Life stage and resistance effects in modelling phosphine fumigation of *Rhyzopertha dominica* (F.)

Thorne, J.*#[1,3], Fulford, G.[1,3], Ridley, A.[2,3], Schlipalius, D.[2,3], Collins, P.[2,3]
[1] School of Mathematical Sciences, Queensland University of Technology QLD.
Email: j1.thorne@student.qut.edu.au
[2] Agri-Science Queensland, DEEDI, 80 Meiers Road, Indooroopilly, QLD
[3] Cooperative Research Centre for National Plant Biosecurity, LPO Box 5012, Bruce, ACT 2617

* Corresponding author
# Presenting author

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Abstract

Resistance to phosphine in insect pests of stored grain is a serious problem and there is a world-wide need for the development of sustainable resistance management strategies. Here we introduce results from a new mathematical model of resistance development that includes all life stages, rates of oviposition, natural mortality and mortality under fumigation in relation to resistant genotype. The example we discuss is phosphine resistance in the lesser grain borer, *Rhyzopertha dominica* where resistance is known to be controlled by two major genes that are close to recessive in expression, so that resistance is not fully expressed unless both resistant genes are present and homozygous. An example of a scenario where this model could be used concerns the repeat application of phosphine in a situation where control of all life stages has not been achieved. We determined a critical interval within which a second fumigation must occur to stop a rapidly recovering population of resistant genotypes. Such scenarios can be readily investigated using this approach to provide the grain industry with resistance management options and strategies.

Keywords: *Rhyzopertha dominica*, Population dynamics, Stored wheat, Phosphine fumigant, Low concentration

1. Introduction

The Australian grain industry has a zero tolerance to the presence of insects in stored grain, and phosphine fumigant is the primary method of control. The genetic resistance of *Rhyzopertha dominica* (F.) to phosphine has driven research to determine how fumigation strategies may effect the proliferation of resistant genotypes, and conversely how to control infestations that contain phosphine resistant pests when increasing the applied dosage may not be practicable. A deterministic two-locus genetics model has been developed using a mating table and a novel death rate under fumigation (Lilford, 2009). The model has had some success in determining the dynamics of the population of *R. dominica* with various levels of resistance to phosphine under phosphine fumigation of varying dosages.

Resistance to phosphine is an inherited trait and two major genes are responsible for the strong phosphine resistance in *R. dominica* (Collins et al., 2002). These two genes act in synergism to display a significantly increased resistance to phosphine compared to any one of the resistance genes on their own (Schlipalius et al., 2008). Phosphine resistance is incompletely recessive at both genes (Schlipalius et al., 2008). That is, individuals that are heterozygous have a phenotypic response similar to susceptible individuals.

Lilford (2009) demonstrated that a two-locus model yielded different conclusions compared to a one-locus model; the single locus approach greatly exaggerates the rate of increase of the strongly resistant individuals compared to the other genotypes.

A greater tolerance to the phosphine fumigant has been observed in the egg and pupa stages (Hole et al., 1976). The Lilford (2009) model does not incorporate any life stage characteristics such as oviposition or stage-specific tolerance to phosphine, therefore a model incorporating life stages was developed to investigate *R. dominica* population dynamics in a more realistic fashion.

For the purposes of the current model, we have assumed that eggs and pupae may be up to three times more tolerant to phosphine than adults. Incorporating life stages into the current model provides us with the opportunity to investigate fumigation strategies that can optimise eradication or be identified as ineffective, in situations where control is not complete and eggs and pupa of the pest survive. In this paper, we outline aspects of the models of interest, and give some preliminary results into the effects of multiple fumigations with the life stage model.

2. Methods: Modelling R. dominica populations in grain silos

The following discussion is concerned with the rates used in the mathematical models. Because the generations are overlapping, the populations are modeled as a continuous function of time. There are thirty-six dependent variables, corresponding to the nine genotypes for each of the four life stages. Therefore, the model consists of thirty-six differential equations with terms corresponding to the complex interactions between genotypes and life stages.

2.1. Genotypical resistance

Similar to Lilford (2009), we model the population of these nine genotypes (i,j each taking values susceptible (s), hybrid (h) or resistant (r) by using an eighty-one times nine mating table (Lilford, 2009) to determine progeny genotype. This mating table permutes every combination of male and female mating, and determines the rate of progeny in a particular genotype when multiplied by the birth rate.

During fumigation, the birth rate (that is; eggs laid per unit time) is considered to be zero, the death rate becomes that due to fumigation and the model becomes a simple exponential relation, that is;

$$N_{ij}(t) = N_{ij}(0).e^{a_{ij}t} \quad (1)$$

where the death rate under fumigation α_{ij} is different for each genotype $\{i, j \in s, h, r\}$ and is dependent on the concentration of phosphine used. The dosage is predominantly dependent on time and concentration of phosphine, and we use Haber's rule (Bunce and Remillard, 2003) to determine a ratio of time and concentration for a toxic effect of phosphine. Haber's rule is $C^n t = k$, where C is concentration, t is the needed time to breathe/absorb the gas in order to produce the toxic effect, k is a constant relating concentration and time for the toxic effect of phosphine on *R. dominica* (in this case), and n is called the toxicity index. Daglish (2004) gives the toxicity index for phosphine and *R. dominica* as n=0.8673 and this value is used in the following calculations.

Collins et al. (2002) determined phosphine concentrations to reduce the population of a particular resistant genotype of *R. dominica* by 50% over a 48-hour fumigation. Using these phosphine concentrations (labeled LC_{50} in the reference but C_H here) and Haber's rule with the toxicity index for phosphine, we derive constants k for each genotype's resistance to phosphine

$$k = 48C_H^n \quad (2)$$

We then take these k values relating concentration and time to toxicity for a particular genotype to get a relation in terms of times (T_H) to kill 50% of the population as follows

$$T_H = \frac{k}{C^n} \quad (3)$$

and then determine the hourly death rate by substituting the time from equation (3), and the fact that the population at this time is half of the initial population (that is $N(t) = N(0)e^{\alpha.T_H} = N(0).0.5$) into equation (1), rearranging and taking logs to get the death rate for varying concentration of phosphine as follows:

$$\alpha(C) = \frac{C^n \ln 0.5}{k} \quad (4)$$

The per capita death rate for each genotype then is determined by the Haber's k constants (determined in equation (2)) and the concentration of phosphine applied. The resistance to phosphine is directly related to the magnitude of k. These values are given in Table 1.

Table 1 Haber's constant for each genotype (s = susceptible, h = hybrid, r = resistance) as derived in Lilford (2009) from data determined in Collins (2002) and Daglish (2004) at 25C and 60% relative humidity. These are used to determine specific death rate under fumigation for genotypes.

Genotype *i, j*	Haber's constant k
ss	0.2088
sh	0.417
sr	1.674
hs	0.2088
hh	0.537
hr	0.537
rs	4.0908
rh	4.0908
rr	50.019

2.2. Life stages and producing offspring

The interest in life stage effects was partly due to the fact that only adults can lay eggs, and also the observation that there is some resistance to phosphine in the egg and pupa life stages. In particular, eggs are resistant to the fumigant and the most numerous life stage, and adults are the easiest to kill (Hole et al., 1976). In this case, a likely scenario after a suboptimal fumigation is that no adults are left, but there are eggs. Egg numbers will initially decrease when this occurs, as there are no adults to lay them, and eggs die naturally or become larvae at a certain rate. So, when there are no adults, how long does it take for egg numbers to reach a minimum, and can this be exploited to maximise the chances of complete control? Figure 1 graphically shows the four life stages as states and shows rates of movement between the states, and rates of movement out of the states that reduce the entire population (death).

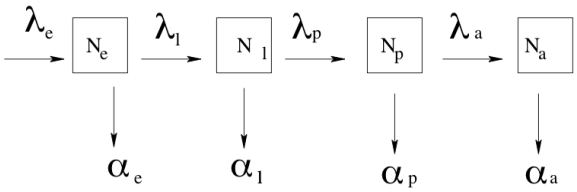

Figure 1 The transition diagram showing rates of population movement between different life stages (λ) and out of the system (α).

2.2.1. Time spent in each life stage

The information presented in Table 2 has been taken from Potter (1935). From Table 2 we can see that out of a beetle's average life span of 100 days, it is only able to lay eggs for less than half of this time.

Table 2 The average time spent in each life stage and a total life span at a temperature of 26°C and relative humidity of 65%, based on Potter (1935).

Description	Dulation (days)
The average time for a laid egg to hatch into a larva	15.5
The average time taken for a larva to develop into a pupa	36
The average time taken for a pupa emerge as an adult	6.5
The average time spent as an egg laying adult	42
Total average life span	100

2.2.2. Determining life stage rates

Table 2 gives expected times in each life stage. However, we need to determine rates into and out of each life stage as per Figure 1, where $\lambda_i, i \in \{e,l,p,a\}$ (egg, larva, pupa, adult) describes the rate into stage *i* from the previous stage, and $\alpha_i, i \in \{e,l,p,a\}$ describes the natural (that is; independent of fumigation) attrition rate out of each life stage. Table 2 was used to determine some of the rates in Figure 1 as follows. For subscript $i \in \{e,l,p,a\}$, let $N_i(t)$ be the number of beetles in life stage i at time t and λ_i be the rate of increase in $N_i(t)$. Now, assuming an exponential distribution for population numbers would imply that the mean time spent in any life stage is given by

$$E(X_i) = \frac{1}{\lambda_i} \quad i \in \{e,l,p,a\} \quad (5)$$

Taking the expected values as the average time spent in the life stages given by Table 2, we can rearrange equation (5) to determine rates from the times in Table 2. However, we also need data for the natural attrition rates (such as the percent of non-viable eggs laid) for each stage. This information specific to *R. dominica* could not be found, so parameters for the Alfalfa weevil were taken from Kuhar et al. (2000) as an approximation. Table 3 combines this information to give the rates used in the model. Under fumigation we assume that there are additional deaths due to the effect of phosphine. The per capita death rate under fumigation is given by adding the fumigation death rate specific to the genotype to the natural death rate specific to the life stage.

Table 3 Transition rates (to 4 decimal places) used in life stage model.

Per capita rate	Symbol	Value (units = per day)
Female adult lays an egg	λ_e	8.3333
Going from egg to larva	λ_l	0.0645
Going from larva to pupa	λ_p	0.0278
Going from pupa to adult	λ_a	0.1538
An egg dies	α_e	0.0363
A larva dies	α_l	0.0095
A pupa dies	α_p	0.0952
An adult dies	α_a	0.0375

2.2.3. Initial populations

We start with an initial total population of 1000, however all the populations for each genotype and life stage are proportional to the total initial population. The model does not rely on whole numbers and so is not affected by the size of the initial population, provided it is positive. This means that if we start with a total initial population of 10,000, the results will all be ten times the result from starting with an initial population of 1000. We do need to disperse the initial total population among the genotypes and life stages. Values for the initial genotype population ratios were taken from Lilford (2009), and the initial ratio of members in the different life stages was determined by running a numerical model for *R. dominica* life stages over a long period of time, and noting the population ratios when they remained stable (Table 4)

Table 4 Ratios of different life stages and genotypes (s = susceptible, h = hybrid, r = resistance) in population

Genotype	Ratio in population (%)	Life stage	Ratio in population (%)
ss	14	Egg	91.05
sh	10	Larva	5.97
sr	4	Pupa	0.65
hs	18	Adult	2.33
hh	17		
hr	6		
rs	6		
rh	21		
rr	4		

3. Results

In this section, we investigate some typical fumigation scenarios to demonstrate how the model might be applied in practice. First, we plot results for a single suboptimal fumigation and the subsequent recovery of *R. dominica*. Second, we plot results for two separate fumigations, interrupted by a certain period and the recovery. Additionally, we explore how long this interruption period can be, how altering the period of the interruption influences the development of resistance, and the ability to control infestations.

One suggestion is that after a fumigation where adults and larvae have been eradicated, but there are still eggs and pupae, the population is shocked into a state of decreasing egg numbers, and as the egg population is the hardest to eradicate, there may be an advantage with starting a second fumigation by the time the egg population reaches a minimum. Figure 2 shows the life stage dynamics with an initial population of 99% eggs, and 1% pupa. These proportions were assumed feasible (under the initial ratios given in Table 4) in a scenario where all larva and adults have been killed by fumigation. In Figure 2, initially the egg numbers decrease as there are no adults left to lay them, but as time goes by, eggs hatch and pass through the larval and pupa stages to adult. Eventually eggs begin to be laid again, and there is a definite recovery in egg numbers around the 6th day. Pupae numbers increase slightly by the 20th day, even though there are no larvae at time zero, and it takes an average of 36 days (by Table 2) for larvae to develop into pupae. This intuitively unrealistic effect occurs because times in Table 2 are averages, the larvae numbers increase quickly, and the more larvae that exist, the greater the possibility that some will develop rapidly into pupae.

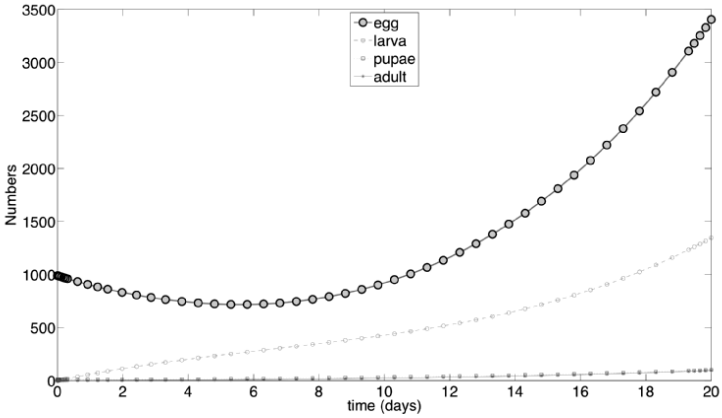

Figure 2 Life stage model showing eggs, larva, pupa and adults with initial population of eggs and pupa only.

Multiple staggered fumigations are simulated with a suboptimal concentration of phosphine as would be the case in a poorly sealed silo that is unable to maintain concentrations for extended periods. The concentration of 0.003 mg/L was chosen to be lower than the labelled dose and not able to eradicate resistant *R. dominica*, but still able to eradicate susceptible *R. dominica* (Collins et al., 2002). The model uses a temperature of 25°C and relative humidity of 60% during fumigation, and 26°C and 65% when fumigation is turned off. These were the conditions when the data for the corresponding rates was obtained, and are consistent with the conditions generally found in grain silos.

Figure 3 represents modelled results of the *R. dominica* population over 70 days where there is a single fumigation and represents a situation where adequate concentration has not been applied due to leakage. This fumigation was maintained for the first 31 days. The model run in Figure 3 gives four different graphs, one for each life stage. The model determines population numbers for nine different genotypes (of varying resistance) of *R. dominica*, portrayed as different types of line in each graph, with the most resistant genotypes as thicker lines with large circle markers. The fumigation period is represented by the shaded area. The fumigation period of 31 days is twice the average period for an egg to develop to the next larval stage (Table 2). After the fumigation, the recovery is different for each of the life stages, with the larva life stage having the greatest initial rate of growth. As would be expected, the resistant genotypes rr, rh and rs all show strong comparative recovery with rh reaching the highest population. Strains hh and hr recover less strongly, and hs does not recover. The susceptible genotypes ss, sh and sr do not recover over the 70 day run.

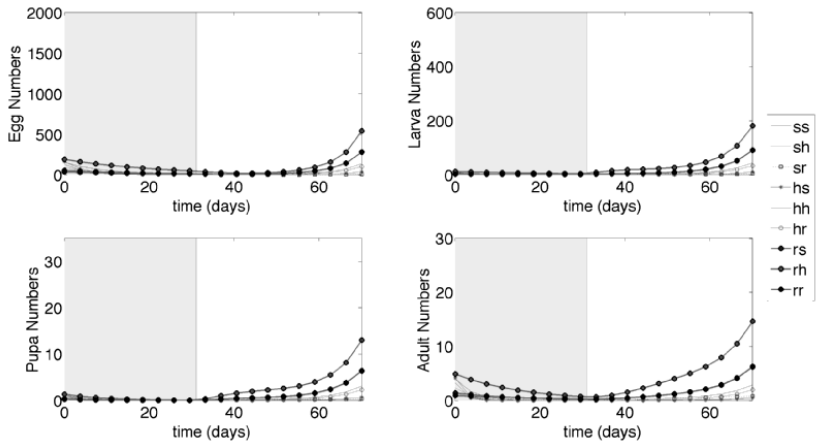

Figure 3 Fumigation at 0.003 mg/L (shaded area) for 31 days.

Figure 4 is a run of the same *R. dominica* population model as depicted in Figure 3 (nine genotypes and four life stages) and the run is also 70 days, but the fumigation has been split into two fumigations of 15.5 days performed 6 days apart. The 15.5 days is taken from Table 2 as the average time an egg takes to turn in to a larva, and 6 days was chosen from Figure 2 as the time taken for *R. dominica* egg numbers to reach a minimum after all adults and larva have been killed. The total duration and concentration of the fumigations run in the two shorter fumigations is the same as in the longer fumigation. Results of the model indicate that there is no observable difference in the total number of insects alive at the end of the 70-day period between the continuous and staggered fumigations. The rate of growth between fumigations is greatest in the larval stage once again. This is related to the population sizes of the immediately previous life stage.

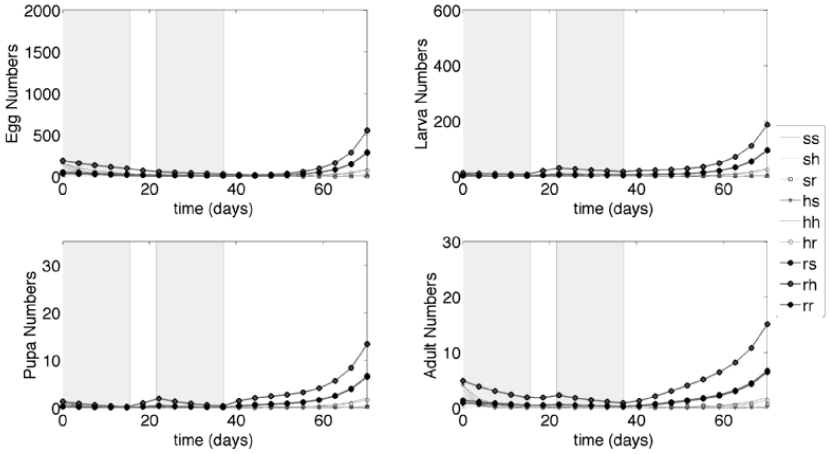

Figure 4 Genotype and life stage model with 2 staggered fumigations (shaded area) 6 days apart.

Figure 5 represents results from a third run of the genotype and life stage model over 70 days with a staggered fumigation with a much longer interval of 26 days between fumigations. The fumigation duration and concentrations were the same as in fig 4. This time, the rate of recovery of the resistant genotypes rr, rh and rs (thick lines with large circle markers) is very rapid. The rh genotype egg number going from around 500 to 2000 in a matter of 13 days. Compare with approximately 50 to 550 in Figure 3, with one long continuous fumigation, and approximately 40 to 550 when the fumigation is staggered with an interval of 6 days between fumigations.

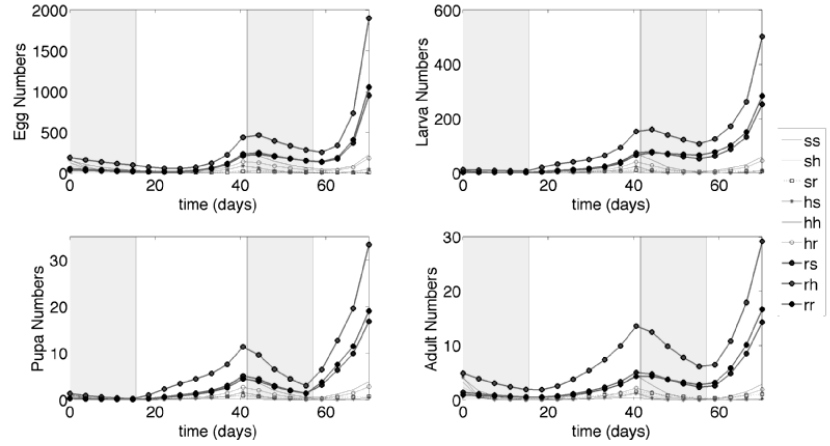

Figure 5 Genotype life stage model with 2 staggered fumigations (shaded area) 26 days apart.

4. Discussion

Analysis of the runs of mathematical models with life stage effects presented here has given effects of different fumigation strategies at low concentrations. The relation between the time a fumigation is run, the time between fumigations and the speed of recovery indicates that a longer interval between fumigations manifests in a faster rate of increase in insect numbers with a high proportion of resistant species after fumigation has ceased.

Our staggered fumigation results indicate a safe period where the population is shocked into dormancy by the first fumigation, and during this period the population is at the greatest risk from phosphine. The

concentration of fumigant in both the first and second fumigation is the same suboptimal concentration in the results presented here to illustrate that the short interval caused no difference compared with the continuous fumigation, but the implication is that if a leak leading to a suboptimal fumigation is discovered, there is a critical period within which the leak must be fixed and a second fumigation of labelled concentration applied to eradicate the pest. Otherwise, there is a risk of increasing proportions of resistant genotypes of pest in the grain that are impossible to eradicate. This critical period is the time taken for egg numbers reaches a minimum after the first fumigation has killed all adults. In results not presented here, the model verified that a second fumigation of labelled dose within this critical period would eradicate the pest.

Presented here are preliminary investigations that illustrate the value of including life stage detail in an effort to determine more effective strategies in controlling pests.

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