3

4   **Reproductive efficiency of the bethylid wasp *Cephalonomia tarsalis:* the influences of**

5   **spatial structure and host density**

6

7   **Panagiotis A. Eliopoulos[1,*], Apostolos Kapranas [1,2,3], Eleftheria G. Givropoulou[1] and Ian**

8   **C.W. Hardy[2]**

9

10   [1]Department of Agricultural Technologists, Technological Educational Institute of Thessaly,

11   Larissa, Greece

12   [2]School of Biosciences, University of Nottingham, Sutton Bonington Campus, Leicestershire,

13   LE12 5RD, UK

14   [3]Department of Biology, National University of Ireland, Maynooth, County Kildare, Ireland

15

16   *Author for correspondence, Fax: +302410613153, Email: eliopoulos@teilar.gr

17

18   *Running head : Reproductive efficacy of C. tarsalis*

19

20

21   **Abstract**

22

23   The parasitoid wasp *Cephalonomia tarsalis* (Ashmead) (Hymenoptera: Bethylidae) is

24   commonly present in stored product facilities. While beneficial, it does not provide a high

25   degree of biological pest control against its host, the saw-toothed beetle *Oryzaephilus*

26   *surinamensis* (L.) (Coleoptera: Silvanidae). A candidate explanation for poor host population

27   suppression is that adult females interfere with each other's foraging and reproductive

28   behavior. We used simple laboratory microcosms to evaluate such mutual interference in

29   terms of its overall effects on offspring production. We varied the density of the hosts and

30   also the spatial structure of the environment, via the extent of population sub-division and the

31   provision of different substrates. Production of *C. tarsalis* offspring was positively influenced

32   by host density and by the isolation of females. With incomplete sub-division within

33   microcosms offspring production was, in contrast, low and even zero. The provision of

34   corrugated paper as a substrate enhanced offspring production and partially mitigated the

35   effects of mutual interference. We recommend simple improvements to mass rearing practice

36   and identify promising areas for further behavioral and chemical studies towards a better

37   understanding of the mechanisms of mutual interference.

38

41

42   **Introduction**

43

44   The bethylid wasp *Cephalonomia tarsalis* (Ashmead) (Hymenoptera: Bethylidae) is a larval

45   ectoparasitoid of beetles, mainly those belonging to the genus *Oryzaephilus*. It is the most

46   common natural enemy of the saw-toothed grain beetle, *Oryzaephilus surinamensis* (L.)

47   (Coleoptera: Silvanidae), which is a very common pest of many agricultural stored products

48   (Sedlacek et al., 1998; Johnson et al., 2000; Asl et al., 2009). Despite its common presence in

49   storage facilities *C. tarsalis* has been regarded as a "poor" biocontrol agent given that

50   significant pest infestation can occur even in cases where wasp population density is very

51   high (Powell, 1938; Eliopoulos et al., 2002a,2002b). One of the first studies of the biology of

52   *C. tarsalis* concluded that, due to its low reproductive output, this wasp was not of economic

53   importance as a biological control agent (Powell, 1938).

54   Pest problems in stored products have persisted and the use of fumigants to control

55   infestations is no longer favored due to associated pollution and the development of

56   insecticidal resistance (e.g. Arbogast & Throne, 1997; Sedlacek et al., 1998; Johnson et al.,

57   2000; Lukáš, 2007; Asl et al., 2009). More recent studies on *C. tarsalis* have taken a more

58   optimistic view of its potential as an agent of biological pest control; some have evaluated

59   aspects of its life-history, behavior and chemistry that are likely to influence its biocontrol

60   potential (e.g. Howard et al., 1998; Johnson et al., 2000; Lukáš, 2007; Collatz & Steidle,

61   2008; Hötling et al., 2014) and others have examined its interactions with other species of

62   parasitoids, mites, parasitic protozoans or fungi that are also natural enemies of *O.*

63   *surinamensis* (Johnson et al., 2000; Lord, 2001, 2006; Žďărkovă et al., 2003; Latifian et al.,

64   2011). Some of these inter-specific interactions appear beneficial, for pest suppression and/or

65   for *C. tarsalis* (Žďărkovă et al., 2003; Lord, 2006) while others are clearly detrimental to *C.*

66   *tarsalis* (Lord, 2001).

67 The reproductive behaviour of *C. tarsalis* shares many commonalities with other species of

68 Bethylids, but exhibits especially elaborate host handling. The wasp locates host habitats, and

69 hosts, using volatile chemical cues deriving from the host's food, hosts themselves and host

70 faeces (Collatz & Steidle, 2008; Hötling et al., 2014). Encountered hosts are recognized by

71 chemical cuticular cues perceived by antennae as well as host movement; vision plays only a

72 limited role in host finding and recognition (Howard et al., 1998). On encountering a host, the

73 female wasp paralyzes it permanently by injecting venom via a sting and then drags it to a

74 shelter (e.g. a hollow wheat grain, cracks in the walls and floor of storage facilities) (Powell,

75 1938; Howard et al., 1998). The female also rubs the host larva all over with the tip of her

76 abdomen and then host feeds (Howard et al., 1998). The female may leave the host and return

77 several times, and may move it to a new location (Howard et al., 1998). Several hosts may be

78 paralysed and hidden before the female commences oviposition and each host is hidden in a

79 separate location (Howard et al., 1998; P.A.E. personal observations). Females may

80 aggressively defend their 'oviposition patch' against conspecific females (Collatz et al.,

81 2009).

82 Eggs are typically laid singly or in pairs onto each host (Powell, 1938; Lukáš, 2007). Very

83 rarely 3-4 eggs may be found on a host (e.g. when hosts are very scarce) but only two of them

84 complete development (Powell, 1938). In cases of single egg deposition, 80% are female

85 whereas in cases of paired eggs there is almost always one male and one female produced;

86 unfertilized eggs produce haploid males (arrhenotoky) (Powell, 1938; Cheng et al., 2003).

87 Development from egg to adult takes between 26.3 days (at 21°C) and 11.4 days (at 30°C)

88 (Lukáš & Stejskal, 2005). Developmental mortality is affected by temperature; at 24-30°C it

89 normally ranges between 20 and 38% but reaches 80-91% at the more extreme low (<18°C)

90 or high (>35°C) temperatures at which assays have been performed (Lukáš, 2007). The most

91 susceptible period for mortality is the transition from larval to pupal stage (Lukáš & Stejskal,

92 2005).

93   Males usually emerge as adults 1 to 2 days earlier than females (Powell, 1938). In cases

94   where a male and female develop on the same host, the male will enter the female's cocoon

95   and copulate with her (Powell, 1938; Zimmerman et al., 2008). However, Cheng et al. (2003)

96   observed males leaving the vicinity of the host after emergence and concluded that sib-

97   mating may not be a common phenomenon in *C. tarsalis*. Collatz et al. (2009) subsequently

98   verified that *C. tarsalis* displays partial local mate competition, i.e. a mixture of sibling and

99   non-sibling mating (Nunney & Luck, 1988; Hardy & Mayhew 1998;). Sex ratios (proportion

100   of offspring that are male) are typically around 0.3-0.5 (Cheng et al., 2003; Lukáš, 2008;

101   Zimmermann et al., 2008; Collatz et al., 2009). Male wasps may live up to 6 days and may

102   copulate with many different females. Males compete directly with each other, via pushing,

103   pulling and grasping, for mating opportunities with females (Cheng et al., 2003). The females

104   live for about 35 days and copulate once or very few times (Powell, 1938; Cheng et al.,

105   2003); the mating frequency of females does not affect the subsequent production of female

106   progeny (Cheng et al., 2004). Females can produce up to around 200 eggs in their lifetime,

107   under laboratory conditions (27°C and plentiful supplies of fresh host) but realized

108   fecundities of 50-100 eggs are more typical and are reduced at higher and lower temperatures

109   (Lukáš, 2007).

110   Interactions with conspecifics are mediated by chemical recognition cues, such as dodecanal,

111   cuticular hydrocarbons and hydrocarbon components secreted by the Dufour gland (Howard

112   & Infante, 1996; Howard, 1998; Howard & Pérez-Lachaud, 2002; Howard & Baker, 2003;

113   Collatz et al., 2009). Stressed adults also release the volatile skatole (3-methylindole)

114   (Goubault et al., 2008) which may disrupt subsequent reproductive behaviour (Gómez et al.,

115   2005; Hardy & Goubault, 2007). Occurence of superparasitism has not been considered as a

116   realistic possibility in our study. Many faunistic studies on the insect fauna in stored grains

117   have revealed hyperparasitoids do not exist in the "closed" environments of grain storage

118   facilities (Eliopoulos et al., 2002a, 2002b).

119   In this study we evaluated the effects of intra-specific interactions between *C. tarsalis*

120   females. A candidate explanation for the poor host population suppression by *C. tarsalis* is

121   that adult females interfere with each other's foraging and reproductive behavior; a

122   phenomenon known as mutual interference (Hassell & May, 1989; Hassell, 2000; Kidd &

123   Jervis, 2005). Among parasitoids in general, mutual interference can have a variety of causes

124   including delayed searching following encounters, host and patch guarding, fighting

125   behaviour, and altered decisions concerning superparasitism, clutch size and sex allocation

126   (e.g. Hassell and May, 1973; Visser et al., 1990; Driessen & Visser, 1997; Meunier &

127   Bernstein, 2002; Goubault et al., 2007; Yazdani & Keller, 2015). Few of these aspects have

128   been directly evaluated in the context of interactions with conspecifics in *C. tarsalis* but it is

129   known that these parasitoids may occur at moderately high density in stored products

130   (Sedlacek et al., 1998) and agonistic interactions between foraging females have been

131   observed (Collatz et al., 2009). Our approach was to evaluate mutual interference, in terms of

132   its overall effects on offspring production, using simple laboratory microcosms; within these

133   we varied the density of the hosts (individuals per unit area) and the spatial structure of the

134   wasps' and host's environment. The effect of spatial structure was explored both by varying

135   the extent of population sub-division and by provision of different substrates. We use our

136   results to recommend improvements to mass rearing practice and to identify promising areas

137   for further work towards improving stored product biological control using *C. tarsalis*.

138

Materials and Methods

140

141   *Insects*

142

143   We studied the wasp *C. tarsalis* and its host the saw-toothed grain beetle, *O. surinamensis*.

144   The beetle was kept in culture in 2-litre clear plastic jars in the laboratory using a mixture of

145 crushed wheat: rolled oats: dried yeast (5:5:1). The wasp was kept in culture using the same

146 rearing medium and jars as the beetle, with a large number of full-grown host larvae. Small

147 pieces (2 cm × 2 cm) of corrugated paper were introduced to the wasp culture jars (15-20 /

148 jar) as "shelters" for the female wasps. All insect cultures were kept under controlled

149 environmental conditions (27°C, 16:8 L:D, 60% R.H.). Paper shelters were replaced every 2-

150 3 days and those with parasitized larvae were transferred to Petri dishes until wasp eclosion.

151 Wasps were collected daily for use in the experimental treatments.

152

153 *Experimental treatments*

154

155 We assessed the effects of spatial structure on *C. tarsalis* reproduction using experimental

156 arenas excavated from opaque plastic blocks with transparent Plexiglas lids (Fig. 1); designs

157 were based on those used by Sreenivas and Hardy (2016). Three different block types were

158 used: Type A blocks were multi-chamber arenas; the diameter each of the 25 chambers was

159 1.78cm. Chambers were interconnected by passages (1cm long × 0.4cm wide). The total area

160 of chambers and passages within the block was 72.7cm^2 (Fig. 1A). Type B blocks were

161 similar to Type A, but there were no passages between the chambers, which were thus

162 isolated from each other, and chambers were of greater diameter (1.92cm) in order to

163 maintain the overall floor area of 72.7cm^2 (Fig. 1B). Type C blocks contained a single

164 circular chamber of diameter 9.62cm, a floor area of 72.7cm^2 (Fig. 1C). All chambers and

165 passages were 0.6cm deep. Spatial structure was further varied by placing within the

166 chambers a single layer of wheat kernels, a small piece of corrugated paper (1cm × 1cm) or

167 no additional substrate (empty chamber). When paper was present, 25 pieces were placed in

168 the single chamber (block type C) or 1 piece per chamber in the multi-chamber blocks (types

169 A & B). Host density was varied by placing either 25 (low density) or 125 (high density)

170 hosts into each block. For single chamber blocks (type C) there were 25 or 125 host larvae in

171   the chamber and for multi-chamber blocks (types A & C) there were either 1 or 5 host larvae

172   in each chamber. There were 10 replications of each of the 18 combinations of experimental

173   conditions, giving 180 replicates in total.

174   Parasitoid density was held constant at 25 adult female wasps per block, with either 25 placed

175   in the single chamber of the block (type C) or one wasp placed into each chamber of the

176   multi-chamber blocks (type A & B). Wasps were briefly anesthetized with CO_2 to place them

177   into the chambers. Female age of *C. tarsalis* at the start of the experiment was 3-5 days.

178   Blocks were inspected daily and adult wasps were removed, once the progeny started

179   reaching pupal stage, to prevent subsequent confusion with adult female offspring. The pupae

180   were collected from each block, counted and transferred to Petri dishes (diameter 9cm) where

181   they were checked daily for emergence of adult offspring. The number of adults and their sex

182   were subsequently recorded. Before proceeding to the main analysis of data on adult numbers

183   we checked that the probability of the collected pupae surviving to adulthood did not differ

184   between experimental treatments: there were 11 combinations of experimental conditions

185   under which some offspring reached pupation and their post-collection survival did not vary

186   significantly across these (logistic ANOVA: $F_{10,93} = 0.55$, $P=0.847$).

187   The experiment was thus essentially a factorial design testing the effects on parasitoid

188   offspring production of host density (low or high), substrate structure (3 types of substrate)

189   and either gross spatial structure (single or multi-chamber blocks) or interconnectedness

190   (passages open or closed). As there could be no passages within single chamber blocks, the

191   analysis was however constrained to proceed in two main steps, after testing for differences

192   across all treatments; the first step using data from single chamber blocks and multi-chamber

193   blocks and the second using data from both types of multi-chamber blocks.

194

195   *Statistical analysis*

196

197   We used generalized linear modelling available in the Genstat statistical package (version 15,

198   VSN International Ltd., Hemel Hempsted). As the key response variable, the number of adult

199   wasps produced per replicate block, consisted of small value integers, we used log-linear

200   models assuming quasi-Poisson error distributions (Crawley, 1993; Zuur et al., 2009).

201   Differences in offspring production across all treatments were tested using one way ANOVA

202   (which used data from all replicates simultaneously but did not allow exploration of

203   potentially important interaction terms). In the two main analytical steps, 3-way factorial

204   ANOVAs were used to explore effects of combinations of spatial structure, substrate,

205   interconnectedness, host density, and their interactions. Significance tests were carried out as

206   terms were sequentially deleted from an initially more complex model and when significant

207   factors with >2 levels were simplified by aggregation to find the minimum adequate model

208   (Crawley, 1993). We illustrate results in terms of parasitoid adults produced per replicate

209   block and also per host per replicate, as the latter pertains to mass rearing efficiency. Logistic

210   modelling, assuming quasi-binomial error distributions, was used for the analysis of pupal-to-

211   adult mortality and sex ratio data (the proportion of offspring that were male) (Crawley,

212   1993; Wilson & Hardy, 2002) and log-linear modelling, assuming quasi-Poisson distributed

213   errors was used for the analysis of male numbers. Quadratic terms were included to test for

214   significant curvilinearity. All statistical testing was 2-tailed.

215   **Results**

216   There were significant differences in production of adult offspring across the 18

217   combinations of experimental conditions (log-linear ANOVA: $F_{17,162}$ =181.28, $P<0.001$,

218   Deviance explained = 95.0%). To explore how production was influenced by host density and

219   the different facets of spatial structure we first compared adult production using data from

220   single chamber blocks plus the open-passage treatments of the multi-chamber blocks. Total

221   adult production was significantly higher among multi-chamber blocks compared to single-

222   chamber blocks (Table 1) and was significantly affected by the substrate provided (Table 1)

223   with adult production particularly enhanced by the presence of corrugated paper and lower

224   when either kernels or no substrate were provided (Figs. 2 & 3). The production of adults

225   was, however, significantly higher when kernels were present than when there was no

226   substrate (attempted model simplification by aggregation of factor levels: $F_{3,113} =11.42$,

227   $P<0.001$). There were significant pairwise interactions between the type of block, the type of

228   substrate and the density of hosts provided (Table 1).

229   We next analyzed adult production data from the multi-chamber blocks only. This allowed us

230   to explore the effect of passages between chambers being open or closed, along with the

231   influence of other main effects and their interactions. Total adult production was significantly

232   higher when host density was high, when passages were closed and when corrugated paper

233   was provided rather than kernels or no substrate (Table 2, Fig. 3). There was no significant

234   difference in production between chambers with a layer or kernels and chambers with no

235   substrate (model simplification by aggregation of factor levels: $F_{3,113} =0.67$, $P=0.571$). There

236   were significant pairwise interactions between host density, the substrate provided within the

237   chambers and with the interconnectedness of the chambers (Table 2).

238   The mean sex ratios produced (proportion of adult offspring that were male) was 0.378 ($\pm$SE

239   = 0.0095) and varied significantly across the 11 experimental combinations under which

240   some adults were produced (logistic ANOVA: $F_{10,93} =12.12$, $P<0.001$), ranging from all-

241   females to 50% of adults being male (Fig. 4). Across all replicates, sex ratios were

242   significantly related to the number of adults produced, generally increasing (logistic

243   regression: $F_{1,102} =34.6$, $P<0.001$) but with significant curvilinearity (quadratic term: $F_{1,101} =$

244   21.1, $P < 0.001$, Fig. 4). This was due to a significant increase in the proportion of male

245   progeny as adult production per block increased up to around 20 (log-linear regression: $F_{1,102}$

246   $=364.99$, $P<0.001$) also in a curvilinear relationship (quadratic term: $F_{1,101} = 122.11$, $P <$

247   0.001, Fig. 5).

248   In single chamber blocks and multi-chamber blocks with open passages (as above) the sex

249   ratios were higher among adults emerging from Type A blocks (logistic factorial ANOVA:

250   $F_{1,40} = 33.39$, $P<0.001$), when host density was high ($F_{1,40} = 6.05$, $P=0.018$) and when

251   corrugated paper was provided as substrate rather than kernels ($F_{1,40} = 11.56$, $P=0.002$; note

252   that no adults were produced when no substrate was provided in these replicates so there

253   were no data on sex ratios). Exploring sex ratios from the multi-chamber blocks confirmed

254   the positive effect of host density ($F_{1,75} = 28.79$, $P<0.001$) but found no significant differences

255   in sex ratio between open and closed passage treatments ($F_{1,75} = 0.03$, $P=0.868$) nor an effect

256   of substrate ($F_{2,75} = 2.60$, $P=0.081$). There were no significant interactions between main

257   effects in any of the above sex ratio analyses.

258

259   **Discussion**

260

261   Production of *C. tarsalis* offspring was strongly influenced by host density and by spatial

262   structure, both in terms of sub-division and the provision of substrate. The greater production

263   of offspring when greater numbers of hosts were provided to isolated females (block type B)

264   is unsurprising because, at 27°C, *C. tarsalis* is capable of laying in excess of 100 eggs across

265   over 25 hosts (Lukáš, 2007). Whether hosts were provided at high or low density, progeny

266   production was generally much lower when there was no sub-division or incomplete sub-

267   division within the microcosms; in many cases no progeny at all were produced. When not

268   isolated, females can experience higher parasitoid densities due to behavioral and/or chemical

269   interactions with other females: these interactions clearly result in mutual interference.

270   Chemically based interference could operate via the release of skatole, the volatile that is

271   produced by adult *C. tarsalis* (Goubault et al., 2008) and which may promote dispersal from

272   areas of resource competition (Gómez et al., 2005). Skatole is likely to be released when

273   females encounter stressors, such as agonistic encounters with conspecifics (Goubault et al.,

274 2008). Agonistic fighting behaviour is well documented between male *C. tarsalis* (Cheng et

275 al., 2003) but at present there are only informal observational reports of female-female

276 fighting (Collatz et al., 2009). Intra- and inter-specific aggression between females competing

277 for oviposition opportunities is, however, well documented in other *Cephalonomia* species

278 (Pérez-Lachaud et al., 2002; Batchelor et al., 2005) and closer examination of agonistic

279 behaviour in *C. tarsalis*, and its potential association with chemical interactions, is thus

280 warranted. Similarly, and given that *C. tarsalis* often co-occurs in storage facilities with

281 *Cephalonomia waterstoni*, other bethylids and also parasitoids in other taxa (Arbogast &

282 Thorne, 1997; Sedlacek et al., 1998; Johnson et al., 2000; Asl et al., 2009), it may be

283 informative to examine the importance of interference competition, whereby species directly

284 reduce each other's survival (Griffith & Poulson 1993; Pérez-Lachaud et al., 2002; Batchelor

285 et al., 2005, 2006).

286 An additional behavioural characteristic of bethylids that would lead to mutual interference is

287 cannibalism of eggs and, in some species, larvae by a conspecific female (Mayhew, 1997;

288 Sreenivas & Hardy, 2016). We know of no documented observations of cannibalism in *C.

289 tarsalis* but it is observed in congeners (Infante et al., 2001; Pérez-Lachaud et al., 2004);

290 examination of the ovicidal and larvicidal propensities of *C. tarsalis* may thus prove

291 informative in terms of both mutual interference and inter-specific interference competition.

292 We found that *C. tarsalis* production was clearly enhanced by the provision of corrugated

293 paper and that its presence could partially offset the negative effects of female interaction.

294 The provision of corrugated paper constitutes a cheap and effective method to enhance the

295 mass rearing of this parasitoid. It has long been established that *C. tarsalis* uses shelters to

296 hide the paralyzed host before oviposition and often halts parasitization in the absence of

297 suitable shelters (Powell, 1938; Howard et al., 1998; Cheng et al., 2003). It seems likely that

298 hiding hosts in paper corrugations would make them harder to find and easier to defend

299 against conspecific females. Given that in *C. tarsalis*, dufors gland secretions have the same

300   hydrocarbon profile as the parasitoid's cuticle (Howard & Baker, 2003), which is different

301   from the profile of the host (Howard, 1998) and that hosts are recognized on the basis of

302   cuticular cues (Howard et al., 1998), we suggest that the possible function of rubbing the host

303   with the abdomen tip (Howard et al., 1998) is to effect olfactory camouflage reducing the

304   probability that hosts are subsequently detected by other females. This hypothesis could be

305   tested in olfactometer experiments, such as those by Collatz and Steidle (2008). The

306   provision of kernels was far less effective than corrugated paper in reducing mutual

307   interference. This may be because chemical cues emanating from kernels attract foraging

308   females (Collatz & Steidle, 2008) and thus paralysed hosts hidden among grains are still

309   likely to be found, whereas attractive cues are unlikely to emanate from corrugated paper

310   (unless parasitoids emerging as adults in culture learn to associate cues from corrugated paper

311   with host locations). We also note that the provision of substrate may affect the outcome of

312   inter-specific interference competition between *C. tarsalis* and, for instance, *C. waterstoni*; as

313   has been found for interactions between other species of *Cephalonomia* (Batchelor et al.

314   2005, 2006).

315   The sex ratios produced by *C. tarsalis* in our study were generally within the relatively

316   narrow range of previous reports (0.3-0.5) but were lower when few adults were produced.

317   Collatz et al. (2009) reported that the sex ratios produced by individual *C. tarsalis* females

318   were uncorrelated with host availability, with 50-400 hosts provided. We found, when

319   providing 25-125 hosts, that the sex ratios collectively produced by 25 females were higher

320   when host density was greater. Our data are compatible with the notion that the sex ratio

321   strategies of individual females are relatively invariant, with the overall female bias selected

322   for by common, but not exclusive, mating between siblings (Hardy & Mayhew, 1998; Collatz

323   et al., 2009). Sex ratio responses to the presence of conspecifics typically involve relatively

324   reduced investment in females, the sex that on maturity attacks hosts, and can thus reduce the

325   natural enemy population's capacity to suppress pests (Ode & Hardy 2008) but given the

326   narrow range of sex ratios observed, sex ratio responses are unlikely to constitute an

327   important component of mutual interference in *C. tarsalis*.

328

329   **Conclusions**

330

331   Our data demonstrate mutual interference in *C. tarsalis* and thus confirm that this is a

332   candidate explanation for its limited biocontrol efficacy. Augmentative and inundative

333   releases of *C. tarsalis* are potential means to enhance pest suppression (Sedlacek et al., 1998;

334   Johnson et al., 2000) but these will rely on efficient mass rearing of parasitoids prior to

335   release. Our data show that when females are not isolated from each other, intra-specific

336   interactions result in a considerable reduction in progeny per female and also per host

337   provided. Mass rearing will thus be most efficient, in terms of parasitoids reared per host,

338   when isolated females are provided with relatively few hosts and are also provided with

339   substrate in which to place the hosts they parasitize.

340   Once parasitoids are released into infested storage facilities they face the challenge of finding

341   their hosts, which they achieve largely by chemical means (Collatz & Steidle, 2008).

342   Synthesized pheromones might be utilized by biocontrol practitioners to attract and retain *C.*

343   *tarsalis* females close to host infestations and thus increase parasitism rates (Hötling et al.,

344   2014). However, our data suggest that higher densities of parasitoids will lead to increased

345   mutual interference, which is likely to be disruptive to biocontrol.

346   We have used simple microcosms to indicate possible population level consequences of intra-

347   specific interactions. While part of a long and useful tradition as a predictor of population

348   processes (e.g. Huffaker, 1958; Infante et al., 2001; Batchelor et al., 2006; de Jong et al.,

349   2011; Sreenivas & Hardy, 2016), such studies do not obviate the value of field-scale

350   experimentation on *C. tarsalis* populations, as suggested by Sedlacek et al. (1998). Our

351   microcosm studies also emphasize a need for further behavioural and chemical studies of

352 interactions, both intra- and inter-specific, between female parasitoids to understand better

353 how these collectively generate the phenomenon of mutual interference and thus how to

354 potentially reduce its occurrence.

355

356 **Acknowledgements**

357

365

366 **References**

367

368 **Arbogast, R.T. & Throne, J.E.** (1997) Insect infestation of farm-stored maize in South

369 Carolina: towards characterization of a habitat. *Journal of Stored Products Research*

370 **33**, 187-198.

371 **Asl, M.H.A., Talebi, A.A., Kamali, H. & Kazemi, S.** (2009) Stored product pests and their

372 parasitoid wasps in Mashhad, Iran. *Advances in Environmental Biology* **3**, 239-243.

373 **Batchelor, T.P., Hardy, I.C.W., Barrera, J.F. & Pérez-Lachaud, G.** (2005) Insect

374 gladiators II: competitive interactions within and between bethylid parasitoid species of

375 the coffee berry borer, *Hypothenemus hampei* (Coleoptera: Scolytidae). *Biological*

376 *Control* **33**, 194-202.

377 **Batchelor, T.P., Hardy, I.C.W. & Barrera, J.F.** (2006) Interactions among bethylid

378   parasitoid species attacking the coffee berry borer, *Hypothenemus hampei* (Coleoptera:

379   Scolytidae). *Biological Control* **36**, 106-118.

380 **Cheng, L., Howard, R.W., Campbell, J.F., Charlton, R.E., Nechols, J.R. & Ramaswamy,**

381   **S.** (2003). Behavioral interaction between males of *Cephalonomia tarsalis* (Ashmead)

382   (Hymenoptera: Bethylidae) competing for females. *Journal of Insect Behaviour* **16**,

383   625-645.

384 **Cheng, L., Howard, R.W., Campbell, J.F., Charlton, R.E., Nechols, J.R. & Ramaswamy,**

385   **S.B.** (2004). Mating behavior of *Cephalonomia tarsalis* and the effect of female mating

386   frequency on offspring production. *Journal of Insect Behaviour* **17**, 227-245.

387 **Collatz, J.; Tolasch, T. & Steidle, J.L.M.** (2009) Mate finding in the parasitic wasp

388   *Cephalonomia tarsalis* (Ashmead): more than one way to a female's heart. *Journal of*

389   *Chemical Ecology* **35**, 761-768.

390 **Collatz, J. & Steidle, J.L.M.** (2008). Hunting for moving hosts: *Cephalonomia tarsalis*, a

391   parasitoid of free-living grain beetles. *Basic and Applied Ecology* **9**, 452-457.

392 **Crawley, M.J.** (1993) *GLIM for Ecologists*. Oxford, Blackwell Scientific Publications.

393 **Jong, P.W. de, Hemerik, L., Gort, G. & van Alphen, J.J.M.** (2011) Rapid establishment of

394   a regular distribution of adult tropical *Drosophila* parasitoids in a multi-patch

395   environment by patch defence behaviour. *PLoS ONE* **6**, e20870.

396 **Driessen, G. & Visser, M.E.** (1997) Components of parasitoid interference. *Oikos* **79**, 209-

397   218.

398 **Eliopoulos, P.A., Athanassiou, C.G. & Buchelos, C.Th.** (2002a). Capture of

399   hymenopterous parasitoids of stored-grain pests, in various trap types. p.76-82 in

400   *Proceedings of the 2nd meeting of Working Group 4 "Biocontrol of Arthropod Pests in*

401   *Stored Products"*, *COST Action 842*, *Prague, 30-31 May 2002,* Research Institute of

402   Crop Production, Prague.

403   **Eliopoulos, P.A., Athanassiou, C.G. & Buchelos, C.Th.** (2002b). Occurrence of

404   Hymeopterous parasitoids of stored product pests in Greece. *IOBC Bulletin* **25 (3)**, 127-

405   139

406   **Griffith, D.M. & Poulson, T.L.** (1993) Mechanisms and consequences of intraspecific

407   competition in a carabid cave beetle. *Ecology* **74**, 1373–1383.

408   **Gómez, J., Barrera, J.F., Rojas, J.C., Macias-Samano, J., Liedo, J.P., Cruz-López, L. &**

409   **Badii, M.H.** (2005) Volatile compounds released by disturbed females of

410   *Cephalonomia stephanoderis*, a bethylid parasitoid of the coffee berry borer

411   *Hypothenemus hampei. Florida Entomologist* **88**, 180–187.

412   **Goubault, M., Mack, A.F.S. & Hardy I.C.W.** (2007) Encountering competitors reduces

413   clutch size and increases offspring size in a parasitoid with female-female fighting.

414   *Proceedings of the Royal Society of London B* **274**, 2571-2577.

415   **Goubault, M., Batchelor, T.P., Romani, R., Linforth, R.S.T., Fritzsche, M., Francke, W.**

416   **& Hardy, I.C.W.** (2008) Volatile chemical release by bethylid wasps: identity,

417   phylogeny, anatomy and behaviour. *Biological Journal of the Linnean Society* **94**, 837-

418   852.

419   **Hardy, I.C.W. & Mayhew, P.J.** (1998) Sex ratio, sexual dimorphism and mating structure

420   in bethylid wasps. *Behavioral Ecology and Sociobiology* **42**, 383-395.

421   **Hardy, I.C.W. & Goubault, M.** (2007) Wasp fights: understanding and utilizing agonistic

422   bethylid behaviour. *Biocontrol News and Information* **28**, 11-15.

423   **Hassell, M.P.** (2000) *The spatial and temporal dynamics of host-parasitoid interactions.*

424   Oxford, Oxford University Press.

425 **Hassell, M.P. & May, R.M.** (1973) Stability in insect host-parasite models. *Journal of*
426   *Animal Ecology* **42**, 693-726.

427 **Hassell, M.P. & May, R.M.** (1989) The population biology of host-parasite and host-
428   parasitoid associations. pp 319-347 in Roughgarden, J., May, R.M. & Levin, S.A.
429   (Eds.) *Perspectives in ecological theory*. Princeton, Princeton University Press.

430 **Hötling, S.; Haberlag, B.; Tamm, M., Collatz, J., Mack, P., Steidle, J.L.M., Vences, M.**
431   **& Schulz, S.** (2014) Identification and synthesis of macrolide pheromones of the grain
432   beetle *Oryzaephilus surinamensis* and the frog *Spinomantis aglavei. Chemistry* **20**,
433   3183-3191.

434 **Howard, R.W.** (1998) Ontogenetic, reproductive, and nutritional effects on the cuticular
435   hydrocarbons of the host-specific ectoparasitoid *Cephalonomia tarsalis* (Hymenoptera:
436   Bethylidae). *Annals of the Entomological Society of America* **91**, 101-112.

437 **Howard, R.W. & Infante, F.** (1996) Cuticular hydrocarbons of the host-specific
438   ectoparasitoid *Cephalonomia stephanoderis* (Hymenoptera: Bethylidae) and its host the
439   coffee berry borer (Coleoptera: Scolytidae). *Annals of the Entomological Society of*
440   *America* **89**, 700–709.

441 **Howard, R.W. & Pérez-Lachaud, G.** (2002) Cuticular hydrocarbons of the ectoparasitic
442   wasp *Cephalonomia hyalinipennis* (Hymenoptera: Bethylidae) and its alternative host,
443   the stored product pest *Caulophilus oryzae* (Coleoptera: Curculionidae). *Archives of*
444   *Insect Biochemistry and Physiology* **50**, 75–84.

445 **Howard, R.W. & Baker, J.E.** (2003) Morphology and chemistry of dufour glands in four
446   ectoparasitoids: *Cephalonomia tarsalis*, *C. waterstoni* (Hymenoptera: Bethylidae),
447   *Anisopteromalus calandrae*, and *Pteromalus cereallae* (Hymenoptera: Pteromalidae).
448   *Comparative Biochemistry and Physiology B* **135B**, 153–167.

449   **Howard, R.W., Charlton, M. & Charlton, R.E.** (1998). Host-finding, host-recognition and

450   host acceptance behavior of *Cephalonomia tarsalis* (Hymenoptera: Bethylidae). *Annals*

451   *of the Entomological Society of America* **91**, 879-889.

452   **Huffaker, C.** (1958) Experimental studies on predation: dispersion factors and predator-prey

453   oscillations. *Hilgardia* **27**, 343-383.

454   **Infante, F., Mumford, J., Baker, P., Barrera, J. & Fowler, S.** (2001) Interspecific

455   competition between *Cephalonomia stephanoderis* and *Prorops nasuta* (Hym.,

456   Bethylidae), parasitoids of the coffee berry borer, *Hypothenemus hampei* (Col.,

457   Scolytidae). *Journal of Applied Entomology* **125**, 63–70.

458   **Johnson, J.A., Valero, K.A., Hannel, M.M. & Gill, R.F.** (2000) Seasonal occurrence of

459   postharvest dried fruit insects and their parasitoids in a culled fig warehouse. *Journal of*

460   *Economic Entomology* **93**, 1380-1390.

461   **Kidd, N.A.C. & Jervis, M.A.** (2005) Population dynamics pp 435-523 in Jervis M.A. (Ed.)

462   *Insects as natural enemies: a practical perspective*. Dordrecht , Springer.

463   **Latifian, M., Soleymannejadian, E., Ghazavy, M., Mosadegh, M.S. & Rad, B.** (2011)

464   Effect of the fungus, Beauveria bassiana (Balsamo) (Asc., Hypocreales) on the

465   functional response and host preference of the parasitoid *Cephalonomia tarsalis*

466   (Ashmead) (Hym., Bethylidae) in larval population of the sawtoothed beetle

467   *Oryzaephilus surinamensis* L. (Col., Silvanidae). *Journal of Entomological Research* **3**,

468   251-264.

469   **Lord, J.C.** (2001) Response of the wasp *Cephalonomia tarsalis* (Hymenoptera: Bethylidae)

470   to *Beauveria bassiana* (Hyphomycetes: Moniliales) as free conidia or infection in its

471   host, the sawtoothed grain beetle, *Oryzaephilus surinamensis* (Coleoptera: Silvanidae).

472   *Biological Control* **21**, 300-304.

473   **Lord, J.C.** (2006) Interaction of *Mattesia oryzaephili* (Neogregarinorida: Lipotrophidae)

474   with Cephalonomia spp. (Hymenoptera: Bethylidae) and their hosts *Cryptolestes*

475   *ferrugineus* (Coleoptera: Laemophloeidae) and *Oryzaephilus surinamensis* (Coleoptera:

476   Silvanidae). *Biological Control* **37**, 167-172.

477   **Lukáš, J.** (2007) Age specific fecundity and survivorship of *Cephalonomia tarsalis*

478   (Ashmead) (Hymenoptera: Bethylidae) at different temperatures. *"Integrated*

479   *Protection of Stored Products" IOBC/WPRS Bulletin* **30**, 45-51.

480   **Lukáš, J.** (2008) *Use of Cephalonomia tarsalis for the biological control of Oryzaephilus*

481   *surinamensis.* Ph.D. Thesis, Prague Research Institute, Prague (In Czech)

482   **Lukáš, J. & Stejskal, V.** (2005) *Cephalonomia tarsalis* – egg, larval and pupal development

483   in dependence on temperature. p.20-21 in *Proceedings of COST 842 meeting*

484   *"Biocontrol of arthropod pests in stored products", Barcelona, 27-31 October 2004.*

485   **Mayhew, P.J.** (1997) Fitness consequences of ovicide in a parasitoid wasp. *Entomologia*

486   *Experimentalis et Applicata* **84**, 115–126.

487   **Meunier, J. & Bernstein, C.** (2002) The influence of local mate competition on host-

488   parasitoid dynamics. *Ecological Modelling* **152**, 77-88.

489   **Nunney, L. & Luck, R.F.** (1988) Factors influencing the optimum sex ratio in a structured

490   population. *Theoretical Population Biology* **33**, 1-30.

491   **Ode, P.J. & Hardy, I.C.W.** (2008) Parasitoid sex ratios and biological control. pp 253-291

492   in: Wajnberg, E., Bernstein, C. & van Alphen, J.J.M. (Eds) *Behavioral ecology of*

493   *insect parasitoids: from theoretical approaches to field applications.* Blackwell

494   Publishing, Oxford.

495 **Pérez-Lachaud, G., Hardy, I.C.W. & Lachaud, J-P.** (2002) Insect gladiators: competitive
496   interactions between three species of bethylid wasps attacking the coffee berry borer,
497   *Hypothenemus hampei* (Coleoptera: Scolytidae). *Biological Control* **25**, 231-238.

498 **Pérez-Lachaud, G., Batchelor, T.P. & Hardy, I.C.W.** (2004) Wasp eat wasp: facultative
499   hyperparasitism and intra-guild predation by bethylid wasps. *Biological Control* **30**,
500   149–155.

501 **Powell, D.** (1938) The biology of *Cephalonomia tarsalis* (Ash.), a vespoid wasp (Bethylidae:
502   Hymenoptera) parasitic on the sawtoothed grain beetle. *Annals of the Entomological*
503   *Society of America* **16**, 44-49.

504 **Sedlacek, J.D., Price, B.D., Sharkey, M.J., Hill, S.J. & Weston, P.A.** (1998) Parasitoids
505   found in on-farm shelled corn in Kentucky. *Journal of Agricultural Entomology* **15**,
506   223-230.

507 **Sreenivas, A.G. & Hardy, I.C.W**. (2016) Mutual interference reduces offspring production
508   in a brood guarding bethylid wasp. *Entomologia Experimentalis et Applicata* (in press)

509 **Visser, M.E., van Alphen, J.J.M. & Nell, H.W.** (1990) Adaptive superparasitism and patch
510   time allocation in solitary parasitoids: The influence of the number of parasitoids
511   depleting a patch. *Behaviour* **114**, 21-36.

512 **Wilson, K. & Hardy, I.C.W.** (2002) Statistical analysis of sex ratios: an introduction. pp 48-
513   92 in Hardy, I.C.W. (Ed.) *Sex ratios: concepts and research methods.* Cambridge,
514   Cambridge University Press.

515 **Yazdani, M. & Keller, M.A.** (2015) Mutual interference in *Dolichogenidea tasmanica*
516   (Cameron) (Hymenoptera: Braconidae) when foraging for patchily-distributed Light
517   Brown Apple Moth. *Biological Control* **86**, 1-6.

518 **Žďărkovă, E., Lukáš, J. & Horăk, P.** (2003) Compatibility of *Cheyletus eruditus* (Schrank)

519   (Acari: Cheyletidae) and *Cephalonomia tarsalis* (Ashmead) (Hymenoptera: Bethylidae)

520   in biological control of stored grain pests. *Plant Protection Science* **39**, 29-34.

521 **Zimmermann, O., Schöller, M. & Prozell, S.** (2008) A new beneficial in storage protection:

522   The use of the bethylid wasp *Cephalonomia tarsalis* against the sawtoothed grain beetle

523   *Oryzaephilus surinamensis*. *DgaaE-Nachrichten* **22**, 46.

524 **Zuur, A.F., Ieno, E.N., Walker, N.J., Saveliev, A.A. & Smith, G.M.** (2009) *Mixed effects*

525   *models and extensions in ecology with R.* New York, Springer.

526

Figure captions

527

528   Fig. 1. Treatment block types[1]

529   Fig. 2. Adult *C. tarsalis* production from single chamber blocks with different substrates and

530   host densities. Production is expressed as total wasps per replicate and as wasps per host.

531

532   Fig. 3. Adult *C. tarsalis* production from multi-chamber blocks with different degrees of

533   isolation, substrates and host densities. Production is expressed as total wasps per replicate

534   and as wasps per host.

535

536   Fig. 4. Sex ratios of adult *C. tarsalis* emerging according to the number of adults produced

537   per replicate. Data are drawn from all experimental conditions and some overlapping points

538   are displaced horizontally to illustrate sample size. The line was fitted by logistic regression

539   including a quadratic term.

540

541   Fig. 5. Number of male *C. tarsalis* emerging according to the number of adults produced per

542   replicate. Data are drawn from all experimental conditions and some overlapping points are

543   displaced horizontally to illustrate sample size. The line was fitted by log-linear regression

544   including a quadratic term.

545

546   Table 1. Influences on adult production in single chamber blocks and multi-chamber blocks

547   with open passages

548

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		

549

550

551

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

553

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		

554

555   Fig. 1. Treatment block types[1]

Block A　　　　**Block B**　　　　**Block C**
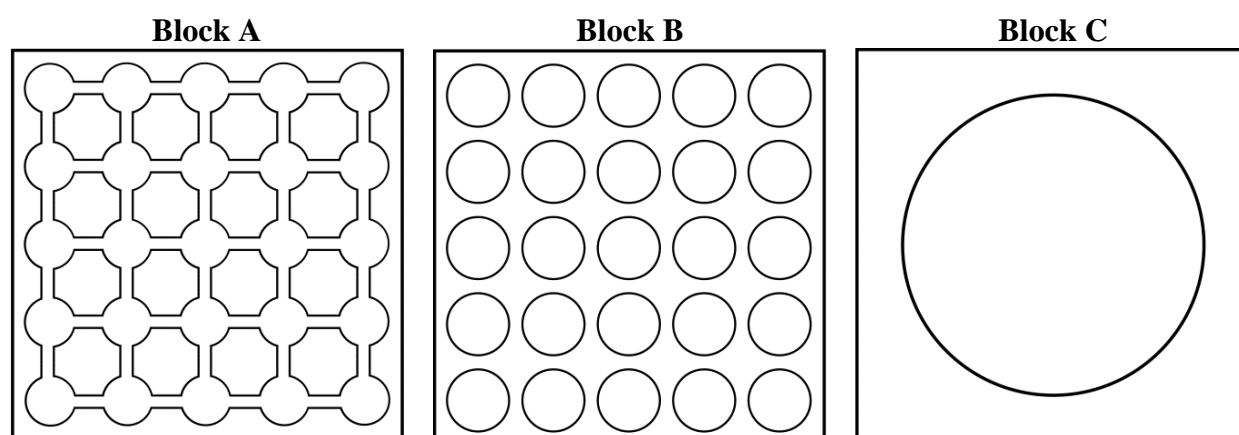
556   [1] The total floor area of the chambers was 72.7cm^2 in all block types.

557

558

559   Fig. 2. Adult *C. tarsalis* production from single chamber blocks with different substrates and

560   host densities. Production is expressed as total wasps per replicate and as wasps per host.
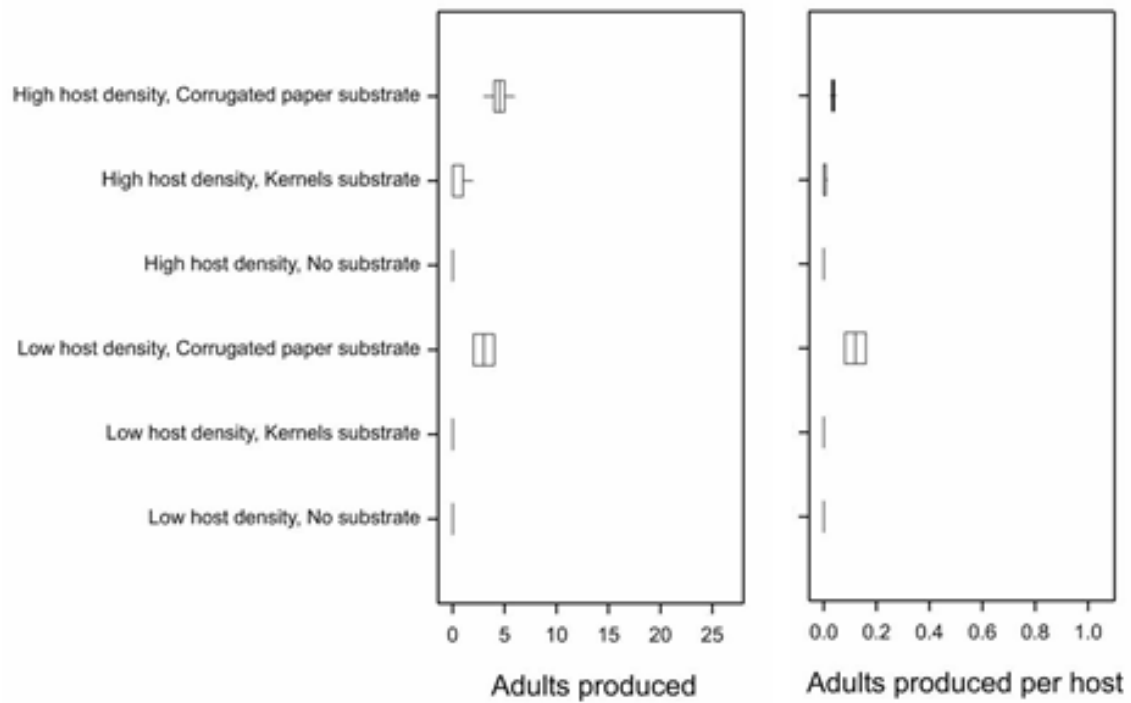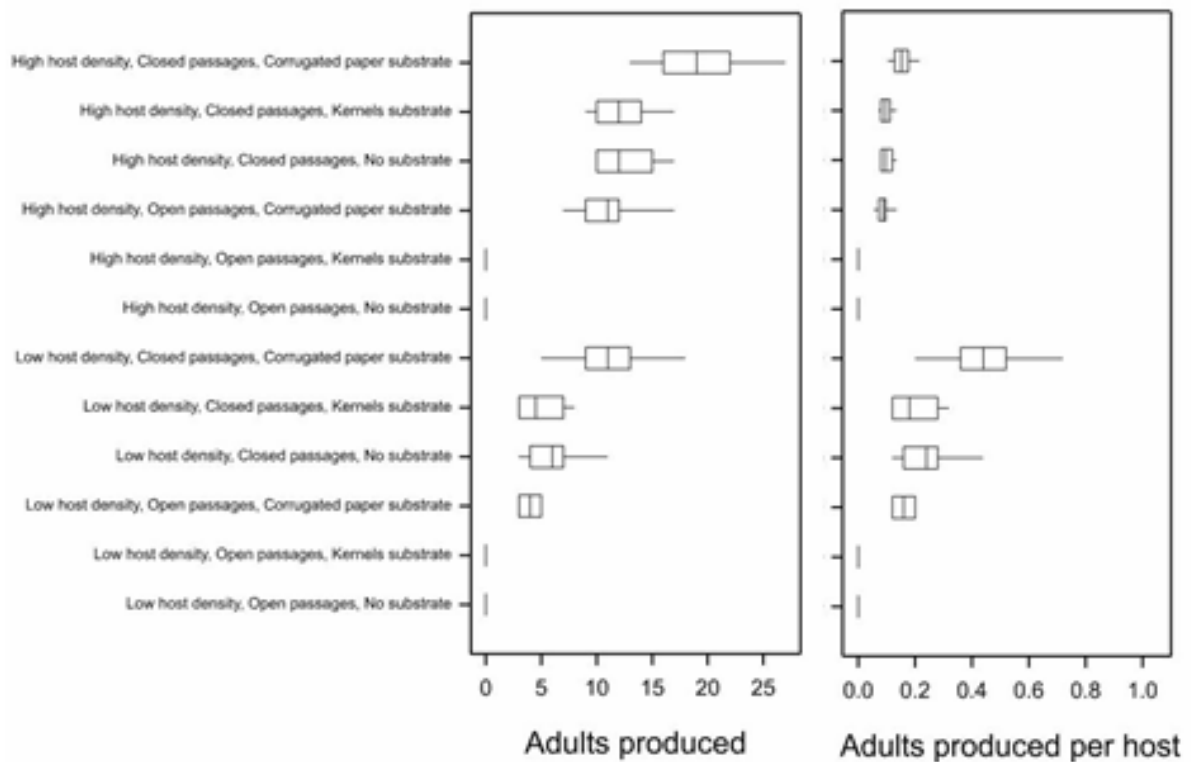

561

562

563   Fig. 3. Adult *C. tarsalis* production from multi-chamber blocks with different degrees of

564   isolation, substrates and host densities. Production is expressed as total wasps per replicate

565   and as wasps per host.

566

567

568

569

570   Fig. 4. Sex ratios of adult *C. tarsalis* emerging according to the number of adults produced

571   per replicate. Data are drawn from all experimental conditions and some overlapping points

572   are displaced horizontally to illustrate sample size. The line was fitted by logistic regression

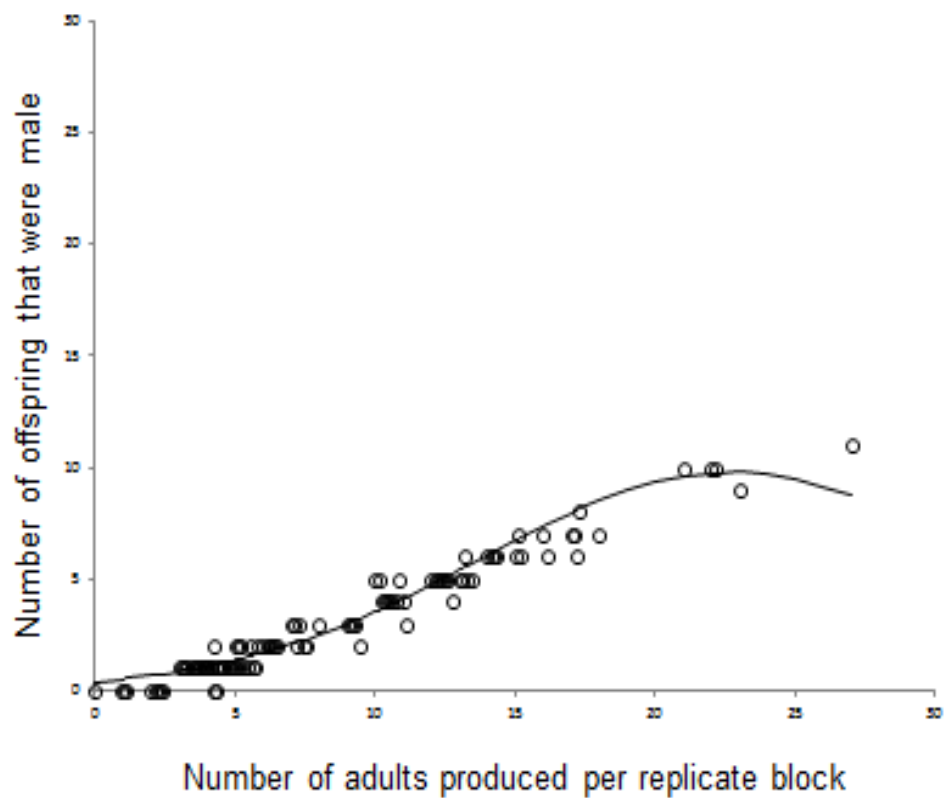573   including a quadratic term.

574
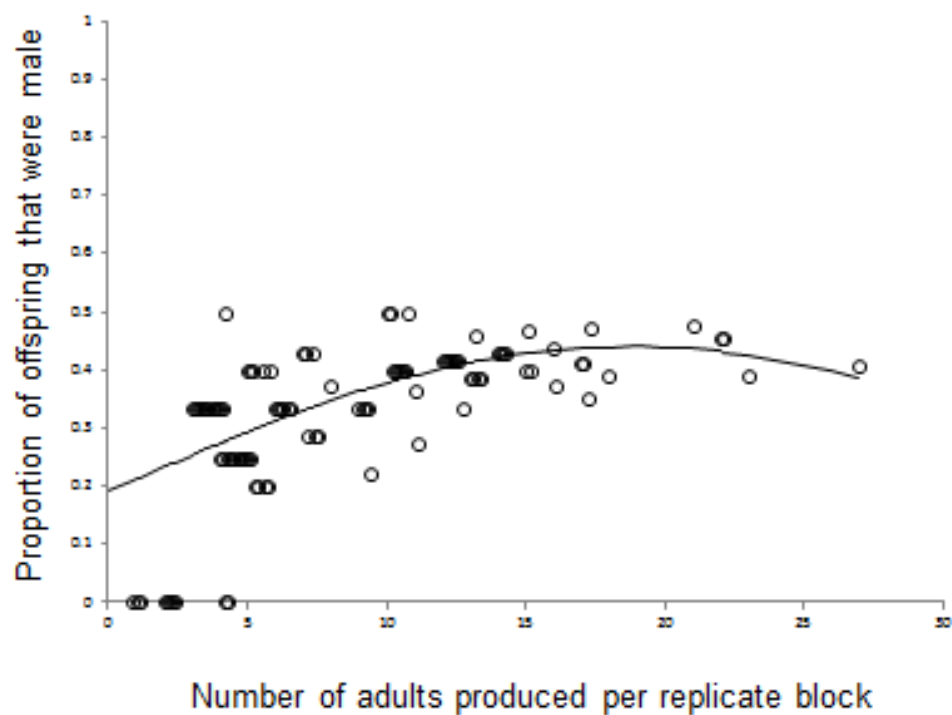
575

576

577

578   Fig. 5. Number of male *C. tarsalis* emerging according to the number of adults produced per

579   replicate. Data are drawn from all experimental conditions and some overlapping points are

580   displaced horizontally to illustrate sample size. The line was fitted by log-linear regression

581   including a quadratic term.

582

583