RECENT DEVELOPMENTS IN THE FUMIGATION OF GRAIN WITH PHOSPHINE

R.G.Winks[a] and R. Ryan[b]
a. CSIRO Division of Entomology, SGRL, PO Box 1700, Canberra, ACT 2601, Australia
b. CIG Pty Ltd. PO Box 288, St. Leonards, N.S.W. 2065, Australia

Abstract

Over the past four years, since the last International Working Conference on Stored-Product Protection, there has been world-wide hardening of attitudes towards pesticide residues in grain. In many cases, this has resulted in greater usage of fumigants, particularly phosphine. This increased usage has the potential to increase selection for phosphine resistance. This paper discusses the important elements of phosphine toxicity focusing on recent studies of phosphine resistance. It also describes some of the recent developments in fumigation techniques aimed at minimising selection and controlling resistant strains. These techniques are based on the use of well-sealed enclosures or the use of controlled-release techniques that are possible with cylinders containing phosphine in carbon dioxide.

Introduction

Over the past few years there have been three major factors that have influenced the usage of phosphine and will influence its use in future years. The first has been the changing attitudes towards pesticide residues in food and the impact this has had on market requirements for grain and grain products. The second has been the increasing incidence of resistance to phosphine and the third has been the reports of phosphine mutagenicity, particularly in the case of humans (Garry *et al.*, 1989).

If phosphine is to retain its place as a major method of pest control in stored food commodities it is essential that it is used in a manner consistent with our current state of knowledge and consistent with current attitudes towards the use of such materials. This paper focuses mainly on the methods of application of phosphine particularly in the context of phosphine resistance.

Principles of phosphine toxicity

Any discussion of methods of application is only meaningful against an understanding of the principal components of phosphine toxicity. These appear to be frequently overlooked or not understood because improper use of phosphine continues with many treatments too short in structures that are too leaky.

Components of phosphine toxicity can be generalised thus:

(i) Time is the critical parameter of dosage

(ii) Tolerance between developmental stages varies greatly

(iii) High concentrations of phosphine induce narcosis at least in adult beetles

(iv) Resistance can be selected easily

(v) Resistance has been selected in every species attempted

(i) Exposure time

The relationship between concentration (C) and exposure time (t) has been shown to fit the general model ($C^n t = k$) where k is a constant for a specified level of mortality (Winks, 1984). The exponent (n) of concentration, is generally less than one and varies between about 0.25 for pupae of *Sitophilus granarius* (Winks, 1986) and 0.9 for adults of *Tribolium castaneum* (Winks,1984). In comparisons between strains it follows that much greater differences will be observed for the same strain, when fixed exposure periods are used as the basis of these comparisons than when fixed concentrations are used. It also follows that the variability of response of the same strain is greater when fixed exposure periods are used compared with fixed concentrations. While these characteristics have most obvious influence on laboratory studies they also have implications for methods of application in the field.

(ii) Tolerance of developmental stages

The tolerance of the egg and pupal stage is considerably greater than that of larvae and adults (Winks, 1986). However, in both eggs and pupae the tolerance decreases with development. The concentrations likely from current dosage recommendations are unlikely to kill early eggs and pupae in these stages. They are only effective if an adequate concentration is maintained long enough to allow the tolerance of these stages to decrease to a level where they will succumb, i.e., as late eggs or first instar larvae or as late pupae or young adults.

The tolerance of late eggs or first instar larvae is generally lower than that of late pupae or young adults. Thus the critical concentration is that which is sufficient to kill adults of the most tolerant species within an enclosure, at a time when all pupae can be expected to have developed to adults and when all eggs should have hatched, at the minimum temperature within the commodity. The minimum exposure period is the longer of the development times of eggs and pupae of the slowest developing species at the temperature in question. This assumes that phosphine does not delay development and that there is little effective uptake within the eggs and pupae. Deviations from both assumptions will mitigate the above statement but will probably tend to offset each other since prolonged development time will increase the minimum exposure while effective uptake in the egg or pupae will tend to decrease the minimum time.

If the foregoing provides a guide to minimum exposure times it is disturbing to note development times for these stages in some of our major species **(Table 1)** particularly at the lower temperatures. With fumigations in which the concentration decays rapidly as in leaky structures or with highly sorptive commodities, there will almost certainly be survival of some of the more tolerant stages and hence selection of the population will occur.

(iii) Phosphine narcosis

At high concentrations phosphine induces a state of narcosis that is coincidentally associated with an increase in tolerance of insects (e.g. Bond, *et al.*, 1969; Nakakita, *et al.*, 1974; Winks, 1984, 1985). At these concentrations longer exposure periods are required than would be expected from the response of the same strain at lower concentrations. Increases in tolerance in the same strain of up to x64 have been observed at high concentration (Winks, 1984). Moreover, the slopes of probit regression lines decrease reflecting the greater variability in response in this region.

The narcosis threshold varies between species and in, for example, a susceptible strain of *Rhyzopertha dominica*, is about 0.05 mg/L (Winks, 1986). In a resistant strain, this

TABLE 1 : The Influence of temperature on the development times of eggs and pupae of a number of major species of stored product Coleoptera

Species	Stage	Temp. (°C)	Mean (days)	Reference
Rhyzopertha dominica	egg	32	5	Birch and Snowball, 1945
		25.5	8	
		22	15	
		18	32	
	egg	28	7.1,7.9	Howe, 1950
		25	11	
	pupa	28	5.5,5.1	Howe, 1950
		25	7.8	
Sitophilus granarius	egg	21	6	Richards, 1947
	pupa	21	10	
Tribolium castaneum	egg	30	3.6	Sokoloff, 1974
		25	6.8	
		20	13.9	
	pupa	30	5.5	Sokoloff, 1974
		25	10.2	
		20	24.4	
Tribolium confusum	egg	30	4.9	Sokoloff, 1974
		25	7.7	
		20	16.9	
	pupa	30	6.1	
		25	10.3	
		20	21.6	

threshold is about 0.5 mg/L. Similarly, in a susceptible strain of *Tribolium castaneum*, the narcosis threshold is about 0.5 mg/L whereas in a resistant strain this threshold is about 5 mg/L (Winks and Waterford, 1986). Although it is tempting to think that insects narcotised quickly have lowered uptake and therefore have a greater chance of surviving, it is those that remain active longest that have the greatest chance of survival, supporting the concept of an active exclusion mechanism. However, once these active ones are knocked down they appear to absorb phosphine normally and are killed. The narcosis thresholds may therefore also be thresholds above which active exclusion becomes a significant factor or is 'switched on'.

The practical implications of narcosis would seem to be as an indicator of concentrations above which insects are more difficult to kill. Above these thresholds of concentration there is also a greater chance of survival and hence selection, because of the increased variability of response.

(iv) and (v) Phosphine resistance

Phosphine resistance has been reported from a number of countries and it is to be expected that this number will increase. Resistance has been detected in most of the major species of stored product insects including *R. dominica, T. castaneum, S. oryzae, Oryzaephilus surinamensis* and *Cryptolestes spp. (Taylor, 1989)*. The distribution of phosphine resistance is not known in detail but it is likely that resistance to phosphine is not an unusual phenomenon in most countries in which the fumigant has been used. Laboratory studies show that resistance to phosphine can be selected easily and with few selections. Moreover, it has been selected in all strains and species in which attempts have been made to select for resistance. This is not consistent with resistance arising from the selection of a rare gene. It would seem more likely that the resistance we have selected is the result of heritable 'rearrangements' of normal genetic material.

Much of the data concerning phosphine resistant strains has been derived from discriminating dosage tests using various exposure periods. Although the FAO test method calls for discriminating dosage tests to be supplemented by graded response studies there are few probit mortality lines describing phosphine-resistant strains and probit planes or their equivalent have so far been little used. Because of this it is difficult to compare strains in any meaningful way or to deduce the full significance of the strains for which some data exist. Even recent mode of action studies such as that by Chaudhry and Price (1990) suffer in part because of the difficulty they had in targeting dosages for uptake studies because of the lack of adequate dosage response models describing the strains they used.

The Stored Grain Research Laboratory has selected strains of a number of species over a minimum of 10 selections. Most showed some resistance when this study commenced. Currently, our most resistant strain is a strain of *R. dominica*, Rd235P10 in which the suffix P10 denotes that the strain has been selected in the laboratory over 10 generations. This strain resembles strain 306 (selected) of Chaudhry and Price (1990), but is not as resistant as their strain BR-2 (Figure 1). Although the tolerance of this strain to phosphine is 'high' compared with other susceptible strains of this species it is not high when compared with other species that form part of the complex of species against which phosphine fumigations are directed. It has been demonstrated in the laboratory and in the field (Winks, unpublished data; Tyler *et al.*, 1983; Taylor, 1989) that these resistant strains can easily be controlled with phosphine providing the fumigation is conducted properly, i.e., that an adequate concentration is maintained long enough by virtue of adequate sealing of the structure or by using SIROFLO, a continuous application technique delivering phosphine, at a constant rate, into a pressurised distribution system.

The key to controlling resistant strains is increased exposure periods using concentrations at which the resistance levels are minimal. Laboratory studies have

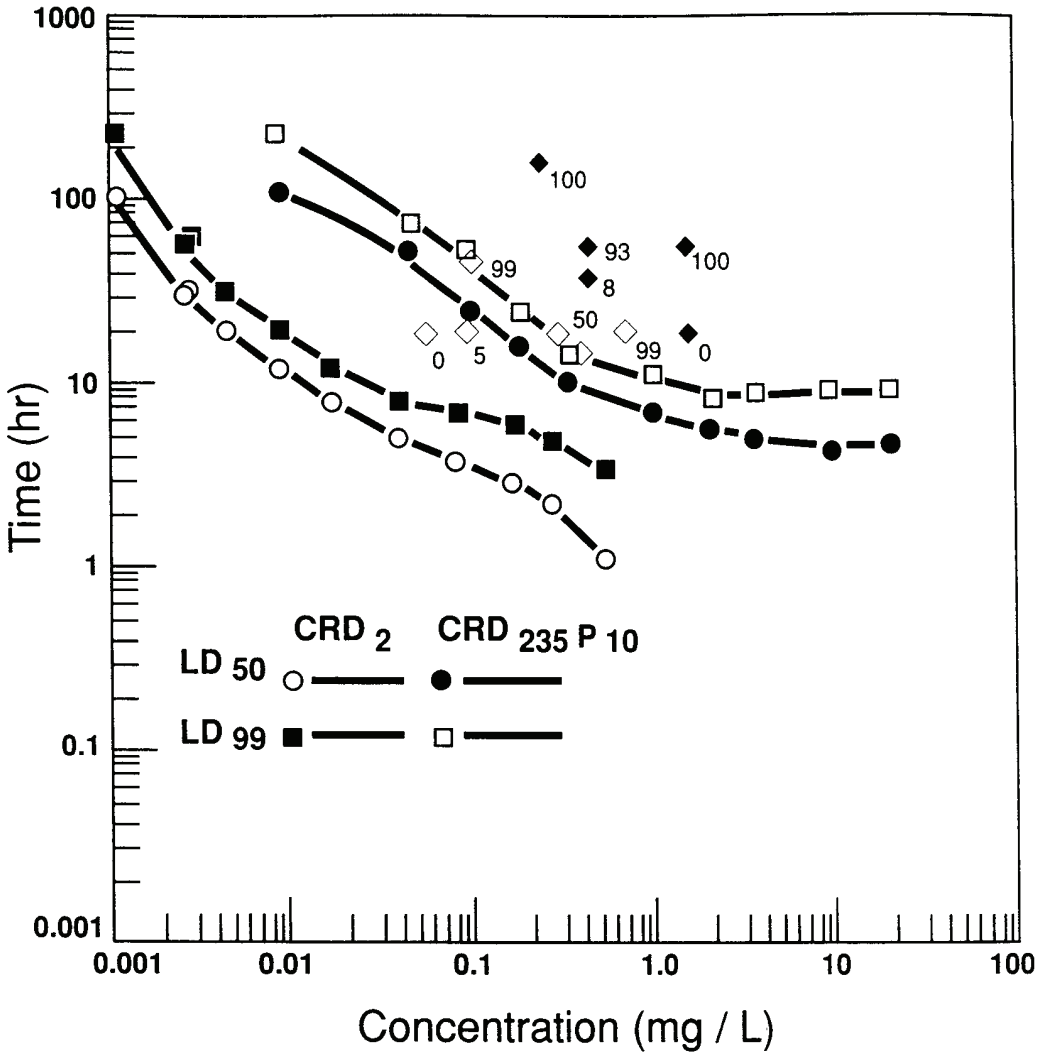

Figure 1 The relationship between concentration and time in the 50% and 99% mortality response of a susceptible and a resistant strain of adults of *Rhyzopertha dominica* exposed to phosphine. Data from Chaudhry and Price (1990) showing single dosage points for strains 306-SEL-Guyana (◇) and BR-2-Bangladesh (◆) with % mortality response adjacent, are also given.

shown that a critical time is required before the active exclusion process breaks down. Because of these characteristics, traditional methods based on the admixture of solid formulations of either aluminium or magnesium phosphide with grain in leaky structures will fail. This is because overall concentrations will not be high enough for long enough or pockets of low concentration will occur as a result of the chimney effect or as a result of the effects of wind on such structures. With phosphine-resistant strains only two approaches are viable, both of which are clearly based on prolonging exposure:

(i) sealed storage or
(ii) SIROFLO

Sealed storage

Storages sealed to the gastightness standard provide an enclosure in which fumigations can be carried out with a high probability of success and at minimum cost. Such structures also provide facilities that should be insect-proof. Their principal disadvantage is the high capital cost where retrosealing is involved and costs of from A$5 a tonne for large structures to A$20 a tonne for smaller structures have recently been estimated for horizontal storages in New South Wales. In addition, for such structures to remain effective, they must be maintained to the prescribed standard.

With sealed storages a range of application methods are available that include solid preparations and cylinders containing phosphine in carbon dioxide (Phosfume - Commonwealth Industrial Gases (CIG), Australia). CIG developed and patented (United States Patent 4,889,708, Great Britain Patent 2177004) Phosfume, a non-flammable mixture of 20g/kg phosphine in liquid carbon dioxide. Phosfume is supplied in 31kg net content high pressure gas cylinders which contain a volume of 16m^3 of 2.6v/v% phosphine in carbon dioxide.

Distribution may be effected with recirculation, with suitable precautions, in vertical silos or by natural convection currents in horizontal storages. In silos with recirculation facilities, introduction of fumigant from cylinders provides the fastest method of establishing a uniform gas concentration throughout the grain mass - the rate of introduction may be adjusted to suit the recirculation rate.

In all sealed storages a technique that we have called SIROFUME may be used. This technique is simply an automatic top-up process that is capable of maintaining a constant concentration in such storages for as long as required and at very low cost. For example, a lethal atmosphere can be maintained for 365 days for little more than 30 cents per tonne. This method will shortly be installed in a 120,000 tonne sub-terminal in New South Wales. Cylinders of Phosfume are ideally suited to this technique and permit the easy introduction of precise amounts of gas.

SIROFLO

SIROFLO is a positive pressure, continuous application technique that also has a high probability of success. It is designed for structures that are not gastight but can be effectively 'sealed' in critical areas. It is based on the continuous introduction of phosphine from a phosphine source such as cylinders of Phosfume, into an air stream that provides the positive pressure within the store. This positive pressure offsets factors that would otherwise give rise to gas loss. The method may be used in a range of storages including vertical silos, horizontal sheds, and farm bins.

In Australia in the coming season it is expected that in excess of 1 million tonnes will be treated with SIROFLO. The capital cost of SIROFLO is low and has been found to range

between about A$1 and A$3 per tonne in vertical storages. This cost includes all equipment and preparation of the silos. Costs in horizontal storages are more variable and are correlated in part with the size of the structures. Operating costs vary depending on the usage of the technique, i.e., single treatment or grain protection system. When used in this latter mode they are still less than those associated with some common grain protectant applications.

One of the particular strengths of SIROFLO is its considerable flexibility. It may be used at both low and high concentrations and unlike other methods, because of the nature of the gas source, e.g. cylinders of Phosfume, concentrations and flows may be adjusted during a fumigation to compensate for unforeseen air ingress through leaks. As with sealed storage, SIROFLO offers a method whereby phosphine-resistant strains may be controlled. In Australia we have used the method on at least two occasions to satisfactorily control resistant strains of *R. dominica*.

Other application techniques

In addition to its support of the Siroflo research project, CIG independently promoted Phosfume as a conventional fumigant to Bulk Grain Authorities and a number of field trials were conducted on gastight bulk grain storages, e.g.:

a) The fumigation of a 20,000m^3 (15,000 tonne of wheat) Co-operative Bulk Handling (CBH) horizontal storage at Jennacubbine, Western Australia required four 31 kg gas cylinders of Phosfume releasing 2.5kg of phosphine to achieve the desired phosphine concentration of 100ppm. Using surface only application techniques, this storage would normally be fumigated using 15kg of aluminium phosphide, liberating 5kg of phosphine, i.e. using Phosfume 50% less phosphine was required to achieve the required concentrations.

By monitoring phosphine concentration, it was observed that the distribution was achieved by using the natural thermal convection currents, i.e. down through the perimeter and up through the middle of the grain. Relatively even distribution was achieved after only 44 hours exposure and remained even, although decaying, throughout the remainder of the fumigation. A phosphine concentration in excess of 100ppm was maintained for 10 days but the cost of 5¢/tonne was in excess of the 1¢/tonne using metallic phosphide tablets. While the time saving (days) in achieving the 100ppm phosphine level was not a perceived benefit in this up-country grain storage it led to trials at seaboard terminals.

b) Further trials were carried out at the CBH Kwinana Grain Terminal where the total capacity of 912,000 tonnes is held in 144 vertical cells (2,200 tonne each) and two horizontal storages (284,000 tonne and 250,000 tonne). For these trials, a single vertical storage (11m diameter x 30m high) containing an integral forced air, closed-circuit recirculation system which draws air from the base of the silo and vents it into the headspace at the top of the silo was used.

The contents of a 15kg cylinder were injected through a restrictor to achieve the required dose during one air volume change of the cell. The required concentration of 100ppm was achieved in five hours; the occupational health and safety concerns of disposal of spent pellets were eliminated; aeration at the completion of the fumigation (100 hours) was completed within 24 hours and the cost of 5¢/tonne compared to 1.5¢/tonne using tablets is acceptable because of the two days time saving.

c) Bunker storage (30,000 tonne tarpaulin covered: 300m long x 50m wide) treatments using Phosfume have been perfected by Bulk Grains Queensland (BGQ). Fumigations at dosages as low as 0.3 g/m^3 were successfully performed by discharging

Phosfume cylinders (15min/cylinder) through a two metre probe which is connected to the Phosfume cylinder by a 30m length of teflon lined, stainless steel braided, high pressure flexible hose. The storage is probed at approximate 10m intervals along its length and the desired concentration of phosphine is achieved within three hours.

The BGQ technique ensures release of phosphine within half an hour; assists distribution by probing along and deep into the storage and achieves peak phosphine concentrations some four times that from aluminium phosphide formulations at equivalent dosages.

d) Vertical silo (2,000 tonne) trials carried out by the Victorian Department of Agriculture and Rural Affairs and the Grain Elevators Board resulted in a Ct of 100gh/m^3 being achieved after 13 days compared to 19 days using the equivalent amount of aluminium phosphide blankets.

Conclusion

The new techniques available for applying phosphine, especially those based on the use of gas from cylinders, enable fumigations to be carried out far more effectively than was the case with earlier methods based on aluminium phosphide preparations in leaky storages. If we are to continue using this fumigant the use of these techniques is essential.

Phosphine-resistance as we currently know it is not the end of the road for this fumigant but rather the start of a new road on which we will have to be more diligent concerning the way in which the gas is used.

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INFLUENCE DE L'AGE DU GRAIN SUR LES EFFETS DU MALATHION ET LA COLONISATION PAR LES INSECTES DES STOCKS DE MAIS DU KENTUCKY

Robert J. BARNEY, John D. SEDLACEK, Bryan D. PRICE et Paul A. WESTON

Kentucky State University
Atwood Research Facility
Frankfort, Kentucky 40601 USA

RESUME

La colonisation par les insectes du maïs en grain a été étudiée pendant la saison de stockage 1988 - 1899. Trois répétitions de quatre combinaisons de traitements ont été examinées : du maïs de 1 et 2 ans, traité ou non au malathion. L'étude a porté sur des silos d'acier de 300 boisseaux (10,5 m^3), contenant 150 boisseaux (5 m^3) de grain à Frankfort, Kentucky, USA. La présence d'insectes a été détectée grâce à 6 pièges-sondes en plastique par cellule, situés dans deux directions (nord et sud) et sur 3 niveaux de profondeur (0,3, 0,75 et 1,5 mètres). Les cinq insectes les plus abondants étaient Typhaea stercorea (L.), Tribolium spp., Cryptolestes spp. Ahasverus advena (Waltl) et Sitotroga cerealella (Olivier). On discutera de l'influence de l'âge du maïs, du malathion, de la direction et de la profondeur des pièges sur ces espèces.