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EFFICACY OF OZONE TO CONTROL INSECTS, MOLDS AND MYCOTOXINS

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ABSTRACT

With the reduced availability of traditional post-harvest storage pesticides, safe alternatives are desperately needed. The long range goal of this research is to find new technologies for controlling insects and mold growth in stored grain and decreasing the likelihood of mycotoxin contamination. With this as the goal, this research project focused on the use of ozone technology in post-harvest grain storage. The specific objectives were to determine the effect of ozone on the survival of insects (*Tribolium confusum* and *Oryzaephilus surinamensis*) and mold (*Aspergillus flavus* and *Fusarium moniliforme*), and the production of mycotoxin in stored corn. Ozone atmospheres (5 ppm) were compared to air environments (controls) in their respective effects on insect mortality, mold radial growth, sporulation and aflatoxin production. For *T. confusum*, 100% mortality was found in 5 d, and for *O. surinamensis* in 3 d. Radial growth of both *A. flavus* and *F. moniliforme* was inhibited for the first 2 d, but after 3 d in the ozone atmosphere growth paralleled that in the control. Sporulation and hyphal growth above the surface of the agar were completely inhibited by the ozone. Aflatoxin production was also reduced by over 99% in the *A. flavus* cultures exposed to ozone.

INTRODUCTION

Insects, molds and vertebrates cause numerous quality problems in stored grains and processed food and feeds. More than 15 billion bushels of grain are stored every year in the United States, and total annual storage losses are estimated at more than $1 billion. Estimated losses due to stored-grain insects exceed $12 million annually in Indiana alone. In the grain storage industry it is essential to have effective pest management programs which protect against economic loss; insect, mold and mycotoxin contamination; and disease due to pest-contaminated foods. Insect pests in storage are currently managed by the application of chemical pesticides. Only two fumigants are still permitted, one of which is methyl bromide (MB). Because of environmental concerns, the US government has dictated that

MB will be eliminated from use by the year 2001. There is no direct substitute for MB that is equally effective and fast-acting, nor is the development of such a corrective tool expected. Additionally, no chemicals except organic acids such as proprionic acid are available for controlling mold growth in stored grain. Unfortunately, in many situations these acids are not suitable. Therefore, new pest management practices are needed in stored grain. The principal investigator (PI), in cooperation with other members of the post-harvest research team, is currently investigating existing residual pesticides (Actellic and Reldan) and alternative pest control strategies (aeration, temperature control and modified atmospheres using carbon dioxide (CO_2)). Ozone (O_3) technology will complement our existing research program.

Ozone is one of the strongest oxidizing agents known. O_3 technology is currently being used in the industrial and medical industries as a disinfectant of microorganisms and viruses and for reducing odor and removing taste, color and environmental pollutants. O_3 is an attractive alternative to other chemicals primarily because it has a short half-life and with it there is an absence of residuals. With a half-life of about 20–50 min in the atmosphere and 1–10 min in water, O_3 rapidly decomposes to diatomic oxygen (a natural component of the atmosphere). O_3 is also attractive because it can be generated on site, eliminating the need for storage.

In recent years, the manufacturers of O_3-generating devices have addressed the possibility of its use in agriculture. Potential applications include deodorizing poultry and swine-waste lagoons and sterilizing the water used to wash chicken carcasses in poultry packaging operations. Published research suggests that the application of O_3 to a stored-grain facility may be a feasible alternative to the fumigant MB. The possibility of controlling mold growth and mycotoxin formation in storage would be additional benefits.

Because of the worldwide use of O_3 for water purification, most of the published research documents its effects on microorganisms in water. Except for a few studies done in the 1960's on its effects on fungal plant pathogens, essentially nothing is known about how O_3 affects fungi (Rich and Tomlinson, 1968). Some effort has been made to determine if ozonation is an effective means of eliminating aflatoxin from peanut and cottonseed meals (Dollear *et al.*, 1968; Maeba *et al.*, 1988), and this research indicated that it can destroy aflatoxin.

There are a few studies suggesting that O_3 has potential for controlling insects. Research on the flour beetles, *Tribolium confusum* and *T. castaneum*, has shown that these insects are sensitive to O_3 (Erdman, 1980). It was found to be lethal to all stages of the insects' life-cycle with the larval and pupal stages being most sensitive.

Both the published literature and our preliminary studies suggest that O_3 can be used for grain pest management. The technology for generating O_3 concentrations capable of fumigating grain storage bins exists today. Our discussions with those in the industry indicate that the generators, already in use for water and air purification, can be adapted for delivering whatever concentration of O_3 is needed. However, some questions need to be answered before this technology can be used. Can O_3 effectively prevent insect and mold growth on stored grain? What concentration, and duration, of O_3 exposure is needed

to control these organisms? Are the benefits of using O_3 great enough to reduce the current use of pesticides in stored grain? The current study is the first step in answering these questions.

RESEARCH OBJECTIVE

The long-range goal of our research is to find new technologies that control insects and mold growth in stored grain and decrease the likelihood of mycotoxin contamination. In accordance with this goal, the research project here reported focused on the use of O_3 technology as a means of controlling insects, molds and mycotoxins in post-harvest grain storage. The specific objectives were to determine the effect of O_3 on insect (*T. confusum* and *Oryzaephilus surinamensis*) and mold (*Aspergillus flavus* and *Fusarium moniliforme*) survival and on mycotoxin production in stored corn.

METHODS

Ozone detection

O_3 levels were measured using SENSIDYNE® detector tubes (4–400 ppm range) in a Gastec Multi-Stroke Gas Sampling Pump. The concentration of O_3 around the insects and petri plates was maintained at 5 ± 1 ppm. Concentrations were confirmed at the beginning and once again either during or at the end of each trial.

Insect mortality

Insects were obtained from a laboratory colony maintained at 21°C, 14:10 L:D photophase. Fifty unsexed adult insects (less than 1 week old) of each species were placed singly in 1.7-ml micro-centrifuge tubes that contained approximately 1 ml flour/cornmeal. The snap-cap lid was replaced with fine mesh fabric. The micro-centrifuge tubes were then placed in a cardboard tray with holes cut to support each tube vertically. The tray was then placed in a 8.3 L (24 × 34 × 12 cm) RUBBERMAID® container. O_3 was then allowed to flow into the container, around the tubes, and out of the container. Mortality counts were determined every 24 h. Control insects were held in tubes under similar temperature and photophase conditions. *T. confusum* trials were replicated four times (200 insects total) and *O. surinamensis* three times (150 insects total).

Mold radial growth, sporulation and aflatoxin production

Petri dishes containing potato dextrose agar (PDA) or coconut agar (CA) medium were inoculated in the center with 5 µl of conidial suspensions of *A. flavus* and *F. moniliforme*. The plates were incubated at 21°C in an atmosphere of either O_3 or air (control). Radial growth was determined daily by measuring the colony diameter. After 5–6 d growth, the PDA-grown cultures plates were flooded with 5 ml of water, and the conidia concentration was determined using a hemacytometer. Simultaneously, aflatoxin production was determined by thin-layer chromatography analysis of extracts taken from the CA culture plates.

RESULTS AND DISCUSSION

Insect mortality

O_3 caused significant mortality in both species examined. On day 3 *T. confusum* mortality was significantly higher than the control, and it continued to increase for the next 2 d (Fig. 1). One hundred percent mortality was achieved by day 5. Only a few of the 200 control insects died during the 5-d test period. O_3 had a much more rapid influence on *O. surinamensis* mortality. Significantly more adults died on day 1 when compared to the control (Fig. 2). One hundred percent mortality was achieved by day 3. These data indicate that O_3 at 5 ppm has a significant influence on adult mortality of *T. confusum* and *O. surinamensis.*

Differential mortality between species has been found in other studies (Erdman, 1980), in which O_3 toxicity ontogenies for *T. confusum* and *T. castaneum* at 45 ppm O_3 were compared. In his study, exposure times were less than 6.5 h and mortality was measured 1 month post-eclosion. He found that *T. castaneum* life stages were more sensitive than those of *T. confusum*; however, both species were equally sensitive in the pupal stage. Our data indicate that O_3 at much lower levels (5 ppm) can cause significant mortality to the adult stage when exposure times are increased (24 h minimum). Experiments underway indicate that even shorter exposures are needed for 100% mortality of other life stages.

Mold radial growth, sporulation and aflatoxin production

At 5 ppm O_3, the radial growth of both *A. flavus* and *F. moniliforme* was inhibited for the first 2 d (Figs. 3 and 4). After 3 d in the O_3 atmosphere, the growth paralleled that of

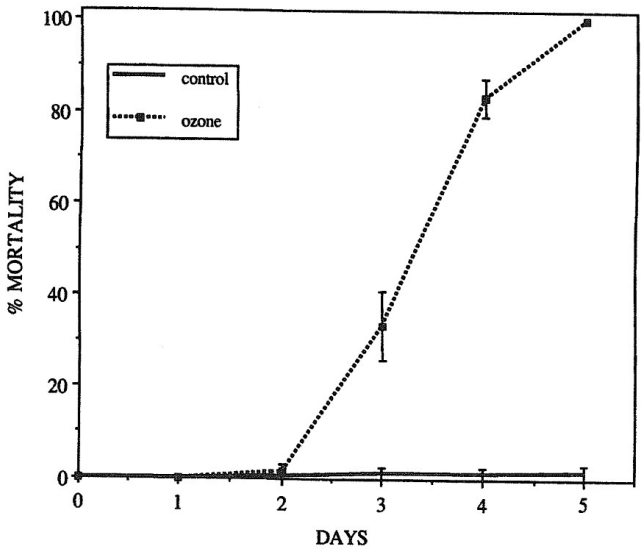

Fig. 1. The effect of ozone on the mortality of *Tribolium confusum* (Bars represent mean ± SE).

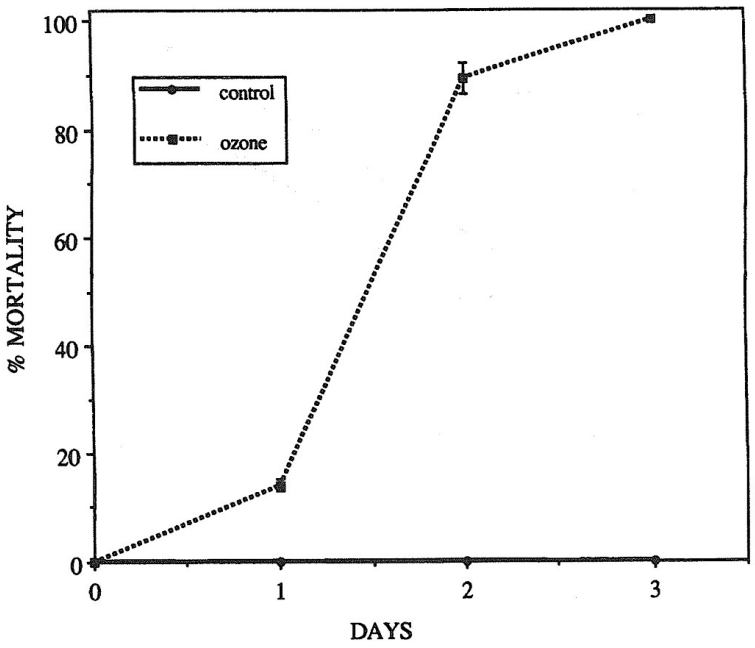

Fig. 2. The effect of ozone on the mortality of *Oryzaephilus surinamensis* (Bars represent mean ± SE).

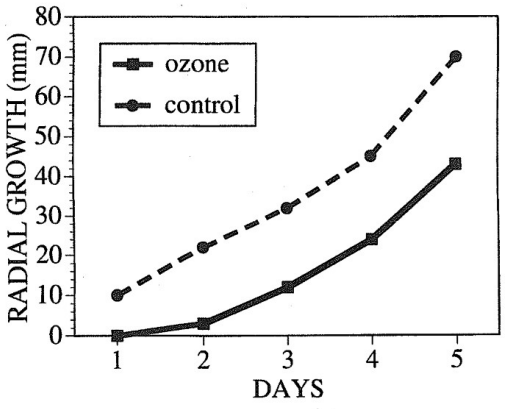

Fig. 3. The effect of ozone on the radial growth of *Fusarium moniliforme*.

the control. Sporulation and hyphal growth above the surface of the agar were completely inhibited by O_3 (Table 1). In contrast, in the control atmosphere there were both profuse sporulation and surface mycelium. These data indicate that O_3 has a direct inhibitory effect on the growth of these fungi and suggest that the gas does not penetrate the surface of the agar medium. Aflatoxin production was also reduced by over 97% in the *A. flavus* cultures exposed to O_3 (Table 1).

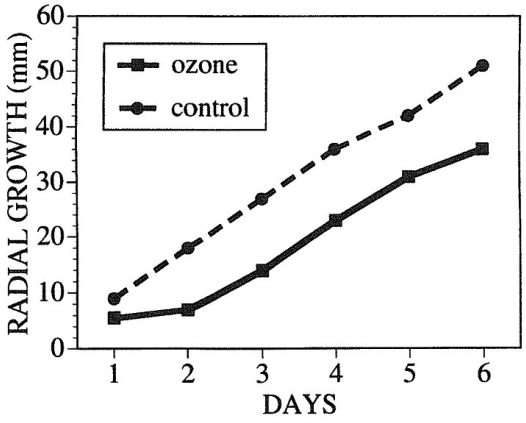

Fig. 4. The effect of ozone on the radial growth of *Aspergillus flavus*.

TABLE 1
The effect of ozone on the sporulation and aflatoxin production by *Aspergillus flavus* and *Fusarium moniliforme*

Mold	Ozone-treated	Control
Aspergillus flavus		
Sporulation	0 conidia/plate	1.0×10^9 conidia/plate
Aflatoxin	32 μg/plate	1,000 μg/plate
Fusarium moniliforme		
Sporulation	0 conidia/plate	1.0×10^8 conidia/plate

SUMMARY

Studies are currently under way to examine additional insect life stages, insect species and O_3 concentrations. In addition, diffusion models are being developed for O_3 within a grain mass. It is hoped that full scale trials will begin later this year.

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