Controlling *Cryptolestes ferrugineus* (Stephens) adults in wheat stored in bolted-metal bins using elevated carbon dioxide

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Alagusundaram, K., Jayas, D.S., White, N.D.G., Muir, W.E. and Sinha, R.N. 1995. **Controlling *Cryptolestes ferrugineus* (Stephens) adults in wheat stored in bolted-metal bins using elevated carbon dioxide.** Can. Agric. Eng. 37:217-223. Experiments were conducted in two 5.56 m-diameter farm bins to determine the mortality of caged adult rusty grain beetles, *Cryptolestes ferrugineus* (Stephens) (Coleoptera: cucujidae), under elevated carbon dioxide (CO_2) concentrations. The bins were filled with wheat to a depth of 2.5 m. Dry ice was used to create high CO_2 concentrations in the wheat bulks. Two different modes of application of dry ice were used: (i) pellets on the grain surface and in the aeration duct and (ii) pellets on the grain surface and blocks in insulated boxes on the grain surface. The pellets exposed to the ambient conditions on the grain surface and in the aeration duct sublimated quickly and had to be replenished at frequent intervals. Dry ice blocks in insulated boxes, however, maintained high CO_2 concentrations without replenishment for over 15 d. In both modes of application, the observed CO_2 concentrations in the intergranular gas were about 15% and 30% (all the CO_2 concentrations given in this article are on a volume basis) at 2.05 m and 0.55 m above the floor, respectively. At 0.55 m above the floor, the mortality of rusty grain beetle adults was more than 90% while in the top portions of the bulk (2.05 m above the floor) the mortality was only 30%. On an average about two thirds of the insects were killed. The use of controlled atmosphere treatment within an integrated pest management context is outlined.

Key words: wheat, storage, rusty grain beetle, controlled atmosphere, carbon dioxide, mortality

Des expériences ont été menées dans deux réservoirs de ferme ayant des diamètres de 5.56m, afin de déterminer la mortalité de coléoptères rouillés *Cryptolestes ferrugineus* (Stephens) (Coleoptera: cucujidae) adultes, dans des grains, et soumis â des concentrations élevées de bioxyde de carbone (CO_2). Les réservoirs ont été remplis à une hauteur de 2.5 m. De la glace sèche a été utilisée pour créer de hautes concentrations de CO_2 dans le chargement de blé. Deux différents modes d'application de la glace sèche ont été utilisés: (i) des boulettes sur la surface des grains et dans le conduit d'aération et (ii) des boulettes sur la surface des grains et des blocs dans des boites isolées sur la surface des grains. Les boulettes exposées aux conditions ambiantes sur la surface des grains et dans le conduit d'aération se sublimaient rapidement et devaient être replacées fréquemment. Quant aux blocs de glace sèche disposés dans les boites isolées, ils maintenaient de hautes concentrations de CO_2 sans être replacés durant 15 J. Pour les deux modes d'application, les concentrations de CO_2 observées dans le gaz entre les grains ont été environ 15% et 30% (toutes les concentrations de CO_2 dans cet article sont fournies sur une base volumique) à des hauteurs respectives de 2.05 m et 0.55 m au-dessus du plancher. A 0.55 m au-dessus du plancher, la mortalité des coléoptères adultes a été de plus de 90%. alors que dans la portion du haut de la charge de grains (2.05 m au-dessus du plancher) la mortalité a été seulement 30%. En moyenne, environ deux tiers des insectes ont été tués. L'utilisation de traitements en atmosphère contrôlée dans un contexte de lutte intégrée est ébauchée.

INTRODUCTION

Increasing consumer awareness of the health risks and environmental damage caused by chemicals is forcing storage managers to reduce or eliminate the use of toxic chemical pesticides for disinfesting stored-grain bulks. The observed selection and genetic resistance of insects to chemicals necessitates the use of doses larger than those used before and exposure periods longer than usual for complete control (Taylor 1989), thereby increasing the chances of health and environmental damage. Because of these problems and the hazards to those applying them, several chemicals have been deregistered worldwide. In Canada, for example, after recent deregistration of several pesticides, phosphine and methyl bromide are the only two remaining fumigants registered for use on or near stored food materials. The present global trend is to minimize the use of chemicals and to identify alternative methods of pest control.

Controlled atmosphere (CA) storage is a potential alternative method for insect control (Banks and Annis 1977). In CA storage, the intergranular air is altered by injecting either carbon dioxide (CO_2) to create high CO_2 atmospheres, or nitrogen (N_2) to create low oxygen (O_2) atmospheres. Over the past 75 years, numerous research studies have been conducted in laboratories and in large grain bulks to determine the effectiveness of controlled atmospheres in controlling stored-product insects (Banks 1979; Annis 1987). The studies conducted elsewhere in the world focused on quantifying the efficacy of CA storage in controlling insects economically important to that region. In the Prairie Provinces of Canada, for example, the rusty grain beetle, *Cryptolestes ferrugineus* (Stephens), is the most common and serious pest of stored grains on farms and in elevators (Sinha and Watters 1985). A knowledge of the efficacy of controlled atmospheres in controlling these beetles will help in planning strategies for reducing the usage of chemical fumigants in Canada.

Only a few studies have been conducted to determine the effectiveness of CA gases for controlling rusty grain beetles (Ganapathy et al. 1993; Rameshbabu et al. 1991; White et al. 1988, 1990). These studies were conducted in laboratories or in small grain bulks with the main objective of determining the effectiveness of various compositions of CA gases on the mortality of rusty grain beetles. Extrapolation of these valuable data to a farm bin situation is not possible because in farm bins additional factors such as the temperature differences in the grain bulk, local weather changes, and gas loss from the structure will affect insect mortality. To achieve the level of understanding required to develop approaches to effective pest management using controlled atmospheres under Canadian conditions, it is essential to test the response of rusty grain beetles to CA gases in full-size farm bins.

The objective of this study was to determine the mortality of adult rusty grain beetles in cages under elevated CO_2 levels in experimental farm bins.

MATERIALS AND METHODS

Two 5.56 m-diameter metal bins filled with wheat to a depth of 2.50 m were used in the tests. The bins were made of corrugated-galvanized steel sections bolted together and placed on a concrete floor on a farm 20 km south of Winnipeg. One bin (Bin 1) was equipped with a 0.46 m-diameter and 4.7 m-long circular duct on its floor (Fig.1). The second bin (Bin 2) had the same dimensions and construction as Bin 1 except that it did not have an aeration duct. The bins were instrumented with gas sampling tubes for drawing intergranular gas samples to determine CO_2 concentrations and copper-constantan thermocouples to monitor grain temperatures. Semi-rigid nylon tubes (3.2 mm-outside diameter, and 0.4 mm-wall thickness) were used as gas sampling tubes. In both bins the gas sampling tubes and thermocouple wires were installed at three levels (0.55 m, 1.30 m, and 2.05 m above the concrete floor) (Fig. 1). In each bin, there were 13 locations at each level for gas samples and temperatures. Both the gas sampling tubes and thermocouple wires were led out of the bin through small holes made in the bin wall. The gas sampling tubes were fitted with rubber septa at their outer ends. The thermocouple wires were connected to a multichannel switch, which in turn was connected to a temperature indicator (Trendicator 410A, Emerson Electric Co., San Diego, CA). The visible holes in the bin wall and the holes made for inserting thermocouple wires and gas sampling tubes were sealed with silicon sealant.

Both bins were used to determine the mortality of 4- to 8-wk old adult insects under elevated CO_2 levels. We chose to test the mortality of adults because they are the most tolerant of all life stages of rusty grain beetles (Ganapathy et al. 1993). When CO_2 was added in Bin 1, Bin 2 was used as the control bin and vice versa. The door of the test bin was sealed by spreading a polyvinylidene chloride (PVDC, made of 3 parts nylon and 4 parts polyethylene) sheet on the inside face and taping it to the bin wall. The door of the control bin was not sealed. Rusty grain beetle adults were taken from laboratory cultures feeding on whole wheat and wheat germ (in the ratio of 19:1 by mass) at 30 ± 1°C and 70 ± 5 % relative humidity. Fifty adults and about 10 g of wheat germ were put in each small bag made of honey straining cloth (0.02 mm^2 aperture openings). These bags were placed in metal tubes of 16.0 mm-inside diameter (hereafter referred to as insect tubes). Perforations for facilitating the entry of CO_2 to the insect bags were made in the insect tubes at points where the insect bags were placed. The insect tubes were inserted into the bins through nipples (19.0 mm-inside diameter and 88.9 mm-long) bolted to the wall. The joints between the nipples and the bin wall were sealed with silicon sealant. Sixty tubes were inserted into each bin (5 tubes at each of the four equally spaced radii at three heights above the floor). The sampling locations for the insects were the same as for CO_2 except that there were two insect bags at sampling location 4 (Fig. 1). In total there were 210 insect bags inserted in each bin (i.e. 10 500 insects per bin).

Carbon dioxide and temperature data were collected every 24 h. Forty two insect bags were removed from the bins every 48 h in experiments 1 and 2 and every 72 h in experiments 3, 4, and 5 (Table I). The insects from the withdrawn bags were allowed to recover at 25 ± 2°C for 48 to 72 h before they were counted and designated as dead or alive.

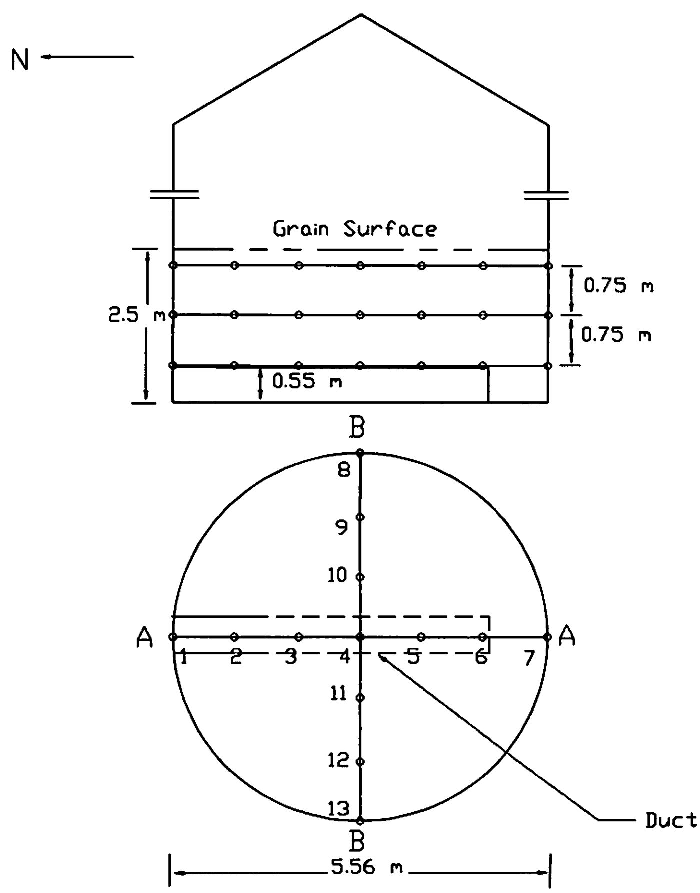

Fig. 1. Schematic diagram of temperature, gas, and insect sampling locations (o) in a 5.56-m-diameter bin with a circular duct on the floor (Bin 1), used for determining mortality of rusty grain beetle adults under elevated CO_2 levels.

Table I: Summary details of experiments conducted in two 5.56-m-diameter bolted-metal bins to determine the mortality of rusty grain beetle adults exposed to elevated CO_2. The bins were filled with wheat to a depth of 2.5 m.

Experiment number	Bin number*	Dry ice application Mass (kg)	Frequency	Starting date	Duration (h)	Mode and point of application of dry ice
1	1	252	28 kg at 0, 24, 48, 72, 96, 152, 168, 192, and 216 h	Sep. 15, 1992	240	14 kg pellets each on the grain surface and in the aeration duct at each time
2	1	240	28 kg at 0, 72, 99, 121, 143, 170, and 218 h; 26 kg at 24 h; 18 kg at 50 h.	Aug. 6, 1993	241	14 kg pellets each on the grain surface and in the aeration duct at all times except at 24 h (12 kg on the grain surface and 14 kg in the duct), and at 50 h (11 kg on the grain surface and 7 kg in the duct)
3	2	240	240** kg at 0 h	Jun. 18, 1993	360	60 kg pellets directly on the grain surface and 180 kg blocks in two insulated boxes on the grain surface
4	2	240	240** kg at 0 h	Jul. 13, 1993	361	60 kg pellets directly on the grain surface and 180 kg blocks in two insulated boxes on the grain surface
5	2	300	300** kg at 0 h	Aug. 23, 1993	360	120 kg pellets directly on the grain surface and 180 kg blocks in two insulated boxes on the grain surface

* Bin 1 had a circular aeration duct on its concrete floor and Bin 2 had a concrete floor with no duct. In each experiment one bin was used as the test bin and the other bin was used as the control bin in which no CO_2 was added.

**Actual amounts of dry ice used were 222 kg in Experiment 3 and 4 and 297 kg in Experiment 5, because the rest of the dry ice remained in the insulated boxes at the end of the experiments.

DATA ANALYSIS

Mortality

The mortality of rusty grain beetle adults was calculated as the percent number of dead insects in relation to the total number of insects in each bag. Whenever the mortality in the control bin exceeded 10%, the mortality in the CO_2-treated bin was corrected using Abbott's (1925) formula:

$$\%\ Corrected\,Mortality = \frac{X-Y}{X} x\ 100 \qquad (1)$$

where:

X = percentage of insects living in control bin (%), and
Y = percentage of insects living in CO_2-treated bin (%).

In experiment 3, the mortality in the control bin exceeded 10% at all sampling times, while in all other experiments the control bin mortality was less than 10%.

Weighted-Volume average CO_2 concentration

For easy graphical representation of the data, weighted-volume average CO_2 concentration was calculated as the sum of the product of measured CO_2 concentration at a sampling point and the space volume represented by this sampling point divided by the total volume. The total volume was taken as the total volume at each level when the weighted-volume average CO2 concentration was calculated for the level or the total volume of the grain bulk when the quantity was calculated for the whole grain bulk. The equation for it is:

$$C_w^{ts} = \frac{1}{V_d} \sum_{i=1}^{n} (CO_2)_i V_i \qquad (2)$$

where:

C_w^{ts} = weighted-volume average CO_2 concentration for sampling time ts (%),
$(CO_2)_i$ = measured CO_2 concentration at a sampling point in volume i (%),
n = number of component volumes represented by one or more sampling points,
V_d = total volume of grain bulk including intergranular air and grain in a layer or the whole grain bulk (m^3), and
V_i = volume of the component region i (m^3).

The grain bulks were divided into 39 to 52 small volumes in such a way that the sampling points were at the geometric centre of the sub-divided region in the horizontal direction. The widths of the regions near the wall were half the width of the regions in the rest of the bulk. The weighted-volume average CO_2 concentrations for each sampling level and for the whole bin at every sampling time were estimated using Eq. 2.

RESULTS AND DISCUSSION

CO_2 distribution

The CO_2 concentrations were higher in the bottom portions of the grain bulk than in the top portions (Fig. 2) regardless of whether CO_2 was introduced on the grain surface (Experiments 3, 4, and 5) or both on the grain surface and in the aeration duct (Experiments 1 and 2). For example, when dry ice was introduced both in the duct and on the grain surface, the average CO_2 concentration over 10 d was 28.9% at 0.55 m above the floor compared to 15.1% at 2.05 m above the floor. This uneven distribution and large gas accumulation near the floor resulted from CO_2 gas being heavier than air (at atmospheric pressure the density of CO_2 at a temperature of 20°C is 1.83 kg/m^3 compared with the density of air of 1.19 kg/m^3 at the same temperature).

Dry ice pellets, introduced directly on the grain surface or in the aeration duct, sublimated quickly. The rapid sublimation and loss of CO_2 from the bin required frequent replenishment (in Experiments 1 and 2). In contrast, dry ice blocks in insulated styrofoam boxes placed on the grain surface maintained CO_2 concentrations for long durations without any additional labour. The observed CO_2 concentrations in both modes of CO_2 application were comparable (Fig. 2). Furthermore, the mass of dry ice required was greater when exposed to the ambient conditions than placed in insulated boxes. For example, the mass of dry ice used was 25 kg/d (Experiment 1) when exposed to the ambient conditions compared with 15 kg/d (Experiment 3 and 4) when in insulated boxes. Thus, introducing dry ice in insulated boxes is less labour intensive and more economical.

Mortality of the rusty grain beetle

In all the experiments the mortality was maximum (greater than 90%) at level 1 (0.55 m above the floor) because of the high CO_2 concentrations in that region. At level 3 (2.05 m above the floor) where the CO_2 concentrations were lower than at levels 1 or 2, the average mortality was also lower than the other two levels. The mortality of the insects at a given temperature and moisture content is influenced by the concentration of the gas and the duration of exposure. In fumigation trials the product of concentration and time (hereinafter referred to as ct-product) is used to represent the dosage (Calderon and Carmi 1973; Wilson et al. 1980). We observed a linear relationship between the mortality of rusty grain beetle adults and the ct-product (Fig. 3), suggesting that increasing the exposure time is likely to ensure complete control (as long as a minimum CO_2 concentration is maintained at all locations in the bin). The Australian recommendation for complete control of most stored-product insects is an initial CO_2 concentration of over 70% declining to nearly 35% in 10 d (an average ct-product of 12600 %•h) at 20°C (Banks and Annis 1980). Although this rule of thumb was verified under Australian conditions, these ranges cannot be assumed to control the rusty grain beetle in Canada because different insects respond differently to CA gases and the environmental conditions in Canada are different.

Laboratory results suggest that adult rusty grain beetles can be controlled in 4 d at 20°C and a CO_2 concentration of 90% (Rameshbabu et al. 1991) (a ct-product of 8640 %•h). In a non-airtight bin, in which CO_2 was lost to the atmosphere and CO_2 layering in the bottom, it was not possible to create or maintain such high CO_2 levels. At low CO_2 levels (about 29%) 2-wk of exposure were required (a ct-product of 9744 %•h) to completely control rusty grain beetles at temperatures declining from 25 to 20°C (White and Jayas 1993). At still lower CO_2 concentrations (about 20%) and at a slightly higher temperature (25 ± 3°C) rusty grain beetles can be controlled in 4-6 wk (ct-products ranging from 13440 to 20160 %•h) (White et al. 1990). Ganapathy et al. (1993) observed complete kill of rusty grain beetle adults in 8 d at

Table II. Average wheat bulk temperatures (°C), and mortality (%) of rusty grain beetle adults at the end of each experiment in controlled atmosphere (CA) and control bins

Experiment number	Wheat bulk temperature (°C) Mean ± S.D.		Mortality (%)							
			Height above the floor						Average for the whole bin	
			0.55 m		1.30 m		2.05 m			
	CA Bin	Control	CA Bin	Control	CA Bin	Control	CA Bin	Control	CA Bin	Control
1	18.9 ± 3.0	18.3 ± 2.0	90.7	4.8	71.3	6.2	32.5	4.1	64.8	5.0
2	20.6 ± 2.0	22.7 ± 1.3	98.6	0.6	78.0	1.0	6.82	1.0	61.1	0.9
3*	20.6 ± 2.7	21.9 ± 2.5	98.5	54.5	79.3	39.5	76.4	20.8	84.7	38.3
4	21.4 ± 1.6	19.9 ± 1.6	92.1	2.0	59.3	8.3	26.0	1.4	59.1	3.9
5	20.8 ± 1.9	19.5 ± 1.5	91.5	5.2	60.7	5.8	51.2	11.2	67.8	7.4

*The mortality at each level was corrected using Abbot's (1925) formula because the mortality in the control bin was more than 10%.

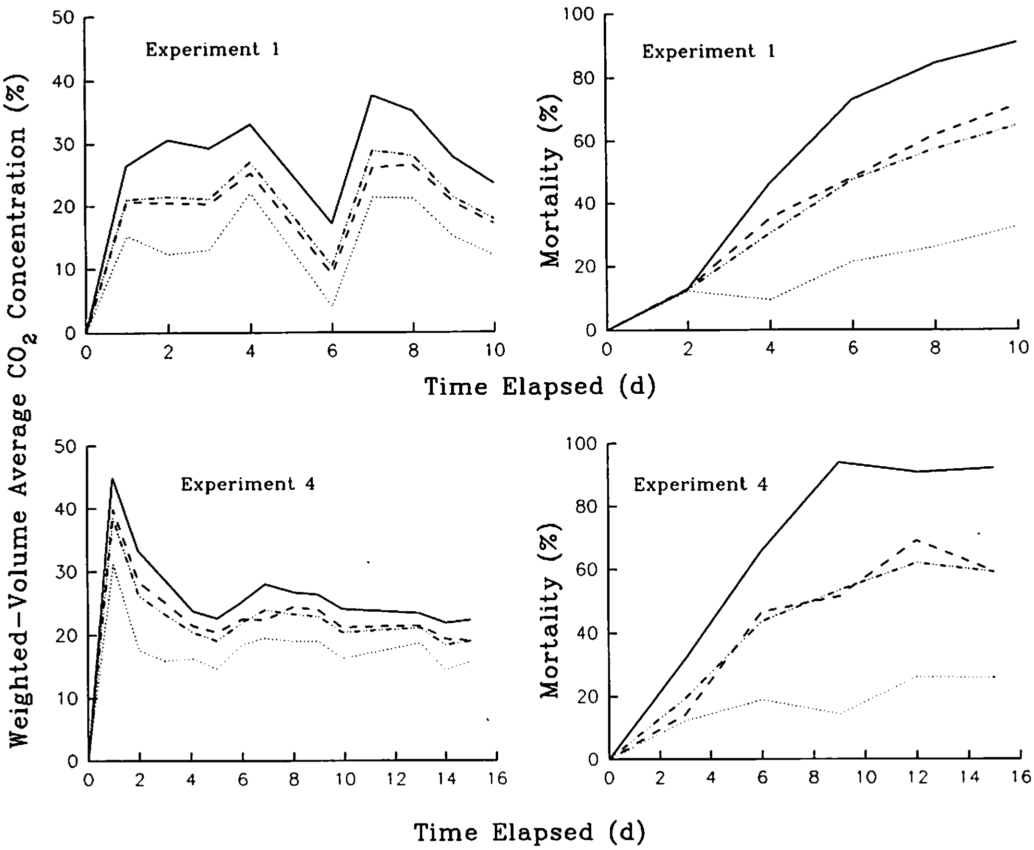

Fig. 2. Changes in weighted-volume average CO_2 concentration with time (left) and mortality with time (right) when dry ice pellets were introduced on the grain surface and in the aeration duct (Experiment 1) and when dry ice pellets were introduced on the grain surface and dry ice blocks in insulated boxes were placed on the grain surface (Experiment 4). The details of the experiments are given in Table 1.
(_______ 0.55 m; — — — 1.3 m; ..... 2.05 m above the floor and — .. — .. — average for whole bin)

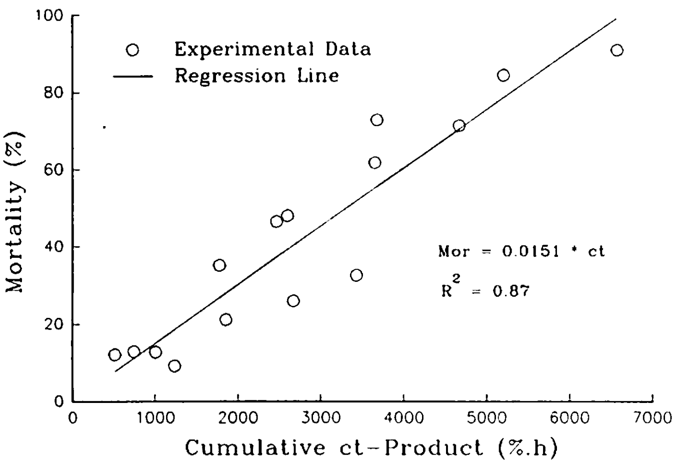

Fig. 3. Increase in mortality of rusty grain beetle adults with cumulative ct-product when dry ice pellets were introduced (14 kg on the grain surface and 14 kg in the duct at 0, 24, 48, 72, 96, 152, 168, 192, and 216 h).

30 to 40% CO_2 (ct-products ranging from 5760 to 7680 %•h).

Thus, to achieve complete control of rusty grain beetle adults, either high CO_2 concentrations (>70%) should be maintained for up to 4 d, or low CO_2 concentrations (20 to 40%) should be maintained for 4-6 wk. In both cases the required minimum CO_2 concentration should be maintained at all locations in the bulk. Based on the CO_2 distribution tests it is obvious that CO_2 concentrations of >70% cannot be created or maintained in the existing bolted metal bins. The maximum ct-products in our experiments ranged from 7000 to 9300 %•h at 0.55-m-above the floor, which are less than the reported dosage for controlling rusty grain beetles. The CO_2 concentrations ranged from 15 to 30% in the grain bulks. If the exposure period was extended up to 4-6 wk, better control of rusty grain beetles in the top levels of the test bin would have occurred. This assumption can be further supported with the observed linear relationship between the mortality (%) and the ct-product (Fig. 3).

COST ANALYSIS

The cost (all costs in Canadian dollars) of phosphine fumigation in Canada is about \$1.20/t of grain (White and Jayas 1993) and in the USA it is about \$0.40/t of grain (Reed et al. 1990). The cost of CO_2 treatment in our experiments ranged from \$1.14 to 1.53/t of wheat (calculated based on a price of

$0.25/kg of dry ice), which is comparable to the cost of phosphine fumigation in Canada. But, to obtain 100% mortality of the rusty grain beetle adults the treatment would have to be continued for 4-6 wk, which would increase the cost of treatment. In our experiments, however, because of the leaks in the bin structure large quantities of introduced CO2 were lost. These bins have to be sealed more effectively to reduce the CO2 loss, thereby reducing the cost of treatment.

Despite these inadequacies, the CO_2 treatment might be quite effective in controlling natural infestations of rusty grain beetle adults because they typically move to the bottom of the grain bulk (White and Loschiavo 1985), where the CO_2 levels were the highest.

INTEGRATED PEST MANAGEMENT STRATEGIES

Based on the observed CO_2 concentrations in these experiments, it is evident that creating and maintaining high levels of CO_2 in leaky bolted-metal bins may not be possible. Under the observed CO_2 levels, on an average two-thirds of the caged rusty grain beetle adults were killed. The legally defined zero tolerance for insects in stored grain in Canada requires that all the stored-product insects be killed before grain is sent for domestic or international markets. Potential health risks of insecticides require reduced or no use of chemicals to control insects. A sound approach to resolve this problem will be to adopt an integrated pest management (IPM) strategy rather than depending on one single method.

For example, the CA storage using high levels of CO_2 can be combined with cold temperature disinfestation. Navarro et al. (1993) observed faster control of *Carpophilus mutilatus* Er. and *Carpophilus hemipterus* L. in dried fruits using 2.8% O_2 in air and low temperatures (-10 to -18°C) than using only CA storage. On farms, where sanitary conditions are relatively poor, grain binned on a hot summer day can be rapidly infested with insects (Madrid et al. 1990). An infested bulk can be treated with CO_2 following the procedure outlined in this manuscript to reduce the initial population and minimize grain damage. In winter the grain can be cooled by aeration or turning. Insects are inactive at temperatures below 8°C (Anonymous 1984). Rusty grain beetles, for example, can be killed in 2 wk at -15°C. If an infestation is detected in the following spring and summer, the grain bulk can be treated with CO_2 one more time for complete control.

Another approach to IPM using CO_2 gas could be to combine chemical fumigants and CO_2. For each 5% increase in CO_2, insect respiration increases by 50%, and this reduces the methyl bromide requirement by 50% (Anonymous 1993). In addition to the reduced chemical requirement, combining CO_2 and chemical fumigants can be advantageous in two other ways:

1. In a leaky storage structure where it is difficult to maintain the required phosphine concentration, a continuous supply of 3% phosphine in CO_2 is effective in controlling insects (Chakrabarti et al. 1991; Bell et al. 1993).
2. In large grain bulks, chemical fumigants can be effectively distributed using CO_2 as a carrier gas (Calderon and Carmi 1973; Viljoen et al. 1984).

CONCLUSIONS

Based on the results of this study, the following conclusions can be drawn:

1. Dry ice pellets introduced directly on the grain surface or in the aeration duct sublimated quickly and had to be replenished frequently. This mode of application was labour intensive.
2. Insulated boxes containing dry ice reduced the rate of sublimation and maintained high CO_2 concentrations (15 to 30%) for long durations (over 15 d), with no additional labour requirement.
3. The mortality of rusty grain beetle adults was maximum (greater than 90%) in the bottom portions of the grain bulk (0.55 m above the floor).
4. Although the costs of treatment in the experiments were comparable to the cost of phosphine fumigation in Canada, only about two thirds of the caged rusty grain beetle adults were killed in 10 or 15 d of exposure.

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