A new method to control stored-product insects using carbon dioxide with high pressure followed by sudden pressure loss

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Abstract

A newly developed method for the control of stored-product insects consisting of a rapid addition of high pressure gas into a chamber, followed by the sudden release of the gas was evaluated using carbon dioxide (CO_2) or helium (He). The effect of various pressure levels and exposure periods was assessed against *Sitophilus zeamais* Motshchulsky, *Rhyzopertha dominica* F., *Tribolium castaneum* (Herbst), and *Lasioderma serricorne* (F.). With CO_2 at 20 kg/cm^2, an exposure time of 5 minutes was sufficient to kill all adults of the four species. However, eggs of *S. zeamais* required treatment at 30 kg/cm^2 of CO_2 for 5 minutes for complete kill. The integument of insects exposed to this treatment was severely damaged because of the expansion of internally dissolved CO_2 in the body when gas pressure was rapidly equilibrated with atmospheric pressure. In contrast, the application of He had no effect on adults of *S. zeamais* even at 70 kg/cm^2. The solubility of gases in the hemolymph appears to be a significant factor in the effectivenessof this method.

Introduction

Many people concerned with control of stored-product insects once believed that methyl bromide and phosphine are ideal chemicals since both can effectively control insects by penetrating into inter- and intragranular spaces within the grain mass without leaving toxic residues. However, a recent movement on methyl bromide has greatly changed the situation since it is considered as one of major chemicals that destroy the earth's ozone layer (Singh et al. 1988). Thus, the limitation of its production level has been internationally determined in the Fourth Meeting of Parties to the Montreal Protocol at Copenhagen in 1992. In addition, although more work is needed, there is a report that phosphine may also have carcinogenic effects on humans (Garry et al. 1989). Thus, safer and more effective means of combating stored-product insects are needed.

As an alternative to conventional fumigants, modified atmospheres using carbon dioxide(CO_2) have recently been widely investigated and developed in many countries such as Australia, the USA and Israel (Shejbal 1980; Ripp et al. 1984; Champ et al. 1990; Calderon and Barkai-Golan 1990). In Japan, the Food Agency has banned the use of methyl bromide or phosphine on domestic rice since 1991 and recommended CO_2 as an alternative. Nevertheless, low temperature warehouses (15°C) have been widely used for rice storage with more than 3 Mt capacity throughout the country.

Carbon dioxide, being a natural component of the atmosphere, is a safe chemical and has been permitted for use as an additive to many types of drinks and foods. A limitation of CO_2-enriched atmospheres for insect control, however, is a longer exposure period compared with those for conventional fumigants (Annis 1989). This limits the use of CO_2 for quarantine and food processing purposes in which quick treatment is necessary. However, several researchers have suggested recently that the limitation of CO_2 can be solved by high-pressure treatment with the gas (Stahl et al. 1985; Reichmuth 1990; Le Torc'h and Fleurat-Lessard 1992). In this study, we investigated conditions for insect control using pressurised gas followed by rapid pressure loss.

Materials and Methods

Insects

The species tested were the maize weevil, *Sitophilus zeamais* Motschulsky, red flour beetle, *Tribolium castaneum* (Herbst), lesser grain borer, *Rhyzopertha dominica* (F.), and cigarette beetle, *Lasioderma serricorne* (F.). All test insects were taken from cultures maintained at 25°C and 75% r.h. in a dark room. Both species of *S. zeamais* and *R. dominica* were reared on whole brown rice; wheat feed was used for *T. castaneum* and *L. serricorne*.

Equipment

The apparatus was composed of a 30 mL cylindrical chamber of 5 mm thickness steel placed between inlet and outlet valves (Fig. 1) (Nitto Koatsu K.K.). The valves can be opened or closed using handles. Test insects, either in test tubes or in grains, were introduced by opening the inlet valve while the outlet valve remain closed. Either CO_2 or helium (He) was then discharged from a pressurised tank into the chamber after closing all valves. After the exposure period, The gas was rapidly discharged to the atmosphere by opening the outlet valve.

Mortality Test

For the test tube test, 25 adults of each of two species were placed in perforated polyethylene tubes, 13 mm in diameter and 28 mm high. For the bulk grain trial, 200 adults of *S. zeamais* were released on 200 g of brown rice and allowed to oviposit for 3 days. From this, 10 g was drawn randomly and mixed with 10 g of fresh rice to make up 20 g test samples of brown rice. Twenty kernels were infested with larvae and another 20 kernels that contained pupae were introduced to the brown rice test medium. These kernels were selected from cultures previously identified to contain larval and pupal stages of the maize weevil. Manifestations of damage such as the presence of frass were used as a basis for judgment. Ages of immature stages used were 25 days for pupae, 15 days for larvae, and 3 days for eggs. Finally 2-week-old unsexed adults were also added to the brown rice . The brown rice containing

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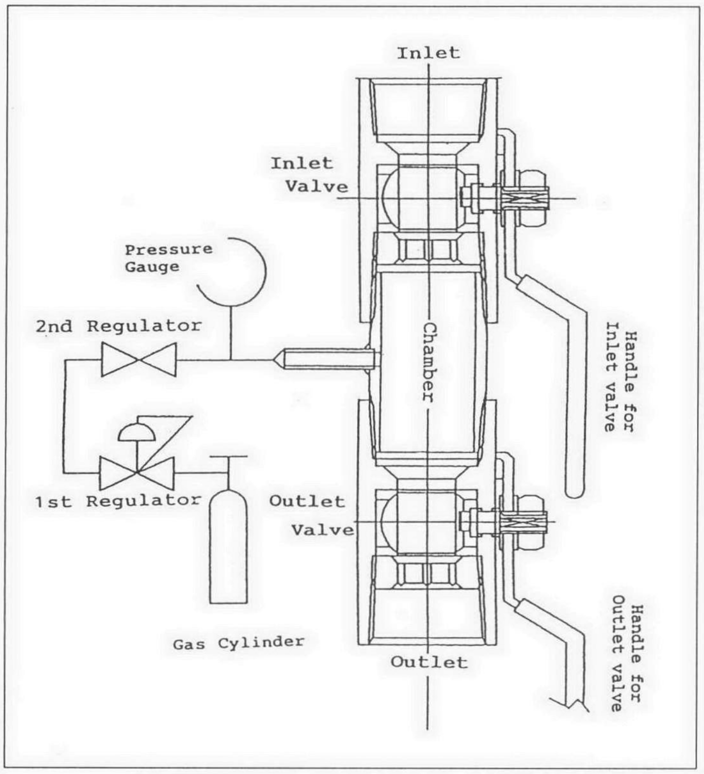

Fig. 1. Schematic diagram of equipment for high pressure gas increase followed by sudden release.

all stages of *S. zeamais* was then placed in the chamber for treatment. After exposure, the insects were transferred to the rearing room for observation. All stages of *S. zeamais* treated in the brown rice were assessed 1 month later to count the number of insect that had emerged. To compare the efficacy of CO_2 with that of another gas, helium was also applied to adults of *S. zeamais*, employing the same method.

Results

Table 1 shows the mortality of adults of four stored product beetles, *S. zeamais*, *R. dominica*, *T. castaneum* and *L. serricorne* resulting from the method after either 5 or 10 minutes exposure to highly purified CO_2 at pressures of 5, 10, 15 and 20 kg/cm^2. Increasing pressure reduced the exposure times needed to obtain higher mortalities although there were differences in susceptibility among species. *R. dominica* was the most susceptible species tested, requiring 5 kg/cm^2 for 10 minutes for a complete kill. *T. castaneum* was the most tolerant species since complete mortality was not obtained until a pressure of 15 kg/cm^2 for 10 minutes was used. All adults of the four species were killed completely in 5 minutes at 20 kg/cm^2.

Figure 2 shows the effect of the treatment on the adults of species. The integument is severely damaged as a result of the bursting of the insect's internal organs.

The eggs and larvae of *S. zeamais* exposed to elevated CO_2 for 5 min were more tolerant than pupae and adults, as shown in Table 2. Eggs were most resistant to the method, with only 21.9% killed at 20 kg/cm^2. A pressure as high as 30 kg/cm^2 was needed to kill all stages of *S. zeamais*. In contrast to CO_2,

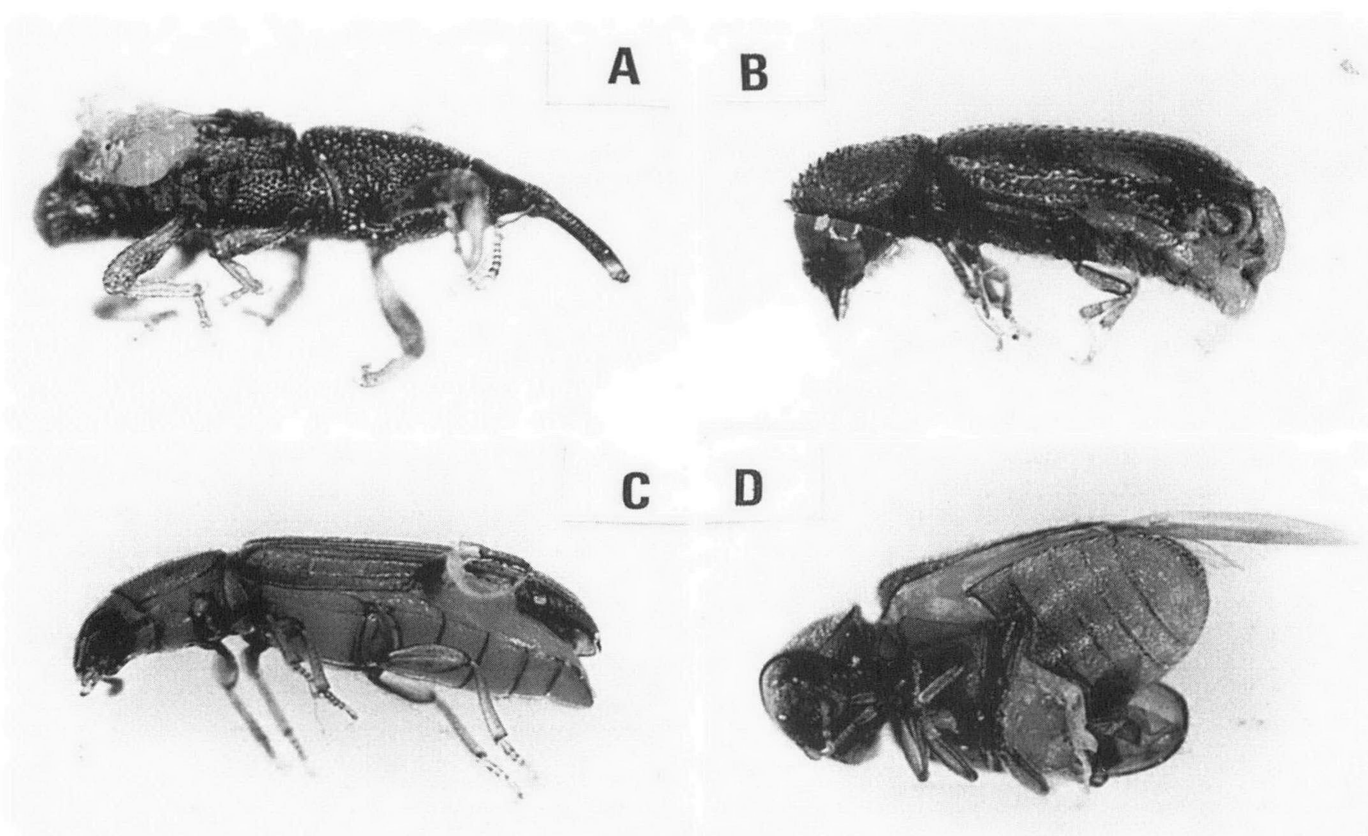

Fig. 2. Physical damage in adult insects killed by high pressure technique: (a) *S. zeamais*, (b) *R. dominica*, (c) *T. castaneum* and (d) *L. serricorne*.

helium produced no significant lethal effect, even at pressures as high as 70 kg/cm^2 (Fig. 3).

Discussion

The rapid increase in CO_2 pressure followed by a quick release of the gas after a 5–10 minutes exposure period appears to be an effective, fast and safe method for the control of stored-product insects.

Comparing it with previously studied control methods, this technique would be almost as rapid as thermal disinfestation procedures such as hot-air fluidised beds (Evans et al. 1983; Fleurat-Lessard 1987) and microwave radiation (Kirkpatrick and Robert 1971; Nakakita et al. 1989). Increasing the pressure could correspondingly shorten the exposure time. Thus, there is a need to establish specific *PT* (pressure × time) products, similar to *CT* (concentration × time) products used for fumigants.

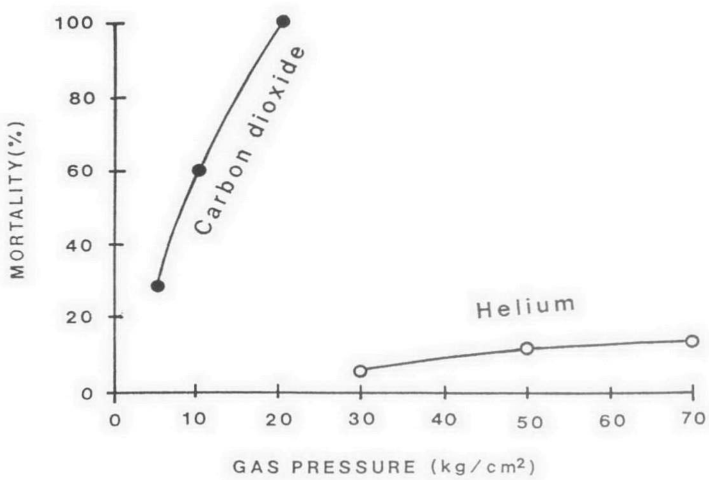

Fig. 3. Comparison of mortality curves of *S. zeamais* adults exposed to high pressure CO_2 or helium for 5 minutes at 25°C. All mortality data were assessed 48 hours after treatment.

Although the application of the method using CO_2 gave rapid control of insects, there were some differences in susceptibilities among species, and among stages of *S. zeamais*. Of the adults of all four species tested, *T. castaneum* was the most tolerant, followed by *S. zeamais*, *R. dominica*, and *L. serricorne*. However, the differences in susceptibility among species are unlike those obtained from conventional CO_2 treatments at atmospheric pressure which involve wide ranges of susceptibility among species (Annis 1989). Application of the method to immature stages of *S. zeamais* showed that the egg was the most resistant life stage, requiring almost three times as much pressure of CO_2 as pupae or adults to achieve complete mortality. In conventional CO_2 treatments, the pupal stage is commonly the most resistant (Navarro 1987). Thus, there must be differences in responses of insects to high pressure and conventional CO_2 methods. These may be due to the different mechanisms involved in the two methods. There would be two actions occurring to achieve insect mortality with the high-pressure method: the physiological action of CO_2 at high concentrations in target sites; and the physical action of high pressure gas in tissues of the insects , causing expansion as the gas is suddenly released to the atmosphere.

Helium had almost no effect on insect mortality even at the extremely high pressure of 70 kg/cm^2. The great difference in mortality between CO_2 and He treatments may be explained by the different solubilities of the gases in the hemolymph of insects, which is mainly water. Carbon dioxide has about an 80-fold greater solubility than helium at 25 kg/cm^2 and 25°C: 16.5 mL and 0.21 mL for CO_2 and He, respectively, in 1 g

Table 1. Effect of CO_2 treatment using high pressure followed by sudden discharge on adults of four stored-product insects.

Species	Pressure (kg/cm^2)							
	5		10		15		20	
	Exposure time (minutes) % mortalities ± SE[a]							
	5	10	5	10	5	10	5	10
S. zeamais	28.0 ± 8.4	36.0 ± 5.6	60.0 ± 10.0	96.0 ± 1.9	100	100	100	100
R. dominica	78.6 ± 7.1	100	100	100	100	100	100	100
T. castaneum	32.0 ± 1.9	45.3 ± 2.9	69.3 ± 1.1	88.0 ± 1.9	85.3 ± 3.9	100	100	100
L. serricorne	81.3 ± 2.1	93.3 ± 2.1	100	100	100	100	100	100

[a]Mean of three replicates.
*Percentage mortality corrected from control mortality by Abbott's (1925) formula.

Table 2. Effects of CO_2 treatment using high pressure followed by sudden discharge on all life stages of *Sitophilus zeamais* (Motsch.).

Pressure (kg/cm^2)	% Mortality ± SE[a]			
	Eggs	Larvae	Pupae	Adults
0	0 (32.0 ± 2.6)[b]	0 (19.3 ± 0.5)[b]	0 (19.6 ± 0.3)[b]	0
10	28.1 ± 10.6	89.6 ± 2.4	100	100
15	32.3 ± 6.1	98.5 ± 1.4	100	100
20	21.9 ± 3.8	100	100	100
30	100	100	100	100

[a]Mean of three replicates.
[b]Value in parenthesis is number of emerged adults.
*Mortality assessed after one month for immature stages; values were calculated by dividing the total number of adults that emerged after the treatment by the total number of adults in the control.

water at normal state (Japan Chemical Society 1966). Helium does not penetrate the bodies of insects; thus no expansion occurs after sudden release of the gas from high pressure. From the viewpoint of water solubility of gases, we may find other gases that are as effective as CO_2.

Applying a gas such as CO_2 using the method described would provide a safe means of insect control leaving no harmful residues on commodities. Thus, the method may apply in places or situations where rapid treatment for insect control is needed such as in quarantine, food processing factories, etc. Further work is, however, necessary to provide information on the relationship between time and pressure, efficacy of this technique on immature stages of different species and quality effects on various commodities. In addition, pilot-scale trials should be carried out determine the commercial applicability and practicability of the technique.

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