THE INFLUENCE OF TEMPERATURE AND INTRODUCTION POINT ON THE DETECTION OF RHYZOPERTHA DOMINICA IN STORED GRAIN

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Keywords

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*Rhyzopertha dominica;* sampling; spatial distribution; stored grain; supply chain model, wheat.
Abstract

The presence of insect pests in grain storages throughout the supply chain is a significant problem for farmers, grain handlers, and distributors world-wide. Insect monitoring and sampling programmes are used in the stored grains industry for the detection and estimation of pest populations. At the low pest densities dictated by economic and commercial requirements, the accuracy of both detection and abundance estimates can be influenced by variations in the spatial structure of pest populations over short distances. Geostatistical analysis of *Rhyzopertha dominica* populations in 2 and 3 dimensions showed that insect numbers were positively correlated over short (0–5 cm) distances, and negatively correlated over longer (≥10 cm) distances. At 35 °C, insects were located significantly further from the grain surface than at 25 and 30 °C. Dispersion metrics showed statistically significant aggregation in all cases.

The observed heterogeneous spatial distribution of *R. dominica* may also be influenced by factors such as the site of initial infestation and disturbance during handling. To account for these additional factors, I significantly extended a simulation model that incorporates both pest growth and movement through a typical stored-grain supply chain. By incorporating the effects of abundance, initial infestation site, grain handling, and treatment on pest spatial distribution, I developed a supply chain model incorporating estimates of pest spatial distribution. This was used to examine several scenarios representative of grain movement through a supply chain, and determine the influence of infestation location and grain disturbance on the sampling intensity required to detect pest infestations at various infestation rates.

This study has investigated the effects of temperature, infestation point, and grain handling on the spatial distribution and detection of *R. dominica*. The proportion of grain infested was found to be dependent upon abundance, initial pest location, and grain handling. Simulation modelling indicated that accounting for these factors when developing sampling strategies for stored grain has the potential to significantly reduce sampling costs while simultaneously improving detection rate, resulting in reduced storage and pest management cost while improving grain quality.
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Statement of Original Authorship

The work contained in this thesis has not been previously submitted to meet requirements for an award at this or any other higher education institution. To the best of my knowledge and belief, the thesis contains no material previously published or written by another person except where due reference is made.

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Acknowledgements

Completion is a joy; it gives me the opportunity to look back over the journey and remember the people who travelled with me along the way.

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For Cynthia.
Chapter 1: Introduction

1.1 GENERAL INTRODUCTION

Wheat is an important agricultural crop world-wide, grown commercially on every continent except Antarctica. Global production in 2011 has been estimated at 694 million tonnes (Taylor and Koo 2012), with approximately 18% (129 million tonnes) of world production traded on international markets. Australia is a major producer of wheat, with production for the 2010/2011 season estimated at over 26 million tonnes (Wheat Exports Australia 2011). Of this, over 15 million tonnes worth AU$5.53 billion was destined for export (Taylor and Koo 2012; Wheat Exports Australia 2011). The position of wheat in both local markets and world trade as a high-value, high-demand crop has lead to development of extensive and highly-efficient domestic and international supply chains.

Since the development of agriculture in pre-historic times, humans have stockpiled produce for more consistent availability (Valamoti and Buckland 1995). Today, as a seasonal crop, wheat is typically held in the supply chain in order to ensure uninterrupted supply and to take advantage of market conditions. Storage takes place in various types of structures – silos, bags, bunkers, warehouses – and wheat may be held for many months. These stockpiles represent a vast untapped resource available to a range of insects which had previously evolved to feed on dry plant material (Cox and Collins 2002; Rees 2004), and some of the same pests that infest stored products today have been found in early storages (Levinson and Levinson 1998; Valamoti and Buckland 1995). Today, over 600 species of Coleoptera and 70 species of Lepidoptera have been recorded as infesting various stored products (Rajendran 2005).

In general terms, these insect pests share many characteristics that are adaptive for survival in food storages. These include wide tolerance of differing environmental conditions such as temperature and relative humidity, a wider range of
food habitats than many other insects, long lifespans with continuous reproductive activity, the ability to withstand long periods without food or adaptability to alternative food resources, high fecundity and the ability to rapidly increase population size, and low detectability due to cryptic behaviour or relatively small size (Cox and Collins 2002; Rees 2004; Throne 1994).

The purpose of this thesis is to investigate the spatial behaviour of a common stored product pest - the lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae) – under temperatures typical of those recorded in wheat storages and supply chains, and in response to the location of infestation. The remainder of this chapter will describe the general biology and life cycle, history and ecology, and economic importance of *R. dominica* in context as a grain pest. Later chapters will describe experimental research undertaken to determine the spatial behaviour of *R. dominica* in stored wheat, the application of this knowledge to improving detection sampling for pests storages, and the development of a simulation model incorporating spatial understanding to inform detection sampling in a grain supply chain.

### 1.2 BIOLOGY AND LIFE CYCLE OF *R. DOMINICA*

The lesser grain borer, *R. dominica*, is a member of the Bostrichidae family of wood-boring insects. The genus *Rhyzopertha* is monotypic. First described by Fabricius as *Synodendron dominicum* in 1792 (Chittenden 1911), the common name of “lesser grain borer” was coined by Chittenden to distinguish it from the larger *Prostephanus truncatus*. *R. dominica*, is a major insect pest of wheat and other stored products in temperate and sub-tropical countries. Adult insects are small reddish-black to black coloured beetles approximately 3.5-4.2mm in length, cylindrical in cross-section and elongated in shape (Edde 2012). As is typical of the Bostrichidae family of borers, the head is tucked under and hidden from above by the prothorax. They are considered to be long-lived, with recorded life spans ranging from 3 to 6 months (Birch 1953; Edde 2012; Edde and Phillips 2006; Rees 2004).
Eggs are laid singly or in small clusters amongst or on grain (Edde 2012). Individual eggs are oval-shaped and nearly oblong, approximately 0.5mm in length and 0.2mm in width (Kučerová and Stejskal 2008). Typically, a female will lay between 300-400 eggs in a lifetime (Schwardt 1933), of which approximately 80% are fertile. Oviposition takes place at temperatures between 18°C and 39°C and above 30% relative humidity (corresponding to approximately 8% wheat moisture content) (Birch 1945a; Birch 1945b; Birch 1953). The incubation period is dependent upon temperature and humidity, ranging from 5 to 11 days (Edde 2012), and may vary somewhat due to incubation beginning before egg deposition (Schwardt 1933).

Upon hatching, larvae are immediately active and begin feeding on grain (Schwardt 1933). Entry to the grain kernel is typically through damage caused by adult feeding activity (Schwardt 1933) or previously cracked grain. Further development continues completely within the grain kernel, with *R. dominica* typically progressing through four larval instars (although this may extend to between five and seven under sub-optimal conditions) (Edde 2012). Larval development takes approximately 16 days under optimal conditions of 34°C and 70%rh (Birch 1945a; Birch 1945b), after which pupation takes place.

Pupation takes place within the grain, in the cavity produced by larval feeding. The pupae is largely immobile, and development time is temperature-dependent. Under optimum conditions, pupation can take as little as 4 days (Birch 1945a; Birch 1945b; Birch 1953). After pupation, newly-formed adults chew their way out of the grain kernel, and may go without feeding for 3-5 days (Schwardt 1933). The minimum development time from oviposition to emergence is 25 days at 34°C and 70%rh (Rees 2004).

1.3 HISTORY AND PEST STATUS OF *R. DOMINICA*

The native range and historical origins of *R. dominica* are uncertain. Although Fabricius described it from material collected in South America (Chittenden 1911) the Indian subcontinent is believed to be the native home, as the region is the focal
point of the majority of Bostrichid species (Chittenden 1911; Schwardt 1933). Early
literature notes it primarily as a grain pest in India, Egypt, and Australia, with
infestations occurring in grain-handling ports in England and Europe as a
consequence of grain shipments from these colonies. It was first recorded in the
United States in 1861 by LeConte (1862), who suggested it had been introduced in
specimens of wheat from Persia (now Iran) distributed by the U.S. Patent Office.
Some years later, Chittenden (1911) described \textit{R. dominica} as being “well
established” in southern U.S. seaports, and recounts several reports of it being
imported in grain and wood from various countries including Peru, Egypt, and Japan.
In addition, there are contemporary reports of it being found in Hawai‘i (Sharp 1910,
p.643). Despite these early reports, \textit{R. dominica} was not considered a major pest in
the U.S. until large numbers were introduced through grain imported from Australia
during and after World War I (Back and Cotton 1922; Doane 1919; Schwardt 1933).
By 1922, Back and Cotton (1922) noted that the “Australian Wheat Weevil”, \textit{R.
dominica}, “bids fair to become a very serious pest of grain throughout the South”,
where it was steadily spreading beyond seaports and into grain storages in
agricultural areas.

The history of \textit{R. dominica} in Australia is less well documented. It is likely
that, like many of the stored-product pests present in Australia today, \textit{R. dominica}
arrived either with the First Fleet in 1788 or during the early years of settlement in
grain transported from South Africa or India (Van Graver and Winks 1994). Beyond
this, little is recorded until its appearance in the shipments of wheat to America noted
above. It is evident, however, that \textit{R. dominica} was considered of secondary
importance to more prevalent pest species such as \textit{Tribolium castaneum} and
\textit{Sitophilus oryzae} until the introduction of malathion as a protectant in 1960-61 (Van
Graver and Winks 1994).

1.4 **ECONOMIC IMPORTANCE OF \textit{R. DOMINICA}**

Post-harvest, growers and handlers typically store wheat for multiple
purposes. Growers may store wheat for future planting, stock feed, or for sale at a
later date to maximise return, while handlers manage their storages in order to ensure
continuous supply and availability for forward contracted sales. It is during this period that the stored wheat is susceptible to infestation, and subsequent volume and quality losses, due to pest activity. Losses caused by stored-product pests are difficult to determine (Hodges et al. 2011) but, on a global scale, it has been estimated that approximately 5% of weight is lost to insect activity in storages (Rajendran 2002).

*R. dominica* is one of the most damaging pests of stored grain, particularly in tropical and temperate zones. Damage is caused by both larval and adult stages boring into the kernel and eating the germ and endosperm. This results in reduced grain weight, protein content, and overall quality. Infestations can, if left untreated, result in considerable damage to wheat. Campbell and Sinha (1976) recorded up to 60% weight loss occurring when single grain kernels were exposed to *R. dominica* adults. Grain damaged by *R. dominica* is susceptible to further damage by secondary feeders such as *Cryptolestes ferrugineus* and *Tribolium castaneum*, as these pests are unable to feed on whole kernels (Rees 2004). In addition, *R. dominica* and other insect pest activity can introduce or facilitate secondary fungal and bacterial infections, further reducing grain quality (Birck et al. 2006).

A developing issue is that of pesticide resistance. Along with many other stored product pests, *R. dominica* has shown remarkable adaptability and increased resistance to a variety of traditional protectants and fumigants. Malathion was first introduced as a protectant in Australian grain production during the 1960-61 wheat harvest. Initially already fairly tolerant of malathion (which itself lead to *R. dominica* supplanting *C. ferrugineus* and *S. oryzae* as the major insect pest of stored grain in Australia), by 1972 resistance to this protectant was observed in *R. dominica* (Van Graver and Winks 1994). Since that time, *R. dominica* has proved either not susceptible, or has developed resistance to, all approved organophosphorus or pyrethroid-based treatments (Collins 2006; Lorini and Galley 1999; Srivastava et al. 2000). This, in addition of the recent appearance of resistance to available hormonal growth regulators such as methoprene (Collins 1998) and fumigants such as phosphine (Collins 2006; Mau et al. 2012) suggest that these treatment may soon similarly lose effectiveness.
1.5 DETECTION OF PESTS IN STORED PRODUCTS

The presence of *R. dominica* and other invertebrate pests in grain storages is not easily determined by direct visual inspection of storages, except in cases where infestation rates are already high. Typically, a combination of sampling within storages, trapping in the immediate vicinity, and inspection of grain during handling or transport is used to detect insects and estimate infestation rates. Detection within storages is hampered by the large bulk (typically many tens to thousands of tonnes) and restricted access to the product (often only from the grain surface or through small sampling ports), and the small size of most stored-grain pests.

Grain is often sampled during storage or transport using hand-operated grain triers or pneumatic (vacuum) probes inserted into the grain mass. Grain moving through a handling system (such as a conveyor belt or auger) may be sampled using a diverter, where a continuous stream or intermittent samples are taken for inspection. A sample of the grain so taken is sieved or otherwise inspected for the presence and abundance of insects. Grain in storages may also be sampled using probe or pitfall traps. As probes and traps are left in the grain for long periods of time they can detect insects where intermittent sampling fails (Hagstrum *et al.* 1998; Lippert and Hagstrum 1987; Vela-Coiffier *et al.* 1997). However, factors such as trap design and location (Subramanyam *et al.* 1989), pest species and temperature (Fargo *et al.* 1989), as well as grain type (Hagstrum *et al.* 1998) can all affect detection rates, and influence the conclusions drawn from sampling results.

In recent times, improvements in pest detection have focussed on the development of techniques such as machine vision, acoustic monitoring, near infrared (NIR), and soft X-ray (Fornal *et al.* 2007; Hagstrum *et al.* 1988; Neethirajan *et al.* 2007; Rees 2004) However, it has been noted that sampling practices have largely been developed on the basis of pragmatic considerations – ease of access to grain bulks, speed of sampling and examination, etc. – rather than on a statistically-robust
foundation (Elmouttie et al. in press; Elmouttie et al. 2010; Jefferies 2000). Most evidently, sampling programmes commonly in use assume that pests are distributed randomly or homogeneously throughout the grain bulk (Hunter and Griffiths 1978; Jefferies 2000; Love et al. 1983), despite evidence to the contrary (Cox and Collins 2002; Loschiavo 1983).

Although not widely implemented, statistically-robust alternative grain pest sampling models that implicitly (Hagstrum et al. 1985) or explicitly (Elmouttie et al. 2010) account for non-heterogeneous spatial distributions have been proposed. Hampering the evaluation and implementation of these and similar models is a lack of detailed knowledge on the spatial distribution of pests within grain bulks. Field-based studies on spatial distribution are limited by the size of and access to the grain bulk, which limits sampling intensity and accuracy. Additionally, the resultant spatial distribution can be affected by uncontrollable biotic and abiotic factors such as interspecies associations (Hagstrum et al. 2010; Nansen et al. 2009) or seasonal variations in moisture and temperature (Flinn et al. 2004; Hagstrum 1987). Conversely, laboratory-based studies have been undertaken, but have focussed on examining the influence of individual environmental factors on insect movement (Collins and Conyers 2009; Flinn and Hagstrum 1998; Jian et al. 2005; Jian et al. 2009; Jian et al. 2003; Plarre 1996) and have not aimed to determine pest occupancy and spatial distribution. Accurate data on the spatial distribution of pest species in grain – specifically, high-resolution occupancy data in three-dimensional grain bulks - suitable for evaluation and development of sampling models has not been available.

1.6 SPATIAL DISTRIBUTION OF STORED PRODUCT PESTS

The spatial distribution of pests within the bulk of a stored product is driven by multiple factors. Non-uniform spatial distribution is dependent on factors such as insect behaviour, moisture and temperature levels and gradients, the presence and location of contaminants, damaged grain, grain dust, the presence of other arthropods, and infestation rates (Cox and Collins 2002). Initial dispersal after emergence, such as observed in *Sitophilus granarius*, can result in the establishment of discrete populations within a single large grain bulk (Stein 1994). *C. ferrugineus* is
known to move downwards from the top of grain masses and aggregate in pockets of damp or damaged grain (Loschiavo 1983), whilst also migrating to warmer locations within the grain mass as grain temperature reduces due to seasonal variation or grain aeration (Flinn and Hagstrum 1998). Other pests, such as *Ahasverus advena* and *Typhaea stercorea*, have been observed to prefer the central regions of grain storages even in the absence of significant temperature and relative humidity gradients (Vela-Coiffier *et al.* 1997).

As noted in the preceding paragraphs, there are many physical, environmental, and ecological constraints that limit the ability of sampling real-world grain storages to provide detailed information of pest spatial distribution. In general, sampling is limited to accessible areas of the grain bulk and sampling intensities are low. For example, a field study taking 36 x 0.5kg samples (18kg total) from a bin containing 80 ~ 120 tonnes of grain (e.g. Hagstrum *et al.* 1985) would be considered ‘intensive’, even though this represents examination of less than 0.03% of the total grain bulk. In the case of the cited study by Hagstrum, the variation in insect count between 2 samples taken concurrently at adjacent locations (30cm apart) was found to be higher than that between non-adjacent locations. This suggests that spatial structuring of pest populations in storages occurs at quite small scales, and highlights the importance of determining spatial structure at a scale appropriate to both the organism and the intended sample size. In practice, this requires intensive sampling of laboratory experiments.

To date, laboratory experiments have tended to focus on the effect of environmental conditions on insect spatial distribution in two (Flinn and Hagstrum 1998; Jian *et al.* 2005; Jian *et al.* 2003; Jian *et al.* 2002; Loschiavo 1983; Parde *et al.* 2004) and three (Jian *et al.* 2011; Jian *et al.* 2012; Surtees 1964d; Surtees 1965a; Surtees 1965b) dimensions. Despite evidence suggesting that at least some pest species distribute heterogeneously in the absence of environmental drivers (Stein 1994; Vela-Coiffier *et al.* 1997), in most cases the underlying distribution pattern is still assumed to be random or homogenous. Additionally, while the few three-dimensional studies undertaken have indicated trends in pest spatial distribution, the sampling methods employed – the use of mesh bags to contain grain by Surtees
(1964b), or sampling with replacement by Jian (2011) may have influenced the results obtained.

1.7 SPECIFIC RESEARCH AIMS

The specific aims of this research are as follows:

1) To develop a method of sampling a system representative of insect pests in grain storages, in three dimensions and with appropriate sampling intensity;

2) To apply this method to examine the underlying spatial distribution of a representative grain pest, *R. dominica*, at a range of temperatures and under conditions typical of grain movement through a supply chain;

3) From the data obtained, develop a predictive model describing the spatial distribution of *R. dominica* in terms suitable for use in a sampling model; and

4) Incorporate the predictive spatial model into an existing but previously-unpublished model of a grain supply chain, with the purpose of examining the influence of pest abundance, and spatial distribution on detection sampling in a representative grain supply chain.

1.8 THESIS PRESENTATION AND STRUCTURE

The structure of this thesis follows QUT rules for a Masters by Research by Publication, which allows thesis examination to be based on the presentation of a body of related published or submitted works, linked together with abbreviated introduction and discussion chapters. Rules can be found at [www.rsc.qut.edu.au](http://www.rsc.qut.edu.au). Only minor formatting changes have been made to the published or submitted works comprising chapters 2 and 3 for the sake of overall consistency. These include: standardisation of numbering of headings, tables and figures, standardisation of citation style, incorporation of figures and tables into text, and compilation of all cited works into a single reference list at the end of the thesis.
Chapter 2: Paper 1 - Geostatistical analysis of adult *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae) in wheat stored at constant temperatures

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Statement of Contribution of Co-Authors for Thesis by Published Paper

The authors listed below have certified* that:

1. they meet the criteria for authorship in that they have participated in the conception, execution, or interpretation, of at least that part of the publication in their field of expertise;
2. they take public responsibility for their part of the publication, except for the responsible author who accepts overall responsibility for the publication;
3. there are no other authors of the publication according to these criteria;
4. potential conflicts of interest have been disclosed to (a) granting bodies, (b) the editor or publisher of journals or other publications, and (c) the head of the responsible academic unit, and
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In the case of this chapter: Paper 1 - Geostatistical analysis of adult *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae) in wheat stored at constant temperatures

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<tr>
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Abstract: Insect monitoring and sampling programmes are used in the stored grains industry for the detection and estimation of insect pests. At the low pest densities dictated by economic and commercial requirements, the accuracy of both detection and abundance estimates can be influenced by variations in the spatial structure of pest populations over short distances. Geostatistical analysis of *Rhyzopertha dominica* populations in 2 dimensions showed that, in both the horizontal and vertical directions and at all temperatures examined, insect numbers were positively correlated over short (0–5 cm) distances, and negatively correlated over longer (≥10 cm) distances. Analysis in 3 dimensions showed a similar pattern, with positive correlations over short distances and negative correlations at longer distances. At 35 °C, insects were located significantly further from the grain surface than at 25 and 30 °C. Dispersion metrics showed statistically significant aggregation in all cases. This is the first research using small sample units, high sampling intensities, and a range of temperatures, to show spatial structuring of *R. dominica* populations over short distances. This research will have significant implications for sampling in the stored grains industry.
2.1 INTRODUCTION

Insect pests of stored products are a major problem worldwide, resulting in both direct loss of production and indirect losses due to secondary infestation and trade restrictions (Adam et al. 2006; Fornal et al. 2007; Oerke 2005). This can result in considerable economic impact to growers, bulk handlers, and distributors (Adam et al. 2010; Hagstrum and Subramanyam 2006 pp14-21). Management practices for pests typically involve monitoring and sampling programmes for the detection of pest insects, or for estimation of the density of insects in grain bulks (Flinn et al. 2007; Flinn and Hagstrum 1990). While practices vary worldwide depending upon country, region and producer (Adam et al. 2006; Jefferies 2000; Kogan 1998), a common requirement is that infestations be detected at low levels in order to minimise the costs of both insect damage and treatment (Adam et al. 2006).

The spatial distribution of insects influences detection and abundance estimates (Athanassiou et al. 2011; Elmouttie et al. 2010; Hagstrum et al. 1985; Trematerra et al. 2007), and this can be especially relevant at low infestation rates (Hagstrum 2000; Taylor 1984). As a result, infestations can remain undetected when abundances are low and sampling effort restricted (Gu and Swihart 2004). As grain commodities are stored in large quantities, and sampling costs increase with the number of samples taken, generally only a small portion of a grain lot is sampled (Adam et al. 2010; Binns and Nyrop 1992).

It is clear that a better understanding of the spatial distribution of pests in stored grain can help to improve both pest detection and treatment methods (Hagstrum et al. 1985; Taylor 1984). However, the method used to resolve spatial distribution impacts on the inferences that can be drawn (Stejskal et al. 2010). For example, previous research in large grain bulks has found that spatial distribution is influenced by factors such as seasonal variations in moisture and temperature (Flinn et al. 2004; Hagstrum 1987), and interspecies associations (Hagstrum et al. 2010; Nansen et al. 2009). While these studies have provided valuable information, assessing exactly which factors are responsible for particular effects can be
challenging, as it is difficult or impossible to control for interactions between these and other environmental factors (Athanassiou et al. 2011).

Conversely, previous laboratory-based studies have focussed on evaluating the influence of individual environmental factors on insect movement. Largely, these have used smaller 2-D systems (Flinn and Hagstrum 1998; e.g. Jian et al. 2005; Jian et al. 2003) or low sampling intensities in larger 3-D systems (Collins and Conyers 2009; Jian et al. 2011; e.g. Plarre 1996). While these studies have also provided valuable information, the restrictions of a 2-D environment or low sampling intensity limit their ability to accurately define pest distributions in 3 dimensions, restricting the understanding of spatial distribution in representative systems at a fine scale. Combining the use of intensive sampling with a representative 3 dimensional system would improve understanding of pest spatial distributions.

To fully understand the effect of individual environmental factors on the spatial distribution of grains pests, establishing a ‘baseline’ spatial distribution for comparison is highly useful. In this study, we develop new methods to create a ‘null model’ using a geostatistical approach to spatial analysis (Davis 1994). We then apply this technique to examine the effect of a single environmental parameter (grain temperature) on the spatial distribution of a typical stored product pest insect. This knowledge may then be used as a reference for future studies examining the effect of other factors on spatial distribution. Ultimately it is expected that the improved understanding of these studies can be applied to the improvement of population and detection sampling models, with the aim of enhancing pest management practices (Phillips and Throne 2010).

2.2 MATERIALS AND METHODS

2.2.1 Insects and Grain

The lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae), was chosen for this study. Both larvae and adults are internal feeders, causing
considerable damage to grain. Due to its wide tolerance of environmental conditions, it is an economically important pest worldwide (Fields et al. 1993; Osuji 1982).

Insects for this study were obtained from the Department of Employment, Economic Development and Innovation, Queensland, Australia. Cultures originated from a wild strain collected during May 2010 near Surat in the western Darling Downs region. Cultures were maintained on whole wheat at 30°C and 55% r.h. (Flinn and Friesen 2010; Hagstrum and Subramanyam 2006). As adult *R. dominica* are difficult or impossible to accurately sex without negatively affecting survival or reproductive potential (Edde 2012; Sinclair 1981), no attempt was made to sex selected adults. To maximise the probability of obtaining an optimum sex ratio while remaining within the confines of typical economically important infestation rates (<2 adults / kg), 30 recently emerged (0-7 day old) adults were randomly selected for use in each experimental replicate.

Certified organic, pesticide-free (CO$_2$-treated) Australian Prime Hard wheat (*Triticum aestivum* (L.)) was used in all experiments. To ensure freedom from live pests and viable eggs, all grain was frozen (-18°C) for 2 weeks, followed by storage at 5 ±1°C until required for use (Fields 1992). At the commencement of experiments, 80 litres (~ 20%) of grain was randomly selected and inspected for pests using a 2mm stainless steel mesh grain hand-sieve. No adult insects were found.

**2.2.2 Lot preparation**

Twenty litre food grade polypropylene containers (26w x 26d x 39h cm) were used for all experiments. A rubber bung, used for the later introduction of CO$_2$ to euthanize insects, was fitted near the base of each container. Grain was transferred from refrigerated storage and 20 litres measured into each container. Containers were then covered with a tight fitting polyester mesh to prevent insect ingress / egress whilst allowing air circulation. These were then transferred to pre-conditioned temperature controlled cabinets and allowed to acclimatise for 48 hours to ensure even temperature distribution throughout the grain mass. Each container formed one experimental lot.
2.2.3 Experimental design

Four experimental treatments were conducted, consisting of three replicates each. A 30°C 2 week control treatment was undertaken as a comparison baseline for other treatments, based on a fixed known population and time. The three remaining treatments were undertaken for 1 generation at 25, 30, and 35°C (Table 2.1). Generation times for these treatments were calculated using existing population and developmental time models (Driscoll et al. 2000; Hagstrum and Milliken 1988; Wagner et al. 1984) and published data (Hagstrum and Subramanyam 2006 p.98).

Thirty insects were introduced to the top centre of the grain surface, and each lot incubated in temperature controlled (± 0.5°C) cabinets for the duration of each treatment. Humidity in the cabinets was controlled to 55% ± 5% r.h. using a saturated sodium bromide solution (Greenspan 1977). Temperature and humidity were monitored using environmental dataloggers.

2.2.4 Sample Preparation

After incubation, insects were euthanized by the introduction of CO₂ gas and refrigeration (5 ±1°C) for a minimum of 10 days. Prior to sample preparation, lots were frozen (-24 ±2°C) for a minimum of 48 hours to assist in preparation.

Granulated gelatine from a commercial supplier was used to prepare a liquid solution (60g/L) according to the two-step process described in Schrieber and Gareis (2007 pp. 138-141). Individual lots were removed from the freezer immediately prior to sample preparation, and the gelatine solution poured evenly to fill the container using a mesh spreader to eliminate disturbance of the grain and insects. Once poured, lots were frozen (-24 ±2°C) for two hours to rapidly set the gel before being held in refrigerated storage (5°C ±1°C) for a minimum of 72 hours.
A wooden form with cutting guide slots spaced at 5cm intervals along opposing sides was used to prepare samples. Once set, the lot was removed from the container and placed in the form, aligned so that slice thickness was referenced to the top surface of the grain, secured in place, and cut into 5cm lateral slices. Each slice was then placed on a cutting board marked with a 5cm² grid, and cut into 5cm cubes. The location of each sample was recorded in an X-Y-Z co-ordinate system before being frozen (-24°C ± 2°C) until examination. A total of 200 samples, accounting for >95% of the total volume, were produced from each lot.

### 2.2.5 Sample Examination

Samples were placed in a large plastic beaker, the gel dissolved with hot water, and the grain transferred to an examination tray for visual inspection. Adult insects recovered outside of grain kernels were counted as ‘loose adults’. Individual grain kernels were then examined for signs of internal insects, and kernels identified were dissected and examined under a microscope. Adult insects (with mature abdominal and elytra colouring) were recorded as “in-grain adults”. The total count of all adults within each sample was used for all analyses.

### 2.2.6 Data Analysis

All data analysis was undertaken using the statistical application and programming language R (R Core Development Team 2011). The standardised Morisita index \( I_p \) (Krebs 1999 pp. 216-217; Smith-Gill 1975) was used to compare dispersion between treatments and replicates. This index ranges from +1 (clumped) to -1 (dispersed), with ±0.5 being the 95% confidence limits around random patterns \( (I_p = 0) \). Clusters were defined as spatially-contiguous groups of samples occupied by at least one insect. Two-level mixed-model nested ANOVAs with post-hoc Tukey’s HSD tests were used to identify significant variations in mean sample abundance between all experiments (top level, fixed effects) and replicates (level 2, random effects). Similarly, to detect significant differences in insect vertical movement, nested ANOVAs were used to identify significant variations in mean per-layer (Z-axis) abundance between experiments and replicates. Figures were created
using R’s ‘lattice’ (Sarkar 2008) and ‘graphics’ (R Core Development Team 2011) packages.

Spatial correlations between sample abundance and inter-sample distance were calculated using the ‘gstat’ package (Pebesma 2004) in R. Correlation coefficients between sample abundances were calculated in one 2-dimensional horizontal (H) plane (X-Y), two 2-dimensional vertical (V) planes (X-Z and Y-Z), and in 3-dimensional (X-Y-Z) space (D). Distances between samples were calculated as the straight line (Euclidean) distance between sample centres; results that were fractions of the sample size were grouped into 5cm categories. Correlation coefficients were calculated between all occupied samples and those located a given distance class away. For example, V(0,[5-10]) represents the correlation coefficient between samples spaced from 5 to 10cm apart in the vertical direction, while D(0,[10-15]) represents the correlation coefficient between samples spaced 10 to 15cm apart in any direction. Significance thresholds for correlation coefficients were determined using the method outlined by Anderson (1942), which accounts for differing spatial distances and sample abundances. As the extra dimension used in 3D analysis influences the significance of correlations compared to 2D analysis, significance for geostatistical analyses were determined at $p=0.10$.

2.3 RESULTS

A total of 2400 samples were examined across the four treatments. Abundance varied with temperature, and the number of occupied samples increased with insect abundance (Table 2.1). Between-replicate variance in abundance increased with temperature (Table 2.1). Irrespective of treatment and replicate, spatial distributions were clumped ($Ip > 0.5$). The number of discrete clusters identified within each replicate was also similar (mean = 2.45, SE = 0.463) across all treatments, with the exception of replicate 1 in the control experiment where 6 distinct clusters were identified.
Sample abundance differed significantly between treatments ($F_{3,84} = 4.8574, p = 0.004$), but no difference was found between replicates within treatments ($F_{8,84} = 0.4096, p = 0.912$). The 30°C treatment was significantly different in sample abundance compared to both the control ($p = 0.004$) and 25°C ($p = 0.019$) treatments (post-hoc Tukey’s HSD). Mean sample abundance between treatments varied significantly with sample depth ($F_{28,64} = 8.2274, p < 0.001$), with post-hoc Tukey’s HSD tests showing that the 35°C treatment was significantly different to the control ($p = 0.005$), 25°C ($p = 0.045$), and 30°C ($p = 0.029$) treatments. Sample abundance at 35°C and >15cm from the top differed significantly ($p < 0.001$) from the control, 25°C and 30°C treatments. In the control, 25°C, and 30°C treatments, abundances were highest at 0-15cm from the grain surface, with insect numbers at these depths accounting for >80% of total insects in each case. At 35°C, abundance peaked at 15-30cm from the grain surface, with > 70% of insects found within this range (Figure 2.1, Figure 2.2).

Examination of between-sample covariance was performed in both 2 and 3 dimensions. Across all treatments, in the horizontal [X-Y] plane there was a trend of decreasing correlations with increasing distance, with results significant ($p \leq 0.10$) at 25°C (10-15cm) and 35°C (0-5cm and 10-15cm) (Table 2.2). This same trend was also evident in the vertical [X-Z] and [Y-Z] planes (Table 2.3, Table 2.4). At 30°C and 35°C, significant ($p \leq 0.10$) positive correlations were found at distances of 0-10cm, while negative correlations were significant at distances $\geq 10$cm in the 25°C, 30°C, and 35°C treatments. This pattern of decreasing correlations with increasing distance was also evident when examined in 3 dimensions (Table 2.5). At 30°C and 35°C, significant ($p \leq 0.10$) positive correlations were found at distances of 0-10cm. Significant ($p \leq 0.10$) negative correlations were found at distances $\geq 10$cm in the 25°C, 30°C, and 35°C treatments.

### 2.4 Discussion

Field based studies of large grain lots are useful to examine the spatial distribution of grain pests in real systems (Hagstrum et al. 1985; Lippert and Hagstrum 1987). Nonetheless, the spatial resolution of data in such studies are
typically limited by factors such as available sampling methods, difficulty in accessing all parts of the grain bulk, and relatively low sampling intensity. Additionally, the effect of individual factors such as temperature and moisture gradients or inter- and intra-species competition on pest spatial distribution has been difficult to isolate. For these reasons, high resolution laboratory studies allow for the collection of data by isolating single factors and gathering data at an appropriate resolution for the particular question at hand. In a recent study, Jian et al. (2011) examined insect movement and spatial structure in a large (1.5 tonne) laboratory volume. The high sampling intensity (~15% of the total volume) employed in that study was sufficient to show spatial structuring of the pest population occurred, but large sample sizes (~15kg) restricted the ability to examine the spatial structure in detail. Again, while this design was appropriate for the questions Jian et al. (2011) were examining, fine-scale spatial structuring of populations could not be assessed.

In the current study, adult R. dominica were found to establish a spatially heterogeneous distribution pattern in grain within 2 weeks of introduction. The observed pattern of horizontal dispersion was similar across all treatments, but vertical dispersion was found to differ considerably at 35°C (Figure 2.1, Figure 2.2), with the majority of insects being found further down in the grain mass. Such spatial structuring, found consistently across replicates in each experiment, is unlikely to occur due to random insect movement. This suggests that behavioural variations due to environmental conditions are an important influence on spatial distribution. Flinn and Hagstrum (2011) showed that R. dominica tends to avoid temperatures above 35°C, favouring areas where the temperature was below 32°C. In the current study, such avoidance was not possible as grain temperature was constant throughout the volume. Our results suggest that where avoidance is impossible vertical dispersion is increased, with insects moving further into the grain mass. R. dominica is known to move deeper into bulk-stored grain than other grain pest species (Flinn et al. 2010). This behaviour appears to be enhanced at higher temperatures, potentially increasing the difficulty of detection and estimation of infestations. While higher temperatures such as these are close to the limit of R. dominica’s environmental tolerance (Hagstrum and Subramanyam 2006; Longstaff 1999), such temperatures can be
found inside bulk grain storages in warmer grain producing regions (Flinn et al. 2004).

Insect abundance had little effect on patterns of spatial distribution. Observed dispersion patterns were consistent within each treatment and at insect densities ranging from approximately 1.5 insects / L to more than 8 insects / L. Abundance in each treatment was found to be lower than predicted by the population model used (Driscoll et al. 2000). This model assumes a stable age structure, which would not be the case within one generation of initial pest introduction, and hence is likely to over-estimate populations in this scenario. However, the low abundance found at 25°C indicated an unexpectedly low population growth rate of only ~50% per generation. Conversely at 35°C there was higher variation in abundance between replicates. While the model used does not predict population variance, it is occasionally accounted for in other models (Hagstrum 1996). In cases where accurate estimation of population after one generation is required, a population growth model accounting for variable age structure would be required.

It was found that analysis in 3 dimensions allowed for the easy identification of strong correlations between sample abundances at varying distances. However, in the absence of a directional component to individual correlations, it was not able to describe the variations from a basic spherical diffusion pattern found. It was also found that correlations in one plane tended to oppose those in other planes, reducing the significance of the overall result. For example, negative correlations at 10-15cm in the vertical direction of the 30°C treatment affected the positive correlations found at this distance in the horizontal direction, reducing the significance of both. Conversely, performing individual 2 dimensional analyses in the X-Y, X-Z, and Y-Z planes, while slightly more complex to undertake and interpret, allowed for the identification and evaluation of the wide horizontal but limited vertical dispersion pattern found. In cases where the direction of dispersion or shape of aggregations is unknown, performing 2 dimensional correlations in multiple planes may provide a more accurate description of the observed spatial pattern when compared to 3 dimensions.
There is little available data on the movement rates of *R. dominica* in stored grain. Field-validated modelling studies (Flinn *et al.* 2004) have suggested a dispersal rate of approximately 1.2 meters per week at 29°C in a 3-dimensional storage. Laboratory studies under controlled conditions (Surtees 1964a; Surtees 1964c) in a 3D volume have suggested a rate of more than 15cm per week (at 25°C), and a spatial distribution approaching homogeneity. In contrast, this study shows considerable difference in insect movement rates in the vertical and horizontal directions, with variations in the resultant spatial structure occurring over distances as short as 10-15cm. This results in a significantly non-random spatial distribution, which in turn can influence the results of sampling and predictions based on an underlying assumption of random spatial distribution.

Several previous studies have examined the spatial structure of insects in grain storages (Athanassiou *et al.* 2011; Flinn *et al.* 2010). While results from these studies indicated similar patterns of insect spatial distribution at larger scales, the relatively large sample sizes and low sampling intensity used did not allow for analysis of variations in population structure over relatively short distances. As our results show, these short distance variations in structure are an important feature of insect clustering. The insect densities used in the current study (1.5 – 8 pests / L) were similar to those required by phytosanitary regulations, commercial requirements, or used in similar studies (Food and Environment Research Agency 2009; Grain Trade Australia 2011; Jian *et al.* 2011). The use of appropriately-sized and regularly spaced samples to examine almost 100% of the grain volume (ensuring an accurate population count) both enhances the ability to detect pest aggregations and minimises the influence of sample edge effect (Davis 1994; Stenseth and Hansson 1979).

An improved understanding of the factors affecting pest spatial distribution can be used to inform not only spatially-explicit population models (Thorpe 1997), but also abundance and detection sampling models (Elmouttie *et al.* 2010; Flinn *et al.* 1992; Hagstrum *et al.* 1985). The observed variation in spatial pattern with
temperature, in particular the differences in mean abundance versus depth found at higher temperatures, potentially increases the difficulty of detection and estimation of infestations. These results highlight the fact that temperature and other environmental factors need to be explicitly considered when developing and choosing methods and protocols for detection and abundance sampling of pests. As such small-scale spatial structuring of populations was previously unknown in *R. dominica*, this suggests that other pest species may also exhibit spatial structure at similar scales. Further study of this aspect of behaviour in other grain pests is required to determine if this may affect detection and abundance sampling for those species.
<table>
<thead>
<tr>
<th>Treatment</th>
<th>Temp (°C)</th>
<th>Duration (days)</th>
<th>No. of adults recovered</th>
<th>Total Insect density (adults / L)</th>
<th>Population Mean &amp; SE</th>
<th>No. of Clusters</th>
<th>No. of occupied samples</th>
<th>Ip</th>
<th>Imor</th>
<th>chi-sq (df = 199)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control R1</td>
<td>30</td>
<td>14</td>
<td>26</td>
<td>1.30</td>
<td>6</td>
<td>19</td>
<td>0.5105</td>
<td>6.7692</td>
<td>343.2308 &lt;0.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control R2</td>
<td>30</td>
<td>14</td>
<td>29</td>
<td>1.45</td>
<td>μ = 28 ± 1</td>
<td>4</td>
<td>22</td>
<td>0.5037</td>
<td>3.9409 281.3448 &lt;0.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control R3</td>
<td>30</td>
<td>14</td>
<td>29</td>
<td>1.45</td>
<td>1</td>
<td>21</td>
<td>0.5087</td>
<td>5.9113</td>
<td>336.5172 &lt;0.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25°C R1</td>
<td>25</td>
<td>59</td>
<td>43</td>
<td>2.15</td>
<td>3</td>
<td>27</td>
<td>0.5129</td>
<td>7.0875</td>
<td>454.6744 &lt;0.0001</td>
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<tr>
<td>25°C R2</td>
<td>25</td>
<td>59</td>
<td>46</td>
<td>2.30</td>
<td>μ = 44 ± 1</td>
<td>2</td>
<td>22</td>
<td>0.5220</td>
<td>10.6280 632.2609 &lt;0.0001</td>
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<tr>
<td>25°C R3</td>
<td>25</td>
<td>59</td>
<td>43</td>
<td>2.15</td>
<td>1</td>
<td>34</td>
<td>0.5012</td>
<td>2.4363</td>
<td>259.3256 0.0026</td>
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<tr>
<td>30°C R1</td>
<td>30</td>
<td>43</td>
<td>161</td>
<td>8.05</td>
<td>4</td>
<td>63</td>
<td>0.5172</td>
<td>8.0901</td>
<td>1333.4100 &lt;0.0001</td>
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<tr>
<td>30°C R2</td>
<td>30</td>
<td>43</td>
<td>163</td>
<td>8.15</td>
<td>μ = 143.67 ± 18.342</td>
<td>3</td>
<td>60</td>
<td>0.5135</td>
<td>6.6349 1111.8470 &lt;0.0001</td>
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<td>30°C R3</td>
<td>30</td>
<td>43</td>
<td>107</td>
<td>5.35</td>
<td>3</td>
<td>50</td>
<td>0.5127</td>
<td>6.4186</td>
<td>773.3738 &lt;0.0001</td>
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<tr>
<td>35°C R1</td>
<td>35</td>
<td>31</td>
<td>105</td>
<td>5.25</td>
<td>μ = 91.7 ±</td>
<td>1</td>
<td>51</td>
<td>0.5065</td>
<td>3.9560 506.4286 &lt;0.0001</td>
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</tbody>
</table>
Table 2.1: Summary of experimental treatments and non-dimensional results. R1-3 are replicates within each treatment. $I_p$ is the scaled Morisita's index of dispersion. $I_{mor}$ is the unscaled Morisita's index, with associated chi-sq and $p$ values.

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Replicate</th>
<th>$I_p$</th>
<th>$I_{mor}$</th>
<th>Chi-sq</th>
<th>Degree of Freedom</th>
<th>$p$ value</th>
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<tr>
<td>35°C R2</td>
<td>35</td>
<td>31</td>
<td>45</td>
<td>2.25</td>
<td>4</td>
<td>0.5089</td>
</tr>
<tr>
<td>35°C R3</td>
<td>35</td>
<td>31</td>
<td>125</td>
<td>6.25</td>
<td>1</td>
<td>0.5071</td>
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Table 2.2: Correlation coefficient of insect densities at different inter-sample distances in the 2-D horizontal X-Y plane.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N_{adult}</th>
<th>H(0,[0-5])</th>
<th>H(0,[5-10])</th>
<th>H(0,[10-15])</th>
<th>H(0,[15-20])</th>
<th>H(0,[20-25])</th>
<th>H(0,[25-30])</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control R1</td>
<td>26</td>
<td>0.4132 *</td>
<td>-0.1679</td>
<td>-0.3246 *</td>
<td>-0.038</td>
<td>-0.2161</td>
<td>N/A</td>
</tr>
<tr>
<td>Control R2</td>
<td>29</td>
<td>-0.1936</td>
<td>0.0199</td>
<td>0.0131</td>
<td>0.2187</td>
<td>0.075</td>
<td>N/A</td>
</tr>
<tr>
<td>Control R3</td>
<td>29</td>
<td>0.1896</td>
<td>-0.2237</td>
<td>-0.0666</td>
<td>0.1081</td>
<td>-0.1941</td>
<td>N/A</td>
</tr>
<tr>
<td>25°C R1</td>
<td>43</td>
<td>0.2397</td>
<td>-0.153</td>
<td>-0.4156 *</td>
<td>-0.2769</td>
<td>-0.1884</td>
<td>N/A</td>
</tr>
<tr>
<td>25°C R2</td>
<td>46</td>
<td>0.2567</td>
<td>-0.3101 *</td>
<td>-0.3997 *</td>
<td>-0.0607</td>
<td>0.1786</td>
<td>N/A</td>
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<tr>
<td>25°C R3</td>
<td>43</td>
<td>0.0873</td>
<td>-0.0074</td>
<td>-0.2227 *</td>
<td>-0.1603</td>
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<tr>
<td>30°C R1</td>
<td>161</td>
<td>0.126</td>
<td>-0.1589</td>
<td>-0.0519</td>
<td>-0.0555</td>
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<td>30°C R2</td>
<td>163</td>
<td>0.1</td>
<td>0.109</td>
<td>-0.0157</td>
<td>-0.0486</td>
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<tr>
<td>30°C R3</td>
<td>107</td>
<td>0.2233</td>
<td>-0.0512</td>
<td>-0.0121</td>
<td>-0.1259</td>
<td>-0.2751 *</td>
<td>N/A</td>
</tr>
<tr>
<td>35°C R1</td>
<td>105</td>
<td>0.2897 *</td>
<td>-0.1355</td>
<td>-0.3302 *</td>
<td>-0.3041 *</td>
<td>0.032</td>
<td>N/A</td>
</tr>
<tr>
<td>35°C R2</td>
<td>45</td>
<td>0.3873 *</td>
<td>-0.0846</td>
<td>-0.0399</td>
<td>-0.2448</td>
<td>-0.4438 *</td>
<td>N/A</td>
</tr>
<tr>
<td>35°C R3</td>
<td>125</td>
<td>0.3662 *</td>
<td>-0.0394</td>
<td>-0.27 *</td>
<td>-0.1994</td>
<td>0.0069</td>
<td>N/A</td>
</tr>
</tbody>
</table>
* significant at $p = 0.10$

N/A = no value calculated due to insufficient observations at this distance.
Table 2.3: Correlation coefficient of insect densities at different inter-sample distances in the 2-D vertical X-Y plane.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N_{adult}</th>
<th>V(0,[0-5])</th>
<th>V(0,[5-10])</th>
<th>V(0,[10-15])</th>
<th>V(0,[15-20])</th>
<th>V(0,[20-25])</th>
<th>V(0,[25-30])</th>
<th>V(0,[30-35])</th>
<th>V(0,[35-40])</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control R1</td>
<td>26</td>
<td>-0.2719</td>
<td>0.0325</td>
<td>-0.0225</td>
<td>-0.1587</td>
<td>0.1153</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Control R2</td>
<td>29</td>
<td>0.0287</td>
<td>-0.2894</td>
<td>-0.114</td>
<td>0.2239</td>
<td>0.071</td>
<td>N/A</td>
<td>-0.405 *</td>
<td>N/A</td>
</tr>
<tr>
<td>Control R3</td>
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<td>-0.2418</td>
<td>-0.1837</td>
<td>-0.1015</td>
<td>0.3714</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>25°C R1</td>
<td>43</td>
<td>0.2596</td>
<td>-0.1175</td>
<td>-0.4312 *</td>
<td>0.0064</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>25°C R2</td>
<td>46</td>
<td>0.3361 *</td>
<td>-0.1375</td>
<td>-0.2497</td>
<td>-0.1845</td>
<td>-0.2223</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>25°C R3</td>
<td>43</td>
<td>0.0513</td>
<td>0.0098</td>
<td>-0.306 **</td>
<td>-0.2881 *</td>
<td>-0.2908</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>30°C R1</td>
<td>161</td>
<td>0.3256 *</td>
<td>-0.0391</td>
<td>0.0564</td>
<td>-0.0676</td>
<td>-0.2679 *</td>
<td>-0.2577</td>
<td>-0.3203</td>
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</tr>
<tr>
<td>30°C R2</td>
<td>163</td>
<td>0.5075 *</td>
<td>0.2654 *</td>
<td>0.1016</td>
<td>-0.1298</td>
<td>-0.2193 *</td>
<td>-0.4179 *</td>
<td>-0.5473 *</td>
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<tr>
<td>30°C R3</td>
<td>107</td>
<td>0.2283</td>
<td>0.1633</td>
<td>-0.0924</td>
<td>-0.0771</td>
<td>-0.3247 *</td>
<td>-0.3204</td>
<td>-0.4483 *</td>
<td>N/A</td>
</tr>
<tr>
<td>35°C R1</td>
<td>105</td>
<td>0.4275 *</td>
<td>0.224 *</td>
<td>-0.215 *</td>
<td>-0.2934 *</td>
<td>-0.3413 *</td>
<td>-0.3294 *</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>35°C R2</td>
<td>45</td>
<td>0.2651</td>
<td>-0.0148</td>
<td>-0.042</td>
<td>-0.2716 *</td>
<td>-0.1791</td>
<td>-0.1112</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>35°C R3</td>
<td>125</td>
<td>0.2613 *</td>
<td>0.1426</td>
<td>-0.1885 *</td>
<td>-0.1862 *</td>
<td>-0.1498</td>
<td>-0.031</td>
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<td>N/A</td>
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</table>
* significant at $p=0.10$

N/A = no value calculated due to insufficient observations at this distance.
Table 2.4: Correlation coefficient of insect densities at different inter-samples distances in the 2-D vertical Y-Z plane.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N_{adult}</th>
<th>V(0,[0-5])</th>
<th>V(0,[5-10])</th>
<th>V(0,[10-15])</th>
<th>V(0,[15-20])</th>
<th>V(0,[20-25])</th>
<th>V(0,[25-30])</th>
<th>V(0,[30-35])</th>
<th>V(0,[35-40])</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control R1</td>
<td>26</td>
<td>0.0819</td>
<td>-0.166</td>
<td>-0.1245</td>
<td>-0.1906</td>
<td>0.2923</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Control R2</td>
<td>29</td>
<td>0.006</td>
<td>0.0363</td>
<td>-0.1663</td>
<td>-0.3511</td>
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<td>-0.3472</td>
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<td>N/A</td>
</tr>
<tr>
<td>Control R3</td>
<td>29</td>
<td>-0.0056</td>
<td>-0.2273</td>
<td>-0.1528</td>
<td>0.0261</td>
<td>-0.1905</td>
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<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>25°C R1</td>
<td>43</td>
<td>-0.1017</td>
<td>-0.0381</td>
<td>-0.2015</td>
<td>-0.2981</td>
<td>-0.1902</td>
<td>-0.4359</td>
<td>-0.4324</td>
<td>N/A</td>
</tr>
<tr>
<td>25°C R2</td>
<td>46</td>
<td>0.2618</td>
<td>-0.2076</td>
<td>-0.2615</td>
<td>-0.1818</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
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<tr>
<td>25°C R3</td>
<td>43</td>
<td>0.2574</td>
<td>0.1104</td>
<td>-0.2305 *</td>
<td>-0.3981 *</td>
<td>-0.3458 *</td>
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<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>30°C R1</td>
<td>161</td>
<td>0.4218 *</td>
<td>0.3162 *</td>
<td>0.0691</td>
<td>-0.0571</td>
<td>-0.2749 *</td>
<td>-0.4603 *</td>
<td>-0.4742 *</td>
<td>-0.5913 *</td>
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<tr>
<td>30°C R2</td>
<td>163</td>
<td>0.3784 *</td>
<td>0.0481</td>
<td>-0.0513</td>
<td>-0.1508</td>
<td>-0.2741 *</td>
<td>-0.3997 *</td>
<td>-0.4517 *</td>
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<td>30°C R3</td>
<td>107</td>
<td>0.4762 *</td>
<td>0.2162</td>
<td>-0.0039</td>
<td>-0.2369 *</td>
<td>-0.297 *</td>
<td>-0.3113</td>
<td>-0.3401 *</td>
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<tr>
<td>35°C R1</td>
<td>105</td>
<td>0.4404 *</td>
<td>0.1011</td>
<td>-0.2621 *</td>
<td>-0.3051 *</td>
<td>-0.2923 *</td>
<td>-0.4217 *</td>
<td>-0.3962</td>
<td>N/A</td>
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<tr>
<td>35°C R2</td>
<td>45</td>
<td>0.3733</td>
<td>0.1203</td>
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<td>0.2412</td>
<td>-0.0507</td>
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<td>0.0857</td>
<td>N/A</td>
</tr>
<tr>
<td>35°C R3</td>
<td>125</td>
<td>0.3342 *</td>
<td>0.0818</td>
<td>-0.067</td>
<td>-0.2863 *</td>
<td>-0.1339</td>
<td>-0.0461</td>
<td>0.1725</td>
<td>N/A</td>
</tr>
</tbody>
</table>
* significant at $p = 0.10$

N/A = no value calculated due to insufficient observations at this distance.
Table 2.5: Correlation coefficient of insect densities at different inter-sample distances in 3-D X-Y-Z space.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N_{adult}</th>
<th>D(0,[0-5])</th>
<th>D(0,[5-10])</th>
<th>D(0,[10-15])</th>
<th>D(0,[15-20])</th>
<th>D(0,[20-25])</th>
<th>D(0,[25-30])</th>
<th>D(0,[30-35])</th>
<th>D(0,[35-40])</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control R1</td>
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<td>0.0905</td>
<td>0.4404</td>
<td>-0.1606</td>
<td>-0.205</td>
<td>-0.1745</td>
<td>0.01874</td>
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<td>N/A</td>
</tr>
<tr>
<td>Control R2</td>
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<td>-0.3948 *</td>
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<td>-0.0068</td>
<td>0.1251</td>
<td>-0.1749</td>
<td>-0.2549</td>
<td>-0.189</td>
</tr>
<tr>
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<td>0.0759</td>
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<td>N/A</td>
<td>N/A</td>
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<td>-0.1208</td>
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<td>N/A</td>
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<tr>
<td>25°C R2</td>
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<td>-0.2977 *</td>
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<td>N/A</td>
<td>N/A</td>
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<td>25°C R3</td>
<td>43</td>
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<td>N/A</td>
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<td>30°C R1</td>
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<td>0.2357 *</td>
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<td>-0.0718</td>
<td>-0.0234</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>30°C R2</td>
<td>163</td>
<td>0.1864 *</td>
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<td>-0.0211</td>
<td>-0.0658</td>
<td>-0.1493 *</td>
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<td>N/A</td>
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<tr>
<td>30°C R3</td>
<td>107</td>
<td>0.101</td>
<td>-0.0714</td>
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<td>-0.0595</td>
<td>-0.0732</td>
<td>-0.132</td>
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<td>N/A</td>
</tr>
<tr>
<td>35°C R1</td>
<td>105</td>
<td>0.3297 *</td>
<td>0.0944</td>
<td>-0.1342 *</td>
<td>-0.2423 *</td>
<td>-0.2488 *</td>
<td>-0.2294 *</td>
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<td>N/A</td>
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<tr>
<td>35°C R2</td>
<td>45</td>
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<td>0.2743 *</td>
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<td>-0.0719</td>
<td>-0.1422</td>
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<td>N/A</td>
</tr>
<tr>
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<td>-0.1002 *</td>
<td>0.0213</td>
<td>N/A</td>
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</tbody>
</table>
* significant at $p = 0.10$

N/A = no value calculated due to insufficient observations at this distance.
Figure 2.1: Mean abundance of adult *Rhyzopertha dominica* in each 5cm layer, for (a) 30°C control, (b) 25°C, (c) 30°C, and (d) 35°C treatments.
Figure 2.2: Representative horizontal (top) and vertical (bottom) spatial distributions, based on the average of 3 replicates. Indicated pest density is relative to the mean pest density of each treatment; darker areas indicate higher densities.
Chapter 3: Paper 2 - Incorporating pest distribution and spatial occupancy in a simple stored-grain supply chain model

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Prepared for submission to Environmental Modelling and Software
Statement of Contribution of Co-Authors for Thesis by Published Paper

The authors listed below have certified* that:

1. they meet the criteria for authorship in that they have participated in the conception, execution, or interpretation, of at least that part of the publication in their field of expertise;
2. they take public responsibility for their part of the publication, except for the responsible author who accepts overall responsibility for the publication;
3. there are no other authors of the publication according to these criteria;
4. potential conflicts of interest have been disclosed to (a) granting bodies, (b) the editor or publisher of journals or other publications, and (c) the head of the responsible academic unit, and
5. they agree to the use of the publication in the student’s thesis and its publication on the QUT ePrints database consistent with any limitations set by publisher requirements.

In the case of this chapter: **Paper 2 - Incorporating pest distribution and spatial occupancy in a simple stored-grain supply chain model**

(Submitted to Environmental Modelling and Software, November 2012)

<table>
<thead>
<tr>
<th>Contributor</th>
<th>Statement of contribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roderic Steel</td>
<td>Primarily responsible for the experimental design, wholly responsible for the laboratory</td>
</tr>
<tr>
<td></td>
<td>studies and data analysis, extensively altered original simulation model, primarily</td>
</tr>
<tr>
<td></td>
<td>responsible for writing manuscript.</td>
</tr>
<tr>
<td>09/11/12</td>
<td></td>
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<tr>
<td>Andreas Kiermeier</td>
<td>Wrote original simulation model, minor manuscript editing.</td>
</tr>
<tr>
<td>Grant Hamilton</td>
<td>Project supervisor, provided advice about experimental design and statistical analyses,</td>
</tr>
<tr>
<td></td>
<td>assisted with writing drafts of manuscript.</td>
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</table>

Principal Supervisor Confirmation

I have sighted email or other correspondence from all Co-authors confirming their certifying authorship.

_______________________ ____________________   ____________________

Name    Signature    Date
Abstract: The presence of insect pests in grain storages throughout the supply chain is a significant problem for farmers, grain handlers, and distributors world-wide. The spatial distribution of pests within the grain bulk is often heterogeneous, and may be affected by factors such as pest behaviour, the site of initial infestation, and disturbance during handling. This in turn may affect the reliability of sampling used for pest detection and population estimates. Typical supply chain models do not include these factors as part of pest population estimates, and so are of limited use when coupled with newer sampling models that rely on knowledge of pest spatial distribution. To account for these factors, we develop a simulation model that incorporates both pest growth and movement through a typical stored-grain supply chain. Using experimental data, we also incorporate the effect of initial infestation site, disturbance during handing, and treatment on pest spatial distribution. We then use these estimates to inform a spatially-aware sampling model, and test several scenarios to examine the influence of infestation location and grain disturbance on the sampling intensity required to detect pest infestations at various infestation rates.
3.1 INTRODUCTION

Stored grain crops, such as wheat, barley, and sorghum, are a major food source for humans and domesticated animals worldwide. As well as being major domestic and export crops, production is variable depending on weather and economic conditions, so grain is often stored in and moved along a complex supply chain. Insect infestations typically occur post-harvest (Flinn et al. 2010; Sinclair 1982), causing quantity and quality losses and reducing economic return (Waterfield and Zilberman 2011).

A variety of systems have been developed for pest management in stored grain systems. These range from relatively simple calendar-based sampling and treatment regimes designed to control pest infestations during storage, up to sophisticated Integrated Pest Management (IPM) tools. IPM tools such as Stored Grain Advisor Pro (Flinn et al. 2007), Integrated Grain Storage Manager, or PestMan (Longstaff 1997) have been designed to inform the decision-making process and recommend management strategies, and depend on accurate information with regards to storage conditions and pest abundance. Typically periodic sampling is undertaken and, in concert with insect growth models and an economic decision-making framework, recommendations are made with regards to pest treatment or grain quality management (Adam et al. 2006; Phillips et al. 2000).

While these and other existing tools perform well for the purpose of pest management within a single storage or facility, they are unable to account for the influence of grain movement through the supply chain on pest detection. Sampling and population models typically operate under the assumption that pest spatial distribution is homogenous (Elmouttie et al. 2010); however, previous studies show that spatial distribution is often heterogeneous (Hagstrum et al. 1985). In a typical supply chain grain moves through multiple storages between production and the end-user, and handling, transport, and pest treatment at each stage can affect spatial distribution and occupancy. In addition, the location of the initial infestation may also affect spatial distribution. Most grain insect pests display some degree of preference for particular regions within a grain mass based on biological or
environmental conditions, and so infestations originating from the bottom of the grain mass due to latent infestations in storages (Hagstrum et al. 2010) or entry via discharge spouts (Reed et al. 2003) may tend to disperse differently than those originating from the top. All these factors can influence the spatial distribution of pests, and so affect the accuracy of sampling models.

At the heart of all such tools is a requirement to accurately estimate and detect pest populations before they reach economically damaging levels. It has previously been reported that current sampling protocols are often limited by physical and economic factors (Jefferies 2000) and may fail to detect or correctly estimate pest abundance at low levels (Rajendran 1999). Alternative sampling schemes have been developed (e.g. Elmouttie et al. 2010; Hagstrum et al. 1985) to improve detection at low levels, however a key requirement of these is an understanding of the spatial distribution of pests within the grain bulk. Therefore, a model that not only simulates movement of grain and pests along a supply chain, but also incorporates the effect of this movement on the spatial distribution of pests, is required to inform detection sampling and improve overall grain pest management.

To develop an appropriate model of spatial distribution in grain storages requires fine scale 3 dimensional data describing pest abundance and location. This is difficult or impossible to obtain from sampling in large grain storages, so experimental data from small representative systems must be used (Steel et al. 2012). By manipulating these systems to simulate differing infestation locations, as well as mixing during handling, it is possible to determine and model the effect of each factor on the resultant pest spatial distribution. This can then be applied, along with existing pest growth and sampling models, to the development of an overall supply chain model.

In this study, a simulation model of a typical stored grain supply chain is developed. This model not only simulates movement of grain along a supply chain, but also incorporates the effect of pest location, disturbance during handling, treatment of pests during storage, and growth of pests on the spatial distribution of
pests at each stage of the chain. Experimental data is generated and used to model the proportion of grain infested for a major grain pest (*Rhyzopertha dominica* (F.)) under various conditions, and is used in the supply chain model to inform a previously published sampling model (Elmouttie *et al*. 2010) to detect pests at the estimated abundance. This extended supply chain model, incorporating the effect of introduction point and mixing on the spatial occupancy of pest populations, is then used to consider several scenarios relating to the effect of each factor on the probability of detection when sampling.

### 3.2 MODEL DESCRIPTION

The model developed is a Monte Carlo simulation of a generalized grain supply chain (Figure 3.1). Movement and allocation of grain (including pests) between farm, regional and export storages, growth of pests during storage, and the effectiveness of pest treatments, are simulated by the model. For each storage the amount of grain held, the infestation rate, the proportion of grain infested, and the number of samples required to detect pests at the estimated infestation rate are calculated. The result is a supply chain model that estimates pest abundance, the proportion of grain infested, and the number of samples required for detection at each step.

Initially, grain is stored at the originating farms in multiple farm silos (storage level A in Figure 3.1). For each individual silo, the weight of grain stored and the pest population are both described by normal distributions. A description of the spatial distribution of pests within the grain, in the form of the proportion of grain infested by pests, is described by a Beta distribution. This information about the grain volume and infestation status of these storages forms the primary inputs to the model.

Regional silos receive grain from each individual farm silo. Transport and handling may influence pest dispersion within the grain bulk, affecting the spatial distribution of pests. This may result in a change in the proportion of grain infested,
so a ‘mixing’ parameter is included to adjust the current proportion infested based on
the proportion infested prior to handling. On delivery, grain may be treated for pests;
the success rate of treatment at each stage is adjustable between 0 and 100%. After
treatment, grain is stored until required for export. During this storage period, pest
numbers can increase or decrease depending on environmental factors such as
temperature and relative humidity. At the conclusion of regional storage, grain from
each individual regional silo may be partly or wholly allocated to one or more export
silos.

Export silos receive grain from the regional silos. As grain from individual
regional silos may be split across multiple export silos, the weight of grain, the
number of pests, and the proportion of grain infested in each export silo is calculated
based on the proportionate contribution of each regional silo. As in regional silos,
‘mixing’ affects the proportion of grain infested, and treatment reduces the number
of pests.

Pest growth or decline during regional and export storage is calculated using an
established population growth model (Driscoll et al. 2000) based on storage duration,
temperature, and relative humidity. As a result, at the end of each model run the final
pest populations and proportion of grain infested are the result of initial conditions,
pest mixing and movement during transport between storages, the effectiveness of
pest treatment, and the environmental conditions and duration of storage at each
stage.

After each run of the model, results are saved. These contain the results from
each Monte Carlo iteration, and consist of the amount of grain stored in each
regional and export silo: the number of pest insects at delivery, post-treatment, and
post-storage in regional and export silos; the proportion of grain infested in each silo,
and the number of samples required to detect infestations at the estimated rate. In
addition, a summary file containing the grain weight, proportion infested, number of
pests, and infestation rate for each export silo is saved.
3.2.1 Grain and pest movement

Grain movement between storage levels (Figure 3.1) is pre-configured prior to model runtime. The contents of each farm silo are allocated to a specific regional storage. Export silos are numbered from 1 to $N$, and receive grain (including pests) from one or more regional silos. Unlike the case of grain movement from farm silos to regional silos, grain from each regional silo can be split across multiple export silos. Consequently, for each combination of regional and export silos, grain movement is determined by the value of the allocation parameter $\theta_{ij}$, which indicates the proportion of grain from each regional silo $j$ that moves to export silo $i$. In this case, $\theta_{ij}$ is also used to describe the allocation of pests from regional silo $j$ to export silo $i$.

3.2.2 Proportion of grain infested

Each farm silo can either contain grain pests or be free from infestation. If there is contamination in the silo, then this is specified via (a) the proportion $p$ of the silo that is infested, and (b) the number of pests that are contained in the infested part. This approach follows that proposed by Elmouttie et al. (2010), where the number of pests is directly related to the rate of contamination $\lambda$. The proportion contaminated is assumed to be distributed according to a Beta distribution with parameters $a$ and $b$, i.e.

$$p \sim B(a, b)$$

as per Johnson et al. (1994).

Based on this specification of the Beta distribution, the mean of $p$ is given by

$$E[p] = \frac{a}{a + b}$$
and standard deviation is

\[ SD[p] = \sqrt{\frac{ab}{(a + b + 1)(a + b)^2}} \]

Where an alternative model describing the proportion contaminated is available, it may be incorporated in the model and used in place of the Beta distribution. The development and implementation of such an occupancy model for *Rhyzopertha dominica* is described in sections 3.2.3 and 3.4.1.

For all species, the proportion infested after storage (farm storage only) may be affected by the location of the point of infestation. When the proportion is specified as a Beta distribution, it is assumed to include any effect of pest location. However, when using an alternative occupancy model, the value of this effect must be specified separately. If this information is available (see section 3.4.2) the proportion infested can be scaled between 0.5 and 1.5 times the original estimate.

The proportion of grain infested may also be affected by insect movement and increased dispersion within the grain bulk during loading, unloading, and transport between storages. Where data quantifying this effect is available (see section 3.4.2) this is incorporated in the model through a user-defined scaling factor, which allows the proportion of grain infested to be increased by a factor of 1 to 2 times. In the model this can occur between farm and regional storages, regional and export storages, or both, and is individually configurable at either stage.

Hence, the total proportion of the grain infested may be calculated as

\[ p_{total} = p_{initial} \times S_{intro} \times S_{mixing} \]
where \( p_{\text{initial}} \) is the initial estimate of the proportion infested, \( S_{\text{intro}} \) is the scaling factor due to the effect of the introduction point, \( S_{\text{mixing}} \) is the scaling factor resulting from the effect of mixing, and \( p_{\text{total}} \) is the final estimate of the proportion infested (limited to 100% of the total grain volume).

### 3.2.3 R. dominica proportion model

For \emph{R. dominica} only, the option is provided to estimate the proportion of grain infested from population parameters. This is based on the experimental results summarized in (Table 3.1), and overrides any parameters describing the infested proportion supplied as part of the initial conditions of the model. The proportion infested \((p)\) is determined by the function described in section 3.4.1. Since pest abundance is known to affect the proportion of grain infested (Steel et al. 2012), this method allows the re-calculation of the proportion infested after pest growth or treatment. This provides a significant advantage over existing models, which assume either homogeneity or a fixed pest distribution within the grain bulks.

### 3.2.4 Detection sampling model

A detection sampling model is also included in the supply chain model. This is an implementation of the sampling model proposed by Elmouttie \emph{et al.} (2010) for pest detection in grain. Given an estimate of the proportion infested \((p)\) and the rate of infestation in that proportion \((\lambda)\), the number of samples \((n)\) required to detect infestation at the estimated rate is

\[
n = \frac{\log \beta}{\log \left(1 - p + pe^{-w\lambda}\right)}
\]

(Elmouttie \emph{et al.} 2010)

where \( \beta \) is the acceptable probability of a Type II error (e.g. 0.05), and \( w \) is the individual sample weight (in kg).
3.3 EXPERIMENTAL

The design of experiments to quantify the effect of mixing and infestation location on the spatial distribution of *R. dominica* followed the method published by Steel *et al.* (2012). For each experiment, three replicate lots consisting of 20 litre food grade polypropylene containers (26w x 26d x 39h cm) were filled with 20 litres (15.8kg) of grain. Australian Prime Hard wheat (*Triticum aestivum* (L.)), certified organic and pesticide-free (CO2-treated), was used in all experiments. All grain used had been frozen (-18°C) for a minimum of 2 weeks, followed by storage at 5 ±1°C until required, to ensure freedom from pests (Fields 1992). A random sample (approx. 1 L) of grain from each lot was inspected for pests using a hand sieve, and no adult insects were found. After preparation, experimental lots were transferred to temperature and humidity controlled (55% relative humidity) cabinets and acclimatised to experimental conditions for a minimum 48hrs.

A total of six experiments were conducted. In each case, thirty 0-7 day old adult *R. dominica* were introduced to each experimental lot. An initial experiment was conducted at 30°C for 14 days to determine spatial distribution at low pest density (approximately 2 pests / litre). To assess spatial distribution at higher pest densities, three experiments of 1 generation duration, were conducted at 25°C for 59 days, 30°C for 43 days, and 35°C for 31 days. For these four experiments, insects were added to the centre top surface of the grain. Each lot was then covered with a tight-fitting mesh cover and incubated for the experimental duration. Data from these experiments was analysed to develop the default occupancy model.

A fifth experiment was undertaken to determine the effect of mixing and an alternative infestation location at the bottom of the grain mass on pest spatial distribution. To simulate grain mixing, prior to the introduction and acclimation of grain, a 40mm hole was drilled in the bottom centre of each experimental container and sealed with a blind rubber grommet. As before, after acclimation insects were added to the top surface of each lot. After the grain and insects had been incubated for 14 days at 30°C, the modified containers were suspended above empty containers and the bung removed to allow grain to pour into the lower container. The lower
containers were then returned to the same temperature controlled cabinet for the remaining 29 days of the generation time (total 43 days).

The final experiment was undertaken to determine the effect of an alternative insect introduction point (e.g. a residual infestation at the bottom of a storage). To simulate this, insects were added directly to the bottom centre of each lot. This was achieved by inserting a 5mm ID glass tube containing a close-fitting glass rod from the top of grain to the bottom. Once positioned the glass rod was withdrawn, a funnel fitted into the glass tube, and insects introduced directly to the bottom centre of the grain mass. The glass tube was then withdrawn, and containers were incubated at 30°C for 43 days (1 generation).

After incubation each container was prepared and sampled using the method described by Steel et al. (2012). This resulted in 200 samples, spatially referenced in 3 dimensions and representing over 95% of the total grain volume, from each container. Each sample was then examined for the presence and number of adult insects internal and external to grain kernels. The total of all adults in each sample was used in all calculations.

3.4 SCENARIOS

After modification of the supply chain model, several scenarios were examined. In each case, grain in 15 farm storages was delivered to one of 3 regional storages, stored, then transferred to the 3 export storages. The disposition of grain between storages is shown in Figure 3.1. At regional and export storages, grain was stored for 10 weeks at 25°C and 55% relative humidity. An initial infestation of 2 pests / kg in all farm storages, and a 95% treatment success at the regional and export storages, was assumed for each scenario. The scenarios examined consisted of (1) no effect of pest introduction point or mixing, (2) a pest introduction point effect at the farm storage, but no mixing effect, (3) no pest introduction point effect, but mixing effects at both regional and export storages, and (4) a pest introduction point effect at the farm storage and mixing effects at regional and export storages. The values for
the introduction and mixing effect used in scenarios 2, 3, and 4 were derived from the experimental data as described in section 3.4.2 and shown in (Table 3.1). For the sampling model, a Type II error probability of $\beta = 0.05$ and sample weight of $w = 0.8\text{kg}$ (typical of grain sampling) was used. For each run of the Monte Carlo simulation model, the number of iterations was fixed at 1000.

3.4.1 Occupancy Model

Summaries of the data from the occupancy experiments are provided in (Table 3.1). The occupancy model was developed using data from the top introduction point treatments only (i.e. no pest introduction point or mixing effects), covering three grain temperatures from 25°C to 35°C with infestation rates in individual replicates ranging from 1.65 to 10.32 pests per kg. A plot of proportion infested vs infestation rate for each replicate (Figure 3.2) suggested a logarithmic relationship, so the ‘nls’ function in R (R Core Development Team 2011) was used to fit an equation to the observed data. This resulted in the function:

$$p_{est} = \log(Z + 2.581) \times 19.493 - 18.494$$

where $Z$ is the overall infestation rate in pests/kg, and $p_{est}$ is the proportion of grain infested (as a percentage of the whole). Using standard diagnostic plots, this function was found to be a good fit to the data (RSS = 26.97, RSE = 1.731 with df = 9) (Figure 3.2).

3.4.2 Mixing and Introduction Point

To assess the effects of mixing and introduction point the occupancy model was used to predict the proportion infestation from the actual infestation rate for each replicate of the six infestation experiments. Then the relative difference in the proportion infested was calculated as

$$\text{Rel. diff.} = \frac{P_{obs} - P_{est}}{P_{est}} \times 100\%$$
where \( p_{\text{obs}} \) is the proportion infested observed in the corresponding experiments. For each of the six scenarios the mean and standard error of the relative differences were calculated (Table 3.1). From this table it can be seen that the model predicted the proportion infested well for the first four experiments, with a relative difference of less than 2.5%. However, for the mixing scenario the model underpredicted the proportion infested by about 24% (mean = 24.12, SE = 3.14), which implies that mixing will substantially spread the infestation through the grain mass. In contrast, introducing pest at the bottom of the grain mass resulted in a 22% (mean = 22.16%, SE = 5.88) smaller proportion infested compared to the predictions from the model, which was developed for infestation from the top.

### 3.4.3 Supply chain model

The pest occupancy model outlined in section 3.4.1, and the sampling model of Elmouttie et al. (Elmouttie et al. 2010), were coded in R and incorporated into the supply chain model. The effects of initial pest location and mixing along the supply chain were incorporated into the model by including GUI controls to modify the estimated pest occupancy. The user interface of the amended model is shown in Figure 3.3.

The four scenarios considered differed only in the effect of infestation point and mixing. In Scenarios 1 and 4, insects were considered to have infested the grain by immigration to the top surface with no additional effect on proportion infested (\( S_{\text{mixing}} = 1 \)); for Scenarios 2 and 4, infestations were considered to be due to residual infestations in storages, reducing the proportion infested by 22% (\( S_{\text{mixing}} = 0.78 \)). Similarly, Scenario 1 assumed no mixing occurred during transport between storages, while in Scenarios 2, 3, and 4, mixing increased the proportion of grain infested by 24% (\( S_{\text{mixing}} = 1.24 \)) at both the farm to regional and regional to export stages.
When initial pest location was assumed to reduce spatial occupancy by 22% (Scenarios 2 and 4), the number of samples required to detect pests increased. For example, at farm storages with an infestation rate of 2 pests/kg, the number of samples required for detection increased 25 to 33; at export storages with a post-treatment infestation rate of 0.36 pests/kg, the number of samples increased from 229 to 294 (Table 3.2). Overall, the effect of a reduction in spatial occupancy by 22% was to increase the number of samples required by 31.51% (s.d. = 4.04). Conversely, when mixing between farm, regional, and export storages was assumed to increase spatial occupancy by 24% at each stage (scenarios 3 and 4), the number of samples required decreased. In scenario 3, at export storages post-treatment with an infestation rate of 0.36 pests/kg, the number of samples required for detection fell from 229 to 149, while at an export post-storage infestation rate of 26 pests/kg the number of samples required was reduced from 5 to 3 (Table 3.2). On average, the effect of an increase in spatial occupancy due to mixing at farm-to-regional and regional-to-export stages was to decrease the number of samples required at regional and export storages by 29.06% (s.d. = 10.31). In combination, the effect of both introduction point and mixing was to decrease the number of samples required by 6.92% (s.d. = 23.26) across farm, regional, and export storages (scenario 4).

3.5 DISCUSSION

Previous attempts to examine the spatial distribution of pests in stored grain have assumed infestation occurs at the grain surface. Although the potential for grain residues in storages to act as sources for re-infestation has previously been noted (Hagstrum et al. 2010; Reed et al. 2003), the effect of introduction site on pest spatial distribution and occupancy has not previously been studied. Similarly, while transport of grain along a supply chain is known to affect pest spatial distribution (Perez-Mendoza et al. 2004), the effect has not been quantified in comparison to undisturbed grain.

This study is the first to examine the effect of both the site of infestation and grain turnover on pest distribution within grain. It was found that an infestation originating from the bottom of the grain bulk reduced the proportion infested by
approximately 22% when compared to an infestation point at the surface. Mixing was expected to increase the proportion of grain infested, and the mixing method used in these experiments resulted in an approximate 24% increase in pest occupancy when compared to model estimates for unmixed grain. In both cases, the estimated sampling intensity required for pest detection differed from that obtained from the scenario where infestations originated at the surface of the grain bulk and were not disturbed by subsequent handling. If the proportion infested is not accounted for there is a risk of under- or over-sampling, which may result in either infestations remaining undetected or incorrect estimates of pest populations. Either of these outcomes can result in sub-optimal or unnecessary pest management actions and associated increase in management costs or decrease in grain quality.

Replicate 2 of the bottom introduction point treatment showed an unexpectedly high population (883) of insects, with a commensurate increase in the infestation rate (55.9 pests/kg) and proportion infested (0.505). Despite being well outside the fitted range of the occupancy model (Figure 3.2), when the effect of introduction point was included in the occupancy model the resultant estimate (0.474) was comparable to the observed occupation rate. The reason for this high infestation rate is unknown, although the proportion of grain infested was consistent with model predictions based on the observed infestation rate and location of infestation. This would suggest that the result was not influenced by residual infestation in the grain or additional pest immigration.

The enhanced supply chain model described in this study indicates that including estimates of pest occupancy can be used to improve detection sampling. Under the scenarios tested, failure to account for the effect of the infestation source located at the bottom of the storage underestimated the number of samples required for pest detection by approximately 31%. Conversely, when the effect of mixing was not accounted for, the number of samples required for detection was overestimated by approximately 29%. Both cases can lead to increased management costs due to oversampling and unnecessary pest treatments, and lower revenues due to undetected pests and product damage (Adam et al. 2006; Phillips et al. 2000).
There is scope for further development of the outlined model. In both the basic and extended supply chain models, storage parameters such as grain temperature, relative humidity, and storage duration are common across all storages at a given regional or export stage. This is unlikely to be the case in real-world storages, due to differing geographical locations and conditions, storage construction (e.g. steel bin, concrete silo, bunker, etc.), grain varieties, and storage durations in individual bins. Extension of the model to allow control of these parameters on a per-bin basis would allow for these variations to be modelled. Similarly, replacement of the existing growth model with a distributed-delay model (such as developed by Hagstrum and Throne (1989) would allow the model to be extended to predict when infestations will reach actionable levels. These and other potential modifications - such as the development of occupancy models for different pest species - would improve the accuracy of the resultant model estimates.

This study has used simplified scenarios to quantify the effects of grain mixing and site of infestation on the spatial distribution of *R. dominica*, and highlighted the potential for these factors to affect decisions made with regards to pest management. Existing models which do not account for these factors risk under- or over-estimating infestation rates in storages, with the result that grain quality may be reduced or unnecessary management actions undertaken. Both of these can result in increased costs to producers, distributors, and consumers. The results obtained in this study highlight the need for these factors to be accounted for when designing sampling protocols and other management tools intended to aid decision making. Further studies could quantify the influence of these factors on a larger scale, resulting in enhanced management tools, more efficient treatment practices, and improved product quality.

3.6 ACKNOWLEDGEMENTS

The authors would like to acknowledge the support of the Australian Government’s Cooperative Research Centres Program.
Table 3.1: Summary of experimental results and model estimates. The predicted proportion infested was estimated by the occupancy model described in section 3.4.1, and does not include the effect of mixing or introduction point.

<table>
<thead>
<tr>
<th>Introduction Point / Treatment</th>
<th>Replicate</th>
<th>Infestation rate (adults / kg)</th>
<th>Observed proportion infested</th>
<th>Predicted proportion infested</th>
<th>% Difference (mean &amp; SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Top (30°C, 14 days)</td>
<td>1</td>
<td>1.646</td>
<td>0.095</td>
<td>0.096</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1.835</td>
<td>0.110</td>
<td>0.105</td>
<td>1.24 ± 1.79</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1.835</td>
<td>0.105</td>
<td>0.105</td>
<td></td>
</tr>
<tr>
<td>Top (25°C, 59 days)</td>
<td>1</td>
<td>2.722</td>
<td>0.135</td>
<td>0.140</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2.911</td>
<td>0.110</td>
<td>0.147</td>
<td>-2.44 ± 13.46</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>2.722</td>
<td>0.170</td>
<td>0.140</td>
<td></td>
</tr>
<tr>
<td>Top (30°C, 43 days)</td>
<td>1</td>
<td>10.19</td>
<td>0.315</td>
<td>0.312</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>10.316</td>
<td>0.300</td>
<td>0.314</td>
<td>-1.30 ± 1.63</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>6.772</td>
<td>0.250</td>
<td>0.251</td>
<td></td>
</tr>
<tr>
<td>Top (35°C, 31 days)</td>
<td>1</td>
<td>6.645</td>
<td>0.255</td>
<td>0.248</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2.848</td>
<td>0.145</td>
<td>0.145</td>
<td>2.41 ± 1.29</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>7.911</td>
<td>0.285</td>
<td>0.273</td>
<td></td>
</tr>
<tr>
<td>Mixed (30°C, 43 days)</td>
<td>1</td>
<td>11.456</td>
<td>0.405</td>
<td>0.330</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>6.772</td>
<td>0.300</td>
<td>0.251</td>
<td>24.12 ± 3.14</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>12.342</td>
<td>0.445</td>
<td>0.342</td>
<td></td>
</tr>
<tr>
<td>Bottom (30°C, 43 days)</td>
<td>1</td>
<td>9.177</td>
<td>0.195</td>
<td>0.295</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>55.886</td>
<td>0.505</td>
<td>0.608</td>
<td>-22.16 ± 5.88</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>11.203</td>
<td>0.275</td>
<td>0.326</td>
<td></td>
</tr>
</tbody>
</table>
Table 3.2: Summary of model simulation results. Pest location 1 indicates insects introduced to grain surface, pest location 2 indicates insects introduced to bottom of grain mass.

<table>
<thead>
<tr>
<th>Scenario</th>
<th>Storage Level</th>
<th>Management Stage</th>
<th>Pests/kg (Mean ± SD)</th>
<th>Prop. Infested (Mean ± SD)</th>
<th>Samples (Mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scenario 1</td>
<td>Farm Post-storage</td>
<td>2 ± 0.20</td>
<td>0.112 ± 0.009</td>
<td>25 ± 2.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Farm → Regional handling : 0% mixing</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Initial</td>
<td>2 ± 0.09</td>
<td>0.112 ± 0.004</td>
<td>25 ± 0.9</td>
<td></td>
</tr>
<tr>
<td>Region Post-storage</td>
<td>0.1 ± 0.00</td>
<td>0.006 ± 0.000</td>
<td>536 ± 18.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Post-storage</td>
<td>7.22 ± 0.31</td>
<td>0.26 ± 0.006</td>
<td>10 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>Regional → Export handling : 0% mixing</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Initial</td>
<td>7.22 ± 0.18</td>
<td>0.26 ± 0.004</td>
<td>10 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>Export Post-treatment</td>
<td>0.36 ± 0.01</td>
<td>0.013 ± 0.000</td>
<td>229 ± 3.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post-storage</td>
<td>26.03 ± 0.65</td>
<td>0.469 ± 0.005</td>
<td>5 ± 0.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scenario 2</td>
<td>Farm Post-storage</td>
<td>2 ± 0.20</td>
<td>0.087 ± 0.007</td>
<td>33 ± 2.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Farm → Regional handling : 0% mixing</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Initial</td>
<td>2 ± 0.09</td>
<td>0.087 ± 0.003</td>
<td>33 ± 1.2</td>
<td></td>
</tr>
<tr>
<td>Region Post-treatment</td>
<td>0.1 ± 0.00</td>
<td>0.004 ± 0.000</td>
<td>687 ± 23.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Post-storage</td>
<td>7.22 ± 0.32</td>
<td>0.203 ± 0.005</td>
<td>13 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>Regional → Export handling : 0% mixing</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Initial</td>
<td>7.22 ± 0.18</td>
<td>0.203 ± 0.003</td>
<td>13 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>Export Post-treatment</td>
<td>0.36 ± 0.01</td>
<td>0.010 ± 0.000</td>
<td>294 ± 5.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post-storage</td>
<td>26.04 ± 0.66</td>
<td>0.366 ± 0.004</td>
<td>7 ± 0.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scenario 3</td>
<td>Farm Post-storage</td>
<td>2 ± 0.20</td>
<td>0.112 ± 0.009</td>
<td>25 ± 2.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Farm → Regional handling : 20% mixing</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Initial</td>
<td>2 ± 0.09</td>
<td>0.138 ± 0.005</td>
<td>20 ± 0.8</td>
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</tr>
<tr>
<td>Region Post-treatment</td>
<td>0.1 ± 0.00</td>
<td>0.007 ± 0.000</td>
<td>432 ± 15.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Post-storage</td>
<td>7.21 ± 0.32</td>
<td>0.322 ± 0.008</td>
<td>8 ± 0.2</td>
<td></td>
</tr>
</tbody>
</table>
### Regional → Export handling: 20% mixing

<table>
<thead>
<tr>
<th></th>
<th>Initial</th>
<th>Post-treatment</th>
<th>Post-storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Export</td>
<td>7.21 ± 0.18</td>
<td>0.36 ± 0.01</td>
<td>26.01 ± 0.66</td>
</tr>
<tr>
<td>Post-storage</td>
<td>0.399 ± 0.006</td>
<td>0.020 ± 0.000</td>
<td>0.694 ± 0.007</td>
</tr>
<tr>
<td></td>
<td>6 ± 0.1</td>
<td>149 ± 2.2</td>
<td>3 ± 0.1</td>
</tr>
</tbody>
</table>

### Farm → Regional handling: 20% mixing

<table>
<thead>
<tr>
<th></th>
<th>Initial</th>
<th>Post-treatment</th>
<th>Post-storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Region</td>
<td>2 ± 0.09</td>
<td>0.10 ± 0.00</td>
<td>7.20 ± 0.33</td>
</tr>
<tr>
<td>Post-storage</td>
<td>0.108 ± 0.004</td>
<td>0.005 ± 0.000</td>
<td>0.265 ± 0.007</td>
</tr>
<tr>
<td></td>
<td>26 ± 1.0</td>
<td>555 ± 20.1</td>
<td>10 ± 0.3</td>
</tr>
</tbody>
</table>

### Regional → Export handling: 20% mixing

<table>
<thead>
<tr>
<th></th>
<th>Initial</th>
<th>Post-treatment</th>
<th>Post-storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Export</td>
<td>7.20 ± 0.19</td>
<td>0.36 ± 0.01</td>
<td>25.97 ± 0.68</td>
</tr>
<tr>
<td>Post-storage</td>
<td>0.328 ± 0.005</td>
<td>0.016 ± 0.000</td>
<td>0.590 ± 0.007</td>
</tr>
<tr>
<td></td>
<td>8 ± 0.1</td>
<td>181 ± 2.7</td>
<td>3 ± 0.1</td>
</tr>
</tbody>
</table>

Scenario 4

(pest location 2, mixing 20%, treatment success 95%)
Figure 3.1: Distribution of infested grain between farm, regional, and export storages in the scenarios tested. Groups of 5 farm silos (Storage Level A) are allocated to each regional storage (Storage Level B); from these, 33% of the grain in each is allocated to each of the 3 export storages (Storage Level C).
Figure 3.2: Infestation rate (pests/kg) vs proportion infested ($p$) for *R. dominica*. Solid line is the fitted model; dotted line is the model projection.
Figure 3.3: Model GUI including options for pest choice and spatial model to use for the simulation, initial pest location, mixing, treatment effectiveness, and storage environmental parameters.
Chapter 4: Discussion

4.1 GENERAL

The spatial distribution of insect pests inside grain storages has been the subject of ongoing interest (Hagstrum et al. 1985; Jian et al. 2011; Jian et al. 2012; Surtees 1963a; Surtees 1963b; Surtees 1963c; Surtees 1964a; Surtees 1964b), as it directly affects both sampling (Elmouttie et al. 2010; Hagstrum et al. 1985) and grain storage models (Thorpe 1997; Thorpe 2008). Temperature, location of infestation, and grain handling are considered to be important influences on the spatial distribution of *R. dominica* in grain storages, but previous research into the spatial distribution of grain pests within storages has focussed on the effect of factors such as temperature and moisture gradients (Flinn and Hagstrum 1998; Flinn and Hagstrum 2011; Jian et al. 2002) and aggregation behaviour (Cox and Collins 2002; Plarre 1996) on pest movement. While these factors undoubtedly have an influence, it is the inherent underlying spatial distribution which is not well understood.

Difficulties in sampling grain storages with the required intensity and spatial accuracy have previously restricted the understanding of spatial distribution of pests in grain storages. The immature stages of many grain pests develop partly or entirely internally within grain kernels, while in particular adult *R. dominica* are also internal feeders. Combined, these factors reduce the accuracy available from sampling techniques traditionally used for detection and estimation of pest infestations. Because of these factors, intensive examination of grain is required for accurate detection and estimates of the size and extent of infestations. The use of intensive sampling of smaller representative systems in laboratory experiments is well established in this field (e.g. Flinn and Hagstrum 1998; Flinn and Hagstrum 2011; Jian et al. 2011; Surtees 1964a), however even in these cases the physical properties of grain – a loose, easily disturbed mass, with insects occupying both individual grains and the interstitial space – restricts the gathering of accurate spatial information.
To overcome these limitations, a novel method of sampling was developed. By encapsulating loose grain prior to sampling, the three dimensional spatial distribution of the lesser grain borer, *Rhyzopertha dominica*, within grain masses was examined with high spatial accuracy at a fine scale. It was found that *R. dominica* populations form distinct spatial patterns within grain, with the particular shape and location of each population dependent on temperature. Using the spatial data obtained, it was shown that the proportion of grain infested was a non-linear function of pest abundance independent of temperature. Additional experiments examined the effect of initial infestation location and mixing during transport or handling on *R. dominica* spatial distribution. Both infestation location and grain handling had a substantial effect on the spatial occupancy of *R. dominica*, decreasing or increasing the proportion of grain infested. A predictive model based on this information was developed and, with a sampling model based on spatial occupancy, was used to significantly extend a simulation model of a grain supply chain. The results of the experimental research, model development, and several simulation modelling scenarios presented in this thesis are discussed in more detail in the following sections.

### 4.2 THE EFFECT OF TEMPERATURE ON *R. DOMINICA* SPATIAL DISTRIBUTION

The results described in Chapter 2 demonstrate that, when *R. dominica* were introduced to the top of the grain mass (simulating a typical infestation scenario of pest immigration through the cap or eave vents of a silo), temperature had a marked effect on the pattern of spatial distribution (Figure 2.2). At each of the three temperatures examined (25°C, 30°C, and 35°C) populations were clustered (Table 2.1), however the actual spatial pattern observed varied with temperature (Figure A2 to Figure A4). At 25°C, *R. dominica* populations were clustered immediately below the point of introduction. At this temperature, the majority of insects were located within 15cm of the grain surface, and horizontal movement was minimal (≤ 5cm). At 30°C, insects were still located within 15cm of the grain surface, but horizontal distribution was more pronounced (≤ 15cm, limited by the size of the containers used). The same pattern was also observed after only 14 days at 30°C, suggesting the observed spatial pattern formed relatively quickly after initial introduction. At 35°C
the population remained clustered but was located further from the grain surface, with the majority of insects located between 10cm and 30cm below the point of introduction.

*R. dominica* was previously considered to distribute relatively homogeneously within both small bulks (Surtees 1964c) and larger grain storages (Flinn *et al.* 2010). In those and similar studies, *R. dominica* was found to disperse rapidly through the grain bulk in a horizontal direction, but vertical dispersion was slower. My research recorded similar patterns of clustering and spatial distribution to those previously found by sampling large storage bulks, but only at the two lower temperatures (25°C and 30°C). The higher vertical dispersion observed at 35°C may be due to increased insect activity at this temperature. The maximum viable temperature recorded for *R. dominica* is 38°C (Birch 1953), and locomotory activity was found to be higher at 35°C than at lower temperatures (Surtees 1964a). As a result, where grain temperatures are close to this, *R. dominica* may move further into the grain mass either due to increased activity or in search of cooler temperatures.

This effect of temperature on spatial pattern and pest location may have important implications for grain sampling and pest management. The majority of existing research on pest detection and storage management has been undertaken in northern hemisphere locations such as North America and Europe, where wheat production is generally restricted to more temperate regions, and harvest and storage occur at cooler temperatures. In Australia wheat is harvested and stored during the late spring or summer. This results in higher temperatures within Australian grain storages, which in turn affects the efficacy of pest management techniques such as sealed storages, fumigation, and grain cooling. In particular, the use of forced aeration to cool grain may be less efficient due to warmer ambient temperatures (Driscoll *et al.* 2000).

My research indicates that these higher temperatures in storages are likely to result in spatial distributions considerably different to those observed in countries where wheat is a winter crop, and this may have implications for the sampling
methods used in such cases. For example, when grain is stored at higher temperatures, the resulting spatial distribution of pests means that techniques for sampling deeper in the grain mass – such as spear samplers or deep probe traps – may improve pest detection and provide better estimates of abundance than surface sampling or pitfall traps.

4.3 DEVELOPMENT OF A SPATIAL OCCUPANCY MODEL FOR *R. DOMINICA*

A recent study by Elmouttie et al. (2010) demonstrated that a sampling model informed by the proportion of grain infested could be used to improve pest detection. This sampling model requires accurate estimates of the overall infestation rate within storages, as well as an estimate of the proportion of the whole infested. While population models to estimate pest abundance within storages exist (e.g. Driscoll et al. 2000; Hagstrum et al. 1985), suitable models of spatial occupancy do not. Due to the total volumes of grain involved (up to hundreds to thousands of tonnes in some storages), the need to sample the grain with minimum disturbance (to avoid accidental movement of pests from infested to uninfested locations), the high sampling intensity required to account for fine-scale variation in pest abundance, and limited access to the majority of the grain mass (restricted to sampling from the surface or through inspection/sampling ports in the storage structure) it is rarely feasible to obtain accurate estimates of spatial occupancy by sampling large storages. In this case, representative systems or models must be used to determine relevant values for the proportion infested.

The high sampling intensity (~95%) and fine scale (5cm resolution) of the sampling method developed in Chapter 2 allowed for accurate determination of the proportion of each experimental lot infested. Multiple regression analysis conducted in preparation for the analysis and modelling in Chapters 2 and 3 indicated that the infestation rate (expressed as the number of pests per kg of total grain weight) was a significant predictor of occupancy (Table B1). On this basis, the non-linear model presented in section 3.4.1 was developed.
The sampling model developed by Elmouttie et al. has been shown to significantly improve detection of pests at low infestations (Elmouttie et al. 2010). When coupled with a suitable population growth model for *R. dominica*, the occupancy model developed in this thesis allows for the inclusion of this sampling model into future stored grain management tools. This will improve pest detection at early stages of infestation and allow development of improved pest management practices, reduce pest management costs throughout the supply chain, reduce resistance development, and improve the quality of grain delivered to domestic and export markets.

### 4.4 THE EFFECTS OF INFESTATION LOCATION AND GRAIN HANDLING ON *R. dominica* SPATIAL DISTRIBUTION

While *R. dominica* is considered to infest grain in storages primarily through immigration to the top surface (Hagstrum 2001), latent infestations in grain handling equipment or storages may result in pest infestation being located at the bottom of the grain mass (Reed *et al.* 2003; Sinclair 1982). The experimental results described in section 3.4.2 demonstrate that initial pest location influences the proportion of grain infested (Table 3.1). Infestations located at the bottom of the grain mass were observed to reduce the proportion of grain infested, and also resulted in a clustered distribution (Figure A6) that differs significantly from those arising from pest infestations at the surface of the grain (Figure A1 to Figure A4).

Dispersion activity of *R. dominica* in grain has previously been observed to be due to random movement influenced by a thigmotactic response to individual kernels, with gradual downwards movement due to gravity and upwards movement evident only at high (200 insects/kg) densities (Surtees 1964a). Although such high overall densities were not encountered in the studies presented here, equivalent individual per-sample densities (12–13 insects/125g) were common in the experiments where infestations were initiated at the bottom of the grain mass. This suggests that in these cases intragroup competition or stimulation is the primary driver of dispersion.
Regardless of the location of pest infestations, handling and movement of grain along a supply chain can disturb pest infestations and affect the proportion of grain infested. Again, the results presented in section 3.4.2 demonstrated a significant effect of grain handling on spatial distribution, with mixing increasing the proportion of grain infested (Table 3.1). It also resulted in a less defined spatial pattern when compared to undisturbed grain, with pests distributed further through the grain mass (Figure A5).

When compared to the estimates derived from the occupancy model described in section 3.4.1, pest infestations originating at the bottom of the grain mass differed significantly from the predicted spatial occupancy. Infestation at the bottom of the grain mass reduced the proportion of grain infested by approximately 22% when compared to model estimates not taking infestation location into account. Conversely, when subjected to simulated handling, the proportion infested was increased by approximately 24% compared to estimates that did not incorporate this factor.

This indicates that both grain mixing and infestation location have significant effects on the proportion of grain infested. These factors have not been accounted for in previous storage or grain supply chain models but, by influencing the proportion of grain infested, can have a disproportionate influence on the sampling intensity required for accurate pest detection and population estimates. Failure to account for these factors when determining the correct sampling strategy for grain storages can result in under- or over-estimation of pest abundance, with associated negative impacts on grain quality and management costs.

4.5 **AN EXTENDED GRAIN SUPPLY CHAIN SIMULATION MODEL**

A simulation model of a grain supply chain was significantly extended using the occupancy model and experimental results from Chapter 3. The proportion of grain infested by *R. dominica*, previously described by a set of fixed distribution parameters, was supplemented by the occupancy model developed in section 3.4.1.
Further extension involved incorporating controls for the effect of pest infestation location and mixing on the proportion of grain infested (section 3.4.2), and the ability to apply pest treatments at each storage level (Figure 3.3). The final supply chain model couples these improvements with the sampling model of Elmouttie et al. (2010) to produce a tool that can estimate *R. dominica* infestation rate, the proportion of grain infested, and the number of samples required to accurately and efficiently detect infestations.

When used to examine scenarios incorporating grain mixing and pest introduction point effects, it was determined that each had a significant effect on sampling. Including a grain mixing factor of 24% in simulations reduced the number of samples required for pest detection by approximately 30%, while infestations at the bottom of the grain mass increased the number of samples required by approximately 30% (section 3.4.3 and Table 3.2). When both of these factors were included, the number of samples required for pest detection varied depending on both the storage stage (i.e. farm, regional, or export storage) and timing (i.e. pre or post treatment, post storage), but was reduced by an average of approximately 7% (section 3.4.3) over the whole supply chain.

These results highlight the importance of incorporating all factors influencing pest spatial distribution in management tools. Early pest detection and control can significantly reduce pest numbers throughout the supply chain, with a resultant improvement in management costs and grain quality. More accurate sampling at later stages in the supply chain can detect pest infestations earlier, improving overall end-to-end control of pests. The availability of accurate simulation models allows for examination of multiple “what if?” scenarios, and result in improved grain quality and reduced management costs at all stages along the supply chain.

### 4.6 CONCLUSIONS AND FUTURE RESEARCH

An understanding of insect spatial behaviour and the relationships between environmental conditions, pest abundance, grain handling and management, and
occupancy is necessary to improve sampling methodologies and pest management in storages (Elmouttie et al. 2010; Hagstrum et al. 1985; Thorpe 1997). The research presented here has revealed spatial patterning occurring in *R. dominica* populations at a fine spatial scale not previously observed. The findings indicate that for *R. dominica* spatial pattern (in the form of the proportion of grain infested) can be modelled as a function of overall pest abundance.

This research has also shown that both the location of infestation and insect disturbance during grain handling can affect both the spatial distribution of *R. dominica* and the proportion of grain infested. A supply chain model incorporating these factors into a spatial occupancy model, and used to inform a sampling model, was developed. Simulations using this model showed that failure to account for differences in pest occupancy due to pest abundance, location, and grain handling has a significant impact on detection sampling and, therefore, pest management decisions and grain quality.

Pressure to minimise product losses and pesticide use, along with the ongoing need to increase product quality and economic returns, demands detection of insect pests at low levels. Because of this, the ability to improve detection, monitoring, and management of pest populations by accounting for spatial distribution may prove to be a key feature of sophisticated pest management tools in the future. While the studies presented here highlight the importance of accounting for spatial distribution when sampling, they are necessarily limited by the small lot sizes required for intensive sampling. Further research on pest distribution within real-world grain storages will be necessary to confirm these findings and validate current and future sampling models.

World markets increasingly demand high quality, pest- and residue-free grain. It is apparent that, while several factors driving the spatial distribution of pests in grain storages have been identified and examined, other factors such as environmental gradients, microclimatic variations, and species composition all affect distribution within these habitats. Further investigation of the effect of such factors
will result in a better understanding of pest distribution within grain storages, and lead to the development of improved simulation and predictive models for use in grain pest management.

The current study indicates that the spatial distribution of *R. dominica* within grain storages is influenced primarily by temperature, but the proportion of grain infested is primarily affected by overall infestation rate. Infestations developing at the bottom of the grain mass occupy a significantly smaller proportion of the grain mass compared to similarly-sized infestations at the top of the grain. Mixing has a significant effect on both the resulting spatial pattern and proportion of grain infested. These factors can feasibly be incorporated into management models for grain storage, with the potential for improving current grain management systems and resulting in higher grain quality and lower management costs.
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Appendix A

3 dimensional plots of observed *R. dominica* spatial distribution
Figure A1: 3D spatial distribution of *R. dominica* after 14 days at 30°C. Individual points represent 1 insect.
Figure A2: 3D spatial distribution of *R. dominica* after 59 days at 25°C. Individual points represent 1 insect.
Experiment 3, Replicate 1  
Experiment 3, Replicate 2  
Experiment 3, Replicate 3

Figure A3: 3D spatial distribution of \textit{R. dominica} after 43 days at 30°C. Individual points represent 1 insect.
Experiment 4, Replicate 1  
Experiment 4, Replicate 2  
Experiment 4, Replicate 3

Figure A4: 3D spatial distribution of *R. dominica* after 31 days at 35°C. Individual points represent 1 insect.
Figure A5: 3D spatial distribution of *R. dominica* after 43 days at 30°C (mixed at 14 days). Individual points represent 1 insect.
Figure A6: 3D spatial distribution of *R. dominica* after 43 days at 30°C (introduced to bottom of grain). Individual points represent 1 insect.
Appendix B

Results of multiple regression analysis between proportion infested and predictor variables
Table B1: Summary of the regression statistics for the predictor variables temperature, duration, infestation rate. Dependent variable = proportion of grain infested.

<table>
<thead>
<tr>
<th>Predictor variable</th>
<th>B</th>
<th>SE (B)</th>
<th>β</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Storage Temp. (°C)</td>
<td>3.345</td>
<td>1.586</td>
<td>0.400</td>
<td>2.109</td>
<td>0.053</td>
</tr>
<tr>
<td>Storage Duration (Days)</td>
<td>0.692</td>
<td>0.336</td>
<td>0.405</td>
<td>2.061</td>
<td>0.058</td>
</tr>
<tr>
<td>Infestation Rate (pests/kg)</td>
<td>1.301</td>
<td>0.315</td>
<td>0.650</td>
<td>4.128</td>
<td>0.001*</td>
</tr>
</tbody>
</table>

B = unstandardized coefficient, SE (B) = standard error, β = standardised beta coefficient, t = t-test statistic, p = significance.

* indicates significant at p = 0.05