Resistance management and the ecology of *Rhyzopertha dominica* (F.) and *Tribolium castaneum* (Herbst) in subtropical Australia

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Abstract

Subtropical Australia is a demonstrated hotspot for phosphine resistance in stored product pests. *Rhyzopertha dominica* and *Tribolium castaneum* are common pests of stored grain in this region and management of these pests is increasingly impeded through the spread of resistance to phosphine, the most desirable control method. A number of field-oriented studies were conducted from the 1970's to 1990's to understand the ecology of these pests in subtropical Australia, including seasonal abundance, flight and population growth in stored grain. To manage the evolution and spread of resistance we require an understanding of movement of these beetles among foci of infestation. This paper presents preliminary analyses of two aspects of new research on these species: (1) a trapping program using pheromones to investigate beetle numbers in spatial and temporal contexts, and (2) characterisation of beetles leaving infested farm silos.

Adults of both species were trapped throughout the year with the lowest numbers corresponding to the coldest part of the year. The coldest trapping period had mean maximum and minimum temperatures of 21.1 and 3.5°C respectively. Trapping also revealed distinct differences between the two species, both in terms of numbers caught and where they were caught. In general, more R. dominica were caught than *T. castaneum*, similar numbers of *R. dominica* were caught near farm silos and in paddocks at least 1 km from the nearest silo, and more *T. castaneum* were caught near silos than in paddocks. Individual adults intercepted flying from farm silos are being characterised in the laboratory, and results to date show that these adults are long-lived, the females have mated before emigrating and are highly fecund. By undertaking research of the type summarised here we aim to develop an understanding of how these two species interact with their environment and how these interactions influence resistance development.

Keywords: Ecology, *Rhyzopertha dominica*, *Tribolium castaneum*, Australia

1. Introduction

The subtropical grain growing region of eastern Australia, comprising southern Queensland and northern New South Wales, is a demonstrated hotspot for phosphine resistance in stored product pests. Two of the most serious pests of stored grain, *Rhyzopertha dominica* (F.) and *Tribolium castaneum* (Herbst), are common pests of stored grain in this region and management of these pests is increasingly impeded through the spread of resistance to phosphine, the most desirable control method. In 1997, a new type of resistance was detected in *R. dominica* in southern Queensland (Collins et al., 2002). After selection of the field strain the resistance factor was ca. 600 times for adults exposed to phosphine for 48 h (Collins et al., 2002), compared with a resistance factor of ca. 30 times for a strain considered to be typical at the time (Daglish, 2004). Currently, about 5% of *R. dominica* populations sampled from southern Queensland and northern NSW contain individuals with this strong resistance (P.J. Collins, personal communication), but the potential of the problem is illustrated by the Brazilian experience where strong resistance is much more common (Lorini et al., 2007). Currently 92% of *T. castaneum* samples from the region contain phosphine resistant individuals although the strength of resistance is lower than in *R. dominica* (M.K. Nayak, personal communication).

An understanding of the ecology of pest insects is critical to effective pest management (Walter, 2003). A number of field-oriented studies were conducted from the 1970's to 1990's to understand the ecology of these pests in subtropical Australia, and provided information such as seasonal abundance, flight and population growth in stored grain (Sinclair and White, 1980; Sinclair 1982; Sinclair and Haddrell, 1985; White, 1988; Daglish, 2005). To manage the evolution and spread of resistance in *R. dominica* and *T. castaneum*, in particular, we require an understanding of their ecology, especially the movement of these beetles among foci of infestation. This paper presents preliminary analyses of two aspects of new research addressing this need: (1) a trapping program using pheromones to investigate beetle numbers in spatial and temporal contexts, and (2) characterisation of beetles leaving infested farm silos.

2. Materials and methods

Trapping was undertaken in a grain growing area in southern Queensland, Australia; where farmers typically grow a winter crop of wheat and a summer crop of sorghum. Bureau of Meteorology 98 year averages show that the coolest and hottest months are typically July (diurnal range 3.6-19.3°C) and January (diurnal range 19.5-33.2°C) respectively. Although rain can fall throughout the year it falls predominantly during summer.

Lindgren four-funnel traps (Contech, Delta, BC, Canada) baited with species-specific pheromone lures (Trece, Adair, OK, USA) were used to monitor flight activity of *R. dominica* and *T. castaneum* in the farm environment. Traps were located either near farm silos (silo traps) or at least 1 km from the nearest stored grain along fences dividing paddocks (paddock traps). Traps were located 1.5 m from the ground and trapped beetles were preserved in propylene glycol. Trapping occurred for one week periods at intervals of 4-6 wk from November 2008 to October 2009. Trap catches were transferred to alcohol in the laboratory, the numbers of *R. dominica* and *T. castaneum* adults were counted.

The mating status, longevity and fecundity of beetles intercepted emigrating from farm silos containing infested wheat were determined in the laboratory at 25°C, 55% r.h. A transparent plastic cone was attached to the base of each farm silo and beetles leaving from the bottom hatch area fell into a glass jar at the base of the cone. Individual beetles were removed immediately and put into individual containers of wheat (10 g). The containers of beetles were taken by car to the laboratory within 48 hours of collection during which time they were stored at ambient conditions. On arrival at the laboratory the containers were placed into controlled environment rooms. *Tribolium castaneum* individuals were placed into 250 g of wheat. The beetles were checked at weekly intervals for mortality and any live beetles were transferred to fresh wheat. Dead *T. castaneum* were sexed based on the presence of the sub-basal setiferous puncture on the anterior femur (Halstead, 1963), and R. dominica were sexed based on the genitalia (Potter, 1935). All containers of wheat in which beetles had been present were incubated for 11 wk and then sieved for the adult progeny.

3. Results

Bureau of Meteorology temperature data are shown in Figure 1. Mean monthly temperatures during the study tended to match long-term means except for maximum temperatures from July to October 2009 which were up to 5.1°C higher than average.

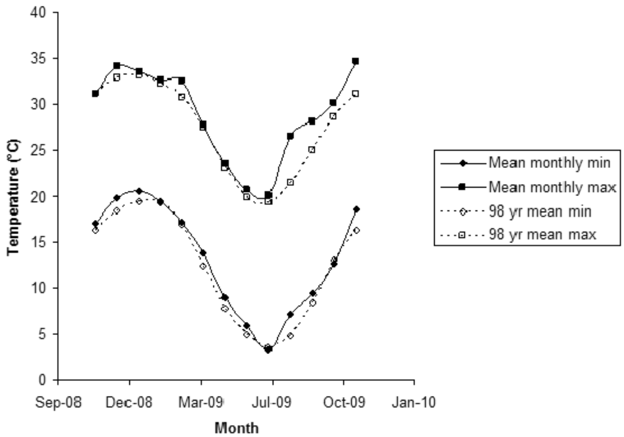

Figure 1 Ambient temperature data for Miles, Australia (source: Australian Bureau of Meteorology).

Figure 2 shows the results of trapping for *R. dominica* in seven sites where traps were located near farm silos and eight sites where traps were located in paddocks. Beetles were trapped throughout the study although few were trapped when traps were set in the winter months of June and July 2009. The mean minimum and maximum temperatures were 7.5 and 20.5°C during the June trapping period and 3.5 and 21.5°C during the July trapping period. The corresponding results for *T. castaneum* are shown in Figure 3. As with *R. dominica*, beetles were trapped throughout the study although few were trapped when traps were set in the months of June and July 2009. The results for *T. castaneum* contrast greatly with those of *R. dominica* in two ways. Mean trap catch tended to be lower and there was an obvious difference between the two categories of trap site, with more beetles being trapped in silos sites than paddock sites.

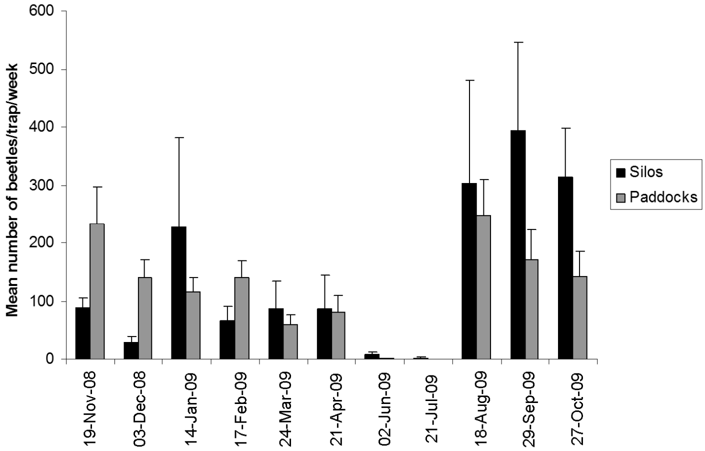

Figure 2 Number (mean & SE) of *Rhyzopertha dominica* adults trapped during 1 wk periods in traps placed near farm silos (n = 7) or in paddocks (n = 8).

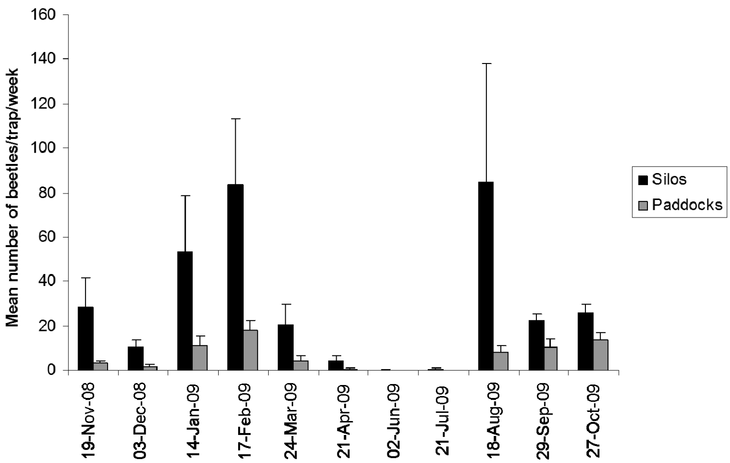

Figure 3 Number (mean & SE) of *Tribolium castaneum* adults trapped during 1 wk periods in traps placed near farm silos (n = 7) or in paddocks (n = 8).

For both species mean trap catch was positively correlated with the variance, indicating that frequency distributions were skewed to the right, and that logarithmic transformation would be needed to normalise the data. There was a strong linear correlation (r11 = 0.935, P < 0.01) between the geometric mean number of *T. castaneum* caught in paddocks and near silos, and approximately three times as many beetles were caught near silos (Fig. 4). Linear correlation was clearly inappropriate for *R. dominica* (Fig. 5) so a non-parametric test was used (Kendal's rank correlation coefficient).

The test showed that there was a weak but significant correlation (τ = 0.382, P < 0.05) between the geometric mean number of *R. dominica* caught in paddocks and near silos.

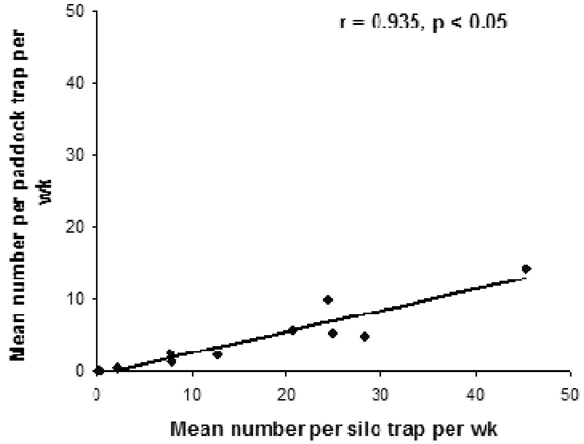

Figure 4 Comparison of geometric mean number of *Tribolium castaneum* adults trapped during 1 wk periods in traps placed near farm silos (n = 7) or in paddocks (n = 8).

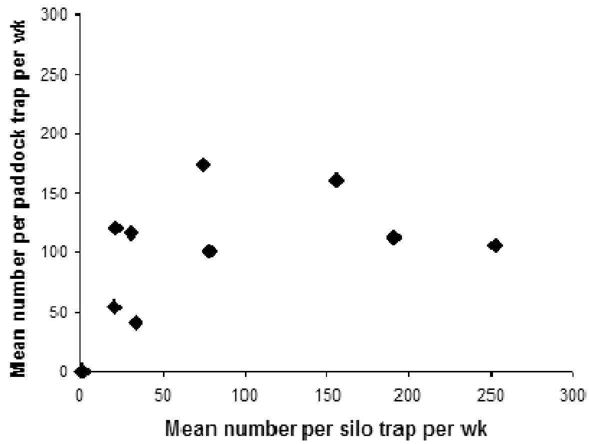

Figure 5 Comparison of geometric mean number of *Rhyzopertha dominica* adults trapped during 1 wk periods in traps placed near farm silos (n = 7) or in paddocks (n = 8).

The study characterising individual beetles emigrating from farm silos has not been completed but preliminary results are informative. The results shown in Table 1 are based on observations on 60 *R. dominica* and 46 *T. castaneum* on wheat at 25°C and 55%rh, yielding data on 31 *R. dominica* females and 28 *T. castaneum* females. Most females had mated before leaving silos, and the average female could reproduce for at least 7 wk without re-mating.

In the case of *R. dominica*, the mean number of offspring per live female declined linearly during the first 7 wk (y = 49.6x – 4.2, r2 = 0.954, F = 104.4, P < 0.001), but this trend was not evident in *T. castaneum*.

Table 1 Reproduction of *Rhyzopertha dominica* and *Tribolium castaneum* females collected emigrating from a farm silo and maintained separately on wheat (25°C, 55% r.h).

Species	Week in Labaratory	Percentage of initial female population producing offspring	Percentage live females producing offspring	Offspring per live female (mean ± SE)
R. dominica[1]	1	96.8	100.0	46.1 ± 3.0
	2	90.6	93.5	39.3 ± 2.4
	3	90.6	96.6	37.2 ± 2.9
	4	84.4	96.4	35.7 ± 2.7
	5	75.0	96.0	28.6 ± 3.1
	6	65.6	87.5	21.5 ± 2.9
	7	56.3	87.7	21.8 ± 3.0
T. castaneum[1]	1	75.0	75.0	4.4 ± 0.8
	2	78.6	81.5	8.2 ± 1.0
	3	78.6	81.5	11.0 ± 1.3
	4	82.1	88.5	13.2 ± 1.4
	5	78.6	84.6	10.1 ± 1.2
	6	75.0	84.0	10.2 ± 1.1
	7	67.9	76.0	8.4 ± 1.3

4. Discussion

The results of pheromone trapping suggest species specific patterns of abundance and distribution for *R. dominica* and *T. castaneum* in subtropical Australia. *Rhyzopertha dominica* has a wide spread distribution of adults that does not appear to be closely linked with stored grain while *T. castaneum* has a much more aggregated pattern of distribution centred around storages. Our results contrast with those of Sinclair and Haddrell (1985) who found no relationship between catch with grain and sticky traps of beetles and nearby infestations except over short distances (less than 100 m).

Two possible explanations of the results obtained so far for *R. dominica* are that beetles are moving significant distances, or that they are reproducing in non-grain hosts away from grain storage. These alternatives are not mutually exclusive and both would have significant implications for managing phosphine resistance. Studies in the USA using pheromones have demonstrated that *R. dominica* can be trapped in open fields and forested sites (Edde et al., 2005; 2006), and there is some evidence that *R. dominica* uses acorns as a non-grain food source resource in the USA (Jia et al., 2008). There is no similar evidence, however, for *R. dominica* using non-grain food sources in Australia.

Changes in trap catch numbers may reflect both changes in abundance or changes in the conditions for flight. Cox et al. (2007) estimated flight thresholds of 20 and 25°C for *R. dominica* and *T. castaneum* respectively in the laboratory, and in Wright and Morton (1995) estimated a threshold for *R. dominica* of 16°C based on diurnal trapping data. Adults of both species were trapped throughout the year although numbers were lower during the two coldest trapping periods, when ambient maximum temperatures were less than 25°C. The large increase in trap catches of both species in August 2009 coincided with unusually high ambient temperatures. Although we have not analysed trap catch data in relation to weather variables, these observations suggest that trap catches in the current study reflect, at least in part, the influence of ambient temperatures on flight.

It would be useful to characterise the beetles being trapped in the rural landscape, both for ecological modelling and modelling resistance development. Preliminary results obtained so far for beetles intercepted leaving farm silos are revealing. The results show that most *R. dominica* and *T. castaneum* females had mated before leaving silos, and that the average female could reproduce for at least 7 wk at moderate temperature without re-mating. This shows the potential of individual females to colonise grain, and suggests a faster spread of resistance genes than would occur if most emigrating females were virgins.

In this paper we have reported preliminary findings from research on the ecology of *R. dominica* and *T. castaneum* in subtropical Australia. We have shown that flight occurs for most of the year, and that beetles are not restricted to the immediate environment around farm storage. Our results suggest that female beetles leaving infested farm are likely to have mated before leaving and are capable of reproducing for many weeks without mating again. Future ecological research will include studies on dispersal, colonisation of grain, the frequency of phosphine resistance, and further characterisation of beetles trapped in the rural landscape. We aim to develop an understanding of how these two species interact with their environment and how these interactions influence resistance development.

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