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- 1 Title
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# ABSTRACT

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- 2 BACKGROUND: The presence of insects in stored grains is a significant problem for grain
- 3 farmers, bulk grain handlers and distributors worldwide. Inspections of bulk grain
- 4 commodities is essential to detect pests and therefore to reduce the risk of their presence in
- 5 exported goods. It has been well documented that insect pests cluster in response to factors
- 6 such as microclimatic conditions within bulk grain. Statistical sampling methodologies for
- 7 grains, however, have typically considered pests and pathogens to be homogeneously
- 8 distributed throughout grain commodities. In this paper we demonstrate a sampling
- 9 methodology that accounts for the heterogeneous distribution of insects in bulk grains.
- 10 RESULTS: We show that failure to account for the heterogeneous distribution of pests may
- lead to overestimates of the capacity for a sampling program to detect insects in bulk grains.
- Our results indicate the importance of the proportion of grain that is infested in addition to
- the density of pests within the infested grain. We also demonstrate that the probability of
- detecting pests in bulk grains increases as the number of sub-samples increases, even when
- the total volume or mass of grain sampled remains constant.
- 16 CONCLUSION: This study demonstrates the importance of considering an appropriate
- 17 biological model when developing sampling methodologies for insect pests. Accounting for
- a heterogeneous distribution of pests leads to a considerable improvement in the detection
- of pests over traditional sampling models.

# Keywords

21 Grains; Stored product pests; Heterogeneity; Sampling; Probability of detection

# 1. INTRODUCTION

- 2 Stored product pests are a major problem in grain supplies globally. Insects in stored grain
- 3 products affect grain quality, their presence is often unacceptable in domestic grain
- 4 supplies, and thus are considered major pests worldwide. They have the potential to cause
- 5 significant economic loss through direct consumption and commodity spoilage and also
- 6 endanger public health through contamination. Secondary losses associated with fungal
- 7 growth and trade restrictions also lead to significant economic costs for growers and bulk
- 8 handlers.<sup>1</sup>

- To minimise commodity loss, a significant emphasis has been placed on developing effective integrated pest management (IPM) strategies for stored products. An essential component
- of IPM is early detection of pest populations, and so extensive insect monitoring and
- sampling programmes form an integral component of management programmes in stored
- 13 grains. <sup>2</sup> Sampling and monitoring techniques often form the basis for treatment and control
- decisions and insect sampling programmes are well established in the grains industry.<sup>3</sup>
- 15 Sampling programmes have been established and are often regulated by major grain
- producing countries as they have recognised the importance of sampling to minimise the
- 17 risk of insects establishing in bulk grain. Typically, they are designed such that a
- 18 representative portion of a larger lot is sampled for analysis or inspection to determine if a
- commodity is free from infestation. While their importance is well established, nonetheless
- 20 considerable variation in the methodology of sampling programmes exists between
- countries, and among different grain producers, grain handlers and regulatory bodies within
- countries.<sup>3</sup> For example, the quantity (generally, measured as the weight) of grain sampled
- from grain bulk varies considerably between sampling programmes.<sup>3</sup> The foundation of

- 1 numerous international and domestic grain sampling programmes relates to historical and
- 2 pragmatic constraints in the supply and distribution network, rather than being designed
- within a robust statistical framework.<sup>3</sup> For example, sampling programmes have been
- 4 developed in relation to grain belt loading speeds at storage and shipping terminals, the size
- of grain trucks or rail cars and the size of storage silos and bunkers.<sup>3</sup> Additionally,
- 6 differences in units in which grains are stored and measured (metric and imperial
- 7 measures), the position within the supply chain at which sampling occurs (e.g. farm silos,
- 8 central storage) and the perception of risk from insect infestation in different geographic
- 9 regions have all contributed to variations to the rate and quantity of commodity sampled in
- sampling programmes.<sup>3</sup> Variation in grain sampling programmes is not only related to the
- quantity of grain sampled, but also to the number of sub-samples taken from the grain bulk
- that contribute to this total. For instance, Grain Trade Australia <sup>5</sup> specifies three 1 litre
- samples to be drawn from lots greater than 10 tonnes in size and an additional 1 litre
- sample to be drawn for every 10 tonne increase in lot size. United Kingdom regulations
- however, prescribe 2 kilogram samples for every 20 tonnes for lots less than 100 t and 1
- kilogram per 20 tonnes for lots greater than 100 tonnes.<sup>3</sup> The intensity of sub-sampling has
- also typically been established on the basis of practical constraints in the supply and
- distribution chain, being influenced by similar factors such as the size of grain rail cars, the
- units in which grains are measured (e.q. bushels) and size of storage facilities.<sup>3</sup>
- 20 As sampling programmes have been established based on practical constraints, the ecology
- of pest species and the influence this has on sampling efficiency has rarely been considered.
- 22 Notably, sampling programmes for grain commodities have been developed assuming that
- 23 insects are distributed homogeneously or randomly throughout the grain bulk. 3,6,7,8

- 1 Statistical models created under this assumption are largely dependent on the proportion of
- 2 grain sampled (the sample fraction) rather than any sub-sampling regime. <sup>6,8</sup> The number of
- 3 sub-samples is not included in these models as the probability of detecting insects is
- 4 assumed to be equal over the entire grain lot.
- 5 Evaluation of sampling programmes has been conducted by numerous grain producing
- 6 countries to ensure grain commodities meet specified standards and thresholds<sup>3</sup> and to
- 7 develop effective IPM strategies.<sup>2</sup> Even though sampling programmes were often found to
- 8 be adequate to detect small infestations in grain bulk, <sup>2,3,6,8</sup> the statistical models used were
- 9 based on the binomial distribution with the implicit assumption that pests were
- 10 homogeneously distributed. Although the validity of assuming a homogenous distribution of
- insects is questionable the performance of models which assume homogeneity under
- 12 heterogeneous conditions has yet to be determined.
- 13 The assumption of homogeneity in stored grains, although widespread, is largely for the
- sake of convenience and is unlikely to be true in bulk grains storage. Ecological and
- behavioural studies on stored product insects suggest that it is more probable that stored
- product insects display a heterogeneous distribution in grain stores. 1,9,10 Conditions within
- 17 grain storage facilities can differ significantly due to the design, size, seasonal temperature
- variation and aspect of storage facility that in turn influences the distribution of pest species
- throughout a grain consignment. <sup>9,10,11</sup> As a consequence, micro-climatic conditions such as
- 20 temperature and relative humidity in relatively small pockets of grain can vary substantially
- and have significant impacts on population growth and structure of stored product pests.<sup>1</sup>
- The age and the quality of grain within a particular storage facility may also vary. 1,10,11 Grains
- can be stored for prolonged periods of time, at either bulk handling facilities or on farm

1 grain storage silos leading to different aged grains of potentially differing quality being

2 mixed. This may have implications for the distribution of infestations within a consignment

as stored product pests are known to select for grains with higher moisture contents (which

is often a function of grain age), with the result that the spatial distribution of insects within

5 the grain will tend to be heterogeneous. 1,10,11

Insects are unlikely to conform to a homogenous distribution within grain bulks, and thus

violate a basic assumption of sampling programmes conducted by major grain producers.

Although insect densities have been statistically fitted to continuous distributions in an

effort to estimate abundance previously, 13,14,15 a generic sampling model that accounts for

the insect clustering behaviour and abundance has not yet been developed. In this paper we

investigate the influence of a heterogeneous distribution of insects on the probability of

detecting insect pests, and consider strategies to improve insect detection. Following

development of a robust sampling model, validation was conducted via estimation of model

parameters and by sampling. We demonstrate the efficacy of this model via sampling of

grains silos, using the model to predict effective sample size for detection. We also compare

our model with a sampling model that does not account for heterogeneity.

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#### 2. MATERIALS AND METHODS

# 2.1 The Model

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- 3 We consider a large grain lot throughout which a target insect may be heterogeneously
- 4 distributed. Our ultimate objective is to determine the probability of detecting insects in
- 5 grain bulk under a given sampling programme using an adaptation of an approach initially
- 6 proposed by Habraken et al. 16 We define the number of sub-samples drawn from a grain lot
- as  $\underline{n}$  and the weight of each of the sub-samples as  $\underline{w}$ ;  $\underline{nw}$  represents the total weight of the
- 8 sample drawn from a grain lot. We assume that any grain lot can be separated into two
- 9 distinct components, which may or may not be contiguous, a proportion  $\underline{p}$  that is infested
- and a proportion (1-p) that is free of infestation. Further, we assume that within the
- infested proportion of the lot, insects are homogeneously distributed according to a Poisson
- 12 distribution<sup>16</sup>.
- 13 Initially, we focus on drawing samples from the infested portion of the grain lot. The
- probability of drawing *X* contaminated samples from *n* total samples is binomially
- 15 distributed:

16 
$$P(X = x) = \binom{n}{x} p^{x} (1 - p)^{n - x}$$
 (1)

- 17 Grain samples are typically measured by mass rather than by volume. For each sub-sample
- that comes from the infested part of the lot, the probability of detecting an insect is
- influenced by the rate of infestation  $\underline{\lambda}$  (where  $\underline{\lambda}$  represents the number of insects present
- per kilogram within the infested portion of the grain lot). Let  $\underline{A}$  be the number of insects in

- 1 the sub-sample conditional on the sub-sample having come from the contaminated part of
- 2 the lot:

$$P(A = a \mid X = x) = \frac{e^{-xw\lambda} (xw\lambda)^a}{a!}$$
 (2)

- 4 However, in the acceptance of a grain lot the situation of key interest is that in which no
- insects are detected and so a = 0. In this situation, equation 2 reduces to:

$$P(A=0 \mid X=x) = e^{-xw\lambda}$$
 (3)

- 7 Consequently, summing over all possible values for <u>X</u> results in the unconditional
- 8 probability:

9 
$$P(A=0) = \sum_{i=0}^{n} P(X=i)P(A=0 \mid X=i)$$

$$= \sum_{i=0}^{n} {n \choose i} p^{i} (1-p)^{n-i} e^{-iw\lambda}$$

11 
$$= \sum_{i=0}^{n} \binom{n}{i} (pe^{-w\lambda})^{i} (1-p)^{n-i}$$

$$= (1 - p + pe^{-w\lambda})^n$$

13 The final step in the equation is derived from the Binomial theorem

$$(a+b)^{n} = \sum_{i=0}^{n} a^{i} b^{n-i}$$

15 Therefore the probability of detection is then given by:

16 
$$P(A > 0) = 1 - P(A = 0) = 1 - (1 - p + pe^{-w\lambda})^n$$
 (4)

#### 2.2 Parameter Estimates and Model Validation

2 2.2.1 Study Sites

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- 3 In order to use equation 4 to determine an appropriate number of samples to take for a
- 4 given probability of detection, the proportion of the lot contaminated  $\underline{p}$ , and the density of
- insects in the contaminated portion  $\lambda$  need to be estimated. Parameter estimation and
- 6 model validation was conducted on a grain farm near Warwick in South East Queensland,
- 7 Australia. Three grain silos of similar design were selected for the study. Each silo held
- 8 approximately 70 tonnes of wheat that had been harvested and placed in storage for four
- 9 months. No insecticide treatments had been conducted in any of the three silos during the
- storage period. All grain sampling from the silos was conducted on a single day.

#### 2.2.2 Parameter Estimates

- One silo was randomly selected, and sampling was conducted in one of the three silos to
- make parameter estimates of  $\underline{\lambda}$  and  $\underline{p}$ . Twenty five 800 gram samples were taken from
- random locations within the silo using a Graintec® Stainless steel grain spear. Note that the
- 15 number of samples taken was consistent both with obtaining good parameter estimates and
- pragmatic considerations of access to the silos. Each sample was individually bagged and
- sieved using a Graintec® 2mm stainless steel grain insect sieve for a standard 10 seconds.
- 18 For each sample the number of Sitophilus oryzae (Rice Weevils), Rhyzopertha dominica
- 19 (Lesser Grain borer) and *Cryptolestes spp.* (Flat Grain Beetle) were recorded. For each
- species  $\underline{p}$  was estimated by dividing the number of samples containing that species by the
- total number of samples taken, and  $\underline{\lambda}$  was estimated as the mean number of insects per
- sample (i.e. the total number of that species sampled divided by the number of samples

- taken). This procedure was replicated three times and mean parameter estimates were
- 2 calculated for use in the validation procedure.
  - 2.2.3 Model Validation

- 4 The model was validated by populating equation 4 with the mean parameter estimates for p
- and  $\lambda$  for each species, and solving to determine the number of samples n that need to be
- taken for each of three detection probabilities (0.75, 0.85, 0.95). This was considered a
- 7 prediction for the maximum sampling intensity that needed to be taken to detect that
- 8 species of insect. Sampling was then undertaken at this intensity at random points within
- 9 the remaining two silos. For each sample taken, the success in detecting the insect species
- within the maximum number of samples was recorded (Table 1). To ensure consistency the
- sample weight  $(\underline{w})$  for each remained a constant 800 grams of wheat.
- 12 This procedure was repeated three times in each of the two silos on which model validation
- was performed. For comparison with existing sampling methods, sample intensity was also
- calculated for each of the probability of detection levels tested above (0.75, 0.85, 0.95)
- under a Binomial sampling model <sup>6</sup> that assumes an homogeneous distribution of insects.
- Note that for the Binomial sampling model the amount of grain sampled (the sample
- fraction) needs to be calculated, and this was done based on the total volume of grain held
- in each silo. Insect density was calculated from initial parameter estimates.

# 3. APPLICATION

- In bulk grain lots, both the rate of infestation  $\underline{\lambda}$ , and the proportion of the lot infested  $\underline{p}$ , will
- 3 vary based on a variety of factors including temperature, humidity, and storage period. We
- 4 consider the effects of these variables on the probability of detection, using a range of
- values for  $\underline{n}$ , the number of sub-samples drawn from the lot and  $\underline{w}$ , the weight of each sub-
- 6 sample.

- 7 The probability of detecting insects within a grain lot increases as both the proportion of
- 8 commodity infested  $\underline{p}$  and the rate of infestation  $\underline{\lambda}$  increases (Figure 1). This can be further
- explored by investigating the probability of detection of insects as both  $\underline{p}$  and  $\underline{\lambda}$  reach their
- respective limits of 1 and infinity. As <u>p</u> approaches one (i.e. a greater proportion of the lot is
- infested),  $\underline{n}$  and  $\underline{w}$  do not effectively act independently. The probability of detection thus
- 12 can be represented as:

13 
$$P(A > 0) = 1 - (1 - p + pe^{-w\lambda})^n$$

$$=1-(e^{-nw\lambda}) \tag{5}$$

- 15 In equation 5 the probability of detection is simply related to the Poisson distribution and
- detection is influenced by the rate of infestation  $\underline{\lambda}$ , and the total weight of grain sampled,
- 17 <u>nw</u> (Figure 2).
- 18 Alternatively, consider the probability of detecting insects as the rate of contamination
- 19 approaches infinity. From equation 4:

20 
$$P(A > 0) = 1 - (1 - p)^n$$
 (6)

- 1 This is the probability of observing a least 1 positive sample for a binomial variable. Under
- 2 this scenario, the probability of detecting an insect will vary with  $\underline{p}$  (Figure 3).
- 3 Although  $\underline{\lambda}$  and  $\underline{p}$  affect the probability of detection of insects within a lot as shown, when a
- 4 grain lot is sampled these will be unknown quantities. The number of sub-samples  $\underline{n}$  can be
- 5 varied, however, and so variations in this parameter may form the basis for sampling
- 6 strategies. We consider here the influence of sub-sampling on the probability of detection
- 7 of insects under various combinations of  $\underline{\lambda}$  and  $\underline{p}$ .
- 8 We initially consider two scenarios to illustrate the effects of changes in sub-sampling on
- 9 the probability of detecting insects for infestation rates of ( $\underline{\lambda}$  = 5) and ( $\underline{\lambda}$  = 0.5). These rates
- are based on estimates for common grain beetle densities in storage <sup>17</sup> and provide
- examples of high and low insect densities for simulation. Note that these rates encompass
- the range of parameter values determined in section 2, however we considered that a wider
- range of parameter values was necessary in order to encompass a broader range of
- 14 conditions. In the first scenario a fixed sample weight (<u>nw</u> = 10kg) was used, representing
- 15 the most intensive sampling rate recommended by Grain Trade Australia.<sup>5</sup> Here, the
- probability of detecting an insect increases as the number of sub-samples increases. The
- level of increase will vary according to the underlying infestation rate (Figures 4a and 4b).
- Similarly, we consider the influence of sub-sampling on probability of detection when w is
- 19 held constant under at three levels of heterogeneity in lot infestation (Figures 5a and 5b).
- 20 As shown in Figures 5a and 5b, the probability of detecting an insect increases as the total
- 21 sample weight increases (shown here by increasing the number of sub-samples with a fixed
- sub-sample weight). The rate of increase in the probability of detection is also significantly
- higher when the total sample weight increases in comparison to when <u>nw</u> remains constant

- 1 (Figures 4a & 5a; 4b & 5b). In all examples the detection curve asymptote is reached
- 2 significantly quicker as <u>p</u> increases, leading to fewer sub-samples being required for
- 3 increased detectability.

# 3.1 Comparison of sampling models

- 5 Hunter and Griffiths <sup>6</sup> proposed an approach based on the Binomial distribution to estimate
- 6 insect population densities in bulk grain lots. Unlike the model presented above, an implicit
- 7 assumption of this approach is that insects are distributed homogenously throughout grain
- 8 lots. Furthermore, the approach does not consider the number and weight of samples
- 9 taken, rather the total sample fraction and follows,

10 
$$P(\psi) = 1 - (1 - \theta)^{\nu}$$
 (7)

Where  $\underline{\psi}$  represents the number of insects in a sample,  $\underline{P(\psi)}$  is the probability of drawing an 11 infested sample,  $\vartheta$  is the total fraction of the bulk grain that is sampled and  $\nu$  represents the 12 13 total number of insects in a lot. For example, if the sample fraction represents 0.0001% of the total grain in a lot and the number of insects in the lot v = 513, solving for equation (7) 14 will give a 5% probability of detection. A further example is presented in Love et al. where 15 16 average insect densities are calculated for given grain bin rejections. Statistical models 17 based on the Binomial that assume homogenous distribution of insects such as Hunter and Griffiths  $^{6}$  form the basis for a number of grain sampling programmes. We therefore 18 compare the performance of the Hunter and Griffiths <sup>6</sup> approach with the model proposed 19 in this paper (Table 1). To do this, we compare sampling results for three insect species 20 Sitophilus oryzae, Rhyzopertha dominica and Cryptolestes spp collected from farm storage. 21

1	As demonstrated in Table 1, the model presented in this paper, $P(A>0)$ , outperforms a
2	model which does not consider insect distribution. This is particularly evident when the
3	proportion of the lot infested is less than 50%, for example, the very poor detection of S.
4	Oryzae by the Hunter and Griffiths <sup>6</sup> model compared with the successful detections with a
5	sampling intensity generated by equation 4 (Table 1).
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#### 4. DISCUSSION & CONCLUSIONS

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2 Given the possible costs of failure to detect pests such as live insects or fungi in grain 3 commodities, it is important to maximise the probability for their detection. Since it is generally impossible or impractical to inspect an entire bulk grain consignment for 4 5 impurities, sampling programmes that are effective and statistically rigorous are required. 6 When considering biological pests as contaminants an understanding of the ecology of the 7 species and how this may influence the distribution of pests through space is required to develop effective sampling strategies. 16,17,18,19 Previous sampling programmes to detect 8 insects in stored grains have been based on the binomial sampling model with the implicit 9 assumption that pests are homogenously distributed throughout the grain bulk. 6,20 In this 10 11 paper, the heterogeneous distribution of insects or other impurities is explicitly considered via the use of an appropriate statistical model. This model better accords with the known 12 biology of grains pests in considering heterogeneity within a grain lot by modelling the lot as 13 having contaminated and un-contaminated portions. As demonstrated via field sampling 14 15 and simulation, our method considerably outperforms traditional sampling methods when the appropriate biological assumption that insects distribute heterogeneously through grain 16 17 bulk is accounted for. Field sampling and simulation experiments demonstrated that the probability of detecting 18 19 insect pests was influenced by both the distribution of pests within consignments and the rate of contamination (Table 1, Figure 1). In field validation studies the number of samples 20 required to detect each insect species was not significantly influenced by the rate of 21 22 infestations as estimates for this parameter were similar for all species (Table 1). The 23 proportion of the silo infested however, did have a significant influence on sampling

- intensity for each species (Table 1). This was in accordance with simulation results
- 2 presented in figures 4a & 5a. Both simulation and field validation studies demonstrated that
- 3 accounting for clustering behaviour is particularly important when only a relative small
- 4 proportion <20% of a lot is infested, irrespective of total insect density.
- 5 The comparison of our model to the Hunter and Griffiths<sup>6</sup> model that does not account for
- 6 the spatial distribution of insects illustrates that current detection probabilities may be
- 7 overestimated when the spatial distribution of insects is not considered (Table 1). If the
- 8 probability of detection of pests is overestimated through the use of an inappropriate
- 9 biological model, management decisions that are based on these estimates may be more
- 10 risky than anticipated, with the potential for unanticipated commodity and economic losses.
- 11 Although the rate of infestation and the proportion of grain bulk infested are biological
- parameters that cannot typically be manipulated directly, it is important to recognise that
- environmental conditions that are known to influence these biological aspects of pest
- infestation will affect pest detection under any given sampling regime.
- Sub-sampling intensity  $\underline{n}$ , and sub-sample weight  $\underline{w}$ , also strongly influence detection
- 16 probabilities of pests. While the rate of infestation and the proportion of grain bulk infested
- may have substantial influence on the probability of detection, these cannot be directly
- changed in a real setting. In contrast, the sampling parameters n and w are critical since
- they can be manipulated in order to achieve the goals of a sampling programme. A key
- 20 observation from this study is that the probability of detection increases when the number
- of sub-samples  $\underline{n}$  increases irrespective of the rate of infestation and proportion of the lot
- infested (Figures 4a & 4b). This will occur despite the total sample weight <u>nw</u>, remaining

- 1 constant. This can be understood as an interaction between sampling and the underlying
- 2 distribution of insects.
- 3 For an insect to be detected in the total sample, a sub-sample must first be drawn from the
- 4 infested portion of grain bulk. Clearly, the probability that at least one sub-sample
- 5 intercepts the infested portion of the lot increases as more sub-samples are drawn. For high
- rates of infestation  $\underline{\lambda}$ , the probability of detecting an insect once the infested portion has
- 7 been intercepted will be high. When the rate of infestation is low, however, the volume
- 8 (weight) of sub-samples will have a greater impact on the probability of detection than the
- 9 number of sub-samples drawn (Figure 4b & 5b). In this scenario, even if the infested portion
- of the grain bulk is intercepted, the probability of actually detecting an insect in that sample
- will be related to the volume of the infested portion that has been sampled.
- 12 As shown here, the intensity of sub-sampling and the weight of sub-samples taken will
- strongly influence the capacity of a sampling regime to detect insects. These variables are
- 14 not considered in traditional grains sampling models, however. The Hunter and Griffiths <sup>6</sup>
- model used as a comparison here considers a single measure, the sample fraction  $\underline{\vartheta}$  which is
- equivalent to  $\underline{nw}$ . Models which do not consider sub-sampling intensity  $\underline{n}$  overestimate the
- probability of detecting insects when tested against a heterogeneous insect distribution.
- 18 This occurs because the probability of insects being in any one sample is assumed to be
- 19 equal.
- 20 Sampling strategies for stored grains have typically been based on lot size. Grain Trade
- 21 Australia<sup>5</sup> standards for example recommend a greater sampling rate as the size of the lot
- increases. This primarily relates to the statistical sampling frameworks failing to consider
- 23 insect distribution. The current study illustrates the importance of developing sampling

- 1 programmes with due consideration of pest ecology, density and distribution rather than lot
- 2 size. Sampling intensity and sample size should be optimised in relation to the rate of
- 3 infestation (density of insects) and the proportion of commodity infested (insect
- 4 distribution) Table 1. Sampling based purely on lot size may not provide valid estimates of
- 5 insect density as small lots may not be sampled adequately if clustering is occurring.
- 6 Conversely, extensive sampling of large lots may be inefficient and leading to added
- 7 monitoring costs.
- 8 The approach taken in this paper was to consider the scenario where no live insects are
- 9 acceptable in a bulk grain lot (i.e. A = 0), a standard that increasingly is being adopted by
- grain handlers for transfers of grain. However, in some scenarios it may be useful to
- consider a sampling regime under an alternative value of  $\underline{A}$ . A primary concern of grain
- handlers and distributors worldwide is the emerging issue of phosphine resistance and the
- reduction of potential alternative insecticide treatments due to regulatory controls. 19,21,22,23
- 14 Here, sampling programmes for grains throughout the production and supply chain might
- take into consideration critical treatment thresholds for insects throughout all stages of
- storage and distribution. That is, with an effective sampling methodology as demonstrated
- 17 here, grain handlers and distributors could administer treatments based on insect density
- thresholds, reducing the potential for over or under utilisation of insecticide treatments.
- 19 Multi-stage sampling strategies could therefore play an important role in determining
- 20 effective treatment times. Furthermore, effective sampling would reduce costs associated
- 21 with control and treatments.
- 22 Monitoring and sampling of stored grains for insect populations has become an integral
- 23 component of IPM strategies.<sup>2,24</sup> The early detection of pest populations is critical for

effective control measures to be implemented.<sup>2</sup> The usefulness of monitoring and sampling 1 2 data however, is dependent on the accuracy of the data so that a realistic representation of 3 the presence of insects can be attained. Ineffective treatment and failure of IPM programmes to control pest populations is often related to failures in detection protocols.<sup>2</sup> 4 5 This study demonstrates that the number of sub-samples and the weight of sub-samples 6 taken will critically influence the detection probabilities of insects in bulk grains. However, 7 the study also highlights the need for flexibility in considering those factors that will 8 influence biological parameters that cannot be easily manipulated, the rate of infestation and the proportion of the lot infested. Further work is required to create sampling 9 10 programmes that can be adapted to account for variations in external temperature and 11 humidity (such as the geographic location of storage and the season in which sampling occurs), the way in which grains are stored (e.g. vertical silos or horizontal warehousing), 12 and the way in which they are transported. This would allow for more efficient, cost-13 effective sampling to occur throughout the production and supply chain leading to improved 14 15 IPM programmes and population management. 16 17 18 19

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Table 1. Comparison of success in detecting three different insect species when silos were sampled under an intensity ( $\underline{n}$ ) predicted by a model that accounts for the heterogeneous distribution of insects (equation 4), and one that does not (Hunter and Griffiths<sup>6</sup>). For each species-probability of detection combination, the required sampling intensity was replicated three times. Detection of that species at the given sampling intensity was counted as a success and so there can be a maximum of three successes from the three replicates.  $\underline{P(\psi)}$  represents the probability of detection for the Hunter and Griffiths<sup>6</sup> model,  $\underline{P(A>0)}$  represents the probability of detection for the model specified above. Parameter estimates of  $\underline{p} = 0.61$ ;  $\underline{\lambda} = 13.1$  for  $\underline{R}$ .  $\underline{dominica}$ ,  $\underline{p} = 0.5$ ;  $\underline{\lambda} = 17.4$  for  $\underline{Cryptolestes}$   $\underline{Spp}$ . and  $\underline{p} = 0.2$ ;  $\underline{\lambda} = 13.04$  for  $\underline{S}$ .  $\underline{oryzae}$  were estimated from sampling used to populate the model (see text for details).

		<u>P(A&gt;0)</u>			<u>P(ψ)</u>		
	Probability of	<u>n</u>	Silo 1	Silo 2	<u>n</u>	Silo 1	Silo 2
	Detection (%)		Successes	Successes		Successes	Successes
<u>R. dominica</u>	95	3	3	3	1	2	3
	85	2	3	3	1	2	3
	75	1	3	3	1	2	3
Cryptolestes	95	5	3	3	1	1	2
<u>Spp.</u>	85	3	3	3	1	1	2
	75	2	3	3	1	1	2
<u>S. oryzae</u>	95	13	3	3	1	2	0
	85	9	2	2	1	2	0
	75	6	2	1	1	2	0

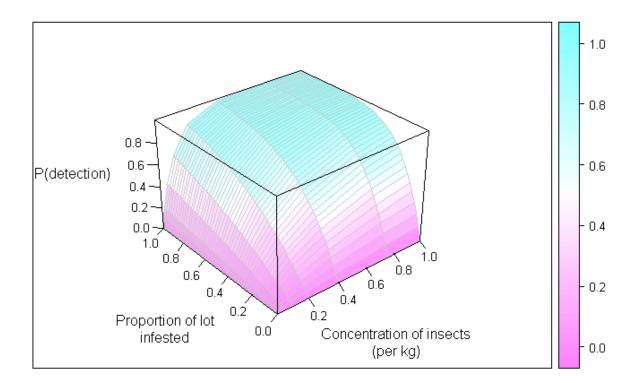


Figure 1. The probability of detecting insects in a grain lot as a function of <u>p</u>, the proportion of lot infested, and
λ. the rate of infestation when number samples <u>n</u> = 10 and the weight of samples <u>w</u> = 1. This represents an
intensive sampling rate for a large lot size.<sup>5</sup>

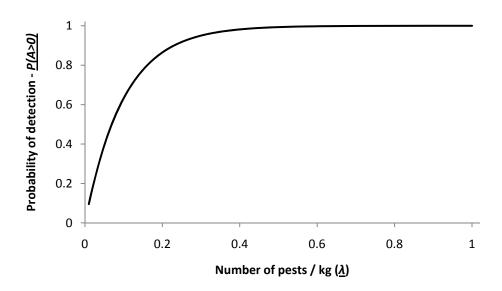


Figure 2. The probability of detection for varying rates of infestation  $\underline{\lambda}$ , when an entire grain lot is infested ( $\underline{p}$  = 1) that is, a homogeneous distribution of insects.

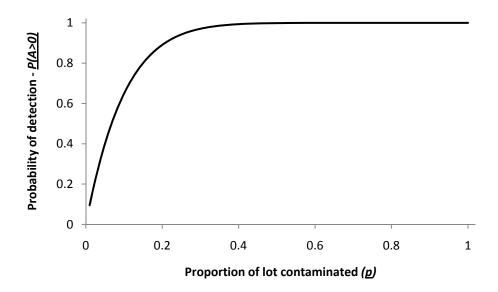


Figure 3. The probability of detecting insects in a grain lot as a function of the proportion of lot infested  $\underline{p}$  as  $\underline{\lambda}$  tends towards infinity.

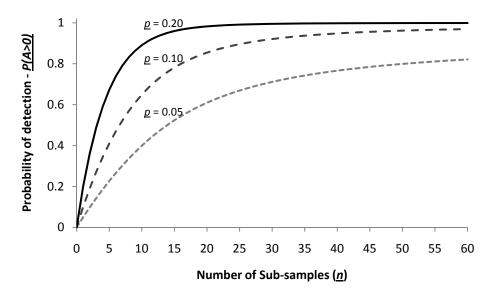


Figure 4a. The probability of detecting insects in a grain lot, in relation to the number of sub-samples drawn for various levels of heterogeneity,  $\underline{p}$ . For the example presented here,  $\underline{nw}$  is held at a constant 10 kg with  $\underline{\lambda}$  = 5.

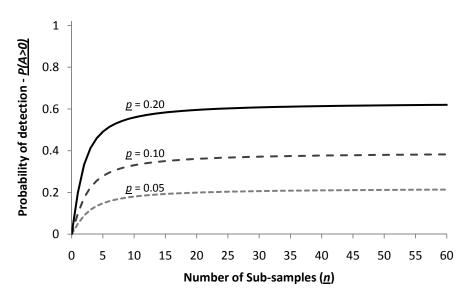


Figure 4b. The probability of detecting insects in a grain lot, in relation to the number of sub-samples drawn for various levels of heterogeneity,  $\underline{p}$ . For the example presented here,  $\underline{nw}$  is held at a constant 10 kg with  $\underline{\lambda}$  = 0.5.

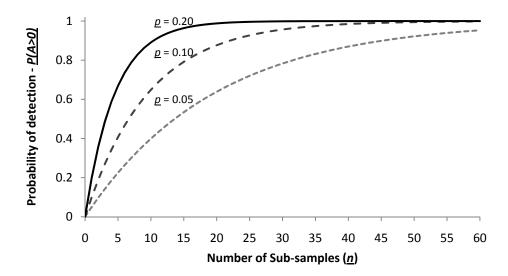


Figure 5a. The probability of detecting insects in a grain lot, in relation to the number of sub-samples drawn for various levels of heterogeneity,  $\underline{p}$ . For the example presented here,  $\underline{w}$  is held at a constant 1 kg with  $\underline{\lambda}$  = 5.

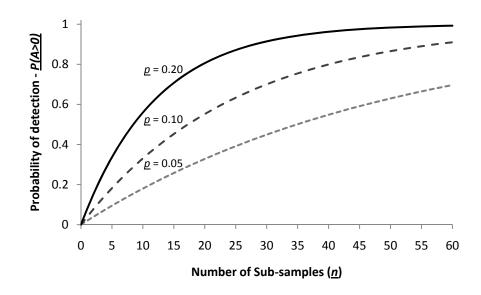


Figure 5b. The probability of detecting insects in a grain lot, in relation to the number of sub-samples drawn for various levels of heterogeneity,  $\underline{p}$ . For the example presented here,  $\underline{w}$  is held at a constant 1 kg with  $\underline{\lambda}$  = 0.5.