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# PHOSPHINE RESISTANCE: WHERE TO NOW?

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## ABSTRACT

The reliability of phosphine ($PH_3$) for the disinfestation of stored-products is under threat due to resistance in several common insect species. This comes at a time when the use of OP grain protectants is losing favour. More importantly, the loss of methyl bromide (MB) for routine disinfestation, under the Montreal Protocol, means that a vital, though unintentional, counter selection pressure will be largely lost. Globally, we will see more $PH_3$ fumigations, many of which will be of dubious quality and will lead to further resistance. The time is overdue for a concerted pro-active strategy to protect $PH_3$ by preventing the development of resistance and eliminating resistant populations where they are known to exist. There is an urgent need for extension services to promote this message so that the required improvement of stack fumigations and bulk commodity fumigations is rapidly achieved. A global resistance survey is urgently required to provide information on the status of the problem in different pest species. The use of rapid resistance tests and the development of national testing centres should be encouraged so that $PH_3$ fumigations can be designed to control known specific resistant populations. The problem must be contained by the latest information and technology if we are not to see resistance as the normal condition in some species/ commodity sectors with the attendant costs and trading problems.

## INTRODUCTION

I have been working in the area of phosphine ($PH_3$) fumigation of stored-products for 32 years. I have had the opportunity to study $PH_3$ toxicity to both susceptible and resistant strains and to participate in many field trials involving commodity stack fumigation and bulk grain fumigation, both in bins and in horizontal storages. It has been both interesting and frustrating. This paper is a personal view of the future for $PH_3$ resistance and, perhaps, for $PH_3$ as a fumigant (Taylor, 1989).

The big issues in $PH_3$ fumigation are the problem of resistance and persistent concerns about its environmental safety, initiated with the Environmental Protection Agency (EPA) in the USA. These problems antagonise each other. On the one hand, there may be a requirement to increase dosage rates to combat resistance so that $PH_3$ can take the place of MB, which is well into phase-out for many uses under the Montreal Protocol. On the other hand, we see an increasing resistance problem, with 12 stored-product insects pests implicated, which will only worsen under the majority of current use methodologies. The resistance problem will also worsen since, with the loss of MB, there will be more $PH_3$ fumigations and, therefore, more sub-standard ones to produce selection pressure. There will not be the, often, fortuitous counter-selection pressure on resistant populations provided by MB in the past.

The aim of this paper is not to give an extensive review of $PH_3$ resistance since it's aim is to look to the future. Excellent reviews on the subject of $PH_3$ resistance are available (Zettler, 1993; Chaudhry, 1997). However, it is necessary to take an overview of the past in order to absorb some important lessons.

**Increase in the frequency of resistant strains**
Table 1 gives data from resistance surveys chosen since they occurred at intervals of time. There is a general increase in the frequency of resistant strains found over time.

TABLE 1
Phosphine resistance survey results over time

| Survey | Date | Country | % of resistant strains | | | |
|---|---|---|---|---|---|---|
| | | | ***R. dominic*** | ***T. castaneum*** | ***S. oryzae*** | ***S. granarius*** |
| Champ and Dyte (FAO) | 1972-73 | Global | 23.4 | 5.6 | 5.9 | 9.4 |
| Taylor and Halliday | 1983-85 | Developing countries | 77.3 | 48.1 | 75.0 | - |
| Pacheco *et al.* | 1986-88 | Sao Paulo State, Brazil | 90.0 | 90.0 | 100 | - |
| Ignatowicz | 1997-98? | Poland | 9.8 | - | - | 21.2 |

In Morocco, the use of $PH_3$ in grain stores at frequencies of up to 5-6 times per year has produced an almost 100% incidence of resistant strains in *Tribolium castaneum, Sitophilus oryzae* and *Rhyzopertha dominica* (H. Ben Halima, this conference). It may be that in Moroccan grain stores resistant strains are the norm.

**Selection for resistance**
Clearly something is going wrong with $PH_3$ fumigation. From our knowledge of the genetics of the resistance (Li and Li, 1994: Mills and Athie, 2000) the development of the resistance should be difficult. The major gene, which controls an active exclusion mechanism (Price, 1984) is semi- recessive. Hence, heterozygotes are little different than susceptibles in tolerance. These should be controlled unless the fumigation is of a very low standard indeed.

The problem is the difficulty in attaining a good level of sealing. In poorly sealed enclosures, there will be gross leakage of the fumigant leading to the inability to reach the necessary exposure periods. However, it is probable that fumigations are generally effective so that control is achieved in the centre of stacks and grain bulks but that survival occurs near to the periphery. This is caused by the entry of air, which dilutes the $PH_3$ and produces selective doses. These can be selective for heterozygotes and later as the proportion of homozygotes in the population increases, for these also. In this situation, there can be very rapid selection for resistance.

**Better fumigation techniques**

It is important that a balance be struck between studying the levels of resistance, genetics etc. and the development of practical fumigation techniques. Tables 2-5 give a breakdown of papers presented at 4 recent conferences, 2 of which were the immediate forerunners of this CAF conference. An attempt has been made to divide them into papers written with resistance in mind and a second group which majors on techniques and background information. It is pleasing that the latter category accounts for 71% of the total (72 out of 101 papers). It appears that workers in the field of fumigation have the message that resistance is an issue and that we need to improve $PH_3$ fumigation techniques.

However, practical dosing technology and methods now must be judged on their likely contribution to the resistance problem. There is a need for methods to be trialed more extensively and peripheral $PH_3$ concentrations carefully measured. If they do not produce good gas distribution then they are obvious failures.

The entry of air is caused by pressure changes so that, if ambient pressures are higher than in the enclosure, air moves into it. The converse also occurs. Enclosures are especially vulnerable in windy conditions since a negative pressure, which sucks $PH_3$ out, occurs. On the windward side there is a positive pressure produced and ingress of air occurs. It is very difficult to seal stacks to the standard required to completely prevent pockets of low concentration, even if the covering sheet is sealed to a base sheet under the stack with adhesive. The problem is even more difficult to overcome with bulk commodities. Gas can be removed from silos by a dramatic 'chimney effect'. Horizontal storages are very difficult to seal

Stacks could be kept under pressure so that slight out-leakage results. A good level of seal combined with a carefully controlled input of compressed air into the centre may solve the problem of air ingress. For long exposures, a higher initial dose would be necessary to ensure that the overall concentration remained effective.

The problem of the effective treatment of grain silos and bins has been solved by the positive pressure system, SIROFLO[R], which is well established. The input of cylinderised formulations of $PH_3$ in conjunction with recirculation is also effective (Wontner-Smith, *et al.,* 1999).

The problem of treating horizontal grain storages remains. SIROFLO has been tried but there is a problem with the chimney effect near the building walls (Winks and Russell, 2000). The use of a peripheral positive pressure recirculation system shows promise in guaranteeing concentrations in all but the strongest winds (Mills, *et al.,* this conference).

TABLE 2
Distribution of papers given at the 1992 CAF Conference, Winnipeg

| Papers specifically on resistance to phosphine | Papers on toxicity to pests, practical phosphine fumigation or background information |
|---|---|
| Response of resistant strains. | Progress in fumigation technology. |
| Review on resistance. | Triple low system, temperature, oxygen and phosphine dose. |
| | Use of ducting for fumigation. |
| | Triple low system. |
| | Release from formulations. |
| | Concentration predictive model. |
| | Use of ducting for fumigation. |
| | Fumigation and decision making. |
| | Cylinder-based phosphine. |
| | Siroflo® |
| | Systems for in-transit fumigation. |
| | Sealed stacks. |
| | Phyto Explo® in-transit. |
| | Double low, generators and use of ducting. |
| | Phyto Explo® in silos |
| 2 papers | 15 papers |

TABLE 3
Distribution of papers given at the 1996 CAF Conference, Nicosia

| Papers specifically on resistance to phosphine | Papers on toxicity to pests, practical phosphine fumigation or background information |
|---|---|
| Use of mixed-age cultures in resistance tests. | Bag stacks. |
| Effect of temperature on resistant insects. | Effect of temperature on bin fumigation. |
| Methyl phosphine against resistant insects. | A new bag to release phosphine. |
| Influence of resistance on fumigation technology. | Active fumigation systems |
| Resistance to phosphine and $CO_2$ mixtures. | Gaseous phosphine. |
| Resistance in the Philippines. | Recirculation. |
| Use and resistance in Poland. | Pressure tests and sealing. |
| | Pressure tests in containers. |
| | Recirculation in concrete silos. |
| | Sealing with sheeting. |
| | Sealing of portable farm silos. |
| | Cool grain and automated dosing. |
| 7 papers | 12 papers |

TABLE 4
Distribution of papers given at the 6th. IWCSSP, Conference 1994, Canberra

| Papers specifically on resistance to phosphine | Papers on toxicity to pests, practical phosphine fumigation or background information |
|---|---|
| Same-day resistance test. | Fumigation – an endangered technology. |
| Farm resistance survey, Australia | Penetration by adding carbon dioxide. |
| *S. oryzae* resistance genetics. | Phyto Explo® in floor stores. |
| Uptake by susceptible and resistant insects. | Improving container gas-tightness. |
| Resistance survey in Malaysia. | Low oxygen phosphine fumigation. |
| Resistance and narcotic response. | Circumfluent fumigation |
| Measurement of resistance. | Phosphate + heat + carbon dioxide for mills. |
| | Fumigation practice survey, Australia. |
| | Status of phosphine fumigation, India |
| | New phosphine-releasing product. |
| | Phosphine + carbon dioxide toxicity. |
| | Detector for continuous concentration monitoring. |
| | Controlled release. |
| | Evolution from formulations. |
| | Fumigation of bag stacks to avoid resistance. |
| | Response of *Bruchus pisorum*. |
| | New formulations for controlled generation. |
| | Poor gas-tightness in the fumigation of field beans. |
| | Sealed silos. |
| | Siroflo® in horizontal storages. |
| | Siroflo® in vertical storages. |
| 7 papers | 21 papers |

**Resistance studies required**

It is vital that resistance surveys be commissioned, either globally or by individual countries. There is much information coming from these surveys but some countries are not carrying them out or are unable to do so, and so assistance will be needed as is already being offered in S.E. Asia to develop national testing centres.

A fuller understanding of the genetics of the resistance is required, backed up by biochemical studies. It is important to study the most resistance strains available. Price and Mills (1986) studied doses required to control all stages of resistant strains. However, a laboratory selected strain of *S. oryzae* was, subsequently, shown to be less tolerant than a strain collected from the field in Maringa, Brazil, and held without selection (Mills and Athie, in press).

Further work is required to develop and refine rapid resistance tests (Savvidou, *et al.*, 1994), particularly for those species not covered by previous investigations. These are very useful in identifying resistance, and allowing a modified fumigation regime to be carried out. Even when resistance is not present, they allow identification of a problem with the fumigation method by elimination of resistance as a contributory cause of failure.

TABLE 5
Distribution of papers given at the 7th. IWCSSP Conference 1998 Beijing

| Papers specifically on resistance to phosphine | Papers on toxicity to pests, practical phosphine fumigation or background information |
|---|---|
| Studies on narcotic response of resistant | Fumigation of stored products – outlook. |
| Improving resistance assays. | Toxicity of cylinderised phosphine. |
| Same-day tests and genetics of resistance. | Toxicity to moth eggs. |
| Quick resistance test. | Relative effects of temperature and dosage |
| Alternative test for high resistance levels. | Mortality from cylinderised phosphine in bins. |
| Cross resistance control strategy. | Effects of oxygen on low dosage fumigation. |
| Relative fitness of resistant strains. | Phosphine at farmer level. |
| Lack of cross resistance to insecticides. | Pressure test techniques. |
| Resistance in Poland. | New technology developments in the UK. |
| Resistance in Asia/Australia. | Phosphine/carbon dioxide in Cyprus. |
| Resistance in India. | New circulation technology. |
| Resistance development and counter measures in China. | Control of mites. |
| Resistance in Vietnam. | Recirculation technology |
| | Silo ducts for fumigation. |
| | Slow release against mites. |
| | Modeling circumfluent silo fumigations. |
| | Phosphine/carbon dioxide against mites. |
| | Phosphine and carbon dioxide generator. |
| | Phosphine generator and circulation. |
| | Gastightness of corn stacks. |
| | Split application to control resistant insects |
| | Intermittent application to control resistance. |
| | Carbon dioxide to control resistant insects. |
| 13 papers | 24 papers |

**The future**

There is a great need to educate fumigators and those storing commodities about the resistance and its consequences. They may find that a single effective fumigation is more cost effective than several ineffective ones. The philosophy of obtaining acceptable control and passing the problem on in the trade chain is unacceptable. Everyone needs to aim for the highest standards for the common good. The pests are easily spread in international trade.

Full use should be made of the latest information technologies to disseminate information. Practical training courses on fumigation, with respect both to efficacy and safety, are an obvious need. More use should be made of pressure and vacuum half-life tests to determine leakage (Navarro, 2000). Use should be made of post-fumigation trapping and the use of the instruments to measure concentrations, especially near the end of a treatment. Care must be given to keep to recommended dosage rates and exposure periods. In particular, exposures should not be terminated prematurely due to lack of planning or for commercial priorities. Full use should be made of cylinderised formulations to maintain concentrations. This time they must be used carefully.

We should not be afraid to propose high technology dosing solutions. They are the only means of guaranteeing pest eradication in some situations. At present some technologies may only be feasible for implementation in some developed countries. However, a holding operation in developing countries (with the objective of arresting resistance build-up) by following best 'low technology' methods, will buy time until these countries can use the more costly but effective alternatives.

The developed countries should not be complacent. Many have temperate climates and consequently the rate of generation turnover of the pests is low. Even though resistance mechanisms are more efficient at higher temperatures, this does not mean that selection is not taking place. Any selective dose anywhere will produce resistance.

Producers of $PH_3$-releasing formulations can play their part by implementing better product stewardship. Experts in the field need to assess the results of surveys and must consider the need to revise dosage schedules and devise pro-active strategies to tackle to problem of resistance.

A serious attempt should be made to introduce methyl phosphine (Chaudhry *et al.,* 1996) as a fumigant. It has the useful property that it is more effective against $PH_3$ resistant strains than against susceptibles ones but can be used for both. It can be used in an alternating use strategy or in mixture with cylinderised $PH_3$.

There is much to be done if we are to prevent $PH_3$ resistance from becoming the norm in some species/commodity sectors; however we must proceed with caution so as not to make the situation worse.

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