# Parasitism of the soybean aphid, *Aphis glycines* by *Binodoxys communis*: the role of aphid defensive behaviour and parasitoid reproductive performance

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## Abstract

The Asian parasitoid, *Binodoxys communis* (Gahan) (Hymenoptera: Braconidae), is a candidate for release against the exotic soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), in North America. In this study, we examined preferences by *B. communis* for the different developmental stages of *A. glycines* and investigated consequences of these preferences for parasitoid fitness. We also determined to what extent aphid defensive behaviours mediate such preferences. We found that *B. communis* readily attacks and successfully develops in the different *A. glycines* developmental stages. *Binodoxys communis* development time gradually increased with aphid developmental stage, and wasps took longest to develop in alates. An average (±SE) of 54.01±0.08% of parasitized *A. glycines* alatoid nymphs transformed into winged adult aphids prior to mummification. No-choice assays showed a higher proportion of successful attacks for immature apterous *A. glycines* nymphs compared to adults and alatoid nymphs. Also, choice trials indicated avoidance and lower attack and oviposition of adults and alatoid nymphs. The different aphid stages exhibited a range of defensive behaviours, including body raising, kicking and body rotation. These defenses were employed most effectively by larger aphids. We discuss implications for the potential establishment, spread and biological control efficacy of *A. glycines* by *B. communis* in the event that it is released in North America.

### Keywords

biological control; host quality; host selection; koinobiont parasitoids; fitness; life history; phoresy

## Introduction

Biological control introductions are being considered against the soybean aphid, *Aphis glycines* Matsumura, an invasive species from Asia that is a destructive pest of soybeans in North America (Heimpel *et al*., 2004; Ragsdale *et al*., 2004; Wu *et al*., 2004; Wyckhuys & Heimpel, 2007; Wyckhuys *et al*., 2007a,b). A strain of *Binodoxys communis* (Gahan) (Hymenopera: Braconidae) that was collected from soybean aphid in China is one of the most promising natural enemies for release against *A. glycines*. This species appears to be very well

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adapted to *A. glycines* in laboratory studies and exhibits fairly high levels of host specificity (Wyckhuys *et al*., 2007b; N. Desneux, personal communication).

The immature stages of insect parasitoids, such as *B. communis*, depend on their host for nutrients, and so adult female parasitoids must accurately assess the suitability of hosts for progeny development (e.g. Sequeira & Mackauer, 1992a; Colinet *et al*., 2005; Henry *et al*., 2005). Many parasitoids preferentially attack certain sizes, ages or stages of a given host species (Mackauer, 1973; Hopper & King, 1984; Liu *et al*., 1984; Wang & Liu, 2002; Lin & Ives, 2003). These preferences influence developmental rate, survival and reproductive capacities of parasitoid offspring (Lewis & Redlinger, 1969; Nechols & Kikuchi, 1985; Hopper, 1986; Sequeira & Mackauer, 1993; Godfray, 1994; Lacoume *et al*., 2006). Host-stage preferences can also strongly influence host behaviour and population growth (Hopper & King, 1984; Murdoch *et al*., 1987; Murdoch *et al*., 2003; Lin & Ives, 2003) and may affect parasitoid efficiency in biological control of aphids (Hågvar & Hofsvang, 1991).

All aphid parasitoids are endoparasitic koinobionts, meaning that eggs are laid within the host and the host continues developing after parasitism. Thus, the host stage used for oviposition generally precedes the host stage(s) used for larval development, complicating oviposition strategies (e.g. Kouamé & Mackauer, 1991; Sequeira & Mackauer, 1992b; Rivero, 2000; Li & Mills, 2004; Jenner & Kuhlmann, 2006). Koinobiosis also may decouple host stages that engage in defensive behaviour from those that support parasitoid development, in some cases allowing development on hosts that would otherwise be inaccessible (Liu *et al*., 1984; Gerling *et al*., 1990; Weisser, 1994, 1995; Chau & Mackauer, 2000). For example, although large aphids contain more nutritional resources than small aphids, they also defend themselves more effectively than smaller ones (Gerling *et al*., 1990; Chau & Mackauer, 1997; Losey & Denno, 1998; Chau & Mackauer, 2000).

Differences in host-stage use also have implications for parasitoid dispersal and establishment in novel environments. Most aphid species, including *A. glycines*, exhibit a polyphenism whereby individuals exhibit both winged (alate) and wingless (apterous) morphs (Dixon, 1998; Ragsdale *et al*., 2004; Hodgson *et al*., 2005). Alates generally leave their natal host plant and colonize new plants, eventually in different environments. Successful parasitism of winged or alatoid morphs could then lead to situations where parasitoid eggs or larvae are carried for considerable distances within their host (Kelly 1917; Hight *et al*., 1972; Rogers *et al*., 1972; Rauwald & Ives, 2001). Such phenomena could be of great importance to establishment, spread and efficacy of potential biological control agents such as *B. communis*.

Here, we investigate *B. communis* parasitism of different developmental stages of the soybean aphid, *A. glycines*, under choice and no-choice settings. Parasitism differences are related to *A. glycines* defensive behaviour, *B. communis* handling costs and oviposition success on each of the *A. glycines* stages. We also assess parasitoid fitness on each of the *A. glycines* instars by measuring key life-history traits, such as development time, survival and sex ratio (e.g. Roitberg *et al*., 2001). This research increases basic knowledge of this host-parasitoid association and helps predict *B. communis* establishment, spread and biological control success upon release in North America.

## Materials and methods

### Study insects

We established a colony of *A. glycines* from individuals that were collected in 2003 from a soybean field in St Paul, Minnesota, USA. This colony has subsequently been maintained in the Minnesota Department of Agriculture/Minnesota Agricultural Experiment Station (MDA/MAES) Quarantine Facility in St Paul with periodic supplementation of aphids from the field.

As aphid colonies were never started from one single aphid and no genetic characterization was done, no estimates are available of the number of clones of which the *A. glycines* colony consisted. The *A. glycines* colony was kept on soybean plants (cultivar M96-D133151), which were grown under greenhouse conditions (25±5°C, 60-80% RH and L16:D8).

A Chinese strain of *Binodoxys communis* was initiated with seven males and 33 females from collections of parasitized *A. glycines* by K. Hoelmer, K. Chen and W. Meikle made in several soybean fields in late August 2002 near Harbin (45°41'27" N, 126°37'42" E) and in Suihua County (45°36'28" N, 126°57'49" E) in the Chinese province of Heilongjiang. Voucher specimens of progeny from the material collected in China are stored frozen in molecular grade ethanol at the USDA-ARS Beneficial Insect Introduction Research Laboratory in Newark, Delaware, USA. We maintained *B. communis* in three subpopulations on *A. glycines* with a geometric mean of 66-68 adult parasitoids for each subpopulation for 26 generations. The parasitoid colony at the MDA/MAES Quarantine Facility was initiated from a total of 102 mummies in 2003, and has since been maintained on *A. glycines*. For experiments, we collected *B. communis* mummies from soybean plants and isolated these in clear gelatin capsules (size 0; Drum Point Pharmacy, Brick, NJ, USA). Adult female wasps were mated within 24 hr of emergence and kept in capsules with a droplet of mixed-flower honey prior to their use in experiments.

## No-choice parasitism trials

In the first experiment, we determined *B. communis* fitness on each of the *A. glycines* developmental stages by measuring overall parasitism levels as well as a set of life-history traits. Hodgson *et al.* (2005) reported *A. glycines* to have a total of four different immature instars, and winged or wingless adult stages. Third and 4th instar nymphs of winged morphs possess wing-pads and are termed alatoid nymphs; of these two morphs, we included 4th instar alatoids in our experiment, yielding a total of seven *A. glycines* types to be tested.

We planted soybean plants in the greenhouse and used them at the V1 stage for our experiments. The V1 developmental stage is characterized by a fully developed first trifoliate leaf and expanded unifoliate leaves (McWilliams *et al.*, 2004). In the laboratory, individually potted live plants were covered with a 0.5 l transparent PETE plastic cup (Solo Cup Company, Urbana, Illinois, USA) from which the bottom was removed. The top of the plastic cups was fitted with a fine nylon mesh, and the entire unit will be referred to as an `experiment cage'. We placed a total of 40 individuals of a given *A. glycines* stage onto each soybean plant using a fine brush. The different aphid stages were determined using an identification key developed by Hodgson *et al.* (2005). We allowed aphids to establish on plants for 1-2 hours before the introduction of parasitoids. Mated female *B. communis* were subsequently transferred to the cages and allowed to parasitize aphids for 4 h. The 4 h period ensured that high numbers of aphid offspring were not produced during the experiment, thereby likely distorting parasitism rates on each of the different aphid stages. Parasitoids were introduced into the cages between 12:00 and 14:00 and were removed after 4 h. The cages were maintained at 25°C, 75% RH and 16:8 L:D and checked on a daily basis for the presence of parasitoid mummies. Mummies were counted upon formation, and the number of days until mummy formation was recorded. Mummies were subsequently placed singly in clear gelatin capsules (size 0) and the number of days until parasitoid emergence was recorded. The sex of emerged parasitoids was determined and sex ratio is expressed as the proportion of adults that were male. We report the parasitism rate as the number of mummies divided by the starting number of aphids (i.e. 40). Although this measure does not distinguish parasitoid acceptance of hosts from host physiological suitability for parasitoid development, it provides a useful assessment of the net effect of parasitoid choice and host suitability on overall parasitism success (Li & Mills, 2004; Colinet *et al.*, 2005). For each of the seven *A. glycines* developmental stages, we carried out a total of ten replicates.

To compare *B. communis* life history traits on the different *A. glycines* developmental stages, we used a Kruskal-Wallis test or One way analysis of variance (ANOVA) with Fisher's protected LSD as post-hoc analysis, according to the normality of the data set.

## No-choice assay of host acceptance

A second experiment was done to quantify *B. communis* acceptance of each of the *A. glycines* developmental stages to determine the nature and extent of defensive behaviour of these stages. The behavioural arena consisted of a single leaflet that was removed from one of the fully expanded leaves of an uninfested V3-5 soybean plant and placed upside down within a 5.8 cm dia. Petri dish filled with moist sand. The leaflet had a diameter of >5 cm and commonly occupied the entire space within the Petri dish. The V3-5 soybean developmental stages are characterized by fully developed and expanded third-fifth trifoliate leaves (McWilliams *et al*., 2004). The Petri dish was then placed under a Leica GZ6E dissecting microscope. On this leaflet, we placed one individual of a given *A. glycines* stage and allowed it to settle for 5 min. Aphids were collected with a fine brush from *A. glycines* colonies on soybean plants of identical phenological stage and (visually) similar quality (Stadler *et al*., 1994). Next, a one-day-old, mated *B. communis* female was gently introduced into a 1 cm dia.×0.65 cm high clear plastic dome. This dome was then placed over the individual aphid within the Petri dish. The observation was started when the parasitoid first encountered the aphid.

We observed both aphid and parasitoid behaviour until a successful oviposition occurred but not longer than 5 min. For *B. communis*, we recorded the time elapsed until oviposition, the number of encounters and the number of probing events. An encounter was defined as the parasitoid making contact with the aphid after having walked away from it for > 5 s for re-encounters. All intervals were timed with a stopwatch to the nearest second. For the different *A. glycines* stages, we recorded defensive behaviour as `kick', `rotate', `walk away' or `cornicle secretion'. Kicking was defined as the aphid raising its body and then contacting the parasitoid with one of its legs. Exposures were replicated 25 times for each aphid stage. For every observation, a different *B. communis* female was used.

For analysis, we computed the number of probing events and the number of encounters as frequencies over the allotted time (i.e. maximum of 5 min or until successful oviposition) (Desneux *et al*., 2004). We compared these frequencies and the time until oviposition for the various aphid developmental stages using a non-parametric Kruskal-Wallis test. Next, pair-wise Mann-Whitney *U* tests were carried out following a Bonferroni correction for multiple comparisons. Proportional measures of attack and successful oviposition were compared between the different *A. glycines* stages using a Chi-square test. The same analysis was used to compare the proportion of aphids exhibiting defensive behaviours among the various stages.

## Choice assay of host acceptance

A third experiment was done to determine whether *B. communis* prefers certain *A. glycines* stages over others and if such preference changes as a parasitoid forages within a patch of aphids of various stages. As in the previous assay, a soybean leaflet was placed upside down within a 5.8 cm dia. Petri dish filled with moist sand and placed under a dissecting microscope. On this leaflet (which will be referred to as the `patch'), we placed a total of five individuals of each of the seven different developmental stages of *A. glycines*, totaling 35 aphids. We allowed the aphids to establish for 5-10 min and then introduced one *B. communis* female. Upon introduction of the parasitoid, the Petri dish was covered with a plastic lid 5.1 cm in diameter and 1.3 cm in height.

We noted the sequence of aphids that were encountered and recorded parasitoid attack and oviposition on each aphid attacked. The observation was terminated when the parasitoid stayed outside the patch for longer than 1 min or when 30 min had elapsed. As *B. communis* did not appear to discriminate against previously-parasitized aphids (see Results), we did not replace parasitized aphids during the course of the observations or treat them differently in subsequent data analysis. However, for each replicate, we composed a new patch using only unparasitized aphids collected from the *A. glycines* colony. The exposure was replicated 25 times.

Procedures for statistical analysis of this experiment were modified from Weisser (1994). Instar preference was measured using the formula of Manly (1974), whereby preference is scored as a deviation of the number of individuals of a given developmental stage selected for a particular behaviour (i.e. encounter, attack and oviposition) from the number of this stage eligible for the action (e.g. number present in the patch, number attacked, etc.). Let $A_i$ be the number of

individuals of a given stage *i* eligible for a particular action by the parasitoid ($\sum_{i=1}^{7} A_i = N =$ total number eligible for this action), and let $x_i$ be the number of stage *i* that have been selected for this particular action and $r_i$ the number that have not been selected (so that $x_i + r_i = A_i$). We considered the case in which an aphid already selected for an interaction is still eligible for this action (Weisser, 1994). Then

$$\beta j = \frac{(xj/Aj)}{\sum\limits_{i=1}^{7} xi/Ai} \quad j = 1, \ldots, 7$$

is Manly's Beta of the *j*th stage for this particular action (with a total of seven different stages being considered). If β*j* is greater than 1/7 for any given developmental stage *j*, then the parasitoid prefers this given stage for the action under consideration. If β*j* is less than 1/7 then the parasitoid avoids this interaction with stage *j* and, finally, if β*j*=1/7, then the parasitoids accept any eligible stage for the action under consideration. This formula is used to determine whether the different stages are encountered, attacked and parasitized in the same proportion as they are present within the patch. To determine whether *B. communis* preference changes with respect to the sequence of aphid attacks, we computed Manly's Beta values for different intervals over the course of the experiment (encounters 0-20, 20-40 and 40-60). We then compared these values between intervals for a select set of actions on each aphid stage (i.e. encounters, attacks or oviposition).

We compared Manly's Beta values for the different *A. glycines* stages using a Kruskal-Wallis test. For data that were normally distributed or could be successfully transformed, a One-way ANOVA was used, followed by Fisher's protected LSD as post-hoc analysis. All statistical analyses were executed using SPSS software (Landau & Everitt, 2004). For datasets that yielded non-significant differences, we performed a power analysis using GPower 3.0.4. (Faul *et al.*, 2007).

## Results

### No-choice parasitism trials

*Binodoxys communis* was able to successfully parasitize and develop on each of the seven stages of *A. glycines*. The number of mummies formed in each of the stages did not show any significant differences (table 1; ANOVA, $F_{6,62}=1.05$, $P=0.40$). However, the achieved power of this analysis was 0.46. Emergence rates of *B. communis* on the different *A. glycines* stages also did not show significant differences (ANOVA, $F_{6,60}=0.91$, $P=0.50$). The power of this analysis was also low, equalling 0.37. Among the 4th instar alatoid nymphs that developed

into mummies, 54.01±0.08% (average±SE) had transformed into winged adults prior to mummification, and the *B. communis* mummies produced from these also possessed wings.

The sex ratio of *B. communis* was highly male-biased on 2nd instar aphids, while female-biased on apterous *A. glycines* adults (table 1). Development time to mummification varied significantly with *A. glycines* stage (Kruskal-Wallis statistic=92.72, *P*<0.001) with mummification taking longest for alate adults. Time to emergence of both female and male *B. communis* also differed among *A. glycines* stages (Kruskal-Wallis statistic=40.41, *P*<0.001; KW statistic=64.27, *P*<0.001, respectively). In the various *A. glycines* stages, parasitoid development time gradually increased with aphid stage up until 4th instar. Parasitoids took longest to develop on alatoid nymphs and alate adults.

## No-choice assays of host acceptance

Although attack by *B. communis* females did not differ among the various *A. glycines* stages in no-choice assays (fig. 1; likelihood ratio $\chi^2$=1.23, *P*=0.97), parasitoid oviposition success did vary with stage (likelihood ratio $\chi^2$=15.65, *P*=0.02). Attacks on apterous adult, alate and alatoid nymphal stages were less likely to succeed than attacks on the apterous nymphal stages. Based on all replicates, we found *B. communis* to encounter 1st instars more frequently than other stages and alate adults less frequently than 2nd instars (table 2; Kruskal-Wallis statistic=16.26, *P*=0.01). Also, parasitoids probed immature *A. glycines* stages more frequently than alatoids and adults (Kruskal-Wallis statistic=18.284, *P*=0.006). First instar *A. glycines* were probed more frequently than other stages. The preferred location for oviposition was the aphid thorax, receiving 70.1% of successful ovipositions, compared to 17.5% for the head region and 12.4% for the abdomen. Aphid instars 1-4 received 72, 65, 72 and 66% of ovipositions in the thorax and 0, 5, 22 and 33% in the head region, respectively. Apterous adults, alatoids and alates received 80, 50 and 100% of oviposition in the thorax and 20, 50 and 0% in the head region, respectively.

The various *A. glycines* stages differed in their defensive behaviour upon attack by *B. communis* (fig. 2). Many of the immature aphid stages did not exhibit any defensive behaviour, and the frequency of inaction varied among *A. glycines* stages (Chi-square $\chi^2$=16.348, *P*=0.012<0.05). Kicking was the most frequently recorded behaviour (seen in 38% of the aphids). The frequency of kicking or body rotation varied among aphid stages (Chi-square $\chi^2$=21.818, *P*=0.001; $\chi^2$=24.718, *P*<0.001, respectively). Some aphids exhibited more than one type of defensive behaviour, commonly combining kicking with body rotation.

## Choice assay of host acceptance

Parasitoid wasps stayed within the patch for 21.64±1.40 min and encountered 43.80±3.67 (average±SE) aphids, indicating that aphids were frequently encountered more than once. *Binodoxys communis* encountered the different *A. glycines* stages to varying extents (fig. 3; ANOVA F6,168=5.71, *P*<0.001). There were also significant differences in attack rates among stages that were encountered and in oviposition rates among stages that were attacked (fig. 3; ANOVA $F_{6,168}$=9.76, *P*<0.001; Kruskal-Wallis $\chi^2$=74.61, *P*<0.001, respectively). Attack rates were highest for the young instars and lowest for alate and apterous adults, given their respective encounter rates. Lastly, 1st and 2nd instar *A. glycines* were also oviposited to highest extent, given their respective rates of attack. Parasitoids did not alter their preference for oviposition of certain aphid stages during the course of the experiment. Manly's Beta values for this action did not differ between the three intervals (Kruskal-Wallis statistic=1.88, *P*=0.39; Kruskal-Wallis statistic=1.60, *P*=0.45; Kruskal-Wallis statistic=3.95, *P*=0.14; Kruskal-Wallis statistic=0.53, *P*=0.77; Kruskal-Wallis statistic=0.30, *P*=0.86; Kruskal-Wallis statistic=1.98, *P*=0.37; Kruskal-Wallis statistic=1.86, *P*=0.39 for 1st, 2nd, 3rd, 4th instar, adult, alatoid and

alate stages, respectively). Also, *B. communis* did not modify its preference for encounter or attack of any of the aphid stages over the allotted 30 min period (fig. 4).

## Discussion

Many parasitoids in the braconid subfamily, Aphidiinae, preferentially parasitize small or intermediate host instars (Liu *et al*., 1984; Sequeira & Mackauer, 1987, 1992a; Weisser, 1994; Mackauer *et al*., 1996; Ives *et al*., 1999; Sharmila & Rajendra, 1999; Chau & Mackauer, 2000, 2001; Perdikis *et al*., 2004). Our research confirms this general pattern for *B. communis*, a parasitoid of the soybean aphid, *A. glycines*. No-choice assays showed a high proportion of successful attacks on all apterous nymphal *A. glycines* instars, while choice trials indicated lower encounter, attack and oviposition of apterous and alate adults, as well as alatoid nymphs. Nevertheless, parasitism trials with exposures over a longer time revealed similar *B. communis* parasitization of the various *A. glycines* stages. This disparity could hint at a lower suitability of young *A. glycines* instars for development of *B. communis*, as indicated below.

Parasitism of the various *A. glycines* stages possibly has major implications for fitness of *B. communis* offspring. Parasitism levels, mummy emergence and parasitoid sex ratio showed little differences among the various aphid stages. However, *B. communis* showed a higher rate of acceptance of young *A. glycines* instars compared to adults or alatoid nymphs. Thus, younger instars may have experienced greater mortality following parasitism (Rakhshani *et al*., 2004; Colinet *et al*., 2005). Alternative explanations are that super-parasitism levels of preferred younger instars is high or that host-stage preferences are not expressed in patches containing a single host stage, particularly for naïve parasitoids. Lastly, additional time of exposure (4 h) during parasitism trials could lead to higher parasitism rates of older stages despite short-term behavioural avoidance. With the exception of the *A. glycines* apterous adult stage, development time of both *B. communis* sexes increased with aphid stage. Various relationships exist between parasitoid development time and host age at oviposition (Hopper, 1986; Colinet *et al*., 2005), with positive associations being occasionally reported (Vinson, 1972; Lawrence *et al*., 1976). Rapid parasitoid development in 1st instar *A. glycines* shows that these hosts provide minimum required nutrient levels for *B. communis* (Henry *et al*., 2005) although parasitoids emerging from young hosts may be smaller. The gradual increase in development time on later *A. glycines* stages may reflect changes in nutritional value of the host, increased aphid resistance and competition of parasitoid larvae with the developing host embryos (Walker & Hoy, 2003; Colinet *et al*., 2005) or increased time necessary for development of a larger parasitoid. No evidence was found of delayed parasitoid development in younger or smaller hosts, a common pattern in koinobiont parasitoids (Harvey, 2005). Younger *A. glycines* instars were much smaller than later developmental stages (Hodgson *et al*., 2005; K. Wyckhuys, personal observation). The interaction between *B. communis* and *A. glycines* is also mediated by host behaviour, particularly aphid defense. In no-choice assays, *A. glycines* exhibited a variety of defensive behaviours, all of which are commonly observed among aphid species (e.g. Gerling *et al*., 1990; Hågvar & Hofsvang, 1991; De Farias & Hopper, 1999; Villagra *et al*., 2002). In no-choice assays, *B. communis* did not refrain from attacking larger or older aphid stages or aphids that exhibited stronger defenses. This could reflect either a low response threshold of *B. communis* for oviposition (Mackauer *et al*., 1996) or acceptance decisions resulting from its lack of previous experience (Henry *et al*., 2005). *Binodoxys communis* females encountered and probed larger aphid stages at a lower frequency and with many probing attempts unsuccessful. Like other members of the genera *Trioxys* and *Binodoxys*, *B. communis* uses a pair of terminal abdominal prongs to grasp the host prior to oviposition (Völkl & Mackauer, 2000), and this grasping is thought to be more effective on smaller instars. Also, as older *A. glycines* stages were less frequently oviposited in and exhibited more body rotation and walking behaviours, thus these defensive behaviours may deter *B. communis* attack. However, 4th instar apterous *A. glycines* exhibited kicking behaviour as frequently as 4th instar alatoid nymphs;

but the former were oviposited in as often as younger instar apterous nymphs, suggesting that this defense does not always work (see fig. 1).

In choice assays, *B. communis* females encountered alate morphs and 1st instars less often than other stages and morphs, while encountering 3rd and 4th instars at a higher rate than other stages and morphs. Although most parasitoids have poor ability to assess host suitability from a distance, they sometimes evaluate aphid shape, size or movement (Battaglia *et al.*, 1995; Le Ralec *et al.*, 2005). Our results suggest that *B. communis* might employ aphid size as an initial criterion to determine host suitability, while increased movement of *A. glycines* alates may act as a release stimulus for attack.

Because *B. communis* successfully develops on all *A. glycines* developmental stages, field releases do not need to target specific phases of aphid infestation. Also, considering that young aphid instars are generally more abundant than older stages in field populations (Hughes, 1963; Chau & Mackauer, 1997; Losey & Denno, 1998), parasitoids are very likely to successfully establish irrespective of *A. glycines* colony composition. Parasitoid preference for younger stages can significantly affect host population growth (e.g. Lin & Ives, 2003), while a sustained attack of older and larger *A. glycines* stages, along with its induction of costly defenses could reduce reproductive capacity of *B. communis* (Nelson & Rosenheim, 2005).

Successful *B. communis* parasitism of *A. glycines* alatoid nymphs and alates and its increased development time on winged aphid hosts suggests the existence of a phoretic association. Such association was suggested by Hoelmer & Kirk (2005), who reported the presence of *B. communis* in association with *A. glycines* at early stages of their colonization of soybean fields in China and hypothesized parasitoid arrival as eggs carried within winged aphids (see also Liu *et al.*, 2004). Also, the finding that *B. communis* does not disrupt the development of wings of *A. glycines* alatoid nymphs (see Demmon *et al.*, 2004; Christiansen-Weniger & Hardie, 2000) indicates that flight of parasitized aphids might be possible. Our findings can have implications for parasitoid establishment, dispersal capability and biological control success upon release in novel environments.

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## References

Battaglia D, Pennacchio F, Romano A, Tranfaglia A. The role of physical cues in the regulation of host recognition and acceptance behavior of *Aphidius ervi* Haliday (Hymenoptera: Braconidae). Journal of Insect Behavior 1995;8:739–750.

Chau A, Mackauer M. Dropping of pea aphids from feeding site: a consequence of parasitism by the wasp, *Monoctonus paulensis*. EntomologiaExperimentalis et Applicata 1997;83:247–252.

Chau A, Mackauer M. Host-instar selection in the aphid parasitoid *Monoctonus paulensis* (Hymenoptera: Braconidae, Aphidiinae): a preference for small aphids. European Journal of Entomology 2000;97:347–353.

Chau A, Mackauer M. Host-instar selection in the aphid parasitoid *Monoctonus paulensis* (Hymenoptera: Braconidae, Aphidiinae): assessing costs and benefits. Canadian Entomologist 2001;133:549–564.

Christiansen-Weniger P, Hardie J. The influence of parasitism on wing development in male and female pea aphids. Journal of Insect Physiology 2000;46:861–867. [PubMed: 10802097]

Colinet H, Salin C, Boivin G, Hance Th. Host age and fitness-related traits in a koinobiont aphid parasitoid. Ecological Entomology 2005;30:473–479.

De Farias AMI, Hopper KR. Oviposition behavior of *Aphelinus asychis* and *Aphidius matricariae* and defense behavior of their host *Diuraphis noxia*. Environmental Entomology 1999;28:858–862.

Demmon AS, Nelson HJ, Ryan PJ, Ives AR, Snyder WE. *Aphidius ervi* (Hymenoptera: Braconidae) increases its adult size by disrupting host wing development. Environmental Entomology 2004;33:1523–1527.

Desneux N, Wajnberg E, Fauverge X, Privet S, Kaiser L. Oviposition behavior and patch-time allocation in two aphid parasitoids exposed to deltamethrin residues. Entomologia Experimental et Applicata 2004;112:227–235.

Dixon, AFG. Aphid Ecology. Chapman & Hall; London, UK: 1998. p. 288

Faul F, Erdfelder E, Lang A-G, Buchner A. G*Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behavior Research Methods 2007;39:175–191. [PubMed: 17695343]

Gerling D, Roitberg BD, Mackauer M. Instar-specific defense of the pea aphid *Acyrthosiphon pisum*: influence on oviposition success of the parasite *Aphelinus asychis* (Hymenoptera: Aphelinidae). Journal of Insect Behavior 1990;3:501–514.

Godfray, HCJ. Parasitoids: Behavioral and Evolutionary Ecology. Princeton University Press; Princeton, NJ, USA: 1994. p. 488

Hågvar EB, Hofsvang T. Aphid parasitoids (Hymenoptera: Aphidiidae): biology, host selection and use in biological control. Biocontrol News and Information 1991;12:13–41.

Harvey JA. Factors affecting the evolution of development strategies in parasitoid wasps: the importance of functional constraints and incorporating complexity. Entomologia Experimentalis et Applicata 2005;117:1–13.

Heimpel GE, Ragsdale DW, Venette R, Hopper KR, O'Neil RJ, Rutledge CE, Wu Z. Prospects for importation biological control of the soybean aphid: anticipating potential costs and benefits. Annals of the Entomological Society of America 2004;97:249–258.

Henry LM, Gillespie DR, Roitberg BD. Does mother really know best? Oviposition preference reduces reproductive performance in the generalist parasitoid *Aphidius ervi*. Entomologia Experimentalis et Applicata 2005;116:167–174.

Hight SC, Eikenbary RD, Miller RJ, Starks KJ. The greenbug and *Lysiphlebus testaceipes*. Environmental Entomology 1972;1:205–209.

Hodgson EW, Venette RC, brahamson M, Ragsdale DW. Alate production of soybean aphid (Homoptera: Aphididae) in Minnesota. Environmental Entomology 2005;34:1456–1463.

Hoelmer KA, Kirk AA. Selecting arthropod biological control agents against arthropod pests: can the science be improved to decrease the risk of releasing ineffective agents? Biological Control 2005;34:255–264.

Hopper KR. Preference, acceptance and fitness components of *Microplitis croceipes* (Hymenoptera: Braconidae) attacking various instars of *Heliothis virescens* (Lepidoptera: Noctuidae). Environmental Entomology 1986;15:274–280.

Hopper KR, King EG. Preference of *Microplitis croceipes* (Hymenoptera: Braconidae) for instars and species of *Heliothis* (Lepidoptera: Noctuidae). Environmental Entomology 1984;13:1145–1150.

Hughes RD. Population dynamics of the cabbage aphid, *Brevicoryne brassicae* (L.). Journal of Animal Ecology 1963;32:393–424.

Ives AR, Schooler SS, Jagar VJ, Knuteson SE, Grbic M, Settle WH. Variability and parasitoid foraging efficiency: a case study of pea aphids and *Aphidius ervi*. American Naturalist 1999;154:652–673.

Jenner W, Kuhlmann U. Significance of host size for a solitary endoparasitoid: a trade-off between fitness parameters. Basic and Applied Ecology 2006;7:461–471.

Kelly EOG. The green-bug (*Toxoptera graminum* Rond.) outbreak of 1916. Journal of Economic Entomology 1917;10:233–248.

Kouamé KL, Mackauer M. Influence of aphid size, age and behaviour on host choice by the parasitoid wasp *Ephedrus californicus*: a test of host-size models. Oecologia 1991;88:197–203.

Lacoume S, Bressac C, Chevrier C. Effect of host size on male fitness in the parasitoid wasp *Dinarmus basalis*. Journal of Insect Physiology 2006;52:249–254. [PubMed: 16359698]

Landau, S.; Everitt, BS. A Handbook of Statistical Analyses Using SPSS. Chapman & Hall/CRC Press; Boca Raton, FL, USA: 2004. p. 354

Lawrence PO, Baranowski RM, Greany PD. Effect of host age on development of *Biosteres longicaudatus*, a parasitoid of the Carribean fruitfly, *Anastrepha suspense*. Florida Entomologist 1976;59:33–39.

Le Ralec A, Curty C, Wajnberg K. Inter-specific variation in the reactive distance of different aphid-parasitoid associations: analysis from automatic tracking of the walking path. Applied Entomology and Zoology 2005;40:413–420.

Lewis WJ, Redlinger LJ. Suitability of eggs of the almond moth *Cadra cautella* of various ages for parasitism by *Trichogramma evanescens*. Annals of the Entomological Society of America 1969;62:1482–1484.

Li BP, Mills N. The influence of temperature on size as an indicator of host quality for the development of a solitary koinobiont parasitoid. Entomologia Eperimentalis et Applicata 2004;110:249–256.

Lin LA, Ives AR. The effect of parasitoid host size preference on host population growth rates: an example of *Aphidius colemani* and *Aphis glycines*. Ecological Entomology 2003;28:542–550.

Liu J, Wu KM, Hopper KR, Zhao KJ. Population dynamics of *Aphis glycines* (Homoptera: Aphididae) and its natural enemies in soybean in northern China. Annals of the Entomological Society of America 2004;97:235–239.

Liu SS, Morton R, Hughes RD. Oviposition preferences of a hymenopterous parasite for certain instars of its aphid host. Entomologia Experimentalis et Applicata 1984;35:249–254.

Losey JE, Denno RF. The escape response of pea aphids to foliar-foraging predators: factors affecting dropping behaviour. Ecological Entomology 1998;23:53–61.

Mackauer, M. Host selection and host suitability in *Aphidius smithi*. In: Lowe, AD., editor. Perspectives in Aphid Biology. Entomological Society of New Zealand; Christchurch, New Zealand: 1973. p. 20-29.

Mackauer M, Michaud JP, ölkl W. Host choice by aphidiid parasitoids (Hymenoptera: Aphidiidae): host recognition, host quality and host value. Canadian Entomologist 1996;128:959–980.

Manly BFJ. A model for certain types of selection experiments. Biometrics 1974;30:281–294.

McWilliams, DA.; Berglund, DR.; Endres, GJ. Soybean growth and management. North Dakota State University; 2004. Extension Publication No. A-1174. http://www.ag.ndsu.edu/pubs/plantsci/rowcrops/a1174/a1174w.htm. Accessed on March 2, 2007

Murdoch WW, Nisbet RM, Blythe SP, Gurney WSC, Reeve JD. An invulnerable age class and stability in delay-differential parasitoid-host models. American Naturalist 1987;129:263–282.

Murdoch, WW.; Briggs, CJ.; Nisbet, RM. Consumer-Resource Dynamics. Monographs in Population Biology. Princeton University Press; Princeton, NJ, USA: 2003. p. 456

Nechols JR, Kikuchi RS. Host selection of the spherical mealybug (Homoptera: Pseudococcidae) by *Anagyrus indicus* (Hymenoptera: Encyrtidae): influence of host age on parasitoid oviposition, development, sex ratio and survival. Environmental Entomology 1985;14:32–37.

Nelson EH, Rosenheim JA. Encounters between aphids and their predators: the relative frequencies of disturbance and consumption. Entomologia Experimentalis et Applicata 2005;118:211–219.

Perdikis DC, Lkouressis DR, Gaantonakis NG, Iatrou SA. Instar preference and parasitization of *Aphis gossypii* and *Myzus persicae* (Hemiptera: Aphididae) by the parasitoid *Aphidius colemani* (Hymenoptera: Aphididae). European Journal of Entomology 2004;101:333–336.

Ragsdale DW, Voegtlin DJ, O'Neil RJ. Soybean aphid biology in North America. Annals of the Entomological Society of America 2004;97:204–208.

Rakhshani E, Talebim AA, Kavllieratos N, Fathipour Y. Host stage preference, juvenile mortality and functional response of *Trioxys pallidus*. Biologia 2004;59:197–203.

Rauwald KS, Ives AR. Biological control in disturbed agricultural systems and the rapid recovery of parasitoid populations. Ecological Applications 2001;11:1224–1234.

Rivero A. The relationship between host selection behaviour and offspring fitness in a koinobiont parasitoid. Ecological Entomology 2000;25:467–472.

Rogers CE, Jackson HB, Eikenbary RD, Starks KJ. Host-parasitoid interaction of *Aphis helianthi* on sunflowers with introduced *Aphelinus asychis, Ephedrus plagiator*, and *Praon gallicum*, and native

*Aphelinus nigritus* and *Lysiphlebus testaceipes*. Annals of the Entomological Society of America 1972;65:38–41.

Roitberg BD, Boivin G, Vet LEM. Fitness, parasitoids and biological control: an opinion. Canadian Entomologist 2001;133:429–438.

Sequeira R, Mackauer M. Host instar preference of the aphid parasite *Praon pequodorum* (Hymenoptera: Aphidiidae). Entomologia Generalis 1987;12:259–265.

Sequeira R, Mackauer M. Covariance of adult size and development time in the parasitoid wasps Aphidius ervi in relation to its host, *Acyrthosiphum pisum*. Evolutionary Ecology 1992a;6:34–44.

Sequeira R, Mackauer M. Nutritional ecology of an insect host parasitoid association - the pea aphid *Aphidius ervi* system. Ecology 1992b;73:183–189.

Sequeira R, Mackauer M. The nutritional ecology of a parasitoid wasp, *Ephedrus californicus* Baker (Hymenoptera: Aphidiidae). Canadian Entomologist 1993;125:423–430.

Sharmila P, Rajendra S. Host size induced variation in progeny sex ratio of an aphid parasitoid *Lysiphlebia myrzai*. Entomologia Experimentalis et Applicata 1999;90:61–67.

Stadler B, Weisser WW, Houston AI. Defense reactions in aphids - the influence of state and future reproductive success. Journal of Animal Ecology 1994;63:419–430.

Villagra CA, Ramirez CC, Niemeyer H. Anti-predator responses of aphids to parasitoids change as function of aphid physiological state. Animal Behaviour 2002;64:677–683.

Vinson SB. Effect of the parasitoid *Campoletis sonorensis* on the growth of its host, *Heliothis virescens*. Journal of Insect Physiology 1972;18:1509–1514.

Völkl W, Mackauer M. Oviposition behaviour of aphidiine wasps (Hymenoptera: Braconidae, Aphidiinae): morphological adaptations and evolutionary trends. Canadian Entomologist 2000;132:197–212.

Walker AM, Hoy MA. Responses of *Lipolexis oregmae* (Hymenoptera: Aphidiidae) to different instars of *Toxoptera citricida* (Homoptera: Aphididae). Journal of Economic Entomology 2003;96:1685–1692. [PubMed: 14977104]

Wang XG, Liu SS. Effects of host age on the performance of *Diadromus collaris*, a pupal parasitoid of *Plutella xylostella*. Biocontrol 2002;47:293–307.

Weisser WW. Age-dependent foraging behaviour and host instar preference of the aphid parasitoid *Lysiphlebus cardui*. Entomologia Experimentalis et Applicata 1994;76:133–141.

Weisser WW. Within-patch foraging behaviour of the aphid parasitoid *Aphidius funebris*: plant architecture, host behaviour and individual variation. EntomologiaExperimentalis et Applicata 1995;76:133–141.

Wu Z, Hopper KR, O'Neil RJ, Voegtlin DJ, Prokrym DR, Heimpel G. Reproductive compatibility and genetic variation between two strains of *Aphelinus albipodus* (Hymenoptera: Aphelinidae), a parasitoid of the soybean aphid, *Aphis glycines* (Homoptera: Aphididae). Biological Control 2004;31:311–319.

Wyckhuys KAG, Heimpel GE. Response of the soybean aphid parasitoid *Binodoxys communis* to olfactory cues from target and non-target host-plant complexes. Entomologia Experimentalis et Applicata 2007;123:149–158.

Wyckhuys KAG, Koch RL, Heimpel GE. Physical and ant-mediated refuges from parasitism: implications for non-target effects in biological control. Biological Control 2007a;40:306–313.

Wyckhuys KAG, Hopper KR, Wu KM, Straub C, Gratton C, Heimpel GE. Predicting potential ecological impact of soybean aphid biological control introductions. CABI Biocontrol News and Information 2007b;28:30N–34N.
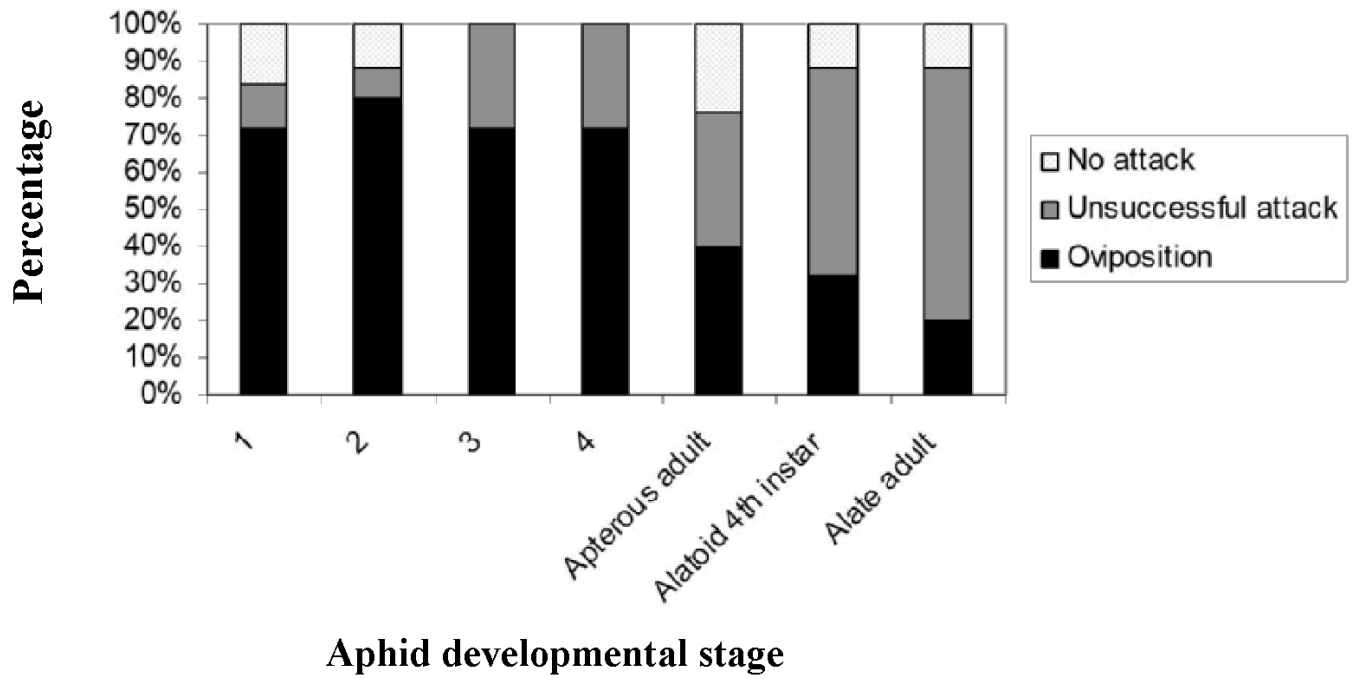
**Fig. 1.**
Outcome of *B. communis* encounters with the various *A. glycines* developmental stages in a no-choice experiment. The graph represents the proportion of aphids (out of 25) of a given stage that were attacked or accepted for oviposition by *B. communis*.
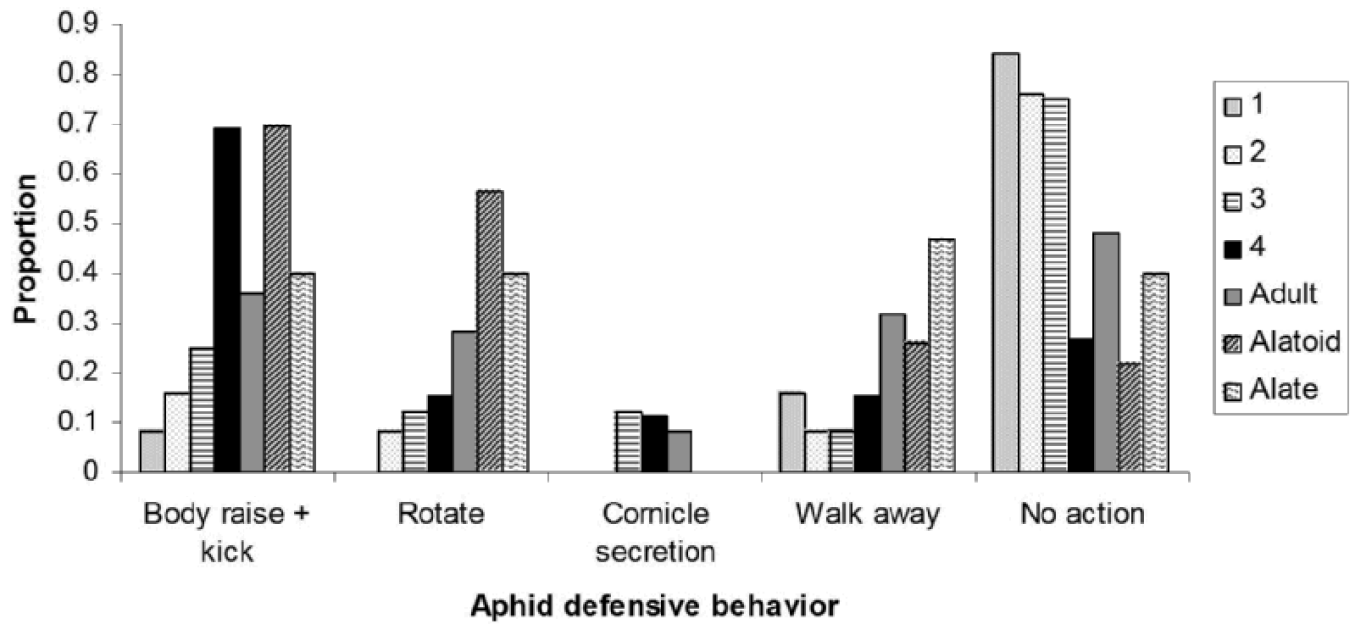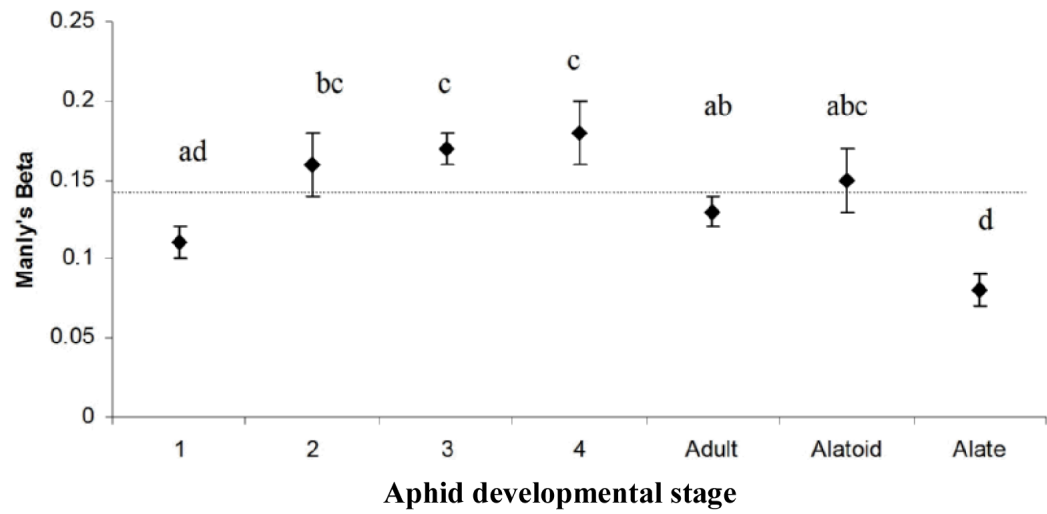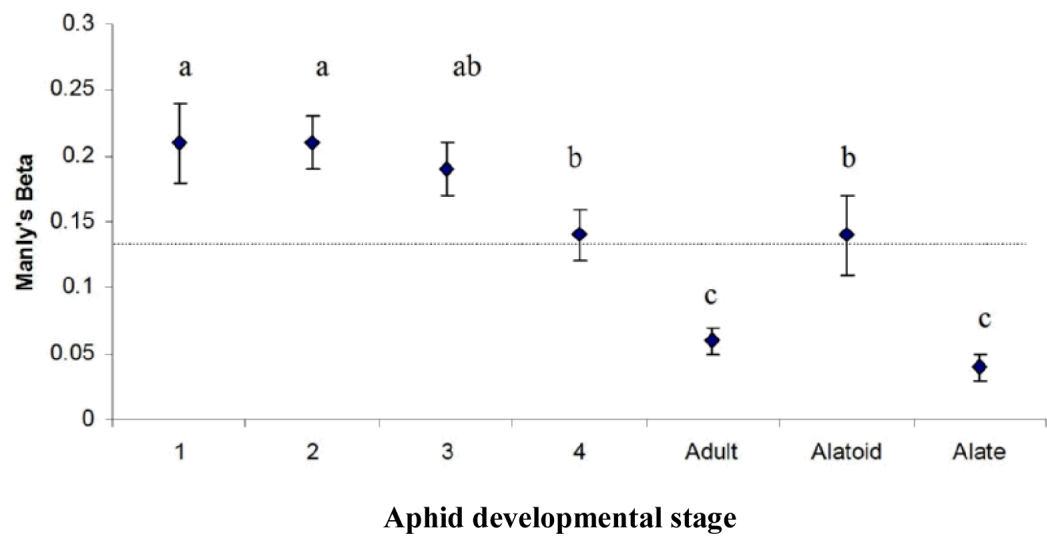
**Fig. 2.**
Aphid defensive behaviours associated with *B. communis* attacking the various developmental stages of the soybean aphid, *Aphis glycines*, in a no-choice experiment. The proportion of aphids (out of 25) of a given instar exhibiting certain behaviours is indicated.
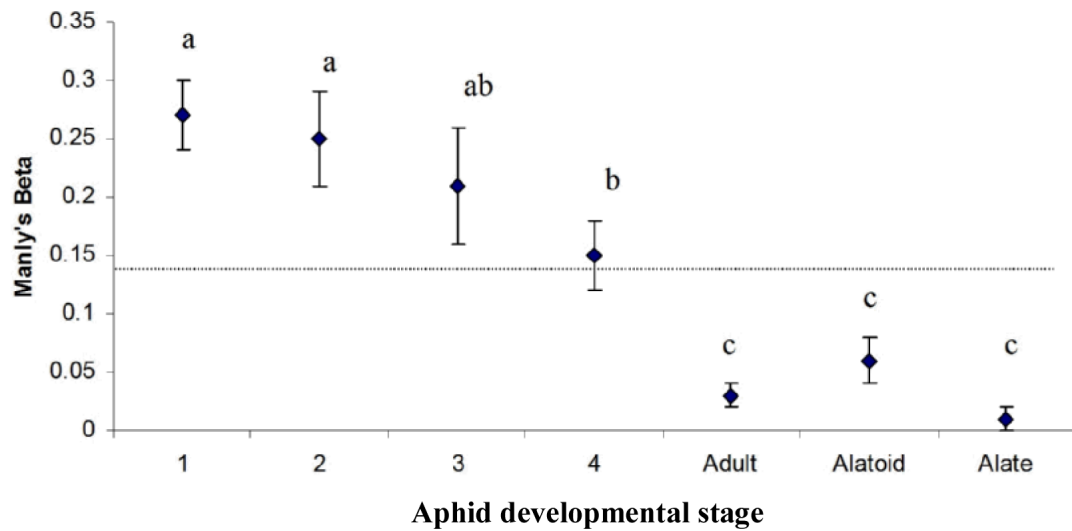
Encounter as a function of # aphids in the patch



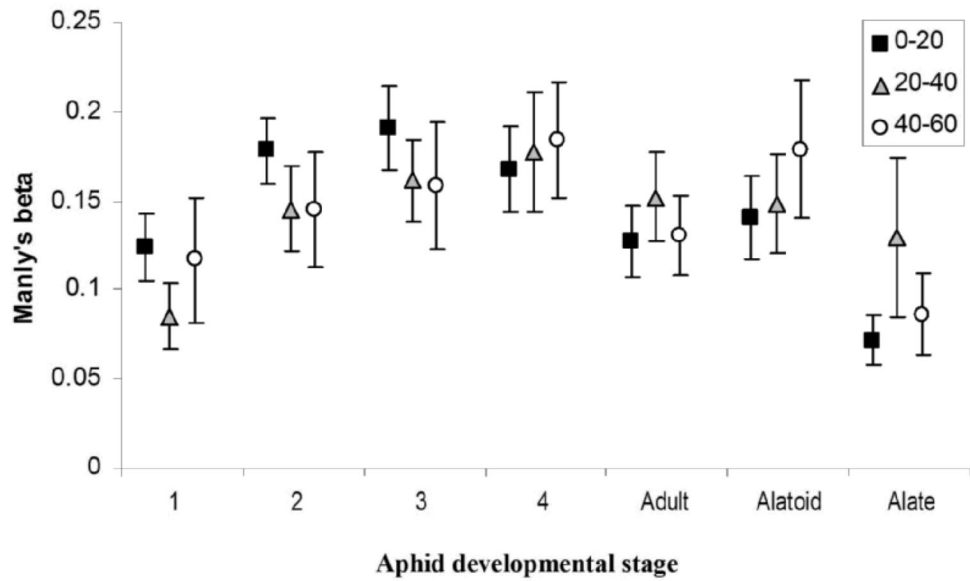Attack as a function of # aphids encountered

## Oviposition as a function of # aphids attacked



**Fig. 3.**
Average (±SE) Manly's Beta values for *B. communis* behaviour in patches with a total of 35 *A. glycines* individuals, equally distributed among all seven different developmental stages. Within each figure, the line represent the baseline value (1/7) against which Manly's Beta values for each stage are compared. Different letters indicate significantly different Manly's Beta values for the subsequent aphid developmental stages (*P*>0.05, one-way ANOVA with Fisher's protected LSD post hoc analysis).

Encounter as a function of # aphids in the patch


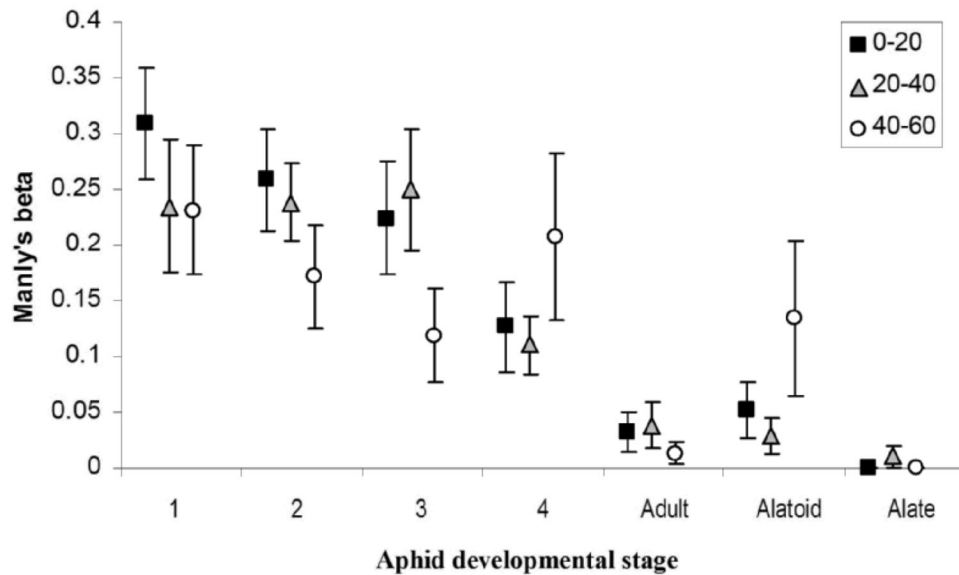
Oviposition as a function of # aphids encountered



**Fig. 4.**
Variation in *B. communis* preference for the different *A. glycines* developmental stages over the course of a 30 min choice experiment. Parasitoid preference for a given stage is represented as Manly's Beta values (±SE), which indicate whether the various stages are either encountered in the same proportion as they are present within the patch or oviposited in the same proportion as they are encountered. Values are computed for the following intervals: encounters 0-20, 20-40 and 40-60.

**Table 1**

Life history traits of *Binodoxys communis* progeny emerging from the various developmental stages of its aphid host, *Aphis glycines*. Only one aphid stage was exposed to each adult female parasitoid in this experiment.

| Host instar | Life history trait | | | | | |
|---|---|---|---|---|---|---|
| | Number of mummies formed | Proportion (of mummified aphids) emerged | Sex ratio (proportion males) | Days until mummification | Female development time | Male development time |
| 1 | 7.22±2.13a | 0.51±0.10a | 0.56±0.14ab | 7.00±0.12ab | 11.25±0.44a | 10.15±0.08a |
| 2 | 10.66±1.85a | 0.62±0.06a | 0.69±0.09b | 7.25±0.16b | 11.45±0.33ab | 11.23±0.28b |
| 3 | 12.60±1.62a | 0.73±0.07a | 0.59±0.11ab | 6.79±0.12a | 11.77±0.29ab | 11.95±0.22cd |
| 4 | 16.40±2.81a | 0.68±0.06a | 0.42±0.11ab | 7.07±0.14ab | 12.12±0.24b | 12.16±0.23d |
| Apterous adult | 10.56±2.90a | 0.61±0.10a | 0.34±0.08a | 6.94±0.11ab | 11.27±0.13a | 11.41±0.13bc |
| Alatoid 4th instar | 9.80±2.14a | 0.53±0.09a | 0.48±0.12ab | 6.80±0.12a | 12.59±0.29bc | 12.88±0.44de |
| Alate adult | 15.70±5.37a | 0.59±0.06a | 0.56±0.11ab | 8.40±0.15c | 13.00±0.26c | 13.03±0.26e |

Mean±SE; values within the same column followed by identical letters are not significantly different ($P>0.05$, one-way ANOVA with Fisher's protected LSD).

## Table 2

Acceptance behaviour of *B. communis* upon encounter with different *A. glycines* developmental stages in a no-choice experiment. Behavioural parameters are reported for all replicates (*N*=25) and for wasps that successfully oviposited in the aphids presented. Number of probing events and number of encounters are indicated as frequencies over the allotted time (i.e. 5 min or until successful oviposition). Parameters include the total number of probing events or encounters and the total time until oviposition.

| All replicates (*N*=25) | | |
|---|---|---|
| Host stage | Number of encounters (min$^{-1}$) | Number of probing events (min$^{-1}$) |
| 1 | 3.27±0.47a* | 3.88±0.60a |
| 2 | 2.34±0.26b | 3.49±0.58ab |
| 3 | 2.27±0.33b | 3.99±0.60a |
| 4 | 2.16±0.23bc | 3.70±0.57ab |
| Apterous adult | 1.79±0.31bc | 2.24±0.53bc |
| Alatoid 4th instar | 1.77±0.19bc | 2.24±0.64bc |
| Alate adult | 1.39±0.12c | 1.70±0.32c |

| Observations where successful oviposition was recorded | | | | |
|---|---|---|---|---|
| Host stage | N | Number of encounters (min$^{-1}$) | Number of probing events (min$^{-1}$) | Time until oviposition (s) |
| 1 | 18 | 3.97±0.55a | 2.25±0.12a | 34.00±8.04a |
| 2 | 20 | 2.69±0.26b | 1.98±0.14a | 58.90±12.21a |
| 3 | 18 | 2.81±0.38b | 2.08±0.17a | 71.83±18.37a |
| 4 | 18 | 2.50±0.27b | 2.10±0.15a | 70.22±12.93a |
| Apterous adult | 10 | 2.91±0.59ab | 2.12±0.17a | 69.10±26.13ab |
| Alatoid 4th instar | 8 | 2.25±0.31b | 2.19±0.31a | 129.75±25.66c |
| Alate adult | 5 | 1.65±0.42b | 1.83±0.08a | 136.80±32.42bc |

Mean±SE; values within the same column followed by identical letters are not significantly different (*P*>0.05, Mann-Whitney *U* test