Report to CRC for National Plant Biosecurity

CONFIDENTIAL

Review and gap analysis of stored grain sampling strategies

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Contents

1. Executive summary

The grain production and export industry represents the largest agricultural industry in Australia. Australia is a world leading grain exporter, of high quality grain products, commanding a premium price for exported products. Australia's reputation for high quality exports stems from the fact that Australian grains are required to meet stringent hygiene standards. The Australian grains industry, however, is facing new and significant pressures in relation to insect management, including phosphine resistance, deregistration of methyl bromide, reduction in use of traditional grain protectants, and an increased awareness in biosecurity worldwide. It is clear that new procedures to detect and manage pests of stored grain in the long term must be considered. The capacity to make these changes must be underpinned by an effective sampling regime.

Similarly to other countries, stored grain sampling regimes currently in use in Australia developed over time in response pragmatic considerations rather than being based on a robust statistical framework. It is thus unsurprising that these sampling regimes were not designed to maximise the power to detect pests. In particular, the ecology of pests in stored grains violates a fundamental assumption of the statistical model used. Current sampling regimes also lack the flexibility to incorporate additional information that would improve sampling power such as season or storage type. The way in which the sampling model is used also hinders the implementation of improved treatment systems, such as the strategic application of fumigants at appropriate times.

Management of post harvest grains in Australia can be substantially improved by the development and implementation of a sampling strategy based on a robust and appropriate statistical model. This model will need to be designed in such a way to allow for the incorporation of additional information that will increase the power of detection of pests and have sufficient flexibility to be strategically for pest management. Such a model is currently under development in the CRC NPB project CRC 30086: Better Grains Sampling.

2. Introduction

The grains industry is the largest agricultural industry in Australia, with grains, pulses and oilseed production accounting for approximately 25% of Australia's gross value of agricultural production (ABARE 2010a). Exports of summer and winter grain, pulses and oil seed represent an annual return of approximately 6 billion dollars AUD (ABARE Statistics) and the industry is expected to steadily grow at a rate of 1.5 % over the coming 5 years (ABARE 2010b). A major concern to grains industries in Australia and abroad is the management of grain pests (Rees 2004, Hagstrum and Subramanyam 2006). The Australian grains industry, although well established, is facing new and significant pressures in relation to insect management. Resistance to phosphine, the most popular fumigant (Nayak *et al.* 2003, Hagstrum and Subramanyam 2006), the imminent deregistration of methyl bromide due to environmental concerns (Hagstrum and Subramanyam 2006), the reduction in use of traditional grain protectants due to residue concerns and the development of resistance (Herron 1990, Donahaye 2000) and an increased awareness in biosecurity globally (Jeffries 2000) are all issues facing the Australian and international stored products industries. It has now become clear that new procedures to detect, control and manage pests in the long term must be considered (Hagstrum *et al.* 1999, Nansen *et al.* 2008).

In this review, current sampling strategies for pests (insects and fungi) within grain storages will be investigated. This will entail an historical overview of sampling leading to a review of sampling strategies that are currently in place. The review will examine if current sampling strategies and statistical sampling frameworks are adequate to ensure accurate detection of pests throughout the grains supply chain. Finally, knowledge gaps in the current framework will be identified together with suggestions as to how these may be overcome.

3. Pests of stored grains

Grain storages provide the ideal environment for a number of insect and mould species to flourish. In some respects this is due to the makeup of storages, as a full grain silo or storage provides a unique habitat that is in many aspects uniform (Nansen *et al.* 2008). Bulk grain storages can hold thousands of tonnes grain, which are semi-protected from external conditions (Toews *et al.* 2005, Hagstrum and Subramanyam 2006). Prior to humans storing organic material however, pest species now associated with stored products were dependent on a number of natural habitat types. Insects utilised habitats under the bark of trees, within seeds, leaf litter, ripening fruit and in wood, however densities were significantly lower than those present within storages (Rees 2004). The quality of grain resources and the vast quantities in which they are stored provide an opportunity for some insect species to reproduce and reach densities which that could not occur in the natural environment (Rees 2004, Nansen *et al.* 2004, Flinn *et al.* 2004, Toews *et al.* 2005 Nansen *et al.* 2008).

There are a number of orders of insects that are associated with stored grains, including beetles (Coleoptera), moths (Lepidoptera), psocids (Psocoptera), bugs (Hemiptera) and parasitic wasps (Hymenoptera) (Rees 2004, Hagstrum and Subramanyam 2006). Some species such as *Rhysopertha dominica* can attack undamaged grain (primary consumers), whilst others species (secondary consumers) depend on a level of spoilage to facilitate infestation and establishment (Rees 2004). Some insects associated with stored grains however, do not depend entirely on grains themselves, rather feeding on other pests within grain storages. Fungal feeders such as beetles in the family Latridiidae, feed on moulds and cannot survive on clean grain (Rees 2004), whilst predators, parasitoids and scavengers prey on other insect species and decaying organic material (Rees 2004, Hagstrum and Subramanyam 2006).

Fungi are also significant problem within stored grains. Fungi provide a primary and supplementary food resource for certain insect species, destroy grain and can produce toxins harmful to humans (Sanchis *et al.* 1982, Barney *et al.* 1995, Rees 2004). There are a number of fungal species associated with stored grains including *Fusarium spp*., *Penicillium spp*., *Rhizopus spp.*, *Asperillus spp.* and *Tilletia spp.* (Williams and Macdonald 1983, Barney *et al.* 1995, Murray and Brennan 1998). The distribution and abundance of each of these species within stored grain, is dependent upon a range of factors including moisture content of the grain, temperature and humidity (Williams and Macdonald 1983, Barney *et al.* 1995).

Prior to grain and other dried organic products being traded, many insect and fungal species associated with stored grains had distinct geographic distributions (Rees 2004). Due to the expansion of trade routes and the thousands of years of unrestricted trade a number of species such as *Furarium spp*., *Ryzopertha dominica*, *Cryptolestes* spp., *Sitophilus oryzae, Oryzaephilus surinamensis* and *Tribolium castineum* now have global distributions (Rees 2004, Hagstrum and Subramanyam 2006). These species and others have become major pests within the grain industry throughout the world (Rees 2004, Hagstrum and Subramanyam 2006). In this review, insects and the sampling procedures to detect insects will be the primary focus. It should be noted however, that sampling procedures for fungi are similar and often occur concurrently (Grain Trade Australia 2009). Further, the basis of sampling plans and statistical and pragmatic assumptions relating to pest distribution are also similar for insects and fungi (Hunter and Griffiths 1978, Hughes *et al.* 1996, Binns *et al.* 2000, Venette *et al.* 2002).

4. Insects in stored grains – historical context

Insects and fungi have been significant pests of stored products since the first early storages were established thousands of years ago (Rees 2004). Over the generations a variety of storages have been developed to protect stored products from pests (Hagstrum and Subramanyam 2006). Traditional storages are designed specifically for pests and usually depend on excluding access to stored products via elevation or placing products into sealed chambers (Hagstrum and Subramanyam 2006). A number of chemical control methods have also been developed, which act to either deter pests from entering a storage or kill pest which are present (Hagstrum and Subramanyam 2006).

Although the methods for storing grain and storages types have improved substantially over time and techniques have been developed to control stored product pests, infestations remain a major concern to grain industries around the world. Severe insect infestations, although rare in developed countries, have the potential to cause significant damage leading to large losses (Rees 2004). Commodity losses in developed countries are still significant however and can be between 5-10% of the total grain stored (Adam *et al.* 2006). In developing countries, severe infestations are of particular concern and are much more common. Estimates of damage in storages in developing countries are high as 50% of total stored products (Adam *et al.* 2006). Smaller insect infestations within storages remain the greatest problem to industry. Small insect infestations are typically more difficult to detect than severe infestations, and have the potential to cause a number of secondary commodity losses to producers and marketers at great expense. These losses include the reduction in product quality, an increased risk of mould growth and issues associated with insect waste products (Rees 2004, Hagstrum and Subramanyam 2006).

Insects have historically been a significant problem in Australia (Herron 1990, Collins *et al.* 2000, Jeffries 2000). The Australian climate provides the perfect environment for stored product pests to flourish (Rees 2004). The dry warm environment and winter crop production leading to grains being stored over the hot summer months have made insect control a significant issue in Australian storages (Rees 2004). Although susceptible to insect infestation it is rare for major commodity losses to occur in Australian storages due to Australia's highly developed well maintained grain production and storage networks (Rees 2004). However, the cost of maintaining protection to grain supplies can be significant and must be ongoing to minimise the risk of destructive infestations (Rees 2004, Hagstrum and Subramanyam 2006).

In recent years, a significant emphasis has been placed on biosecurity by countries around the world. Measures have been adopted by numerous countries to minimise the risk of importing foreign biological material that, if successful in colonising and establishing, could have significant impacts on the local environment and economy. Quarantine services are responsible for the inspection of goods entering a country, however a responsibility also exists for the exporting country and company to ensure goods entering the destination country are free from pests and disease from their origin.

Over 170 countries are signatories to the International Plant Protection Convention (IPPC 2010). The IPPC convention was developed to minimise the introduction and spread of pests into cultivated and wild plants (IPPC 2010). As part of the convention exporting countries are obliged to provide phytosanitary certificates. Phytosanitary certificates act by providing assurance to importing countries that commodities meet required standards, being either absent of pests or absent of specified quarantine pests by importing country (IPPC 2010).

As a signatory, countries must provide details of the measures used to inspect and treat commodities. The issuance of a phytosanitary certificate can only occur if the plant protection regulation of the importing country can be met (IPPC 2010). It should be noted

IPPC regulation do not specify sampling or inspection procedures (SCA Working Paper 1981). Rather, to the onus is on the exporting country to ensure that their sampling and inspection procedures meet the required standards of the importing country.

Impurities such as insects or fungi detected prior to departure may be treated with a fumigant or an alternative chemical acceptable to the purchaser of the goods. Detection of impurities on intake by the importing country can lead to the shipment being rejected, a reduced sale price or treatment costs being imposed on the distributor. Large bulk commodities such as grains are therefore of particular interest to quarantine services, as they have the potential to harbour numerous pests, pathogens and weed seeds. This has lead to the requirement for export grains to be sampled and subsequently treated so as to minimise the risk of pests and weeds being delivered with commodities (Jeffries 2000).

As the emphasis on biosecurity has grown globally the financial costs associated with the loss of market access due to the detection of contaminated shipments by quarantine services has become a major concern to industry. Furthermore, increased scrutiny from quarantine services can also lead to buyers purchasing bulk commodities from alternative suppliers to minimise stock flow disruptions on import. This has led to producers and bulk grain handlers now placing a far greater focus on ensuring grain commodities are free from live insects or at least meet standards of the buyer concerned (SCA Working Paper 1981, Jeffries 2000). Effective insect management strategies are therefore now fundamental to the success of grain production and distribution to maintain market access and product quality.

Although Australian grain production is significantly lower than other producers, due to low domestic consumption Australia exports approximately 80% of grain produced (Collins *et al.* 2000). As the quantity of grain exported is significantly lower than major export countries such as the USA, Canada and the European Union, Australian producers endeavour to market a high quality product (Collins *et al.* 2000). The production of such a high quality commodity has allowed Australian grain producers and distributors to command the highest market price (Pheloung and Macbeth 2000). The Commonwealth government agency, Australian Quarantine and Inspection Service (AQIS), is responsible for maintaining the high standards associated with Australia's export grain commodities, particularly with regards to hygiene (Pheloung and Macbeth 2000). AQIS performs export inspection and phytosanitary certification of grain products leaving Australian terminals and these certifications have significantly elevated Australia's standings in the international community as a quality grain exporter (Pheloung and Macbeth 2000). Australia's export sampling programme represents one of the most intense export sampling programmes globally (Jeffries 2000).

The certification of Australian grain commodities by AQIS assists Australian grain producers and distributors to command a premium price for exported grains in the world market (Pheloung and Macbeth 2000). Maintenance of strict biosecurity regulations and standards is therefore essential for Australian grain exporters so they can maintain the confidence of overseas consumer markets. As it is impossible to inspect the entire commodity being sampled biosecurity measures are only as effective as the sampling programme which backs them. To be effective, a sampling programme must be based on an understanding of how impurities enter a commodity, how they distribute within the commodity and the density at which they may be present (Stephens 2001).

5. Challenges for insect management in bulk stored grains

While significant advances have been made in insect control and management within grain bulk in the past 40 years, keeping commodities free from insect pests is still a substantial problem (Rees 2004, Hagstrum and Subramanyam 2006). Although control technologies differ, difficulties associated with insect control and management in stored grains exist in both developed and developing countries. Insects have therefore become a significant concern for producers, distributors and exporters, which has led to significant research being targeted at understanding stored product pests around the world (Hagstrum 1987, White 1987, Driscoll *et al.* 1999, Stejskal *et al.* 2003, Flinn *et al.* 2010).

Within developed countries numerous factors contribute to the difficulties in controlling insects in bulk grains. In part, this is due to the complexity of the grains production, supply and marketing network. In large grain producing countries, such as Australia, Canada and the USA, grain production, supply and distribution can occur over large geographic areas covering vastly different environmental conditions (Rees 2004, Hagstrum and Subramanyam 2006). Grains may be transported multiple times prior to final sale and be stored in numerous types of storages for various periods of time (Rees 2004, Hagstrum and Subramanyam 2006). Due to the variation in storage condition, storage types and climatic variation control strategies for insects vary significantly, with control typically being more difficult in warmer regions (Hagstrum *et al.* 1999).

A further complication is that grain supplies are often produced, stored and marketed by separate individuals or corporate entities that may differ in sampling and treatment regimes. Grain sampling techniques differ significantly between countries, domestic producers and bulk handlers such that no uniform sampling procedure exists. Grains shipments are typically sampled on outturn or when received to determine if insects are present (Jeffries 2000). Insects can be transported between storage facilities, leading to infestations at the receiving facilities storages even though they may have been free from insects prior to receiving the shipment.

6. Insect control strategies

Grains are stored in a variety of storage types, from large bunkers, upright concrete storage silos and grain sheds to smaller farm silos and silo bags (Rees 2004, Hagstrum and Subramanyam 2006). Although farm storage, bulk storage and export facilities differ substantially in terms of the quantity of grains held, the types of grains available and the period at which grains are stored, the techniques used to control pests are similar (Rees 2004, Hagstrum and Subramanyam 2006). Manipulation of storage atmosphere via fumigation or aeration, biological control, the application of residual insecticides, sanitation and exclusion, modification of storage temperature and impact and removal are all common methods of control (Hagstrum and Subramanyam 2006).

6.1. Non chemical controls

There are a number of natural enemies of stored product pests that can act as biological control agents (Rees 2004). For example, parasitic wasps in the Order Hymenoptera, attack juvenile stages of beetles and moth pests (Rees 2004). Although biological control agents may be affective at suppressing pest populations (Hagstrum and Subramanyam 2006) and in the correct conditions may self perpetuate (Borgemeister *et al.* 1997) full commodity control is rarely achievable. Further, the presence of natural enemies in shipped grain commodities is often undesirable (Jeffries 2000) and hence their presence would lead to shipment rejection. It is also difficult to utilise biological control techniques with other strategies as their implementation will have adverse consequences for the biological control agents.

Aeration is a strategy becoming more common within storages particularly farm storages. Aeration acts by moving cool ambient air through storages to lower grain temperature such conditions become sub-optimal for insect populations residing within the storages (Flinn *et al.* 1997). Within cooler environments aeration can lead to 100% mortality of insect pests, however higher ambient temperatures such as those in Australia make it more difficult to achieve such outcomes (Flinn *et al.* 1997). Poor aeration techniques can have adverse consequences, however. Uneven distribution and flows can lead to localised moisture build up, encouraging mould and grain spoilage (Hagstrum and Subramanyam 2006). Overuse can also lead to a reduction in grain moisture content, ultimately leading to reduced grain weight and sale price (Hagstrum and Subramanyam 2006).

Implementation of sound hygiene and exclusion practices are fundamental to long-term successful insect management. Although sanitation and exclusion practices alone will rarely lead to 100% control and insects may enter a storage undetected, failing to maintain high standards of hygiene will ultimately lead to the failure of any integrated management programme (Hagstrum and Subramanyam 2006). Maintaining hygiene standards however can be expensive, as storages require constant maintenance and harbourage areas require regular attention (Hagstrum and Subramanyam 2006). Hygiene standards also require all individuals involved in storage operations to maintain facility standards and protocols.

Intense impact can be used as a method kill insects within stored grains (Banks and Field 1995, Paliwal *et al.* 1999). The movement of grains on conveyers, specialised impact machines or the act of sieving are techniques which can cause sufficient damage to insects causing mortality. Although not common as a standalone management strategy, most bulk handling, export and large farm facilities turn grain. Turning grain creates high impact, with grain being vigorously mixed, moved and poured from one storage to another leading to insect mortality (Hagstrum and Subramanyam 2006). This technique however, has a number of disadvantages. Impact may lead to commodity being damaged, and turning and sieving grain just to reduce insect numbers is prohibitively expensive and unrealistic (Hagstrum and Subramanyam 2006). Further, impact alone does not provide a long term solution as not all insect within storage will die and due to the expense and damage to grain regular repetition is not feasible (Hagstrum and Subramanyam 2006).

6.2. Chemical based control

Chemical based or insecticide control is the most common form of control utilised within grain storages. Unlike chemical free control measures, the application of insecticides leads to an immediate reduction in insect population size, and depending upon the chemical used, extended control due to the residual nature of the compound. Insecticides typically are broad spectrum and act on a range of pests, and for many insecticides application is not difficult or expensive (Herron 1990, Hagstrum and Subramanyam 2006). Certain insecticide treatments may also be used as a preventative control measure with application occurring in storage structures prior to the arrival of grain (Nayak *et al.* 1998, Collins *et al.* 2000, Nayak *et al.* 2002, Nayak *et al.* 2003).

Insecticides used as preventive control measures tend to have long residual properties on solid surfaces. Malathion (butanedioic acid diethyl ester) was an early organophosphate used as a grain protectant in Australia and around the world (Herron 1990). Malathion is a contact insecticide, applied directly to grain or storage in liquid or dust form. On contact or via ingestion the chemical binds irreversibly to cholinesterase disrupting the nervous system of the insect causing death (Hagstrum *et al.* 1999). Malathion provided a viable insect control option for bulk grains however, within 8 years of its introduction to Australia, resistance was detected, with the product being withdrawn from use 18 years after its introduction (Murray 1979).

Insecticide resistance is the phenomenon where insects are no longer are susceptible to a given insecticide. Typically it occurs due to genetic selection of less susceptible individuals over a period time where a single insecticide is used or multiple insecticides with similar toxicities and modes of actions (Roush and McKenzie 1987). Insecticide resistance is prevalent in numerous agricultural industries (Roush and McKenzie 1987) and is of particular concern of the grains industry (Hagstrum and Subramanyam 2006)

In response to resistance developing to malathion, numerous alternative grain protectants have been developed. Chemical compounds ranging from stronger organophosphates to synthetic and natural pyrethroid products have been developed and registered for use within grains storages as grain protectants. Similarly to malathion however, overuse has led to the development of insect resistance (Hagstrum and Subramanyam 2006). In response, prescribed application rates have needed to increase to ensure high mortality and thus to prolong the period for which these chemical compounds are available to industry (Herron 1990, Hagstrum and Subramanyam 2006).

Increased dose rates and the development of new stronger residual chemistries have lead to major concerns with chemical residues (Donahaye 2000). Alternatives to residual insecticides are being sought due in part to pressure being placed on legislators to ensure minimal or zero residues on food products (Donahaye 2000). Many developed countries including EU countries (*i.e.* UK, France, Germany) and Asian countries (*i.e.* Japan) the USA and Australia have stringent restriction relating to residue levels for certain insecticides, in some instances prescribing that products are required to be residue free (Donahaye 2000). These regulations therefore prohibit the application of residual insecticides in grain storages placing a greater reliance on non residual control (Donahaye 2000, Jeffries 2000).

Fumigants are an effective alternative to residual insecticides. Similarly to residual insecticides, fumigants provide rapid knockdown of insect pests. In addition, the limited residual characteristics of fumigants do not pose the same public health and trade restrictions (Donahaye 2000). Fumigants are also easily applied, and can penetrate the entire grain bulk if storages are sealed correctly to maintain fumigant pressure, providing an inexpensive control method (Hagstrum and Subramanyam 2006)

Significant concerns exist with the longevity of certain fumigant products. Methyl bromide, an effective fumigant, is likely to be phased out within the coming years as methyl bromide has been shown to deplete atmospheric ozone (Donahaye 2000, Hagstrum and Subramanyam 2006). Concerns also exist for the longevity of phosphine as a high level of resistance to the fumigant has developed in some stored product pest species (Nayak *et al.* 2003).

Phosphine resistance is a major concern to the grains industry. Phosphine is the most commonly used fumigant for grains worldwide (Hagstrum and Subramanyam 2006). When initially developed, phosphine provided grain handlers and producers a cheap, easily applied fumigant, effective on a range of insect species (Hagstrum and Subramanyam 2006). However excessive use has seen resistance build to major beetle (Coleoptera) pests and psocids (Psocoptera)(Nayak *et al.* 2003, Hagstrum and Subramanyam 2006, Lilford *et al.* 2009). The probability of insects developing resistance to phosphine increases with the number of times that they are exposed to the fumigant (Hemingway *et al.* 2002). With increasing concerns about phosphine resistance in Australia and worldwide, it has been recognised that *ad hoc* applications of phosphine contribute to the resistance problem, and that this chemical must now be applied strategically to prolong its useful life for the grains industry.

Typically insecticides and fumigants have been applied on a calendar based system or applied just prior to sale to reduce insect loads and ensure grain shipments are not rejected on intake. This strategy fails to consider the number of insects within a grain bulk, how insect density may respond to factors such as temperature and humidity (Driscoll *et al.* 1999), if an insecticide treatment is necessary, and the effectiveness of other control strategies which may be in place (Rees 2004, Hagstrum and Subramanyam 2006). Since sampling is not undertaken, under these application schedules insecticides can be applied even when insect numbers are very low. These types of application strategies can lead to overuse, increasing the chance of resistance developing.

As insecticide resistance is a major concern for the grains industry methods to maintain effective chemicals must be developed. The lack of development of new fumigants and insecticide due to developmental regulatory costs coupled with international protocols banning certain chemicals due to the human and environmental risks has forced industry to consider broader options for insect control (Nansen *et al.* 2008). Sulfural fluoride (Profume) has recently been registered in Australia (APVMA 2010) with a further alternative fumigant being considered for registration. If these chemicals are to be effective for a prolonged period it is imperative that industry develop effective long term chemical management strategies, which consider the necessity of treatment, rather than solely being based on a calendar system. For this it is necessary to understand whether there are sufficient insects within a grain bulk to justify treatment, and thus essential to develop effective grains sampling strategies.

7. Grains sampling programmes

Sampling programmes for bulk commodities such as grains are typically designed such that representative portion of a larger lot is sampled for analysis or inspection in order to determine if a commodity is free from infestation (Stephens 2001). While the importance of effective robust sampling is well recognised, it is interesting to note that considerable variation in the methodology of grain sampling programmes exists internationally and domestically (Jeffries 2000). This variation is related to numerous factors, both pragmatic and historical (Jeffries 2000).

Grain sampling occurs for numerous reasons. Samples are taken to determine quality traits (protein & moisture), purity, seed borne pathogens, foreign objects, weeds seeds and insects. Sampling and subsequent testing is performed in part to meet specific export standards, buyer's requirements or to meet international seed certification (Morrison 1999, Jeffries 2000, Armitage 2003).

Although insects have been problematic in grain storage for thousands of years, sampling for insects is a relatively recent development (Rees 2004, Jeffries 2000). Sampling programmes have been established and are often regulated by major grain producers as they have recognised the importance of sampling to minimise the risk of insects establishing in grain bulk (Nansen *et al.* 2008). This is typically done prior to sale or movement between facilities to ensure grains meet required criteria in relation to accepted insect densities within shipments or to ensure insects are not being transported and incorporated into clean storages (Jeffries 2000). The way in which grains are sampled however, differs from country to country and even within domestic producers and bulk handlers (Jeffries 2000).

7.1. Export sampling in Australia

Throughout the 1950s and early 1960s Australian grain exports were developing a poor reputation for insect infestation (Jefferies 2000, Pheloung and Macbeth 2000). In response to this the Australian government introduced the first regulatory controls relating to insects in grain products. The regulations outlined initially in the Export Grain Regulation (1963) were concerned only with wheat exports (SCA working paper 1981). In 1968 barley and oats were added to the regulation and sorghum in 1970 (SCA working paper 1981). The addition of barley, sorghum and oats to the regulations was related to issues of cross contamination in storage and shipping facilities. The regulations now include wheat, oats, barley, canola, field peas, lentils, vetch, chickpeas, faba beans and mung beans, soybeans and lupins (Jeffries 2000).

The Export Grain Regulations (1963), now under the Export Control Act prescribe that export grains are required to be free from live insects to be fit for export (SCA Working paper 1981, Love *et al.* 1983, Jeffries 2000, Pheloung and Macbeth 2000). Initially under the act, the department of Primary industries would be responsible for sampling and certifying export products (SCA Working Paper 1981), however AQIS are now responsible for grain inspection and certification in Australia (Jeffries 2000). A sampling rate of 2.25 litres per 33 tonnes, irrespective of grain type, was established. This rate was established based primarily on belt loading speeds (400 tonnes per hour at the time) that allowed inspectors five minutes to manually draw a sample, sieve and inspect for insects (Jefferies 2000, Pheloung and Macbeth 2000).The rate was thus established solely on pragmatic considerations rather than being based on a statistical framework to ensure the effective detection of insects (SCA Working Paper 1981, Jeffries 2000).

As belt loading speeds have increased, now being in excess of 1000 tonnes per hour, it has become impractical and dangerous to draw samples manually. A number of new methods have been developed to draw samples, to both increase safety and reliability of sample procedure. Automated sampling and sieving mechanisms have been developed and incorporated into all shipping ports (Jeffries 2000). The sampling rate of 2.25 litres per 33 tonnes has been maintained and remains the export standard today (Jeffries 2000).

7.2. International export sampling protocols

Significant variation exists between all international sampling programmes. The quantity of grain sampled the number of samples drawn per tonne and the number of sub-samples drawn to form an overall sample all vary between major grain producers (Jeffries 2000). Sampling equipment and where samples are drawn (*i.e.* at loading or prior to loading of ships) and also varies between countries (Jeffries 2000). A further complication is variation in how many insects may be present with the bulk. For example, Australia operates on a zero tolerance dictating that no live insect should be present in samples drawn at the prescribed rate (Pheloung and Macbeth 2000). The United States in comparison works on a dual decision based system, where tolerances vary for different pest species (Jeffries 2000). Although international sampling procedures and rates are significantly different (Table 1), there has been no pressure to move to an international uniform standard (Jeffries 2000).

Table 1: A comparison of International bulk grain export sampling rates. For ease of comparison all sampling rates have been converted to sample quantity per 33 tonnes. Further as Australia is the only exporter to sample based on a volume rather than weight, a standardised weight based on wheat has been presented for the Australian rate.

Country	Sample rate per 33 tonnes (kg)
Australia	1.6
Canada	0.37
United States of America	0.3
United Kingdom	0.82 - 2.64

Similarly to the Australian grain sampling programme, international sampling rates have not been designed within a robust statistical framework. Variation in international grain sampling programmes relates in part to historical and pragmatic constraints in the storage, supply and distribution networks (Jeffries 2000). Sampling programmes for example have been developed in consideration of grain belt loading speeds at storage and shipping terminals, the size of transport vehicles or rail cars and the type and size of storage structures (Jefferies 2000). Differences in sampling programmes can also be attributed to the position in the supply chain that grains are sampled and the use of imperial or metric measurements (Jeffries 2000). For example, in Australia sampling occurs at ship loading with 2.25 litres of grain being drawn every 33 tonnes, whilst in the USA sampling occurs prior to ship loading with 2.5kg being drawn from every 10000 bushels (Jeffries 2000).

Additionally, grain producers vary sampling protocols based on perceived risk of infestation (Jeffries 2000). Canada samples less grain per tonnage than in Australia, based in part on lower risk of insect infestation (Jeffries 2000). Grain production in Australia and the USA occurs over large geographic areas and it has been well documented that insect growth and reproduction biology varies substantially due to climatic variation (White 1987, Driscoll *et al.* 1999, Hagstrum *et al.* 1999). In the USA treatment and sampling regimes have been shown to differ in relation to perceived pest risk and geographic location, with greater emphasis being placed on insect management and detection southern areas of the USA (Hagstrum *et al.* 1999).

7.3. Domestic sampling programmes

Unlike export sampling programmes, sampling protocols throughout the domestic supply and storage network are rarely regulated. Often sampling guidelines exist, which may be developed by an industry body or dictated by individual bulk handlers or grain producers. For pragmatic reasons these sampling rates and protocols are closely related to the export sampling rates and aim to ensure products going to export facilities will meet export standards. Typically sampling rates aim to exceed the export sampling rate, so that bulk handlers can with some level of certainty, ensure product will pass inspection at port.

In Australia, Grain Trade Australia (2009) has developed a set of guidelines for grain sampling. These guidelines cover both methodology and intensity for grain sampling relating to insect and other quality measures. Although not all bulk handlers adhere rigorously to the guidelines, typically their grain sampling rates are similar. Grain Trade Australia (GTA) guidelines stipulate that three 1 litre samples be drawn from lots greater than 10 tonnes in size with an additional 1 litre sample to be drawn for every 10 tonne increase in lot size up to 100 tonnes. Whilst this rate is not equivalent to the Australian export rate of 2.25 litres per 33 tonnes it was constructed to be more intensive than the export rate, such that detections could be made prior to export. Further in the In the United Kingdom, the export sampling rate is 4kg per 10 minutes (equivalent to 0.82 – 2.64 kilograms per 33 tonnes) whilst domestic storage sampling guidelines suggest that 2 kilogram samples are to be drawn from storages every 20 tonnes for lots less than 100 tonnes and 1 kilogram per 20 tonnes for lots greater than 100 tonnes (Jeffries 2000).

7.4. On farm grain sampling

Due to the deregulation of the grains industry in Australia, on farm storage capacity has grown significantly. Farmers now can choose to hold and sell grains to distributors independently of bulk handlers. This has led to grains being held on farm for longer periods and therefore increased the need for effective long-term management.

Historically growers have not had a formally developed sampling protocol, and sampling tends to be *ad hoc*. Sampling from silos may occur during storage, however there is no evidence that a set rate is adhered to. Anecdotal reports suggest that samples may be drawn from the top of upright storage, by releasing grain from the bottom of silos, or sampling on outturn. These methods or the localities from which samples are drawn are deemed to be areas of highest risk, thus it is assumed that if insects are present they will be found within the sample drawn.

8. Effectiveness of sampling protocols

Numerous countries including Australia, the United Kingdom and New Zealand have considered the efficacy of sampling protocols to detect insects at a given statistical confidence. Programmes have been evaluated to ensure grain commodities meet specified standards and thresholds (Jeffries 2000). Wilkin (1991) evaluated the United Kingdom sampling protocol of 4kg per 10 minutes and determined that an infestation of 1 insect per kg could be detected on 95% of occasions. Love *et al.* (1983) demonstrated a statistical 95% confidence in the detection of an infestation of 25 insects per tonne using the Australian sampling rate.

Although international sampling programmes that have been evaluated have been theoretically justified, statistical models to evaluate programmes have been based on the assumption that insects are distributed homogeneously or randomly throughout the grain bulk (Hunter and Griffiths 1978, Johnston 1979, Love *et al.* 1983, Jeffries 2000). The assumption of homogeneity in grains, although widespread in numerous detection and acceptance sampling models, was not informed by knowledge of pest species ecology or behaviour. While models that assume homogeneity are often simpler, with less need for estimation of unknown parameters, it is unlikely that sampling programmes based on these models will be more accurate than those based on more complex models.

9. Binomial sampling models

Acceptance or detection sampling is the process of selecting a representative portion of a larger lot for analysis or inspection in order to determine if consignment meets required standards (Stephens 2001). Sampling methodologies are designed to determine the probability of a accepting a consignment under certain condition or detecting impurities at a given level, that is, that a consignment is free of impurities and can be passed as adequate under a given sampling intensity. Probability functions, such as the Binomial, Poisson and Hypergeometric are regularly used to evaluate and develop sampling programmes (Stephens 2001).

The Hypergeometric function is a three parameter function commonly used when sampling finite lots without replacement (Stephens 2001). The function considers the number of defectives (*i.e.* sub-samples with insects) in a given sample (*i.e.* total sample) from a total lot size containing a number of non conforming units (total consignment). The Binomial is a two parameter function that is commonly used to determine the number of defectives within a lot. It is commonly used as a close approximation to the Hypergeometric function. The Binomial should only be used to approximate the Hypergeometric when lot size is substantially larger than the sample drawn (Stephens 2001). The function denotes the probability of non conforming units being drawn from a sample drawn from a process with fraction of non conforming units. The Poison probability function is a single parameter function commonly used when sampling from fixed volumes (Stephens 2001). The Poisson also provides a close approximation of the Binomial and Hypergeometric function where large lot sizes are being sampled and where only a single parameter is necessary (*i.e.* the number of defectives).

It is appropriate to use different statistical models in different circumstances (Stephens 2001). Acceptance or detection sampling programmes are commonly based on the binomial distribution (Stephens 2001, Wintle *et al.* 2004, Nansen *et al.* 2008) because of its applicability to the interpretation of continuous processes from which large lots emanate (Stephens 2001). Computation of the Binomial is also simpler than most other probability functions (Habraken *et al.* 1986, Cameron and Baldock 1997)

Grains sampling programmes have also typically been evaluated using a Binomial sampling model. However rather than using the model to determine sampling intensity, the Binomial model has been used to determine if a given sample fraction would adequately detect insects at a particular density (Love *et al.* 1983):

$$P(detection) = 1 - (1 - \theta)^{\nu} \quad (1)$$

Where θ represents the sample fraction (the fraction of the lot taken for sampling) and v represents the number of insects in the lot.

For example, Love *et al.* (1983) cites an example in which insects are detected in 5.7% of 2000 tonne grain lots (*i.e. P(detection)* = 0.057), the sample fraction represents 0.00006% of the total grain in a lot, then this equation can be solved for the number of insects (ν = 980). This model considers the distribution of insects to be homogeneously distributed throughout grain lots, with any sample having an equal probability of detecting insects.

Hunter and Griffiths (1978) provided justification of the above approach using a Bayesian approximation method. Unlike the basic binomial approach presented above, the method provides estimates of uncertainly relating to estimates in the confidence intervals. It is important to incorporate uncertainty, particularly when dealing with biological systems and species behaviour is variable. Estimates of uncertainty can aid in minimising different kinds of statistical errors, type one errors (*e.g.* concluding that a grain bulk contains insects when it does not) and type two errors (*e.g.* concluding a grain bulk is insect free when it is in fact positive). The authors demonstrate that the methodology can be used to detect insects at relatively low numbers. Hunter and Griffiths (1978) suggest that the method demonstrated had been used for similar problems however they do not consider insect distribution within grain bulks and do not justify if a homogeneous distribution is a reasonable assumption within stored grains.

Although sampling programmes in countries such USA, United Kingdom and New Zealand were found to be adequate to detect small infestation in grain bulk (Jefferies 2000), the statistical models used were based on the Binomial distribution and also did not consider insect distribution.

As shown above, the development of grains sampling programmes, particularly those relating to export, were typically based on practical constraints in the supply and distribution networks. From a statistical perspective, the Binomial model is simple and has well understood properties, and has traditionally been widely used for sampling. It is perhaps understandable therefore, that this model has been transferred to the sampling in stored grains. Nonetheless, an essential difference between sampling in stored grains and other commodities is that the targets for detection are living and therefore may exhibit characteristics that contravene the underlying assumptions of the Binomial model.

10. Distribution of insects in grain lots

Depending upon the commodity, impurities, pests and pathogens will be distributed through space in different ways (Hagstrum *et al.* 1985, Barney *et al.* 1995, Stephens 2001, Nansen *et al.* 2008). Biological systems are inherently complex due to the way that organisms within them distribute throughout space and time (Hagstrum *et al.* 1985, Ergon *et al.* 2001). An organism's distribution is rarely consistent over time and space as the factors that may influence its distribution such as climate and resources also vary across time and space (Ergon *et al.* 2001, Nansen *et al.* 2008).

Stored product insects can respond to environmental conditions by altering reproductive outputs, entering periods of diapause or increasing growth rate (Hagstrum *et al.* 1999, Hagstrum and Subramanyam 2006). 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 (Driscoll *et al.* 1999). 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 (Rees 2004). Armitage (1984) 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.

In Australia, as in other countries, different grain types are typically stored individually with batches of similar quality and variety being stored in the same bunker, shed or silo. Grains storages may therefore be considered to be significantly less complex than natural systems and represent a system which is largely uniform. Storages do not, however, represent a homogeneous environment for insects. Conditions within 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 bulk (Hagstrum 1987, Cuperus *et al.* 1990). As a consequence, 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 (Rees 2004, Hagstrum and Subramanyam 2006). For example, Stejskel *et al.* (2003) suggested that beetle populations were harder 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 (Hagstrum 1987, Rees 2004, Hagstrum and Subramanyam 2006). 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 pests are known to select for grains with higher moisture contents, often a function of grain age (Rees 2004, Hagstrum and Subramanyam 2006).

The spatial distribution of impurities, pests or pathogens through a commodity will influence the results from sampling programmes (Stephens 2001). The assumption of homogeneity in stored grains, although widespread, is largely for the sake of convenience and simplicity and has been demonstrated to be inaccurate in bulk grains storage (Hagstrum *et al.* 1985, Lippert and Hagstrum 1987, Hagstrum *et al.* 1988). The implications of such an assumption and the validity of sampling programmes and their effectiveness have yet to be established however.

11. Drawbacks of current sampling programmes

Maximising the probability of detection of target species is an important problem in grain sampling. While the statistical models on which sampling programmes have been based are statistically sound in the right circumstances, when sampling living organisms it is necessary to consider whether the biology of the target organism violates the underlying assumptions of the model. While it has been convenient in grains sampling programmes to assume that target species are distributed homogeneously throughout the environment, many studies have demonstrated that this assumption is unlikely to be realistic (Hagstrum *et al.* 1985, Cuperus *et al.* 1990, Athanassiou *et al.* 2003). In turn, this violates an important assumption of the Binomial statistical model on which sampling programmes are based which is likely to deleteriously impact on the accuracy of pest detection in bulk grains. Note that while Hagstrum *et al.* (1985) ostensibly developed a model to estimate heterogeneous insect distributions to a continuous statistical distribution, it is complex and not suitable to determine sampling rates for a required detection probability.

In addition to fundamental concerns with the appropriateness of the statistical models on which current grains sampling programmes are based, remarkably limited flexibility is currently available to incorporate known risk factors that increase the probability of insects being present. For example, the season in which sampling occurs, the area in which grain is grown and stored, and the type of grain storage have all been shown to affect pest distribution and abundance (Hagstrum *et al.* 1985, Cuperus *et al.* 1990, Athanassiou *et al.* 2003 Stejskel *et al.* 2003). Ignoring these factors means that valuable information is lost that could increase the power of a sampling strategy to detect pests.

11.1. Strategic sampling as a component of integrated grains management

Sampling strategies are currently designed such that the individual or organisation sampling the grain consignment can be confident that the grain consignment meets the standards of the buyer, to prevent rejection. Grain sampling for insects therefore occurs predominantly prior to sale, on movement of consignments and at export. For export, and often for transport among domestic storages, a zero tolerance threshold is the desired objective, with detections leading to a treatment such as an insecticide being administered.

Since bulk grain sales may occur with little warning, it has been traditionally been argued that regular applications of insecticides (so called calendar based applications) are also necessary to ensure that insects are kept to a minimum at all times. As concern grows in regards to resistance to insecticides, particularly phosphine, these treatment regimes have been bought into question however (Flinn *et al.* 2003, Nansen *et al.* 2008). Responses to insecticide resistance in grains and other cropping systems have historically been countered with the development of new insecticides based on chemistry sufficiently different to existing insecticides to ensure that resistant insects can be killed. To do this, the newly developed chemicals are either stronger than those which the insects became resistant too, or act on a different neurological or physiological pathway. However due tighter regulatory controls in both public and environmental health and the cost of development, new chemistries are less common. Furthermore, chemicals that remain effective (*i.e.* methyl bromide) are being discontinued or withdrawn from certain markets due environmental and health issues (Roush and McKenzie 1987). Agricultural Industries are thus now attempting to better manage chemicals by encouraging more responsible usage to increase product longevity (Hemingway *et al.* 2002).

Integrated pest management programmes (IPM) have been developed to counter such issues. A fundamental component of successful IPM is early detection of pest populations, accurate determination of pest density and long term monitoring (Nansen *et al.* 2008). Integrated pest management programmes are effective when treatments are based on critical thresholds rather than simple detection or calendar application. These strategies are designed not only to increase control, but minimise chemical use and prolong the longevity of chemical compounds.

Until recently, little consideration has been placed and developing robust estimates to determine to the presence of insects within storages prior to treatment. This is an important concept as when considering treatment not only should the presence of insects be considered, but also if a treatment is economically viable and necessary to maintain product quality and long term control. Therefore sampling programmes should be designed independently for IPM and be based upon treatment thresholds.

Current sampling programmes for bulk grains for export are designed around a zero limit threshold, and this standard is increasingly being adopted by grain handlers for transfers of grain. However, if the grain commodity is being sampled not at the point of sale but rather to determine if treatment is necessary it may be useful to consider a sampling regime under an alternative value of detection. Alternative values for detection would allow sampling programmes and critical treatment thresholds for insects throughout all stages of storage and distribution. That is, with an effective sampling methodology, grain handlers and distributors could administer treatments based on insect density thresholds, reducing the potential for over or under utilisation of insecticide treatments. Multi-stage sampling strategies could therefore play an important role in determining effective treatment times. Furthermore, effective sampling would reduce costs associated with control and treatments.

12. Gap analysis

There are inherent limitations in the current bulk grains sampling schemes used in Australia, and three clear areas in which changes are required to improve these schemes for industry. These relate to:

i) limitations of the underlying statistical methodology on which current sampling of an individual grain bulk is based;
ii) the lack of flexibility with which an effective statistical sampling model can be applied, and;
iii) the need to consider sampling for thresholds as part of integrated management in order to more effectively use existing insecticides.

12.1. Limitations of the current statistical methodology

Current sampling schemes are based on the Binomial model, and not on a robust statistical model that accounts for the ecology of pests in stored grains bulks. Violation of the assumptions on which statistical models are based means that the accuracy of current sampling programmes is less than optimal. The fundamental requirement for an improved sampling strategy is the development and testing of a robust statistical model that accounts for the heterogeneous distribution of pests in bulk grains

In addition, current sampling methods do not incorporate estimates of uncertainty. It is essential that a comprehensive statistical framework provide robust estimates which incorporate uncertainty such that end users can be confident regarding decisions in relation to treatment and distribution.

12.2. Lack of flexibility

Current schemes are unable to account for a wide variety of factors that will influence the likelihood of pests being present in grains, and their density and distribution at any given time. Among other possible factors, this includes:

i) Variation in storage environment due to seasonal conditions – climatic conditions influence the survival and growth rate of insects;

ii) Variation in storage environment due to geographic location - grains are stored over a vast area in Australia, and different geographic areas will be subject to different regimes of temperature and rainfall that are known to influence pest dynamics;

iii) Grain storage type - storage type will influence the extent to which external conditions can influence pest populations within bulk grains;

A capacity to introduce these factors will increase the power of a sampling methodology to detect pests.

An additional important point is that the current sampling protocols were based on export sampling programmes. These protocols were based on sampling large bulk lots which do not necessarily represent smaller bulk storages or farm storages. Any sampling framework that is developed must therefore be sufficiently robust to adequately detect pests in large lots but be flexible enough to consider smaller lot sizes.

12.3. Alternative sampling thresholds

Current sampling protocols are based on a zero tolerance threshold. This is primarily related to sampling programmes being developed for export grains commodities and subsequently be modified for use throughout the supply chain. As the need to consider appropriate IPM programmes grows, detecting pests at alternative thresholds will be essential to better manage the application of treatments and to save money for the industry. Statistical sampling frameworks must therefore have the capacity to easily calculate required sampling intensity for a given detection probability, for a range of thresholds depending upon end user requirements.

13. Conclusions

Stored product pests can enter the production and distribution system in on farm storages, at bulk handling facilities and at export storage facilities (Rees 2004, Hagstrum and Subramanyam 2006). If effective management and detection is to be achieved it is imperative that effective statistically robust sampling strategies are developed so that insect populations can be accurately determined.

A significant feature of current grains sampling protocols is the use of statistical sampling models that assume the homogeneous distribution of insects and fungi through grain bulks. Since these organisms show a clumped distribution in grains, this violates a fundamental assumption of the statistical models used and will lower the efficiency of the sampling programme in detecting insects. In addition, current sampling frameworks are not sufficiently flexible to account for grain storage type, season, geographical location and other factors that may increase the risk of pest infestation. Accounting for these factors is essential to maximise the probability of pest detection and to develop effective sampling strategies.

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