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CONTROL OF STORED GRAIN INSECTS BY USING NITROGEN IN LARGE CONCRETE SILOS IN CYPRUS

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ABSTRACT

Nitrogen (N₂) was applied in three concrete silos each containing 2,400 tonnes of grain for controlling stored grain insects. Structural sealing was carried out and air tight valves were installed to improve the air tightness of the bins. Pressure decay tests (250-125 Pa) carried out in full bins showed decay time from 120 s up to 290 s. Using a PSA N₂ generator the oxygen (O₂) concentration was reduced in 44-56 h below 0.9% and after that it was continuously maintained between 0.2-0.9% up to 23.8 days. Treatment of 18.7 and 23.8 days on grain temperature of 26°C and 22°C, respectively, was effective for the control of the adults of important stored grain insects *Oryzaephilus surinamensis* (L.), *Tribolium confusum* J. du Val and *Rhyzopertha dominica* (F.) placed above and inside grain mass. In separate bioassays, which were placed in different zones right above the grain mass complete (100%) mortality of *T. confusum* (all life stages), *O. surinamensis* (larvae and adults), *Sitophilus granarius* (L.) (adults) and *R. dominica* (adults) was achieved in 18.7 and 23.8 days treatment as well. When applied to large quantities of grain, the N₂ modified atmosphere (MA) technology by using a N₂ generator proved to be cost competitive with the analyzed other treatment methods. Under trial conditions the cost of annual treatment of 40,000 tonnes using the PSA of EcO₂ system is about 1.6 €/tonnes in comparison to 0.5-0.8 €/tonnes by using Siroflo/ECO₂FUME, 0.4-0.8 €/tonnes by using contact insecticides and 0.40 €/tonnes for aeration. By increasing the annually treated quantity to 130,000 tonnes the treatment cost with N₂ MA EcO₂ system is estimated 0.80 €/tonnes. The N₂ MA provided a full control of studied stored grain insects, free-residues products, no environment chemical contamination, low occupational hazard, no-dependence on insect resistance, no need for registration and full control and automation of the treatment operation. On the basis of these trials the N₂ MA technology is a successful alternative to phosphine Siroflo/ECO₂FUME and contact insecticides for the control of stored grain insects in large concrete silos.

Key words: Nitrogen, modified atmospheres, grain protection, grain storage, stored-grain insects, silo sealing, post harvest systems, non-chemical alternatives, oxygen, phosphine, contact insecticides, eco-friendly methods

INTRODUCTION

Phosphine and contact insecticides are still the main means used around the world for protection and disinfestations of grains stored in silos. On the other hand, there is an increasing need in new grain protection methods which should be friendlier to the environment, safer to products and employees and more effective for insect control on an acceptable cost basis. Modified atmosphere (MA) by using nitrogen (N_2) is one of the most promising alternative methods providing effective and residue-free insect control in sealed storage structures with reduced hazard to employees, no need for registration and no contamination of environment. The effects of low oxygen concentrations by using N_2 to control stored grain insects was reported in many works (Navarro, 1978; 2006; Jay, 1984; Banks and Annis, 1990). The method requires sufficiently sealed storage structures. The cost and difficulties of sealing large grain bins and the cost of N_2 supply, in combination with the widespread use of phosphine and liquid insecticides, have delayed the implementation of N_2 in large grain silo bins. During the last years there is an increasing interest in introduction of N_2 Modified Atmosphere technology for protection of stored grains in silos (Cassells et al., 1994; Banks and Annis, 1997; Timlick et al., 2002; Navarro, 2006). Liquid nitrogen from tank has been commercially and routinely used for grain treatment in 1800-tonnes sealed concrete silos of at least 5 min half life pressure decay time at Newcastle export terminal in Australia; the combination of IPM and nitrogen has been reported to be very effective (Clamp and Moore, 2000; Clamp and Banks, 2000).

The Siroflo/ECO₂FUME fumigation technology by using phosphine from cylinders in unsealed silos is an alternative to solid phosphine and liquid insecticides (Winks, 1992; Winks and Russell, 1994; Varnava et al., 1998). The Siroflo/ECO₂FUME was introduced to Cyprus in 1996; it was installed and successfully used in unsealed vertical metal and concrete grain silos. On the other hand, the need in increased doses of phosphine by Siroflo system and the non re-registration of ECO₂FUME in EU created the necessity for alternative solution. This trial was conducted at Cyprus Grain Commission's port concrete silo where Siroflo/ECO₂FUME fumigation system is used and aimed to demonstrate the application of N_2 MA technology as alternative to Siroflo/ECO₂FUME and define the real requirements, effectiveness and cost of sealing and using this technology. The results of this study are presented in this article.

MATERIALS AND METHODS

N_2 was applied in three bins at Cyprus Grain Commission's Limassol port silo. Bins are made of concrete with conical base floor (bin diameter 10.5 m, height to eaves 33.4 m, depth of cone 5.4 m, total storage capacity 3,046 m³). Each bin is connected to a Siroflo/ECO₂FUME flow-through fumigation system and to an aeration system via two aeration ducts entering bins from the bottom. The bins were not constructed to be used with modified atmosphere.

Sealing works were carried out to improve gas tightness of bins A, B and C. At the bottom of bin B aeration duct inlets were sealed by installing two gas tight valves; the grain outlet gate was also replaced by a gas tight knife-type valve; at the bottom of the other two bins polyethylene sheet, multipurpose aerosol adhesive spray, tape and silicone were used for improving sealing at these places.

After this preparation, bins were loaded with grain up to about 1 m below bin roof leaving about 150 m³ head space above grain. In bin A 2200 tonnes of barley (m.c. 12.9% wet

basis) and in each of bins B and C 2400 tonnes of feed wheat (m.c. 11.8% wet basis) were stored.

Additional sealing works were carried out at the top of bins. Sealing of the cracks and crevices inside silo bins between the roof and the wall joints was carried out from inside bins by using expandable foam polyurethane, plaster, gastight coating, tissue and sealing silicone. Aeration duct outlets were sealed using polyethylene sheet and tape; manhole inlets were covered and sealed with a temporary board, tissue, gastight coating and silicone; on board an over/under pressure valve, oxygen analyzer tube and temperature sensor were installed for measurement at different levels inside each bin and above grain. The top of each silo bin is equipped with two loading ports; silicone was used to seal the loading ports; in the case of bins B and C the second loading port (x-type valve) was removed and the opening was closed with a board, tissue, gastight coating and silicone; the x-valve of the bin A was sealed from inside bin using polyethylene sheet, multipurpose aerosol adhesive spray and tape.

For studying insect mortality two separate trials were carried out. In the first trial, test insects were separated from infested grain taken from commercial storages in Cyprus. Adults of *O. surinamensis*, *T. confusum* and *R. dominica* were placed in tubes (1.5 cm in diameter, 10 cm in height) with metal mesh walls and about 5 g of feed (flour and whole wheat kernels). The tubes containing the insects were placed on grain surface, at 0.5 m and 3 m below and at 1 m above grain surface, at the bin's centre.

In the second trial, the mortality of *T. confusum* (all life stages), *O. surinamensis* (larvae and adults), *S. granarius* (adults) and *R. dominica* (adults) was studied. Test insects were taken from laboratory cultures of *T. confusum* reared on wheat flour, *O. surinamensis* on cracked oats and *R. dominica* with *S. granarius* on whole wheat kernels. All adults used in the bioassays were <1 month old, while all larvae <2 week old and eggs of *T. confusum* were <1 day old. Twenty individuals from each species/life stage were placed in small cylindrical plastic vials (2.5 cm in diameter, 8 cm in height). About 0.3 g of diet was added to each vial before they were closed, but equipped with small openings in the lid to allow sufficient aeration. For each bin, there were 9 vials for each species and life stage combination. Three of them were placed in the center of the bin, three at the median of the radius and three at the edge, close to the bin walls, on grain surface.

Adults of *T. confusum* and *R. dominica* were placed in the control bin. After the termination of the N₂ treatment, all tubes and vials with insects were transferred to the laboratory and examined for surviving individuals.

The temperature inside bins was monitored using thermocouple cables at different locations 1 m above, on grain surface and 0.5 m and 3 m below grain surface, at bin's centre, before starting treatment and after completing it. The oxygen (O₂) concentration was monitored by taking gas samples at 1 m above and 3 m below grain surface and analyzing them using a portable meter. Additional measurements of oxygen and temperature inside treated bins at grain surface, at bin's centre, were taken by the Eco2 system during treatment continuously every 10 min.

Before starting treatment with N₂ a pressure test of full bins was carried out using the "half life pressure decay time method 250-125 Pa" to determine the bins gas tightness level.

The EcO₂ system was used for producing N₂ from ambient air and purging it into bins from the bottom. The EcO₂ generator was connected to two different points of the silo bins: a) from the top of the silo bins via the gas sampling tube and temperature sensor; and b) from the bottom of the silo bin via 25 mm (internal diameter) flexible tube connected to an already prepared 25 mm steel connection. The tube served for purging the silo bin using N₂ from the EcO₂ generator.

The EcO₂ generator used was based on air-to-N₂ production using pressure swing adsorption (PSA) technology and the system was installed in a mobile 6 m container with necessary equipment and control devices.

After reaching O₂ concentration below 0.9% in bins A, B and C, this concentration was continuously maintained by the EcO₂ system below 0.9% for 516 h, 399 h and 273 h, respectively.

Cost analysis data was carried out to compare N₂ based MA application using the EcO₂ system and other stored grain protection methods used in Cyprus Grain Commission (CGC) grain silos (Siroflo/ECO₂FUME, contact insecticides, aeration).

RESULTS AND DISCUSSION

Cost effectiveness of sealing an existing silo is an important factor for making a decision on which grain protection method to use. The use of gas tight valves improved sealing. The cost of sealing bins including the use of gas tight valves, and the half life pressure decay time achieved after sealing is shown in Table 1.

Table 1. Cost for sealing three 2400-tonne capacity concrete silo bins and gas tight pressure tests results in full bins after sealing

Bin	Sealing details	Cost (€)	Pressure decay test (250-125 Pa) time
A	Structural sealing works only*	2,500	2 min 20 s
	Structural sealing works *	2,500	
B	2 gas-tight valves at aeration inlet ducts +	2,000	4 min 50 s
	1 gas-tight knife-type valve at grain out loading port	11,200	
	Total cost for bin B	15,700	
C	Structural sealing works only*	2,500	2 min

* structural sealing works include sealing of the cracks and crevices inside silo bins between the roof and the wall joints, sealing of aeration exhaust vents and grain inlet ducts and manholes and other works except the installation of three gas tight valves.

The use of gas tight valves at aeration inlet ducts and at grain unloading port improved considerably the half life pressure decay time, but the sealing cost also increased close to 15,700 € per bin. Although the half life pressure decay time was at the border line of the acceptable levels for using MA, even in the bin where three gas tight valves were installed (4 min 50 s), in all bins the O₂ dropped below 0.9%. The time, the volume of N₂ and the energy needed by the EcO₂ generator to produce the required volume of N₂ is shown in Table 2.

The O₂ concentration was maintained between 0.1-0.9% for a period of 523 h in bin A, 404 h in bin B and 261 h in bin C. These values are comparable with the reports from previous studies from other parts of the world (Cassells et al., 1994; Clamp and Moore, 2000; Timlick et al., 2002). The purge time, maintenance time, the volume of N₂ produced, the power consumed by the EcO₂ generator and energy cost under trial conditions are presented in Table 3.

Table 2. Purge time, nitrogen volume and energy needed to reduce the oxygen concentration to below 0.9 % in three full silo bins using the EcO₂ system

Bin	Time (h)	Volume of N ₂ (m ³)	Energy** to produce Nitrogen (kWh)	Cost*** for energy (€)
A	49	3709	1780	356
B*	44	2859	1372	274
C	56	3571	1714	343

* sealing includes structural works and 3 gas tight valves.

** energy consumption by EcO₂ converter during trials up to 0.48 kWh/m³ N₂

*** average electricity cost 0.20 €/kWh

Table 3. Treatment duration, volume of nitrogen, energy and cost for treatment of three silo bins using EcO₂ system (O₂<0.9%)

Bin	Grain quantity (tonnes)	Total duration of treatment including purge, h (d)	Total vol. of N ₂ (m ³)	Total volume of N ₂ (L t ⁻¹ d ⁻¹)	Total energy to produce N ₂ ** (kWh)	Total cost for energy*** (€)	Total cost for energy*** (€/t)
A	2200	572 (23.8)	17957	342	8619	1724	0.78
B*	2400	448 (18.7)	13381	298	6423	1285	0.54
C	2400	317 (13.2)	11399	360	5472	1094	0.46

* sealing includes structural works and 3 gas tight valves

** energy consumption by EcO₂ generator during trials up to 0.48 kWh/m³ N₂

*** average electricity cost 0.20 €/kWh

Regardless of differences in the gas tightness of the bins and that the half life pressure decay time was lower than 5 min, in all bins the O₂ concentration was continuously maintained below 0.9% for the specified exposure time. Therefore, the difference among the bins was in the level of gas tightness that was reflected mainly on the amount of N₂ used to achieve O₂ concentrations below 0.9% and the same level of insect mortality; the more gastight was the bin, the less gas was used. Thus, the level of gas tightness affected the cost of treatment to achieve the same mortality level. Insect mortality of bioassays are shown in Tables 4 and 5. The trials were carried out for various exposure times to N₂ treatment, from 13 to 24 days. Table 4 shows that complete mortality of *R. dominica* adults, could be achieved when the treatment was 23.8 days but some survivals were observed when treatment was 18.7 days. The EcO₂ system was effective in maintaining the O₂ below 0.9%, which controlled the tested life stages of *O. surinamensis*, *T. confusum*, *R. dominica* and *S. granarius* (Tables 4 and 5).

After completing the treatment, operating the aeration system of the bins for 1-2 h was enough to restore the treated bins to atmospheric O₂ level. The cost of treatment using EcO₂ system in sealed silos mainly depends on: a) the fixed cost for rental of EcO₂ system, including maintenance and using the EcO₂ system control software and central communication system, b) cost of electricity for generating N₂, c) expenses for sealing a bin and depreciation of sealing, d) transportation of grain from one bin to a sealed bin for treatment with N₂, e) other factors like quantity (tonnes) of treated grain per year, the number

of gas tight valves and the expected life of bin sealing, the duration of treatment and other minor technical and logistical costs.

Table 4. Influence of different exposure times to oxygen concentration below 0.9% on mortality of adult insects in three bins treated with nitrogen generated by EcO₂ system

Bin	Total duration of treatment incl. purge time** (d)	Grain*** temper. (°C)	Air*** temper. (°C)	Oxygen concentr. during treatment (%)	Insect species in tubes at central zone of bin at different locations****	Total insects in tubes and sample	Insect mortality % (± SD)
A	23.8	22	15	0.2-0.9	<i>O. surinamensis</i>	63	100 ± 0.0
					<i>T. confusum</i>	265	100 ± 0.0
					<i>R. dominica</i>	263	100 ± 0.0
B*	18.7	26	16	0.5-0.9	<i>O. surinamensis</i>	203	100 ± 0.0
					<i>T. confusum</i>	109	100 ± 0.0
					<i>R. dominica</i>	308	91.6 ± 6.8
C	13.2	19	17	0.3-0.9	<i>O. surinamensis</i>	72	100 ± 0.0

* sealing of this bin includes structural works + 3 gas tight valves

** 44-56 h were required to fill bins with N₂ and reach O₂<0.9%

*** Air and grain temperature is the average of measurements taken before and after completing treatment, at bin's centre, at 1 m above and on grain surface and at 0.5 m and 3 m below grain surface respectively; in bin C it is the average of measurements taken before starting and after completing treatment, at bin's centre, at 1 m above and 0.5 m below grain surface, respectively.

**** in bins A and B tubes with metal mesh walls containing insects and feed were placed 1 m above and on grain surface and 0.5 m and 3 m below grain, at bin's centre; in bin C on grain surface.

Estimated cost for 18-day treatment of 2,400 tonnes grain in sealed concrete bins using the EcO₂ system under the Cyprus Grain Commission conditions at Limassol port silo is shown in Table 6. Estimated cost of about 1.6 €/tonnes is based on current data. Cost for rental, maintenance and use of EcO₂ system represents about 60% of the total cost of treatment with N₂. The electricity expenses for the generation of N₂ represent about 30% of the total cost. The rest of the cost that includes expenses for bin sealing and depreciation structural works, two gas tight valves at aeration inlet ducts and one gas tight valve at grain out loading port was about 10% of total cost.

A cost comparison of four different stored grain protection methods is shown in table 7. Cost estimates were based on 40,000 tonnes of grain treated annually. Since the EcO₂ system rental cost is fixed (about 38,000 €/year), the more grain is treated, the less the cost of the treatment is. For 40,000 tonnes of grain, the cost per treated tonne with N₂ MA using the PSA EcO₂ system including expenses for the rental of N₂ converter, electricity and sealing depreciation is about 1.6 €/tonne, in comparison to 0.5-0.8 €/tonne by using phosphine by Siroflo/ECO₂FUME, 0.4-0.8 €/tonne by using contact insecticides, and 0.40 €/tonne by cooling using aeration. By increasing the annually treated quantity to 130,000 tonnes the treatment cost with N₂ MA technology is reduced to 0.80 €/tonne making it cost competitive with any other available treatment method (Table 7).

Table 5. Insect mortality under different durations of oxygen concentration below 0.9% in two bins treated with nitrogen generated by EcO₂ system and in a non treated bin

Bin	Total treatment duration incl. purge time** (d)	Grain*** (°C)	Air*** (°C)	Insect species in vials at different locations on grain surface in bin****	Insect life stage	Central zone, insect mortality % (± SD)	Median zone, insect mortality % (± SD)	Peripheral zone, insect mortality % (± SD)
B* and A	18.7 (B*) and 23,8 (A)	26 (B) and 22 (A)	16 (B) and 15 (A)	<i>T. confusum</i>	eggs	100 ± 0.0	100 ± 0.0	100 ± 0.0
				<i>T. confusum</i>	pupae	100 ± 0.0	100 ± 0.0	100 ± 0.0
				<i>T. confusum</i>	larvae	100 ± 0.0	100 ± 0.0	100 ± 0.0
				<i>T. confusum</i>	adults	100 ± 0.0	100 ± 0.0	100 ± 0.0
				<i>O.surinamensis</i>	larvae	100 ± 0.0	100 ± 0.0	100 ± 0.0
				<i>O.surinamensis</i>	adults	100 ± 0.0	100 ± 0.0	100 ± 0.0
				<i>S. granarius</i>	adults	100 ± 0.0	100 ± 0.0	100 ± 0.0
				<i>R. dominica</i>	adults	100 ± 0.0	100 ± 0.0	100 ± 0.0
				Control (non treated bin)	24 d, O ₂ =20.5%	17	15	<i>T. confusum</i>
<i>T. confusum</i>	pupae	9.4 ± 4.4	14.2 ± 4.9					11.9 ± 6.5
<i>T. confusum</i>	larvae	17.4 ± 6.7	13.8 ± 7.4					9.8 ± 4.5
<i>T. confusum</i>	adults	5.9 ± 3.1	2.9 ± 1.3					6.8 ± 2.1
<i>O.surinamensis</i>	larvae	24.2 ± 7.8	24.8 ± 10.1					13.2 ± 6.5
<i>O.surinamensis</i>	adults	19.3 ± 8.3	23.4 ± 4.3					15.4 ± 4.4
<i>S. granarius</i>	adults	7.9 ± 5.8	8.9 ± 4.5					13.3 ± 4.7
<i>R. dominica</i>	adults	5.7 ± 3.2	7.3 ± 4.3					3.4 ± 1.6

* sealing of this bin includes structural works + 3 gas tight valves

** 44-56 h were required to fill bins with N₂ to reach O₂<0.9%

*** Air and grain temperature is the average of measurements taken before and after completing treatment, at bin's centre, at 1 m above and on grain surface and at 0.5 m and 3 m below grain surface respectively; in the control (non treated bin) it is the average of measurements at 1 m above and 0.5 m below grain surface at bin's centre, taken before placing and after removing vials.

**** vials containing insects and feed were placed on grain surface

Table 6. Estimated expenses for 18-d treatment of 2,400 tonnes grain in sealed concrete bins using the EcO₂ system based on trial results, €/tonne

Cost parameter	Cost (€/t)	Share of the cost (%)
Rental, maintenance and operation of EcO ₂ Nitrogen generator and system	0.95	60
Electricity for the production of used Nitrogen by EcO ₂ generator and system	0.425	27
Depreciation of expenses for sealing a bin	0.16	10
Transfer of grain from one bin to a sealed bin for treatment with Nitrogen	0.06	3
Total expenses per treated tonne	1.6	100

Conditions/Assumptions: 18 d treatment including 2 d for purging with N₂; O₂<0.9%; electricity consumption by N₂ EcO₂ generator 0.35 kWh/ m³ N₂; a group of four sealed bins with N₂ MA system; sealing includes structural works and three gas tight valves; expected life of sealing 10 years; rental, cost for maintenance and use of EcO₂ generator and system 38,000 €/year; average electricity cost 0.22 €/kWh; 0.3 m³ N₂/tonne/day; 40,000 tonnes of grain treated using N₂ per year of which 20,000 tonnes will have to be transferred from another bin at a cost of 0.12 €/tonne.

Since the total annual sales of CGC is around 300,000 tonnes, the treatment of only 40,000 tonnes will contribute to prevent spread of infestation with a minor increase of 0.08% of total grain selling price by 0.21 €/tonne.

Another possibility is to use a smaller N₂ generator at a reduced rental cost. Although it might not be feasible to base all the treatments on a single technology, it is clear that increasing the amount of grain to be treated or reducing the rental cost, places the N₂ MA EcO₂ system in cost competition with all other analyzed treatment methods.

The main conclusions of this study are comparable with the conclusions of trials with liquid nitrogen MA in Newcastle grain terminal in Australia (Clamp and Moore, 2000). The N₂ MA technology proved to be cost competitive when applied to large quantities of grain and provided a full control of the tested various stages of stored grain insects, providing residue-free products, without environment chemical contamination, with contribution to improve occupational hazard, without the risk of insect resistance and full control and automation of the treatment operation. On the basis of these trials the N₂ MA technology was evaluated as a successful alternative to phosphine Siroflo/ECO₂FUME and contact insecticides for the control of stored grain insects in large concrete silos. On this basis the Cyprus Grain Commission is planning to replace the Siroflo/ECO₂FUME fumigation system by implementing the N₂ MA technology in four concrete silo sealed bins.

Table 7. Cost comparison of four stored grain protection methods, €/tonne

Calculations based on quantity of annually treated grain	Nitrogen MA using a PSA EcO ₂ system in concrete sealed silos*	Siroflo/ Eco2fume**	Contact insecticides***	Aeration****
40,000 tonnes/year	1.60	0.50-0.80	0.40-0.76-0.80	0.40
130,000 tonnes/year	0.80	0.50-0.80	0.40-0.76-0.80	0.40

* Cost includes 18 days treatment with O₂<0.9% using a rental EcO₂ nitrogen converter and operation system, four bins sealing cost depreciation (structural works and 3 gas tight valves), electricity for producing N₂ and partial transfer of grain.

** Dose 65-100 ppm PH₃ for 15 days. No grain transfer is needed.

*** The lower cost for 5 ppm Actellic EC 50; the middle cost for 0.5 ppm Spinosad Tracer 48 EC; the higher cost for 0.25 ppm K-Obiol ULV 6. Cost for transfer of grain from one bin to another 0.12 €/tonne is included.

**** 100 h aeration, 30 kW fan. No grain transfer is needed.

CONCLUSIONS

Each grain protection technology has its own strong and weak points. In comparison to Siroflo/ECO₂FUME, contact insecticides and aeration, the N₂ MA technology using a rental N₂ converter, when applied to limited quantity of grain, has the disadvantage being the most costly. By increasing the amount of treated grain makes the technology cost competitive with other conventional methods. Use of phosphine and contact insecticides face the problem of worldwide increasing insect resistance and the need for higher doses leading to higher expenses per tonne. The MA technology by using N₂ is a very effective and residue-free protection method, friendly to environment, contributes to improve occupational safety, suitable to organic commodities and without the potential insect resistance. These are important advantages that comply with increasing demand of the market and global environment protection and meet the expectations of a modern society.

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