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CARBON DIOXIDE UNDER HIGH PRESSURE TO CONTROL THE TOBACCO BEETLE *LASIODERMA SERRICORNE*

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ABSTRACT

Pressurized carbon dioxide (CO_2) can control most of the important insect and mite pests within a few hours. A rapid increase from atmospheric pressure to 20 bar for several hours and subsequent decrease back to atmospheric pressure within a few minutes reduces the lethal exposure time to under 1 h. The short treatment time makes this method attractive for pest control, and it is especially feasible with high value products. Pressure tight chambers of up to 30 m^3 with CO_2 recapture apparatus to reduce gas emission are used for short-exposure pest control.

The lethal effect seems due to a combination of increased CO_2 solution in the insect tissues, leading to reduction of pH or increase in acidity, and to the rupture of cell membranes following depressurisation.

Laboratory and practical results are presented describing the possibility of controlling the tobacco beetle *Lasioderma serricorne*, which causes severe losses in the tobacco processing industry, at various CO_2 pressures at various temperatures.

INTRODUCTION

Control of stored-product insects with inert atmospheres at atmospheric pressure requires days or even weeks, depending upon the developmental stage and species, the commodity and, above all, the temperature. In contrast, effective disinfestation of stored products with carbon dioxide (CO_2) under increased pressure lasts only a few hours. Due to the relatively high treatment costs involved, this method is more suitable for such high-value commodities as spices, drugs of plant origin, tobacco, cocoa beans and hazel nuts. Stahl *et al.* (1985a, b) determined for the first time that insects could be controlled within minutes or hours by exposure to CO_2 under 10–50 bar pressure. In experiments by Gerard *et al.* (1988), the tobacco beetle *Lasioderma serricorne* proved one of the most tolerant insect species. The lethal effect is presumably due to the combined effects of pressure and CO_2. In addition to the physiological stress — increased pressure, especially during the process of quick

pressure build-up, and subsequently decreased pressure at the end of the treatment — caused to their cells, the insects also suffered from lack of O_2 and from changes in cellular acidity caused by sorption of CO_2.

The present paper tries to answer several questions concerning the differences in sensitivity of the different developmental stages of the tobacco beetle to CO_2 high-pressure treatment, the extent to which mortality is dependent on temperature, the effect on mortality of the speed of build-up and decrease in pressure, the possibility of delayed mortality among insects surviving the treatment and the correspondence between laboratory mortality results and those of a field trial.

MATERIAL AND METHODS

The experimental high-pressure chamber was originally designed by Dr R. Wohlgemuth, the former director of the Institute for Stored-Product Protection. The 8-mm steel cylindrical chamber has an inner diameter of 55 mm and a height of 80 mm, with a volume of 200 ml. It is sealed with a thick brass screw and a rubber gasket. The temperature in the chamber can be controlled by using water that is heated or cooled in a bath connected to copper tubes which surround the steel cylinder which in turn is embedded in styrofoam. A thermosensor inside the chamber and a pressure sensor in the gas supply tube were used to monitor the experiments.

The eggs, larvae and adults of the tobacco beetle *L. serricorne* were obtained from an established culture reared on tobacco leaves, bran and yeast at 25°C and 65% r.h. at the Institute for Stored-Product Protection in Berlin.

Experiments on eggs were undertaken at 15 and 25°C and at 25, 30, 35 and 40 bar over a range of exposure times. Because the beetles hide their eggs by sticking them on the tobacco, they could not be counted in advance. Therefore, it was only possible to distinguish between complete and incomplete control. For all other results, an LT_{95}, derived from probit analysis of the mortality results, was calculated.

Larvae and adults were examined at 15, 25 and 35°C, and at 15, 20, 25, 30, 35, 40 and 45 bar, over a range of exposure times; additional insects served as controls.

The effect of temperature on adult beetles was investigated at 15, 25, 30, 35 and 45°C and at 20 bar of CO_2.

In further experiments, the speed of pressure change from 1 to 20 bar was varied between 1 and 2 min. In five replications a total of 400 adult beetles were exposed at 25°C to the four possible alternative combinations of slow and quick build-up and decrease of pressure. The treatment period of 5 min at a final pressure of 20 bar was constant. The bioassay was carried out 24 h after treatment by counting living insects.

There were three investigations into delayed mortality during the post-treatment period. Replicates of 100 adults were treated with exposure periods of 5, 10 and 15 min at 20 bar and 25°C, with build-up and decrease of pressure within 2 min. After treatment, both the treated beetles and untreated control insects were placed on tobacco and bran at 25°C and 65% r.h. and checked daily for mortality.

In the commercial scale trial, 12 cages containing insect samples were placed in the centres of three compressed 2-m^3 tobacco bales. They were treated for 17 h at 2 to 4°C with a pressure build-up of 100 min and depressurisation within 15 min. The samples were regularly examined for surviving insects for a period of 10 weeks after the treatment.

RESULTS AND DISCUSSION

Figure 1 shows the relationship between lethal exposure period and CO_2 pressure at two temperatures for the eggs of *L. serricorne*. At 25°C complete control was obtained after 80, 52, 36 and 20 min, at 25, 30, 35 and 40 bar, respectively. For comparison, Gerard *et al.* (1988) report a lethal exposure time of 50 min at 30 bar and about 10°C, in a trial using a 3.3-m^3 chamber, for complete control of all stages including eggs of *L. serricorne*. Eggs of *Plodia interpunctella* failed to survive a 15 min treatment with only 20 bar at 25°C; thus, they seem to be more susceptible than the eggs of *L. serricorne* (Reichmuth and Wohlgemuth, 1994).

As shown in Figs. 2 and 3, the larvae and adults of the tobacco beetle are less tolerant of this treatment. For example, at 25 bar and 25°C, adults and larvae are more sensitive than the eggs (Fig. 1).

Figure 4 shows the influence of temperature on lethal exposure times for adults; 90% mortality was obtained within 1, 4, 15, 24 and 35 min at 15, 25, 30, 35 and 45°C, respectively.

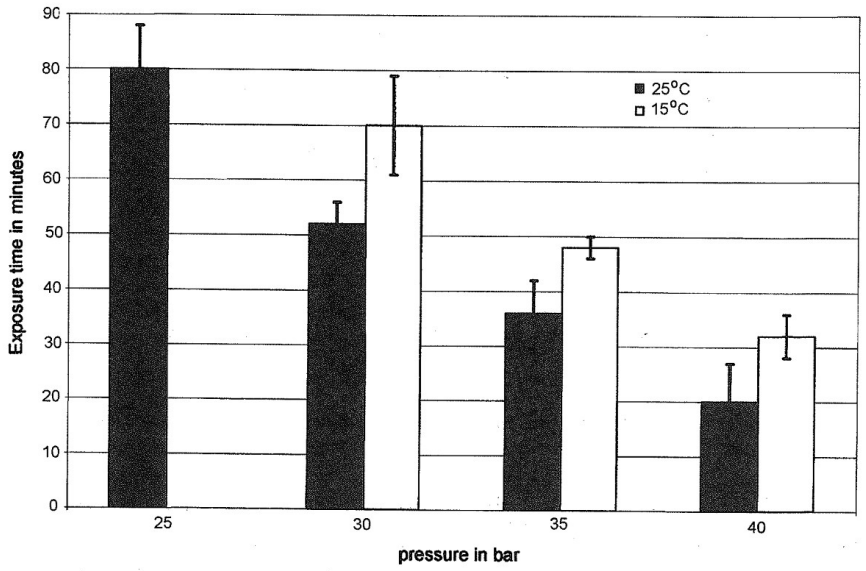

Fig. 1. Exposure time to control the eggs of *Lasioderma serricorne* with CO_2 at 15°C and 25°C under various pressures.

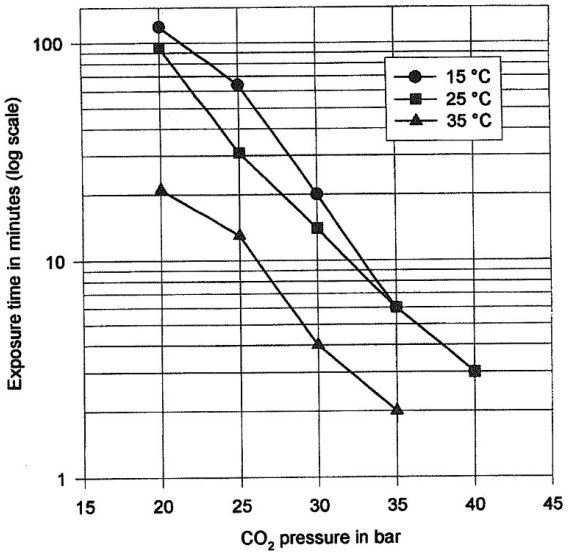

Fig. 2. LT_{95} for various CO_2 pressures to control larvae of *Lasioderma serricorne*.

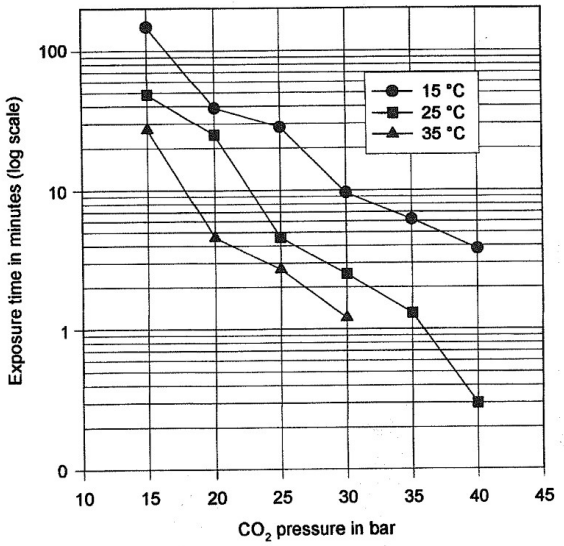

Fig. 3. LT_{95} for various CO_2 pressures to control adults of *Lasioderma serricorne*.

From Fig. 5 it can be seen that the speed of pressure change exercises a strong influence on the mortality of treated tobacco beetle eggs (Ulrichs, 1994). The paramount influence of quick depressurisation has often been postulated (Caliboso *et al.*, 1994; Nakakita and Kawashima, 1994; Ulrichs, 1994; Prozell and Reichmuth, 1992); it was demonstrated here for the first time. In treated products, the egg cells might even burst

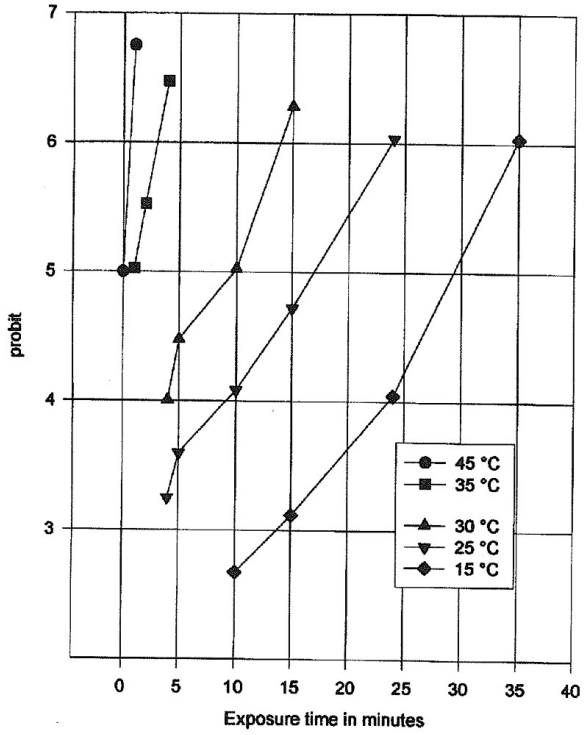

Fig. 4. Influence of the temperature on the mortality of adult *Lasioderma serricorne* during treatment with 20 bar of CO_2.

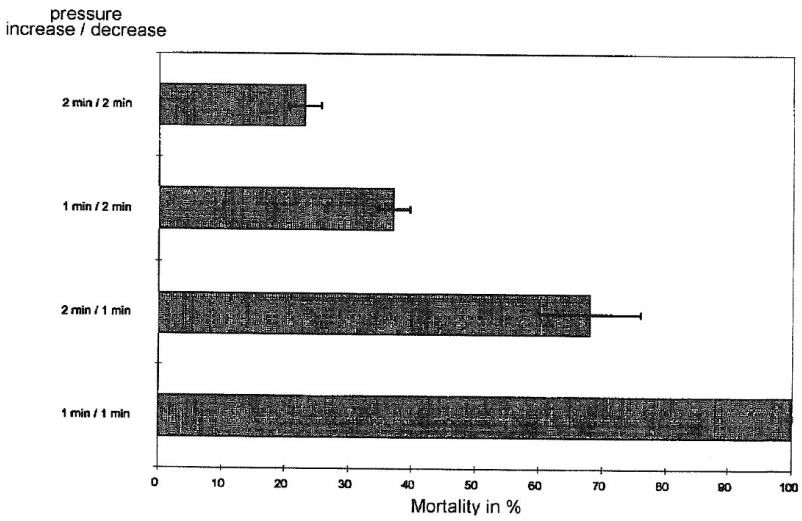

Fig. 5. Effects of different speeds of pressure increase and decrease using CO_2 treatments of 5 min at 20 bar and 25°C on the mortality of adult *Lasioderma serricorne*.

after very rapid expansion (Gerard *et al.*, 1988). With a constant exposure period of 5 min at the set experimental pressure of 20 bar, a difference of only 1 min in the speed of pressure change caused pronounced changes in mortality. Independently of how rapid the build-up of pressure was, pressure decay of 2 min caused significantly less mortality than decay of only 1 min. Rapid build-up of pressure, however, was more effective than was slower pressure build-up.

Figure 6 shows the principal difference in the characteristics of exposure to pressure. Considering CO_2 as behaving like a typical fumigant, it may be expected that when pressure build-up is slow, the insects should be exposed for a longer time. The integral of the corresponding characteristic can be calculated as: 20 bar min (build-up) + (5 × 20) bar min (exposure at 20 bar for 5 min) + 20 bar min (decrease of pressure) = 140 bar min to express a product resulting from pressure × time (Pt-product). In the case of quicker build-up of pressure this Pt-product is reduced to: 10 bar min (build-up) + (5 × 20) bar min (exposure at 20 bar for 5 min) + 10 bar min (decrease of pressure) = 120 bar min.

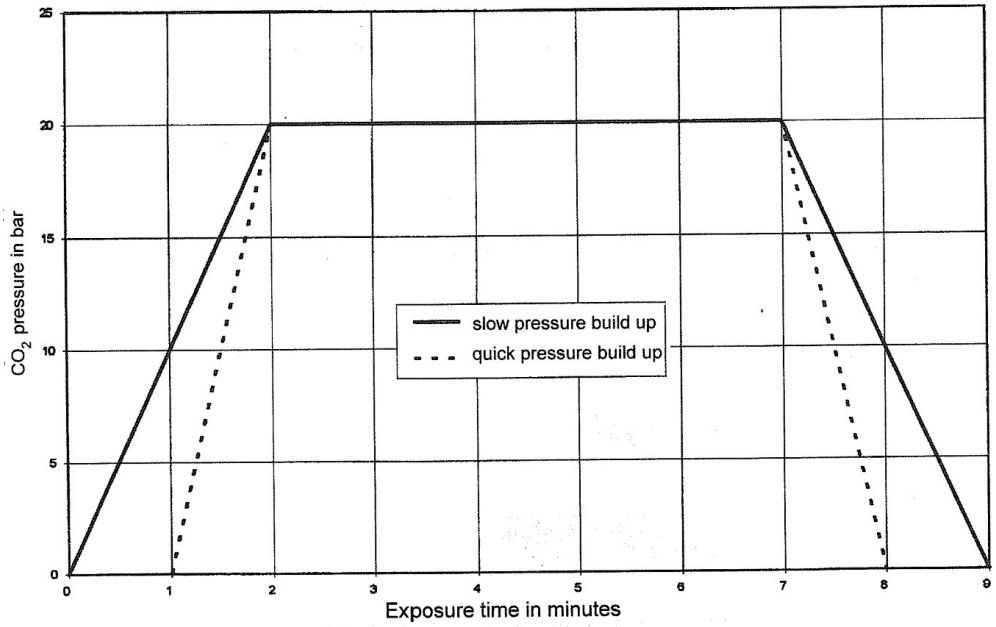

Fig. 6. Model of the treatment with 20 bar of CO_2 for 5 min with 1 min or 2 min pressure change at the beginning and end of the fumigation.

Despite the higher Pt-product, the shorter pressure change time is more effective in obtaining control. The effect of quick depressurisation is known as divers' disease ("the bends") and is caused by the transition of the dissolved gas from liquid to gaseous phase with a concomitent sudden expansion in volume. This internal pressure change leads to the shattering of cell membranes. The rapid entry of the gaseous CO_2 into solution also seems to lead to additional lethal effects.

For practical application, it can be deduced that large-diameter supply pipes will cause more rapid lethal effects when pressure build-up and decay are effected within a shorter time. To ensure success, the chosen exposure time was extended in order to compensate for both slower penetration into the tobacco and lower temperatures.

The results of the field trial with compressed tobacco showed 100% mortality. This supports the feasibility of applying the CO_2 high-pressure disinfestation method to bulky, high-density products.

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