. Insects and fungi

The three species of stored product insects used in the efficacy trials were reared as previously described (Strait, 1998). Twenty last instar Indian meal moth (IMM) larvae, 50 adult red flour beetles (RFB), and 50 adult maize weevils (MW) were placed into separate (10 cm × 3 cm o.d.) cages containing approximately 100 whole kernels of yellow food grade maize.

Aspergillus parasiticus Speare strain ATCC 24551 produces averufin, a metabolite in the aflatoxin biosynthetic pathway that is easily identifiable because this orange metabolite accumulates when grown on potato dextrose agar (PDA) medium. Conidia harvested from a colony maintained on PDA were suspended in 0.05% Triton X-100 at 2.5×10^7 conidia per ml and 1 ml was added to 30 g of maize kernels and dispersed by shaking. Inoculated maize was placed into cages similar to those used for the insects.

Ten cages for each insect species and *A. parasiticus* were placed in a control bin and 10 in the ozone-treated bin during the field study only. Cages were positioned vertically in the maize just below the surface and covered with about 2 cm of maize. The experiment was repeated three times at treatments of 50 ppm ozone for 3 d and 25 ppm for 5 d. After the treatment, the cages were pulled from the bins and the numbers of live and dead insects were determined. Maize in the cages was washed with 100 ml of 0.05% Triton X-100 to remove surface fungi. The wash was serially diluted and then plated on PDA medium to count the colony forming units (CFU) that developed. Statistical analyses were carried out with SAS procedures (SAS Institute, 1990). Significant differences in percentage mortality (insects) or numbers of CFU (fungi) among treated and the control bin were determined using analysis of variance (general linear model).

Table 1
Effects of ozone on insect mortality in 8.9 tonnes of maize

Species[c]	Ozone conc (ppm)	Exposure time (d)	N[a]	Percent mortality[b]	
				Treated	Control
IMM	25	5	25	77.0 ± 2.1a	13.6 ± 2.8b
	50	3	10	94.5 ± 2.2a	9.2 ± 1.6b
MW	25	5	30	99.9 ± 0.1a	1.1 ± 0.3b
	50	3	30	100.0 ± 0.0a	3.2 ± 0.7b
RFB	25	5	30	91.4 ± 1.5a	4.5 ± 0.9b
	50	3	30	92.2 ± 1.3a	5.6 ± 0.7b

[a] Number of cages.
[b] Average ± standard error. Different letters within each row indicate a significant difference between treated and control ($P < 0.05$).
[c] IMM — Indian meal moth larvae; MW — Maize weevil adults; RFB — red flour beetle adults.

3. Results and discussion

3.1. Ozone efficacy against insects

Two to three days are required to complete a grain fumigation with phosphine and laboratory studies have indicated that 50 ppm ozone for 3 d killed adult insects commonly found in stored grain (Strait, 1998). Under field conditions, ozone fumigation treatments at 50 ppm for 3 d and 25 ppm for 5 d significantly increased mortality of IMM, MW, and RFB compared with the control (Table 1). Mortality levels above 90% were observed except for IMM in maize treated at 25 ppm for 5 d. At 50 ppm, MW were more susceptible than RFB, however the majority of surviving RFB after ozone treatment displayed altered behaviors such as more than one pair of legs failing to move or a lack of coordinated movement in all legs. Strait (1998) observed similar behavioral effects as well as delayed mortality after the insects survived initial ozone treatments. Kernels in the cages were whole and undamaged. To determine the effect of broken kernels on IMM insect mortality, cages were filled with either whole or cracked kernels. Almost 60% of IMM in cracked maize survived an ozone treatment of 50 ppm for 3 d in contrast to only 5% in whole grain. This decreased efficacy may be due to reduced airflow caused by the increased amount of fine material or more ozone reaction sites due to exposed germ and endosperm.

Natural populations of insects were also suppressed by ozone treatment. Two weeks prior to ozonation, IMM were detected by pheromone traps in both bins; however, no moths were trapped in the ozone-treated bin following treatment. In contrast, traps in the control bin caught 62 and 57 moths in the first and second week, respectively. Also, the numbers of hairy fungus beetles (*Typhaea stercorea* (L.)) and foreign grain beetle (*Ahasverus advena* (Waltl)) captured in pitfall probe traps were lower in the ozone-treated bin compared with the control bin (data not shown).

Table 2
Effects of ozone on viability of *Aspergillus parasiticus*

Ozone conc. (ppm)	Exposure time (d)	N[a]	Colony forming units ($\times 10^5$)[b]	
			Treated	Control
25	5	30	43.7 ± 4.9a	51.5 ± 3.1a
50	3	30	16.1 ± 2.2a	43.0 ± 2.2b

[a] Number of cages.
[b] Average ± standard error. Different letters within each row indicate a significant difference between treated and control ($P < 0.05$).

3.2. Ozone efficacy against A. parasiticus

The number of viable *A. parasiticus* conidia on the grain surface was reduced by 63% when grain was exposed to 50 ppm ozone for 3 d (Table 2), whereas 25 ppm for 5 d failed to significantly reduce spore viability. At 50 ppm, 99% of the colonies recovered from ozone-treated grain were *A. parasiticus* strain ATCC 24551. In contrast, many other species of fungi also survived the 25 ppm ozone treatment as indicated by the large numbers growing on dilution plates. These data indicate that a 50 ppm ozone fumigation targeted at an insect infestation should have the added benefit of reducing the populations of fungi on the grain surface.

3.3. Ozone generator performance

The maximum airflow from the generator producing 50 ppm ozone was 0.021 m^3/s (or 1.14 m/s output velocity). This produced a maximum apparent air velocity of 0.0036 m/s through the maize mass in the field-study bins and approximately 0.056 m/s in the laboratory columns. Theoretical calculation of the velocity through the laboratory column given the generator fan capacity was 0.081 m/s. However, the actual velocity was reduced because of some air leakage from the generator, ducting and barrels, and because of a greater actual pressure drop through the maize than the theoretical equations predicted.

Generator performance in the laboratory and field experiments was affected by environmental factors, such as temperature, relative humidity and air density. Generally, as the day progressed from morning to afternoon, the air velocity (and thus the air volume) through the duct increased by approximately 10% because of the decreased density of the warmer afternoon air. In turn, the increased air volume across the ozone generator reduced the ozone concentration by 5–8 ppm. Because of variability in performance, ozone concentration at any given depth was recorded as a percentage of the ozone measured at the 0.3 m level in the grain. The 0.3 m level was selected as the bench-mark because concentrations were similar to those in the headspace and they fluctuated less.

Desired ozone concentrations were maintained during windy conditions in the field study. However, wind sometimes caused fluctuations in the ozone concentration at different depths in the grain resulting in higher concentrations at the bottom of the grain bin compared with the middle of the bin. These fluctuations were temporary since the continual flow of fresh ozone into

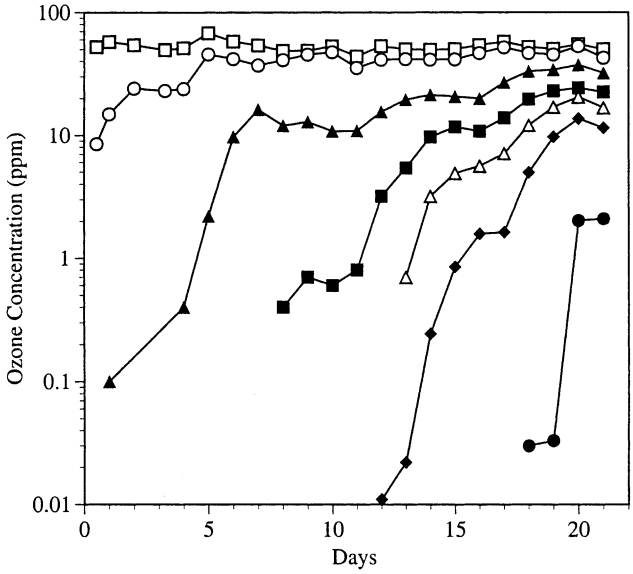

Fig. 1. Ozone concentration at various depths in the grain bin when introduced to the headspace at apparent velocities of 0.0036 m/s. Headspace (□), 0.3 m center (○), 0.9 m center (▲), 1.5 m center (◆), 1.8 m center (●), 1.5 m south wall (■), and 1.8 m south wall (△).

the bin quickly re-established the proper concentrations. The ability of ozone to maintain fumigation concentration during windy conditions is a considerable advantage over passive fumigations with phosphine.

3.4. Ozone penetration of grain mass

The generator produced 50 ppm of ozone at 0.0030–0.0036 m/s apparent velocity in the field study with 8.9 tonnes of maize in a 12.7 tonne capacity storage bin. Ozone was detected within 0.5 d at 0.3 m depth in the center, 1 d at 0.9 m center, 12 d at 1.5 m center, 18 d at 1.8 m center, 8 d at 1.5 m south wall, and after 13 d at 1.8 m south wall of the bin (Fig. 1). The ozone levels stabilized at 20 d, but the concentration was lower with increasing grain depth. Additionally, ozone penetrated the periphery (south wall) of the maize mass more readily than the center, presumably because of the higher level of fine material in the core of the maize mass. After 20 days, readings in the center of the grain mass reached 10 and 1.5 ppm at 1.5 and 1.8 m, respectively, and 70% of the generator output was measured at a grain depth of 0.9 m. The ozone generator was adjusted after 21 d to produce 25 ppm and ozone was detected at the bottom of the bin (data not shown).

The main goal of this ozone fumigation was to introduce 50 ppm ozone into a grain mass as fast as possible. We selected this dose because previous laboratory experiments to determine dose/time effects of ozone on insect mortality indicated that 50 ppm for 3 d resulted in 100% mortality of adult RFB and MW, and greatly reduced emergence of larval IMM (Strait, 1998). Introducing ozone through the top of the bin is preferable because most insects typically are in the headspace

and the top 2 m of the grain mass. This area in the bin also has the greatest potential for damage caused by fungi due to potential higher grain moisture content resulting from condensation and leaks. A previous study indicated that ozone fumigation of grain has two distinct phases (Strait, 1998). Maize not previously treated with ozone has inherent sites on its surface that react with ozone during initial fumigation (Phase 1). Ozone is degraded as it reacts with these sites and eliminates them. This phenomenon has been described as the ozone demand of the medium (Kim et al., 1999). Once these sites have reacted with ozone (Phase 2), the rate of ozone degradation decreases.

Finding the optimal velocity to minimize the time needed for Phase 1 of the fumigation is necessary to promote ozone as an effective fumigant. Completion of Phase 1 was not achieved with the ozone generator used in the field study. However, smaller diameter columns in the laboratory (0.57 vs. 3 m) allowed higher air velocities with the ozone generator set for 50 ppm. Three velocities were selected to evaluate the ability (and time required) for ozone to penetrate the grain mass. The three velocities were 0.02, 0.03, and 0.04 m/s. At a velocity of 0.02 m/s, more than 75% of the ozone introduced into the top of the column penetrated to a depth of 2.7 m within 3.7 d (95% CI: 3.6, 3.8) (Fig. 2A). In the same number of days (3.7 d), ozone concentrations were 93, 86, and 80% at 0.9, 1.5, and 2.1 m, respectively. When the apparent velocity was increased to 0.03 and 0.04 m/s the same amount of ozone (75% of the ozone introduced into the top of the column) was attained at a depth of 2.7 m within 1.3 ± 0.1 d. At this time, ozone concentrations were 94, 88, and 81% for both 0.03 and 0.04 m/s at 0.9, 1.5, and 2.1 m, respectively (Fig. 2B and C).

Apparent velocities greater than 0.02 m/s during Phase 1 of fumigation were required to effectively penetrate grain and establish stable concentrations of ozone at all measured depths in the grain. Apparent velocities of 0.03 m/s or higher permitted more than 85% of the ozone to penetrate 2.7 m of grain (Fig. 2A and B). Increasing velocity from 0.2 to 0.3 m/s decreased from 3 to 0.8 d the period of time required to achieve greater than 75% of the ozone concentration at 2.7 m.

It will be necessary to characterize the dynamics of ozone movement through the various grain types before ozone generators can be developed for use on large commercial storage bins. Our experimental data with maize indicated that ozone concentrations stabilized in a linear relationship between concentration and depth in the grain column. We have termed this linear relationship the stable degradation rate. Based on airflow and ozone degradation, a stable degradation rate of 1 ppm ozone per 0.30 m depth is an attainable rate during Phase 1 fumigation. Theoretically, this would allow a killing zone (>25 ppm) to penetrate about 8 m of grain. To determine the time required to achieve this stable degradation rate, we calculated the point in time when the linear slope equals 1 ppm per 0.3 m, at each velocity and day.

for 0.02 m/s

$$y' = -7.2(95\%\ \mathrm{CI}: -8.5, -5.7)\ \mathrm{Log}\ x + 9.6(95\%\ \mathrm{CI}: 8.7,\ 10.6),\quad P < 0.001,$$

for 0.03 m/s

$$y' = -7.4(95\%\ \mathrm{CI}: -8.5,\ -6.3)\ \mathrm{Log}\ x + 6.2(95\%\ \mathrm{CI}: 5.5,\ 6.9),\quad P < 0.001,$$

and for 0.04 m/s

$$y' = -7.6(95\%\ \mathrm{CI}: -8.7,\ -6.4)\ \mathrm{Log}\ x + 6.0(95\%\ \mathrm{CI}:\ 5.4,\ 6.7),\quad P < 0.001.$$

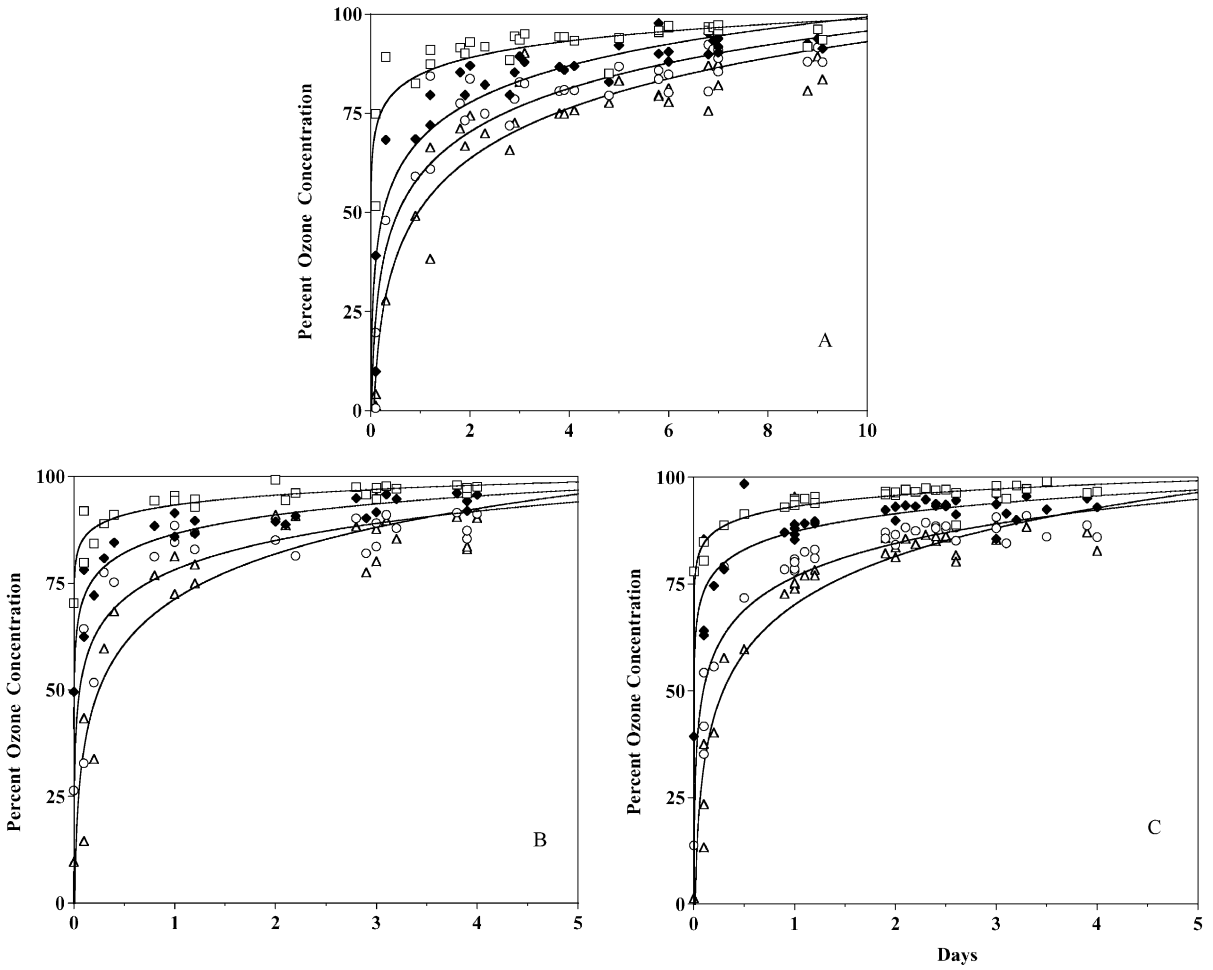

Fig. 2. Ozone concentration at various depths in the grain column when introduced to the headspace at apparent velocities of: (A) 0.02 m/s; (B) 0.03 m/s; and (C) 0.04 m/s. Data collected at 0.9 m (□), 1.5 m (◆), 2.1 m center (○), and 2.7 m (△) are percentage ozone concentration relative to that at 0.3 m.

Over time, changes in ozone degradation between velocities of 0.03 and 0.04 m/s were not significant (Fig. 3). Between 0.02 and 0.03 (or 0.04) m/s the slopes were not significant, but there was a significant difference in intercept.

The change in ozone degradation over time was similar at all three velocities, but it took longer to achieve a stable degradation rate of 1 ppm per 0.3 m at 0.02 m/s than at either 0.03 or 0.04 m/s. An air velocity of 0.02 m/s required 15.5 ± 1.7 d to reach a stable degradation rate of 1 ppm per 0.3 m, but only 5.0 ± 2.1 d at 0.03, and 4.6 ± 1.9 at 0.04 m/s (Fig. 3). These calculations indicate that an apparent velocity of 0.03 m/s is necessary to push the ozone front through the grain in a reasonable time. Air velocity calculations (data not shown) were made for a range of standard

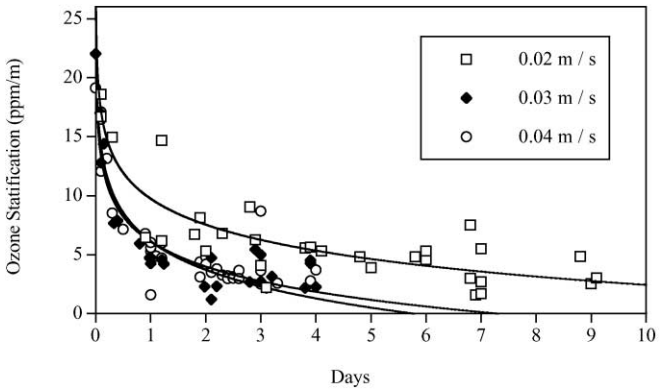

Fig. 3. Effect of apparent velocity on the stratification of ozone in the grain column (ozone concentration versus depth).

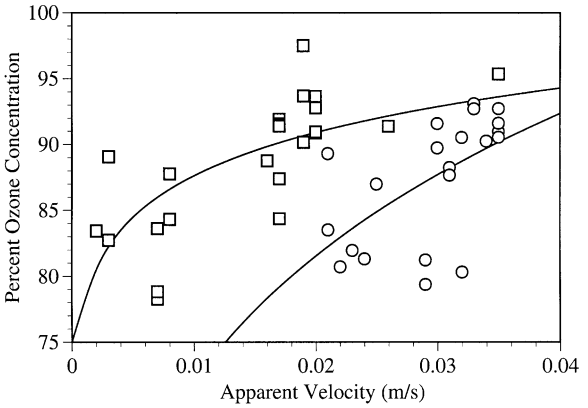

Fig. 4. Ozone concentration at 2.7 m relative to 0.3 m in depth during Phase 1 (○) and Phase 2 (□) fumigations when ozone was introduced into the headspace at various apparent velocities.

aeration fan and storage bin configurations, which confirmed that a velocity of 0.03 m/s would be achievable.

The parameters of Phase 2 fumigation were studied in the laboratory after the apparent Phase 1 reaction sites were eliminated. During Phase 2, only 4% of ozone was lost through the 2.7 m columns. The air velocity was reduced step-wise from 0.04 m/s to a minimum of 0.002 m/s. Significantly less velocity was required to maintain the ozone concentration during Phase 2 compared with Phase 1 (Fig. 4).

Phase 1 fumigation:

$$y' = 29.9(95\%\text{CI: } 23.5,\ 36.4)\ \text{Log}\ x + 79.9(95\%\text{CI: } 77.6,\ 82.2),\quad P < 0.001,$$

Phase 2 fumigation:

$$y' = 11.0(95\%\text{CI: } 6.9, 15.0)\ \text{Log}\ x + 90.6(95\%\text{CI: } 89.1, 92.0),\quad P < 0.05.$$

During Phase 1, a velocity of 0.02 m/s was required to achieve 80% of the generator output at 2.7 m while only 0.002 m/s was required during Phase 2. A velocity of 0.04 m/s was required during Phase 1 to achieve 90% of generator output at 2.7 m compared to 0.018 m/s during Phase 2. Based on these data, velocities could be lowered during Phase 2 fumigation to achieve the same ozone penetration. Another advantage of Phase 2 fumigation is that the time required to re-establish the target concentration of 50 ppm ozone is less than 0.5 h during subsequent fumigations.

Commercial formulations of phosphine penetrate the kernel to kill internal infestations and do not leave a residue. By definition, it may not be correct to refer to ozone as a fumigant since, at the concentration used in this study, ozone does not penetrate the pericarp enough to kill insects or fungi within the kernel (Strait, 1998). In addition to being residue free, ozone fumigation (ozonation) has advantages over existing fumigation technologies. Ozone can reduce the number of insects and fungal spores in grain in as little as 3 d. During windy conditions, ozone concentrations are maintained through continual and automatically regulated production during the fumigation (ozonation) process. The short half-life of ozone and its degradation to molecular oxygen eliminates environmental contamination evident with current fumigants. The challenge with ozone fumigation is Phase 1; to produce sufficiently large concentrations of ozone to thoroughly penetrate the full depth of a grain mass in a short time. In these studies, 0.03 m/s apparent velocity was the optimum fumigation velocity required. This velocity is attainable with current aeration equipment. Another challenge of ozonation at 50 ppm is that 100% mortality was not achieved during field trials. This suggests the recommended concentration of ozone may need to be increased if 100% mortality is desired. We also have shown that subsequent fumigations of pre-ozonated dry grains should be done at the same velocity, but concentrations in the grain mass will re-establish more rapidly due to Phase 2 fumigation characteristics.

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