2007

# Using ozone to control fungi in high moisture corn

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**Using ozone to control fungi in high moisture corn**

by

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A thesis submitted to the graduate faculty

in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Co-Majors: Agricultural Engineering; Biorenewable Resources & Technology

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Ames, Iowa

2007

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**ACKNOWLEDGEMENTS**

I would like to start by thanking my major professors, Drs. Carl Bern and Hans van Leeuwen, for their help and suggestions. Their experiences and expertise was useful in deciding what directions to take in my research.

In addition, I would like to thank Dr. Theodore Bailey and Dr. Thomas Brumm for serving on my program of study committee.

There were several people who helped me with technical issues involved with my research and with other aspects of graduate school. I would like to thank Patrick Murphy for his help with adjusting to graduate school, the late night humor and company in the lab, and assistance in understanding and applying statistics. I also greatly appreciate the help Dr. Sam Beattie gave me in plating and identifying fungal samples. I also have to thank my brother, Jeremy White, who was dragged into the lab on several occasions to help me when I was short on time. I am also grateful for the work done by Clayton Thacker in helping me with the experiments.

I would like to end by thanking the Iowa Energy Center and William Haman for the financial support that allowed my research to take place.

## ABSTRACT

One of the main problems with harvesting high moisture corn is the rapid dry matter loss due to fungi. Ozone was looked at as a possible way to control fungi growth in high moisture corn. Two experiments were performed to test the effect of ozone on high moisture corn dry matter loss and damage kernel total. A system was designed for the first experiment to monitor effects of ozone on high moisture corn during aerated storage in a lab. The corn was stored in an environmental chamber that controlled the relative humidity and temperature of the corn in glass tubes. A continuous airflow was used to deliver the ozone to the tubes and to measure the carbon dioxide leaving the grain. The carbon dioxide concentrations were used to estimate the dry matter loss of the corn. A Visual Basic 6.0 program was used with a PMD 1208LS microcontroller to collect data from several sensors and to sample the air from individual tubes.

The first experiment stored 22% moisture content corn under high temperature conditions ($32^{o}$C) for 9 days. Corn was treated for either for the initial 24 h, 5 h, or every 3 d. Dry matter loss was estimated from glucose oxidation into carbon dioxide. Ozone had little impact on the overall storage time of the high moisture corn, based on dry matter loss and damage kernel total evaluations. The second experiment stored 26% moisture content corn under low temperature conditions ($15.5^{o}$C) for 30 d. Ozone was applied over the initial 24 h or once every 3, 6, or 12 d. Ozone did have a significant effect on dry matter loss of high moisture corn, but made no impact on the damage kernel total.

The effectiveness of ozone for fungi control in high moisture corn was studied by enumerating fungi. Three moisture contents (18, 22, 26%) were treated with ozone at (0, 50, 500, 1000, 15000 ppm) for 1 h and an airflow rate of 0.47 L / min. The results showed that increasing ozone concentrations increased the number of uninfected kernels. These findings would indicate that ozone causes fungi inactivation and could have applications in corn storage or fungi control. Ozone appeared to have an inhibiting effect on fungi genera in the following order (highest to lowest): *Penicillium*, *Mucor*, other genera, *Aspergillus*, *Fusarium*, and *Rhizopus*.

# CHAPTER 1.

# GENERAL INTRODUCTION

## Thesis Organization

The information presented in this thesis is organized into four chapters. The first chapter is a general introduction, which contains sections on the thesis organization, objectives, and literature review. The second chapter contains a paper entitled "Respirometer and ozone control system for monitoring fungi activity in high moisture corn." The third chapter contains a paper entitled "Using ozone to control fungi in high-moisture corn." The fourth chapter contains a paper entitled "Enumeration of fungi from high moisture corn treated with ozone." The final chapter contains general conclusions which are derived from information contained in chapter three.

Chapter three was prepared for publication in the Journal of Stored Products Research and follows general format guidelines for submission to the journal. Chapter four was prepared for publication in Food Microbiology.

## Literature Review

### Corn Production

Corn is one of the world's most abundant agricultural commodities. There was an estimated $6.83 \times 10^8$ Mg ($2.69 \times 10^{10}$ bushels) of corn produced globally during 2005-06. US production of $2.82 \times 10^8$ Mg accounts for 41% of global production for 2005-06 (USDA/Foreign Agricultural Service 2006). The Corn Refiners Association estimates that

corn is an ingredient in almost 4,000 products (Iowa Corn Growers Association 2006). The large quantities of corn produced globally incur some damage at every stage of handling, storage and processing. There is an estimated loss of more than $500 million each year due to damage caused by fungi and insects to stored grain in the United States (Kells et al. 2001). Some countries have losses approaching 50% for their stored grain (Allen et al. 2003).

**Corn Development**

Corn kernels attain maximum dry matter weight when they reach physiological maturity, usually at a wet basis moisture level between 35 and 25%[1] (Bern 1998). After corn has reached physiological maturity, kernel moisture content decreases until harvest, usually at moisture levels between 25 and 17%. Once harvested, temperature and moisture conditions favor rapid growth of fungi in stored corn, making it necessary to either dry the corn or use some other preservation process (Bern 1998).

**Low Moisture Corn**

In an artificial drying system, corn should be dried to 15.5% moisture content for storage times up to six months, and 13% for periods longer than six months (Hellevang 1994; Bern 1998). A recommendation by Brook suggests that corn stored for winter should have a maximum moisture content of 15%, a maximum moisture content of 14% if stored past the following summer, and 13% or lower if stored longer than a year (Munkvold 2003). The most common dying methods use forced-air systems that move either natural air or heated air through the grain. While drying corn is effective at extending the storage life and slowing

deterioration, it is energy intensive. Another determent to quickly drying corn with heated air is the tendency to form stress cracks as the shell of the kernel dries faster than in inside, causing it to shrink and crack (Bern et al. 2003).

About 87% of the Iowa corn crop is preserved by drying after harvesting (Bern 1998). The drying process is very energy intensive because of the high latent heat of vaporization of water and dryer inefficiency. This energy usually comes from direct combustion of liquefied petroleum gas (LPG) or natural gas, plus electricity to run the equipment. It takes the equivalent of about 96 million L (25.5 million gallons) of LPG, plus 30 million kWh of electricity to remove each percentage point of moisture from the Iowa corn crop, and nearly 533 million L (141 million gallons) of LPG, plus nearly 165 million kWh of electrical energy to dry the crop from the average harvest moisture of 20.5% to 15% moisture for storage (Bern 1998).

An estimate of the cost of drying was done using 80% of this US corn dried from 20% moisture to 15%, or around $11.3 \times 10^6$ Mg of water evaporated. Using an estimate that conventional dryers would require at least 250% of the latent heat of vaporization (2400 kJ/kg) or $76 \times 10^{12}$ kJ ($73 \times 10^{12}$ Btu) per year to complete this drying and natural gas priced at $7.50 per MBTU (NYSE, first half of 2007), the cost of energy for drying would exceed $545 million in the US. If 600 million kWh of electricity at $0.10/kWh is also included, and the estimated total energy cost for drying would amount to more than $600 million per year.

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[1] All moistures are % wet basis

The high cost associated with drying corn could make other preservation methods encouraging.

**High Moisture Corn**

There are several reasons that make harvesting high moisture (>17% moisture content) corn appealing. "Harvesting corn at high moisture content reduces field pest attacks, avoids bad-weather consequences, and minimizes field losses" (Aljinovic et al. 1994). The corn can be harvested from the field 2 to 3 weeks earlier than corn harvested for dry storage (Miller, 2002). Harvesting earlier places corn closer to physiological maturity and maximum dry matter at harvest. The earlier harvest time can avoid a 3 to 8% dry matter loss that would accrue if corn was left in the field to dry (Miller 2002). The earlier harvesting also opens the possibility of having a higher quality residue left in the field (Miller 2002). Disadvantages include rapid deterioration from fungal activity after harvest, and higher drying costs (Aljinovic et al. 1994). High moisture corn also has a lower market flexibility compared to dry corn (Miller 2002).

## Fungi in Corn

While many microorganisms influence stored grain quality, only some fungal species are important. Most bacteria and yeast have little impact on corn storage outside of very high moisture conditions (Paulsen et al. 2003). Fungi are loosely defined as "eukaryotic, spore-producing, achlorophyllous organisms with absorptive nutrition that generally reproduce both sexually and asexually and whose usually filamentous, branched somatic structures, known as hyphae, typically are surrounded by cell walls" (Alexopoulos et al. 2004). Storage fungi species found in corn will grow in temperatures between -2 and 50$^{o}$C with an optimal growth

temperature between 20 to 35°C depending on the species (Paulsen et al. 2003). Fungi found in corn also need access to water in order to grow. The lower limit for growth conditions in most species is between 70 and 81% relative humidity or 13.5 to16.5% moisture in the corn (Paulsen et al. 2003). The main fungal species and conditions for growth that affect corn storage are in table 1.1. The main conditions that affect fungal growth are temperature, relative humidity and oxygen content of air surrounding the grain, physical conditions of the corn kernels, mold inoculum level, and previous grain storage history (Bern et al. 2002; Paulsen et al. 2003).

Mold growth in corn involves the mycelium on the surface of the grain tissue (Paulsen et al. 2003). Mycelium is the vegetative part of a fungus that is made of a mass of branching, threadlike hyphae (Madigan et al. 2006; Jay et al. 2005). Fungal enzymes are excreted and digest the grain structures, providing a source of energy for the mold (Paulsen et al. 2003). Heat, carbon dioxide ($CO_2$), and moisture are the byproducts of aerobic respiration from mold activity (Paulsen et al. 2003; Bern et al. 2002).

Table 1.1 Temperature and relative humidty conditions of growth for storage fungi on corn (Paulsen et al. 2003).

| | Growth Temperature (°C) | | | Relative Humidity (%) | |
|---|---|---|---|---|---|
| Fungal Species | Lower Limit | Optimum | Upper Limit | Lower Limit | Lower Corn Moisture Equivalent |
| Storage Species | | | | | |
| *Aspergillus restrictus* | 5 - 10 | 30 - 35 | 40 - 45 | 70 | 13.5 - 14.5 |
| *A. glaucus* | 0 - 5 | 30 - 35 | 40 - 45 | 73 | 14.0 - 14.5 |
| *A. candidus* | 10 - 15 | 45 - 50 | 50 - 55 | 80 | 15.0 - 15.5 |
| *Penicillium cyclopium* | -2 | 20 - 24 | 30 - 32 | 81 | 16.0 - 16.5 |
| *P. brevi-compactum* | -2 | 20 - 24 | 30 - 32 | 81 | 16.0 - 16.5 |
| *P. viridicatum* | -2 | 20 - 24 | 34 - 36 | 81 | 16.0 - 16.5 |
| Storage and Field Species | | | | | |
| *A. flavus* | 10 - 15 | 40 - 45 | 48 - 50 | 81 | 16.0 - 16.5 |
| Field Fungi | | | | | |
| *P. oxalicum* | 8 | 31 - 33 | 35 - 37 | 86 | 17.0 |
| *P. funiculosum* | 8 | 31 - 33 | 35 - 37 | 91 | 19.0 |
| *Alternaria* | -4 | 20 | 36 - 40 | 91 | 19.0 - 20.0 |
| *Gibberella zeae (Fusarium graminearum)* | 4 | 24 | 32 | 94 | 20.0 - 21.0 |
| *F. moniliforme* | 4 | 28 | 36 | 91 | 19.0 - 20.0 |

**Modeling Deterioration of Stored Corn**

The characteristics that affect growth of fungi are also used to model shelled corn deterioration: storage time, kernel moisture, kernel temperature, kernel visible mechanical damage level, genetic susceptibility to storage fungi, and other factors (Bern et al. 2002; Paulsen et al. 2003). These factors also influence the acceptable dry matter loss that is allowed by grain users. Steele and Saul (1969) observed that shelled corn can, on average, experience a 0.5% dry matter loss due to storage fungi before its USDA grade is reduced by one USDA level. While not precise, the 0.5% dry matter loss limit for shelled corn deterioration is widely accepted.

It is possible to predict the storage time of corn to reach 0.5% dry matter loss based on moisture content, temperature, visible mechanical damage, genetic traits, and fungicidal application (equation 1.1; Bern et al. 2002). Table 1.2 (Bern et al. 2002; ASABE Standard, 2005) shows the predicted storage times for corn to lose 0.5% dry matter using equation 1.1.

Predicted corn storage time:

$$t_n = t_s M_M M_T M_D M_H M_F \qquad (1.1)$$

Where:

$t_n$ = time (h) under non-reference conditions
$t_s$ = time (h) under reference conditions (15.6$^o$C, 25% moisture, 30% wt visible mechanical damage) = 230 h
$M_m$ = moisture multiplier
$M_T$ = temperature multiplier
$M_D$ = damage multiplier
$M_H$ = hybrid multiplier
$M_F$ = fungicide multiplier

Table 1.2 Shelled corn storage time for 0.5 % dry matter loss, in days (Bern et al. 2002; ASABE, 2005)

| Corn temp | | Corn moisture, % wet basis | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| °F | °C | 16 | 18 | 20 | 22 | 24 | 26 | 28 | 30 | 32 | 34 |
| 35 | 1.7 | 1144 | 437 | 216 | 128 | 86 | 63 | 50 | 41 | 35 | 31 |
| 40 | 4.4 | 763 | 291 | 144 | 85 | 57 | 42 | 33 | 27 | 24 | 21 |
| 45 | 7.2 | 509 | 194 | 96 | 57 | 38 | 28 | 22 | 18 | 16 | 14 |
| 50 | 10.0 | 339 | 130 | 64 | 38 | 26 | 19 | 15 | 12 | 10 | 9 |
| 55 | 12.8 | 226 | 86 | 43 | 25 | 17 | 13 | 10 | 8 | 7 | 6 |
| 60 | 15.6 | 151 | 58 | 29 | 17 | 11 | 8 | 7 | 5 | 5 | 4 |
| 65 | 18.3 | 113 | 43 | 22 | 13 | 9 | 7 | 5 | 4 | 4 | 3 |
| 70 | 21.1 | 85 | 32 | 16 | 10 | 7 | 5 | 4 | 4 | 3 | 3 |
| 75 | 23.9 | 63 | 24 | 12 | 8 | 5 | 4 | 3 | 3 | 2 | 2 |
| 80 | 26.7 | 47 | 18 | 9 | 6 | 4 | 3 | 3 | 2 | 2 | 2 |
| 85 | 29.4 | 35 | 14 | 7 | 5 | 3 | 3 | 2 | 2 | 2 | 1 |
| 90 | 32.2 | 26 | 10 | 5 | 4 | 3 | 2 | 2 | 2 | 1 | 1 |
| 95 | 35.0 | 20 | 8 | 4 | 3 | 2 | 2 | 2 | 1 | 1 | 1 |
| 100 | 37.8 | 15 | 6 | 3 | 2 | 2 | 2 | 2 | 1 | 1 | 1 |
| 105 | 40.6 | 11 | 4 | 3 | 2 | 2 | 2 | 1 | 1 | 1 | 1 |
| 110 | 43.3 | 8 | 3 | 2 | 2 | 2 | 1 | 1 | 1 | 1 | 1 |
| 115 | 46.1 | 6 | 2 | 2 | 2 | 1 | 1 | 1 | 1 | 1 | 1 |
| 120 | 48.9 | 5 | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |

[a] D = 30%, $M_D = M_H = M_F = 1$, Times calculated using °F temperature values.

## Methods for Determining Fungal Activity

Because of the role fungi play in grain storage, it is possible to monitor the fungal growth in order to determine the allowable grain storage time. Wadsö (1997) stated that "Growth is usually measured as a radial growth, mass increase or as number of spores produced." In addition to monitoring direct fungal growth, it is also possible to predict fungal growth based on off-gas composition, environmental conditions, and changes in corn dry matter.

### *Carbon Dioxide Respiration*

Deterioration of corn can be tracked by measuring the carbon dioxide being produced by fungi on the corn (Steele et al. 1969). Fungal respiration is often modeled as oxidation of glucose (equation 1.2). According to the model, $CO_2$ produced is directly proportional to dry

matter loss of corn. With a balanced chemical equation, the carbon from glucose is involved in an energy producing reaction that ends with the carbon going from glucose to $CO_2$. Based on the oxidation of glucose model, a 0.5% dry matter (glucose) loss corresponds to 7.35 g of $CO_2$ per kg of corn or 14.7 g of $CO_2$ per kg of corn for a 1.0% dry matter loss. Based off of experimental results from Steele et al. (1969), the carbon dioxide from corn at 0.5% dry matter loss is represented in equation 1.3.

Chemical equation of oxidation of glucose:

$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + 2835$ kJ / gram mole (1.2)

Empirical equation of carbon dioxide evolution from corn at reference conditions:

$$Y = 1.3\left(e^{0.006t_s} - 1\right) + 0.015t_s \quad (1.3)$$

Where:
Y = g $CO_2$ per kg dry matter
$t_s$ = time (h) under reference conditions (15.6°C, 25% moisture, 30% wt visible mechanical damage)

When: $t = t_s = 230$h, Y = 7.35 g / kg

Rukunudin et al. (2004), Dugba et al. (1996), Aljinovic et al. (1995), and Al-Yahya et al. (1993) used similar systems to monitor grain deterioration based on carbon dioxide evolution. The deterioration of stored grain was determined from carbon dioxide being produced by fungi on the corn. Rukunudin used soybeans, while the other three tested corn treated with chemicals. In each case, compressed air was first stripped of carbon dioxide using potassium hydroxide. Then the air was bubbled through water and salt solution in gas-washing bottles to obtain a desired relative humidity needed to maintain the corn moisture

content. Once the air stream had attained the desired relative humidity, it was passed through the grain. Carbon dioxide produced from deterioration of grain was mixed into the air at this point. Next air left the grain and had the moisture removed by first passing through Drierite (anhydrous $CaSO_4$), then $Mg[ClO_4]_2$. Carbon dioxide was then absorbed in sulaimanite (a mixture of vermiculite and potassium hydroxide solution). The amount of carbon dioxide produced was then calculated by measuring the weight gain of the sulaimanite.

## Detection of Fungi

There are several ways to determine if there is fungal growth on grain, including visual inspection, direct plating, and dilution plating.

### Direct Examination

The first method to detect fungi, direct examination of the foodstuff, is done prior to processing the product using the naked eye or a stereomiscope. This is often sufficient because of the size of fungal colonies and that fungal growth usually occurs on the product surface. Improved direct examination can be done preparing slides and using the aid of a microscope. The visible fungi then are transferred to plates for further analysis (Samson et al. 2004).

When direct examination is used as a method to determine the USDA grade of corn, brown discoloration, “blue-eye,” or other signs of mold invasion of the kernel are some of the damage types evaluated when determining the damage kernel total (DKT) percentage (Paulsen et al. 2003). USDA Grade corn grades of 1, 2, 3, 4, and 5 have maximum DKT

limits of 3, 5, 7, 10, and 15%, respectively. Direct examination of grain to determine USDA grade is preformed by licensed inspectors per US Grain Standards Act. A minimum of 125 g of corn is hand inspected and compared to interpretive slides. The DKT % is then determined by dividing the weight of damaged corn kernels by the weight of the total corn sample.

**Direct Plating**

Direct plating is used to obtain a more valuable mycological assessment and is more effective than dilution plating in detecting fungal species (Tournas et al. 1998). Direct plating is useful to identify microflora in the food and indicating a percentage of food infection. In the case of most grains and nuts, a surface disinfection should be done so that only fungi that invaded the food are enumerated. Surface disinfection is achieved by vigorously shaking 100 particles of the food product in a 0.4% freshly prepared sodium hypochlorite solution for 2 min. The chlorine is then removed from the food product by rinsing with sterile distilled water (Samson et al. 2004; Tournas et al. 1998). Non-surface disinfected samples can be plated to determine if the mold is from an internal or external invasion (Tournas et al. 1998). The food particles are transferred using flame-sterilized forceps to plates at a rate of 5 to 10 particles per plate. The plates are incubated for 5 d at 25$^{o}$C with a constant atmosphere composition. The results provide a percentage fungi infected particle. These results can be broken down further into genera and species using a stereomicroscope or plating on a selective media (Samson et al. 2004; Tournas et al. 1998).

### Dilution Plating

Dilution plating is used to determine the colony forming units (CFU) of fungi in a food product (Tournas et al. 1998). Dilution plating is done by suspending the fungi on a food sample in a liquid substrate, then diluting, plating, and incubating the suspended fungi (Samson et al. 2004; Tournas et al. 1998). Samson et al. (2004) recommends using a 5-g sample when working with homogeneous foods (i.e. sugar or flour) and a 40 g sample for non-homogenous food products (i.e. corn or wheat). A non-homogeneous sample may need to be soaked in a 0.1% peptone solution for 30 minutes to extract deep seated or internal fungi. The initial dilution of the material is done by adding one sample part to a 0.1% peptone solution at a rate of nine parts by weight (1:10 dilution). Further dilutions are done in the presence of large fungi concentrations, while lower dilution ratios are used for small concentrations of fungi. After the dilution has been thoroughly mixed, 0.1 mL of the inocula is spread over a plate and incubated upright for 5 d at 25$^{o}$C. At the end of the incubation period, the colonies are counted (CFU / g food) to determine the fungi population (Samson et al. 2004; Tournas et al. 1998).

## Methods for Storing High Moisture Corn

The options for storing high moisture corn are usually chemical treatments or oxygen-limiting conditions. Ionizing energy has also been shown to successfully decrease fungi populations in high moisture corn (Bern et al. 1994). Each of these methods is used on a small portion of the overall corn stored each year. Approximately 5% of corn in Iowa is stored using oxygen-limiting conditions, while another 1% is stored using chemical treatments (Bern 1998).

## Oxygen-limiting Storage

Corn with moisture contents between 25 and 28% can be preserved by being ensiled in a sealed structure such as a silo, bunker, or plastic bag. Anaerobic conditions set in once the oxygen supply inside the structure is consumed by microorganisms. The lack of oxygen prevents further fungal activity while allowing anaerobic bacteria to dominate. Activity of bacteria results in some dry matter loss as they consume carbohydrates and produce organic acids. Production of organic acids lowers pH, eventually causing bacteria growth to stop around a pH of 3.8 (Bern et al. 2003).

## Chemical Preservation

Chemicals that have been tested for preserving high moisture corn from fungi include propionic acid, ammonia, iprodione, and sulfur dioxide (Aljinovic et al. 1994, Bern 2000). Methyl bromide and phosphine are fumigants that are also used for corn storage (Mason et al. 2003). Each of these treatments has several negative traits that make chemical treatment for preservation unpopular for common use. Propionic acid, sulfur dioxide, and phosphine are highly corrosive. The cost associated with chemical preservation can also be significantly higher compared to drying corn to lower moistures for storage. Ammonia treatments affect kernel color and decrease dry matter. Methyl bromide was set to be phased out of use by 2005 due to the Montreal Protocol.

### *Iprodione*

A study done by Dugba et al. (1996) looked into using iprodione (3-(3,5-dichlorophenyl)-N-(1-methylethyl)2,4-dioxo-1-imidazoline-carboximide) as a preservative in shelled corn in three experiments. In each experiment, each sample consisted of one kg of wet corn stored

maintained at a temperature of 20$^{o}$C inside a 5.08-cm x 91.4-cm Plexiglas tube. Corn moisture content was controlled by bubbling the air stream through a glycerin solution. Deterioration of the corn was calculated using the cumulative weight of $CO_2$ measured in the exhaust air. The storage time for each test was set at the estimated allowable storage time for untreated corn stored under the moisture content and temperature used for each experiment.

The first experiment tested the effects of moisture and fungicide solution rates. It used two moisture contents (18 and 22.5%) with five liquid application levels: 0 ppm in 3.29 mL / kg wet corn, 0 ppm in 5.48 mL / kg wet corn, 20 ppm in 3.29 mL / kg wet corn, 20 ppm in 5.48 mL / kg wet corn, and 20 ppm in 3.29 mL / kg wet corn with 0.25% addition of an activating agent. The results showed that iprodione treated corn could be stored significantly longer than untreated corn. A possible increase in storage time of 21% for corn at 18% moisture content and 13% increase in storage time for corn at 22.5 % moisture were obtained. There was a noticeable effect between fungicide and moisture, fungicide and liquid application rate, and moisture and application rate. When damaged kernel total (DKT %) was considered, the fungicide treatment, liquid application level, or activating agent had no significant effect.

The second and third experiments tested effects of different fungicide rates. Experiment two used five concentrations of iprodione (0, 20, 50, 100, and 200 ppm) applied at the same rate of 5.48 mL / kg of corn at 23.5% moisture content. The third experiment performed by Dugba used four treatments (0, 20, half of the sample treated at 20, and half sample of the sample treated at 40 ppm) using the same application rate and moisture content as the second experiment. In the treatments where only half of the sample was treated, the two halves were

mixed for 5 min directly after liquid application. The results showed that the maximum increase in storage attainable with iprodione was around 25%.

## Ozone

Another chemical that may be useful in storing high moisture corn is ozone. It has several properties that are desirable when compared to the chemicals already discussed for corn preservation. Ozone is currently used as a disinfectant and reactant in several processes such as in water treatment, wastewater treatment, odor elimination, and pesticide removal (EPA 2002).

### Ozone Properties

Ozone ($O_3$) is an unstable triatomic, allotropic structure of oxygen ($O_2$). The structural instability of the oxygen-oxygen bonds causes ozone to be a strong oxidizer. An oxidizing agent is a substance that causes the oxidation, that is, the loss of one or more electrons, from the atoms of another substance (Brown et al. 2006). The ability of a substance to act as an oxidizing agent is referred to as its oxidizing potential and is measured in volts. Ozone has an oxidizing potential of 2.07 volts at a temperature of 25$^{o}$C, which is 150% of the oxidizing potential of chlorine (Bran 2001; Malik et al. 2000; Novazone 2006; table 1.3). Ozone has an affinity for the olefinic double bond that allows it to react with a large number of chemical groups (McKenzie et al. 1997).

Table 1.3 Comparative oxidizing potentials at 25°C (Novazone, 2006)

| Chemical Compound | Oxidizing Potential (volts) |
|---|---|
| Fluorine ($F_2$) | 2.87 |
| Ozone ($O_3$) | 2.07 |
| Hydrogen Peroxide ($H_2O_2$) | 1.78 |
| Potassium Permanganate ($KMnO_4$) | 1.70 |
| Hyprobromous Acid (HOBr) | 1.59 |
| Hypochlorous Acid (HOCl) | 1.49 |
| Chlorine ($Cl_2$) | 1.36 |
| Chlorine Dioxide ($ClO_2$) | 1.27 |
| Oxygen ($O_2$) | 1.23 |
| Chromic Acid ($H_2CrO_4$) | 1.21 |
| Bromine ($Br_2$) | 1.09 |
| Nitric Acid ($HNO_3$) | 0.94 |
| Iodine ($I_2$) | 0.54 |

**Ozone Production**

The instability of ozone makes it necessary to generate ozone near its point of application. Methods of generating ozone include ultraviolet (UV) light, cold plasma, corona-discharge, chemical, thermal, chemonuclear, and electrolytic methods (Kim et al. 1999). Of the options to produce ozone, corona-discharge units are most common (EPA 1999) because they can produce greater concentrations of ozone, have longer unit stability, and are more cost effective than the other production methods (Linntech 2005; Ozone Solutions Inc. 2006).

***Corona-Discharge Ozone Generation***

In a corona-discharge system, also called "hot-spark" production and electrical discharge method, the corona-discharge element builds a capacitive load as a high-voltage alternating

current (VAC) is applied across a discharge gap (Kim et al. 1999; EPA 1999; Linntech 2005). Corona-discharge systems contain a dielectric layer to control the electrical discharge across the gas stream (Linntech 2005; Ozone Solutions Inc. 2006). Figure 1.1 illustrates a corona-discharge ozone generator. Suslow et al. states that the voltage in corona-discharge systems is greater than 5000 VAC. As oxygen molecules ($O_2$) pass through the corona-discharge element, an electrical discharge breaks oxygen-oxygen double bonds, producing two oxygen radicals ($O^{-2}$) as shown in equation 1.4. The oxygen radicals then combine with oxygen molecules to form ozone (equation 1.5; Šimek et al. 2002; Linntech 2005; Ozone Solutions Inc. 2006) and can produce ozone concentrations up to 4% (Kim et al. 1999) or 0.5% to 3.0% by weight with an air feed gas (EPA 1999). Using pure oxygen as the feed gas increases the possible concentrations by two to four times the concentrations observed with an air feed gas (EPA 1999).

It is possible achieve ozone concentrations between 10 to 18% using a combination of technology (Kim et al. 1999). It is also possible to produce concentrated ozone at 30% continuously or 80% in batch processes by liquefying the oxygen/ozone mixture and then using the difference in boiling points to increase the ozone concentrations (Koike et al. 2000). Šimek et al. (2002) stated that micro-discharges in air-fed generators could attain a maximum efficiency for ozone production of 100 g $O_3$ / kWh, while Zhang et al. (2003) found a production efficiency of 118 g $O_3$ / kWh for a miniature oxygen-fed ozone generator. Eliasson et al. (1991) stated that if energy ion dissipation could be avoided, a maximum predicted efficiency of 400 g $O_3$ / kWh could be reached, while optimal experimental conditions can reach an efficiency of 250 g $O_3$ / kWh. The typical electrical power

requirement for industrial ozone generation amounts to 12 to 18 kWh / kg $O_3$ (Gottschalk et al. 2000).

Oxygen breaks into oxygen radicals as follows:

$$O_2 \xrightarrow{e^-} 2O \qquad (1.4)$$

Oxygen radicals form ozone as follows:

$$O_2 + O \rightarrow O_3 \qquad (1.5)$$

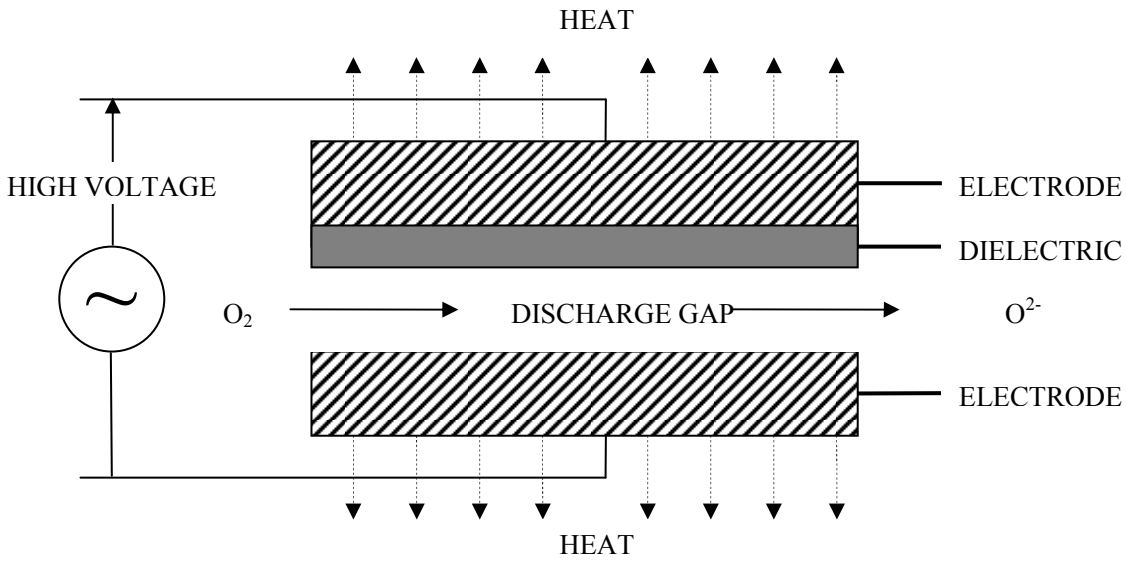


Figure 1.1 Corona-discharge ozone generation (Lenntech, 2005)

The disadvantage of corona-discharge systems is that they produce large amounts of heat, needing a water coolant to remove the excess heat (Linntech 2005). Another possible setback to corona-discharge systems is that reactions with nitrogen can occure when non-dry air is used (Kim et al. 1999; Šimek et al. 2002). Šimek et al. (2002) noted that there are

several species produced from atomic nitrogen and nitrogen oxides during the discharge and post-discharge (equations 1.6 – 1.10). These species cause reactions that lead to the reduction of ozone concentrations (Šimek et al. 2002) and can cause metal surfaces to corrode within the generator (Kim et al. 1999).

Nitrogen based reactions with oxygen and ozone:

$N + O_3 \rightarrow NO + O_2$ (1.6)
$NO + O_3 \rightarrow NO_2 + O_2$ (1.7)
$NO_2 + O \rightarrow NO + O_2$ (1.8)
$NO_2 + O_3 \rightarrow NO_3 + O_2$ (1.9)
$NO_3 + O \rightarrow NO_2 + O_2$ (1.10)

***UV-Light Ozone Generation***

UV-light ozone generators produce ozone in a process called the Chapman reaction caused by radiation of 185 nm wavelength (NASA, 2000; Kim et al. 1999). The Chapman reaction is a photocatalytic oxidation that occurs when high energy UV photons are absorbed by oxygen molecules (Haslow et al. 1993; NASA, 2000). The photons cause oxygen molecules dissociate into oxygen radicals and produce the same chemical reaction previously described for a Corona-discharge generator. UV-light generators are the cheapest and have the lowest maintenance (Haslow et al. 1993) cost of the three methods discussed to produce ozone but are only able to produce low concentrations. The disadvantage of a UV-light ozone system is that the oxygen requires a longer exposure time to the UV light, leading to a lower throughput of oxygen and production of ozone compared to Corona-discharge systems (Linntech 2005; Ozone Solutions Inc. 2006).

### *Cold Plasma Ozone Generation*

Cold plasma ozone production uses two hollow glass rods containing a noble gas separated by a gap, with the addition of a dielectric layer. An electrostatic plasma field is formed between the two rods when the gas is energized, emitting high intensity UV radiation (Moreno et al. 2005). Oxygen anions are produced once pure oxygen is passed between the glass rods. The oxygen anions are then capable of producing ozone and several other short lived allotropes such as $O_4$. The disadvantage of cold plasma ozone generators is that they are expensive compared to corona-discharge generators with the same ozone production capabilities (Malik et al. 2000).

### Measurement of Ozone

Methods of measuring ozone can be categorized into three areas: physical, physicochemical, and chemical. Physical methods use measurement of an ozone property to quantify concentrations. Physical methods of measurement include using UV, visible, or infrared absorption. Physicochemical methods use reagents and then measure the effects physical effects of the ozone reaction. Physicochemical methods might measure the chemiluminescence or heat of the reaction. Chemical methods quantify the products of a chemical reaction with a reagent. Chemical methods include potassium iodine titrations and polymer molecular weight reductions. The most accurate method to measure gaseous ozone is UV spectrophotometrics (Kim et al. 1999).

## Ozone as a Disinfectant

A review by Kim et al. (1999) noted that ozone is capable of decreasing microbial populations, the chemical and biological oxygen demand, and the quantity of toxic organic compounds within the treated environment. Ozone has been considered for many applications including food preservation, artificial aging of beverages, odor control, and medical therapy. The main uses of ozone are currently drinking water treatment and municipal and industrial wastewater treatment (Graham 1997).

**Ozone as a Food Preservative**

Ozone displays several characteristics that make it ideal for use as a fumigant for foodstuffs. Gaseous ozone has been known to posses antimicrobial traits for over 120 years (Jay et al. 2005, pp. 312-314). As a powerful oxidant, ozone quickly inactivates microorganisms such as viruses, bacteria, and fungi (Majchrowicz 1998) and kills small invertebrates such as insects (Mendez et al. 2003). Ozone is also capable of decreasing levels of toxic organic compounds (Kim et al. 1999). The effects of ozone can be short-lived as it quickly dissipates into $O_2$ as shown in equation 1.11 (Bran 2001) and has a limited residual effect (Majchrowicz 1998). The quick dissipation time coupled with the absence of residual toxins makes ozone a well-suited treatment for most food materials (FDA 2002) and it has been shown to extend the shelf life of certain food (Jay et al. 2005, pp. 55-56). These are also reasons why ozone was given a "generally recognized as safe" (GRAS) classification from the FDA first in 1982 with limitations (Kim et al. 1999) and then more recently on June 26, 2001 (FDA 2002; Suslow et al. 2004). Ozone has been approved for use on food in Australia, France, and Japan (Jay et al. 2005, pp. 55-56; Graham 1997).

Decomposition of ozone:

$$O_3 + O \rightarrow 2O_2 \qquad (1.11)$$

It has also been shown that ozone has no measurable effect on the nutritional content or germination of treated grain samples of wheat, corn, or soybeans (Mendez et al. 2003) but can cause changes in other food products such as an increase in rancidity in high-lipid-content foods (Jay et al. 2005, pp. 55-56) and changes in volatile oil constituents in ground pepper (Zhao and Cranston 1995). According to Khadre et al. (2001), "Presence of organic substances with high ozone demand may compete with microorganisms for ozone. Viruses and bacteria associated with cells, cell debris, or feces are resistant to ozone, but purified viruses are readily inactivated with the sanitizer." The composition of foodstuffs may provide competing reactions that would make ozone less effective on microorganisms.

There are also some negative aspects of ozone. The oxidizing effects of ozone make it toxic at high doses (Bran 2001, table 1.4) and steps may be necessary to destroy off-gases to prevent worker exposure (EPA 1999). The highly reactive nature of ozone also requires the use of corrosion-resistant equipment such as stainless steel.

Table 1.4 Health effects for humans at various ozone concentrations (Novazone 2006)

| | Concentration (ppm) | Duration of Exposure | Effect |
|---|---|---|---|
| ACCEPTABLE ZONE | 0.01-0.04 | - | Odor threshold. |
| | 0.1 | - | Minor eye, nose and throat irritation. |
| | 0.1 | *8 hour average exposure limit* | |
| HAZARDOUS ZONE | >0.1 | few minutes | Continuous headache, shortness of breath. |
| | 0.25-0.5 | 2-5 hours | Reduction in lung function and the ability to do physical work (for persons with a history of heart or lung disease). |
| | 0.3 | *15 minute exposure limit* | |
| | 0.4 | 2 hours | Reduction in lung function during moderate work for all persons. |
| | >0.6 | 1-2 hours | Chest pain, dry cough. |
| | 1 | 1-2 hours | Lung irritation (coughing), severe fatigue. |
| | >1.5 | 2 hours | Reduced ability to think clearly. Continuing cough and extreme tiredness maybe lasting for 2 weeks. |
| | | | Severe lung irritation with fluid build-up. |
| | 9 | intermittent | Severe pneumonia (arc welders). |
| CRITICAL ZONE | 10 | *Immediately dangerous to life & health* | |
| | 11 | 15 minutes | Rapid unconsciousness. |
| | 50 | 30 minutes | Expected to be fatal. |

Mendez et al. (2002) applied gaseous ozone to whole grains in order to determine if characteristics of the grain were changed. Ozone was applied to samples of hard wheat, soft wheat, corn, and soybeans at a concentration of 50 ppm and an airflow rate of 0.02, 0.03, and 0.04 m/s. The grain samples were stored in four steel barrels (208 L each) bolted together, with final dimensions of 0.57m dia. x 3m. Ozone concentrations were measured 1 to 4 times per day at five depths within the head space, plenum, and at depths of 0.3, 0.9, 1.5, 2.1, and 2.7 m (setup previously used for Kells et al. 2001). Data suggested that ozone had no effect on the adhesiveness of rice, popping volume of popcorn, saturated or unsaturated fatty acids of maize, soybeans, or wheat. The milling efficiency of soft and hard wheat also remained unchanged when compared to control samples. These results suggest that ozone did not penetrate the grain.

While ozone can be effective in controlling microorganisms, integrating ozone into foodstuff preservation can be more challenging. There have been several studies done that prove that ozone is effective in inactivating fungi in food, acting to reduce fungal spore production on food surfaces and to decrease spread of fungi to adjacent produce. The use of gaseous ozone appears to be the most effective in cooler temperature storage (temperature range not provided) and a relative humidity between 85 to 95% (Suslow 2004). Gaseous ozone is only effective as a surface treatment because it does not penetrate natural openings or wounds in sufficient amounts to control microorganisms (Suslow 2004) and antimicrobial action occurs primarily on the surface with water phase food because of the rapid decomposition of the ozone (Kim et al. 1999).

**Ozone Used for Insect Control**

Ozone used as a fumigant to treat stored maize is effective in controlling insects and fungi. Tests preformed by Kells et al. (2001) showed the effect of gaseous ozone on three insect species and on the fungal specie *Aspergillus parasiticus*, Speare strain ATCC 24551. A sample size of 30 g of corn was first seeded with *A. parasiticus* and then divided into cages. The cages were then placed 2 cm below the surface of a 12.7-Mg capacity steel grain bin filled with corn. The corn was then treated with 50 ppm ozone for 3 d or 25 ppm for 5 d. The same corn and grain bin was used for all treatments. The same corn and grain bin was used for all treatments. The test found that corn treated with 50 ppm ozone for 3 d resulted in 92 to 100% insect mortality. Temperature, corn moisture content, and relative humidity were several factors impacting the effectiveness of ozone but were not stated in the research paper.

Kells described a two-phase reaction progression when ozone was exposed to corn in a field grain bin study and lab column study. The lab column study used four steel barrels (208 L each) bolted together, with final dimensions of 0.57m dia. x 3m. Air samples were measured 1 to 4 times per day in the head space, plenum, and at depths of 0.3, 0.9, 1.5, 2.1, and 2.7 m. The lab column study looked at three air velocities: 0.02, 0.03, and 0.04 m / s. At an air velocity of 0.02 m / s, 75% of the ozone passed a depth of 2.7 m in 3.7 d. When a velocity of 0.03 m / s was used, the same concentration of ozone at 2.7 m was reached in 1.3 d. In this phase I, ozone rapidly degraded and slowly moved through the corn. In the phase II of treatment, the ozone passed through the corn freely with minor losses to concentrations. Phase I is marked by a drop in ozone concentrations as the ozone reacts with active sites throughout the corn. These reactive sites likely consist of fungi, bacteria, and the kernel shell. In phase II, ozone passes through the grain mass with only minor ozone degradation. While phase II was never reached in the field study, the lab column study showed that a degradation rate of 1 ppm ozone / 0.3 m occurred in phase I fumigation leading to phase II. Kells predicted that this degradation rate would allow an insect and fungi "killing zone," described as a concentration above 25 ppm, to reach a depth of 8 m of grain.

**Ozone Used for Microorganism Control**

Concentrations of ozone between 0.1 and 0.5 ppm for short periods of time have been proven to be effective against gram-positive and gram-negative bacteria[2], viruses, and protozoa (Jay et al. 2005, pp. 312-314; Kim et al. 1999). Khadre et al. (2001) states that **"**Inactivation of

[2] Gram-positive bacteria normally have thick cell walls that contain large amounts of teichoic acids; Gram-negative bacteria, in contrast, have a more complex cell walls with layers of peptidoglycan and the secondary cell membrane (Prescott et al. 1999)

microorganisms by ozone is a complex process because of the multiple cellular sites which ozone can affect. These sites include proteins, unsaturated lipids and respiratory enzymes in cell membranes, peptidoglycans in cell envelopes, enzymes and nucleic acids in the cytoplasm, and proteins and peptidoglycan in spore coats and virus capsids."

***Ozone Effect on Cell Envelopes***

One common theory is that ozone inactivates microorganisms by targeting cell membranes, thereby disrupting permeability functions (Jay et al. 2005, pp. 312-314; Kim et al. 1999). Kim et al. (1999) detected cell leakage from bacterial cells treated with ozone, indicating a change in the cell permeability and the possibility of lysis of the cell wall. The double bonds of unsaturated lipids that are part of the cell envelope are mentioned as the primary sites for ozone reaction in bacteria. Another probable action site for gram-negative bacteria is the lipoprotein and lipopolysaccharide layers.

***Ozone Effect on Spores and Oocyst***

Microorganisms have the ability to survive longer in a dormant state, such as spores and oocyst than in an active state (Jay et al. 2005, pp.687-688). An oocyst is the spore phase of certain protists (Prescott et al. 1999, pp. 822). In the case of spores, the cells are more resistant to environmental stresses including heat, ultraviolet radiation, chemical disinfectants, and desiccation (Prescott et al. 1999, pp. 66). While ozone does work on oocysts, a higher dosage and treatment time is needed (Jay et al. 2005, pp.687-688). A review by Khadre et al. (2001) stated that "Ozone is generally more effective against vegetative bacterial cells than bacterial and fungal spores." One example in a study done on

mold spores (*Neosartorya fischeri*) found an intermediate resistance to ozone. In another case, spores of 8 *Bacillus* spp., *B. stearothermophilus* where shown to have the highest level of resistance among all the tested species (Khadre et al. 2001). Smilanick (2003) reported that 1 h of 200 ppm ozone was sufficient to deactivate spores *Penicillium digitatum* (green mold), *P. italicum* (blue mold), and *Geotrichum citri-auranatii* (sour rot).

**Ozone Used for Fungi Control**

A review of food use of ozone by Kim et al. 1999 stated that ozone could be an effective fungicidal agent. Fungal spores have a microbicidal activity threshold to aqueous ozone (0.3 to 0.5 mg / L) at 90 to 180 minutes exposure for *Aspergillus* spores, 45 to 60 minutes exposure for *Penicillium* spores, and 5 to 10 minutes exposure for *Candida paracreus* spores.

Ozone was considered as a possible substitute for gaseous ethylene oxide to decontaminate pepper (Zhao and Cranston 1995). Whole peppercorn (*Piper nigrum* L) and ground black pepper was used to determine the effects of ozone on the volatile oil constituents and microbial populations. In all of the experiments, ozone concentrations were kept at 6.7 mg / L and an airflow of 6 L / min (reactor volume not provided). In the first experiment, 500 mL suspensions of $10^{11}$ to $10^{12}$ cfu / L cultures of *Escherichia coli*, *Salmonella* ssp, *Staphylococcus aureus*, *Bacillus cereus*, *Penicillium* ssp, and *Aspergillus* ssp were sparged with ozone. A 10-mL aliquot of each treated suspension was then removed and enumerated using standard plate count methods for the aerobic bacteria, *Penicillium* ssp, and *Aspergillus* ssp. Anaerobic bacteria and mesophilic aerobic sporeformers were also enumerated using standard plate count methods with the exceptions that the anaerobic bacteria were incubated

under anaerobic conditions and tryptone glucose extract agar was used for the mesophilic aerobic sporeformers. A five log reduction, effectively a 99.99% inactivation, was achieved for each organism after the following ozone concentration was applied: *E. coli*, 0.4 g / L; *Salmonella* ssp, 0.4 g / L; *S. aureu*s, 1.2 g / L; *B. cereus*, 1.2 g / L; *Aspergillus* ssp, 0.4 g / L; and *Penicillium* ssp, 1.8 g / L. The concentrations found for the two fungi would indicate that *Aspergillus* is more susceptible to ozone than *Penicillium*. In the second experiment whole peppercorn samples of 200 g were immersed in 500 mL distilled water. The water-spice mixture was then sparged with ozone for various times. A 50 g sample was then removed for enumeration and for gas chromatography (GC) tests. A 3 to 4 log reduction in total aerobic bacteria, total anaerobic bacteria, and mesophilic aerobic sporeformers was realized after 10 min of ozone treatment. The GC of the volatile oil showed that ozone caused only a slight variation from untreated samples and that no new compounds were created. In the third experiment 500 g of ground black pepper was sparged with ozone in a rotating flask for various times. Three moisture contents (39.0, 104.0, and 176.0 g water / kg ground black pepper) were tested to determine effects of gaseous ozone. A 50 g sample was then removed for enumeration and for gas chromatography (GC) tests. A 3 log or greater reduction in *Salmonella* ssp and *E. coli* was realized after 60 min of ozonation at 40 mg / min and an airflow rate of 6 L / min. A similar reduction was seen in *Penicillium* ssp after 40 min and a greater than 4 log reduction was seen in *Aspergillus* ssp after 10 min. The three moisture contents showed that the higher moisture had the greatest reduction of the microbial load. The effects of moisture only became evident after 2 h of ozonation. The GC tests of volatile oils showed that ozone caused several changes in the final composition of the ground pepper. Ozone eliminated 16 components that were detected in the untreated samples.

Ozone was also responsible for the creation of 14 new components that were only found in the ozone treated samples. The concentrations of several individual components found in both the treated and non-treated sample were considerably different. The study concluded that ozone application to ground pepper is not a viable alternative to current chemical practices due to the chemical composition change, but ozone may be useful in treating un-ground peppercorn.

A study conducted by Beuchat et al. (1999) used aqueous ozone to inactivate aflatoxigenic species of *Aspergillus flavus* (NRRL 3357) and *Aspergillus parasiticus* (NRRL 2999) suspended in a phosphate buffer solution with one of two pH conditions (pH 5.5 or 7.0). Suspensions of 0.1 mL of conidia in sterile 1mM phosphate buffer had a gas stream bubbled through at an airflow rate of 0.8 L / min with 21 mg $O_3$ L / min (ca. 21-ppm / min). The ozone concentration in the conidia suspension was then controlled at 1.74 ppm for the duration of the treatment. A 0.5 mL of the suspension was withdrawn for testing at intervals of 2, 4, and 6 min after inoculation. The withdrawn suspension was then 10-fold serially diluted in phosphate buffer and surface plated on PDA. The CFU for each plate were counted after 3 d of incubation at 25$^{o}$C. The times required for 90% inactivation of the fungi conidia (D-values) were then determined from slopes of the regression lines using a general linear model of the Statistical Analysis System. The results showed that the D-value for *A. flavus* conidia treated with 1.74-ppm ozone was 1.72 min in pH 7.0 and 1.54 min in pH 5.5. The D-value for *A. parasiticus* with the same ozone treatment was 2.08 min in pH 7.0 and 1.71 min in pH 5.5. There was not a significant difference ($P > 0.05$) between the D-values

in relation to the pH conditions for both fungi tested. There was also no significant difference between the D-values for each of the two fungi tested.

A study done by Li and Wang (2003) tested the effectiveness of ozone as a surface disinfectant by exposing agar plates of *Escherichia coli*, *Bacillus subtilis*, *Candida famata*, and *Penicillium citrinum* to several ozone dosages. Each of the four organisms is different from the others: *E. coli* is a gram-negative, non-spore-forming bacterium; *B. subtilis* is a gram-positive, endospore-forming bacterium; *P. citrinum* is a mold; and *C. famata* is a yeast. In each case, the microorganism suspension was diluted to $10^5$ CFU / mL. Then 0.2 mL of the dilution was spread on either trypticase soy agar (TSA) or malt extract agar (MEA), producing roughly <100 CFU / plate. The effective range of ozone concentrations were determined and then applied to each organism for a set period (see table 1.5 for ozone dosages and treatment times for each microorganism). The airflows used for each test were not listed in the literature. After the ozone treatment, the plates were incubated for 24 or 48 h at a temperature of 37 or 25$^{o}$C depending on the media used. Ozone treated plates were then compared to the non-treated plates and a survival fraction was calculated for each microorganism using equation 1.12. Two ranges of relative humidity (55 - 60 and 85 - 90%) were used to test the influence it had on the survival fraction of ozone treated microorganisms.

Determination of survival fraction of fungi:

$$Survival\_Fraction = \frac{Ns}{No} \times e^{-KD} \qquad (1.12)$$

Where:
No = CFU per plate of microorganism unexposed to ozone (CFU / plate)
Ns = DFU per plate of microorganism exposed to ozone (CFU / plate)
D = ozone dosage based on ozone concentration, flow rate, and exposure time (mg)
K = microorganism susceptibility factor (1 / mg)

The survival fraction for each of the four organism tested are shown in figure 1.5. There was an exponential decline in the survival fraction with increases in ozone concentrations. Another observation was that the germicidal efficiency of ozone on the surface increased as relative humidity increased. When the four microorganisms were compared, *E. coli* was found to be the most susceptible to ozone (2 – 2.5 and 3.5 – 4 mg ozone for 50 and 80% inactivation) while *B. subtilis* was the least susceptible (45 – 70 and 145 – 150 mg ozone for 50 and 80% inactivation).

Table 1.5 Survival fractions for four organisms treated with ozone (Li and Wang 2003)

| | | Relative Humidity (%) | |
|---|---|---|---|
| Microorganism | Ozone Treatment | 55-60 | 85-90 |
| *Escherichia coli* | 600 ppb for 120 min | 0.29 | 0.27 |
| | 900 ppb for 90 min | 0.03 | 0.07 |
| | 1200 ppb for 90 min | 0.004 | |
| *Bacillus Subtilis* | 8 ppm for 150 min | 0.42 | 0.27 |
| | 12 ppm for 150 min | 0.27 | 0.24 |
| | 16 ppm for 150 min | 0.23 | |
| *Candida famata* | 1.2 ppm for 150 min | 0.33 | 0.07 |
| | 1.8 ppm for 120 min | 0.21 | 0.09 |
| | 2.4 ppm for 90 min | 0.19 | |
| *Penicillium citrinum* | 8 ppm for 150 min | 0.41 | 0.01 |
| | 12 ppm for 150 min | 0.21 | 0.15 |
| | 16 ppm for 120 min | 0.14 | |

Hibben et al. (1969) conducted a study on the germination effects of ozone on fungi spores. They used the following fungi grown on V-8 juice agar: *Trichoderma viride*, *Aspergillus terreus*, *Aspergillus niger*, *Penicillium egyptiacum*, *Botrytis allii*, *Stemphylium sarcinaeforme*, *Stemphylium loti*, *Fusarium oxysporum*, *Alternaria* sp., *Verticillium albo-atrum*, *Verticillium dahliae*, *Colletotrichum lagenarium*, *Rhizopus stolonifer*, and *Chaetomium* sp. Spores were then harvested off of slants and concentrated to 4 x $10^6$ to 7 x $10^6$ spores / mL by centrifugation and re-suspended in distilled water. Spore suspensions of 0.05 mL were looped onto yeast extract disks. The disks were then exposed to ozone concentrations of 10, 25, 50, or 100 pphm for time intervals of 1, 2, 4, and 6 h inside a 25 cm wide x 28 cm deep x 25 cm high plexiglass chamber. The chambers were then stored in an incubator maintained at 22 C and 95 to 99% relative humidity. After 36 h incubation at 30 C, at least 100 spores per disk were examined at 600X magnification using a microscope. The results showed that ozone had a minimal effect (germination range of 86 to 100%) on spores of *Chaetomium* sp. *S. sarcinaeforme*, *S. loti*, and *Alternaria* sp. after 6 h exposure to 100 pphm. Ozone had moderate effect on spores of *T. viride, A. terreus, A. niger*, *P. egyptiacum, B. allii,* and *R. stolonifer* after 100 and 50 ppmh for 4 and 6 h. Spores of *F. oxysporum, C. lagenarium, V. albo-atrum*, and *V. dahliae* were either inhibited or significantly reduced after 100 pphm ozone for 2, 4, and 6 h. They also noticed that low levels of ozone could stimulate germination of some fungi spores, specifically *T. viride, A. terreus, P. egyptiacum, R. stolonifer*, and *V. dahliae*. The results also showed that low concentrations of ozone for an extended time was almost as efficient as short exposures and higher concentrations at reducing spore germination. Examination of the fungi also showed that ozone was also effective in suppressing aerial hyphae.

***Ozone Used in Fruit Storage***

The effect of a continuous exposure to a low dose of ozone was tested on peaches and table grapes by Palou et al. (2002). Both fruits were harvested at commercial maturity from the San Joaquin Valley. After being harvested, the fruit was superficially disinfected by submersion in a diluted bleach solution (0.5 % sodium hypochlorite) for 1 minute. To test the effect of ozone on wounded fruit, peaches were wounded with a 1-mm probe tip and inoculated with *Monilinia fructicola, Botrytis cinerea, Mucor piriformis, or Penicillium expansum*. Four 20-fruit trays inoculated with a pathogen were placed in a storage room held at 20$^{o}$C, 90% relative humidity, and 0.3 ppm ozone for 4 weeks. To test the effects of ozone on wounded grapes, grapes were inoculated with spores of *B. cinerea* by spraying one sample set and injecting the spores into another sample set. The grapes were then stored for 7 weeks in the same conditions as the peaches. Another test was done with peaches and grapes to test for the physiological response of the fruit to 0.3-ppm ozone exposure compared to ambient air under 20$^{o}$C and 90% relative humidity conditions over several weeks. The results showed that 0.3 ppm was effective in inhibiting the normal aerial growth of mycelia and preventing spores from being produced and spread in the wounded peaches, but had no noticeable effect on the pathogen activity within the wounds. Ozone also did not reduce mold incidence on the inoculated grapes. In the physiological test with peaches, the ozone exposed fruit lost more weight. That would indicate that the ozone might have damaged the peaches' cuticle or epidermal tissue. It was also noted in all the experiments that airflow plays a crucial part in the effectiveness of ozone gas. More mycelia growth was seen where ozone was partially stopped from contacting fruit because of the plastic trays used to hold the fruit.

In a previous study using table grapes, Sarig et al. (1996) showed that 8 mg of ozone per min for 20 min was effective in controlling fungi, yeast, and bacteria. Table grapes were collected directly after being harvested in Israel and inoculated with *Rhizopus stolonifer* at a concentration of $10^7$ spores per mL distilled water. Ozone was applied to 2-kg samples at a rate of 8 mg per min for time intervals between 0 and 80 min. They found that ozone applied at that rate for 20 min was effective in reducing fungi colony forming units from 40 to less than 10.

Palou et al. (2003) showed that the capability of ozone to deactivate microorganisms is heavily dependant on ability of the gas to come into contact with the microorganisms. This was tested by treating navel oranges (*Citrus sinensis* (L.) Osbeck) in different storage conditions with ozone. Four types of packaging were used: standard corrugated fiberboard citrus cartons (2.6% vented surface area), returnable plastic containers with uncovered fruit (35.9% vented surface area), returnable plastic containers with bagged fruit (0.7% vented surface area), and corrugated fiberboard Master carton with bagged fruit (2.9% vented surface area). The oranges were inoculated with a $10^6$ spores / mL suspension of either *Penicillium digitatum* or *Penicillium italicum*. After a 24-h inoculation period, the oranges were stored in either of two 678 m3 cold storage room held at 12.8$^{o}$C for 14 d. One storage room had a continuous exposure to ozone that was discharged into the room at a rate of 2.5 g / h. The other room was used as a control and received no ozone. There were no spores present on the oranges stored uncovered in the returnable plastic containers, which also had an 81.9% ozone penetration. The other storage methods had lower vented surface areas, impacting ozone penetration (>17%) and 5 to 60% spore coverage on the fruit.

***Ozone Used in Grain Storage***

A study by Allen et al. (2003) used gaseous ozone to inactivate fungi in barley grain. They tested the effects of four different ozone doses on barley using four moisture contents (19, 22, 25, and 30%), and three temperatures (0, 20, and 40$^{o}$C). Ozone application rates were 0.98, 0.16, 0.08, and 0.04 mg / g barley min. After five minutes of ozonation at a rate of 0.16 mg of ozone / g barley min, the ozone had caused a 96% inactivation of spores. The inactivation of spores increased as ozone dose increased, going from around 60% spore survival at an ozone application of 0.04 mg / g barley – min, to less than 4% spore survival at an ozone application of 0.98 mg / g barley – min (figure 1.2). They also noticed that higher temperatures and water activity increased the inactivation percentage of fungi on the barley. With a 0.98 mg / g barley-min and a temperature of 20$^{o}$C, a 19.6% spore survival rate occurred at 19% moisture while less than 4.2 % spore survival rate occurred for 30% moisture. The reaction to temperatures of 0, 20, and 40$^{o}$C had respective spore survival rates of 36.2%, 13.95% and, 4.2% with an ozone rate of 0.98 mg / g barley-min and 19% moisture content.

A study by Raila et al. (2006) used an ozone-air mixture to dry 23.2% moisture content wheat. The 22 kg samples of wheat were stored in five cylinders of 0.18 m inner diameter and 1.2 m height. An aeration rate of 12 m / s was applied to 4 of the cylinders for 8 h a day over 8 d. Ozone concentrations of 280 ppb and 700 ppb were applied to wheat in two cylinders. At the end of 8 d, 10 g of each sample were ground and diluted in 90 mL sterile water. The dilution was then spread on agar plates for micromycetes evaluation after

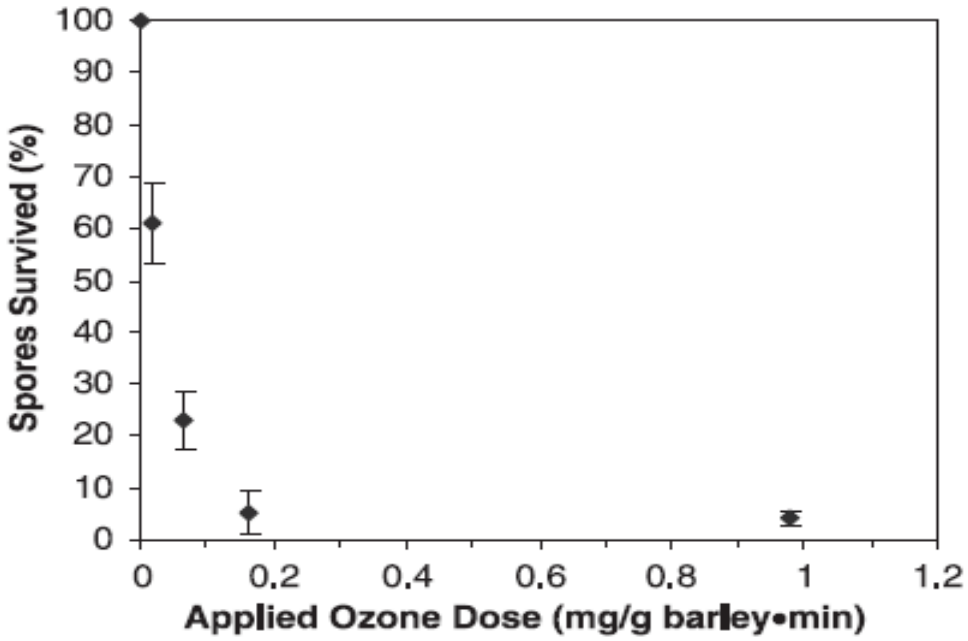


Figure 1.2 Effect of applied ozone dose on inactivation of fungal spores in barley (Allen et al. 2003)

incubation periods of 3, 5, and 7 d. Their results suggest that ozone has a positive effect on grain drying (a 20% reduction in drying time) and decreasing mycological populations. A general observation of fungi genera at the end of the experiment showed that *Fusarium*, *Geotrichum*, *Myrothecium*, and *Mucor* were significantly retarded by ozone. Ozone was less effective on fungi genera *Alternaria* and *Verticillium*, and species of *Penicillium* and *Aspergillus* were the most likely to survive ozone treatments. The journal article was unclear if dry air was used for the ozone generators or if any excess heat was produced by the ozone generators, both factors that would influence the results. Fungi genera populations were also made from observations instead of population counts.

In the previously mentioned study, Kells et al. (2001) looked into the effect of gaseous ozone on three insect species and the fungal species *Aspergillus parasiticus*, Speare strain ATCC 24551 on corn. They were able to identify that particular strand of *A. parasiticus* based on the production of a metabolite in the aflatoxin biosynthetic pathway, averufin, which can be identified by an orange color when grown on potato dextrose agar medium. A 30 g sample of corn was first seeded with *A. parasiticus* and then divided into screen cages. The cages were then placed 2 cm below the surface of a 12.7 Mg capacity steel grain bin filled with corn. The corn was then treated with 50-ppm ozone for 3 d or 25 ppm for 5 d. The temperature, corn moisture content, and relative humidity were several factors that would impact the effectiveness of ozone but were not provided in the research paper. After the treatment, the corn was washed with a Triton X-100 solution to remove fungi, and then plated on the dextrose agar. Colony counts from the plates showed that a 63% reduction in *A. parasiticus* occurred after the 3 d treatment of 50-ppm ozone. The 25-ppm treatment for 5 days failed to significantly reduce the fungi counts when compared to control samples.

While effects of fumigating corn with ozone have been tested, reports on effects of using ozone to limit dry matter losses remain unexplored. Ozone quantities needed to keep dry matter losses below 0.5% need to be determined before ozone treatments can be considered as a method of managing fungi in corn.

**Degradation of Mycotoxins**

Another possible benefit of ozone is its ability to degrade and detoxify some mycotoxins. Mycotoxins are secondary chemical metabolites produced by a few filamentous fungal

species (Frisvad et al. 2004). When vertebrate animals consume or inhale these toxins, they can cause severe diseases of internal organs, the nervous system, and the circulatory system, or even be fatal. Mycotoxins are usually found in produce, such as grain, that was harvested at high moisture content or stored under high relative humidity conditions that support the growth of mycotoxin producing fungi (Agrios 2005, pp. 39-40). The economic impact of mycotoxins is potentially large with estimates that 25 to 50% of the world's food supply affected with some level of mycotoxins. It is estimated that the crop damages from mycotoxins in the United States could be in excess of $932 million annually. The importance of mycotoxins has led to regulations in minimum of 77 countries (Dohlman 2004).

The most common cases of mycotoxicoses are caused by fungi species belonging to subdivision Ascomycotina in the genera *Aspergillus*, *Penicillium*, *Fusarium,* and *Alternaria* (Agrios 2005, pp. 559; Frisvad et al. 2004, table 1.6). Of these, mycotoxins produced by *Aspergillus* and *Penicillium* can be found in stored seeds, hay, and commercial processed foods, while *Fusarium* produces toxins mainly in grains infected in the field or during harvest (Agrios 2005, pp. 559). *Alternaria* is a field fungus that is capable of producing mycotoxins. In foods, it can be found on wheat, red meats, and may appear as a black rot on stone fruits, apples, and figs (Jay et al. 2005, pp. 28). The most important mycotoxin producing food-borne species in temperate regions are *Fusarium graminearum, F. culmorum, P. verrucosum, P. nodicum, P. freii, P. cyclopium, P. expansum*, and *P. crustosum*. The most important mycotoxin producing food-borne species in subtropical

regions are *Aspergillus flavus, F. verticillioides, P. polonicum, P. viridicatum, P. islandicum*, and *P. oxalicum* (Frisvad et al. 2004).

Table 1.6 Mycotoxins (Koenning et al. 1999)

| Mycotoxin | Fungi Associated | Symptoms/Toxicology |
|---|---|---|
| Aflatoxin | *Aspergillus flavus, A. parasiticus* | liver necrosis, liver tumors, reduced growth, depressed immune response, carcinogen |
| Fumonisin | *Fusarium moniliforme, F. proliferatum* | equine leukoencephalomalacia, porcine pulmonary edema |
| Deoxynivalenol (DON) | *F. graminearum* | feed refusal, reduced weight gain, diarrhea, vomiting |
| Trichothecenes | *F. graminearum, F. culmorum, F. poae* | alimentary toxic aleukia, necrosis, hemorrhages, oral lesion in broiler chickens |
| Ochratoxin | *Penicillium verrucosum, A. ochraceus* | porcine nephropathy, various symptoms in poultry |
| Citrinin | *Penicillium sp., Aspergillus sp.* | kidney damage |
| Cyclopiazonic Acid | *Penicillium sp., Aspergillus sp.* | neurotoxin |
| Sterigmatocystin | *Aspergillus sp., and others* | carcinogen, mutagen |

### *Aspergillus and Penicillium Toxins*

*Aspergillus flavus, A. nomius*, and *A. parasiticus* are known producers of aflatoxins in foods (Frisvad et al. 2004). As of 1997, Aflatoxins were the sole mycotoxins formally regulated in the United States (McKenzie et al. 1997). Aflatoxins are found at low concentrations of about 50 ppb in infected cereal seeds and most legumes. Certain years of corn production have concentration levels more than 100 ppb, while peanuts, cottonseed, fishmeal, and Brazil nuts grown in warm and humid areas have aflatoxin concentrations upward of 1000 ppb (Agrios 2005, pp. 559).

*Aspergillus* and *Penicillium* are both capable of producing the following mycotoxins: ochratoxins, patulin, and xanthomegnin. Species of *Penicillium* will also produce yellowed-rice toxins (citreoviridin, citrinin, and luteoskyrin) in stored rice, barley, corn, and dried fish (Agrios 2005, pp. 560).

***Fusarium Toxins***

Three common mycotoxins are produced by genera of *Fusarium* are zearalenones, trichothecenes, and fumonisins. *Fusarium verticillioides, F. proliferatum,* and *F. nygamai* are known producers of fumonisins (Frisvad et al. 2004). *Fusarium moniliforme* also produces fumonisins in corn, referred to as Fusarium ear rot of corn. It can affect large areas of a cornfield (90%) and can causes blind staggers in horses, pulmonary edema in swine, and cancer in humans (Agrios 2005, pp. 559-560).

There are more than a 170 different forms of trichothecins produced by *Fusarium* species and several other fungi species (Agrios 2005, pp. 560; Frisvad et al. 2004). Trichothecins are divided into two categories: macrocyclic trichothecenes and non-macrocyclic trichothecenes. The non-macrocyclic trichothecenes is subdivided into type A and type B based on the chemical structure. Two common forms of type A trichothecins, T-2 toxin and diacetoxyscirpenol, are produced by *F. sporotrichiodes, F. poae, F. acuminatum, F. equiseti*, and *F. sambucinum*. Deoxynivalenol (also known as vomitoxin or DON) and nivalenol are two forms of type B trichothecins produced by three species of *Fusarium; F. cerealis, F. culmorum*, and *F. graminearum* (Frisvad et al. 2004). The fungus *Gibberella zeae*

(anamorph *Fusarium graminearum*) is also responsible for producing deoxynivalenol in a condition called Gibberella ear rot in corn and head blight in wheat (Agrios 2005, pp. 560).

**Limiting Mycotoxins in Grain**

The temperature, moisture, infestation of the grain by insects and mites, and grain quality are all important in controlling mycotoxins in stored grain. Fungi growth is rapid at temperatures between 30 and 55$^{o}$C, slow between 12 to 15$^{o}$C, and stops at 5 to 8$^{o}$C. Fungi growth also is rapid at higher moisture contents, usually requiring a minimum of at least 14% in some cereal grains. Keeping the temperature and moisture as low as possible is necessary if mycotoxin production is a concern. Grain fumigation should be used to control insects and mites that can infect the grain by spreading the fungi that produce mycotoxins. The seed coat integrity is important because cracked and damaged grain is more susceptible to infection by storage fungi (Agrios 2005, pp. 560).

There have been successful tests to reduce mycotoxins present in grain. Hydrated sodium calcium aluminosilicate can be added to infected corn. It considerably reduces the effects of the toxin by binding to the mycotoxin (Agrios 2005, pp.560). A study by McKenzie et al. (1997) tested the ability of ozone to degrade aflatoxins $B_1$, $B_2$, $G_1$, $G_2$, cyclopiazonic acid, fumonisin $B_1$, ochratoxin A, patulin, secalonic acid D, and zearalenone. In one experiment, 32 μM equimolar content for each toxin in aqueous solutions of 4 mL was treated with ozone at 10% or 20% by weight for 15 seconds. In the cases of aflatoxins $B_1$ and $G_1$ a rapid degradation was seen with a 2% by weight treatment of ozone for 15 seconds, and a total degradation occurred using a 20% by weight ozone treatment. Aflatoxins $B_2$ and $G_2$ saw a

rapid degradation only at the 20% ozone concentration. The differences between the $B_1$ and $G_1$ compared to the $B_2$ and $G_2$ degradation rates is likely due to the presence of the C8-C9 double bond present in the $B_1$ and $G_1$ toxins (figure 1.3 for aflatoxin degradation reactions). In the other toxins tested, the 10% ozone treatment resulted in a reduction in the toxin to undetectable levels by HPLC. There was also evidence to suggest that ozone degradation of compounds led to the formation of water-soluble products like organic acids, volatile compounds, and mineralization products that include carbon dioxide, oxygen, and water.

## OBJECTIVE

The objective of chapter 2 was to develop a system to control and monitor grain storage conditions (relative humidity, temperature) while monitoring carbon dioxide level coming from the grain. The system must also be capable of delivering ozone to the grain.

The objective of chapter 3 was to study the effectiveness of using ozone for preservation of high-moisture corn in two experiments. The objective of experiment I was to find the effective of ozone on dry matter loss and damage kernel total of corn stored under high temperature and high moisture conditions with continuous aeration. The objective of experiment II was to find the effect of ozone on dry matter loss and damage kernel total of corn stored under low temperature and high moisture conditions.

The objective of chapter 4 was to test by enumeration the effect of four ozone concentrations on fungi from corn at three different moisture contents.

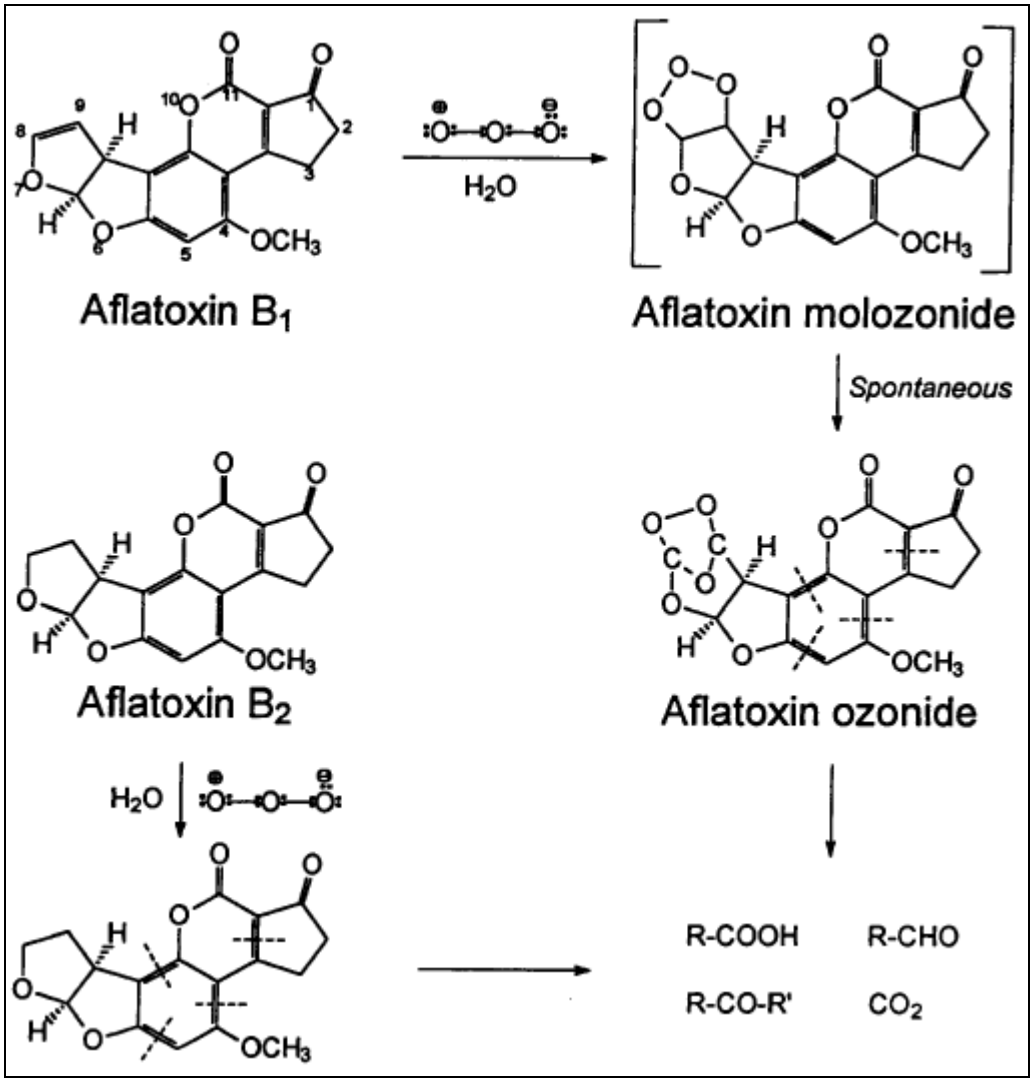


Figure 1.3 Possible degradation of aflatoxins with ozone from McKenzie et al. (1997)

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# CHAPTER 2.

# RESPIROMETER CONTROL SYSTEM

***Abstract.*** *A system was designed to monitor effects of ozone on high moisture corn during aerated storage in a lab. The corn was stored in an environmental chamber that controlled the relative humidity and temperature of the corn in glass tubes. A continuous airflow was used to deliver the ozone to the tubes and to measure the carbon dioxide leaving the grain. The carbon dioxide concentrations were used to estimate the dry matter loss of the corn. A Visual Basic 6.0 program was used with a PMD 1208LS microcontroller to collect data from several sensors and to sample the air from individual tubes.*

***Keywords.*** *Corn storage, Dry matter loss, Ozone, Carbon dioxide evolution*

## Introduction

A system was needed that could control environmental conditions of the corn while monitoring the growth characteristics of the microorganisms. In addition, the system must be capable of treating corn with gaseous ozone to investigate its effect on corn storage time. With the capabilities of readily available computer microcontrollers and data-loggers, it is possible to monitor and control multiple sensors, output devices, and record the information needed to estimate the dry matter loss of corn as time progresses.

## Literature Review

### High Moisture Corn

There are several reasons that make harvesting high moisture (>17% moisture content) corn appealing. "Harvesting corn at high moisture content reduces field pest attacks, avoids bad-weather consequences, and minimizes field losses" (Aljinovic et al. 1994). The corn can be harvested from the field 2 to 3 weeks earlier than corn harvested for dry storage (Miller, 2002). Harvesting earlier places corn closer to physiological maturity and maximum dry

matter at harvest. The earlier harvest time can avoid a 3 to 8% dry matter loss that would accrue if corn was left in the field to dry (Miller 2002). The earlier harvesting also opens the possibility of having a higher quality residue left in the field (Miller 2002). Disadvantages include rapid deterioration from fungal activity after harvest, and higher drying costs (Aljinovic et al. 1994). High moisture corn also has a lower market flexibility compared to dry corn (Miller 2002).

## Fungi in Corn

While many microorganisms influence stored grain quality, only some fungal species are important. Most bacteria and yeast have little impact on corn storage outside of very high moisture conditions (Paulsen et al. 2003). Storage fungi species found in corn will grow in temperatures between -2 and 50$^{o}$C with an optimal growth temperature between 20 to 35$^{o}$C depending on the species (Paulsen et al. 2003). The lower limit for growth conditions in most species is between 70 and 81% relative humidity or 13.5 to16.5% moisture in the corn (Paulsen et al. 2003). The main conditions that affect fungal growth are temperature, relative humidity and oxygen content of air surrounding the grain, physical conditions of the corn kernels, mold inoculum level, and previous grain storage history (Bern et al. 2002; Paulsen et al. 2003).

Mold growth in corn involves the mycelium on the surface of the grain tissue (Paulsen et al. 2003). Mycelium is the vegetative part of a fungus that is made of a mass of branching, threadlike hyphae (Madigan et al. 2006; Jay et al. 2005). Fungal enzymes are excreted and digest the grain structures, providing a source of energy for the mold (Paulsen et al. 2003).

Heat, carbon dioxide ($CO_2$), and moisture are the byproducts of aerobic respiration from mold activity (Paulsen et al. 2003; Bern et al. 2002).

The characteristics that affect growth of fungi are also used to model shelled corn deterioration: storage time, kernel moisture, kernel temperature, kernel visible mechanical damage level, genetic susceptibility to storage fungi, and other factors (Bern et al. 2002; Paulsen et al. 2003). These factors also influence the acceptable dry matter loss that is allowed by grain users. Steele and Saul (1969) observed that shelled corn can, on average, experience a 0.5% dry matter loss due to storage fungi before its USDA grade is reduced by one USDA level. While not precise, the 0.5% dry matter loss limit for shelled corn deterioration is widely accepted.

It is possible to predict the storage time of corn to reach 0.5% dry matter loss based on moisture content, temperature, visible mechanical damage, genetic traits, and fungicidal application (equation 2.1; Bern et al. 2002).

Predicted corn storage time:

$$t_n = t_s M_M M_T M_D M_H M_F \qquad (2.1)$$

Where:
$t_n$ = time (h) under non-reference conditions
$t_s$ = time (h) under reference conditions (15.6$^o$C, 25% moisture, 30% wt visible mechanical damage) = 230 h
$M_m$ = moisture multiplier
$M_T$ = temperature multiplier
$M_D$ = damage multiplier
$M_H$ = hybrid multiplier
$M_F$ = fungicide multiplier

## Methods for Determining Fungal Activity

Because of the role fungi play in grain storage, it is possible to monitor the fungal growth in order to determine the allowable grain storage time. Wadsö (1997) stated that "Growth is usually measured as a radial growth, mass increase or as number of spores produced." In addition to monitoring direct fungal growth, it is also possible to predict fungal growth based on off-gas composition, environmental conditions, and changes in corn dry matter.

### *Carbon Dioxide Respiration*

Deterioration of corn can be tracked by measuring the carbon dioxide being produced by fungi on the corn (Steele et al. 1969). Fungal respiration is often modeled as oxidation of glucose (equation 2.2). According to the model, $CO_2$ produced is directly proportional to dry matter loss of corn. With a balanced chemical equation, the carbon from glucose is involved in an energy producing reaction that ends with the carbon going from glucose to $CO_2$. Based on the oxidation of glucose model, a 0.5% dry matter (glucose) loss corresponds to 7.35 g of $CO_2$ per kg of corn or 14.7 g of $CO_2$ per kg of corn for a 1.0% dry matter loss. Based off of experimental results from Steele et al. (1969), the carbon dioxide from corn at 0.5% dry matter loss is represented in equation 2.3.

Chemical equation of oxidation of glucose:

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + 2835 \text{ kJ / gram mole} \qquad (2.2)$$

Empirical equation of carbon dioxide evolution from corn at reference conditions:

$$Y = 1.3\left(e^{0.006t_s} - 1\right) + 0.015t_s \quad (2.3)$$

Where:
Y = g $CO_2$ per kg dry matter
$t_s$ = time (h) under reference conditions (15.6°C, 25% moisture, 30% wt visible mechanical damage)

When: $t = t_s = 230$h, Y = 7.35 g / kg

Rukunudin et al. (2004), Dugba et al. (1996), Aljinovic et al. (1995), and Al-Yahya et al. (1993) used similar systems to monitor grain deterioration based on carbon dioxide evolution. The deterioration of stored grain was determined from carbon dioxide being produced by fungi on the corn. Rukunudin used soybeans, while the other three tested corn treated with chemicals. In each case, compressed air was first stripped of carbon dioxide using potassium hydroxide. Then the air was bubbled through water and salt solution in gas-washing bottles to obtain a desired relative humidity needed to maintain the corn moisture content. Once the air stream had attained the desired relative humidity, it was passed through the grain. Carbon dioxide produced from deterioration of grain was mixed into the air at this point. Next air left the grain and had the moisture removed by first passing through Drierite (anhydrous $CaSO_4$), then $Mg[ClO_4]_2$. Carbon dioxide was then absorbed in sulaimanite (a mixture of vermiculite and potassium hydroxide solution). The amount of carbon dioxide produced was then calculated by measuring the weight gain of the sulaimanite.

## Methods for Storing High Moisture Corn

The options for storing high moisture corn are usually chemical treatments or oxygen-limiting conditions. Ionizing energy has also been shown to successfully decrease fungi populations in high moisture corn (Bern et al. 1994). Each of these methods is used on a small portion of the overall corn stored each year. Approximately 5% of corn in Iowa is stored using oxygen-limiting conditions, while another 1% is stored using chemical treatments (Bern 1998).

## Ozone

A chemical that may be useful in storing high moisture corn is ozone. It has several properties that are desirable when compared to the chemicals already discussed for corn preservation. Ozone is currently used as a disinfectant and reactant in several processes such as in water treatment, wastewater treatment, odor elimination, and pesticide removal (EPA, 2002).

### Ozone Properties

Ozone ($O_3$) is an unstable triatomic, allotropic structure of oxygen ($O_2$). The structural instability of the oxygen-oxygen bonds causes ozone to be a strong oxidizer. An oxidizing agent is a substance that causes the oxidation, that is, the loss of one or more electrons, from the atoms of another substance (Brown et al. 2006). Ozone has an oxidizing potential of 2.07 volts at a temperature of 25$^{o}$C, which is 150% of the oxidizing potential of chlorine (Bran, 2001; Malik et al. 2000; Novazone, 2006). Ozone has an affinity for the olefinic double bond that allows it to react with a large number of chemical groups (McKenzie et al. 1997).

## Ozone Production

The instability of ozone makes it necessary to generate ozone at its point of application. Methods of generating ozone include ultraviolet (UV) light, cold plasma, corona-discharge, chemical, thermal, chemonuclear, and electrolytic methods (Kim et al. 1999). Of the options to produce ozone, corona-discharge units are most common (EPA 1999) because they can produce greater concentrations of ozone, have longer unit stability, and are more cost effective than the other production methods(Linntech, 2005; Ozone Solutions, Inc., 2006).

### *Corona-Discharge Ozone Generation*

In a corona-discharge system, also called "hot-spark" production and electrical discharge method, the corona-discharge element builds a capacitive load as a high-voltage alternating current (VAC) is applied across a discharge gap (Kim et al. 1999; EPA 1999; Linntech, 2005). Corona-discharge systems contain a dielectric layer to control the electrical discharge across the gas stream (Linntech, 2005; Ozone Solutions, Inc., 2006). Suslow et al. states that the voltage in corona-discharge systems is greater than 5000 VAC. As oxygen molecules ($O_2$) pass through the corona-discharge element, an electrical discharge breaks oxygen-oxygen double bonds, producing two oxygen radicals ($O^{-2}$) as shown in equation 2.4. The oxygen radicals then combine with oxygen molecules to form ozone (equation 2.5; Šimek et al. 2002; Linntech, 2005; Ozone Solutions, Inc., 2006) and can produce ozone concentrations up to 4% (Kim et al. 1999) or 0.5% to 3.0% by weight with an air feed gas (EPA 1999). Using pure oxygen as the feed gas increases the possible concentrations by two to four times the concentrations observed with an air feed gas (EPA 1999).

It is possible achieve ozone concentrations between 10 to 18% using a combination of technology (Kim et al. 1999). It is also possible to produce concentrated ozone at 30% continuously or 80% in batch processes by liquefying the oxygen/ozone mixture and then using the difference in boiling points to increase the ozone concentrations (Koike et al. 2000). Šimek et al. (2002) stated that micro-discharges in air-fed generators could attain a maximum efficiency for ozone production of 100 g $O_3$ / kWh, while Zhang et al. (2003) found a production efficiency of 118 g $O_3$ / kWh for a miniature oxygen-fed ozone generator. Eliasson et al. (1991) stated that if energy ion dissipation could be avoided, a maximum predicted efficiency of 400 g $O_3$ / kWh could be reached, while optimal experimental conditions can reach an efficiency of 250 g $O_3$ / kWh. The typical electrical power requirement for industrial ozone generation amounts to 12 to 18 kWh / kg $O_3$ (Gottschalk et al. 2000).

Oxygen breaks into oxygen radicals as follows:

$$O_2 \xrightarrow{e^-} 2O \qquad (2.4)$$

Oxygen radicals form ozone as follows:

$$O_2 + O \rightarrow O_3 \qquad (2.5)$$

The disadvantage of corona-discharge systems is that they produce large amounts of heat, needing a water coolant to remove the excess heat (Linntech, 2005). Another possible setback to corona-discharge systems is that reactions with nitrogen can occure when non-dry air is used (Kim et al. 1999; Šimek et al. 2002). Šimek et al. (2002) noted that there are several species produced from atomic nitrogen and nitrogen oxides during the discharge and

post-discharge (equations 2.6 – 2.10). These species cause reactions that lead to the reduction of ozone concentrations (Šimek et al. 2002) and can cause metal surfaces to corrode within the generator (Kim et al. 1999).

Nitrogen based reactions with oxygen and ozone:

$N + O_3 \rightarrow NO + O_2$ (2.6)
$NO + O_3 \rightarrow NO_2 + O_2$ (2.7)
$NO_2 + O \rightarrow NO + O_2$ (2.8)
$NO_2 + O_3 \rightarrow NO_3 + O_2$ (2.9)
$NO_3 + O \rightarrow NO_2 + O_2$ (2.10)

**Measurement of Ozone**

Methods of measuring ozone can be categorized into three areas: physical, physicochemical, and chemical. Physical methods use measurement of an ozone property to quantify concentrations. Physical methods of measurement include using UV, visible, or infrared absorption. Physicochemical methods use reagents and then measure the effects physical effects of the ozone reaction. Physicochemical methods might measure the chemiluminescence or heat of the reaction. Chemical methods quantify the products of a chemical reaction with a reagent. Chemical methods include potassium iodine titrations and polymer molecular weight reductions. The most accurate method to measure gaseous ozone is UV spectrophotometrics (Kim et al. 1999).

## Objective

The objective of this project was to develop a system to control and monitor grain storage conditions (relative humidity, temperature) while monitoring carbon dioxide level coming from the grain. The system must also be capable of delivering ozone to the grain.

## Procedure

The control system (figure 2.1) consists of several components. The environmental components include an environmental chamber and grain storage units. The environmental chamber controls the relative humidity, temperature, and lighting. The grain storage units were glass tubes that were placed inside the environmental chamber. The airflow system includes ozone treatment to the corn samples, monitoring the airflow to each sample with flow meters, and airflow regulation with valves. The integrated circuits components are part of a computer system that issues commands that control sample selection, data collection, and monitors the corn environment.

### Environmental Components

#### Environmental Chamber

The environmental chamber was a model I-35LLVL Incubator (Percival Scientific, Inc., Boone, Iowa; figure 2.2). The relative humidity was controlled using an atomizer type humidifier located on the base of the environmental chamber compartment. A dehumidifier was also used with the humidifier to keep within a +/- 5% range between 40 and 90% relative humidity. The environmental chamber also had a controlled temperature range of 2 to 44$^{o}$C. The chamber was also equipped with florescent lights that were kept off during the

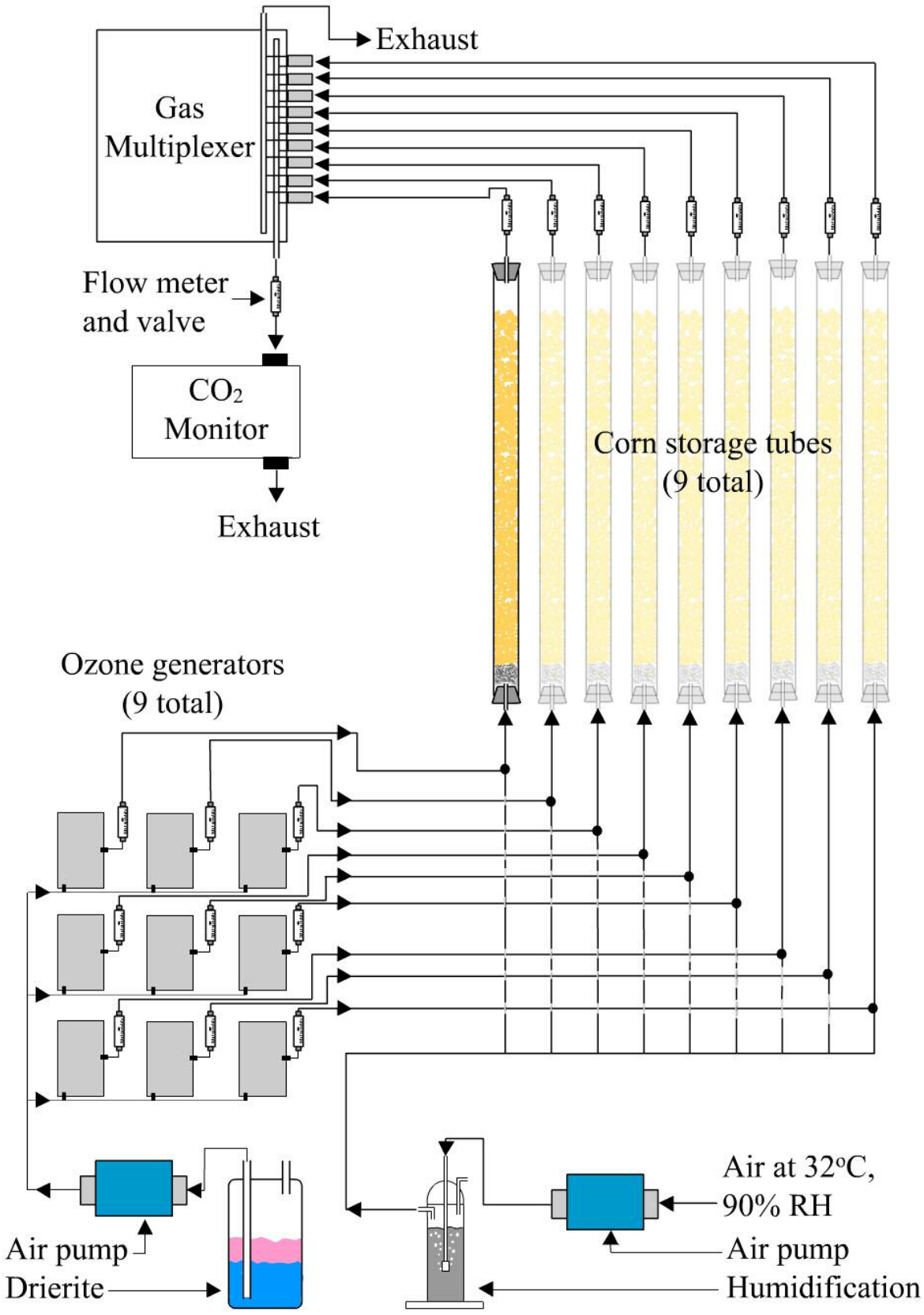


Figure 2.1 Respirometer control system

experiment. For the short-term high-temperature experiment discussed in chapter 3, the environmental chamber was set at 32$^{o}$C and 90% relative humidity.

### Grain Storage Units

The grain samples were placed inside 1-m long, 47.2-mm inner diameter glass tubes that were mounted vertically inside the environmental chamber (figure 2.2). Each glass tube was washed with soap, rinsed with water three times, and then autoclaved at 121$^{o}$C for 30 min. Rubber stoppers with a glass tube through the center were placed at each end of the glass tube. Fiberglass (Angel hair) was placed at the bottom of each tube to ensure equal air pressure over the bottom corn surface. Each tube held about 500-g dry matter weight of corn.

## Airflow Components

### Ozone Generation

Ozone ($O_3$) was generated using Enaly OZX-300U (B) Ozone Generators (Enaly Trade Co. Ltd, Coquitlam, BC, Canada; figure 2.3). The ozone generators were ambient air cooled and use the corona-discharge method to produce ozone at a maximum rate of 200 to 300 mg / h. Each ozone generator was equipped with an internal pump that had an output of about 2 L / min. The internal pump was disabled and airflow was controlled using an external pump in order to get a constant airflow. The generator was modified by removing a flipper-valve that disabled the internal pump. A Gast Oilless Diaphragm Pump, model MOA-P122-AA (Gast Manufacturing Inc, Benton Harbor, MI) was used to provide a stable airflow to the ozone generators. The airflow to the ozone generators flows through Drierite (anhydrous $CaSO_4$)

Figure 2.2 Environmental chamber with grain storage units (environmental chamber lights on only for purpose of picture)

that removes water from the air. After the air passed through the Drierite, it passed through the air pump and to a manifold where the airflow was divided into 9 air lines. Each air line

then was connected to an ozone generator. After exiting the ozone generators, each line passed through a Matheson Instruments PM flowmeter, model PM-1000 (Matheson Instruments, Montgomeryville, PA) and an adjustable valve to control the airflow.

Figure 2.3 Ozone generators and flow meters

***Ozone Calibration***

Changing the ozone output from each generator was accomplished by altering the voltage from 60 to 120 Vac with a Variable Autotransformer, type 2PF1010 (Staco Energy Products Co, Dayton, OH). Two methods were used to calibrate the ozone generators. In the first set of calibrations, the ozone production was calculated from determining the oxidant demand of

a potassium iodide, KI, solution that had ozone bubbled through it and was then titrated with sodium thiosulfate, $Na_2S_2O_3$ (Appendix H; American Water Works Association, 2001). A calibration curve was fit to a data set made for each ozone generator by testing the ozone output at four voltage levels, with 3 reps per voltage level. Ozone output was then determined by setting the ozone generators to the corresponding voltage taken from the calibration curve. The second method of calibration used a PCI-Wedeco Ozone Analyzer, model MC-400 (Wedeco ITT Industries, Charlotte, NC) to determine the ozone concentrations. The ozone concentrations of each generator were measured with the ozone analyzer and the voltage was adjusted until the desired concentration was being produced. Titration was only used until the ozone analyzer was procured because the ozone analyzer has higher accuracy and measurement repeatability, and a shorter testing time. Titration was less accurate because each ozone generator preformed differently and did not always have the same ozone output for each voltage. Ozone output from the generators was stable, but varied between each operation. Figure 2.4 shows two calibration curves for ozone generator #2 using the two methods.

The ozone analyzer determined ozone concentrations from 0 to 1200 ppm by comparing the ultraviolet (UV) absorption of a sample to a zero gas. The concentration is then derived using Beer-Lambert Law (equation 2.12) using light path length, ozone concentration, and wavelength of the light. The ozone analyzer had a resolution of 0.1 ppm by volume and an accuracy of +/- 3% (operational manual).

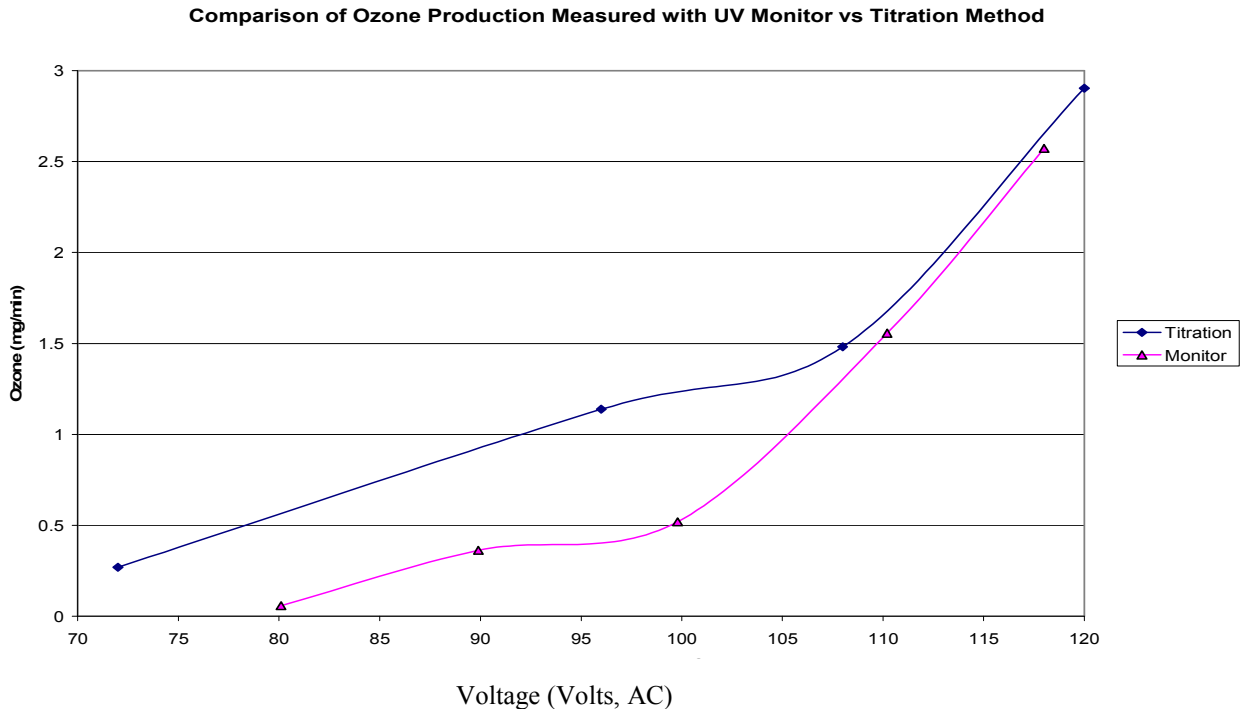


Figure 2.4 Calibration curves for ozone generator #2

Light attenuation expressed by the Beer-Lambert Law:

$$I = I_0 e^{-xLC} \qquad (2.12)$$

Where:
$I_0$ = Light intensity at reference concentration
I = Light intensity at sample concentration
x = Specific absorption coefficient
L = Path length
C = Difference in ozone concentration

**Airflow**

A Gast Oilless Diaphragm Pump, model DOA-P135-AA (Gast Manufacturing Inc, Benton Harbor, MI) was used to provide a stable airflow to each glass tube. Air was taken from the environmental chamber, passed through the pump, and then bubbled through a gas diffuser to increase the relative humidity to around 92 to 95% relative humidity. After the air passed

through the gas diffuser, the air line went to a manifold that split into 9 lines. Then air from the ozone generator was mixed with the high relative humidity and connected to the glass tubes at the base of the grain storage units. Another line was connected to the glass tubes at the top of the grain storage units. Each line passed through a Matheson Instruments PM flowmeter, model PM-1000 (Matheson Instruments, Montgomeryville, PA) and a valve to control the airflow at 0.94 L / min (2 $ft^3$ / h) before entering the gas multiplexer. All tubing used for the air lines was 0.25-inch Tygon Tubing, type 14-169-1J (Saint-Gobain Performance Plastics Corporation, Akron, Ohio).

## Integrated Circuits Components

### Computer and Control Program

A Gateway E-3200 computer (Gateway, Inc., Irvine, CA) running on Windows 95 operating system (Microsoft Corporation, Redmond, WA) was used for the respirometer control system. A program was written using Visual Basic 6.0 (Microsoft Corporation, Redmond, WA). Schneider (2001) was used as a reference in writing the program and setting up the interface. The program (Appendix A) used an interface that required text fields identified as the title, identified tests, and ozone application times to be entered (figure 2.5). After the text fields had been filled in, the program was initiated by clicking on “Start” button. Starting the program created a file in the C: directory of the computer with the “title” text field as the file name. All data collected data were stored in this file.

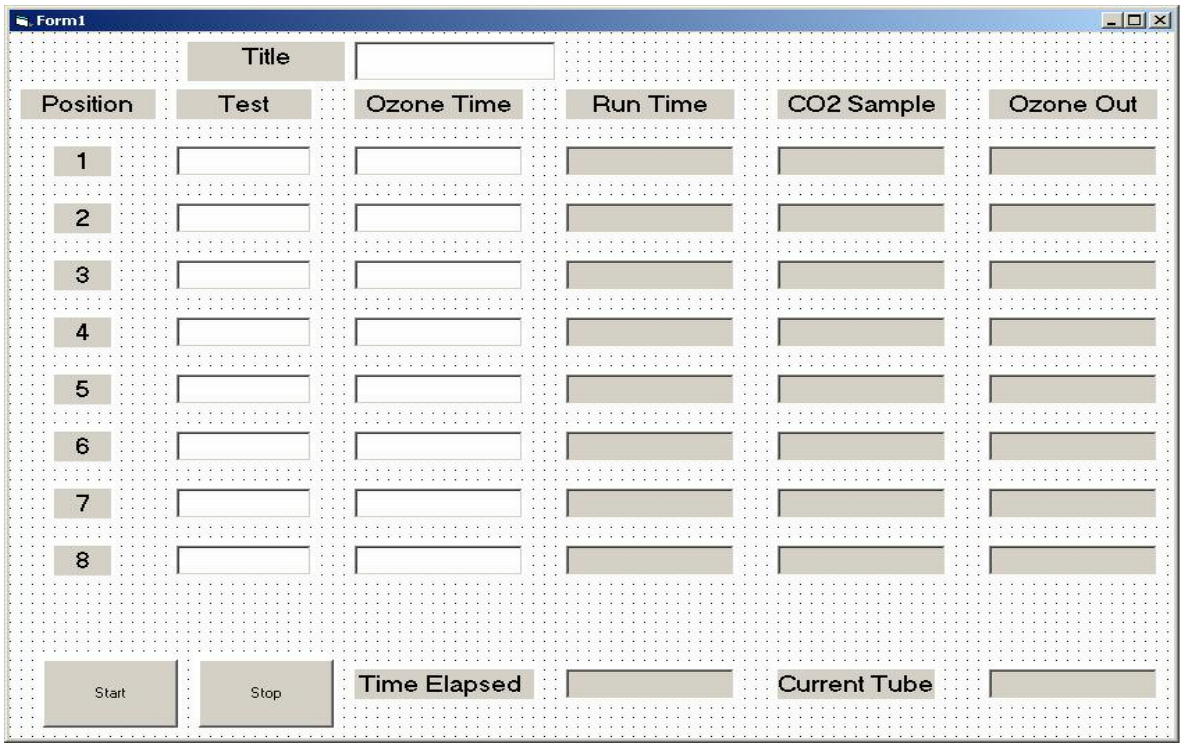


Figure 2.5 Respirometer control system program interface

**Microcontroller**

Once the program had created a storage file, the program prompted the computer to access a microcontroller through the universal serial bus (USB) port of the computer. The main controller used for the operations of switching samples and recording data was a Personal Measurement Device (PMD) 1208LS microcontroller (Measurement Computing Corp, Norton, MA). The PMD has 12 bit resolution on 6 differentially-ended import channels. Figure 2.6 outlines the electrical connections used in the PMD. Channel 0 was connected to a $CO_2$ monitor, which outputs a 0 to 5 Vdc analog signal. Channel 1 was connected to the $O_3$ monitor with a 5 Vdc voltage gain to adjust the $O_3$ output analog signal of 0 to 1 Vdc into a 0-5 Vdc signal. Channel 2 was connected to an AD592 temperature transducer that outputs a

0 to 1Vdc analog signal when a 1k resistor is connected to ground in series with the transducer. A 5 Vdc voltage gain was used on the temperature sensor to increase voltage range to 0 to 5 Vdc and improve the resolution to 0.32$^{o}$C / bit. Channel 3 was connected to a HM1500 relative humidity sensor that outputs a linear analog signal from 0 to 5 Vdc relative in relation to relative humidity ranging from 0 to 98%.

**Gas Multiplexer**

A gas multiplexer was used to select one sample at a time to be analyzed. A row of solenoid valves was placed parallel to each other. The air from all the samples was diverted to one of two output lines. A 5 Vdc signal from the PMD was sent to one solenoid at a time, which would open the solenoid to divert the air stream to the carbon dioxide sensor. The air in the other output line was exhausted into a fume hood. A 4-min pause was written into the program to allow any of the previous air samples to be flushed from the system and to allow all the sensors time to make measurements.

The gas multiplexer was initially designed by Robert Cogdill in 2000 with significant apparatus testing done by Jeremy Hansen in 2004. The multiplexer hardware was left intact while the control system for the apparatus was redesigned for use with ozone.

**Carbon Dioxide**

The carbon dioxide ($CO_2$) level in air from selected sample tube was monitored using a Rosemount Analytical Model 880A Non-Dispersive Infrared Analyzer (Emerson Process Management, Orrville, Ohio) gas analyzer that uses optic sensors to determine the $CO_2$

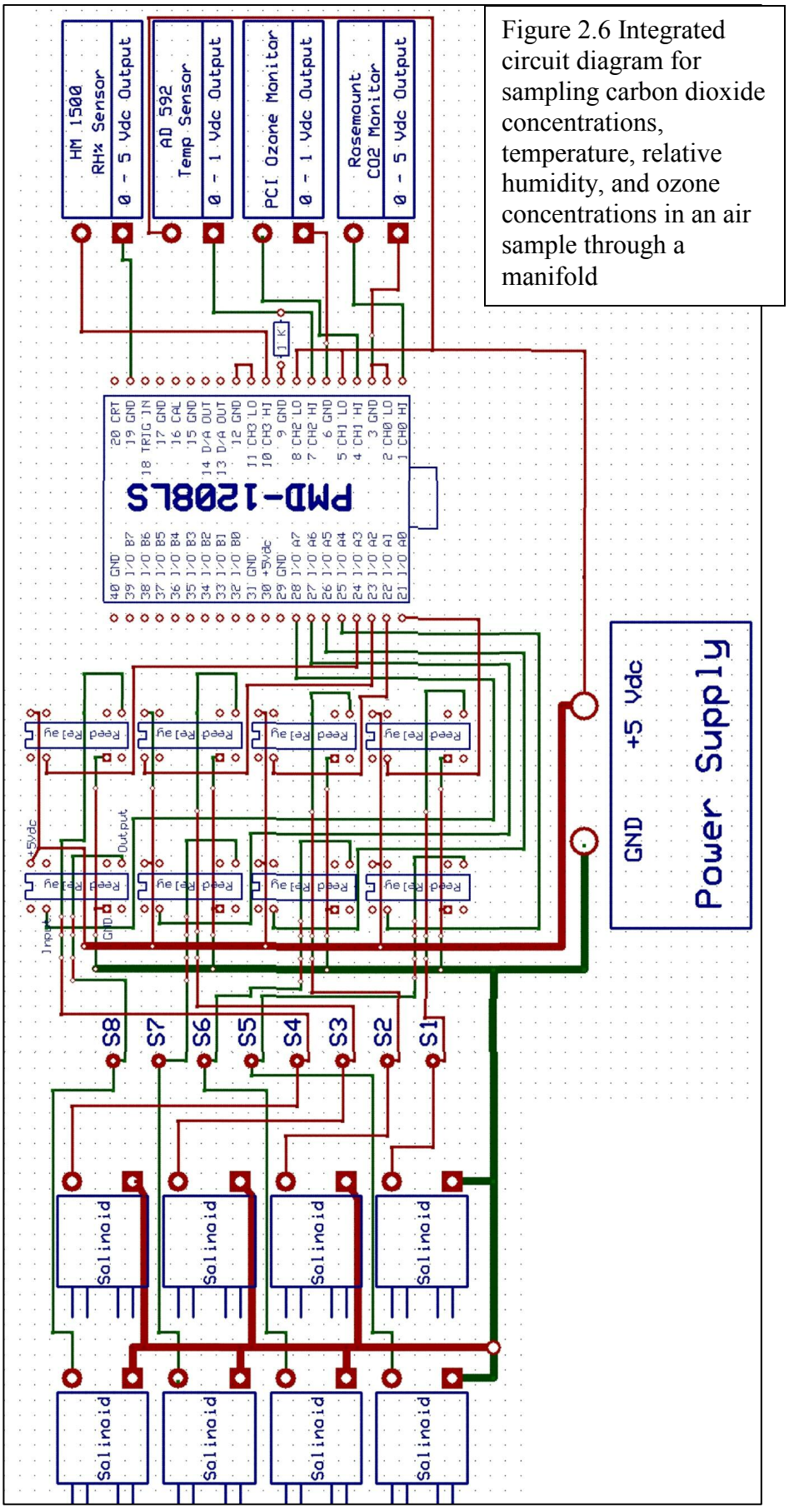


Figure 2.6 Integrated circuit diagram for sampling carbon dioxide concentrations, temperature, relative humidity, and ozone concentrations in an air sample through a manifold

concentrations in gaseous samples (figure 2.7). Infrared radiation is interrupted by a 5 Hz chopper and then sent through the gas sample. The portion of infrared radiation that is absorbed is proportional to the $CO_2$ concentration. The energy difference between the sample cell and a reference cell is then used to determine a capacitance change that is used with the $CO_2$ proportional concentration to determine the final $CO_2$ concentration.

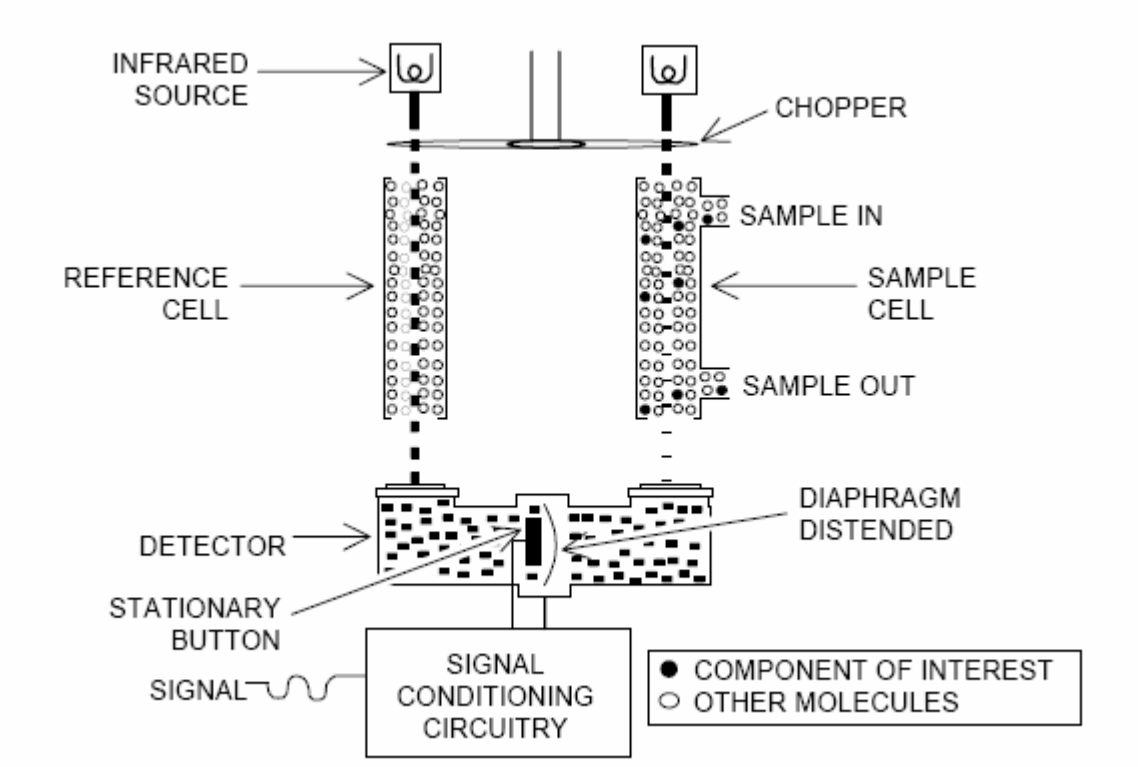


Figure 2.7 Rosemount Analytical model 880A infrared $CO_2$ detection system (Emerson Process Management, 2002).

A 1200 ppm $CO_2$ gas mixture was used to calibrate the full scale range of 1500 ppm and nitrogen gas was used to calibrate the zero set point. The $CO_2$ monitor has an accuracy of +/- 1% full scale, or 15 ppm. The $CO_2$ monitor was recalibrated once a day to limit drift, +/- 1% full-scale span and zero for every 24 h of constant operation. The $CO_2$ measurements were

recorded by the PMD from a 0 to 5 Vdc analog output from the $CO_2$ monitor. Figure 2.8 shows the signal resolution calculated using equations 2.13 – 2.16.

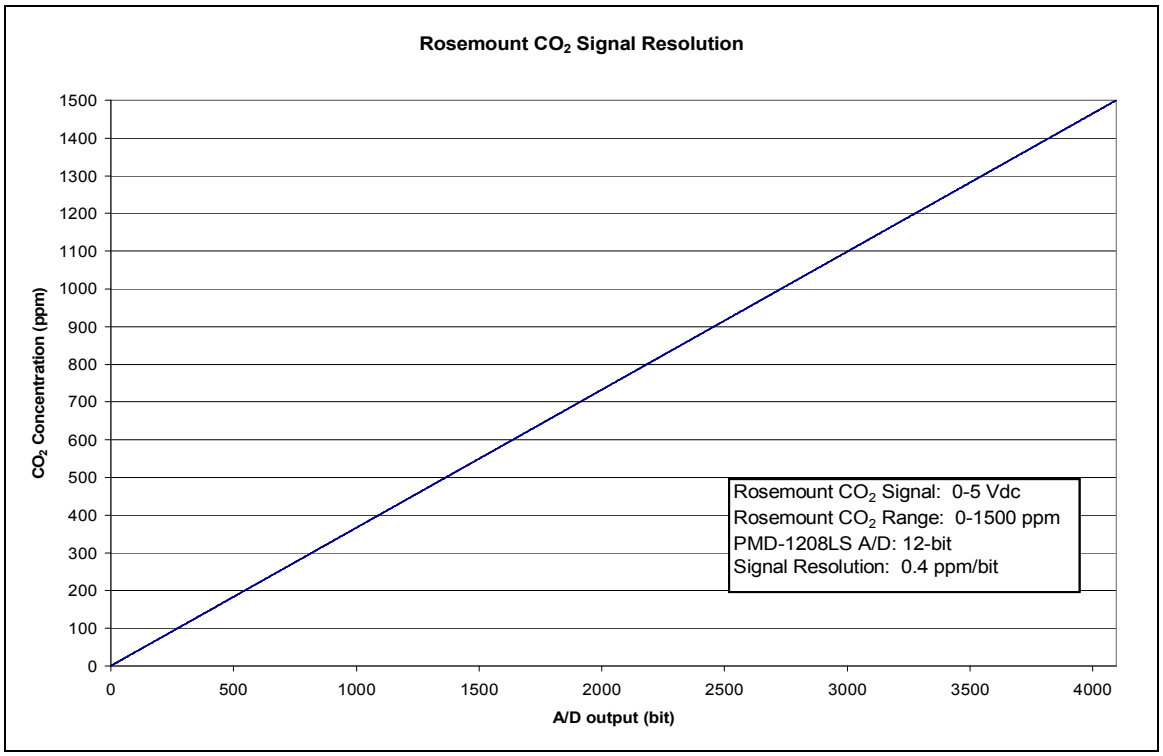


Figure 2.8 Carbon dioxide sensing range and resolution.

Calculation of sensor resolution:

$$\text{Sensor Resolution} = \frac{Range}{Voltage} = \frac{1500\,ppm}{5Vdc} = 300\,ppm/Vdc \qquad (2.13)$$

$$\text{Total Bits} = 2^{12} = 4096 \text{ bits} \qquad (2.14)$$

$$\text{PMD Resolution} = \frac{Voltage}{Bits} = \frac{5Vdc}{4096bits} = 0.001221Vdc/bit \qquad (2.15)$$

Signal Resolution = Voltage Resolution x PMD Resolution (2.16)

= 300 ppm / Vdc x 0.001221 Vdc / bit = 0.3662 ppm / bit ~ <u>0.4 ppm / bit</u>

The 0.94 L / min airflow (1.7 ($ft^3$ / min) / bu) used in the respirometer control system was determined based on the maximum carbon dioxide levels seen in trial experiments. At airflow rates less than 0.94 L / min, the $CO_2$ was liable to exceed the 1500 ppm maximum measurable concentration.

After an individual air sample was selected from the gas multiplexer, it was diverted to the $CO_2$ monitor. The $CO_2$ concentrations were then transmitted to the PMD and recorded by the computer.

## Results & Discussion

The respirometer control system functioned as intended for finding the effects of ozone on the storage time of high moisture corn. One problem in the system that should be addressed in the future is the buildup of condensation in the air lines. As the air left the environmental chamber, it encountered cooler air that caused condensation to build in the air lines. If left unattended for long periods of time (>15 h), the lines were liable to become clogged with water leading to the loss of data until the water was emptied from the air lines. This problem could be remedied by placing solenoid valves at the lowest point along the air lines outside the environmental chamber and introducing a "flushing" step into the program. The "flushing" step would open all the solenoid valves at certain time intervals and remove the condensation that had built up in the lines.

Another problem came up when the ozone monitor was used to test the air samples after they exited the carbon dioxide analyzer. The monitor was used to determine the residual ozone

leaving the corn. The $O_3$ concentrations were then transmitted to the PMD and recorded by the computer. These concentrations were not accurate due to the cycle time necessary for the ozone monitor to measure a sample exceeded the 4-min pause written into the program. Extending the pause in the program would allow the monitor to function correctly.

## Conclusions

- The respirometer control system in a satisfactory manner controlled environmental conditions (relative humidity, temperature)
- The respirometer control system monitored carbon dioxide levels in air samples in a satisfactory manner
- The system allowed dry matter loss to be estimated from the carbon dioxide levels from the corn

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# CHAPTER 3

# USING OZONE TO CONTROL FUNGI IN HIGH MOISTURE CORN

A paper to be submitted to Journal of Stored Products Research

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***Abstract.*** *One of the main problems with harvesting high moisture corn is the rapid deterioration of the corn due to fungi growth. Ozone was tested in two experiments to determine the effectiveness of using ozone to control fungi in high moisture corn. The first experiment was an accelerated test using 22% moisture content corn stored under high temperature conditions (32$^{o}$C) for 9 d. Corn was treated for three treatment times of either 24 h, 5 h, or every 3 d. Dry matter loss was estimated from carbon dioxide evolution. Ozone had little impact on the overall storage time of the high moisture corn, based on dry matter loss and damage kernel total evaluations. The second experiment stored 26% moisture content corn under low temperature conditions (15.5$^{o}$C) for 30 d. Ozone was applied over the initial 24 h or once every 3, 6, or 12 d. In the second experiment, ozone did have an impact on dry matter loss, but had no effect on the damage kernel total.*

***Keywords.*** *Corn Storage, Dry matter loss, Damage kernel total, Ozone, Carbon dioxide evolution*

## Introduction

### Corn Production

Corn is one of the world's most abundant agricultural commodities. There was an estimated 6.83 x $10^{8}$ Mg (2.69 x $10^{10}$ bushels) of corn produced globally during 2005-06. US production of 2.82 x $10^{8}$ Mg accounts for 41% of global production for 2005-06 (USDA/Foreign Agricultural Service 2006). There is an estimated loss of more than $500 million each year due to damage caused by fungi and insects to stored grain in the United States (Kells et al. 2001). Some countries have losses approaching 50% for their stored grain (Allen et al. 2003).

Corn kernels attain maximum dry matter weight when they reach physiological maturity, usually at a wet basis moisture level between 35 and 25%[3] (Bern 1998). After corn has reached physiological maturity, kernel moisture content decreases until harvest, usually at moisture levels between 25 and 17%. Once harvested, temperature and moisture conditions favor rapid growth of fungi in stored corn, making it necessary to either dry the corn or use some other preservation process (Bern 1998).

**Low Moisture Corn**

In an artificial drying system, corn should be dried to 15.5% moisture content for storage times up to six months, and 13% for periods longer than six months (Hellevang 1994; Bern 1998). A recommendation by Brook suggests that corn stored for winter should have a maximum moisture content of 15%, a maximum moisture content of 14% if stored past the following summer, and 13% or lower if stored longer than a year (Munkvold 2003). The most common dying methods use forced-air systems that move either natural air or heated air through the grain. While drying corn is effective at extending the storage life and slowing deterioration, it is energy intensive. Another determent to quickly drying corn with heated air is the tendency to form stress cracks as the shell of the kernel dries faster than in inside, causing it to shrink and crack (Bern et al. 2003).

About 87% of the Iowa corn crop is preserved by drying after harvesting (Bern 1998). The drying process is very energy intensive because of the high latent heat of vaporization of

water and dryer inefficiency. This energy usually comes from direct combustion of liquefied petroleum gas (LPG) or natural gas, plus electricity to run the equipment. It takes the equivalent of about 96 million L (25.5 million gallons) of LPG, plus 30 million kWh of electricity to remove each percentage point of moisture from the Iowa corn crop, and nearly 533 million L (141 million gallons) of LPG, plus nearly 165 million kWh of electrical energy to dry the crop from the average harvest moisture of 20.5% to 15% moisture for storage (Bern 1998).

An estimate of the cost of drying was done using 80% of this US corn dried from 20% moisture to 15%, or around $11.3 \times 10^6$ Mg of water evaporated. Using an estimate that conventional dryers would require at least 250% of the latent heat of vaporization (2400 kJ/kg) or $76 \times 10^{12}$ kJ ($73 \times 10^{12}$ Btu) per year to complete this drying and natural gas priced at $7.50 per MBTU (NYSE, first half of 2007), the cost of energy for drying would exceed $545 million in the US. If 600 million kWh of electricity at $0.10/kWh is also included, and the estimated total energy cost for drying would amount to more than $600 million per year. The high cost associated with drying corn could make other preservation methods encouraging.

**High Moisture Corn**

There are several reasons that make harvesting high moisture (>17% moisture content) corn appealing. "Harvesting corn at high moisture content reduces field pest attacks, avoids bad-weather consequences, and minimizes field losses" (Aljinovic et al. 1994). The corn can be

[3] All moistures are % wet basis

harvested from the field 2 to 3 weeks earlier than corn harvested for dry storage (Miller, 2002). Harvesting earlier places corn closer to physiological maturity and maximum dry matter at harvest. The earlier harvest time can avoid a 3 to 8% dry matter loss that would accrue if corn was left in the field to dry (Miller 2002). The earlier harvesting also opens the possibility of having a higher quality residue left in the field (Miller 2002). Disadvantages include rapid deterioration from fungal activity after harvest, and higher drying costs (Aljinovic et al. 1994). High moisture corn also has a lower market flexibility compared to dry corn (Miller 2002).

**Fungi in Corn**

Storage fungi species found in corn will grow in temperatures between -2 and 50$^{o}$C with an optimal growth temperature between 20 to 35$^{o}$C depending on the species (Paulsen et al. 2003). The fungi found in corn also need access to water in order to grow. The lower limit for growth conditions in most species is between 70 and 81% relative humidity or 13.5 to16.5% moisture in the corn (Paulsen et al. 2003).

While many microorganisms influence stored grain quality, only some fungal species are important. Storage fungi species found in corn will grow in temperatures between -2 and 50$^{o}$C with an optimal growth temperature between 20 to 35$^{o}$C depending on the species (Paulsen et al. 2003). The lower limit for growth conditions in most species is between 70 and 81% relative humidity or 13.5 to16.5% moisture in the corn (Paulsen et al. 2003). The main conditions that affect fungal growth are temperature, relative humidity and oxygen

content of air surrounding the grain, physical conditions of the corn kernels, mold inoculum level, and previous grain storage history (Bern et al. 2002; Paulsen et al. 2003).

**Modeling Deterioration of Stored Corn**

The characteristics that affect growth of fungi are also used to model shelled corn deterioration: storage time, kernel moisture, kernel temperature, kernel visible mechanical damage level, genetic susceptibility to storage fungi, and other factors (Bern et al. 2002; Paulsen et al. 2003). These factors also influence the acceptable dry matter loss that is allowed by grain users. Steele and Saul (1969) observed that shelled corn can, on average, experience a 0.5% dry matter loss due to storage fungi before its USDA grade is reduced by one USDA level. While not precise, the 0.5% dry matter loss limit for shelled corn deterioration is widely accepted.

It is possible to predict the storage time of corn to reach 0.5% dry matter loss based on moisture content, temperature, visible mechanical damage, genetic traits, and fungicidal application (equation 3.1; Bern et al. 2002).

Predicted corn storage time:

$$t_n = t_s M_M M_T M_D M_H M_F \qquad (3.1)$$

Where:
$t_n$ = time (h) under non-reference conditions
$t_s$ = time (h) under reference conditions (15.6$^o$C, 25% moisture, 30% wt visible mechanical damage) = 230 h
$M_m$ = moisture multiplier
$M_T$ = temperature multiplier
$M_D$ = damage multiplier
$M_H$ = hybrid multiplier
$M_F$ = fungicide multiplier

## Methods for Determining Fungal Activity

Because of the role fungi play in grain storage, it is possible to monitor the fungal growth in order to determine the allowable grain storage time. Wadsö (1997) stated that "Growth is usually measured as a radial growth, mass increase or as number of spores produced." In addition to monitoring direct fungal growth, it is also possible to predict fungal growth based on off-gas composition, environmental conditions, and changes in corn dry matter.

### *Carbon Dioxide Respiration*

Deterioration of corn can be tracked by measuring the carbon dioxide being produced by fungi on the corn (Steele et al. 1969). Fungal respiration is often modeled as oxidation of glucose (equation 3.2). According to the model, $CO_2$ produced is directly proportional to dry matter loss of corn. With a balanced chemical equation, the carbon from glucose is involved in an energy producing reaction that ends with the carbon going from glucose to $CO_2$. Based on the oxidation of glucose model, a 0.5% dry matter (glucose) loss corresponds to 7.35 g of $CO_2$ per kg of corn or 14.7 g of $CO_2$ per kg of corn for a 1.0% dry matter loss.

Chemical equation of oxidation of glucose:

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + 2835 \text{ kJ / gram mole} \qquad (3.2)$$

Rukunudin et al. (2004), Dugba et al. (1996), Aljinovic et al. (1995), and Al-Yahya et al. (1993) used similar systems to monitor grain deterioration based on carbon dioxide

evolution. The deterioration of stored grain was determined from carbon dioxide being produced by fungi on the corn. Rukunudin used soybeans, while the other three tested corn treated with chemicals. In each case, compressed air was first stripped of carbon dioxide using potassium hydroxide. Then the air was bubbled through water and salt solution in gas-washing bottles to obtain a desired relative humidity needed to maintain the corn moisture content. Once the air stream had attained the desired relative humidity, it was passed through the grain. Carbon dioxide produced from deterioration of grain was mixed into the air at this point. Next air left the grain and had the moisture removed by first passing through Drierite (anhydrous $CaSO_4$), then $Mg[ClO_4]_2$. Carbon dioxide was then absorbed in sulaimanite (a mixture of vermiculite and potassium hydroxide solution). The amount of carbon dioxide produced was then calculated by measuring the weight gain of the sulaimanite.

**Direct Examination**

Direct examination of the foodstuff is done prior to processing the product using the naked eye or a stereomiscope. This is often sufficient because of the size of fungal colonies and that fungal growth usually occurs on the product surface. Improved direct examination can be done preparing slides and using the aid of a microscope. The visible fungi then are transferred to plates for further analysis (Samson et al. 2004).

When direct examination is used as a method to determine the USDA grade of corn, brown discoloration, “blue-eye,” or other signs of mold invasion of the kernel are some of the damage types evaluated when determining the damage kernel total (DKT) percentage (Paulsen et al. 2003). USDA Grade corn grades of 1, 2, 3, 4, and 5 have maximum DKT

limits of 3, 5, 7, 10, and 15%, respectively. Direct examination of grain to determine USDA grade is preformed by licensed inspectors per US Grain Standards Act. A minimum of 125 g of corn is hand inspected and compared to interpretive slides. The DKT % is then determined by dividing the weight of damaged corn kernels by the weight of the total corn sample.

## Methods for Storing High Moisture Corn

The options for storing high moisture corn are usually chemical treatments or oxygen-limiting conditions. Ionizing energy has also been shown to successfully decrease fungi populations in high moisture corn (Bern et al. 1994). Each of these methods is used on a small portion of the overall corn stored each year. Approximately 5% of corn in Iowa is stored using oxygen-limiting conditions, while another 1% is stored using chemical treatments (Bern 1998).

### Oxygen-limiting Storage

Corn with moisture contents between 25 to 28% can be preserved by being ensiled in a sealed structure such as a silo, bunker, or plastic bag. Anaerobic conditions set in once the oxygen supply inside the structure is consumed by microorganisms. The lack of oxygen prevents further fungal activity while allowing anaerobic bacteria to dominate. The activity of the bacteria results in some dry matter loss as the bacteria consume carbohydrates and produce acid. The production of acid lowers the pH, eventually causing bacteria growth to stop around a pH of 3.8 (Bern et al. 2003).

### Chemical Preservation

Chemicals that have been tested for preserving high moisture corn from fungi include propionic acid, ammonia, iprodione, and sulfur dioxide (Aljinovic et al. 1994, Bern 2000). Methyl bromide and phosphine are fumigants that are also used for corn storage (Mason et al. 2003). Each of these treatments has several negative traits that make chemical treatment for preservation unpopular for common use. Propionic acid, sulfur dioxide, and phosphine are highly corrosive. The cost associated with chemical preservation can also be significantly higher compared to drying corn to lower moistures for storage. Ammonia treatments affect kernel color and decrease dry matter. Methyl bromide was set to be phased out of use by 2005 due to the Montreal Protocol.

## Ozone

Another chemical that may be useful in storing high moisture corn is ozone. It has several properties that are desirable when compared to other chemicals that have been tested for corn preservation. Ozone is currently used as a disinfectant and reactant in several processes such as in water treatment, wastewater treatment, odor elimination, and pesticide removal (EPA, 2002).

### Ozone Properties

Ozone ($O_3$) is an unstable triatomic, allotropic structure of oxygen ($O_2$). The structural instability of the oxygen-oxygen bonds causes ozone to be a strong oxidizer. Ozone has an

oxidizing potential of 2.07 volts at a temperature of 25$^{o}$C, which is 150% of the oxidizing potential of chlorine (Bran, 2001; Malik et al. 2000; Novazone, 2006). Ozone has an affinity for the olefinic double bond that allows it to react with a large number of chemical groups (McKenzie et al. 1997).

## Ozone as a Disinfectant

A review by Kim et al. (1999) noted that ozone is capable of decreasing microbial populations, the chemical and biological oxygen demand, and the quantity of toxic organic compounds within the treated environment. Ozone has been looked at for many applications including food preservation, artificial aging of beverages, odor control, and medical therapy. The main uses of ozone are currently drinking water treatment and municipal and industrial wastewater treatment (Graham 1997).

Khadre et al. 2001 made the following statement, "Presence of organic substances with high ozone demand may compete with microorganisms for ozone. Viruses and bacteria associated with cells, cell debris, or feces are resistant to ozone, but purified viruses are readily inactivated with the sanitizer."

### Ozone as a Food Preservative

Ozone displays several characteristics that make it ideal for use as a fumigant in foodstuff. Gaseous ozone has been known to posses antimicrobial traits for over 120 years (Jay et al. 2005, pp. 312-314). As a powerful oxidant, ozone quickly deactivates microorganisms such as viruses, bacteria, and fungi (Majchrowicz, 1998) and kills small vertebrates such as insects

(Mendez et al. 2003). Ozone is also capable of decreasing levels of toxic organic compounds (Kim et al. 1999). The effects of ozone can be short-lived as it quickly dissipates into $O_2$ (Bran, 2001) and has a limited residual effect (Majchrowicz, 1998). The quick dissipation time coupled with the absence of residual toxins makes ozone a well-suited treatment for most food materials (FDA, 2002) and it has been shown to extend the shelf life of certain food (Jay et al. 2005, pp. 55-56). These are also reasons why ozone was given a "generally recognized as safe" (GRAS) classification from the FDA first in 1982 with limitations (Kim et al. 1999) and then more recently on June 26, 2001 (FDA, 2002; Suslow et al. 2004). Ozone has been approved for food use in Australia, France, and Japan (Jay et al. 2005, pp. 55-56; Graham 1997). It has also been noticed that ozone has no measurable effect on the nutritional content or germination of treated grain samples of wheat, corn, or soybeans (Mendez et al. 2003) but can cause changes in other food products such as an increase in rancidity in high-lipid-content foods (Jay et al. 2005, pp. 55-56) and changes in volatile oil constituents in ground pepper (Zhao and Cranston 1995). A few negative aspects of ozone are that the oxidizing effects of ozone make it toxic at high doses (Bran, 2001) and steps may be necessary to destroy off-gases to prevent worker exposure (EPA 1999). The highly reactive nature of ozone also requires the use of corrosion-resistant equipment such as stainless steel.

Mendez et al. (2002) applied gaseous ozone to whole grains in order to determine if the characteristics of the grain were changed. Ozone was applied to samples of hard wheat, soft wheat, corn, and soybeans at a concentration of 50 ppm and an airflow rate of 0.02, 0.03, and 0.04 m/s. The grain samples were stored in four steel barrels (208 L each) bolted together,

with final dimensions of 0.57m dia. x 3m. Ozone concentrations were measured 1 to 4 times per day at five depths within the head space, plenum, and at depths of 0.3, 0.9, 1.5, 2.1, and 2.7 m (setup previously used for Kells et al. 2001). Data suggested that ozone had no effect on the adhesiveness of rice, popping volume of popcorn, saturated or unsaturated fatty acids of maize, soybeans, or wheat. The milling efficiency of soft and hard wheat also remained unchanged when compared to control samples. These results would suggest that ozone did not penetrate the grain.

While ozone can be effective on controlling microorganisms, integrating ozone into foodstuff preservation can be more challenging. There have been several studies done that prove that ozone is effective in inactivating fungi in food, acting to reduce fungal spore production on food surfaces and to decrease spread of fungi to adjacent produce. The use of gaseous ozone appears to be the most effective in cooler temperature storage (temp range not provided) and a relative humidity between 85 to 95% (Suslow 2004). Gaseous ozone is only effective as a surface treatment because it does not penetrate natural openings or wounds in sufficient amounts to control microorganisms (Suslow 2004) and antimicrobial action occurs primarily on the surface with water phase food because of the rapid decomposition of the ozone (Kim et al. 1999).

**Ozone Used for Insect Control**

Ozone used as a fumigant to treat stored corn is effective in controlling insects and fungi. Tests preformed by Kells et al. (2001) showed the effect of gaseous ozone on three insect species and on the fungal specie *Aspergillus parasiticus*, Speare strain ATCC 24551. A

sample size of 30 g of corn was first seeded with *A. parasiticus* and then divided into 10 cages for each insect species and fungi. The cages were then placed 2 cm below the surface of a 12.7-Mg capacity steel grain bin filled with corn. The corn was then treated with 50 ppm ozone for 3 d or 25 ppm for 5 d. The same corn and grain bin was used for all treatments. The test found that corn treated with 50 ppm ozone for 3 d resulted in 92 to 100% insect mortality. The temperature, corn moisture content, and relative humidity were several factors that would impact the effectiveness of ozone that were not provided in the research paper.

Kells described a two-phase reaction progression when ozone was exposed to corn in a field grain bin study and lab column study. The lab column study used four steel barrels (208 L each) bolted together, with final dimensions of 0.57 m dia. x 3 m. Air samples were measured 1 to 4 times per day in the head space, plenum, and at depths of 0.3, 0.9, 1.5, 2.1, and 2.7 m. The lab column study looked at three air velocities: 0.02, 0.03, and 0.04 m / s. At an air velocity of 0.02 m / s, 75% of the ozone passed a depth of 2.7 m in 3.7 d. When a velocity of 0.03 m / s was used, the same concentration of ozone at 2.7 m was reached in 1.3 d. In the phase I, ozone rapidly degraded and slowly moved through the corn. In the phase II of treatment, the ozone passed through the corn freely with minor losses to concentrations. Phase I is marked by a drop in ozone concentrations as the ozone reacts with active sites throughout the corn. These reactive sites likely consist of fungi, bacteria, and the kernel shell. In phase II, ozone passes through the grain mass with only minor ozone degradation. While phase II was never reached in the field study, the lab column study showed that a degradation rate of 1 ppm ozone / 0.3 m occurred in phase I fumigation leading to phase II.

Kells predicted that this degradation rate would allow an insect and fungi "killing zone," described as a concentration above 25 ppm, to reach a depth of 8 m of grain.

**Ozone Used for Microorganism Control**

Concentrations of ozone between 0.1 and 0.5 ppm for short periods of time have been proven to be effective against gram-positive and gram-negative bacteria, viruses, and protozoa (Jay et al. 2005, pp. 312-314; Kim et al. 1999). Khadre et al. (2001) states that **"**Inactivation of microorganisms by ozone is a complex process because of the multiple cellular sites which ozone can affect. These sites include proteins, unsaturated lipids and respiratory enzymes in cell membranes, peptidoglycans in cell envelopes, enzymes and nucleic acids in the cytoplasm, and proteins and peptidoglycan in spore coats and virus capsids."

**Ozone Used for Fungi Control**

A review of food use of ozone by Kim et al. 1999 stated that ozone could be an effective fungicidal agent. Fungal spores have a microbicidal activity threshold to aqueous ozone (0.3 to 0.5 mg / L) at 90 to 180 minutes exposure for *Aspergillus* spores, 45 to 60 minutes exposure for *Penicillium* spores, and 5 to 10 minutes exposure for *Candida paracreus* spores.

***Ozone Used in Fruit Storage***

The effect of a continuous exposure to a low dose of ozone was tested on peaches and table grapes by Palou et al. (2002). Both fruits were harvested at commercial maturity from the San Joaquin Valley. After being harvested, the fruit was superficially disinfected by submersion in a diluted bleach solution (0.5 % sodium hypochlorite) for 1 minute. To test the effect of ozone on wounded fruit, peaches were wounded with a 1-mm probe tip and

inoculated with *Monilinia fructicola, Botrytis cinerea, Mucor piriformis, or Penicillium expansum*. Four 20-fruit trays inoculated with a pathogen were placed in a storage room held at 20°C, 90% relative humidity, and 0.3 ppm ozone for 4 weeks. To test the effects of ozone on wounded grapes, grapes were inoculated with spores of *B. cinerea* by spraying one sample set and injecting the spores into another sample set. The grapes were then stored for 7 weeks in the same conditions as the peaches. Another test was done with peaches and grapes to test for the physiological response of the fruit to 0.3-ppm ozone exposure compared to ambient air under 20°C and 90% relative humidity conditions over several weeks. The results showed that 0.3 ppm was effective in inhibiting the normal aerial growth of mycelia and preventing spores from being produced and spread in the wounded peaches, but had no noticeable effect on the pathogen activity within the wounds. Ozone also did not reduce mold incidence on the inoculated grapes. In the physiological test with peaches, the ozone exposed fruit lost more weight. That would indicate that the ozone might have damaged the peaches' cuticle or epidermal tissue. It was also noted in all the experiments that airflow plays a crucial part in the effectiveness of ozone gas. More mycelia growth was seen where ozone was partially stopped from contacting fruit because of the plastic trays used to hold the fruit.

In a previous study using table grapes, Sarig et al. (1996) showed that 8 mg of ozone per min for 20 min was effective in controlling fungi, yeast, and bacteria. Table grapes were collected directly after being harvested in Israel and inoculated with *Rhizopus stolonifer* at a concentration of $10^7$ spores per mL distilled water. Ozone was applied to 2-kg samples at a rate of 8 mg per min for time intervals between 0 and 80 min. They found that ozone applied

at that rate for 20 min was effective in reducing fungi colony forming units from 40 to less than 10.

Palou et al. (2003) showed that the capability of ozone to deactivate microorganisms is heavily dependant on ability of the gas to come into contact with the microorganisms. This was tested by treating navel oranges (*Citrus sinensis* (L.) Osbeck) in different storage conditions with ozone. Four types of packaging were used: standard corrugated fiberboard citrus cartons (2.6% vented surface area), returnable plastic containers with uncovered fruit (35.9% vented surface area), returnable plastic containers with bagged fruit (0.7% vented surface area), and corrugated fiberboard Master carton with bagged fruit (2.9% vented surface area). The oranges were inoculated with a $10^6$ spores / mL suspension of either *Penicillium digitatum* or *Penicillium italicum*. After a 24-h inoculation period, the oranges were stored in either of two 678 m3 cold storage room held at 12.8$^{o}$C for 14 d. One storage room had a continuous exposure to ozone that was discharged into the room at a rate of 2.5 g / h. The other room was used as a control and received no ozone. There were no spores present on the oranges stored uncovered in the returnable plastic containers, which also had an 81.9% ozone penetration. The other storage methods had lower vented surface areas, impacting ozone penetration (>17%) and 5 to 60% spore coverage on the fruit.

***Ozone Used in Grain Storage***

A study by Allen et al. (2003) used gaseous ozone to inactivate fungi in barley grain. They tested the effects of four different ozone doses on barley using four moisture contents (19, 22, 25, and 30%), and three temperatures (0, 20, and 40$^{o}$C). Ozone application rates were

0.98, 0.16, 0.08, and 0.04 mg / g barley min. After five minutes of ozonation at a rate of 0.16 mg of ozone / g barley min, the ozone had caused a 96% inactivation of spores. The inactivation of spores increased as ozone dose increased, going from around 60% spore survival at an ozone application of 0.04 mg / g barley – min, to less than 4% spore survival at an ozone application of 0.98 mg / g barley – min. They also noticed that higher temperatures and water activity increased the inactivation percentage of fungi on the barley. With a 0.98 mg / g barley-min and a temperature of 20$^{o}$C, a 19.6% spore survival rate occurred at 19% moisture while less than 4.2 % spore survival rate occurred for 30% moisture. The reaction to temperatures of 0, 20, and 40$^{o}$C had respective spore survival rates of 36.2%, 13.95% and, 4.2% with an ozone rate of 0.98 mg / g barley-min and 19% moisture content.

In the previously mentioned study, Kells et al. (2001) looked into the effect of gaseous ozone on three insect species and the fungal species *Aspergillus parasiticus*, Speare strain ATCC 24551 on corn. They were able to identify that particular strand of *A. parasiticus* based on the production of a metabolite in the aflatoxin biosynthetic pathway, averufin, which can be identified by an orange color when grown on potato dextrose agar medium. A sample size of 30 g of corn was first seeded with *A. parasiticus* and then divided into screen cages. The cages were then placed 2 cm below the surface of a 12.7 Mg capacity steel grain bin filled with corn. The corn was then treated with 50-ppm ozone for 3 d or 25 ppm for 5 d. The same corn and grain bin was used for all treatments. The temperature, corn moisture content, and relative humidity were several factors that would impact the effectiveness of ozone but were not provided in research paper. After the treatment, the corn was washed with a Triton X-100 solution to remove fungi, and then plated on the dextrose agar. Colony counts from

the plates showed that a 63% reduction in *A. parasiticus* occurred after the 3 d treatment of 50-ppm ozone. The 25-ppm treatment for 5 days failed to significantly reduce the fungi counts when compared to control samples.

While effects of fumigating corn with ozone have been tested, the effects of using ozone to limit dry matter losses remain unexplored. Ozone quantities needed to keep dry matter losses below 0.5% need to be determined before ozone treatments can become a useful method of managing fungi in corn.

## EXPERIMENT I: HIGH-TEMPERATURE HIGH-MOISTURE CORN STORAGE WITH CONTINUOUS AIRFLOW

### Objective

The objective of experiment I was to find the effective of ozone on dry matter loss and damage kernel total of corn stored under high temperature and high moisture conditions with continuous aeration.

### Procedure

#### Sample Origin and Preparation

Corn used in the high temperature storage was Curry 4825 hybrid harvested in the fall of 2005 in Calhoun County, Iowa. Harvest moisture content of the corn was about 16% as measured with a Dickey-John GAC 2000 grain analysis computer (Dickey-John Corporation, Auburn, Illinois). After the corn was harvested, it was cleaned using a 4.67-mm (12/64-inch)

diameter round-hole screen in a CEA Carter-Day Dockage Tester (CEA Carter-Day Co., Minneapolis, Minnesota) and then stored at 5$^{o}$C until the start of the experiment on November 13, 2006. Then three 100-g samples of corn were inspected kernel by kernel to determine visible mechanical damage which averaged 8%.

Target moisture content for the high temperature experiment was 22%. To increase moisture content, corn was placed in sealed polyethylene bags and then distilled water was added with a spray bottle. The sealed bags were then placed in 5$^{o}$C storage for at least 2 d. The corn was then mixed and divided into 0.6-kg dry matter sets. Then the starting moisture content of the corn was determined using the standard oven method of 103$^{o}$C for 72 h (ASABE 2003).

**Assembly**

Once the corn had attained the desired moisture content, it was placed inside sterile 1-m long, 47.2-mm inside diameter autoclaved glass tubes that were mounted vertically inside a controlled environmental chamber. The environmental chamber was a model I-35LLVL Incubator (Percival Scientific, Inc., Boone, Iowa) kept at 32$^{o}$C and 90% relative humidity. A Gast Oil less Diaphragm Pump, model DOA-P135-AA (Gast Manufacturing Inc, Benton Harbor, MI) was used to provide a stable airflow to each glass tube (figure 3.1). Airflow to each tube was maintained at 0.94 L / min (2 $ft^3$ / h) and an aeration rate of 1.32 $m^3$ / min-Mg (1.19 ($ft^3$ / min) / bu) by valves connected to a Matheson Instruments PM flowmeters, model PM-1000 (Matheson Instruments, Montgomeryville, PA). Air was taken from the environmental chamber, passed through the pump, and then bubbled through a gas diffuser to

increase the relative humidity to between 92 and 95% relative humidity. After the air passed through the humidifier (gas diffuser), the air line went to a manifold that split into 9 lines. Then air from the ozone generators was mixed with the high relative humidity air and routed to the glass tubes at the base of the grain storage units. Another line was connected to the glass tubes at the top of the grain storage units. Each line then passed through the gas multiplexer that was able to select one line at a time to analyze. A 4-min pause between tubes was used to purge the previous air sample. All tubing used for the air lines was 0.25-inch Tygon Tubing, type 14-169-1J (Saint-Gobain Performance Plastics Corporation, Akron, Ohio).

Carbon dioxide ($CO_2$) levels in air from a selected tube was monitored using a Rosemount Analytical Model 880A Non-Dispersive Infrared Analyzer (Emerson Process Management, Orrville, Ohio) gas analyzer that uses optic sensors to determine the $CO_2$ concentrations in gas leaving the corn. Carbon dioxide levels were then used to calculate dry matter loss of the corn. To calculate the dry matter loss, the ambient air carbon dioxide levels were subtracted from the sample air to determine the $CO_2$ being produced in the corn. The $CO_2$ concentrations (ppm) were then converted into mass (g $CO_2$) and then moles (44.01 g / mol). Using equation 3.2, a molar ratio of 6 moles $CO_2$ to 1 mole of glucose, providing the number of moles of glucose that would have been used to produce the $CO_2$. The moles of glucose were then converted into mass by using 180.16 g / mol. The mass of glucose was then used to determine the dry matter loss of the corn. The airflow used in the respirometer control system was determined based on the maximum carbon dioxide levels seen in trial experiments and the maximum measurable concentration of 1500 ppm. At airflow rates less

than 0.94 L / min, the $CO_2$ was liable to exceed the 1500 ppm maximum measurable concentration.

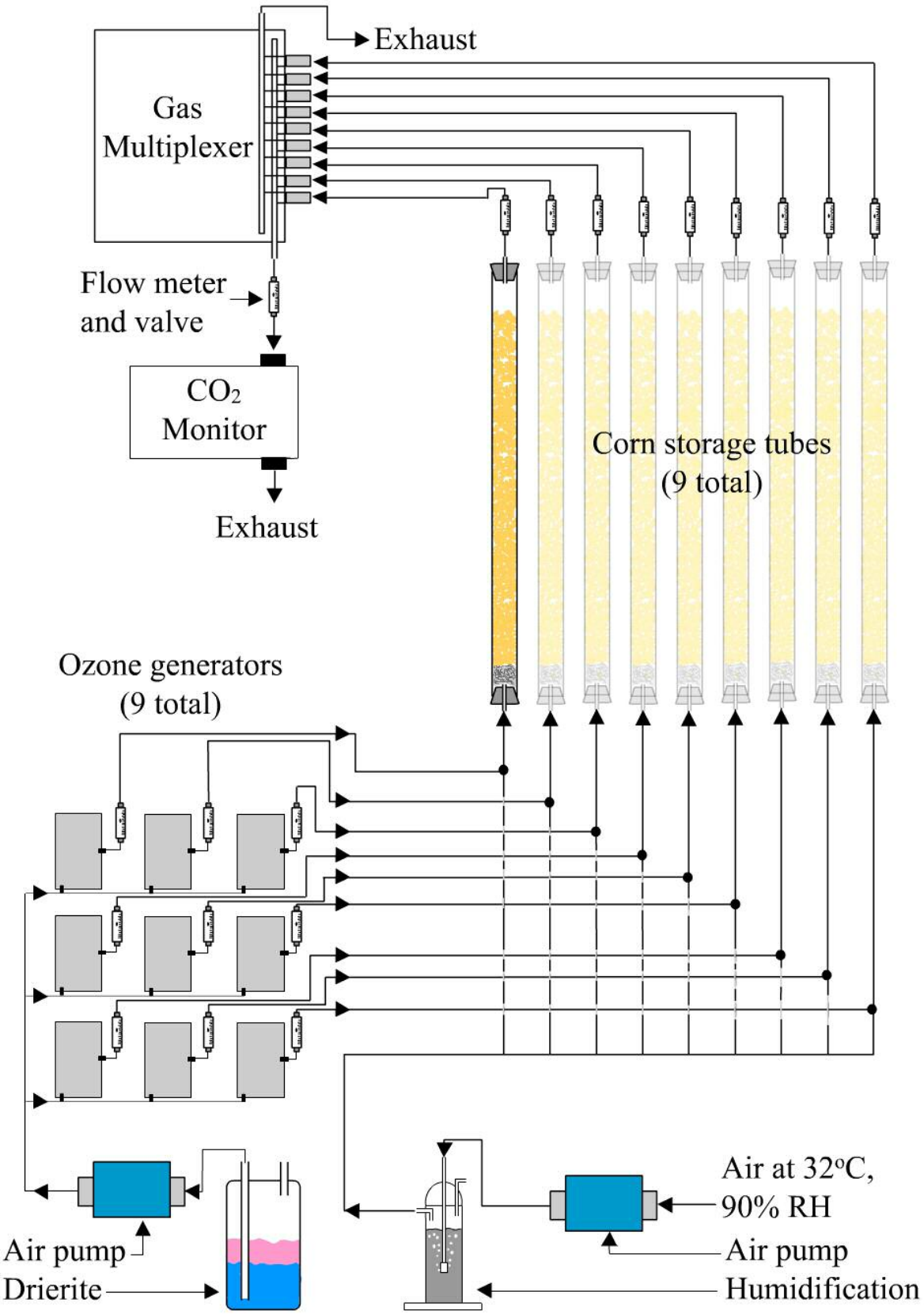


Figure 3.1 High temperature short term continuous aeration system diagram

Ozone ($O_3$) was generated using Enaly OZX-300U (B) Ozone Generators (Enaly Trade Co. Ltd, Coquitlam, BC, Canada). The ozone generators were ambient air cooled and use the corona-discharge method to produce ozone at a maximum rate of 200 to 300 mg / h. Each ozone generator was equipped with an internal pump that had an output of about 2 L / min. The internal pump was disabled and airflow was controlled using an external pump in order to get a constant airflow. The generator was modified by removing a flipper-valve that disabled the internal pump. A Gast Oilless Diaphragm Pump, model MOA-P122-AA (Gast Manufacturing Inc, Benton Harbor, MI) was used to provide a stable airflow to the ozone generators. The air to the ozone generators flowed through Drierite (anhydrous $CaSO_4$) to remove water from the air. After the air passed through the Drierite, it passed through the air pump and to a manifold where the airflow was divided into 9 air lines. Each air line then was connected to an ozone generator. After exiting the ozone generators, each line passed through a Matheson Instruments PM flowmeter, model PM-1000 (Matheson Instruments, Montgomeryville, PA) and an adjustable valve to control the airflow (figure 3.1). The air lines containing ozone were then merged with the airlines containing high humidity air.

**Experimental Treatments**

Eight different ozone concentration levels at two different application times were tested. A test using repeated ozone treatments was done using a rate of 1.2 mg ozone / min for 24 h initially and an additional 6 h treatment ever 3 d. Each test consisted of nine tubes of corn treated with ozone (see first four columns of table 3.1). Each treatment level and time was replicated three times. The treatment rates are listed in mg ozone per min, the treatment times are listed in hours (either 5 h or 24 h of treatment). After the ozone treatment time had

ended, the corn continued to be aerated for the remainder of the 9 d test. The test time was determined by the amount of time taken for 22% moisture, 32°C temperature, 8% visible mechanical damage, and an 0.88 hybrid multiplier corn content corn to reach 1% dry matter loss (equation 3.1; Bern et al. 2002).

After 9 d of storage, a final weight measurement was taken and the ending moisture content of the corn was determined using the standard oven method (ASABE 2003). The remainder of the corn was then spread two kernel deep over paper towels and allowed to air dry before being sent to Central Iowa Grain Inspection Corporation (Des Moines, IA) for damage kernel total determination.

**Statistical Analysis**

Experiment 1 was designed as an incomplete block design. Each block contained two experimental runs. The 16 treatments had three replicates with one replicate per block. A non-treated tube of corn was included in each experimental run, for six replicates. Data were subjected to the general linear model (GLM) procedure in SAS statistical software (SAS Institute Inc., Cary, NC). Differences between treatments were determined by means of an F test or least significant difference (LSD) with significance established at $p > F = 0.05$. The SAS code and results are located in Appendix C.

## Results and Discussion

Dry matter loss for the 5 h ozone treatments are shown in figure 3.2 and the dry matter loss for the 24 h ozone treatments are shown in figure 3.3.

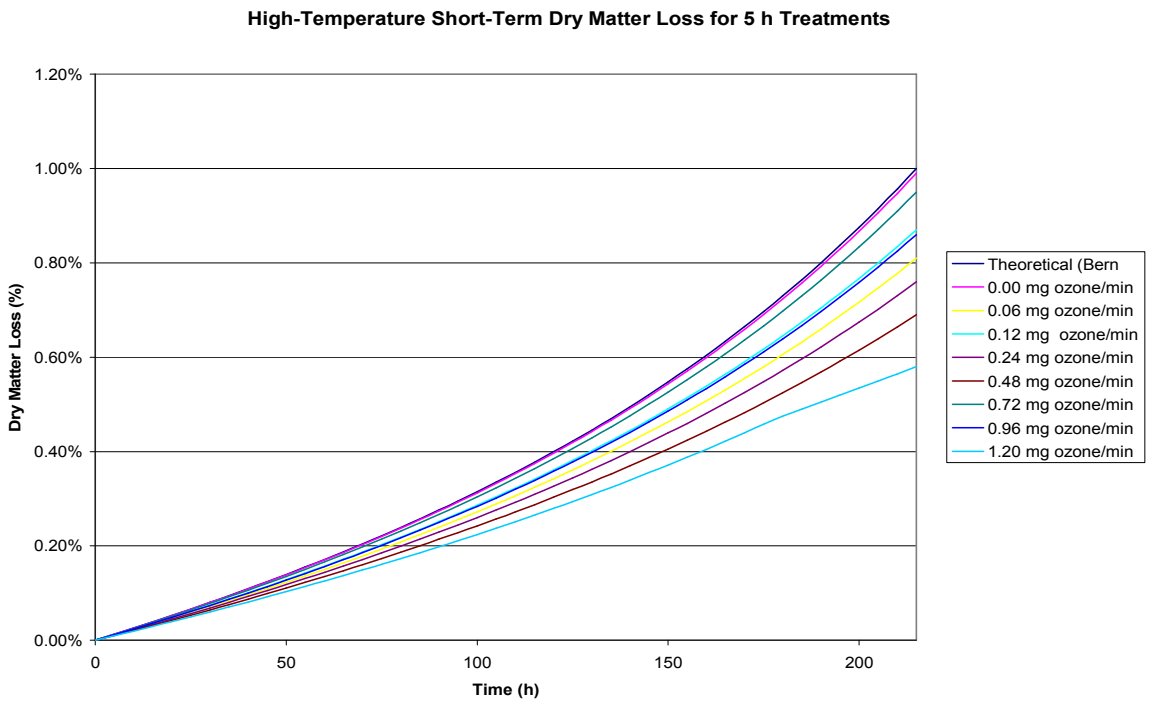


Figure 3.2 Dry matter loss of 5 h ozone treatments of corn estimated from carbon dioxide (each line represents 3 reps)

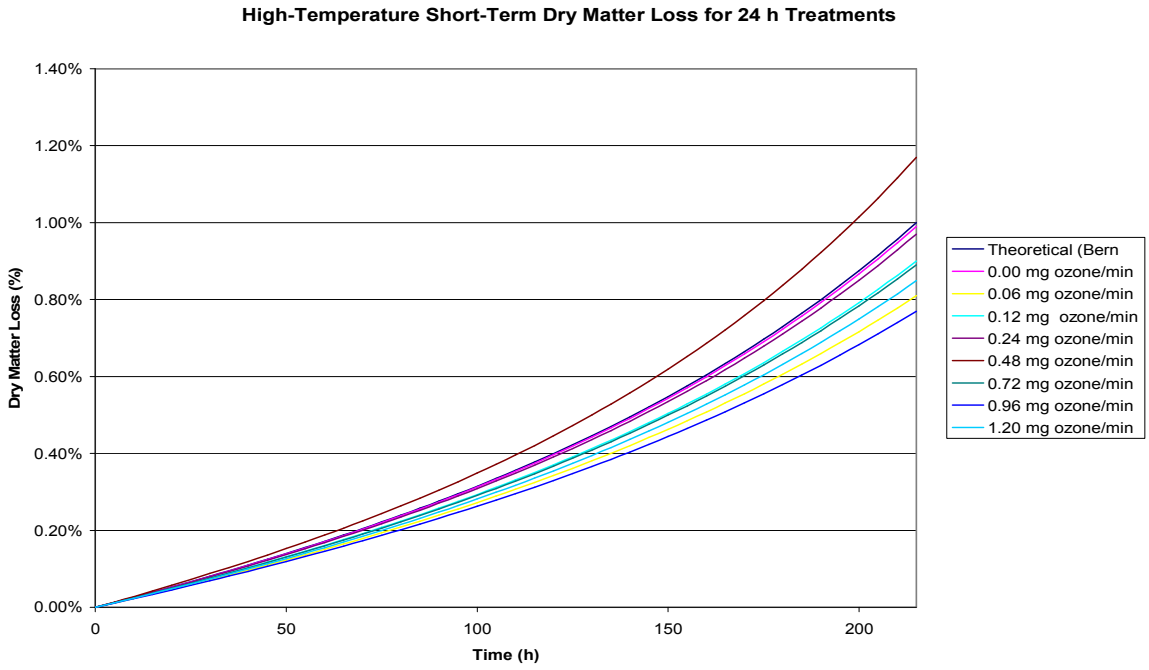


Figure 3.3 Dry matter loss of 24 h ozone treatments of corn estimated from carbon dioxide (each line represents 3 reps)

The dry matter loss and damage kernel total (DKT) for the short-term high-temperature tests were adjusted using SAS based on the final moisture content to minimize the difference caused by variance in moisture content. The final moisture content fluctuated between samples and was used as a covariate. The adjusted values are listed in table 3.1, and the raw data can be found in Appendix B. Figures 3.4 and 3.5 show the adjusted DKT and dry matter loss, where each point represents an average of 3 reps.

Table 3.1 Treatment levels and adjusted dry matter loss and damage kernel total for high-temp short-term experiment (each number represents 3 reps)

| Rate (mg ozone / min) | Time (h) | Total Ozone (mg) | Applied ozone per sample (mg ozone / g corn) | DM Loss (%) | DKT (%) |
|---|---|---|---|---|---|
| 0 | 0 | 0 | 0.00 | 0.99 | 9.9 |
| 0.06 | 5 | 18 | 0.03 | 0.81 | 8.0 |
| | 24 | 86 | 0.14 | 0.81 | 8.5 |
| 0.12 | 5 | 36 | 0.06 | 0.87 | 13.7 |
| | 24 | 173 | 0.29 | 0.90 | 8.2 |
| 0.24 | 5 | 72 | 0.12 | 0.76 | 9.4 |
| | 24 | 345 | 0.58 | 0.97 | 11.9 |
| 0.48 | 5 | 144 | 0.24 | 0.69 | 10.3 |
| | 24 | 691 | 1.15 | 1.17 | 9.0 |
| 0.72 | 5 | 216 | 0.36 | 0.95 | 16.3 |
| | 24 | 1036 | 1.73 | 0.89 | 13.1 |
| 0.96 | 5 | 288 | 0.48 | 0.86 | 15.0 |
| | 24 | 1382 | 2.30 | 0.78 | 12.3 |
| 1.2 | 5 | 360 | 0.60 | 0.58 | 8.1 |
| | 24 | 1728 | 2.88 | 0.84 | 16.2 |
| Rpt of 1.2 every 3 d | 42 | 2880 | 4.80 | 0.90 | 7.4 |

There was no significant effect ($p > 0.05$) between any of the treated corn compared to the non-treated corn for dry matter loss or damage kernel total (table 3.2). There was also no significant effect ($p > 0.05$) between the 5 h and 24 h ozone treatments for dry matter loss or damage kernel total. These results indicate that a single dose of ozone may not deactivate

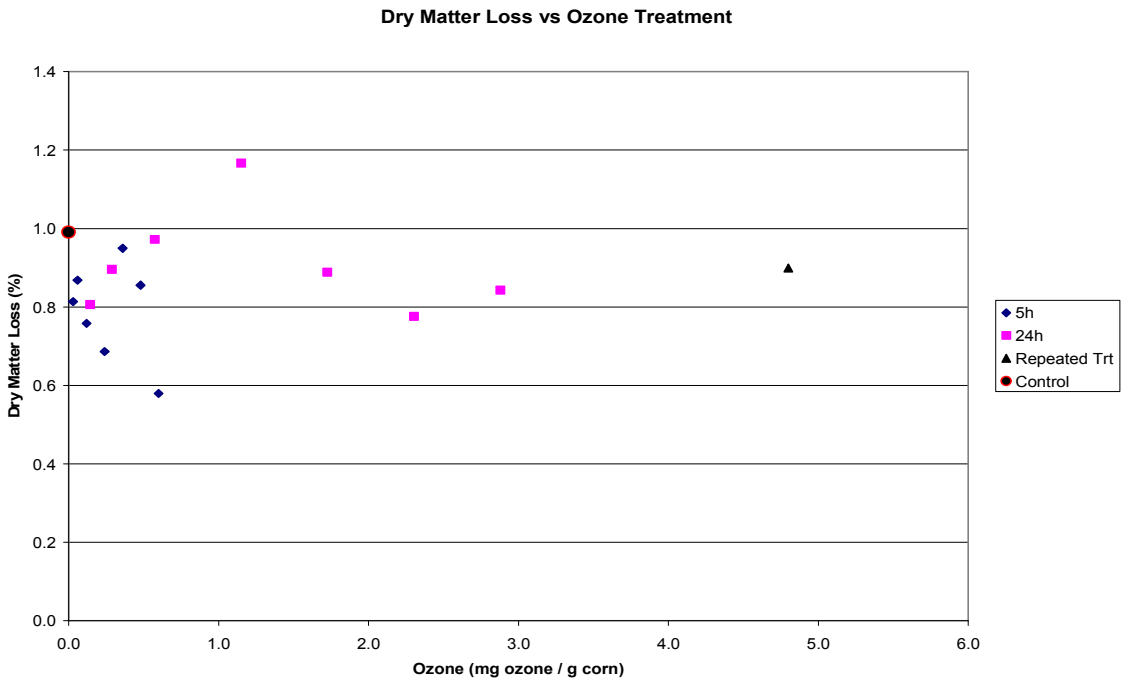


Figure 3.4 High-temp short-term dry matter loss (each point represents 3 reps)

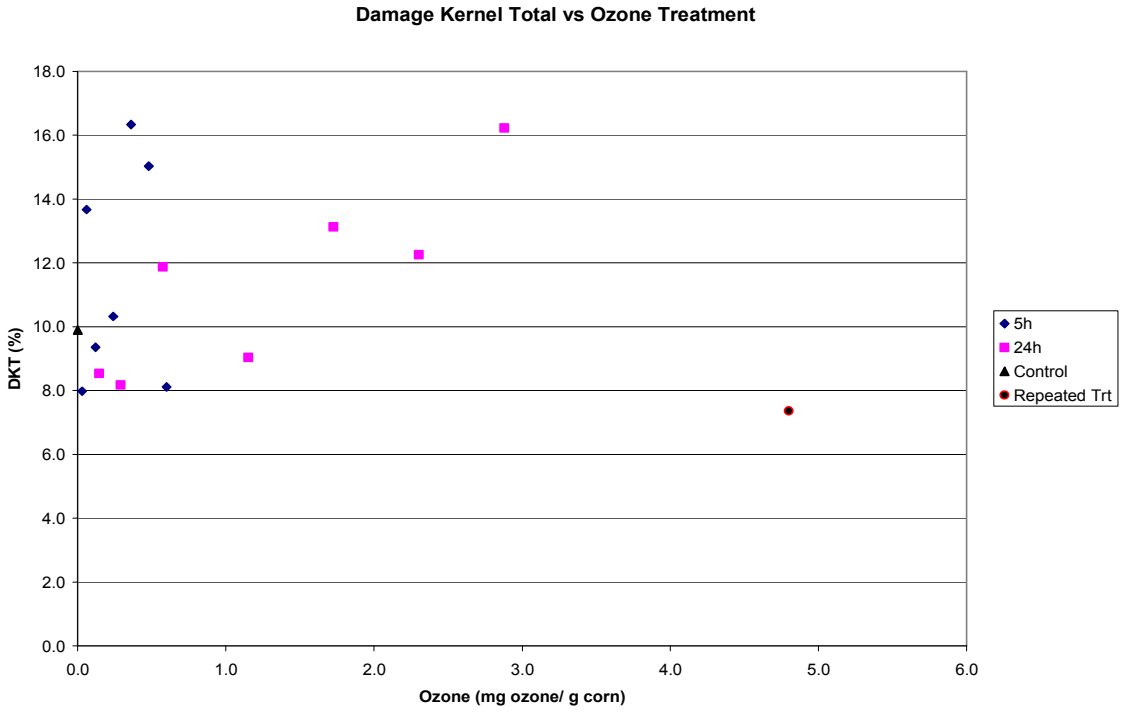


Figure 3.5 High-temp short-term damage kernel total (each point represents 3 reps)

sufficient levels of fungi to impact dry matter loss or damage kernel total. The repeated treatment also failed to have a significant effect ($p > 0.05$) on the dry matter loss or damage kernel total. Contrasts comparing each treatment were also not significant. These results indicate that repeated ozone treatments may not be effective in high moisture corn (22% moisture) at 32$^{o}$C and continuous aeration. Continuous ozone application or higher doses of ozone may have an effect, but these results do not support those possibilities. The shortened residual time of ozone under elevated temperatures and humidity coupled with the rapid growth rate of the fungi are likely factors for why storage of high moisture corn would be impractical.

Table 3.2 High-temp short-term experiment comparisons

| Variable | Contrast | *p* value | Estimate | Standard Error |
|---|---|---|---|---|
| DML | 5 h vs 24 h Treatment | 0.2175 | -0.11 | 0.08 |
| | Treated vs Control | 0.2650 | 0.13 | 0.12 |
| | 1.2 mg/min for 5h, 24h, repeated | 0.3347 | 0.19 | 0.19 |
| | | | | |
| DKT | 5 h vs 24 h Treatment | 0.8756 | 0.23 | 1.43 |
| | Treated vs Control | 0.5355 | -1.26 | 2.02 |
| | 1.2 mg/min for 5h, 24h, repeated | 0.1527 | -4.81 | 3.28 |

Even though the airflow was bottom to top, most visible fungal growth was first noted at the top of each corn tube, and progressed down over time. Possibly the corn located lower in the glass tube was subjected to more ozone than the corn at the top. This idea is drawn from how ozone might react and decompose as it progressed through the corn, reacting with active sites throughout the corn as noticed by Kells et al. (2001).

# EXPERIMENT II. LOW-TEMPERATURE HIGH-MOISTURE CORN STORAGE

## Objective

The objective of experiment II was to find the effect of ozone on dry matter loss and damage kernel total of corn stored under low temperature, and high moisture conditions.

## Procedure

### Sample Origin and Preparation

Corn samples used in the low temperature storage were Fontanelle 8R394 hybrid. Corn was harvested using a JD 4420 combine on October 2, 2006 from the Iowa State University Agronomy-Agricultural Engineering Research Farm, west of Ames Iowa. The initial moisture content of the corn was between 19 and 22% as measured with a Dickey-John GAC 2000 grain analysis computer (Dickey-John Corporation, Auburn, Illinois). After corn was harvested, it was cleaned using a 4.67-mm (12/64-inch) diameter round-hole screen in a CEA Carter-Day Dockage Tester (CEA Carter-Day Co., Minneapolis, Minnesota). The corn had 21% visible mechanical damage after cleaning. The corn was then stored at 5$^{o}$C until the start of the experiment on November 13, 2006.

Target moisture content for the low-temperature experiment was 26%. To increase the moisture content of the corn, it was placed in sealed bags and then distilled water was added with a spray bottle till the water content was achieved. Sealed bags were then placed in 5$^{o}$C storage for 2 d. The corn was then mixed and divided into 1.8-kg DM sample sets. The moisture content of the corn was determined using the standard oven method (ASABE 2003).

**Assembly**

Each sample was placed in a 2.4-L Rubbermaid Servin' Saver Canister with a perforated bottom. The 2.4-L canister was then suspended in a 5.0-L Rubbermaid Food Storage Select Canister (figure 3.6). The 5.0-L canisters contain at least 5 cm of distilled water to sustain the relative humidity of the air space inside the container, maintaining the equilibrium moisture content of the corn in the 2.4-L canister.

**Experimental Treatments**

Ozone was generated using Enaly OZX-300U (B) Ozone Generators (Enaly Trade Co. Ltd, Coquitlam, BC, Canada). There were five ozone treatment levels (0.0, 0.6, 1.2, 2.4, and 4.8 mg ozone / min) with three reps of each. Ozone was applied to the corn over a 24 h time period at an airflow rate of 0.47 L / min at a temperature of 23°C. In addition to the five ozone treatment levels, there were three repeated treatments done with an initial treatment of 4.8 mg ozone / min followed by an additional 1 h treatment of ozone at 4.8 mg ozone / min every 3, 6, or 12 d (first four columns of table 3.3). Three environmental chambers maintained at 15.5$^{o}$C were used, with one rep per chamber.

The samples were weighed every 3 d after the ozone treatment. Weighing the corn also allowed air to enter the sample in order to keep the system aerobic. The ending storage time

Figure 3.6 Low temperature corn storage container

was set at 30 d, based on calculations for corn at 26% moisture content, 21% visible mechanical damage, stored at 15.5°C to lose 5.2% dry matter loss (Bern et al. 2002). After 30 d of storage, a final weight measurement was taken and the ending moisture content of the corn was determined using the standard oven method (ASABE 2003). The remainder of the sample was then spread two kernel-deep over paper towels and allowed to air dry before being sent to Central Iowa Grain Inspection Corporation for determination of damage kernel

total. Dry matter loss was calculated by subtracting the ending dry matter from the starting dry matter.

**Statistical Analysis**

The experiment was setup as a randomized complete block design (RCBD). The three environmental chambers each acted as a block with one replicate of the eight treatments per block. Data were subjected to the general linear model (GLM) procedure in SAS statistical software (SAS Institute Inc., Cary, NC). Differences between treatments were determined by means of an F test or least significant difference (LSD) with significance established at $p > F = 0.05$. The SAS code and results are located in Appendix E.

## Results and Discussion

The results for experiment II are shown in table 3.3, figures 3.7 and 3.8. Ozone treatments did have an effect ($p < 0.05$) on dry matter loss when compared to non-treated corn (table 3.4). There was also difference between the dry matter loss of repeated treatment and a single treatment samples at the same ozone rate ($p > 0.05$). There was a difference between the dry matter loss of repeated ozone treatments compared to non-treated corn ($p > 0.05$) while there was no difference between single ozone treatments and non-treated corn ($p > 0.05$). There was no difference in damage kernel total between any of the treatment combinations compared to non-treated corn ($p > 0.05$).

This information indicates that ozone might be used to decrease dry matter loss under lower temperatures and non-aerated conditions. One possible reason why ozone had effect is that

ozone has a longer residual time at lower temperatures and fungi have a slower growth rate. The non-aerated conditions might have also had a positive impact on the storage of the corn

Table 3.3 Experiment II: Low-temp treatments and results
(each number represents 3 reps)

| Rate (mg ozone / min) | Time (h) | Total Ozone (mg) | Applied ozone per sample (mg ozone / g corn) | Dry Matter Loss (%) | Damage Kernel Total (%) |
|---|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 2.94 | 31.03 |
| 0.6 | 24 | 864 | 0.48 | 2.68 | 31.47 |
| 1.2 | 24 | 1728 | 0.96 | 2.25 | 26.87 |
| 2.4 | 24 | 3456 | 1.92 | 1.63 | 20.17 |
| 4.8 | 24 | 6912 | 3.84 | 1.72 | 31.67 |
| Rpt of 4.8 ever 12 d | 25 | 7776 | 4.32 | 1.32 | 31.63 |
| Rpt of 4.8 ever 6 d | 26 | 8928 | 4.96 | 1.26 | 22.80 |
| Rpt of 4.8 ever 3 d | 27 | 10656 | 5.92 | 2.06 | 36.33 |

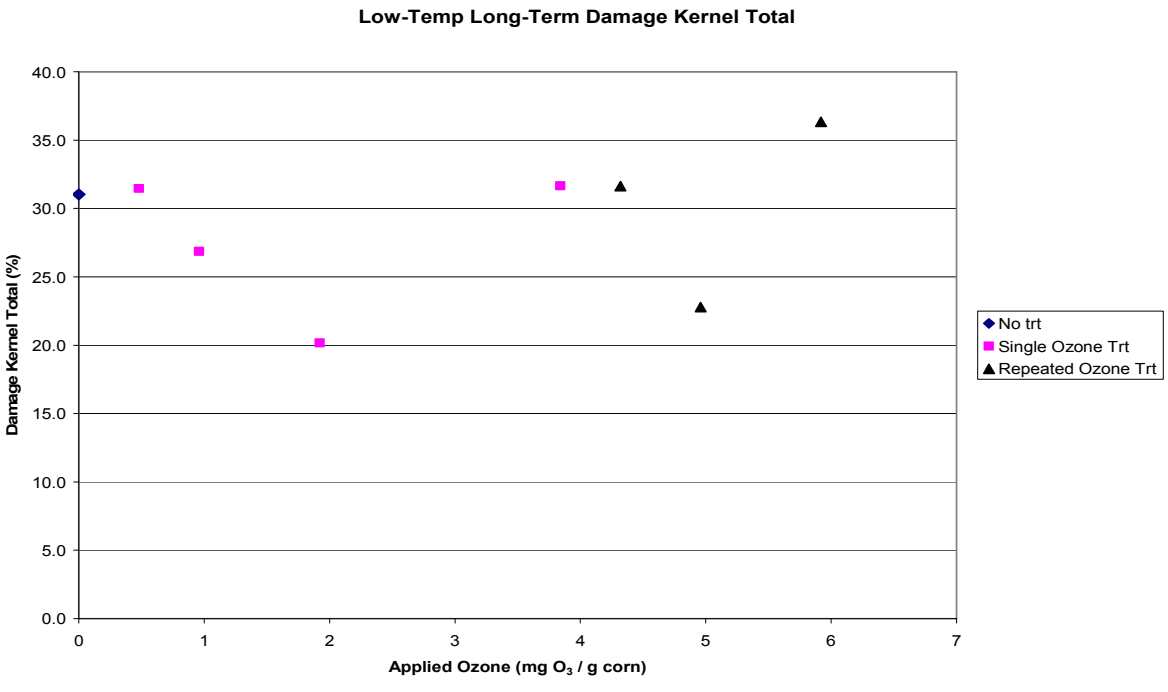

Figure 3.7 Low-temp (15.5°C) long-term (30 d) damage kernel total for 26% moisture corn (each point represents three replicates)

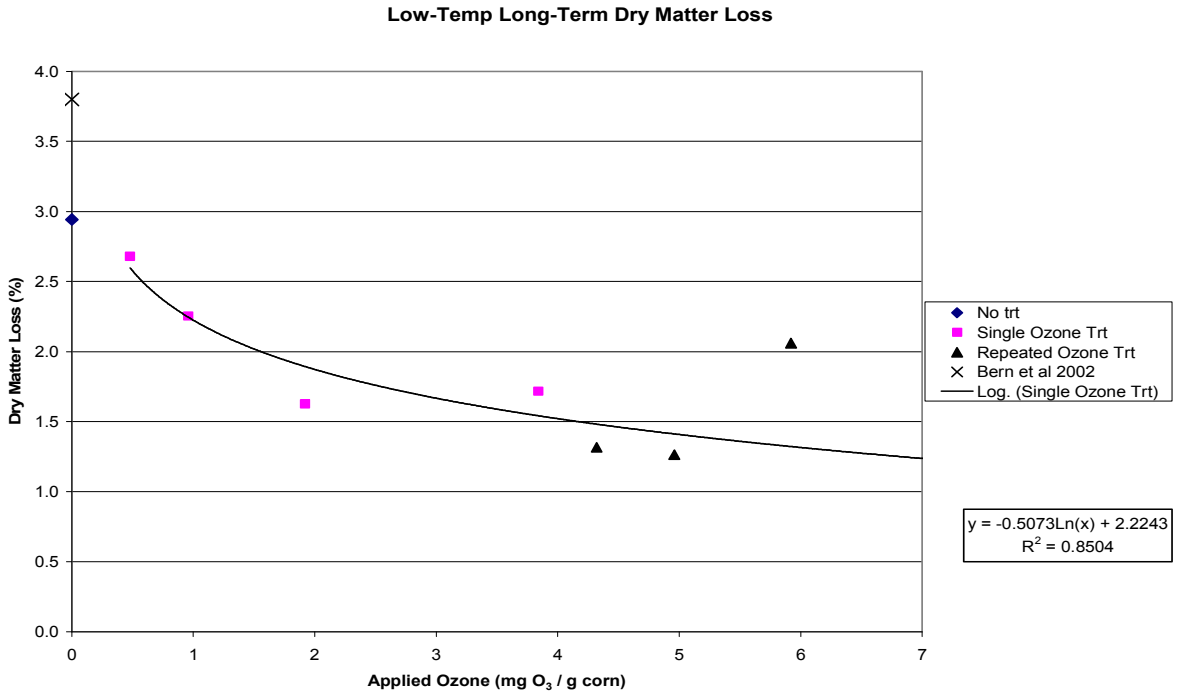


Figure 3.8 Low-temp (15.5°C) long-term (30 d) dry matter loss 26% moisture corn (each point represents three replicates)

Table 3.4 Low-temp (15.5°C) long-term (30 d) 26% moisture corn comparisons

| Variable | Parameter | Estimate | Standard Error | t Value | *p* |
|---|---|---|---|---|---|
| Dry Matter Loss | control vs trt | 1.098 | 0.428 | 2.570 | 0.022 |
| | 4.8 vs repeated 4.8 | 0.170 | 0.462 | 0.370 | 0.719 |
| | control vs single trt | 0.874 | 0.448 | 1.950 | 0.071 |
| | control vs repeated trt | 1.397 | 0.462 | 3.020 | 0.009 |
| | | | | | |
| Damage Kernel Total | control vs trt | 2.329 | 5.659 | 0.410 | 0.687 |
| | 4.8 vs repeated 4.8 | 1.411 | 6.113 | 0.230 | 0.821 |
| | control vs single trt | 3.492 | 5.918 | 0.590 | 0.565 |
| | control vs repeated trt | 0.778 | 6.113 | 0.130 | 0.901 |

when compared to the aerated experiment I. Under non-aerated conditions, outside fungi cannot enter the system. Another possibility is that fungi movement within the sample might be increased under aerated conditions, allowing fungi to spread quickly to areas that had lowered fungi levels due to ozone treatment.

## Conclusions

- Ozone appears to have a greater effect of controlling fungi under cooler conditions (15.5$^{o}$C) compared to warmer conditions (32$^{o}$C)
- Under continuous aeration and a higher temperature (32$^{o}$C), ozone failed to cause any significant effect on dry matter loss and dry matter loss
- In the high-temperature (32$^{o}$C) short-term (9 d) test and the low-temperature (15.5$^{o}$C) long-term (30 d) test, ozone had no impact on DKT values at any treatment level

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# CHAPTER 4

# ENUMERATION OF FUNGI FROM HIGH MOISTURE CORN TREATED WITH OZONE

A paper to be submitted to Food Microbiology

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***Abstract****. The effectiveness of ozone for preserving stored corn was studied by enumerating fungi in high moisture corn. Three moisture contents (18, 22, 26%) were treated with ozone at (0, 50, 500, 1000, 15000 ppm) for 1 h and an airflow rate of 0.47 L / min. The results showed that increasing ozone concentrations increased the number of uninfected kernels. These findings would indicate that ozone causes fungi inactivation and could have applications in corn storage or fungi control. Ozone appeared to have an increasingly inhibing effect on fungi genera in the following order: Penicillium, Mucor, other genera, Aspergillus, Fusarium, and Rhizopus.*

***Keywords.*** *High moisture corn, Ozone, Fungi enumeration, Fungi inactivation*

## Introduction

### Corn Production

Corn is one of the world's most abundant agricultural commodities. There was an estimated $6.83 \times 10^8$ Mg ($2.69 \times 10^{10}$ bushels) of corn produced globally during 2005-06. US production of $2.82 \times 10^8$ Mg accounts for 41% of global production for 2005-06 (USDA/Foreign Agricultural Service, 2006). The large quantities of corn produced globally incur some damage at every stage of handling, storage and processing. There is an estimated loss of more than $500 million each year due to damage caused by fungi and insects to grain stored in the United States (Kells et al. 2001). Some countries have losses approaching 50% for their stored grain (Allen et al. 2003).

**Corn Development**

Corn kernels attain maximum dry matter weight when they reach physiological maturity, usually at a wet basis moisture level between 35 and 25%[4] (Bern, 1998). After corn has reached physiological maturity, kernel moisture content decreases until harvest, usually at moisture levels between 25 and 17%. Once harvested, temperature and moisture conditions favor rapid growth of fungi in stored corn, making it necessary to either dry the corn or use some other preservation process (Bern, 1998).

**High Moisture Corn**

There are several reasons to look at harvesting high moisture corn. "Harvesting corn at high moisture content reduces field pest attacks, avoids bad-weather consequences, and minimizes field losses" (Aljinovic et al. 1994). The corn can be harvested from the field 2 to 3 weeks earlier than corn harvested for dry storage (Miller, 2002). Harvesting earlier puts the corn closer to the physiological maturity and subsequent maximum dry matter. The earlier harvest time can avoid a 3 to 8% dry matter loss that would accrue if the corn was left in the field to dry (Miller, 2002). The earlier harvesting also opens the possibility of having a higher quality residue left in the field (Miller, 2002).

High moisture corn also has several disadvantages, mainly rapid deterioration from fungal activity after harvest, and higher drying costs (Aljinovic et al. 1994). High moisture corn also has a lower market flexibility compared to dry corn (Miller, 2002). Storage fungi

species found in corn will grow in temperatures between -2 and 50$^{o}$C with an optimal growth temperature between 20 to 35$^{o}$C depending on the species (Paulsen et al. 2003). The fungi found in corn also need access to water in order to grow. The lower limit for growth conditions in most species is between 70 and 81% relative humidity or 13.5 to16.5% moisture in the corn (Paulsen et al. 2003).

## Fungi in Corn

While many microorganisms damage stored grain, the concern is mainly a few fungal species. Most bacteria and yeasts have little impact on corn storage outside of very high moisture conditions (Paulsen et al. 2003). Fungi are loosely defined as "eukaryotic, spore-producing, achlorophyllous organisms with absorptive nutrition that generally reproduce both sexually and asexually and whose usually filamentous, branched somatic structures, known as hyphae, typically are surrounded by cell walls" (Alexopoulos et al. 2004, pp. 2). The main conditions that affect fungal growth are air temperature, relative humidity and oxygen content of air surrounding the grain, conditions of the corn kernels, mold inoculum level, and previous grain storage history (Bern et al. 2002; Paulsen et al. 2003).

Mold growth in corn involves the mycelium on the surface of the grain tissue (Paulsen et al. 2003). Mycelium is the vegetative part of a fungus that is made of a mass of branching, threadlike hyphae (Madigan et al. 2006; Jay et al. 2005). Fungal enzymes are excreted and digest the grain structures, providing a source of energy for the mold (Paulsen et al. 2003).

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[4] All moistures are % wet basis

Heat, carbon dioxide ($CO_2$), and moisture are the byproducts of aerobic respiration from mold activity (Paulsen et al. 2003; Bern et al. 2002).

## Methods for Determining Fungal Activity

Because of the role fungi plays in grain storage, it is possible to monitor the fungal growth in order to determine the allowable grain storage time. Wadsö (1997) stated that "Growth is usually measured as a radial growth, mass increase or as number of spores produced." In addition to monitoring direct fungal growth, it is also possible to predict fungal growth based on off-gas composition, environmental conditions, and changes in corn dry matter.

## Detection of Fungi

There are several ways to determine if there is fungal growth on grain, including visual inspection, direct plating, and dilution plating.

### Direct Plating

Direct plating is used to obtain a more valuable mycological assessment and is more effective than dilution plating in detecting fungal species (Tournas et al. 1998). Direct plating is useful to identify microflora in the food and indicating a percentage of food infection. In the case of most grains and nuts, a surface disinfection should be done so that only fungi that invaded the food are enumerated. Surface disinfection is achieved by vigorously shaking 100 particles of the food product in a 0.4% freshly prepared sodium hypochlorite solution for 2 min. The chlorine is then removed from the food product by rinsing with sterile distilled water (Samson et al. 2004; Tournas et al. 1998). Non-surface disinfected samples can be plated to determine if the mold is from an internal or external invasion (Tournas et al. 1998).

The food particles are transferred using flame-sterilized forceps to plates at a rate of 5 to 10 particles per plate. The plates are incubated for 5 d at 25$^{o}$C with a constant atmosphere composition. The results provide a percentage fungi infected particle. These results can be broken down further into genera and species using a stereomicroscope or plating on a selective media (Samson et al. 2004; Tournas et al. 1998).

## Methods for Storing High Moisture Corn

The options for storing high moisture corn are restricted to chemical treatments or oxygen-limiting conditions. Each of these methods is used on a small portion of the overall corn stored each year. Approximately 5% of corn in Iowa is stored using oxygen-limiting conditions, while another 1% is stored using chemical treatments (Bern 1998).

## Ozone

A chemical that may be useful in storing high moisture corn is ozone. It has several properties that are desirable when compared to other chemicals used for corn preservation. Ozone is currently used as a disinfectant and reactant in several processes such as in water treatment, wastewater treatment, odor elimination, and pesticide removal (EPA, 2002).

### Ozone Properties

Ozone ($O_3$) is an unstable triatomic, allotropic structure of oxygen ($O_2$). The structural instability of the oxygen-oxygen bonds causes ozone to be a strong oxidizer. An oxidizing agent is a substance that causes the oxidation, that is, the loss of one or more electrons, from the atoms of another substance (Brown et al. 2006). The ability of a substance to act as an

oxidizing agent is referred to as its oxidizing potential and is measured in volts. Ozone has an oxidizing potential of 2.07 volts at a temperature of 25$^{o}$C, which is 150% of the oxidizing potential of chlorine (Bran, 2001; Malik et al. 2000; Novazone, 2006). Ozone has an affinity for the olefinic double bond that allows it to react with a large number of chemical groups (McKenzie et al. 1997).

### Ozone Production

The instability of ozone makes it necessary to generate ozone at its point of application. Methods of generating ozone include ultraviolet (UV) light, cold plasma, corona-discharge, chemical, thermal, chemonuclear, and electrolytic methods (Kim et al. 1999). Of the options to produce ozone, corona-discharge units are most common (EPA 1999) because they can produce greater concentrations of ozone, have longer unit stability, and are more cost effective than the other production methods(Linntech, 2005; Ozone Solutions, Inc., 2006).

## Ozone as a Disinfectant

A review by Kim et al. (1999) noted that ozone is capable of decreasing microbial populations, the chemical and biological oxygen demand, and the quantity of toxic organic compounds within the treated environment. Ozone has been considered at for many applications including food preservation, artificial aging of beverages, odor control, and medical therapy. The main uses of ozone are currently drinking water treatment and municipal and industrial wastewater treatment (Graham 1997).

Khadre et al. (2001) made the following statement, "Presence of organic substances with high ozone demand may compete with microorganisms for ozone. Viruses and bacteria associated with cells, cell debris, or feces are resistant to ozone, but purified viruses are readily inactivated with the sanitizer."

**Ozone as a Food Preservative**

Ozone displays several characteristics that make it ideal for use as a fumigant in foodstuff. Gaseous ozone has been known to posses antimicrobial traits for over 120 years (Jay et al. 2005, pp. 312-314). As a powerful oxidant, ozone quickly inactivates microorganisms such as viruses, bacteria, and fungi (Majchrowicz, 1998) and kills small invertebrates such as insects (Mendez et al. 2003). Ozone is also capable of decreasing levels of toxic organic compounds (Kim et al. 1999). The effects of ozone can be short-lived as it quickly dissipates into $O_2$ (Bran, 2001) and has a limited residual effect (Majchrowicz, 1998). The quick dissipation time coupled with the absence of residual toxins makes ozone a well-suited treatment for most food materials (FDA, 2002) and it has been shown to extend the shelf life of certain food (Jay et al. 2005, pp. 55-56). These are also reasons why ozone was given a "generally recognized as safe" (GRAS) classification from the FDA (FDA, 2002; Suslow et al. 2004). Ozone has been approved for food use in Australia, France, and Japan (Jay et al. 2005, pp. 55-56; Graham 1997). It has also been noticed that ozone has no measurable effect on the nutritional content or germination of treated grain samples of wheat, corn, or soybeans (Mendez et al. 2003) but can cause changes in other food products such as an increase in rancidity in high-lipid-content foods (Jay et al. 2005, pp. 55-56) and changes in volatile oil constituents in ground pepper (Zhao and Cranston 1995). A few negative aspects of ozone

are that the oxidizing effects of ozone make it toxic at high doses (Bran, 2001) and steps may be necessary to destroy off-gases to prevent worker exposure (EPA 1999). The highly reactive nature of ozone also requires the use of corrosion-resistant equipment such as stainless steel.

While ozone can be effective on controlling microorganisms, integrating ozone into foodstuff preservation can be more challenging. There have been several studies done that prove that ozone is effective in inactivating fungi in food, acting to reduce fungal spore production on food surfaces and to decrease spread of fungi to adjacent produce. The use of gaseous ozone appears to be the most effective in cooler temperature storage (temperature range not provided) and a relative humidity between 85 to 95% (Suslow 2004). Gaseous ozone is only effective as a surface treatment because it does not penetrate natural openings or wounds in sufficient amounts to control microorganisms (Suslow 2004) and antimicrobial action occurs primarily on the surface with water phase food because of the rapid decomposition of the ozone (Kim et al. 1999).

**Ozone Used for Microorganism Control**

Concentrations of ozone between 0.1 and 0.5 ppm for short periods of time have been proven to be effective against gram-positive and gram-negative bacteria, viruses, and protozoa (Jay et al. 2005, pp. 312-314; Kim et al. 1999). Khadre et al. (2001) states that **"**Inactivation of microorganisms by ozone is a complex process because of the multiple cellular sites which ozone can affect. These sites include proteins, unsaturated lipids and respiratory enzymes in

cell membranes, peptidoglycans in cell envelopes, enzymes and nucleic acids in the cytoplasm, and proteins and peptidoglycan in spore coats and virus capsids."

***Ozone Effect on Cell Envelopes***

One common theory is that ozone inactivates microorganisms by targeting cell membranes, thereby disrupting permeability functions (Jay et al. 2005, pp. 312-314; Kim et al. 1999). In one case, cell leakage was detected from bacterial cells treated with ozone, indicating a change in the cell permeability and the possibility of lysis of the cell wall (Kim et al. 1999). The double bonds of unsaturated lipids that are part of the cell envelope are mentioned as the primary site for ozone reaction in bacteria. Another proposed action site for gram-negative bacteria is the lipoprotein and lipopolysaccharide layers.

***Ozone Effect on Spores and Oocyst***

Microorganisms have the ability to survive longer in a dormant, such as spores and oocyst than in an active state (Jay et al. 2005, pp.687-688). An oocyst is the spore phase of certain protists (Prescott et al. 1999, pp. 822). In the case of spores, the cells are more resistant to environmental stresses including heat, ultraviolet radiation, chemical disinfectants, and desiccation (Prescott et al. 1999, pp. 66). While ozone does work on oocysts, a higher dosage and treatment time is needed (Jay et al. 2005, pp.687-688). A review by Khadre et al. (2001) stated that "Ozone is generally more effective against vegetative bacterial cells than bacterial and fungal spores." One example is in a study done on mold spores (*Neosartorya fischeri*) found an intermediate resistance to ozone. In another case, spores of 8 *Bacillus* spp., *B. stearothermophilus* where shown to have the highest level of resistance among all

the tested species (Khadre et al. 2001). Smilanick (2003) reported that 1 h of 200 ppm ozone was sufficient to deactivate spores *Penicillium digitatum* (green mold), *P. italicum* (blue mold), and *Geotrichum citri-auranatii* (sour rot).

**Ozone Used for Fungi Control**

A review of food use of ozone by Kim et al. 1999 stated that ozone could be an effective fungicidal agent. Fungal spores have a microbicidal activity threshold to aqueous ozone (0.3 to 0.5 mg / L) at 90 to 180 minutes exposure for *Aspergillus* spores, 45 to 60 minutes exposure for *Penicillium* spores, and 5 to 10 minutes exposure for *Candida paracreus* spores.

Ozone was looked at as a possible substitute for gaseous ethylene oxide to decontaminate pepper (Zhao and Cranston 1995). Whole peppercorn (*Piper nigrum* L) and ground black pepper was used to determine the effects of ozone on the volatile oil constituents and microbial populations. In all of the experiments, ozone concentrations were kept at 6.7 mg / L and an airflow of 6 L / min (the volume of the reaction vessel was not provided). In the first experiment, 500 mL suspensions of $10^{11}$ to $10^{12}$ cfu / L cultures of *Escherichia coli*, *Salmonella* ssp, *Staphylococcus aureus*, *Bacillus cereus*, *Penicillium* ssp, and *Aspergillus* ssp were sparged with ozone. A 10-mL aliquot of each treated suspension was then removed and enumerated using standard plate count methods for the aerobic bacteria, *Penicillium* ssp, and *Aspergillus* ssp. Anaerobic bacteria and mesophilic aerobic sporeformers were also enumerated using standard plate count methods with the exceptions that the anaerobic bacteria were incubated under anaerobic conditions and tryptone glucose extract agar was used for the mesophilic aerobic sporeformers. A five log reduction, effectively a 99.99%

inactivation, was achieved for each organism after the following ozone concentration was applied: *E. coli*, 0.4 g / L; *Salmonella* ssp, 0.4 g / L; *S. aureu*s, 1.2 g / L; *B. cereus*, 1.2 g / L; *Aspergillus* ssp, 0.4 g / L; and *Penicillium* ssp, 1.8 g / L. The concentrations found for the two fungi would indicate that *Aspergillus* is more susceptible to ozone than *Penicillium*. In the second experiment whole peppercorn samples of 200 g were immersed in 500 mL distilled water. The water-spice mixture was then sparged with ozone for various times. A 50 g sample was then removed for enumeration and for gas chromatography (GC) tests. A 3 to 4 log reduction in total aerobic bacteria, total anaerobic bacteria, and mesophilic aerobic sporeformers was realized after 10 min of ozone treatment. The GC of the volatile oil showed that ozone caused only a slight variation from untreated samples and that no new compounds were created. In the third experiment 500 g of ground black pepper was sparged with ozone in a rotating flask for various times. Three moisture contents (39.0, 104.0, and 176.0 g water / kg ground black pepper) were tested to determine its effects with gaseous ozone. A 50 g sample was then removed for enumeration and for gas chromatography (GC) tests. A 3 log or greater reduction in *Salmonella* ssp and *E. coli* was realized after 60 min of ozonation at 40 mg / min and an airflow rate of 6 L / min. A similar reduction was seen in *Penicillium* ssp after 40 min and a greater than 4 log reduction was seen in *Aspergillus* ssp after 10 min. The three moisture contents showed that the higher moisture had the greatest reduction of the microbial load. The effects of moisture only became evident after 2 h of ozonation. The GC tests of volatile oils showed that ozone caused several changes in the final composition of the ground pepper. Ozone eliminated 16 components that were detected in the untreated samples. Ozone was also responsible for the creation of 14 new components that were only found in the ozone treated samples. The concentrations of several individual

components found in both the treated and non-treated sample were considerably different. The study concluded that ozone application to ground pepper is not a viable alternative to current chemical practices due to the chemical composition change, but ozone may be useful in treating un-ground peppercorn.

A study conducted by Beuchat et al. (1999) used aqueous ozone to inactivate aflatoxigenic species of *Aspergillus flavus* (NRRL 3357) and *Aspergillus parasiticus* (NRRL 2999) suspended in a phosphate buffer solution with one of two pH conditions (pH 5.5 or 7.0). Suspensions of 0.1 mL of conidia in sterile 1mM phosphate buffer had a gas stream bubbled through at an airflow rate of 0.8 L / min with 21 mg $O_3$ L / min (ca. 21-ppm / min). The ozone concentration in the conidia suspension was then controlled at 1.74 ppm for the duration of the treatment. A 0.5 mL of the suspension was withdrawn for testing at intervals of 2, 4, and 6 min after inoculation. The withdrawn suspension was then 10-fold serially diluted in phosphate buffer and surface plated on PDA. The CFU for each plate were counted after 3 d of incubation at 25$^{o}$C. The times required for 90% inactivation of the fungi conidia (D-values) were then determined from slopes of the regression lines using a general linear model of the Statistical Analysis System. The results showed that the D-value for *A. flavus* conidia treated with 1.74-ppm ozone was 1.72 min in pH 7.0 and 1.54 min in pH 5.5. The D-value for *A. parasiticus* with the same ozone treatment was 2.08 min in pH 7.0 and 1.71 min in pH 5.5. There was not a significant difference ($P > 0.05$) between the D-values in relation to the pH conditions for both fungi tested. There was also no significant difference between the D-values for each of the two fungi tested.

A study done by Li and Wang (2003) tested the effectiveness of ozone as a surface disinfectant by exposing agar plates of *Escherichia coli*, *Bacillus subtilis*, *Candida famata*, and *Penicillium citrinum* to several ozone dosages. Each of the four organisms is different from the others: *E. coli* is a gram-negative, non-spore-forming bacterium; *B. subtilis* is a gram-positive, endospore-forming bacterium; *P. citrinum* is a mold; and *C. famata* is a yeast. In each case, the microorganism suspension was diluted to $10^5$ CFU / mL. Then 0.2 mL of the dilution was spread on either trypticase soy agar (TSA) or malt extract agar (MEA), producing roughly <100 CFU / plate. The effective range of ozone concentrations were determined and then applied to each organism for a set period for ozone dosages and treatment times for each microorganism). The airflows used for each test were not listed in the literature. After the ozone treatment, the plates were incubated for 24 or 48 h at a temperature of 37 or 25$^{o}$C depending on the media used. Ozone treated plates were then compared to the non-treated plates and a survival fraction was calculated for each microorganism. Two ranges of relative humidity (55 - 60 and 85 - 90%) were used to test the influence it had on the survival fraction of ozone treated microorganisms.

There was an exponential decline in the survival fraction with increases in ozone concentrations. Another observation was that the germicidal efficiency of ozone on the surface increased as relative humidity increased. When the four microorganisms were compared, *E. coli* was found to be the most susceptible to ozone (2 – 2.5 and 3.5 – 4 mg ozone for 50 and 80% inactivation) while *B. subtilis* was the least susceptible (45 – 70 and 145 – 150 mg ozone for 50 and 80% inactivation).

***Ozone Used in Fruit Storage***

The effect of a continuous exposure to a low dose of ozone was tested on peaches and table grapes by Palou et al. (2002). Both fruits were harvested at commercial maturity from the San Joaquin Valley. After being harvested, the fruit was superficially disinfected by submersion in a diluted bleach solution (0.5 % sodium hypochlorite) for 1 minute. To test the effect of ozone on wounded fruit, peaches were then wounded with a 1-mm probe tip and inoculated with *Monilinia fructicola, Botrytis cinerea, Mucor piriformis, or Penicillium expansum*. Four 20-fruit trays inoculated with a pathogen were placed in a storage room held at 20$^{o}$C, 90% relative humidity, and 0.3 ppm ozone for 4 weeks. To test the effects of ozone on wounded grapes, grapes were inoculated with spores of *B. cinerea* by spraying one sample set and injecting the spores into another sample set. The grapes were then stored for 7 weeks in the same conditions as the peaches. Another test was done with peaches and grapes to test for the physiological response of the fruit to 0.3-ppm ozone exposure compared to ambient air under 20$^{o}$C and 90% relative humidity conditions over several weeks. The results showed that 0.3 ppm was effective in inhibiting the normal aerial growth of mycelia and preventing spores from being produced and spread in the wounded peaches, but had no noticeable effect on the pathogen activity within the wounds. Ozone also did not reduce mold incidence on the inoculated grapes. In the physiological test with peaches, the ozone exposed fruit lost more weight. That would indicate that the ozone might have damaged the peaches' cuticle or epidermal tissue. It was also noted in all the experiments that airflow plays a crucial part in

the effectiveness of ozone gas. More mycelia growth was seen where ozone was partially stopped from contacting fruit because of the plastic trays used to hold the fruit.

In a previous study using table grapes, Sarig et al. (1996) showed that 8 mg of ozone per min for 20 min was effective in controlling fungi, yeast, and bacteria. Table grapes were collected directly after being harvested in Israel and inoculated with *Rhizopus stolonifer* at a concentration of $10^7$ spores per mL distilled water. Ozone was applied to 2-kg samples at a rate of 8 mg per min for time intervals between 0 and 80 min. They found that ozone applied at that rate for 20 min was effective in reducing fungi colony forming units from 40 to less than 10.

Palou et al. (2003) showed that the capability of ozone to deactivate microorganisms is heavily dependant on ability of the gas to come into contact with the microorganisms. This was tested by treating navel oranges (*Citrus sinensis* (L.) Osbeck) in different storage conditions with ozone. Four types of packaging were used: standard corrugated fiberboard citrus cartons (2.6% vented surface area), returnable plastic containers with uncovered fruit (35.9% vented surface area), returnable plastic containers with bagged fruit (0.7% vented surface area), and corrugated fiberboard Master carton with bagged fruit (2.9% vented surface area). The oranges were inoculated with a $10^6$ spores / mL suspension of either *Penicillium digitatum* or *Penicillium italicum*. After a 24-h inoculation period, the oranges were stored in either of two 678 m3 cold storage room held at 12.8$^{o}$C for 14 d. One storage room had a continuous exposure to ozone that was discharged into the room at a rate of 2.5 g / h. The other room was used as a control and received no ozone. There were no spores

present on the oranges stored uncovered in the returnable plastic containers, which also had an 81.9% ozone penetration. The other storage methods had lower vented surface areas, impacting ozone penetration (>17%) and 5 to 60% spore coverage on the fruit.

***Ozone Used in Grain Storage***

A study by Allen et al. (2003) used gaseous ozone to inactivate fungi in barley grain. They tested the effects of four different ozone doses on barley using four moisture contents (19, 22, 25, and 30%), and three temperatures (0, 20, and 40$^{o}$C). Ozone application rates were 0.98, 0.16, 0.08, and 0.04 mg / g barley min. After five minutes of ozonation at a rate of 0.16 mg of ozone / g barley min, the ozone had caused a 96% inactivation of spores. The inactivation of spores increased as ozone dose increased, going from around 60% spore survival at an ozone application of 0.04 mg / g barley – min, to less than 4% spore survival at an ozone application of 0.98 mg / g barley – min. They also noticed that higher temperatures and water activity increased the inactivation percentage of fungi on the barley. With a 0.98 mg / g barley-min and a temperature of 20$^{o}$C, a 19.6% spore survival rate occurred at 19% moisture while less than 4.2 % spore survival rate occurred for 30% moisture. The reaction to temperatures of 0, 20, and 40$^{o}$C had respective spore survival rates of 36.2%, 13.95% and, 4.2% with an ozone rate of 0.98 mg / g barley-min and 19% moisture content.

Kells et al. (2001) looked into the effect of gaseous ozone on three insect species and the fungal species *Aspergillus parasiticus*, Speare strain ATCC 24551 on corn. They were able to identify that particular strand of *A. parasiticus* based on the production of a metabolite in the aflatoxin biosynthetic pathway, averufin, which can be identified by an orange color

when grown on potato dextrose agar medium. A 30 g sample of corn was first seeded with *A. parasiticus* and then divided into screen cages. The cages were then placed 2 cm below the surface of a 12.7 Mg capacity steel grain bin filled with corn. The corn was then treated with 50-ppm ozone for 3 d or 25 ppm for 5 d. The same corn and grain bin was used for all treatments. The temperature, corn moisture content, and relative humidity were several factors that would impact the effectiveness of ozone but were not provided in the research paper. After the treatment, the corn was washed with a Triton X-100 solution to remove fungi, and then plated on the dextrose agar. Colony counts from the plates showed that a 63% reduction in *A. parasiticus* occurred after the 3 d treatment of 50-ppm ozone. The 25-ppm treatment for 5 days failed to significantly reduce the fungi counts when compared to control samples.

While effects of fumigating corn with ozone have been tested, reports on effects of using ozone on different fungi genera in corn have not been found.

## Objective

The objective of this experiment was to test by enumeration the effect of four ozone concentrations on fungi from corn at three different moisture contents.

## Procedure

### Sample Origin and Preparation

The corn samples used in the fungi enumeration were Fontanelle 8R394 hybrid. The natural fungi populations were tested in the enumeration study. The corn was harvested using a JD

4420 combine on October 2, 2006 from the Iowa State University Agronomy-Agricultural Engineering Research Farm, west of Ames Iowa. The initial moisture content of the corn was between 19 and 22% as measured with a Dickey-John GAC 2000 grain analysis computer (Dickey-John Corporation, Auburn, Illinois). After the corn was harvested, it was cleaned using a 4.67-mm (12/64-inch) diameter round-hole screen in a CEA Carter-Day Dockage Tester (CEA Carter-Day Co., Minneapolis, Minnesota). The corn was then stored at 5$^{o}$C until the start of the experiment in March 2007. The moisture content of the corn had changed to 17.5% at the start of the experiment.

There were three moisture contents (26, 22, 18%) used in the fungi enumeration. To increase the moisture content of the samples, the corn was placed in sealed bags and then distilled water was added with a spray bottle till the desired water content was achieved. The sealed bags were then placed in 5$^{o}$C storage for 2 d. The moisture content of the corn was determined using the standard oven method, ASABE standard S352.2 (2003).

**Assembly**

The treatment containers (figure 4.1) used for the fungi enumeration were 6.6 x 28.9-cm (2 5/8 x 11 3/8 inches) inner dimensions molded polycarbonate cylinders (W.A. Hammond Drierite Co. Xenia, OH). These containers were fitted with 6.4-mm (¼ inch) barbs at the top and bottom that allowed airflow through the container. The corn was suspended 2.5 cm from the base of the cylinder with a metal screen.


Figure 4.1 Fungi enumeration container

**Experimental Treatments**

Ozone was applied at rates of 0, 50, 500, 1000, and 15000 ppm for 1 h at an airflow rate of 0.47 L / min to corn in the treatment chamber. Ozone concentrations of 0, 50, 500, and 1000 ppm were generated using Enaly OZX-300U (B) Ozone Generators (Enaly Trade Co. Ltd, Coquitlam, BC, Canada). An Ozat Ozone Generator, type CF-0 (Ozonia, Ltd., Switzerland) was used for the 15,000 ppm ozone concentration. The 50 ppm concentration was only tested at the 18% moisture content level because initial data indicated that ozone at this level

had little effect. The containers were held at a temperature of 23 °C for the duration of the experiment. After the treatment time, the containers were closed and allowed to sit for 15 min. Then the containers were moved to a sterile biological hood for processing.

**Enumeration**

After the ozone treatment, the corn samples were surface disinfected by vigorous shaking in a solution of 5250 ppm sodium hypochlorite (10% bleach) for 2 min. After the corn had been surface disinfected, it was rinsed three times with sterilized distilled water to remove any remainder of the sodium hypochlorite solution. Then 100 kernels were planted into modified malt extract agar (MEA) plates, with 5 kernels per plate. The modified MEA (product number: M 8927, Sigma, Sigma-Aldrich, Inc., Saint Louis, Missouri) was prepared according to product instructions. The plates were then incubated at a temperature of 25°C for 5 days in a dark environment. After the incubation period, fungi colonies were counted and identified according to genus (identification key in Appendix I). The number of uninfected kernels (kernels that had no fungi present after the incubation period) was also recorded. The number of fungi present was also totaled as the sum of infections. Additional plates were made on Czapek Dox agar and modified MEA for identification. The Czapek Dox agar (product number: 70185, Fluka, Sigma-Aldrich, Inc., Saint Louis, Missouri) was prepared according to product instructions.

**Statistical Analysis**

The experiment was a completely randomized design (CRD) with three replications of each treatment by moisture combination. Data were subjected to the general linear model (GLM)

procedure in SAS statistical software (SAS Institute Inc., Cary, NC). Differences between treatments were determined by means of an F test or least significant difference (LSD) with significance established at $p > F = 0.05$. The SAS code and results can be found in Appendix E.

## Results and Discussion

### Uninfected kernel percent

The uninfected kernel percent at each treatment level and moisture content is shown in figure 4.2. Statistic comparison results for ozone rate, moisture content, and ozone rate by moisture content are shown in table 4.1. The effect of the rate of ozone applied was significant ($p < 0.0001$) in uninfected kernel % at all moisture contents. As the ozone concentration increased the number of uninfected kernels also increased. The difference among the three moisture content levels of the corn was also significant ($p = .0019$). A decrease in the number of uninfected kernels was seen as the moisture content increased. The same results were found when looking at the sum of infections (figure 4.3). A decrease in uninfected kernels as moisture increases is different than the effect seen in barley (Allen et al. 2003) and ground black pepper (Zhao and Cranston 1995) where an increase in moisture content led to a decrease in spore survival. A possible reason for this could be that airflow characteristics through barley, ground pepper, and corn are different due to the different sizes and shapes of each material. Other possibilities could be the competing chemical reactions may be different for various treated products or the seed coat of a corn kernel may also be less protective than in barley, being more likely to offer fungi shelter in damaged sections (cracks) that ozone would not penetrate.

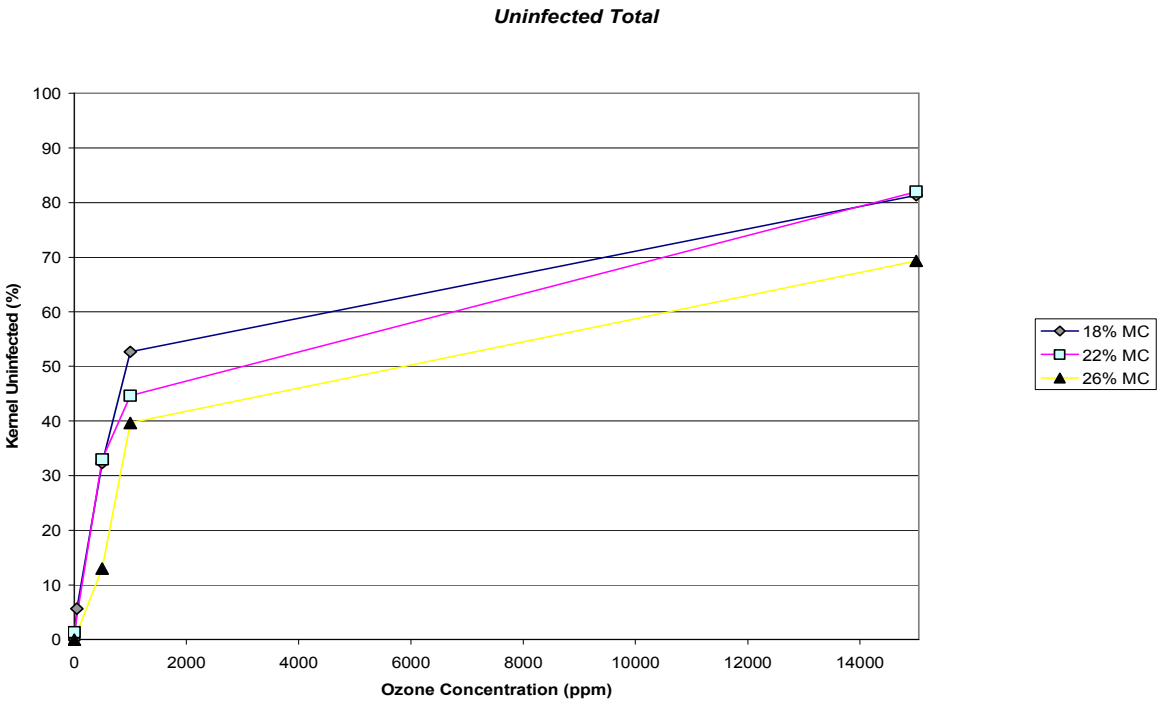


Figure 4.2 The effect of ozone concentration on uninfected kernels (each point is the average of 3 replications)

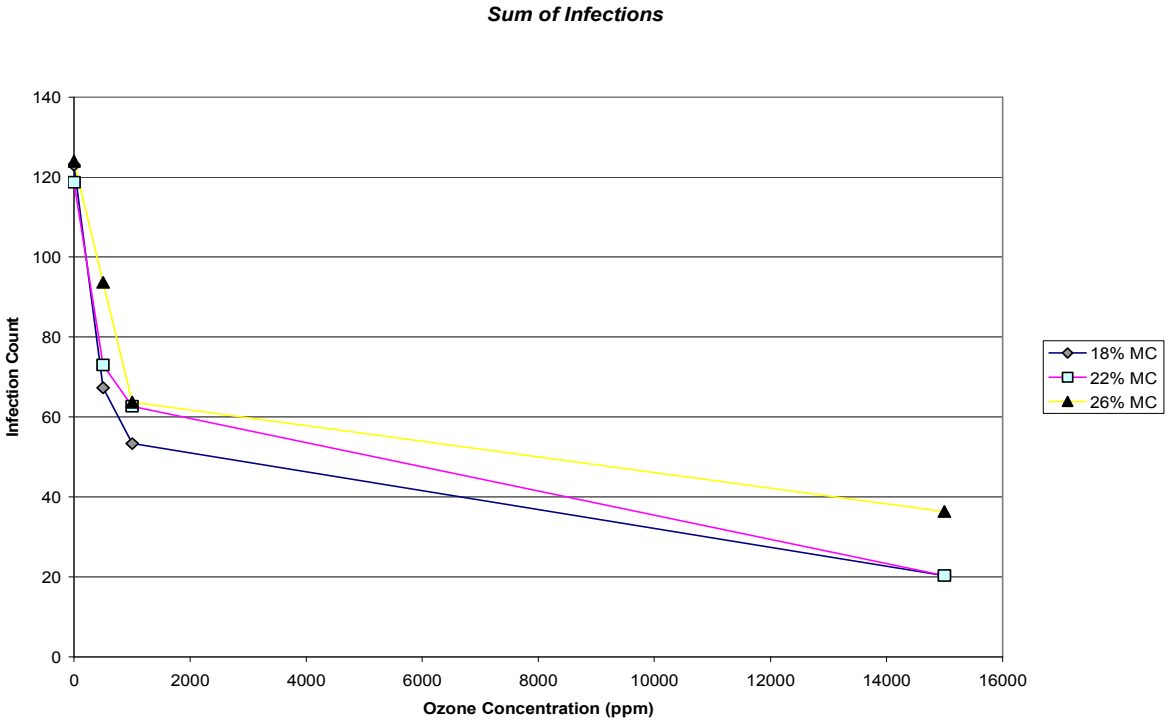


Figure 4.3 The effect of ozone concentration on fungal infections on corn (each point is the average of 3 replications)

**Influence of ozone on genera**

Genera that appeared frequently in this experiment were *Aspergillus*, *Fusarium*, *Rhizopus*, *Penicillium*, and *Mucor*. In all of these genuses, the rate of ozone applied was significant ($p < 0.0001$). All non-frequent genera were grouped together as "other" and also showed that ozone rate had an effect ($p = 0.0092$). This indicates that ozone has the effect of decreasing the fungi that were present on the corn. Figures 4.4 to 4.8 show the number of times each genus was present in at each treatment. The moisture content was only significant in *Mucor* ($p = 0.0132$), *Penicillium* ($p = 0.0115$), and *Rhizopus* ($p < 0.0001$). Figure 4.9 shows the number of infections for each genus at each ozone treatment level. The moister contents were pooled together because there was no interaction between moisture content and treatment levels.

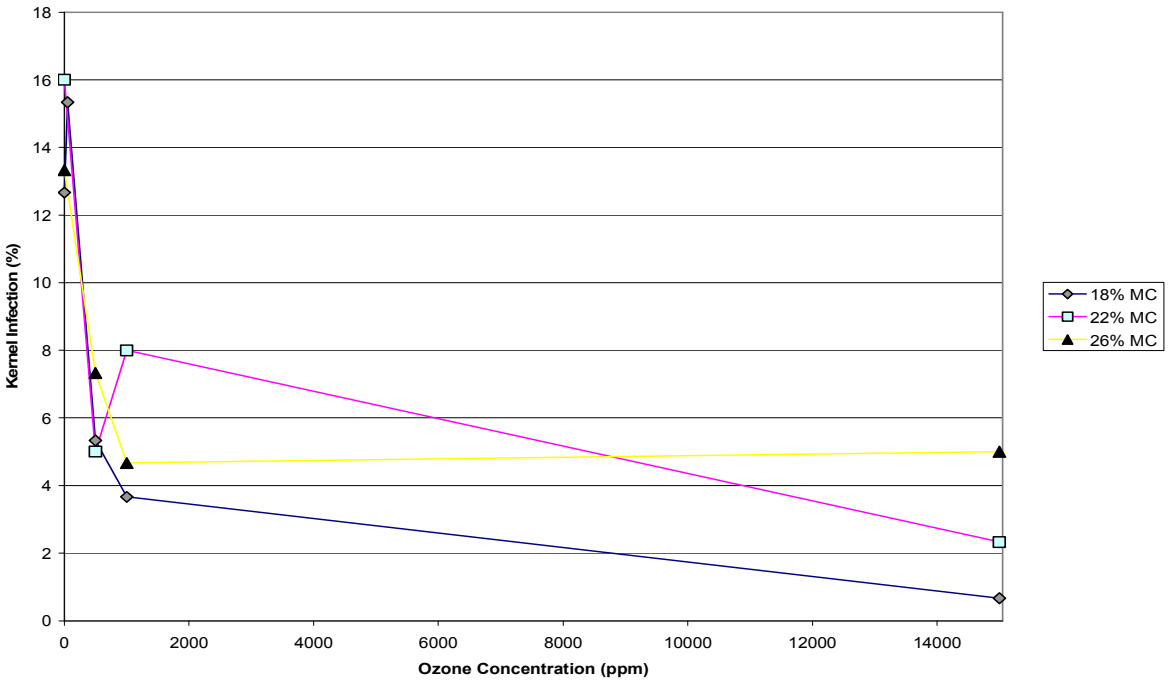


Figure 4.4 The effect of ozone on *Aspergillus* infections on corn (each point is the average of 3 replications)

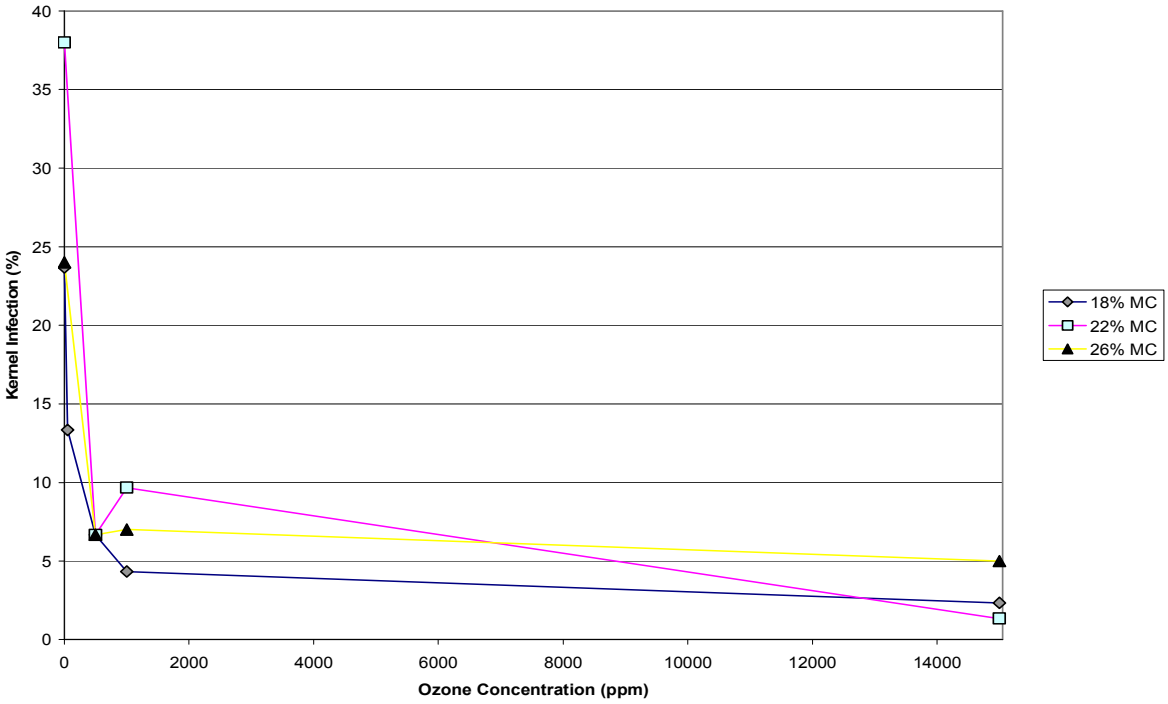


Figure 4.5 The effect of ozone on *Fusarium* infections on corn (each point is the average of 3 replications)

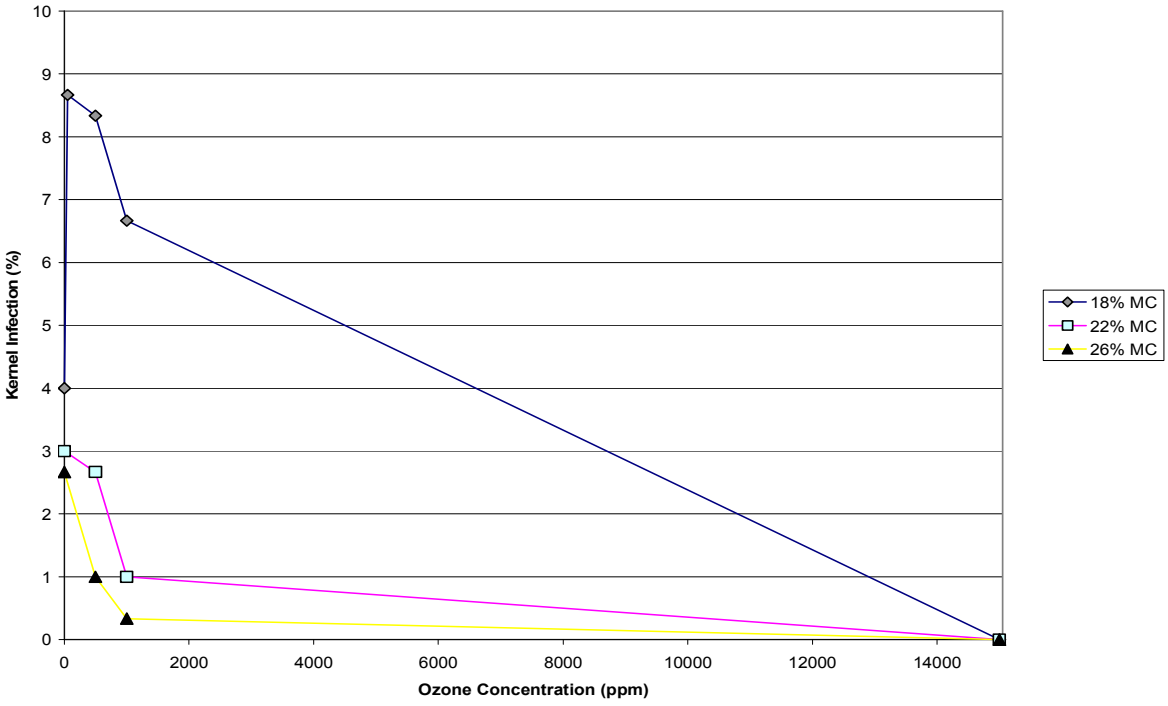


Figure 4.6 The effect of ozone on *Rhizopus* infections on corn (each point is the average of 3 replications)

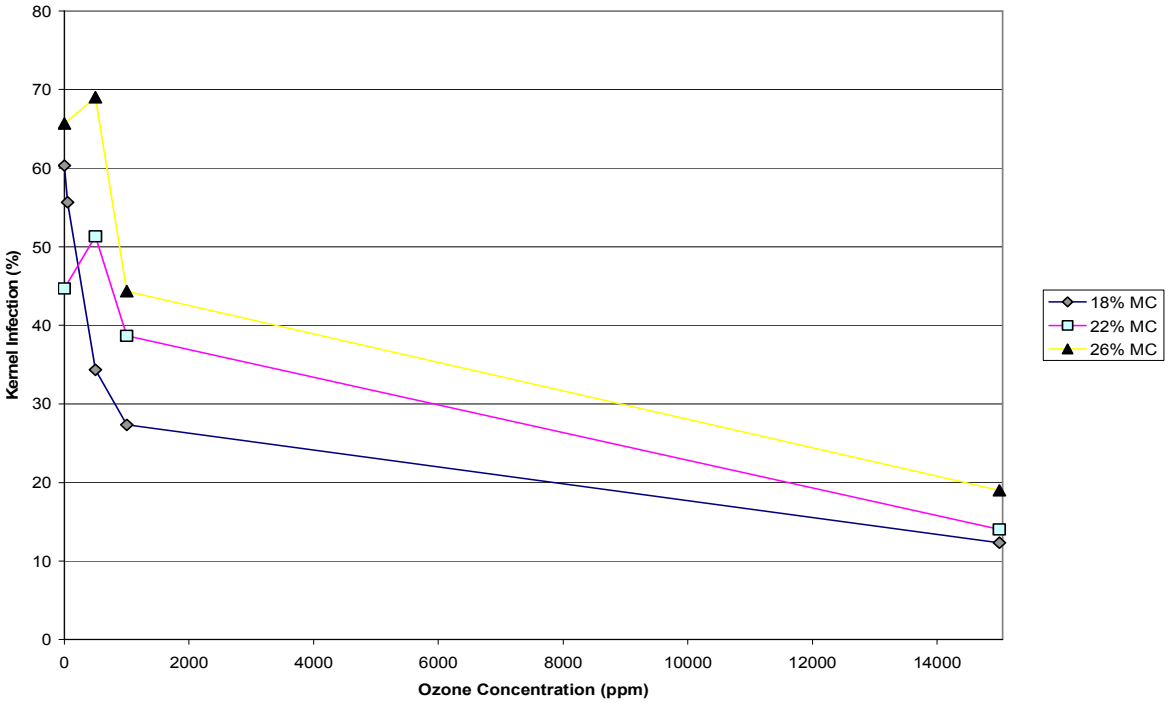


Figure 4.7 The effect of ozone on *Penicillium* infections on corn (each point is the average of 3 replications)

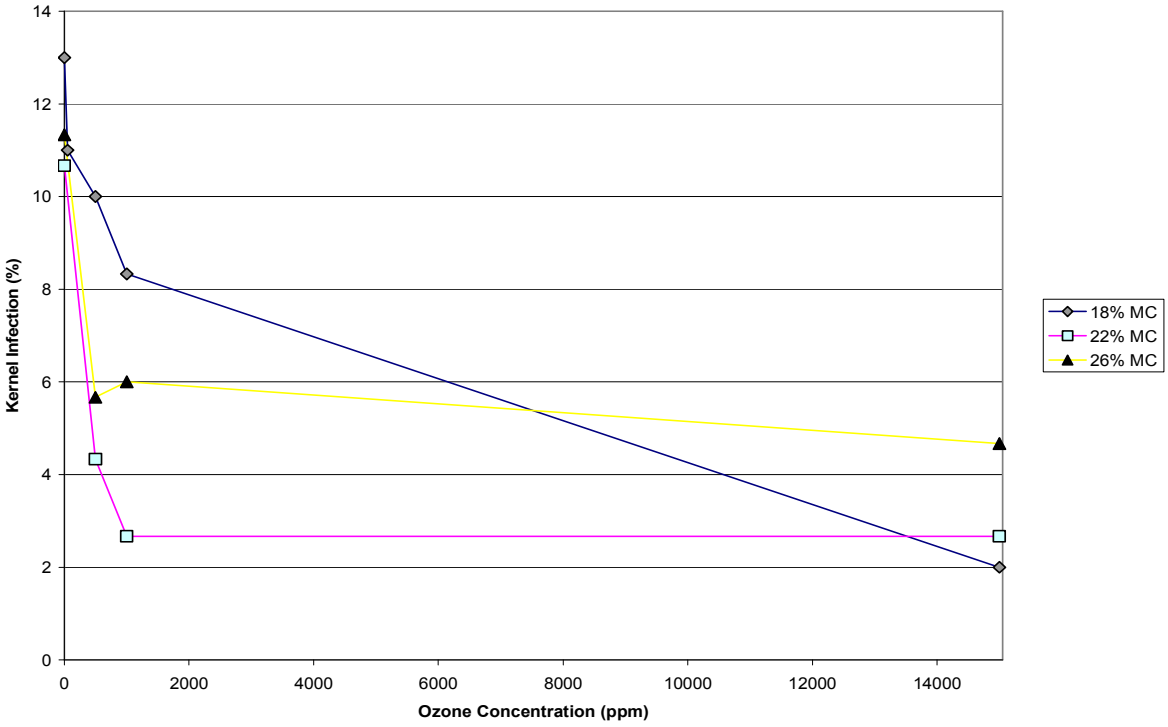


Figure 4.8 The effect of ozone on *Mucor* infections on corn (each point is the average of 3 replications)

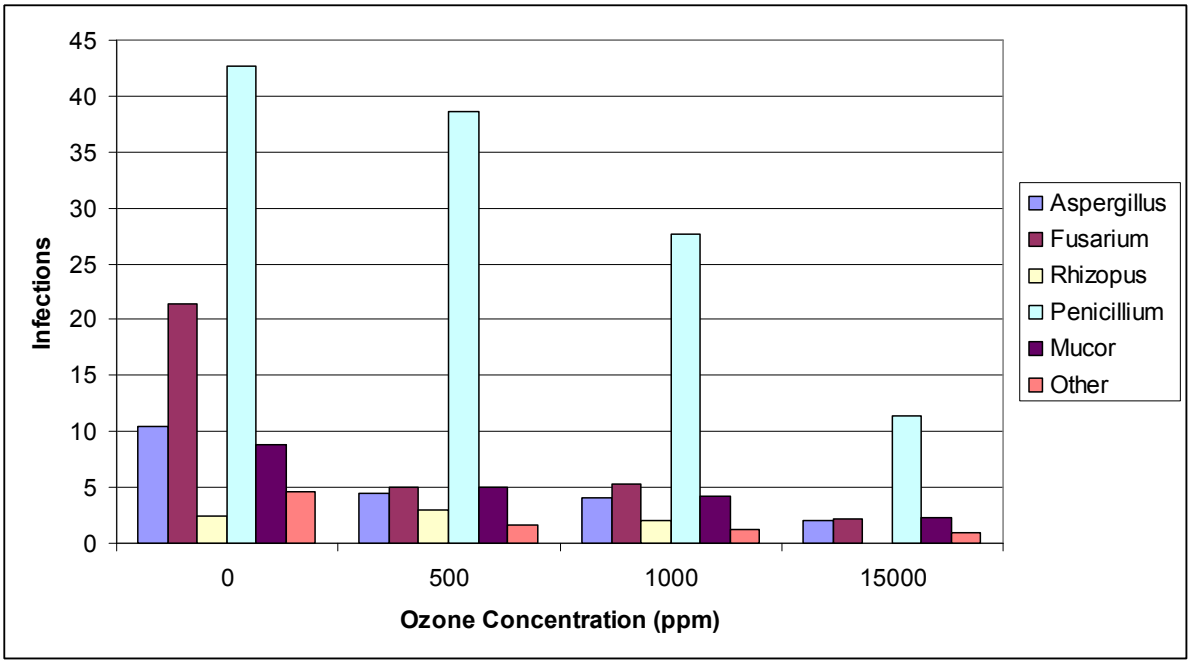


Figure 4.9 Genera population change in response to ozone (each bar is the average of the 3 replications at the 3 moisture contents)

**Change from starting fungi levels with respect to genus**

The survival percentage of each genus was determined by pooling data from all moisture contents. The levels of genera were then compared to control values to observe changes (figure 4.10). Ozone effects on fungi genera fell in the following order (greatest to least): *Rhizopus*, *Fusarium*, *Aspergillus*, other genenera, *Mucor*, and *Penicillium*. These findings agree with those of Zhao and Cranston (1995), who found that that some *Aspergillus* was more susceptible to ozone than *Penicillium.*

Table 4.1 Statistic Comparisons for Ozone Enumeration

| Dependent Variable | Source | p value |
|---|---|---|
| Uninfected Kernels | Ozone Rate | <0.0001 |
| | Moisture Content | 0.0019 |
| | Rate x MC | 0.2498 |
| *Aspergillus* | Ozone Rate | <0.0001 |
| | Moisture Content | 0.2248 |
| | Rate x MC | 0.6218 |
| *Fusarium* | Ozone Rate | <0.0001 |
| | Moisture Content | 0.1698 |
| | Rate x MC | 0.2129 |
| *Rhizopus* | Ozone Rate | <0.0001 |
| | Moisture Content | <0.0001 |
| | Rate x MC | 0.0139 |
| *Penicillium* | Ozone Rate | <0.0001 |
| | Moisture Content | 0.0115 |
| | Rate x MC | 0.2343 |
| *Mucor* | Ozone Rate | <0.0001 |
| | Moisture Content | 0.0132 |
| | Rate x MC | 0.2006 |
| Other | Ozone Rate | 0.0092 |
| | Moisture Content | 0.5742 |
| | Rate x MC | 0.8967 |

It appears that ozone may be useful in decreasing fungi populations in high moisture corn. While ozone may work for certain applications, information from chapter 3 indicates that a single dose of ozone is not enough to control fungi and increase storage time because the high growth rate associated with conditions of high moisture corn allow the fungi to quickly re-infect the corn to non-treated levels.

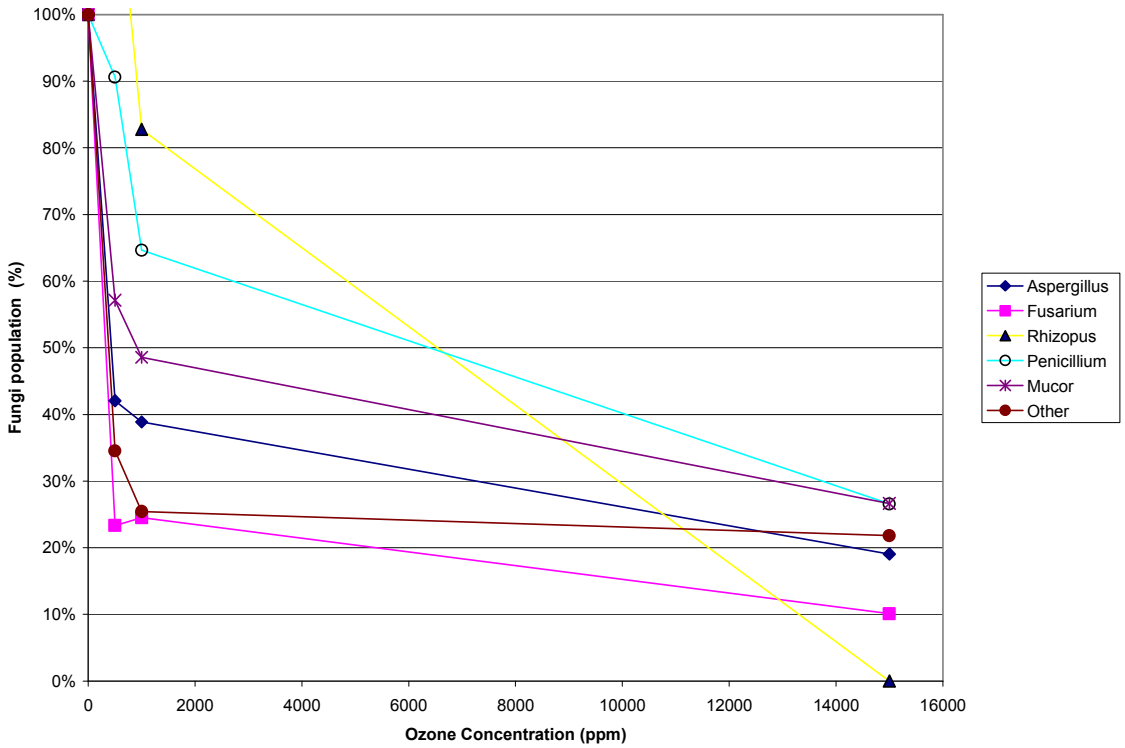


Figure 4.10 Genera population change in response to ozone (each point is the average of the 3 replications at the 3 moisture contents)

## Conclusions

- Increasing ozone concentration from 0 ppm to 15000 ppm decreased the total fungi population present on corn kernels
- Increasing ozone concentrations increased the percentage of uninfected corn
- Increasing ozone concentrations decreased each fungi genus population
- There was an increase in infected kernels with increased corn moisture content (18, 22, 26% moisture content)
- Ozone effect on fungi genera occurs in the following order (most to least): *Rhizopus, Fusarium, Aspergillus*, other genenera, *Mucor*, and *Penicillium*

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# CHAPTER 5

# GENERAL CONCLUSIONS

## Storage Study

While ozone can inactive fungi found in corn, it appears that the favorable growth conditions (high moisture content) of the fungi and the limitations of ozone make it less effective for decreasing the dry matter loss of high moisture corn in storage. The limitations of ozone include a limited residual time and the lack of penetration into damaged corn that could harbor fungi. In the high-temperature short-term continuous airflow experiment, ozone had no measurable effect on dry matter loss or damage kernel total. In the low-temperature long-term experiment, ozone did have an effect on the dry matter loss but was ineffective at improving the damage kernel total. Under the cooler conditions, the fungi grow at a slower rate and ozone has a long residual time. That would indicate that the ozone may have been able to inactivate more fungi and the fungi that remained after ozonation grew slower. In tests, the damage kernel total and the dry matter loss did result in a loss of at least one USDA grade for corn.

## Enumeration Study

In the enumeration study, increasing ozone concentrations decreased the total fungi population present on corn kernels. This was shown by the increase in the percentage of uninfected corn and a decrease in the total fungi population for each sample as ozone concentrations were increased. There was an increase in infected kernels with increased corn moisture content (18, 22, 26% moisture content). A possible reason for this could be airflow characteristics through barley, ground pepper, and corn are different. Other possibilities

could be the competing chemical reactions may be different for various treated products or the seed coat of a corn kernel may also be less protective than in barley, being more likely to offer fungi shelter in damaged sections (cracks) that ozone would not penetrate. Another observation from the enumeration test was that ozone has a greater effect on fungi genera in the following order: *Rhizopus, Fusarium, Aspergillus*, other genenera, *Mucor*, and *Penicillium.* Ozone might be useful in areas where *Fusarium* and *Aspergillus* (producers of mycotoxins) are problematic because they were both responsive to ozone treatments.

## Future Studies

One possible problem with using ozone to treat corn is that gaseous ozone does not contact all of the seed coat. Tests should be done to see if seed movement would improve ozone contact and reduce fungi more than in a stationary situation. Another option that might work would be using aqueous ozone. Aqueous ozone might be able to penetrate damaged kernels to deactivate fungi that gaseous ozone likely would not affect.

The enumeration study showed that ozone can have different effectiveness on fungi genus. Because some fungi species produce mycotoxins, it would be useful to test fungi species for ozone sensitivity. More fungi enumeration tests at ozone concentrations between 1000 and 15000 ppm would also give a better idea of how each fungi genera reacts to ozone. The different results for changing the moisture content of corn compared to other grains should also be investigated. Doing a comparison between inoculations of fungi on a sterile material the same size as corn or a corn sample that has all the damaged corn removed and a sample of corn containing some damage might be able to show the effects of cracks in corn.

## APPENDIX A

## PMD PROGRAM CODE IN VISUAL BASIC 6.0 FOR CHAPTER 2

```
'
'_____________________________________________________________________________
'--------------------------------------Ozone & CO2-------------------------------------
'Written By Steven White 2006
'
'Table of Variables
'---Integers---
'x :    Routing Integer
'Kill : End Program, associated with Stop Button
'a :    Loop Posetion Integer, notes when to retrieve samples
'b :    Loop Position Integer, notes when to print samples to file/screen
'c :    Loop Position Integer, notes when to check ozone status
'
'-----------------------------------------Variables-----------------------------------------
'StartTime :     Used to determine when program started
'i :             Sample Number
'SampleNumber : Used to indicate first loop of program
'TimeOfSample : Used to determine when loop completed
'WaitTime :      Time to wait until next sample, compared to TimeOfSample
'CO2(x) :        Storage for CO2 values until dumped to file
'O3(x) :         Storage for O3 values until dumped to file
'TimeSample : Start of timing for next sample reading, valve opened at this time
'StartSample :  Set to compare time change against
'NewSample :  CO2 and O3 Sample Time check
'TimeStep :      Time that must elapse before samples taken
'StartMin :      Convert time into minutes for comparison
'NewMin :       Re-Set Variable
'Difference :    Used to hold time elapsed over 60 minutes
'-------------------------------------------------------------------------------------------
'
_____________________________________________________________________________
Const BoardNum% = 1                                    'Identify PMD number
Dim x, j, Kill, a, b, c, L As Integer                  'Set integers and variables
Dim StartTime, i, SampleNumber, TimeOfSample, WaitTime, CO2(20) As Double
Dim TimeSample, StartSample, NewSample, TimeStep As Double
Dim StartMin, NewMin, Difference As Double
Dim T1(20), T2(20), O3(20) As Double
Dim retval As Long
Sub Start()                                            'Begin a subprogram
   ulStat% = cbDeclareRevision(CURRENTREVNUM)
   ulStat% = cbErrHandling(PRINTALL, DONTSTOP)
      If ulStat% <> 0 Then Stop:
```

```
ulStat% = cbDConfigPort(BoardNum%, FIRSTPORTA, DIGITALOUT)
      If ulStat% <> 0 Then Stop
      ulStat% = cbDConfigPort(BoardNum%, FIRSTPORTB, DIGITALOUT)
      If ulStat% <> 0 Then Stop

ulStat% = cbDOut(BoardNum%, FIRSTPORTA, 0)
      If ulStat% <> 0 Then Stop

ulStat% = cbDOut(BoardNum%, FIRSTPORTB, 0)
      If ulStat% <> 0 Then Stop

ulStat% = cbAOut(BoardNum%, Chan%, UNI5VOLTS, DataValue%)
      If ulStat% <> 0 Then Stop

   newHour = Hour(Now())
   newMinute = Minute(Now())
   newsecond = Second(Now())
   StartTime = TimeSerial(newHour, newMinute, newsecond)

StartMin = Minute(Now()) + Second(Now()) / 60
Difference = 0
newsecond2 = 0
newsecond = 0

For L = 0 To 1
If txtOzone(L) > 0 Then
ulStat% = cbDBitOut(BoardNum%, FIRSTPORTA, L + 14, 1)
Else: ulStat% = cbDBitOut(BoardNum%, FIRSTPORTA, L + 14, 0)
End If
Next L

For i = 0 To 8
   picOzonetime(i).Cls
   picOzoneOut(i).Cls
   picCO2(i).Cls
Next i
Call Main

End Sub

Sub Main()                                  'Start the "Main" subprogram
Do                                          'Begin do loop
If a = 0 Then Call GetSamples
If b = 0 Then Call SamplePrint
DoEvents
```

```
If Kill = 1 Then Call cmdStop_Click
a = 0
b = 0
Loop                                                    ‘Return to beginning of loop
End Sub                                                 ‘
Private Sub cmdStart_Click()
x = x + 1
1   'Control loop
   If x = 4 Then x = 2

   If x = 1 Then
      cmdStart.Caption = "Hit to Pause"
      Call Start

   ElseIf x = 2 Then
      cmdStart.Caption = "Hit to Restart"
      GoTo 2

   ElseIf x = 3 Then
      cmdStart.Caption = "Hit to Pause"
      a = 0
      b = 0
      Call Main
End If

2 'Pause
DoEvents
GoTo 1

End Sub
Sub GetSamples()
   a = 1
   For i = 0 To 13                                      'reset all valves to close
   ulStat% = cbDBitOut(BoardNum%, FIRSTPORTA, i, 0)
   Next i

   For i = 0 To 13
      picCurrentTube.Cls
      picCurrentTube.Print i + 1
      TimeSample = 0

      ulStat% = cbDBitOut(BoardNum%, FIRSTPORTA, i, 1)       'open valve

      StartSample = Minute(Now()) + Second(Now()) / 60
         'pause for set time
```

```
        Do While TimeSample < 4
           NewSample = Minute(Now()) + Second(Now()) / 60
           If NewSample < StartSample Then
              Difference = 60 - StartSample
              TimeSample = Difference + NewSample
           Else
              TimeSample = NewSample - StartSample
           End If
           DoEvents
           Loop

'------------------------------------------------------------------------
'This Loop Controls the Ozone Generators using ports 14-15
NewMin = Minute(Now()) + Second(Now()) / 60
If NewMin < StartMin Then
Difference = 60 - StartMin
TimeStep = Difference + NewMin + TimeStep
Else
TimeStep = NewMin - StartMin + TimeStep
End If
picCurrentTime.Cls
picCurrentTime.Print TimeStep / 60

'--if ozone generator control is used, turn on optional code below
For L = 0 To 1
If TimeStep >= txtOzone(L) * 60 Then
ulStat% = cbDBitOut(BoardNum%, FIRSTPORTA, L + 14, 0)
picOzonetime(L).Cls
picOzonetime(L).Print "Off"
ElseIf TimeStep < txtOzone(L) * 60 Then
ulStat% = cbDBitOut(BoardNum%, FIRSTPORTA, L + 14, 1)
picOzonetime(L).Cls
picOzonetime(L).Print TimeStep / 60
End If
DoEvents
Next L

StartMin = Minute(Now()) + Second(Now()) / 60
'------------------------------------------------------------------------

      ulStat% = cbAIn(BoardNum%, 0, BIP5VOLTS, Ch0Binary)     'Get CO2 voltage
      CO2(i) = ((Ch0Binary / 4096) * 10 - 5) * (1500 / 4.99)
      ulStat% = cbAIn(BoardNum, 1, BIP5VOLTS, Ch1Binary)      'Get O3 voltage
      O3(i) = ((Ch1Binary / 4096) * 10 - 5)
      ulStat% = cbDBitOut(BoardNum%, FIRSTPORTA, i, 0)        'close valve
```

```
        ulStat% = cbAIn(BoardNum, 2, BIP10VOLTS, Ch2Binary)     'Temp
        T1(i) = ((Ch2Binary / 4096) * 10 - 5) * (997 / 8) - 273
        ulStat% = cbAIn(BoardNum, 3, BIP5VOLTS, Ch3Binary)      'RH
        T2(i) = (((Ch3Binary / 4096) * 10 - 5) * 1000) * 0.03892 - 42.017



    DoEvents
    picCO2(i).Cls
    picOzoneOut(i).Cls
    picCurrentTime.Cls
    picRH.Cls
    picTemp.Cls
    picRH.Print T2(i)
    picTemp.Print T1(i)
    picCO2(i).Print CO2(i)
    picOzoneOut(i).Print O3(i)
    picCurrentTime.Print TimeStep / 60
    Next i
Call Main
End Sub
Sub SamplePrint()  'Portion of Code that Writes all data to a file
b = 1
    T1(15) = (T1(0) + T1(1) + T1(2) + T1(3) + T1(4) + T1(5) + T1(6)) / 7
    T2(15) = (T2(0) + T2(1) + T2(2) + T2(3) + T2(4) + T2(5) + T2(6)) / 7

    If SampleNumber = 0 Then       'On first loop, initialize file and post starting information
        Title = "c:/" & txtTitle & "CO2" & ".txt"  'Create CO2 file
        Title2 = "c:/" & txtTitle & "O3" & ".txt"  'Create O3 file
        Title3 = "c:/" & txtTitle & "Temp" & ".txt" 'Create temp & RH file

        Open Title For Output As #1
        Write #1, Title, StartTime
        Write #1, "Sample", 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14; "Hours"
        Write #1, "Treatment", txtTest(0), txtTest(1), txtTest(2), txtTest(3), txtTest(4),
txtTest(5), txtTest(6), txtTest(7), txtTest(8), txtTest(9), txtTest(10), txtTest(11), txtTest(12),
txtTest(13)

        Open Title2 For Output As #2
        Write #2, Title, StartTime
        Write #2, "Sample", 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14; "Hours"
        Write #2, "Treatment", txtTest(0), txtTest(1), txtTest(2), txtTest(3), txtTest(4),
txtTest(5), txtTest(6), txtTest(7), txtTest(8), txtTest(9), txtTest(10), txtTest(11), txtTest(12),
txtTest(13)
```

```
        Open Title3 For Output As #3
        Write #3, Title, StartTime
        Write #3, "Seqence", "TempAverage", "RHAverage", "Hours"
    End If

SampleNumber = SampleNumber + 1
Write #1, SampleNumber, CO2(0), CO2(1), CO2(2), CO2(3), CO2(4), CO2(5), CO2(6),
CO2(7), CO2(8), CO2(9), CO2(10), CO2(11), CO2(12), CO2(13); TimeStep / 60
Write #2, SampleNumber, O3(0), O3(1), O3(2), O3(3), O3(4), O3(5), O3(6), O3(7), O3(8),
O3(9), O3(10), O3(11), O3(12), O3(13), TimeStep / 60
Write #3, SampleNumber, T1(15), T2(15)

Call Main
End Sub
Private Sub cmdStop_Click()
Kill = 1
Stop
End Sub
```

# APPENDIX B

## EXPERIMENTAL DATA FOR HIGH-TEMPERATURE EXPERIMENT

| Rep | Rate, mg/min | Time, hr | Ending MC, % | DM loss | Damage, % |
|---|---|---|---|---|---|
| 1 | 0.00 | 0.00 | 19.80 | 1.25 | 13.5 |
| 1 | 0.00 | 0.00 | 21.9 | 1.25 | 9.2 |
| 2 | 0.00 | 0.00 | 18.9 | 0.89 | 10.80 |
| 2 | 0.00 | 0.00 | 16.4 | 1.14 | 15.90 |
| 3 | 0.00 | 0.00 | 18.90 | 1.16 | 12.30 |
| 3 | 0.00 | 0.00 | 16.40 | 0.85 | 10.70 |
| 1 | 0.06 | 5.00 | 22.10 | 0.94 | 7.10 |
| 2 | 0.06 | 5.00 | 20.20 | 1.11 | 13.10 |
| 3 | 0.06 | 5.00 | 14.70 | 0.35 | 3.60 |
| 1 | 0.12 | 5.00 | 20.80 | 1.09 | 18.80 |
| 2 | 0.12 | 5.00 | 14.90 | 0.51 | 9.10 |
| 3 | 0.12 | 5.00 | 16.00 | 0.79 | 11.40 |
| 1 | 0.24 | 5.00 | 14.80 | 0.38 | 3.90 |
| 2 | 0.24 | 5.00 | 15.00 | 0.41 | 9.60 |
| 3 | 0.24 | 5.00 | 21.00 | 0.53 | 12.60 |
| 1 | 0.48 | 5.00 | 25.50 | 1.40 | 15.80 |
| 2 | 0.48 | 5.00 | 16.10 | 0.63 | 9.50 |
| 3 | 0.48 | 5.00 | 15.00 | 0.26 | 5.40 |
| 1 | 0.72 | 5.00 | 24.30 | 0.95 | 18.10 |
| 2 | 0.72 | 5.00 | 22.30 | 1.30 | 14.80 |
| 3 | 0.72 | 5.00 | 24.50 | 1.17 | 20.10 |
| 1 | 0.96 | 5.00 | 20.80 | 1.00 | 20.30 |
| 2 | 0.96 | 5.00 | 16.00 | 0.66 | 11.40 |
| 3 | 0.96 | 5.00 | 20.40 | 0.90 | 13.30 |
| 1 | 1.20 | 5.00 | 20.30 | 0.88 | 6.40 |
| 2 | 1.20 | 5.00 | 19.00 | 0.77 | 13.80 |
| 3 | 1.20 | 5.00 | 16.00 | 0.34 | 3.50 |
| 1 | 0.06 | 24.00 | 17.70 | 0.93 | 12.10 |
| 2 | 0.06 | 24.00 | 22.10 | 1.38 | 11.30 |
| 3 | 0.06 | 24.00 | 15.00 | 0.46 | 1.40 |
| 1 | 0.12 | 24.00 | 23.80 | 1.10 | 6.70 |
| 2 | 0.12 | 24.00 | 19.30 | 1.11 | 10.70 |
| 3 | 0.12 | 24.00 | 15.00 | 0.36 | 7.30 |
| 1 | 0.24 | 24.00 | 24.80 | 0.98 | 7.70 |
| 2 | 0.24 | 24.00 | 20.60 | 1.03 | 8.50 |
| 3 | 0.24 | 24.00 | 23.90 | 1.33 | 22.90 |
| 1 | 0.48 | 24.00 | 24.10 | 1.28 | 12.40 |
| 2 | 0.48 | 24.00 | 15.00 | 0.30 | 3.30 |
| 3 | 0.48 | 24.00 | 18.40 | 1.12 | 11.40 |

| Rep | Rate, mg/min | Time, hr | Ending MC, % | DM loss | Damage, % |
|---|---|---|---|---|---|
| 1 | 0.72 | 24.00 | 17.40 | 0.77 | 15.60 |
| 2 | 0.72 | 24.00 | 19.70 | 1.05 | 15.40 |
| 3 | 0.72 | 24.00 | 15.00 | 0.48 | 6.80 |
| 1 | 0.96 | 24.00 | 22.80 | 1.25 | 16.70 |
| 2 | 0.96 | 24.00 | 19.80 | 0.86 | 15.70 |
| 3 | 0.96 | 24.00 | 15.00 | 0.22 | 4.40 |
| 1 | 1.20 | 24.00 | 18.30 | 0.73 | 14.60 |
| 2 | 1.20 | 24.00 | 18.30 | 0.76 | 18.30 |
| 3 | 1.20 | 24.00 | 21.00 | 0.70 | 15.80 |
| 1 | 1.2 rtrt | 34.00 | 18.40 | 0.88 | 6.30 |
| 2 | 1.2 rtrt | 34.00 | 15.50 | 0.67 | 6.00 |
| 3 | 1.2 rtrt | 34.00 | 24.70 | 0.96 | 10.10 |

## APPENDIX C

## SAS CODE FOR HIGH-TEMPERATURE EXPERIMENT CHAPTER 3

```
/* Using ozone to control fungi in high moisture corn
/* Short term, High temperature experiment
/* Steven White*/

options nocenter ls=89 ps=51 pageno=1;
title 'Short term,  High Temp Experiment';


/*      Treatment Table
 1      0.06 mg / min ozone applied for 5 h
 2      0.12 mg / min ozone applied for 5 h
 3      0.24 mg / min ozone applied for 5 h
 4      0.48 mg / min ozone applied for 5 h
 5      0.72 mg / min ozone applied for 5 h
 6      0.96 mg / min ozone applied for 5 h
 7      1.20 mg / min ozone applied for 5 h
 8      0.06 mg / min ozone applied for 24 h
 9      0.12 mg / min ozone applied for 24 h
10      0.24 mg / min ozone applied for 24 h
11      0.48 mg / min ozone applied for 24 h
12      0.72 mg / min ozone applied for 24 h
13      0.96 mg / min ozone applied for 24 h
14      1.20 mg / min ozone applied for 24 h
ctrl    no ozone application
rtrt    1.20 mg / min ozone applied for 24 h,
        additional 6 h of ozone applied every 3 d
        at 1.20 mg / min
*/
data b;
label  emc = 'moisture content, % wb';
label dml = 'dry matter loss, %';
label dkt = 'damage, % wb';

input blk trt $ rate time dml dkt emc;
infile 'hightempMC.txt';
proc glm;
class trt;
model dml dkt = trt emc / ss3;
contrast 'effect of time' trt 1 -1 -1 -1 -1 -1 1 1 1 1 1 1 1 -1 -1 0 0;
estimate 'effect of time' trt 1 -1 -1 -1 -1 -1 1 1 1 1 1 1 1 -1 -1 0 0 / divisor = 7;
contrast 'effect of ozone rate' trt 1 0 0 0 0 0 -1 0 0 0 0 0 0 1 -1 0 0,
```

```
                                trt 1 -1 0 0 0 0 0 -1 0 0 0 0 1 0 0 0,
                                trt 1 0 -1 0 0 0 0 0 -1 0 0 0 1 0 0 0,
                                trt 1 0 0 -1 0 0 0 0 0 -1 0 0 1 0 0 0,
                                trt 1 0 0 0 -1 0 0 0 0 0 -1 0 1 0 0 0,
                                trt 1 0 0 0 0 -1 0 0 0 0 0 -1 1 0 0 0;
contrast 'time X ozone rate'    trt 1 0 0 0 0 0 -1 0 0 0 0 0 -1 1 0 0,
                                trt 1 1 0 0 0 0 0 -1 0 0 0 0 -1 0 0 0,
                                trt 1 0 1 0 0 0 0 0 -1 0 0 0 -1 0 0 0,
                                trt 1 0 0 1 0 0 0 0 0 -1 0 0 -1 0 0 0,
                                trt 1 0 0 0 1 0 0 0 0 0 -1 0 -1 0 0 0,
                                trt 1 0 0 0 0 1 0 0 0 0 0 -1 -1 0 0 0;
contrast 'ctrl vs. treated' trt -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 15 -1;
estimate 'ctrl vs. treated' trt -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 15 -1 / divisor = 15;
contrast '1.2 mg/hr 5 and 24 hr vs. rtrt' trt 0 0 0 0 0 -1 0 0 0 0 0 -1 0 0 0 2;
estimate '1.2 mg/hr 5 and 24 hr vs. rtrt' trt 0 0 0 0 0 -1 0 0 0 0 0 -1 0 0 0 2 / divisor = 2;
lsmeans trt;
run;
quit;
```

**ANOVA TABLES FOR CHAPTER 3 HIGH-TEMPERATURE EXPERIMENT**

Short term, High Temp Experiment 19:44 Saturday, July 7, 2007 1

The GLM Procedure

Class Level Information

| Class | Levels | Values |
|---|---|---|
| blk | 3 | 1 2 3 |
| trt | 16 | 1 10 11 12 13 14 2 3 4 5 6 7 8 9 ctrl rtrt |

Number of Observations Read 51
Number of Observations Used 51

Short term, High Temp Experiment 19:44 Saturday, July 7, 2007 2

Dependent Variable: dml dry matter loss, %

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Model | 18 | 1.92973671 | 0.10720760 | 1.46 | 0.1708 |
| Error | 32 | 2.35012211 | 0.07344132 | | |
| Corrected Total | 50 | 4.27985882 | | | |

| R-Square | Coeff Var | Root MSE | dml Mean |
|---|---|---|---|
| 0.450888 | 31.04454 | 0.271001 | 0.872941 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| blk | 2 | 0.56374026 | 0.28187013 | 3.84 | 0.0321 |
| trt | 15 | 0.82231775 | 0.05482118 | 0.75 | 0.7215 |
| emc | 1 | 0.17489848 | 0.17489848 | 2.38 | 0.1326 |

| Contrast | DF | Contrast SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|

| | | | | | |
|---|---|---|---|---|---|
| effect of time | 1 | 0.11623447 | 0.11623447 | 1.58 | 0.2175 |
| effect of ozone rate | 6 | 0.19317098 | 0.03219516 | 0.44 | 0.8476 |
| time X ozone rate | 6 | 0.41929146 | 0.06988191 | 0.95 | 0.4729 |
| ctrl vs. treated | 1 | 0.09454891 | 0.09454891 | 1.29 | 0.2650 |
| 1.2 mg/hr 5,24 hr vs. rtrt | 1 | 0.07045068 | 0.07045068 | 0.96 | 0.3347 |

| Parameter | Estimate | Standard Error | t Value | Pr > \|t\| |
|---|---|---|---|---|
| effect of time | -0.10538979 | 0.08377246 | -1.26 | 0.2175 |
| ctrl vs. treated | 0.13386747 | 0.11798234 | 1.13 | 0.2650 |
| 1.2 mg/hr 5, 24 hr vs. rtrt | 0.18793830 | 0.19188585 | 0.98 | 0.3347 |

Short term, High Temp Experiment 19:44 Saturday, July 7, 2007 3

Dependent Variable: dkt damage, % wb

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Model | 18 | 561.591344 | 31.199519 | 1.45 | 0.1748 |
| Error | 32 | 688.381597 | 21.511925 | | |
| Corrected Total | 50 | 1249.972941 | | | |

| R-Square | Coeff Var | Root MSE | dkt Mean |
|---|---|---|---|
| 0.449283 | 42.11945 | 4.638095 | 11.01176 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| blk | 2 | 37.3211861 | 18.6605930 | 0.87 | 0.4297 |
| trt | 15 | 408.8289634 | 27.2552642 | 1.27 | 0.2778 |
| emc | 1 | 32.9607561 | 32.9607561 | 1.53 | 0.2248 |

| Contrast | DF | Contrast SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| effect of time | 1 | 0.5360281 | 0.5360281 | 0.02 | 0.8756 |
| effect of ozone rate | 6 | 179.2707600 | 29.8784600 | 1.39 | 0.2492 |

| | | | | |
|---|---|---|---|---|
| time X ozone rate | 6 | 178.7423730 | 29.7903955 | 1.38 0.2508 |
| ctrl vs. treated | 1 | 8.4424687 | 8.4424687 | 0.39 0.5355 |
| 1.2 mg/hr 5, 24 hr vs. rtrt | 1 | 46.1728873 | 46.1728873 | 2.15 0.1527 |

| Parameter | Estimate | Standard Error | t Value | Pr > \|t\| |
|---|---|---|---|---|
| effect of time | 0.22632099 | 1.43374099 | 0.16 | 0.8756 |
| ctrl vs. treated | -1.26497376 | 2.01923295 | -0.63 | 0.5355 |
| 1.2 mg/hr 5, 24 hr vs. rtrt | -4.81134213 | 3.28406966 | -1.47 | 0.1527 |



Least Squares Means

| trt | dml LSMEAN | dkt LSMEAN |
|---|---|---|
| 1 | 0.91366448 | 7.9836391 |
| 10 | 0.97225726 | 11.8745656 |
| 11 | 1.16675091 | 9.0344898 |
| 12 | 0.88875082 | 13.1319692 |
| 13 | 0.77603486 | 12.2579933 |
| 14 | 0.84270153 | 16.2246599 |
| 2 | 0.86828168 | 13.6712887 |
| 3 | 0.75805945 | 9.3597575 |
| 4 | 0.68652867 | 10.3229586 |
| 5 | 0.94936840 | 16.3309613 |
| 6 | 0.85556572 | 15.0306461 |
| 7 | 0.57917063 | 8.1174135 |
| 8 | 0.80608420 | 8.5332295 |
| 9 | 0.89578795 | 8.1755106 |
| ctrl | 0.99105953 | 9.8956114 |
| rtrt | 0.89887438 | 7.3596946 |

# APPENDIX D

## EXPERIMENTAL DATA FOR LOW-TEMPERATURE EXPERIMENT

| Storage Chamber | Treatment (mg ozone/g corn) | DM Loss | DKT |
|---|---|---|---|
| 1 | 0 | 3.23 | 31.1 |
| 2 | 0 | 2.59 | 34.2 |
| 3 | 0 | 3.01 | 27.8 |
| 1 | 0.6 | 1.58 | 33.6 |
| 2 | 0.6 | 3.31 | 29.5 |
| 3 | 0.6 | 3.81 | 31.3 |
| 1 | 1.2 | 3.51 | 32.6 |
| 2 | 1.2 | 1.90 | 25.7 |
| 3 | 1.2 | 1.35 | 22.3 |
| 1 | 2.4 | 1.55 | 19.3 |
| 2 | 2.4 | 1.80 | 17.9 |
| 3 | 2.4 | 1.53 | 23.3 |
| 3 | 4.8 | 2.07 | 38.2 |
| 2 | 4.8 | 1.76 | 44.3 |
| 1 | 4.8 | 1.32 | 12.5 |
| 1 | Repeated 4.8 ever 3 days | 1.51 | 32.9 |
| 2 | Repeated 4.8 ever 3 days | 1.86 | 39.5 |
| 3 | Repeated 4.8 ever 3 days | 2.81 | 36.6 |
| 2 | Repeated 4.8 ever 6 days | 1.04 | 21.1 |
| 1 | Repeated 4.8 ever 6 days | 1.25 | 20.4 |
| 3 | Repeated 4.8 ever 6 days | 1.50 | 26.9 |
| 1 | Repeated 4.8 ever 12 days | 1.13 | 27.0 |
| 2 | Repeated 4.8 ever 12 days | 2.08 | 51.7 |
| 3 | Repeated 4.8 ever 12 days | 0.74 | 16.2 |

# APPENDIX E

## DATA ANALYSIS AND SAS CODE FOR LOW-TEMPERATURE HIGH-MOIURE TEST IN CHAPTER 3

```
/* Using ozone to control fungi in high moisture corn
/* Long term, Low temperature experiment
/* Steven White*/

options nocenter ls=89 ps=51 pageno=1;
title 'Long Term Low Temp Experiment';
title2 ' Randomized Complete Block Design';

/*      Treatment Table
/* 1    Control, 0 mg ozone/min
/* 2    0.6 mg ozone/ min
/* 3    1.2 mg ozone/ min
/* 4    2.4 mg ozone/ min
/* 5    4.8 mg ozone/ min
/* 6    4.8 mg ozone/ min every 3 days
/* 7    4.8 mg ozone/ min every 6 days
/* 8    4.8 mg ozone/ min every 12 days
*/

Data A;
input trt cham dml dkt emc;
label dml = '% Dry Matter Loss';
label dkt = '% Damage Kernel Total';
label emc = 'Ending Moisture Content %';
datalines;
1       1       3.23    31.1    27.2
1       2       2.59    34.2    27.7
1       3       3.01    27.8    27.7
2       1       1.58    33.6    26.4
2       2       3.31    29.5    26.1
2       3       3.15    31.3    26.0
3       1       3.51    32.6    27.4
3       2       1.90    25.7    26.0
3       3       1.35    22.3    25.9
4       1       1.55    19.3    25.6
4       2       1.80    17.9    25.4
```

```
4       3       1.53    23.3    26.1
5       3       2.07    38.2    26.9
5       2       1.76    44.3    26.6
5       1       1.32    12.5    27.5
6       1       1.51    32.9    27.3
6       2       1.86    39.5    27.6
6       3       2.81    36.6    27.4
7       2       1.04    21.1    27.0
7       1       1.25    20.4    27.5
7       3       1.50    26.9    27.8
8       1       1.13    27.0    27.5
8       2       2.08    51.7    27.6
8       3       0.74    16.2    27.2
;

proc glm data=A;
class cham trt;
model dml dkt = cham trt/ ss3;
contrast 'control vs trt' trt 7 -1 -1 -1 -1 -1 -1 -1;
contrast '4.8 vs repeated 4.8' trt 0 0 0 0 3 -1 -1 -1;
contrast 'control vs single trt' trt 4 -1 -1 -1 -1 0 0 0;
contrast 'control vs repeated trt' trt 3 0 0 0 0 -1 -1 -1;
estimate 'control vs trt' trt 7 -1 -1 -1 -1 -1 -1 -1 / divisor = 7;
estimate '4.8 vs repeated 4.8' trt 0 0 0 0 3 -1 -1 -1 / divisor = 3;
estimate 'control vs single trt' trt 4 -1 -1 -1 -1 0 0 0 / divisor = 4;
estimate 'control vs repeated trt' trt 3 0 0 0 0 -1 -1 -1 / divisor = 3;
means trt;
run;
```

**Long Term Low Temp Experiment** 11:54 Wednesday, April 4, 2007 1
RCBD DML ANOVA

The GLM Procedure

Class Level Information

| Class | Levels | Values |
|---|---|---|
| cham | 3 | 1 2 3 |
| trt | 8 | 1 2 3 4 5 6 7 8 |

| | |
|---|---|
| Number of Observations Read | 24 |
| Number of Observations Used | 24 |

Dependent Variable: dml % Dry Matter Loss

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Model | 9 | 10.56036555 | 1.17337395 | 2.10 | 0.1033 |
| Error | 14 | 7.82899695 | 0.55921407 | | |
| Corrected Total | 23 | 18.38936250 | | | |

| R-Square | Coeff Var | Root MSE | dml Mean |
|---|---|---|---|
| 0.574265 | 36.72467 | 0.747806 | 2.036250 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| cham | 2 | 0.28020305 | 0.14010152 | 0.25 | 0.7818 |
| trt | 7 | 10.04534590 | 1.43504941 | 2.57 | 0.0632 |

| Contrast | DF | Contrast SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| control vs trt | 1 | 4.04361257 | 4.04361257 | 7.23 | 0.0176 |
| 4.8 vs repeated 4.8 | 1 | 0.06502500 | 0.06502500 | 0.12 | 0.7382 |
| control vs single trt | 1 | 2.42975781 | 2.42975781 | 4.34 | 0.0559 |
| control vs repeated trt | 1 | 5.52480648 | 5.52480648 | 9.88 | 0.0072 |

t Tests (LSD) for dml

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

| | |
|---|---|
| Alpha | 0.05 |
| Error Degrees of Freedom | 14 |
| Error Mean Square | 0.559214 |
| Critical Value of t | 2.14479 |
| Least Significant Difference | 1.3096 |

Means with the same letter are not significantly different.

| t Grouping | Mean | N | trt |
|---|---|---|---|
| A | 3.1533 | 3 | 1 |
| A | | | |
| B A | 2.9000 | 3 | 2 |
| B A | | | |
| B A C | 2.2533 | 3 | 3 |
| B A C | | | |
| B A C | 2.0600 | 3 | 6 |
| B C | | | |
| B C | 1.7167 | 3 | 5 |
| B C | | | |
| B C | 1.6267 | 3 | 4 |
| C | | | |
| C | 1.3167 | 3 | 8 |
| C | | | |
| C | 1.2633 | 3 | 7 |

Class Level Information

| Class | Levels | Values |
|---|---|---|
| cham | 3 | 1 2 3 |
| trt | 8 | 1 2 3 4 5 6 7 8 |

| | |
|---|---|
| Number of Observations Read | 24 |
| Number of Observations Used | 24 |

Dependent Variable: dkt % Damage Kernel Total

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Model | 9 | 872.202552 | 96.911395 | 1.16 | 0.3883 |
| Error | 14 | 1170.997448 | 83.642675 | | |
| Corrected Total | 23 | 2043.200000 | | | |

| R-Square | Coeff Var | Root MSE | dkt Mean |
|---|---|---|---|
| 0.426881 | 31.10761 | 9.145637 | 29.40000 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| cham | 2 | 208.0492190 | 104.0246095 | 1.24 | 0.3183 |
| trt | 7 | 621.2407270 | 88.7486753 | 1.06 | 0.4356 |

| Contrast | DF | Contrast SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| control vs trt | 1 | 38.29120317 | 38.29120317 | 0.46 | 0.5097 |
| 4.8 vs repeated 4.8 | 1 | 4.48027778 | 4.48027778 | 0.05 | 0.8203 |
| control vs single trt | 1 | 58.90705274 | 58.90705274 | 0.70 | 0.4155 |
| control vs repeated trt | 1 | 12.31450574 | 12.31450574 | 0.15 | 0.7070 |

t Tests (LSD) for dkt

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

| | |
|---|---|
| Alpha | 0.05 |
| Error Degrees of Freedom | 14 |
| Error Mean Square | 83.64267 |
| Critical Value of t | 2.14479 |
| Least Significant Difference | 16.016 |

Means with the same letter are not significantly different.

| t Grouping | | Mean | N | trt |
|---|---|---|---|---|
| | A | 36.333 | 3 | 6 |
| | A | | | |
| B | A | 34.267 | 3 | 1 |
| B | A | | | |
| B | A | 31.667 | 3 | 5 |
| B | A | | | |
| B | A | 31.633 | 3 | 8 |
| B | A | | | |
| B | A | 31.467 | 3 | 2 |
| B | A | | | |
| B | A | 26.867 | 3 | 3 |
| B | A | | | |
| B | A | 22.800 | 3 | 7 |
| B | | | | |
| B | | 20.167 | 3 | 4 |

# APPENDIX F

## EXPERIMENTAL DATA FOR FUNGI ENUMERATION

| Ozone Trt (ppm) | MC (%) | Rep | Unifect. | *Asp.* | *Fus.* | *Rhi.* | *Pen.* | *Cur.* | *Muc.* | *Cla.* | Other | Sum Occ. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 18 | 1 | 0 | 7 | 24 | 3 | 79 | 2 | 10 | 1 | 5 | 131 |
| 0 | 18 | 2 | 0 | 10 | 23 | 5 | 61 | 3 | 16 | 3 | 7 | 128 |
| 0 | 18 | 3 | 0 | 21 | 24 | 4 | 41 | 0 | 13 | 1 | 6 | 110 |
| 50 | 18 | 1 | 6 | 19 | 19 | 6 | 60 | 1 | 12 | 0 | 0 | 117 |
| 50 | 18 | 2 | 6 | 9 | 10 | 10 | 61 | 1 | 13 | 0 | 2 | 106 |
| 50 | 18 | 3 | 5 | 18 | 11 | 10 | 46 | 3 | 8 | 1 | 3 | 100 |
| 500 | 18 | 1 | 49 | 2 | 10 | 10 | 14 | 0 | 8 | 0 | 1 | 45 |
| 500 | 18 | 2 | 24 | 7 | 3 | 11 | 43 | 3 | 10 | 0 | 1 | 78 |
| 500 | 18 | 3 | 24 | 7 | 7 | 4 | 46 | 2 | 12 | 0 | 1 | 79 |
| 1000 | 18 | 1 | 55 | 2 | 6 | 9 | 24 | 0 | 9 | 0 | 1 | 51 |
| 1000 | 18 | 2 | 53 | 4 | 1 | 6 | 29 | 1 | 6 | 0 | 1 | 48 |
| 1000 | 18 | 3 | 50 | 5 | 6 | 5 | 29 | 1 | 10 | 1 | 4 | 61 |
| 15000 | 18 | 1 | 83 | 0 | 3 | 0 | 12 | 1 | 3 | 0 | 1 | 20 |
| 15000 | 18 | 2 | 81 | 0 | 2 | 0 | 13 | 0 | 1 | 1 | 3 | 20 |
| 15000 | 18 | 3 | 80 | 2 | 2 | 0 | 12 | 0 | 2 | 1 | 2 | 21 |
| 0 | 22 | 1 | 4 | 13 | 28 | 2 | 37 | 0 | 11 | 0 | 11 | 102 |
| 0 | 22 | 2 | 0 | 18 | 58 | 1 | 47 | 2 | 7 | 0 | 1 | 134 |
| 0 | 22 | 3 | 0 | 17 | 28 | 6 | 50 | 0 | 14 | 0 | 5 | 120 |
| 500 | 22 | 1 | 19 | 6 | 7 | 1 | 69 | 0 | 1 | 0 | 2 | 86 |
| 500 | 22 | 2 | 41 | 6 | 8 | 4 | 33 | 1 | 8 | 3 | 1 | 64 |
| 500 | 22 | 3 | 39 | 3 | 5 | 3 | 52 | 0 | 4 | 1 | 1 | 69 |
| 1000 | 22 | 1 | 51 | 6 | 5 | 1 | 38 | 1 | 2 | 0 | 1 | 54 |
| 1000 | 22 | 2 | 36 | 9 | 14 | 0 | 51 | 1 | 1 | 0 | 0 | 76 |
| 1000 | 22 | 3 | 47 | 9 | 10 | 2 | 27 | 0 | 5 | 1 | 4 | 58 |
| 15000 | 22 | 1 | 85 | 2 | 0 | 0 | 12 | 0 | 2 | 0 | 0 | 16 |
| 15000 | 22 | 2 | 80 | 2 | 1 | 0 | 16 | 0 | 5 | 0 | 0 | 24 |
| 15000 | 22 | 3 | 81 | 3 | 3 | 0 | 14 | 0 | 1 | 0 | 0 | 21 |
| 0 | 26 | 1 | 0 | 12 | 22 | 4 | 68 | 1 | 9 | 0 | 4 | 120 |
| 0 | 26 | 2 | 0 | 19 | 16 | 4 | 77 | 0 | 12 | 0 | 1 | 129 |
| 0 | 26 | 3 | 0 | 9 | 34 | 0 | 52 | 0 | 13 | 0 | 15 | 123 |
| 500 | 26 | 1 | 4 | 7 | 5 | 0 | 92 | 0 | 3 | 0 | 2 | 109 |
| 500 | 26 | 2 | 10 | 8 | 6 | 3 | 68 | 0 | 6 | 0 | 2 | 93 |
| 500 | 26 | 3 | 25 | 7 | 9 | 0 | 47 | 0 | 8 | 0 | 8 | 79 |
| 1000 | 26 | 1 | 48 | 2 | 9 | 1 | 35 | 0 | 9 | 1 | 0 | 57 |
| 1000 | 26 | 2 | 44 | 4 | 4 | 0 | 44 | 0 | 6 | 0 | 2 | 60 |
| 1000 | 26 | 3 | 27 | 8 | 8 | 0 | 54 | 0 | 3 | 0 | 1 | 74 |
| 15000 | 26 | 1 | 63 | 5 | 5 | 0 | 22 | 1 | 6 | 0 | 1 | 40 |
| 15000 | 26 | 2 | 74 | 3 | 3 | 0 | 15 | 1 | 3 | 0 | 4 | 29 |
| 15000 | 26 | 3 | 71 | 7 | 7 | 0 | 20 | 0 | 5 | 0 | 1 | 40 |

## MEAN VALUES

| Ozone Trt (ppm) | MC (%) | Uninfect. | *Asp.* | *Fus.* | *Rhi.* | *Pen.* | *Cur.* | *Muc.* | *Cla.* | Other | Sum Occ. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0.0 | 18.0 | 0.0 | 12.7 | 23.7 | 4.0 | 60.3 | 1.7 | 13.0 | 1.7 | 6.0 | 123.0 |
| 50.0 | 18.0 | 5.7 | 15.3 | 13.3 | 8.7 | 55.7 | 1.7 | 11.0 | 0.3 | 1.7 | 107.7 |
| 500.0 | 18.0 | 32.3 | 5.3 | 6.7 | 8.3 | 34.3 | 1.7 | 10.0 | 0.0 | 1.0 | 67.3 |
| 1000.0 | 18.0 | 52.7 | 3.7 | 4.3 | 6.7 | 27.3 | 0.7 | 8.3 | 0.3 | 2.0 | 53.3 |
| 15000.0 | 18.0 | 81.3 | 0.7 | 2.3 | 0.0 | 12.3 | 0.3 | 2.0 | 0.7 | 2.0 | 20.3 |
| 0.0 | 22.0 | 1.3 | 16.0 | 38.0 | 3.0 | 44.7 | 0.7 | 10.7 | 0.0 | 5.7 | 118.7 |
| 500.0 | 22.0 | 33.0 | 5.0 | 6.7 | 2.7 | 51.3 | 0.3 | 4.3 | 1.3 | 1.3 | 73.0 |
| 1000.0 | 22.0 | 44.7 | 8.0 | 9.7 | 1.0 | 38.7 | 0.7 | 2.7 | 0.3 | 1.7 | 62.7 |
| 15000.0 | 22.0 | 82.0 | 2.3 | 1.3 | 0.0 | 14.0 | 0.0 | 2.7 | 0.0 | 0.0 | 20.3 |
| 0.0 | 26.0 | 0.0 | 13.3 | 24.0 | 2.7 | 65.7 | 0.3 | 11.3 | 0.0 | 6.7 | 124.0 |
| 500.0 | 26.0 | 13.0 | 7.3 | 6.7 | 1.0 | 69.0 | 0.0 | 5.7 | 0.0 | 4.0 | 93.7 |
| 1000.0 | 26.0 | 39.7 | 4.7 | 7.0 | 0.3 | 44.3 | 0.0 | 6.0 | 0.3 | 1.0 | 63.7 |
| 15000.0 | 26.0 | 69.3 | 5.0 | 5.0 | 0.0 | 19.0 | 0.7 | 4.7 | 0.0 | 2.0 | 36.3 |

## COMPARISON TO NON-TREATED FUNGI LEVELS

| Ozone Trt (ppm) | Uninfected | *Asp.* | *Fus.* | *Rhi.* | *Pen.* | *Cur.* | *Muc.* | *Cla.* | Other |
|---|---|---|---|---|---|---|---|---|---|
| 0 | 100% | 100% | 100% | 100% | 100% | 100% | 100% | 100% | 100% |
| 500 | 42% | 23% | 124% | 91% | 75% | 57% | 80% | 35% | 64% |
| 1000 | 39% | 25% | 83% | 65% | 50% | 49% | 60% | 25% | 49% |
| 15000 | 19% | 10% | 0% | 27% | 38% | 27% | 40% | 22% | 21% |

## APPENDIX G

## DATA ANALYSIS AND SAS CODE FOR FUNGI ENUMERATION TEST IN CHAPTER 4

```
title 'High-moisture storage of corn using ozone';

data a;
label rate  = 'Ozone Concentration, ppm';
label mc   = 'moisture content, % wb';
label unin = 'Uninfected, %';
label asp  = 'Aspergillus';
label fus  = 'Fusarium';
label rhi  = 'Rhizopus';
label pen  = 'Penicillium';
label cur  = 'Curvularia';
label muc  = 'Mucor';
label cla  = 'Cladosporium';
label oth  = 'Other';
label sum  = 'Sum of all Infections';

input rate mc unin asp fus rhi pen cur muc cla oth sum;
datalines;
0     18    0     7     24    3     79    2     10    1     5     131
0     18    0     10    23    5     61    3     16    3     7     128
0     18    0     21    24    4     41    0     13    1     6     110
50    18    6     19    19    6     60    1     12    0     0     117
50    18    6     9     10    10    61    1     13    0     2     106
50    18    5     18    11    10    46    3     8     1     3     100
500   18    49    2     10    10    14    0     8     0     1     45
500   18    24    7     3     11    43    3     10    0     1     78
500   18    24    7     7     4     46    2     12    0     1     79
1000  18    55    2     6     9     24    0     9     0     1     51
1000  18    53    4     1     6     29    1     6     0     1     48
1000  18    50    5     6     5     29    1     10    1     4     61
15000 18    83    0     3     0     12    1     3     0     1     20
15000 18    81    0     2     0     13    0     1     1     3     20
15000 18    80    2     2     0     12    0     2     1     2     21
0     22    4     13    28    2     37    0     11    0     11    102
0     22    0     18    58    1     47    2     7     0     1     134
0     22    0     17    28    6     50    0     14    0     5     120
500   22    19    6     7     1     69    0     1     0     2     86
500   22    41    6     8     4     33    1     8     3     1     64
```

```
500     22      39      3       5       3       52      0       4       1       1       69
1000    22      51      6       5       1       38      1       2       0       1       54
1000    22      36      9       14      0       51      1       1       0       0       76
1000    22      47      9       10      2       27      0       5       1       4       58
15000   22      85      2       0       0       12      0       2       0       0       16
15000   22      80      2       1       0       16      0       5       0       0       24
15000   22      81      3       3       0       14      0       1       0       0       21
0       26      0       12      22      4       68      1       9       0       4       120
0       26      0       19      16      4       77      0       12      0       1       129
0       26      0       9       34      0       52      0       13      0       15      123
500     26      4       7       5       0       92      0       3       0       2       109
500     26      10      8       6       3       68      0       6       0       2       93
500     26      25      7       9       0       47      0       8       0       8       79
1000    26      48      2       9       1       35      0       9       1       0       57
1000    26      44      4       4       0       44      0       6       0       2       60
1000    26      27      8       8       0       54      0       3       0       1       74
15000   26      63      5       5       0       22      1       6       0       1       40
15000   26      74      3       3       0       15      1       3       0       4       29
15000   26      71      7       7       0       20      0       5       0       1       40
;

proc glm data=a;
class rate mc;
model unin asp fus rhi pen cur muc cla oth = rate mc rate*mc;
lsmeans rate*mc;
run;
quit;
```

High-moisture storage of corn using ozone 15:07 Friday, July 6, 2007

The GLM Procedure

Class Level Information

| Class | Levels | Values |
|---|---|---|
| rate | 5 | 0 50 500 1000 15000 |
| mc | 3 | 18 22 26 |

| | |
|---|---|
| Number of Observations Read | 39 |
| Number of Observations Used | 39 |
| High-moisture storage of corn using ozone | 2 |

Dependent Variable: unin Uninfected, %

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Model | 12 | 32702.66667 | 2725.22222 | 49.78 | <.0001 |
| Error | 26 | 1423.33333 | 54.74359 | | |
| Corrected Total | 38 | 34126.00000 | | | |

| R-Square | Coeff Var | Root MSE | unin Mean |
|---|---|---|---|
| 0.958292 | 21.13969 | 7.398891 | 35.00000 |

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| rate | 4 | 31362.00000 | 7840.50000 | 143.22 | <.0001 |
| mc | 2 | 878.72222 | 439.36111 | 8.03 | 0.0019 |
| rate*mc | 6 | 461.94444 | 76.99074 | 1.41 | 0.2498 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| rate | 4 | 31661.57222 | 7915.39306 | 144.59 | <.0001 |
| mc | 2 | 878.72222 | 439.36111 | 8.03 | 0.0019 |
| rate*mc | 6 | 461.94444 | 76.99074 | 1.41 | 0.2498 |

Dependent Variable: asp Aspergillus

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Model | 12 | 922.974359 | 76.914530 | 6.67 | <.0001 |
| Error | 26 | 300.000000 | 11.538462 | | |
| Corrected Total | 38 | 1222.974359 | | | |

| R-Square | Coeff Var | Root MSE | asp Mean |
|---|---|---|---|
| 0.754696 | 44.45517 | 3.396831 | 7.641026 |

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| rate | 4 | 835.1965812 | 208.7991453 | 18.10 | <.0001 |
| mc | 2 | 36.5000000 | 18.2500000 | 1.58 | 0.2248 |
| rate*mc | 6 | 51.2777778 | 8.5462963 | 0.74 | 0.6218 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| rate | 4 | 871.0388889 | 217.7597222 | 18.87 | <.0001 |
| mc | 2 | 36.5000000 | 18.2500000 | 1.58 | 0.2248 |
| rate*mc | 6 | 51.2777778 | 8.5462963 | 0.74 | 0.6218 |

Dependent Variable: fus Fusarium

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Model | 12 | 4153.589744 | 346.132479 | 9.57 | <.0001 |
| Error | 26 | 940.000000 | 36.153846 | | |
| Corrected Total | 38 | 5093.589744 | | | |

| R-Square | Coeff Var | Root MSE | fus Mean |
|---|---|---|---|
| 0.815454 | 52.57836 | 6.012807 | 11.43590 |

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| rate | 4 | 3687.811966 | 921.952991 | 25.50 | <.0001 |
| mc | 2 | 137.388889 | 68.694444 | 1.90 | 0.1698 |
| rate*mc | 6 | 328.388889 | 54.731481 | 1.51 | 0.2129 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| rate | 4 | 3716.127778 | 929.031944 | 25.70 | <.0001 |
| mc | 2 | 137.388889 | 68.694444 | 1.90 | 0.1698 |
| rate*mc | 6 | 328.388889 | 54.731481 | 1.51 | 0.2129 |

Dependent Variable: rhi Rhizopus

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Model | 12 | 351.8974359 | 29.3247863 | 8.66 | <.0001 |
| Error | 26 | 88.0000000 | 3.3846154 | | |
| Corrected Total | 38 | 439.8974359 | | | |

| R-Square | Coeff Var | Root MSE | rhi Mean |
|---|---|---|---|
| 0.799953 | 62.39093 | 1.839732 | 2.948718 |

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| rate | 4 | 187.6752137 | 46.9188034 | 13.86 | <.0001 |
| mc | 2 | 96.0555556 | 48.0277778 | 14.19 | <.0001 |
| rate*mc | 6 | 68.1666667 | 11.3611111 | 3.36 | 0.0139 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| rate | 4 | 118.2333333 | 29.5583333 | 8.73 | 0.0001 |
| mc | 2 | 96.0555556 | 48.0277778 | 14.19 | <.0001 |
| rate*mc | 6 | 68.1666667 | 11.3611111 | 3.36 | 0.0139 |

Dependent Variable: pen Penicillium

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Model | 12 | 13149.23077 | 1095.76923 | 6.97 | <.0001 |
| Error | 26 | 4084.66667 | 157.10256 | | |
| Corrected Total | 38 | 17233.89744 | | | |

| R-Square | Coeff Var | Root MSE | pen Mean |
|---|---|---|---|
| 0.762986 | 30.36200 | 12.53406 | 41.28205 |

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| rate | 4 | 10109.67521 | 2527.41880 | 16.09 | <.0001 |
| mc | 2 | 1673.16667 | 836.58333 | 5.33 | 0.0115 |
| rate*mc | 6 | 1366.38889 | 227.73148 | 1.45 | 0.2343 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| rate | 4 | 10607.61111 | 2651.90278 | 16.88 | <.0001 |
| mc | 2 | 1673.16667 | 836.58333 | 5.33 | 0.0115 |
| rate*mc | 6 | 1366.38889 | 227.73148 | 1.45 | 0.2343 |

Dependent Variable: cur Curvularia

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Model | 12 | 14.00000000 | 1.16666667 | 1.62 | 0.1454 |
| Error | 26 | 18.66666667 | 0.71794872 | | |
| Corrected Total | 38 | 32.66666667 | | | |

| R-Square | Coeff Var | Root MSE | cur Mean |
|---|---|---|---|
| 0.428571 | 127.0978 | 0.847319 | 0.666667 |

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| rate | 4 | 4.88888889 | 1.22222222 | 1.70 | 0.1797 |
| mc | 2 | 4.66666667 | 2.33333333 | 3.25 | 0.0550 |
| rate*mc | 6 | 4.44444444 | 0.74074074 | 1.03 | 0.4274 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| rate | 4 | 2.45555556 | 0.61388889 | 0.86 | 0.5037 |
| mc | 2 | 4.66666667 | 2.33333333 | 3.25 | 0.0550 |
| rate*mc | 6 | 4.44444444 | 0.74074074 | 1.03 | 0.4274 |

Dependent Variable: muc Mucor

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Model | 12 | 518.2564103 | 43.1880342 | 6.96 | <.0001 |
| Error | 26 | 161.3333333 | 6.2051282 | | |
| Corrected Total | 38 | 679.5897436 | | | |

| R-Square | Coeff Var | Root MSE | muc Mean |
|---|---|---|---|
| 0.762602 | 35.07197 | 2.491009 | 7.102564 |

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| rate | 4 | 396.7008547 | 99.1752137 | 15.98 | <.0001 |
| mc | 2 | 63.7222222 | 31.8611111 | 5.13 | 0.0132 |
| rate*mc | 6 | 57.8333333 | 9.6388889 | 1.55 | 0.2006 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| rate | 4 | 364.4000000 | 91.1000000 | 14.68 | <.0001 |
| mc | 2 | 63.7222222 | 31.8611111 | 5.13 | 0.0132 |

| | | | | | |
|---|---|---|---|---|---|
| rate*mc | 6 | 57.8333333 | 9.6388889 | 1.55 | 0.2006 |

Dependent Variable: cla Cladosporium

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Model | 12 | 10.56410256 | 0.88034188 | 2.15 | 0.0502 |
| Error | 26 | 10.66666667 | 0.41025641 | | |
| Corrected Total | 38 | 21.23076923 | | | |

| R-Square | Coeff Var | Root MSE | cla Mean |
|---|---|---|---|
| 0.497585 | 166.5333 | 0.640513 | 0.384615 |

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| rate | 4 | 0.56410256 | 0.14102564 | 0.34 | 0.8459 |
| mc | 2 | 2.05555556 | 1.02777778 | 2.51 | 0.1012 |
| rate*mc | 6 | 7.94444444 | 1.32407407 | 3.23 | 0.0167 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| rate | 4 | 0.82222222 | 0.20555556 | 0.50 | 0.7352 |
| mc | 2 | 2.05555556 | 1.02777778 | 2.51 | 0.1012 |
| rate*mc | 6 | 7.94444444 | 1.32407407 | 3.23 | 0.0167 |

Dependent Variable: oth Other

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Model | 12 | 166.9743590 | 13.9145299 | 1.68 | 0.1301 |
| Error | 26 | 215.3333333 | 8.2820513 | | |
| Corrected Total | 38 | 382.3076923 | | | |

| R-Square | Coeff Var | Root MSE | oth Mean |
|---|---|---|---|
| 0.436754 | 106.8918 | 2.877855 | 2.692308 |

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| rate | 4 | 139.6410256 | 34.9102564 | 4.22 | 0.0092 |
| mc | 2 | 9.3888889 | 4.6944444 | 0.57 | 0.5742 |
| rate*mc | 6 | 17.9444444 | 2.9907407 | 0.36 | 0.8967 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| rate | 4 | 139.0388889 | 34.7597222 | 4.20 | 0.0094 |
| mc | 2 | 9.3888889 | 4.6944444 | 0.57 | 0.5742 |
| rate*mc | 6 | 17.9444444 | 2.9907407 | 0.36 | 0.8967 |

## APPENDIX H: OZONE DEMAND/REQUIREMENT – SEMI-BATCH METHOD

2350 Oxidant demand/requirement. Standard Methods for the Examination of Water and Wastewater, 21st Edition

The following chemicals must be prepared to run titrations: ozone free water, sulfuric acid, potassium iodide, sodium thiosulfate, sodium thiosulfate dilution, and a starch indicator.

Ozone Free Water

- Ozonate water for 1 hr
- Purge with nitrogen gas for 1 hr

Sulfuric Acid ($H_2SO_4$)

- Add 56 mL conc sulfuric acid to 800 mL ozone-demand-free water in a 1-L flask
- Mix
- Add water to 1 L mark

Potassium Iodide (KI)

- Dissolve 20 g KI in 800 mL ozone-demand-free water
- Mix
- Cool
- Fill to 1L with ozone-demand-free water

Sodium Thiosulfate Titrant ($Na_2S_2O_3$) .1N

- Dissolve 25g $NA_2S_2O_3*5H_2O$ in 1 L freshly boiled distilled water
- Add a few ml of chloroform ($CHCl_3$)
- Let the solution sit for 2 weeks

Sodium Thiosulfate Titrant .005N

- Dilute 50 ml of .1N to 1L total volume

Starch Indicator Solution

- 5 g starch
- Add a little chilled water and mix to a paste
- Pour into 1L of boiling distilled water
- Stir
- Settle overnight
- Use supernate
- Preserve with one of the following:
    - 1.25g salicylic acid
    - 4g zinc chloride

Titration Instructions

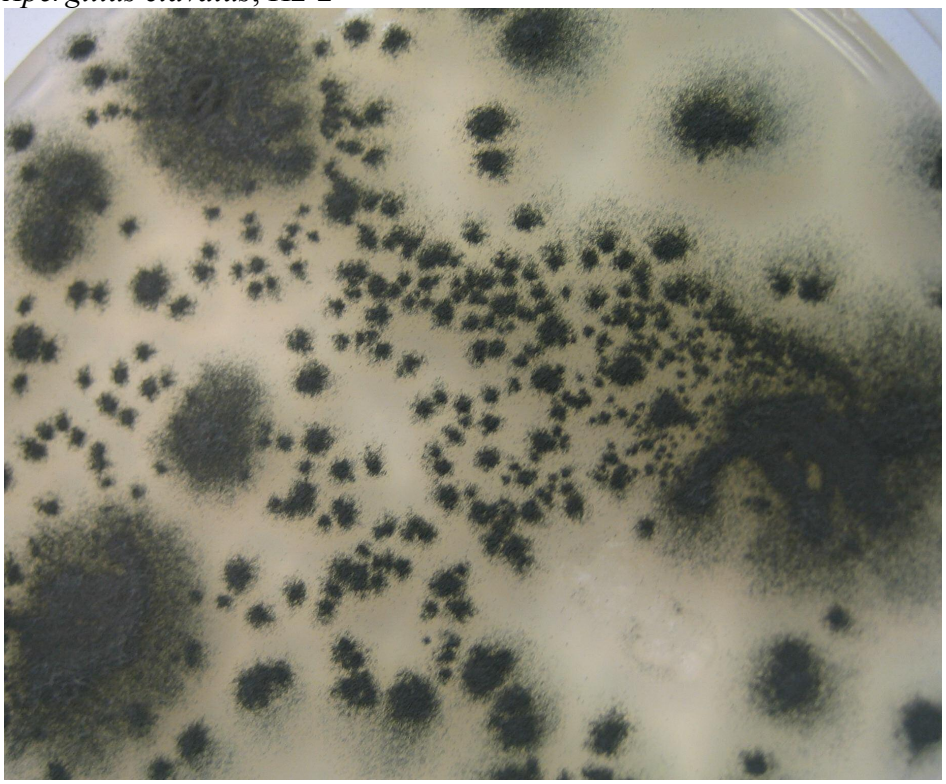
1. Run Ozone through KI trap for ~10 min. at 1 L/min
    a. KI trap must have at least 200mL of 2% KI
2. Add 5 ml of 2N $H_2SO_4$
3. Titrate with .005N $Na_2S_2O_3$ until yellow disappears
4. Add 1~2 mL starch indicator solution
5. Titrate until solution becomes visually clear (the blue hue disappears)

## APPENDIX I. FUNGI IDENTIFICATION KEY

### *Aspergillus*

Kingdom: Fungi
Phylum: Ascomycota
Class: Eurotiomycetes
Order: Eurotiales
Family: Trichocomaceae
Genus: *Aspergillus*

*Apergillus clavatus*, H2-2



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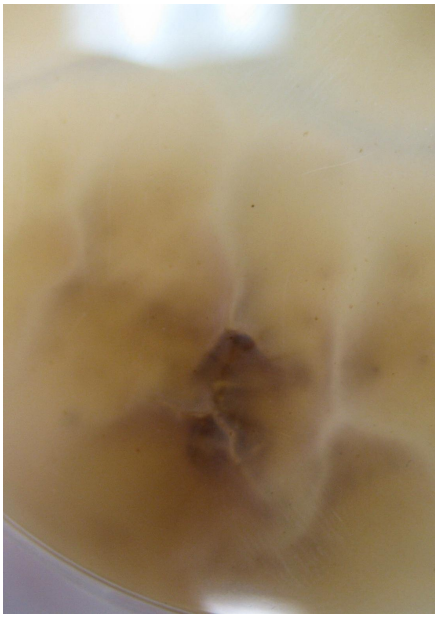
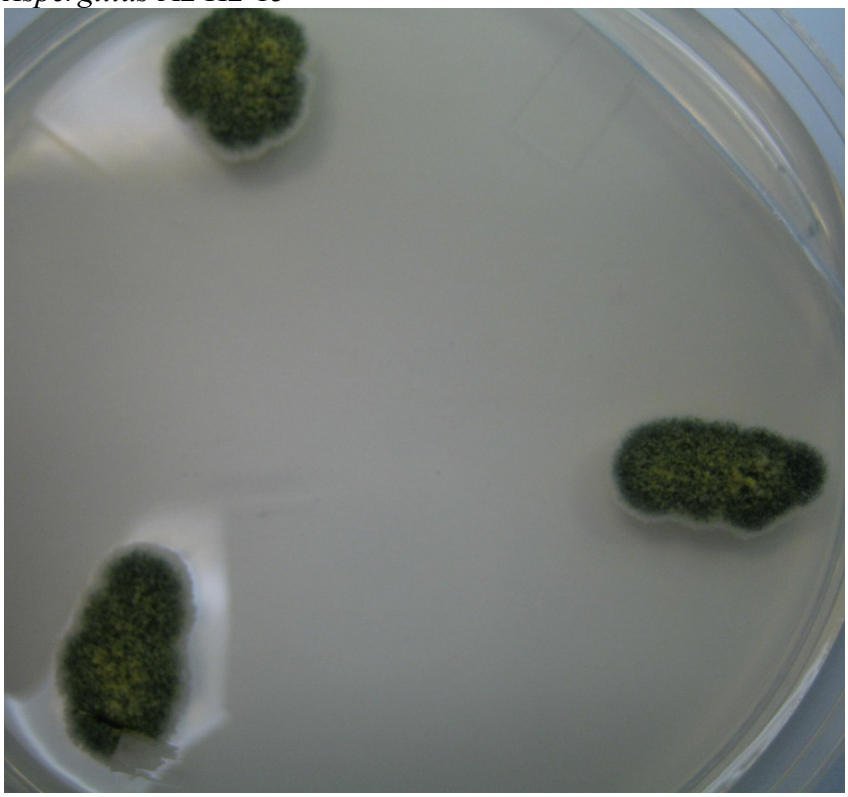


Czapek Dox Agar – Growth: Light brown/grey, Underside: Rigid grooves (resembles cracks in dried clay)

*Aspergillus* A2 H2-15



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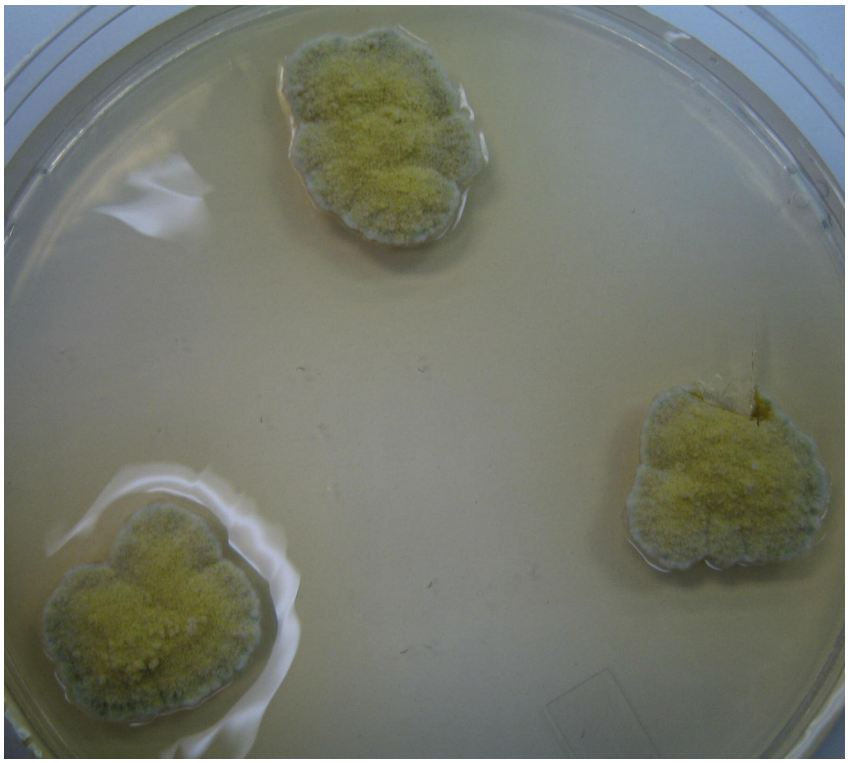
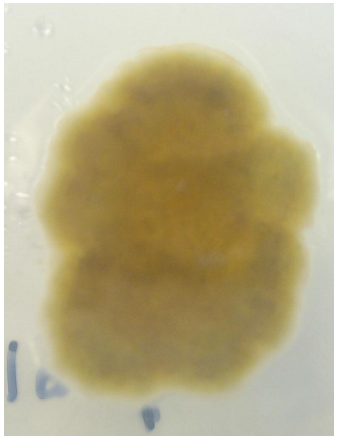
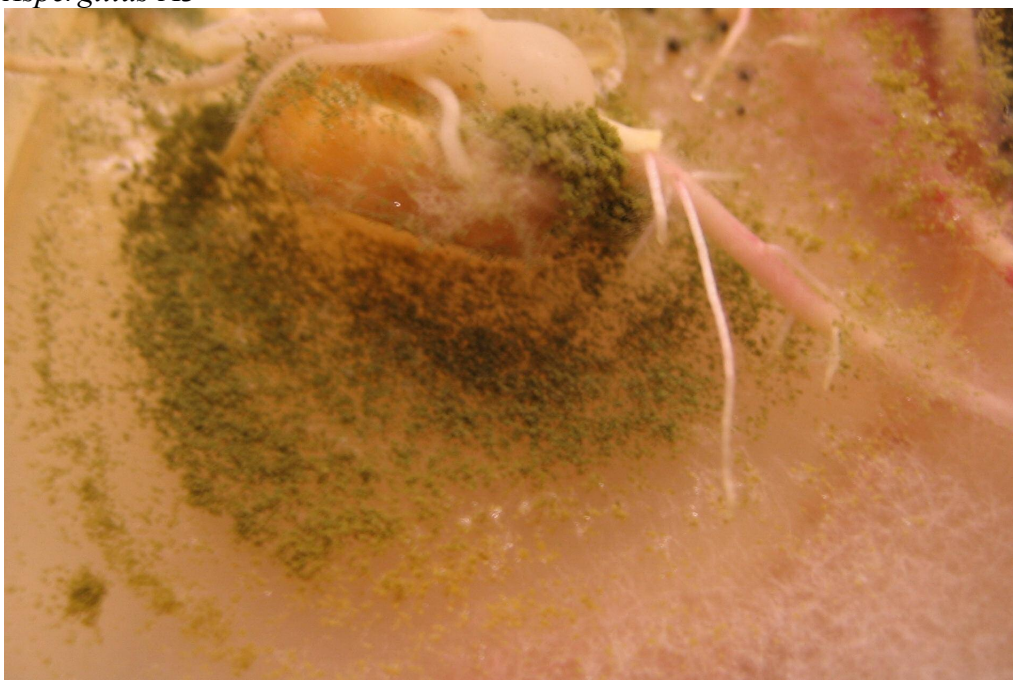




Czopek Dox Agar

*Aspergillus* A3



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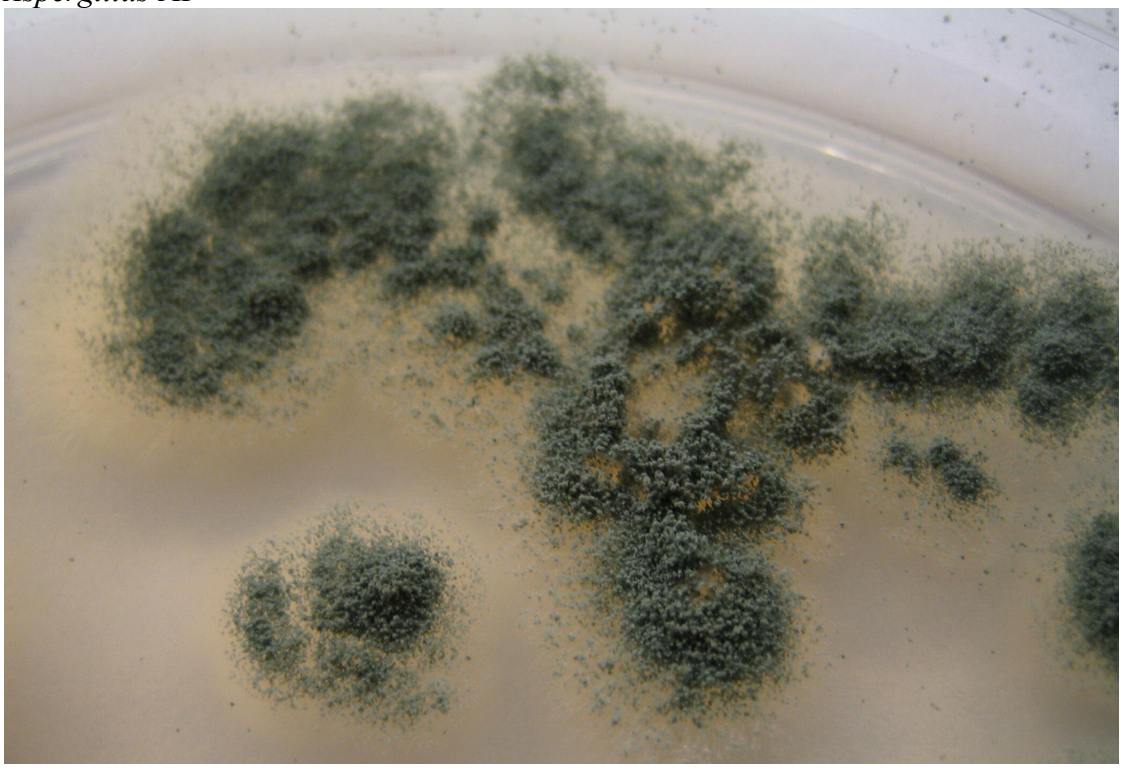
MEA

*Aspergillus* AF



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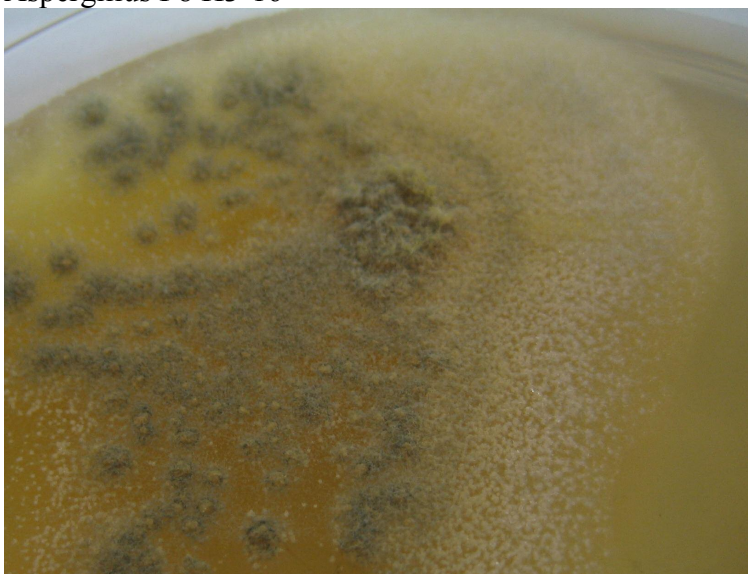
Czopek Dox Agar

Aspergillus P8 H3-16



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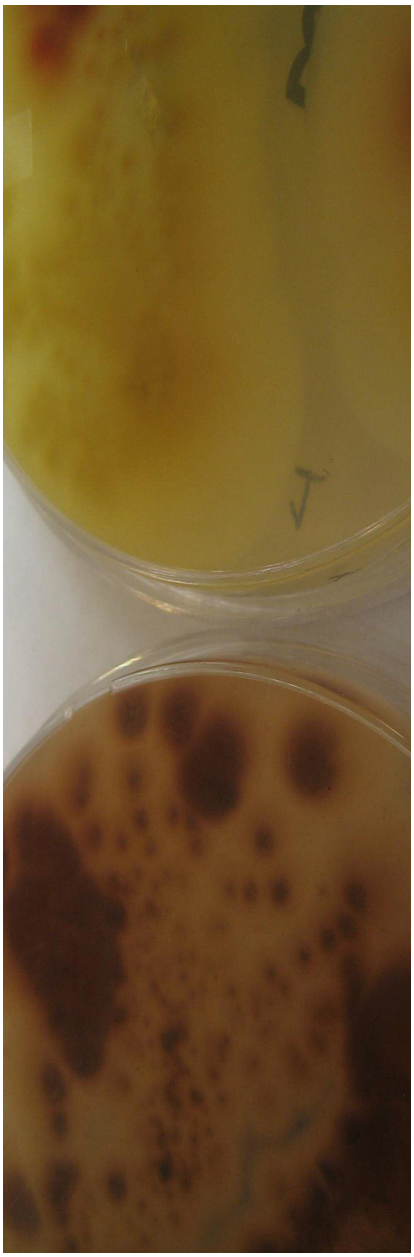
Czopek Dox Agar

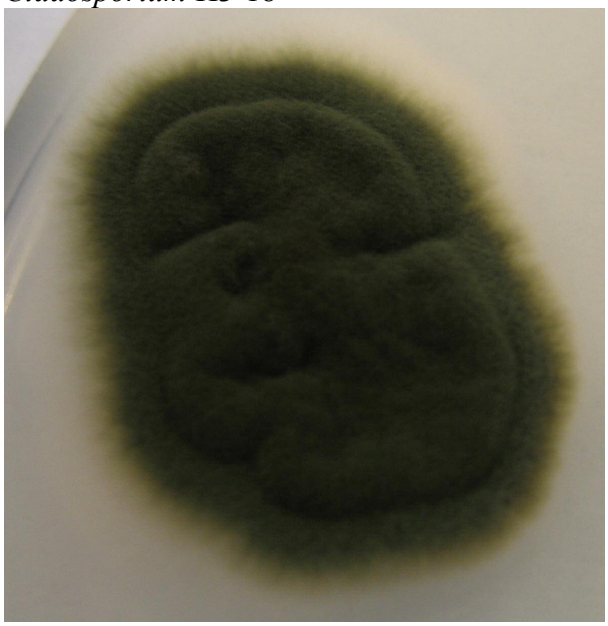


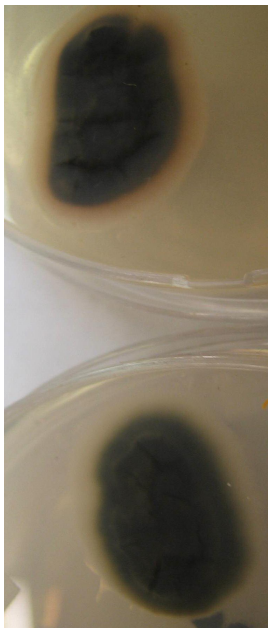
***Cladosporium***

Kingdom: Fungi
Division: Deuteromycota
Order: Moniliales
Family: Dematiaceae
Genus: *Cladosporium*

Information: *Cladosporium* species produce no major mycotoxins of concern, but does produce volatile organic compounds (VOCs).

*Cladosporium* H3-18





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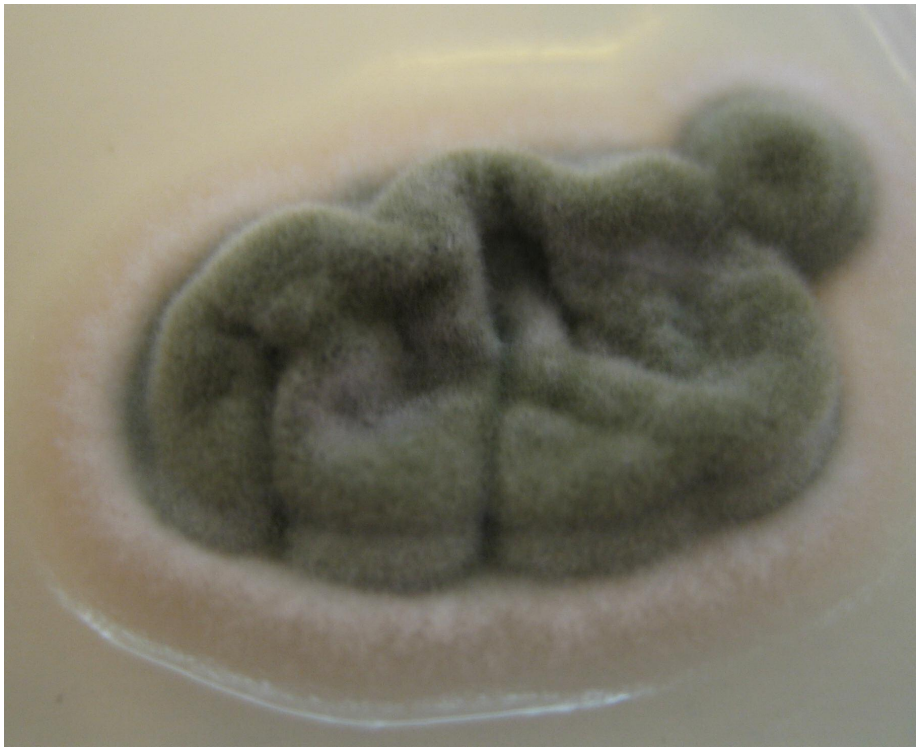
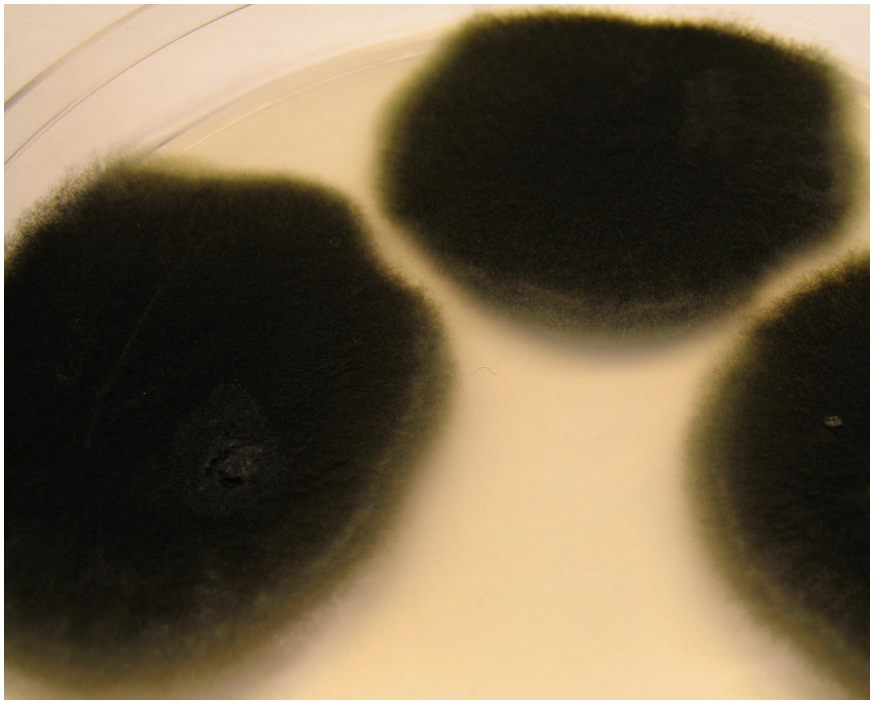


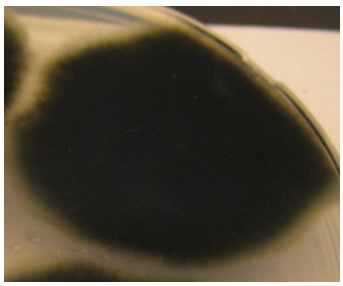
Czopek Dox

***Curvularia***

Kingdom: Fungi
Phylum: Ascomycota
Class: Euascomycetes
Order: Pleosporales
Genus: *Curvularia*

*Curvularia* S1-9-1





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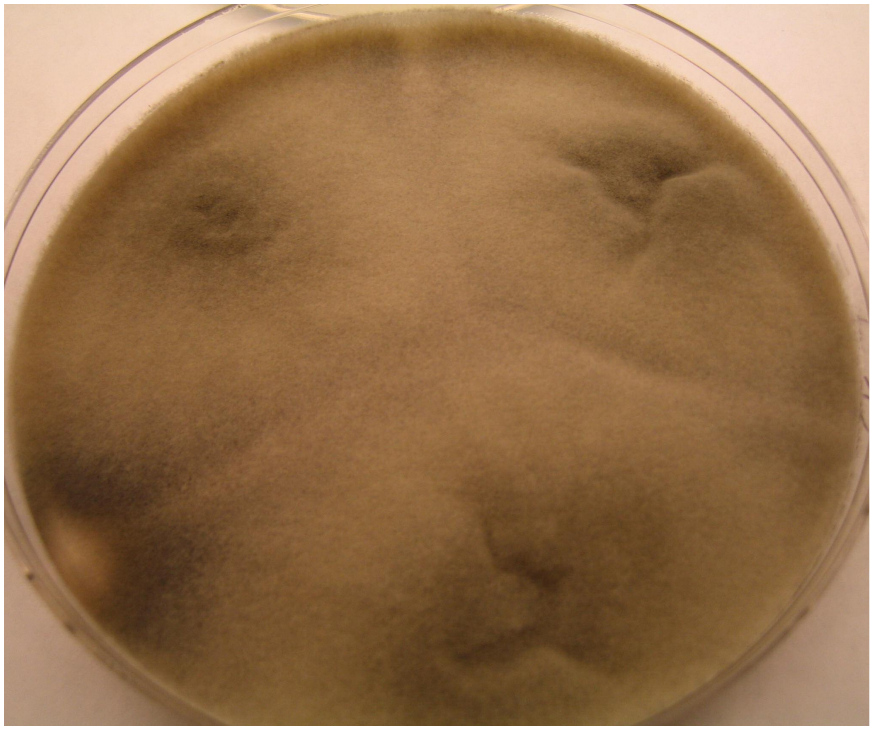
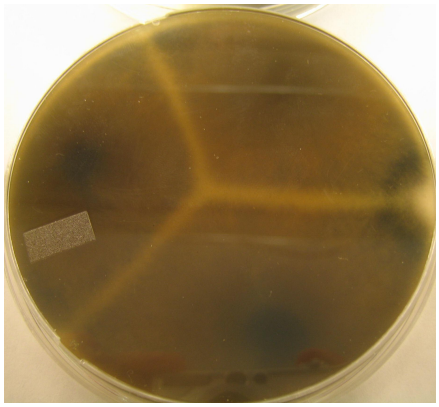




Czopek Dox

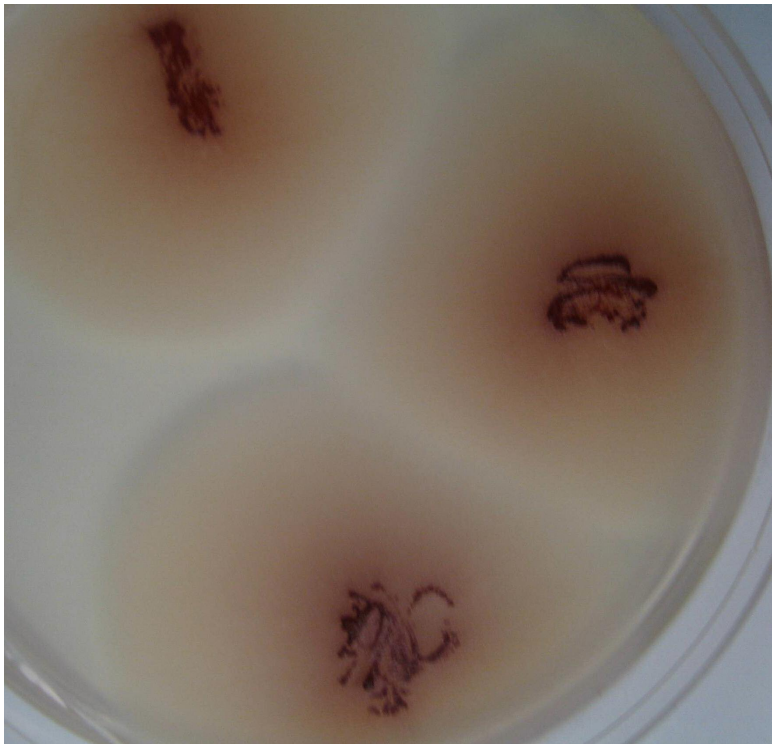
# *Fusarium*

Kingdom: Fungi
Phylum: Ascomycota
Class: Sordariomycetes
Order: Hypocreales
Genus: *Fusarium*

*Fusarium* is a large genus with world-wide distribution. It is a filamentous fungi that is usually harmless, but some species are plant pathogens that may cause root rot, stem rot, vascular wilts, fruit rot, infect seeds. Furthermore, *fusarium* produces three of the five mycotoxins that are internationally regulated.

**Appearance:**
On PDA, colonies will have a fast growth with a white, cream color, yellowish, brownish, pink, reddish, or violet complexion. The surface will appear "cottony," while the underside will have a distinct red appearance.

*Fusarium* S1-5



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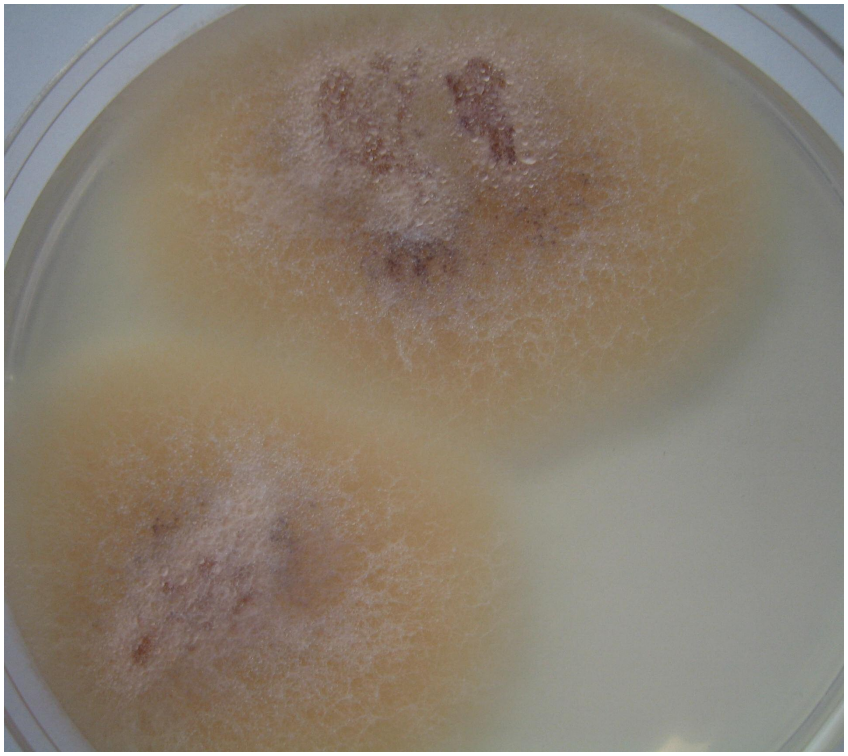
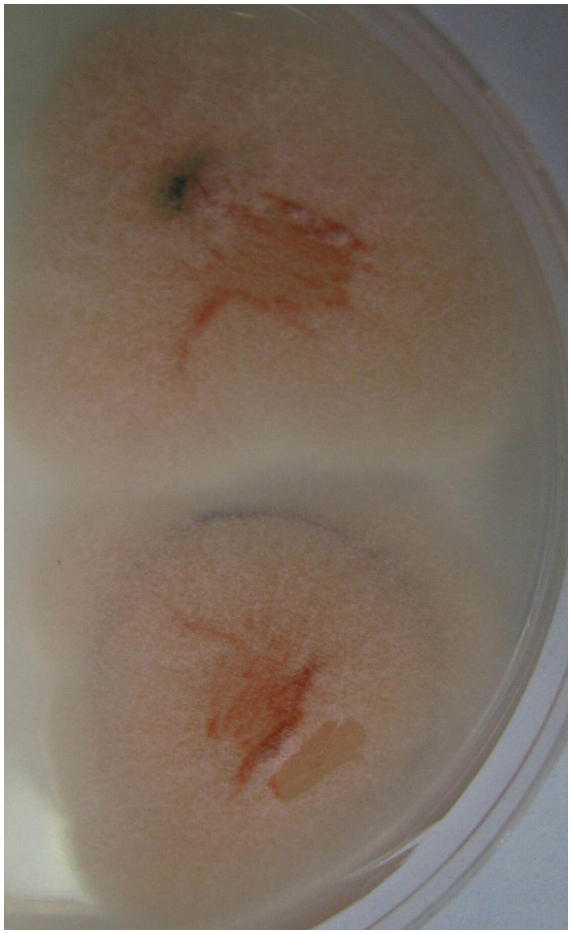


PDA

*Fusarium* S1-3-4



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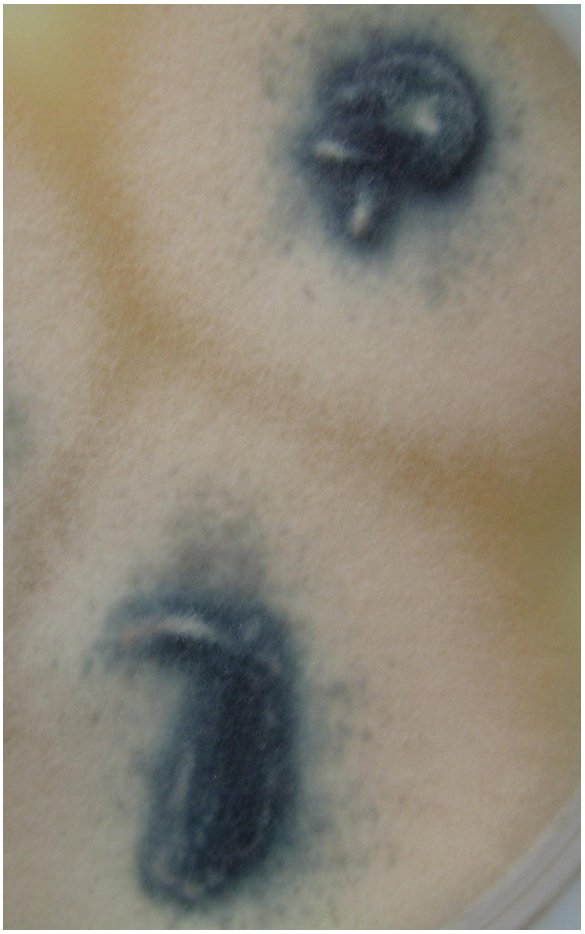


Czopek Dox

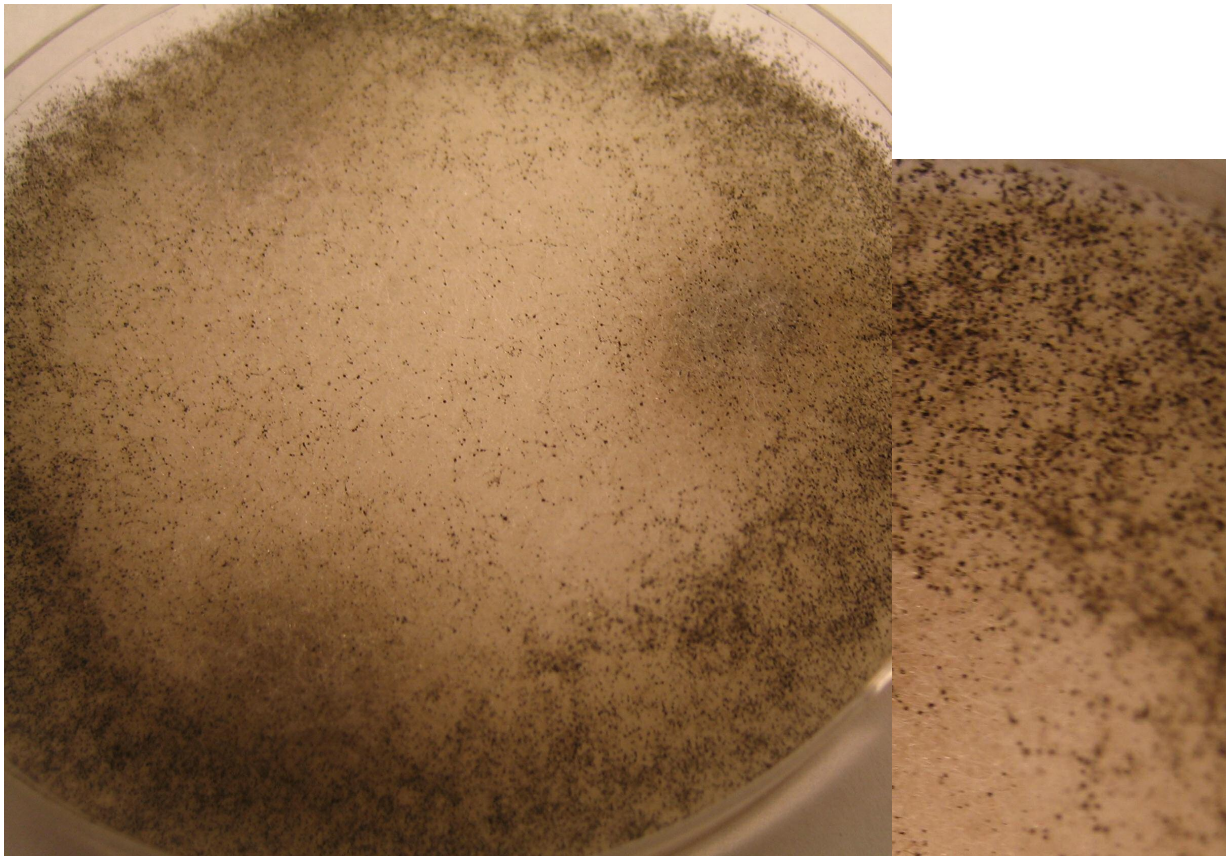
PDA

***Mucor***

Kingdom: Fungi
Division: Zygomycota
Class: Zygomycetes
Order: Mucorales
Family: Mucoraceae
Genus: *Mucor*

Information: Colonies typically white to beige or gray and fast-growing (usually covering entire plate)

*Mucor* S0-12-1



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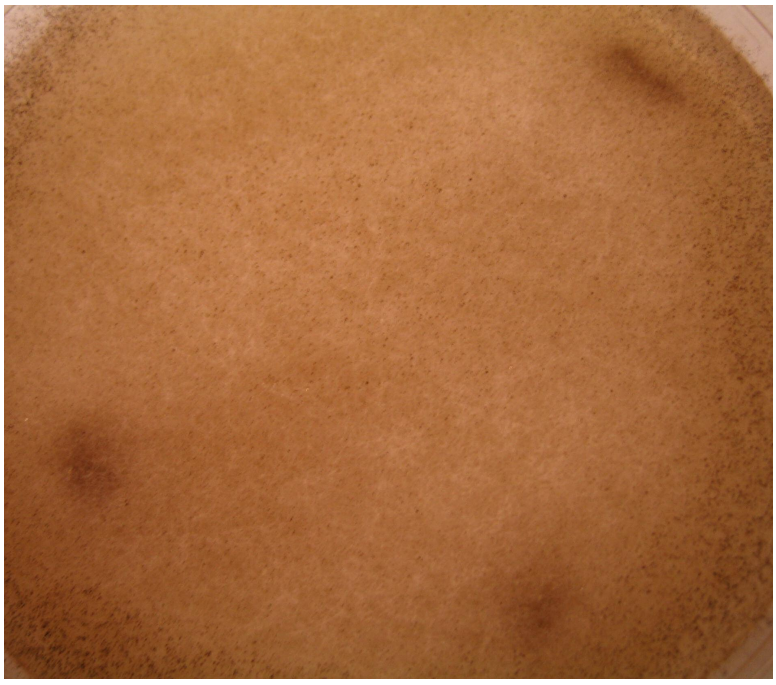

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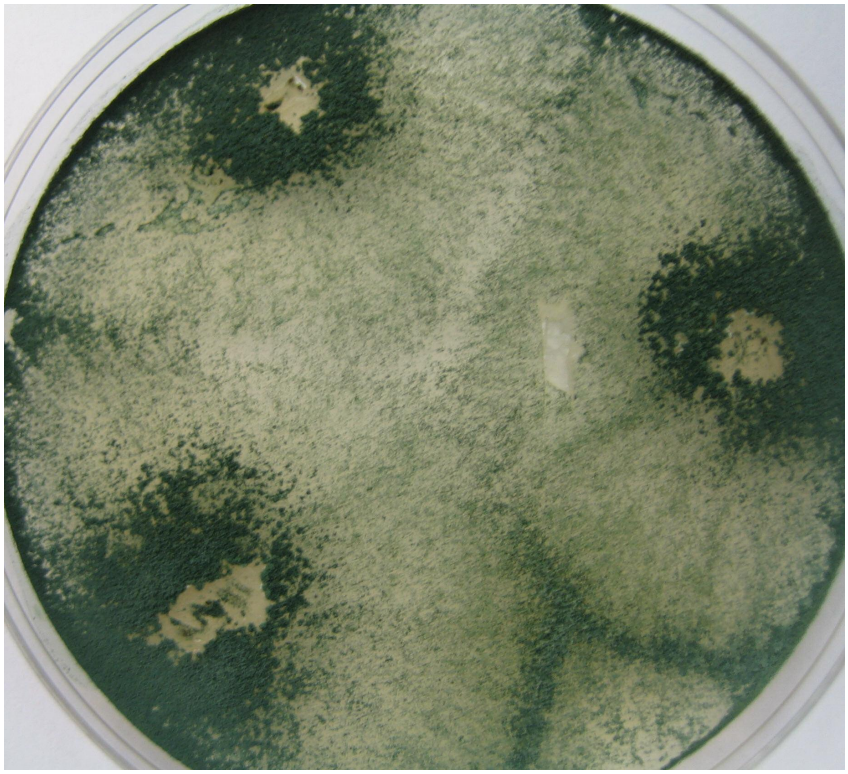
*Mucor* S1-8-2



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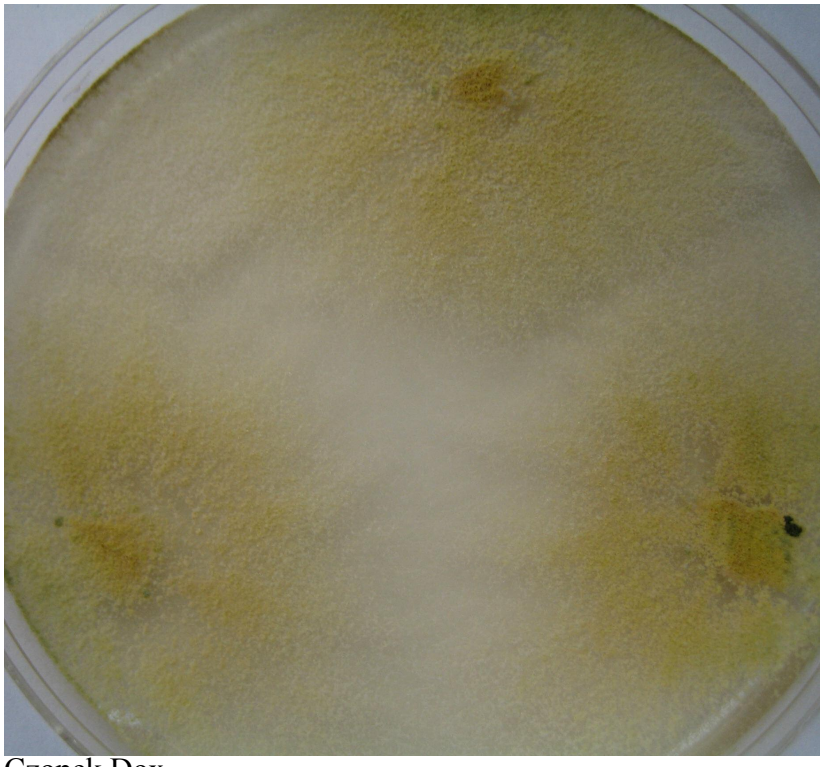
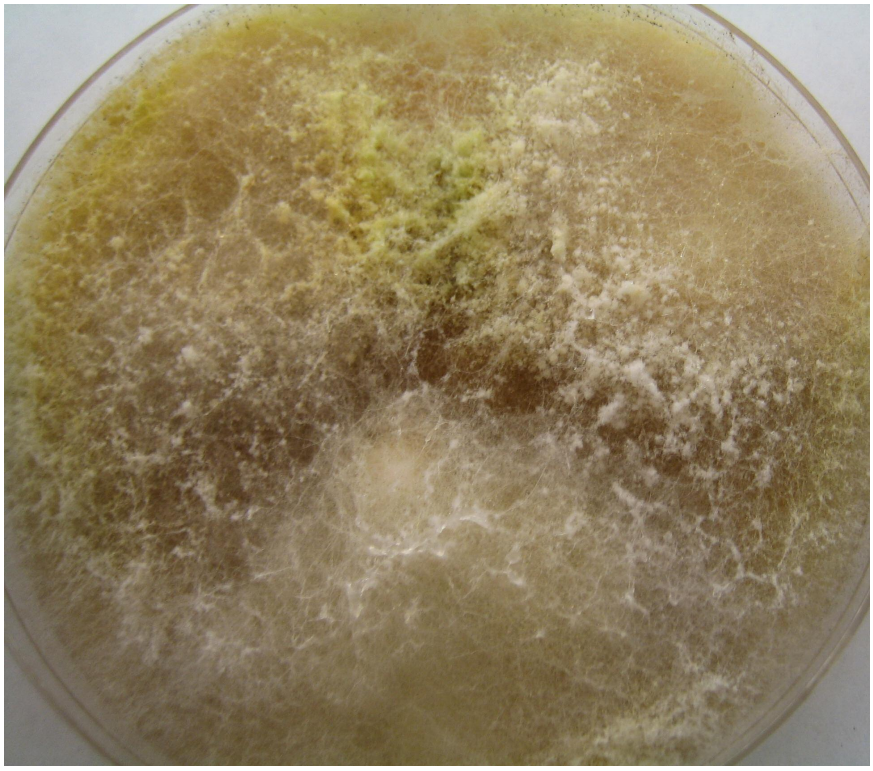
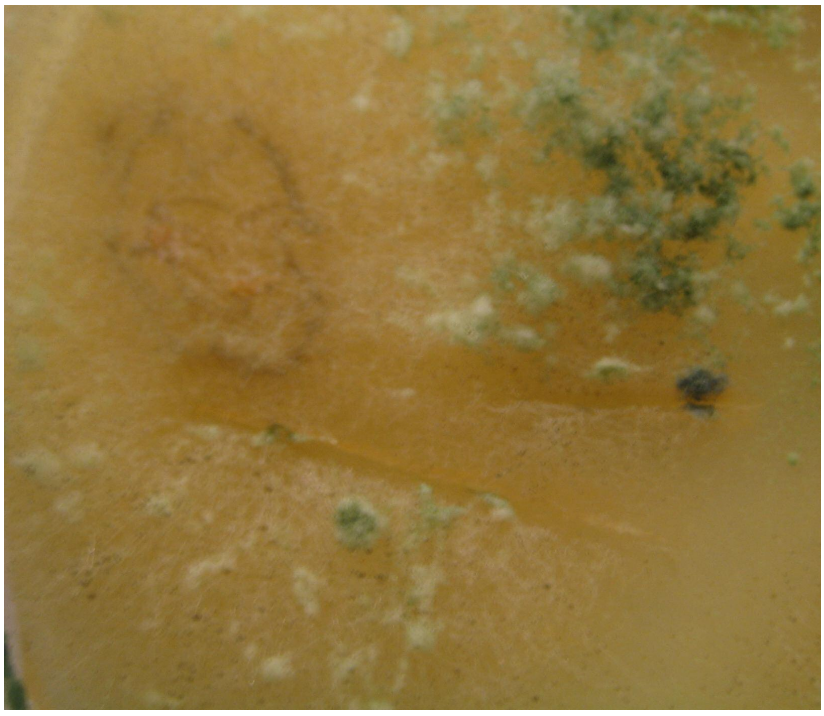


Czopek Dox

*Mucoral* II



Czopek Dox



MEA

Mucoral S3-7

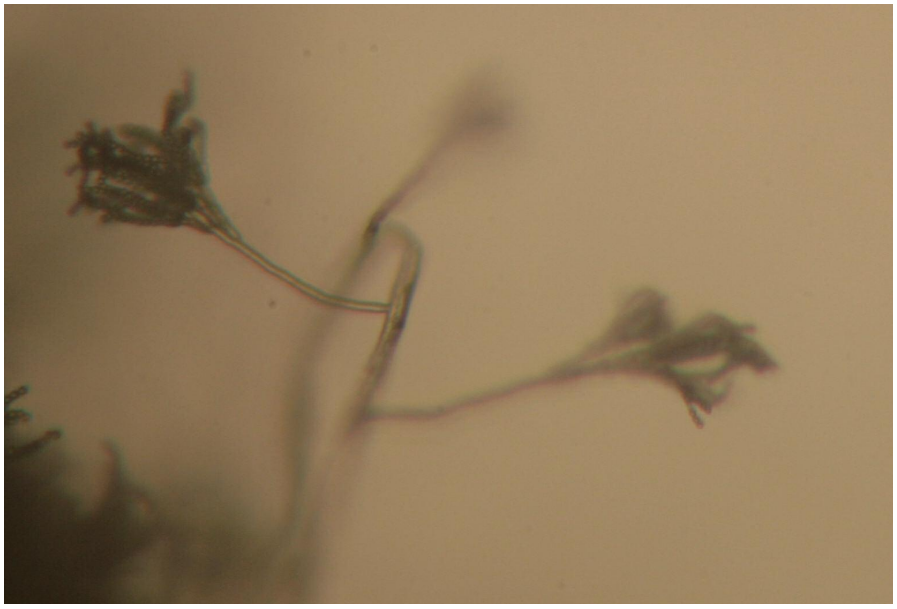
MEA

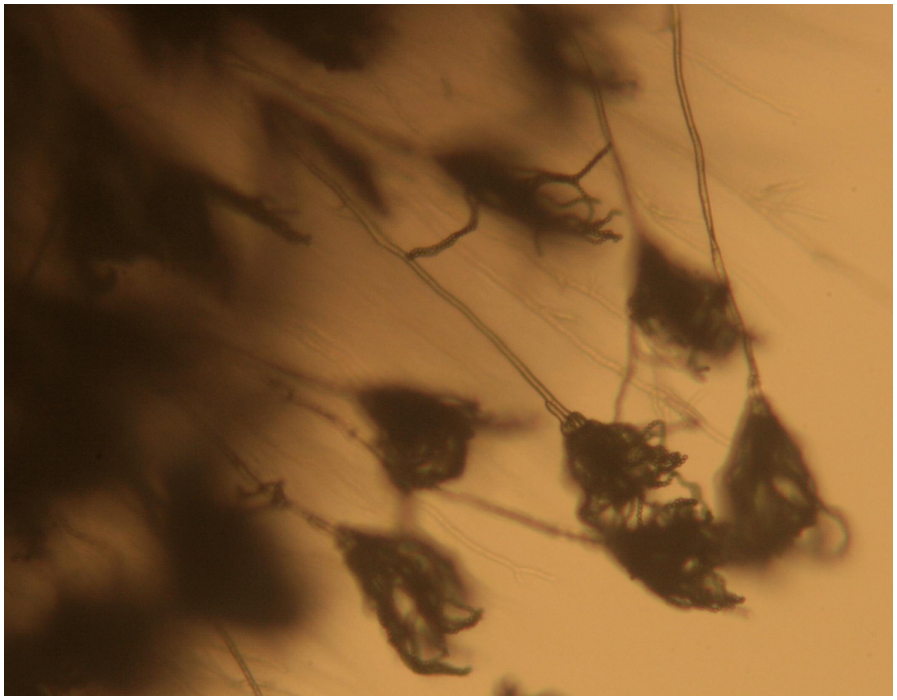
Czopek Dox

*Zygomycetes*

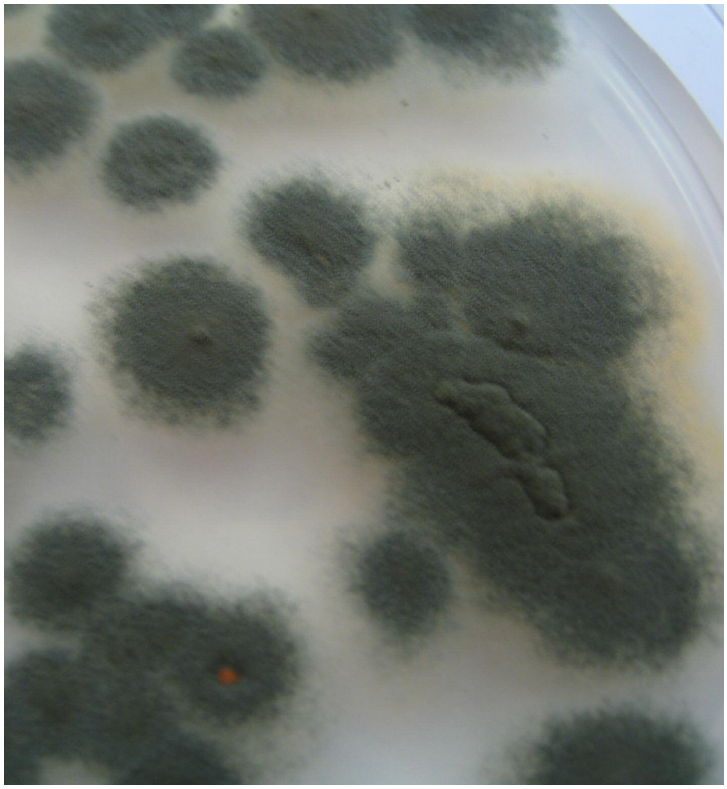
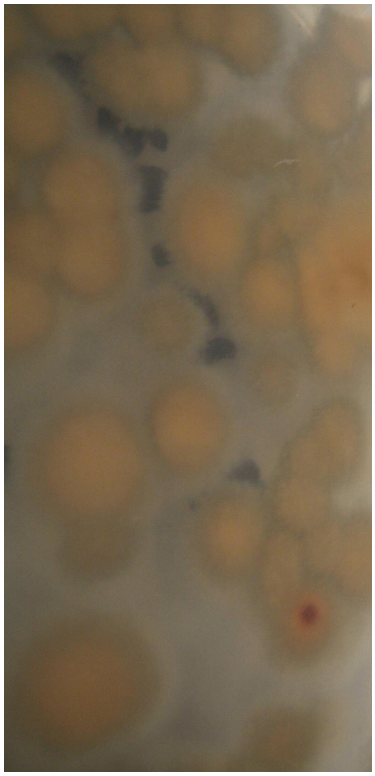
***Penicillium***

Kingdom: Fungi
Phylum: Ascomycota
Class: Eurotiomycetes
Order: Eurotiales
Family: Trichocomaceae
Genus: *Penicillium*

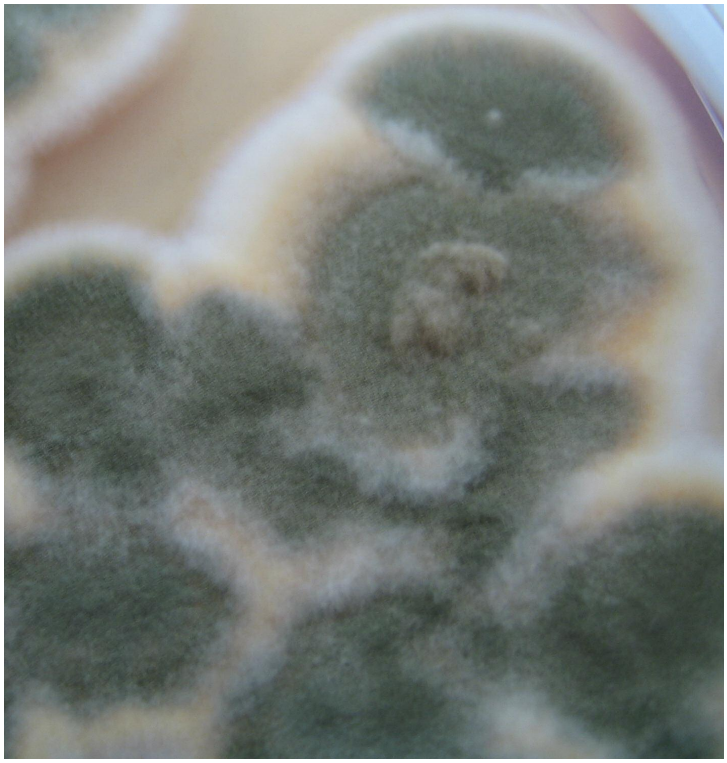
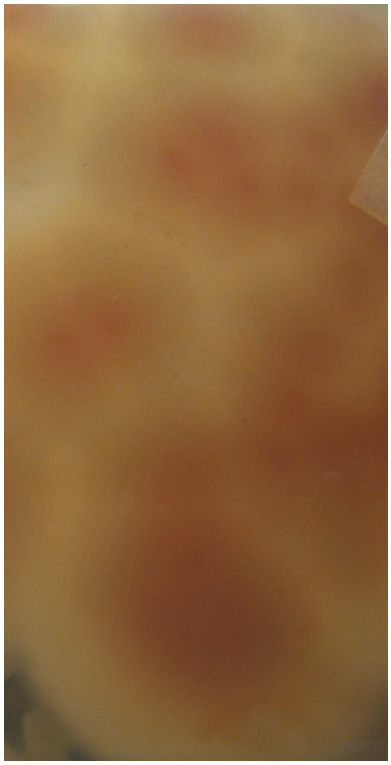




*Penicillium* P5 H3-15

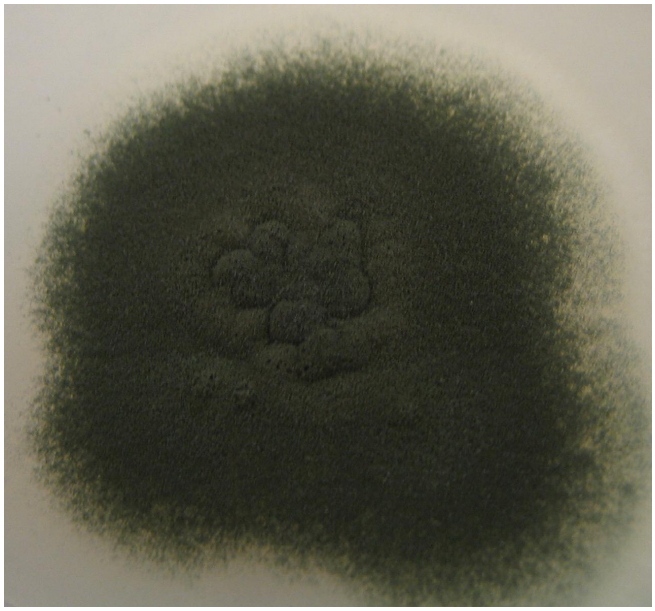



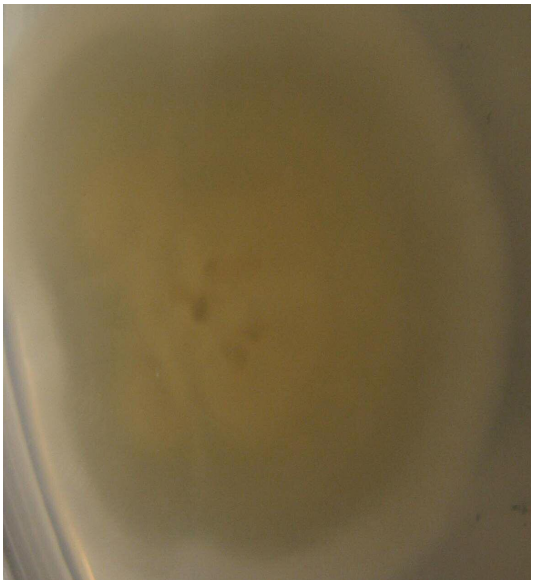
MEA

Czopek Dox

*Penicillium* P6 H3-16





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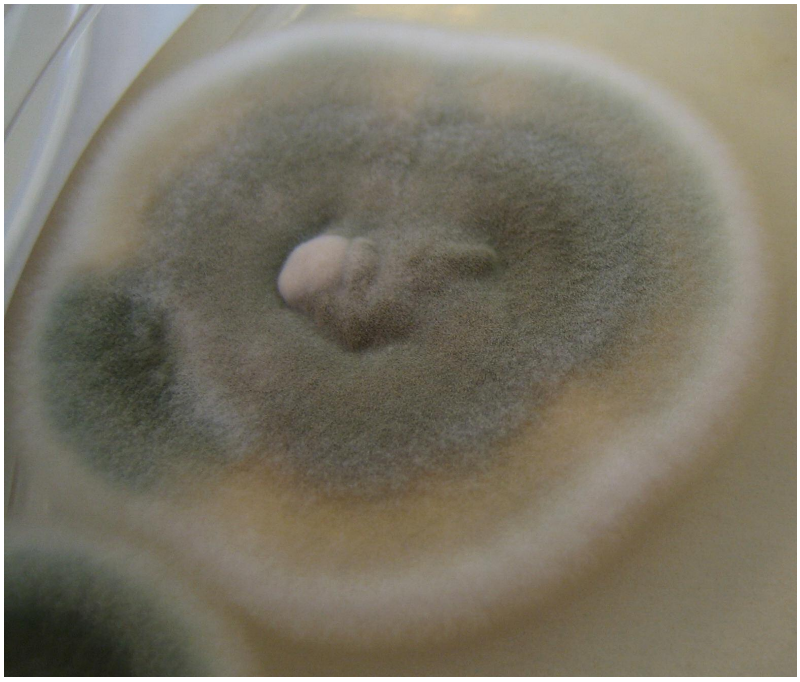
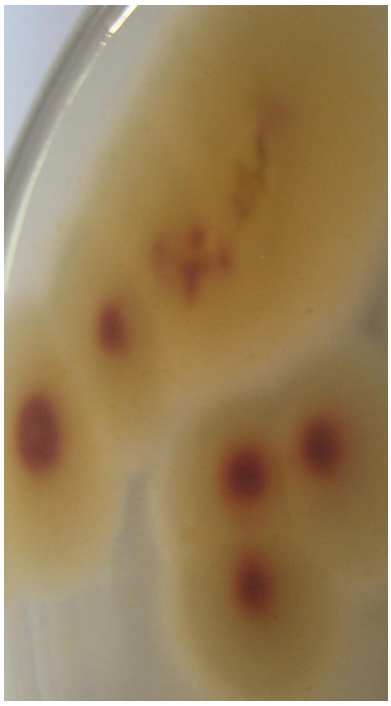
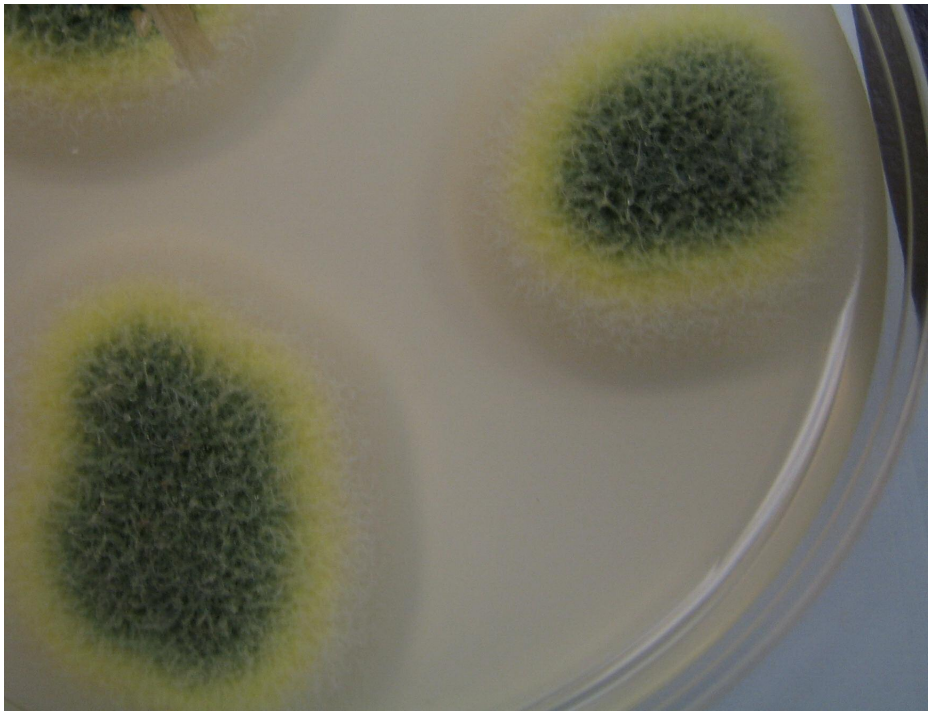




Czopek Dox

*Penicillium* H2-19-1



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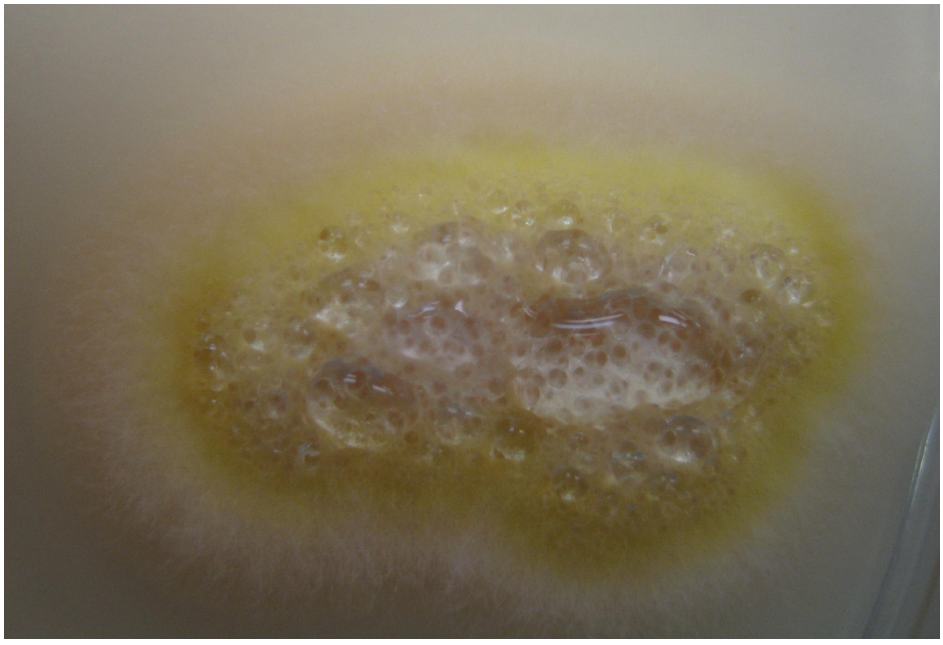
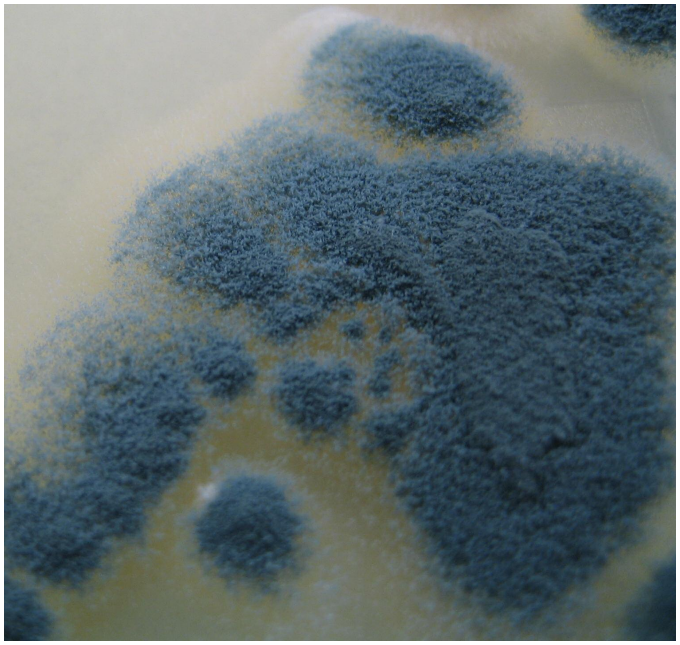
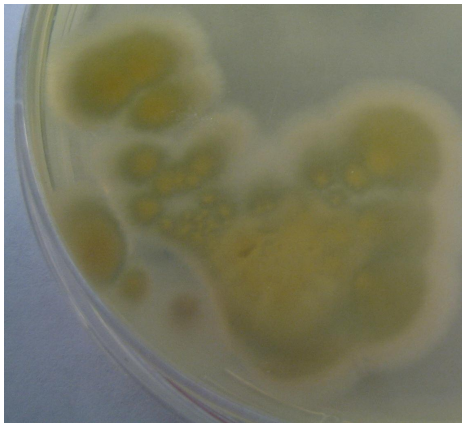


Czopek Dox

*Penicillium* P1 S1-3-1

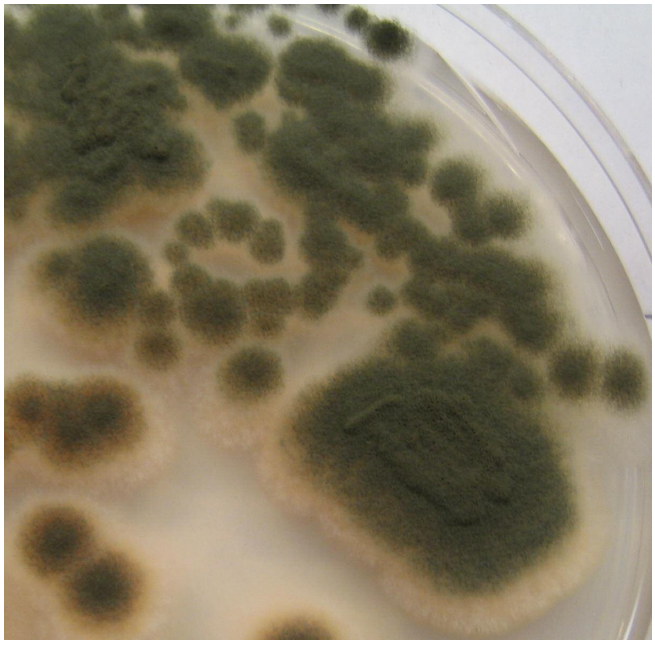


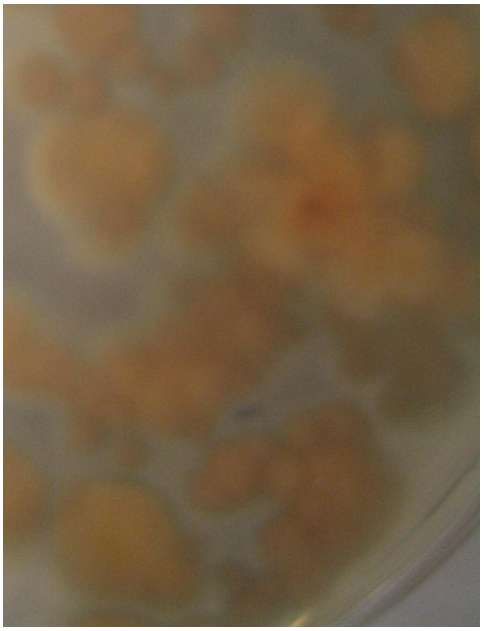


MEA

Czopek Dox

*Penicillium* S1-3-2





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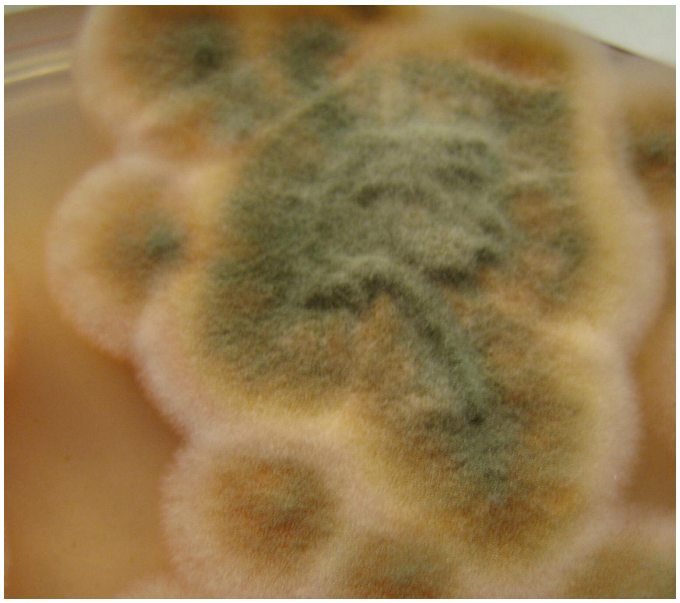
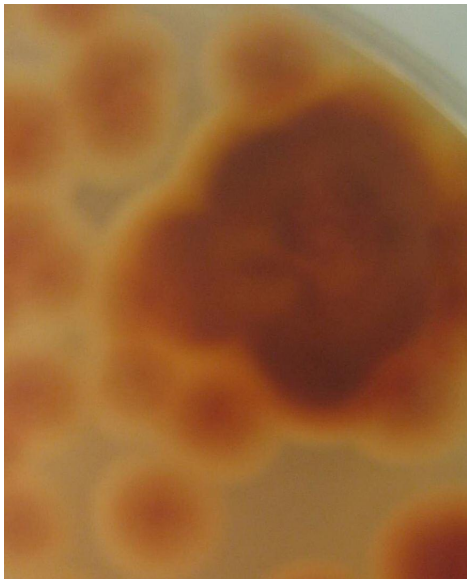
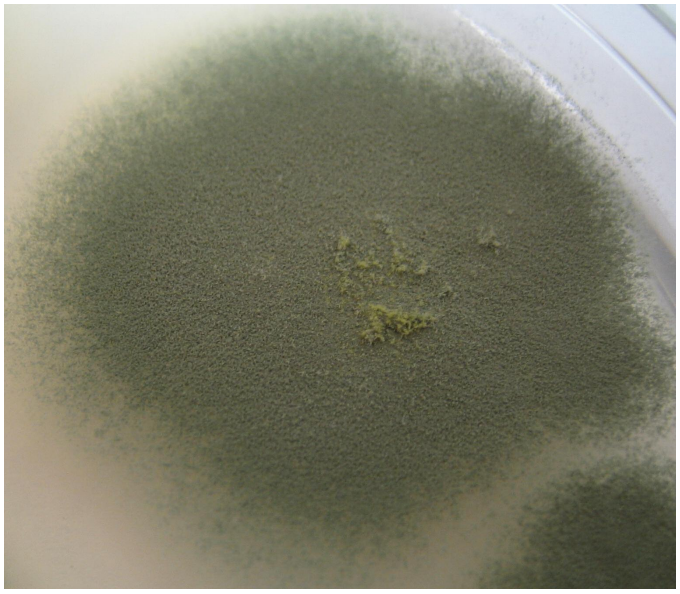




Czopek Dox

*Penicillium* P7 H3-14



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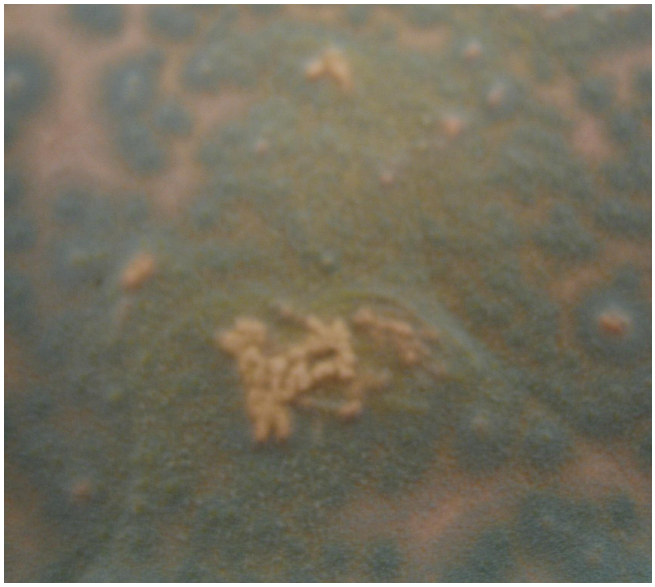

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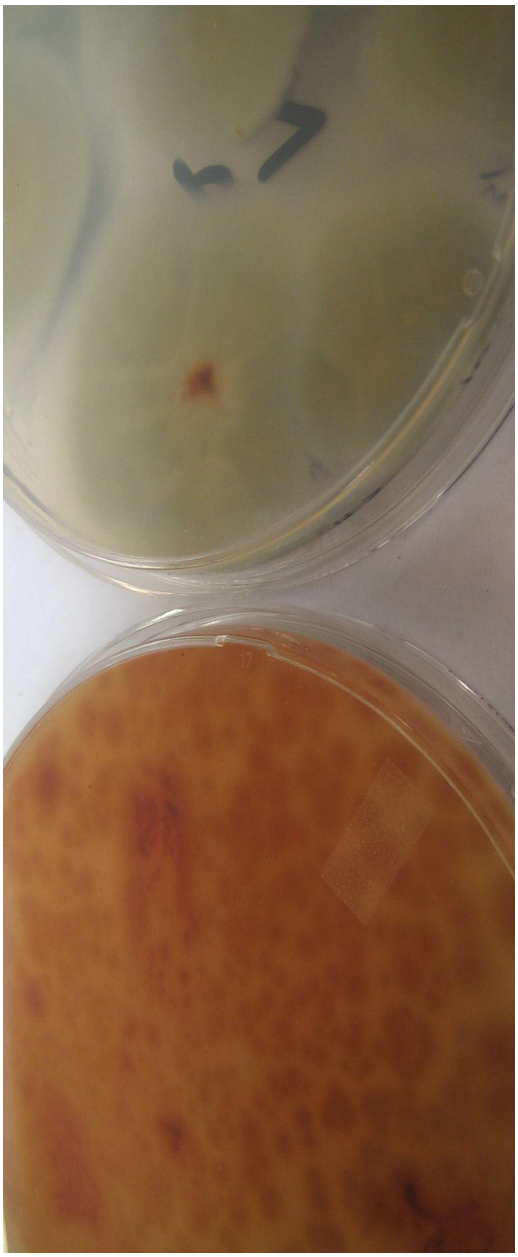
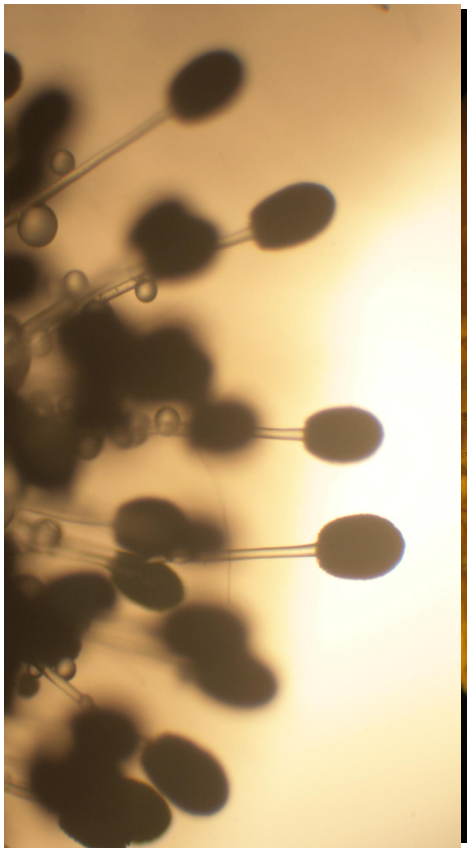
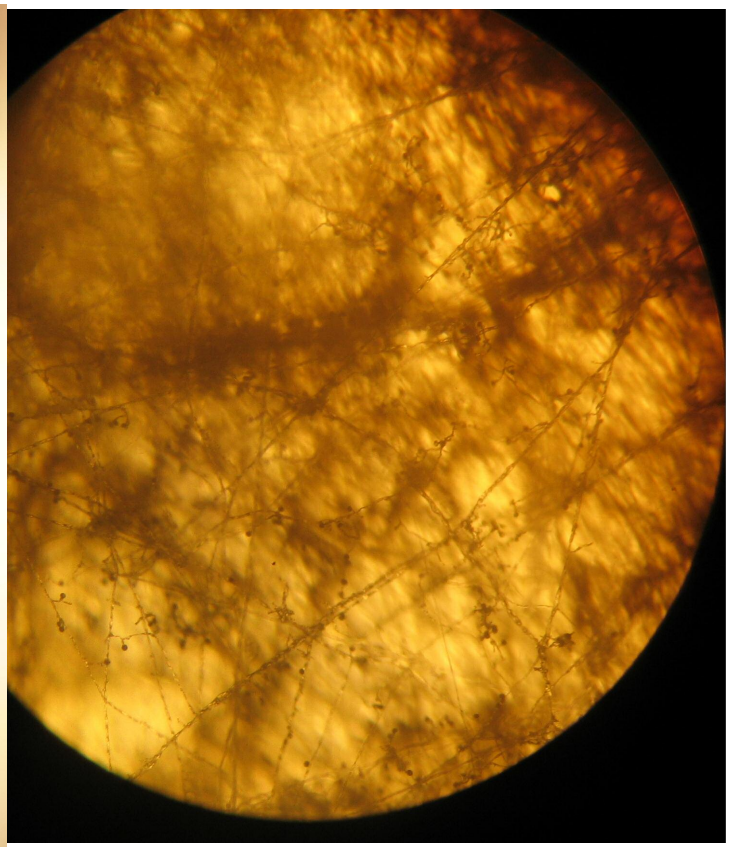


***Rhizopus***

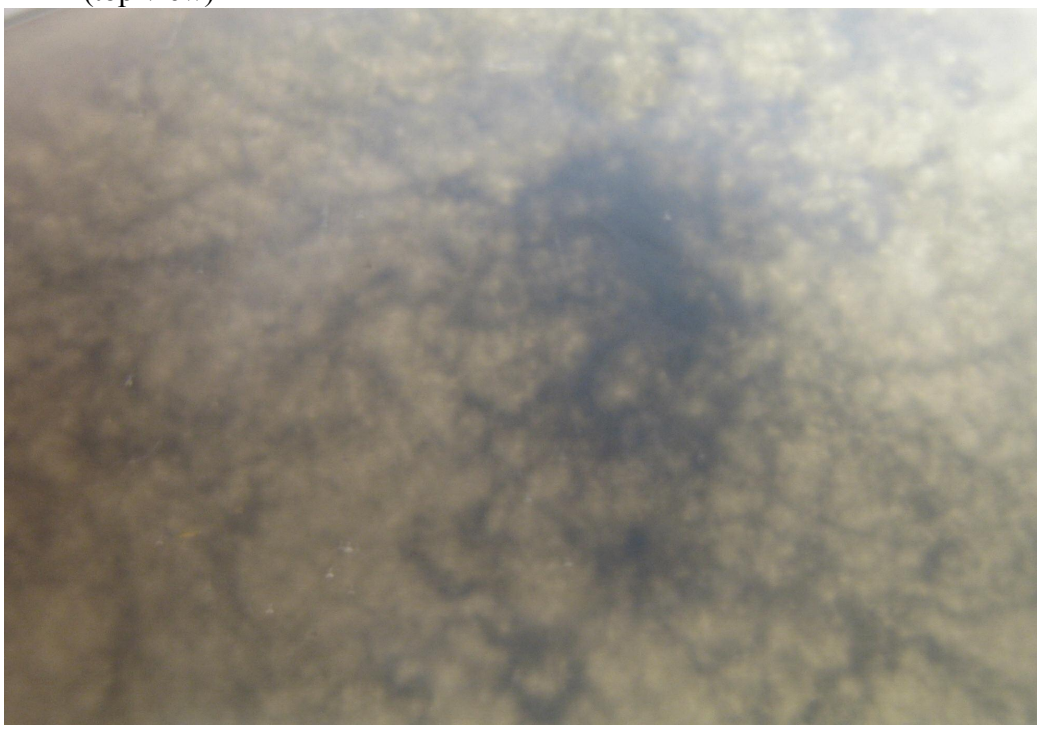
Kingdom: Fungi
Division: Zygomycota
Class: Zygomycetes
Order: Mucorales
Family: Mucoraceae
Genus: *Rhizopus*





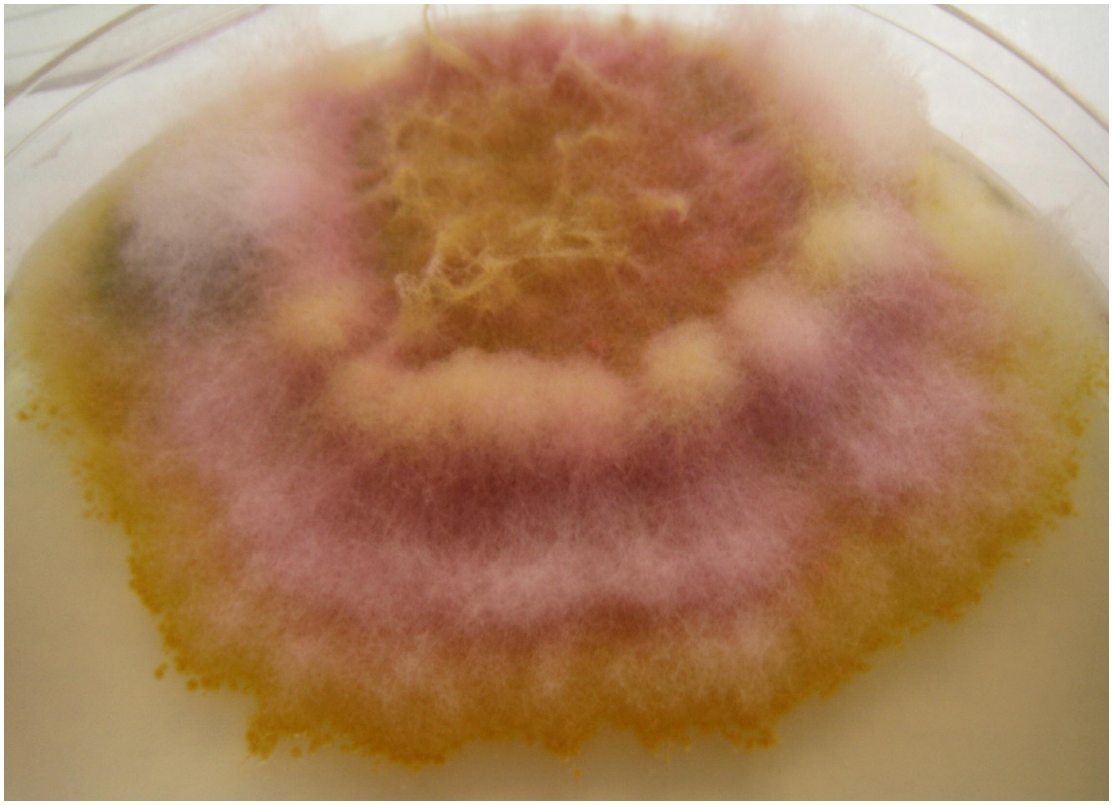
*Rhizopus* Rx1

MEA (top view)

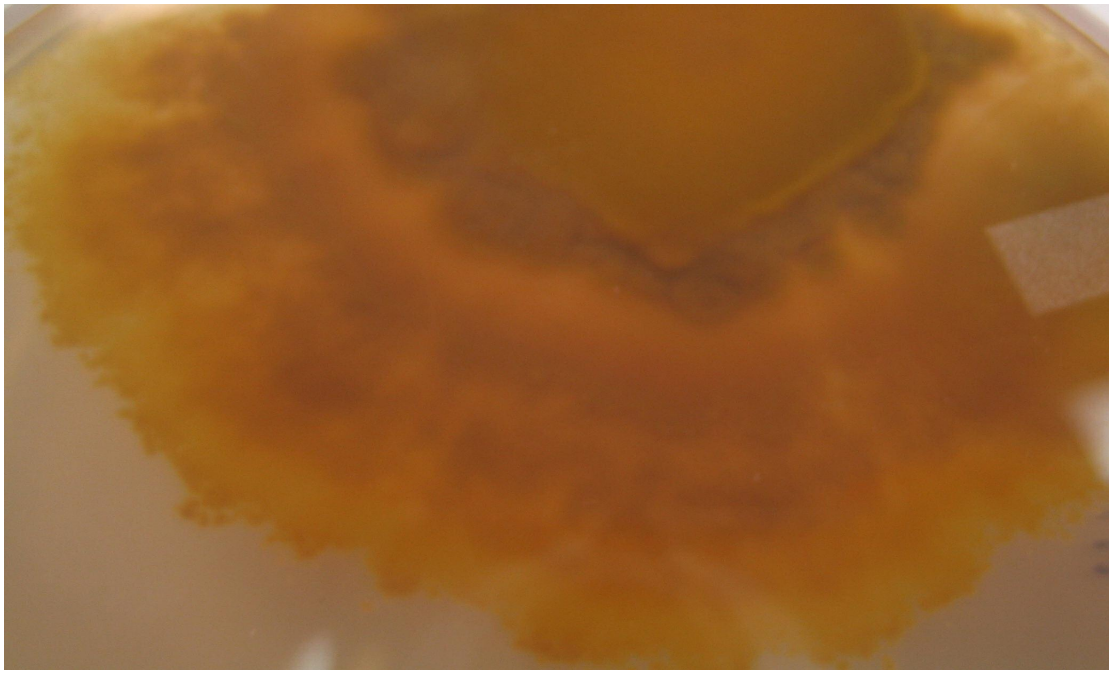


MEA (bottom view)

Unidentified 1



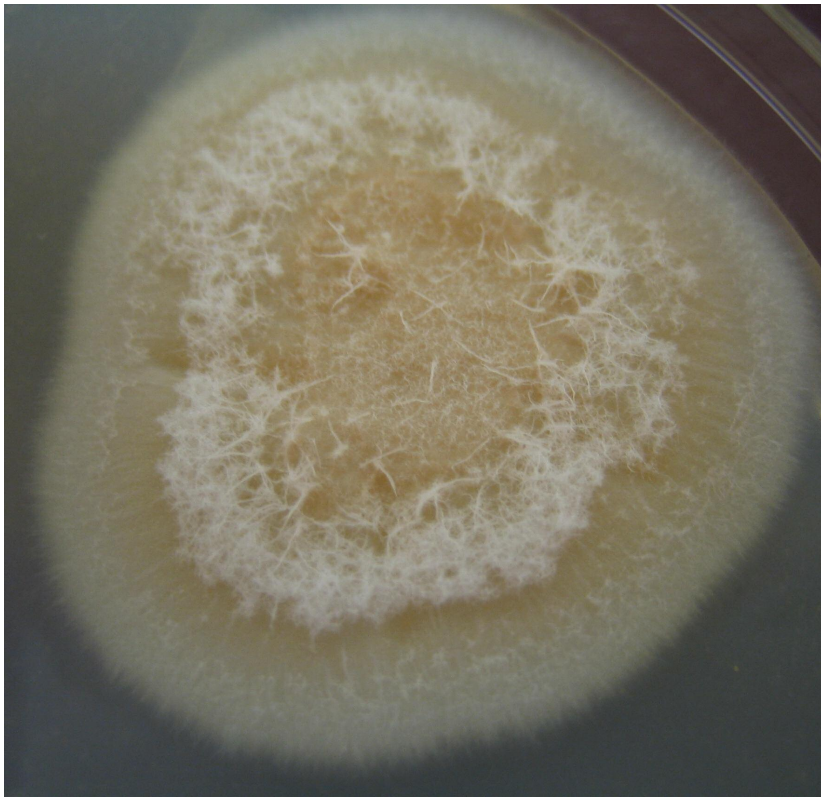
MEA (top view)



MEA (bottom view)

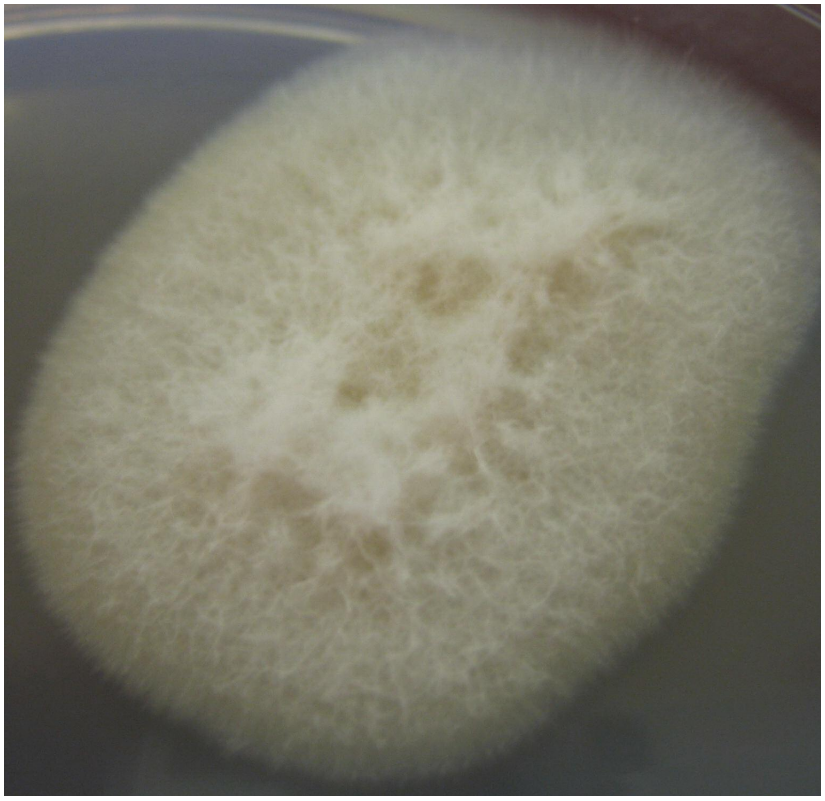
Unidentified 2 3A-H3-10

MEA



Czopek Dox

Unidentified 3 4A-H3-17

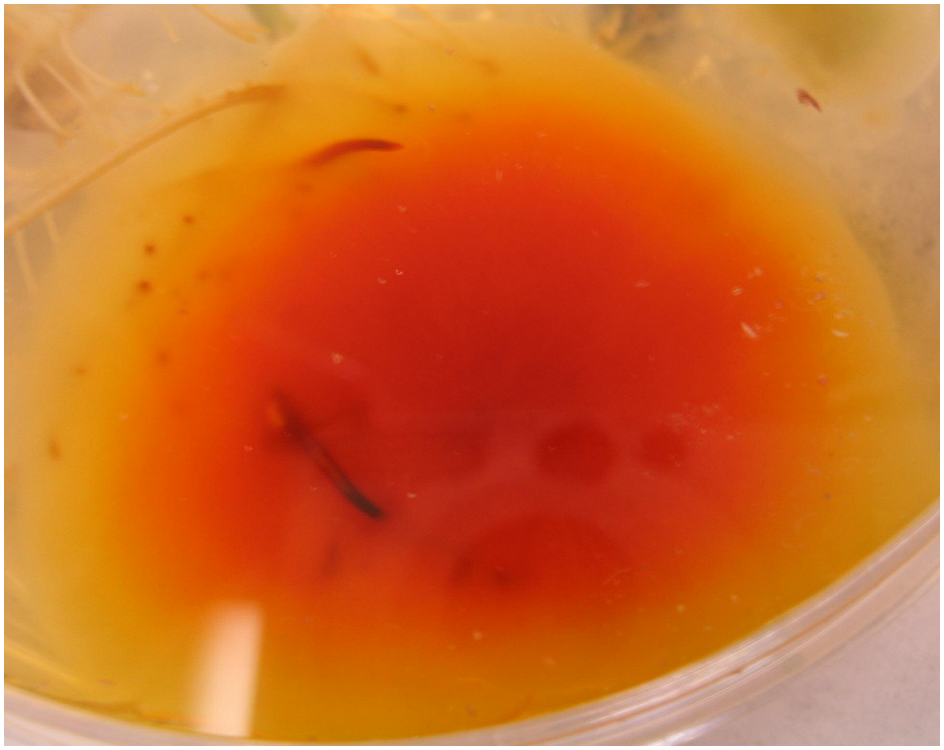


MEA

Czopek Dox

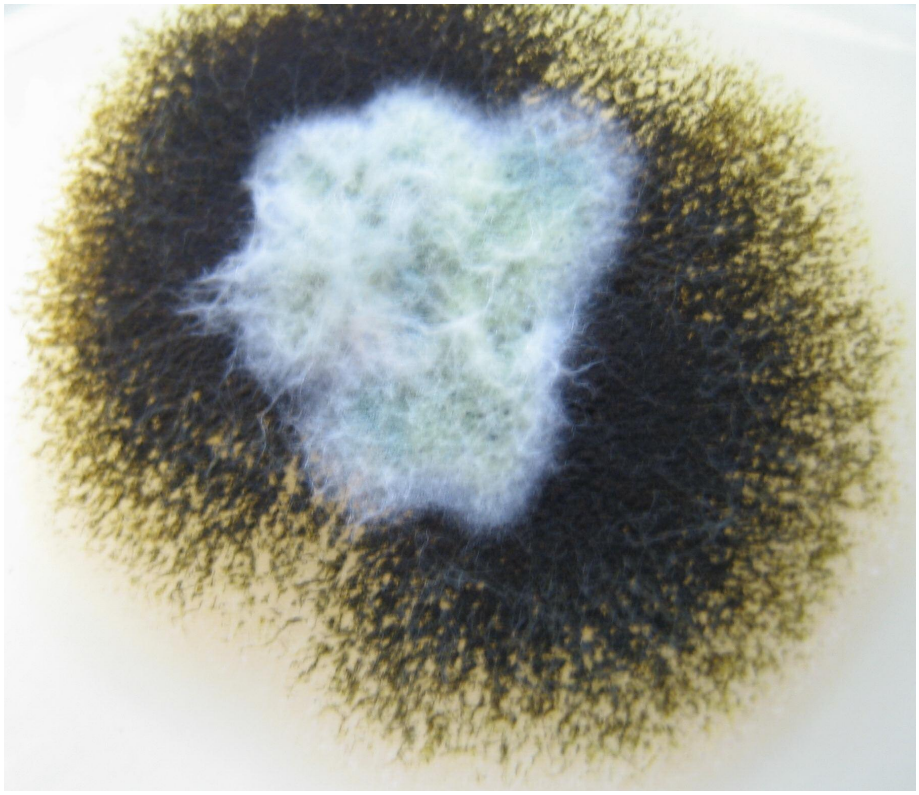
Unidentified 4 UNK-IA

MEA (top view)



MEA (bottom view)

Unidentified 5 J7-S3-19-1



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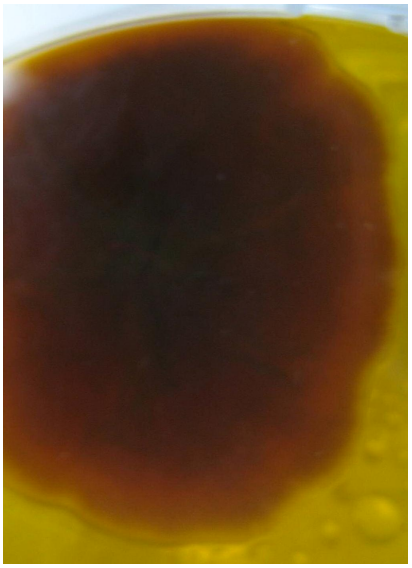
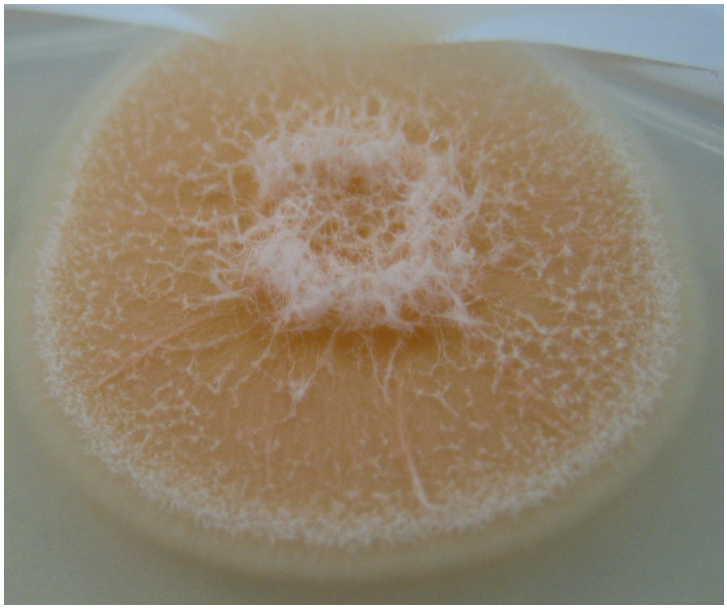


Czopek Dox

Unidentified 6 H1-14-Z



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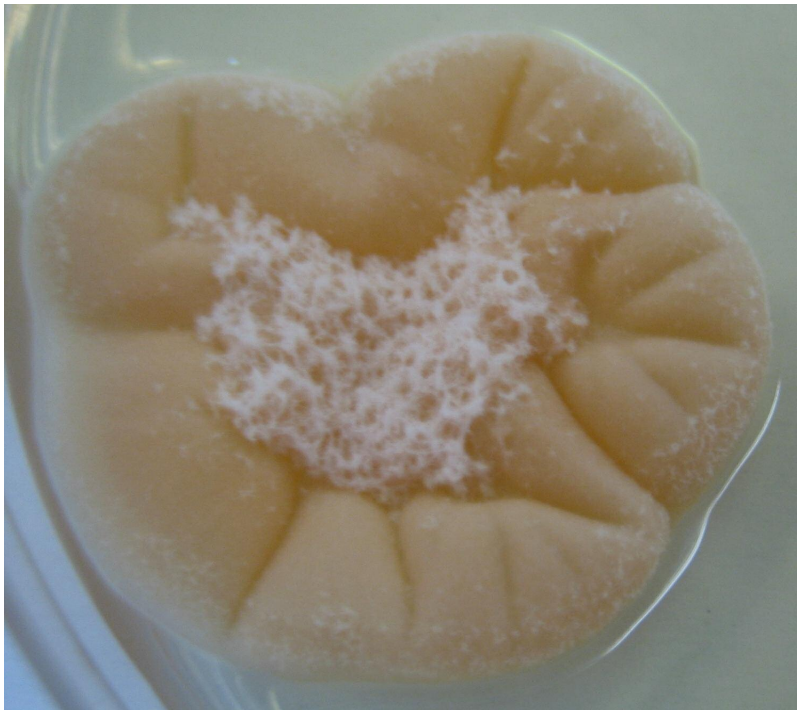

Czopek Dox