Fumigation of stored-grain insects — a two locus model of phosphine resistance

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Abstract: The standard pest control methodology for insect pests in grain storage is fumigation with phosphine gas. However phosphine resistance is a serious problem which threatens Australia’s grain industries. There are several questions regarding how to determine the most effective strategies for fumigation using phosphine to prevent the emergence of resistant strains.

Linkage analysis and molecular techniques have provided strong evidence that resistance is conferred by two genes on separate chromosomes for the insect species \textit{Rhyzopertha dominica}. For this species resistance for those homozygous with both copies of the sensitive gene has been determined to be well over \textit{250}\times those with no copies of the resistance genes, whereas there is a resistance factor of \textit{2.5\times to 30\times} if the resistance genes are present in only one of the two locations, depending on which location. A further important question arises as to whether single-locus population genetics models are adequate to give an accurate representation of the population dynamics under fumigation or whether two-locus models are needed.

This paper describes the development of a two-locus model for the population genetics for \textit{R. dominica} for fumigation under a given concentration of phosphine gas. The mathematical model consists of nine sub-populations, corresponding to the nine genotypes, modelled by a system of nonlinear ordinary differential equations. These are solved numerically using standard techniques.

Using the model, some different fumigation strategies are investigated; for fumigation switched on for a given period and switched off for a given period. The two-locus model is compared with a single-locus model. Preliminary results of this model have identified situations where a single-locus model gives different qualitative conclusions to the two-locus model.

Keywords: Two-locus inheritance, pest resistance, grain, genetics
1. INTRODUCTION

Resistance to chemical treatments in stored grain insect pests is a potential major threat to the Australian grain industry. Currently, phosphine treatments are the most effective control methods, however, pest resistance to phosphine means that significantly higher concentrations of phosphine will be required to control insect populations in the future and, in some cases, complete control may no longer be possible.

Control of the lesser grain borer, *Rhyzopertha dominica*, is particularly pertinent in Australia where a zero tolerance to pests in export grain has been observed since the Export (Grains) Regulations were passed in the early 1960s (Rees, 1998). The number of pest populations exhibiting resistance to phosphine has been slowly increasing worldwide since it was first noted in the Food and Agriculture Organization’s (FAO) 1972/1973 global survey on pesticide resistance (Champ and Dyte, 1976). In Australia, White and Lambkin (1990) first recorded a mild level of resistance in *R. dominica* in 1990. By the turn of the millennium, experiments had shown that some Australian strains were highly resistant to phosphine, compared both to susceptible insects and to the previously-discovered mildly-resistant strains (Collins, 1998).

Collins et al. (2002) discuss two observed resistance types, or phenotypes, currently found in Australian strains of *R. dominica*. They begin with a null hypothesis of a single gene controlling resistance in both phenotypes; the possibility of this monogenic inheritance arose in earlier work on a Brazilian strain of *R. dominica* also exhibiting weak resistance (Ansell, 1992). The experiments conducted by Collins et al. (2002) give evidence to reject this null hypothesis for both weakly and strongly-resistant beetles and conclude that phosphine resistance in both phenotypes is controlled by multiple genes, at least one of which contributes a major factor to resistance in each type. Resistance is characterised by incompletely recessive alleles on these major genes.

When a trait is specified by a recessive allele, an individual must possess identical copies of that allele for the trait to be expressed in their phenotype. For incompletely recessive alleles, heterozygous individuals show a limited expression of the trait — in this case, a lower level of resistance similar to susceptible insects.

A detailed genetic analysis at the molecular level by Schlipalius et al. (2002) confirms that there are two positions, or loci, on different chromosomes, of the strongly-resistant strain that carry resistance alleles. Both Collins et al. (2002) and Schlipalius et al. (2002) conclude that one of the genes determining resistance in the strongly-resistant strain is also present in the weakly-resistant strain. More recently, Schlipalius et al. (2006) propose that the gene shared between the weakly-resistant strain and the strongly-resistant strain (which they label *rph*1) was responsible for the initial emergence of phosphine resistance in Australia. Selection of the recessive allele for *rph*1 under fumigation subsequently caused the selection of the recessive allele at an additional, secondary resistance gene, labelled *rph*2. In the absence of *rph*1, homozygosity for this recessive allele at *rph*2 would convey only a modest survival advantage and heterozygosity almost no advantage at all over susceptible insects. It is the presence of resistance alleles on both *rph*1 and *rph*2 that is responsible for the strong level of resistance first noted in Collins (1998).

2. TWO-LOCUS MODEL

The mathematical model consists of nine ordinary differential equations for the nine genotypes; where we denote *N*ss as the total number of insects with both loci as the susceptible type, *N*sh as those with the first locus as the susceptible type and the second locus as the hybrid, through to *N*rr as the numbers with both loci carrying resistance alleles. The differential equations are obtained by constructing an offspring table which gives the proportions of offspring of the different genotypes for all the different mating combinations. The complete set of equations for the nine genotypes in the two-locus inheritance model is given in Figure 1. The parameters in the model that need to be specified are β, the per-capita birth rate (assumed to be the same for each genotype), and α1(t), α2(t) ... α9(t), which are the per-capita death rates for each of the nine genotypes.

Driscoll et al. (2000) have tabulated values of the intrinsic growth rate *r* = β − α, in the absence of fumigation, for a range of temperatures and humidity values. We have used the value *r* = 0.392 as an indicative value corresponding to a temperature of 24°C and humidity of 70%. The per-capita death rate, for all genotypes in the absence of phosphine fumigation, is set to a value of α = 1/13 per week for each genotype. This number has been estimated from the reciprocal of the average life-span of *R. dominica*, which is given by Rees and Rangsi (2004) as 13 weeks.

In the presence of fumigation the per-capita death rates are concentration dependent, such that the insects with resistance alleles have smaller per-capita death rates than the susceptible insects. A separate per-capita death rate has been defined for each of the genotypes in this model — for example, αss gives the number of deaths per week, per female insect, of insects with the doubly susceptible genotype ss. Daglish (2004) measure the
Two-locus model differential equations.

\[
\begin{align*}
\frac{dN_{ss}}{dt} &= \frac{\beta}{16N} \left( 4N_{ss} + 2N_{sh} + 2N_{sh} + N_{hh} \right)^2 - \alpha_{ss}N_{ss} \\
\frac{dN_{hh}}{dt} &= \frac{\beta}{8N} \left( 2N_{ss} + N_{sh} + 4N_{sr} + 2N_{sr} \right) \left( 4N_{ss} + 2N_{sh} + 2N_{sr} + N_{hh} \right) - \alpha_{hh}N_{hh} \\
\frac{dN_{sr}}{dt} &= \frac{\beta}{16N} \left( 2N_{ss} + N_{sh} + 4N_{sr} + 2N_{sr} \right)^2 - \alpha_{sr}N_{sr} \\
\frac{dN_{rs}}{dt} &= \frac{\beta}{8N} \left( 2N_{sh} + N_{sh} + 4N_{sr} + 2N_{sr} \right) \left( 4N_{ss} + 2N_{sh} + 2N_{sr} + N_{hh} \right) - \alpha_{rs}N_{rs} \\
\frac{dN_{sh}}{dt} &= \frac{\beta}{8N} \left( N_{hh} + 2N_{sh} + 2N_{sr} + 4N_{sr} \right) \left( 2N_{ss} + N_{sh} + 4N_{sr} + 2N_{sr} \right) \left( 4N_{ss} + 4N_{sr} + N_{hh} \right) - \alpha_{sh}N_{sh} \\
\frac{dN_{hr}}{dt} &= \frac{\beta}{8N} \left( N_{hh} + 2N_{sh} + 2N_{sr} + 4N_{sr} \right) \left( 2N_{sh} + N_{sh} + 4N_{sr} + 2N_{sr} \right) - \alpha_{hr}N_{hr} \\
\frac{dN_{rr}}{dt} &= \frac{\beta}{16N} \left( N_{hh} + 2N_{sh} + 2N_{sr} + 4N_{sr} \right)^2 - \alpha_{rr}N_{rr}.
\end{align*}
\]

where the total population \( N(t) \) is given by

\[
N = N_{ss} + N_{ss} + N_{sh} + N_{sh} + N_{sh} + N_{sh} + N_{sr} + N_{sr} + N_{sr}.
\]

**Figure 1.** The full set of nine differential equations for the 2-locus model.

Concentrations for the population to fall to 50% of their original value, denoted \( LC_{50} \), of some of these genotypes and their successive offspring. Remaining \( LC_{50} \) values were obtained from Collins et al. (2002). All \( LC_{50} \) values were taken from controlled fumigations of duration 48 hours. An exponential relationship between concentration and exposure time, as determined by Daglish (2004) and assuming exponential growth, is used to determine \( \alpha \) as \( \alpha = 168 \log(2)C_k^h/k_2 \) where \( k_1 = 0.8673 \) and \( k_2 \), for the nine genotypes, have been determined from the \( LC_{50} \) data, and are given in Table 1. However, this equation is based on controlled laboratory experiments so the mortality rates for insects in the field may be different. The resistance factor, \( f \), the ratio of \( LC_{50} \) relative to the susceptible genotype. For the strongly resistant genotype (which has the recessive gene in both locations) has a significantly higher value of \( f \) than the weakly resistant genotypes, with the recessive gene occurring in only one of the two loci. In Schlipalius et al. (2006) this was given as greater than \( f = 240x \), and later, in Schlipalius et al. (2008) as \( f = 616x \), which is the value we adopt here, see Table 1.

**Table 1.** \( LC_{50} \) values from the literature (Collins et al., 2002; Schlipalius et al., 2008; Daglish, 2004). Also given are the resistance factor \( f \) which are the \( LC_{50} \) values relative to the \( LC_{50} \) values for the ss genotype, and the estimated values for \( k_2 \) parameter for the per-capita death rate under fumigation.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>ss,hs</th>
<th>sh</th>
<th>hh, hr</th>
<th>sr</th>
<th>rs, th</th>
<th>rr</th>
</tr>
</thead>
<tbody>
<tr>
<td>( LC_{50} )</td>
<td>0.0017</td>
<td>0.0042</td>
<td>0.00563</td>
<td>0.0204</td>
<td>0.032</td>
<td>1.049</td>
</tr>
<tr>
<td>( f )</td>
<td>3</td>
<td>2.5</td>
<td>3</td>
<td>12</td>
<td>30</td>
<td>616</td>
</tr>
<tr>
<td>( k_1 )</td>
<td>0.2088</td>
<td>0.417</td>
<td>0.537</td>
<td>1.674</td>
<td>4.0908</td>
<td>50.019</td>
</tr>
</tbody>
</table>

The differential equations in Figure 1 were solved using the ODE45 solver in MATLAB. This is an adaptive time-stepping method based on the Runge-Kutta method. Firstly, we look at a typical hypothetical control strategy then we compare the results of this two-locus model with a single-locus model by aggregating the nine genotypes into three roughly equivalent phenotypes.

**3. RESULTS**

In Figure 2 the nine genotype proportions, \( N_{ss}(t)/N(t), N_{sh}(t)/N(t) \ldots N_{rr}(t)/N(t) \), where \( N(t) \) is the total population, are plotted against time, \( t \). In this scenario the fumigation time is over two weeks, occurring at the second week. The population initially is assumed to consist predominantly of weakly resistant and susceptible insects. To obtain the initial genotype proportions under this assumption, it was assumed that...
alleles for susceptibility and resistance were equally likely on the first locus, and that of all alleles present in the population at $rph_2$ only 5% were the allele for resistance. It can be shown that these values produce an initial proportion of the $rr$ genotype of only 0.025. Additionally, using allele proportions allows the establishment of Hardy-Weinberg equilibrium between the genotype proportions, as is demonstrated in Figure 2 for weeks 0–2.

During the fumigation period of two weeks an idealised phosphine concentration of 0.075 mg l$^{-1}$ was applied, which is 150% of the registered application rate in Australia in 2000 (Collins et al., 2000). We consider ideal conditions where the concentration remains constant during the duration of the fumigation, i.e. no leakage from the container. After fumigation, the three genotypes, $rr$, $rs$ and $rh$ dominated the proportions of the population, and the $rh$ genotype accounted for around half the population. These three genotypes had the highest resistance factors. Most importantly it can be seen that the $rr$ genotype, associated with strong phosphine resistance, has gone from a 2.5% presence in the population to close to 20%. This result explains how strong resistance might have been selected for in $R. \text{dominica}$, as indeed it was by 1997 in Australia (Collins et al., 2002). Collins et al. (2000) recommended higher concentrations be used to prevent the growth of the resistant strains; this graph shows clearly the danger in ignoring this recommendation.

In Figure 3 a comparison is made of the two-locus inheritance genetics model with a possible single-locus model of inheritance. Although it is now well known that phosphine resistance in $Rhyzopertha \text{dominica}$ is conferred by two genes many existing models of pest resistance employ only a single-locus approach. The differential equations for single-locus inheritance are constructed using a similar approach to that used to construct the two-locus inheritance model, and is explored extensively by Hoppensteadt (1975). The nine genotypes from the two-locus model have been aggregated into three groups for a direct comparison with a single-locus model. The aggregation is as follows.

- **Susceptible type.** The ss and hs genotypes are grouped (labelled $S_2$) for comparison with the susceptible homozygous genotype $s$ in the single-locus model (labelled $S_1$), and $S_1$ is assumed to share the same capita mortality rate as these two genotypes. So $S_2 = N_{ss} + N_{hs}$ and $S_1 = N_s$.

- **Weakly resistant type.** The genotypes rs, sh, hh, rh, sr, and hr are aggregated into the second group (labelled $H_2$) and are compared with the single-locus hybrid genotype (labelled $H_1$). The per-capita mortality rate for the single-locus hybrid type is the one corresponding to the resistance factor of $f = 2.5$, from Table 1. It may be argued that under a single-locus inheritance assumption, resistance would be incompletely recessive. However, resistance factors for the hybrid type would be very close to that for the susceptible type, so the $f = 2.5$ value from Table 1 is the most appropriate one to choose. So here $H_2 = N_{rs} + N_{sh} + N_{hh} + N_{rh} + N_{sr} + N_{hr}$ and $H_1 = N_h$.

- **Strongly resistant type.** The two-locus strongly resistant type $rr$ (labelled $R_2$) is compared with the single-locus resistant genotype $r$ (labelled $R_1$), both using a mortality rate consistent with the resistance factor $f = 616$. Here $R_2 = N_{rr}$ and $R_1 = N_r$.
The *R. dominica* population is now considered to have been in similar conditions for long enough to establish proportions of genotypes in Hardy-Weinberg equilibrium, assuming that the resistance allele on the first gene accounts for 40% of alleles on this gene and that the resistance allele on the second gene accounts for 15% of alleles on this gene. These values are chosen as an approximation to proportions of resistance types that have been seen in field studies.

Figure 3. Comparison of single-locus inheritance with two-loci inheritance for two different concentration levels of phosphine. The top plots assume the application rate was the registered rate in Australia in 2000, with (a) showing single-locus results and (b) the two-loci results after aggregation of genotypes. The bottom plots simulate an under-dosage of phosphine, showing in (c) the single-locus results and (d) the two-loci results after aggregation. Here $S_2$, $H_2$, $R_2$ denote the proportions of the population corresponding to aggregation of the 2-locus genotype into a susceptible, hybrid and strongly resistant groups, as described in the text, with $S_1$, $H_1$ and $R_1$ being the corresponding one-locus groups. Two concentrations were investigated; an idealised concentration of 0.05 mg l$^{-1}$ over 2 weeks and an ineffective concentration of 0.01 mg l$^{-1}$ over 2 weeks.

In Figure 3, plots (a) and (b) show the effects on the population proportions after applying a concentration of 0.05 mg l$^{-1}$ over 2 weeks, one of the previously registered application rates in Australia (Collins et al., 2000). Plot (a) shows the results from the single-locus model ($S_1$ to $R_1$) and (b) the two-loci model after aggregation of the genotypes $S_2$ to $R_2$). In Figure 3, plots (c) and (d) emulate the situation where the applied concentration rate is not reached within the storage facility. This may arise through inadequate (or no) sealing of a silo or incorrect application of the gas. The concentration over two weeks is chosen to be $C = 0.01$ mg l$^{-1}$ which is 20% of the previously recommended dosage from the year 2000, and could correspond to very leaky container of to an area of the container which receives an inadequate dose due to poor flow conditions.

Both two-locus and single-locus models show the increase of the resistant proportions, but it is clear that the single-locus model greatly exaggerates the rate of increase of resistant insects compared to the two-loci model, as well as the rate of decrease of hybrids ($H_1$ compared to $H_2$). This results, in the single-locus case, in a post-fumigation population consisting entirely of resistant insects whereas plot (b) indicates that rather the resistant insect proportion begins to decrease after fumigation under the (correct) two-loci assumption.

Under the inadequate-dose fumigation scenario the single- and two-loci models show similar qualitative behaviour. Both exhibit a sharp decline in the proportions of susceptible insects and indeed both models have
a susceptible proportion of effectively zero after the first week of fumigation. Additionally, both models exhibit rapidly-increasing proportions of heterozygotes, and comparatively very slowly-increasing populations of resistant insects. However, when fumigation ceases after two weeks the two-locus and single-locus models give contrasting results. Note the sudden decrease in heterozygote proportions in the single-locus case compared to the slight increase for the same in the two-loci case. More importantly, the single-locus model over-exaggerates the proportions of both susceptible and resistant insects showing an increase in both over time, while the two-loci model indicates that the resistant proportions are dwindling and the susceptible proportion effectively zero.

In Figure 3, plot (c), the single-locus model results are shown and in plot (d) the two-loci model aggregated results are shown. The single-locus model again exaggerates the rate of increase of the resistant proportions, this time resulting in a proportion nearly 10 times that of the resistant proportion in the two-loci model by the end of fumigation. The resulting post-fumigation overall proportions of the population are now completely reversed in the single-locus model when compared to the two-loci inheritance results. Where the hybrids make up over 90% of the population after fumigation when two-loci inheritance of resistance is assumed, it is the resistant insects that comprise greater than 90% of the population under a single-locus assumption.

4. CONCLUSIONS
This study has described the development of a two-locus model for phosphine resistance in stored grain insects and compared the two-locus model with a one-locus model. Complex resistance inheritance has driven the creation of the more explicit two-loci based model. Both the models were run under equivalent conditions. The results highlight the importance of incorporating two-locus inheritance in models used for investigating suitability of different phosphine fumigation strategies.

As discussed by Collins et al. (2000), the old previous registered application concentrations of phosphine in Australia, from 2000, appeared insufficient to control resistant strains of R. dominica; a conclusion supported by the results of our model. However, we do note that our model is a simplified prototype, and that further study and validation will be required before this model can be used to make reliable recommendations regarding fumigation strategies. The mortality rates for insects in the field may not depend on phosphine concentration in the same way as for the laboratory experiments on which our mortality rates are based, so caution should be exercised in interpreting results except to compare the effects of high verses lower ineffective concentrations. Our final model will also need to incorporate such influences as age-structure, temperature and humidity effects on birth and mortality rates and stochastic effects for very small populations. Also, account of leakage of phosphine from storage containers and sorption of phosphine into the grain needs to be taken into account. A comprehensive validation of the model should be undertaken before the results can be relied upon in practice. Nevertheless, the importance of including the appropriate two-locus genetics has been demonstrated here.

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NOTATIONS AND UNITS

<table>
<thead>
<tr>
<th>Symbol</th>
<th>description</th>
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<tbody>
<tr>
<td>$N_t$</td>
<td>total population, at time $t$, relative to initial population</td>
</tr>
<tr>
<td>$N_{ss}$, $N_{sh}$, $N_{sr}$</td>
<td>susceptible, hybrid and resistant genotypes, at locus 1 or 2</td>
</tr>
<tr>
<td>$N_{s1}$, $N_{h1}$, $N_{r1}$</td>
<td>number of insects of first three of the nine genotypes, relative to initial total population</td>
</tr>
<tr>
<td>$\beta$</td>
<td>per-capita birth rate (weeks$^{-1}$)</td>
</tr>
<tr>
<td>$\alpha_{ss}$, $\alpha_{sh}$, $\alpha_{sr}$</td>
<td>per-capita death rates for the first three genotypes, in weeks$^{-1}$</td>
</tr>
<tr>
<td>$S_2$, $H_2$, $R_2$</td>
<td>two-locus aggregated groups, susceptible, hybrid (weakly resistant and strongly resistant)</td>
</tr>
<tr>
<td>$S_1$, $H_1$, $R_1$</td>
<td>one-locus groups, susceptible, hybrid and strongly resistant</td>
</tr>
<tr>
<td>$LC_{50}$</td>
<td>phosphine concentration required to time to reduce population to 50% of its original value</td>
</tr>
<tr>
<td>$f$</td>
<td>resistance factor, defined as ratio of $LC_{50}$ values relative to ss genotype</td>
</tr>
</tbody>
</table>

REFERENCES


Daglish, G.J. (2004), Effect of exposure period on degree of dominance of phosphine resistance in adults of *Rhyzopertha dominica* (Coleoptera: Bostrychidae) and *Sitophilus oryzae* (Coleoptera: Curculionidae). *Pest Management Science*, 60(8), 822–826.


