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

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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.

**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.
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 distort- ing 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. x 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 (\( \sum_{i=1}^{\text{Ix}} A_i = N = \text{total number eligible for this action} \)), and let \( x_i \) be the number of stage \( i \) that have been selected for this
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.

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

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).

particular action and \(r_i\) the number that have not been selected (so that \(x_i + r_i = A_j\)). We considered the case in which an aphid already selected for an interaction is still eligible for this action (Weisser, 1994).

Then

\[
\beta_j = \frac{(x_j/A_j)}{\sum_{i=1}^{7} x_i/A_i}
\]

is Manly’s Beta of the \(j\)th stage for this particular action (with a total of seven different stages being considered). If \(\beta_j\) is greater than \(1/7\) for any given developmental stage \(j\), then the parasitoid prefers this given stage for the action under consideration. If \(\beta_j\) is less than \(1/7\) then the parasitoid avoids this interaction with stage \(j\) and, finally, if \(\beta_j = 1/7\), 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, \(F_{6,62} = 0.91, P = 0.50\)). 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 alatoid *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...
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)

<table>
<thead>
<tr>
<th>Host stage</th>
<th>Number of encounters (min⁻¹)</th>
<th>Number of probing events (min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.27 ± 0.47a</td>
<td>3.88 ± 0.60a</td>
</tr>
<tr>
<td>2</td>
<td>2.34 ± 0.26b</td>
<td>3.49 ± 0.58ab</td>
</tr>
<tr>
<td>3</td>
<td>2.27 ± 0.33b</td>
<td>3.99 ± 0.66a</td>
</tr>
<tr>
<td>4</td>
<td>2.16 ± 0.23bc</td>
<td>3.70 ± 0.57ab</td>
</tr>
<tr>
<td>Apterous adult</td>
<td>1.79 ± 0.31bc</td>
<td>2.24 ± 0.53bc</td>
</tr>
<tr>
<td>Alatoid 4th instar</td>
<td>1.77 ± 0.19bc</td>
<td>2.24 ± 0.64bc</td>
</tr>
<tr>
<td>Alate adult</td>
<td>1.39 ± 0.12c</td>
<td>1.70 ± 0.32c</td>
</tr>
</tbody>
</table>

Observations where successful oviposition was recorded

<table>
<thead>
<tr>
<th>Host stage</th>
<th>Number of encounters (min⁻¹)</th>
<th>Number of probing events (min⁻¹)</th>
<th>Time until oviposition (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.97 ± 0.55a</td>
<td>2.25 ± 0.12a</td>
<td>34.00 ± 8.04a</td>
</tr>
<tr>
<td>2</td>
<td>2.69 ± 0.26b</td>
<td>1.98 ± 0.14a</td>
<td>58.90 ± 12.21a</td>
</tr>
<tr>
<td>3</td>
<td>2.81 ± 0.38b</td>
<td>2.08 ± 0.17a</td>
<td>71.83 ± 18.37a</td>
</tr>
<tr>
<td>4</td>
<td>2.50 ± 0.27b</td>
<td>2.10 ± 0.15a</td>
<td>70.22 ± 12.93a</td>
</tr>
<tr>
<td>Apterous adult</td>
<td>2.91 ± 0.59ab</td>
<td>2.12 ± 0.17a</td>
<td>69.10 ± 26.13ab</td>
</tr>
<tr>
<td>Alatoid 4th instar</td>
<td>2.25 ± 0.31b</td>
<td>2.19 ± 0.31a</td>
<td>129.75 ± 25.66c</td>
</tr>
<tr>
<td>Alate adult</td>
<td>1.65 ± 0.42b</td>
<td>1.83 ± 0.08a</td>
<td>136.80 ± 32.42bc</td>
</tr>
</tbody>
</table>

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

Parasitoid oviposition success did vary with stage (likelihood ratio χ² = 15.65, *P* = 0.02). Attacks on alate adults and alatoids were more 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 alate 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 χ² = 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 χ² = 21.818, *P* = 0.001; χ² = 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**

Parasitoids 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 *F* = 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* = 9.76, *P* < 0.001; Kruskal-Wallis χ² = 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, $B. \text{communis}$ 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 &
Parasitism of *Aphis glycines* by *Binodoxys communis*

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 (4h) 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 distance, they sometimes evaluate aphid shape, size or *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).

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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**


