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DISINFESTATION OF STORED GRAINS USING HIGH- PRESSURE CARBON DIOXIDE

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ABSTRACT

Although low temperature storage is widely used in Japan for protecting rice from both insect infestation and fungus infection, CA storage with CO_2 has been used occasionally for insect disinfestation of commodities in room temperature storage, as an alternative to the commonly used treatment with phosphine. CA would be one of the safest control methods for the stored-product protection. However, it needs longer exposure times to control the insect pests compared with those required for conventional fumigants. To overcome this, we developed the application of CO_2 at a high pressure, followed by a sudden pressure loss, that kills insects quickly due to both the high concentration of CO_2 and damage to internal tissues. The method was successfully scaled up using a rotary valve system, which allowed continuous treatment of large amounts of grain in a short time. Thus the method is considered as a promising alternative to fumigants.

INTRODUCTION

Application of high-pressure carbon dioxide (CO_2) for disinfestation of stored-product insects has attracted attention since the method is capable of killing insects within extremely short exposure periods, compared to those of conventional controlled atmosphere (CA) treatments with CO_2. The first attempt to use this method was described by Mitsura *et al*.,(1973) who reported that compressed CO_2 had a potent disinfesting effect against the grain mite, *Tyrophagus putrescentiae* in foodstuffs. This test was conducted using a 200-mL autoclave at various pressures and exposure times with different gases, namely, CO_2, nitrous oxide, nitrogen, and hydrogen. Mitsura *et al.,* (1973) recommended a CO_2 treatment at 16 bar pressure for 10 minutes at 40% CO_2. However, in those days, there was little restriction on the use of fumigants and consequently this method was not put into practice. Stahl *et al.,* (1985) demonstrated that some of the stored-product insects infesting medicinal plants can be eradicated with high-pressure CO_2 within a short period. The first model chamber based on this method was manufactured in 1988 by Kohlensaure-

Werke Rud Buse Co. (Pohlen *et al.,* 1989) and has been commercially applied to disinfest herbs, spices, and pet foods in Germany.

At present, high-pressure CO_2 is of considerable interest to researchers for controlling insects that infest grains or other agricultural products as an alternative to methyl bromide (MB). However, to the best of our knowledge, trials on a commercial scale using this system have not been conducted, except for a recent trial in Japan. In this report, we review the lethal effects of high-pressure CO_2 on insect pests in stored-grains, and discuss our recent laboratory data on the lethal effects of CO_2 on eggs and larvae of some major insect pests of grains. We also describe a newly developed high-pressure CO_2 'disinfester' (disinfesting apparatus) with rotary valves that can continuously treat a moving stream of grains.

LETHAL EFFECTS OF HIGH-PRESSURE CO_2 ON STORED-GRAIN INSECTS

Over the last decade, some 10 papers have been published describing the lethal effects on stored-grain insects produced by the high-pressure CO_2 method. The compiled information presented in Table 1 shows the pressures and the exposure times required to produce a sufficient lethal effect on the developmental stages of different species of stored-grain insects, together with other experimental conditions such as temperature, equipment size and how the gas is released.

For *Sitophilus* spp, which are major pests of cereal grains, data on all stages of *Sitophilus oryzae* (Locatelli *et al*., 1999), *S. zeamais* (Nakakita and Kawashima, 1994) and *S. granarius* (Prozell and Reichmusth, 1991), have been collected. Among these species, *S. zeamais* seems to be the most susceptible since the exposure time needed to kill *S. zeamais* was much shorter than for the other two species. Moreover, our results indicated that the egg is the most tolerant stage, requiring 30 bar pressure for 5 min exposure for complete kill. The tolerance levels are progressively lower for larvae, adults and pupae. In *S. oryzae*, the sensitivity of the adults was very high, whereas all other stages were killed under the same conditions of 20 bar and 45 min exposure. With *S. granarius,* all stages required a pressure of 20 bar for 60 min to achieve complete mortality. However, the exact differences in tolerance levels among stages of *S. granarius* were not established because exposure periods of less than 60 min were not carried out in this study. Nevertheless, it was shown that lowering of the temperature reduced the efficacy of the high-pressure treatment, especially for the egg stage.

Lethal effects of CO_2 under high-pressure have been determined for developmental stages of other species of stored-grain insects, including *Tribolium castaneum* (Caliboso *et al.* 1994), *Lasioderma serricorne* (Ulrichs *et al*., 1997; Ulrichs, 1994), *Oryzaephilus surinamensis* (Locatelli *et al*., 1999), *Plodia interpunctella* (Reichmuth and Wohlgemuth, 1994) and *Corcyra cephalonica* (Caliboso *et al*, 1994). It was found that the egg stage was the most resistant in all the species. In particular, for *L. serricorne*, which rarely infests grains, the eggs stage was the most difficult to kill, requiring an exposure of 40 bar for 20 min at 25°C to achieve complete kill. Moreover, it again became clear that the high-pressure method

is less effective at lower temperatures, since it required almost 50% longer exposure periods to kill the eggs of *L. serricorne* when the temperature was lowered from 25 to 15°C (Ulrichs *et al.*, 1997). According to Yoshihashi and Caliboso (personal communication), the results obtained on *Trogoderma granarium* under high-pressure CO_2 in the Philippines, showed that 30 bar for 30 min exposure at 30°C were required for a 100% kill of eggs.

Compared to the data reviewed in Table 1, the data from our studies with several insect species always showed a greater lethal effect. We used a 30 mL capacity chamber, which was able to release the compressed gas instantly. The greater lethal effect might have been due to tissue destruction, which is caused by rapid CO_2 expansion during the instantaneous pressure release (Ulrichs, 1994; Caliboso *et al.,* 1994; Nakakita and Kawashima, 1994; Ulrichs *et al.,* 1997). However, conflicting results were observed with eggs of *P. interpunctella* (Reichmuth and Wohlgemuth 1994).

LETHAL EFFFECTS OF HIGH-PRESSURE CO_2 ON IMMATURE STAGES OF STORED-GRAIN INSECTS

A perusal of the lethal doses given in Table 1 indicates that the eggs are the most tolerant stage to high-pressure CO_2, followed by larvae, adult and pupae. To obtain detailed mortality curves of both eggs and larvae of the major grain pests, we used a newly designed 1-L chamber as shown in Plate 1, in which CO_2 pressure and exposure time are automatically regulated and the gas is rapidly released within 1sec when the exposure period is completed. Figure 1 shows the mortality curves depicting both pressures and exposure times required for 100% mortality of eggs 1-3 days after oviposition, of 6 species of stored-product insects: *S. zeamais*, *S. oryzae*, *Rhyzopertha dominica*, T. *castaneum*, *P. interpunctella* and *Sitotroga cerealella*. Although all mortality curves were inversely proportional between pressures and exposure time, their gradients differed greatly between species. The most tolerant eggs were those of *S. zeamais*, followed by *S. oryzae, S. cerealella*, *T. castaneum*, *R. dominica* and *P. interpunctella*.

Figure 2 shows the mortality curves of late instar larvae of *S. zeamais*, *S. oryzae*, *R. dominica* and *P. interpunctella*. The higher pressures effectively decreased the exposure periods required to kill the larvae, but larvae of *R. dominica* were extremely difficult to control. The order in species tolerance of the larvae was completely different from that of the eggs; *R. dominica* was the most tolerant species, followed by *S. oryzae*, *P. interpunctella* and *S. zeamais*.

Ulrichs *et al.,* (1997) recognized that temperature has an intense influence on the lethal effects of high-pressure CO_2 on the eggs of *L. serricorne*. A similar result was obtained when eggs of *S. zeamais* were subjected to 20, 25 and 30°C under high-pressure CO_2. As shown in Fig. 3, treatment at 30°C enabled the exposure time needed to kill these eggs to be shortened in comparison with exposure at 25°C. However, there was not much influence on the gradient of mortality curves between 25 and 20°C, although the lower temperature did require a longer exposure time.

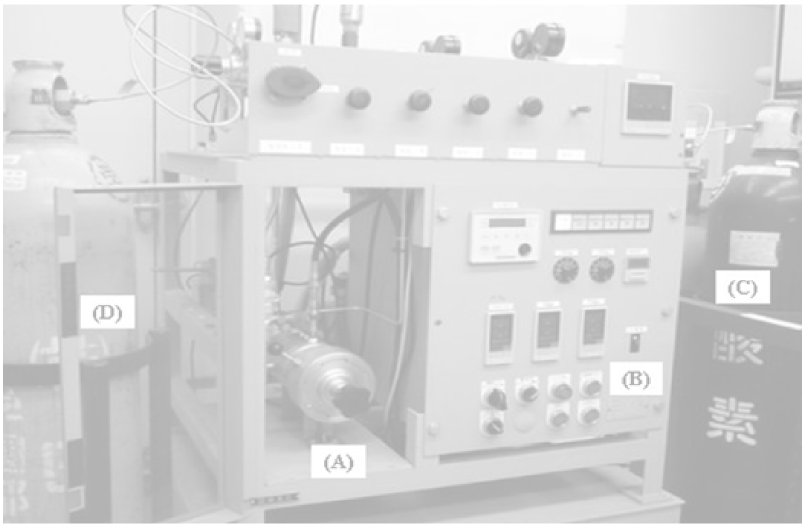

Plate 1: showing newly designed high-pressure chamber

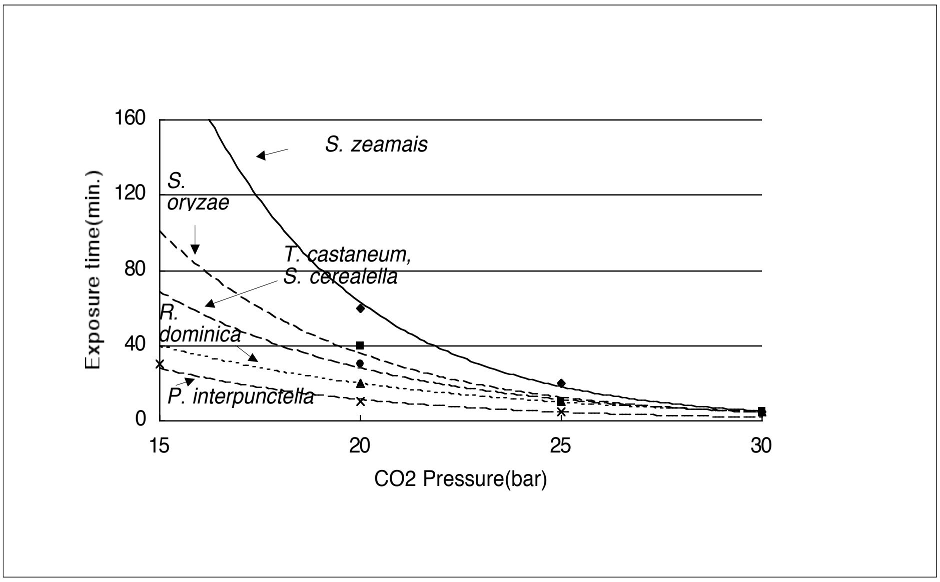

Fig. 1. Relationship between pressure and exposure time for 100% mortality of eggs of stored grain insects

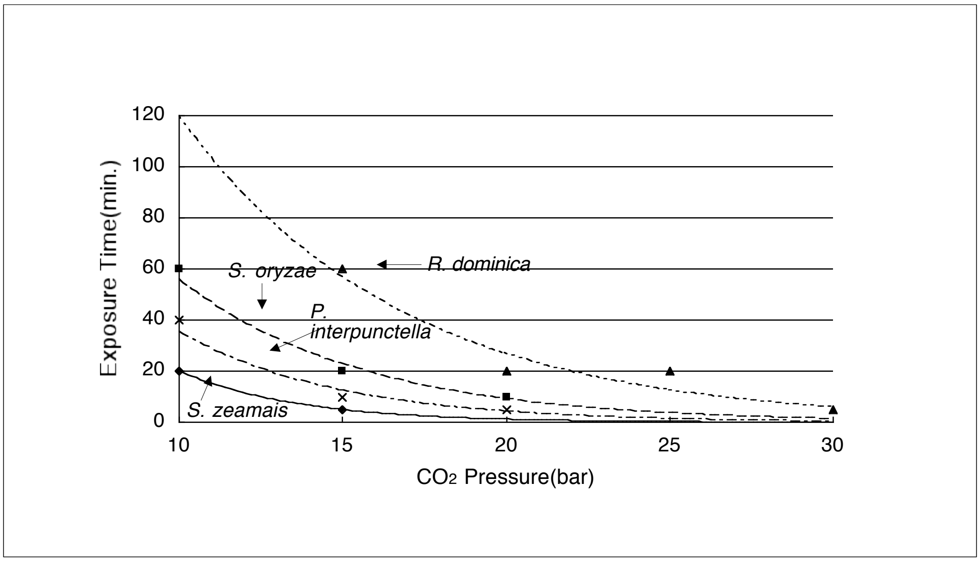

Fig. 2. Relationship between pressure and exposure time for 100% mortality of larvae of stored grain insects

DISINFESTATION OF GRAINS WITH A ROTARY VALVE SYSTEM OF HIGH-PRESSURE CO_2

Based on the data we have accumulated, we devised a practical method for using high-pressure CO_2 to disinfest stored grains. The batch-type compression treatment using a large chamber, of up to 30 m^3 capacity has already been used commercially for disinfestation of herbs in Germany (Pohlen *et al.,* 1989; Hirano and Nakakita 1995; Reichmuth, 1997). However, this chamber is not very efficient for disinfestation of large quantities of grain because it is highly labor-intensive. This problem was solved by the design of a high-pressure 'disinfester' with rotary valves, recently developed by Mitsubishi Heavy Industries under the Seiken Organization in Japan (Plate 2). This equipment can continuously treat grains that are transported on a belt conveyer, by a process of compression-decompression. Figure 4 shows the principles and structure of this piece of equipment. The rotary valve has 14 chambers called pockets (2.1-L capacity each) fixed radially around the axis of rotation. The circumferential surface of each pocket is lined with ceramics, which have a packing effect, and the rotary part is contained in a sealed vessel called the body. The grain feeding ports are placed above and below the body, and the pockets are serially filled with grains from the upper port. When the pocket reaches the sealed part, CO_2 is injected from the gas conduit to increase the pressure in the pocket. When the pocket reaches its lowest position, the grains fall into the disinfestation tank filled with compressed gas. The grains are driven in the tank horizontally by a screw conveyer and reach the pocket of the lower rotary valve, the gas escapes through the gas outlet on the side of the pocket as the valve rotates, and the CO_2-treated grains are recovered from the opening at the bottom of the machine. In our tests, all stages of major stored-grain insects, including the tolerant stages of both eggs of *S. zeamais* and larvae of *R. dominica* were completely controlled with this 'disinfester' at conditions of 30 bar for 15 min exposure at 25°C.

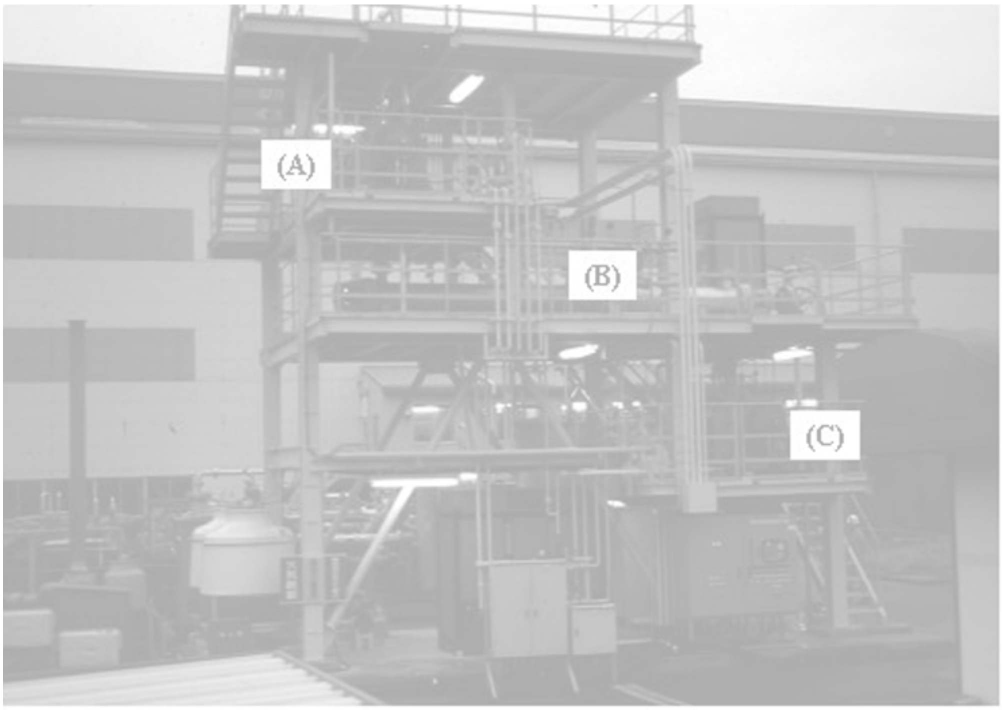

Plate 2. Commercial scale high-pressure CO_2 rotary valve insect 'disinfester'.

CONCLUSION

On the basis of our findings using small capacity high-pressure chambers, we successfully completed a scale-up to the development of a rotary valve 'disinfester' processing 6 tonnes/h (to a maximum of 40 tonnes/h) for cereal grains under high-pressure CO_2. In Japan, insect infestations in brown rice are presently suppressed by storing the rice at low temperatures (15°C). However, this temperature is not lethal, and there are claims of insect contamination of rice during the distribution process after polishing. Therefore, by installing this high-pressure CO_2 'disinfester' before the polishing process any problems caused by insect infestations could be eliminated. The costs of this equipment, including its installation, and based on 10 years continuous use, was calculated by Mitsubishi to be approximately US$ 13/tonne.

This cost is reasonable for treating stored milled rice because the market price of one tonne of milled rice can be as high as US$ 3,000 in Japan.

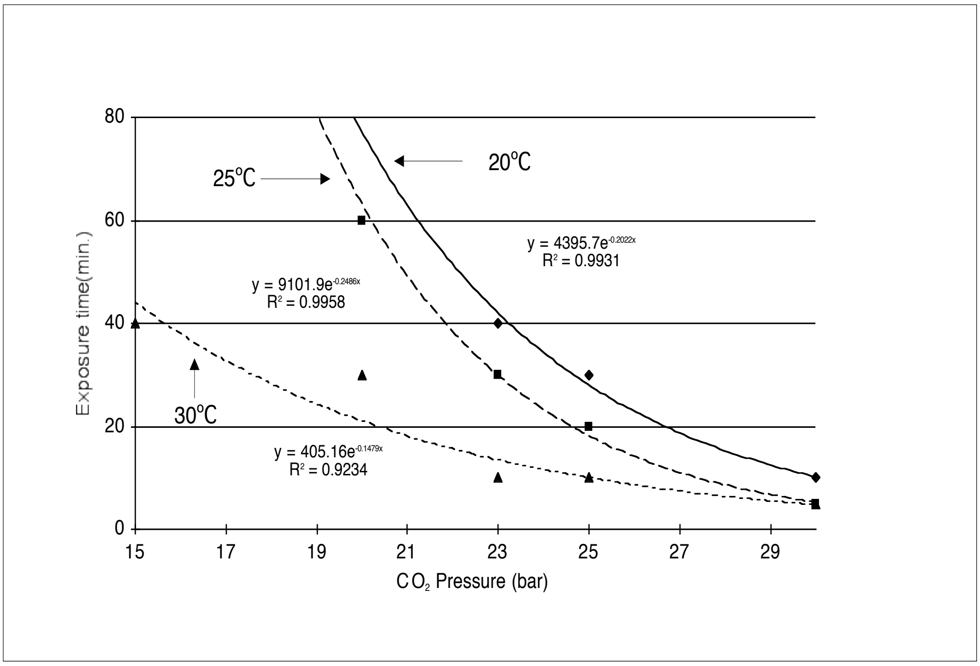

Fig. 3. Lethal curves for eggs of *Sitophilus granarius* under high pressure at different temperatures.

Another potential application is in quarantine where methyl bromide has been extensively used in Japan. Japan imports over 30 million tons of grains and beans from foreign countries annually, and about half of these consignments are contaminated by stored-product insects. The newly developed rotary valve 'disinfester' described here is an alternative to methyl bromide for quarantine treatments, since it can be installed as an on-line unit for imported commodities while they are transported by conveyer from ship to warehouse. However, the current design and operation of the 'disinfester' may not be sufficient to eradicate eggs of *T. granarium,* which is the most important quarantine pest. To eradicate *T. granarium* under high-pressure CO_2, it may be necessary to combine CO_2 with a small dose of a fumigant.

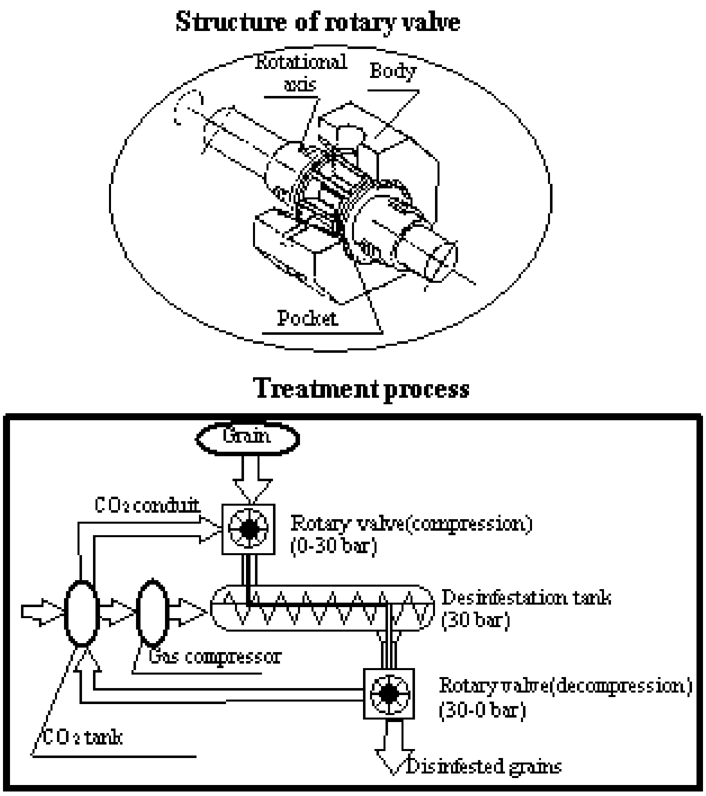

Fig. 4. Details of the commercial scale rotory-valve high pressure CO_2 insect 'disinfester'.

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