Phytoprotection

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Action toxique de la phosphine chez le ciron des champignons (*Tyrophagus putrescentiae*) [Astigmata : Acaridae] adulte

F. Jian, D.S. Jayas and N.D.G. White

Volume 81, Number 1, 2000

URI: https://id.erudit.org/iderudit/706197ar
DOI: https://doi.org/10.7202/706197ar

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Publisher(s)

Société de protection des plantes du Québec (SPPQ)l

ISSN

0031-9511 (print)
1710-1603 (digital)

Explore this journal

Cite this article

Jian, F., Jayas, D. & White, N. (2000). Toxic action of phosphine on the adults of copra mite *Tyrophagus putrescentiae* [Astigmata : Acaridae]. *Phytoprotection*, *81*(1), 23–28. https://doi.org/10.7202/706197ar

Article abstract

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Toxic action of phosphine on the adults of the copra mite *Tyrophagus putrescentiae* [Astigmata : Acaridae]

Fuji Jian[1] , Digvir S. Jayas[1], and Noel D.G. White[2]

Received 2000-03-04; accepted 2000-05-17

PHYTOPROTECTION 81 : 23-28

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[Action toxique de la phosphine chez le ciron des champignons (*Tyrophagus putrescentiae*) [Astigmata : Acaridae] adulte]

Nous avons mesuré chez des cirons des champignons (*Tyrophagus putrescentiae*) adultes l'absorption de phosphine et étudié *in vivo* et *in vitro* les effets inhibiteurs de ce gaz sur la cytochrome C-oxydase et la catalase. Nous avons constaté que la phosphine a réduit l'activité des deux enzymes, les valeurs d'inhibition relative des enzymes pouvant être décrites comme suit : catalase *in vivo* > catalase *in vitro*, cytochrome C-oxydase *in vivo* < cytochrome C-oxydase *in vitro*; les effets inhibiteurs *in vitro* étaient proportionnels à la durée de l'exposition des extraits cytosoliques à la phosphine. L'absorption de phosphine chez les cirons des champignons adultes augmente lorsque la concentration du gaz et la durée de l'exposition s'élèvent, mais la vitesse d'absorption est alors moins élevée que lorsque la concentration est faible et l'exposition courte. Il semble que le mécanisme d'absorption de la phosphine ne soit pas le même chez les acariens que chez les insectes.

1. Department of Biosystems Engineering, University of Manitoba, Winnipeg, Manitoba, Canada R3T 5V6; e-mail : jianfj1963@hotmail.com
2. Cereal Research Centre, Agriculture and Agri-Food Canada, 195 Dafoe Road, Winnipeg, Manitoba, Canada R3T 2M9

INTRODUCTION

Phosphine (PH_3) is the most commonly used fumigant in the world to control stored-product insects and mites. Controlling stored-product mites always requires higher concentrations of phosphine and longer exposure periods than are needed to control insects (Jalil *et al.* 1974). In many circumstances, such as with short exposure and low gas concentration, the mite populations will rapidly increase after fumigation.

The main toxic action of phosphine on insects is as a respiratory inhibitor (Bond *et al.* 1969; Chefurka *et al.* 1976; Hobbs and Bond 1989; Nakakita 1976; Nakakita *et al.* 1971; Price 1980). In studies on stored-product beetles, inhibitory effects of the gas on *in vitro* cytochrome C oxidase was found; however, no effects on this enzyme were detected in susceptible or resistant insects after phosphine treatment *in vivo* (Price and Dance 1983). Price *et al.* (1982) reported that catalase both *in vivo* and *in vitro* was inhibited by the gas, and Price (1984) proposed that active exclusion of the toxic gas by resistant insects was likely. There is little research currently reported about the uptake and toxic action of phosphine on stored-product mites, which are common in stored food throughout the world (Hughes 1976).

The objectives of this research were to determine the uptake of phosphine, the inhibitory effect on cytochrome C oxidase and catalase of mites exposed to the toxic gas, and the relationship between gas uptake and enzyme inhibition.

MATERIALS AND METHODS

Mites

The mites, *Tyrophagus putrescentiae* (Schrank) [Astigmata : Acaridae], were obtained from the Zhengzhou Alcohol Factory (Zhengzhou, Peoples Republic of China) (lat. 34°7' N, long. 113°8' E) and reared at 27 ± 1°C, 75 ± 5% RH on a diet of whole-wheat flour with 5% yeast for about 150 d. Eggs were separated through a 180 mesh (30 μm) sieve and were reared to adults on the same diet and condition. Adults 15 ± 1 d old were used in the experiment.

Phosphine

Phosphine was generated by immersing 75.21% (w:w) zinc phosphide (Shenyang Pesticide Factory, Shenyang, P. R. of China) in 5% (w:v) sulfuric acid solution (China Chemical Co. Beijing, P. R. of China) and collecting the generated fumigant in a glass burette by displacement of sulfuric acid solution (Kashi and Bond 1975). The phosphine was retained in the burette for more than one wk.

The concentration of the phosphine was measured according to the method of Dumas (1964) using a DaoJin GC-5A model gas chromatograph (Japan) equipped with 2.7 m x 3.2 mm stainless steel column packed with chromosorb W and 30% Apiezon L. The nitrogen flow rate and column temperature were the same as in the method of Kashi and Bond (1975).

Fumigation chamber

Wide-necked glass bottles of approximately 40-mL capacity fitted with two glass tubes and a stopper were used as fumigation chambers. The chambers were sealed at the periphery of the stopper with melted paraffin wax. Two short rubber tubes were fit to the glass tubes to facilitate dosing and sampling. The chambers were humidified using 3 mL aqueous NaCl saturated solution (Winston and Bates 1960). This method maintained 75 ± 5% RH and the same absorption volume of phosphine in every chamber.

Testing for uptake of phosphine

Uptake of phosphine was determined by the indirect method of measuring the depletion of gas from the fumigation chambers as it was absorbed by the mites (Bond *et al.* 1969). About 20 mg of pre-weighed mites (without food) were put in a 1-cm diam and 4-cm long tube and it was suspended in the fumigation chamber. Twenty-four hour later, before the required volume of phosphine was injected into the chamber through one of the rubber tubes from a Hamilton gas-tight syringe, an equal

volume of air was evacuated from the chamber through the other rubber tube. Chambers were dosed at 0.05 mg L^{-1} and at 0.45 mg L^{-1} which were respectively equivalent to LD_{30} and LD_{50} in 48 h exposure. After 24 h or 48 h, the gas concentrations in the chamber were measured by gas chromatography (Dumas 1964). All experiments were carried out at 27 ± 1°C and 75 ± 5% RH. There were three replicates for each of two concentrations of phosphine and each experiment was repeated at least three times.

Testing of enzyme activity

In vitro

About 30 mg of frozen mites were immersed in buffer I (0.25 mol L^{-1} sucrose, 0.5 mmol L^{-1} ethylenediamine tetraacetic acid (EDTA), and 10 mmol L^{-1} Tris, pH 7.4) for testing cytochrome C oxidase or buffer II (sodium biphosphate - potassium dihydrogen phosphate, pH 7.4) for testing catalase. Mites were homogenized immediately using a Polytron homogenizer (Brinkmann Instruments, Westbury, N.J.) with a PT-10 generator (1 cm diam) at approximately 13 000 rpm (Hobbs and Bond 1989). The supernatant was filtered through Whatman No. 4 qualitative filter paper to remove fatty material. The protein concentration was determined by the dye-binding method of Brandford (1976), and was adjusted to 1 mg L^{-1} by adding buffer I or II. To facilitate the maximum inhibitory rate of enzymes by phosphine, and in order to compare the inhibitory rate with published data and with *in vivo* enzymes, the cytosolic extract solution was treated by bubbling phosphine gas in it using a Hamilton gastight syringe. To aid the dissolving of the gas, stirring was done for 30 min for every 1 h until 3 h or 6 h at 25 ± 1°C. This method maintained 120 to 200 mg L^{-1} phosphine concentration in the extract solution (Hobbs and Bond 1989). Similar extracts that were not treated with phosphine served as controls. After 3 or 6 h exposure, the enzyme activity was tested at 0, 7 and 14 d (for catalase activity) or at 0, 5 and 7 d (for cytochrome C oxidase activity) by using the methods of Price *et al.* (1982) and Price and Dance (1983). There were five replicates per treatment.

In vivo

About 30 mg of mites were exposed to 2.0 mg L^{-1} phosphine at 25 ± 1°C for 6 h (about 80% of mites were killed). Mites were homogenized, cytosolic extracts were filtered, protein was determined and enzyme activity was measured as described earlier. Enzymatic activity in phosphine-exposed mites was always compared to that in untreated controls. There were five replicates per treatment. The cytosolic extract solutions and standard enzymes (bovine liver catalase, standard cytochrome C oxidase) were kept at 0 to 4°C.

RESULTS

Uptake of phosphine

The uptake by the adult mites in the high concentration of PH_3 was higher than at the lower concentration; uptake was higher at 48 h exposure than at 24 h exposure (Table 1). However, the ratio of uptake was lower than the ratio of phosphine concentration, and the uptake increased minimally as the exposure time doubled.

Table 1. Uptake of phosphine by the adults of *Tyrophagus putrescentiae* (n = 5) [a]

PH_3 (mg L^{-1})	Uptake of PH_3 (mean ± S.E. µg g^{-1} mites)		
	24 h	48 h	Ratio (48/24 = 2)
0.05	1.1 ± 0.9	1.5 ± 0.7	1.4
0.45	5.8 ± 0.7	6.3 ± 0.5	1.1
Ratio (0.45/0.05 = 9)	5.3	4.2	

[a] The change of the phosphine concentration in the controls (without mites) was in 0.0001 mg L^{-1} in 24 h and 48 h at both phosphine concentrations.

Effect of phosphine on Cytochrome C oxidase and catalase

The activity of cytochrome C oxidase was reduced after phosphine treatment both *in vitro* and *in vivo,* and the activity of the enzyme *in vitro* was lower than that *in vivo* (Table 2). The inhibition of the enzyme *in vitro* was dependent on the length of phosphine treatment period. With 3 h exposure *in vitro,* the enzyme activity declined 43% in comparison with the control; with 6 h exposure *in vitro,* the enzyme activity decreased 57%. The *in vivo* enzyme activity only decreased 32%. ANOVA analyses and t-test showed that there were significant differences ($P < 0.05$) in enzyme activity between treated and control, and between mites treated *in vivo* or *in vitro.* There were no differences between treatments on 7 d.

The activity of catalase of the mites was reduced by phosphine both *in vitro* and *in vivo,* and the inhibition rate of the enzyme activity *in vivo* was higher than that *in vitro* (Table 2). The inhibition rate of *in vitro* enzyme activity was also proportional to the treatment time applied to the cytosolic extracts. ANOVA analyses and t-test showed that there were significant differences ($P < 0.01$) in enzyme activity between treated and control mites, between mites treated *in vivo* or *in vitro* on 0 d. There were no differences between treatments on 7 and 14 d.

The activities of the enzymes decreased along with the increase of incubation time except at 6 h exposure *in vitro* cytochrome C oxidase (Table 2). As incubation time increased, the activities of the treated enzymes were the same as the controls on 7 d (cytochrome C oxidase) or on 14 d (catalase).

DISCUSSION

Inhibition of oxygen consumption of insects by phosphine has been consistently reported (Bang and Telford 1966; Qureshi *et al.* 1965). These results indicate that phosphine is a respiration inhibitor (Bond *et al.* 1969; Chefurka *et al.* 1976; Hobbs and Bond 1989; Nakakita 1976; Nakakita *et al.* 1971; Price 1980). The mode of action of this gas has been further explained by its inhibition of mitochondrial activity from rat liver (Nakakita *et al.* 1971) and insects (Chefurka *et al.* 1976; Price 1980). Phosphine specifically inhibited cytochrome C oxidase, therefore this enzyme may be a target site in insects (Chefurka *et al.* 1976). Furthermore, Price and Dance (1983) found that *in vivo* exposure to phosphine of the lesser grain borer *Rhyzopertha dominica* (F.) [Coleoptera : Bostrichidae] did not inhibit cytochrome

Table 2. Enzyme activity in cytosolic extracts of adults of *Tyrophagus putrescentiae* treated with phosphine (n = 5)

Enzyme	Time[a]	Enzyme activity (mean ± S.E. µmol mg^{-1} min^{-1})				
		Control (no PH_3)		PH_3 exposure *in vitro*		PH_3 exposure *in vivo*
	(d)	3 h	6 h	3 h[b]	6 h[b]	6 h[b]
Cytochrome C-oxidase	0	30.8 ± 3.2	30.8 ± 2.1	17.6 ± 2.3**	13.2 ± 0.2*	20.9 ± 1.1**
	5	18.2 ± 2.8	18.1 ± 3.3	15.9 ± 1.4*	15.9 ± 0.5**	17.3 ± 0.7**
	7	15.4 ± 1.7	15.3 ± 2.2	15.3 ± 0.6	15.2 ± 0.2	14.3 ± 0.8
Catalase	0	14.10 ± 0.42	14.10 ± 0.16	10.84 ± 0.09**	7.70 ± 0.09**	7.15 ± 0.07**
	7	2.01 ± 0.13	2.00 ± 0.21	1.45 ± 0.04	1.15 ± 0.04	1.20 ± 0.16
	14	1.05 ± 0.04	1.03 ± 0.04	1.05 ± 0.04	1.05 ± 0.03	1.05 ± 0.02

[a] Time after treatment.

[b] Means that are significantly different from their controls at $P < 0.05$*, and $P < 0.01$** respectively, using t-test.

C oxidase in the same way as *in vitro* exposure caused non-competitive inhibition of cytochrome C oxidase. This suggests that it is important whether phosphine or its derivatives can pass through the double membrane system of mitochondria. If the phosphine or its derivatives can pass through the double membrane, it will have a chance to inhibit cytochrome C oxidase. The present results show that *in vivo* and *in vitro* exposure to phosphine inhibited cytochrome C oxidase, and the inhibition rate by *in vitro* exposure was higher than the *in vivo* exposure. This indirectly suggests that mitochondrial penetration of phosphine or its derivatives was reduced, or mites actively absorbed less phosphine and thus cytochrome C oxidase was inhibited less in the body than in the homogenate of mites. Therefore, cytochrome C oxidase in mites may not be the primary target or initial target.

There are many reports about inhibition of catalase of insects by phosphine (Hobbs and Bond 1989; Price *et al.*1982). These papers show that catalase both *in vivo* and *in vitro* was inhibited and the inhibition rate of *in vivo* catalase was higher than that of *in vitro* catalase. This evidence seems to support the argument for a connection between the inhibition of catalase and the toxicity of phosphine. Price (1984) found that resistant *R. dominica* appeared to actively exclude phosphine, which resulted in a lower inhibition rate of catalase in the cytosolic extracts of the resistant *R. dominica*. Hobbs and Bond (1989) reported that the activity of catalase recovered 2 wk after phosphine exposure. The present study agrees with results from the literature related to insects. The inhibition of catalase, which results in the accumulation of O_2^- in mite cells, may be one of the toxic effects of phosphine. This might explain why knocked-down mites can recover after a short period, and why phosphine fails to follow the concentration-time-mortality relationships (Fuji *et al.* 1992) that are characteristic of many other fumigants.

The factors governing uptake and toxicity of phosphine are complex and different between insects (Bond *et al.* 1969). The phosphine uptake of the adults of *Sitophilus granarius* (L.) [Coleoptera : Curculionidae] and *Tribolium confusum* DuVal [Coleoptera : Tenebrionidae] increases about 1.5 fold when phosphine concentration increases from 0.006 to 0.009 mg L^{-1} (1.5 fold) (Kashi and Bond 1975). At 20°C, 5 h exposure and in 0.2 mg L^{-1} phosphine, the adults of susceptible *R. dominica* can absorb 8.92 $\mu g\ g^{-1}$, and the resistant *R. dominica* can absorb 1.02 $\mu g\ g^{-1}$ (Price 1984). Compared with these data of phosphine uptake by insects, the uptake by mites was lower and was not greatly altered by increasing concentration (Table 1). Slower uptake of phosphine by mites might explain why longer exposures to phosphine are needed to control mites, compared with insects. Many mites, such as *Tyrophagus*, respire directly through their soft cuticle (Hughes 1976), unlike insects which respire through a tracheal system. The relationships among this respiration difference, uptake and the toxicity of phosphine are unclear.

ACKNOWLEDGEMENTS

The authors thanks professor Qizong Chen and professor Anbang Lu, Department of Grain and Oil Storage, Zhengzhou Grain College (P.R. of China), for advice during the experiments and data compilation.

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