EFFECTS OF PERIODICALLY ELEVATED CARBON DIOXIDE ON STORED-WHEAT ECOSYSTEMS AT COOL TEMPERATURES

N. D. G. WHITE AND D. S. JAYAS[1]

Agriculture Canada Research Station, Winnipeg, Manitoba R3T 2M9, Canada

Abstract

Carbon dioxide concentration above 15%, with occasional surges up to 50% in 322-kg bulks of wheat stored at 15 to 12°C, controlled insect and mite populations in non-airtight bins in 42 days. Unsealed control bins, sealed but untreated bins, and bins with 6-day or 14-day half-lives for carbon dioxide loss were purged with compressed carbon dioxide at days 0, 7, 14, and 28. The purges for the two treatments, replicated three times, resulted in carbon dioxide levels of: 15, 40, 40, 50% or 19, 40, 45, 50%, respectively, in bottom samples. Oxygen levels did not fall below 11%. Populations of the insects *Cryptolestes ferrugineus* (Stephens), *Tribolium castaneum* (Herbst) and *Ahasverus advena* (Waltl) and the mites *Tarsonemus granarius* Lindquist, *Paratriophtydeus coineaui* André, *Lepidoglyphus destructor* (Schrank), and *Aeroglyphus robustus* (Banks) were sharply reduced in all seven sampling locations per bin by 28 days in carbon dioxide treatments. A few *A. robustus* survived for 84 days in bins with the most rapid carbon dioxide loss. Seed germination and fungal infection by *Aspergillus glaucus* group and *Penicillium* spp. were not directly affected by the carbon dioxide in 84 days, but germination was occasionally lower in control bins because of insect feeding.

Introduction

Modified atmospheres having elevated carbon dioxide (>60%) or nitrogen, and depleted oxygen (<1%) (Bailey and Banks 1980) are effective for controlling insects and mites in stored grain (Annis 1986; Navarro *et al.* 1985). Carbon dioxide is more effective than nitrogen at lower concentrations (Bell *et al.* 1980) largely because of desiccation due to opening of spiracles and a direct physiological action rather than simple anoxia (Nicolas and Sillans 1989). Atmospheres containing 10 to 30% carbon dioxide, 0.5 to 2.6% oxygen, and a balance of nitrogen, produced by the combustion of hydrocarbon fuels, are also effective in controlling stored-product insects (Storey 1980).

A major limitation to using carbon dioxide on farms is that most storage bins are not air-tight, therefore maintaining high carbon dioxide levels (60-95%) for days (Keever 1989; Annis 1986) or lower levels (>20%) for weeks (White *et al.* 1990) becomes difficult. Attempts can be made to seal storage structures to a suitable level (Williams *et al.* 1980) to minimize gas loss caused by diffusion, wind, convection currents, and changing barometric pressure (Banks and Annis 1980, 1983).

Numerous species of insects and mites typically occur together in farm-stored grain in western Canada (Madrid *et al.* 1990; White and Sinha 1990) and responses to modified

[1]*Department of Agricultural Engineering, University of Manitoba, Winnipeg, Manitoba, Canada R3T 2N2.*

atmospheres vary considerably among species (Krishnamurthy *et al.* 1986). Within several months of harvest, grain temperatures on farms rarely exceed 20°C in the centre of 5-m-diam bins (Sinha and Wallace 1977) unless active spoilage is occurring, and the low temperatures slow the effectiveness of toxic gases. Our study was done to evaluate the effects of four purges of carbon dioxide at ≤50% by volume, into experimental bins with 6- or 14-day half-lives for carbon dioxide loss, on control of insects and mites in stored wheat, and the effects on seed germination and microfloral infection under cool (<15°C) conditions.

Materials and Methods

Storage Bins. Twelve cylindrical steel bins (444 litres, 168 by 58 cm) were constructed by welding two steel oil drums together end-to-end. A removable lid was attached to the top of the bin with a circular band of steel and a bolt tightener. The bins were placed on their ends and supported on top of 20-cm-high concrete blocks. There were six equispaced grain-sampling and gas-sampling ports made in a spiral pattern along the sides of the bins with one additional sampling port at the bottom centre (White *et al.* 1990).

Grain Storage. About 3,864 kg of Canadian western red spring wheat (*Triticum aestivum* L., cv. Katepwa) containing about 0.8% by weight dockage (chaff, broken kernels) was obtained at 14.8% moisture content. Each bin was filled with 322 kg of wheat (431 litres) to a level 5 cm below the top.

The bins containing wheat were kept in a room with modified temperatures. Relative humidity in the room varied from 40-65%.

Initially, 1,110 adult *C. ferrugineus*, (1:1 sex ratio) were added to the wheat at the top of each bin. The insects were taken from laboratory cultures on whole wheat and wheat germ (19:1, wt/wt) maintained at 30±1°C and 70±5% RH. Once insects were added, the top lid of each bin was sealed at the periphery and coated with silicone caulking compound. Open holes (6 cm diameter) near the side in the lid were covered with fine screens to permit ventilation. The grain contained natural low level infestations of the red flour beetle, *Tribolium castaneum* (Herbst) and the foreign grain beetle, *Ahasverus advena* (Waltl), and the mites *Tarsonemus granarius* Lindquist (Tarsonemidae), *Paratriophtydeus coineaui* André (Tydeidae), *Lepidoglyphus destructor* (Schrank) (Glycyphagidae), and *Aeroglyphus robustus* (Banks) (Glycyphagidae). The insects and mites were allowed to multiply and develop at 30±2°C for 5 wk.

Addition of Carbon Dioxide. Carbon dioxide from a compressed-gas cylinder was introduced at a rate of 40 L/min through the bottom gas sampling port. In each experiment, three replicate bins did not receive carbon dioxide and had an open hole covered with screen in the top (controls).

Carbon dioxide was added to six treatment bins on days 0, 7, 14, and 28. Three of the bins had a CO_2 half-life of 6 days and three had a CO_2 half-life of 14 days. Another three bins were tightly sealed by adding a screw-on cap to the hole in the top and caulking with silicone, but no carbon dioxide was added. All treatment bins were sealed by closing the top ventilation hole with a screw-on cap and sealing with silicone.

Sampling Procedure. Before gas was initially added, measurements of carbon dioxide and oxygen concentrations, temperatures, grain moisture and germination, incidence of seed-borne microflora, and insect and mite numbers were made at each sample location (White *et al.* 1990). Thereafter, sampling for carbon dioxide, oxygen and temperatures was

done every 7 days for 84 days. Moisture and insect and mite counts were done every 14 days, and seed germination and seed-borne microfloral counts were done on days 0 and 84.

Data Analysis. All data were analyzed with Statistical Analysis Systems programs (SAS Institute 1985). Data were transformed using $\log_{10}X + 1$ (insect and mite numbers), $\sqrt{x + 1}$ (CO_2, O_2, moisture), or arcsin $\sqrt{X/100}$ (germination, *A. glaucus* group, *Penicillium*). Analysis of variance (ANOVA) was done on transformed pooled data from all seven sampling dates.

Results and Discussion

Temperatures in the bins were held relatively constant during and after the 5-week incubation period at 30±2°C. Once the experiment began, temperatures were 15±2°C at the tops of bins declining to 12±2°C at the bottoms. During the experiment, at cool temperatures, grain moisture content remained constant at 13.0±0.2% at the tops of the bins and 16.0±0.2% at the bottoms.

After the initial incubation period and prior to sealing the bins, there were considerable numbers of *C. ferrugineus, T. castaneum*, and *A. advena* adults and larvae; and *T. granarius, P. coineaui, L. destructor,* and *A. robustus* per 100 ml of wheat at most sampling locations in all 12 bins (Table 1). Mean seed germination ranged from 83 to 96% in top samples and 79 to 91% in bottom samples. Mean *A. glaucus* group infection ranged from 57 to 71% of seeds in top samples and 52 to 59% in bottom samples. Mean *Penicillium* infection ranged from 0 to 5% in top samples and 0 to 3% in bottom samples.

Mean carbon dioxide and oxygen levels in the grain are given for the top and bottom locations based on three bins for each treatment (Fig. 1). The trends in the graphs are representative for all sampling locations in each treatment. Initially, carbon dioxide levels were about 2% and oxygen levels were near 20%. The initial levels of carbon dioxide were caused by both microfloral (White *et al.* 1982) and insect respiration and reflect levels found in infested farm granaries (Sinha *et al.* 1986).

In the unsealed, control bins, *C. ferrugineus, A. advena, T. granarius, P. coineaui,* and *L. destructor* were more common in bottom than top grain samples, while *T. castaneum* and *A. robustus* were more common in top samples (Table 1). *A. advena*, and *T. granarius* decreased appreciably during 84 days in the control bins. Arthropods in the sealed but untreated bins followed similar distribution patterns and numbers per sample, although there were fewer *T. castaneum*.

There was little survival of arthropod populations in bins with the 6-day half-life for carbon dioxide following the third purge of gas. By day 84 only a mean of one *C. ferrugineus* adult was found and 19 *A. robustus*.

In the bins with the 14-day half-life for carbon dioxide, virtually all arthropods were killed after the final gas purge on day 28. Prior to day 28, considerable numbers of *C. ferrugineus* adults had survived in bottom samples (Table 1).

During the 84 days of the study, seed germination did not decrease in top samples from any treatment but did decrease to 71% in bottom samples in the control bins where *C. ferrugineus* were relatively abundant. *A. glaucus* group infection increased during the study in all treatments from 50-60% at day 0 to about 90% at day 84, while *Penicillium* infection was relatively unaffected by storage time and remained at low levels (< 5%).

Analysis of variance indicated that among days, all variables were significantly different except *Aeroglyphus* and germination. All variables were significantly different for

Table 1. Mean numbers[1] of insects and mites in 100 g wheat samples from tops (T, location 1) or bottoms (B, location 10) of bins[2], with or without carbon dioxide, holding 322 kg of wheat at ca. 15°C, 13% MC at the top or 12°C, 16%MC at the bottom.

Treatment	Storage period (days)									
	0		14		28		42		84	
	T	B	T	B	T	B	T	B	T	B
Control (unsealed bins)										
C.f.A.[3]	2	37	7	31	5	8	6	23	7	76
C.f.L.	3	4	6	3	3	8	2	8	1	15
T.c.A.	2	1	12	1	39	1	24	0	34	0
T.c.L.	1	1	1	0	3	1	1	0	1	0
A.a.A.	0	11	1	10	0	0	1	1	0	0
A.a.L.	1	14	0	12	1	3	0	2	0	0
T.g.	66	1404	13	146	5	64	6	44	0	8
P.c.	34	155	37	47	9	125	2	33	1	208
L.d.	0	29	0	47	1	538	0	694	0	155
A.r.	32	0	44	0	68	0	170	0	157	0
Sealed bins (no added CO_2)										
C.f.A.	10	7	6	19	8	20	11	10	4	73
C.f.L.	1	3	1	1	1	1	1	2	1	3
T.c.A.	1	1	9	1	20	0	29	0	6	0
T.c.L.	2	0	1	0	1	0	2	0	0	0
A.a.A.	1	28	0	6	0	1	0	0	0	0
A.a.L.	0	8	2	6	0	1	0	1	0	0
T.g.	41	1141	4	124	1	83	6	27	0	10
P.c.	39	686	5	70	1	46	5	39	1	34
L.d.	0	5	0	39	4	210	3	407	1	576
A.r.	61	14	23	0	47	1	70	0	56	0
6-day half-life										
C.f.A.	2	15	0	23	1	2	0	1	0	1
C.f.L.	2	3	2	1	1	0	1	0	0	0
T.c.A.	1	0	0	0	1	0	0	0	0	0
T.c.L.	0	0	1	0	0	0	0	0	0	0
A.a.A.	0	9	0	1	0	0	0	0	0	0
A.a.L.	0	5	1	1	0	0	0	1	0	0
T.g.	36	1700	0	4	0	1	0	0	0	0
P.c.	28	288	0	2	1	1	0	0	0	0
L.d.	3	23	1	1	0	1	0	0	0	0
A.r.	18	2	14	0	10	0	7	0	19	0
14-day half-life										
C.f.A.	3	5	2	30	0	13	0	0	0	0
C.f.L.	1	3	3	4	1	0	0	0	0	0
T.c.A.	1	1	2	1	1	0	0	0	0	0
T.c.L.	0	1	1	0	1	0	0	0	0	0
A.a.A.	1	14	0	1	0	0	0	0	0	0
A.a.L.	1	3	0	1	0	0	0	0	0	0
T.g.	96	2114	1	57	1	0	0	0	0	0
P.c.	12	59	0	4	1	1	0	0	0	0
L.d.	0	29	1	12	1	0	0	0	0	1
A.r.	15	0	18	0	1	0	0	0	0	0

[1] n = 3 bins.

[2] CO_2 introduced on days 0, 7, 14, 28.

[3] *C.f.*A. = *Cryptolestes ferrugineus* adults; *C.f.*L. = *C. ferrugineus* larvae; *T.c.*A. = *Tribolium castaneum* adults; *T.c.*L. = *T. castaneum* larvae; *A.a.*A. = *Ahasverus advena* adults; *A.a.*L. = *A. advena* larvae; *T.g.* = *Tarsonemus granarius*; *P.c.* = *Paratriophytdeus coineaui*; *L.d.* = *Lepidoglyphus destructor*; *A.r.* = *Aeroglyphus robustus*. 6-day half-life: 15/40/40/50% CO_2; 14-day half-life: 19/40/45/50% CO_2 at bin bottom.

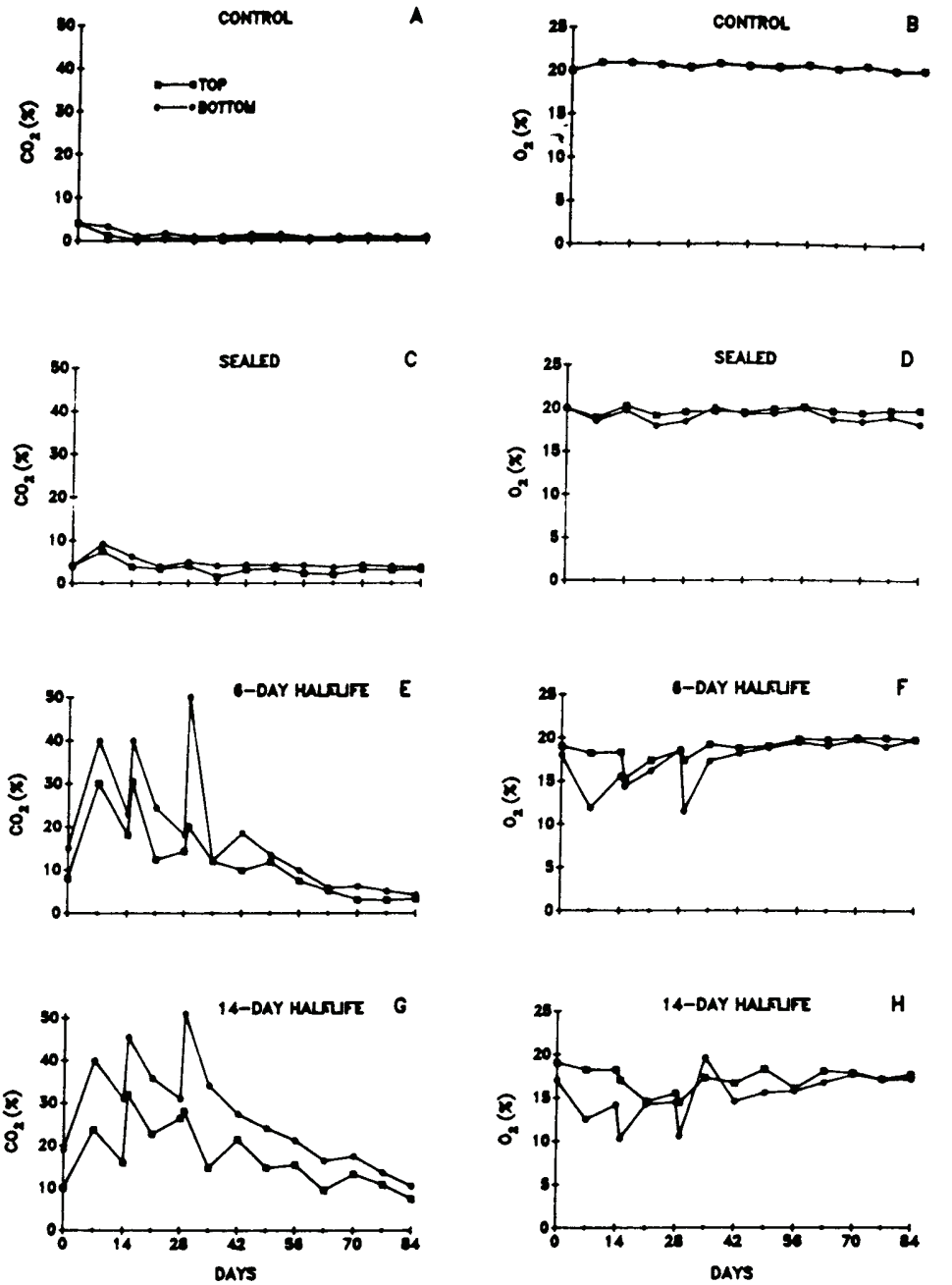

Fig. 1. Mean carbon dioxide and oxygen levels at top and bottom sampling locations in three replicate bins holding 322 kg of wheat, that were unsealed (controls), sealed, or had 6-day or 14-day half-lives for carbon dioxide levels.

treatments except germination and *A. glaucus* group infection. All variables were significantly different at different bin locations except *Penicillium*. The interaction of treatments and days had no significant effect on moisture, *Tribolium* larvae, *Aeroglyphus*, germination, and *A. glaucus* group infection.

Effectiveness of Carbon Dioxide. Carbon dioxide is less toxic to arthropods at temperatures below 20°C than at warmer temperatures (White *et al.* 1988). Nonetheless, long-term exposure (12 weeks) to carbon dioxide levels declining from 20 to 9% can control *C. ferrugineus* populations at temperatures declining from 21 to 7°C (White *et al.* 1990). The present experiment indicates that all of the arthropods studied could be killed in about half that time if surges in carbon dioxide between 30 and 50% occur and levels of this gas remain above 15%, even if oxygen levels remain above 11%.

It is impractical to make most farm granaries air-tight, but if efforts are made to reduce gas loss, such as by placing plastic sheets over the grain-bulk surface (McGaughey and Akins 1989) and by limited caulking of bin walls, it should be possible to control arthropod infestations with prolonged exposure to relatively low carbon dioxide levels. This could be especially effective in welded steel hopper bins, or in polythethylene, temporary grain bins which are used in years of large harvests (Muir *et al.* 1973). Integration of three-dimensional heat transfer models (Alagusundaram *et al.* 1990*a*, *b*) with CO_2 diffusion models (Jayas *et al.* 1988) and mortality responses of insect species to CO_2 under controlled conditions in the laboratory (Rameshbabu *et al.* 1989, 1990) will soon lead to accurate prediction of conditions for pest control. Although cool grain requires a longer exposure to carbon dioxide than warm grain, this method of pest control should be practical and have no effects on seed germination or microfloral infection of seeds.

Acknowledgment

We thank R.J. Bell for technical assistance. This research was funded in part by the Natural Sciences and Engineering Research Council of Canada.

References

Alagusundaram, K., D.S. Jayas, N.D.G. White and W.E. Muir. 1990*a*. Three dimensional finite element heat transfer model of temperature distribution in grain storage bins. Trans. Amer. Soc. Agric. Eng. (In press).

Alagusundaram, K., D.S. Jayas, N.D.G. White and W.E. Muir. 1990*b*. Finite difference model of three dimensional heat transfer in grain bins. Can. Agric. Eng. (In press).

Annis, P. C. 1986. Towards rational controlled atmosphere dosage schedules: a review of current knowledge, pp. 128-148. *In* E. Donahaye and S. Navarro [eds.], Proceedings 4th International Working Conference on Stored Product Protection, Tel Aviv, Israel, Maor-Wallach, Jerusalem.

Bailey, S. W. and H. J. Banks. 1980. A review of recent studies on the effects of controlled atmospheres on stored product pests, pp. 101-118. *In* J. Shejbal [ed.] Controlled atmosphere storage of grains. Elsevier, Amsterdam.

Banks, H. J. and P. C. Annis. 1980. Conversion of existing grain storage structures for modified atmosphere use, pp. 461-473. *In* J. Shejbal [ed], Controlled atmosphere storage of grains. Elsevier, Amsterdam.

Banks, H. J. and P. C. Annis. 1983. Importance of processes of natural ventilation to fumigation and controlled atmosphere storage. Proc. International Symposium on Practical Aspects of Controlled Atmosphere and Fumigation in Grain Storage. Perth, W. Australia.

Bell, C. H., E. C. Spratt and D. J. Mitchell. 1980. The effects of nitrogen and carbon dioxide on eggs of *Ephestia cautella* (Walker) and *E. kuehniella* (Zeller) (Lepidoptera: Pyralidae). Bull. Entomol. Res. 70: 293-298.

Jayas, D. S., W. E. Muir and N. D. G. White. 1988. Modelling the diffusion of carbon dioxide in stored grain. Paper 88-6013. 12 pp. Am. Soc. Agric. Eng., St. Joseph, Mich.

Keever, D. W. 1989. Use of carbon dioxide to disinfest a tobacco warehouse of the cigarette beetle. J. Agric. Entomol. 6: 43-51.

Krishnamurthy, T. S, E. C. Spratt and C. H. Bell. 1986. The toxicity of carbon dioxide to adult beetles in low oxygen atmosphere. J. Stored Prod. Res. 22: 145-151.

Madrid, F. J., N. D. G. White and S. R. Loschiavo. 1990. Insects in stored cereals, and their association with farming practices in southern Manitoba. Can. Entomol. 122: 515-523.

McGaughey, W. H. and R. G. Akins. 1989. Application of modified atmospheres in farm grain storage bins. J. Stored Prod. Res. 25: 201-210.

Muir, W. E., R. N. Sinha and H. A. H. Wallace. 1973. Abiotic and biotic characteristics of grain stored in temporary farm bins. Can. Agric. Eng. 15: 35-42.

Navarro, S., O. Lider and U. Gerson. 1985a. Response of adults of the grain mite *Acarus siro* L. to modified atmospheres. J. Agric. Entomol. 2: 61-68.

Nicolas, G. and D. Sillans. 1989. Immediate and latent effects of carbon dioxide on insects. Ann. Rev. Entomol. 34: 97-116.

Rameshbabu, M., D.S. Jayas and N.D.G. White. 1989. Design and fabrication of a controlled atmosphere unit for laboratory studies on control of stored product pests. Paper 89-6033 (20 pp.), Amer. Soc. Agric. Eng., St. Joseph, Mich.

Rameshbabu, M., D.S. Jayas and N.D.G. White. 1990. Mortality of *Cryptolestes ferrugineus* (Stephens) adults and eggs in elevated CO_2 and depleted O_2 atmospheres. J. Stored Prod. Res. (In press).

SAS Institute. 1985. SAS user's guide: statistics. SAS Institute, Cary, N.C.

Sinha, R. N. and H. A. H. Wallace. 1977. Storage stability of farm-stored rapeseed and barley. Can. J. Plant Sci. 57: 351-365.

Sinha, R. N., D. Watterer and W. E. Muir. 1986. Carbon dioxide concentrations associated with insect infestations of stored grain. 1. Natural infestation of corn, barley, and wheat in farm granaries. Sci. des Aliments 6: 91-98.

Storey, C. L. 1980. Mortality of various stored product insects in low oxygen atmospheres produced by an exothermic inert atmosphere generator, pp. 85-92. *In* J. Shejbal [ed.]. Controlled atmosphere storage of grain. Elsevier, Amsterdam.

White, N. D. G. and R. N. Sinha. 1990. Effect of chlorpyrifos - methyl on oat ecosystems in farm granaries. J. Econ. Entomol. 83: 1128-1134.

White, N. D. G., D. S Jayas and R. N. Sinha. 1988. Interaction of carbon dioxide and oxygen levels, and temperature on adult survival and multiplication of *Cryptolestes ferrugineus* (Coleoptera: Cucujidae) in stored wheat. Phytoprotection 69: 31-39.

White, N. D. G., D. S. Jayas and R. N. Sinha. 1990. Carbon dioxide as a control agent for the rusty grain beetle (Coleoptera: Cucujidae) in stored wheat. J. Econ. Entomol. 83: 277-288.

White, N. D. G., R. N. Sinha and W. E. Muir. 1982. Intergranular carbon dioxide as an indicator of biological activity associated with the spoilage of stored wheat. Can. Agric. Eng. 24: 35-42.

Williams, P., W. Minett, S. Navarro and T. G. Amos. 1980. Sealing a farm silo for insect control by nitrogen swamping or fumigation. Aust. J. Exp. Agric. Anim. Husb. 20: 108-114.

LES EFFETS D'UNE ELEVATION PERMANENTE DU TAUX DE DIOXYDE DE CARBONE SUR LES ECOSYSTEMES DES STOCKS DE BLE STOCKE A BASSE TEMPERATURE

N.D.G. WHITE (1) et D.S. JAYAS (2)

(1) Agriculture Canada Research Station,
195 Dafoe Road, Winnipeg,
Manitoba R3T 2M9, Canada
(2) Department of Agricultural Enginnering
University of Manitoba, Winnipeg,
Manitoba R3T 2N2, Canada

RESUME

Des concentrations de dioxyde de carbone supérieures à 14 %, avec des pics de moins de 50 % pour 322 kg de blé en vrac stockés à des températures de 12 à 15° C, ont éliminé les populations d'insectes et d'acariens dans des enceintes non étanches en 42 jours. Des enceintes témoins non étanches, des enceintes étanches mais non traitées et des enceintes avec une perte de dioxyde de carbone d'une demi-vie de 6 et 14 jours, ont été purgées au dioxyde de carbone comprimé à 0, 7, 14 et 28 jours. Les purges des deux traitements, recommencées trois fois, ont donné des taux de dioxyde de carbone de 5, 40, 40 et 50 % ou 5, 40, 45 et 50 %, respectivement, dans les échantillons du fond. Les teneurs en oxygène ne sont pas tombées au-dessous de 11 %. Les populations d'insectes *Cryptolestes ferrugineus* (Stephens), *Tribolium castaneum* (Herbst) et *Ahasverus advena* (Waltl) et des acariens *Tarsonemus granarius* (Lindquist), *Paratriophtydeus coineaui* (Andre), *Lepidoglyphus destructor* (Schrank) et *Aeroglyphus robustus* (Banks) ont été sévèrement réduites dans les sept emplacements d'échantillonnage par enceinte en 28 jours après traitement au dioxyde de carbone. Quelques *Aeroglyphus robustus* ont survécu pendant 84 jours dans les enceintes présentant la perte de dioxyde de carbone la plus rapide. La germination des grains et l'infection fongique par les groupes *Aspergillus glaucus* et *Penicillium spp.* n'ont pas été directement affectées par le dioxyde de carbone pendant 84 jours alors que la germination a diminué occasionnellement dans les enceintes témoins en raison de l'alimentation des insectes.