

Stewart Postharvest Review

An international journal for reviews in postharvest biology and technology

Insect distributions and sampling protocols for stored commodities

Grant Hamilton^{1,2*} and David Elmouttie^{1,2}

¹Cooperative Research Centre for National Plant Biosecurity, Bruce, Australia

²Discipline of Biogeosciences, Queensland University of Technology, Brisbane, Queensland, Australia

Abstract

Purpose of review: This review provides an overview on the importance of characterising and considering insect distribution information for designing stored commodity sampling protocols.

Findings: Sampling protocols are influenced by a number of factors including government regulations, management practices, new technology and current perceptions of the status of insect pest damage. The spatial distribution of insects in stored commodities influences the efficiency of sampling protocols; these can vary in response to season, treatment and other factors. It is important to use sampling designs based on robust statistics suitable for the purpose.

Future research: The development of sampling protocols based on flexible, robust statistics allows for accuracy across a range of spatial distributions. Additionally, power can be added to sampling protocols through the integration of external information such as treatment history and climate. Bayesian analysis provides a coherent and well understood means to achieve this.

Keywords: statistical model; grains; pests; detection; abundance estimation; statistical distribution

Abbreviations

ELISA Enzyme-linked Immunoassays
IPM Integrated Pest Management

***Correspondence to:** Grant Hamilton, Discipline of Biogeosciences, Queensland University of Technology, GPO Box 2434, Brisbane, Queensland, Australia, 4001. Phone: + 61 7 3138 1325 ; Fax: + 61 7 3138 1535; email: g.hamilton@qut.edu.au

Stewart Postharvest Review 2011, 3:6

Published online 01 December 2011

doi: 10.2212/spr.2011.3.6

Introduction

Storage structures provide the ideal environment for insect populations to flourish, primarily since they provide a high resource environment protected from external conditions [1, 2**, 3]. Insect infestations have the potential to cause significant damage leading to large commodity losses of 5–10% in developed countries and as high as 50% of total stored products in developing countries [4]. Other possible losses include reduction in product quality, an increased risk of mould growth and issues associated with insect contamination [1, 5] and additional economic losses related to trade and regulatory issues. In the current review we focus primarily on stored grains; however, the information presented here can also be applied to other stored commodities.

Detection or abundance estimation

Sampling consists of the examination of a representative portion of a lot. By far the widespread “approved” detection technique used commercially involves the removal and manual processing of the stored commodity to detect or estimate stored commodity pests. However, alternative technologies are available for screening samples for insects such as

species-specific enzyme-linked immunoassays (ELISA) [6] and near infra-red technologies [7].

Effective sampling is crucial to ensure early detection and ongoing management of insect infestations in stored commodities. To be accurate and effective, sampling protocols must be based on appropriate and robust statistics [5]. The form of sampling protocol selected will be broadly determined by its purpose, either to maximise the probability of detection of insects or to accurately estimate populations [8**]. However, there are a number of other factors that will influence the choice of sampling protocol.

Regulatory requirements of importing or exporting countries, stored commodity management practices and a range of other factors may influence the appropriate sampling protocol to use. For on-going management and the implementation of Integrated Pest Management (IPM) [9*], estimation of insect densities is most important [10*]. In countries where legislation demands pest-free exports, commodities can be rejected if pests are detected. Whether for insect detection or abundance estimation, the statistical models underlying the sampling methods are based on assumptions. The appropriateness and accuracy of a given sampling protocol in any circumstance will depend on the statistical model assumptions, any violations of the assumptions and the robustness of the method relative to those violations.

It is also important to consider the implications of models designed for different purposes and how they are employed. For example, Hagstrum *et al.* [8**] formulated and compared both estimation and detection models using samples drawn from wheat bins. Here they defined the probability of detecting an insect infestation of a given species as the fraction of samples with that species present. They found that when the number of insects per sample was low, fewer samples were required to estimate the mean of density of insects than for detection of the infestation. The opposite of this was found when the number of insects per sample was high. The results of comparisons such as these will depend on the nature of the models used and the assumptions underpinning those models.

Factors influencing the spatial and temporal distribution of insects

Depending on the commodity, foreign material, moisture and temperature, insect pests may be distributed through space in different ways [3, 8**, 11*, 12]. Although grain storages appear to be homogeneous, significant variation exists throughout any storage structure, which in turn influences insect distribution [13*, 14**]. For example, temperature and moisture profiles will vary depending on the season, position within the bulk-stored commodity, grain quality and climatic conditions at harvest [1, 5, 12]. Hagstrum *et al.* [10*] noted that management practices vary in different regions of the USA because insects are a greater problem in the south than in the north. Hence, insects commonly display varied distributions within and among storages [8**, 13*, 14**, 15**].

Interspecific associations among stored grain beetles can affect spatial distribution, with the density and distribution of certain species being influenced by the presence of others [16**].

Stored-product insects can respond to environmental conditions by altering reproductive outputs, entering periods of diapause or increasing their growth rate [10*]. Within a variable environment, insects will tend to aggregate in areas that provide climatic conditions and resources that are most suitable for reproduction and growth [17]. Insects therefore tend to be found in clusters within an environment, in which certain areas are highly sought and utilised with other not so favourable areas less densely populated [1]. Armitage [18**] demonstrated that mite populations clustered in relation to moisture content, even when variation in moisture content was minimal due to atmospheric controls being in place in storages.

Conditions within storage facilities can differ significantly due to the design, size, seasonal temperature variation and aspect of storage facility, which in turn influences the distribution of pest species throughout a grain bulk [14**, 19]. Consequently, micro-climatic conditions such as 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 [1, 5]. For example Stejskal *et al.* [20] suggested that beetle populations were harder to control in horizontal flat stores in comparison to vertical silo stores as it was more difficult to regulate temperature in horizontal stores.

Grain age and quality within a particular storage facility can also vary, with resulting impacts on the spatial distribution of insects [1, 19]. Grains can be stored for prolonged periods of time, at either bulk-handling facilities or on-farm grain storage silos leading to different aged grains of potentially differing quality being mixed. This may have implications for the distribution of infestations within a consignment as stored-product insect pests are known to select for grains with higher moisture contents, often a function of grain age [1, 5].

Statistical distributions and sampling plans

Sampling programmes are typically designed such that a representative portion of a larger lot is sampled for analysis or inspection in order to determine if a commodity is free from infestation or to estimate abundance [21]. Given that the entire lot is not sampled, the intensity of sampling (ie, the number of samples taken and the total mass of the commodity sampled) is designed for a fixed type II statistical error, usually 95%. For example, in a detection strategy this would be the probability that sampling finds no insects when insects in fact are present.

Sampling inferences are valid if, amongst other things, the sample that is tested is representative given the statistical approach taken. It can be difficult to determine what comprises a representative sample, however. For a sample to be

representative of the overall lot an understanding of how pests are distributed throughout the lot is required [21]. Furthermore, the sampling statistics used must aim to capture the spatial distribution of pests within the commodity being sampled [21]. Most common probability functions such as binomial, Poisson and hypergeometric functions are used to evaluate and develop sampling programmes [21]. The hypergeometric function considers the probability of sampling a particular number of positive samples (eg, six samples containing insects) from a total number samples drawn from a finite population without replacement (eg, a total of 30 samples drawn from a grain bin). In practice the hypergeometric function can be difficult to evaluate and so is rarely used. The binomial or Poisson functions form adequate and more easily calculated replacements [21].

The binomial probability function denotes the probability of non-conforming units being drawn from a sample. The Poisson probability function in contrast is a single parameter function commonly used when sampling from fixed volumes [21, 22**]. Binomial and Poisson sampling programmes have been developed to determine the probability of detecting pests in stored commodities and relate the proportion of samples with insects and mean density [22**]. In the case of detection, Poisson and binomial models may not be ideal because they fail to account for species clustering behaviour [23**].

A range of indices have been developed to describe dispersion patterns, including Iwao's patch regression [24] and the standardised Morisita Index [24]. Taylor's power law [25] has been used in a number of studies to accurately describe the dispersion pattern of insects within storages [8**, 26, 27, 28*], and has been used in the development of successful sequential sampling plans [29].

Alternative approaches

It is important to understand how the spatial distribution of insects will influence alternative statistical sampling models. Hagstrum *et al.* [8**] evaluated the frequency distributions of a range of insect pests sampled from stored wheat. They fitted these to negative binomial, binomial and Poisson distributions (among others), and introduced a double-log model. Unlike simple binomial and Poisson approaches, the double-logarithmic model and negative binomial approaches explicitly consider sample-to-sample variation. For the negative binomial model, the dispersion parameter k is used to account for the variation between sample units and can be estimated using Taylor's Power law [25]. In the case of the double-logarithmic model, sample-to-sample variation is accounted for as a process of "the logarithmic increase in sample units occupied by more than one insect with an increase in mean density" and the "logarithmic increase in the number of insects occupying the infested sample units" [8**]. This model can be useful for mean abundance estimation and insect detection.

Subramanyam *et al.* [27] demonstrated the accuracy of the model, showing that mean densities could be predicted based

on the relative proportion of sample units containing insects. The model explained 84–90% of variation in trap catches. This approach has formed the basis of a number of sampling programmes [13*, 27].

Hagstrum *et al.* [30], developed a generic approach for sampling stored product insects. Unlike previous methodologies predicting the variance for a mean insect density which traditionally was based on a linear relationship formed when the logarithm of the variance is regressed against the logarithm of the mean, this approach considered a non linear relationship. The study illustrated that a generic non linear regression equation could be used to calculate the precision of mean insect density estimates. Moreover, the approach was shown to be applicable over a range of insect densities, for multiple species and using multiple capture methodologies.

More recently, Elmouttie *et al.* [23**] adopted an alternative methodology to maximise the detection of insect pests within grain storages. Unlike the double-logarithmic model or negative binomial model, the approach proposed by Elmouttie *et al.* [23**] explicitly considers that stored grain can be conceptualised as two discrete components, infested and uninfested portions of the lot, and that the density within the infested portion should be accounted for. They demonstrated a method for determining sampling effort accounting for the density of individuals and the level of heterogeneity within a sampled area. For example, consider a scenario where 20% of the grain lot is infested and the mean density of insects in the infested portion of the lot is two. If the threshold for detection is set to zero, approximately 16 one kilogram samples would need to be drawn to be 95% confident of detecting an insect.

An additional benefit of the approach proposed by Elmouttie *et al.* [23**] is that both the number of samples required to maximise detection and the size of samples is considered. They showed that detection at a given infestation rate is directly related to not only the number of samples drawn but also the size (which can be expressed as either weight or volume depending on units used in the sampling programme; see Figures 4 and 5 in [23**]). Therefore, the approach allows for sampling plans to be optimised for a given set of conditions. Furthermore, unlike previous methods which incorporate clustering or heterogeneity, model parameters can be easily estimated as they are direct estimates of density and spatial occupancy across the area of grain being sampled. This approach allows for prior information in the form of environmental or other data to be used to inform appropriate sampling intensity.

Determining insect spatial distribution

Sampling allows for analysis of sample means and sample-to-sample variation to infer insect spatial distributions. While useful, this approach assumes that the information gathered from sampling (such as the mean-variance ratio) is a sufficient descriptor of the characteristics of spatial distribution to

enable sampling plans to be made. Sampling can also be useful for examining the three dimensional spatial distribution of insects. For example, Hagstrum [31] used five different sampling methods to examine the distribution of insects in wheat bins in Kansas. Results showed that the rusty grain beetle *Cryptolestes ferrugineus* (Stephens) was consistently more abundant in the centre of the grain mass. Results such as this can aid the design of sampling programs.

Information on insect density, species and age-structure, spatial distribution, and population growth rate can be gathered from sampling. As the resolution of sampling becomes finer, greater inferences regarding the spatial distribution of insects can be made. Recently Jian *et al.* [32*] used a sampling approach to assess the three dimensional spatial and temporal distribution of *C. ferrugineus*. They used large sample units (15 or 45 kg) under pilot-scale laboratory conditions. Using geostatistical analysis, they found that insect aggregation was higher at lower insect densities or grain temperatures.

An alternative is to attempt to directly examine grain being sampled to accurately assess locations where insects are present. Once techniques for this are established, the influence of different factors such as temperature on dispersion patterns can be made. Perhaps the first attempt to do this was by Surtees [33], who recognised that a method needed to be developed where the position within the grain bulk could be noted and that such experiments “precluded the use of sampling techniques commonly used in the detection of pest insects”, because these disturbed the grain altering spatial distribution of insects. Surtees [33] used mesh nets that retained grain but allowed the free passage of insects. He suggested that different insects could be classified according to their dispersion patterns. Recent developments by the authors have established techniques to directly map insect locations at a fine scale. Preliminary results by the authors suggest that the fine scale aggregation characteristics of the lesser grain borer, *Rhyzopertha dominica* (F.) in stored wheat can be influenced by temperature.

The future of sampling

Sampling stored grains is essential either for insect detection or for abundance estimation. With an increasing recognition of the factors that influence the accuracy of sampling protocols comes the possibility that sampling intensity may be inadequate for the desired type II error. Sampling comes at a cost, however, and so methods to increase the power of analysis will be essential. Methods such as Bayesian analyses [34] allow for a range of information (for example, climate) to be taken into account when designing sampling protocols. The theoretical basis is well understood, and because of its utility it has become widespread in a range of other disciplines such as economics and ecology.

Conclusions

Sampling protocols are essential for insect detection or estimation, especially for making IPM decisions. The reliability

of the protocols used will be determined by the statistical assumptions underpinning them and the distribution of insects within a commodity. The reasons for the implementation of sampling protocols should be regularly reviewed, since the management of new technologies and even perceptions of what constitutes a pest species damage requiring intervention can change.

Acknowledgements

The authors would like to acknowledge the support of the Australian Government’s Cooperative Research Centres Program. We thank Drs. Bhadriraju Subramanyam and David Hagstrum for reviewing an earlier version of this article.

References

Papers of interest have been highlighted as:

* Marginal importance

** Essential reading

- 1 Rees D. Insects of stored products 2004, Melbourne Victoria: CSIRO Publishing.
- 2 Toews MD, Phillips TW and Payton ME. Estimating populations of grain beetles using probe traps in wheat-filled concrete silos. *Environmental Entomology* 2005; 34(3): 712–718.
- ** This paper investigates the usefulness of probe traps for estimating insect density in concrete silos. Multiple regression analysis was used to estimate population density by considering capture density and grain temperature. The authors found that probe traps could be a viable tool however trap placement was essential in assuring accuracy of estimates.
- 3 Nansen C, Meikle WG, Campbell J, Phillips TW and Subramanyam BA. Binomial and Species-Independent Approach to Trap Capture Analysis of Flying Insects. *Journal of Economic Entomology* 2008; 101(6): 1719–1728.
- 4 Adam BDP, TW and Flinn PW. The economics of IPM in stored grain: Why don’t more grain handlers use IPM? in 9th International Working Conference on Stored Product Protection. 2006. Campinas, Brazil.
- 5 Hagstrum DW and Subramanyam B. Fundamentals in stored-product entomology 2006, St Paul, Minnesota: AACC International Press.
- 6 Dunn JA, Thind BB, Danks C and Chambers J. Rapid method for the detection of storage mites in cereals: feasibility of an ELISA based approach. *Bulletin of Entomological Research* 2008; 98(2): 207–213.
- 7 Throne JE, Dowell FE, Perez-Mendoza J and Baker JE. Entomological applications of near-infrared spectroscopy. *Advances in Stored Product Protection*, PF Credland (ed), *et al.* 2003: 131–134.
- 8 Hagstrum DW, Milliken GA and Waddell MS. Insect distribution in bulk-stored wheat in relation to detection or estimation of abundance. *Environmental Entomology* 1985; 14(6): 655–661.
- **The role and importance of species distribution and density within grain bulk was considered in relation to sampling detection and estimation of mean abundance. This manuscript illustrated the importance of considering both the density of insects collected within samples and the variation in species abundance among samples when estimating mean abundances throughout grain bulk.
- 9 Phillips TW and Throne JE. Biorational Approaches to Managing Stored-Product Insects. *Annual Review of Entomology* 2010; 55: 375–397.
- *The review investigates the latest techniques and developments used for IPM in stored products. Particular emphasis is placed on the importance of sampling and population estimation in IPM and considers how the decision threshold of IPM should be formulated.
- 10 Hagstrum DW, Reed C and Kenkel P. Management of stored wheat insect pests in the USA. *Integrated Pest Management Reviews* 1999; 4: 127–142.

*This review considers a range of factors which will influence the development

and ultimately the success of IPM programmes. The importance of effective sampling strategies and a thorough understanding of pest species ecology and population dynamics is emphasised by the authors.

- 11 Barney RJ, Price BD, Sedlacek JD and Siddiqui M. Fungal species composition and abundance on stored corn as influenced by several management-practices and maize weevil (Coleoptera, Curculionidae). *Crop Protection* 1995; 14(2): 159–164.
- 12 Athanassiou CG, Kavallieratos NG, Palyvos NE and Buchelos CT. Three-dimensional distribution and sampling indices of insects and mites in horizontally-stored wheat. *Applied Entomology and Zoology* 2003; 38 (3): 413–426.

* This paper investigates the distribution of insects and mite species in large flat grain storages. Similar to previous studies pests were typically found to be present in the upper zone of grain storages. Interestingly, results suggest greater effort is required when estimating the mean density of mites when compared to insects.

- 13 Lippert GE and Hagstrum DW. Detection or estimation of insect populations in bulk-stored wheat with probe traps. *Journal of Economic Entomology* 1987; 80(3): 601–604.

* This study compares the use of grain probes and insect traps in detecting and estimating insect population densities in storages. The authors illustrate that traps are more effective than probes in detecting insects; however the number of traps required is dependent on the target insect density.

- 14 Cuperus GW, Fargo WS, Flinn PW and Hagstrum DW Variables affecting capture of stored-grain insects in probe traps. *Journal of the Kansas Entomological Society* 1990; 63: 486–489.
- 15 Flinn PW, Hagstrum DW, Reed C and Phillips TW. Insect population dynamics in commercial grain elevators. *Journal of Stored Products Research* 2010; 46(1): 43–47.

**This study investigates the distribution of insect species within large elevator silos storing wheat. Results indicate that insects are distributed towards the top of the grain mass and are rarely found at depths below 12 m. This pattern was found to be consistent even at high insect densities.

- 16 Nansen C, Flinn P, Hagstrum D, Toews MD and Meikle WG. Interspecific associations among stored-grain beetles. *Journal of Stored Products Research* 2009; 45(4): 254–260.

**This paper considers in the interspecific association among common grain beetles. Results suggest that population densities of the beetle species are related to the presence of other species. However, the effect of the inter-specific associations were not consistent across species, with certain beetles species displaying positive responses and increasing in density in association with other species while others displayed negative associations.

- 17 Driscoll R, Longstaff BC and Beckett S. Prediction of insect populations in grain storage. *Journal of Stored Products Research* 2000; 36(2): 131–151.
- 18 Armitage DM (ed). The vertical distribution of mites in stored produce. *Acarology VI*, DAGACE Bowman (eds). Vol. 2. 1984. 1006–1013.
- 19 Hagstrum DW. Seasonal-variation of stored wheat environment and insect populations. *Environmental Entomology* 1987; 16(1): 77–83.

**The importance of both abiotic factors (moisture and temperature) within grain storages and inter-specific associations are considered in relation to the distribution of insects over time. The author illustrates different pest species favour alternative areas of storages in relation to conditions and that fecundity is also influenced by storage conditions.

- 20 Stejskal V, Hubert J, Kucerova Z, Munzbergova Z, Lukas J and Zd'arkova E. The influence of the type of storage on pest infestation of stored grain in the Czech Republic. *Plant Soil and Environment* 2003; 49(2): 55–62.
- 21 Stephens KS. The handbook of applied acceptance sampling: plans, principles and procedures 2001, Milwaukee, Wisconsin: American Society for Quality; 2001.
- 22 Subramanyam B and Hagstrum DW. Integrated management of insects in

stored products 1996, New York.: Marcel Dekker, Inc. New York.

**This text provide a comprehensive review and synthesis of the literature (at date of publication) relating to the management of insects in stored products. Over 9 chapters, pest species, ecology, sampling, control methods, resistance management and IPM are individually considered in a clear and concise fashion.

- 23 Elmoultie D, Kiermeier A and Hamilton G. Improving detection probabilities for pests in stored grain. *Pest Management Science* 2010; 66(12): 1280–1286.

**This paper proposes a novel methodology to maximise the probability of detection of target pests within grain storages. Unlike previous methods, the methodology proposed explicitly considers heterogeneity in insect distribution by separating grain bulks in infested and uninfested portions. The authors illustrate using simulation and field estimates that the method is effective over a range of insect densities and infestation levels.

- 24 Smith-Gill SJ. Cytophysiological basis of disruptive pigmentary patterns in the leopard frog, *Rana pipiens*. II. Wild type and mutant cell specific patterns. *Journal of Morphology* 1975(146): 35–54.
- 25 Taylor LR. Aggregation, Variance and the Mean. *Nature* 1961; 189: 732–735.
- 26 Subramanyam B, Harein PK and Cutkomp LK. Organo-Phosphate Resistance in Adults of Red Flour Beetle (Coleoptera, Tenebrionidae) and Sawtoothed Grain Beetle (Coleoptera, Cucujidae) Infesting Barley Stored on Farms in Minnesota. *Journal of Economic Entomology* 1989; 82(4): 989–995.
- 27 Subramanyam BH, Hagstrum DW and Schenk TC. Sampling adult beetles (Coleoptera) associated with stored grain – comparing detection and mean trap catch efficiency of 2 types of probe traps. *Environmental Entomology* 1993; 22(1): 33–42.
- 28 Obit GP, Throne JE and Flinn PW. Sampling Plans for the Psocids *Liposcelis entomophila* and *Liposcelis decolor* (Psocoptera: Liposcelididae) in Steel Bins Containing Wheat. *Journal of Economic Entomology* 2009; 102(4): 1714–1722.
- 29 Subramanyam B, Hagstrum DW, Meagher RL, Burkness EC, Hutchison WD and Naranjo SE. Development and evaluation of sequential sampling plans for *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Cucujidae) infesting farm-stored wheat. *Journal of Stored Products Research* 1997; 33(4): 321–329.
- 30 Hagstrum DW, Subramanyam B and Flinn PW. Nonlinearity of a generic variance-mean equation for stored-grain insect sampling data. *Environmental Entomology* 1997; 26(6): 1213–1223.
- 31 Hagstrum DW. Using five sampling methods to measure insect distribution and abundance in bins storing wheat. *Journal of Stored Products Research* 2000; 36(3): 253–262.
- 32 Jian F, Larson R, Jayas DS, White NDG. Three dimensional spatial distribution of adults of *Cryptolestes ferrugineus* (Coleoptera: Laemophloeidae) in stored wheat under different temperatures, moisture contents, and adult densities. *Journal of Stored Products Research* 2011; 47(4): 293–305.

** This paper describes the spatial distribution of adult grain beetles in fully controlled experimental systems where approximately 15% of the grain mass was sampled intensively, leading to a higher resolution than previous studies. Aggregation behaviour *C. ferrugineus* was shown to be greater when insect density was lower and was suggested to be a result of intra-specific competition among adults. The authors also illustrated that insect numbers were more highly correlated to the vertical distribution than any horizontal aggregation in the samples.

- 33 Surtees G. Laboratory studies on dispersion behaviour of adult beetles in grain VI. Three-dimensional analysis of dispersion of five species in a uniform bulk. *Bulletin of Entomological Research* 1964; 55(1): 161–171.
- 34 Gelman A, Carlin JB, Stern HS, Rubin DB. *Bayesian Data Analysis* 2003: CRC Press.