



Screenivas, A.G. and Hardy, Ian C.W. (2016) Mutual interference reduces offspring production in a brood-guarding bethylid wasp. *Entomologia Experimentalis et Applicata*, 159 (2). pp. 260-269. ISSN 1570-7458

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1 *Entomologia Experimentalis et Applicata*

2 **Special Issue: Mark Jervis Memorial**

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4 **Mutual interference reduces offspring production in a brood-**
5 **guarding bethylid wasp**

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17 **Running title:** *Mutual interference in a bethylid wasp*

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19 *Key words:* parasitoid, laboratory microcosm, female offspring production, mass rearing,
20 *Goniozus nephantidis*, Hymenoptera, Bethyilidae

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22 **Accepted: 16 September 2015**

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1 **Abstract**

2 Parasitoids have the potential to suppress populations of their hosts and thus may play an
3 important role in influencing the temporal and spatial dynamics of pest arthropods.

4 Behavioural interactions between foraging females, collectively constituting ‘mutual
5 interference’, can reduce host suppression. We use laboratory microcosms to assess the
6 prevalence and consequences of mutual interference behaviour in a bethylid wasp, *Goniozus*
7 *nepantidis* (Muesebeck) (Hymenoptera: Bethylidae), which is known to brood guard and to
8 engage in agonistic contests for individual hosts and which is also an agent of biological pest
9 control. We hold host and parasitoid numbers constant and vary the degree of female-female
10 contact that can occur. Mutual interference is manifest in a considerable reduction in the
11 number of offspring produced when females are not fully isolated from each other, due to
12 effects operating at the early stages of offspring production. This mutual interference may
13 contribute towards the limited degree of host population suppression achieved when some
14 species of bethylids are deployed as agents of biological pest control and also has clear
15 potential to influence the efficiency of mass rearing of parasitoids prior to field release.

16

1 **Introduction**

2 Parasitoid wasps are free-living as adults: females search for and attack hosts, usually the
3 immature stage of another arthropod, laying their eggs on or in these hosts which then form
4 the only food resource for their developing, parasitic, offspring, leading to the death of the
5 host (Godfray, 1994; Jervis, 2005). Parasitoid efficiency in host attack, and consequently
6 offspring production, may be tempered by behavioural interactions with other foraging
7 females, termed ‘mutual interference’ (Hassell & Varley, 1969; Hassell, 1971, 2000; Rogers
8 & Hassell, 1974; Beddington, 1975; Free et al., 1977; Begon et al., 1996; Driessen & Visser,
9 1997; Fellowes et al., 2005; Jervis et al., 2005; Kidd & Jervis, 2005).

10 Interference interactions can take a variety of forms; seen broadly they include not
11 only the rate of parasitism of the current generation of hosts but also influences on the size
12 and sexual composition of the next generation of parasitoids (Visser & Driessen, 1991; Visser
13 et al., 1999). Examples include time-wasting disruption of foraging for hosts without
14 explicitly agonistic interactions between foraging females (Hassell, 1971, 2000; Cronin &
15 Strong, 1993; Field et al., 1998; Wajnberg et al., 2004; Le Lann et al., 2011; Yazdani &
16 Keller, 2015), aggressive patch or brood guarding (Hassell, 1971; Waage, 1982; Field et al.,
17 1997; Goubault et al., 2005; Nakamatsu et al., 2009; Venkatesan et al., 2009a,b; de Jong et
18 al., 2011; Hardy et al., 2013; Mohamad et al., 2015), clutch size and superparasitism
19 decisions differing in the presence, or anticipated presence, of competitors (van Alphen &
20 Visser, 1990; Visser & Driessen, 1991; Visser et al., 1992; Visser, 1996; Field et al., 1998;
21 Goubault et al., 2007a), and sex allocation decisions contingent on the number of ovipositing
22 ‘foundress’ females present (Hamilton, 1967; Waage, 1982; Meunier & Bernstein, 2002;
23 Irvin & Hoddle, 2006; Ode & Hardy, 2008; Luo et al., 2014).

24 Empirical investigations of factors contributing to mutual interference have often been
25 conducted from the perspective of behavioural ecology but there has also been great interest
26 in their consequences for host–parasitoid population dynamics, in part because this
27 contributes towards explaining population phenomena on the basis of natural selection
28 (Hassell & May, 1973, 1989; Anderson, 1989; Cronin & Strong, 1993; Driessen & Visser,
29 1997; Visser et al., 1999; Bernstein, 2000; Meunier & Bernstein, 2002). Typically, mutual
30 interference interactions reduce the per-host production of female offspring (the sex that
31 attacks future generations of hosts) and will also be more prevalent at higher parasitoid
32 densities: this density-dependent effect may be expected to contribute to the dynamic stability
33 of host–parasitoid populations, but extreme effects of interference can also lead to predictions
34 that host populations will not be regulated (sufficiently suppressed) by parasitism (Rogers &

1 Hassell, 1974; Begon et al., 1996; Bernstein, 2000; Hassell, 2000; Kidd & Jervis, 2005). To
2 practitioners of biological control, who deploy parasitoids against agricultural pests, the
3 importance of mutual interference is thus that it reduces the likelihood that pest populations
4 will be depressed below thresholds of economic damage (Anderson, 1989; Hassell & May,
5 1989). Although there have been many successful biocontrol programmes, attempts have also
6 frequently failed because parasitoids have not established and because established parasitoids
7 have not depressed the pest populations sufficiently (e.g., Mills, 2000; Kapranas & Hardy,
8 2014). In particular, parasitoids are often mass reared and inundatively released at high
9 densities, which may artificially promote the occurrence of economically detrimental mutual
10 interference interactions (Irvin & Hoddle, 2006; Ode & Hardy, 2008).

11 In this study we use simple laboratory microcosms to assess the prevalence and
12 consequences of mutual interference behaviour in a bethylid wasp, *Goniozus nephantidis*
13 (Muesebeck) (Hymenoptera: Bethyridae). This parasitoid species is known to guard
14 individual hosts and developing broods, to engage in agonistic contests for hosts prior to and
15 following oviposition (Hardy et al., 2013), and is also mass reared for release in biological
16 control programmes against coconut pests (Shameer et al., 2002; Mohan & Shameer, 2003;
17 Venkatesan et al., 2003, 2007, 2009a,b; Lyla et al., 2006). Our purpose is to understand better
18 the competitive and reproductive biology of brood-guarding parasitoids for which prior most
19 investigations have been confined to dyadic interactions (Hardy et al., 2013), and to in turn
20 suggest approaches towards enhancing the efficiency of their mass rearing and field release
21 when utilized as agents of biological pest control. Venkatesan et al. (2009b) have already
22 reported that individual *G. nephantidis* females produced more progeny (per host and also in
23 total) when provided with just one host rather than multiple hosts simultaneously within a
24 single chamber (a glass vial). They also reported that when the number of females within the
25 chamber to which a single host was presented was varied, progeny production was greatest
26 when just one female wasp was present: offspring production was thus greatest (per female
27 and also in total) when one host and one female *G. nephantidis* were present (Venkatesan et
28 al., 2009b). Here, we hold the number of hosts available per female (resource-weighted
29 density; Lewontin & Levins, 1989) constant, at unity, and experimentally vary both spatial
30 density (individuals per unit area) and the degree of physical sub-division between chambers
31 within microcosms (the latter allowing parasitoid behaviour to determine the organism-
32 weighted density; Lewontin & Levins, 1989). The overall hypothesis is that parasitoids will
33 produce fewer offspring when there is more contact possible between reproductive adult
34 females, even though in all cases one host per female is provided.

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Biology of Goniozus nephantidis

Goniozus nephantidis is a gregarious larval ectoparasitoid for which many aspects of behaviour and life history have been documented (Hardy et al., 1992; Cook, 1993; Hardy & Cook, 1995; Humphries et al., 2006; Goubault et al., 2007a,b, 2008; Venkatesan et al., 2009a,b). It is naturally associated with the coconut pest, *Opisina arenosella* Walker (Lepidoptera: Oecophoridae) in the Indian sub-continent (Mohan et al., 2004; Venkatesan et al., 2007, 2009a,b). *Opisina arenosella* larvae feed on the vascular tissues of coconut fronds by chewing, leading to affected leaves showing a burnt appearance, and also construct protective tunnels and galleries with silk, leaf parts, and frass (Mohan et al., 2004; Venkatesan et al., 2009a). Coconut crop yield losses due to *O. arenosella* can be as high as 45% and it can take up to 4 years for palms to regain normal yield after heavy infestation (Mohan et al., 2010). Adult *G. nephantidis* females forage for hosts by entering the galleries. On encountering a host larva, the adult female attacks it by injecting venom whereupon the host becomes paralysed within minutes and their development is arrested (idiobiontism). Eggs are laid onto the host 1-4 days later (Jayaratnam, 1941; Goubault et al., 2007b), the intervening period possibly being used to mature eggs (Stokkebo & Hardy, 2000; but see Goubault et al., 2007b). The number of eggs laid on a host is positively correlated with host size, with clutches ranging up to ca. 18 (Hardy et al., 1992). Eggs hatch about 1 day after oviposition and the larvae begin to feed on the host through punctures in its integument.

During the period between paralysis and oviposition, females remain in close physical association with their hosts, aggressively guarding them against intruding conspecific females which would otherwise utilize the unguarded host (Hardy & Blackburn, 1991; Petersen & Hardy, 1996). Mothers also remain with their broods after laying eggs, typically until the offspring pupate around 7 days later, and during this period guard their eggs and developing larvae against intruders (Hardy & Blackburn, 1991; Goubault et al., 2007b; Venkatesan et al., 2009a). Mothers continue to mature eggs within their abdomens whilst guarding and surviving mothers have an increasing tendency to leave their broods, possibly to forage for further hosts (Goubault et al., 2007b).

Although prior-ownership is an advantage in host-ownership contests, other factors, especially body size differences, contribute to determining the outcomes of agonistic encounters (Petersen & Hardy, 1996; Humphries et al., 2006; Hardy et al., 2013) and prior owners can thus be driven away from hosts they have paralysed (fighting is rarely fatal). Intruders that displace mothers will often destroy the brood and then lay their own eggs, but

1 the probability of destruction followed by oviposition declines as the developmental stage of
2 the original brood increases (Hardy & Blackburn, 1991; Goubault et al., 2007b).

3 Some offspring fail to mature, even in clutches that are not destroyed by intruders,
4 with mortality of guarded offspring estimated at around 28% (Hardy & Cook, 1995), mainly
5 due to low juvenile survival during earlier developmental stages. Surviving offspring eclose
6 as adults approximately 2 weeks after oviposition: males eclose first and mate with their
7 sisters, then adults disperse from the natal site over the following few days (Hardy et al.,
8 1999). The sex ratios of broods at eclosion and dispersal are usually female biased (Hardy &
9 Cook, 1995; Hardy et al., 1999; Venkatesan et al., 2009a,b).

11 **Materials and methods**

12 *Goniozus nephantidis* cultures were maintained using the rice moth, *Corcyra cephalonica*
13 Stainton (Lepidoptera: Pyralidae), as a factitious host (following Hardy & Blackburn, 1991;
14 Hardy et al., 1992; Petersen & Hardy, 1996). *Corcyra cephalonica* was reared on a diet of
15 glycerol, honey, corn meal, wheat bran, and yeast (Lizé et al., 2012). All cultures and
16 experiments were carried out in a climate room at 27 °C, L12:D12, with relative humidity
17 maintained at *ca.* 35% by evaporation from a water bath.

18 We assessed the effects of host and parasitoid density and of spatial sub-division on
19 parasitoid reproduction using experimental arenas excavated from polyethylene blocks and
20 with transparent Plexiglas lids, with designs derived from the simpler contest arenas used and
21 illustrated in Petersen & Hardy (1996) and Goubault et al. (2006). We used four types of
22 arenas, termed block A-D (Figure 1), all excavated to a depth of 6 mm. Block A contained 25
23 chambers and each chamber was 1.7 cm in diameter. The total excavated area (floor area)
24 within the block of the chamber was 56.8 cm². As wasps could not pass between the
25 chambers, block A served as a control treatment in which interactions between adult females
26 wasps were prevented. Block B was the same as A except that each chamber was connected
27 to the others around it by a 1 cm long and 0.1 cm wide slot, through which adult *G.*
28 *nephantidis* can pass with ease. Block B thus had an extra 4 cm² of floor area, an increase of
29 7% compared to A, but retained the same chamber size as block A and also as in previous
30 studies (Petersen & Hardy, 1996; Goubault et al., 2006). Block C had the same total
31 excavated area as A but in the form of a single chamber with no spatial sub-division. Block D
32 also had a single chamber but with half the total excavated area (28.4 cm²).

33 We selected larval *C. cephalonica* weighing between 30-40 mg (mean = 31.4) from
34 our cultures and presented them individually to an adult female *G. nephantidis* until the larva

1 was paralysed (but with no eggs laid on its integument), and then the paralysing female wasp
2 was discarded. Twenty-five paralysed *C. cephalonica* larvae were placed into each replicate
3 block, one per chamber in blocks A and B, and all in the single chamber in C and D. In the
4 latter block types we attempted to arrange the larvae into a regular array within the single
5 chamber. The total weights of the host larvae at the start of the experiment did not differ
6 significantly across block types (ANOVA: $F_{3,44} = 0.54$, $P = 0.66$, $r^2 = 0.035$).

7 We then selected female *G. nephantidis* aged 3-5 days since emergence as adults in
8 broods containing at least one male (females had thus mated) and which had had no
9 experience of attacking or ovipositing on hosts; we anesthetized them with CO₂ gas and then
10 placed them into the chambers within the blocks (one female per chamber in A and B, and all
11 25 females in the centre of the chamber in C and D). The Plexiglas lid was then placed over
12 the block. Anesthetized wasps regained their activity within 2 min. Each block was inspected
13 daily until adult offspring matured (adult emergence from cocoons).

14 From each replicate block we recorded the numbers of hosts on which eggs were laid,
15 the clutch sizes laid onto each host, and the numbers of offspring reaching the larval, pupal,
16 and adult stages. Due to ectoparasitic development, immature *G. nephantidis* are observable
17 at all stages; data on numbers of eggs laid are, however, likely to have been underestimates as
18 some eggs could have been eaten between oviposition and observation. Adult female wasps
19 (mothers) were removed from each block once parasitoid offspring had reached the pupal
20 stage; this avoided confusion between generations during subsequent weight measurements.
21 The numbers of adult male and female offspring were recorded and we obtained measures of
22 the mean dry weight of adult male and of adult female offspring. There were 12 replicates for
23 each of the four block types.

24

25 ***Statistical analysis***

26 Data were mainly analysed using generalized linear modelling (GLM) techniques (Crawley,
27 1993; Zuur et al., 2009). In most analyses, the sampling unit was taken as the summed
28 observations deriving from an individual replicate block, but we also carried out mixed
29 effects modelling (GLMM) on clutch size, in which the replicate within which individual
30 observations derived was fitted as a random factor (Zuur et al., 2009). We adopted quasi-
31 Poisson errors and a log-link function for log-linear analyses of count data and quasi-
32 binomial errors and a logit-link function for logistic analyses of data on proportions, in both
33 cases using empirically estimated scale parameters to correct for overdispersion (Crawley,
34 1993; Wilson & Hardy, 2002; Zuur et al., 2009; Warton & Hui, 2011). When significant

1 differences across treatments were found from fitting an initial GLM, the parsimonious
2 model was identified by aggregation of factor levels (Crawley, 1993). For the analysis of
3 adult dry weight, errors were non-normally distributed so we employed non-parametric
4 Kruskal-Wallis ANOVAs (Siegel & Castellan, 1988). All statistical testing was 2-tailed. All
5 analyses were carried out using the GENSTAT statistical package (v. 15.1; VSN
6 International, Hemel Hempstead, UK).

7

8 **Results**

9 The proportion of hosts on which eggs were observed varied significantly between block
10 types (logistic ANOVA: $F_{3,40} = 8.02$, $P < 0.001$; Figure 2): the probability of a host being
11 oviposited on was highest in blocks of type A and did not differ significantly among blocks
12 of the other three types (model simplification by aggregation of factor levels: $F_{2,42} = 0.85$, $P =$
13 0.43). Among those hosts on which eggs were observed, clutch sizes varied significantly
14 across block types (log-linear GLMM: Wald statistic = 70.08, $F_{3,42.8} = 23.36$, $P < 0.001$), with
15 larger clutches in A [mean (+SE, -SE); A: 7.98 (+0.263, -0.254); B, 5.78 (+0.352, -0.331); C:
16 4.42 (+0.348, -0.323); D: 4.35 (+0.344, -0.319)]. In terms of the total numbers of eggs,
17 larvae, pupae, and adults produced per replicate, there were no significant differences among
18 B, C, and D, but production was significantly greater in A (Table 1). Ultimately, around 4× as
19 many adults emerged from type A than from the other block types (Figure 3).

20 The mean (+SE, -SE) proportion of offspring that survived from the egg to the adult
21 stage was 0.59 (+0.033, -0.032) and was not significantly influenced by block type (logistic
22 ANOVA: $F_{3,42} = 2.11$, $P = 0.11$), but note that this estimate is constrained to use the observed
23 maximum number of eggs per replicate and the true probability of survival in some replicates
24 could have been lower due to ovicide (see above). However, the probability of (observed)
25 eggs surviving to the larval stage was affected by block type ($F_{3,42} = 4.05$, $P = 0.013$) and was
26 greatest in A and not significantly different among B, C, and D (aggregation of factor levels:
27 $F_{2,44} = 0.50$, $P = 0.61$). The probability of larvae surviving to the pupal stage was not
28 significantly influenced by block type ($F_{3,42} = 1.20$, $P = 0.32$), nor was the pupal to adult
29 survival ($F_{3,42} = 2.74$, $P = 0.055$).

30 The mean (+SE, -SE) overall sex ratio of adult offspring (proportion that were male)
31 was 0.32 (+0.021, -0.020) and was not influenced by block type (logistic ANOVA: $F_{3,42} =$
32 1.24 , $P = 0.31$). The mean dry weights of adult male offspring, female offspring, or males and
33 females pooled did not differ across the block types (Kruskal-Wallis ANOVA; males: $H =$
34 7.013 , $P = 0.071$; females: $H = 5.338$, $P = 0.15$; pooled: $H = 4.717$, $P = 0.19$; all d.f. = 3).

1

2 **Discussion**

3 Offspring production by *G. nephantidis* females is negatively affected by direct or indirect
4 contact with conspecific females: only around one quarter as many adults developed under
5 incomplete separation of broods compared to complete separation. Given that one host per
6 female was always provided, the effect is not dependent on resource-weighted density, nor is
7 it due to variation in spatial density (which was either held constant or had non-significant
8 effect), but can be viewed as due to variation in organism-weighted density (Lewontin &
9 Levins, 1989). That is, when not isolated, all females can experience higher parasitoid
10 densities due to behavioural and/or chemical (Goubault et al., 2008) interactions with other
11 females. It is notable that interference interactions occurred even when each female was
12 initially placed in a separate chamber containing a host. A similar regular distribution of
13 females across host patches was observed as the end result of agonistic interactions followed
14 by dispersal of the loser in *Asobara citri* (Fischer), a braconid parasitoid of *Drosophila* spp.
15 larvae, when females were experimentally released at a central point (de Jong et al., 2011).
16 The fact that *G. nephantidis* females interacted with each other despite being placed in a
17 regular array (block type B) suggests that the perceived host-patch is larger than a single
18 experimental chamber. This accords with prior observation that although brood-guarding *G.*
19 *nephantidis* females spend most of their time in the close vicinity of their hosts, they also
20 patrol beyond the confines of the chamber (Goubault et al., 2007b) and it is during such
21 patrolling behaviour that they are likely to encounter other females. Direct physical
22 encounters can lead to the release of volatile chemicals (Goubault et al., 2008) which may in
23 turn influence the behaviour of further females indirectly.

24 The differences in *G. nephantidis* offspring production were principally due to effects
25 operating at the early stages of offspring production: far fewer hosts were oviposited onto and
26 also fewer eggs per host were observed when more than one female had access to each host.
27 We pose three candidate explanations, which are not mutually exclusive. The first, a
28 mechanistic explanation, is that females that were not completely isolated were continually
29 disturbed, and possibly displaced from hosts, prior to and during oviposition. This is
30 supported by observations of mutual attack and disruption of oviposition when one host was
31 presented to several *G. nephantidis* females within a chamber (Venkatesan et al., 2009b). The
32 second, an adaptive explanation, is that game-theoretic reasoning predicts females should lay
33 smaller clutches after having encountered competitors, due to anticipation of a more
34 competitive future environment for their offspring coupled with an increased size of offspring

1 that develop from smaller clutches on given-sized hosts (Petersen & Hardy, 1996; Mesterton-
2 Gibbons & Hardy, 2004). This gains some support from prior observation of the required
3 effects in *G. nephantidis*: when females with hosts weighing <30 mg experienced 1-4
4 competitors for 30 min, their subsequent clutch sizes were reduced by ca. 18% and offspring
5 sizes were consequently enhanced (Goubault et al., 2007a). In our present experiment, the
6 reduction in observed clutch size in response to encountering competitors was more marked
7 (from ca. 8 to ca. 4-6 eggs per host) but so was the likely occurrence of female-female
8 encounters. However, we found no evidence for effects on the size of maturing offspring –
9 we used hosts of 30-40 mg, and Goubault et al. (2007a) also found no effects using hosts >30
10 mg. The lack of difference in sex ratios across experimental treatments also suggests that
11 mothers did not adaptively adjust their reproductive decisions according to the density of
12 ovipositing females (Hamilton, 1967; Legner & Warkentin, 1988; Irvin & Hoddle, 2006; Ode
13 & Hardy, 2008), probably because aggressive host and brood guarding naturally lead to
14 broods being produced by single foundresses (Hardy & Cook, 1995; Hardy et al., 2013)
15 resulting in little selection for sex-ratio adjustment to foundress number (Herre, 1987).

16 The third explanation is that oviposition occurred to a similar extent in all
17 experimental conditions but fewer eggs were observed on non-isolated hosts because some
18 were eaten prior to being counted. Ovicidal behaviour is well documented in *G. nephantidis*,
19 its congeners, and some other bethylids (Venkatraman & Chacko, 1961; Goertzen & Doult,
20 1975; Hardy & Blackburn, 1991; Mayhew, 1997; Infante et al., 2001; Pérez-Lachaud et al.,
21 2002; Goubault et al., 2007b; Bentley et al., 2009; Venkatesan et al., 2009b), occurring when
22 clutches of eggs are not attended by a guarding mother, and the lower probability of egg-to-
23 larval survival in blocks of type B, C, and D supports this. As with the mechanistic disruption
24 hypothesis above, females that oviposited may have been intruded upon by such a number of
25 other females that they were unable to defend successfully their clutches (for analogous
26 arguments see Waage, 1982; van Alphen & Visser, 1990; Moore & Greeff, 2003; de Jong et
27 al., 2011). The similar probabilities of the survival of offspring between the larval stages and
28 adulthood, across experimental treatments, accords with prior reports of conspecific larvicide
29 being absent or rare in *G. nephantidis* and congeners and, if it occurs, to be limited to the
30 smaller larval stadia (Venkatraman & Chako, 1961; Goertzen & Doult, 1975; Hardy &
31 Blackburn, 1991; Goubault et al., 2007b; Bentley et al., 2009; Venkatesan et al., 2009a).

32 Irrespective of the particular mechanism of mutual interference, the number of *G.*
33 *nephantidis* offspring produced is negatively affected by contact between adult females
34 during the host- and clutch-guarding phases. We now consider the potential consequences of

1 this for biological pest control programmes using *G. nephantidis*, its congeners, and other
2 bethylids. Members of the genus *Goniozus* have been used in several biocontrol programs
3 (Dharmaraju, 1963; Legner & Silveira-Guido, 1983; Gothilf & Mazor, 1987; Legner &
4 Warkentin, 1988; Legner & Gordh, 1992; Mohan & Shameer, 2003; Lyla et al., 2006;
5 Venkatesan et al., 2007, 2009a,b) and others are regarded as having pest control potential
6 (Witethom & Gordh, 1994; Conlong, 1994; Berry, 1998; Abbas et al., 2008; Etiam, 2001;
7 Sadeghi et al., 2012). For instance, estimates of the percentage of *O. arenosella* parasitized
8 by *G. nephantidis* in coconut plantations range from around 4% to nearly 60% (Lyla et al.,
9 2006; Venkatesan et al., 2007) and almond pests have been shown to be regulated by
10 *Goniozus legneri* Gordh following classical biocontrol introduction (Legner & Gordh, 1992).
11 Across all biocontrol programmes using bethylids, about half have resulted in parasitoid
12 population establishment and around 10% have achieved partial control of the target pests but
13 records typically indicate that pest suppression is not sufficient to no longer require further
14 control measures (Greathead & Greathead, 1992; Hardy & Goubault, 2007). Mutual
15 interference behaviour could clearly be a contributory factor to a limited suppression of hosts
16 once bethylid populations are established and parasitoid density increases such that foraging
17 females frequently encounter each other at hosts. Even if mutual interference effects in the
18 field are weaker than indicated by our experiment (given that laboratory studies typically
19 over-estimate mutual interference due to the use of artificially high parasitoid densities; Free
20 et al., 1977), our data do suggest that field release of mass-reared *G. nephantidis* females may
21 be more effective (on a per parasitoid basis) when females are released at lower densities,
22 with lower female-female encounter rates, than at higher densities. However, as females
23 spend much time brood-guarding during which they could be foraging for further hosts, their
24 ability to suppress large numbers of hosts will be limited (Venkatesan et al., 2009b). A
25 contrasting suggestion is that, by leading to a regular distribution of parasitism across hosts,
26 female-female fighting behaviour in *A. citri* might promote host population suppression (de
27 Jong et al., 2011). It also contrasts with expectation when deploying bethylids of the genus
28 *Sclerodermus* (Yang et al., 2014): in *Sclerodermus*, the presence of other females can
29 enhance the probability of offspring production because females do not interact with each
30 other aggressively and exhibit cooperative brood care after collectively suppressing and
31 ovipositing on an individual host (Hu et al., 2012; Tang et al., 2014; Kapranas et al., 2016).
32 Mutual interference interactions are also likely to influence the efficiency of mass
33 rearing of *G. nephantidis* and its congeners. Our data, and the prior results of Venkatesan et
34 al. (2009b), clearly suggest that mass-rearing programmes will not maximize the production

1 of female offspring if multiple adult females are in contact with each other once presented
2 with hosts: using one vial for each female wasp plus one host will thus likely be more
3 effective than using larger communal containers. Irvin & Hoddle (2006) reached the same
4 conclusion when considering the economics of mass rearing mymarid parasitoids which
5 adjust their progeny sex ratios when ovipositing in the presence of conspecifics, and several
6 other studies have similarly concluded that mass production will not be optimized by using
7 high parasitoid densities (e.g., Luo et al., 2014). In contrast to our results, Abbas (1999)
8 reported that in the congener *G. legneri* there was no reduction in offspring production when
9 multiple hosts were provided to multiple females rather than individual females being
10 presented with hosts separately; we have no ready explanation for this, especially as the
11 reproductive and competitive behaviours of *G. nephantidis* and *G. legneri* are generally very
12 similar (Hardy et al., 2013). More in accord with our results is that Legner & Warkentin
13 (1988) found that for *G. legneri* the production of offspring per adult female ‘foundress’ was
14 reduced by an increase in foundress number and increased by an increase in the number of
15 hosts provided. They further found that the largest number of offspring per host was
16 produced when just one foundress was present (Legner & Warkentin, 1988).

17 The use of simple laboratory microcosms to suggest population-level consequences of
18 behaviour has its drawbacks, given that there is neither the fine control achieved by
19 experiments on the behaviours of one or a few individuals, nor the realism of field-scale
20 evaluations. Nonetheless, the value of microcosms to examine the summed effects of spatial
21 complexity and/or agonistic interactions has been recognised at least since the work of
22 Huffaker (1958; Kareiva, 1989; Hassell, 2000; Infante et al., 2001; Batchelor et al., 2006; de
23 Jong et al., 2011). Adopting the microcosm approach in this study has shown that interactions
24 between *G. nephantidis* females extend beyond the immediate vicinity of provided hosts, and
25 generate increased organism-weighted density when females are not completely separated.
26 These interactions constitute mutual interference, manifest as a considerable reduction in the
27 number of offspring produced. This mutual interference may contribute towards the limited
28 degree of host population suppression achieved when some species of bethylids are deployed
29 as agents of biological pest control and also has clear potential to influence the efficiency of
30 mass rearing of female parasitoids prior to field release.

31

32 **Acknowledgements**

33 We thank the late Dr Mark Jervis, who we visited to discuss this work: we will not forget the
34 hospitality shown by Mark and his family during our stay in Cardiff. We also thank Sahand

1 Khidr for discussion, Julietta Marquez for practical help, Bryan Morris for making the
2 treatment blocks, and four anonymous referees for constructive comments on an earlier
3 version. This research was funded by a Commonwealth Academic Staff Fellowship to AGS,
4 held at the University of Nottingham.
5

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34

1 **Table 1** Effects of experimental treatment on total offspring production at progressive life-
 2 history stages

Stage	Log-linear ANOVA			Model simplification ¹	
	F _{3,44}	P	% deviance explained	F _{2,46}	P
Eggs	13.65	<0.001	48.21	2.27	0.12
Larvae	16.65	<0.001	49.97	1.30	0.28
Pupae	13.63	<0.001	48.16	1.35	0.27
Adult females	9.45	<0.001	39.18	0.62	0.55
Adult males	13.75	<0.001	48.40	2.80	0.072
Adults (total)	12.28	<0.001	45.56	1.25	0.30

3 ¹At every life-history stage, the overall significant difference in offspring production was due
 4 to greater numbers in block type A than in the other block types (see Figure 1): model
 5 simplification by aggregation of factor levels showed that there were no significant
 6 differences between block types B, C, and D.

7
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 9

10 **Figure captions**

11 **Figure 1** Illustration of treatment blocks to vary sub-division and parasitoid density.

12

13 **Figure 2** The effect of treatment on the proportion of hosts within a replicate that had eggs
 14 laid onto them. Bars represent standard errors of the means and are asymmetrical due to back
 15 transformation from the logit scale. Differences between block types B, C and D were not
 16 significant.

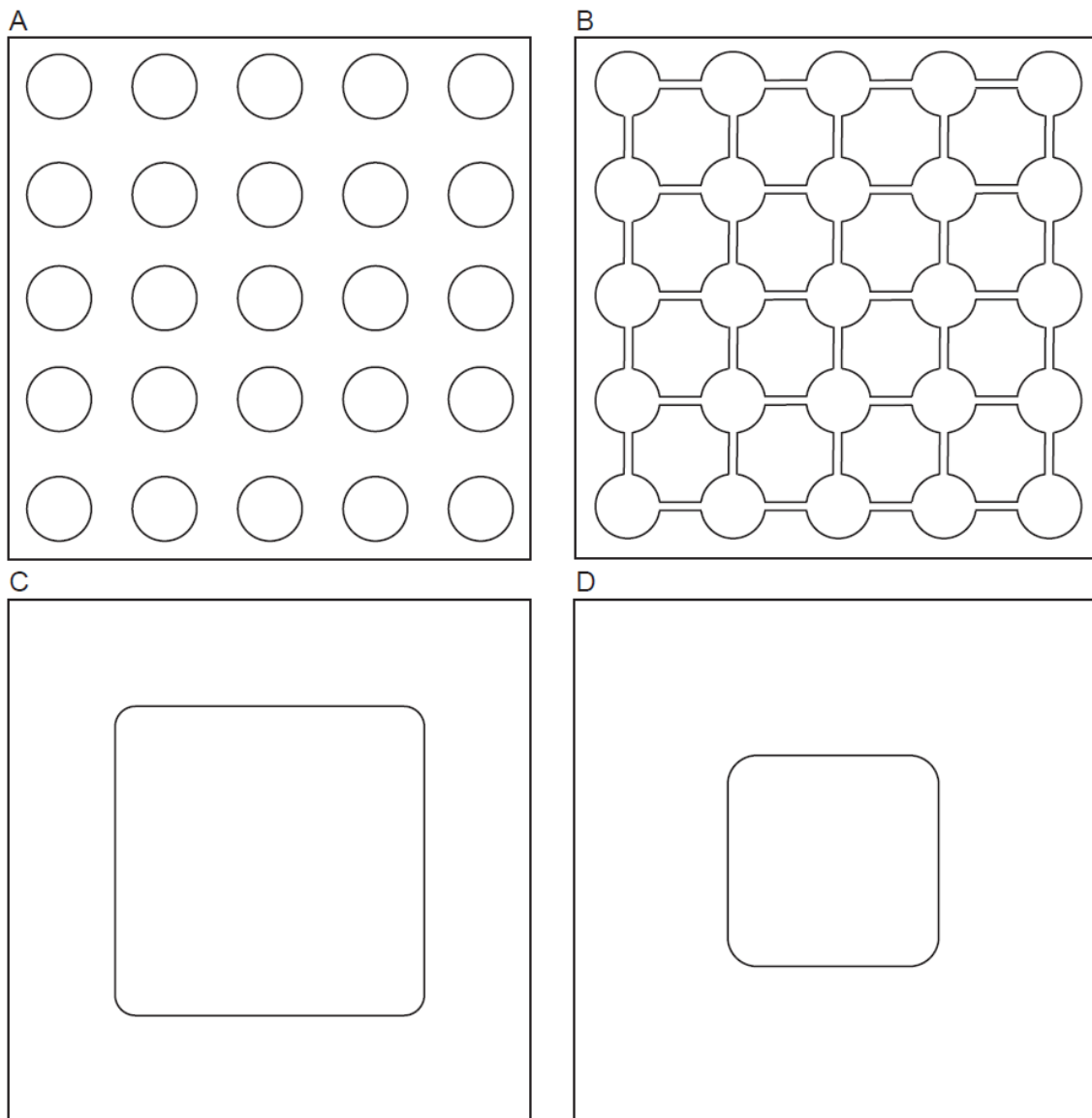
17

18 **Figure 3** The effect of treatment on the number of adult progeny produced per replicate. Bars
 19 represent standard errors of the means and are asymmetrical due to back transformation from
 20 the log scale. Differences between block types B, C and D were not significant.

21

1 Figure1.

2

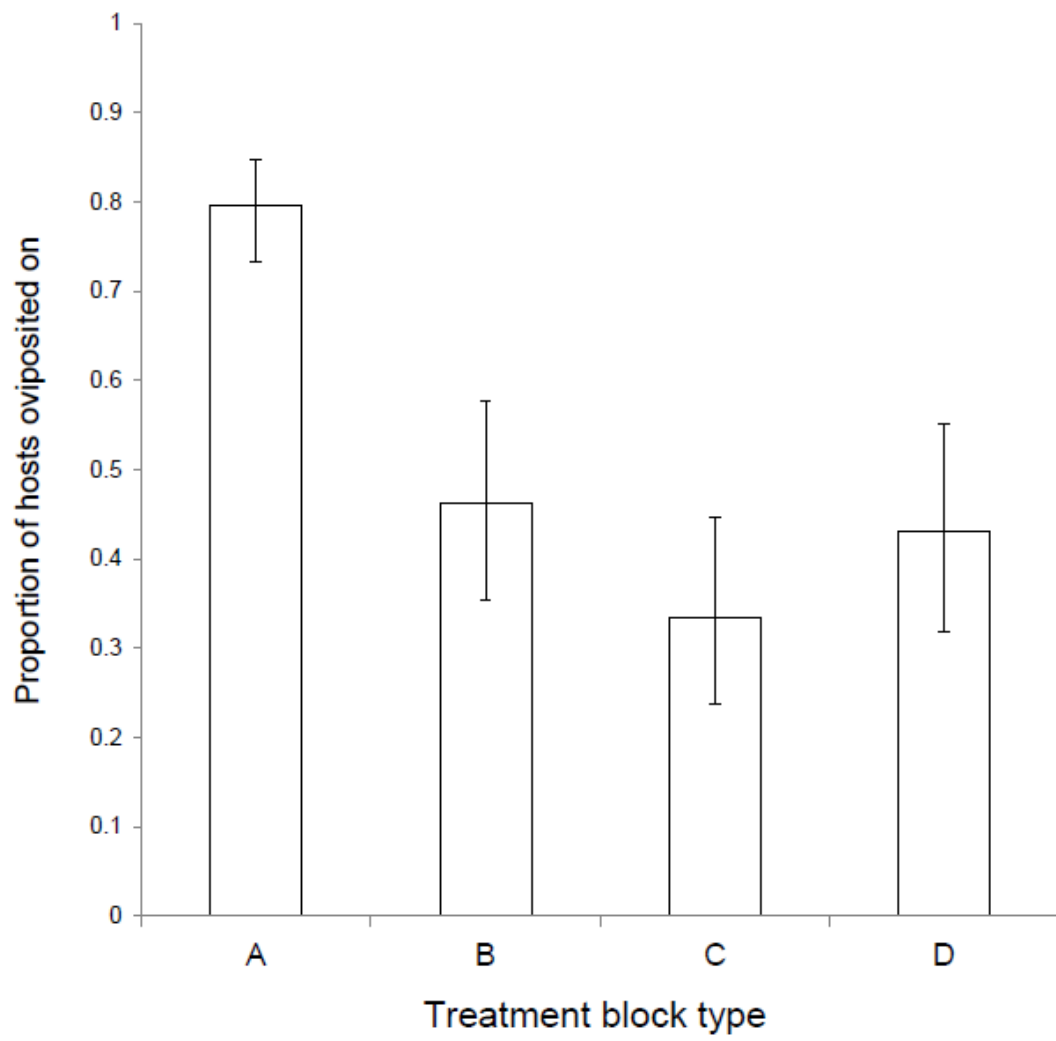


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6 Figure 2.

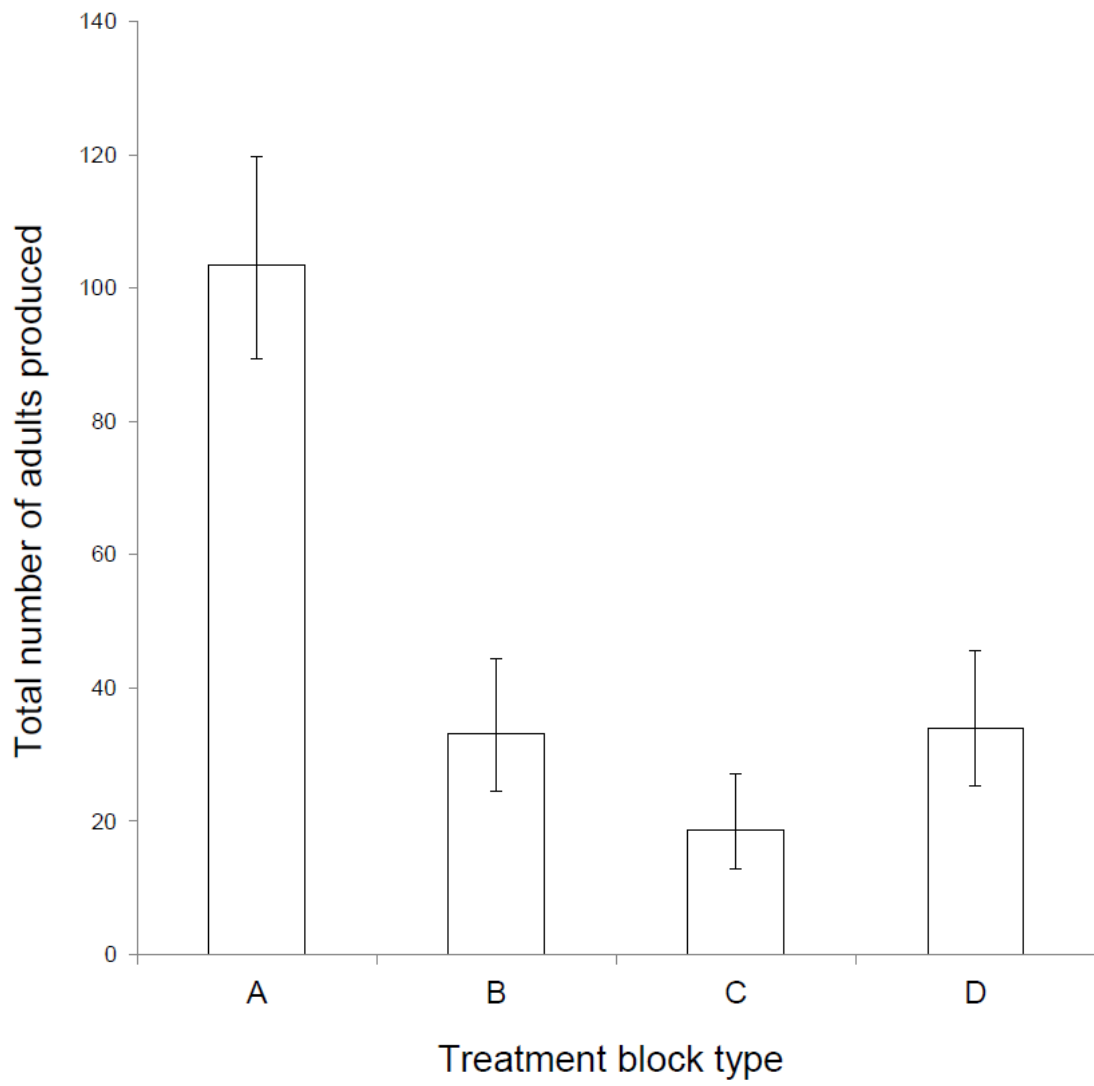


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3 Figure 3.

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