# The Mortality Response of *Sitophilus oryzae* (L.) Eggs to Diurnal Interrupted Doses of Phosphine (PH3)

Stephen Beckett, CSIRO Entomology, GPO Box 1700, Canberra, ACT 2601, Australia.
E-mail: Stephen.Beckett@csiro.au

**Abstract** — Fumigant distribution in grain is inherently variable with movement occurring by gas expansion and contraction, convection and diffusion. Diurnal changes in temperature and wind velocity, and weather changes more broadly, drive movement which is further influenced by the type of storage facility and the degree of gas tightness. Near boundaries, in particular, this can prevent lethal concentrations being maintained. Such conditions may have implications for resistance selection in stored grain insects. However, there is limited information on insect mortality responses to variable concentrations similar to those often confronting industry. This paper reports on initial results from a study that investigates the impact of repeated sub-lethal doses of phosphine on the mortality of eggs from three strains of *Sitophilus oryzae* that are susceptible, slightly resistant and moderately resistant to the fumigant. The treatments used are designed to represent fumigant environments undergoing simple diurnal fluctuations. Results are compared to those obtained from equivalent continuous treatments and discussed in reference to phosphine toxicology and with regard to the practical implications for successful fumigation.

Key words: phosphine, diurnal interrupted doses, *Sitophilus oryzae,* eggs, mortality

## Introduction

Considerable variation in phosphine dosage occurs during industrial fumigations throughout a large range of storage structures. Reasons include gas dispersal, the level of gas-tightness, grain sorption and weather conditions. The effects of gas expansion and contraction, convection and diffusion can lead to uneven concentrations which are particularly noticeable at the storage boundaries when sealing is poor. This can prevent lethal concentrations being maintained and may have implications for resistance selection in stored grain insects.

Diurnal patterns of gas expansion and contraction occur due to the daily temperature cycle. The amount of fumigant at certain locations can follow these oscillating conditions causing a repeated interrupted treatment, particularly near the periphery. This phenomenon has been reported by several authors [3; 13]. Certain weather conditions [2] that also have a diurnal pattern [11] can further affect distribution and leakage. Environmental events with a uniform cycle [2] that have similar impacts on fumigant movement may coincide, such as lower temperatures and wind velocities. This can continually cause, for example, a period of several hours each morning of minimal gas expansion, movement and leakage where uniform fumigant dispersal is optimum.

Most data on insect mortality due to phosphine have been determined using continuous treatments at constant concentrations. However, there have been some investigations of

mortality under variable concentrations reflecting those occurring in industrial fumigations. These studies were reviewed by Daglish et al [6]. Attempts to determine mortality in terms of the Concentration X time (Ct) product have not on the whole been very fruitful. However, Daglish et al. [6] were able to relate the mortality of *Rhyzopertha dominica* to $C^n t = k$. Nevertheless, predicting insect mortality under variable or interrupted fumigant concentrations remains inadequate.

Specific information on the mortality response of stored grain insects to repeated sub-lethal doses or interrupted treatments of phosphine is small. Bond and Upitis[4] found that two sub-lethal 5h doses of phosphine within 24 hours caused greater mortality of *Tribolium confusum* and *Sitophilus granarius* adults than one 10h treatment at the same concentration, and Hobbs and Bond[9] reported a similar response in *T. castaneum*.

This paper reports on initial results of an investigation exploring the response of insects to the effects of diurnally interrupted doses of phosphine. Data are presented on the mortality response of eggs of *S. oryzae* which represent a development stage tolerant to phosphine[12] and allow for a specific definition of the mortality response. The treatments were designed to reflect simple fumigant environments that might occur under conditions described above. The results give insight into overall treatment times required under such circumstances and demonstrate the particular toxicological effects of phosphine on insect physiology.

## Materials and Methods

*Bioassays*

Three strains of *S. oryzae* were compared. These were a long standing phosphine susceptible strain (LS2), a weakly resistant strain (QQSO1111) and a moderately resistant strain (QSO335). Levels of resistance were determined by the FAO method for resistance measurement [7] with moderate resistance considered commercially significant [7]. Eggs between 24 and 48 hours old were obtained for bioassay by allowing adult insects to lay for 24h on soft wheat at 30°C/12% grain moisture content (mc) 48h before treatment. The adults were then discarded and replicates were setup for treatment by placing 25g of infested grain in 50 ml jars.

*Fumigation*

Fumigations were conducted in closed desiccators (2.5–6.4L) at 30°C/60% relative humidity (r.h.). Humidity was generated with 100ml of aqueous glycerol solution (specific gravity 1.185-1.190) held in a dish at the bottom of each desiccator. Phosphine was produced by adding aluminium phosphide tablets to 10% (v/v) aqueous sulfuric acid [1]. The concentration of this source gas was established using a Gow Mac (Model 11-625) gas density balance in a Tracor (MT-150) gas chromatograph fitted with a HayeSep Q 80/100 mesh column. The volume of gas required for fumigation was determined with respect to desiccator volume, gas purity and atmospheric pressure. The appropriate dose was then injected into each desiccator through a septum in the lid with a gastight syringe. The concentration of each dose was then confirmed from peak areas calibrated with 4 standards and integrated automatically using Varian Star software. The standards were

established using a Tracor (MT-150) gas chromatograph fitted with a flame photometric detector and a GSQ column (30 m, 0.53 mm i.d.) run at 110°C, or on a Varian CP3800 with a pulse flame photometric detector AT/Q column (30 m, 0.53 mm i.d ) run at 200°C.

*Treatments*

Diurnally interrupted treatments covered a range of phosphine doses from 36h at 0.67mg/l to 6h at 4mg/l and are set out in Table 1. In this way, all treatments had the same concentration X time (Ct) product. However, each treatment ran over three days by dividing the treatment time into three equal parts with each part administered at the same time each day (e.g. 12 X 3 = 36h) (figure 1). Fifteen replicates were setup at the start of each diurnal treatment. At the end of each day's treatment, 5 replicates were removed to be assessed for mortality. Thus, one set of replicates was treated on day 1, one set was treated on day 1 and 2, and one set was treated on day 1, 2 and 3. By measuring a reduction in emergence against controls as a surrogate for mortality after each day as dose accumulated, lethal time (LT) predictions could be made as to the ultimate number of diurnal doses needed for a given %mortality.

An equivalent continuous treatment was also conducted for each interrupted treatment to determine the degree to which interruption affected efficacy (figure 1). Continuous treatments were comparable not only in Ct product but also in terms of time at the points of assessment during the treatment (Table 1). For example, if the series of doses under an interrupted treatment regime were 1mg/l for 8h, or 8h on two successive days or 8h on 3 successive days; under the continuous treatment, doses were 1mg/l for 8h, or 16h, or 24h. The same number of replicates was used for both interrupted and continuous treatments.

Table 1. Phosphate doses for diurnal interrupted treatments over 3 days and equivalent continuous treatments

| | | Diurnal interrupted treatments | | Continuous treatments | |
|---|---|---|---|---|---|
| mg/l | PPM | Days/treat. | Duration time/day (h) | Duration times/treatment (h) | Ct (mg h/l) |
| 0.67 | 480 | 3 | 12 | 12, 24, 36 | 24 |
| 1.00 | 720 | 3 | 8 | 8, 16, 24 | 24 |
| 1.33 | 960 | 3 | 6 | 6, 12, 18 | 24 |
| 2.00 | 1440 | 3 | 4 | 4, 8, 12 | 24 |
| 4.00 | 2880 | 3 | 2 | 2, 4, 6 | 24 |

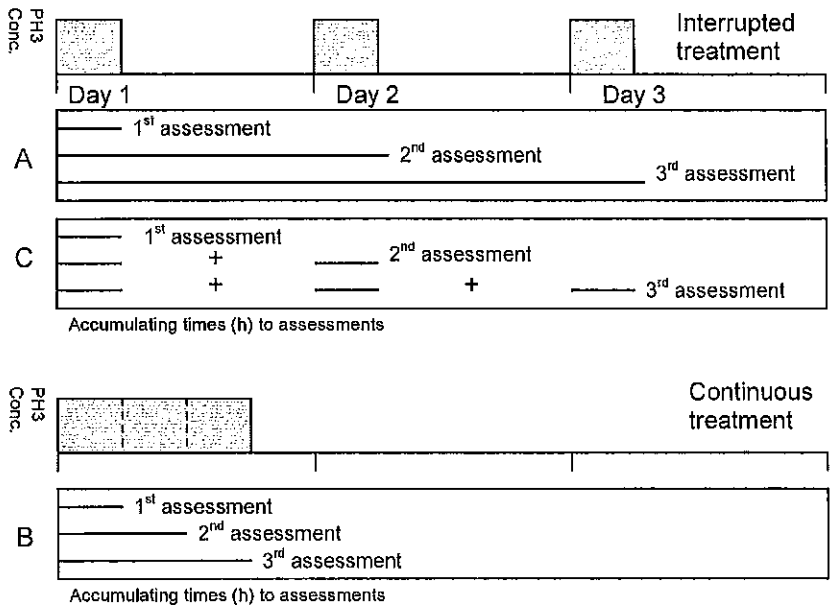



Figure 1. Schematic description treatment conditions and assessment times

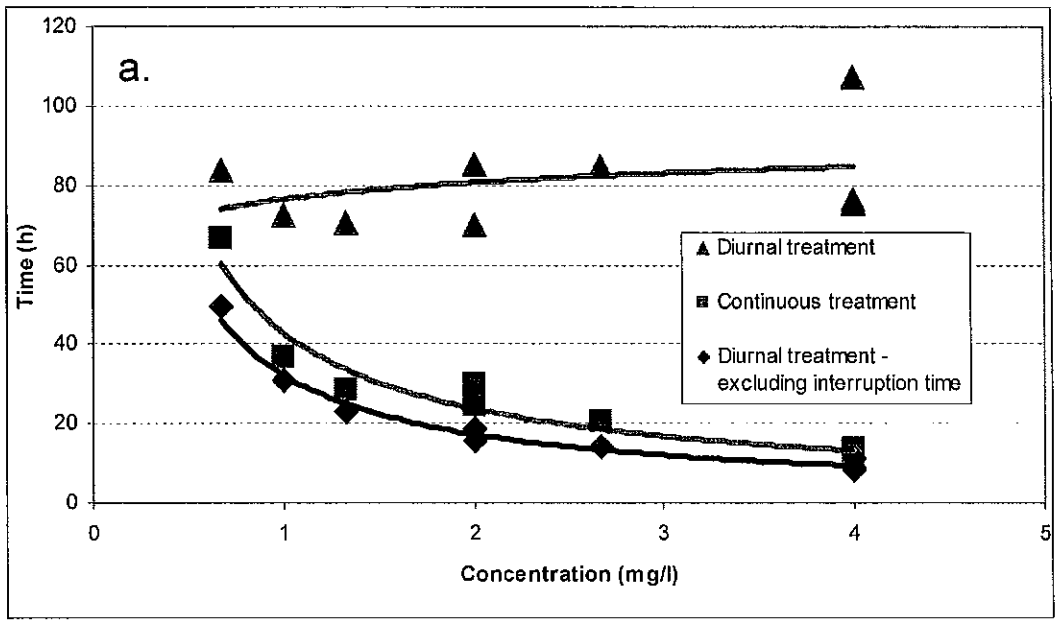
For the first 5 days of development at 30°C/60% r.h., *S. oryzae* appears to have roughly the same level of phosphine tolerance (Beckett and Seneviratna, unpublished data). Thus, the same degree of tolerance was assumed for eggs exposed to both interrupted and continuous treatments. After fumigation, desiccators were aired for at least 6 hours under a fume-hood. The insects were then either held at 30°C 60% r.h. before receiving another treatment the following day or incubated at 26°C/60% r.h. for about 5 weeks to be assessed as emerging adults relative to controls. Mortality data were analyzed with probit transformations [8] using a program on Excel (Annis, unpublished).

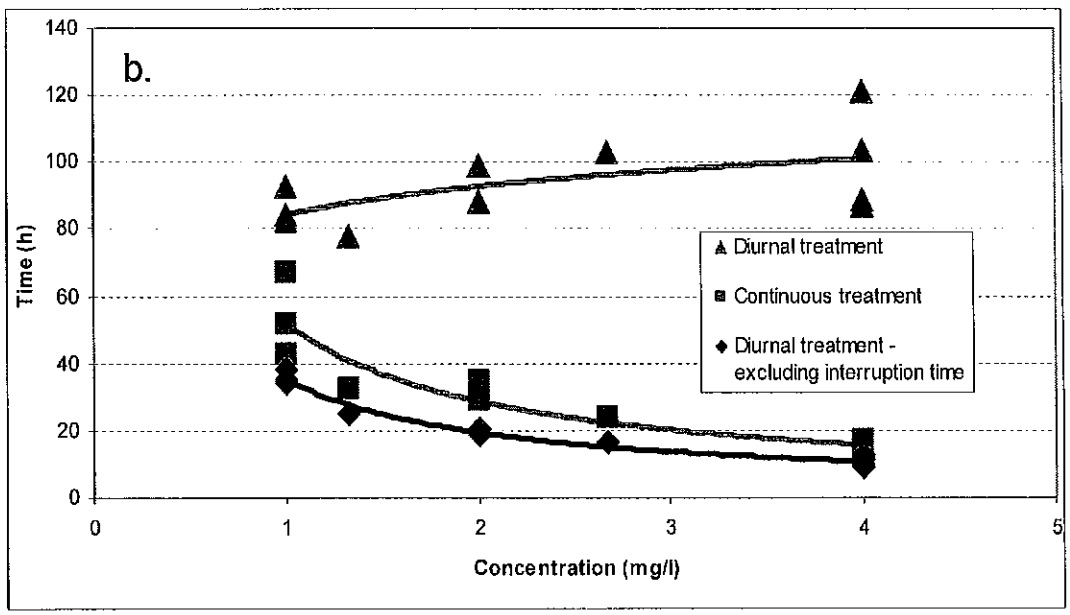
## Results

Predicted LT99.9 values for eggs of the three strains of *S. oryzae* exposed to the range of phosphine concentrations shown in Table 1, which were administered either as diurnal interrupted treatments (figure 1 A) or as continuous treatments (figure 1 B) are shown in figure 2a, b and c. Results are also shown for diurnal interrupted treatments with the time where no fumigation occurred excluded from the probit predictions (figure 1 C).

LT99.9 values for each strain subjected to diurnal interrupted treatments tend to be greater at higher concentrations for shorter treatment times. LT99.9 values for the moderately resistant strain (QSO335) were much greater than those for the slightly resistant strain (QQSO1111) and the susceptible strain (LS2) (147.2h cf. 84.4h cf. 76.5h at 1mg/l respectively and 212.5h cf. 101.1h cf. 84.7h at 4mg/l respectively). LT99.9 values of each strain subjected to continuous treatments were lesser at higher concentrations for shorter treatment times. LT99.9 values for all treatments were greatest for the moderately resistant strain and least for the susceptible strain (69.7h cf.42.8h at 1mg/l and 19.4h cf. 13.2h at 4mg/l). LT99.9 values for each strain subjected to diurnal interrupted treatments where the times without fumigation were excluded were also lesser

at higher concentrations for shorter treatment times. Again, LT99.9 values for all treatments were greatest for the moderately resistant strain and least for the susceptible strain (54.2h cf.32.1h at 1gm/l and 20.2h cf. 9.3h at 4mg/l). LT99.9 values determined for diurnal interrupted treatments in this way were usually less than those for the equivalent continuous treatment.



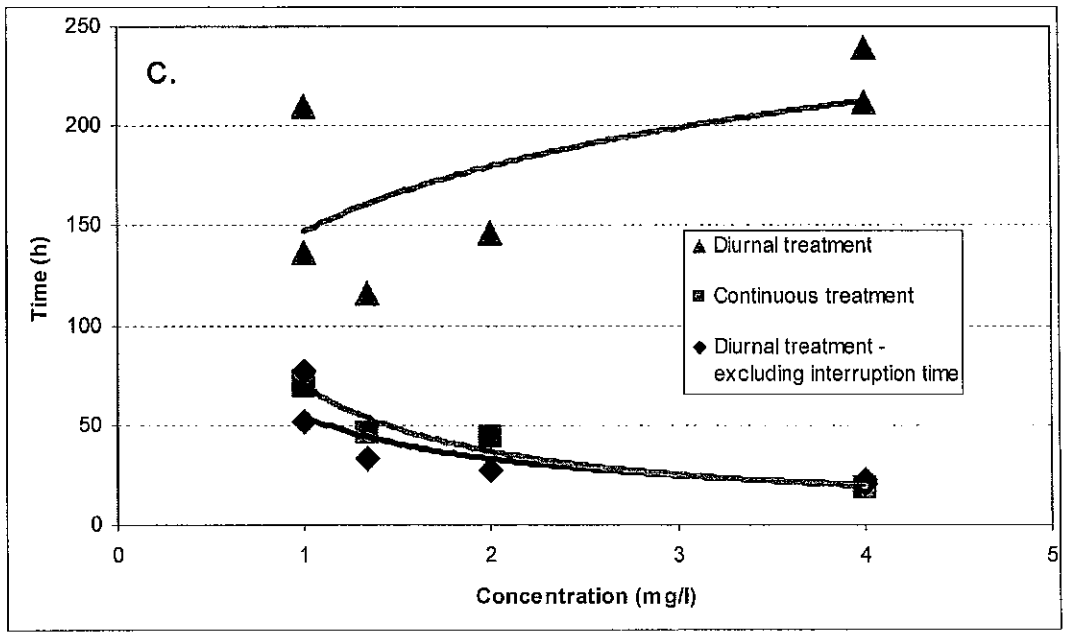



Figure 2. LT99.9 values for eggs of a) susceptible strain, b) mildly resistant strain and c) moderately resistant strain of *S. oryzae* exposed to a range of phosphine concentrations administered as either diurnal interrupted treatments or as continuous treatments. Results for diurnal interrupted treatments are also shown with the periods of interruption excluded from analysis.

## Discussion

Results presented in this paper are preliminary. However, they show that a phosphine treatment administered as a simple interrupted dose over several days greatly extends the time required for 99.9% mortality of *S. oryzae* eggs relative to an equivalent continuous dose (figure 2a, b and c). Over the range of concentration X time combinations that were used, all giving a CT product of 24mg h/l, the predicted time required to obtain LT99.9 appeared to increase for all three insect strains at treatments that have higher phosphine concentrations and shorter treatment times. The relative difference in predicted treatment time between diurnal interrupted treatments and continuous treatments for a given concentration is therefore greater at higher concentrations, since under continuous treatment, the time needed to obtain LT99.9 is less at higher concentrations. The greatest relative difference in LT99.9 values between the two treatment regimes was exhibited by QSO335 (147.2h cf. 69.7h at 1mg/l and 212.5h cf. 19.4h at 4mg/l) followed by QQSO1111 (84.4h cf. 52.0h at 1mg/l and 101.1h cf. 15.8h at 4mg/l) and LS2 (76.5h cf. 42.8h at 1mg/l and 84.7h cf. 13.2h at 4mg/l).

If LT99.9 values are determined for diurnal interrupted treatments where only the time when eggs were under fumigation is used for the $y$ axis, the correlation of LT99.9 to concentration and time is like that occurring during continuous treatments with treatment time decreasing at increased concentrations and $C^n t = k$ where $n < 1$. However, the values of LT99.9 determined for interrupted treatments are generally lower for the three strains

relative to those for the equivalent continuous treatment. This not only indicates that the toxic affects of phosphine do not decline in the period between fumigations in the diurnal cycle (which in this study can be up to 22h/d) but also implies that efficacy may be increased by the event. A possible explanation why this may be so is that breaks in dosage may bring more fumigant to the cell over time since the rate of absorption decreases over time as saturation approaches [5]. Phosphine toxicity involves the build up of hydrogen peroxide in the mitochondria due to the inhibition of enzymes such as catalase [9] and there is evidence that respiration is greater after fumigation than during it [10]. Therefore, diurnal interrupted treatments increase mitochondrial hydrogen peroxide due to the presence of greater amounts of oxygen and hydrogen phosphide.

While interrupting fumigation may have some toxicological benefits at the doses tested in this study, in reality, the extended time required for sufficient insect mortality under diurnally fluctuating conditions means serious control problems may arise if major variations in dosage are not sufficiently minimized. However, at the lower phosphine concentrations, diurnally fluctuating doses do not extend the necessary treatment time by as much. This is evident by the apparent convergence of the two LT99.9 data sets as concentration decreases. Further work is required at lower phosphine concentrations where treatment times are extensive and efficacy relies on tolerant insect development stages progressing to susceptible stages. The impact of diurnally fluctuating doses on the effectiveness of phosphine fumigation is important to clarify because any reduction in efficacy will have implications for selection of insect resistance.

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