Investigation of dispersal and spatio-temporal distributions of stored grain insects in Australia using ecological and molecular tools

Daglish, G.J.*#[1,2], Ridley, A.W.[1,2], Hereward, J.P.[3], Emery, R.N.[1,4], Holloway, J.C.[1,5], Raghu, S.[6], Walter, G.H.[1,3]

[1]Plant Biosecurity Cooperative Research Centre, GPO Box 5012, Bruce, ACT 2617, Australia
[2]Department of Agriculture, Fisheries and Forestry, Queensland, Ecosciences Precinct, GPO Box 267, Brisbane, QLD 4001, Australia
[3]School of Biological Sciences, The University of Queensland, Brisbane, QLD 4072, Australia
[4]Department of Agriculture and Food, 3 Baron-Hay Court, South Perth, WA 6151, Australia
[5]Department of Primary Industries, Private Mail Bag, Wagga Wagga, NSW 2650, Australia
[6]CSIRO Ecosystem Sciences, Ecosciences Precinct, Dutton Park, QLD 4102, Australia

*Corresponding author, Email: greg.daglish@daff.qld.gov.au
#Presenting author, Email: greg.daglish@daff.qld.gov.au

DOI: 10.14455/DOA.res.2014.16

Abstract

Since 2008, there has been a concerted and on-going effort in Australia to investigate the dispersal and spatio-temporal distributions of major insect pests of stored grain. The aim is to understand the spread of pests, and potentially resistance alleles, and to use this knowledge to develop better pest and resistance management strategies. A significant thrust of this research has been trapping with pheromone lures, but additional valuable information is coming from determination of patterns of gene flow. The best studied species are *Rhyzopertha dominica* (F.) and *Tribolium castaneum* (Herbst). We have strong evidence that individuals of these species disperse considerable distances across rural landscapes, with enough gene flow on the district scale (ca. 100 km), at least, to render the gene pool across this area homogeneous. *Rhyzopertha dominica* has even been trapped in forests many kilometers from grain storages. In tropical and sub-tropical grain growing areas of eastern Australia, *R. dominica* and *T. castaneum* were trapped year-round although numbers were lower in the cooler months. In contrast, there was little or no flight for several months a year in southwestern Australia when mean daily temperatures were often 15°C or less. Extensive trapping of *Sitophilus oryzae* (L.), has yielded very few of individuals, suggesting that flight is uncommon in this species or flying adults are not attracted to the pheromone lure. Limited data on *Cryptolestes ferrugineus* (Stephens) trapped without a pheromone lure suggest that this species can disperse across the rural landscape. Studies like these are providing insights into the ecology of key pests of stored grain with relevance to Australia, although conclusions are applicable elsewhere. These studies are providing farmers and others who store grain information on the threat posed by flying stored grain insects, and the geographic scale on which pest and resistance management needs to be applied.

Keywords: dispersal, spatio-temporal, distribution, gene flow, *Rhyzopertha*, *Tribolium*, *Sitophilus*, *Cryptolestes*

1. Introduction

Australian grain growers aim to produce high quality grain for export and domestic markets, but because of Australia's climate, temperatures at harvest and during storage are often favourable to major insect pests. A zero tolerance to live insects at the point of export has been in place since 1963 and a key to meeting this standard has been the use of phosphine fumigation and grain protectants. There is however, the ever-present threat of resistance with many instances of insects developing resistance to phosphine or grain protectants, including strong phosphine resistance in *Cryptolestes ferrugineus* (Stephens) (Nayak et al., 2013) and methoprene resistance in *Rhyzopertha dominica* (F.) (Daglish et al., 2013). Australia has responded well to this threat by keeping informed of resistance trends, and developing new treatments or treatment protocols, but the threat remains.

Ecology represents an untapped potential. It offers a change of perspective by shifting the focus onto the insects themselves raising questions about why, when, where and how? Importantly, it also moves the focus from the immediate storage environment to the broader environment. Benefits include informed decision-making by growers and other sectors of the grain industry and potentially new ecologically-based tools (e.g. pheromone trapping for monitoring or pest suppression). In recent years, there has been considerable research effort investigating the dispersal and spatio-temporal distributions of major insect pests of stored grain in Australia. The aim is to understand the spread of pests, and potentially resistance alleles they carry, and to use this knowledge to improve pest and resistance management. A significant thrust of this research has been trapping with pheromone lures, but additional valuable information is coming from determination of patterns of gene flow. In this paper we give an overview of this research.

2. Materials and Methods

2.1. Trapping

This paper reports results from four trapping studies in three grain growing states of Australia. The four study areas encompass a wide range of seasonal temperature patterns (Table 1). Beetles were trapped in Lindgren four-funnel traps (Contech, Inc, BC, Canada) suspended 1.5 m above the ground and propylene glycol was used to preserve trapped beetles for identification and in some cases DNA extraction. Temperature data were obtained from the nearest Bureau of Meteorology site.

Table 1 Mean daily minimum and maximum temperatures (°C) for January and July in the study areas in Queensland (Qld), New South Wales (NSW) and Western Australia (WA).

State	Town	Location	January		July	
			Min	Max	Min	Max
Qld	Emerald	23.53˚S, 148.18˚E	22.2	34.4	8.9	23.2
Qld	Miles	26.72˚S, 150.15˚E	19.5	33.2	3.6	19.3
WA	Brookton	32.37˚S, 117.01˚E	15.5	33.0	4.5	16.1
NSW	Wagga Wagga	35.12˚S, 147.37˚E	16.3	31.7	2.7	12.7

2.1.1. Queensland trapping

Trapping occurred during a 12 month period from 2008 to 2009 in southern Queensland, using aggregation pheromone lures for *T. castaneum* and *R. dominica* (Trece Inc, USA). The study area was near the town of Miles. As well as providing information on flight activity, the trapping provided samples for gene flow analysis (see 2.2). Traps were situated next to farm storage, in fields, at bulk grain depots and in native vegetation reserves. Trapping period was 1 week and trapping occurred 11 times during the study. The results for *T. castaneum* have been published (Ridley et al., 2011), and a preliminary analysis is available for *R. dominica* (Daglish et al., 2010).

Trapping also occurred during a 12 month period from 2011 to 2012 in central Queensland, using *T. castaneum* and *R. dominica* pheromone lures (Trece Inc, USA). Trapping occurred at 2 week intervals along a 28.4 km linear transect that included sites at bulk grain depots and sites away from stored grain. The study transect included the town of Emerald.

2.1.2. Western Australian trapping

Since 2011, trapping has been taking place in the Western Australian wheat belt located in the south west of the state. In this paper we focus on results from trapping at approximately 2 week intervals at a large bulk grain depot in the town of Brookton and a protected native vegetation site called Boyagin Rock Reserve. Traps were baited with *T. castaneum* and *R. dominica* pheromone lures (Trece Inc, OK, USA) during 2011 to 2013. Subsequently, traps were baited with the *Sitophilus* aggregation pheromone lure (Insects Limited Inc, USA) and a food volatile lure (Insects Limited Inc, USA).

2.1.3. New South Wales trapping

In 2014, a trapping study began in a temperate region of New South Wales near the town of Wagga Wagga. Trapping has been occurring at approximately monthly intervals with paired traps baited with and without the *Sitophilus* pheromone lure (Insects Limited Inc, USA). Traps are located near farm storage or in fields away from storage.

2.2. Gene flow analysis

Molecular population genetics was used to determine gene flow in *T. castaneum* and *R. dominica* in the southern Queensland trapping study area. The results for *T. castaneum* have been published (Ridley et al., 2011) and publication of the results for *R. dominica* is planned. DNA was extracted from approximately 30 individuals per sample and the DNA from each individual was screened for at least 11 highly polymorphic neutral DNA markers. Standard analytical techniques were used such as analysis of molecular variance (AMOVA) and Bayesian clustering. Grain is rarely moved between farms in this study area, so dispersal by flight would be a major contributor to gene flow.

3. Results and Discussion

3.1. Trapping

Trapping with *T. castaneum* and *R. dominica* pheromone lures has provided a detailed picture of flight activity in these species in different grain growing regions of Australia. In the first study, in southern Queensland, beetles were not limited to the immediate storage environment but were also caught in fields and in native bushland (Daglish et al., 2010; Ridley et al., 2011). To our knowledge, this is the first time this has been demonstrated in *T. castaneum*, although similar findings have been reported for *R. dominica* in Oklahoma and Kansas in the USA (e.g. Edde et al., 2006; Toews et al., 2006; Mahroof et al., 2010). Other studies in Western Australia and central Queensland, also using *T. castaneum* and *R. dominica* pheromone lures, confirmed the initial finding that *T. castaneum* and *R. dominica* flight is not limited to the immediate storage environment. Trapping in Western Australia showed that these beetles were active at a major bulk grain depot as well as a native vegetation reserve many kilometres from the nearest stored grain. The number of fortnights in 2012, that *T. castaneum* was caught was not significantly different between the depot site (14 times) and reserve site (15 times) (chi-square = 0.84, $p > 0.05$). Likewise with *R. dominica*, there was no significant difference between the number of times this species was caught at the depot site (20 times) and reserve site (17 times) (chi-square = 0.08, $p > 0.05$).

Trapping along a 28.4 km transect in central Queensland showed that *T. castaneum* and *R. dominica* were caught every fortnight of trapping, and they were active at major bulk grain depots as well as in areas away from storages that were dominated by native vegetation. In December, for example, there was no significant difference between *R. dominica* catch from the depot or non-depot traps ($t = 1.32$, $P > 0.05$), as was the case with *T. castaneum* ($t = 0.856$, $P > 0.05$).

Extensive trapping in Western Australia using the *Sitophilus* pheromone lure has yielded very few *S. oryzae* beetles, suggesting that flight is uncommon in this species or flying adults are not attracted to the pheromone lure. Preliminary data from New South Wales support this conclusion. Trapping at eight bulk grain depots in Western Australia for a cumulative period of almost 5,000 days has yielded a total of 10 *S. oryzae* beetles, nine of which were caught on one occasion at one depot.

A commercially available lure is not available for *C. ferrugineus* but small numbers of beetles of this species have been trapped enabling some tentative conclusions to be drawn. The trapping study under way in farmland in New South Wales is using the *Sitophilus* lure only, but the insect by-catch has included some *C. ferrugineus* showing that this species can be caught away from storage. For example, in the trap catch from eight 4 wk trapping periods analysed to date, *C. ferrugineus* have been caught in traps next to farm silos on five occasions and in traps in fields on four occasions (Chi-square = 0.254, $P > 0.05$).

In the southern Queensland study, *T. castaneum* and *R. dominica* flight occurred year-round, although few beetles were caught in the coldest months of June and July (Daglish et al., 2010; Ridley et al., 2011). Perhaps not surprisingly, in the central Queensland study, with a warmer climate, flight also occurred year-round. A major finding from the Western Australian study was that *T. castaneum* and *R. dominica* were rarely caught during the coldest months of the year (June-August) when mean daily temperatures were often 15°C or less. In the New South Wales study, flight activity of *T. castaneum, R. dominica* and *C. ferrugineus* was also limited during the coldest montsh of the year. To our knowledge, there is no similar published information on flight activity in *T. castaneum* or *C. ferrugineus* in other countries. In *R. dominica*, however, flight ceased for several months during studies in Kansas and Oklahoma, where temperatures can be much lower than in Queensland, often falling to below 0°C (Edde et al., 2006; Toews et al., 2006).

3.2. Gene flow

The trapping studies showed that *T. castaneum* and *R. dominica* are not only in the immediate storage environment but in the broader farm and natural landscape. Molecular population genetic tools provide an opportunity to determine the levels of gene flow across the environment. In the case of *T. castaneum*, there was significant gene flow across the southern Queensland trapping study area which covered farmland and native vegetation sites (Ridley et al., 2011). Similarly, preliminary analysis of *R. dominica* samples from the same study shows that there was significant gene flow in this species. In this study area, we know that grain is rarely transported between farms although grain can be transported from farms to bulk grain depots. We conclude, therefore, that dispersal by flight must be a major contributor to gene flow.

4. Conclusions

Ecological tools (e.g. pheromone trapping) and molecular population genetics tools can be used to develop a better understanding about dispersal and spatio-temporal distributions of key pests across agricultural and natural landscapes. Although ecological or population genetics tools alone are useful, there are circumstances where using both can provide better understanding than either alone can provide. Results such the ones presented in this paper have implications for pest and resistance management (e.g. timing and geographic scale of actions).

Acknowledgements

The authors gratefully acknowledge the support of Plant Biosecurity Cooperative Research Centre (Project No: PBCRC3039) and the National Plant Biosecurity Cooperative Research Centre (Project No's: NPBCRC50089 and NPBCRC50149), established and supported under the Australian Government's Cooperative Research Centres Program (http://www.crcplantbiosecurity.com.au). We gratefully acknowledge GrainCorp Ltd and CBH Ltd, and the farmers and their families for access to their properties. We thank Mr P. Burrill, Ms V. Ooi, Mr M. Whitehouse, Ms M. Saunders, Mr D. Cousins and staff from GrainCorpLtd and CBH Ltd for their help with the trapping studies.

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