Donahaye, E.J., Navarro, S. and Varnava, A. [Eds.] (1997) Proc. Int. Conf. Controlled Atmosphere and Fumigation in Stored Products, 21–26 April 1996, Printco Ltd., Nicosia, Cyprus, pp. 519–525

THERE IS NO RESISTANCE OF STORED-PRODUCT MOTHS AGAINST TREATMENT WITH CARBON DIOXIDE UNDER HIGH PRESSURE!

C. REICHMUTH
Federal Biological Research Centre for Agriculture and Forestry,
Institute for Stored-Product Protection,
Königin-Luise-Straße 19, D-14195 Berlin, Germany

ABSTRACT

Several moth species of the family Pyralidae, such as *Ephestia* spp. and *Plodia interpunctella*, regularly infest various stored products. Warehouse keepers and food factories throughout the world suffer from losses ranging in the billions of DM annually. In addition to direct losses, further financial loss is caused both by claims on the part of consumers, retailers and importers for replacement and by the need to dispose of infested commodities. The current strategies for overcoming this problem include intensive hygienic measures and the treatment of all raw products entering the storage structure. The high turnover of trade and production and, in some cases — such as herbs and spices — the high value of the commodity, require quick disinfestation procedures that do not damage the goods. A recent new approach is treatment with carbon dioxide (CO_2) under high pressure (about 20 bar) over 1–3 h. Nearly all developing stages of insect pests and mites fail to survive such treatment.

Official authorization according to the plant protection law exists in Germany for the use of CO_2 in this type of application. The results presented here deal with the possible build-up of resistance in the eggs of *P. interpunctella*. Twelve generations stemming from eggs of adults surviving this treatment were exposed at the 50% lethal dose (LD_{50}) level. Only a slight, insignificant change in mortality occured during this 9-fold replicated series of experiments.

INTRODUCTION

A very recent innovative, and feasible, approach to the control of stored-product insects by combining chemical and physical methods was developed by Stahl *et al.* (1985a, b). Supercritical carbon dioxide (CO_2) had been previously used for extracting chemical compounds from drugs, so techniques for handling this gas under pressure were already well-developed for routine procedures. The use of combinations of fumigants, both excluding and including CO_2, under pressure, for controlling insects was reviewed by Ferguson and Hawkins (1949). The surprise was that CO_2 had a much more rapid effect than many other gases under increased pressure (Anon., 1989a, b; Gerard *et al.*, 1988a, b and 1990;

Mitsura, 1973; Pohlen *et al.*, 1989; Rau, 1985). Presumably, this is due to its solubility in water or other liquids, which, combined with changes in pH and the lesion of cell membranes during decompression of the treated and infested products, accounts for its rapid lethal effect (Gerard *et al.*, 1988a; Nakakita and Kawashima, 1994; Prozell and Reichmuth, 1991; Reichmuth, 1991). Caliboso *et al.* (1994) and Ulrichs (1994) observed ruptures of cell membranes of *Lasioderma serricorne* after exposure to CO_2 at a high pressure (20 bar) with subsequent rapid depressurisation. Eggs of *Plodia interpunctella* failed to survive treatment at 20 bar for 15 min with subsequent decompression within 1 min (Reichmuth and Wohlgemuth, 1994).

In Germany, the USA and Japan, where steel chambers of up to 30 m^3 are in commercial use, this new technique is generating much interest. Patents for bigger chambers for treatment of bulk products have been registered (Corinth and Reichmuth, 1991).

Some failures to completely control all insects or mites might be explained by either too short an exposure time, especially when treated goods are held at low temperatures, or lack of even and rapid distribution of the CO_2, especially when bulk commodities (such as several kg of palleted dried figs in cardboard cartons) are treated. Prozell addressed this problem at this meeting of the CAF Conference (Prozell *et al.*, 1997).

On the other hand, due to their short life cycles, insects are known to be able to develop resistance to nearly all the toxic substances to which they are exposed. This raises the question of whether unsuccessful treatments can be linked to the development of resistance. This paper reports on work done to determine whether eggs of the Indian meal moth *P. interpunctella*, which are known to be the most tolerant developmental stage to treatment with CO_2 under high pressure (Reichmuth and Wohlgemuth, 1994), develop resistance when exposed for several consecutive generations to treatments that produce 50% mortality (LD_{50} conditions).

MATERIALS AND METHODS

Insects were taken from the stock culture of the Institute for Stored-Product Protection of the Federal Biological Research Centre for Agriculture and Forestry in Berlin, where they had been reared for more than 25 years at constant conditions of 25°C and 65% r.h. under a 16:8 light:dark photoperiod. One-day-old eggs of *P. interpunctella* were collected after exposing several hundred adult moths of both sexes overnight under culture conditions in an oviposition chamber made of 2-mm-aperture steel-wire mesh. Prior to exposure the eggs were aged 14 ± 12 h. Previous experiments (Reichmuth and Wohlgemuth, 1994) showed this age group to be the most tolerant, with LD_{50} achieved in 5 min at 20 bar and 25°C. Table 1 shows the experimental design for one generation of eggs treated for selection.

Four petri dishes (3-cm diameter, 1-cm rim), each containing 100 eggs, were stacked in the fumigation chamber, as described by Reichmuth and Wohlgemuth (1994) and Ulrichs (1994), and treated with CO_2 for 5 min at 20 bar. An additional 100 eggs of the same age from the same generation served as control to establish if hatch occured normally. This experimental set of treated eggs was repeated four times for a total of 1,600 treated eggs and

TABLE 1
Experimental design for one generation of eggs treated for selection

	Number of eggs in each set of replicates			
	Set 1	Set 2	Set 3	Set 4
Replicate 1	100	100	100	100
Replicate 2	100	100	100	100
Replicate 3	100	100	100	100
Replicate 4	100	100	100	100
Untreated	100			

Experiment 1 (four sets repeated nine times) comprises the trials with eggs of the first generation from parents of the unselected stock culture. *P. interpunctella* eggs, 14 ± 12-h-old were exposed for 5 min at 20 bar CO_2.

100 control eggs. After treatment, the four lots of treated eggs and the control eggs from the fifth petri dish which did not undergo pressure and CO_2 treatment were all transferred into small gauze cages with substrate. Subsequently, all were checked for larval hatch. Trials were repeated with a set of 1,700 eggs from each of nine generations. The surviving larvae from untreated eggs were discarded. The surviving larvae from the 5-min treatment were reared to maturity and delivered the eggs for the next set of 1,700 eggs for the experiment on the second generation. After treatment of 11 sequential generations had been carried out in this manner, an additional series of experiments comprising threefold repeated investigations with 100 eggs was carried out for each sample, exposure time and replicate at five different exposure periods (2, 4, 5, 8 and 16 min) to determine precisely if a pronounced tendency to increased tolerance could be observed. A set of eggs from the 12th generation of the unselected stock culture was similarly tested.

RESULTS AND DISCUSSION

In all experiments, the 100 untreated eggs, laid by parents that had been selected, developed normally. There was an average hatch rate of 97%.

The mortality rates of the 9-fold repeated experiments on the eggs after 5-min exposure to 20 bar CO_2 at 25°C are summarised in Table 2, the last column of which shows the averages of the nine replications. Figure 1 shows the average results, including standard deviations, for the 12 subsequently selected generations. The deviations ranged from 5.1 to 9.4%. The average mortality of 30.19%, after 12 successive selections, with an upper limit of +7.39% lies within the lower part of the range of average mortality of the stock culture (40.47 – 7.91%).

An examination of the standard deviations reveals that for the most part the average mortality results can not be distinguished significantly from each other. Over the whole range of 12 generations, after an initial increase there was a slight tendency to decrease in the sensitivity of the eggs to successively repeated CO_2 high-pressure treatment. This

TABLE 2

Mortality results (±SD) of investigations with 11 sequential generations of 1,600 × 9 eggs each of *Plodia interpunctella,* all treated at 25°C with 20-bar CO_2 for 5 min corresponding to LD_{50}

	Percentage mortality of eggs of *P. interpunctella*									
	Replicate									Averages
Generation of eggs	1	2	3	4	5	6	7	8	9	1–9
Experiment 1 (F_1) (eggs of stock culture)	34.9 ±5.9	35.4 ±7.9	39.4 ±10.3	34.4 ±6.9	42.1 ±8.0	42.1 ±8.3	41.2 ±6.0	42.8 ±10.9	51.9 ±7.0	40.47 ±7.91
Experiment 2 (F_2) (LD_{50} survivors of F_1)	49.6 ±10.6	69.6 ±8.2	38.7 ±6.1	54.3 ±8.4	45.7 ±8.5	37.4 ±6.1	42.0 ±10.1	49.4 ±13.5	44.8 ±13.3	47.94 ±9.42
Experiment 3 (F_3) (LD_{50} survivors of F_2)	42.6 ±7.7	40.4 ±7.1	47.9 ±9.8	51.6 ±7.6	39.0 ±9.5	45.9 ±9.5	45.8 ±6.9	35.5 ±6.6	51.9 ±7.7	44.51 ±8.04
Experiment 4 (F_4) (LD_{50} survivors of F_3)	56.8 ±10.8	43.8 ±7.6	46.6 ±7.6	38.8 ±8.9	48.6 ±7.7	57.1 ±11.3	49.6 ±8.2	60.4 ±7.9	48.9 ±5.8	50.07 ±8.42
Experiment 5 (F_5) (LD_{50} survivors of F_4)	41.1 ±6.5	38.0 ±6.4	50.2 ±7.5	40.1 ±7.0	39.4 ±8.0	46.7 ±6.8	40.4 ±7.3	49.6 ±7.9	52.4 ±10.0	44.21 ±7.49
Experiment 6 (F_6) (LD_{50} survivors of F_5)	28.8 ±6.6	37.3 ±7.1	35.4 ±9.7	42.6 ±7.0	38.2 ±9.6	44.9 ±9.9	38.5 ±9.5	43.6 ±8.2	42.9 ±5.3	39.13 ±8.10
Experiment 7 (F_7) (LD_{50} survivors of F_6)	35.3 ±5.4	40.3 ±5.0	34.8 ±10.1	19.4 ±4.3	24.9 ±4.5	21.3 ±4.8	25.9 ±3.0	30.8 ±4.7	20.3 ±4.4	28.11 ±5.13
Experiment 8 (F_8) (LD_{50} survivors of F_7)	36.5 ±7.2	27.4 ±5.9	25.4 ±5.5	30.0 ±6.0	29.0 ±4.2	23.6 ±5.2	30.8 ±4.4	29.6 ±4.0	20.6 ±5.2	28.10 ±5.29
Experiment 9 (F_9) (LD_{50} survivors of F_8)	34.4 ±4.3	29.9 ±6.5	47.5 ±3.9	31.2 ±4.6	31.5 ±5.4	31.0 ±6.6	27.2 ±6.7	32.7 ±8.5	36.8 ±3.8	33.58 ±5.59
Experiment 10 (F_{10}) (LD_{50} survivors of F_9)	32.1 ±4.8	42.1 ±6.1	34.9 ±6.2	39.8 ±7.2	38.7 ±5.6	36.5 ±6.7	31.3 ±5.0	31.7 ±4.1	33.6 ±7.3	35.63 ±5.89
Experiment 11(F_{11}) (LD_{50} survivors of F_{10})	25.4 ±3.2	39.4 ±4.3	27.4 ±9.3	31.3 ±5.1	48.1 ±7.0	41.3 ±8.1	29.4 ±6.3	44.4 ±6.8	48.7 ±4.9	37.27 ±6.11
Experiment 12 (F_{12}) (LD_{50} survivors of F_{11})	27.6 ±8.2	25.2 ±6.4	26.2 ±5.2	36.4 ±9.8	39.9 ±9.4	31.0 ±11.4	26.6 ±5.7	26.4 ±5.6	32.4 ±3.7	30.19 ±7.27

[1]After 5-min exposure to 20 bar CO_2 at 25°C.

tendency fluctuated during the process of selection and is therefore not enough to reach the conclusion that there was a quick build-up of resistance. It would be of interest to check further selection pressure and investigate the stability of the increased tolerance without further selection. From the results of the first three selected generations it can be concluded that the chosen sublethal conditions of 5 min at 20 bar of CO_2 were slightly below the real LD_{50}. Figure 2 supports this statement. After 12 selections the eggs respond

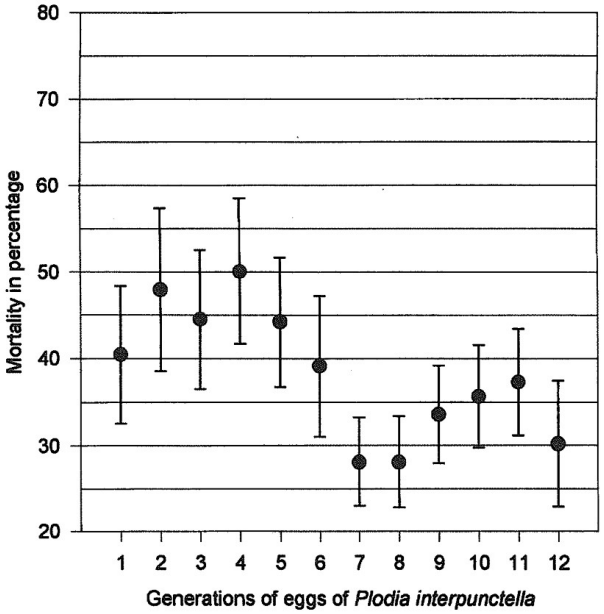

Fig. 1. Repeated selection using 14,400 eggs per generation of *Plodia interpunctella* with CO_2 at 20 bar at 25°C for 5 min treatment (vertical bars are SD values).

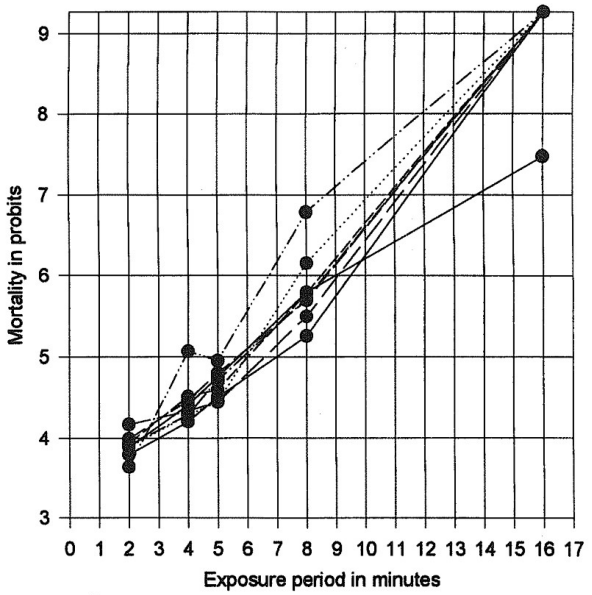

Fig. 2. Dose mortality response to CO_2 at 20 bar at 25°C of 13,500 eggs of the 12th generation of selections of *Plodia interpunctella.* n = 9.

to treatment with CO_2 at 20 bar in a similar manner as does the stock culture. The LT_{50} (probit 5) at about 6 min is slightly longer than, but still very close to, the 5 min of the stock culture. Standard deviations are not included in this graph.

These findings lead to the conclusion that the use of high pressure CO_2 treatment to control stored-product pests is not endangered by the threat of quick insect-resistance build-up. Where eggs of stored-product pests are exposed for several generations to sublethal conditions, subsequent treatment at normally lethal conditions still causes complete mortality.

ACKNOWLEDGEMENT

My assistant, Mrs Agnes Paul, is hereby thanked for her excellence, patience and accuracy in doing tedious laboratory work.

REFERENCES

Anon. (1989a) Pest control with carbon dioxide. *Food Tech.* **52.**

Anon. (1989b) Schädlingsbekämpfung mit Kohlendioxid [Pest control with carbon dioxide]. *Der praktische Schädlingsbekämpfer* **41,** 200.

Caliboso, F.M., Nakakita, H. and Kawashima, K. (1994) A preliminary evaluation of carbon dioxoide under high pressure for rapid fumigation. In: *Proc. 6th Int. Working Conf. on Stored-Product Protection* (Edited by Highley, E., Wright, E.J., Banks, H.J. and Champ, B.R.), Canberra, Australia, 17–23 April 1994, CAB International, Wallingford, Oxon, UK, **1,** 45–47.

Corinth, H.-G. and Reichmuth, Ch. (1991) Verfahren und Einrichtung zum Entwesen von organischem Schüttgut [Procedure and installation to disinfest organic bulk material]. Patent of the Federal Republic of Germany, No. 39 30 470 [1992, new No. 04 17 430].

Ferguson, J. and Hawkins, S.W. (1949) Toxic action of some simple gases at high pressure. *Nature* **164,** 963–964.

Gerard, D., Kraus, J., Fröhlingsdorf, C.J. and Dallüge, A. (1990) Rückstandsfreier Vorratsschutz für Arznei- und Teedrogen [Residue-free stored product protection for medical and tea drugs]. *Deutsche Apotheker Zeitung* **130,** 2014–2018.

Gerard, D., Kraus, J., Quirin, K.-W. and Wohlgemuth, R. (1988a) Anwendung von Kohlendioxid (CO_2) unter Druck zur Bekämpfung vorratsschädlicher Insekten und Milben [Use of carbon dioxide (CO_2) to control stored-product pest insects and mites]. *Pharm. Ind.* **50,** 1298–1300.

Gerard, D., Kraus, J. and Quirin, K.-W. (1988b) Rückstandsfreie Druckentwesung mit natürlicher Kohlensäure [Residue-free pressure disinfestation with natural carbon dioxide]. *Gordian* **88,** 90–94.

Mitsura, A., Amano, R. and Tanabe, H. (1973) The acaricidal effects of compressed gas treatments on the grain mite *Tyrophagus putrescentiae*. *Shokuhin Eisagaki-zasski* **14,** 511–516.

Nakakita, H. and Kawashima, K. (1994) A new method to control stored product insects using carbon dioxide with high pressure followed by sudden pressure loss. In: *Proc. 6th Int. Working Conf. on Stored-Product Protection* (Edited by Highley, E., Wright, E.J., Banks, H.J. and Champ, B.R.), Canberra, Australia, 17–23 April 1994, CAB International, Wallingford, Oxon, UK, **1,** 126–129.

Pohlen, W., Rau, G. and Finkenzeller, F. (1989) Erste praktische Erfahrungen mit einem Verfahren zur Druckentwesung mit Kohlendioxid [First practical experiences with a procedure to disinfest with pressurized carbon dioxide]. *Pharm. Ind.* **51,** 917–918.

Prozell, S. and Reichmuth, Ch. (1991) Response of the granary weevil *Sitophilus granarius* (L.) (Col.: Curculionidae) to controlled atmospheres under high pressure. In: *Proc. 5th Int. Working Conf. on Stored-Product Protection* (Edited by Fleurat-Lessard, F. and Ducom, P.), Bordeaux, France, 9–14 September 1990, **2,** 911–918.

Prozell, S., Reichmuth, Ch., Ziegleder, G., Schartmann, B., Matissek, R., Kraus, J., Gerard, D. and Rogg, S. (1997) Control of pests and quality aspects in cocoa beans and hazelnuts and diffusion experiments in compressed tobacco with carbon dioxide under high pressure. In: *Proc. Int. Conf. on Controlled Atmosphere and Fumigation in Stored Products* (Edited by Donahaye, E.J., Navarro, S. and Varnava, A.), Nicosia, Cyprus, 21–26 April 1996, Printco Ltd., Nicosia, Cyprus, 325–333.

Rau, G. (1985) Die Anwendung von verdichtetem Kohlendioxid zur Qualitätsverbesserung von Drogen [Application of pressurized carbon dioxide to improve the quality of drugs]. Ph. D. Thesis, University of the Saarland, Saarbrücken, Germany. 100 pp.

Reichmuth, Ch. (1991) New techniques in fumigation research today. In: *Proc. 5th Int. Working Conf. on Stored-Product Protection* (Edited by Fleurat-Lessard, F. and Ducom, P.), Bordeaux, France, 9–14 September 1990, **2,** 709–725.

Reichmuth, Ch. and Wohlgemuth, R. (1994) 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. In: *Proc. 6th Int. Working Conf. on Stored-Product Protection* (Edited by Highley, E., Wright, E.J., Banks, H.J. and Champ, B.R.), Canberra, Australia, 17–23 April 1994, CAB International, Wallingford, Oxon, UK, **1,** 163–172.

Stahl, E., Rau, G. and Adolphi, H. (1985a) Entwesung von Drogen durch Kohlendioxid-Druckbehandlung (PEX-Verfahren) [Disinfestation of drugs by pressure treatment with carbon dioxide (PEX procedure)]. *Pharm. Ind.* **47,** 528–530.

Stahl, E. and Rau, G. (1985b) Neues Verfahren zur Entwesung [A new method for disinfestation]. *Anz. Schädlingskde., Pflanzenschutz, Umweltschutz* **58,** 133–136.

Ulrichs, Ch. (1994) Effects of different speed of build up and decrease of pressure with carbon dioxide on the adults of the tobacco beetle *Lasioderma serricorne* (Fabricius) (Coleoptera: Anobiidae). In: *Proc. 6th Int. Working Conf. on Stored-Product Protection* (Edited by Highley, E., Wright, E.J., Banks, H.J. and Champ, B.R.), Canberra, Australia, 17–23 April 1994, CAB International, Wallingford, Oxon, UK, **1,** 214–216.