RESPONSES OF STORED-PRODUCTS INSECTS TO PHOSPHINE

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ABSTRACT

Phosphine, a major fumigant currently in use for grain protection has two drawbacks: (1) the relatively long exposure period required, and (2) the development of insect resistance. In recent years, considerable work has been done on the influence of phosphine on stored products insect pests: (1) to elucidate its mechanism of toxic action; (2) to understand the mechanisms by which the insects detoxify and develop resistance to the fumigant; and (3) to maximise the efficacy of phosphine in the control strategy. Based on recent literature and the author's own findings, the various responses, including mortality, narcosis, active exclusion/ reduced uptake of phosphine, inhibition of respiration, reproduction, and changes in development and behaviour are discussed. The influence of other fumigants, atmospheric gases, and irradiation on phosphine toxicity to insects are also included. The mode of action of phosphine is not simple as revealed by *in vitro* and *in vivo* studies which do not always concur . The responses of phosphine-treated insects differ from those exposed to HCN or nitrogen (anoxia). The mortality response to phosphine dosages varies widely between species and between life stages of the same species and demonstrates heterogeneity at the higher response levels. The response of insects to constant and changing concentrations of phosphine needs further attention. Only carbon dioxide up to certain concentration limits improved the toxic action of phosphine. Although resistance occurs readily following repeated phosphine treatments, it is not expressed at the same level in all stages of the same species.

INTRODUCTION

Phosphine released from metal phosphide preparations is currently the major fumigant in use for the protection of stored-products world-wide. Phosphine is the preferred fumigant for routine treatments especially in developing countries where other control techniques including controlled atmosphere storage will be expensive and therefore cannot be readily adopted. The fumigant does not modify the criteria of food safety, as revealed by

several rat feeding trials on phosphine fumigated foods (e.g., Cabrol Telle, *et al.*, 1985). On many parameters, like application, quality aspects of fumigated commodities (Bond, 1984; AFHB/ACIAR, 1989), financial, toxicological (non-target organisms) and environmental considerations (Reichmuth, 1990), phosphine surpasses other fumigants (Table 1). Nevertheless, the long exposure period is one of the limiting factors for the fumigant. The exposure period cannot be reduced by increasing the dose as the fumigant has its own characteristic toxic effects on insects. Following the first detailed report by Champ and Dyte (1976) on the incidence of strains resistant to phosphine among field collected samples from different countries, the field occurrence of phosphine resistance associated with control failures has been documented (Champ, 1985; Taylor, 1986; Rajendran, 1989). Hence increasing attention has been paid to understanding the biological properties of phosphine against stored products insects: (1) to identify/establish the site of action; and (2) to maximise its insecticidal efficacy. Some brief reviews have been published on specific topics, e.g., toxicity (Howe, 1974; Banks, 1975), biochemical effects (Price and Chambers, 1990), resistance (Champ, 1985), and many other aspects (GASGA, 1986; WHO, 1988). In this paper, the various responses of stored products insects to phosphine are discussed with their implications for insect control.

Table 1: Merits and drawbacks of phosphine.

Parameter	Characteristics
Quality of treated commodities	- No adverse effect on quality, organoleptic property, germination, and end-use parameters - No tainting - Low residue levels
Application aspects	- Easy to store, handle and apply - Devices to detect at hygienic levels available - Not applicable for vacuum fumigation - Corrodes metals, especially copper
Entomological aspects	- Large differences in susceptibility between species/developmental stages - Insects develop resistance quickly - Ineffective at low temperatures - Longer exposure period necessary
Financial aspects	- Very economical, especially for routine treatments
Toxicological aspects (non-target organisms)	- No cumulative toxicity - Does not modify the criteria of food safety
Environmental aspects	- Rapidly degraded in the atmosphere

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RESPIRATION AND GAS UPTAKE

Inhibition of respiration in insects exposed to phosphine has long been established. The inhibition is greater at higher phosphine concentrations and is partly attributed to reduced phosphine uptake by insects. Oxygen is essential for the toxic action of phosphine: in the absence of oxygen the uptake of phosphine does not take place (Bond, *et al.,* 1969). To a certain extent, phosphine uptake appears to be independent of opening or closing of spiracles or insect respiration (Nakakita and Kuroda, 1986). The amount and rate of fumigant uptake are known to vary between species, between strains, and between the developmental stages of a species. Active stages absorb relatively more phosphine than eggs or pupae. Adults of *Sitophilus granarius* L. absorb less phosphine and at a slower rate (Bond *et al.,* 1969). Banks (1975) postulated that the defensive secretions play a role in the higher gas uptake in *Tribolium castaneum* Herbst. The levels of phosphine uptake beyond certain limits do not correlate with a mortality response (Bond, 1980). The uptake was shown to be higher when the insects were treated in the presence of increasing carbon dioxide levels (Kashi and Bond, 1975).

Resistant strains absorb less phosphine (Price and Dance, 1983). Chaudhry and Price (1992) suggested the presence of a phosphine carrier-protein in the tracheal walls of insects, the level of which may vary between species and between susceptible and resistant strains. The carrier-protein may play a vital role in preventing phosphine from reaching the target site of action.

RESPONSES AT THE BIOCHEMICAL LEVEL

The interactions of phosphine with the biochemical processes are given in Table 2. Since phosphine resembles HCN in its inhibition of insect respiration, it was thought that like HCN, phosphine attacks the electron transport chain, especially cytochrome c oxidase in mitochondria (Chefurka *et al.,* 1976). *In vitro* and *in vivo* studies indicated changes in the mitochondria configuration (Kashi, 1974; Price and Bell, 1981). However, the *in vivo* inhibition of cytochrome c oxidase in phosphine-fumigated insects was not established (Price, 1980). Then it was suggested that insects might succumb to phosphine due to the action of the fumigant on catalase, another haemprotein (Price and Dance, 1983). In several insect species, *in vivo* inhibition of catalase activity has been demonstrated. However, *in vitro* studies have not shown any inhibition except for *T. castaneum* (Hobbs and Bond, 1989; Price and Chambers, 1990). Later investigations questioned that the significance of catalase activity in insects and the inhibition might be an indirect effect of the fumigant. Price and Walter (1987) failed to achieve an increased mortality response in insects that were fed 3-amino-1,2,4-triazole, a semi-specific inhibitor of catalase synthesis. The involvement of superoxide dismutase, an antioxidant enzyme, was proposed by Nakakita and Kuroda

(1986) following *in vitro* studies on *T. castaneum* adults. However, Price and Chambers (1990) claimed that the increased level of activity of superoxide dismutase in *T. castaneum* was an artifact of the combination of insect and buffer used. The implications of antioxidant enzymes in the toxicity of phosphine were stressed also by Bolter and Chefurka (1990). In *in vitro* studies on *S. granarius,* the latter linked the biochemical responses of lowered catalase and peroxidase activities with elevated superoxide dismutase and peroxidase activities and assumed the production of hydroxyl radicals that eventually destroy the cell integrity.

Glutathione S-transferase activity was increased in exposed larvae of *Trogoderma granarium* Everts, but the level of reduced glutathione was not decreased proportionately (Shivanandappa and Rajendran, 1987). However, the role of glutathione in the susceptibility of insects was ruled out as there was no significant change in mortality of *Rhyzopertha dominica* F. adults previously fed on buthionine sulphoximine, a specific inhibitor of γ-glutamylcysteine synthetase which consequently lowers tissue GSH levels (Chaudhry and Price, 1992). Acetylcholinesterase inhibition has been reported in *Ephestia cautella* Walker, although its significance in the action of phosphine is not known (Al-Hakkak *et al.,* 1989). Phosphine absorbed by the insects is oxidised to hypophosphite. Nevertheless, the pH of haemolymph is not reported to change indicating the good buffering capacity of the insect haemolymph (Chaudhry and Price, 1989).

Thus, the action of phosphine on insects is not as simple and straightforward as considered earlier. Price and Chambers (1990) are of the opinion that a number of options are available for biochemical action of phosphine on insects and subsequent exertion of its toxic effect.

COMPARATIVE RESPONSES TO HCN, ANOXIA AND PHOSPHINE

Newly laid (0-6 hr-old) eggs of *E. cautella* and *E. kuehniella* Zeller are very sensitive to 100% nitrogen (anoxia), showing an apparent uniform 1-2 days delay in hatching. By contrast, newly laid eggs are highly tolerant to phosphine and the delay in hatching is short, occurring only at phosphine concentrations causing more than 30% kill. Furthermore, anoxia causes a general disruption of cellular integrity but phosphine produces specific changes in the ultrastructure of mitochondria in the eggs (Price and Bell, 1981). *In vivo* studies on the adults of *R. dominica* established the differences in the biochemical effects of phosphine, anoxia and HCN. Pyruvate and lactate levels increased following exposure to anoxia or HCN, but not to phosphine. However, exposure to phosphine or HCN depleted the ATP level in the tissues (Price and Walter, 1987). HCN inhibits cytochrome c oxidase *in vivo* and *in vitro* in insects and mammals, whilst phosphine does not inhibit it *in vivo* (Price, 1980). UV and visible absorption spectra of mitochondria (from maize or rat liver) incubated with phosphine showed peaks at 602 and

445 nm, whereas the mitochondria incubated with HCN or held under anaerobic conditions showed peaks at 605 and 443 nm only (Nakakita, 1976). It is evident, therefore, that the responses to phosphine differ from those to anoxia or HCN.

Table 2: Interaction of phosphine with the probable target sites/detoxifying systems.

Source	Reaction	Effect	References
HAEMPROTEINS			
Cytochrome C oxidase			
Rat liver	*in vitro*	Inhibited	Nakakita *et al.*, 1971
Mouse liver	*in vitro*	Inhibited	Chefurka *et al.*, 1976
Housefly flight muscle	*in vitro*	Inhibited	Chefurka *et al.*, 1976
Granary weevil	*in vitro*	Inhibited	Chefurka *et al.*, 1976
Lesser grain borer	*in vitro*	Inhibited	Price and Dance, 1983
Rust-red grain beetle	*in vitro*	Inhibited	Price and Dance, 1983
Saw-toothed grain beetle	*in vitro*	Inhibited	Price and Dance, 1983
Saw-toothed grain beetle	*in vivo*	Inhibited	Price and Dance, 1983
Rust-red grain beetle	*in vivo*	Not inhibited	Price and Dance, 1983
Lesser grain borer	*in vivo*	Not inhibited	Price and Dance, 1983
Catalase			
Lesser grain borer	*in vitro*	Inhibited	Price and Dance, 1983
Granary weevil	*in vivo*	Inhibited	Bond, 1963
Saw-toothed grain beetle	*in vivo*	Inhibited	Price and Dance, 1983
Lesser grain borer	*in vivo*	Inhibited	Price and Dance, 1983
Granary weevil	*in vivo*	Inhibited	Bolter and Chefurka, 1990
Red flour beetle	*in vivo*	Inhibited	Hobbs and Bond, 1989
Red flour beetle	*in vitro*	Inhibited	Hobbs and Bond, 1989
Bovine liver	*in vitro*	No spectral change	Chaudhry and Price, 1990b
Peroxidase			
Granary weevil	*in vivo*	Inhibited	Bolter and Chefurka, 1990
Lesser grain borer	*in vivo*	Inhibited	Chaudhry and Price, 1992
Haemoglobin			
Rat	*in vitro*	No change	Trimborn and Klimmer, 1962
Rat	*in vivo*	No change	Klimmer, 1969
Human	*in vitro*	Spectral change	Chaudhry and Price, 1990b
Myoglobin			
Equine skeletal muscle	*in vitro*	Spectral change	Chaudhry and Price, 1990b

Table 2: Interaction of phosphine with the probable target sites/detoxifying systems (cont.)

Source	Reaction	Effect	References
SUPEROXIDE DISMUTASE			
Red flour beetle	*in vitro*	Increased	Nakakita and Kuroda, 1986
Granary weevil	*in vivo*	Increased	Bolter and Chefurka, 1990
Lesser grain borer	*in vivo*	Increased	Chaudhry and Price, 1992
LIPID PEROXIDASE			
Lesser grain borer	*in vivo*	Increased	Chaudhry and Price, 1992
GLUTATHIONE S-TRANSFERASE			
Khapra larvae	*in vivo*	Increased	Shivanandappa and Rajendran, 1987
ACETYLCHOLINESTERASE			
Almond moth	*in vivo*	Inhibited	Al-Hakkak *et al.*, 1989
Almond moth	*in vitro*	Inhibited	Al-Hakkak *et al.*, 1989

MORTALITY

The mortality response caused by phosphine is influenced by its concentration, level of oxygen and carbon dioxide present, exposure time, and temperature (Howe, 1974). Large differences in the susceptibility of the species, strains, and developmental stages in a species and a high heterogeneity at the higher response levels (>90% kill) are characteristic of phosphine toxicity (Nakakita and Winks, 1981; Winks, 1987). Besides diapausing larvae of *T. granarium* and *Ephestia* spp., eggs and pupae among the developmental stages are generally tolerant (Hole *et al.*, 1976; Bell, 1977). It is an established fact that insects respond better to lower concentrations with longer exposure period than to higher concentrations with shorter exposure period. Time is important both as a dosage factor and as a response factor (Winks, 1986a). Treatment temperature and/or lengths of exposure time sometimes determine the tolerant stage in a species. For instance, in *Lasioderma serricorne* F., eggs are tolerant to phosphine at >25OC but at 15OC the pupae are more tolerant than the eggs. In *Ptinus tectus* (Boield.), at 10-25OC, pupal stage is tolerant during short exposures whereas eggs are tolerant during longer exposures (Hole, *et al.*, 1976). Diapausing larvae of *E. elutella* Hübner respond better at a lower temperature (15OC) than at a higher temperature (20OC) when exposed to high or low phosphine concentrations (Bell and Mills, 1983).

Phosphine exhibits cumulative toxicity in insects that varies from one species to the other (Bond and Upitis, 1973; Hobbs and Bond, 1989). Insects

exposed previously to sublethal doses of phosphine are more susceptible in subsequent treatments, with response increasing the shorter the treatment interval. Differences in the responses of stored products insects to fixed or constant, and changing concentrations of phosphine have been reported. The developmental stages of *S. granarius* are susceptible to constant rather than changing phosphine concentrations (Reichmuth, 1986). In bioassay involving a rise and subsequent decline in phosphine concentrations, the mortality response of *Cryptolestes ferrugineus* Stephens was related to the gas concentration remaining at the end of exposure with an advantage if concentrations fell as late as possible, in terms of improved response (Bell *et al.*, 1990). Rajendran (unpublished) tested a phosphine-resistant strain (x 4.5 resistance factor at LD$_{50}$) of *T. castaneum* at 50% (ct = 0.55 mg hr/L) and 90% (ct = 1.65 mg hr/L) response levels with 6-48 hour exposure periods (Fig. 1). No significant change in the mortality of the adults exposed to constant, increasing and decreasing concentrations of phosphine, and decreasing concentrations of phosphine was evident. Further tests involving tolerant stages will help in understanding the necessary changes in application techniques.
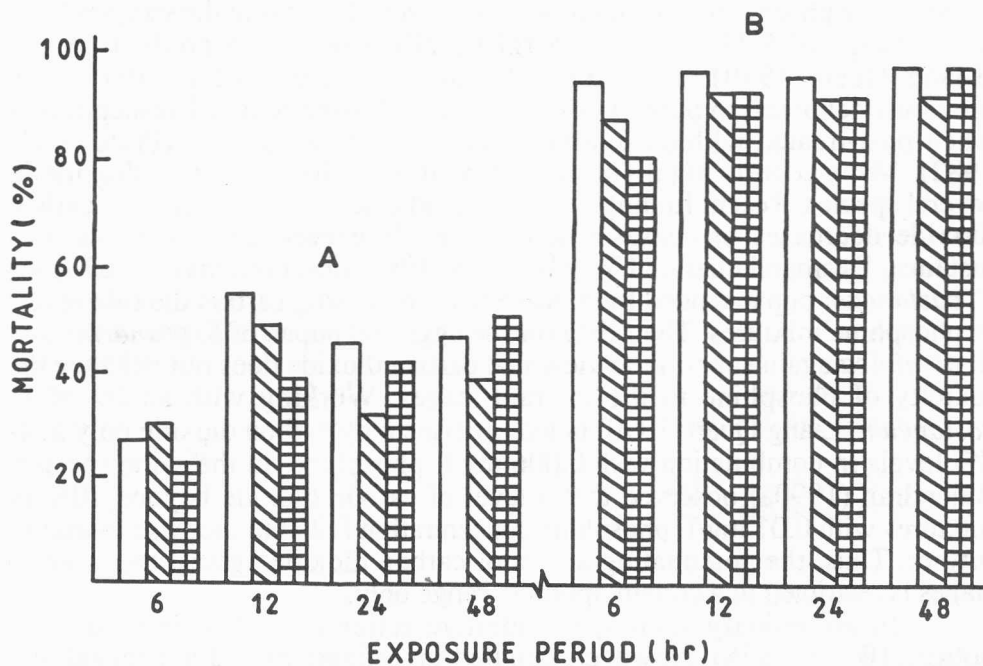

Fig. 1: Response of *T. castaneum* adults to constant (▢), increasing (◩) and decreasing (⊞) concentrations of phosphine at a ct product of (A) 0.55 mg hr/L and (B) 1.65 mg hr/L at 25±1°C and 70-80% r.h.

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NARCOSIS

Insects are narcotised by high concentrations of phosphine. The leveling off in dose-mortality relationship above 90% kill is partly attributed to narcosis (Bond *et al.*, 1969; Winks, 1985). Exposure time needed to induce narcosis is shorter at higher concentrations. Mortality of the insects exposed to narcotic range of concentrations, i.e., 0.5 mg/L and above, is strongly dependent on extended exposure periods. Reichmuth (1986) speculated that exposure of insects to changing concentrations induced them to narcosis even at a subnarcotic concentration range (<0.5 mg/L). Narcosis threshold concentration is always higher in phosphine-resistant strains (Winks, 1986a). Narcosis caused by higher concentrations of phosphine is believed to be due to the effect of phosphine on nerves (Nakakita *et al.*, 1974; Kashi, 1981).

PHOSPHINE IN MIXTURES

The influences of atmospheric gases and other fumigants either as pre- or post-treatments or simultaneously with phosphine against insects have been studied (Table 3). The necessity of oxygen to elicit the mortality response by phosphine is well known. However, decreasing the oxygen level to the range of 5-12% has a synergistic effect on stored product beetles (Liang Quan, 1990). In an exceptional case, adults of *R. dominica,* previously exposed to pure oxygen for 2 days showed reduced susceptibility when post-treated with phosphine at the discriminating dose (Price *et al.,* 1982). Many reports highlight the potentiating action of carbon dioxide in several species. Recent findings, however, indicate that the efficacy of carbon dioxide decreases or ceases when the levels exceed certain limits. For instance, Desmarchelier and Wohlgemuth (1984) observed that the LT_{99} for *T. castaneum* pupae tended to increase with increasing carbon dioxide levels in phosphine mixtures. Their data for the eggs and pupae of *S. granarius* and *R. dominica* again support the view that carbon dioxide does not enhance the toxicity of phosphine to the tolerant stages. Working with adults of *T. castaneum* Liang Quan (1990) noted synergism by carbon dioxide only at 4-8% levels in combination with 0.006 mg/L phosphine. In the same species, Rajendran (1990a) observed that a level of carbon dioxide beyond 10% in mixtures with 0.01 mg/L phosphine concentration failed to increase mortality further. Thus, the potentiating action of carbon dioxide against the tolerant stages is restricted to a certain optimum range only.

In preliminary studies, the additive action of methyl bromide was noticed (Bond, 1978). However, detailed investigations did not reveal any advantage in terms of toxicity of the mixtures containing methyl bromide and phosphine (Bond and Morse, 1982; Rajendran and Muthu, 1989). Gamma irradiation before or after phosphine treatment does not influence the toxicity of phosphine (Saxena and Bhatia, 1981).

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Table 3: Effect of pre-, post- and simultaneous-treatments with atmospheric gases,
fumigants and irradiation on phosphine toxicity to insects.

Insect	Life stage tested	Mortality response		References
		Increased	Reduced or no change	
OXYGEN				
Pre-treatment				
S. granarius	Adult	*		Bond, 1963
T. mauritanicus	Larva	*		Bond, 1963
R. dominica	Adult		*	Price *et al.,* 1982
Post-treatment				
S. granarius	Adult	*		Bond, 1963
T. mauritanicus	Larva	*		Bond, 1963
T. castaneum	Adult	*		Hobbs and Bond, 1989
Simultaneous				
T. castaneum	Adult	*		Liang Quan, 1990
NITROGEN				
Pre-treatment				
S. granarius	Adult		*	Bond, 1963
T. mauritanicus	Larva		*	Bond, 1963
Post-treatment				
S. granarius	Adult		*	Bond, 1963
T. mauritanicus	Larva		*	Bond, 1963
T. castaneum	Adult		*	Bond *et al.,* 1967
Simultaneous				
C. chinensis	Adult		*	Sato and Suwanai, 1973
S. zeamais	Adult		*	Sato and Suwanai, 1973
Several species	Adult		*	Kashi, 1981
CARBON DIOXIDE				
Pre-treatment .				
S. granarius	Adult		*	Kashi and Bond, 1975
T. confusum	Adult		*	Kashi and Bond, 1975
Post-treatment				
S. granarius	Adult		*	Kashi and Bond, 1975
T. confusum	Adult		*	Kashi and Bond, 1975
Simultaneous				
S. granarius	Adult	*		Kashi and Bond, 1975
Several species	All stages	*		Desmarchelier and Wohlgemuth, 1984
T. castaneum	Egg	*		Rajendran and Muthu, 1989
T. castaneum	Pupa	*		Rajendran, 1990a
T. castaneum	Adult	*		Liang Quan, 1990

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Table 3: Effect of pre-, post- and simultaneous-treatments with atmospheric gases, fumigants and irradiation on phosphine toxicity to insects (cont.)

Insect	Life stage tested	Mortality response		References
		Increased	Reduced or no change	
T. granarium	Larva (diapausing)	*		El-Lakwah *et al.*, 1989
METHYL BROMIDE				
Post-treatment				
E. elutella	Larva (diapausing)	*		Bell and Mills, 1983
Simultaneous				
S. granarius	Adult	*		Bond, 1978
T. castaneum	Adult	*		Bell and Mills, 1983
T. granarium	Larva	*		El-Lakwah, 1978
T. granarium	Larva (diapausing)	*		Bell *et al.*, 1984
T. castaneum	Adult		*	Bond and Morse, 1982
T. castaneum	Egg		*	Rajendran and Muthu, 1989
T. castaneum	Pupa		*	Rajendran, 1990a
HYDROGEN CYANIDE				
Pre-treatment				
T. castaneum	Adult		*	Hobbs and Bond, 1989
Post-treatment				
T. castaneum	Adult		*	Hobbs and Bond, 1989
Simultaneous				
T. castaneum	Adult		*	Hobbs and Bond, 1989
OTHER FUMIGANTS[1]				
T. castaneum	Egg		*	Rajendran and Muthu, 1989
T. castaneum	Pupa		*	Rajendran, 1990a
IRRADIATION				
Pre-treatment				
T. castaneum	Adult		*	Saxena and Bhatia, 1981
Post-treatment				
T. castaneum	Adult		*	Saxena and Bhatia, 1981

[1] Methyl iodide, methyl chloroform and ethylene dibromide

RESISTANCE

Many laboratory selection studies have shown clearly that in general, the stored-products insects develop resistance to phosphine quickly (Mills, 1978; Winks, 1986b). However, the level of resistance a species can develop varies, and the highest known level of resistance from field collected insects was noted for *R. dominica* (Mills, 1983; Rajendran, 1989). Within a species, resistance build-up differs between strains (Winks, 1986b). Once developed, resistance is quite stable (Taylor, 1989). Resistance is expressed in all stages, whether the specimens are laboratory-selected or field-collected (Bell *et al.,* 1977; Nakakita and Winks, 1981; Mills, 1983; Bakward, 1984; Rajendran, 1989). In a selection experiment with *T. castaneum,* Rajendran (1992) established that insects surviving repeated phosphine treatments, whether they are the tolerant pupae and eggs or the susceptible adults and larvae, can give rise to resistant strains (Figs. 2 and 3). The tolerant stages produce relatively high levels of phosphine resistance. Moreover, among the life stages of *T. castaneum* , the adult stage is a relatively poor indicator of phosphine resistance.

Whether selected in the laboratory or occurring in the field phosphine resistant strains show no cross-resistance to methyl bromide (Dyte, *et al.*, 1983; Rajendran, 1992) and to insecticides (Bakward, 1984; Kem, 1979). However, in some strains, multiple resistances to pesticides including phosphine can occur (Attia, 1984). Resistant strains are normal in their susceptibility to gamma-irradiation (Saxena and Bhatia, 1981; Nakakita, *et al.*, 1985). Some of the changes in biological parameters observed in the resistant strains include a reduction in body weight, respiratory rate (Nakakita and Winks, 1981), and reproduction potential (Saxena and Bhatia, 1980; Bakward, 1984). However, the reduced fecundity or fertility is not correlated with the level of resistance (Winks, 1986b). Differences in locomotor activity of the resistant strains are also noticed (Nadarajan, *et al.*, 1984; Bell, 1987).

Reduced uptake and active exclusion of phosphine, and enhanced narcosis threshold concentration levels are some of the characteristics of resistant strains. Though respiration and activity were normal, resistant adults absorbed less phosphine (Price and Dance, 1983). Chaudhry and Price (1990a) are of the opinion that apart from reduced uptake of phosphine, some other metabolic detoxification may be affecting the toxicity of phosphine in resistant strains. Lower phosphine uptake may also be due to some barrier in tracheal permeability (periplasmic carrier protein?) to phosphine (Chaudry and Price, 1992). Arriving at the concept of "net phosphine balance", Price (1986) demonstrated active exclusion of phosphine during short exposures in resistant strains of many stored product insects. The significance of active exclusion in the resistance mechanism still warrants examination. The shift in narcosis threshold range in resistant strains is important from a control point of view (Reichmuth, 1986; Winks, 1986b). In resistant *T. castaneum* and *R. dominica*, the narcosis threshold increased tenfold. Differences in the
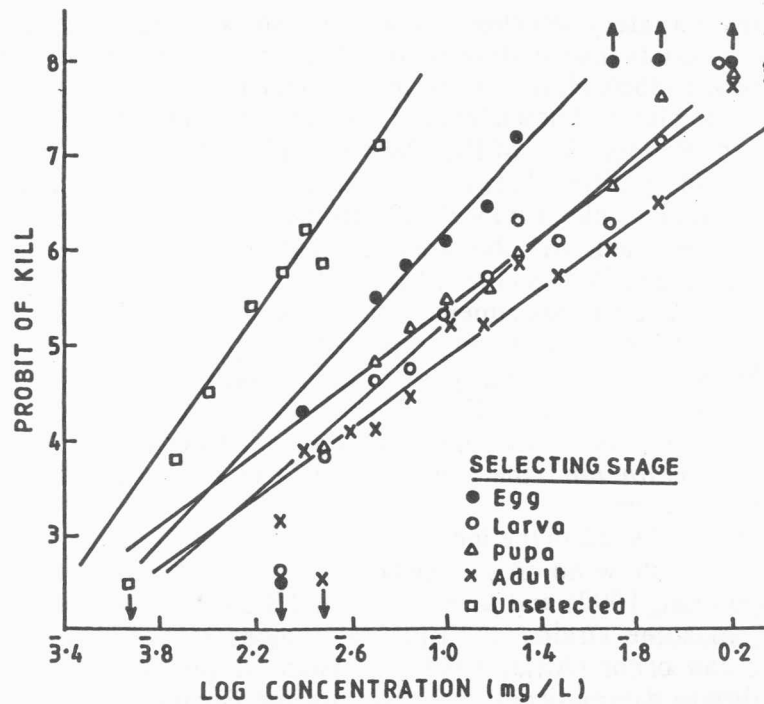
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Fig. 2: Responses of *T. castaneum* larvae exposed to phosphine for 24 hr at 25 ± 1°C and 70-80% r.h. after laboratory selection pressure for six generations.

basal levels of the activity of enzymes, believed to be involved in the toxicity/metabolism of phosphine including catalase, cytochrome c oxidase, peroxidase, and superoxide dismutase, were noticed (Price and Dance, 1983; Bolter and Chefurka, 1990; Chaudhry and Price, 1990a). By contrast to susceptible strains, the activities of antioxidant enzymes were not affected in resistant adults of *S. granarius* exposed to phosphine at the LD_{30} of the susceptible strain i.e. 0.035 mg/L for 5 hours (Bolter and Chefurka, 1990).

To overcome these specific mechanisms of active exclusion and enhanced narcosis threshold, lengthening of the exposure period has been strongly recommended. Winks (1987) proved the effectiveness of increased exposure periods rather than increases in phosphine concentrations against resistant strains of *T. castaneum* and *R. dominica*.

BEHAVIOUR

A study of the impact of fumigants on the behaviour of insects, such as avoidance and disturbance, is important in control strategies. During

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fumigation under an enclosure, some insects are likely to move away from lethal concentrations towards leakage spots, thereby enhancing their chances of survival. High concentrations of phosphine (0.5 mg/L) are repellent to *S. granarius*, but not to *Oryzaephilus surinamensis* L. or *T. castaneum* (Bell, 1987). Further investigations using other stages are needed in this area.
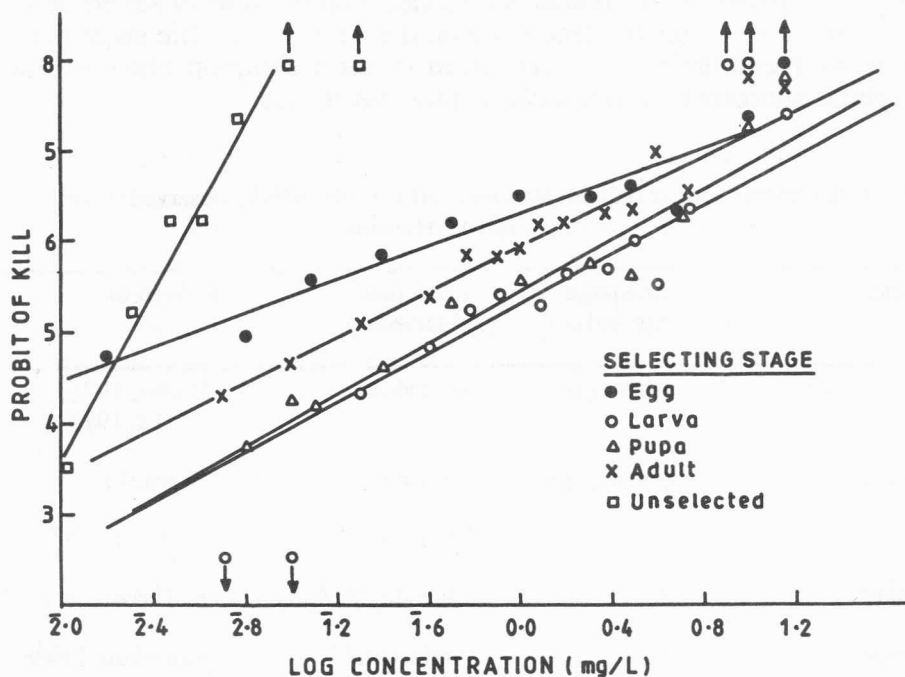

Fig. 3: Responses of *T. castaneum* pupae exposed to phosphine for 24 hr at 25 ± 1°C and 70-80% r.h. after laboratory selection pressure for six generations.

DEVELOPMENT, LONGEVITY AND REPRODUCTION

The development of insects is generally not affected by phosphine. Actually, during the fumigation period insect development proceeds until the tolerant eggs and pupae reach the susceptible stages and succumb (Howe, 1973). Nevertheless in a few cases, delay in development has been noted (e.g., in *Tenebroides mauritanicus* L.) (Bond and Upitis, 1973). Working with the eggs of *T. castaneum*, Rajendran and Muthu (1991) recorded a slight increase in the number of stragglers, i.e., larvae that failed to reach the adult stage within 45 days from the day of oviposition, in fumigated batches as compared with controls. Very little information is available on the longevity of the fumigated insects. The longevity of adults of *E. cautella* and *S . granarius* surviving exposure as pupae was not affected (Al-Hakkak *et*

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al., 1985; Howe, 1973), whereas the longevity of *Tribolium castaneum* adults was reduced following exposure to 2.07 mg/ (Winks, 1973).

An ideal fumigant need not have sterilising property or other effects which directly or indirectly affect the multiplication of survivors, yet such an effect would be advantageous to a fumigant, because control failures and resultant insect survival are not uncommon in commercial fumigations. Phosphine inhibits the reproductive/multiplication potential of stored-products insects to a certain extent, depending on the dose and the life stage exposed (Table 4). Nevertheless, this reduction is not a common phenomenon in phosphine fumigation, to forestall repetitive treatments.

Table 4: Observations of reduced fecundity/fertility/productivity observed in survivors of phosphine treatments.

Species	Life stage exposed	Reduction observed in	References
T. castaneum	All stages	Fecundity	Winks, 1971; Winks, 1973
S. granarius	Larva, pupa	Fecundity	Howe, 1973
R. dominica	Adult	Fecundity	Bakward, 1984
E. cautella	Pupa	Fecundity Fertility	Al-Hakkak, *et al* 1985
S. oryzae	Adult	Productivity	Rajendran, 1990b
T. granarium	Larva	Productivity	Rajendran, 1990b

CONCLUSION

From the foregoing reports it is obvious that the interactions of phosphine with target insects are still not completely understood. Phosphine uptake at high concentrations is low, thereby enabling survival. The reasons for lower uptake of phosphine in high concentrations, and the mode of action are yet to be elucidated. The cumulative toxicity of phosphine needs further investigation using tolerant stages and resistant strains. Multiple dosing (Friendship *et al.*, 1986) or intermittent application of phosphine (Liang Quan, 1990) that has the advantage of cumulative toxic effect, may prove effective. Comparative responses of insects to changing and constant concentrations also warrant examination, especially against the tolerant insect stages and at different climatic conditions.

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