

## **INFLUENCE OF RESISTANCE ON FUTURE FUMIGATION TECHNOLOGY**

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### **ABSTRACT**

Although global dependence on fumigation continues to increase, the many constraints on this technology could lead to its extinction. One of these constraints is resistance. Even though phosphine resistance continues to increase in frequency and intensity among pest populations, control failures rarely occur. When they do, however, they can be managed by using other existing, alternative methods or technologies. In spite of our ability to control these resistant populations, future pest-control efforts will become more complex and challenging. Newly developing technologies like genetic engineering could threaten our pest-control security and undermine our efforts to manage resistance. The presence of transgenic food products will increase in the marketplace and have an impact on pest-management technologies in the future.

### **INTRODUCTION**

Fumigation represents our last line of defense against insect infestation and remains the major pest-disinfestation control method worldwide. Dependence on this methodology is increasing for a variety of reasons.

Only two fumigants, methyl bromide (MB) and phosphine (PH<sub>3</sub>), remain in widespread use globally. Each of these chemicals possesses certain characteristics that make it suitable for a particular use. MB, the quick action of which requires relatively short exposure periods, is important in fumigation of perishable products; PH<sub>3</sub>, with its relatively long exposure period, is better suited for use with durable commodities. Many countries require that imported commodities be fumigated to meet phytosanitary requirements. Many countries during the last decade have (either voluntarily or in obedience to the law) reduced pesticide usage, particularly usage of insecticidal protectants (Matteson, 1995), thereby making fumigation more critical. Australia, for example, has enforced residue-free standards on its grain for many years. The US Environmental Protection Agency has announced a partnership among the EPA, the USDA, the US Food and Drug Administration and volunteer grower groups and utility companies to promote environmental

stewardship in pesticide use. In 1984 the USDA announced a separate initiative to help farmers implement Integrated Pest Management (IPM) on 75% of total crop acreage by the year 2000. Several state agencies in the US no longer recommend insecticidal protectants on grain; instead, they are relying on other, nonchemical methods of maintaining commodity quality. Some large food processing companies in the US have committed themselves to moving towards a less chemical-intensive pest-management program. This is due to litigation about illegal residues, as well as to public opinion (Hegele, 1994). All of this has contributed to more frequent insect infestations in grain stores, and this trend is likely to continue pending implementation of suitable IPM strategies.

Thus, at a time when great dependence is being placed on conventional fumigants, the technology is being attacked on many fronts. MB, because of its ozone-depletion potential, will be banned in the US in 2001 (EPA, 1993). There is also great concern about  $\text{PH}_3$  causing chromosomal aberrations and being carcinogenic (Garry *et al.*, 1989; Alavanja *et al.*, 1990). More stringent regulations on  $\text{PH}_3$  emissions have raised costs and at times are difficult to implement (Keever, 1990; Keever and Hamm, 1996). Resistance to  $\text{PH}_3$  is also increasing both in frequency and intensity. It is fair to say that fumigation as we know it is becoming an endangered technology (Banks, 1994).

Of all the potential threats outlined above, resistance is probably the least important. Nonetheless, because it is the only one over which we have some control, its importance is considerable.

#### FUMIGANT RESISTANCE

Table 1 shows the results of  $\text{PH}_3$ -resistance surveys. Several recent reports indicate that fumigant resistance globally is continuing to develop in intensity and frequency (Irshad *et al.*, 1992; Emery, 1994; Rajendran and Narasimhan, 1994; Suliaman *et al.*, 1994; Bell and Wilson, 1995). One of these reports showed that in Indian tobacco stores, control failure with  $\text{PH}_3$  was the result of  $\text{PH}_3$ -resistance in the cigarette beetle (Rajendran and Narasimhan, 1994). Despite increased resistance in these beetles, control failures, as in earlier instances, were overcome by increasing both dosage and exposure time.

#### COMBATING RESISTANCE

No cases of MB control failures due to resistance have been reported. This fumigant seems to remain effective although its regulatory future is uncertain.  $\text{PH}_3$ -resistance, on the other hand, has caused control failures. Often these resistant populations have been dealt with by MB fumigation or increasing the  $\text{PH}_3$  dose by lengthening the exposure period or increasing the concentration, or both. Lengthening the exposure period is generally most effective (Winks, 1980). These methods are, of course, used only after ensuring that the affected commodity can be sufficiently sealed to contain the gas for the required increase in the Ct (concentration  $\times$  time) product.

TABLE 1  
Incidences of phosphine resistance by region and species\*

Region	Number of strains tested (left) and number of strains resistant (right) for each species									
	<i>Tribolium castaneum</i>	<i>Rhyzopertha dominica</i>	<i>Sitophilus spp.</i>	<i>Oryzaephilus spp.</i>	<i>Tribolium confusum</i>	<i>Cryptolestes spp.</i>	<i>Lasioderma serricorne</i>	<i>Plodia interpunctella</i>	<i>Cadra cautella</i>	<i>Trogoderma granarium</i>
N Africa	3	1	4	2	1	0				
Sub-Sahara <sup>9</sup>	23	7	7	1						1
S Asia	17	11	3	1		2	2			1
SE Asia	12	0	3	0		2	1			
S America <sup>1</sup>	30	24	22	21		14	14			
Australia <sup>2</sup>	1,330	224	210	58	27	3				
US <sup>3</sup>	59	22	21	8	17	3	20	8	18	3
UK <sup>4</sup>	14	5	39	17	4	1				
Malaysia <sup>5</sup>	22	5	17	2		30	4			
Pakistan <sup>6</sup>	33	5	25	11						
India <sup>7</sup>	5	5	3	1		1	0			
France <sup>8</sup>	1	1					2	2		

\*After Taylor (1989) and Zettler (1992).

<sup>1</sup>Sartori *et al.* (1991); <sup>2</sup>Attia and Greening (1981), Herron (1990) and White and Lambkin (1990); <sup>3</sup>Zettler (1991); <sup>4</sup>Mills (1986); <sup>5</sup>Suliaman *et al.* (1994); <sup>6</sup>Irshad *et al.* (1992); <sup>7</sup>Rajendran (1989), Rajendran and Narasimhan (1994); <sup>8</sup>Becton *et al.* (1988); <sup>9</sup>Bell and Wilson (1995).

### INCREASING EXPOSURE TIME

Following the first reported case of control failure with  $\text{PH}_3$ , an analysis of resistance levels in the affected insects showed that an effective  $\text{PH}_3$  dose for the resistant insects would be 150 mg L/h for a minimum of 3 d (Tyler *et al.*, 1983). Treatment schedules for  $\text{PH}_3$ -resistant insect populations given by Mills (1986) required a minimum exposure time of 6 d at 25°C. The effective dose for  $\text{PH}_3$ -resistant cigarette beetles reported by Rajendran and Narasimhan (1994) is a Ct of 480 mg L/h for a minimum of 10 d at 30°C.

Thus, while it is possible to control  $\text{PH}_3$ -resistant populations with  $\text{PH}_3$  by increasing the exposure time, clearly at some point in the future there will be a practical limit to the extent of such an increase. Most fumigated structures are not sufficiently gastight to provide adequate fumigation for pesticide-susceptible insects. How long, then, can we expect to be able to simply increase the exposure period in order to deal with resistance? Given the present state of storage facilities in most parts of the world, it is reasonable to expect resistance to continue to increase, forcing us to concomitantly increase exposure times.

### OTHER FUMIGATION TECHNIQUES

Dealing with fumigant resistance is not yet an uncontrollable problem. Resistance can be managed by effective pest control. Many suitable technologies and methodologies exist to deal with it. However, most resistance occurs in developing countries where suitable equipment and sanitation practices are generally lacking. Despite these limitations, economic advances have been made in the proper fumigation of sheeted bag-stacks. Both laminated PVC sheets and large sandbags (Taylor and Harris, 1994) and laminated wall and floor coverings (Xu and Wang, 1993) can greatly improve fumigation efficiency.

Hermetic storage, because of its simplicity, is ideal for use in developing countries. It is environmentally friendly and has global applications for protection of stored commodities in both developing and advanced countries (Navarro *et al.*, 1994).

Fumigant recirculation technology has been known for more than 50 years and used for HCN and MB fumigations. This technology has been applied to modified atmospheres (MA's) (Wilson *et al.*, 1980) and  $\text{PH}_3$  fumigations (Cook, 1984). Recent technological advances include the closed-loop system used in the US (Kenkel *et al.*, 1993; Noyes and Kenkel, 1994), the circumfluent system used in China (Sun *et al.*, 1993; Lu *et al.*, 1994), the PHYTO-EXPLO® system (Chakrabarti *et al.*, 1994; Vacquer *et al.*, 1993a, b) and J-SYSTEM® (Degesch) for fumigation of large, flat bulk storages, silos and holds of export grain ships (Zettler *et al.*, 1984; Leesch *et al.*, 1986). These technologies usually reduce the dose of fumigant required to produce a lethal Ct product in grain pests and thus improve the efficiency of conventional types of  $\text{PH}_3$  fumigation.

Flow-through systems like SIRO-FLO® (Winks, 1993) and cylinder-based  $\text{PH}_3/\text{CO}_2$  mixtures (Bell *et al.*, 1993; Chakrabarti *et al.*, 1994) are efficient because they rely on the increased susceptibility of pest insects to continuous steady-state concentrations of  $\text{PH}_3$  rather than on increasing/decreasing the concentrations experienced during normal conventional fumigations. In addition, the concentrations required are lower than normal and



thus more economical and also more environmentally friendly. This allows the extension of exposure periods for control of resistant populations at no additional expense. Finally, these technologies are forgiving in that they can be effective even in leaky storages, although this is certainly not to recommend that they be used in leaky situations in lieu of properly sealing the store.

Combination treatments of  $\text{PH}_3$  with carbon dioxide ( $\text{CO}_2$ ) (Carmi *et al.*, 1990, 1994) and with  $\text{CO}_2$  and heat (Mueller, 1993) have proved useful under some circumstances for fumigating large silos and flour mills.  $\text{CO}_2$  helps the movement of  $\text{PH}_3$  through commodities (Leesch, 1990), and the addition of heat lowers the effective dose of  $\text{PH}_3$  required for a lethal Ct product.

The list of alternative fumigants is small. Several new compounds being tested or retested were listed by Banks (1994). Carbonyl sulfide (Desmarchelier, 1994) and methylisothiocyanate (Ducom, 1994) have shown promise in stored-grain situations but they are not labeled for use on any commodity.

#### BIOTECHNOLOGY AND THE FUTURE OF POSTHARVEST PEST CONTROL

Biotechnology has the potential to significantly affect postharvest pest control via genetic engineering. This branch of molecular biology is developing rapidly. The first engineered gene to successfully confer insect resistance on plants was constructed less than 15 years ago (Meussen and Warren, 1989). Today, more than 50 plant species have been genetically manipulated for some specific purpose (Gasser and Fraley, 1989), i.e. resistance to disease, resistance to insect predation and herbicides or an increase in shelf-life of fresh fruits and vegetables.

Three areas of research are presently being actively investigated with the goal of improving stored-product insect pest management.

##### DNA fingerprinting

Polymerase chain reaction (PCR) is a technique that amplifies or clones DNA fragments which can then be utilized as a fingerprint for that particular DNA. This DNA fingerprint is unique for each individual or population within a species (Beeman, 1994). Recently a variation of the PCR technique called random amplification of polymorphic DNA (RAPD) has been used to fingerprint populations of *Plodia interpunctella*. Comparisons of RAPD fingerprints within regions could provide new insights into population movements and infestation sources which would be potentially useful in tracking the global movement of resistant populations of stored-product pests.

##### Resistance detection

PCR can be used to fingerprint resistance genes. For example, the lindane resistance gene, prevalent throughout the world, has recently been cloned and its structure determined (French-Constant, 1993). Shortly thereafter, a PCR-based resistance detection assay was developed and tested on *Tribolium castaneum* (Andreev *et al.*, 1994). This

assay system should be useful in detecting other types of resistance once the relevant genes are identified and molecularly characterized.

### **Insect-resistant seeds**

Transgenic corn, incorporating the delta endotoxin of *Bacillus thuringiensis* (Bt), has been approved by the EPA (Anon., 1995). Similarly, the cotton plant has been genetically engineered to incorporate the 'Bollgard gene,' derived from Bt, that is toxic to cotton bollworm, tobacco budworm and pink bollworm (Barton, 1995). A transgenic potato plant has also been engineered to contain a Bt gene which is toxic to the Colorado potato beetle (Wassell and Knorr, 1995).

These insect-resistant crops all contain Bt endotoxin genes which may in actuality be just another chemical insecticide to which insect pests will develop resistance (Oberlander, 1995). The controversy raised by this possibility has led to considerable concern about resistance management (McGaughey, 1990; McGaughey and Whalon, 1992; Tabashnik and McGaughey, 1994).

The insecticidal components of these transgenic plants are manifested in the foliage. However, they can also be manipulated to be manifested in the seeds themselves. Indeed, a genetically engineered garden pea has been developed which contains an amylase inhibitor protein (Shade *et al.*, 1994). The gene for this protein was taken from the common bean that, by virtue of its amylase inhibitor protein, is normally tolerant to the cowpea weevil, *Callosobruchus maculatus*, and the Azuki bean weevil, *C. chinensis*. The potential to insert this gene into seeds of other crop plants exists. Other candidate insect control proteins that might be used to confer insect resistance to seeds of other transgenic plants include Bt toxins, proteinase inhibitors, vitamin binding proteins, venoms and chitinases (Beeman, 1994).

What are the implications of these biotechnological advances for postharvest entomology? Some day our silos and warehouses may be filled with transgenic grains and oilseeds containing a variety of pest-control agents. Based on previous history, one can assume that the resistance problem will not be alleviated but is instead likely to increase. The insecticides or pesticides to which pests will be exposed will likely be more environmentally friendly, and perhaps more selective in their toxicities, than those available today. However, insects are likely to develop resistance to them. This translates into higher pest infestations and more pest-control failures. Consequently, there is likely to be a continued need for conventional and MA fumigation, even in the transgenic arena.

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