Report to CRC for National Plant Biosecurity

CONFIDENTIAL

Case Study 1

Comparison of Statistical Sampling Models for the Detection of Pests in Stored Grain

Project - CRC 30086

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Executive Summary

Developing robust sampling strategies for cereal grains, based on a coherent statistical framework, is becoming increasingly important. These methods are developed in an attempt to ensure internationally and domestically traded grain commodities are free from unwanted pests and pathogens. Developing sampling strategies to target insect pests and pathogens in grains is inherently difficult however, as these organisms can display varied spatial distributions in relation to conditions within storages.

Statistical sampling models are generally developed on an underlying statistical distribution, selected on the basis of how closely the distribution approximates the known distribution of the target pests. The Binomial, Negative Binomial or Poisson distributions have formed the basis of many sampling models, due to their simplicity in part and their underlying characteristics. In grain sampling and the broader ecological literature, both the Negative Binomial and Poisson distribution have often been selected to represent situations where targets are highly aggregated or rare. However, the behaviour of these models under the broad range of insect distributions common in grain bulks is relatively unknown.

In this case study we compare four sampling models, the compound model, proposed by Elmouttie et al 2010, a model based on the Negative Binomial proposed by Hagstrum et al. (1985) and Green and Young (1993), a Poisson model proposed by Green and Young (1993) and a Binomial approach proposed by Hunter and Griffiths (1978). Using data collected from farm silos in Australia and the USA we demonstrate that the compound model proposed by Elmouttie et al. (2010) performs better than alternative models in meeting a specified level of detection. We also illustrate the conditions under which each model fails in achieving this desired level of detection.

2

Background - Sampling

An efficient sampling programme is an imperative component of any quality assurance programme, whether it be for manufacturing, exporting or in the agricultural sector. Sampling programmes form the basis for the acceptance or rejection of goods to be sold or traded, are used to determine the quality of sampled goods and can lead to the initiation of treatment or management regimes. It is therefore important that a sampling programme is adequate with respect to achieving its desired goal, such as pest detection.

The fundamental component to any sampling programme is determining how intensively a commodity should be sampled to detect defects or unwanted items. Determining sampling intensity however, is inherently difficult. The intensity at which a commodity is required to be sampled will vary in relation to a range of factors including the commodity itself, the ease at which it can be sampled, the density of the target and the type of target which is being sampled. No single or standard sampling protocol or programme can be consistently used inserted across a range of systems.

Sampling programmes have historically been developed based upon an underlying statistical distribution. A statistical distribution is used to approximate the distribution of sampling target within the commodity being sampled. From this, sampling intensity can be determined based on factors such as target density and the approximated distribution. There are a number of statistical distributions used to develop sampling programmes, namely, the Poisson, Binomial or Negative Binomial. Each distribution is selected on the basis of how well it may approximate the system being sampled with selection of individual distribution based on the type of system being sampled.

Sampling - grains

Robust sampling strategies are becoming increasingly important to ensure the detection of pests and pathogens in stored commodities such as cereal grain. There is a growing emphasis on developing statistically robust sampling programmes that maximise detection of target species due to biosecurity and food shortage concerns globally. In a pest management context, sampling typically has two main objectives, to either estimate the presence or quantity of a target species within an area, or determine the spatial distribution that the species displays within the sampled area (Pillar 1998). Whatever the objective however, it is important to have a basic understand of the target species biology so that sampling plans can be tailored specifically to the particular management situation.

Similarly to sampling programmes for other commodities, sampling programmes for biological systems have been developed based on an underlying statistical distribution which aims to best approximate the 'known' distribution of the target species. A number of sampling programmes based on a range of statistical distribution have been adopted and modified for use in stored grains sampling (Hunter and Griffiths, Love et al. 1983, Hagstrum et al. 1985). The statistical models chosen in each scenario and modifications made attempt to capture the variability within biological systems which stem from factors such as environment and species' behaviours. However as it is difficult to find a single distribution which adequately describes the natural variation that exists, distribution may be chosen and assumptions are made for convenience, often without scientific support.

As with the broader field of sampling, most common statistical sampling programmes for biological systems have been based on the Binomial, Negative Binomial and Poisson distributions (Hunter and Griffith 1978, Hagstrum et al. 1985, Green and Young 1993,

4

Southwood and Henderson 2000). The usage and basis for selection of each of these distributions to develop sampling programmes however has typically been derived from quality assurance and modified for ecological requirements rather than being primarily developed for a biological system (Stephens 2001).

Developing robust sampling programmes for biological systems (such as stored grains) that capture the range variability in the system in question is inherently more complex than capturing the variability in a manufacturing process. Target species can be difficult to detect for numerous reasons. For example, species distributions can vary over space and time, influencing detection rates. Further the area being sampled may not be conducive to sampling. It is therefore necessary that the sampling programmes established and the statistical framework developed best approximates the actual system as closely as possible.

The issue of species aggregation and clustering behaviour (i.e. spatially heterogeneous distributions) has been of particular concern to biologist when developing sampling programmes. Sampling plans have been developed attempting to account for species clustering behaviour (Hagstrum et al. 1985, Green and Young 1993, Elmouttie et al. 2010). In such instances, statistical models have most commonly been based on either the Negative Binomial or Poisson probability functions. Although neither function implicitly considers heterogeneity they do provide a good approximation of rarity, in case of the Poisson and clustering, in the case of the Negative Binomial (Green and Young 1993, Subramanyam and Hagstrum 1996).

An area which has seen significant research with regards to sampling ecology has been stored grain insects (Hagstrum et al. 1995, Lippert and Hagstrum 1987, Elmouttie et al. 2010). The presence of grain beetles within storages is problematic, as they lead to restrictions in biosecurity and trade, commodity losses and spoilage (Rees 2004, Hagstrum and Subramanyam 2006). Thus a significant emphasis has been placed on developing techniques to detect insect pests early within grain storages (Hagstrum et al 1985, Lippert and Hagstrum 1987, Hagstrum et al. 1988, Elmouttie et al. 2010).

Although grain storages appear to be homogeneous, insect distributions within them often display varied distributions. The distribution of insects within a storage will vary from species to species, between storage types, and in relation to external and internal climatic conditions (Cuperus et al. 1990, Athanassiou et al. 2003, Nansen et al. 2009). It is therefore logical that sampling programmes account for species clustering behaviour and not consider the grain mass as a homogenous entity to be sampled. Not all sampling programmes have been developed to account for species distribution, however.

In this case study we aim to compare a range of sampling models. We aim to test each model over a range of insect densities to determine where each model performs well and where they perform poorly. In order to make an extensive comparison, we use data collected both from Australian on farm storages by Dr. David Elmouttie, and data collected from an intensive sampling programme of on farm storages in the USA supplied by Dr. Paul Flinn (USDA). This ensures that a wide range of possible storage conditions, environmental and management practices are covered in the comparison. Given that insects pests occur at a range of densities in a range of environmental conditions, this comparison has been made to determine which models are most appropriate to use under a variety of environmental conditions.

6

Methodology

Data Collection

<u>Australia</u>

A grain silo holding approximately 70 tonnes of wheat that had been harvested and placed in storage for four months was sampled. No insecticide treatments had been administered during the storage period. All grain sampling from the silo was conducted on a single day. Twenty five 800 gram samples were taken from random locations within the silo using a Graintec[®] Stainless steel grain spear. Each sample was individually bagged and sieved using a Graintec[®] 2mm stainless steel grain insect sieve for a standard 10 seconds. For each sample the number of *Sitophilus oryzae* (Rice Weevils), *Rhyzopertha dominica* (Lesser Grain borer) and *Cryptolestes spp.* (Flat Grain Beetle) were recorded. This procedure was replicated three times such that three data sets for each species was available for simulation.

<u>USA</u>

Data used for model comparisons was collected from four independent vertical Silos approximately 4.75m in diameter containing on average 30 tonnes of wheat in Kansas USA were sampled monthly for seven consecutive months commencing in July 2003 as described in (Flinn et al. 2004). Silos were separated in relation to three distinct treatments, Control (no treatment), Aeration (Aerated silos). Each treatment was administered over two independent silos. Sampling was conducted using a pneumatic grain sampler (Pro2e-A-Vac®, Cargill Minneapolis, MN). Twenty one, 3 kg samples were drawn from each Silo during in sampling period of three height strata (0-0.8m, 0.8-1.6m, 1-6-2.4m). All samples were processed using an Insectomat[®] motorized inclined sieve (Samplex LTD, Willow Park, UK), to separate insects from grain. The number of live adult insects was counted in each sample immediately after extraction. Three major grain insect pest species were the target of the study, *Rhyzopertha dominica, Cryptolestes spp* and *Tribolium spp*.

Model Parameter estimation and simulations

In general, the aim of a grains sampling protocol is to detect insects at a given power, that is, to ensure with a given probability that insects are detected when they are in fact present. In this section, four sampling models were compared: i) a Compound model proposed by Elmouttie et al (2010; equation 1); ii) a Poisson model proposed for sampling by Green and Young (1993; equation 2); iii) a Negative Binomial model proposed by Green and Young (1993) and Hagstrum et al. (1985)(equation 3); and iv) and a Binomial approach proposed by Hunter and Griffiths (1978;equation 4) (equations 1-4 below). Data from each silo was used to calculate parameter estimates for each model at each sampling period for each species. These data were then used to populate each model to determined estimated sampling intensity, *n*. In the following models *n* represents the number of samples required for a given power $(1 - \beta)$, β represents the probability of the Type 2 error (the probability of failing to detect a species after n samples are drawn), and *m* represents the mean density of the target.

(equation 1 – Elmouttie et al. 2010 – Compound Model)

where *p* is the proportion of the silo infested, *w* represents the weight of the sample drawn and λ represents mean density of insects in the infested portion of the silo.

(equation 2: – Green and Young 1993 - Poisson)

------ (equation 3 – Green and Young 1993, Hagstrum et al. 1985 – Negative

Binomial)

In equation 4, k represents a dispersion parameter. The dispersion parameter was estimated using the method presented by Southwood and Henderson (2000)

— equation 3.1

where *s* represents the standard deviation.

(equation 4 – Hunter and Griffiths 1978 - Binomial)

where ψ represents the number of insects in a sample, $P(\psi)$ is the probability of drawing an infested sample, θ is the total fraction of the bulk grain that is sampled and v represents the total number of insects in a lot

Model Comparisons

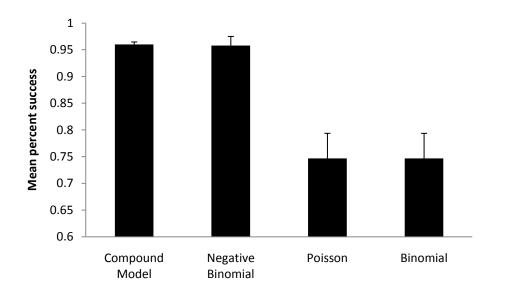
Parameter estimates were generated for each model for each treatment, species and sampling period for both Australian and USA data sets. These data were then used to populate each model to determine the required sampling intensity *n* for a 95% probability of detection. The data from which the estimates were generated from were then randomly sampled in a Monte Carlo simulation with replacement for 10 000 iterations and intensity *n* for each model. A detection was recorded when *n* samples taken from the data set resulted in at least one insect being found. The number of detections from the 10 000 simulation was recorded and percentage success rate determined for each trial simulation.

Standard errors were calculated based on mean percentage success for combined simulation results. This formed the basis for model comparisons with models in which standard errors did not overlap being considered different.

Results

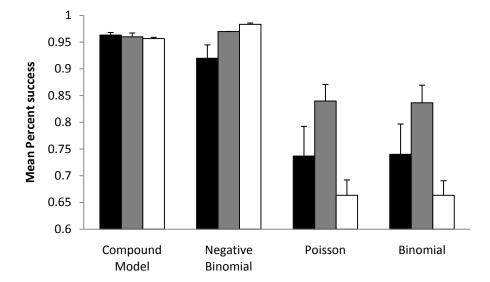
Australia data

The performance of the four models differed significantly for combined species data simulations. On average, the Negative Binomial model and Compound model proposed by Elmouttie et al. (2010) out performed both the Poisson and Binomial approaches with both models achieving the desired 95% mean success rate (Figure 1). Interestingly, the performance of both the Binomial approach and Poisson approach were almost identical with mean simulation results (\pm S.E.) of 0.774 \pm 0.12_{Poisson}, 0.774 \pm 0.11_{Binomial} (Figure 1).



<u>Figure 1</u>: Mean percentage success (± 1 S.E) for each sampling model for combined species data. This pattern was consistent across the three species sampled (Figure 2). In all simulations, The Poisson and Binomial models were outperformed by the Negative Binomial and Compound model and did not achieve the desired 95% detection rate. The Negative

Binomial model did however fall just below the 95% threshold for the Sitophilus oryzae trial



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(0.92±0.25)(Figure 2)
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Figure 2: Mean percentage success (\pm 1 S.E) for each sampling model for each species. (\blacksquare = Sitophilus oryzae, \blacksquare = Rhyzopertha dominica, \square = Cryptolestes spp)

<u>USA data</u>

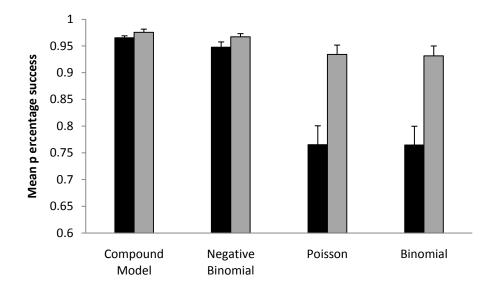
Simulations conducted with the data above only consider sampling from a single silo over a narrow time frame. However, insect densities and distribution are known to vary over time due to microclimatic conditions. It is useful therefore to evaluate how different sampling models perform over a range of densities and spatial patterns. Here we will use data collected over 7 months in 4 independent farm silos in Kansas USA, to determine how different spatial arrangement and densities affect sampling efficiencies of 4 proposed models.

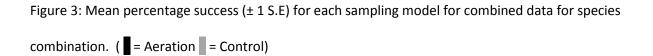
Mean insect densities were calculated for all combinations of time period, species and treatment used in analysis. Insect densities varied between treatments, sampling periods and insect species (Table 1) such that a range of combinations existed to test model predictions. In general however, insect population densities increased throughout experimental periods peaking in warmer summer months (Table 1). This pattern was also evident in the number of samples containing insects, *ie*. positive subsamples in total sample (Table 1). Table 1: Mean (±SD) insect densities across all treatments within four independent grain Silos. The number in parenthesis represent the total number of subsamples (3kg) containing insects of the 21 samples drawn in each sampling event.

Species	Time	Aeration		Control	
		Silo 1	Silo2	Silo 3	Silo4
Rhyzopertha	1	0.00 (0)	0.00 (0)	0.047±0.21 (1)	0.00 (0)
dominica	2	0.33±0.57 (6)	0.28±0.46 (6)	0.66±0.85 (10)	0.23±0.43 (5)
	3	2.81±4.33 (8)	10.19±19.77 (16)	28±14.18 (21)	25.47±17.39 (21)
	4	24.47±19.03 (21)	45.19±40.58 (20)	169±88.26 (21)	176.33±127.38 (21)
	5	15.76±15.63 (17)	58.8±107.42 (21)	96.14±68.53 (21)	138.28±100.69 (21)
	6	0.00 (0)	24.38±72.79 (5)	43.52±33.20 (21)	109.61±68.23 (21)
	7	1.47±4.47 (4)	16.66±49.62 (6)	45.90±46.37 (21)	127.80±125.67 (21)
Cryptolestes spp	1	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)
	2	0.14±0.35 (3)	0.14±0.35 (3)	0.23±0.53 (4)	0.047±0.21 (1)
	3	0.95±1.32 (10)	0.76±1.37 (7)	3.85±3.59 (16)	0.85±0.85 (13)
	4	5.09±5.82 (14)	4.42±5.97 (16)	37.66±25.12 (21)	9.61±8.07 (18)
	5	0.42±1.16 (4)	0.66±1.27 (5)	89.09±81.49 (21)	44.47±28.41 (21)
	6	0.00 (0)	0.33±1.06 (2)	96.04±115.12 (20)	76.90±101.48 (21)
	7	0.095±0.300 (2)	0.00 (0)	388.95±861.21 (19)	65.47±68.95 (19)
Tribolium spp	1	0.00 (0)	0.00 (0)	0.00 (0)	0.047±0.21 (1)
	2	0.47±1.03 (5)	0.66±1.01 (8)	1.76±3.49 (11)	0.23±0.53 (4)
	3	2.38±3.16 (14)	2.09±3.85 (13)	3.33±3.29 (18)	7.66±7.53 (20)
	4	1.66±1.90 (15)	2.61±4.34 (15)	12.00±14.18 (17)	12.57±12.78 (19)
	5	1.00±1.78 (10)	2.61±5.28 (13)	9.38±16.59 (20)	12.85±23.81 (19)
	6	0.00 (0)	1.00±1.34 (10)	6.00±10.55 (14)	13.80±28.19 (15)
	7	0.095±0.30 (2)	0.47±0.98 (5)	30.23±83.82 (19)	5.09±3.65 (18)

Simulation trials were only conducted through time periods 2-7 for Aeration trail as no insects were present in both silos for first sampling period. Similarly simulations were only conducted in time periods 1-3 for control silos as beyond this point insect population densities were exceedingly high making all model prediction for *n* equivalent, *i.e.* draw 1 sample). Mean percentage success rate for trial simulations, combining all simulation results

through sampling periods 2-7 for aeration trial and 1-3 for control trials, were similar for both the Negative Binomial ($0.948\pm0.01_{Aeration}$, $0.967\pm0.006_{Control}$) and Compound model ($0.965\pm0.004_{Aeration}$, $0.975\pm0.006_{Control}$). The Poison model ($0.765\pm0.035_{Aeration}$, $0.934\pm0.018_{Control}$) and Binomial model ($0.765\pm0.035_{Aeration}$, $0.934\pm0.019_{Control}$) were also similar however differed from The Negative Binomial and Compound model (Figure 3).





Similarly to the data from Australian trial, this pattern was found to be consistent for all species with Binomial and Poisson model predications being consistently similar to each other however lower than the expected 0.95 probability of detection threshold (Figure 4). Mean percent success rate for simulation from the Negative Binomial model were however also slightly below the desired 0.95 probability of detection for *Rhyzopertha dominica* (0.934±0.024) and *Cryptolestes spp* (0.938±0.014) (Figure 4).

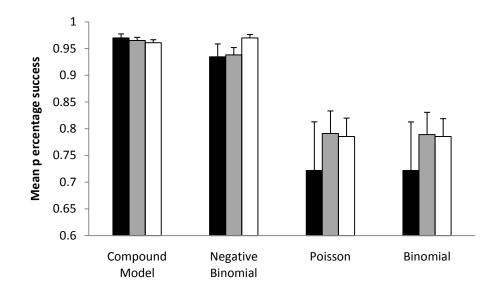


Figure 4: Mean percentage success (\pm 1 S.E) for each sampling model for each individual species using data from aeration trial. (\blacksquare = *Rhyzopertha dominica*, \blacksquare = *Cryptolestes spp*, \square = *Tribolium spp*. To this point simulation results have been grouped across all time periods. However, insect densities varied significantly over time during the Aeration trial as did the distribution of insects in the grain silos (inferred from the number of positive samples) (Table 1). Figure 5 illustrates that although mean percentage success was constantly lower for the Poisson and Binomial models, predictions were most accurate during the second sampling period. During all sampling periods the Compound model performed well and consistently met the desired 0.95 probability of detection threshold (Figure 5). The Negative Binomial model performed well in sampling periods 2 through 5, with mean success rates at the desired 0.95 probability of detection (Figure 3). In sampling periods 6-7 however, prediction fell well below the 0.95 threshold (0.846±0.553_{period 6}, 0.912±0.666_{period 7})(Figure 5).

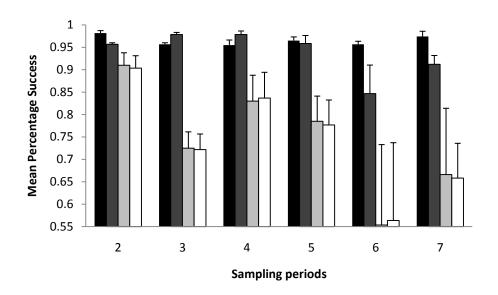


Figure 5: Simulation mean percentage success (\pm 1 S.E) for each sampling model at each sampling period for Aeration data trial (\blacksquare = Compound model, \blacksquare = Negative Binomial, \blacksquare = Poisson, \square = Binomial).

Discussion

Maximising the probability of detecting a target pest is a significant problem in agriculture, particularly in heavily traded commodities such as cereal grains. In Australia and other developed economies detection or non detection of pest forms the basis of treatment strategies, dictates whether commodities can be moved or sold and often has a significant implication in relation to trade (Jeffries 2000). It is therefore imperative that commodity sampling programmes meet the required objective of detecting target species when they are present.

There are a number of difficulties however when developing sampling programmes for biological/agricultural systems. This primarily relates to the environment in which we sample often being heterogeneous leading to irregular usage of space by the target species. This is further complicated by the fact that the level of heterogeneity is often difficult to perceive or quantify. Secondly, target species have specific behaviours which in itself will give rise to varied distribution and densities of a target species within the overall target area. It is therefore important to develop sampling protocols based on an understanding of pest species ecology and behaviour and a statistical design which adequately describes the system.

In this case study we have compared four sampling models, models which have been developed specifically for use in agricultural/biological systems. Each of these models has been developed or adapted for use in biological system and attempt to capture the known distribution of target species. Each model deals with the phenomena of pest distribution and density in alternative manner however.

The binomial model tested simplifies the area being sampled by considering the distribution of pests within the sampled area to be homogenous. In contrast, the Poisson approach attempts to capture species distribution patterns by considering detection as a rare event. The approach based on the Negative Binomial distribution (Hagstrum et al. 1985, Green and Young) attempts to describe the level of aggregation within a population or group of individual by considering the relationship between the sample mean and sample variance. In contrast the model proposed by Elmouttie et al. (2010) attempts to directly consider heterogeneity, delineating occupied and unoccupied portions of a sampled area.

The Binomial and Poisson approaches examined performed the most poorly of all models. Interestingly although each model operates under different assumptions they booth perform poorly in relation to similar data ranges. This is a direct of the both models being reliant on an estimate of target density parameters *m* and *v* and not considering special distribution. This is problematic for insect sampling as although insects within grain commodities may only be present in confined areas within the grain mass, population densities within those areas may be high (Table 1). Therefore density based approaches as the Binomial and Poisson approaches over estimated the probability of detecting a target pest within the sample and neither implicitly considers species distribution, rather being based on homogeneity (Binomial) and rarity (Poisson). As both approaches are also based solely on single parameter (a density derived parameter) they perform adequately only when insect density and distribution is low. This is illustrated by figure 5, where model predictions were found to be most accurate in sampling period 2, where both species density and the proportion of positive samples was low (Table 1). However as mean density increased prediction became less accurate, irrespective of the proportion of contaminated samples or heterogeneity (Table 1, Figure 5).

In contrast, both the Negative Binomial and Compound model approach performed well, with prediction typically reaching the desired 0.95 probability of detection threshold. The Negative Binomial approach however was not as robust where data was highly skewed by the variance. In Figure 5, mean detection success declined for the Negative Binomial based approaches in sampling periods 6 and 7 in the aeration trial. This corresponded to data which represented very high population densities within a small number of samples. Therefore caution should be used when using a Negative Binomial sampling model when sampling systems which may have over inflated variances due to high population densities.

The method used to estimate the dispersion parameter k for the Negative Binomial used in this study (equation 3.1) is based on the mean-variance relationship of the number of insects in a sample, and is the most common methodology used in the literature. It should be noted however that alternative methods have been developed. Although these methods produce modified outcomes under particular scenarios they still are influenced by the relationship of the mean to the variance of the sample. Further the alternative methods can be difficult to compute and hence do not provide a useful method for end-users.

The Compound model performed consistently well over a broad range of data attributes and was found not to be overly influenced by population mean, or variance attributes. An added benefit of the model is the direct biological relevance of the parameters. Alternative models exist to estimate sampling intensity, however the parameters required are often difficult to estimate. This is may be in part why Poisson and Binomial based approaches have gained such popularity in the literature (Stephens 2001). Although these approaches often do not best describe the system, parameters are easily estimated and biologically quantifiable.

Similarly to a Negative Binomial model, the Compound model proposed by Elmouttie et al. (2010) requires two parameters to be estimated p, the proportion of infestation and λ the mean density within infested portion of the site. In a Negative Binomial framework mean insect density must also be calculated however the second parameter, k, is often more difficult to calculate. Although in this case study parameter estimates could be made from sample data, in many situations such data are not available. An advantage of the Compound model is that estimates for model parameters p and λ could be developed from prior information or expert opinion as they have a direct biological relevance (Martin et al. 2005). This would be significantly more difficult when developing estimates for k parameter in the Negative Binomial model as it is based on an interaction between multiple factors not solely a biological characteristic. Here we have illustrated importance of selecting a robust model, particularly when dealing with data that may be highly variable. It should be recognised however that no one model is perfect and depending on the system alternative approaches may be favourable. The Compound model however conforms well to the known biology of insect pests within grain storages, contains simple parameters that can be estimated via multiple methods by end users and performs well over a wide range of data types.

This study has also demonstrated the importance of developing a sampling framework based on a thorough understanding of the system and extensive sampling data. Using data from the USA we were able to show the importance of considering season as insect distributions vary in relation external factors. In a large grain producing country such as Australia, these factors will not only be influenced by season but also geography. It is therefore important to acquire robust sampling data across a geographic and seasonal continuum so we can better inform sampling programmes, making them both more efficient and robust.

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