PERGAMON

Journal of Stored Products Research 40 (2004) 197–205

Journal of STORED PRODUCTS RESEARCH

www.elsevier.com/locate/jspr

Effectiveness of short exposures of propylene oxide alone and in combination with low pressure or carbon dioxide against *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae)

S. Navarro[a,*], A.A. Isikber[b], S. Finkelman[a], M. Rindner[a], A. Azrieli[a], R. Dias[a]

[a] *Department of Stored Products, Agricultural Research Organization, The Volcani Center, P.O. Box 6, Bet-Dagan 50250, Israel*

[b] *Department of Plant Protection, Faculty of Agriculture, University of Kahramanmaras Sutcu Imam, Kahramanmaras 46060, Turkey*

Accepted 25 October 2002

Abstract

Toxicity of the fumigant propylene oxide (PPO) alone and in combination with low pressure (100 mm Hg) or 92% CO_2 to all life stages of *Tribolium castaneum* using short exposure times (4 and 8 h) at 30°C was studied. Results indicated that PPO was moderately toxic with Ct products ranging from 120 to 608 mg h/l required to obtain complete mortality of the different life stages. A marked difference in susceptibility between life stages was recorded. Eggs were the most sensitive with a LD_{99} value of 30.1 mg/l for 4 h, whereas pupae were the most tolerant with a LD_{99} value of 146.5 mg/l. It was shown that an increase in exposure time from 4 to 8 h resulted in 23%, 42%, 48% and 47% reductions of LD_{99} values for eggs, larvae, pupae and adults, respectively.

There was no or very limited mortality of all stages except the egg (53% to 62%), when exposed to either 100 mm Hg or 92% CO_2 for 4 h. However, when 100 mm Hg or 92% CO_2 were combined with PPO, the LD_{50} and LD_{99} values for PPO in all stages except the egg were significantly reduced. Combinations of PPO with 100 mm Hg or 92% CO_2 produced equal reductions in the LD_{99} value from 146.5 to about 22 mg/l for the most tolerant pupal stage. Both combinations also produced significant reductions in the LD_{99} values for larvae and adults (6.3- to 6.6-fold) compared with those exposed to PPO alone. These results indicated that 100 mm Hg and 92% CO_2 each had a synergistic effect on the toxicity of PPO to *T. castaneum*. The combination of PPO with vacuum or CO_2 can thus provide a potential alternative to methyl bromide.

Keywords: Propylene oxide; Carbon dioxide; Vacuum fumigation; *Tribolium castaneum*; Synergistic effect

*Corresponding author. Tel.: +972-3-9683-587; fax: +972-3-9683-583.
E-mail address: snavarro@volcani.agri.gov.il (S. Navarro).

0022-474X/03/$ - see front matter
doi:10.1016/S0022-474X(02)00097-8

1. Introduction

Propylene oxide (PPO) is an FDA approved fumigant to control microbial contamination in dry and shelled walnuts, cocoa powder and spices. PPO boils at 35°C and is a liquid at normal temperature and pressure. It is a safe fumigant for use on food as a sterilant because it is quickly converted to non-toxic glycols in the stomach. Therefore, it has been used for food sterilization since 1958 (Griffith, 1999). It is also not an ozone depleter and is environmentally benign. PPO has been recently demonstrated by preliminary tests to have insecticidal properties under low pressure by killing all stages of the confused flour beetle (*Tribolium confusum* du Val), the Indian meal moth (*Plodia interpunctella* (Hübner)) and the warehouse beetle (*Trogoderma variabile* Everts) at concentrations as low as 100 g of PPO/m^3 (Griffith, 1999). However, PPO is flammable from 3% to 37% by volume in air. Elimination of the flammability hazard of PPO can be achieved by applying it under low pressure or in a carbon dioxide (CO_2) enriched atmosphere. However, PPO effectiveness at low pressure or in combination with CO_2 requires elucidation to clarify the efficiency of each treatment separately or in combination.

Several studies on the use of low pressure to reduce flammability of fumigants (Bond, 1984) and improve penetration, and also to enable the application of lower dosages and reduction in exposure time, have been investigated. In some cases, insects were found to be more susceptible at reduced pressure by factors of between two and three (El-Nahal, 1953; Monro, 1959; Monro et al., 1966; Calderon and Leesch, 1983). Additionally, the use of low pressure for the storage of high value commodities has gained renewed interest and has recently been reviewed by Navarro et al. (2001).

The use of CO_2 together with conventional fumigants has also been studied. The advantages of using CO_2 in the mixture are to increase the toxicity of the fumigant, improve the distribution pattern, limit the levels of harmful residues in the treated commodity, and also eliminate the flammable hazard of some fumigants. Several general studies on fumigant/CO_2 mixtures have been made in the past (Cotton and Young, 1929; Jones, 1938), and these were followed by investigations which showed that the addition of CO_2 to methyl bromide (MB) resulted in an increase in the susceptibilities of some stored-product insects (AliNiazee and Lindgren, 1969; Calderon and Leesch, 1983; Williams, 1985). It was also reported that the flammable hazard of some fumigants such as ethylene oxide and ethylene dichloride was eliminated by using CO_2 and fumigant mixtures (Hashiguchi et al., 1967; Berck, 1974; Bond, 1984).

MB is now the only fumigant available for quarantine treatment of commodities where rapid disinfestation techniques are essential. Since MB is being phased out as a fumigant under the Montreal Protocol (UNEP, 1995), alternatives to MB treatments must be found, especially for quarantine purposes, where rapid and complete control is required. Griffith (1999) in preliminary tests on some stored-product insects showed that PPO has a potential to control stored-product pests; however, only limited information is available on the efficacy of PPO against a wide range of storage pests under a variety of fumigation conditions, including combination with low pressure or CO_2. Nor has the use of PPO been considered previously for rapid disinfestation of durable stored-products as a replacement for MB.

With this objective, our study was designed to evaluate the relative effectiveness of low pressure and CO_2 on the efficacy of PPO against all life stages of *T. castaneum* (Herbst) at short exposure durations.

2. Materials and methods

2.1. Test insects

Tests were carried out on all life stages of red flour beetle, *T. castaneum*. All stages were obtained from cultures reared at 28 ± 2°C and 65 ± 5% relative humidity (r.h.) on a diet of ground wheat and yeast using standard culture techniques (Donahaye, 1990). Cultures were started from eggs obtained by sieving from 750 ml oviposition jars containing adults in 400 g of wheat flour. Eggs were separated from oviposition jars by sieving daily. Eggs for exposure to treatments were transferred into "pits" drilled into Perspex exposure slides, each slide containing 50 pits. When filled, the slides were covered with a perforated cover glass to retain the eggs (Navarro and Gonen, 1970). Two slides containing 100 eggs aged 1–2 d were exposed to each treatment. Larvae were removed from culture jars and exposed to the treatments 12 d after oviposition. Two-day-old pupae were obtained by daily separation from culture jars and holding in wheat flour for 24 h before the exposure. Newly emerged adults were held in pre-exposure jars containing wheat flour, and were exposed to treatments 7–10 d after emergence.

2.2. Fumigation chambers

Test chambers consisted of 2.64-l desiccators, each capped with a ground-glass stopper equipped with entry and exit tubing. A magnetic stirrer placed in the bottom well beneath a wire-mesh disc served to mix the air with the fumigant. Two pieces of rubber tubing, 5 cm long, 6.2 mm ID, were attached to the tubing and sealed with pinch clamps. The desiccators were sealed with silicone vacuum grease.

2.3. The fumigant

The fumigant was 99% + pure liquid PPO that was withdrawn from a sealed vial fitted with a rubber septum, using a gas-tight syringe. CO_2 was supplied from a cylinder and was 99% + pure.

2.4. Dosing and fumigation procedures

Propylene oxide was introduced as a liquid into the desiccators using 50 or 250 µl gas-tight syringes. CO_2 was transferred from the supply cylinder through a pipe equipped with a regulator valve. Pressure in each desiccator was measured using a 0 to 800 mm Hg Celesco, USA vacuum gauge (model SE-2000). The 100 mm Hg measure referred to herein is absolute pressure, with 760 mm Hg considered as atmospheric pressure. Prior to each test, 50 larvae, pupae or adults were confined, separately, inside 3 cm diameter by 8 cm long wire-mesh cages. For eggs, two exposure slides each holding 50 eggs were used per fumigation.

For PPO fumigation, the calculated volumes of liquid were withdrawn from the PPO vial and injected into the exposure chamber containing the insects. Concentrations of 9, 17, 26, 35, 69, 103 and 138 mg/l were tested for each stage of the insect at exposure times of 4 and 8 h. For fumigations under low pressure, the insects were first placed in the desiccators and then, prior to the introduction of the required PPO concentration, 100 mm Hg was obtained by evacuating air.

For the treatments with PPO in a CO_2 atmosphere, the insects were first placed in the desiccators. Then, the desiccators were briefly evacuated to 60.8 mm Hg followed by flushing with CO_2 until restoration of atmospheric pressure so as to obtain a uniform concentration of 92% CO_2. This process lasted about 10 min.

Each of the combinations of PPO with low pressure or CO_2 was tested at four to six dosages: 4, 7, 17, 26, 35 and 45 mg/l. In addition, separate exposures to 100 mm Hg and to 92% CO_2 alone were made, and untreated control insects were exposed to atmospheric conditions. Each test was replicated at least twice. Whereas exposure times were 4 and 8 h for PPO alone, for PPO in combination with 100 mm Hg and 92% CO_2 only the 4-h exposure was used. The gas mixtures in the desiccators were stirred for at least 20 min. For all fumigations, r.h. and temperature were maintained at $62 \pm 5\%$ and $30 \pm 2^{\circ}C$, respectively, at atmospheric pressure. The r.h. in the desiccators was established by adding a wick imbued with 2.7 g of saturated salt solution of $NaNO_2$ (Budavari et al., 1989). However, the r.h. decreased to $55 \pm 5\%$ at the initial stage of the treatment when 100 mm Hg was applied, but returned to $62 \pm 5\%$ within 0.5 h after the treatment.

Pressure inside the desiccators was checked after injecting the PPO and at the end of each test. Concentrations of CO_2 inside the desiccators were checked by withdrawing 5 ml gas samples from exposure chambers, which were analyzed in a gas chromatograph (GC) equipped with thermal conductivity cells and dual columns packed with 'Poropak Q;' and 'molecular sieve 5A' (SRI 8610C, SRI Instruments USA). Relative humidity during fumigations was measured by placing small hygrometers within the desiccators. Concentrations of PPO in each desiccator were checked by withdrawing 15 μl gas samples using a 50 μl gas-tight syringe. The gas concentration of PPO was measured using a Shimadzu 17A GC fitted with an Flame Ionization Detector and an EC™–WAX capillary column (30 m length × 0.25 mm ID × 0.25 μm film thickness) run at 170°C isothermal.

2.5. Data processing and analysis

After each treatment, larvae, pupae, and adults were transferred to 200-ml jars containing food medium and were held at $28 \pm 2^{\circ}C$ and $65 \pm 5\%$ r.h. until examined for mortality. The eggs in their Perspex slides were held under the same conditions until they were examined for egg hatch.

Mortality counts for adults were made 4–5 d after exposure; for larvae they were based on those insects that had failed to pupate 9 d after exposure; pupal mortality was based on those pupae that failed to produce adults 9 d after exposure, and egg hatch was counted 7 d after treatment. Results obtained from each insect stage for all three treatments were subjected to probit analysis using a program written by Daum (1970). Required concentrations × time (Ct) products to obtain 50% and 99% mortality of all insect stages of the insect were calculated using the LD_{50} and LD_{99} concentrations derived from probit analyses.

3. Results and discussion

The LD_{50} and LD_{99} levels expressed as mg/l of all stages of *T. castaneum* when exposed to PPO alone for 4 and 8 h, are presented in Table 1 and show a marked difference in susceptibility between the different stages. Whilst the eggs were the most sensitive stage with LD_{99} values of 30

Table 1
Toxicity of propylene oxide to all life stages of *T. castaneum* when exposed alone for 4 and 8 h

Life stage	Exposure time (h)	χ^2 (d.f.)	Slope ± SE	LD_{50}[a] (mg/l)	LD_{99}[a] (mg/l)
Egg	4	12.59 (6)	12.70 ± 1.31	19.7 (18.86–20.54)	30.1 (27.78–33.56)
	8	9.49 (4)	7.78 ± 1.05	11.7 (9.80–13.14)	23.2 (19.54–32.71)
Larva	4	12.59 (6)	6.44 ± 0.57	36.3 (33.67–38.80)	83.5 (72.54–101.40)
	8	7.81 (4)	7.53 ± 1.30	23.7 (20.06–26.04)	48.2 (42.31–61.49)
Pupa	4	9.48 (6)	4.50 ± 0.41	44.5 (40.80–48.66)	146.5 (119.21–195.48)
	8	7.81(4)	10.79 ± 2.16	46.3 (34.95–54.85)	76.0 (61.74–165.03)
Adult	4	11.07 (6)	9.01 ± 1.12	30.6 (28.31–33.42)	55.4 (47.53–70.54)
	8	9.49 (4)	18.11 ± 2.15	21.7 (20.92-22.44)	29.2 (27.47–32.01)

[a] Numbers in brackets give the 95% confidence range.

and 23 mg/l for 4 and 8 h, respectively, for the pupae, which was the most tolerant stage, LD_{99} levels at 4 and 8 h exposure were only achieved at concentrations of 147 and 76 mg/l, respectively. The order of tolerance of the stages at both the LD_{50} and LD_{99} was: pupa > larva > adult > egg. It was found that the increase in exposure time from 4 to 8 h resulted in 23%, 42%, 48% and 47% reductions in LD_{99} values for eggs, larvae, pupae and adults, respectively. There was also high variation in Ct products of different life stages required for LD_{50} and LD_{99}. Calculations of Ct products reveal a high Ct product of 586 mg h/l required to obtain 99% kill of pupae, whereas for eggs a low Ct product of 120 mg h/l was required for 99% kill in a 4-h exposure.

These findings may be compared with several studies on the two most commonly used fumigants, MB and phosphine for control of *T. castaneum*. Whereas MB requires Ct products of 62, 59 and 168 mg h/l to obtain 95% of kill of adults, larvae and pupae, respectively, at 27°C (Lindgren and Vincent, 1965; Rajendran, 1990), phosphine requires Ct products of 12, 47 and 56 mg h/l to achieve 90% of kill of adults, larvae and pupae, respectively, at 24°C (Bang and Telford, 1966; Williams, 1985), and only 2.2 mg h/l to achieve 99% kill of all stages including eggs in a 7-d exposure at 25°C (Hole et al., 1976). Tests on adults of *T. castaneum* of other fumigants such as ethylene dichloride and carbon tetrachloride produced 90% mortality with Ct products of 462 and 600 mg h/l, respectively, whereas ethylene oxide required a Ct product of 135 mg h/l to obtain 95% mortality (Bang and Telford, 1966; Busvine, 1938). It appears therefore that PPO is less toxic to *T. castaneum* than phosphine, MB and ethylene oxide, but is more toxic than ethylene dichloride and carbon tetrachloride.

A low pressure of 100 mm Hg and exposure to 92% CO_2 alone did not produce any mortality of pupae and adults of *T. castaneum*, but both resulted in relatively high mortality of eggs (53–62%) and a very limited mortality of larvae of 5–7.7% (Table 2). The responses of the four life stages to PPO in combination with 100 mm Hg or 92% CO_2 are shown separately in Tables 3–6. The tables show that PPO in combination with 100 mm Hg or 92% CO_2 reduced the LD_{50} and LD_{99} values of all life stages. Both combination treatments produced a significant decrease in the LD_{99} values for the larvae, pupae and adults (6.2- to 7.1-fold) compared with the PPO treatment alone. However, the combination treatments gave only a 1.2- (CO_2) to 1.4-fold (low pressure) reduction

Table 2
Mortality (%) of all life stages of *T. castaneum* when exposed to low pressure (100 mm Hg) and 92% CO_2 alone for 4 h

Life stage	92% CO_2	100 mm Hg
Egg	62	53
Larva	5	7.7
Pupa	0	0
Adult	0	0

Table 3
Toxicity of propylene oxide to the egg stage of *T. castaneum* when exposed to PPO alone and in combination with low pressure (100 mm Hg) or 92% CO_2 for 4 h

Treatments	χ^2 (d.f.)	Slope ± SE	LD_{50} (mg/l)[a]	LD_{99} (mg/l)[a]	Ratio of PPO to	
					LD_{50}	LD_{99}
PPO alone	12.6 (6)	12.7 ± 1.31	19.7 (18.86–20.54)	30.1 (27.78–33.56)	—	—
PPO + 92% CO_2	7.8 (4)	2.80 ± 0.36	3.7 (2.71–4.43)	24.7 (19.15–37.27)	5.3	1.2
PPO at 100 mm Hg	6.0 (3)	2.61 ± 0.84	4.6 (2.62–5.91)	22.4 (19.42–27.30)	4.2	1.4

[a] Numbers in brackets give the 95% confidence range.

Table 4
Toxicity of propylene oxide to the larval stage of *T. castaneum* when exposed to PPO alone and in combination with low pressure (100 mm Hg) or 92% CO_2 for 4 h

Treatments	χ^2 (d.f.)	Slope ± SE	LD_{50} (mg/l)[a]	LD_{99} (mg/l)[a]	Ratio of PPO to	
					LD_{50}	LD_{99}
PPO alone	12.6 (6)	6.44 ± 0.57	36.3 (33.67–38.80)	83.5 (72.54–101.40)	—	—
PPO + 92% CO_2	7.8 (4)	7.64 ± 0.79	6.5 (6.01–7.13)	13.2 (11.58–16.00)	5.6	6.3
PPO at 100 mm Hg	9.5 (4)	5.13 ± 0.53	4.6 (4.10–4.96)	12.9 (10.71–16.76)	7.9	6.5

[a] Numbers in brackets give the 95% confidence range.

Table 5
Toxicity of propylene oxide to the pupal stage of *T. castaneum* when exposed to PPO alone and in combination with low pressure (100 mm Hg) or 92% CO_2 for 4 h

Treatments	χ^2 (d.f.)	Slope ± SE	LD_{50} (mg/l)[a]	LD_{99} (mg/l)[a]	Ratio of PPO to	
					LD_{50}	LD_{99}
PPO alone	9.48 (6)	4.5 ± 0.41	44.5 (40.80–48.66)	146.5 (119.21–195.48)	—	—
PPO + 92% CO_2	7.8 (4)	9.20 ± 0.96	12.4 (11.60–13.49)	22.1 (20.06–25.43)	3.6	6.6
PPO at 100 mm Hg	6.0 (4)	5.55 ± 0.65	8.4 (6.96–9.90)	22.0 (17.85–29.73)	5.3	6.6

[a] Numbers in brackets give the 95% confidence range.

Table 6
Toxicity of propylene oxide to the adult stage of *T. castaneum* when exposed to PPO alone and in combination with low pressure (100 mm Hg) or 92% CO_2 for 4 h

Treatments	χ^2 (d.f.)	Slope ± SE	LD_{50} (mg/l)[a]	LD_{99} (mg/l)[a]	Ratio of PPO to	
					LD_{50}	LD_{99}
PPO alone	11.07 (6)	9.0 ± 1.12	30.6 (28.31–33.42)	55.4 (47.53–70.54)	—	—
PPO + 92% CO_2	7.8 (4)	2.6 ± 0.39	1.0 (0.06–1.32)	7.8 (5.39–14.78)	30.6	7.1
PPO at 100 mm Hg	6.0 (4)	12.5 ± 1.61	5.8 (5.34–6.37)	8.9 (7.86–10.86)	5.3	6.2

[a] Numbers in brackets give the 95% confidence range.

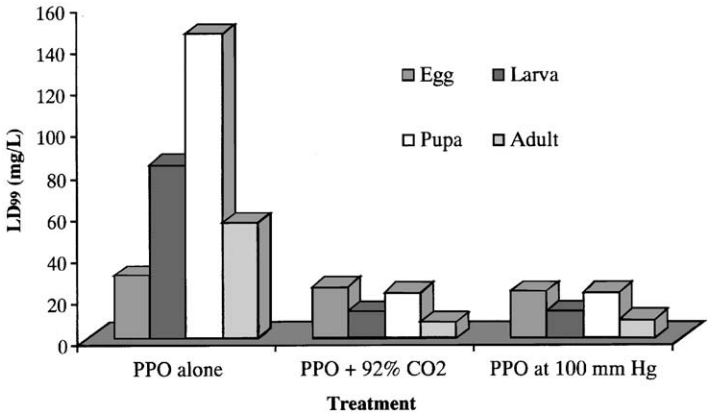

Fig. 1. LD_{99} values for fumigation of all life stages of *T. castaneum* with propylene oxide alone and in combination with low pressure (100 mm Hg) or 92% CO_2 at 4-h exposure time.

in LD_{99} values for the eggs (Table 3). Although PPO in combination with 100 mm Hg or 92% CO_2 resulted in very similar reductions in LD_{99} values for all life stages, the reduction in LD_{50} values varied with the combination treatments and the insect stages. However, although the reductions in LD_{50} values were smaller than those for LD_{99} values, the LD_{50} values obtained from PPO in combination with 100 mm Hg or 92% CO_2 were still reduced by 3.6- to 7.9-fold for all life stages, except the adult stage (30.6-fold for PPO with 92% CO_2), compared with PPO alone.

From this study, it can be seen that the use of 100 mm Hg or 92% CO_2 with PPO clearly resulted in significant reductions of LD_{50} and LD_{99} values for almost all life stages (Fig. 1). This was particularly effective for the most tolerant pupal stage where combining PPO with 100 mm Hg or 92% CO_2 decreased the LD_{99} value from 147 to 22 mg/l. It might be argued that low O_2 concentrations could influence the potentiating effect of CO_2 and reduced pressure. However, data without PPO indicated that there was no, or only limited mortality of all stages except the egg, on exposure to 100 mm Hg or 92% CO_2 alone for 4 h. Therefore, the results suggest that low pressure and CO_2 have a synergistic effect on the test insect when employed together with PPO.

Other studies have shown that vacuum fumigation or the admixture of CO_2 could increase the toxicity of fumigants, mainly MB and phosphine (Monro et al., 1966; Dumas et al., 1969;

Calderon and Leesch, 1983; Williams, 1985; Donahaye and Navarro, 1989). In all these studies the susceptibilities of test insects to fumigants combined with CO_2 or vacuum were found to increase by only a factor of one to three. However, the results obtained from our studies reveal that reductions in LD_{50} and LD_{99} values for post-embryonic stages caused by PPO in combination with CO_2 or low pressure are much higher than those reported by the above authors.

In conclusion, PPO alone was found to be effective against all the life stages of the common stored-product insect, *T. castaneum*, at moderate concentrations (30–150 mg/l) and short exposure times (4–8 h). It is however less toxic to *T. castaneum* than MB and phosphine. The use of a low pressure of 100 mm Hg, or 92% CO_2 appears to have a synergistic effect on this species as evidenced by significant reductions in LD_{50} and LD_{99} values for all life stages. These results indicate that the combination of PPO with low pressure or CO_2 can render the fumigant a potential replacement for MB. Clearly, further research is needed to obtain toxicity data on other stored-product insects, on its absorption by different commodities, and on its power of penetration into bulk commodities.

Acknowledgements

We are grateful to Dr Jonathan Donahaye, for his helpful suggestions during the course of this work, and for his review of the manuscript. We also thank the Ministry of Foreign Affairs of Israel (MASHAV) and the University of Kahramanmaras Sutcu Imam Turkey, for providing the funds that enabled Dr A. Isikber to participate in this study during a post-doctorate fellowship undertaken at Agricultural Research Organization, The Volcani Center. This work was funded in part by a grant from the United States—Israel Science and Technology Foundation (USISTF), ARO project number 417-0384-01.

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