

## Development of an Organic Stored Product Pest Control Treatment Station Utilizing Nitrogen for Shipment Containers

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**Abstract:** There are few non-chemical alternatives existing for pest control in stored organic grains and grain-based products. One available technology that appears timely to develop and demonstrate is nitrogen-based modified atmosphere. This study was undertaken to demonstrate that nitrogen treatment works utilizing a transportable nitrogen-based modified atmosphere treatment station by conducting a full-scale trial with bagged grain in a shipping container. Initial tests were conducted using 0.71 m<sup>3</sup> bags made from a five-layer oxygen barrier extruded plastic film filled with soybeans and purged with 99.9% nitrogen until the oxygen level was near zero. Stored product insect bioassays were placed inside the bags containing adult maize weevil (MW), red flour beetle (RFB), lesser grain borer (LGB), and Indian meal moth (IMM) adults and larvae. Bioassays were kept for 3, 7, and 21 days inside the nitrogen-purged bags. Results showed 100% mortality for all insects as oxygen level inside a bag was below 0.13% after 3 days of exposure, which was maintained for 7 and 21 days. Maintaining a good seal on the bag, in addition to the proper selection of plastic film material, were essential contributors to the success of this study. Preparing a larger bag for the 6.1 m long container was a substantial challenge. Nevertheless, trials with a shipping container using corn-filled tote bags and purged for 7 days with 99.9% nitrogen showed 100% mortality of RFB, MW and LGB adults.

### Introduction

To maintain agricultural competitiveness, emerging value-added opportunities such as organic grains and grain-based products need to be supported through applied research and demonstration. Organic crop production is one diversification strategy to enhance the viability of U. S. producers and organic crops are an innovative source to meet the demand for nutritious and healthy foods. The U. S. organic farming system has expanded rapidly since the 1990's in response to a demand increase from local and national markets. As of 2001 (latest data), Indiana had at least 2000 certified organic hectares with 82% planted with field crops and hay. About 500 hectares each were utilized for corn and soybeans and almost another 500 hectares for wheat and oats<sup>[1]</sup>. Additionally, hectares are dedicated to organic popcorn and tofu soybeans for export primarily to Japan<sup>[2]</sup>. One key challenge facing Indiana producers and processors of organic grains is pest control consistent with organic criteria during post-harvest handling to ensure quality and avoid costly rejection at the point of sale/receipt. At least one major snack food manufac-

turing plant is faced with this challenge since it began receiving organic food corn from a supplier<sup>[3]</sup>.

Few non-chemical alternatives exist for pest control in stored organic grains and grain-based products<sup>[4]</sup>. Available technologies that have been explored include refrigeration of product to -18°C for 6 days (effective but costly and time consuming), heating of equipment and structures to 45°C for 16 - 24 h (product cannot be heated as it reduces quality), ozonation of bulk product (controls external pests but neither internal infesters nor bagged product), use of insect growth regulators (works for certain pests only), pheromones (limited effectiveness as it only attracts male insects into traps), PyGanic (a pyrethrin derived from a natural product; a grain protectant that cannot be used for control at the time of shipment or receipt), Diatomaceous earth (a silica-based grain protectant that cannot be used for control at the time of shipment or receipt), and modified atmosphere using 45% - 60% CO<sub>2</sub> for 4 - 21 days (effective but costly and time consuming; and the primary treatment competing with the nitrogen-based technology proposed in this project).

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In sealed containers purged with nitrogen, a progressive hypoxia or anoxia causes mortality in stored product pests<sup>[5]</sup>. Navarro<sup>[6]</sup> documented 95% mortality of almost all life stages of *Ephesia cautella*, *Sitophilus oryzae*, and *Tribolium castaneum* when exposed to 99% nitrogen (1% oxygen) for at least 6.5 days and 99.9% nitrogen (0.1% oxygen) for 9 days. Interestingly, pupae of *Sitophilus oryzae* required at least 20 and 14 days at 0.1% and 1% oxygen, respectively. A number of additional studies showed similar efficacy using nitrogen-based modified atmosphere treatment for a range of durable and perishable products such as for pears<sup>[7]</sup>; for snow pea<sup>[8]</sup>; for cacao<sup>[9]</sup>; and for Dhakki dates<sup>[10]</sup>.

Based on the successful research results documented in the literature, discussion with other researchers<sup>[11]</sup> who have conducted some of this successful research recently, current advancements in the development of nitrogen generators, plastic liners and gas monitoring equipment as well as the current needs of Indiana producers and processors and interest by the leading Indiana pest control service supplier, the time appeared right to develop and demonstrate a nitrogen-based modified atmosphere pest control treatment system for organic grains and grain-based products in Indiana.

This project focused on the development and demonstration of a nitrogen-based modified atmosphere treatment station for the control of stored product pests in bagged organic grains and grain-based products grown in Indiana (and surrounding states) and designated for sale in domestic and overseas markets. The objectives of this study were:

- 1 To construct a transportable nitrogen-based modified atmosphere treatment station and conduct a pilot-scale trial with bagged grain in sealed stacks.

- 2 To demonstrate the transportable nitrogen-based modified atmosphere treatment station by conducting a full-scale trial with bagged grain in a shipping container.

## Materials and Methods

### Materials and Procedure for Constructing the Oxygen Barrier Plastic Liner

Key to a successful modified atmosphere treatment with nitrogen is the availability of an airtight enclosure. Typical shipping containers are not sufficiently airtight to meet this need. Specially designed plastic material that acts as a barrier to oxygen can be used to create brick-

shaped liners that fit inside a shipping container. The liner material for this project was obtained from Germany. The plastic material is 5m wide extruded 6mm thick sheeting that consists of five layers with the middle layer designed as an oxygen barrier.

For both objective experiments, the plastic film was cut into sheets just large enough to minimize waste and the number of seams that had to be welded. Each sheet was folded once across its width to allow welding of the two longer sides. This created a brick-shaped bag that could be filled with product before welding the third seam and sealing the bag. Before the final seam was welded, two inexpensive plastic valves were installed on each bagone was used for pumping in the nitrogen and the other for bleeding out the air. For the Objective 1 experiments, the 5m wide plastic liner was cut into 2m long sheets; for the Objective 2 experiments, the liner was cut into 8.5m long sheets. A commercially available plastic belt sealer was modified by adding 2.54cm diameter wheels that run along a 10.2cm wide and 2.5m long aluminum channel that served as a guiding track. After initial folding, each plastic sheet to be sealed was laid on a table with the aluminum track fastened in parallel to the table. The plastic sealer self-propelled along the track while welding the first seam of the bag. Then the bag was turned over on the table and the sealer was allowed to weld the second seam. Once a bag was in place and filled with product, excess material was trimmed and the sealer was used manually to weld the final seam and seal the bag. Creating and welding the smaller bags was relatively easy while creating and welding the large bags to fit inside the container proved to be a substantial challenge. Handling of all bags was done carefully to minimize folds and seal breakage in order to avoid any source for air leakage.

### Objective 1: Construction and Testing of Transportable Treatment Station with Sealed Stacks

*Treatment Station Construction* A Pressure Swing Adsorption Nitrogen generator Model HPN - 25 was made available by Innoventor (Maryland Heights, Missouri) that only required electrical power. Castor wheels were added to make the unit transportable so that it could be placed at a pest control service supplier for treatment of infested organic products shipped to their location, or the unit could be shipped via truck to the location of an organic producer,

marketer or processor for the treatment of infested organic products before shipping or upon receipt.

**Sealed Product Stacks** Two stacks of soybean-filled bags were used. One open-top cube-shaped plastic liner was placed on each pallet and stacked full with 0.5 kg product bags. Subsequently, temperature sensors and insect cages were placed among the soybean bags. Then the top of the cube-shaped plastic liners were sealed. Access valves for the inlet purging and outlet recirculation lines were incorporated into the seam as the liners were welded shut.

**Nitrogen Treatment** Nitrogen was introduced on one side of the liner enclosing each product stack to purge its content with 99.9% nitrogen which was then maintained for 3, 7 and 21 days. Once achieved, the generator was shut off, and the supply hose was removed. A treatment was considered complete once the required exposure time had been reached. Each treatment was repeated three times. An additional three stacks without any liners were used as control.

**Temperature and Oxygen Monitoring** Temperature was monitored continuously at several points in each bagged stack. Oxygen concentration in each stack was monitored using zirconium type oxygen analyzers. The AirDac data acquisition and control software developed by the Purdue Agricultural Air Quality Laboratory was adapted for daily gas concentration monitoring.

**Insect Bioassays** Bioassays of live insects (adults of red flour beetles (RFB), lesser grain borer (LGB), maize weevil (MW), and Indian meal moth (IMM) and larvae of Indian meal moth) were placed among the bags in each stack. For each sealed and control stack of soybeans, insect bioassays were placed in three locations each inside and outside the product bags. These were collected at the end of each treatment and live *versus* dead insects were counted.

## **Objective 2: Demonstration of Transportable Treatment Station with A Shipping Container**

**Shipping Container Preparation** A 6.1 m long shipping container was used to hold 14 maize-filled 455 kg tote bags placed inside the large brick-shaped plastic liner bag. The shipping container doors were left cracked open for the nitrogen supply line to connect to the inlet valve installed on the sealed liner bag. The container was filled to allow access to the totes for data monitoring. The shipping container re-

mained on site at Purdue University and was not transported.

**Sealed Product Stacks** Before filling the container, carpet padding was placed on the floor of the container and then the large brick-shaped liner bag was placed on the floor at the far end of the container. The top-portion of the liner was attached to the ceiling with two-sided tape. The open front of the liner bag was aligned with the front doors of the container. A second layer of carpet padding was placed on the floor of the liner and then a layer of 1.27 cm thick plywood was placed on the carpet padding. This permitted a fork lift to drive in and out of the container to place pallets carrying the totes inside the container. Tote bags were stacked two high side-by-side and from back to front in the container. The container was not completely filled to allow access around the totes and placement of the data monitoring equipment and laptop computer. Subsequently, temperature sensors, gas monitors, and insect bioassays were placed among the tote bags.

**Nitrogen Treatment** Nitrogen was introduced to the back of the liner by an extended inlet tube to purge its content with 99.9% nitrogen from back to front and then maintain near zero oxygen for seven days. A bleeder valve at the seam was used to control the purging process. The generator was continuously operating when the liner was sealed. A treatment was considered complete once the required exposure time of seven days at < 0.1% oxygen had been reached.

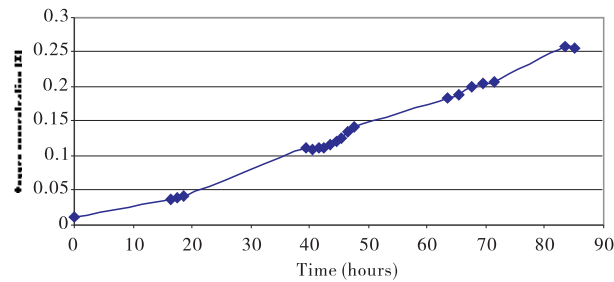
**Temperature and Nitrogen Monitoring, and Insect Bioassays Preparation and Placement** were similar as described in Objective 1, except no IMM larvae and adults were used in the bioassays.

## **Results and Discussion**

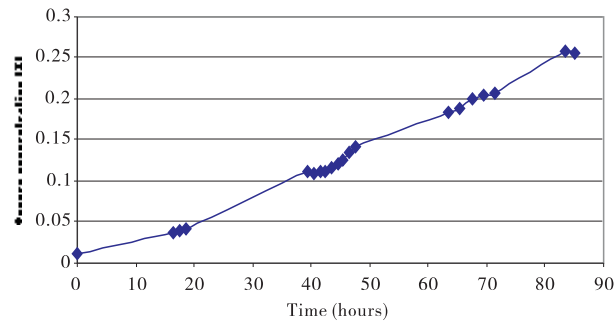
### **Objective 1: Construction and Testing of Transportable Treatment Station with Sealed Stacks**

Purging the 0.71 m<sup>3</sup> bag stacks with 99.9% nitrogen took about 3.5 h to reduce oxygen to near zero. The nitrogen generator was operated to supply 6.8 m<sup>3</sup>/s and two sealed bag stacks were purged at the same time. An essential part of each experiment was maintaining the seal of the liner in order to achieve and maintain a near-zero oxygen level inside each bag. Figures 1 to 3 show changes in oxygen level inside the plastic liners after 3, 7, and 21 days of storage. Based on these figures, oxygen level in-

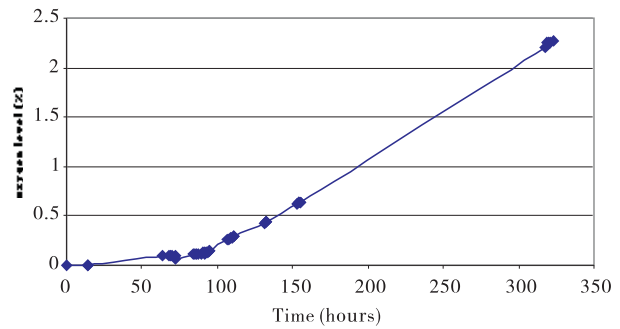
creased with length of storage from 0.25% for 3 days to 2.25% after 21 days.



**Fig. 1 Change in oxygen level for 3 days inside a sealed bag liner initially purged with nitrogen.**



**Fig. 2 Change in oxygen level for 7 days inside a sealed bag liner initially purged with nitrogen.**



**Fig. 3 Change in oxygen level for 21 days inside a sealed bag liner initially purged with nitrogen.**

Insect mortality of adult MW, LGB, RFB, IMM and IMM larvae exposed for the different treatment times is presented in Table 1. All treatments yielded 100% mortality for all insects considered except for the MW in one of the replications in the 3 day exposure. In that replication, only 67% mortality was observed but oxygen level in that bag liner at the end of the 3 day period was 0.24%. When oxygen level was maintained below 0.13% for the first three days of any treatment period, mortality reached consistently 100% for all species and life stages. No dead insects were found in the control bioassays.

**Table 1. Percent mortality of insects in cages for different treatment times under MA with nitrogen.**

Storage Time	MW adult	LGB adult	RFB adult	IMM adult	IMM larvae
3 days (trial 1)	100	100	100	100	100
3 days (trial 2)	67 *	100	100	100	100
3 days (trial 3)	100	100	100	100	100
7 days (3 trials)	100	100	100	100	100
21 days (3 trials)	100	100	100	No data	No data

\* Oxygen level was 0.24% for 67% mortality compared to  $\leq 0.13\%$  for 100% mortality.

Part of the success of this system depends on using plastic material impervious to oxygen. The plastic obtained from Germany performed well in terms of maintaining low oxygen levels. Other plastics tested were purchased from local hardware stores. These plastic materials were thinner (i. e., 4 mm vs 6 mm) and not as strong as the extruded 5 – layer German plastic.

We conducted a simple test which consisted of placing a fast green dye solution inside small plastic bags and then placing them in a 1 liter beaker filled with water. In each case, the dye passed through these inexpensive plastics, indicating that they would also be more permeable to the smaller oxygen molecules. On the other hand, performing this test with the 5 – layer German plastic did not show any leak of the

dye.

Another plastic material tested was one used for silage bags. This material is thicker (9mm) than the German liner material and was designed to be a barrier for oxygen. The thickness of the material made it harder for the sealer to produce a good seal along the seams. At the same time, extra care in handling of the plastic during folding and sealing was needed to prevent creases that reduced the sealing integrity of the material. Testing a small cube made from the silage bag material and filling it with soybean sample bags showed the oxygen level reaching 0.55% at 30 hours after nitrogen purging ended. This oxygen level was sufficient to allow insects such as MW to survive.

**Objective 2: Demonstration of Trans-**

## portable Treatment Station with a Shipping Container

The initial trial with the 6.1m shipping container required 4.5 days or about 108 hours to purge the brick-shaped liner with 99.9% nitrogen. Lining the container took skill and ingenuity in order to properly secure the plastic material along the ceiling and walls of the container. A fan was used to blow air into the container to inflate the plastic liner and double-sided tape was used to keep the liner attached to the ceiling and walls. Care was taken in loading the tote bags to make sure that the plastic did not tear. Unfortunately, the large liner was not perfectly sealed as leakage was detected after the generator was turned off. As a result, it was run continuously to maintain the desired near zero oxygen treatment effect.

After seven days of near zero oxygen exposure, no live adult MW, RFB, and LGB were found in the insect bioassays. As a result, replicates two and three were also purged continuously with nitrogen, which also resulted in 100% mortality of all adult insects.

## Conclusions

This study shows that the application of nitrogen for modified atmosphere storage proved to be effective in controlling maize weevil, red flour beetle, Indian meal moth, and lesser grain borer. Its effectiveness relies on the utilization of specially designed oxygen barrier plastic material and the integrity of sealing the plastic liner in order to maintain near-zero oxygen concentrations for the duration of the treatment. After 3 days of treatment, oxygen had to be less than 0.13% to achieve 100% mortality. For longer treatment periods, the oxygen level could be as high as 0.4% oxygen for 7 days and up to 2% for 21 days.

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