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Performance of *Sclerodermus brevicornis,* a parasitoid of invasive longhorn beetles, when reared on rice moth larvae

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Abstract

Biological control efficiency can be improved by developing effective mass-rearing systems to produce large numbers of high-quality parasitoids. This study explored an alternative host for rearing *Sclerodermus brevicornis* (Kieffer) (Hymenoptera: Bethylidae), a potential biocontrol agent for the suppression of exotic and invasive wood-boring longhorn beetle (Coleoptera: Cerambycidae) populations in the European agroforestry ecosystems. We tested larvae of the rice moth, *Corcyra cephalonica* Stainton (Lepidoptera: Pyralidae), as host for the parasitoid. We quantified the probability and timing of host attack and parasitism as well as reproductive success, offspring production, and the characteristics of adult offspring. As *S. brevicornis* is a quasi-social species (multiple females, communally produced offspring broods), we also explored the effects of varying the number of females to which individual hosts were presented, with the aim of determining the optimal female-to-host ratio. As time to host attack can be a limiting factor in *S. brevicornis* rearing protocols, we tested the use of adult females of another bethylid species, *Goniozus legneri* Gordh, to paralyse *C. cephalonica* larvae prior to presentation. We identified the conditions within our experiment that maximized offspring production per host and offspring production per adult female parasitoid. We found that *C. cephalonica* is suitable as a factitious host and, as it is considerably more straightforward for laboratory rearing than cerambycid species, it is a good candidate for adoption by future *S. brevicornis* mass-rearing and release programmes.

Introduction

Members of the genus *Sclerodermus* (Hymenoptera: Bethylidae) are small (1.5–6 mm) parasitoid wasps (Evans, 1978). There are approximately 81 species worldwide (Gordh & Móczár, 1990; Lanes & Azevedo, 2008). Species of this genus are idiobiont ectoparasitoids (Li & Sun, 2011; Hu et al., 2012), typically of coleopteran larvae. Morphological characteristics of *Sclerodermus* enable them

to find and attack their hosts in enclosed spaces, such as feeding tunnels (Kühne & Becker, 1974; Evans, 1978; Yang et al., 2012a; Baena & Zuzarte, 2013; Jiang et al., 2015). Some species are of considerable economic importance, as they are used in biological control programmes (Yang et al., 2014) whereas others may be medically detrimental (Evans, 1978; Papini, 2014; Yang et al., 2014; Skvarla, 2018; Men et al., 2019).

In China, *Sclerodermus* species have formed an integral part of effective and successful management programmes of forest pest beetles (Coleoptera) (Chen & Cheng, 2000; Yang, 2004; Kaishu, 2006; Tang et al., 2012; Yang et al., 2014; Jiang et al., 2015). Among the pests are the pine sawyer beetle, *Monochamus alternatus* Hope, a vector of the

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pine wood nematode *Bursaphelenchus xylophilus* (Steiner & Buhrer) (Lai et al., 2012; Zhang et al., 2012; Yang et al., 2012b, 2013, 2014), the oak longhorn beetle, *Massicus raddei* Blessig and *Aromia bungii* (Faldermann) (all Cerambycidae) (Wu et al., 2008; Wang et al., 2010; Tang et al., 2012; Yang et al., 2012a; Men et al., 2019), and the emerald ash borer, *Agrilus planipennis* Fairmaire (Buprestidae) (Wu et al., 2008; Yang et al., 2012a). Several Asian species of longhorn beetles have invaded Europe (Jucker & Lupi, 2011) and some of the 17 recorded species of *Sclerodermus* (André, 1881; Kieffer, 1914a, 1914b) have now been reported as associated with these invasive pests (Hérard et al., 2005; Lupi et al., 2014). One of these European natives, *Sclerodermus brevicornis* (Kieffer), was first reported as associated with the longhorn beetle *Oxypleurus nodieri* Mulsant approximately 1 century ago (Kieffer, 1914a), and in 2011 it was reported in association with the exotic Asian longhorn beetle *Psacothea hilaris hilaris* (Pascoe) (both Cerambycidae) (Lupi et al., 2014). It was subsequently shown that *S. brevicornis* can be reared in the laboratory on *P. hilaris hilaris* and two other invasive longhorn beetle species and that it has potential for deployment in biocontrol programmes (Lupi et al., 2017).

Although rearing *S. brevicornis* on longhorn beetles is entirely possible, the process of rearing the hosts is time-consuming and physically demanding (Lupi et al., 2015, 2017). The success of parasitoids used in biological control programmes is often determined by finding suitable alternative hosts used for their mass-rearing (Lemos et al., 2003; Ramalho & Dias, 2003; Pratissoli et al., 2004). Such hosts should ideally have low production costs and no adverse effects on the reproductive and behavioural performance of the parasitoids (Pereira, 2006). Therefore, attempts have been made to find a suitable factitious host for *S. brevicornis*. Thus far, it has been found that female *S. brevicornis* do not successfully parasitize pupae of the mealworm, *Tenebrio molitor* L., or late-instars of the greater wax moth, *Galleria mellonella* L. (D Lupi, unpubl.).

Here, we evaluated the potential use of larvae of the rice moth, *Corcyra cephalonica* Stainton (Lepidoptera: Pyralidae), as host for *S. brevicornis*. Besides being a pest insect, the rice moth is commonly used as a factitious host for rearing approximately 75 species of natural enemies (Chaudhuri & Senapati, 2017), including parasitoids and predators (Hardy & Blackburn, 1991; Jalali & Singh, 1992; Sahayaraj, 2002; Ballal et al., 2003; Jalali et al., 2003; Lenin & Rajan, 2016; Subandi et al., 2017). Apart from few cases (e.g., Amiresmaeili et al., 2018), it is generally easier to produce biocontrol agents on factitious hosts rather than on their natural ones (Kumar & Murthy, 2000). We explored the performance of *S. brevicornis,* when presented with *C. cephalonica* larvae, by quantifying the probability and

timing of host attack and parasitism as well as reproductive success and offspring production characteristics. As *S. brevicornis* is a quasi-social species – multiple females, termed 'foundresses', attack single hosts and produce offspring broods on them communally – we also explored the effects of varying the number of females to which individual hosts are presented, with the aim of determining the optimal female-to-host ratio (Wei et al., 2017). As time to host attack can be a limiting factor in *S. brevicornis* rearing protocols, we tested the use of adult females of another bethylid wasp species, *Goniozus legneri* Gordh, to paralyse *C. cephalonica* larvae prior to their presentation to *Sclerodermus* females. *Goniozus legneri* is a gregarious ectoparasitoid present in several agroecosystems and easy to rear in the laboratory (Legner & Silveira-Guido, 1983; Legner & Gordh, 1992; Steffan et al., 2001; Zaviezo et al., 2007); it is also known to typically attack and paralyse *C. cephalonica* larvae within minutes of presentation (I Hardy, pers. obs.).

Materials and methods

All cultures and experiments were carried out in a climate room at 26.0 ± 0.5 °C, L16:D8 photoperiod, and 70 ± 5% r.h. in a laboratory at the University of Milan, Italy.

Host and parasitoid cultures

A stock culture of *C. cephalonica* was set up in Plexiglas cages (36 × 26 × 25 cm) where the adults could fly and mate. To obtain eggs, females were captured and placed into a small container to allow oviposition. Eggs were then collected and transferred into Petri dishes (15 cm diameter, 2 cm deep) containing a diet used for rearing pyralid moths (following Limonta et al., 2009). Petri dishes were kept closed until used for experiments or until pupation to obtain new adults for the stock culture.

Sclerodermus brevicornis were maintained on the larvae of the longhorn beetle *P. hilaris hilaris*. Two systems for rearing *P. hilaris hilaris* were set up according to the instar (pre-imaginal or adult) following published protocols (Lupi et al., 2015; Favaro et al., 2017). To obtain new offspring of *S. brevicornis*, 6-8 newly emerged females were presented with a single late instar of the long-horned beetle in plastic containers (5 cm diameter, 4 cm deep) on a bed (ca. 1.5 mm thick) of cork oak granules, following Lupi et al. (2017).

A culture of *G. legneri* was reared on *C. cephalonica*. Individual female parasitoids were presented with single host larvae in glass vials of 10 ml with a base of 1.4 mm. The strain of *G. legneri* was obtained from the University

of Nottingham, UK, in 2016, and is referred to as 'U' in Khidr et al. (2013).

Experiments

All experiments were carried out using *C. cephalonica* as a host, selecting larvae weighing between 0.030 and 0.040 g. One of the two procedures was adopted before *C. cephalonica* were presented to *S. brevicornis*: either *S. brevicornis* females were presented with unparalysed hosts or were presented with pre-paralysed larvae by female *G. legneri*, which were removed before the larvae were presented to *S. brevicornis*. Then, in each replicate, a host was placed into a plastic vial (10 cm long, 1.3 cm diameter) and either 1, 2, 3, 4, 5, or 6 adult *S. brevicornis* females (foundresses) were added into each vial. The vials were closed with a gauze and cotton wool. To give adequate overall statistical power (Smith et al., 2011), the trial contained the same number of replicates per female at time zero (n = 100) but when, prior to the paralysis, some parasitoids died inside the vial, the foundress number for the replicate was reclassified. The result was that we set up 92, 224, 111, 54, 50, and 54 replicates for foundress numbers of 1, 2, 3, 4, 5, and 6, respectively, giving a total of 585 replicates. We ensured that a balanced range of pre-paralysed or unparalysed hosts was represented within the replicates of the first four foundress groups. Five- and 6-foundress groups were not presented with pre-paralysed hosts.

Vials were observed daily to record host paralysis (occurrence and timing), egg laying (occurrence and time of first oviposition to begin a clutch), pupation (timing of first within a brood), emergence of adult offspring (timing, number, and sex), and whether offspring were alate or apterous (*S. brevicornis* is wing dimorphic in both sexes according to Lupi et al., 2017). Observations were made while attempting not to disturb the wasps and in consequence some offspring may have been overlooked at earlier developmental stages. If a host was oviposited on, it was considered to have been parasitized successfully. Parasitism rate was defined as the proportion of replicates in which oviposition occurred out of the total number of replicates in which hosts were paralysed. The production of at least one adult offspring in a replicate was defined as reproductive success.

Statistical analysis

Data were analysed with Generalized Linear Models (GLM; Aitkin et al., 1989; Crawley, 1993; Faraway, 2006) and statistical software package GenStat (v.17; VSN International, Hemel Hempstead, UK).

The explanatory variables 'foundress number' and the 'use of *G. legneri*' (as a pre-host-paralysing agent) were fitted as factors. For some analyses, foundress number was initially treated as a factor with six levels but, because there was only variation in *G. legneri* use among 1- to 4-foundress replicates, analyses including consideration of pre-paralysis excluded 5- and 6-foundress replicates and thus fitted foundress number as a factor with four levels. In some analyses, we moreover explored results by fitting foundress number as a continuous variable.

The response variables were the parameters recorded during the daily observations and at the end of the experiment. Logistic analyses (GLM) were used to explore binary data on host paralysis, oviposition, and foundresses' reproductive success; these analyses assumed binomially distributed errors (the reported test statistic is change in deviance, G, which is approximately χ^2 distributed; Crawley, 1993). Other logistic analyses of proportional data with denominators >1 (e.g., proportion of alate males or females, offspring sex ratio, etc.) assumed quasi-binomial error distributions (the reported test statistic is the F-ratio; Crawley, 1993; Wilson & Hardy, 2002). All logistic analyses adopted a logit link function. Log-linear analyses were used to analyse count data (e.g., number of offspring produced) assuming quasi-Poisson error distributions and a log-link function (Crawley, 1993). Data derived from integers (e.g., per-foundress brood size) that were likely to be approximately Poisson-distributed were also analysed using log-linear models (Faraway, 2006; Tang et al., 2014). Following logistic and log-linear analyses, we give the percentage deviance explained (%Dev) as a descriptor analogous to r^2.

Parametric cohort survival analyses with censoring (Aitkin et al., 1989; Crawley, 1993) were used to analyse the influences on times-to-events, such as time to host paralysis, oviposition, larval hatching, spinning cocoons, and adult offspring emergence.

Statistical significance was assessed by model simplification (Crawley, 1993; Wilson & Hardy, 2002), whereby interactions, variates, and factors were progressively removed from initially complex models, and multiple levels of factors were simplified by aggregation, to obtain parsimonious models.

Results

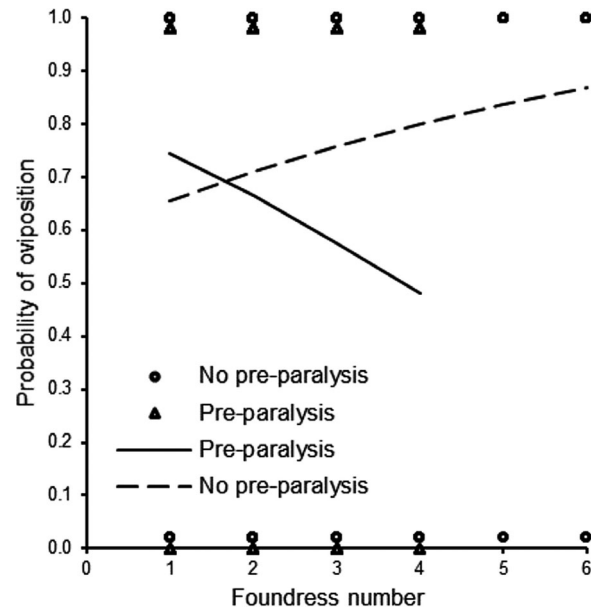### Probability of host utilization and developmental success

Probability of paralysis. The mean probability of a host (which had not been pre-paralysed by *G. legneri*) being paralysed by *S. brevicornis* was 0.754 (SE = +0.021, −0.022; n = 411). The probability was greater when there were multiple foundresses (logistic ANOVA: G_5 = 3.11, P = 0.008, %Dev = 3.39; Figure 1). The model was simplified by aggregating foundress number categories to find the minimal adequate model (Crawley, 1993): this
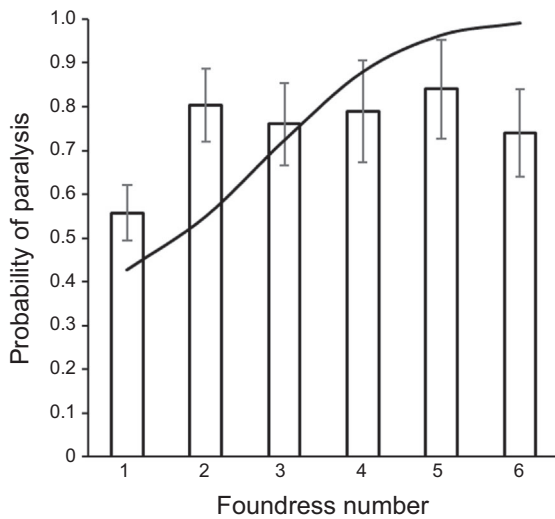
Figure 1 Effect of foundress number on the probability of paralysis. Mean ($\pm$ SE) probability of a host – which had not been pre-paralysed by *Goniozus legneri* – being paralysed by *Sclerodermus brevicornis* was greater when there were multiple foundresses. The line shows the fitted quadratic logistic regression: probability of paralysis = $1/(1+\{1/\exp [(0.081 \times \text{foundress number}) + (0.137 \times \text{foundress number}^2) - 0.515]\})$.


Figure 2 Effect of foundress number on the probability of oviposition. Oviposition probability for previously unparalysed hosts increased with an increase in foundress number, but declined when hosts were pre-paralysed by *Goniozus legneri*. Logistic regression lines fitted separately across the range of foundress numbers evaluated.

showed that there was no significant difference between the multi-foundress replicates ($G_4 = 0.51$, P = 0.73, %Dev = 0.57; n = 350) and that the probability of paralysis was higher among these than among single-foundress replicates ($G_1 = 13.51$, P<0.001, %Dev = 2.95). Similarly, if foundress number was fitted as a continuous variable, the probability of paralysis increased in a curvilinear relationship with increasing foundress number (logistic regression: $G_1 = 3.15$, P = 0.076, %Dev = 0.69; quadratic term: $G_1 = 7.48$, P = 0.006, %Dev = 1.63; Figure 1).

Probability of oviposition. The mean overall probability of a presented host being oviposited on by *S. brevicornis* was 0.610 ($\pm$ 0.020 SE; n = 585). The interaction between foundress number and pre-paralysis treatment was significant: for unparalysed hosts, oviposition probability increased with foundress number but for pre-paralysed hosts, it declined (logistic ANCOVA: $G_3 = 6.50$, P<0.001, %Dev = 2.98; n = 481). For hosts that were paralysed (whether by *G. legneri* or by *S. brevicornis*), the mean probability of subsequent oviposition by *S. brevicornis* was 0.7454 (SE = +0.019, −0.020). Again, the interaction between foundress number and pre-paralysis treatment was significant (logistic ANCOVA: $G_3 = 6.75$, P<0.001, %Dev = 4.21; Figure 2).

Probabilities of development towards adulthood. Among the 357 replicates in which eggs were laid, the mean probability of at least one egg hatching was 0.7956 (SE = +0.020, −0.022). This probability was affected by foundress number (logistic analysis: $G_5 = 2.49$, P = 0.029, %Dev = 3.45). The model was simplified by aggregating foundress number categories to find the minimal adequate model, showing that there was no significant difference among groups of 1, 2, 3, 4, and 6 foundresses ($G_4 = 0.83$, P = 0.51, %Dev = 1.11) and the probability was lower when eggs were laid by 5-foundress groups ($G_1 = 9.16$, P = 0.002, %Dev = 2.53). Egg hatching probability was not influenced by the use of *G. legneri* ($G_1 = 2.09$, P = 0.15, %Dev = 0.79; pre-paralysis*foundress number interaction: $G_3 = 0.04$, P = 0.99, %Dev = 0.05).

In replicates with hatched larvae, the mean probability of some developing to the pupal stage was 0.8416 (SE = +0.021, −0.023; n = 284). Larvae produced by 4-foundress groups pupated significantly more successfully than those for 2- or 3-foundress groups, whereas pupation success of offspring of other foundress group sizes was intermediate (logistic analysis: $G_5 = 2.79$, P = 0.016, %Dev = 5.62). Pupation probability was unaffected by the use of *G. legneri* (G_1<0.01, P = 0.95, %Dev = 0.002; pre-paralysis*foundress number interaction: $G_3 = 0.02$, P = 0.99, %Dev = 0.02).
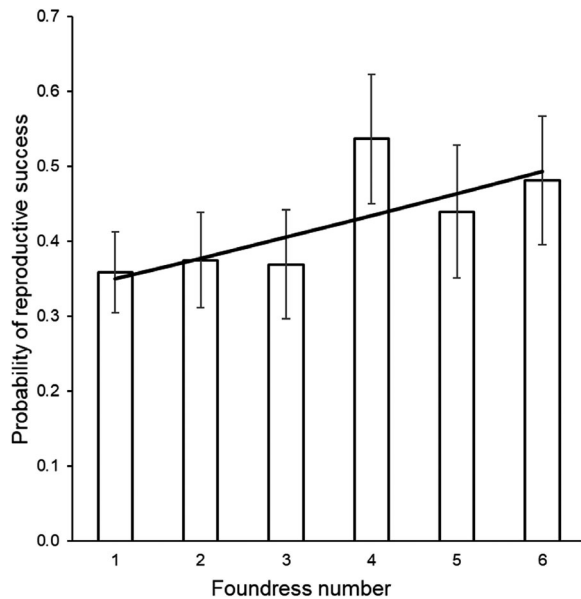
Figure 3 Overall probability of reproductive success. Mean (±
SE) probability of success did not differ significantly between
groups (foundress number treated as a factor) but increased
significantly when foundress number was fitted as a variate;
logistic regression line, probability = $1/(1+\{1/\exp$
$[(0.119 \times$ foundress number$) - 0.739]\})$.

Among replicates in which some offspring reached the
pupal stage (n = 239), the mean probability of at least one
adult offspring emerging was 0.983 (SE = +0.007, −0.011);
this was unaffected by foundress number ($G_5 = 1.11$,
P = 0.36, %Dev = 13.60), the use of *G. legneri* ($G_1 = 0.48$,
P = 0.49, %Dev = 1.24), or their interaction ($G_3 = 1.54$,
P = 0.20, %Dev = 11.88).

Overall probability of reproductive success. The mean
overall probability of the successful production of at least
one adult offspring from a presented host was 0.402 (±
0.020 SE). Success did not differ across all foundress
number treatments (logistic ANOVA: $G_5 = 1.52$,
P = 0.18, %Dev = 0.96; n = 585) but there was a positive
relationship when foundress number was fitted as a variate
(logistic regression: $G_1 = 4.52$, P = 0.033, %Dev = 0.57;
Figure 3). Among foundress number treatments where
pre-paralysis was also varied, there was a significant
interaction (pre-paralysis: $G_1 = 0.03$, P = 0.86, %Dev =
0.005; interaction: $G_3 = 2.72$, P = 0.043, %Dev = 1.23;
n = 481) such that reproductive success increased with
increase of foundress number when *G. legneri* was not
utilized but decreased when hosts had been pre-paralysed:
this interaction was due to the use of *G. legneri* affecting
the probability of oviposition (Figure 2). Considering

only the hosts that were oviposited on, adult offspring
were produced in two-thirds of the cases (mean = 0.658,
SE = +0.025, −0.026); the probability of post-oviposition
success was affected by foundress number ($G_5 = 2.23$,
P = 0.048, %Dev = 2.43) but there was no overall trend
as foundress number increased (logistic regression:
$G_1 = 0.16$, P = 0.69, %Dev = 0.04) and the probability
was unaffected by the use of *G. legneri* (main effect:
$G_1 = 0.75$, P = 0.39, %Dev = 0.21; n = 280; interaction:
$G_3 = 0.22$, P = 0.88, %Dev = 0.18).

Timing of host use and offspring development
Descriptive statistics for timings under all experimental
treatment combinations are given in Table 1.

Time to paralysis. Among the 411 replicates in which
unparalysed hosts were presented to *S. brevicornis* females,
the time to paralyse hosts ranged between 1 and 13 days
(mean ± SE = 3.95 ± 0.12) but not all hosts became
paralysed (see above). The time taken to paralysis was
affected by foundress number (survival analysis with hosts
unparalysed by day 13 treated as censors: $G_5 = 28$,
P<0.001, %Dev = 4.48). Aggregation of factor levels
indicated that timing was not different among multi-
foundress treatments ($G_4 = 3$, P = 0.56, %Dev = 0.47)
but was slower in single-foundress replicates ($G_1 = 25$,
P<0.001, %Dev = 4.15; Figure 4).

*Time to oviposition.* The time from host presentation to
first observation of eggs ranged from 3 to 18 days
(mean ± SE = 7.37 ± 0.14) and varied according to the
number of foundresses present ($G_5 = 35$, P<0.001,
%Dev = 3.77; n = 582); larger foundress groups typically
started ovipositing earlier. There was a significant interaction
between foundress number and the use of *G. legneri*
($G_3 = 22$, P<0.001, %Dev = 3.01; n = 478); groups of three
foundresses took longer to lay eggs on hosts that were pre-
paralysed compared to other treatment combinations.

Once a host was paralysed, the time until oviposition
ranged from 1 to 17 days (mean ± SE = 4.22 ± 0.15)
and varied according to foundress number ($G_5 = 88$,
P<0.001, %Dev = 11.59; n = 476), with eggs typically laid
earlier by larger foundress groups (Figure 5). Times were
shorter when *G. legneri* foundresses were not utilized for
pre-host-paralysis ($G_1 = 18$, P<0.001, %Dev = 2.83;
n = 394) and, as above, there was a significant interaction
between foundress number and the use of *G. legneri*
($G_3 = 21$, P<0.001, %Dev = 3.3).

Time to egg hatching. The time from when at least one
egg was first observed on a host to the first observation
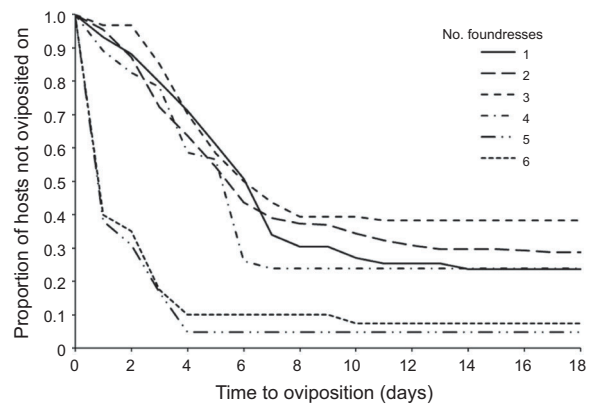of larvae on the host ranged from 1 to 12 days

Table 1 Mean (± SE) number of days taken for host use and offspring development

No. foundresses	*Goniozus legneri* used to pre-paralyse the host?	Host presentation to paralysis	Host presentation to oviposition	Paralysis to oviposition	Oviposition to hatching	Hatching to pupation	Pupation to emergence	Total egg-to-adult development
1	Yes	1.70 ± 0.21	9.05 ± 0.58	7.25 ± 0.54	6.35 ± 0.80	7.07 ± 0.81	15.08 ± 0.84	26.92 ± 1.05
	No	5.50 ± 0.50	9.50 ± 0.51	4.27 ± 0.47	5.04 ± 0.59	5.10 ± 0.48	17.00 ± 0.58	25.90 ± 0.80
2	Yes	1.08 ± 0.12	8.60 ± 0.33	7.36 ± 0.31	4.54 ± 0.31	6.06 ± 0.42	13.69 ± 0.46	24.28 ± 0.59
	No	3.76 ± 0.24	7.24 ± 0.29	3.42 ± 0.27	4.67 ± 0.28	6.08 ± 0.37	14.00 ± 0.57	24.50 ± 0.75
3	Yes	0.66 ± 0.14	8.60 ± 0.72	6.00 ± 0.67	4.36 ± 0.53	6.00 ± 1.24	16.22 ± 1.01	26.11 ± 0.79
	No	2.98 ± 0.23	7.36 ± 0.39	4.44 ± 0.36	3.63 ± 0.27	5.09 ± 0.44	17.34 ± 0.51	26.16 ± 0.50
4	Yes	2.01 ± 0.27	7.00 ± 0.72	5.00 ± 0.67	4.60 ± 0.54	8.00 ± 0.72	15.70 ± 0.63	28.30 ± 0.30
	No	4.10 ± 0.40	7.36 ± 0.55	3.68 ± 0.52	4.90 ± 0.48	4.84 ± 0.56	17.00 ± 0.71	26.95 ± 0.71
5	No	4.14 ± 0.15	5.78 ± 0.41	1.68 ± 0.38	4.83 ± 0.28	6.09 ± 0.46	17.00 ± 0.81	27.59 ± 0.84
6	No	4.18 ± 0.12	5.89 ± 0.43	1.87 ± 0.40	4.16 ± 0.29	6.31 ± 0.28	16.00 ± 0.54	26.42 ± 0.45
Overall mean		3.01 ± 0.24	7.64 ± 0.49	4.50 ± 0.46	4.71 ± 0.44	6.06 ± 0.58	15.90 ± 0.66	26.31 ± 0.68
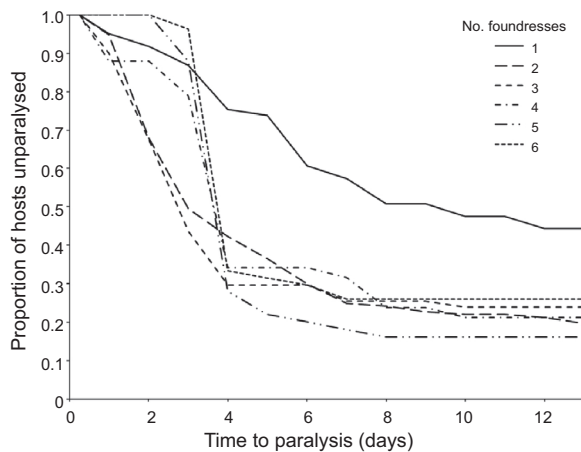
Figure 4 Effect of foundress number (1–6) on time taken to paralyse hosts. Single foundresses took significantly longer to paralyse hosts than did multiple foundresses. Among multiple foundress replicates, the number of foundresses did not affect timing significantly.

Figure 5 Influence of time to egg laying on paralysed hosts. Smaller foundress groups (1–4) took significantly longer to oviposit on hosts than did groups of five or six foundresses.

(mean ± SE = 4.43 ± 0.12). This timing varied across foundress number treatments ($G_5 = 23$, P<0.001, %Dev = 4.33; n = 354); eggs produced by groups of five foundresses typically took the longest time to hatch. Eggs hatched earlier when hosts had not been pre-paralysed by *G. legneri* ($G_1 = 4$, P = 0.046, %Dev = 0.81; n = 277) but there was no interaction with foundress number ($G_3 = 0.00$, P = 1.0, %Dev = 0.17).

Time to pupation. Pupae were first observed between 4 and 17 days (mean ± SE = 5.91 ± 0.16) after the first observation of larvae on a host. Offspring of smaller

foundress groups pupated earlier ($G_5 = 14$, P = 0.016, %Dev = 3.60; n = 282). Time to pupation was unaffected by the use of *G. legneri* ($G_1 = 2$, P = 0.16, %Dev = 0.57; interaction with foundress number: $G_3 = 2$, P = 0.57, %Dev = 0.72; n = 227).

Time to adult emergence. Adult offspring of *S. brevicornis* emerged from pupae between 6 and 26 days (mean ± SE = 15.56 ± 0.223) after the first observation of pupae in the brood. This period did not differ across foundress number treatments ($G_5 = 7$, P = 0.22, %Dev = 3.99; n = 237). Pupae produced from hosts that were pre-paralysed by *G. legneri* developed to adult offspring within a shorter time ($G_1 = 13$, P<0.001, %Dev = 6.15; n = 189). There was no interaction effect between

Table 2 Mean (± SE) offspring production according to the combinations of conditions trialled

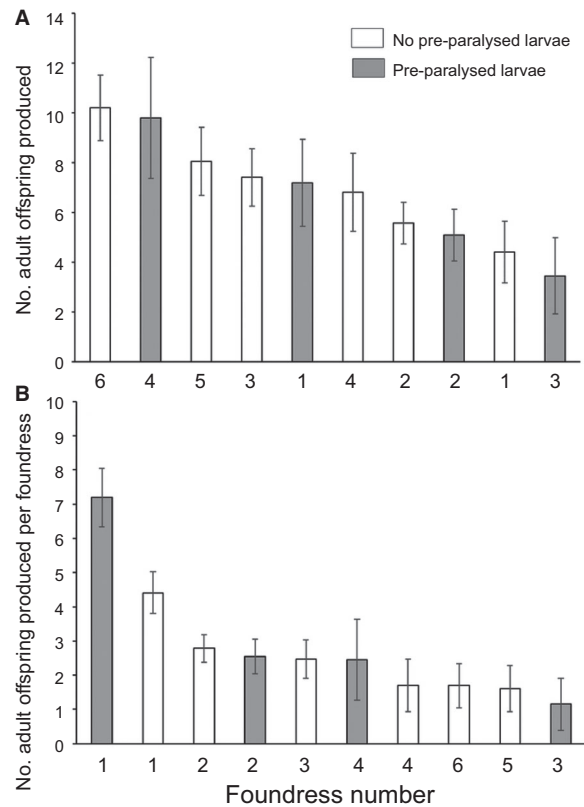| No. foundresses	Pre-paralysis treatment	Total adult offspring	Females		Males		Per-foundress adult offspring
			Apterous	Alate	Apterous	Alate	
1	Yes	7.194 ± 1.744	5.839 ± 1.601	0.129 ± 0.084	0.032 ± 0.028	1.194 ± 0.282	7.194 ± 0.854
	No	4.409 ± 1.243	3.393 ± 1.141	0.001 ± 0.060	0.067 ± 0.020	0.590 ± 0.201	4.409 ± 0.609
2	Yes	5.092 ± 1.041	4.529 ± 0.956	0.001 ± 0.050	0.001 ± 0.017	0.563 ± 0.168	2.546 ± 0.510
	No	5.569 ± 0.830	5.102 ± 0.762	0.015 ± 0.040	0.001 ± 0.013	0.453 ± 0.134	2.785 ± 0.406
3	Yes	3.450 ± 1.535	3.025 ± 1.409	0.025 ± 0.074	0.025 ± 0.024	0.375 ± 0.248	1.150 ± 0.752
	No	7.423 ± 1.152	6.634 ± 1.058	0.169 ± 0.055	0.014 ± 0.018	0.606 ± 0.186	2.474 ± 0.564
4	Yes	9.812 ± 2.428	8.000 ± 2.228	0.001 ± 0.116	0.001 ± 0.039	1.813 ± 0.392	2.453 ± 1.188
	No	6.816 ± 1.575	6.026 ± 1.446	0.158 ± 0.076	0.026 ± 0.025	0.605 ± 0.255	1.704 ± 0.771
5	No	8.060 ± 1.373	7.500 ± 1.261	0.020 ± 0.066	0.001 ± 0.022	0.540 ± 0.222	1.612 ± 0.672
6	No	10.204 ± 1.321	9.593 ± 1.213	0.001 ± 0.063	0.001 ± 0.021	0.611 ± 0.214	1.701 ± 0.647

foundress number and the use of *G. legneri* ($G_3 = 7$, $P = 0.57$, %Dev = 1.27).

Time from egg-to-adult development. Overall egg-to-adult development ranged between 17 and 40 days (mean ± SE = 25.85 ± 0.25). Developmental time was shorter when eggs were produced by 4-foundress groups ($G_5 = 12$, $P = 0.035$, %Dev = 2.22; $n = 354$). This time was not affected by the use of *G. legneri* as a pre-host-paralysing agent ($G_1 = 1$, $P = 0.32$, %Dev = 0.18; interaction with foundress number: $G_3 = 1$, $P = 0.80$, %Dev = 0.16; $n = 277$).

**Number and characteristics of offspring produced**

Number of offspring. A mean of 6.34 (SE = +0.40, −0.38) offspring were produced per host across all 585 replicates. Offspring production did not differ across all foundress number treatments (log-linear ANOVA: $F_{5,579} = 1.95$, $P = 0.084$, %Dev = 1.66; $n = 585$). However, among the treatments where pre-paralysis was also varied, there was an interaction between pre-paralysis and foundress number (pre-paralysis: $F_{1,474} = 3.84$, $P = 0.051$, %Dev = 0.77; interaction: $F_{3,476} = 3.58$, $P = 0.014$, %Dev = 2.16; $n = 481$). Overall, a mean of 7.56 (SE = +0.90, −0.80; $n = 174$) offspring were produced when hosts were pre-paralysed and 5.84 (SE = +0.52, −0.48; $n = 307$) were produced when hosts were not pre-paralysed. The overall mean (± SE) offspring production per host was at a maximum of 10.20 ± 1.32 when there were six foundresses and the host was not pre-paralysed (Table 2, Figure 6A).

When replicates with no offspring production were excluded, a mean of 15.79 (SE = +0.63, −0.61) offspring were produced per host. The number of offspring produced was affected by foundress number (log-linear ANOVA: $F_{5,229} = 3.31$, $P = 0.007$, %Dev = 6.74; $n = 235$) in a curvilinear relationship (quadratic log-linear

Figure 6 Offspring production according to the combinations of conditions trialled. Mean (± SE) number of adult offspring produced (A) per host in total, and (B) per foundress. White bars indicate treatments without pre-paralysis and grey bars indicate pre-paralysis treatments.

regression: $F_{1,234} = 4.07$, $P = 0.045$, %Dev = 1.72; Figure 7). Among this restricted set of replicates, production was higher when hosts were pre-paralysed (pre-paralysis: $F_{1,184} = 10.32$, $P = 0.002$, %Dev = 5.17; $n = 187$). There
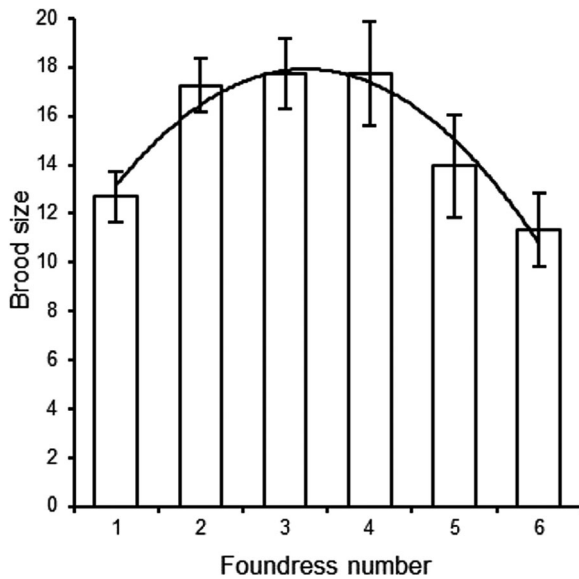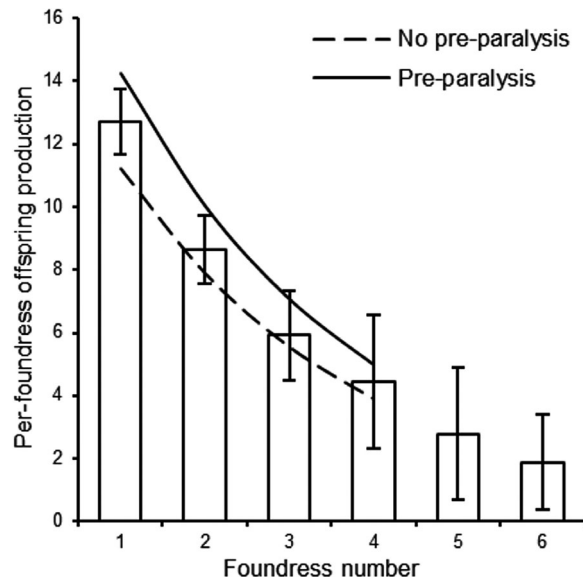
Figure 7 Number of adult offspring produced. Mean ($\pm$ SE) brood size was larger in intermediate sized foundress groups than in smaller or larger groups. Quadratic log-linear regression: brood size $= (-0.942 \times$ foundress number$^2) + (6.1177 \times$ foundress number$) + 7.991$.


Figure 8 Effect of foundress number and pre-paralysis on per-foundress offspring production. Mean ($\pm$ SE) number of offspring produced per foundress per host (across replicates in which there was some reproductive success) was typically lower when foundress number was greater. Regression curves indicate trends in per-foundress offspring production in pre-paralysed and paralysed hosts.

was no interaction between foundress number and the use of *G. legneri* ($F_{3,182} = 0.80$, P = 0.49, %Dev = 1.20).

The mean number of offspring produced per foundress per host across all 585 replicates (i.e., including those with complete reproductive failure) was 2.78 (SE = +0.18, −0.17) and was typically lower when foundress number was greater (log-linear analysis: $F_{5,579} = 8.67$, P<0.001, %Dev = 6.97), or when hosts were not pre-paralysed ($F_{1,477} = 4.97$, P = 0.026, %Dev = 0.99; n = 481). There was no interaction effect between foundress number and pre-paralysis treatment ($F_{3,476} = 2.49$, P = 0.060, %Dev = 1.49). The overall mean ($\pm$ SE) offspring production per foundress per host was at a maximum of 7.194 $\pm$ 0.854 when there was a single foundress and the host was pre-paralysed (Table 2, Figure 6B).

Similar patterns were observed among the replicates in which there was some reproductive success: per-foundress offspring produced per host (mean = 6.91, SE = +0.34, −0.33) declined with foundress number ($F_{5,229} = 37.29$, P<0.001, %Dev = 44.88; n = 235), or when hosts were not pre-paralysed ($F_{1,183} = 8.99$, P = 0.003, %Dev = 3.45; n = 187; Figure 8). There was no interaction between foundress number and *G. legneri* use as pre-paralysis treatment ($F_{3,182} = 0.97$, P = 0.41, %Dev = 1.12).

Sexual composition of broods. Three of the 235 broods that produced adult offspring contained male offspring

only: a 1-foundress brood of 13 males, a 2-foundress brood of 15 males, and a 3-foundress brood of just one male. As all-male broods typically suggest maternal virginity, and thus a lack of sex allocation control, these replicates were excluded from further sex ratio analysis, following Hardy & Cook (1995) and Kapranas et al. (2016a). Two broods with exceptionally high sex ratios were also excluded: a single-foundress replicate of 0.89 (a sex ratio 3$\times$ higher than any other mixed sex brood, suggesting lack of sex ratio control) and a 5-foundress replicate of 0.90 (several foundresses may have been virgins). Data remained from 31 1-foundress broods, 83 2-foundress broods, 40 3-foundress broods, 29 4-foundress broods, 21 5-foundress broods, and 26 6-foundress broods.

Brood sex ratios (= proportion of offspring that were male, m/[m + f]) were very strongly female biased (mean = 0.0838, SE = +0.0051, −0.0049). Sex ratios were not influenced by pre-paralysis treatment ($F_{1,175} = 0.98$, P = 0.32, %Dev = 0.40; n = 182) or its interaction with foundress number ($F_{3,177} = 0.56$, P = 0.64, %Dev = 0.69). Sex ratios were typically more female biased in broods produced by larger numbers of foundresses (logistic analysis: $F_{5,226} = 12.44$, P<0.001, %Dev = 21.58; Figure 9). Sex ratios were also affected by brood size and
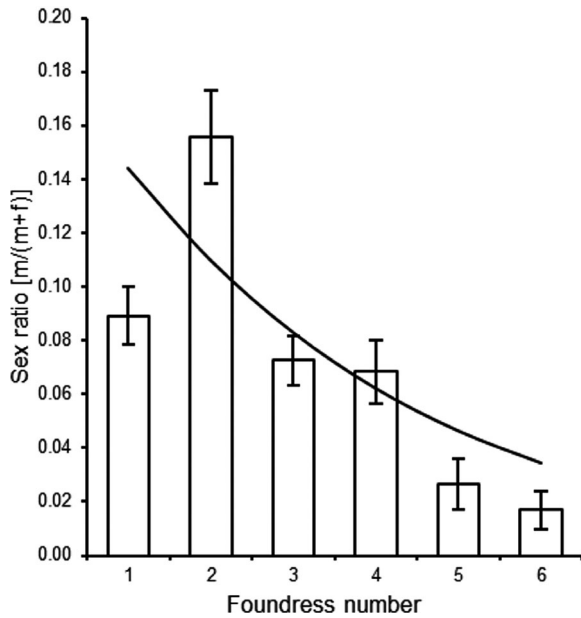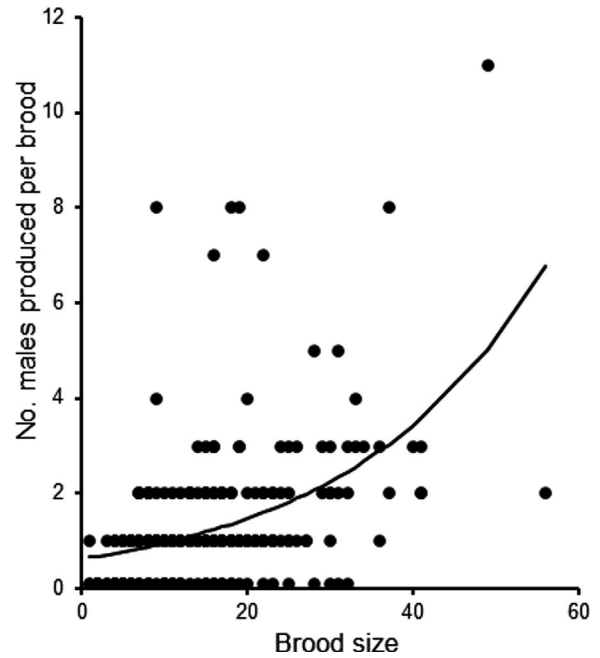
Figure 9 Mean (± SE) sex ratios (proportion sons) in relation to foundress number. Broods produced by larger foundress groups were typically more female biased. Logistic regression: sex ratio = $1/(1+\{1/\exp[(-0.3106 \times \text{foundress number}) - 1.471]\})$.
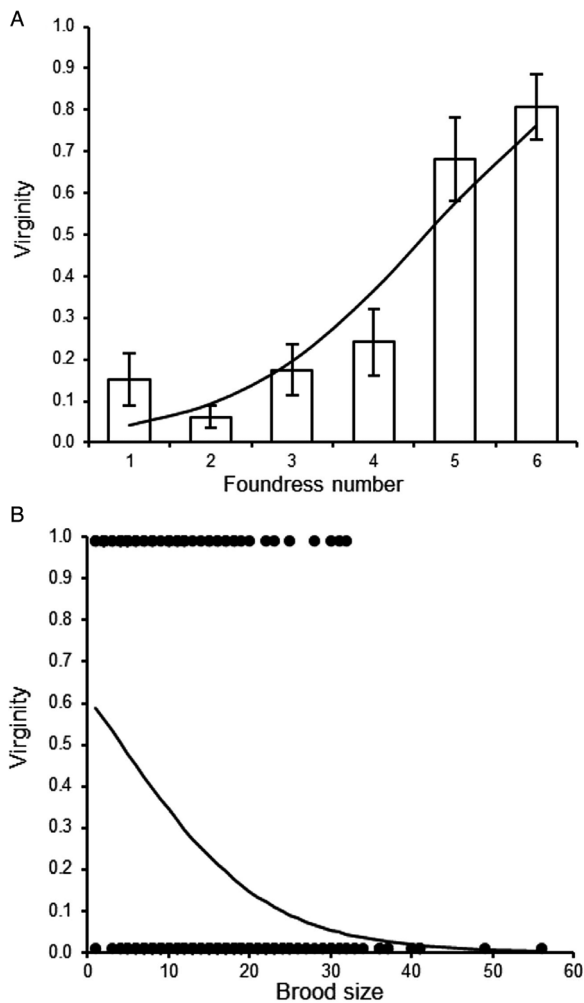

Figure 10 Effect of brood size on per-brood male production. The number of males produced per brood increased with an increase of brood size. The line representing the trend of number of males per brood in relation to brood size was fitted with logistic regression.

by its interaction with foundress number (brood size: $F_{1,221} = 7.77$, $P = 0.006$, %Dev = 2.42; interaction: $F_{5,225} = 4.92$, P<0.001, %Dev = 7.64), such that sex ratios declined with an increase of brood size among broods produced by 1, 2, 3, or 5 females and increased among broods produced by 4 or 6 foundresses.

The number of males produced per brood decreased significantly as foundress number increased (log-linear analysis: $F_{5,223} = 9.89$, P<0.001, %Dev = 15.16) and increased with an increase of brood size ($F_{1,219} = 49.78$, P<0.001, %Dev = 15.26; Figure 10) as there was an interaction between foundress number and brood size ($F_{5,223} = 3.85$, P = 0.002, %Dev = 5.90). The mean per-foundress number of males in a brood produced decreased as foundress number increased ($F_{5,223} = 18.54$, P<0.001, %Dev = 18.43) and increased with brood size ($F_{1,219} = 4.88$, P<0.001, %Dev = 8.52), with generally greater increases when foundress numbers were greater (foundress number*brood size interaction: $F_{5,223} = 3.32$, P = 0.007, %Dev = 3.30).

The proportion of broods that were all female ('virgin broods', according to Kapranas et al., 2016b) generally increased with increasing foundress number (logistic analysis: $G_5 = 2.67$, P = 0.020, %Dev = 5.06; Figure 11A) and also decreased with an increase of brood size ($G_1 = 22.21$, P<0.001, %Dev = 8.41; Figure 11B); there

was a significant interaction between foundress numbers and brood size ($G_5 = 3.21$, P = 0.007, %Dev = 6.07). Virginity was not affected by the use of *G. legneri* to pre-paralyse the host ($G_1 = 0.04$, P = 0.85, %Dev = 0.02; n = 182), nor by its interaction with foundress number ($G_3 = 2.22$, P = 0.083, %Dev = 2.91).

Wing dimorphism. Wing dimorphism occurred in both female and male offspring (Table 3). Of the 230 broods containing at least one adult female, there were no instances of exclusively alate (winged) females. In 216 broods, all females were apterous (wingless) whereas 14 broods contained a mixture of apterous (81%) and alate (19%) females. Adult males were produced in 170 broods: 164 contained exclusively alate males and six contained a mix of apterous (25.8%) and alate (74.2%) males. Male aptery only occurred when all or most females in a brood were also apterous.
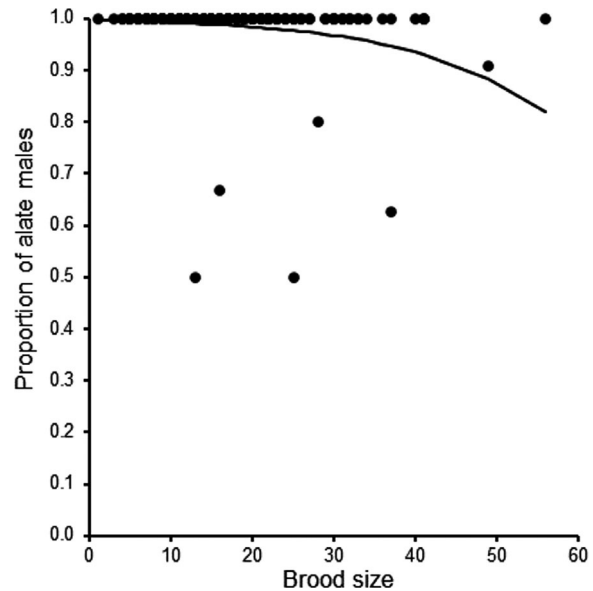
Table 3 Wing dimorphism among adult offspring

Sex	Total offspring	Alate	Apterous
Male	306	298 (97.4%)	8 (2.6%)
Female	3346	46 (1.4%)	3300 (98.6%)

Figure 12 Relationship between the proportion of winged males and brood size. Line fitted with logistic regression.

Figure 11 Prevalence of virgin broods. (A) Mean (± SE) proportion of broods that were all female increased with an increase in foundress number. (B) Virginity decreased with an increase of brood size. Lines representing the trend of virginity considering the two factors (foundress number and brood size) were fitted with logistic regression.

The proportion of females that were alate differed among foundress number treatments ($F_{5,230} = 2.41$, $P = 0.038$, %Dev = 4.73; n = 232), such that adult female offspring produced by single-foundress groups contained significantly more alate members. This proportion declined as total brood size increased ($F_{1,226} = 114.78$, $P<0.001$, %Dev = 5.81) and also as the number of females in a brood increased ($F_{1,231} = 10.86$, $P = 0.001$, %Dev = 4.34). There was no interaction between foundress number and either brood ($F_{5,225} = 2.06$, $P = 0.072$, %Dev = 4.05) or female offspring number ($F_{5,225} = 1.87$, $P = 0.10$, %Dev = 3.73).

The proportion of males that were alate was generally high but differed according to foundress number, being lowest when broods were produced by three foundresses ($F_{5,170} = 7.79$, $P<0.001$, %Dev = 16.60; n = 172). This proportion also declined with an increase in brood size ($F_{1,161} = 17.47$, $P<0.001$, %Dev = 7.44; Figure 12) but there was no interaction between foundress number and brood size ($F_{5,165} = 1.19$, $P = 0.32$, %Dev = 2.53). Alate males were also less common when there were more males in a brood ($F_{1,166} = 14.71$, $P<0.001$, %Dev = 5.37) and there was an interaction between foundress number and the number of males in a given brood ($F_{5,165} = 7.90$, $P<0.001$, %Dev = 14.41).

Discussion

We have shown that larvae of the rice moth, *C. cephalonica*, can be utilized as factitious hosts for *S. brevicornis*. This is in contrast to a previous attempt to rear *S. brevicornis* on larvae of *G. mellonella* (D Lupi, unpubl.) and it is noteworthy that *Sclerodermus* species normally develop on coleopterans (Gordh & Móczár, 1990). Approximately 75% of hosts were attacked and paralysed (similar to estimates obtained from *S. brevicornis* attack on equally sized longhorn beetle larvae; Abdi et al., 2020), and around 60% of all hosts presented had eggs laid onto them (compared to 73% when provided with beetle larvae; Abdi et al., 2020). Oviposition was disrupted by the pre-paralysis of hosts using *G. legneri* (possibly due to changes in host odour). However, the probabilities of paralysis and

oviposition increased with foundress number, as did the probability of a host yielding at least one parasitoid offspring as found by Tang et al. (2014). Our finding that approximately half of the hosts presented yielded offspring is consistent with data from *S. brevicornis* reared on larvae of *P. hilaris hilaris* (Lupi et al., 2017) and similar to estimates from other *Sclerodermus* species (Tang et al., 2014).

Timing of offspring production

Lupi et al. (2017) reported that the developmental time of *S. brevicornis* on beetle larvae is approximately 1 month. Our data indicate that overall development times on *C. cephalonica* are similar. The time required to successfully parasitize hosts decreased with an increase in foundress numbers, as also found by Tang et al. (2014). We also found that *S. brevicornis* foundresses took more time to oviposit on hosts (and for their eggs to eclose) when hosts were pre-paralysed by *G. legneri* females; we suggest that this may be related to altered odours when hosts are paralysed. In terms of the timing of parasitoid production, there appeared to be no major negative effects associated with using *C. cephalonica* as a factitious host.

Numerical offspring production

Across all hosts provided, the numerical production of adult offspring (ca. 6.5 per host) was not affected by the number of foundresses present but, if hosts yielding no offspring were excluded, production was greatest at intermediate numbers of foundresses (peaking at ca. 18 per host). Our data suggest that to maximize *S. brevicornis* offspring production per foundress per host, hosts should be pre-paralysed and should be presented to a single foundress. Under these conditions, approximately 7.2 offspring per foundress per host can be recruited into the next generation. This is represented by the parameter c in classical discrete-time host-population models: typically, c is negatively associated with equilibrium host density (Hassell, 2000; Heimpel, 2000) and, thus, better biocontrol performance. Previous studies on *S. harmandi* (Tang et al., 2014) and *S. pupariae* (Wei et al., 2017) have found that the probability of producing adult offspring from a given host is enhanced when foundress groups were larger than one. However, the sizes of hosts considered by those studies were substantial [ranging up to 0.64 g (*S. harmandi*) and 0.24 mg (*S. pupariae*), whereas the maximum size of *C. cephalonica* hosts studied here was 0.040 g – compared to cerambycid larvae, *C. cephalonica* larvae are small hosts].

Offspring properties

Female-biased sex ratios generally a desirable property among parasitoids that are biological control agents

because are females, not males, that attack the target pest (Ode & Hardy, 2008; Li et al., 2010; Boulton et al., 2015). Most adult offspring produced by *S. brevicornis* were females (92%) which agrees with prior reports in this species (Lupi et al., 2017) and congeners (Kühne & Becker, 1974; Li & Sun, 2011; Liu et al., 2011; Hu et al., 2012; Tang et al., 2014; Wei et al., 2014; Gao et al., 2016; Kapranas et al., 2016b). It is thought that sex-ratio bias in *Sclerodermus* is due to local resource enhancement (LRE), a mutually beneficial female–female interaction that increases the reproductive value of daughters, rather than local mate competition (LMC) which is the more commonly applied explanation of female-biased sex ratios among parasitoids (Tang et al., 2014). Here, we found a slight increase in bias as foundress number increased, whereas other studies have found the opposite (Tang et al., 2014; Wang et al., 2016). This strongly female-biased sex ratio in *S. brevicornis*, whether caused by LRE or LMC, is considered an asset for biological control programmes (Ode & Hardy, 2008). In addition, cooperation among *S. brevicornis* co-foundresses as a correlate of local resource enhancement (LRE) is likely to increase the range of successfully attackable host sizes (Tang et al., 2014; Kapranas et al., 2016b; Abdi et al., 2020) and, in consequence, is likely to improve the biocontrol potential of the parasitoid.

Associated with the highly female-biased sex ratio, some broods contained no adult male offspring at all (virgin broods), which is generally undesirable in rearing programmes because, as a consequence of arrhenotoky, unmated females are constrained to produce only male offspring in the following generation. As found in prior studies on *S. brevicornis* (Lupi et al., 2017) and in congeners (Kapranas et al., 2016b), the prevalence of all-male broods declined strongly with brood size and is likely to be associated with mortality of offspring within developing broods. However, we found that virginity was more prevalent when broods were produced by more foundresses, whereas Kapranas et al. (2016b) found the opposite effect of foundress number for *S. harmandi*.

Wing dimorphism was present among the adult offspring of both sexes, although the vast majority (ca. 99%) of females produced were apterous whereas alate morphs dominated among the males (ca. 97%), as observed throughout the genus (Kühne & Becker, 1974; Evans, 1978; Tang et al., 2014; Wang et al., 2016; Lupi et al., 2017; Wei et al., 2017). Apterous males were most common when brood sizes were larger and we found that the production of alate females was associated with single-foundress broods. This is consistent with *S. pupariae* in which the proportion of winged female progeny decreases as foundress number increases (Wang et al., 2016). Often wingless forms are confined to a given emergence site

whereas winged morphs have greater potential to colonize hosts at greater distances, which is likely to influence the efficacy of mass release programmes.

Caveats

Although we have shown that *S. brevicornis* can be reared on *C. cephalonica*, our present study has not evaluated all aspects of parasitoid 'quality': many aspects of parasitoid performance can be influenced by laboratory-rearing environments, including the use of factitious hosts (van Lenteren, 2003). For instance, we have evaluated the numerical production of adult offspring but not their size, and size is known to affect the ability of females of some parasitoid species to forage for hosts in the field (Visser, 1994; Luck & Forster, 2003), their survival, and also fecundity (Hardy et al., 1992; Godfray, 1994; Gao et al., 2016). There may also be a variation in parasitoid performance over generations of rearing on a given host (Nunney, 2003; van Lenteren et al., 2003; Pratissoli et al., 2004; Li et al., 2010), possibly due to associative learning of cues emanating with the factitious host, rather than the target host (Vet et al., 2003; Men et al., 2019). Such effects remain largely unassessed for *S. brevicornis*, although some evidence suggests that females reared on *C. cephalonica* attack and lay eggs on *C. cephalonica* larvae less rapidly than do those reared on a coleopteran host (Abdi et al., 2020). Further investigations, ideally beyond the laboratory environment (Steinberg & Cain, 2003; van Lenteren, 2003), are thus required to evaluate the performance of laboratory-reared *S. brevicornis* and thus better assess its potential in augmentative biological control programmes.

Conclusion

Species of *Sclerodermus* are considered among the most effective bethylids used in biocontrol programmes (Yang, 2004; Yang et al., 2014; Jiang et al., 2015). *Sclerodermus brevicornis* is native to Europe and has potential to counter invasion by Asian wood-boring longhorn beetles, especially if mass-reared for subsequent inundative field release (Lupi et al., 2017). However, *S. brevicornis* rearing systems have depended on using larvae of longhorn beetles that require considerable effort and time to maintain in laboratory cultures (Lupi et al., 2017). *Corcyra cephalonica* is simple and inexpensive to mass rear and is a commonly used factitious host for a range of parasitoid species but has not previously been used for *Sclerodermus*. We conclude that, despite being a relatively small lepidopteran, rather than a large coleopteran, *C. cephalonica* is suitable for the maintenance of laboratory cultures of *S. brevicornis*. Further, the procedure of pre-paralysis is

not required; although it can enhance production of offspring per host, it would be labour-intensive in mass-culture facilities and, taking human labour into account, will likely lead to its exclusion from optimal rearing protocols. Although not all *C. cephalonica* hosts presented to *S. brevicornis* females ultimately yield the next generation of parasitoid offspring, it should be possible to obtain outputs of up to around 10 parasitoids per host, the vast majority of which will be mated females. Figure 8 summarizes the relative outputs for the combinations of culturing conditions trialled. The development of practicable and efficient mass-rearing systems brings the active deployment of European *Sclerodermus* against invasive longhorn beetles closer to implementation. However, the performance of laboratory-reared *S. brevicornis* in the field has yet to be evaluated.

Acknowledgements

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