A preliminary evaluation of carbon dioxide under high pressure for rapid fumigation

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Abstract

Fumigation is a widely used practice for commodity disinfestation but there are few available fumigants. In addition, there are environmental concerns in regard to the use of methyl bromide, and a low level of resistance to phosphine exists in stored-product insects.

Numerous studies have been carried out on the use of carbon dioxide for grain fumigation but most require lengthy exposure intervals, typically 10 days or longer. The current work involved the use of carbon dioxide at high pressures and concentrations for short time intervals.

Exposure of relevant development stages of major pests of stored grain to high concentrations of carbon dioxide (around 98%) for short intervals (5–20 minutes) produced complete mortality. In general, beetle species were more difficult to control than moths, and eggs were the most tolerant stage. At pressures up to 30 kg/cm^2 and 5–20 minutes exposure there was no significant effect on the viability of rice. The technique appears to have considerable potential for quarantine disinfestation.

Introduction

Fumigation is a widely used practice for commodity disinfestation but there are few available fumigants. In addition, there are environmental concerns in regard to the use of methyl bromide and a low level of resistance to phosphine currently exists.

Numerous studies have been carried out on the use of carbon dioxide for grain fumigation but most strategies require lengthy exposure intervals, typically 10 days or longer.

Exposure to CO_2 requires over 10 days to kill insects at 20–25°C (Annis 1987; Jay et al. 1990). Although exposure periods can be reduced as temperatures are raised (Bailey and Banks 1980; Jay 1986), the duration is still impracticable for quarantine treatments. Likewise, alternative methods such as holding insects under high vacuum alone requires over a week for acceptable kill even at warm temperatures (Calderon and Navarro 1968; Cline and Highland 1987; Locatelli and Traversa 1989). A combination of CO_2 under low pressure and raised temperatures can achieve complete kill at best within 12 hours at 40°C, but longer (18–54 hours) at lower temperatures (Locatelli and Daolio 1993).

The new process of using carbon dioxide under high pressure offers an exciting and novel alternative to conventional CO_2 application. It was first described by Stahl et al. (1985). According to Gerard et al. (1988) and Pohlen et al. (1989), the quality of the treated commodities is not adversely affected. The required amounts of CO_2 are minute compared with the natural carbon dioxide emanating from the surface of the earth (Reichmuth 1990). The high pressure CO_2 treatment combines most of the advantages of CA technology and at the same time addresses its most serious drawback that is it requires extremely short durations for lethal exposure, within the range of a few minutes. This renders the new method highly promising for quarantine treatment and rapid disinfestation of valuable products.

Survival of eggs of *Plodia interpunctella* was completely prevented when these were exposed to high pressure CO_2 at 30 bar for 10 minutes (Gerard et al. 1988). Likewise, adult and immature *Lasioderma serricorne* were controlled after 15 minutes at 30 bar and 90 minutes at 20 bar.

The current work involved the application of carbon dioxide at high pressures for short time intervals.

Materials and Methods

Relevant developmental stages of four species of storage insects, i.e., *Sitophilus oryzae*, *Rhyzopertha dominica*, *Tribolium castaneum* and *Corcyra cephalonica* were challenged with carbon dioxide released at high pressures for short intervals.

Eggs, larvae and pupae of the test insects were exposed to carbon dioxide under pressures of 5, 10, 20 and 30 kg/cm^2 for 5 or 10 minutes in the pressurised chamber illustrated on Figure 1. The cylinder measured 17.3 cm high with external diameter of 3.8 cm to yield a volume of 30 mL. Pure (>99%) food-grade carbon dioxide was used. Pressure was adjusted by carefully moving the first regulator; the second regulator is further used for finer adjustment to attain the desired level of pressure. Although the actual CO_2 level inside the chamber was not measured and monitored, CO_2 concentration was calculated to be more than 90%.

Each larval or pupal sample contained 10 individuals However for the egg stage, each replicate had 20 individuals. Each treatment combination was replicated three times for the larval and pupal stages while the trials on eggs were replicated six times.

The insects were brought back to their respective culture rooms immediately after treatment. All experiments were conducted at 20–25°C and 70–75% r.h.

The cultures were assessed for adult emergence. This was continued for 30 days in the case of eggs to ensure that there had no extreme delay in eclosion had occurred. On the other

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hand, mortality of the larvae and pupae was assayed 48 hours after exposure. Moribund insects were considered dead. The development of the remaining live pupae to adult was also observed.

Results and discussion

Among the various species tested, the rice moth (*C. cephalonica*) was the most susceptible. It was highly sensitive to the treatment with the pupae succumbing to the lethal atmosphere even at the lowest dosage of 5 kg/cm^2 pressure and exposure period of 5 minutes (Table 1). All the coleopteran species tested exhibited similar levels of tolerance, with complete control achieved at 30 kg/cm^2 within 5 minutes (Table 2).

In terms of developmental stage, the egg was the most tolerant, followed by the larva. A pressure of 30 kg/cm^2 was required to prevent egg survival. The pupal stage was the most susceptible, in most cases a complete kill resulting from exposure to 20 kg/cm^2 pressure for 5 minutes.

Increasing pressure had a more pronounced effect on insect mortality than did duration of exposure. As evidence of this, doubling the pressure from, say, 10 to 20 kg/cm^2 resulted in 50% or more reduction in survival; the same degree of increase in exposure period failed to produce a corresponding quantum response in insect survival or mortality.

Gerard et al. (1988) reported 100% mortality in eggs of *Plodia interpunctella* after exposure to 20 bar (4.04 kg/cm^2) for 30 minutes or 30 bar (6.06 kg/cm^2) for 10 minutes at room temperature. In the same study complete kills of beetle species such as *Stegobium paniceum* and *Tribolium confusum* required 120 minutes exposure at 20 bar. Increasing this to 30 bar shortened the exposure time necessary for complete kill to 15 minutes in *S. paniceum* and 40 minutes in *T. confusum*. A further increase in pressure to 40 bar (8.08 kg/cm^2) correspondingly cut the lethal time to 10 miutes in both species. Although the pressures utilised in this work were much lower than the ones reported in the current work, high levels of mortality were likewise obtained. This could be attributed to the low temperature —10°C— utilised by Gerard et al.

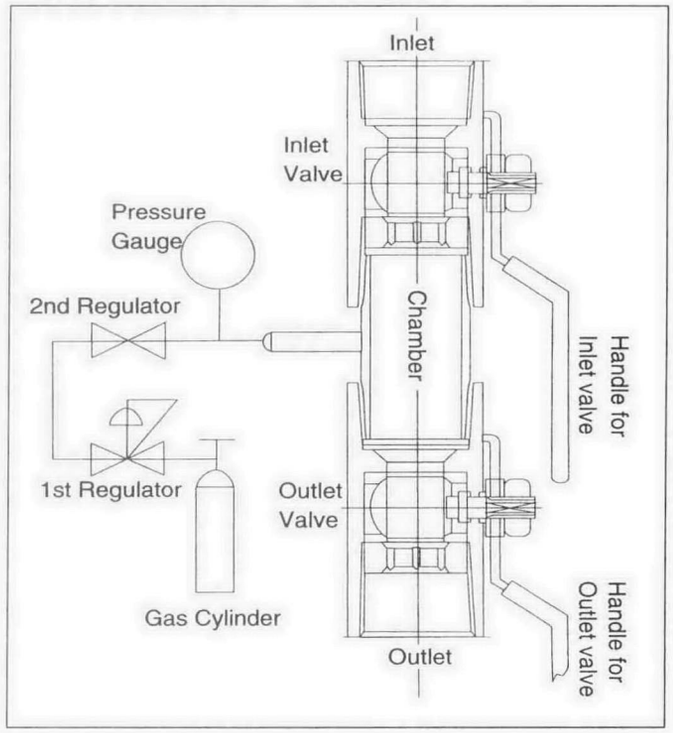

Fig. 1. Schematic diagram of pressurized cylinder and experimental set-up for CO_2 treatment under high pressure.

Experiments were also carried out to determine the effect of the new technique on the viability of rice seeds. Mean lifespan of the seeds was retained at carbon dioxide pressures of 10 and 20 kg/cm^2 for up to 60 minutes exposure while it declined slightly at 30 kg/cm^2 after a 10 minutes duration although this was not significant.

Table 1. Mean percent[a] survival of immature stage of *Corcyra cephalonica* exposed to high pressure carbon dioxide.

	Egg[b]		Larva[b]		Pupa[b]	
	Exposure time		Exposure time		Exposure time	
Pressure (kg/cm^2)	5 min	10 min	5 min	10 min	5 min	10 min
5	53.33	48.33	96.67	96.67	0.00	0.00
10	40.00	33.33	63.00	71.67	0.00	0.00
20	0.00	0.00	0.00	0.00	0.00	0.00
30	0.00	0.00	0.00	0.00	0.00	0.00

[a]Mean of 3 replicates.
[b]Means of control are 61.67%, 100% and 93.33% for egg, larva and pupa, respectively.

Table 2. Mean percent[a] survival of immature stage of *Tribolium castaneum* exposed to high pressure carbon dioxide.

	Egg[b]		Larva[b]		Pupa[b]	
	Exposure time		Exposure time		Exposure time	
Pressure (kg/cm^2)	5 min	10 min	5 min	10 min	5 min	10 min
5	60.00	63.33	76.67	66.67	16.67	16.67
10	75.00	63.33	16.67	13.33	3.33	6.67
20	60.00	23.33	0.00	0.00	0.00	0.00
30	0.00	0.00	0.00	0.00	0.00	0.00

[a]Mean of 3 replicates.
[b]Means of control are 68.33%, 100% and 100% for egg, larva and pupa, respectively.

Conclusion/Recommendation

Survival of any stage of all species herein investigated can be completely suppressed by CO_2 treatment released at a high pressure of 30 kg/cm^2 for 5 minutes.

The mechanism of action of CO_2 released at high pressure on target insects is not yet fully understood. However, it is well known that the solubility of gases increases at higher pressures. Thus, the toxic action of carbon dioxide could have been enhanced by its greater solubility in the insect hemolymph leading to a better penetration of, and possibly greater interaction with, the sites of action. Stahl et al. (1985) explained that the treatment acts by increasing the respiration and solution in intestinal liquids. In addition, it destroys the cell membranes during rapid decompression.

The results obtained further support the strong potential of this novel procedure as a feasible alternative for quick disinfestation of agricultural commodities, in particular for quarantine treatment of exported and imported grain commodities. In addition, this technique poses minimal risk to workers, leaves no harmful residue and is environmentally safe.

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