

Effectiveness of carbon dioxide in compressed gas or solid formulation for the control of insects and mites in stored wheat and barley

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Résumé de l'article

Le bioxyde de carbone peut être utilisé comme un fumigant efficace des grains entreposés dans des mini-silos relativement étanches. Du CO₂ a été ajouté à du blé (*Triticum aestivum*) sous forme de gaz comprimé, ainsi qu'à de l'orge (*Hordeum vulgare*) sous forme solide (glace sèche) dans des amas de grains de 322 kg. Le blé a été entreposé à une température passant de 18 à 10°C au cours d'une période de 12 semaines. Les mini-réservoirs de blé ont été laissés ouverts, scellés sans ajout de CO₂ ou avec ajout de CO₂, à des concentrations de 25, 34 ou 46%. L'orge a été entreposé à une température passant de 25 à 20°C au cours d'une période de 8 semaines. Les mini-silos d'orge ont été laissés ouverts, scellés sans ajout de CO₂ ou avec ajout de CO₂, à des concentrations de 23, 29 ou 34%. Les teneurs en humidité du blé et de l'orge ont été de 14,5-16,3% et de 14,5-16,1%, respectivement. Les teneurs en O₂ du blé ont reflété le déplacement de l'air par le CO₂; cependant les niveaux plus faibles de CO₂ dans l'orge ont reflété une combinaison du déplacement de l'air par le CO₂ et de l'utilisation d'O₂ pour la respiration des grains et des microorganismes aux températures plus élevées. Les insectes *Cryptolestes ferrugineus* et *Tribolium castaneum* ont été réprimés en 2 semaines à 34% de CO₂ et 15% d'O₂ à une température passant de 18 à 10°C, ou à 29% de CO₂ et 3% d'O₂ à une température passant de 25 à 20°C. Les acariens *Tarsonemus granarius*, *Lepidoglyphus destructor* et *Aeroglyphus robustus* ont été réprimés en moins de 2 semaines à ces concentrations de CO₂. La germination des grains et la microflore n'ont pas été affectées par tous ces environnements gazeux.

Effectiveness of carbon dioxide in compressed gas or solid formulation for the control of insects and mites in stored wheat and barley

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Carbon dioxide can be used as an effective stored-grain fumigant in relatively air-tight bins. Carbon dioxide was added to wheat (*Triticum aestivum*) as a compressed gas and to barley (*Hordeum vulgare*) as a solid (dry ice) in 322-kg grain bulks. Wheat was stored at temperature decreasing from 18 to 10°C over a 12-wk period. Bins were left open, sealed without CO₂ added, or with CO₂ supplemented at 25, 34, and 46% levels. Barley was stored at temperature decreasing from 25 to 20°C over an 8-wk period. Bins were left open, sealed without CO₂ added, or with CO₂ treatments of 23, 29, and 34%. The wheat and barley moisture content were 14.5-16.3% and 14.5-16.1%, respectively. Oxygen levels in the wheat reflected air displacement with CO₂, but lower O₂ levels in the barley reflected a combination of air displacement by CO₂ and consumption of O₂ by respiring grain and microorganisms at the warmer temperatures. The insects *Cryptolestes ferrugineus* and *Tribolium castaneum* were controlled in 2 wk at 34% CO₂ and 15% O₂ at temperature decreasing from 18 to 10°C, or 29% CO₂ and 3% O₂ at temperature decreasing from 25 to 20°C. The mites *Tarsonemus granarius*, *Lepidoglyphus destructor*, and *Aeroglyphus robustus* were killed in less than 2 wk at these CO₂ levels. Seed germination and microflora were unaffected by all gaseous environments.

White, N.D.G. et D.S. Jayas. 1993. Efficacité du bioxyde de carbone sous forme gazeuse ou solide pour la lutte aux insectes et acariens dans le blé et l'orge entreposés. PHYTOPROTECTION 74: 101-111.

Le bioxyde de carbone peut être utilisé comme un fumigant efficace des grains entreposés dans des mini-silos relativement étanches. Du CO₂ a été ajouté à du blé (*Triticum aestivum*) sous forme de gaz comprimé, ainsi qu'à de l'orge (*Hordeum vulgare*) sous forme solide (glace sèche) dans des amas de grains de 322 kg. Le blé a été entreposé à une température passant de 18 à 10°C au cours d'une période de 12 semaines. Les mini-réservoirs de blé ont été laissés ouverts, scellés sans ajout de CO₂ ou avec ajout de CO₂, à des concentrations de 25, 34 ou 46%. L'orge a été entreposé à une température passant de 25 à 20°C au cours d'une période de 8 semaines. Les mini-silos d'orge ont été laissés ouverts, scellés sans ajout de CO₂ ou avec ajout de CO₂, à des concentrations de 23, 29 ou 34%. Les teneurs en humidité du blé et de l'orge ont été de 14,5-16,3% et de 14,5-16,1%, respectivement. Les teneurs en O₂ du blé ont reflété le déplacement de l'air par le CO₂; cependant les niveaux plus faibles de CO₂

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dans l'orge ont reflété une combinaison du déplacement de l'air par le CO_2 et de l'utilisation d' O_2 pour la respiration des grains et des microorganismes aux températures plus élevées. Les insectes *Cryptolestes ferrugineus* et *Tribolium castaneum* ont été réprimés en 2 semaines à 34% de CO_2 et 15% d' O_2 à une température passant de 18 à 10°C, ou à 29% de CO_2 et 3% d' O_2 à une température passant de 25 à 20°C. Les acariens *Tarsonemus granarius*, *Lepidoglyphus destructor* et *Aeroglyphus robustus* ont été réprimés en moins de 2 semaines à ces concentrations de CO_2 . La germination des grains et la microflore n'ont pas été affectées par tous ces environnements gazeux.

INTRODUCTION

Controlled atmospheres with high levels of carbon dioxide (CO_2), or nitrogen (N_2) and low levels of oxygen (O_2) are effective for controlling insects and mites in stored grain (Jayas *et al.* 1991). The toxicity of CO_2 and N_2 is dependent on temperature and relative humidity. Longer exposures to these gases than to synthetic neurotoxic fumigants are required to kill pests (Annis 1986). Currently, methyl bromide and phosphine gas are the only fumigants used on stored products in North America. These fumigants may leave chemical residues in stored food and chemically react with structural materials (Bond 1984). Phosphine is a potential mutagen in humans (Garry *et al.* 1989; Potter *et al.* 1991), and methyl bromide can decrease seed germination and reacts intensely with atmospheric ozone. Resistance to fumigants by insects is slowly increasing throughout the world (Taylor 1989). Methyl bromide and phosphine are currently under regulatory review in the United States and methyl bromide is being phased out in Canada. The possibility of losing these fumigants in Canada could result in \$20-\$160 million losses annually because of the effects of pest infestation on grain quantity and quality (Waithe 1991). Alternate methods, such as controlled atmospheres, could be used to prevent these losses.

Carbon dioxide is a more effective gas than nitrogen in a controlled atmosphere, because virtual elimination of oxygen is not necessary with CO_2 . Carbon dioxide also causes desiccation of insects when spiracles are opened and causes direct physiological action rather than simple suffocation (Nicholas and Sillans 1989). The utility and efficacy of CO_2

applied as both compressed gas and solid (dry ice) has been studied in the past (Jay 1980; Jay and D'Orazio 1984).

Carbon dioxide, which is registered for fumigation of stored cereals in Canada, does not leave chemical residues in food, is less likely to produce highly resistant insects, and can be economical (White and Jayas 1991). Prolonged controlled atmosphere storage may also control fungal growth and does not reduce germination of stored grain (White and Jayas 1993).

A major limitation to using CO_2 on farms is that most storage bins are not air-tight. Attempts can be made to seal storage structures and to increase bin air-tightness to minimize gas loss (Banks and Annis 1980). Controlled atmospheres also can be made practical by modifying storage atmospheres by a series of gas infusions or a continuous gas flow to obtain required gas concentrations and gradients predicted with computer models (White *et al.* 1993). The most cost-efficient and effective method of fumigation, however, is with air-tight bins.

Several species of insects and mites typically occur together in farm-stored grain in western Canada (Madrid *et al.* 1990; White and Sinha 1990). Responses to controlled atmospheres vary among species (Jay 1984; Krishnamurthy *et al.* 1986). Grain temperatures rarely exceed 20°C in the centre of 5-m-diam bins within several months of harvest in western Canada (Sinha and Wallace 1977) unless active spoilage is occurring. The low temperatures slow the effectiveness of all toxic gases because of the lowered metabolism of insects and mites. The zero tolerance for detectable insects in Canadian grain makes disinfestation at cool temperatures a concern. It is necessary

to determine a practical technique for generating CO₂ and the levels and exposure times for effective pest control under simulated conditions. The aim of this study was to determine the effectiveness of injected or sublimated CO₂ on control of insects and mites frequently found in western Canada at typical autumn temperatures of stored grain which can be harvested warm (>30°C) but will gradually cool.

MATERIALS AND METHODS

Storage bins

Fifteen cylindrical steel bins (444 L, 58 cm diam x 168 cm high) were each constructed by welding two steel oil drums together end to end. A removable lid was attached to the top of the bins with a circular band of steel and a bolt tightener. The bins were placed on 20-cm-high concrete blocks. Six equally spaced grain and gas sampling ports were made in a spiral pattern along the vertical sides of the bins, with an additional sampling port in the centre of the bottom (White *et al.* 1990). A copper-constantan thermocouple was positioned beside each gas sampling tube.

Grain storage

Injected CO₂ - wheat

Each bin was filled with 322 kg (431 L) of Canada western red spring wheat (*Triticum aestivum* L.) with a 15-16% moisture content and containing $\approx$ 0.8%, by weight, dockage (chaff, broken kernels).

One thousand adults of both the rusty grain beetle, *Cryptolestes ferrugineus* (Stephens) [Coleoptera: Cucujidae], and the red flour beetle, *Tribolium castaneum* (Herbst) [Coleoptera: Tenebrionidae], were added to the tops of all bins with ventilation holes (6 cm in diam covered with wire mesh) in the lids and left for 5 wk at 30°C to multiply. The mites *Tarsonemus granarius* Lindquist [Acarina: Tarsonemidae], *Aeroglyphus robustus* (Banks) [Acarina: Glycyphagidae], *Lepidoglyphus destructor* (Schrank) [Acarina: Glycyphagidae], and some Tydeidae spp. also were naturally present in low numbers. All treatments were replicated three times. The five treatments were: bins that remained as open con-

trols, bins tightly sealed but with no CO₂ added, bins each receiving 2 min of gas flow (25% CO₂ treatment), bins each receiving 4 min of gas flow (34% CO₂ treatment), and bins each receiving 6 min of gas flow (46% CO₂ treatment). The CO₂ was obtained from a compressed-gas cylinder holding 22.7 kg of CO₂ and introduced at a rate of 40 L min⁻¹ through the bottom gas-sampling port. The top port was temporarily opened to permit pressure equalization. Temperatures in the storage room gradually declined from 18 to 10°C during the 12-wk study.

Gas and grain samples as well as temperatures were taken from seven vertically-arranged sample locations per bin initially and then bi-weekly for 12 wk (7 samples per bin; 21 samples (200 mL of grain) per treatment x 5 treatments = 105 samples per date).

Dry Ice (CO₂) - barley

The same 15 bins were later emptied, thoroughly cleaned and refilled with barley (*Hordeum vulgare* L.) at 15% moisture content. One thousand adults of *C. ferrugineus* and *T. castaneum*, and several thousand adults of the mite *A. robustus* were added to each bin and allowed to multiply for 5 wk at 30°C in bins which all had ventilated lids. The mite *L. destructor* was also naturally present in the grain. The treatments were: bins that remained open, bins sealed tightly but with no CO₂ added, bins each receiving 96 g of dry ice as pellets ($\approx$ 30% CO₂), bins each receiving 128 g dry ice ($\approx$ 40% CO₂) and bins each receiving 160 g dry ice ($\approx$ 50% CO₂). All treatments were replicated three times. The dry ice was added to the top of the grain bulks and a bottom grain-sampling septum was removed until gas pressure in the drums equalled atmospheric pressure. The experiment was conducted at temperatures gradually declining from 25 to 20°C over an 8-wk period. Gas and grain samples as well as temperatures were taken at the top, middle, and bottom of each bin initially and then once a week for 8 wk, with the exception of week 7 (3 samples per bin; 9 samples per treatment x 5 treatments = 45 samples per week).

Sampling procedures

Methods for monitoring variables, sam-

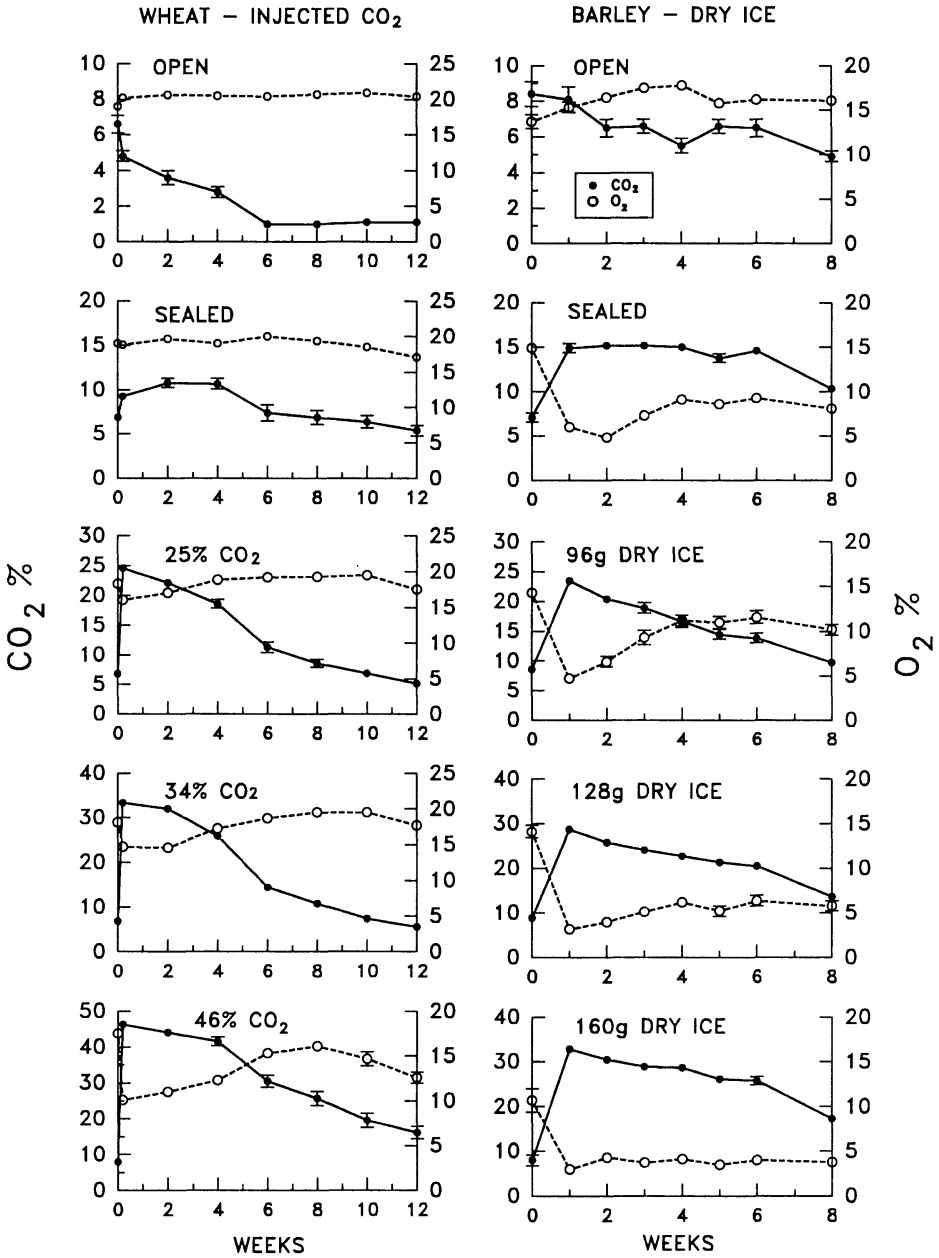


Figure 1. Mean ($\pm$ SE) of CO₂ and O₂ levels of wheat in bins with open lids, sealed lids, or treated with compressed CO₂ (25%, 34% or 46%) (n = 7 locations per bin, 3 bins per treatment); and mean ($\pm$ SE) CO₂ and O₂ levels of barley in bins with open lids, sealed lids, or treated with 96 g (23% CO₂), 128 g (29% CO₂), or 160 g (34% CO₂) dry ice (n = 3 locations per bin, 3 bins per treatment).

pling procedures, and grain sample analyses were previously reported (White *et al.* 1990). The variables monitored included CO₂ and O₂ (gas chromatography; Matheson gas chromatograph, -model 8430, 1 mL fixed volume sample loops, thermal conductivity detector), temperature (thermocouples, potentiometer), grain moisture content (oven-dry methodology, 130°C for 19 h), numbers of insects and mites per 150 mL grain sample (Berlese funnel extraction), and seed germination and microfloral infection at the beginning and end of the study (incubation at 22°C on water-saturated or NaCl solution-saturated filter papers). All data were summarized and analyzed (ANOVA) using SAS procedure GLM (SAS Institute 1985).

RESULTS AND DISCUSSION

Injected CO₂ - wheat

Moisture content of the wheat did not change during the study, but there were significant differences ($P \leq 0.01$) in moisture content between locations in bins and treatments. Wheat was drier at top than at bottom locations of the bins and grain in the open bins was drier than all other treatments. Means and standard errors for moisture contents of grain at top and bottom locations were 14.5 ± 0.1 and 15.9 ± 0.2 for open bins, 15.3 ± 0.1 and 16.3 ± 0.1 for sealed bins, 15.8 ± 0.2 and 16.3 ± 0.1 for 25% CO₂ treatment, 15.3 ± 0.2 and 16.3 ± 0.1 for 34% CO₂ treatment, and 15.5 ± 0.1 and 16.3 ± 0.1 for 46% CO₂ treatment.

Carbon dioxide levels were near 5% at the beginning of the study (Fig. 1) because of insect, microflora, and grain respiration after 5 wk incubation at 30°C (White *et al.* 1982). There were significant differences ($P \leq 0.01$) in both CO₂ and O₂ levels with time (bin leakage) among locations within bins and among treatments. There was usually 1-3% more CO₂ at bin bottoms than tops, because CO₂ density is greater than that of air.

The mean gas levels from all samples in a treatment showed that CO₂ declined from about 5% to 1% in the open bins after 12 wk (ambient air levels are 0.03% CO₂) and O₂ levels remained near

ambient-air levels of 20.9% by volume (Fig. 1). The sealed bins had CO₂ levels near 11% by the second week, declining to 5% after 12 wk with O₂ levels usually near 19%. The initial 25, 34, and 46% CO₂ treatments had corresponding O₂ levels of 16, 15, and 10% (Fig. 1) at the beginning of the experiment. The CO₂ levels declined by 80, 85, and 65% from initial values after 12 wk for the 25, 34, and 46% CO₂ treatments, respectively, because of gas loss during grain sampling.

At time 0 there were between 2 and 22 adults, and 2.5 and 10 larvae of *C. ferrugineus* in 150 mL of wheat from the various treatments (Fig. 2). The total numbers of adults and larvae per bin did not change appreciably over 12 wk in the open bins or the sealed bins because the cool temperatures inhibited reproduction and development (Sinha and Watters 1985). However, the mean number of *C. ferrugineus* adults per sample increased sharply at 6 wk because most of the adults in the bins moved to the bottom sample of the sealed bins without added CO₂ as compared to previous even distribution throughout the grain bulk (White and Jayas 1991) (Fig. 2). Larvae are much less mobile and tend to stay inside the germ of seeds so their distribution did not change. Virtually all adults and larvae were killed in 2 to 4 wk in all CO₂ treatments including the lowest CO₂ treatment which had CO₂ concentration declining from 25 to 18%, and O₂ concentration rising from 16 to 19%. A few *T. castaneum* adults and larvae survived 12 wk even at the highest CO₂ level (46% declining to 16% CO₂) (Fig. 3). Throughout the study, *C. ferrugineus* adults and larvae were more common in lower regions of the bins, while *T. castaneum* adults and larvae were more common near the upper levels of the bins. There were 0.4 ± 0.3 *T. granarius*, 25 ± 10 Tydeidae, 0.1 ± 0.1 *L. destructor*, and 0.2 ± 0.1 *A. robustus* per 150 mL wheat at the beginning of the study. By the second week, all mites were absent from treatments with injected CO₂, but at 12 wk, there were 50.7 ± 25.2 and 18.5 ± 6.6 *T. granarius* and 0.7 ± 0.5 and 1.4 ± 1.0 *A. robustus* per sample in the open bins and the sealed bins, respectively.

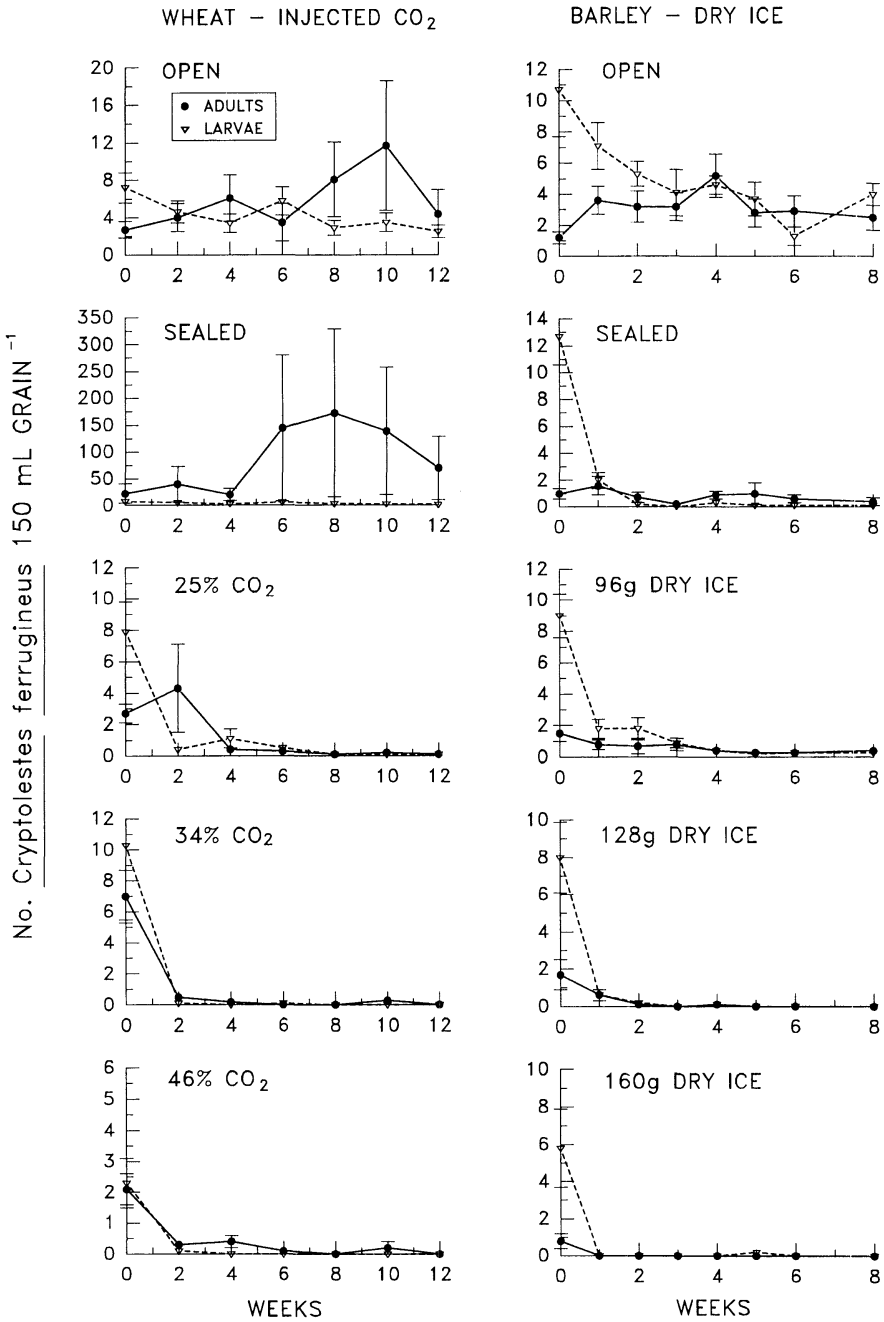


Figure 2. Mean ($\pm$ SE) numbers of *Cryptolestes ferrugineus* adults and larvae per 150 mL of wheat in bins with ventilated lids, sealed lids, or treated with compressed CO₂ (25%, 34% or 46%) ($n = 7$ locations per bin, 3 bins per treatment); and mean ($\pm$ SE) numbers of *C. ferrugineus* adults and larvae per 150 mL barley in bins with open lids, sealed lids, or treated with 96 g (23% CO₂), 128 g (29% CO₂), or 160 g (34% CO₂) dry ice ($n = 3$ locations per bin, 3 bins per treatment).

Germination of all wheat seeds was very low at the beginning of the study because of prolonged storage at warm temperatures prior to obtaining the grain. It did not change significantly during the 12-wk period (Table 1). Infection by fungi, *Penicillium* spp., remained relatively low and did not change significantly over 12 wk (Table 1). *Aspergillus glaucus* group fungi were common, initially infecting 70–92% of seeds (Table 1) and the frequency of infection declined over 12 wk.

Dry ice (CO₂) – barley

Moisture content of the barley did not change during the study. The top of the grain in the open bins was significantly ($P \leq 0.01$) drier than in other treatments. Means and standard errors for moisture contents of grain at top and bottom locations were 14.5 ± 0.4 and 14.9 ± 0.3 for open bins, 15.4 ± 0.1 and 14.8 ± 0.1 for sealed bins, 15.4 ± 0.1 and 14.8 ± 0.1 for 96-g dry ice bins, 15.2 ± 0.1 and 15.5 ± 0.6 for 128-g dry ice bins, and 15.3 ± 0.1 and $16.1 \pm 0.3\%$ for 160-g dry ice bins.

Carbon dioxide levels following 5 wk of insect incubation at 30°C and prior to adding CO₂, were near 8% because of intense insect, microflora, and grain res-

piration (Fig. 1). Once the experiment began at 25°C, CO₂ gradually declined to about 5% and O₂ increased to about 16% by week 8 in the open bins (Fig. 1). In the sealed bins, mean CO₂ levels rose to about 15% and remained at that level until week 6. Oxygen levels fell as low as 5% reflecting hermetic storage conditions for relatively moist grain at warm temperatures (Penteado *et al.* 1990). There were significant differences ($P \leq 0.01$) in both CO₂ and O₂ level changes with time (bin leakage), among locations within the bins (1–3% more CO₂ at bin bottoms than tops), and among treatments. The addition of dry ice did not change grain temperatures. Calculated CO₂ levels were 30, 40, and 50%; actual mean CO₂ levels were 23, 29, and 34%, corresponding to 77, 73, and 68% of the calculated levels, respectively. The lower levels were probably caused by gas adsorption by the grain (Cofie-Agblor *et al.* 1993) and gas pressure increase in the bin which resulted in forced air movement while the bins were vented during gas introduction.

The O₂ levels in the dry ice treatments were lower than would be caused by displacement of air with CO₂ and resulted from a combination of CO₂ introduction

Table 1. Initial and final ($\bar{X} \pm$ SE) seed germination and frequency of microfloral infection of seeds in wheat stored at temperatures decreasing from 18 to 10°C under various CO₂ enriched atmospheres for 12 wk^a

Treatment	CO ₂ : O ₂ concentration at 1 h (%)	Storage period (wk)	Seed germination (%)	Frequency of seed infection	
				<i>Aspergillus glaucus</i> group (%)	<i>Penicillium</i> spp. (%)
Open bin	4.8 : 20	0	5.0 ± 3.9	86.1 ± 2.7	9.7 ± 1.8
		12	3.4 ± 2.0	73.8 ± 4.8	7.2 ± 1.6
Sealed bin	9 : 19	0	1.5 ± 0.5	85.9 ± 2.6	7.0 ± 1.3
		12	0	70.4 ± 6.3	4.6 ± 2.0
24% CO ₂ bin	24 : 16	0	0	69.9 ± 4.1	27.9 ± 3.6
		12	0.1 ± 0.1	58.0 ± 4.5	17.4 ± 3.4
34% CO ₂ bin	34 : 15	0	3.8 ± 1.3	91.8 ± 2.2	5.1 ± 1.1
		12	6.3 ± 2.3	74.5 ± 3.6	7.6 ± 1.6
46% CO ₂ bin	46 : 10	0	2.9 ± 1.3	89.6 ± 2.2	12.3 ± 2.2
		12	2.3 ± 0.8	68.0 ± 4.0	9.1 ± 2.8

^a n = 21 sample locations per treatment, 7 samples per bin; 25 seeds per location. CO₂ was added as compressed gas.

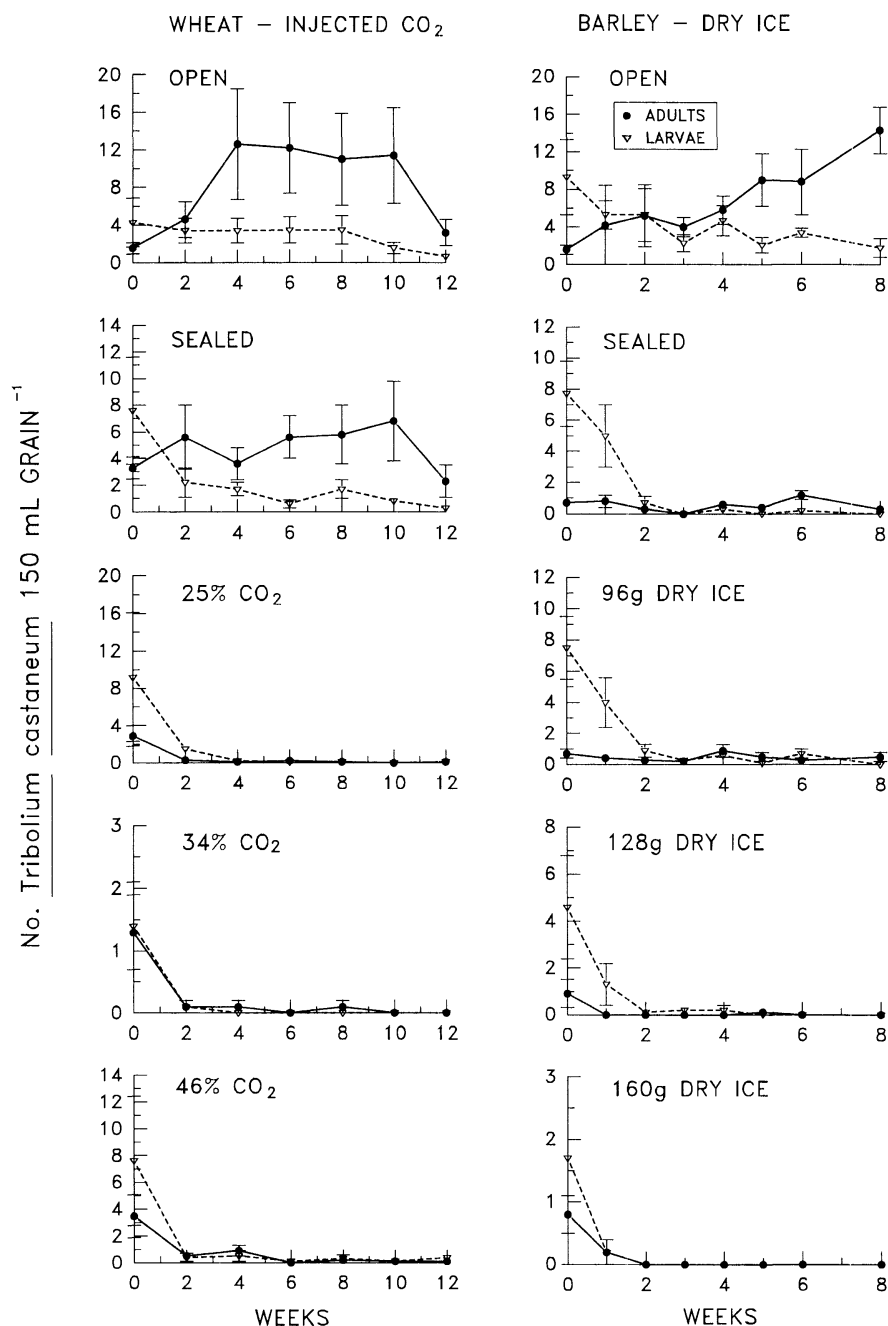


Figure 3. Mean ($\pm$ SE) numbers of *Tribolium castaneum* adults and larvae per 150 mL of wheat in bins with open lids, sealed lids, or treated with compressed CO₂ (25%, 34% or 46%) ($n = 7$ locations per bin, 3 bins per treatment); and mean ($\pm$ SE) numbers of *T. castaneum* adults and larvae per 150 mL barley in bins with open lids, sealed lids, or treated with 96 g (23% CO₂), 128 g (29% CO₂), or 160 g (34% CO₂) dry ice ($n = 3$ locations per bin, 3 bins per treatment).

and remaining O₂ consumption by respiring grain and microorganisms at warm temperatures (White *et al.* 1982).

At time 0, there was a mean of one adult and 6-13 larvae of *C. ferrugineus* per 150 mL of barley as well as one adult and 2-9 larvae of *T. castaneum* (Figs. 2 and 3). In the open bins, the number of *C. ferrugineus* adults remained fairly constant for 8 wk and the numbers of larvae gradually declined (Fig. 2). The number of *T. castaneum* adults rose to over 14 per 150 mL barley at week 8, while larval numbers decreased. The sealed bins offered good insect regulation in 3 wk which was equivalent to the 96-g dry ice treatment (Figs. 2 and 3). Insects were controlled in the sealed bins more effectively than in the wheat experiment sealed bins because temperatures in the barley were warmer and there was more CO₂. The 128-g dry ice treatment (29% CO₂, 3% O₂) eliminated most insects in 2 wk although a few *T. castaneum* adults survived for 5 wk (Figs. 2 and 3). The 160-g dry ice treatment (34% CO₂, 3% O₂) eliminated *C. ferrugineus* in 1 wk and *T. castaneum* in 2 wk (Figs. 2 and 3).

As in the earlier study using wheat, *C. ferrugineus* were more numerous in the lower half of the grain columns and *T. castaneum* were more numerous in the top half of the grain columns throughout the experiments. At week 0, no *L. destructor* were noted in grain samples, but there were 22 ± 6 *A. robustus* per 150 mL of barley. At 8 wk, in the open bins, there were 33 ± 15 *L. destructor* and 21 ± 11 *A. robustus* per 150 mL barley. A few *L. destructor* survived in top samples of the sealed bins at 8 wk (0.3 ± 0.2 per 150 mL barley), but *A. robustus* did not survive more than 4 wk. The occasional *L. destructor* or *A. robustus* were detected for up to 8 wk in the 96 g dry ice treatment. The two higher CO₂ treatments had no mites after 1 wk.

Germination of all seeds was initially between 88 and 93% after 8 wk declined significantly ($P \leq 0.5$) only in the open bins (Table 2) where insects were most common. There were no significant changes in *Penicillium* (<3% of the seeds infected) or *A. glaucus* group infections in any treatment during 8-wk period (Table 2).

Table 2. Initial and final ($\bar{X} \pm SE$) seed germination and frequency of microfloral infection of seeds in barley stored at temperatures declining from 25 to 20°C under various CO₂ enriched atmospheres for 8 wk^a

Treatment	CO ₂ : O ₂ concentration at 1 h (%)	Storage period (wk)	Seed germination (%)	Frequency of seed infection	
				<i>Aspergillus glaucus</i> group (%)	<i>Penicillium</i> spp. (%)
Open bin	8.5 : 15	0	88.2 $\pm$ 2.2	51.1 $\pm$ 5.5	0.7 $\pm$ 0.3
		8	81.8 $\pm$ 2.0	55.4 $\pm$ 8.9	0.9 $\pm$ 0.5
Sealed bin	15 : 6	0	90.7 $\pm$ 0.8	50.7 $\pm$ 5.6	2.0 $\pm$ 0.7
		8	84.9 $\pm$ 2.9	61.8 $\pm$ 7.7	3.0 $\pm$ 0.9
96 g bin ⁻¹	23 : 5	0	93.0 $\pm$ 1.1	60.5 $\pm$ 6.5	2.0 $\pm$ 0.6
		8	90.7 $\pm$ 1.6	47.7 $\pm$ 5.0	1.7 $\pm$ 0.8
128 g bin ⁻¹	29 : 3	0	89.3 $\pm$ 2.2	46.4 $\pm$ 6.4	0.9 $\pm$ 0.5
		8	89.3 $\pm$ 1.8	54.2 $\pm$ 7.2	0.9 $\pm$ 0.6
160 g bin ⁻¹	34 : 3	0	90.0 $\pm$ 1.3	47.0 $\pm$ 6.1	0.3 $\pm$ 0.3
		8	90.7 $\pm$ 2.7	52.3 $\pm$ 9.4	2.0 $\pm$ 0.9

^a n = 9 sample locations per treatment, 3 samples per bin; 50 seeds per location.
CO₂ was added as dry ice.

Based on the range of CO₂ levels tested, this study indicated that moderate levels of CO₂ (34% CO₂ and 15% O₂ at temperatures decreasing from 18 to 10°C, or 29% CO₂ and 3% O₂ with temperature decreasing from 25 to 20°C) were very effective in controlling insects and mites in wheat and barley in relatively air-tight bins in 2 wk. Lower levels of CO₂ are adequate for insect control if longer exposure is maintained while higher temperatures could increase the rate of the toxic action of the gas (White *et al.* 1988).

While solid CO₂ is often more convenient to handle and to weigh accurate quantities of gas applied to grain, it is considerably more expensive than compressed CO₂. Regardless of the formulation, care must be taken in generating desired gas levels because of gas adsorption by grain (Banks 1993; Cofie-Agblor *et al.* 1993). It is impractical to make most farm granaries airtight, but if efforts are made to reduce gas loss, such as by placing plastic sheets impermeable to CO₂ over the grain bulk surface and caulking bin walls, it should be possible to control arthropod infestations. Carbon dioxide would be most effective in welded steel hopper bins, hopper railcars, or in polyethylene temporary grain bins, which are used in years of large harvests.

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