Donahaye, E.J., Navarro, S. and Varnava, A. [Eds.] (1997) Proc. Int. Conf. Controlled Atmosphere and Fumigation in Stored Products, 21–26 April 1996, Printco Ltd., Nicosia, Cyprus, pp. 73–81

THE EFFECT OF MODIFIED ATMOSPHERES ON THE JUVENILE STAGES OF SIX GRAIN BEETLES

S.T. CONYERS AND C.H. BELL
Central Science Laboratory, Ministry of Agriculture, Fisheries and Food, London Road, Slough, Berks, SL3 7HJ, UK

ABSTRACT

The adult emergence of six species of grain beetle, *Cryptolestes ferrugineus*, *Oryzaephilus surinamensis*, *Rhyzopertha dominica*, *Sitophilus granarius*, *S. oryzae* and *Tribolium castaneum*, was assessed after the exposure of the juvenile stages to three different modified atmospheres (MA's) at 15°C and 70% r.h. The use of this low temperature was important as it is typical of the conditions prevalent in grain stores at the start of the UK storage season.

A range of juvenile stages was exposed to three MA's, one based on nitrogen (N_2), one on simulated burner gas and one on carbon dioxide (CO_2), with 0.5% oxygen. The three internal grain feeders, *R. dominica*, *S. granarius* and *S. oryzae*, were capable of emerging even after 30 d exposure to CO_2, the most effective of the MA's. N_2 was the least effective of the MA's. *S. granarius,* the most tolerant species, required a 60-d exposure for complete control of all its stages. Of the other species tested, *T. castaneum* was the most tolerant; its pupae required 9 or 10 d exposure to prevent emergence in all three atmospheres.

INTRODUCTION

Modified atmospheres (MA's) provide an alternative method, particularly with pesticide admixture, for insect control in stored grain. The technology involves the alteration of the ratio of atmospheric gases, nitrogen (N_2), oxygen (O_2) and carbon dioxide (CO_2), to produce conditions lethal to pests in stores (Banks and Fields, 1995). MA's are now used in both agriculture and food industries in order to disinfest and protect raw materials from the harvest through to the packaging of finished products (Bell and Armitage, 1992). Easily combined with the present strategies of drying and cooling, MA's could play an important role in integrated systems for the protection of grain, particularly where residue-free *in situ* treatments are desired (Banks *et al.*, 1991). Sophisticated markets expect pesticide-free foods, and with the development of resistance by target pests the position of insecticides as the main tool of pest control is becoming ever more problematical (Banks and Fields, 1995). Legislative restrictions also make other alternative treatments, such as fumigation, much more expensive (Banks *et al.*, 1991). Adoption of a N_2-based MA treatment at the main grain export terminal in Australia was motivated by such problems (Banks, 1994).

The use of MA's does not adversely affect the quality of the stored grain. The use of N_2 for continuous storage for up to a year did not have a detrimental effect on the germination or end-use properties of wheat, rice or barley (Ouye, 1984). Germination potential, so important for the maltsters, is much more likely to be lost because of such climatic conditions of storage as high temperature than because of MA storage (Fleurat-Lessard *et al.*, 1994). MA's may also be advantageous as they can extend the storage life of grain at levels of moisture which are normally considered marginal for safe storage and do not allow fungi to multiply at lower water activities (Banks *et al.*, 1991).

Various methods are available for generating MA's from gas or liquid sources (Banks *et al.*, 1980; Annis, 1990; Bell *et al.*, 1993a). On-site generation of gas using a separating mechanism which removes O_2 from air, leaving pure N_2, has an obvious advantage over bulk liquid supplies of CO_2 or N_2. An alternative system produces an O_2-deficient atmosphere by burning propane in air (Storey, 1973; Fleurat-Lessard and Le Torc'h, 1987; Bell *et al.*, 1993b). However, in order to evaluate the suitability of each system, it is important to test the efficacy of the MA's in the laboratory. Many such tests have been carried out (Annis, 1987; Reichmuth, 1987; Jayas *et al.*, 1991); however, the results do not cover all the environmental conditions required.

The current work was carried out in order to assess the relative efficacy of different MA's on various stored-grain pests. There were two ways to reduce the time which would have been needed to cover each stage separately: using the most tolerant juvenile stage and using a mixed culture with a known age range, as described by Annis and Dowsett (1993). The juvenile stages of *Cryptolestes ferrugineus* (Stephens) (rust-red grain beetle), *Oryzaephilus surinamensis* (L.) (saw-toothed grain beetle), *Rhyzopertha dominica* (F.) (lesser grain borer), *S. granarius* (granary weevil), *S. oryzae* (L.) (rice weevil) and *Tribolium castaneum* (Herbst) (rust-red flour beetle) were treated with three MA's: CO_2, simulated burner gas and N_2, at the temperature usual at the beginning of the UK grain storage season.

MATERIALS AND METHODS

Insect rearing

The six beetle species were obtained from stock cultures set up by placing 100 adults of mixed ages in a glass culturing jar (diameter: 7.5 cm, height: 14 cm) about one-third full of food. *R. dominica*, *S. granarius* and *S. oryzae* were raised on whole wheat, *C. ferrugineus* on a mixture of rolled oats, wholemeal flour and 5% brewer's yeast, *O. surinamensis* on rolled oats and *T. castaneum* on a mixture of wholemeal wheat flour and 5% brewer's yeast. The stock cultures, excepting *C. ferrugineus* which was reared at 30°C, were maintained at 25°C and 70% r.h. Two different strains of each species were used to determine if there were any differences in their tolerance of the MA's. Standard laboratory strains and malathion-resistant strains of *R. dominica*, *S. granarius* and *S. oryzae*, and a phosphine-resistant strain of each of the other species, were used.

For *R. dominica*, *S. granarius* and *S. oryzae,* where juveniles develop within the wheat grains, the cultures were divided among the containers used for the exposures. For the other species, either 50 pupae or 50 late fourth instar larvae were counted into each container. These containers were one-third full of fresh culture medium. Three containers were used for each exposure period and another six (three kept in the same room as the exposure apparatus and three remaining in the rearing conditions) were used as controls. The exposures took place in glass tubes (diameter: 2.5 cm, height: 7.5 cm) sealed with squares of nylon mesh held in place by sections of rubber pipe which were inserted in the openings of the tubes.

All the insects, including the controls, were lowered to the exposure temperature of 15°C in daily 5°C stages (so that they could acclimatise) while humidity was held constant at 70% r.h. At the completion of the longest exposure period, they were again raised to their rearing temperatures, the duration and temperature stages being parallel. Then they were checked regularly for adult emergence. The results were used to calculate the mean emergence for each exposure time as a percentage of the emergence from the control samples for *S. granarius*, *S. oryzae* and *R. dominica.* For the other species correction for other sources of mortality was made from the controls. The percentage emergence of each treated sample was calculated as a proportion of the emergence of the controls.

MA's

The three gas mixtures (0.5% O_2 and 99.5% N_2; 0.5% O_2, 10% CO_2 and 89.5% N_2 — burner gas; and 0.5% O_2 and 99.5% CO_2) were produced using a three-channel gas blender (Signal Instrument Co. Ltd., Camberley, Surrey) supplied with high purity gases from cylinders. After the gases were mixed to the required proportions, the gas stream was split into eight, each gas flow being limited to 100 ml/min by means of flow control valves. The eight streams were then humidified to 70% by passing the gas over solutions of potassium chloride (Winston and Bates, 1960). The humidified gas stream then passed via a tube into the bottom of the exposure chamber, a 5-L desiccator. The exposure containers were then placed on a layer of wire mesh supported above the bottom of the desiccator.

A vent in the top effected a flow-through system. This set-up ensured a constant gas mixture, with no change through leakage or respiration, within the exposure chamber. The apparatus was kept in a controlled-environment room at 15°C and 70% r.h. O_2, CO_2 and humidity levels were monitored every day, using a Model 570A paramagnetic O_2 analyser (Servomex Ltd., Crowborough, Sussex), a Model PA 404 infra-red CO_2 analyser (Servomex Ltd.) and a Protimeter DP680 (Protimeter Ltd., Marlow, Bucks.), respectively, and were adjusted if required.

At the end of the longest exposure period for each species the exposure containers and the controls were moved back to their culturing temperatures by reversing the steps used to acclimatise the insects before exposure. The containers were then checked once a week for any adult emergence. This procedure was continued until all possible emergence had taken place. A comparison was then made with the mean exposure results for each time period for the experimental insects and for the controls. Thus, the level of mortality produced by the MA's was determined.

RESULTS

Nitrogen

From previous experience it was expected that *S. granarius* would be the species most tolerant to this mixture. A 55-d exposure period killed most of the juveniles, indicating that there was no difference in strain tolerance. A 60-d exposure period was required to kill all stages (Table 1).

There was little difference in mortality between the strains of *S. oryzae*. An exposure period much longer than any tested would be required for complete control; this was also true for *R. dominica*, the cultures of which were at a younger developmental stage. It appears that a complete kill of the more tolerant pupal stage of this beetle would require a longer exposure than that required for *S. oryzae*.

In this test the resistant strain (Res) of *R. dominica* appeared more tolerant of the atmosphere than the laboratory strain (Lab). This may be notable only because the longest exposure times used gave survivals of approximately 50%.

The remaining three species, and particularly *T. castaneum* Lab, had poor emergence due to the low temperature in some of the control replicates. These results call into

TABLE 1
A comparison of the mean percentage emergence of resistant (Res) and laboratory (Lab) strains of six species of grain beetle after varying lengths of juvenile exposure to 0.5% O_2 and 99.5% N_2 at 15°C and 70% r.h.

Species	*S. granarius*		*S. oryzae*		*R. dominica*		*T. castaneum*		*C. ferru-gineus*		*O. surina-mensis*	
Strain	Lab	Res	Lab	Res	Lab	Res	Lab	Res	Lab	Res	Lab	Res
Age (wks)	0–5	0–5	0–3	0–3	0–3	0–2	5–6	5–6	3–4	3–4	4–5	4–5
Time (d)												
3									92	52	6	9
5									52	42	3	0
6							56	28				
8							17	28				
9							0	0				
38			39	32	63	70						
44			34	34	49	66						
55	0	2										
60	0	0										

question the accuracy of the emergence results from the exposed juveniles. There was little difference between the strains of *T. castaneum*, which was more tolerant than the remaining two species. *O. surinamensis* was the least tolerant of the three species; only a short extension of the 5-d exposure period would be required for complete control, whereas a period closer to the longest exposure of 9 d used for *T. castaneum* would be needed for *C. ferrugineus*.

Burner gas

There was little difference between the strains of *S. granarius* in the tolerance to burner gas (Table 2). Complete control was achieved in 65 d, which was not significantly longer than the period required using the N_2-based atmosphere. For *S. oryzae* and *R. dominica* the results were better than those achieved with N_2, and a far higher level of control was achieved in a shorter time period. This occurred even though the *R. dominica* cultures were much older than those used for the N_2 test cultures and contained pupae, which are more tolerant of these atmospheres than are larvae. There was some evidence that the Res strain of *S. oryzae* was more tolerant than the Lab strain.

Results of the burner gas and N_2-based atmospheres were generally similar for *T. castaneum* and *O. surinamensis*.

TABLE 2
A comparison of the mean percentage emergence of six species of grain beetle after varying lengths of juvenile exposure to 0.5% O_2, 10% CO_2 and 89.5% N_2 at 15°C and 70% r.h.

Species	*S. granarius*		*S. oryzae*		*R. dominica*		*T. castaneum*		*C. ferrugineus*		*O. surinamensis*	
Strain	Lab	Res	Lab	Res	Lab	Res	Lab	Res	Lab	Res	Lab	Res
Age (wks)	3–4	3–4	0–3	0–3	7–9	0–9	5–6	5–6	3–4	3–4	4–5	4–5
Time (d)												
4												11
5											0	0
9							0	0	0	0		
28			3	19	6	3						
36			3	11	4	1						
55	2	0										
65	0	0										

Carbon dioxide

For *S. granarius* 38 d was required for complete mortality (Table 3). This is a much shorter time than that required for both N_2 and burner gas. A similar pattern was seen with *S. oryzae* and *R. dominica* although the difference was much less in comparison with the results obtained from burner gas. However, the *R. dominica* stages used for the burner gas

TABLE 3
A comparison of the mean percentage emergence of six species of grain beetle after varying lengths of juvenile exposure to 0.5% O_2 and 99.5% CO_2 at 15°C and 70% r.h.

Species	*S. granarius*		*S. oryzae*		*R. dominica*		*T. castaneum*		*C. ferru-gineus*		*O. surina-mensis*	
Strain	Lab	Res	Lab	Res	Lab	Res	Lab	Res	Lab	Res	Lab	Res
Age (wks)	0–5	0–5	0–3	0–3	0–3	0–2	5–6	5–6	3–4	3–4	4–5	4–5
Time (d)												
3											25	31
5									33	82	3	0
6							22	28				
8							0	3				
10							0	0				
28			1	1	0	1						
32			2	1	0	0						
34	0	1										
38	0	0										

MA test were much older than those used for the CO_2 test and the time required to control the tolerant pupal stage with CO_2 was sometimes much longer than the 32 d needed to kill the younger stages (Table 3).

With the other three species there was little variance from the previous results. A strain difference was evident for *C. ferrugineus* where Res was the more tolerant. An extension of the range of exposure times tested to achieve complete control of this species is required. Such additional tests are needed for both the CO_2 and the N_2-based atmospheres.

DISCUSSION

For MA treatments to become established, it is essential that they effect complete control of any insects present, whatever their stage. In general, results have indicated that CO_2 atmospheres are more toxic than O_2-deficient ones (Navarro and Donahaye, 1990). This is because CO_2 relies not on anoxia only to be lethal but also on acidification of body fluids and inhibition of glycolysis (Adler, 1994). For *S. granarius*, one of the most tolerant insects, 60 d were required to kill the most tolerant pupal stage with N_2 but only 38 d were required with CO_2. Reichmuth (1990) achieved an LT_{95} with 99% N_2 in 45 d and a similar result with 90% CO_2 in only 35 d. Adler (1994) gave 50 d for 97–100% N_2 and these differences demonstrate the importance of monitoring tolerances of pest populations in order to determine the correct time period needed for control. Differences in tolerance to MA's were demonstrated by Adler (1991), working with many strains of the same species.

The juvenile stages of beetles showed a marked difference between the internal grain feeders, *R. dominica*, *S. granarius* and *S. oryzae*, and the other three, the external feeders, in their tolerance of all MA's, the latter being much more susceptible. Ten days was the maximum exposure required for the external feeders for all three MA's, whereas *R. dominica* and *S. oryzae* required 32 d exposure with CO_2, at least 36 d with burner gas, and more than 44 d with N_2. The tolerance of MA's occurs when consumption of O_2 is lowest, often during the egg and pupal stages (Reichmuth, 1987). Clearly, these insects must be controlled before their development has reached the most tolerant stage. However, there are differences in response among other strains of these species (Jay, 1984); Jay used a slightly higher temperature of 16°C and a lower 55–60% r.h. that produced 100% mortality with 60% CO_2, and 91% and 86% mortality with 99% N_2, with *R. dominica* and *S. oryzae*, respectively, after exposures of only 2 weeks.

Similar differences were seen among the external feeders. White and Jayas (1993), using mixed populations of *C. ferrugineus* and *T. castaneum*, achieved control in 14 d with 34% CO_2 and the temperature decreasing from 18 to 10°C. A 100% CO_2 atmosphere controlled *T. castaneum* pupae in 14 d at 15.6°C and 38% r.h. (Aliniazee, 1971), and 100% N_2 controlled the same stage under the same conditions in only 5 d (Aliniazee, 1972). This species was also studied in one of the few published investigations using burner gas. Storey (1977) used a mixture of <1% O_2 and 9–9.5% CO_2 in N_2 at 18°C, effecting an LT_{95} of 4.5 d for pupae. The last species, *O. surinamensis,* was the least tolerant of all; it succumbed within 5 d to all MA's. Jay (1984) used 98% CO_2 at 16°C and 50% r.h., effecting 100% mortality in 3 d for all stages. Any differences in the environmental conditions would have significantly influenced the results in all these comparisons.

In conclusion, in this brief survey CO_2 has proven to be the most effective MA, followed by burner gas and N_2. Pupae of *Sitophilus* spp. showed the highest tolerance to these atmospheres, followed by the other internal grain feeder *R. dominica.* The other three species, the external grain feeders, were much less tolerant.

ACKNOWLEDGEMENTS

This work was funded by the Home-Grown Cereals Authority, London, UK.

REFERENCES

Adler, C.S. (1991) Efficacy of controlled atmospheres on ten strains of the granary weevil *Sitophilus granarius* (L.) from different places of origin. In: *Proc. 5th Int. Working Conf. on Stored-Product Protection* (Edited by Fleurat-Lessard, F. and Ducom, P.), Bordeaux, France, 9–14 September 1990, 727–735.

Adler, C.S. (1994) A comparison of the efficacy of CO_2-rich and N_2-rich atmospheres against the granary weevil *Sitophilus granarius* (L.) (Coleoptera: Curculionidae). In: *Proc. 6th Int. Working Conf. on Stored-Product Protection* (Edited by Highley, E., Wright, E.J., Banks, H.J. and Champ, B.R.), Canberra, Australia, 17–23 April 1994, CAB International, Wallingford, Oxon, UK.

Aliniazee, M.T. (1971) The effect of carbon dioxide gas alone or in combination on the mortality of *Tribolium castaneum* (Herbst) and *T. confusum* du Val (Coleoptera, Tenebrionidae). *J. Stored Prod. Res.* **7,** 243–252.

Aliniazee, M.T. (1972) Susceptibility of the confused and red flour beetles to anoxia produced by helium and nitrogen at various temperatures. *J. Econ. Entomol.* **65,** 60–64.

Annis, P.C. (1987) Towards rational controlled atmosphere dosage schedules: a review of current knowledge. In: *Proc. 4th Int. Working Conf. on Stored-Product Protection* (Edited by Donahaye, E. and Navarro, S.), Tel Aviv, Israel, 21–26 September 1986, Maor-Wallach Press, Jerusalem, 208–217.

Annis, P.C. (1990) Requirements for fumigation and controlled atmospheres as options for pest and quality control in stored grain. In: *Fumigation and Controlled Atmosphere Storage of Grain: Proc. Int. Conf.* (Edited by Champ, B.R. *et al.*), Singapore, ACIAR Proc. No. 25, 83–91.

Annis, P.C. and Dowsett, H.A. (1993) Low oxygen disinfestation of grain: exposure periods needed for high mortality. In: *Proc. Int. Conf. on Controlled Atmospheres and Fumigation in Grain Storages* (Edited by Navarro, S. and Donahaye, E.), Winnipeg, Canada, 11–13 June 1992, Caspit Press Ltd., Jerusalem, 71–83.

Banks, H.J. (1994) Fumigation — an endangered technology? In: *Proc. 6th Int. Working Conf. on Stored-Product Protection* (Edited by Highley, E., Wright, E.J., Banks, H.J. and Champ, B.R.), Canberra, Australia, 17–23 April 1994, CAB International, Wallingford, Oxon, UK, 2–6.

Banks, H.J. and Fields, P. (1995) Physical methods for insect control in stored ecosystems. In: *Stored-Grain Ecosystems* (Edited by Jayas, D.S. *et al.*). Marcel Dekker Inc., New York, USA, 353–409.

Banks, H.J., Annis, P.C., Henning, R.C. and Wilson, A.D. (1980) Experimental and commercial modified atmosphere treatments of stored grain in Australia. In: *Controlled Atmosphere Storage of Grains* (Edited by Shejbal, J.). Elsevier Sci. Publ. Co., Amsterdam, Holland, 207–224.

Banks, H.J., Annis, P.C. and Rigby, G.R. (1991) Controlled atmosphere storage of grain: the known and the future. In: *Proc. 5th Int. Working Conf. on Stored-Product Protection* (Edited by Fleurat-Lessard, F. and Ducom, P.), Bordeaux, France, 9–14 September 1990, 695–706.

Bell, C.H. and Armitage, D.M. (1992) Alternative storage practices. In: *Storage of Cereal Grains and their Products* (Edited by Sauer, D.B.). American Association of Cereal Chemists Inc., St. Paul, USA, 249–311.

Bell, C.H., Chakrabarti, B., Llewellin, B.E. and Wontner-Smith, T.J. (1993a) A comparison of carbon dioxide and burner gas as replacement atmospheres for control of grain pests in a welded steel silo. *Postharvest Biol. Technol.* **2,** 241–253.

Bell, C.H., Chakrabarti, B., Conyers, S.T., Wontner-Smith, T.J. and Llewellin, B.E. (1993b) Flow rates of controlled atmospheres required for maintenance of gas levels in bolted metal farm bins. In: *Proc. Int. Conf. on Controlled Atmospheres and Fumigation in Grain Storages* (Edited by Navarro, S. and Donahaye, E.), Winnipeg, Canada, 11–13 June 1992, Caspit Press Ltd., Jerusalem, 315–326.

Fleurat-Lessard, F. and Le Torc'h, J.-M. (1987) Practical approach to purging grain with low oxygen atmosphere for disinfestation of large wheat bins against granary weevil. In: *Proc. 4th Int. Working Conf. on Stored-Product Protection* (Edited by Donahaye, E. and Navarro, S.), Tel Aviv, Israel, 21–26 September 1986, Maor-Wallach Press, Jerusalem, 208–217.

Fleurat-Lessard, F., Just, D., Barrieu, P., Le Torc'h, J.-M., Raymond, P. and Saglio, P. (1994) Effect of modified atmosphere storage on wheat seed germination vigour and on physiological criteria of the ageing process. In: *Proc. 6th Int. Working Conf. on Stored-Product Protection* (Edited by Highley, E., Wright, E.J., Banks, H.J. and Champ, B.R.), Canberra, Australia, 17–23 April 1994, CAB International, Wallingford, Oxon, UK, 695–700.

Jay, E.G. (1984) Imperfections in our current knowledge of insect biology as related to their response to controlled atmospheres. In: *Controlled Atmosphere and Fumigation in Grain Storages* (Edited by Ripp, B.E. *et al.*). Elsevier Sci. Publ. Co., Amsterdam, Holland, 493–508.

Jayas, D.S., Khangura, B. and White, N.D.G. (1991) Controlled atmosphere storage of grains. *Postharvest News Inf.* **2**, 423–427.

Navarro, S. and Donahaye, E. (1990) Generation and application of modified atmospheres and fumigants for the control of storage insects. In: *Fumigation and Controlled Atmosphere Storage of Grain: Proc. Int. Conf.*, Singapore (Edited by Champ, B.R. *et al.*), ACIAR Proc. No. 25, 152–165.

Ouye, M.T. (1984) An overview of post-harvest insect research performed by USDA, ARS laboratories. In: *Insect Management for Food Storage and Processing* (Edited by Baur, F.J.). American Association of Cereal Chemists Inc., St. Paul, USA, 203–224.

Reichmuth, C. (1987) Low oxygen content to control stored-product insects. In: *Proc. 4th Int. Working Conf. on Stored-Product Protection* (Edited by Donahaye, E. and Navarro, S.), Tel Aviv, Israel, 21–26 September 1986, Maor-Wallach Press, Jerusalem, 194–207.

Reichmuth, C. (1990) Toxic gas treatment responses of insect pests of stored products and impact on the environment. In: *Fumigation and Controlled Atmosphere Storage of Grain: Proc. Int. Conf., Singapore* (Edited by Champ, B.R. *et al.*), ACIAR Proc. No. 25, 56–69.

Storey, C.L. (1973) Exothermic inert-atmosphere generators for control of insects in stored wheat. *J. Econ. Entomol.* **66,** 511–514.

Storey, C.L. (1977) Effect of low oxygen atmospheres on mortality of red and confused flour beetles. *J. Econ. Entomol.* **70,** 253–255.

Winston, P.W. and Bates, D.H. (1960) Saturated solutions for control of humidity in biological research. *Ecology* **41,** 232–237.

White, N.D.G. and Jayas, D.S. (1993) Effectiveness of carbon dioxide in compressed gas or solid formulation for control of insects and mites in stored wheat and barley. *Phytoprotection* **74,** 101–111.