Phosphine resistance in the Asia/Australia region

Mervyn Bengston¹, Miriam A. Acda², Gregory J. Daglish¹ and Patrick J. Collins¹

Abstract
Phosphine resistance is emerging as a major threat to current pest control measures in grain storages in the Asia/Australia region. Currently it appears that all of the phosphine-resistant strains can be controlled by phosphine in fumigations carried out to a high standard. However, in Australia, recent detection of a strain of Rhyzopertha dominica (Fabricius) with resistance characteristics similar to strains in Asia has led to an increase in the phosphine concentration recommended in the Siroflo® continuous application system. Development of strains with a higher level of resistance must be anticipated. Recent studies have shown that in strains of three major pest species from Australia, with low level resistance (∗10), the resistance is semidominant and is controlled by at least 2 major genes. Phosphine-resistant strains have not shown cross-resistance to grain protectant insecticides or the chloropicrin or ethyl formate fumigants in resistant R. dominica from the Philippines, adults are at least as difficult to control as the immature stages. Over the phosphine concentration range likely to be encountered in field fumigations the response data fit the equation \( c^n t = k \) where 'c' is concentration (mg/L), 't' is time (h), and 'n' is a constant here approximately 0.4 and 'k' is a constant, here 124 mg h/L, at the LC 99 level. Such an approximation may be useful in calculating a modified concentration by time product to assess the efficacy of field fumigations with fluctuating concentrations against resistant strains.

Introduction
Phosphine fumigation is the major method of pest control in the Asia/Australia region. It is the most practical method of pest control in bag storage and it is often the method of choice in bulk storage. Phosphine-resistance has been known from the region for over 20 years (Champ and Dyte, 1976). However, the situation is now such that a revision of some of the existing fumigation protocols has been required. This paper discusses the significance of recent developments.

Recent Reports of Resistance
Phosphine-resistance has been reported in virtually all countries of the Asia/Australia region. Among the major pest species, some of the more significant occurrences include Rhyzopertha dominica (Fabricius) in China with resistance levels in the FAO test of ∗606 (Ren et al., 1994), in India ∗380 (Rajendran and Narasimhan, 1994) and in the Philippines ∗2036 (Acda et al., in press); Sitophilus oryzae (Lamnaeus) in China ∗63.7 (Lang, 1990). More recently, a strain of R. dominica with responses similar to strains in Asia has been reported from Australia (Collins, P. J., unpublished).

Most Tolerant Stage
Eggs and pupae generally are more tolerant to phosphine than are adults and larvae (Anon, 1989). However, in the case of resistant strain of Tribolium castaneum (Herbst) from Pakistan (Ansell, 1992) and in the case of R. dominica from the Philippines (Acda et al., in press) the adults were more tolerant than the immature stages. Such a finding underlines the need to determine the response of all stages of strains with new resistances.

Genetics
Studies of the genetics of strains with the relatively low levels of resistance prevalent in Australia indicate that the resistance is controlled by a minimum of two genes. This is true for R. dominica with a resistance level ∗23 in the FAO test (Bengston et al. unpublished), S. oryzae ∗12 (Bengston et al. unpublished) and T. castaneum ∗13 (Bengston et al. in press). In all cases the resistance was incompletely dominant. There was considerable overlap in the response of the log dose/probit mortality lines for the F1 hybrid (presumably rss) with the parental strains. The response of the mixed hybrids that result from backcrossing the F1 hybrid to either the resistant or to the susceptible parent could not be determined directly, but the response was between that of the respective parents. Field strains are likely to contain mixtures of these genetic hybrids and estimation of the response of such a strain will give a range...
of values between that of the homozygous susceptible and resistant strains. Such field strains will increase in resistance under selection and perhaps the most important practical aspect is the response of the homozygous resistant strain.

Strains with the much higher resistance levels prevalent in Asia also have resistance controlled by at least two genes, e.g., *S. oryzae* (Li and Li, 1994), *T. castaneum* and *R. dominica* (Ansell, 1992) and it seems reasonable to infer that at least three genes are involved.

### Cross Resistance

To date, detailed studies have not indicated that phosphine-resistance confers cross-resistance to grain protectant insecticides. These include the organophosphorus compounds chlorpyrifos-methyl, fenthion, malathion, or parathion-methyl and the synthetic pyrethroid compounds boresmethrin and deltamethrin (Liang Yongsheng, Ran Li, Cao Yang and Collins P J, unpublished data). Similarly, there is no evidence of cross-resistance to the fumigants chloropicrin (Liang Quan, unpublished data) or ethyl formate (Collins, P J, unpublished data). It should be noted that some of the strains studied had resistances to more than one compound but the finding of other strains with only one resistance indicated that the resistances were independent.

The absence of cross-resistance suggests that efficacy of any of the above compounds is not affected by phosphine-resistance and they could be used in control programs against resistant strains subject to the practicalities of the specific situation. Each could also be used in a resistance management strategy involving rotation of treatments and aimed at delaying the further development of resistance. The benefits of such a strategy would of course depend on the relative fitness of the susceptible and resistant strains.

### Fitness

To date, there have been relatively few studies reported regarding the fitness of phosphine-resistant strains compared to the susceptible strains. It is likely that no single method will provide a definitive answer to the question. One option is to measure the population growth parameters of the strains. Strains of *T. castaneum* from China with differing levels of phosphine-resistance have been shown to differ in population growth potential but it is not clear that the differences are directly related to resistance (Cao Yang and Collins P J, unpublished data). An alternative approach is to use repeated backcrossing and selection of the resistant strain to produce isogenic lines and then to assess fitness which would be directly associated with the resistance gene but no such studies have been noted. A further approach is to set up a competitive situation containing a known proportion of susceptible and resistant insects and then to monitor the resistance frequency over successive generations. Such an approach has been commenced in our laboratory.

### Assessment of Response of Resistant Strains

Most assessments of the response of strains to phosphine have been carried out using the FAO method (Anon, 1975) which involves exposing adults to phosphine for 20 hours. As is well known, this test is effective in detecting resistance but the results do not directly indicate the likely success or failure of field fumigations where typical exposures may be 7 days.

Several authors have modified the FAO test by using extended exposure periods and resistance factors from these tests have tended to be lower than those with the standard FAO test (Acad, M A and Daglish, G J, unpublished data). Since the longer exposure periods involve lower phosphine concentrations, such modified tests may avoid the problems of narcotic response which may occur with higher concentrations. Where adults are the most tolerant stage, tests with 6 day exposures may give a conservative estimate of the concentration likely to control of the resistant strains under field conditions in the typical 7 day fumigation.

Where data have been obtained using the FAO methodology at several exposure times and the log dose/probit mortality lines do not reject the hypothesis of parallelism, then the response data may be used to fit the generalised dosage response equation (Winks and Hyne, 1994)

\[ c^n t = k \]

where 'c' is concentration (mg/L), 'n' is the toxicity index, 't' is time (h), 'k' is a constant (mg h /L).

### Fumigation Protocols and Field Dosages

Some fumigation protocols have been based on the dosages necessary to achieve complete control of pupae of *Trogoderma granarium* which are reportedly the most tolerant stage of the most tolerant insect. As resistance develops, other species and stages may become more difficult to control and their responses will need to be considered in setting fumigation protocols. Clearly it would be a major advantage to relate laboratory data directly to the field situation.

This is straightforward in the case of continuous application systems such as Siroflo® which deliver a
constant concentration of phosphine for a specified period of time. Laboratory apparatus such as flow-through equipment can be used to determine time to population extinction for all stages of relevant strains under specified conditions (Winks and Hyne, 1997) Such data can be used directly to develop field fumigation protocols. In Australia, the recent recognition of a strain of \textit{R. dominica} with a level of resistance approaching that of the Asian strains (Collins, P J, unpublished data) has resulted in an increase in the concentrations recommended in Siroflo fumigations.

The problem is more complex when the phosphine is derived from a phosphine-generating formulation applied to the commodity in a fumigation enclosure since the concentration of the phosphine varies throughout the fumigation interval. The usual approach in determining the effective phosphine dosage has been to calculate the concentration by time (ct) product. This approach assumes that the toxicity index ($n$) is 1.

Values of the toxicity index $n$ for the resistant strain of \textit{R. dominica} Philippines strain were approximately 0.4 (Acda et al., in press) and the value of $k$ at the LD 99 level was 124 mg/L. An approximation of the effective dose was derived as a modified concentration by time product calculated using the observed value of $n$ in the generalised dosage response equation. Based on published data (Bengston et al. 1997) modified concentration by time products calculated for bag stack fumigations of milled rice carried out to a good standard in Indonesia were in the range 177–222 mg/L. On this basis, the resistant strain of \textit{R. dominica} from the Philippines will be fully controlled in a fumigation which meets the Australian criterion of a phosphine concentration of 1 mg/L remaining at the end of a 7 days.

**Future Directions**

Further development of phosphine resistance appears inevitable but every effort should be made to delay its development as long as possible. Resistance surveys should be continued with emphasis on the collection of insects that appear to have survived fumigations of a good standard. Laboratory studies on the response of new strains should be conducted in a manner that indicates whether or not the resistant strains will be fully controlled under recommended fumigation protocols. The protocols should be amended as necessary.

All phosphine fumigations should aim to produce a complete kill of treated insects. To this end, pressure testing to ensure an appropriate degree of gastightness should be carried out in all storages or enclosures in which that is practical. Phosphine concentrations should be monitored to determine that the target concentrations are being achieved.

**References**


Anon 1989 Suggested recommendations for the fumigation of grain in the ASEAN region Part 1 Principles and general practice Kuala Lumpur, ASEAN Food Handling Bureau, 131 p


