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NITROGEN APPLICATION OFFERS FOR BOTH CONTROL OF INSECT AND GRAIN QUALITY

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ABSTRACT

Nitrogen (N₂) based atmosphere has been re-evaluated as cheaper, reliable and high efficient nitrogen generators (PSA – Pressure Swing Absorption and MNS – Membrane Nitrogen Separator) are available for grain industry. The systematic laboratory bioassay were conducted on four species of mixed-age cultures of *Sitophilus oryzae* (L.), *Tribolium castaneum* (Herbst), *Rhizopertha dominica* (F.) and *Trogoderma variabile* (Ballion) in five representative grains (wheat, barley, oats, Lupine and canola), and adult stages of Ladybird and Bronzed field beetle (*Adelium brevicorne*) in canola at 95-99% N₂ and 25-35°C for 1 day to 4 weeks exposure. The techniques were tested on farmer storage using farm bins - Lake Grace (33°06'00"S 118°27'40"E) and Cooperative Bulk Handling Albany export terminal (35°1'50.90"S 117°53'10.54"E). This research successfully developed clean high nitrogen technology at a commercial scale for offering an immediate answer to the growing problem of insect resistance to phosphine and satisfies growing market demand for grain free of pest and chemical residues.

Key words: grain storage, controlled atmosphere, nitrogen, PSA nitrogen generator, low oxygen, insect control, grain quality

INTRODUCTION

The Australian grain industry relies heavily on the internationally accepted treatment of fumigation with phosphine (PH₃) for grain treatment to maintain its harvest, free from insect infestation - an important criterion for market access. Currently, phosphine is the only fumigant available to treat bulk grains and oil seeds (more than 85% grains were treated / re-treated with phosphine) in each of the linkages from on-farm storage to the grain terminal. However, three factors are challenging the fate of using phosphine for treatment of grain; 1) OH&S and environmental issues, 2) a restrictive Codex Maximum Residue Limit - MRLs (marketing requirement and consumer safety) of 0.1 mg/kg, with some countries reducing the MRL from 0.1 to 0.05 or 0 mg/kg and 3) increasing resistance of insects. In the past 20 years,

however, resistance to phosphine in target insect pests has developed to such an extent that it now threatens effective control and, as a consequence, jeopardises market access.

Although controlled atmosphere storage has been in use for decades, but these did not lead to the adoption of the technique, as liquid nitrogen source and on site nitrogen generator is very costly. However, cheaper, reliable and high efficient nitrogen generators (PSA – Pressure Swing Absorption and MNS – Membrane Nitrogen Separator) are available, it is appropriate that N₂-based atmosphere be re-evaluated. Nitrogen based CA has several operational advantages over fumigation, particularly management of phosphine resistance and chemical residues issue.

- N₂ constitutes 78% of air, free source of nitrogen
- N₂ is not toxic, greatly reduced OHS&E risks
- Provides “organic” and truly residue-free grain
- No resistance problems
- No reaction with construction materials
- No need for ventilation before grain can be marketed
- No need for product registration

Our aim of this project is to develop cost-effective, viable non-chemical options to the current stand alone system based on phosphine. Research focus on development of nitrogen application technology to the “ready for adoption” stage, which can function as a direct replacement for specific uses of phosphine or eliminate resistant outbreaks.

MATERIALS AND METHODS

Grain samples

Newly harvested five representative grains (wheat, barley, oats, Lupine and canola) were used at moisture content of 9.9, 10.0, 8.4, 11.2 and 4.7%, w/w respectively. The moisture content of the grains was determined by using a Grintec HE 50 electronic moisture meter. The results obtained were expressed as a percentage calculated from replicates.

Insects and bioassays

Four species of stored product insects were used for bioassays. They were mixed-age cultures of *Sitophilus oryzae* (L.), *Tribolium castaneum* (Herbst), *Rhyzopertha dominica* (F.) and *Trogoderma variabile* (Ballion) which were established by adding adults (400-500) to media (1 kg) at 25°C and 65% r.h. The adults were left on the media (sterilised wheat for *S. oryzae* and *R. dominica*, wheat flour+yeast for *T. castaneum* and sterilised crushed canola for *T. variabile*) for 4-5 weeks, by which time there were representative numbers from each stage - egg, larva, pupa, and adult – based on knowledge of development rates (Howe, 1952; Beckett et al., 1994). The insects were sourced from susceptible strains MUWTC 8, MUWSO8, MUWRD 7, MUTV 11 of *T. castaneum*, *S. oryzae* (L.), *R. dominica* and *Trogoderma variabile* (Ballion) respectively held at the Murdoch University Post harvest Plant Biosecurity Laboratory, Perth, Australia. The resistant strains of *T. castaneum*, *S. oryzae* (L.) and *R. dominica* were also used for bioassays. Culturing and general handling techniques followed those described in Winks (1982). Data from these strains can be compared with laboratory bioassays and field results on several species of insects.

Bioassays were conducted by placing muslin bag (150 mm × 60 mm) at a depth of 6 and 23 cm within the fumigation chamber. Each bag contained 20 g of mixed-age cultures, in

standard laboratory culture medium, of approximately 100-120 adults, and an unknown quantity of eggs, larvae and pupae. The control bags containing a high population of insects (400-500 adults) was placed in a bottle containing unfumigated grain. The bagged insects were initially kept in controlled conditions of 25°C and 60% r.h. for one day before being placed in the fumigation chamber. The bioassay samples were retrieved at the end of the fumigation period, the adult insects counted and removed, and the remaining mixed-age cultures incubated at 25°C and 55-60% r.h. Subsequent-emerging adult insects were counted weekly for a period of 5 weeks with live and dead adults being removed at each count.

Nitrogen gas and apparatus

Food grade nitrogen was sourced from BOC Gases Australia. The laboratory bioassays were conducted at Murdoch University Post Harvest Plant Biosecurity laboratory. A gas purging flow system (Fig 1) was used to treat the insects with constant concentrations of nitrogen and oxygen and maintain low carbon dioxide concentrations during the period of treatment. Concentrations of nitrogen, oxygen and carbon dioxide were monitored twice a day during the 1-4 weeks treatment period.



Fig 1- A gas purging flow system was used to treat the insects with constant concentrations of nitrogen and oxygen and maintained low carbon dioxide concentrations during the period of treatment. All 15 cylinders were filled with a known amount (1.8-2 kg) of grain (wheat, barley, oats, lupine and canola).

The range of nitrogen concentrations were 97-99% balanced with oxygen for treatment of all stages of *S. oryzae*, *T. castaneum*, *R. dominica* and *T. variabile* in wheat, barley, oats, lupine and canola, and adult stages of Ladybird and Bronzed field beetle (*Adelium brevicorne*) in canola at 20-30°C.

Oxygen and carbon dioxide were analysed with Witt OXYBABY® 6.0 (WIT-Gasetchnik GmbH & Co KG T, Germany). Accuracy 0.1-100% O₂/0.01-100% carbon dioxide.

Before and after exposure to nitrogen, grain moisture content, protein, oil content, starch or seed colour were analysed using a FOSS Infratec, 1241 Grain Analyzer (FOSS Analytical, Denmark).

Lake Grace farm bin trials

Farm bin-scale trials were conducted on the property of Doug Clarke near Lake Grace (-33.117, 118.607), Western Australia (Figure 2).



Fig. 2- The farm bin-scale nitrogen application trials were conducted at Doug Clarke's farm near Lake Grace (33.117, 118.607), Western Australia. A pressure swing adsorption (PSA) nitrogen generator (capacity of 30 m³ of 99.5% N₂/hour) was used for purging nitrogen to wheat and canola bines (capacity of 75 tonne).

A pressure swing adsorption (PSA) nitrogen generator with a capacity of 30 m³ of 99.5% N₂/hour was used to protect the grain retained on farm for sale. Nitrogen was applied to wheat and canola held in 75 tonne gas-tight storages (P_{1/2} ≥ 180s). The trial was conducted on wheat at 20°C. The final in-store nitrogen concentration was 97-98% for 3 weeks exposure and for canola trial at 35°C for 7 days. Caged mixed-age culture (40-50 g) containing 100-120 of *R. dominica*, *S. oryzae* and *T. castaneum* adults and *T. variable* larvae were used for bioassays. The cages were at different locations within the silo.

CBH Albany grain export terminal trials

A 350 m³ of 99.5% N₂/hour PSA nitrogen generator has been installed at CBH Albany grain export terminal (35°1'50.90"S 117°53'10.54"E). The generator is plumbed to a bank of 10 × 10,000 tonne concrete cells. The project has conducted and completed trials on 5 × 10,000 tonne concrete cells containing newly harvested canola and barley at 30-32°C (Figure 3). The grain was naturally infested with *T. castaneum*, Ladybirds and Bronzed field beetles. The final in-store nitrogen concentration was 97-98% for 2 weeks.

RESULTS

Laboratory bioassays.

- a) Laboratory bioassays show that adults of *T. variabile*, *T. castaneum*, *R. dominica* and *S. oryzae* in wheat, barley, oats, lupins and canola at 99, 98, and 97% N₂ complete control was achieved at 24±2°C for 1, 3 and 7 days, at 30±2°C, for 1, 2 and 5 days, and at 35±2°C for 1, 2 and 4 days exposure periods.



Fig. 3- A 350 m³/hour PSA nitrogen generator has been installed at the CBH Albany grain export terminal (35°1'50.90"S 117°53'10.54"E). The generator is plumbed to a bank of 10 × 10,000 tonne concrete cells.

- b) Laboratory bioassays show that all immature stages of *R. dominica*, *S. oryzae* and *T. castaneum* in wheat, barley, oats, lupins and canola at 99, 98 and 97% N₂ complete

control was achieved at $24\pm 2^{\circ}\text{C}$ for 2, 3 and 4 weeks, at $30\pm 2^{\circ}\text{C}$ for 10 days, 2 weeks and 3 weeks, and at $35\pm 2^{\circ}\text{C}$ for 1, 2 and 3 weeks exposure period. For *T. variabile* at 98-99% N_2 complete control was achieved at 25, 30 and 35°C for 4, 3 and 2 weeks exposure period.

- c) Laboratory bioassays show that all adult and all immature stages of *R. dominica*, *S. oryzae*, *T. castaneum* and *T. variabile* were controlled using the nitrogen treatment and there was no significant difference on mortality between phosphine-resistant and susceptible strains of these insects.
- d) Adult stages of Ladybirds and Bronzed field beetles in canola were completely controlled at 95, 97 and 99% N_2 25°C for 6, 5 and 1 day exposure period, respectively.
- e) The mortality of all stages of all insects tested increased with decreasing levels of oxygen, and increasing exposure time and temperature.
- f) In comparison with wheat, barley, oats and lupin, high concentrations of nitrogen or low oxygen in canola kills all stages of all tested insects with higher efficacy.

Efficacy of high nitrogen (low oxygen) atmosphere on grain quality

Samples of wheat, barley, oats, lupins and canola were analysed both before and after exposure to 97, 98 and 99% N_2 for period of 1-5 weeks at 25, 30 and 35°C . There was no change in moisture content, protein, oil content, starch or seed colour.

Lake Grace farm bin trials

The trials on wheat at 20°C and 97-98% N_2 , all adults of *R. dominica*, *S. oryzae* and *T. castaneum* were killed after one week and complete extinction of all life stages occurred after 3 weeks exposure, but 6-10% of *T. variabile* larvae survived. The trials in canola at 35°C shows that with 7 days exposure to nitrogen at 97% all Bronzed field beetles and Ladybirds were eliminated, and after 2 weeks exposure all stages of *R. dominica*, *S. oryzae*, *T. castaneum* and *T. variabile* were killed. Canola seed colour, oil content and levels of free fatty acid did not change during the 2 month storage period. This storage process of canola significantly contributed to maintaining quality by inhibiting the respiration process that can lead to rapid localised heating and prevented the oxidation that leads to seed deterioration at this high temperature.

Various atmospheric purging methods were evaluated during the trials. The most efficient method was to pump nitrogen into the base of the bin with the top lid closed and air purging from the silo through a pipe connected to headspace, exiting at ground level. The purge continued until the exhaust air contained 98% N_2 . After one day, 1-1.5% O_2 desorbed from grain, requiring the storage to be topped up until the exhaust air again contained 98% N_2 .

CBH Albany grain export terminal trials

After 2-3 weeks treatment with 98% N_2 , all barley and canola was inspected for export with no live insect pests found. The bioassay with mixed age cultures show that all stages of tested *T. castaneum*, *S. oryzae* and *R. dominica* were killed after 2-3 weeks exposure. The treated barley and canola had no change in moisture content, protein, starch, oil content and level of free fatty acid and seed colour.

CBH Albany grain export terminal now incorporate the use of nitrogen as a management tool for grain coming in from up country that has been treated with phosphine. This means that effectively all grain exported from Albany will only be treated with phosphine once, or not at all, with the use of nitrogen only at port. The introduction of

nitrogen at CBH Albany grain export terminal has offered solutions for management of phosphine resistance an alternative to phosphine treatment and a grain quality control method.

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