Prospects for predicting insect mortality in relation to changing phosphine concentrations

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Abstract

Typically, in bag-stack or silo fumigations the concentration of phosphine is not constant, and yet most of what is known about phosphine efficacy against grain insects comes from studies with fixed concentrations. Indeed, where changing concentration experiments have been performed, researchers have been unable to explain observed efficacy on the basis of data from fixed concentrations. The ability to predict insect mortality in relation to changing phosphine concentrations would facilitate the development of effective fumigation protocols. In this paper, we explore the prospects for making such predictions. After reviewing published and new results, we conclude that the commonly used concentration × time (*Ct*) product is unreliable for this purpose. New results, for a strongly resistant strain of *Rhyzopertha dominica* from Australia, suggest that the relationship $C^n t = k$ may be useful for predicting mortality of this type of insect in changing concentrations. However, in the case of a strain of *Sitophilus oryzae* with a type of resistance common in Australian *S. oryzae*, the relationship $C^n t = k$ proved to be less reliable.

Keywords: Phosphine; Changing concentrations; *Rhyzopertha dominica*; *Sitophilus oryzae*; Resistance

Introduction

In fumigations using aluminium phosphide as a source of phosphine gas, the concentration of phosphine is not constant, but typically rises to a peak and then declines over time (e.g. Taylor et al., 1994; Bengston et al., 1997; Rajendran and Muralidharan, 2001). There have been few published studies on phosphine efficacy in relation to changing concentration, and a predictive method has yet to be established (Reichmuth, 1986; Bell et al., 1990; Rajendran, 1994; Rajendran and Gunasekaran, 2002; Annis and Dowsett, 2002). The aim of this paper is to explore the prospects for predicting insect mortality in relation to changing phosphine concentrations. We first review the published papers and then present the results of experiments on phosphine-resistant *Rhyzopertha dominica* (F.) and *Sitophilus oryzae* (L.) from Australia.

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Review of published data

Reichmuth (1986) exposed various life stages of susceptible *Sitophilus granarius* (L.) to phosphine concentrations that increased to a maximum and then declined in exposures lasting up to 72 h. He then calculated the concentration × time (*Ct*) product required to kill insects in changing concentrations, and also the *Ct* product from the lowest constant concentration required to kill insects within the same exposure period. The *Ct* product for the constant concentration was much lower than the *Ct* product for the changing concentration. He speculated that narcosis, as described by Winks (1984, 1985), might render high concentrations less effective than expected.

In experiments with constant or changing concentration regimes, Bell et al. (1990) found that mortality of the various life stages of resistant *Cryptolestes ferrugineus* (Stephens) was related to the concentration at the end of 10 days exposure rather than to the *Ct* product. They also noted that mortality was enhanced when concentrations fell later, rather than earlier. They therefore suggested that improved sealing or a system of repeated or continuous introduction of gas would improve efficacy.

Rajendran (1994) investigated the effects of changing concentrations on resistant *Tribolium castaneum* (Herbst) and *R. dominica* from India. Eggs, pupae and adults of *T. castaneum* and adults of *R. dominica* were exposed to fixed *Ct* products with constant, rising or falling concentrations for up to 72 h. The *Ct* product was an unreliable predictor of mortality. Whether or not there were differences in mortality between constant, rising or falling concentrations depended on the *Ct* product chosen and the species. Rajendran concluded that it was important to maintain a sufficiently high concentration of phosphine during fumigation to avoid the chances of survival resulting from the reduced effectiveness of changing concentrations.

In another study on resistant insects from India, Rajendran and Gunasekaran (2002) exposed mixed-age cultures of *R. dominica* and *S. oryzae* to fixed *Ct* products with constant, rising or falling concentrations for seven days. They found that fumigation with rising concentrations resulted in fewer survivors than constant or falling concentrations, and that this was more pronounced in *S. oryzae*. They suggested that the advantage of rising concentrations could be exploited by using slow release or multiple applications.

Annis and Dowsett (2002) exposed mixed-age cultures of a resistant strain of *R. dominica* from Australia in a fumigation lasting 10 days, during which the concentration rose to a peak and then fell. They calculated the *Ct* product for 100% control, which occurred after six days of exposure. They then estimated the constant concentration that would yield the same *Ct* product. The estimated constant concentration was similar to the average experimental concentration during the six days of exposure. However, had they done the opposite and estimated the lowest constant concentration required for 100% control in six days, the resulting *Ct* product probably would have been much less than the observed *Ct* product. This is because, for a given level of mortality, lower constant concentrations tend to yield smaller *Ct* products than higher concentrations.

Although all of these studies reported interesting results, none suggests a method for predicting insect mortality in relation to changing phosphine concentrations. In four of the five studies reviewed, the *Ct* product was unreliable in this respect (Reichmuth, 1986; Bell et al., 1990; Rajendran, 1994; Rajendran and Gunasekaran, 2002), so another approach is needed.

New data

Experiments were conducted aimed at elucidating the effects of changing concentrations on mortality of phosphine-resistant *R. dominica* and *S. oryzae* from Australia. The strains of *R. dominica* and *S. oryzae* used in this study were QRD569 and QSO335, respectively. Both strains were derived from field samples that had undergone laboratory selection until only resistant genotypes remained. Mixed-age cultures, i.e. batches of wheat containing all life stages, were fumigated using a modified version of the method used by Winks and Hyne (1997), which is described in Daglish et al. (in press). Essentially, a mixture of phosphine and air was passed through fumigation vessels containing infested wheat. There were four phosphine concentration profiles (Table 1), and samples were removed at intervals during each fumigation. Adults were sieved from each sample, added to fresh wheat and assessed for mortality two weeks later. The remaining grain was incubated for eight weeks, when the number of live adults was counted. Controls were treated as described, except that they were not fumigated. The fumigation conditions were 25°C and 57% r.h., and the post-fumigation conditions were 25–28°C and 55–60% r.h. Under these conditions the mean number of live adults emerging from the untreated controls was 2284 (range 1995–2574) for *R. dominica* and 5663 (range 4908–6254) for *S. oryzae*.

We decided to see whether the relationship $C^n t = k$ could be used to predict time to population extinction of mixed-age cultures in relation to changing phosphine concentrations. The effects of time (d) and constant concentration (mg/L) at 25°C have been quantified for the strains of *R. dominica* (Collins, unpublished data) and *S. oryzae* (Daglish et al., in press) used in this study. The following equations for 99.9% control were used:

R. dominica: $C^{0.4269}t = 4.2741$
(over the range 0.2–1.25 mg/L) (1)

S. oryzae: $C^{0.4812}t = 4.1371$
(over the range 0.02–2 mg/L) (2)

$C^n t$ was calculated for each day of exposure and a cumulative $C^n t$ was then calculated. The day on which the cumulative $C^n t$ was >4.2741 for *R. dominica,* or >4.1371 for *S. oryzae*, was the day on which population extinction was predicted to occur.

Table 2 shows predicted and observed days of exposure required for 99.9% control of mixed-age cultures. The predictions for *R. dominica* were very good, suggesting that the method may be suitable for practical purposes. In contrast, the method overestimated the time required to control *S. oryzae* in each experiment. This difference between the two species is unexpected because equations (1) and (2) produce very similar estimates for both strains for concentrations in the range 0.2–1 mg/L.

Re-analysis of published data

The strain of *R. dominica* used by Annis and Dowsett (2002) was supplied from our laboratory. As this strain is the same strain of *R. dominica* used in our experiments, we decided to re-analyse their data as a further test of our method. In their experiment, concentration changed continuously over 10 days, so we had to use the mean concentration on each day. As mean concentration was not shown in their results, it had to be calculated from their *Ct* product calculations. Having obtained the mean concentration for each day of exposure, we then used equation (1) to estimate the day on which 99.9% control should have occurred. We also converted their results from numbers of insects emerging to percentage

Table 1. Phosphine concentration (mg/L) during experiments in which resistant *R. dominica* and *S. oryzae* from Australia were exposed to changing concentrations.

Day of exposure	Experiment			
	1	2	3	4
1	0.2	0.4	0.2	0.4
2	1	1	1	1
3	1	1	1	1
4	1	1	0.2	0.43
5	0.2	0.4	0.2	0.43
6	0.2	0.4	0.2	0.43
7	0.2	0.4	0.2	0.43
8	–	–	0.2	0.43

Table 2. Days of exposure required for 99.9% control of mixed-age cultures of resistant *R. dominica* and *S. oryzae* from Australia exposed to changing concentration regimes.

Species	Experiment	Predicted	Observed	Difference
R. dominica	1	6	6	0
	2	5	4	+1
	3	7	8	–1
	4	6	5	+1
				Mean +0.25
S. oryzae	1	6	4	+2
	2	5	4	+1
	3	7	5	+2
	4	6	4	+2
				Mean +1.75

reduction relative to their controls. The cumulative C^nt should have exceeded 4.2741 after five days exposure, meaning that >99.9% control would be expected. Our estimate agrees very well with their results, with 99.4% and 100% control after five and six days exposure, respectively.

Discussion

We examined the prospects for predicting insect mortality in relation to changing phosphine concentrations. It is clear from the literature that the *Ct* product is unreliable for this purpose (Reichmuth, 1986; Bell et al., 1990; Rajendran, 1994; Rajendran and Gunasekaran, 2002; Annis and Dowsett, 2002). The fundamental problem with the *Ct* product is that it depends on the concentration tested. Generally speaking, lower constant concentrations result in smaller *Ct* products than higher concentrations. So the problem arises as to what *Ct* product is to be used to judge the efficacy in a fumigation in which phosphine concentration is not constant.

The possibility of using the relationship $C^nt = k$ for predicting phosphine efficacy in practical conditions, where concentrations are rarely constant, was investigated in four laboratory experiments at 25°C. The lowest concentration tested was 0.2 mg/L and the highest 1 mg/L, both of which would be within the range of concentration experienced by insects in well-conducted commercial fumigations. The results of these experiments suggest that the relationship $C^nt = k$ may be useful for predicting mortality of strongly resistant *R. dominica,* found in Australia, in changing concentrations. However, this relationship appears to be less useful for *S. oryzae* with a type of resistance common in Australia. Unexpectedly, the *S. oryzae* strain was easier to control in all four experiments than the *R. dominica* strain. The current results suggest that, in parts of Australia where *R. dominica* and *S. oryzae* coexist, changing concentration profiles that are expected to control strongly resistant *R. dominica* will also control resistant *S. oryzae.* Further research is needed to confirm this.

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