



Foraging abilities and competitive interactions between two egg parasitoids of bagrada bug in California

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Abstract

Bagrada bug, *Bagrada hilaris* (Burmeister) (Hemiptera: Pentatomidae), is an invasive pest of cole crops in the United States. Because it also feeds on widespread weeds and persists in natural habitats surrounding crop fields, conventional control strategies are often ineffective at providing long-term control. One egg parasitoid, *Gryon aetherium* Talamas (Hymenoptera: Scelionidae), is a promising biological control candidate because of its ability to parasitise *B. hilaris* buried eggs. Recently, adventive populations of *G. aetherium* were recovered from sentinel eggs in California along with a native egg parasitoid, *Ooencyrtus californicus* Girault (Hymenoptera: Encyrtidae). A better understanding of these parasitoid species' spatial preference for foraging and their possible competitive interactions will help evaluate their host suppression potential. We compared the foraging abilities of these two parasitoid species for eggs deposited below and above ground. We also investigated the effect of interspecific competition on host suppression and the ability of *O. californicus* to parasitise eggs previously parasitised by *G. aetherium*. *G. aetherium* parasitised naturally and manually buried eggs, whereas *O. californicus* did not. In another experiment, *O. californicus* parasitised eggs glued to cards, but not in the presence of sand. Results suggest that *G. aetherium* may be negatively affected by the presence of *O. californicus*, and there was a slight but significant reduction in total host mortality when the parasitoids were present together. However, the inability of *O. californicus* to forage in soil likely limits negative interactions between these two species, and the two parasitoids may ultimately complement each other.

Introduction

Bagrada bug, *Bagrada hilaris* (Burmeister) (Hemiptera: Pentatomidae), is an invasive stink bug from southern Asia that rapidly spread throughout the southwestern United States and Hawaii after it arrived in Southern California in 2008 (Palumbo and Natwick, 2010; Bundy *et al.*, 2012; Reed *et al.*, 2013; Palumbo *et al.*, 2016). Both adults and nymphs feed on vegetable crops in the Brassicaceae family (cabbage, broccoli, kale, cauliflower, arugula, etc.), as well as brassicaceous ornamental plants and weeds such as mustards (Palumbo and Natwick, 2010; Reed *et al.*, 2013). Because of its persistence in natural habitats surrounding crop fields, conventional control strategies are often ineffective at providing long-term control of this pest, in addition to being costly and negatively impacting the environment and human health (Stark and Banks, 2003).

In its native range, *B. hilaris* appears to be under the control of natural enemies with parasitism by egg parasitoids reaching up to 30% (Mahmood *et al.*, 2015). *B. hilaris* has a rather unusual oviposition behaviour for a pentatomid as females bury their eggs in the soil (Taylor *et al.*, 2015) rather than depositing them in clusters on the underside of leaves (Javahery, 1994). Burying eggs likely provides protection from natural enemies (Fatouros *et al.*, 2020). Eggs are usually oviposited singly or in small groups of 2–13 at an average depth of 2 mm (Martel and Sforza, 2021). However, some eggs can also be found on plant stems, leaves, and inflorescences, as well as partially or completely exposed on the surface of the soil because of the female's failed attempt to bury them (Taylor *et al.*, 2014).

One egg parasitoid from Pakistan, *Gryon aetherium* Talamas (Hymenoptera: Scelionidae), previously referred to as *Gryon gonikopalense* Sharma (Talamas *et al.*, 2021), has been identified as a promising biological control candidate because of its ability to find and parasitise eggs buried in the soil (Tofangsazi *et al.*, 2020; Martel and Sforza, 2021). In 2017–2020, sentinel eggs were deployed in northern and central California to assess the presence of egg parasitoids attacking *B. hilaris* (Hogg *et al.*, 2021, 2023). Two egg parasitoid species were recovered: *G. aetherium*, found for the first time in the United States; and *Ooencyrtus californicus* Girault (Hymenoptera: Encyrtidae), a native parasitoid previously described as *Ooencyrtus lucidus* Triapitsyn & Ganjisaffar (Triapitsyn *et al.*, 2021). This species is suspected to have switched from native hosts such as the green stink bug *Chinavia hilaris* (Say) to *B. hilaris*

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(Triapitsyn *et al.*, 2020), and may compete with *G. aetherium* for *B. hiliaris* eggs when the two species forage the same host patches. A better understanding of parasitoid species' spatial preference for foraging and their possible competitive interactions will help evaluate their potential for host suppression (Cusumano *et al.*, 2016; Schulz *et al.*, 2019; Ode *et al.*, 2022).

In this study, we compared the foraging abilities of the two parasitoid species in a laboratory. First, we looked at their foraging abilities below ground to confirm that adventive populations of *G. aetherium* can parasitise buried eggs of *B. hiliaris*, just like its exotic counterpart. Then, we compared their foraging abilities on and above the ground surface to better understand the species' spatial preferences. Since both species can parasitise eggs above ground, we looked at the effect of interspecific competition on host suppression when the two species forage simultaneously. Finally, we looked at the ability of *O. californicus* to parasitise eggs previously parasitised by *G. aetherium* (multiparasitism) as this could negatively affect the success of *G. aetherium* in controlling *B. hiliaris*.

Materials and methods

Insect colonies

The *B. hiliaris* colony was established from individuals collected from 2015 to 2018 in Solano and Monterey counties in California. Adults and nymphs were kept in bug dorms (61 × 61 × 61 cm³, BioQuip Products Inc., Rancho Dominguez, California) at 28–30°C, 30–40% relative humidity (RH), and 16L:8D photoperiod, and fed with store-bought organic broccoli and kale. The eggs used for parasitoid rearing and in the experiments below were produced in separate oviposition boxes consisting of custom laser-cut ventilated Plexiglas boxes (28 × 16.5 × 14 cm³), in which uncovered sand-filled Petri dishes (diameter: 47 mm, height: 5 mm) were exposed to mating pairs of *B. hiliaris*. Before use, the sand (Quikrete Brown Play Sand, American Canyon, CA) was sieved with a No. 35 sieve (Humboldt Manufacturing Co., Elgin, IL; mesh size: 500 μm). The Petri dishes were sheltered under a laser-cut Plexiglas shade structure (14 × 7 × 2.5 cm³) and organic broccoli and kale was provided to the bugs. After exposure and unless otherwise noted, the sand was sieved again to collect the eggs.

Both egg parasitoid colonies were established from parasitised *B. hiliaris* sentinel eggs deployed near Davis, California, in 2020.

Table 1. Egg distribution, parasitism success, and per cent parasitism by *G. aetherium* on eggs manually placed on the surface of sand (surface), 2 mm below the surface of sand (buried), and left undisturbed after natural egg-laying (undisturbed)

Treatment	<i>n</i>	No. of eggs	Parasitism success	Parasitism (%)
Surface	15	18.3 ± 1.8 ^a	15 (100%) ^a	82.3 ± 5.5 ^a
Buried	16	18.6 ± 1.5 ^a	3 (19%) ^b	2.6 ± 1.5 ^c
Undisturbed	15	28.6 ± 4.8 ^b	10 (67%) ^b	11.0 ± 2.5 ^b
Undisturbed surface		3.7 ± 1.6 ^A	8 (53%) ^A	73.1 ± 11.2 ^A
Undisturbed buried		24.9 ± 3.7 ^B	9 (60%) ^A	6.0 ± 1.5 ^B

For the undisturbed treatment, distinction between eggs deposited on the surface and buried were analysed separately. Means followed by different letters differ significantly (Tukey's HSD test, *P* < 0.05).

The parasitoids were identified by Dr Elijah Talamas (Florida Department of Agriculture and Consumer Services) and Dr Robert Zuparko (California Academy of Sciences). Parasitoid adults were kept in glass vials (diameter: 25 mm, height: 95 mm) in a laboratory at 20–24°C, 40–60% RH, and 16L:8D photoperiod with organic raw honey spread on the inside of the stopper as a food source. About 2–4 times a week, 20–40 freshly sieved *B. hiliaris* eggs (≤24 h old) were glued to rectangular pieces of cardstock (20 × 60 mm²) using Elmer School Glue (Elmer's Products Inc., Columbus, OH) and exposed to parasitoids adults in glass vials for 1–3 days. After the exposure, the egg cards were incubated in glass vials under the same conditions until adult emergence (21–30 days later). The experiments below were conducted under the same conditions. Experimental parasitoid females were assumed mated because they emerged in the presence of males and were housed with males within 1 day of their emergence until used for experiments. We used freshly sieved *B. hiliaris* eggs (≤24 h old) for all the experiments.

Foraging abilities on and below ground

The ability to parasitise eggs on and below the ground surface was investigated using sand. Naïve 4–6 day-old-female wasps of both species were released singly inside a round Plexiglas enclosure (diameter: 10.2 cm, height: 7.6 cm) containing one uncovered Petri dish filled with sand and randomly assigned to one of the following treatments: (1) sieved *B. hiliaris* eggs manually deposited on the surface of clean sand ('surface'), (2) sieved *B. hiliaris* eggs manually buried under a 2 mm layer of clean sand ('buried'), or (3) naturally laid *B. hiliaris* eggs ('undisturbed'). We used both manually buried and naturally buried eggs to test whether the parasitoids follow a chemical trail left by ovipositing females to find the eggs in the soil.

For each trial date, the available eggs were divided equally between replicates in the surface and buried treatments, while it was not possible to know the number of eggs in the undisturbed treatment prior to exposure to parasitoids (see table 1 for the number of eggs in each treatment). The accuracy of depth in the buried treatment was ensured by first filling the Petri dish with clean sand until a three-dimensional-printed spacer (2 mm thick) sat flush with the dish edge. Then, the spacer was removed and sieved eggs were manually deposited in a random pattern on this levelled surface. More sand then was gently added until levelled with the dish edge. The undisturbed treatment consisted of sand-filled Petri dishes previously exposed to mating pairs of *B. hiliaris* for 24 h. The sand was neither refreshed nor disturbed in this treatment and contained both surface and buried eggs. Small drops of honey were spread on the arena ceiling and floor, and care was taken to release the wasps on the sand surface. After 48 h the eggs were retrieved by sieving the sand and placed in glass vials (one glass vial per replicate) for incubation. Before sieving the eggs in the undisturbed treatment, the eggs on the sand surface were removed with featherweight forceps under a microscope and placed in separate vials. After 30 days, the number of emerged wasps and *B. hiliaris* nymphs was recorded. All unhatched eggs were dissected to record the number of unhatched (dead) *B. hiliaris* nymphs and unemerged (dead) parasitoid adults. Replicates where females were found dead at the end of the exposure were excluded from the analyses, resulting in a total of 16 replicates for each treatment/species conducted over eight trial dates.

Foraging abilities above ground

Similarly to the previous experiment, naïve 1–5 day-old-female wasps were released singly into a round Plexiglas enclosure containing a card ($2 \times 4 \text{ cm}^2$) with ten *B. hiliaris* eggs glued to it. The egg card was either (1) placed in an uncovered Petri dish centred in the middle of the enclosure floor ('ground'), (2) same as (1) but a thin layer of sand was added between the eggs which remained unburied ('sand'), or (3) attached to a small stand 4 cm above the arena floor ('elevated'). The elevated treatment was included to mimic eggs deposited above the ground on the stem or leaves of the host plant.

Small drops of honey were spread on the arena ceiling and floor and care was taken to release the wasp on the egg cards. After 48 h the egg cards were incubated in glass vials until the emergence of *B. hiliaris* or parasitoids. After 30 days the number of emerged wasps and *B. hiliaris* nymphs was recorded. All unhatched eggs were dissected to record the number of unhatched (dead) *B. hiliaris* nymphs and unemerged (dead) parasitoid adults. Replicates where females were found dead at the end of the exposure were excluded from the analyses, resulting in a total of 14 replicates for each treatment/species conducted over eight trial dates.

Competition when foraging simultaneously

Since both species can potentially forage the same host patch on or above ground, the effect of interspecific competition on host suppression was investigated with an additive-series design (Schmitz, 2007). Egg cards with 20 or 30 eggs were randomly assigned to one of the following treatments: (1) exposure to one *G. aetherium* female ('G only'), (2) exposure to one *O. californicus* female ('O only'), (3) exposure to one female of each species simultaneously ('G + O'), and (4) no exposure to parasitoids (control). Using this experimental design, intraspecific competition is kept constant (i.e. the number of adult parasitoids of each species is the same in the single species and two species treatments). These exposures were done in small Petri dishes with either 30 (3 replicates) or 20 (13 replicates) *B. hiliaris* eggs and lasted either 3 h (30 eggs) or 2 h (20 eggs), with all treatments within a replicate receiving the same numbers of eggs. The reduction from 30 eggs/3 h to 20 eggs/2 h was dictated by a temporary decrease in egg production in our *B. hiliaris* colony. These combinations of numbers of eggs and exposure times were selected to ensure an excess of hosts. All parasitoids tested were naïve 1–5 days old. After the exposure, the egg cards were incubated in glass vials until the emergence of *B. hiliaris* nymphs or parasitoid adults. After 30 days, all unhatched eggs were dissected to determine the presence of a recognisable *B. hiliaris* nymph or parasitoid adult. Replicates where females were found dead at the end of the exposure were excluded from the analyses, resulting in a total of 16 replicates conducted over seven trial dates.

Multiparasitism by *O. californicus*

The ability of *O. californicus* to parasitise eggs previously parasitised by *G. aetherium* was also investigated since multiparasitism could negatively affect the success of *G. aetherium* in controlling *B. hiliaris*. In this experiment, egg cards with ten eggs each were first exposed to two–three female *G. aetherium* (1–3 days old) for 24 h to ensure maximum parasitism. Then, half of the egg cards were exposed to one *O. californicus* female (1–4 days old)

for another 24 h while the other cards were not (control). The exposures were done in small Petri dishes and all egg cards were incubated in glass vials after the exposure until the emergence of *B. hiliaris* nymphs or parasitoids. After 30 days, all unhatched eggs were dissected to determine the presence of a recognisable *B. hiliaris* nymph or parasitoid adult. Replicates where females were found dead at the end of the exposure were excluded from the analyses, resulting in a total of 20 replicates conducted over four trial dates.

Data analysis

Because it was not possible to control the number of eggs in the undisturbed treatment in the first experiment (foraging abilities on and below ground), the numbers of host eggs in each treatment were compared using a mixed-model generalised linear model (GLM) with Poisson errors, with treatment and species as explanatory variables along with a species \times treatment interaction term, and including trial date as a random factor, using the glmer function in R version 4.0.2 (R Development Core Team, 2020). For both foraging experiments (below and above ground), parasitism success was calculated as the proportion of replicates where at least one egg was parasitised, and proportion parasitism was calculated as the sum of emerged and unemerged parasitoids divided by the number of eggs. For the latter calculation, we assumed that no more than one adult emerged from each host egg for both species. Indeed, *G. aetherium* appears to avoid conspecific superparasitism (Hougardy and Hogg, 2021). And, while some *Ooencyrtus* spp. can sometimes be facultatively gregarious (Mohammadpour *et al.*, 2014), when this happens, we should expect the size of the co-emerged adults to be considerably reduced. Since no obvious difference in size has been observed among emerged *O. californicus* in our experiments, we rejected the possibility of gregarious development. Parasitism success and proportion parasitism were then compared between species (when relevant) and treatments using mixed-model GLMs with binomial errors, including trial date as a random factor. For foraging below ground, an additional mixed-model GLM was conducted for the undisturbed treatment to compare parasitism success and proportion parasitism by *G. aetherium* between eggs deposited on the surface and buried. Egg number was included as an offset in analyses for the experiment testing foraging abilities on and below ground, to account for higher numbers of eggs in the undisturbed treatment (for comparing treatments and for comparing surface and buried eggs within the undisturbed treatment). For the competition experiment (when foraging simultaneously), effects on host mortality rates, calculated as the total number of host eggs minus the number of *B. hiliaris* nymphs (hatched or unhatched) divided by the total number of host eggs, emergence rates, calculated as the number of each parasitoid's offspring divided by the total number of eggs, and egg mortality rates, the proportion of unclosed eggs, were assessed using a 2×2 mixed-model factorial GLM, with the addition of *G. aetherium* and *O. californicus* and their interaction as factors, and including trial date as a random factor. Finally, we compared the observed host mortality in the two species treatment with an expected mortality value calculated across replicates using the following multiplicative risk model:

$$H_{\text{exp}} = H_G + H_O - (H_G \times H_O),$$

where H_{exp} is the expected host mortality when the host is exposed to the two species simultaneously, and H_G and H_O are the observed host mortality in the *G. aetherium* (G) and *O. californicus* (O) single-species treatments, respectively. The observed and expected host mortalities were then compared across replicates using a mixed-model GLM with replicate and trial date as random factors. If the interspecific interactions between species do not affect host suppression, observed and expected mortalities should not be significantly different. To examine possible effects of competition on parasitoids, proportion emergence (number of emerged parasitoids divided by number of eggs) of each species was compared between the respective single-species treatments and the combined-species treatments using mixed-model GLMs with binomial errors. For the multiparasitism experiment, proportion parasitism by *G. aetherium* was compared between the treatment and control using a mixed-model GLM including trial date as a random factor. All multiple comparisons between treatments were performed using Tukey's honestly significant difference (HSD) tests using the pairs function in the emmeans package in R.

Results

Foraging abilities on and below ground

The distribution of *B. hilaris* eggs varied between treatments (GLM, $\chi^2 = 145.79$; $df = 2$; $P < 0.001$) but not between species ($\chi^2 = 0.02$; $df = 1$; $P = 0.89$), and the distribution of eggs within each treatment was consistent between species (no interaction between the two factors: $\chi^2 = 3.53$; $df = 2$; $P = 0.17$). Overall, the average number of eggs in the undisturbed treatment (27.6 ± 3.0 , range = 5–70) was significantly higher than the average number of eggs in the surface treatment (17.1 ± 1.0 , range = 9–35) and buried treatment (16.6 ± 0.9 , range = 9–32) (Tukey's HSD test, $P < 0.05$). Within the undisturbed treatment, significantly more eggs were buried (24.4 ± 4.4 eggs; range = 4–55) than deposited on the surface (3.3 ± 0.6 eggs; range = 0–24) ($\chi^2 = 28.03$; $df = 1$; $P < 0.001$).

O. californicus females did not parasitise any eggs in any of the treatments while *G. aetherium* females did in all three treatments. Therefore, the following analyses were conducted for *G. aetherium* only. There were significant differences in parasitism success and proportion parasitism between treatments (GLM, parasitism success: $\chi^2 = 26.67$, $df = 2$, $P < 0.001$; proportion parasitism: $\chi^2 =$

388.75, $df = 2$, $P < 0.001$), with greater success in the surface and undisturbed treatments than in the buried treatment, although only the surface treatment was significantly higher, and the highest proportion parasitism in the surface treatment, followed by the undisturbed treatment, and the lowest in the buried treatment (table 1) (Tukey's HSD tests, $P < 0.05$). Within the undisturbed treatment, proportion parasitism was higher on surface than buried eggs ($\chi^2 = 55.17$, $df = 1$, $P < 0.001$; table 1), although there was no significant difference in parasitism success ($\chi^2 < 0.01$; $df = 1$; $P > 0.99$; table 1).

Foraging abilities above ground

G. aetherium parasitised eggs in all three treatments (ground, sand, and elevated) while *O. californicus* did in only two; no eggs were parasitised by *O. californicus* in the sand treatment (table 2). When both species were included in analyses, parasitism success and proportion parasitism differed among treatments (GLM, parasitism success: $\chi^2 = 17.14$, $df = 2$, $P < 0.001$; proportion parasitism: $\chi^2 = 44.67$, $df = 2$, $P < 0.001$) and between species (parasitism success: $\chi^2 = 11.59$, $df = 1$, $P < 0.001$; proportion parasitism: $\chi^2 = 106.87$, $df = 1$, $P < 0.001$). The effect of treatment was consistent between species (no interaction between the two factors, parasitism success: $\chi^2 = 3.04$, $df = 2$, $P = 0.22$; proportion parasitism: $\chi^2 = 2.07$, $df = 2$, $P < 0.36$). When the effect of treatment was analysed for the two species separately, treatment effects for parasitism success were only marginally significant for *G. aetherium* ($\chi^2 = 5.27$, $df = 2$, $P = 0.07$) but were significant for *O. californicus* ($\chi^2 = 15.27$, $df = 2$, $P < 0.001$), although pairwise comparisons uncovered no significant differences between treatments (Tukey's HSD tests, $P > 0.05$; table 2). Proportion parasitism differed between treatments for *G. aetherium* ($\chi^2 = 45.91$, $df = 2$, $P < 0.001$) and *O. californicus* ($\chi^2 = 72.08$, $df = 2$, $P < 0.001$), with lower levels of parasitism in the sand treatment for both species (Tukey's HSD tests, $P < 0.05$).

Competition when foraging simultaneously

The competition experiment confirmed that *G. aetherium* is a highly efficient parasitoid of *B. hilaris* causing 50.3% of host mortality when operating alone. This translated to parasitising up to 29 eggs in 3 h (average: 10.5 ± 2.0). Host mortality by *O. californicus* alone was lower, reaching 9.3% which corresponded to an average of 2.4 eggs parasitised, with a maximum of five eggs. Host mortality caused by the two parasitoid species differed greatly and was significantly affected by the presence of *G. aetherium* (GLM, $\chi^2 = 235.89$, $df = 1$, $P < 0.001$) but not *O. californicus* ($\chi^2 = 1.37$, $df = 1$, $P = 0.24$). The occurrence of a marginally significant *G. aetherium* \times *O. californicus* interaction ($\chi^2 = 3.84$, $df = 1$, $P = 0.05$) suggests that interactions between the two species affected host mortality (fig. 1). The observed host mortality when the two species foraged simultaneously was significantly lower than the expected mortality calculated using the multiplicative risk model ($\chi^2 = 10.21$, $df = 1$, $P = 0.001$; fig. 1). The presence of *O. californicus* negatively affected proportion emergence of *G. aetherium* ($\chi^2 = 23.38$, $df = 1$, $P = 0.001$; $41.98 \pm 6.60\%$ in the G alone treatment vs. $27.19 \pm 5.86\%$ in the G + O treatment), while the presence of *G. aetherium* did not significantly impact proportion emergence of *O. californicus* ($\chi^2 = 0.61$, $df = 1$, $P = 0.44$; $4.48 \pm 1.76\%$ in the O treatment vs. $2.92 \pm 1.28\%$ in the G + O treatment). When egg mortality rate (proportion of uneclosed eggs) was analysed, *G. aetherium* had a significant effect (GLM,

Table 2. Parasitism success and per cent parasitism by *G. aetherium* and *O. californicus* on egg cards containing ten eggs placed on the arena floor with (sand) or without sand (ground) and elevated 4 cm above the floor of the arena (elevated)

Species	Treatment	<i>n</i>	Parasitism success	Parasitism (%)
<i>G. aetherium</i>	Ground	13	10 (77%) ^a	66.3 \pm 10.8 ^a
	Sand	13	6 (46%) ^a	36.6 \pm 11.8 ^b
	Elevated	13	11 (85%) ^a	73.1 \pm 11.3 ^a
<i>O. californicus</i>	Ground	14	8 (57%) ^a	29.3 \pm 8.5 ^a
	Sand	14	0 (0) ^a	0.0 \pm 0.0 ^b
	Elevated	14	7 (50%) ^a	27.1 \pm 8.9 ^a

Within each species, means followed by different letters do differ significantly (Tukey's HSD, $P < 0.05$).

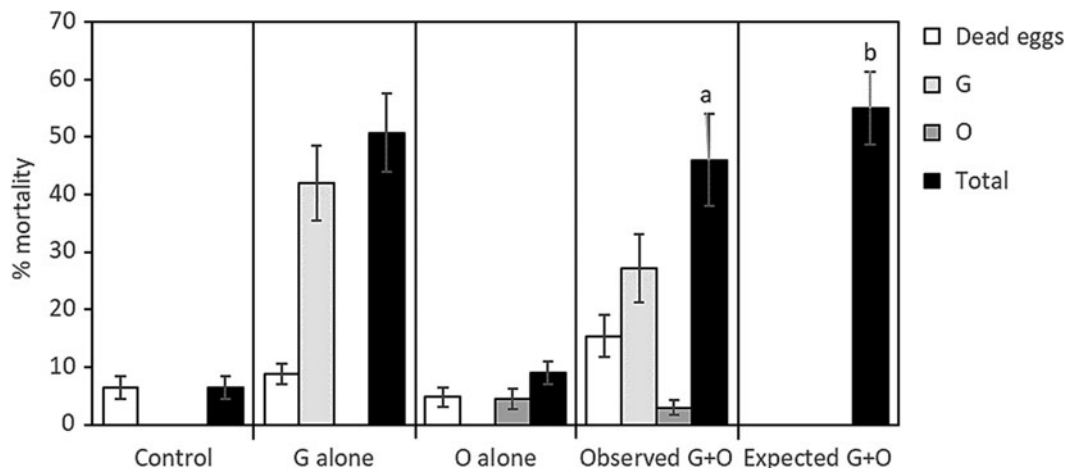


Figure 1. Per cent host mortality inflicted by *G. aetherium* (G) and *O. californicus* (O) when released alone or together (observed G + O) in an additive design experiment. Dead eggs are unclosed eggs that did not develop. Control treatment is the natural host mortality in the absence of parasitism. Expected mortality (expected G + O) was calculated according to the multiplicative risk model. Data are mean ± SE. Bars with similar letters do not differ significantly.

$\chi^2 = 11.96$, $df = 1$, $P < 0.001$) but *O. californicus* did not ($\chi^2 = 2.09$, $df = 1$, $P = 0.15$) and the *G. aetherium* × *O. californicus* interaction was significant ($\chi^2 = 6.48$, $df = 1$, $P = 0.01$). Proportions of dead eggs were highest in the G + O treatment (fig. 1).

Multiparasitism by *O. californicus*

O. californicus successfully multiparasitised hosts previously parasitised by *G. aetherium*, significantly reducing the number of emerging *G. aetherium* (fig. 2): proportion parasitism by *G. aetherium* was significantly lower under multiparasitism (GLM, $\chi^2 = 31.10$, $df = 1$, $P < 0.001$).

Discussion

Our results confirmed that the adventive population of *G. aetherium* in California can parasitise *B. hiliaris* eggs deposited on and below the ground surface. In the first experiment, parasitism success and proportion parasitism were higher in the surface treatment, most likely because surface eggs were easier to find and parasitise compared with buried eggs. Despite its specialised foraging abilities, searching for buried eggs is likely to be time-

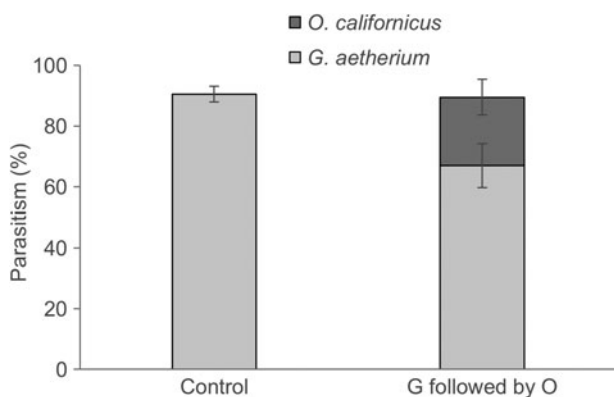


Figure 2. Per cent parasitism by *G. aetherium* (G) and *O. californicus* (O) when eggs are exposed to *G. aetherium* only (control) or subsequently to *O. californicus*. Data are mean ± SE.

consuming and labour-intensive for *G. aetherium*. For the two treatments containing buried eggs (i.e. buried and undisturbed treatments), proportion parasitism was significantly higher in the undisturbed treatment compared to the manually buried treatment. A study by Martel and Sforza (2021) conducted with a Pakistani population of *G. aetherium* found similar results. There was a 20–30% reduction in parasitism rates when *G. aetherium* was exposed to manually buried eggs in clean sand vs. sand that was exposed to ovipositing *B. hiliaris* females (but disturbed in this case). Chemical cues have been shown to facilitate egg discovery by other parasitoid species attacking stinkbug eggs (Conti and Colazza, 2012) and *G. aetherium* likely also relied on host-derived cues to locate eggs under the sand surface. These cues, possibly chemical trails left by ovipositing *B. hiliaris* females, were left intact in the undisturbed treatment but were absent in the buried treatment. The presence of a few surface eggs in some of the replicates in the undisturbed treatment could have alerted *G. aetherium* to the presence of buried eggs. However, *G. aetherium* also parasitised buried eggs in arenas that lacked eggs on the surface. Furthermore, the ability of *G. aetherium* to parasitise at least some manually buried eggs suggests that the parasitoid females use chemical cues emitted by the eggs themselves in addition to the possible chemical trail left in the sand. Egg parasitoids of pentatomid hosts usually use contact kairomones and physical cues, such as shape and size, to locate host eggs (Conti and Colazza, 2012). Since these types of cues are not directly available to *G. aetherium* because of the sand/soil barrier, volatile compounds may be involved. In the undisturbed treatment, *G. aetherium* females locate equally well eggs on the surface or buried (53 vs. 60%) but parasitism rate may have been lower for buried eggs because the total number of underground hosts was greater. Conversely, the higher underground host density in the undisturbed treatment could have accelerated host location by reducing travel time between hosts relative to the surface and buried treatments. More study of the effects of host density on *G. aetherium* parasitism would be necessary to assess this possibility.

The results of the second experiment showed that *O. californicus* can parasitise both ground-level and elevated eggs, but it did not parasitise any eggs deposited on sand, suggesting that either the females did not forage or could not locate eggs on sand. *O.*

californicus is a native parasitoid that parasitises eggs of native pentatomid hosts that lay their eggs on the underside of leaves, not on the soil (Javahery, 1994). Evolutionary pressure must have shaped its foraging abilities and responses to environmental cues to focus on the plant or other surfaces and away from the ground. *G. aetherium* was not limited to eggs on the ground but also foraged for eggs above the ground. In the field, it will likely also parasitise eggs deposited on plant stems and foliage, and probably travels these short distances between ground and foliage by walking. In a previous study, *G. aetherium* was never seen flying in the cages and appeared to locate hosts by walking rather than by flying (Tofangsazi *et al.*, 2020).

The multiplicative risk model analysis indicated a reduced combined impact of *G. aetherium* and *O. californicus* on *B. hiliaris*. Our results showed that any disruption in parasitism by *O. californicus* would have had only a negligible impact on host mortality, given its low overall parasitism rate. A previous study estimated that *O. californicus* females laid on average six eggs per day (Ganjisaffar and Perring, 2020). The disruption we observed likely occurred through reduced parasitism by *G. aetherium*. Indeed, the reduction in *G. aetherium* emergence in the presence of *O. californicus* indicates that *O. californicus* negatively impacted either foraging by *G. aetherium* and/or the successful development of *G. aetherium* larvae. Behavioural observations in a previous study showed that *G. aetherium* was quite indifferent to the presence of *Trissolcus hyalinipennis* Rajmohana & Narendran (Hymenoptera: Scelionidae) (Hougardy and Hogg, 2021), another egg parasitoid attacking *B. hiliaris* eggs in Pakistan, often ovipositing right next to *T. hyalinipennis* and sometimes even engaging in aggressive behaviour to secure access to a host egg (Hougardy and Hogg, 2021). Unfortunately, we do not have comparable observations of *O. californicus* oviposition behaviour, but *O. californicus* could be a more aggressive forager than *T. hyalinipennis*, interrupting and displacing ovipositing *G. aetherium*. It is also possible that multiparasitism negatively affected overall host mortality by reducing the total number of parasitoid offspring. The proportion of dead eggs was highest in the combined treatments and could be the result of failed multiparasitism (when all the parasitoid larvae died before emergence).

Ooencyrtus spp. are generally superior intrinsic competitors (Cusumano *et al.*, 2011; Peri *et al.*, 2011; Mohammadpour *et al.*, 2014) and the usurpation of eggs is less time-sensitive and can happen when females encounter previously parasitised eggs later. Our last experiment confirmed that *O. californicus* can successfully parasitise eggs previously parasitised by *G. aetherium*, leading to a significant decrease in *G. aetherium* parasitism, but not affecting host mortality. Multiparasitism is common in nature, especially between unrelated species (Turlings *et al.*, 1985), and the ability to defeat larval competitors varies between species and the timing of the ovipositions. Usually, the first species in place has a developmental advantage, although the larvae of superior intrinsic competitor species are capable of finding and destroying competitor larvae even if their oviposition happens second in the sequence (Cusumano *et al.*, 2012; Harvey *et al.*, 2013). Larval competition can also be mediated by maternal factors, such as the injection of fluids that will inhibit egg hatching and larval development of the competitor (Paul *et al.*, 2023). When the time interval between parasitism is large, such that the larva of the first species has consumed the entire ooplasm, some competitive species can become facultative hyperparasitoids, developing on the body of the first species. Some *Ooencyrtus* spp. are known to be facultative hyperparasitoids (Cusumano *et al.*,

2013; Mohammadpour *et al.*, 2014; Triapitsyn *et al.*, 2020), and this possibility still needs to be investigated in our system.

Coexistence between competing species on a shared resource is often explained by counter-balanced competitive abilities: one species, usually a specialist, being superior in extrinsic competition (competition at the adult stage), while the other species, usually a species with a wider host range, is a superior intrinsic competitor (competition between immature stages). This has been demonstrated in several parasitoid systems where two species share a common host resource, including egg parasitoids (Cingolani *et al.*, 2013; Mohammadpour *et al.*, 2014; Peri *et al.*, 2014). A similar counter-balanced strategy may operate in interactions between *G. aetherium* and *O. californicus*. Our study demonstrated, on the one hand, the highly specialised searching behaviour of *G. aetherium* under the sand surface along with its high parasitism success on *B. hiliaris* and, on the other, successful multiparasitism by *O. californicus*.

While the presence of *O. californicus* negatively affected the emergence of *G. aetherium*, this was compensated by an increase in the proportion of dead eggs in the combined species treatment, resulting in only a slight decrease in overall host mortality. In addition, since *O. californicus* cannot forage in soil, some resource partitioning is likely to limit the negative interactions between these two parasitoid species and support their coexistence. We suspect that their effect on host population is ultimately additive.

Data. The data presented in this study are available on request from the corresponding author.

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