Monitoring carbon dioxide concentration for early detection of spoilage in stored grain

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Abstract

Field experiments were conducted in storage silos to evaluate carbon dioxide sensors to monitor spoilage in grain prior to spoilage detection by traditional methods such as visual inspections and temperature cables. Carbon dioxide concentrations in the storage silo were monitored up to eight months and correlated to the presence of stored-product insects, molds and mycotoxin levels in the stored grain. The data showed that safe grain storage was observed at CO_2 concentrations of 400 to 500 ppm. Higher concentrations of CO_2 clearly showed mold spoilage or insect activity inside the grain storage silo. Carbon dioxide concentrations of 500 to 1200 ppm indicated onset of mold infection where as CO_2 concentrations of 1500 to 4000 ppm and beyond clearly indicated severe mold infection or stored-product insects infestation. The percent kernel infection was in the range of 30% for CO_2 concentrations of 500 to 1000 ppm to 90% for CO_2 concentrations of 9000 ppm. Fungal concentrations were in the range of 2.0×10^2 colony forming units per gram (cfu/g) at 500 ppm CO_2 concentration to 6.5×10^7 cfu/g at 9000 ppm CO_2 concentration. Fungi of genera *Aspergillus* spp., *Penicillium* spp., and *Fusarium* spp. were isolated from spoiled grain. High concentration of fungi and presence of mycotoxins (aflatoxin: 2 ppb and Deoxynivalenol (DON): 1 ppm) were correlated with high CO_2 concentration in the silos. The findings from this research will be helpful in providing more timely information regarding safe storage limits, aeration requirements and costs of spoilage mitigation measures such as turning, aerating and fumigating grain. Additionally, it will provide information on preventive stored grain quality management practices that should reduce residue levels of mycotoxins, pesticides and other foreign material in our food supply. The CO_2 monitoring technology will increase the quality and quantity of stored grain, while saving the U.S. and global grain production, handling and processing industry millions of dollars annually.

Keywords**:** Carbon dioxide, Grain storage, Stored-product insects, Mold and mycotoxin

1. Introduction

Temperature, relative humidity and moisture content (m.c.) of the stored grain are the most important factors that influence stored-product insect activity, mold growth and subsequent production of mycotoxins in storage. Maintaining optimum temperature, relative humidity and proper moisture content are the challenges faced because of the seasonal and daily climate fluctuations, the economics of drying grain, and the need to process grain at higher moistures. The optimum temperature range for mold growth is 25-30°C, and temperatures above 15°C are ideal for insect growth and reproduction. Insect metabolic activity in dry commodities (below 15% m.c.) can result in heating up to 42°C (Mills 1989). A major contributor to the spoilage of grain is growth of various mold species, including several that produce mycotoxins. Mycotoxins are natural chemicals produced by fungi that are detrimental to the health of both animals and humans. The U.S. Food and Drug Administration has placed an action level for mycotoxins in stored grain and other food products including milk. As a result, millions of dollars are spent each year to screen food that includes stored grain, processed food, milk and animal feed. The earlier methodologies such as human sensory exposure and temperature cables have their own limitations and drawbacks in monitoring grain spoilage during storage. New management practices are needed that will allow grain processors to maintain high quality grain free of stored-product insects, fungi and mycotoxins. Previous studies have shown that CO_2 sensors can be effectively used to monitor early detection of spoilage during storage (Zagrebenyev et al., 2001; Maier et al., 2002; Bhat et al., 2003;

Maier et al., 2006; Bartosik et al., 2008). The goal of this project was to refine the existing CO_2 based technology for its accuracy and consistency in real time monitoring of grain spoilage prior to detection by traditional methods such as visual, smell and temperature sensors.

2. Materials and methods

2.1. Site selection and installation of CO_2 sensors

To monitor CO_2 concentration for early detection of spoilage due to mold and stored-product insects, we selected a corrugated steel bin containing 254 tons of maize located near Manhattan, Kansas. The CO_2 sensor box (BinTech Company, Denver, CO, USA) box was installed on the roof of the silo close to a vent by cutting a 10.7 cm diameter hole using a metal cutter. Care was taken to seal the gaps around the sensor box to protect water infiltration and to avoid CO_2 leakage. Once the sensor box was installed the CO_2 sensor was released inside the silo by maintaining roughly one meter distance above the stored grain. The control box comprising the battery and display board was mounted on the outside wall of the silo about 1.50 m above ground. The CO_2 sensor and the control boxes were connected and tested for wireless telephone signals and its connectivity to the main server. During grain storage, the CO_2 data were transmitted to the main server (BinTech Company) in the form of digital codes using the wireless telephone network.

2.2. Monitoring changes in CO_2 concentrations

Carbon dioxide concentrations in all storage bins were monitored from February to August 2009. The maize samples were collected and analyzed for grain quality parameters, stored-product insect incidence, presence of molds, and mycotoxin contamination. A log book was maintained to document all grain storage activities including information on battery change and dates of sample collection. Furthermore, details of the storage silo including size, number of fans, pesticide usage, storage start date and contact details of the cooperator were recorded. The silo was inspected and maize samples were collected based on high CO_2 concentration readings and correlated to mold spoilage or stored-product insect activity in the silo. Two sets of grain samples in replicates were collected by probing with a grain sampler. One set was used to analyze molds, mycotoxins and insects in the Grain and Feed Microbiology and Toxicology Laboratory (Department of Grain Science and Industry, Kansas State University, Manhattan, KS, USA) while the second set of grain samples was sent to the Kansas Grain Inspection Service Lab (Topeka, KS, USA) to determine grain quality parameters such as moisture content, dockage and damaged kernels.

2.3. Isolation, enumeration and identification of insects and molds

Grain samples collected during this study were immediately brought to the lab and sieved (480-µm openings) to separate all live insects. These insects were identified, counted and expressed as number of insects per kilogram (kg) of grain. For isolation of molds from grain samples we followed the procedure described by Samson et al. (1996). Twenty five grams of representative sample was soaked in 250 mL of sterile peptone (0.1%) water for 30 min before stomaching for two mins. One mL of the sample, serially diluted in 9 mL of peptone water and a 100-µL sample from serial dilutions, was drop-plated on Dichloron Glycerol-18 (DG-18) agar medium (Oxoid Chemicals, Hampshire, UK) and incubated at 30°C for 4-5 d in an upright position. After incubation, the colony forming units were recorded to determine the number of molds per gram of grain (cfu/g). To confirm the species level, the isolates were observed under microscope for the morphology of spores and mycelia. The observations were recorded and matched with descriptions given by Samson et al. (1996) to confirm genus and species.

2.4. Detection of mycotoxins using ELISA

The levels of aflatoxin, fumonisin, and Deoxynivalenol (DON) in maize grain samples were quantified using the AOAC International, Gaithersburg, MD, USA) approved method based on an Enzyme Linked Immmunosorbent Assay (ELISA) (AgraQuant® Mycotoxin ELISA Test Kits, Romer Labs Inc., Union, MO, USA). Twenty grams of representative sample was grounded and extracted using 70/30 (v/v) methanol/water. For DON analysis the grains were extracted with 100 mL water. The extract was mixed and added to the antibody-coated microwell. Mycotoxins in samples and control standards were allowed to compete with enzyme-conjugated mycotoxins for the antibody binding sites. After the washing step, an enzyme substrate was added for color (blue) development. A stop solution was added to stop the reaction which changed the color from blue to yellow which was measured optically (450 nm)

using a microplate reader (Stat Fax® 303+ Microstrip Reader, Awareness Technology, Inc., Palm City, FL, USA) to determine the concentration of mycotoxins in a sample which was expressed in ppb or ppm.

3. Results

The storage silo selected for this study had spoilage before the CO_2 sensor was installed. It can be observed in Fig. 1 that high CO_2 readings (>3000 ppm) were detected by the sensors in March of 2009. We inspected the bin and found an inch thick of spoiled grain on the surface. We took samples and brought this to the attention of the cooperator and recommended the removal of the moldy grain. As time elapsed, CO_2 readings remained stable until late May of 2009. As ambient and headspace temperatures increased, CO_2 readings in June rose to around 1000 ppm and above, which clearly indicated mold or insect activity. In early July, CO_2 readings began to increase up to 5000 ppm and on inspection of the silo, mold development and spoiled grain on the surface layer were noticed. A sudden drop in concentration of CO_2 on 8 July, 2009 was the result of an attempt to further clean out the top layer of spoiled grain. This did not work because CO_2 readings shot up even higher than before to 7000 ppm and above. The bin was finally emptied in the middle of August. Samples were analyzed and as expected the maize grain was heavily damaged due to mold. We observed a strong correlation between the rise in headspace CO_2 concentrations verses mold and stored-product insects activities in stored maize. Analysis showed a high concentration of mold (6.5×10^7 cfu/g) per gram of maize (Table 1). The percent kernel infections assay showed that 90.0% of the maize kernels were infested by molds at CO_2 concentrations of 9000 ppm and above.

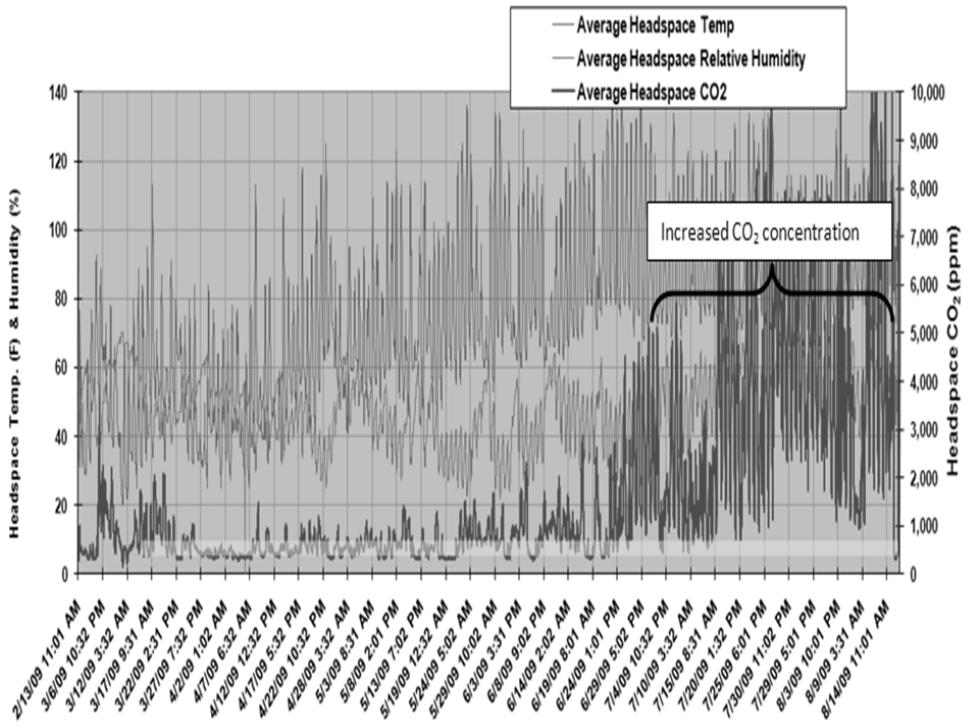

Figure 1 Change in headspace CO_2 concentration, relative humidity and temperature during grain storage.

Table 1 Grain quality parameters and incidences of stored-product insects, molds and mycotoxins in maize during storage

Time (2009)	Grain Moisture (%)	Air Temperature (ºC)	Relative Humidity (%)	Stored-product Insects (No. insects/kg)	Percent Infections (%)	Kernel cfu/g	Molds Total aflatoxins (ppb)	Mycotoxins Fumonisins (ppm)	Mycotoxins Deoxynivalenol (ppm)
February	14.5	16	60	0	30	$2.0 \pm 0.2 \times 10^2$	0	0	0
March	NA	26	60	1	70	$5.0 \pm 0.1 \times 10^6$	0	2	0
April	NA	26	50	2	NA	$2.2 \pm 0.0 \times 10^2$	0	0	0
May	NA	33	50	4	NA	$2.5 \pm 0.2 \times 10^3$	0	0	0
June	NA	43	52	10	NA	NA	0	0	0
July	13.5	50	59	18	80	$4.2 \pm 0.3 \times 10^6$	1	0	0
August	13.7	49	62	27	90	$6.5 \pm 0.3 \times 10^7$	2	0	1

NA: Data not available

We detected 2 ppb of aflatoxins and 1 ppm of DON in the unloaded maize (ending) sample. Mold concentration in the maize correlated with high CO_2 readings in the silo. Also, heavy stored-product insect infestation was noticed. These insects were identified as flat grain beetle*, Cryptolestes pusillus* (Schönherr) (Coleloptera: Laemophoeidae) which is a mold feeder, and maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) which feed on the maize. Upon enumeration we noticed 27 live insects per kg of maize (Table 1). Nearly 100 tones (40% of stored maize) of spoiled and damaged maize was separated and used for animal feed.

4. Discussion

It is essential for the grain storage industry to have effective management programs to protect against economic loss due to contamination from stored-product insects, molds and mycotoxins. Manual grain inspection (human sensory exposure) and measuring grain temperature are the main tools used by the farmers and the grain industry for monitoring proper storage conditions (Bartosik et al., 2008). Human sensory exposure literally means having personnel "walk" the grain mass, smell the grain, smell the aeration discharge stream and look at the grain. Human sensory exposure for mold spoilage and other quality parameters could be biased and it varies from person to person. Temperature cables are routinely placed in modern grain bins. Unfortunately, a temperature cable will not detect the fungal growth several feet away from the cable until the size of the spoiling grain mass is large enough to raise the temperature around the volume of the temperature cable. These limitations are overcome with the CO_2 sensors. Fungi and their related mycotoxin contamination problem is one that is not easily resolved by the food, feed and grain processing industry (CAST, 1989; Miller and Trenholm, 1994). Only organic acids are available for controlling fungal growth. Unfortunately, these acids are not suitable for many situations because they severely limit the number of end uses available for the treated grain. Our experiment clearly demonstrated that CO_2 sensors can be effectively used to detect stored-product insects infestation and grain spoilage due to mold infections well before spoilage detection by traditional methods such as visual inspections, smell and temperature cables. Production of carbon dioxide has been a method used for many years to predict the storability of grains under laboratory and field conditions (Stroshine and Yang, 1990; Maier et al., 2006). In this study, we successfully used the CO_2 sensors under field conditions for early detection of spoilage in maize due to molds and stored-product insects. Further, in this study we refined the CO_2 sensor technology that provides accurate and consistent results.

Carbon-dioxide-based, spoilage-detection devices are expected to save grain producing, handling and processing industry millions of dollars annually. Reducing spoilage would lower residue levels of mycotoxins, pesticides and other foreign materials in our food supply, and maintain the quality and quantity of stored grain, while minimizing storage and handling costs. Such an early warning system would provide more timely information to farmers to make the correct management decision to avoid the costs of spoilage mitigation measures such as turning, aeration, and fumigation. This would help in continuing to store grain or market it early to avoid further quality deterioration.

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