Report to CRC for National Plant Biosecurity

CONFIDENTIAL

Case Study 3

Sampling grain based on detection

thresholds: implications for integrated

pest management

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Summary

Integrated Pest Management (IPM) strategies lead to the cost effective management of pests in stored grains. Although significant advances have been made in fumigation technologies, storage types and insecticide treatment regimes worldwide, numerous countries still do not have a single cohesive integrated approach to pest management. This in part is due to the lack of simple, robust statistical sampling techniques for use in IPM, for example to detect insects at a pre-determined treatment threshold. In this study we extend theory previously developed in this project to consider detection thresholds of greater than zero, and show that this provides a sound basis for IPM decisions. Using a case study we demonstrate that setting a treatment threshold for fumigation of grain and using the new methodology for sampling can reduce the number of fumigations applied and thus substantially cut costs by up 17% depending on environmental conditions. The new methods we have developed and present in this report provide an important step towards evidence based IPM in Australia.

Introduction

Improving insect control methods has been has been a primary focus of stored product research for many years (Hagstrum and Subramanyam 2006). While chemical controls have often been central to management of insect pest within storages (Longstaff 1994), reliance on chemical control methods can be costly in the long term. Some chemical controls are expensive making them not cost effective as the sole control strategy, whilst the use of residual insecticides can lead to trade restriction (Hagstrum and Subramanyam 1996). In the longer term, the critical problem of over reliance on chemicals as the sole control mechanism for insect management is the development of resistance to control agents (Herron 1990). Resistance, particularly to cost effective fumigants such as Phosphine, is one of the greatest threats to the global grains supply and production industry (Herron 1990).

In recent years stored product research has placed a greater focus on developing alternative technologies to control insects, in part as a response to issues arising from fumigant resistance (Hagstrum and Subramanyam 2006). This has culminated in the development of integrated pest management (IPM) strategies which aim to use a range of technologies to achieve a higher level of control than could be achieved using one control method in isolation (Scholler *et al.* 1997, Kogan 1998, Hagstrum *et al.* 1999). IPM programmes for stored products use a broad range of techniques including fumigation, insecticide application, aerations, hygiene and improved storage as a means to maximise control outcomes (Hagstrum and Subramanyam 2006).

The combination of control techniques used within IPM programmes are intended to efficiently manage individual species within specific regions at specific periods of time

(Kogan 1998). It is therefore important to determine when and where particular techniques are most effective (Kogan 1998). For example, aeration is a strategy which aims to maintain grain temperature at a level that is suboptimal for pest development and is most effective in cooler conditions prior to insect populations reaching high densities. While fumigants, in contrast, can be applied year round, they will be most cost effective when insect densities reach a threshold where damage is economically important (Hagstrum and Subramanyam 1996). Therefore, as the efficacy of treatments varies depending on conditions and pest pressure IPM programmes aim to utilise various treatments when they are most efficacious to maximise control outcomes (Kogan 1998).

Although the need and benefits of IPM are broadly accepted (Rees 2004), many grain producing nations still do not have an industry wide IPM control strategy. Control strategies vary significantly and are often more concerned with the immediate availability of grain than long term control success. For example, countries which base sampling on a zero tolerance framework such as Australia and Canada may manage grain bulks by sampling on purchase and sale of the grain, and undertake regular (calendar based) fumigations during the time in which the grain stored. This can be costly in the short term because there is a high likelihood that fumigations will be undertaken when none are needed (Subramanyam and Hagstrum 2006). In the longer term, there is a strong possibility that such strategies can lead to large, industry wide losses because excessive fumigation is closely related to insecticide resistance in grain pests (Collins *et al.* 2000).

Treatment thresholds, where treatments are only initiated when pests reach a specified density, can be used to ensure fumigations are only conducted when required. IPM programmes under this regime need to be based on a robust sampling programme with a

pre determined threshold, which allows for detection of pests at particular population densities (Kogan 1998). As such, sampling programmes provide a means to determine when particular control methods should be used (Kogan 1998). Within grain storages for example, pest density as well as other external factors such as temperature and humidity will determine the type of control strategy that will be most effective. If insect densities are low and conditions are not conducive to rapid insect growth, control may be delayed or may be undertaken via the use of a technique such as aeration, which has lower efficacy than phosphine fumigation or residual chemical use, but still provides an effective method to maintain population densities at substantially lower cost. In contrast, if insect densities are high, a more costly strategy with higher efficacy (e.g. chemical application or fumigation) may be used. When particular strategies are initiated is thus dependent on the density of pests and an effective sampling programme to identify that density. For example, in United States grain storages, insect densities greater than 2 insect per kilogram are required to be treated (Subramanyam et al. 1997, Hagstrum et al. 1999) and therefore effective sampling is required to detect at that level.

Sampling strategies for IPM must maximise detection and estimate the density of the species of interest accurately within the environment (Kogan 1998). In stored grains a number of sampling plans have been developed based on a fixed number of samples to determine mean density estimates within storages (Hagstrum *et al.* 1985, Lippert and Hagstrum 1987, Subramanyam *et al.* 1993). Further, Subramanyam *et al.* (1997) developed a sequential sampling plan to estimate population density for management. Although effective, robust parameter estimates for these approaches are heavily reliant on the

availability of existing data with models being parameterised specifically for individual species. Furthermore models can be complex for end users to interpret and use.

Alternative sampling models have been developed, that focus on maximising the probability of detecting insect for a zero tolerance approach, *i.e.* to determine the freedom of insects from a bulk (Hunter and Griffiths, Love *et al.* 1983, Jeffries 2000). While these methods do not depend on specific pre-existing data to parameterise models, they are not suitable for use in IPM where estimating a threshold density is more relevant than simply determining if insects are present within storage. It is therefore valuable to develop methods to detect pest species at thresholds greater than zero for use in IPM which allow managers to simply identify pest levels in bulks at minimal costs.

Elmouttie *et al.* (2010) proposed a methodology to determine the number of samples required to maximise detection of insect pests within storages for zero tolerance management. Similar to previous methodology (Hunter and Griffiths 1978, Hagstrum *et al.* 1985) this approach considered insect density however also explicitly accounted for the potential insect infestations to be heterogeneously distributed within a grain lot. Thus rather than being based on parameters to describe insect spacing behaviour (Hagstrum *et al.* 1985) or the relationship between sample means and variances that may be difficult to calculate (Hagstrum *et al.* 1985, Lippert and Hagstrum 1987, Subramanyam *et al.* 1993) the model was based on two biologically relevant parameters, the proportion of grain infested and the density of the infestation within the infested portion.

This study aims to expand the approach proposed by Elmouttie *et al.* (2010) by investigating the influence of alternative detection thresholds on sampling intensity. We investigate how

model behaviour is affected by alternative detection threshold and what effect this has on the probability of detection. We demonstrate that sampling with a methodology which considers alternative detection thresholds increases the accuracy of sampling programmes for detecting targets pests at a treatment threshold when compared to a zero tolerance approach and thus has important implications for IPM. Finally we demonstrate that the approach developed here can be used to implement management strategies when most required and that significant cost savings can be achieved when treatments are administered based on a threshold rather than on a calendar based system.

The Model

Model Development

Elmouttie *et al.* (2010) proposed an approach for sampling insects in grain storages in which insect distribution is heterogeneous. Unlike other approaches, this approach explicitly divided grain lots into insect infested (*p*) and un-infested areas (*1-p*). Probability of detection is related to the proportion of grain infested (*p*), the weight of samples drawn, (*w*) and the density of insects (λ) within the infested portion of the lot, (*p*) and given by:

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

The principle focus for model development was to detect the presence of insects and therefore the method was developed to consider the probability of sampling a heterogeneous grain lot and detecting no insects *i.e.* when a = 0.

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

Thus the probability of detection can be shown to be:

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

When developing IPM strategies detection at alternative detection thresholds may be of interest, that is when $a \neq 0$. Thus the probability of detection when $a \neq 0$ is given by:

$$P(A \le a) = \sum_{j=0}^{a} \sum_{i=0}^{n} P(x=i) PA = j | X = i)$$

= $\sum_{i=0}^{n} {n \choose i} p^{i} (1-p)^{n-i} \frac{e^{-xw\lambda} (xw\lambda)^{j}}{j!}$ (Equation 2)

The method allows for various detection thresholds to be established. Detection is given when the number of insects throughout the grain sampled is equal to or greater than the detection threshold.

Model behaviour and cost analysis

Sampling intensity (the number of samples drawn to detect insects) is of primary importance to end users. In both equations 1 and 2 sampling intensity (n) will vary in relation to a number of factors including the proportion of the lot infested (p), the density of infestation (λ) and the size of the sample drawn (w). However in equation 2 detection thresholds will also affect sampling intensity (n). We therefore examine how alternative thresholds (a) influence sampling intensity. To do so we examine the effect of three thresholds, a > 0 (more than 0 insects detected), $a \ge 2$, (2 or more insects are detected) and $a \ge 5$ (5 or more insects are detected). Finally we consider the implications of treatments (fumigations) being based on insect detection thresholds rather than as a regular schedule that ignores infestation levels. We do so by considering three scenarios: a) treatment occurring on a regular quarterly bases (4 fumigations per year) with no sampling; b) an intensive sampling programme, where treatment is based on detection of insects at predetermined treatment threshold and sampling occurs on a monthly basis commencing 30 days from fumigation clearance; and c) a low intensity sampling programme, where treatment is based on detection at a threshold and sampling occurs 60 days after fumigation clearance. Cost benefit analysis is conducted using current cost estimates for fumigation using phosphine from the Australian production region and a fixed hourly labour rate.

Model Application

When sampling grain for either IPM or for detection, of primary interest is the number of samples required to achieve a given level of confidence. We therefore investigate the effect of various sampling thresholds (a = 0, a = 2, a = 5) on the number of samples required for a probability of detection of 0.95. Estimates for insect density (λ) in the infested portion of the lot are set at 2 and 10, whilst initially we consider a scenario where p = 0.2, that is, 20% of the lot is infested. Note that an infestation of 10 insect per kilogram ($\lambda = 10$) over 20% of the lot equivalent to 2 insect per kilogram over the entire lot which represents the threshold for treatment in the USA.

a)



b)



Figure 1 a and b. The probability of detecting insects at three alternative detection thresholds (a = 0, a= 2, a=5) when (a) insect density $\lambda = 2$ and (b) insect density $\lambda = 10$. The weight of samples is held constant (w = 1kg) as is the proportion of the lot infested (p = 0.2). ($a = 0^{-...}, a = 2^{-..-}, a=5^{--}$)

As the probability of detection increases the number of samples required to detect insects also increases irrespective of detection thresholds. When the density of insects is low however ($\lambda = 2$), detection at higher thresholds requires substantially more samples to be drawn to achieve the same probability of detection (figure 1a). In contrast, when the density of insects is high ($\lambda = 10$) the number of samples required to detect is equivalent for all thresholds (figure 1b). In this example the proportion of the lot infested remained constant (p = 0.2). However the proportion of the lot infested p, may also influence the probability of detection at alternative threshold. We now consider the probability of detection in a situation where the infestation is more widespread in the grain bulk, where p = 0.5 and infestation rates of $\lambda = 2$ and $\lambda = 10$ (Figure 2a and b).

a)



b)



Figure 2 a and b. The probability of detecting insects at three alternative detection thresholds (a = 0, a= 2, a=5) when (a) insect density $\lambda = 2$ and (b) insect density $\lambda = 10$. The weight of samples is held constant (w = 1) and the proportion of the lot infested also is held constant (p = 0.5). ($a = 0^{-...}$, $a = 2^{-...}$, $a=5^{--}$)

When insect density is low ($\lambda = 2$) the number of samples required to detect insects increases as the detection thresholds increases (figure 2a). However similarly to the first example, when the density of insects in the lot is high ($\lambda = 10$) the number of samples required to detect is equivalent across all thresholds. Of interest however, when a zero tolerance threshold is set, the number of samples required to detect high and low densities of insects within the infested portion of the lot is the same (Figures 1 a and b, Figure 2 a and b). Detection thresholds therefore provide a mechanism to discriminate between treatment thresholds based on both the density of the infestation (λ) and the proportion of the lot infested (p) (Figures 1 a and b, Figure 2 a and b). As demonstrated, if a threshold of a = 0 is set, it is impossible to determine if the density within the infested portion of the lot is two (λ = 2) or greater as the number of samples required to detect these thresholds is equivalent (Figures 1 a and b, Figure 2 a and b). This lack of discrimination occurs because at a threshold of a = 0, a detection is recorded whether 1 or 50 insects is found within a sample and additional insects are not accounted for. In the examples provided, sampling at a threshold of a = 5 when the density in the infested portion of the lot is high ($\lambda = 10$) does not require more samples to be taken to maximise detection for a given probability of detection (e.g. 0.95) than the other thresholds examined. However, when the density in the infested portion of the lot is low, more samples are required to achieve the equivalent probability of detection for higher thresholds. This occurs as the density of insects within the infested portion of the lot increases, the probability that samples will contain multiple insects also increases. However, as the density in the infested portion of the lot decreases there is a corresponding decrease in the probability of detecting a greater number of insects within the samples taken. As such, detection thresholds provide end-users a method to ensure that the proportion of the lot infested and the density within that infested portion corresponds to the pre-determined treatment threshold.

In the above examples both the density of the infestation (λ) and the proportion of the lot infested (p) affected the probability of detection at a given sampling intensity (n) and sample size (w). Below we examine these factors independently on the probability of detection. We explore how these factors affect the probability of detection at three

detection thresholds (a=0, a=2, a=5). (Figure 3 a and b).

a)







Figure 3 a and b. Probability of detecting insects within a grain lot at various detection thresholds as a function of (a) infestation rate where (p) is held constant at 1 and (b) the proportion of the lot infested where insect density (λ) is held constant at 1.

Across all detection thresholds an increase in the density of infestation or the proportion of the lot infested leads to an increase in the probability of detection (Figure 3 a & b). However at higher detection threshold the probability of detection increases more slowly than when thresholds are lower. Although not surprising this has significant implications for developing IPM strategies. From a management perspective the density of pests and the distribution will influence growth and therefore dictate treatment regimes. As such detection thresholds should be based on both insect distribution and insect density.

Sampling and Treatment costs

A key factor that will drive decision making along the grain supply chain is the costs or savings that will result from changed management practices. Here we consider the costs of sampling and treatment at an arbitrary action threshold basing results on current Australian practices and costs. It is common practice for fumigations to be conducted on a calendar basis with approximately four fumigations conducted per year (every three months) with no pre-fumigation sampling. This fumigation pattern will be used as the baseline for cost benefit analysis. As economic treatment thresholds have not yet been determined for Australian storages we consider a scenario in which treatment is initiated when 5 insects are detected (threshold a = 5) using infestation parameters of 20% of the grain being infested (p = 0.2) and a mean density of insects in infested portions equal to 10 ($\lambda = 10$). As shown in Elmouttie *et al.* (2010), these parameters are realistic for Australian conditions. This also equates to 2 insects per kilo (as per the USA treatment threshold) (Hagstrum *et al.* 1999). A threshold of a = 5 is selected as this provides the greatest certainty that the density of insects within the infested portion of the lot is equal to 10 however does not result in added sampling effort. This is illustrated in figure 1 a and b where sampling intensity (number of samples to be drawn) is no different between a = 0, a = 2 or a = 5 when $\lambda = 10$ for the equivalent probability of detection. As such, setting a higher threshold provides no added cost however provides greater confirmation that the density of insects is at the level expected. Detection at these parameters, would require 15 x 1kg samples to be drawn at a 95% probability of detection (Figure 1b).

To illustrate potential cost savings we consider sampling and fumigation under 3 different scenarios: Scenario 1 is a calendar based fumigation plan where fumigations are conducted on a three monthly basis and no sampling is undertaken; Scenario 2 where sampling is conducted monthly commencing 30 days after fumigation clearance and detection at the threshold (a = 5) is required for treatment; and Scenario 3 where sampling is conducted monthly commencing 60 days after fumigation clearance and detection at the threshold (a = 5) is required for treatment; and Scenario 3 where sampling is conducted monthly commencing 60 days after fumigation clearance and detection at the threshold (a = 5) is required for treatment. For simplicity we consider that *Rhyzopertha dominica* to be the only target pest and sampling is conducted in a 20 000 tonne bunker sealed bunker with no emigration or immigration of insects. We assume initial population density within the bunker is 0.025 insects per kilogram, and grain temperature is maintained at 25°C and grain moisture at 11.5%. Population growth rate is predicted as per (Hagstrum 1996).

Table1. Costs associated with fumigation and sampling under 3 sampling scenario, a) calendar based fumigation with no sampling, b) sampling one month after fumigation and fumigation on detection at threshold, and c) sampling 2 month after fumigation and fumigation on detection at threshold. Fumigation costs are based on industry estimates for labour associated with application of Vaporphos at 1 gram per tonne for a 20,000 tonne bunker, plus bunker clearance and monitoring costs. Sampling costs are based on 4 hours labour at current rates (\$ 50AUD/ hour). (N = No action take, S = Sampling conducted, D = detection at threshold a = 5, F = Fumigation)

	Scenario 1			Scenario 2			Scenario 3		
Month	Sample	Treat	Cost	Sample	Treat	Cost	Sample	Treat	Cost
1	Ν	Ν		N	Ν		N	Ν	
2	Ν	Ν		S	Ν	200	Ν	Ν	
3	Ν	F	3800	S	Ν	200	S	Ν	200
4	Ν	Ν		SD	F	4000	SD	F	4000
5	Ν	Ν		Ν	Ν		Ν	N	
6	Ν	F	3800	S	Ν	200	Ν	N	
7	Ν	Ν		S	Ν	200	S	N	
8	Ν	Ν		SD	F	4000	SD	N	4000
9	Ν	F	3800	Ν	Ν		Ν	Ν	
10	Ν	Ν		S	Ν	200	Ν	N	
11	Ν	Ν		S	Ν	200	S	N	200
12	Ν	F	3800	SD	F	4000	SD	Ν	4000
Total	0	4	15200	6	3	13200	5	2	12600

Table 1 illustrates even when sampling time and cost are unrealistically high (\$200 / 4 hours for 15 samples) if less than 4 fumigation treatments are used this will provide a cost saving. In fact, Scenario 2 and 3 shows that if fumigation is reduced by only a single fumigation

treatment annually, substantial cost savings can be achieved (up to 17%) even if sampling is conducted over 9 months of the year.

Discussion

In this study we have developed a robust methodology for determining the sampling intensity needed to detect insects at an arbitrary threshold. As we show, this can be particularly useful for developing sampling plans and assessing treatment thresholds for use in stored grains IPM strategies. Results from this study illustrate that the addition of alternative detection thresholds into the statistical sampling model proposed by Elmouttie *et al.* (2010) provides a means to ensure treatments are administered during periods that they will be most effective. Most significantly, when this method is coupled with an appropriate threshold for treatment, we show that costs of managing bulk grain can be substantially decreased. Clearly the capacity to do this will depend on the growth rate of insects in bulk grain, which in turn will depend on temperature and humidity (Hagstrum 1996). Nonetheless, the scenarios we have presented here are realistic for Australian conditions and based on growth models of (Hagstrum 1996). In the cost comparison we show that if sampling to an action threshold leads to the reduction of even a single fumigation, a reduction in costs of up to 17% per bunker can be made.

In this study we have illustrated that the rate of infestation and the proportion of the lot infested will have a significant effect on the probability of detection for various detection thresholds (figures 1 a and b and figure 2 a and b). This occurs as irrespective of detection threshold, the probability of detection will involve both sampling an infested portion of the lot and detecting insects within the infested area. This has significant implications for storages managers. Typically IPM programmes have been designed considering a mean density of insects across a grain bulk (Hagstrum *et al.* 1985, Subramanyam *et al.* 1993, Hagstrum *et al.* 1997). Unlike mean based approaches the methodology proposed in this

study involves detection thresholds being based on both the proportion of the lot infested and the density of insect pests. As such when developing effective treatment thresholds, consideration of both parameters will be required.

Although developing thresholds based on 2 parameters may seem initially to be more complex it does allow variation based on different environmental conditions to be incorporated into IPM programme. It has been well established that storage type, climatic condition and grain moisture will effect population growth and distribution (Hagstrum 1996, Athanassiou *et al.* 2003, Nansen *et al.* 2009). As such IPM programmes should be based not only on a mean estimate of insects, but also consider factors which may influence population growth in the area (Kogan 1998). In Australia for example, where grain moisture, storage temperature and grain type vary significantly across the continent (Rees 2004), thresholds developed for specific regions based not only on density of the infested portion of the lot (λ) but also on the proportion of the lot infested (p) may provide greater benefits in relation to management.

A key finding of this study is that when an appropriate threshold is chosen for a target density within the infested portion of the lot, sampling intensity is not greater than when sampling at lower thresholds (figure 1 a and b and 2 a and b). This provides significant benefits for grain storage manages, as there is not a requirement to take more samples, or inspect individual samples independently. Rather, the sampler need only count how many insects are in the total sample amount taken. It is important to recognise however, that the threshold selected is directly related to the density of insects that is to be targeted. For example, if the mean density of insects to be targeted was 5 ($\lambda = 5$) over a portion of the lot, setting a threshold of 5 or greater would result in substantial increase in samples for no

added detection benefit. This occurs as the density of insect decreases the probability of detecting multiple insects within a sample also decreases, and hence the number of samples required to detect that multiple insect will increase. As such, the detection threshold needs to be optimised in relation to the density of insects targeted within the infested portion of the lot.

A number of sampling programmes have been developed for stored grains based on a fixed number of sample units to estimate insect density (Hagstrum *et al.* 1985, Subramanyam *et al.* 1993). These programmes have aided in the development of IPM strategies, however are species specific in parameterisation and require extensive data to generate parameter estimates. Subramanyam *et al.* (1997) developed a sequential sampling programme specifically for IPM. This approach, unlike approaches based on a fixed number of samples, considers the number of contaminated samples to maximise the probability of detecting insects. Thus each sample within the total grain volume sampled needs to be examined independently.

Although best practice, the examination of sample units independently by end users would be difficult to initiate due to time limitations. This is of particular concern when grain is sampled prior to being moved from site to site via rail or road transportation, which typically occurs during the most hectic periods during the grain production and storage cycle. In such scenarios, examining each sample unit independently may not be viable. In this paper we have demonstrated that detection probabilities can be maximised by setting a pre-determined threshold. This allows grain handlers not only to have flexibility in their sampling protocols but also negates the need to examine multiple sample units independently, rather designs are made based on a cumulative detection threshold. Using the method proposed here, a fixed number of sample units may be drawn, grouped and examined, saving time, and costs whilst maximising detection at target threshold.

The cost comparison presented on Table 1 illustrates that sampling can significantly lower cost per bunker even when only a single fumigation treatment is removed. This has significant implication to industry as cost associated with treatment are increasing due to associated labour charges and the cost of newer more efficacious fumigants. Further the cost associated with sampling is not significant such that savings could be achieved even if only a portion of bunkers were fumigated less than occurs on a calendar cycle.

In the cost analysis example presented, zero percent insect mortality was assumed as was a constant grain temperature. As such cost savings could be substantially increased when the effects of insect mortality and alternative treatments where incorporated into the model. Additionally cost savings associated with the better management of fumigants to manage resistance where not considered. Phosphine resistance in grain beetles is a growing concern globally (Collins *et al.* 2002, Schlipalius *et al.* 2002). Poor fumigation strategies and frequency of fumigations has been seen to be the principle cause of phosphine resistance (Collins *et al.* 2000). Integrated pest management strategies based on robust statistical sampling plans can therefore also aid in managing pesticide resistance as they provide a mechanism to determine when treatments are actually needed and minimising chemical usage.

This study highlights the need for flexible sampling programmes for use in IPM. Although a number of countries (including the USA) have predetermined thresholds in which IPM should be administered (Hagstrum *et al.* 1999), a number large grain producing nations

continue to base IPM on a zero tolerance threshold, originally initiated for export (Jeffries 2000). Such strategies are not only inefficient but also jeopardise the longevity and efficacy of current available controls (*i.e.* fumigants) due to the build up of resistance from excessive use (Herron 1990), are an expensive means of control and are not based on a sound ecological framework. Further research needs to determine the economic thresholds for pests within particular regions followed by the implementation of an effective and accurate sampling model.

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