Interspecific interactions between beetles in multi-species infestations

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Abstract

We evaluated the interspecific interactions between three stored common pests in stored wheat: *Rhyzopertha dominica* (Fabricius), *Tribolium castaneum* (Herbst) and *Cryptolestes ferrugineus* (Stephens). The adult survival, vertical movement, progeny production and progeny distribution were studied in vertical columns (7.6 cm internal diameter and 56 cm high). Adults were introduced alone and together at the top of columns with a uniform moisture content (grain + 5% flour: 12.5 ± 0.1%) at 30 ± 0.5°C and 60 ± 2% r.h. The vertical distribution of the three species varied, but was independent of the other species presence. *Cryptolestes ferrugineus* demonstrated the fastest downward displacement, with uniform distribution throughout the column within 7 days in both treatments. The population growth of *T. castaneum* was promoted by the presence of *R. dominica*. However, the presence of *T. castaneum* did not impede the population growth of *R. dominica*, suggesting the presence of *R. dominica* resulted in a positive interaction for *T. castaneum* through habitat modification. *Cryptolestes ferrugineus* was unable to successfully reproduce under the current rearing conditions. *Tribolium castaneum* exhibited escalated downward distribution after progeny production when reared alone and together. This increase was attributed to resource depletion and not competition. The analysis of the three species in the vertical columns is discussed from an ecological perspective.

Keywords: multi-species, interspecific interactions, *Rhyzopertha dominica*, *Cryptolestes ferrugineus*, *Tribolium castaneum*

1. Introduction

The primary feeder *Rhyzopertha dominica* (Fabricius), and secondary feeders *Cryptolestes ferrugineus* (Stephens) and *Tribolium castaneum* (Herbst) are cosmopolitan beetle species of significant economic importance to stored grain commodities. Their presence within stored products causes significant loss through spoilage and consumption (White, 1983; Loschiavo, 1983; Throne, 1987). In order to effectively control these beetle pests and mitigate their impact, knowledge of their ecological behaviour within storage ecosystems is fundamental to effective management. Data obtained from such studies is useful when evaluating realistic population modelling and control strategies for stored grain beetles (Hagstrum and Throne, 1989; Flinn et al., 2004).

Several laboratory studies have investigated the ecology of single species under various environmental conditions (i.e. commodity, temperature, relative humidity and moisture content) (Parde et al., 2004; Jian et al., 2011, Steel et al., 2012). These small and large scale studies provided important information on intraspecific behaviour of species. However, these studies do not represent field conditions where multi-species infestations and resultant interspecific associations are commonplace (Nansen et al., 2009). Interspecific associations can be termed as direct and indirect interactions between species that have positive or negative effects (Moon et al., 2010). The negative interactions (i.e. competition/predation) between grain beetles has attracted the attention of most grain ecosystem research (Hulasere

et al., 2012; Suresh et al., 2009), whilst the 'positive interactions' between co-existing species has been widely ignored.

Direct and indirect positive interactions and feedback mechanisms are central forces in natural community organisation and dynamics (Bertness and Callaway, 1994). Therefore, these interactions could also be pivotal to spatial distribution and population structure in stored grain ecosystems. In recent years more research has tried to assess interspecific associations from sampling of commercial stores, which provide needed knowledge of populations dynamics derived from existing infestations (Nansen et al., 2004; 2009). However, such studies have no control over environment parameters and are limited in the assessment of species interactions due to the scale of sampling required (Athanassiou, 2011; Steel et al., 2012). Few laboratory studies have attempted to investigate the co-existence of multiple stored product beetle species (Lefkovitch, 1968; Cieselska, 1975; Cui et al., 2006).

Under rearing conditions with limited resources and high insect densities, competition between beetle species has been suggested to impact their respective population growth. In particular, *R. dominica's* population growth has been proposed to be impeded by the presence of *T. Castaneum* under conditions of limited space and resources (Ciao et al., 2004). Additionally, *C. ferrugineus* has been indicated to hinder the population growth of *T. castaneum* (Suresh, 2001; Hulasere, et al., 2012). It is hypothesised that at low initial densities and with ample resources; the negative interactions previously reported will be absent. Furthermore, the co-existence of beetle species may result in positive interactions beneficial to one or more species. This assessment was achieved by rearing *R. dominica*, *T. castaneum* and *C. ferrugineus* alone and together in vertical experimental columns. The insects were kept at low densities, with ample host commodity, and under constant rearing conditions. The objective of this project was to investigate the effect of interspecific interactions on the adult survival, vertical distribution, progeny production and progeny distribution of the different species.

2. Materials and Methods

2.1. Insects

Adults of *R. dominica, T. castaneum* and *C. ferrugineus* were supplied from wild strain cultures maintained at the Department of Agriculture, Fisheries and Fisheries, Queensland. All adult insects used for experiments were 7-14-days old. The *Rhyzopertha dominica* stock culture was maintained 0n hard wheat 12% m. at 30°C and 55% r.h. *Tribolium castaneum* was maintained on organic wholemeal flour + 5% Brewer's Yeast 12% m.c. at 30°C and 55% r.h. *Cryptolestes ferrugineus* was maintained on a diet comprised of rolled oats + sorghum powder + yeast 12 % m.c. at 30°C and 75% r.h. (Jagadeesan et al., 2013).

2.2. Experimental columns and conditions

Purpose built experimental columns were designed and constructed of transparent Perspex rings (8cm in height and 7.6 cm internal diameter). Nine of these sections were connected together with air-tight flanges, to form a vertical column measuring 72 in height. The columns were capped at the bottom, and had a removable Perspex flange lid with a 3 cm (diameter) hole drilled in the centre, which was covered with filter paper to prevent escapees and enable adequate gas exchange (Figure 1). Columns were filled with ~2 kg of medium comprised of *Katana* whole grain wheat + 5% organic wholemeal flour (uniform 12.5% m.c.) to the top of the second highest ring, which left a headspace of 8 cm. Thirteen columns were constructed, with 12 columns used for single and mixed species rearing experiments and an additional column served as a reference column for continuous monitoring of temperature and humidity. Temperature and humidity were monitored in the reference column using data loggers (Lascar, Australia) buried within the bottom, middle

and grain surface strata. The wheat and flour used for experiments was conditioned at 30°C and 60% r.h. for 14 days prior to introduction to the columns.

Experiments were initiated by introducing (unsexed) adult *R. dominica*, *T. castaneum* and *C. ferrugineus* at the top of columns, and then sealing them with the Perspex lid. Insect density for single species experiments was 10 adults, and10 of each species in the multi-species treatments. Each experiment consisted of three replicates. All columns were kept in a temperature and humidity controlled cabinet (Thermo Fisher, Australia) at 30°C and 60% r.h. for 8 weeks. Data from the reference tower revealed temperature and humidity (30 ± 0.5°C and $60 \pm 1\%$ r.h.) was uniform throughout the columns for the duration of the experiment.
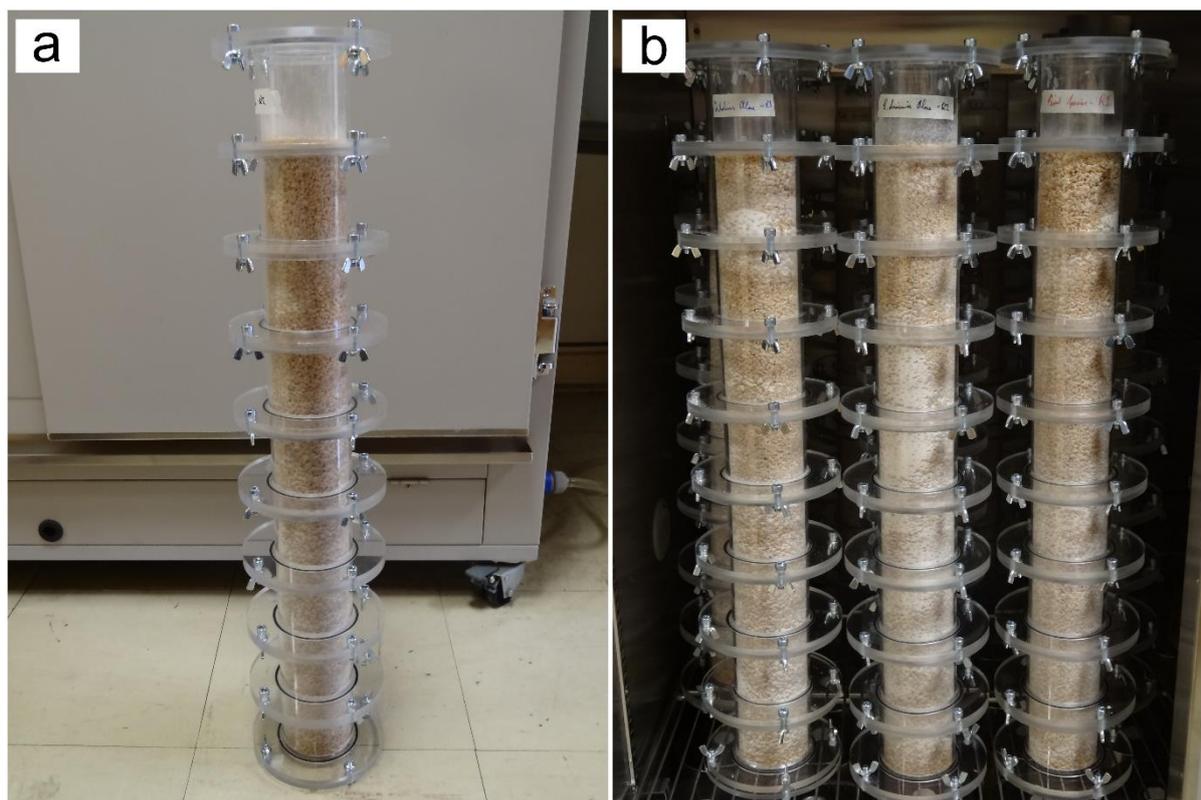

Figure 1 Experimental columns used for mixed and single species rearing experiments a) experimental column and b) experimental columns within rearing chamber.

2.3. Population survival and vertical distribution

To investigate interspecific associations between species, survival and vertical movement within columns was obtained for single and multi-species treatments. Adult survival for single and mixed species treatments was recorded on sample days 7, 14 and 28. Sampling involved column section flange bolts being loosened and metal plates (8.5 x 15 cm) inserted between flanges connecting strata. This enabled the top stratum to be removed and its contents poured into holding glass jars. The medium from each stratum was then sieved and the number of live and dead insects recorded. Dead insects were removed and all contents and live insects returned to the stratum of origin. Simultaneously, the number of live and dead insects present in different vertical locations of the columns was also recorded. The analysis of vertical displacement was conducted using a covariance model based on methods described by Vardemann et al. (2006). This involved each of the column sections filled with medium being assigned a number from one to eight, with one being the top stratum. This number is then multiplied by the number of live insects present in the corresponding stratum and summed

over all strata. The sum was then divided by the total number of live insects in each column to obtain a relative index of net vertical displacement at sample intervals. The higher value indicates increased movement from the introduction point.

2.4. Progeny production and progeny distribution

Adult progeny produced in all experimental columns was recorded after 56 days of rearing. The density of insect numbers in each stratum was also recorded, to assess if spatial distribution of the progeny population differed between single and multi-species treatments.

2.5. Statistical analysis

Insect counts were square root transformed (if not normally distributed) and analysed using ANOVA. Insect number was the dependent variable, and species (separate or combined) were independent variables. ANOVAs were also used to identify significant variations in means per strata between rearing conditions for vertical distribution analysis. Treatment means from the different analysis were grouped into homogeneous subsets with the Waller–Duncan test (type I/type II error ratio = 100). Conditions within a subset were considered to be not significantly different. All statistical analyses were performed using SPSS software (SPSS for Windows, Release 22.0.0., SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Population survival

Table 1 displays adult survival of the three species at the three designated sample intervals. There was no significant difference in adult survival at sample days 7, 14 and 28 ($F_{(1, 4)} = 0.07$; p = 0.79, $F_{(1, 4)} = 0.10$; p = 0.76 and $F_{(1, 4)} = 0.06$; p = 0.81 respectively) between *R. dominica* reared alone or in mixed species cultures. Similarly, *T. Castaneum* showed no adult mortality in either treatment on day 7, and no significant difference between treatments on sample days 14 and 28 ($F_{(1, 4)} = 4.0$; p = 0.11 and F (1, 4) = 4.0; p = 0.11 respectively). There were no significant differences in survival of adults of *C. ferrugineus* when cultured alone or with other species on sample days 7 and 14 ($F_{(1, 4)} = 1.8$; p = 0.27 and $F_{(1, 4)} = 2.4$; p = 0.21 respectively). However, on sample day 28 the adult *C. ferrugineus* population was significantly higher in the mixed species column ($F_{(1, 4)} = 8.0$; p = 0.04).

Table 1 Adult population survival for *Rhyzopertha dominica*, *Tribolium castaneum* and *Cryptolestes ferrugineus* sampled on days 7, 14 & 28 in mixed and single species treatments. (mean ± SD)[a].

Treatment		Survival (%)		
		Day 7	**Day 14**	**Day 28**
R dominica	(mixed)	90.0 ± 17.3Aa	86.7 ± 15.3Aa	80.0 ± 17.3Aa
	(alone)	93.3 ± 11.5Aa	90.0 ± 10.0Aa	83.3 ± 15.3Aa
T. castaneum	(mixed)	100 ± 0Aa	93.3 ± 5.8Aa	93.3 ± 5.8Aa
	(alone)	100 ± 0Aa	100 ± 0Aa	100 ± 0Aa
C. ferrugineus	(mixed)	100 ± 0Aa	96.7 ± 5.8Aa	93.3 ± 5.8Aa
	(alone)	93.3 ± 5.8Aa	90.0 ± 0Aa	66.7 ± 15.3Bb

(*n* = 3)
[a]Means followed by different uppercase letters are significantly different within species conditions (mixed/alone); and means followed by different lowercase letters are significantly different between species under all conditions (P < 0.05, Waller–Duncan k-ratio t-test).

3.2. Vertical distribution

Figures 2-4 display the vertical movement of the three species, reared together and alone on days 7, 14 and 28. The vertical movement of *R. dominica* recorded within 7 days revealed that 100% of the population remained in the top two layers of the column (<16 cm), with no significant difference between single and mixed species treatments (ANOVA; P > 0.05) (Fig. 2a). On day 14 adults had dispersed further down the column as far as the fifth layer (40 cm), and were distributed evenly with no significant difference (ANOVA; P > 0.05)between occupied strata or treatments (Fig 2b). On day 28 adults in both treatments had dispersed throughout the column with a uniform distribution, and no significant difference between strata or treatments (ANOVA; P > 0.05) (Fig. 2c). *Tribolium castaneum* exhibited no substantial downward dispersal at any sample interval and ~ 100% of the population remained near the introduction point in the top two strata (< 18 cm), with no significant difference between treatments (ANOVA; P > 0.05) (Fig. 3a-c). *Cryptolestes ferrugineus* was distributed throughout the column within 7 days and remained relatively uniformly dispersed on days 14 & 28; with no significant difference between strata and sample interval (ANOVA P > 0.05) (Fig. 4a-c). These results show that there was no significant differences in the vertical displacement within species treatments (mixed v alone), there were significant differences in the vertical displacement between species which is represented in Table 2.
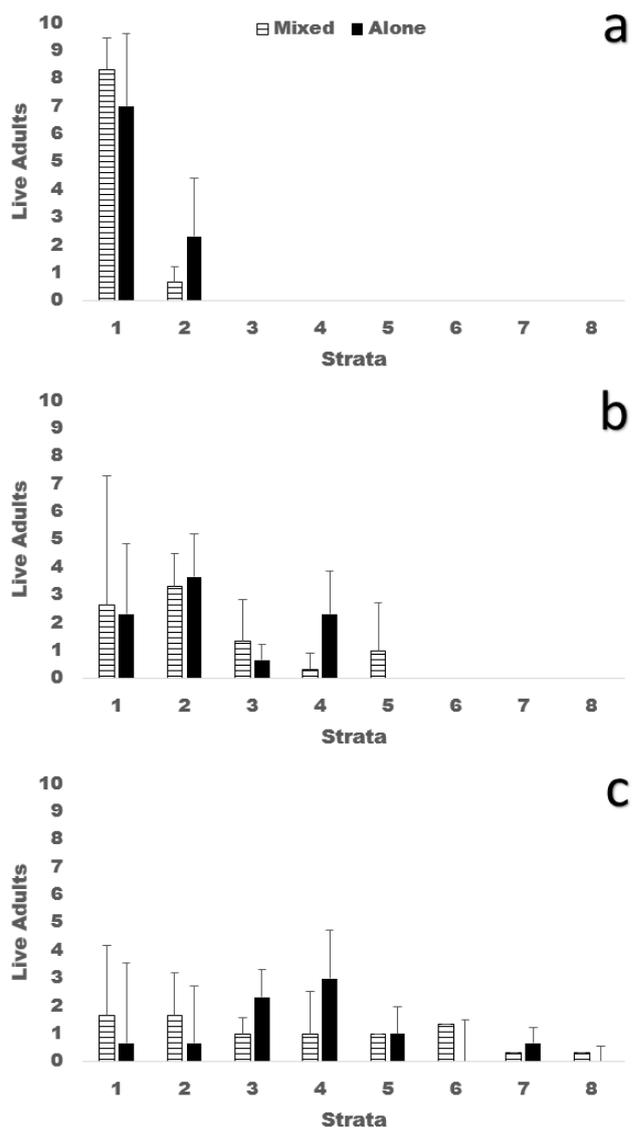
Figure 2 Distribution of *Rhyzopertha dominica* live adult insects in vertical strata, sampled on days 7 (a), 14 (b) & 28 (c) in mixed and single species treatments. (mean ± SD), * Above column bars at respective stratum indicates significant difference between treatments (P < 0.05, Waller–Duncan k-ratio t-test).
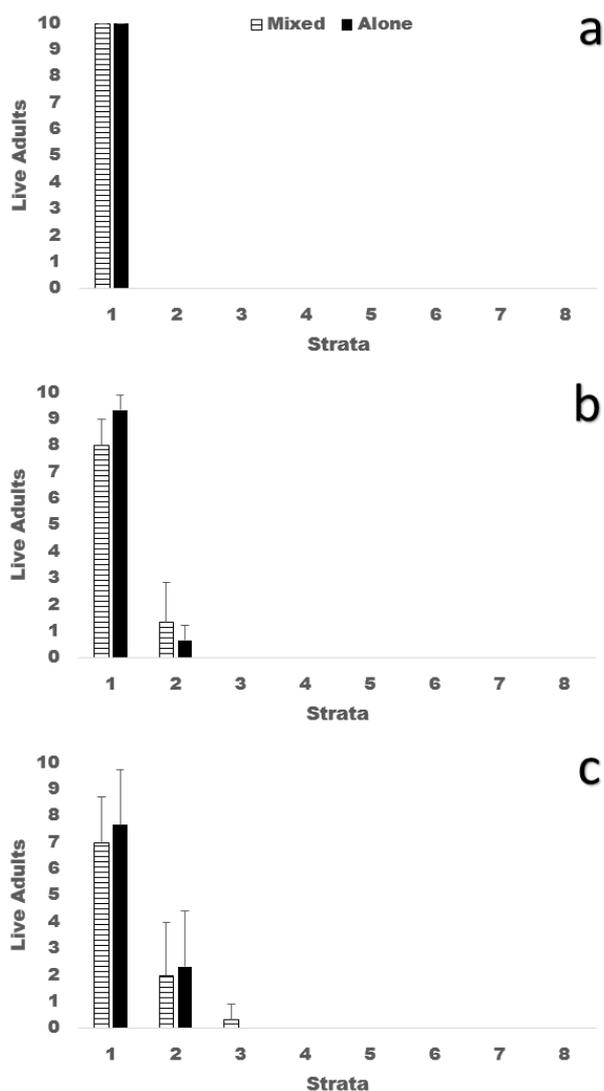
Figure 3 Distribution of *Tribolium castaneum* live adult insects in vertical strata, sampled on days 7 (a), 14 (b) & 28 (c) in mixed and single species treatments. (mean ± SD), * Above column bars at respective stratum indicates significant difference between treatments (P < 0.05, Waller–Duncan k-ratio t-test).
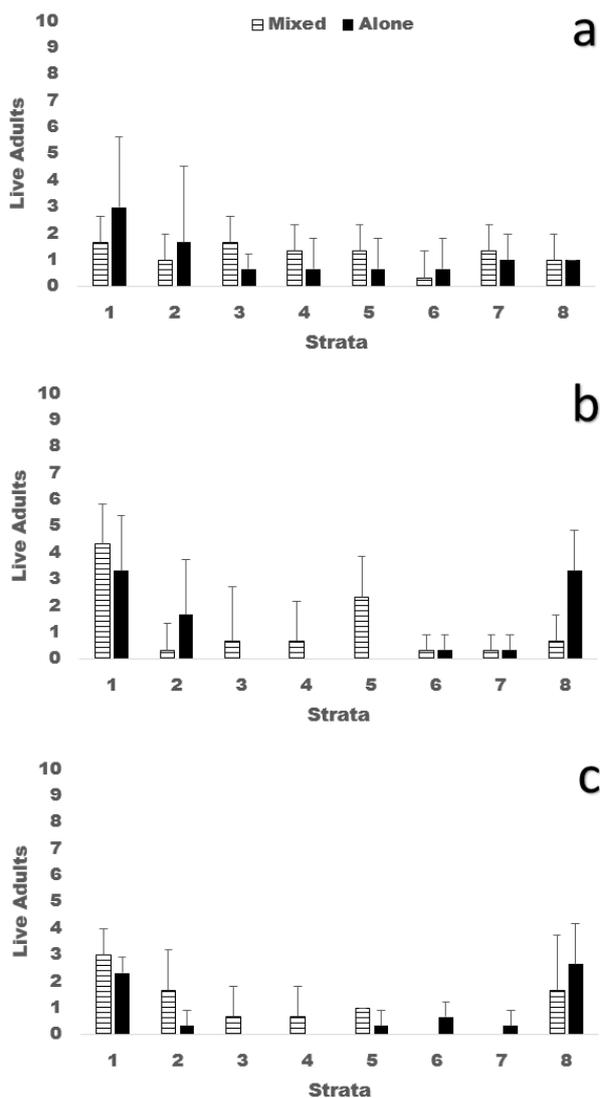
Figure 4 Distribution of *Cryptolestes ferrugineus* live adult insects in vertical strata, sampled on days 7 (a), 14 (b) & 28 (c) in mixed and single species treatments. (mean ± SD), *Above column bars at respective stratum indicates significant difference between treatments (P < 0.05, Waller–Duncan k-ratio t-test).

Table 2 Index of vertical displacement of live adult *Rhyzopertha dominica, Tribolium castaneum* and *Cryptolestes ferrugineus* in mixed and single species treatments at different sample intervals. (mean ± SD)[a].

Treatment		Vertical Displacement		
		Day 7	Day 14	Day 28
R. dominica	(mixed)	1.1 ± 0.1Aa	2.3 ± 1.1Aa	3.7 ± 1.9Aa
	(alone)	1.3 ± 0.2Aa	2.3 ± 0.5Aa	3.6 ± 1.0Aa
T. castaneum	(mixed)	1.0 ± 0.0Aa	1.1 ± 0.2Ab	1.3 ± 0.2Ab
	(alone)	1.0 ± 0.0Aa	1.1 ± 0.1Ab	1.2 ± 0.2Ab
C .ferrugineus	(mixed)	4.2 ± 0.6Ab	3.9 ± 1.0Aa	3.3 ± 1.3Aa
	(alone)	3.5 ± 0.8Ab	4.2 ± 0.9Aa	4.7 ± 1.2Aa

($n = 3$)
[a]Means followed by different uppercase letters are significantly different within species conditions (mixed/alone); and means followed by different lowercase letters are significantly different between species under all conditions ($P < 0.05$, Waller–Duncan k-ratio t-test).

3.3. Progeny production

Table 3 displays the mean values for progeny production for all three species reared alone and in mixed culture treatments after 56 days. *Tribolium castaneum* exhibited the largest population growth of the three species, with a significant difference between treatments and larger population growth in the mixed species culture ($F_{(1, 4)} = 8.07$; p = 0.04). *Rhyzopertha dominica* was not significantly affected between single and mixed species rearing conditions ($F_{(1, 4)} = 2.75$; p = 1.72). *Cryptolestes ferrugineus* was incapable of progeny production under the rearing conditions, with no significant difference between treatments ($F_{(1, 4)} = 2.70$; p = 1.76). Unfortunately, the lack of progeny production by *C. ferrugineus* resulted in the species being omitted from distribution analysis.

Table 3 Live progeny produced by *Rhyzopertha dominica*, *Tribolium castaneum* and *Cryptolestes ferrugineus* after 56 days of rearing under single and mixed species conditions. (mean ± SD)[a].

Treatment		Progeny Production
R. dominica	(mixed)	183.7 ± 35.0Aa
	(alone)	236.7 ± 42.8Aa
T. castaneum	(alone)	361.0 ± 68.7Bb
	(mixed)	220.0 ± 45.3Aa
C. ferrugineus	(alone)	6.7 ± 7.02Ac
	(mixed)	0 ± 0.00Ac

($n= 3$)
[a] Means followed by different uppercase letters are significantly different within species conditions (mixed/alone); and means followed by different lowercase letters are significantly different between species under both rearing conditions (P < 0.05, Waller–Duncan k-ratio t-test).

3.4. Progeny distribution

The distribution of *R. dominica* progeny distribution was relatively homogenous, with the only significant difference between treatments occurring in the third stratum ($F_{(1, 4)} = 36.11$; p = 0.004). The greater portion of *T. castaneum* populations was located in the top strata, however there was increased distribution throughout lower strata in the mixed species culture. With a significant difference between the treatments in strata five ($F_{(1, 4)} = 19.83$; p = 0.01), six ($F_{(1, 4)} = 8.93$; p = 0.04) and eight ($F_{(1, 4)} = 15.09$; p = 0.01) (Fig. 5a-b.).
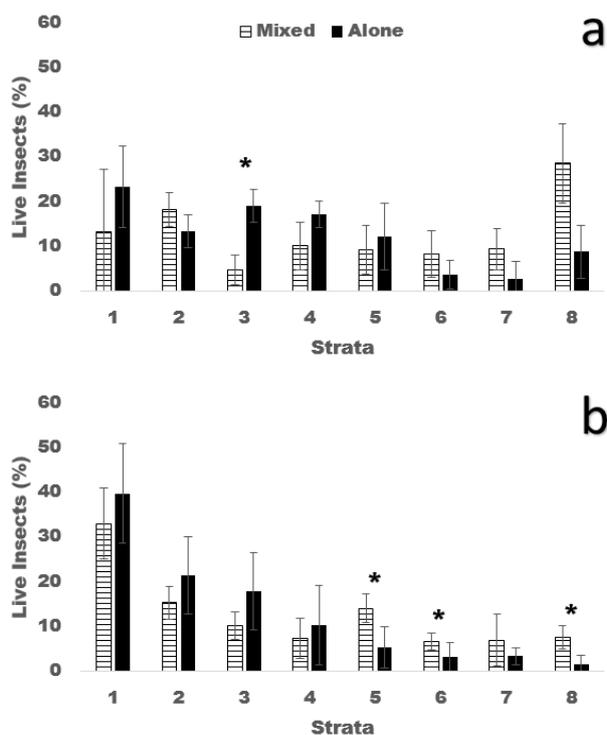

Figure 5 Distribution of (a) *Rhyzopertha dominica* and (b) *Tribolium castaneum* live progeny in vertical strata, sampled after 56 days of rearing in mixed and single species treatments (mean±SD).* Above column bars at respective stratum indicates significant difference between treatments (P < 0.05, Waller–Duncan k-ratio t-test).

4. Discussion

The results from our study supported the hypothesis that in the absence of resource depletion, beetle species may co-exist in stored grain environments without any direct or indirect negative interactions. Positive interactions between beetle species may promote population growth in storage ecosystems. Adult populations of *R. dominica* and *T. castaneum* exhibited no significant mortality in single or mixed species conditions. However, *C. ferrugineus* did suffer significantly higher mortality in the single species treatment. The environmental conditions and diet provided may have been inadequate for this species; which is susceptible to suboptimal temperature, humidity, moisture content (Smith, 1962; Currie, 1967) and diet (Jagadeesan et al., 2013).The addition of wholemeal flour was hoped to enhance diet suitability and simulate dockage in the bulk grain.

Vertical distribution varied between the three species, and appeared to be independent of interspecific interactions throughout the first 28 days of the test. *R. dominica* dispersed homogenously throughout the grain columns within 14 days. As abiotic factors were uniform in these experiments, it is likely this behaviour was due to intraspecific interaction. The

dispersal could be driven by search for mates and movement post oviposition (Edde, 2012). *Tribolium castaneum* had limited vertical dispersal in the first 28 days of culture, and nearly 100% of insects occurred exclusively within <18 cm of the grain surface, irrespective of intra or interspecific interactions. This preference for surface layers has been observed in farm bins (Hagstrum, 2000) and in laboratory experiments (Surtee, 1965). Jian et al. (2005) suggested vertical distribution of *T. castaneum* could be impeded by the small granular space in bulk wheat. However, distribution of progeny in these experiments indicated that a large proportion of the population had distributed throughout the tower, with large numbers at the bottom of towers in both single and mixed species treatments. The dispersal of *T. castaneum* to the bottom of columns in comparison with middle strata would have required increased fitness cost, and suggests that distribution may be driven by depletion of resources and increasing density. This dispersal may have been stimulated by an increased emission of benzoquinones, which *T. castaneum* release as anti-aggregation pheromones in response to density and stress (Duehl et al., 2011).

Cryptolestes ferrugineus distributed relatively homogenously throughout the vertical column within 7 days, and displayed no preference for vertical depth under the present experimental conditions of uniform moisture content and temperature. Flinn and Hagstrum (1998) similarly reported no spatial preference in the absence of a temperature gradient in this species. Increased vertical distribution of *C. ferrugineus*, in small and large grain columns has been attributed to varying moisture content, temperature gradients, drift effect and size of columns (Jian et al., 2004, 2006, 2009). Increased dispersal of *C. ferrugineus* has also been attributed to high densities, whereas low densities (0.1 & 1/kg) cause the insects to cluster (Jian et al., 2011). However, in the current study we observed that *C. ferrugineus* was distributed throughout the column with relatively small initial densities (~3/kg). Furthermore, Nansen (2009) noted that in commercial sites *C. ferrugineus* crowding did not appear to elicit dispersal at high densities and presence of other species. It must be noted that both studies were under extremely different conditions, making direct comparisons difficult.

In the present study, there were no adverse effects observed on progeny production of *R. dominica,* when reared in mixed species conditions. This was the case even though *T. castaneum* had an accelerated reproduction rate when reared with *R. dominica*. It is likely that *T. castaneum* benefitted from damage to the grain caused by *R. dominica* feeding and reproductive processes (Edde, 2012). This habitat modification by the primary pest could be viewed as an indirect positive interaction between the two beetle species. Additionally, under abundant conditions the relationship between the two species could be described a form of 'commensalism'. This is defined as a relationship between different species where one organism gains benefit from the relationship and the other is unaffected (Stachowicz, 2001; Moon et al., 2004). The similar reproduction rate of *R. dominica* in both mixed and single species indicates co-existence was not detrimental to this species. However, other studies under limited space and food resources have indicated that *T. castaneum* impedes the reproductive rate of *R. Dominica* (Cui et al., 2004). In those studies, food resources would have become depleted, possibly resulting in the omnivorous *T. castaneum* consuming immature stages of *R. dominica*.

With resources and space being abundant in most grain storage environments, competition would not be the principle interaction determining densities and spatial distribution of insect populations. Conversely, if shared resources (host commodity) becomes a limiting factor, there would be a negative effect on one species resulting in resource depletion mediated competition (Moon and Keagy, 2010). In many laboratory studies of interspecific associations, this could be inadvertently occurring through experimental design. Numerous studies indicating traditional 'competition' between beetle species, may have really been

observing resource dependant competition induced by high insect densities, limited space and food resources. A grain storage facility is an abundant resource that is very difficult to replicate in laboratory studies. Nevertheless concentrated efforts must be taken in experimental design, to avoid erroneous conclusions drawn from what amount to artificial interspecific associations; driven by crowded conditions in laboratory experiments. This is especially important if the data obtained is to be used in the integrated management of storage pests.

5. Conclusion

This preliminary study demonstrated that positive interactions can occur between species co-existing in stored grain ecosystems and provides further understanding into the ecology of stored grain beetles, and the interactions which drive community structure and population dynamics. Future work should investigate interspecific conditions between species at different introduction points, and also under various environmental conditions. Understanding of the inter-specific interactions in multi-species infestations is important, when evaluating realistic population modelling and control strategies for stored grain pests.

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