Reproductive efficiency of the bethylid wasp *Cephalonomia tarsalis:* the influences of spatial structure and host density

P.A. Eliopoulos[1]*, A. Kapranas[1,2,3], E.G. Givropoulou[1] and I.C.W. Hardy[2]

[1]Department of Agricultural Technologists, Technological Educational Institute of Thessaly, Larissa, Greece: [2]School of Biosciences, University of Nottingham, Sutton Bonington Campus, Leicestershire, LE12 5RD, UK: [3]Department of Biology, National University of Ireland, Maynooth, County Kildare, Ireland

Abstract

The parasitoid wasp *Cephalonomia tarsalis* (Ashmead) (Hymenoptera: Bethylidae) is commonly present in stored product facilities. While beneficial, it does not provide a high degree of biological pest control against its host, the saw-toothed beetle *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae). A candidate explanation for poor host population suppression is that adult females interfere with each other's foraging and reproductive behavior. We used simple laboratory microcosms to evaluate such mutual interference in terms of its overall effects on offspring production. We varied the density of the hosts and also the spatial structure of the environment, via the extent of population sub-division and the provision of different substrates. Production of *C. tarsalis* offspring was positively influenced by host density and by the isolation of females. With incomplete sub-division within microcosms offspring production was, in contrast, low and even zero. The provision of corrugated paper as a substrate enhanced offspring production and partially mitigated the effects of mutual interference. We recommend simple improvements to mass rearing practice and identify promising areas for further behavioral and chemical studies towards a better understanding of the mechanisms of mutual interference.

Keywords: mutual interference, *Cephalonomia tarsalis*, *Oryzaephilus surinamensis*, stored products, behavioural and chemical interactions

Introduction

The bethylid wasp *Cephalonomia tarsalis* (Ashmead) (Hymenoptera: Bethylidae) is a larval ectoparasitoid of beetles, mainly those belonging to the genus *Oryzaephilus*. It is the most common natural enemy of the saw-toothed grain beetle, *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae),

which is a very common pest of many agricultural stored products (Sedlacek *et al.*, 1998; Johnson *et al.*, 2000; Asl *et al.*, 2009). Despite its common presence in storage facilities *C. tarsalis* has been regarded as a 'poor' biocontrol agent given that significant pest infestation can occur even in cases where wasp population density is very high (Powell, 1938; Eliopoulos *et al.*, 2002*a*, *b*). One of the first studies of the biology of *C. tarsalis* concluded that, due to its low reproductive output, this wasp was not of economic importance as a biological control agent (Powell, 1938).

Pest problems in stored products have persisted and the use of fumigants to control infestations is no longer favored due to associated pollution and the development of

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*Author for correspondence
Phone: +302410684280
Fax: +302410613153
E-mail: eliopoulos@teilar.gr

insecticidal resistance (e.g. Arbogast & Throne, 1997; Sedlacek *et al.*, 1998; Johnson *et al.*, 2000; Lukáš, 2007; Asl *et al.*, 2009). More recent studies on *C. tarsalis* have taken a more optimistic view of its potential as an agent of biological pest control; some have evaluated aspects of its life-history, behavior and chemistry that are likely to influence its biocontrol potential (e.g. Howard *et al.*, 1998; Johnson *et al.*, 2000; Lukáš, 2007; Collatz & Steidle, 2008; Hötling *et al.*, 2014) and others have examined its interactions with other species of parasitoids, mites, parasitic protozoans or fungi that are also natural enemies of *O. surinamensis* (Johnson *et al.*, 2000; Lord, 2001, 2006; Žďárková *et al.*, 2003; Latifian *et al.*, 2011). Some of these inter-specific interactions appear beneficial, for pest suppression and/or for *C. tarsalis* (Žďárková *et al.*, 2003; Lord, 2006) while others are clearly detrimental to *C. tarsalis* (Lord, 2001).

The reproductive behaviour of *C. tarsalis* shares many commonalities with other species of Bethylids, but exhibits especially elaborate host handling. The wasp locates host habitats, and hosts, using volatile chemical cues deriving from the host's food, hosts themselves and host faeces (Collatz & Steidle, 2008; Hötling *et al.*, 2014). Encountered hosts are recognized by chemical cuticular cues perceived by antennae as well as host movement; vision plays only a limited role in host finding and recognition (Howard *et al.*, 1998). On encountering a host, the female wasp paralyzes it permanently by injecting venom via a sting and then drags it to a shelter (e.g. a hollow wheat grain, cracks in the walls and floor of storage facilities) (Powell, 1938; Howard *et al.*, 1998). The female also rubs the host larva all over with the tip of her abdomen and then host feeds (Howard *et al.*, 1998). The female may leave the host and return several times, and may move it to a new location (Howard *et al.*, 1998). Several hosts may be paralyzed and hidden before the female commences oviposition and each host is hidden in a separate location (Howard *et al.*, 1998; P.A.E. personal observations). Females may aggressively defend their 'oviposition patch' against conspecific females (Collatz *et al.*, 2009).

Eggs are typically laid singly or in pairs onto each host (Powell, 1938; Lukáš, 2007). Very rarely 3–4 eggs may be found on a host (e.g. when hosts are very scarce) but only two of them complete development (Powell, 1938). In cases of single egg deposition, 80% are female whereas in cases of paired eggs there is almost always one male and one female produced; unfertilized eggs produce haploid males (arrhenotoky) (Powell, 1938; Cheng *et al.*, 2003). Development from egg to adult takes between 26.3 days (at 21°C) and 11.4 days (at 30°C) (Lukáš & Stejskal, 2005). Developmental mortality is affected by temperature; at 24–30°C it normally ranges between 20 and 38% but reaches 80–91% at the more extreme low (<18°C) or high (>35°C) temperatures at which assays have been performed (Lukáš, 2007). The most susceptible period for mortality is the transition from larval to pupal stage (Lukáš & Stejskal, 2005).

Males usually emerge as adults 1–2 days earlier than females (Powell, 1938). In cases where a male and female develop on the same host, the male will enter the female's cocoon and copulate with her (Powell, 1938; Zimmermann *et al.*, 2008). However, Cheng *et al.* (2003) observed males leaving the vicinity of the host after emergence and concluded that sib-mating may not be a common phenomenon in *C. tarsalis*. Collatz *et al.* (2009) subsequently verified that *C. tarsalis* displays partial local mate competition, i.e. a mixture of sibling and non-sibling mating (Nunney & Luck, 1988; Hardy & Mayhew, 1998). Sex ratios (proportion of offspring that are

male) are typically around 0.3–0.5 (Cheng *et al.*, 2003; Lukáš, 2008; Zimmermann *et al.*, 2008; Collatz *et al.*, 2009). Male wasps may live up to 6 days and may copulate with many different females. Males compete directly with each other, via pushing, pulling and grasping, for mating opportunities with females (Cheng *et al.*, 2003). The females live for about 35 days and copulate once or very few times (Powell, 1938; Cheng *et al.*, 2003); the mating frequency of females does not affect the subsequent production of female progeny (Cheng *et al.*, 2004). Females can produce up to around 200 eggs in their lifetime, under laboratory conditions (27°C and plentiful supplies of fresh host) but realized fecundities of 50–100 eggs are more typical and are reduced at higher and lower temperatures (Lukáš, 2007).

Interactions with conspecifics are mediated by chemical recognition cues, such as dodecanal, cuticular hydrocarbons and hydrocarbon components secreted by the Dufour gland (Howard & Infante, 1996; Howard, 1998; Howard & Pérez-Lachaud, 2002; Howard & Baker, 2003; Collatz *et al.*, 2009). Stressed adults also release the volatile skatole (3-methylindole) (Goubault *et al.*, 2008), which may disrupt subsequent reproductive behavior (Gómez *et al.*, 2005; Hardy & Goubault, 2007). Occurence of superparasitism has not been considered as a realistic possibility in our study. Many faunistic studies on the insect fauna in stored grains have revealed hyperparasitoids do not exist in the 'closed' environments of grain storage facilities (Eliopoulos *et al.*, 2002*a*, *b*).

In this study we evaluated the effects of intra-specific interactions between *C. tarsalis* females. A candidate explanation for the poor host population suppression by *C. tarsalis* is that adult females interfere with each other's foraging and reproductive behavior; a phenomenon known as mutual interference (Hassell & May, 1989; Hassell, 2000; Kidd & Jervis, 2005). Among parasitoids in general, mutual interference can have a variety of causes including delayed searching following encounters, host and patch guarding, fighting behavior, and altered decisions concerning superparasitism, clutch size and sex allocation (e.g. Hassell & May, 1973; Visser *et al.*, 1990; Driessen & Visser, 1997; Meunier & Bernstein, 2002; Goubault *et al.*, 2007; Yazdani & Keller, 2015). Few of these aspects have been directly evaluated in the context of interactions with conspecifics in *C. tarsalis* but it is known that these parasitoids may occur at moderately high density in stored products (Sedlacek *et al.*, 1998) and agonistic interactions between foraging females have been observed (Collatz *et al.*, 2009). Our approach was to evaluate mutual interference, in terms of its overall effects on offspring production, using simple laboratory microcosms; within these we varied the density of the hosts (individuals per unit area) and the spatial structure of the wasps' and host's environment. The effect of spatial structure was explored both by varying the extent of population sub-division and by provision of different substrates. We use our results to recommend improvements to mass rearing practice and to identify promising areas for further work towards improving stored product biological control using *C. tarsalis*.

Materials and methods

Insects

We studied the wasp *C. tarsalis* and its host the saw-toothed grain beetle, *O. surinamensis*. The beetle was kept in culture in
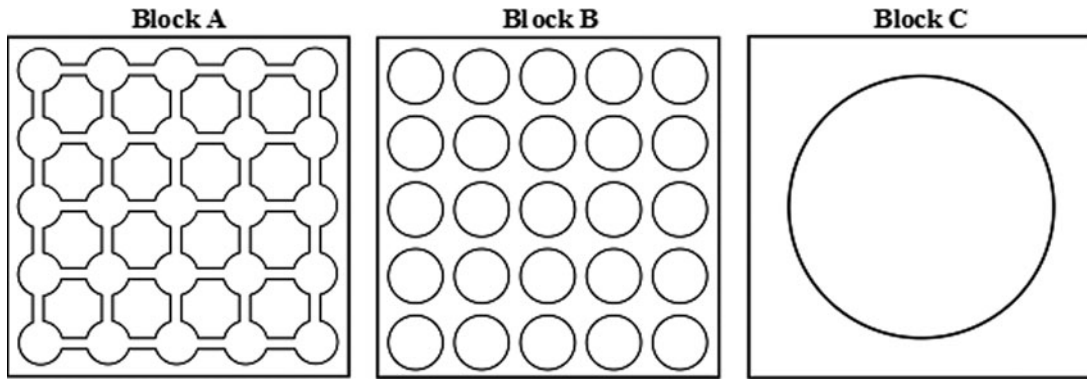
Fig. 1. Treatment block types. The total floor area of the chambers was 72.7 cm^2 in all block types.

2-litre clear plastic jars in the laboratory using a mixture of crushed wheat: rolled oats: dried yeast (5:5:1). The wasp was kept in culture using the same rearing medium and jars as the beetle, with a large number of full-grown host larvae. Small pieces (2 cm × 2 cm) of corrugated paper were introduced to the wasp culture jars (15–20/jar) as 'shelters' for the female wasps. All insect cultures were kept under controlled environmental conditions (27°C, 16:8 L:D, 60% R.H.). Paper shelters were replaced every 2–3 days and those with parasitized larvae were transferred to Petri dishes until wasp eclosion. Wasps were collected daily for use in the experimental treatments.

Experimental treatments

We assessed the effects of spatial structure on *C. tarsalis* reproduction using experimental arenas excavated from opaque plastic blocks with transparent Plexiglas lids (fig. 1); designs were based on those used by Sreenivas & Hardy (2016). Three different block types were used: Type A blocks were multi-chamber arenas; the diameter each of the 25 chambers was 1.78 cm. Chambers were interconnected by passages (1 cm long × 0.4 cm wide). The total area of chambers and passages within the block was 72.7 cm^2 (fig. 1a). Type B blocks were similar to Type A, but there were no passages between the chambers, which were thus isolated from each other, and chambers were of greater diameter (1.92 cm) in order to maintain the overall floor area of 72.7 cm^2 (fig. 1b). Type C blocks contained a single circular chamber of diameter 9.62 cm, a floor area of 72.7 cm^2 (fig. 1c). All chambers and passages were 0.6 cm deep. Spatial structure was further varied by placing within the chambers a single layer of wheat kernels, a small piece of corrugated paper (1 cm × 1 cm) or no additional substrate (empty chamber). When paper was present, 25 pieces were placed in the single chamber (block type C) or 1 piece per chamber in the multi-chamber blocks (types A & B). Host density was varied by placing either 25 (low density) or 125 (high density) hosts into each block. For single chamber blocks (type C) there were 25 or 125 host larvae in the chamber and for multi-chamber blocks (types A & C) there were either 1 or 5 host larvae in each chamber. There were 10 replications of each of the 18 combinations of experimental conditions, giving 180 replicates in total.

Parasitoid density was held constant at 25 adult female wasps per block, with either 25 placed in the single chamber of the block (type C) or one wasp placed into each chamber

of the multi-chamber blocks (type A & B). Wasps were briefly anesthetized with CO_2 to place them into the chambers. Female age of *C. tarsalis* at the start of the experiment was 3–5 days. Blocks were inspected daily and adult wasps were removed, once the progeny started reaching pupal stage, to prevent subsequent confusion with adult female offspring. The pupae were collected from each block, counted and transferred to Petri dishes (diameter 9 cm) where they were checked daily for emergence of adult offspring. The number of adults and their sex were subsequently recorded. Before proceeding to the main analysis of data on adult numbers we checked that the probability of the collected pupae surviving to adulthood did not differ between experimental treatments: there were 11 combinations of experimental conditions under which some offspring reached pupation and their post-collection survival did not vary significantly across these (logistic ANOVA: $F_{10,93} = 0.55$, $P = 0.847$).

The experiment was thus essentially a factorial design testing the effects on parasitoid offspring production of host density (low or high), substrate structure (3 types of substrate) and either gross spatial structure (single or multi-chamber blocks) or interconnectedness (passages open or closed). As there could be no passages within single chamber blocks, the analysis was however constrained to proceed in two main steps, after testing for differences across all treatments; the first step using data from single chamber blocks and multi-chamber blocks and the second using data from both types of multi-chamber blocks.

Statistical analysis

We used generalized linear modeling available in the Genstat statistical package (version 15, VSN International Ltd., Hemel Hempsted). As the key response variable, the number of adult wasps produced per replicate block, consisted of small value integers, we used log-linear models assuming quasi-Poisson error distributions (Crawley, 1993; Zuur *et al.*, 2009). Differences in offspring production across all treatments were tested using one way ANOVA (which used data from all replicates simultaneously but did not allow exploration of potentially important interaction terms). In the two main analytical steps, 3-way factorial ANOVAs were used to explore effects of combinations of spatial structure, substrate, interconnectedness, host density, and their interactions. Significance tests were carried out as terms were sequentially deleted from an initially more complex model

Table 1. Influences on adult production in single chamber blocks and multi-chamber blocks with open passages.

| Source	d.f.	Deviance	Mean deviance	*F-ratio*	*P*
Block type	1	22.38	22.38	110.59	<0.001
Host density	1	38.88	38.88	192.13	<0.001
Substrate	2	455.03	227.51	1124.24	<0.001
Block type × Host density interaction	1	3.99	3.99	19.74	<0.001
Block type × Substrate interaction	2	12.06	6.03	29.78	<0.001
Host density × Substrate interaction	2	4.77	2.38	11.78	<0.001
Block type × Host density × Substrate interaction	2	0.0006	0.0003	∼0.00	∼1.000
Residual	108	22.26	0.20		
Total	119	556.97	4.68		
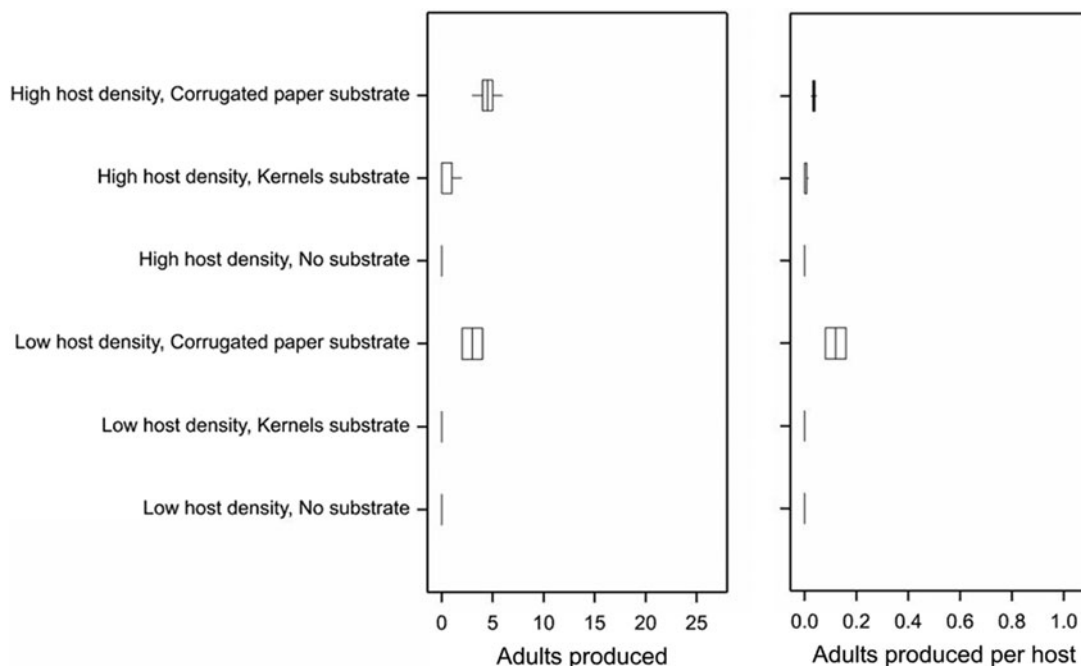
Fig. 2. Adult *C. tarsalis* production from single chamber blocks with different substrates and host densities. Production is expressed as total wasps per replicate and as wasps per host.

and when significant factors with >2 levels were simplified by aggregation to find the minimum adequate model (Crawley, 1993). We illustrate results in terms of parasitoid adults produced per replicate block and also per host per replicate, as the latter pertains to mass rearing efficiency. Logistic modeling, assuming quasi-binomial error distributions, was used for the analysis of pupal-to-adult mortality and sex ratio data (the proportion of offspring that were male) (Crawley, 1993; Wilson & Hardy, 2002) and log-linear modeling, assuming quasi-Poisson distributed errors was used for the analysis of male numbers. Quadratic terms were included to test for significant curvilinearity. All statistical testing was 2-tailed.

Results

There were significant differences in production of adult offspring across the 18 combinations of experimental conditions (log-linear ANOVA: $F_{17,162} = 181.28$, $P < 0.001$, Deviance explained = 95.0%). To explore how production was influenced by host density and the different facets of spatial structure we first compared adult production using data from single chamber blocks plus the open-passage treatments of the multi-chamber blocks. Total adult production was significantly higher among multi-chamber blocks compared with single-chamber blocks (table 1) and was significantly affected by the substrate provided (table 1) with adult production particularly enhanced by the presence of corrugated paper and lower when either kernels or no substrate were provided (figs 2 and 3). The production of adults was, however, significantly higher when kernels were present than when there was no substrate (attempted model simplification by aggregation of factor levels: $F_{3,113} = 11.42$, $P < 0.001$). There were significant pairwise interactions between the type of block, the type of substrate and the density of hosts provided (table 1).

We next analyzed adult production data from the multi-chamber blocks only. This allowed us to explore the effect of passages between chambers being open or closed, along with the influence of other main effects and their interactions.
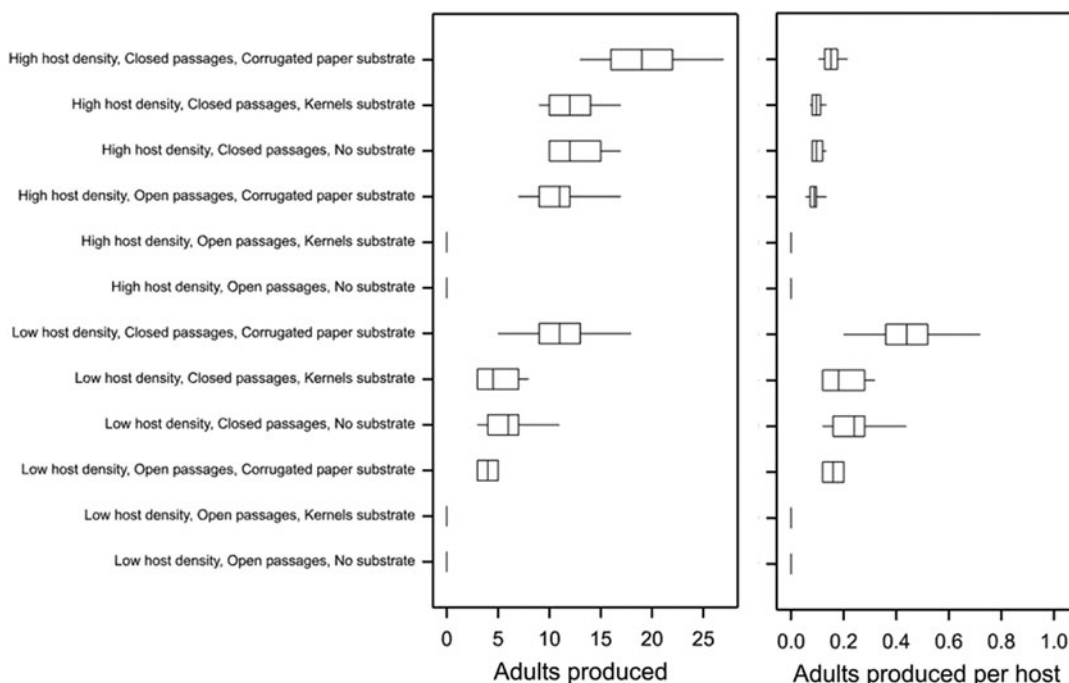
Fig. 3. Adult *C. tarsalis* production from multi-chamber blocks with different degrees of isolation, substrates and host densities. Production is expressed as total wasps per replicate and as wasps per host.

Table 2. Influences on adult production in multi-chamber blocks.

Source	d.f.	Deviance	Mean deviance	F-ratio	P
Host density	1	110.38	110.38	226.77	<0.001
Passages	1	343.72	343.72	706.16	<0.001
Substrate	2	170.72	85.36	175.38	<0.001
Host density × Passages interaction	1	4.95	4.95	10.16	0.002
Host density × Substrate interaction	2	3.21	3.21	3.30	0.041
Passages × Substrate interaction	2	204.23	102.11	209.79	<0.001
Host density × Passages × Substrate interaction	2	2	0.0003	0.0001	∼1.000
Residual	108	5256	0.49		
Total	119	885.63	7.44		

Total adult production was significantly higher when host density was high, when passages were closed and when corrugated paper was provided rather than kernels or no substrate (table 2, fig. 3). There was no significant difference in production between chambers with a layer or kernels and chambers with no substrate (model simplification by aggregation of factor levels: $F_{3,113} = 0.67$, $P = 0.571$). There were significant pairwise interactions between host density, the substrate provided within the chambers and with the interconnectedness of the chambers (table 2).

The mean sex ratios produced (proportion of adult offspring that were male) was 0.378 (±SE = 0.0095) and varied significantly across the 11 experimental combinations under which some adults were produced (logistic ANOVA: $F_{10,93} = 12.12$, $P < 0.001$), ranging from all-females to 50% of adults being male (fig. 4). Across all replicates, sex ratios were significantly related to the number of adults produced, generally increasing (logistic regression: $F_{1,102} = 34.6$, $P < 0.001$) but with significant

curvilinearity (quadratic term: $F_{1,101} = 21.1$, $P < 0.001$, fig. 4). This was due to a significant increase in the proportion of male progeny as adult production per block increased up to around 20 (log-linear regression: $F_{1,102} = 364.99$, $P < 0.001$) also in a curvilinear relationship (quadratic term: $F_{1,101} = 122.11$, $P < 0.001$, fig. 5).

In single chamber blocks and multi-chamber blocks with open passages (as above) the sex ratios were higher among adults emerging from Type A blocks (logistic factorial ANOVA: $F_{1,40} = 33.39$, $P < 0.001$), when host density was high ($F_{1,40} = 6.05$, $P = 0.018$) and when corrugated paper was provided as substrate rather than kernels ($F_{1,40} = 11.56$, $P = 0.002$; note that no adults were produced when no substrate was provided in these replicates so there were no data on sex ratios). Exploring sex ratios from the multi-chamber blocks confirmed the positive effect of host density ($F_{1,75} = 28.79$, $P < 0.001$) but found no significant differences in sex ratio between open and closed passage treatments
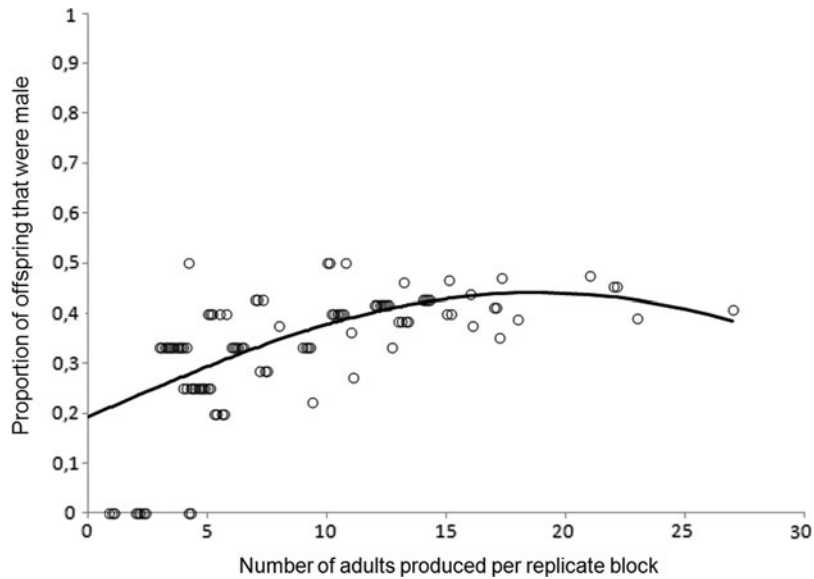
Fig. 4. Sex ratios of adult *C. tarsalis* emerging according to the number of adults produced per replicate. Data are drawn from all experimental conditions and some overlapping points are displaced horizontally to illustrate sample size. The line was fitted by logistic regression including a quadratic term.
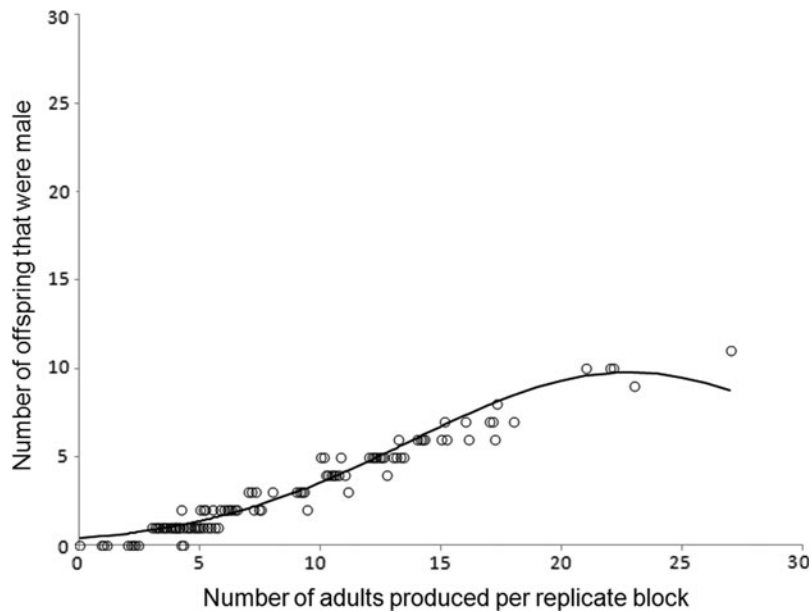

Fig. 5. Number of male *C. tarsalis* emerging according to the number of adults produced per replicate. Data are drawn from all experimental conditions and some overlapping points are displaced horizontally to illustrate sample size. The line was fitted by log-linear regression including a quadratic term.

($F_{1,75} = 0.03$, $P = 0.868$) nor an effect of substrate ($F_{2,75} = 2.60$, $P = 0.081$). There were no significant interactions between main effects in any of the above sex ratio analyses.

Discussion

Production of *C. tarsalis* offspring was strongly influenced by host density and by spatial structure, both in terms of sub-division and the provision of substrate. The greater production of offspring when greater numbers of hosts were provided to isolated females (block type B) is unsurprising because, at 27°C, *C. tarsalis* is capable of laying in excess of 100 eggs across over 25 hosts (Lukáš, 2007). Whether hosts were provided at high or low density, progeny production was generally much lower when there was no sub-division or incomplete sub-division within the microcosms; in many cases no

progeny at all were produced. When not isolated, females can experience higher parasitoid densities due to behavioral and/or chemical interactions with other females: these interactions clearly result in mutual interference.

Chemically-based interference could operate via the release of skatole, the volatile that is produced by adult *C. tarsalis* (Goubault *et al.*, 2008) and which may promote dispersal from areas of resource competition (Gómez *et al.*, 2005). Skatole is likely to be released when females encounter stressors, such as agonistic encounters with conspecifics (Goubault *et al.*, 2008). Agonistic fighting behavior is well documented between male *C. tarsalis* (Cheng *et al.*, 2003) but at present there are only informal observational reports of female–female fighting (Collatz *et al.*, 2009). Intra- and inter-specific aggression between females competing for oviposition opportunities is, however, well documented in other *Cephalonomia* species (Pérez-Lachaud *et al.*, 2002; Batchelor *et al.*, 2005) and closer examination of agonistic behavior in *C. tarsalis*, and its potential association with chemical interactions, is thus warranted. Similarly, and given that *C. tarsalis* often co-occurs in storage facilities with *Cephalonomia waterstoni*, other bethylids and also parasitoids in other taxa (Arbogast & Throne, 1997; Sedlacek *et al.*, 1998; Johnson *et al.*, 2000; Asl *et al.*, 2009), it may be informative to examine the importance of interference competition, whereby species directly reduce each other's survival (Griffith & Poulson, 1993; Pérez-Lachaud *et al.*, 2002; Batchelor *et al.*, 2005, 2006).

An additional behavioral characteristic of bethylids that would lead to mutual interference is cannibalism of eggs and, in some species, larvae by a conspecific female (Mayhew, 1997; Sreenivas & Hardy, 2016). We know of no documented observations of cannibalism in *C. tarsalis* but it is observed in congeners (Infante *et al.*, 2001; Pérez-Lachaud *et al.*, 2004); examination of the ovicidal and larvicidal propensities of *C. tarsalis* may thus prove informative in terms of both mutual interference and inter-specific interference competition.

We found that *C. tarsalis* production was clearly enhanced by the provision of corrugated paper and that its presence could partially offset the negative effects of female interaction. The provision of corrugated paper constitutes a cheap and effective method to enhance the mass rearing of this parasitoid. It has long been established that *C. tarsalis* uses shelters to hide the paralyzed host before oviposition and often halts parasitization in the absence of suitable shelters (Powell, 1938; Howard *et al.*, 1998; Cheng *et al.*, 2003). It seems likely that hiding hosts in paper corrugations would make them harder to find and easier to defend against conspecific females. Given that in *C. tarsalis*, dufors gland secretions have the same hydrocarbon profile as the parasitoid's cuticle (Howard & Baker, 2003), which is different from the profile of the host (Howard, 1998) and that hosts are recognized on the basis of cuticular cues (Howard *et al.*, 1998), we suggest that the possible function of rubbing the host with the abdomen tip (Howard *et al.*, 1998) is to effect olfactory camouflage reducing the probability that hosts are subsequently detected by other females. This hypothesis could be tested in olfactometer experiments, such as those by Collatz & Steidle (2008). The provision of kernels was far less effective than corrugated paper in reducing mutual interference. This may be because chemical cues emanating from kernels attract foraging females (Collatz & Steidle, 2008) and thus paralyzed hosts hidden among grains are still likely to be found, whereas attractive cues are unlikely to emanate from corrugated paper (unless parasitoids emerging as adults in culture learn to associate cues from corrugated paper with host locations). We also note that the provision of substrate may affect the outcome of inter-specific interference competition between *C. tarsalis* and, for instance, *C. waterstoni*; as has been found for interactions between other species of *Cephalonomia* (Batchelor *et al.*, 2005, 2006).

The sex ratios produced by *C. tarsalis* in our study were generally within the relatively narrow range of previous reports (0.3–0.5) but were lower when few adults were produced. Collatz *et al.* (2009) reported that the sex ratios produced by individual *C. tarsalis* females were uncorrelated with host availability, with 50–400 hosts provided. We found, when providing 25–125 hosts, that the sex ratios collectively produced by 25 females were higher when host density was greater. Our data are compatible with the notion that the sex ratio strategies of individual females are relatively invariant, with the overall female bias selected for by common, but not exclusive, mating between siblings (Hardy & Mayhew, 1998; Collatz *et al.*, 2009). Sex ratio responses to the presence of conspecifics typically involve relatively reduced investment in females, the sex that on maturity attacks hosts, and can thus reduce the natural enemy population's capacity to suppress pests (Ode & Hardy, 2008) but given the narrow range of sex ratios observed, sex ratio responses are unlikely to constitute an important component of mutual interference in *C. tarsalis*.

Conclusions

Our data demonstrate mutual interference in *C. tarsalis* and thus confirm that this is a candidate explanation for its limited biocontrol efficacy. Augmentative and inundative releases of *C. tarsalis* are potential means to enhance pest suppression (Sedlacek *et al.*, 1998; Johnson *et al.*, 2000) but these will rely on efficient mass rearing of parasitoids prior to release. Our data show that when females are not isolated from each other, intra-specific interactions result in a considerable reduction in progeny per female and also per host provided. Mass rearing will thus be most efficient, in terms of parasitoids reared per host, when isolated females are provided with relatively few hosts and are also provided with substrate in which to place the hosts they parasitize.

Once parasitoids are released into infested storage facilities they face the challenge of finding their hosts, which they achieve largely by chemical means (Collatz & Steidle, 2008). Synthesized pheromones might be utilized by biocontrol practitioners to attract and retain *C. tarsalis* females close to host infestations and thus increase parasitism rates (Hötling *et al.*, 2014). However, our data suggest that higher densities of parasitoids will lead to increased mutual interference, which is likely to be disruptive to biocontrol.

We have used simple microcosms to indicate possible population level consequences of intra-specific interactions. While part of a long and useful tradition as a predictor of population processes (e.g. Huffaker, 1958; Infante *et al.*, 2001; Batchelor *et al.*, 2006; de Jong *et al.*, 2011; Sreenivas & Hardy, 2016), such studies do not obviate the value of field-scale experimentation on *C. tarsalis* populations, as suggested by Sedlacek *et al.* (1998). Our microcosm studies also emphasize a need for further behavioral and chemical studies of interactions, both intra- and inter-specific, between female parasitoids to understand better how these collectively generate the phenomenon of mutual interference and thus how to potentially reduce its occurrence.

Acknowledgements

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