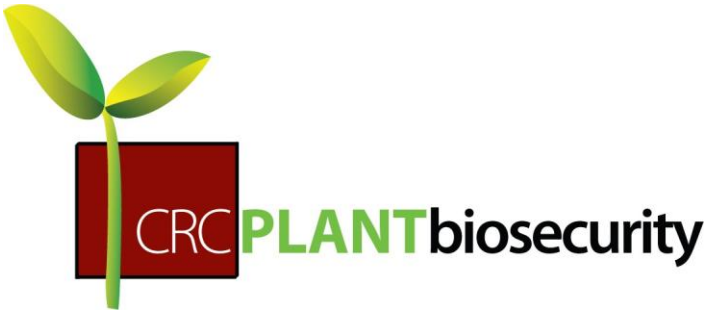

Cooperative Research Centre
for National Plant Biosecurity

Final Report

CRC50147

Alternatives to Phosphine

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1. Executive Summary

The use of nitrogen as a method for grain storage has been known for more than 30 years but only recently with the development of lower priced Pressure Swing Adsorption (PSA) and Membrane Separation (MS) Nitrogen generators, has it been possible to consider the technique as a viable grain protection system. This project 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.

Development research for high nitrogen application techniques were conducted at the Murdoch University Post Harvest Plant Biosecurity laboratory. The techniques were developed in collaboration with the principal end-users, such as grain growers using farm bins - Lake Grace (33°06'00"S 118°27'40"E) and Cooperative BulkHandling Albany export terminal (35°1'50.90"S 117°53'10.54"E) Both parties were involved in validation field trials.

1.1. Laboratory evaluation of efficacy of high nitrogen (low oxygen) atmosphere against insect pests and effect on grain quality

1.1.1. Efficacy of high nitrogen (low oxygen) atmosphere against insect pests

The laboratory bioassays were conducted at Murdoch University Post Harvest Plant Biosecurity laboratory. A gas purging flow system (Figure 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.

The range of nitrogen concentrations were 95-99% balanced with oxygen for treatment of all stages of *Sitophilus oryzae* (L.), *Tribolium castaneum* (Herbst), *Rhyzopertha dominica* (F.) and *Trogoderma variabile* (Ballion) in wheat, barley, oats, lupins and canola, and adult stages of Ladybird and Bronzed field beetle (Adelium brevicorne) in canola at 20-30°C. Bioassay samples were retrieved at the end of the exposure period, the adult insects were counted and removed and the remaining grain incubated at 25±1°C and 65% RH. Subsequent emerging adult insects were counted weekly for a period of five weeks, with live and dead adults removed at each count.

a) Laboratory bioassays were conducted on adults of *Trogoderma variabile*, *Tribolium castaneum* and *Rhyzopertha dominica* and *Sitophilus oryzae* in wheat, barley, oats, lupins and canola at 99, 98 and 97% nitrogen, complete control was achieved at 24±1°C for five days, two weeks and three weeks; at 30±1°C for three, 10 and 15 days; and at 35±1°C for two, six and 10 days exposure periods.

b) Laboratory bioassays were conducted on all immature stages of *R. dominica, S. oryzae* and *T. castaneum* in wheat, barley, oats, lupins and canola at 99, 98 and 97% nitrogen, complete control was achieved at 24±1°C for two, three and four weeks; at 30±1°C for 10 days, two weeks and three weeks; and at 35±1°C for one, two and three weeks exposure period.

   Bioassays were conducted on all immature stages of *T. variabile* in wheat, barley, oats, lupins and canola 98-99% nitrogen and at 25, 30 and 35°C for four, three and two 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 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% of nitrogen and 25°C for six, five and three days 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.

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

Samples of wheat, barley, oats, lupins and canola were analysed using a FOSS Infratec, 1241 Grain Analyzer both before and after exposure to 97, 98 and 99% nitrogen for period of 1-4 weeks at 25, 30 and 35°C. There was no change in moisture content, protein, oil content, starch or seed colour.

1.2. 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). The farmer was sufficiently interested in the process that he invested in a pressure swing adsorption (PSA) nitrogen generator to protect the grain retained on farm for sale. Nitrogen was applied to wheat and canola held in 75 tonne gas-tight storages (P½ = >180s) using a PSA generator with a capacity of 30 m^3 of 99.5% N_2/hour. Efficacy against insect pests and effect of the storage atmosphere on grain quality were evaluated. The initial trial was conducted on wheat with a temperature of 20°C. The final in-store nitrogen concentration was 97-98%. All adults of *R. dominica, S. oryzae* and *T. castaneum* were killed after one week and complete extinction of all life stages occurred after three weeks exposure, but 6-10% of *Trogoderma variabile* larvae survived.

A subsequent trial in canola at 35°C showed that with seven days exposure to nitrogen at 97% all Bronzed field beetles and Ladybirds were eliminated, and after two 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 two 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% nitrogen. After one day, 1-1.5% oxygen desorbed from grain, requiring the storage to be topped up until the exhaust air again contained 98% nitrogen.

1.3. CBH Albany grain export terminal trials

A 350 m^3/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 x 10,000 tonne concrete cells. The project has conducted and completed trials on 5 x 10.000 tonne concrete cells containing newly harvested canola and barley at 30-32ºC (Figure 3). The grain was naturally infested with *T. castaneum,* Ladybirds and Bronzed field beetles.

After 2-3 weeks treatment with 98% nitrogen only, 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's fumigation protocols state that grain must be re-fumigated after three months storage. 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 (Table 1).

1.4. Chinese commercial scale trials in large horizontal storage

The laboratory has collaborated with Professor Cao Yang (Stored Grain Research Lab, Chinese Academy of Cereal Science) and Chinese Research Institute of Membrane Separation Technology to explore a membrane separation (MS) nitrogen generator for grain storage. Commercial scale trials were conducted in Zhengding (38.145358,114.528351) China. Two units of 150 m^3/hour membrane separation nitrogen generator were used to apply 99.5% purity of nitrogen to a

10,000 tonne horizontal wheat storage. Recirculation was used to return the exhausted air containing >85% nitrogen to the storage which created significant savings in energy. In addition, the trial was able to attain oxygen levels of less than1% at the base of storage – an excellent result as this is normally the weak point for controlled atmosphere technology.

The grain was naturally infested with *T. castaneum* and Psocids. After four weeks treatment with 98.5% nitrogen at 26ºC, there were no live insect pests found. The bioassay with mixed age cultures shown that all stages of tested *T. castaneum*, *S. granarius* and *R. dominica* were completely killed after four weeks exposure.

2. Aims and objectives

The project aims and objectives were to undertake a national scoping study to develop cost-effective and readily adoptable chemical alternatives using nitrogen-based controlled atmosphere technology to protect and disinfest stored grain. This technology aims to produce insect-free grain at out-turn and control phosphine-resistant insects which has the potential to prolong the useful life of phosphine, complement current management practices and comply with market requirements and environmental and work-place health and safety standards.

The goal to is have Nitrogen based controlled atmosphere technology adopted by the collaborating grain companies CBH, Vitarra and GrainCorp. However, it is likely that once proven other larger storage organizations and growers will adopt the technology.

3. Key findings

3.1. Knowledge of insect response (toxicity) to low O_2/high N_2 concentrations and its relationship to phosphine resistance

a) Laboratory bioassays were conducted on adults and all immature stages of *Trogoderma variabile*, *Tribolium castaneum*, *Rhyzopertha dominica* and *Sitophilus oryzae* in wheat, barley, oats, lupins and canola at 99, 98 and 97% nitrogen and at 25, 30 and 35°C for four, three and two weeks exposure period. The mortality of all stages of all insects tested increased with decreasing levels of oxygen, and increasing exposure time and temperature. In comparison with wheat, barley, oats and lupin, in canola high concentrations of nitrogen or low oxygen kills all stages of all tested insects with higher efficacy (Table 1).

   Adult stages of Ladybirds and Bronzed field beetles in canola were completely controlled at 95, 97 and 99% of nitrogen and 25°C for six, five and three days exposure period, respectively (Table 1).

c) Laboratory bioassays show that there is no difference in mortality under nitrogen treatment between phosphine-resistant and susceptible strains of

adults and all immature stages of *R. dominica, S. oryzae, T. castaneum* and *T. variabile*.

3.2. Knowledge of the effects of low O_2/high N_2 concentrations on grain quality

There was no effect of nitrogen on moisture content, protein, oil content, starch and seed colour at 97, 98 and 99% for period of 1-4 weeks at 25, 30 and 35°C. Particularly, canola seed colour, oil content and level of free fatty acid did not change during the two month storage period at 35ºC. Canola stored under high N2 maintained 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.

3.3. Demonstrated and adopted nitrogen-based controlled atmosphere grain storage system using pressure-swing absorbtion technology

a) The most efficient method to purge nitrogen was through the base of the bin with the top lid closed and air purging from the silo through a pipe connected to the headspace, exiting at ground level. The exhaust air was measured until it reached 98% nitrogen. After one day, 1-1.5% oxygen was desorbed from grain, requiring the storage to be topped up with nitrogen until the exhaust air again contained 98% nitrogen.

b) A recirculation technique can reuse the exhausted air contained >85% nitrogen with significant savings in energy. In addition, we were able to attain oxygen levels of less than1% at the base of storage – an excellent result as this is normally the weak point for CA technology.

4. Implications for stakeholders

Although controlled atmosphere storage has been in use for decades, Australia has become the first country to successfully develop clean high nitrogen/low oxygen technology as an alternative to fumigants such as phosphine. The technology offers an alternative, affordably priced control technology to growers and the grain export industry to keep stored cereal grains and canola free from pests and in peak condition without chemical residues. Additionally there is benefit in the management of phosphine resistance which is seen as an important advance in helping to shield Australian rural exports worth $6 billion a year.

The availability of high efficient and low-cost nitrogen generators from China and India has given nitrogen technology a boost and made this a much more practical technology. Including capital and operating costs the technology is only a little more expensive in the long term than using phosphine – but without the added issues of insect resistance. The system is of considerably lower cost than heat disinfestation and other non-chemical methods, meets OHS&E requirements and it is expected that over time will become a significant feature of buyer preferences.

An important advantage of this technology is that all the key aspects of grain quality, protein, viablity, starch, colour and oil content – are preserved in a nitrogen atmosphere. Storing oilseeds in a low oxygen atmosphere prevents the risk of self generated heat and fire.

This technology will significantly contribute to further enhance the Australian grain industry reputation on the domestic and international market. For example, the CBH Albany grain export terminal now incorporates the use of nitrogen as a management tool for grain coming in from up country that has been treated with Phosphine. It is projected that all grain exported from Albany will only be treated with Phosphine once, or not at all, using only nitrogen. This technique has offered solutions for management of phosphine resistance potentially removing a reliance on only one chemical for control of storage pests.

5. Recommendations

5.1. To evaluate and demonstrate the membrane separation nitrogen generator and recirculation system.

5.2. To extend PSA and/or MS nitrogen technology at both grower and bulk handler level.

5.3. To evaluate high temperature (50-50ºC) + nitrogen (95-99%) for Khapra beetle and other insect pests on grain and fruit and to develop application technologies.

5.4. To investigate increasing the efficacy of nitrogen by heating it for reducing the temperature required for efficacy or for applying CA during heat treatment under anoxic conditions to reduce the time of the treatment should be evaluated at high priority.

5.5. To conduct systematic laboratory and field trials to evaluate protection of colour for pulses and malting quality and colour of barley.

Figure 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, lupin and canola). A sealed sachet made of muslin cloth with 40-50 g of mixed age culture of stored grain insects with >200 adults were inserted at a depth of 6 and 23 cm.

Figure 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 m^3 of 99.5% N_2/hour) was used for purging nitrogen to wheat and canola bines (capacity of 75 tonne).

Figure 3. A 350 m^3/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 x 10,000 tonne concrete cells.

Figure 4. Commercial scale trials were conducted in Zhengding grain depot (38.145358,114.528351), China. Two units of 150 m^3/hour membrane separation nitrogen generator were used to apply 99.5% purity of nitrogen to horizontal wheat storage (capacity of 10,000 tonne).

Table 1. Recommended dosage of nitrogen and exposure period at different temperatures

Storage Type	Commodity	Insect Species	*Dosage Rate of Nitrogen	*Dosage Rate of Nitrogen (Insect Infested)	#Exposure Period of Nitrogen at Different Grain Temperatures	Monitoring Interval (Days)	Minimum Level of Nitrogen Required	Holding Period for Outloading
Cells	Canola	All species of adult insect pests	99% Nitrogen (1% Oxygen) by volume to be topped up as necessary	99% Nitrogen (1% Oxygen) by volume to be topped up as necessary	≥ 35ºC & 2 days ≥ 30ºC & 3 days ≥ 25ºC & 5 days ≥ 20ºC & 7 days	**Daily**	98% in Headspace and bottom	No witholding
		Harvest Beetles and weevils			≥ 35ºC & 2 days ≥ 30ºC & 3 days ≥ 25ºC & 6 days			
		All species of insect pests at all stages (no warehouse beetle present)			≥ 30ºC & 14 days ≥ 20ºC & 21 days			
		All stage warehouse beetle			≥ 35ºC & 14 days ≥ 30ºC & 18 days			

					≥ 25ºC & 25days			
Cells	Wheat Barley	All species of adult insect pests	99% Nitrogen (1% Oxygen) by volume to be topped up as necessary	99% Nitrogen (1% Oxygen) by volume to be topped up as necessary	≥ 35ºC & 2 days ≥ 30ºC & 3 days ≥ 25ºC & 5 days ≥ 20ºC & 7 days	**Daily**	98% in Headspace and bottom	No withholding
		All species of insect pests at all stages (no warehouse beetle present)			≥ 35ºC & 10 days ≥ 30ºC & 15 days ≥ 25ºC & 20 days ≥ 20ºC & 25 days			
		All stage warehouse beetle			≥ 35ºC & 17 days ≥ 30ºC & 21 days ≥ 25ºC & 28days			

TABLE 2. MINIMUM FUMIGATION RECOMMENDATIONS

Storage Type	Fumigant	*Dosage Rate of Fumigant	*Dosage Rate of Fumigant (Insect Infested)	#Exposure Period of Fumigant (Days)	Monitoring Interval (Days)	Minimum PPM Required
Sealed Storages	Phosphine	0.5 grams per tonne storage capacity	0.66 grams per tonne storage capacity	28 Days	Weekly ideally at 7, 14 & 21 days Minimum 3 Readings	100 ppm at 14 days
Sealed Storage (Containing >40% Canola, Oats or Peas)	Phosphine	0.66 grams per tonne storage capacity	Liaise with Grain Protection Supervisor	28 Days	As Above	100 ppm at 14 days
Internal Tarping (All commodities)	Phosphine	1.5 grams per tonne	Liaise with Grain Protection Supervisor	28 Days	As Above	100 ppm at 14 days
Open Bulkheads	Phosphine	0.75 grams per tonne	1.5 grams per tonne	28 Days	As Above	100 ppm at 14 days
Open Bulkheads (Containing canola, oats or peas)	Phosphine	1.5 grams per tonne	Liaise with Grain Protection Supervisor	28 Days	As Above	100 ppm at 14 days
Cells	Phosphine	0.5 grams per tonne storage capacity	0.66 grams per tonne storage capacity	14 days	As Above	100 ppm at 14 days
Cells (Rapid Fumigation)	Phosphine	0.5 grams per tonne storage capacity	0.66 grams per tonne storage capacity	7 Days	Daily	350 ppm for 7 days

Dosage rate can be varied providing minimum PPM requirements are met.

Monitoring: To be carried out using

approved monitoring equipment.

Exposure period may be varied with formal permission from the Grain Protection Department.

FORMULAE

SEALED STORAGE

- Dosage Rate x Storage Capacity ÷ 500 gms (Tablets)
- Dosage Rate x Storage Capacity ÷ 1122 gms (Blankets)
- Dosage Rate x Storage Capacity ÷ 620gms (Cylinders)

OPEN BULKHEAD

- Dosage Rate x Tonnage ÷ 500 gms (Tablets)
- Dosage Rate x Tonnage ÷ 620 gms (Cylinders)

INTERNAL TARPING

- Dosage Rate x Tonnage ÷ 500 gms (Tablets)
- Dosage Rate x Tonnage ÷ 620 gms (Cylinders)

SIROFLO

- 80ppm for min 28 days

VAPORPHOS

Dosage Rate x Storage Capacity ÷ 1000 = kg required

- PPM Increase x Storage Capacity ÷ 800 (for fumigation top up)

PHOSPHINE

Tablets 1.5kg	evolves	500gm ph3
Blankets 3.4kg	evolves	1122gm ph3
G Cylinders	contains	620gm ph3
E Cylinders	contains	300gm ph3
Vaporphos cylinder	contains	22000gms ph3

TABLE 2. MINIMUM FUMIGATION RECOMMENDATIONS

Storage Type	Fumigant	*Dosage Rate of Fumigant	*Dosage Rate of Fumigant (Insect Infested)	#Exposure Period of Fumigant (Days)	Monitoring Interval (Days)	Minimum PPM Required
Cells	Nitrogen	99% Nitrogen (1% Oxygen) by volume to be topped up as necessary	99% Nitrogen (1% Oxygen) by volume to be topped up as necessary	Adult Insects 7 Days All Life Stages 25 Days	**Daily**	98% in Headspace Ideally ≥ 20ºC Grain Temp
Cells	Carbon Dioxide	95% initial concentration by volume to be topped up as necessary	95% Initial concentration by volume	Minimum 14 days	**Daily**	35% @ 14 Days
Monitoring: To be carried out using approved monitoring equipment.			*Dosage rate can be varied to help achieve minimum PPM requirements.			

Exposure period may be varied with permission from Grain Protection Department.

6. Abbreviations/glossary

ABBREVIATION	FULL TITLE
CRCNPB	Cooperative Research Centre for National Plant Biosecurity
CBH	Co-operative Bulk Handling Group
MS	Membrane Separation nitrogen generator
N_2	Nitrogen
O_2	Oxygen
PH_3	Phosphine
PSA	Pressure Swing Adsorption nitrogen generator

7. Plain English website summary

CRC project no:	CRC50147
Project title:	Nitrogen as Alternatives to Phosphine
Project leader:	YongLin Ren
Project team:	James Newman, Manjree Agarwal and Hui Cheng
Research outcomes:	• Establish rate of desorption of air from grain under nitrogen. • New knowledge of insect response (toxicity, behavioural) to low O_2/high N_2 concentrations and its relationship to phosphine resistance. • New knowledge of the effects on grain quality of storage under nitrogen. • New nitrogen application method.
Research implications:	This research has led to development of cost-effective, readily adoptable phosphine alternatives that will control resistant insects and comply with industry and market standards which significantly contribute to further enhance Australian grain industry reputation in both domestic and international market.
Research publications:	• Ren, Y.L. and Newman, C. (2010). Post Harvest Grain storage - From Farmer to Market. Global Biosecurity 2010: safeguarding agriculture and the environment 28 February – 3 March 2010, Brisbane Convention and Exhibition Centre. • Ren, Y.L., Manjree Agarwal, James Newman and Hui Cheng. (2011). Alternative to phosphine – nitrogen storage. CRCNPB Science Exchange, 8-11 February

	2011, Adelaide, Australia. • Ren, Y.L., James Newman, Manjree Agarwal and Hui Cheng. (2012). Alternative to phosphine – nitrogen storage. CRCNPB Science Exchange, 22-25 May 2012, Perth, Australia. • Ren, Y.L., James Newman, Manjree Agarwal and Hui Cheng. (2012). Nitrogen application offers for both control of insect and grain quality. Proceedings of an International Conference on Controlled Atmosphere and Fumigation in Stored Products 2012. Submitted
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