Carbon dioxide under high pressure of 15 bar and 20 bar to control the eggs of the Indianmeal moth *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae) as the most tolerant stage at 25°C

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Abstract

The combination of carbon dioxide and high pressure has been described and applied for stored-product pest control over the last ten years. The method requires autoclaves to hold pressures of about 20 to 40 bar. The greatest advantage compared to conventional control measures is the short lethal exposure period, in the range of minutes to a few hours depending on the pest species and stage of development.

The high cost of autoclaving restricts its use to high value products such as cocoa beans, nuts, almonds, drugs and spices, but this method could be adapted for grain in export and quarantine situations.

The present study describes in detail the efficacy of this new method on eggs of the Indianmeal moth *Plodia interpunctella*, the major insect pest in the German food industry. The experiments were carried out in the laboratory in a special chamber of 200 mL volume at controlled temperature of 25°C.

Very young eggs proved to be most tolerant, and 100% mortality was achieved within 40 minutes at 20 bar under carbon dioxide. 1 to 4-day-old eggs required about 20 minutes of exposure to 20 bar for complete kill.

Introduction

In the course of the extended study of the action of 'physically toxic' substances on the granary weevil by Ferguson and Pirie (1948), gases were applied at pressures much above atmospheric to attain the required lethal effects (Ferguson and Hawkins 1949). The authors pointed out very clearly that the actual concentration within the organism is the most important criterion to judge the toxicity of a substance. Most of the poisons proved to have a combined physical and chemical effect which was supported by narcosis of the organism. Unfortunately, the authors did not test carbon dioxide.

Mitsura et al. (1973) described treatments of the grain mite *Tyrophagus putrescentiae* with four different gases. For the first time, carbon dioxide was identified to be lethal within less than 60 minutes of exposure to all investigated stages within a pressure range of 6 to 26 bar. Dinitrogen monoxide, in contrast, caused only incomplete mortality, and hydrogen and nitrogen had very little effect.

The initial approach of Stahl and co-workers (Stahl and Rau 1985; Stahl et al. 1985) to control pest insects in drugs with pressurised carbon dioxide was prompted by the ban of ethylene oxide in Germany. During the search for alternative quick methods of disinfestation, among many gases tested, CO_2 under pressure showed the surprising property to kill all stages of the tested insects within less than 3 hours. Sometimes only minutes were needed. The group worked in the area of extraction of natural products with supercritical fluids and tried to control microorganisms with high pressure treatment (Rau 1985). Therefore, the step to apply carbon dioxide to insects was within range.

In the meantime, this method is common in Germany (Gerard et al. 1988a, b; Anon. 1989; Pohlen et al. 1989; Gerard et al. 1990; Corinth and Reichmuth 1991; Finkenzeller 1991; Reichmuth 1991, 1993; Rau 1993) and is registered as a stored–product protection procedure by several firms[1]. To be precise, it is the carbon dioxide under high pressure which is registered as a substance. Side effects on the quality of the treated products have been investigated and seem to be negligible (Pohlen et al. 1989). Still, the mode of action is not fully understood. It must be linked to the high solubility of carbon dioxide in water which enables a dramatic increase in uptake under high pressure. The question arises as to how much the rapidity of expansion after the pressurisation is responsible for a quick evaporation of the gas from the liquid leading to lesions of cell membranes (diver's disease). Gerard et al. (1988b) determined insect eggs to be the most tolerant stage, which supports the hypothesis: eggs have a low water content and very few cell membranes and are most stable as spheres.

This study reports on experiments on the efficacy of pressurised carbon dioxide against eggs of the Indianmeal moth *Plodia interpunctella*, one of the currently most important pest insects in the German food processing industry. This insect also causes losses in drugs and spices, and leads to various customer complaints.

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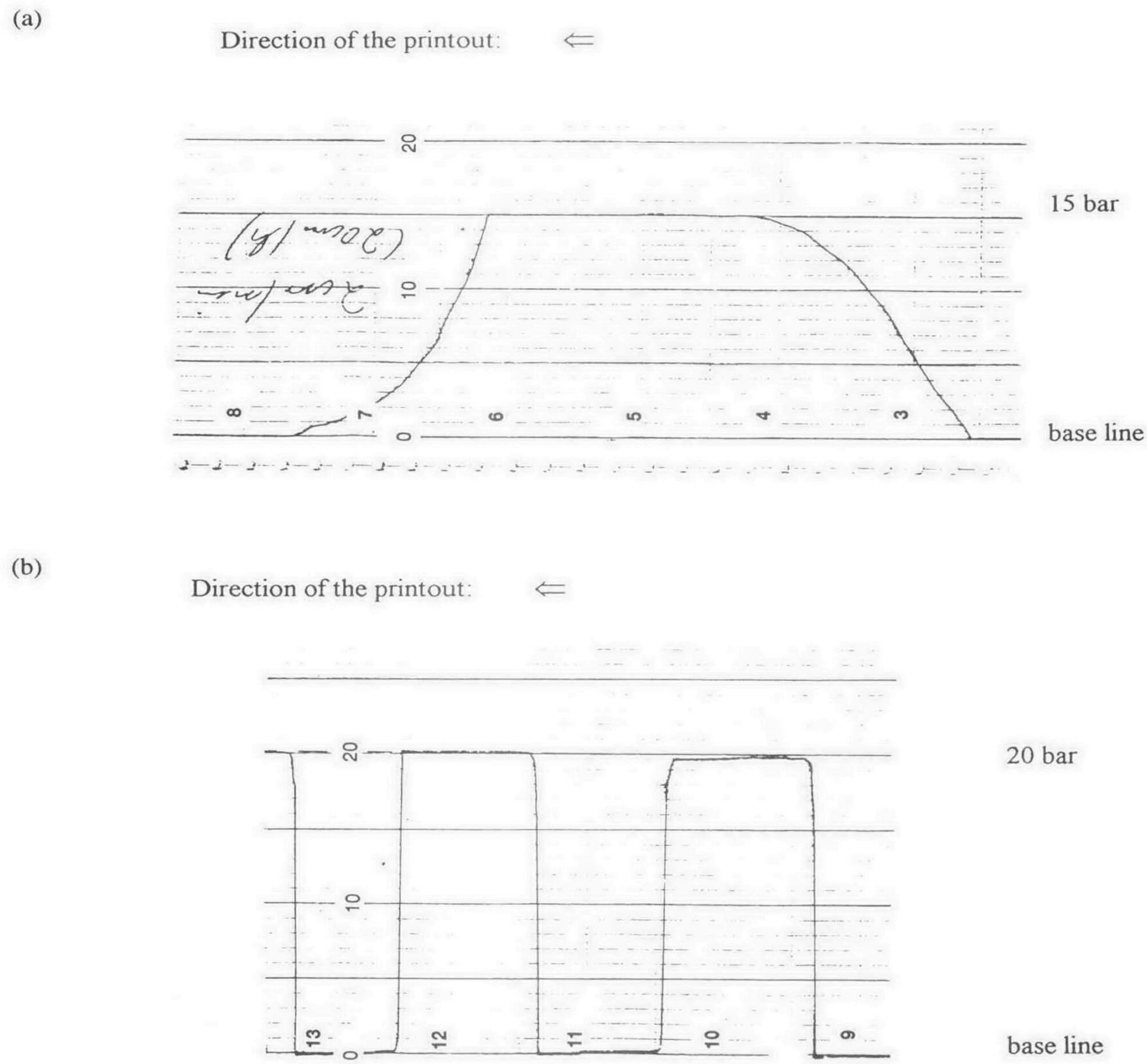

Fig. 1. Recorder printout of the speed of pressure build up and decay in the experimental CO_2 fumigation chamber, paper speed: 2 cm/ minute (a) low speed (s=slow) 1 minute, (b) high speed (q=quick) 1 second. Direction of printout ←

Materials and Methods

Insects

The eggs of *Plodia interpunctella* were taken from a strain which has been cultured at 25°C and 75% r.h. in the Institute for Stored Product Protection in Berlin, for more than 30 years. Adult moths laid their eggs during a sharply controlled time span of 22 hours or 2 hours, respectively. Egg age was adjusted by storing the freshly laid eggs for different times at 25°C.

The treated eggs were transferred into small glass rings of 13 mm diameter with nylon gauze-covered bottom and closed by a stop cock. The mesh of 250 µm allowed the hatching larvae to escape into the wheat bran outside the glass ring inside a Petri dish. One week later, dead eggs and husks of eggs which had been left behind by the hatched larvae were counted.

Fumigation procedure

After counting the eggs into small Petri dishes, batches of 4 with 100 eggs each were exposed to the pressurised carbon dioxide in a small laboratory pressure-tight chamber made of brass and of 400 mL volume. Water from a thermostat was pumped through copper tubes around the pressure cell to adjust the temperature at 25°C. The changes of pressure caused an increase to 32°C during pressurising and a decrease to about 18°C during depressurisation. These short-term temperature deviations, however, were readjusted to 25°C within seconds. Since the pure gas was taken from a cylinder, humidity was very low. The speed of pressure build up and decrease could be adjusted. Two types of pressure change were distinguished: quick pressure change within 1 second and slow pressure change within 1 minute to and from the required value (see Fig. 1). In both cases, only the exposure time at the final pressure was recorded. The final pressure and the speed of pressurisation and depressurisation were adjusted manually with valves and regulators before an experiment. By opening and closing of taps after introducing the eggs into the chamber, pressure was adjusted and recorded together with temperature. After the given exposure time aeration was started.

Pressure range tested

Previous experiments have shown that a pressure of 20 bars gives control of most of the insects within less than 2 hours (Prozell and Reichmuth 1990, 1991). Bearing in mind that each further bar requires the addition of an amount of carbon dioxide corresponding to the free volume in the chamber, the cost of treatment escalates rapidly with increasing pressure. To ensure the mechanical stability of the chamber for increasing pressure, the thickness of the walls of the chamber has to be increased. Safety and economy thus restrict the pressure range to less than about 40 bar. If sterilisation is the goal, higher pressures will be needed (Rau 1985). To use the chamber overnight or at weekends requires the determination of the lethal carbon dioxide pressure for longer exposure times.

Pressures of 15 and 20 bar were therefore selected to demonstrate some basic dependencies between exposure time, speed of pressure change, age of eggs, and mortality.

Results

Figure 2 contains information on the influence of the age of *Plodia interpunctella* eggs on the speed of control at 15 bar and 25°C . Quite clearly, the susceptibility is increasing with age from 1 to 4 days. The data in Figure 2(a) and (b) indicate that a slow change in pressure is more effective than the quick change. Because the quick change led to longer lethal exposure times, we identified the quick change as the experimental condition which resulted in the 'safest' determination of the exposure time needed to control eggs at a given age and pressure of CO_2. In other experiments not reported here quick pressure increase and slow pressure decrease, and converse conditions, were tested and found to support the quick change as the least effective (Reichmuth and Wohlgemuth, unpublished data). Comparing Figure 2 with corresponding data in Figure 3 shows the reduction in lethal exposure time from 30 to 10 minutes by pressure increase from 15 to 20 bar.

The mortality data in Figure 2 show pronouned scattering. This tendency was confirmed by data at 20 bar (Figure 3). Experiments were therefore continued at 15 bar with more precise differentiation between the ages. Young eggs had proven to be most tolerant. Accordingly, further treatments were carried out with 0–2, 2–4, 4–6, 6–8, and 22–24-hour-old eggs (see Figure 4). The scatter in mortality results was much reduced. Complete mortality of all tested eggs with an age of less than 8 hours occurred within 35 minutes of exposure. The acceleration of the control procedure by slower pressure changes can again be seen in Figure 4. Differences between the responses of these four different aged eggs were not very pronounced. Only the slightly older eggs in Figure 4(e) seemed to be more susceptible than the other tested young eggs. But they were more tolerant than the 1-day-old eggs in Figure 2(a).

Discussion

The egg stage of *Plodia interpunctella* is relatively tolerant to treatment with carbon dioxide under pressure compared with other stages (Gerard et al. 1988b). By differentiating the age of the eggs stepwise down to a Δ t of 2 hours, it could be shown that eggs less than 1 day old can survive exposure to CO_2 pressure of 15 bar for up to 40 minutes. The strongly changing susceptibility with age seems to be the reason for pronounced variations in response of eggs if they are not within well-defined age groups. If very young eggs are not included in the experiments to determine the necessary lethal exposure time, a recommendation of too-short exposure periods may result. The lethal effect is strongly dependent on the length of exposure and not on the speed of depressurisation. On the contrary, when pressure is slowly increased or decreased, the exposure to these slowly changing CO_2 pressures, increased above atmospheric, has already toxic effects. These seem to add to the effects caused at the final experimental pressure and reduce the lethal exposure period, compared with exposures when the pressure is very quickly adjusted within seconds. Temperature was not tested in this context, but it may play a role (Prozell and Reichmuth 1991). This supports the presumption that solubility in liquids, transport and reaction of carbon dioxide, and number of membranes which are temperature dependent, will all have a strong effect on the efficacy of pressurised CO_2 on insects.

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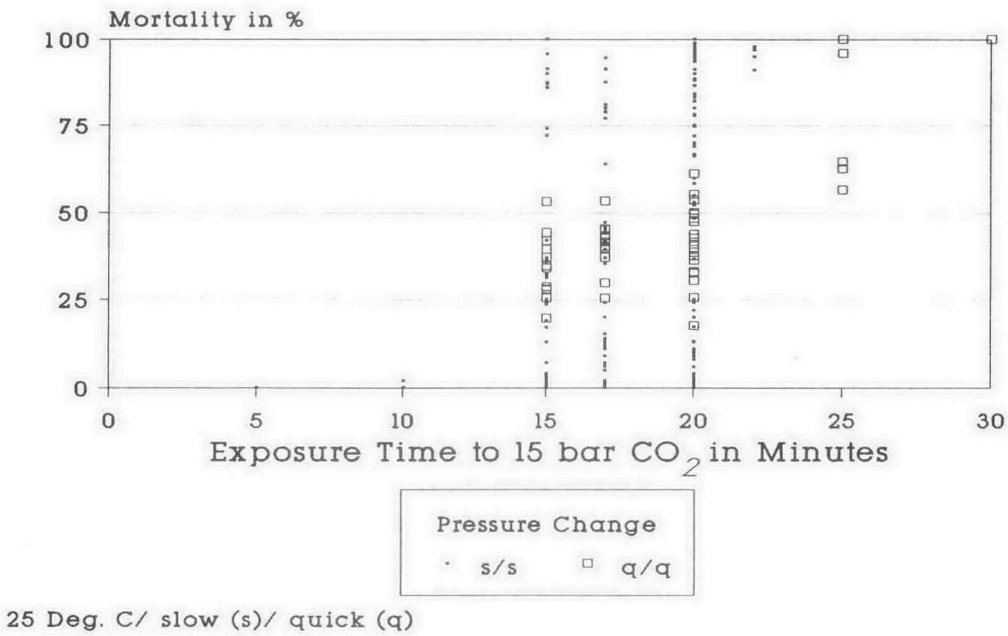
(a)



(b)

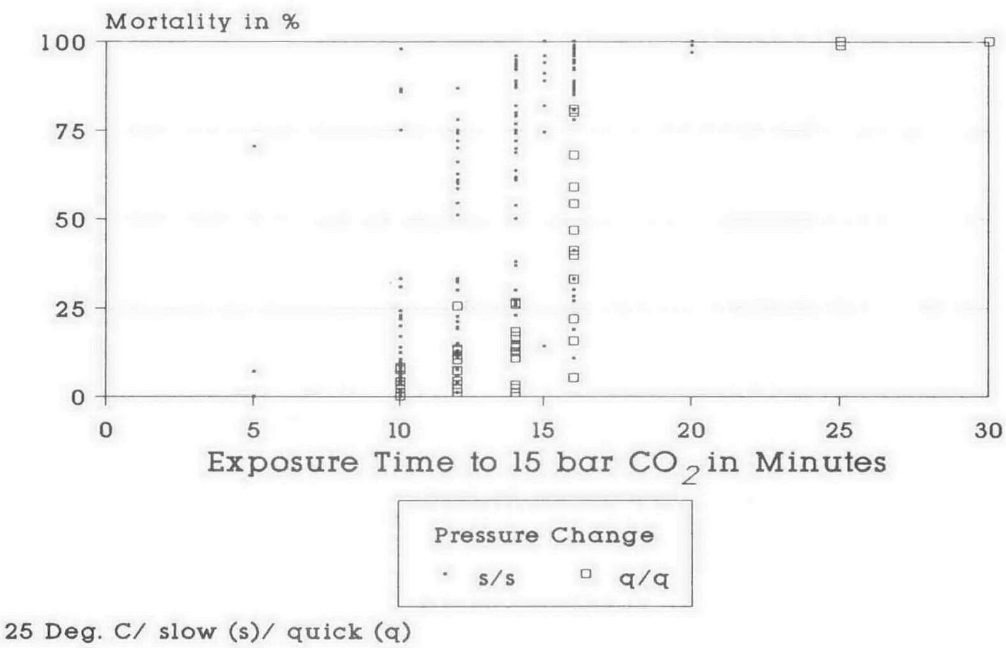
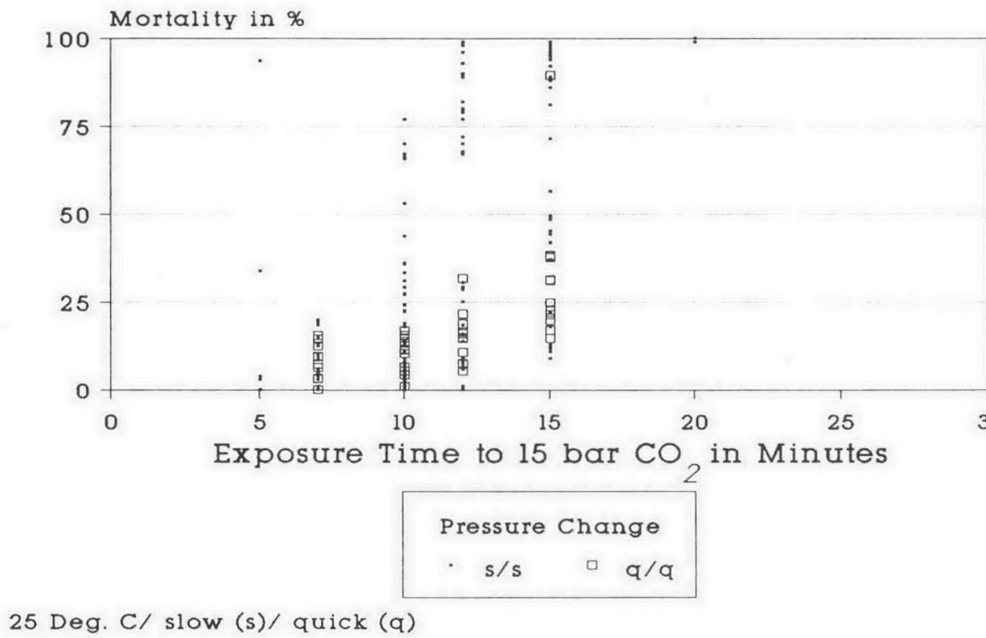


Fig. 2. Mortality results for the treatment of (a) 1 day- and (b) 2-day-old eggs of the Indianmeal moth *Plodia interpunctella* with 15 bar carbon dioxide at 25°C.

(c)



(d)

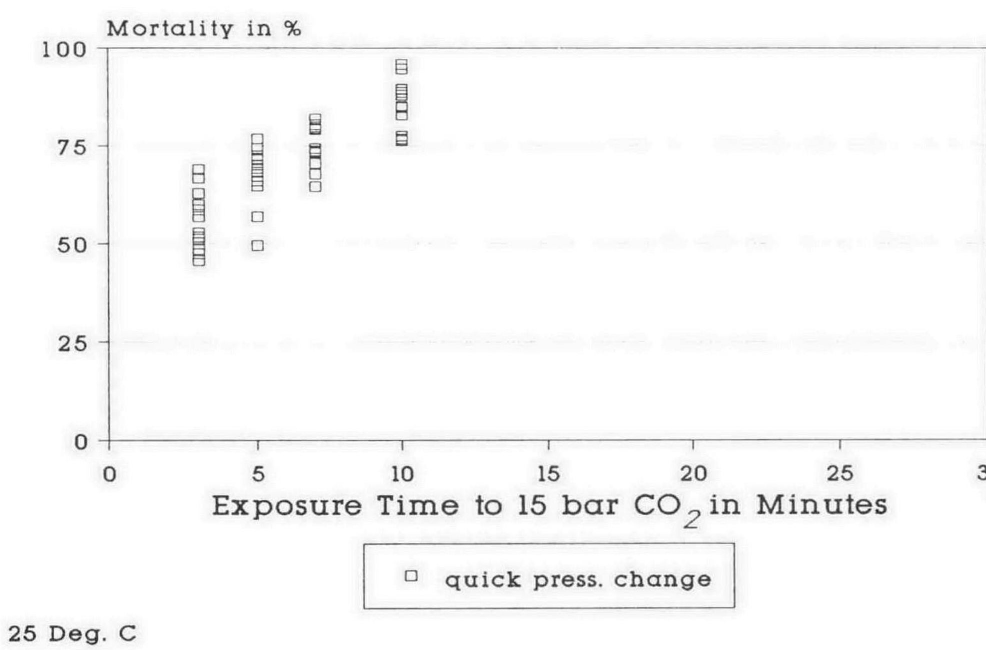
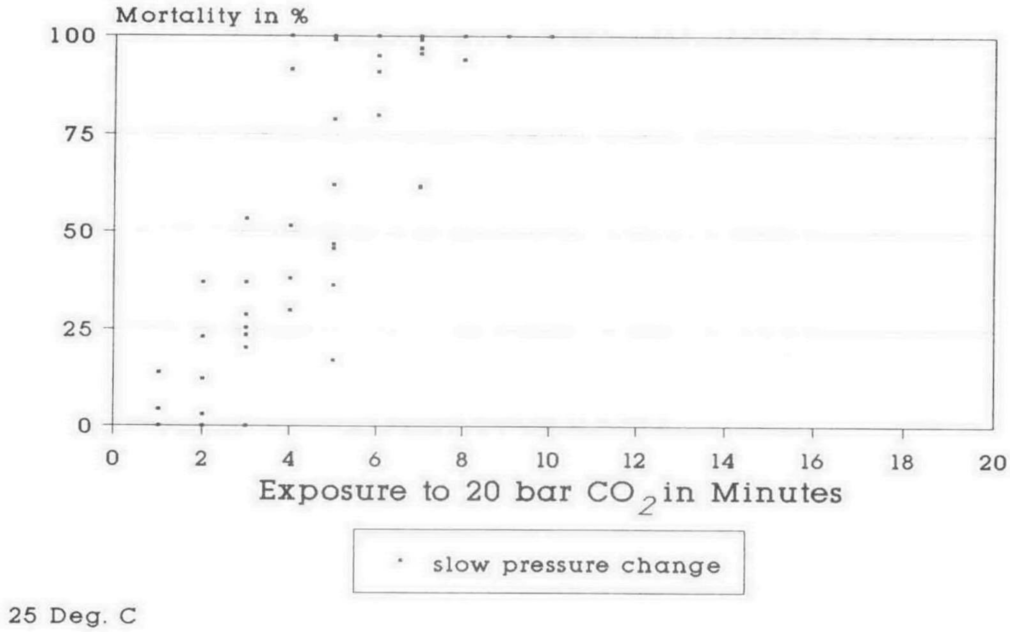


Fig. 2 – cont'd . Mortality results for the treatment of (c) 3 day- and (d) 4-day-old eggs of the Indianmeal moth *Plodia interpunctella* with 15 bar carbon dioxide at 25°C.

(a)



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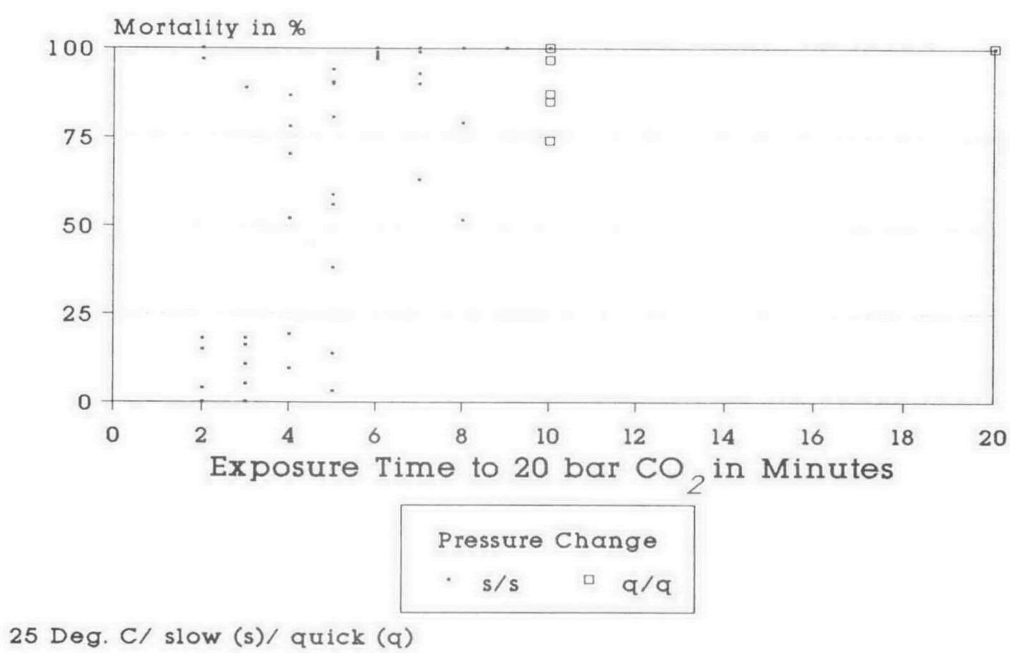
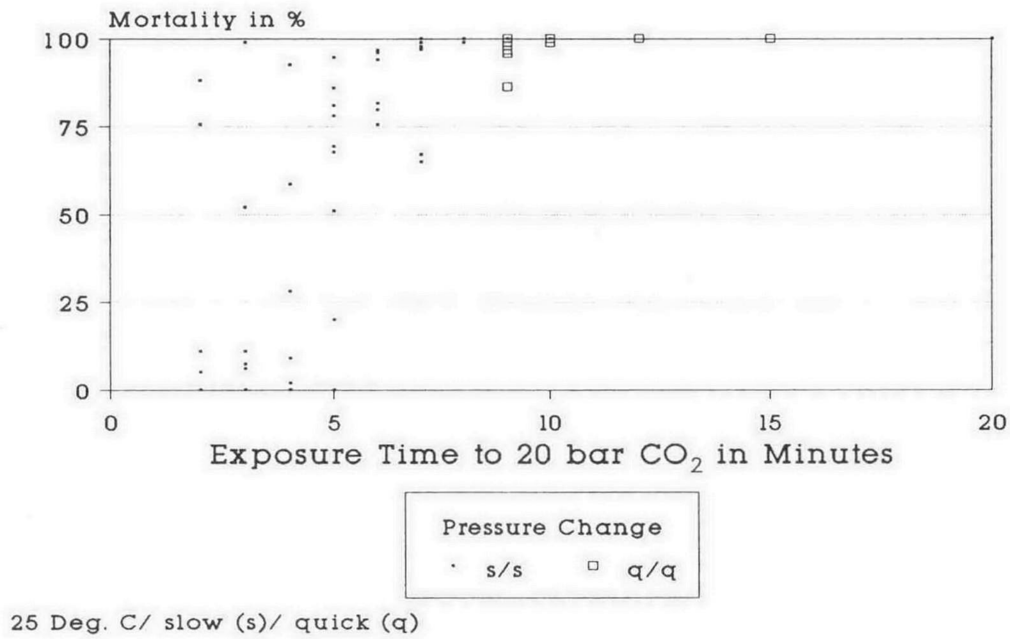


Fig. 3. Mortality results for the treatment of (a) 1 day- and (b) 2-day-old eggs of the Indianmeal moth *Plodia interpunctella* with 20 bar carbon dioxide at 25°C.

(c)



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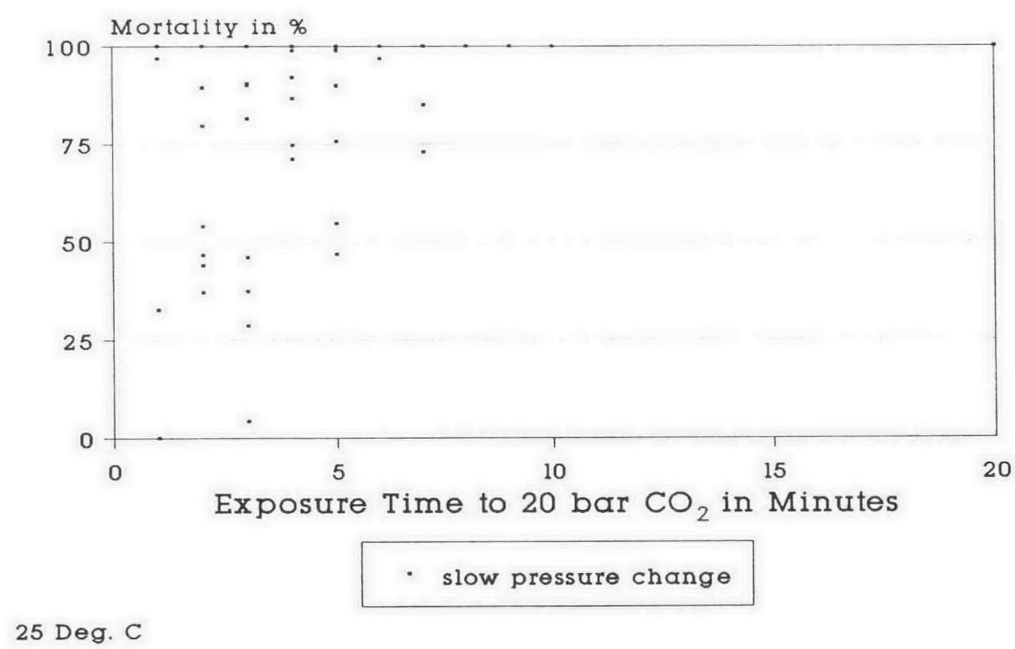
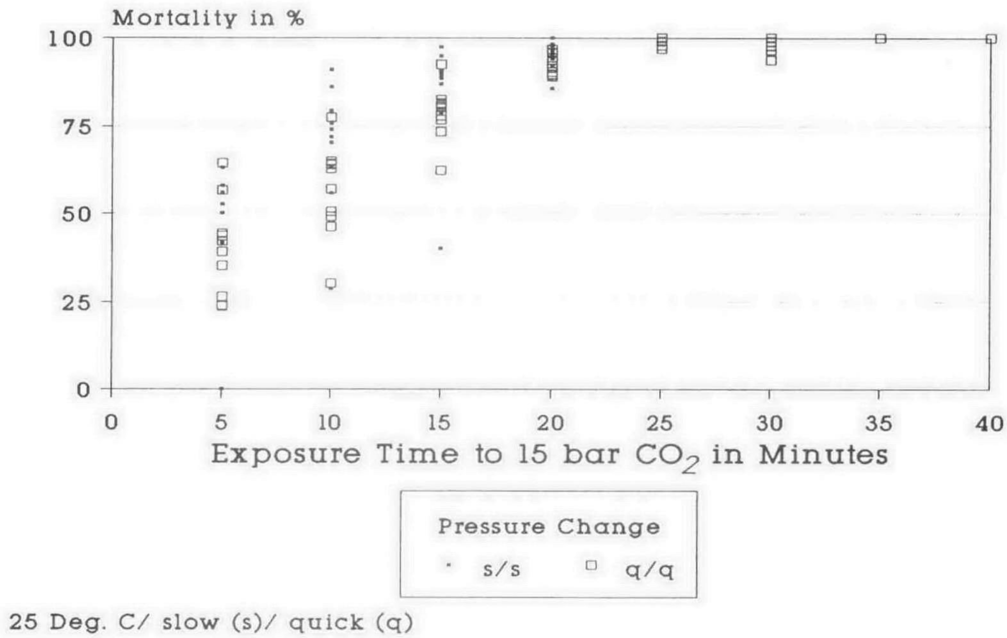


Fig. 3. – cont'd. Mortality results for the treatment of (c) 3 day- and (d) 4-day-old eggs of the Indianmeal moth *Plodia interpunctella* with 20 bar carbon dioxide at 25°C.

(a)



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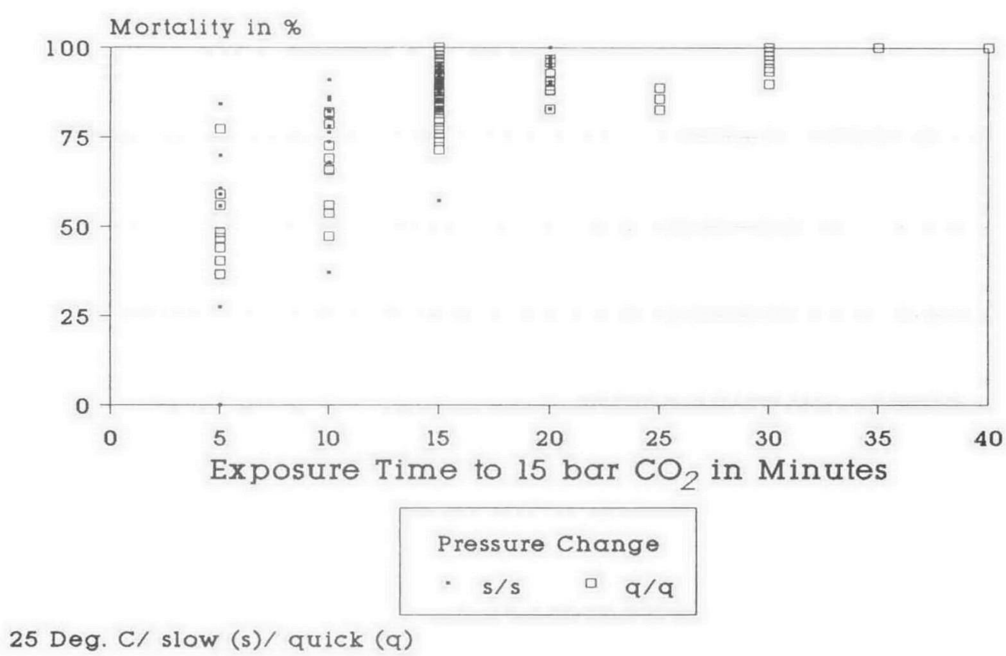
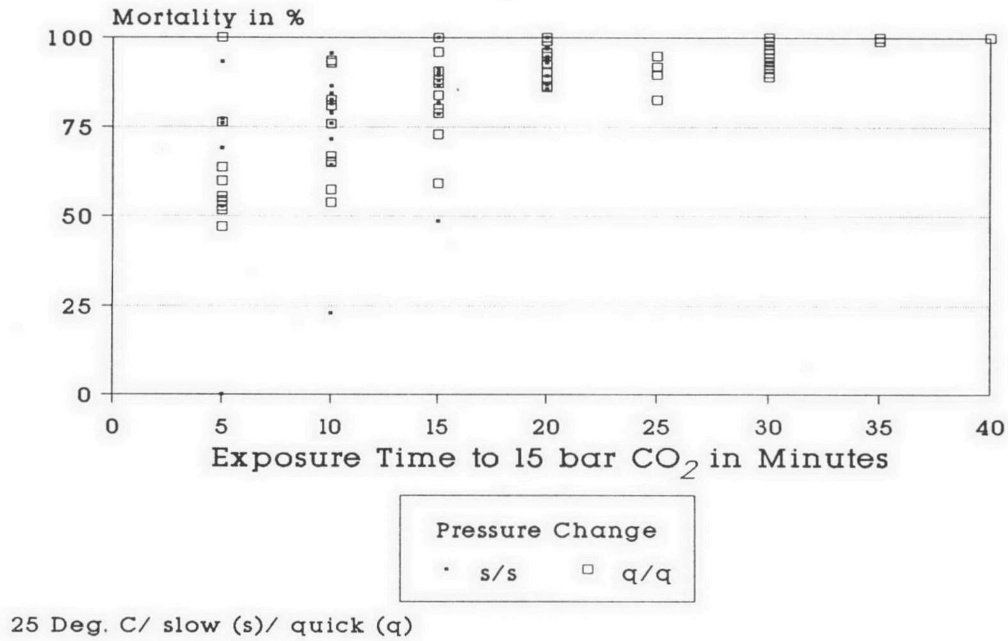


Fig. 4. Mortality results for the treatment of (a) 0–2 hour- and (b) 2–4 hour-old eggs of the Indianmeal moth *Plodia interpunctella* with 15 bar carbon dioxide at 25°C.

(c)



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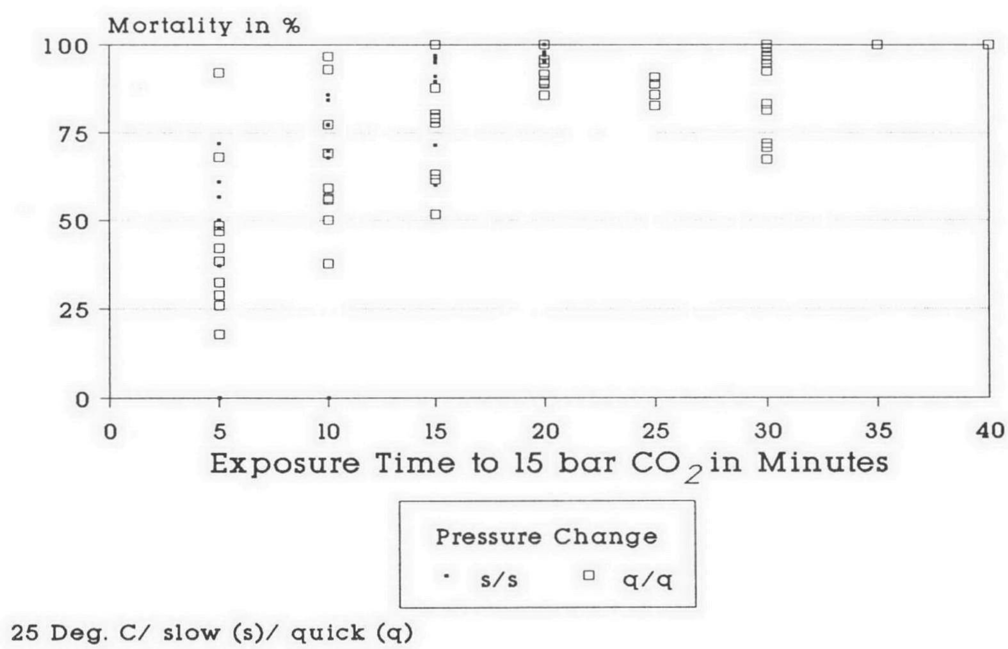

Fig. 4. – cont'd. Mortality results for the treatment of (c) 4–6 hour- and (d) 6–8 hour-old eggs of the Indianmeal moth *Plodia interpunctella* with 15 bar carbon dioxide at 25°C

(e)

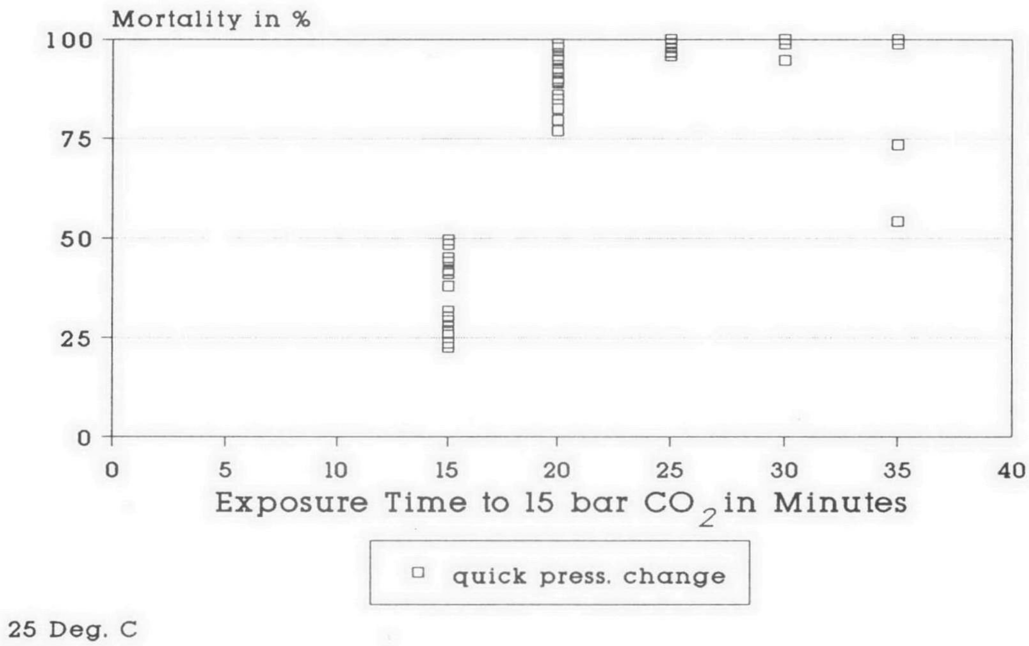

Fig. 4. – cont'd. Mortality results for the treatment of (e) 22–24-hour-old eggs of the Indianmeal moth *Plodia interpunctella* with 15 bar carbon dioxide at 25°C.

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