Parasitism of Autumnal Morphs of the Soybean Aphid (Hemiptera: Aphididae) by *Binodoxys communis* (Hymenoptera: Braconidae) on Buckthorn

Author(s): Mark K. Asplen, Kris A. G. Wyckhuys, and George E. Heimpel
Published By: Entomological Society of America
DOI: 10.1603/AN10172
Parasitism of Autumnal Morphs of the Soybean Aphid (Hemiptera: Aphididae) by *Binodoxys communis* (Hymenoptera: Braconidae) on Buckthorn

MARK K. ASPLEN, KRIS A. G. WYCKHUYS, AND GEORGE E. HEIMPEL

**ABSTRACT** The soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), is both heteroecious and holocyclic, seasonally alternating between buckthorn (*Rhamnus* spp.), (the primary, overwintering host) and soybean, *Glycine max* (L.) Merr. (the secondary host). Recently, a classical biological control program for this invasive pest has been implemented in North America using the Asian aphidiine braconid wasp *Binodoxys communis* Gahan. Two critical, related questions regarding the overwintering biology of *B. communis* are 1) does the parasitoid maintain fidelity to *A. glycines* throughout the aphid life cycle and follow it to its primary host; and, if it does, 2) is parasitoid migration facilitated by phoretic movement within buckthorn-specific winged aphids? In the laboratory, we compared *B. communis* parasitism on several different autumnal morphs of *A. glycines*: winged gynoparae (fall migrants) and their oviparous offspring on buckthorn, fourth-instar alatoid nymphs that would form either gynoparae or summer migrants on soybean, and third-instar gynoparous alatoid nymphs on soybean. We also introduced gynoparae and *B. communis* onto caged buckthorn plants in southeastern Minnesota to examine autumnal parasitism under natural conditions. In both the laboratory and field, parasitism rates of oviparae were much higher than those of gynoparae. In addition, *B. communis* rarely completed development on fourth-instar alatoid nymphs. Although wasps successfully developed on third-instar gynoparous nymphs, these hosts mummified before forming wings. These results suggest that although at least one buckthorn-specific morph of *A. glycines* seems suitable for *B. communis* parasitism, it is unlikely that alate-mediated dispersal of immature parasitoids is an adaptive strategy to locate *Rhamnus* in this species.

**KEY WORDS** classical biological control, dispersal, host alternation, overwintering, soybean aphid

Common buckthorn, *Rhamnus cathartica* L., is an invasive shrub that is native to Europe and serves as the main overwintering host for the soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), an Asian species that has become a severe pest in North America (Ragsdale et al. 2007 and 2011, Johnson et al. 2009). The *A. glycines* life cycle (Fig. 1) begins with fundatrices hatching each spring from overwintering eggs and clonally reproducing on buckthorn (Ragsdale et al. 2004). By the third generation, spring migrants are formed that migrate to the secondary host, soybean, *Glycine max* (L.) Merr. These alates and subsequent generations clonally propagate on soybean to produce both apterous and alate offspring. In the fall, females of a new winged morph (gynoparae) and winged males are formed, which then migrate back to *Rhamnus*. Subsequently, gynoparae clonally produce wingless oviparae, which mate with the later arriving males and lay overwintering eggs on buckthorn stems and branches.

Because current *A. glycines* management programs rely heavily upon broad-spectrum insecticides (Ragsdale et al. 2007, Ohnesorg et al. 2009), classical biological control may prove a particularly attractive alternative (Heimpel et al. 2004, Wu et al. 2004, Hoelmer and Kirk 2005). The Asian parasitoid wasp *Binodoxys communis* (Gahan) (Braconidae: Aphidiinae) was approved for release in 2007 against *A. glycines* in the north central United States after quarantine testing of potential efficacy and safety (Wyckhuys et al. 2007, 2008; Wyckhuys and Heimpel 2007; Desneux et al. 2009). Although data from open-field releases of *B. communis* continue to be collected, early studies suggest that factors such as biotic interference (Chacón et al. 2008, Chacón and Heimpel 2010) and parasitoid dispersal (Asplen et al., in prep.) may strongly impact the efficacy of this classical biological control agent.

Although laboratory experiments and initial field releases have increased our understanding of the potential for *B. communis* to control *A. glycines* populations, the overwintering biology of *B. communis* remains a key unknown characteristic in the interaction...
between these two species. In particular, a critical question is whether or not *B. communis* parasitizes *A. glycines* throughout its life cycle, thus following it to *Rhamnus* during the aphid’s fall host alternation. A connection between buckthorn and *B. communis* has been implied based upon observed *A. glycines* parasitism in early-season soybean in China (Hoelmer and Kirk 2005, Wyckhuys et al. 2008). Hoelmer and Kirk (2005) hypothesized that this pattern is potentially explained by dispersal of parasitoid eggs within emigrating spring migrants of *A. glycines*. All aphidline braconids are endoparasitic koinobionts (Brodeur and McNeil 1989) before hatching from the substrate (Brodeur and McNeil 1989) before hatching from the substrate. Evidence of immature parasitoid dispersal within alate aphids has been observed (Rauwald and Kirk 2005, Hoelmer and Kirk 2005). Hoelmer and Kirk’s (2005) observations result from spring parasitism by *B. communis* on buckthorn, it is possible that all or some of the ovipositing females immigrate from alternative overwintering sites.

Here, we examined the potential for *B. communis* to use fall stages of *A. glycines*, specifically gynoparous and oviparous (preliminary trials suggested that alate males were unsuitable hosts; Heimpel et al. 2010). First, we examined the performance of *B. communis* when encountering fall colonies on buckthorn in both the laboratory and in field cages (study 1). Second, we examined the relative suitability of fourth-instar gynoparous and summer alatoid nymphs on soybean for *B. communis* oviposition and development by using no-choice laboratory experiments (study 2). Finally, we examined the potential for third-instar gynoparous alatoids to support *B. communis* oviposition and development and the probability that parasitized third-instar alatoids molt into alates before mummification (study 3). These experiments shed light on two critical parts of the buckthorn overwintering hypothesis: 1) the suitability of fall, buckthorn-specific *A. glycines* stages for *B. communis* parasitism, and 2) the likelihood that gynoparous females immigrate from alternative overwintering sites.

Materials and Methods

Plants. Mature, fully unfolded buckthorn leaves were collected from transplanted *Rhamnus* seedlings (<30 cm in height), obtained from wild populations in Waseca, MN (summer 2008) and St. Paul, MN (spring and fall 2009; spring 2010). Leaves from Waseca-collected plants were used in study 1 (see below), whereas leaves from the St. Paul-collected plants were used in studies 2 and 3 (see below). All buckthorn plants were kept under greenhouse conditions (≥25–30°C and a photoperiod of 16:8 [L:D] h) in 14- by 14-cm pots filled with SunGro Metro Mix 200 potting soil (SunGro Horticulture Canada Ltd., Seba Beach, AB, Canada) and pruned periodically to regulate the sizes of the shrubs. *Rhamnus* shrubs were maintained until they reached a maximum height of ≥60 cm. Soybean leaves (“NK S19 R5”) were obtained from first trifoliate stage seedlings grown under identical greenhouse conditions to the *Rhamnus* plants.

Insects. All laboratory experiments were conducted in the Minnesota Department of Agriculture/Minnesota Agricultural Experiment Station Quarantine Facility (MDA/MAES) on the St. Paul Campus of the University of Minnesota. Except where noted, all insects were reared in growth chambers at 25°C, ~75% RH, and a photoperiod of 16:8 (L:D) h.

Aphids. Experimental *A. glycines* originated from a clonally propagated colony initiated from field-collected viviparous from a soybean field in St. Paul, MN, in 2003 and periodically supplemented with field-caught aphids from the St. Paul campus. Colonies were maintained on whole soybean plants (NK S19 R5).
Soybean-specific aphid stages were collected from this colony for further experimentation.

Gynoparous *A. glycines* were obtained in the laboratory through manipulation of temperature and photoperiod, after Yoo et al. (2005). More specifically, *A. glycines* apterai from the MDA/MAES source colony were transferred onto potted soybean plants (two to three plants at the first unifoliate stage per 311-mm² pot), and subjected to fall environmental conditions (i.e., 16°C, 55–75% RH, and a photoperiod of 10:14 [L:D] h) in a growth chamber. As gynoparous and summer alate/alate *A. glycines* are difficult to distinguish morphologically (Voegtlin et al. 2004), we developed bioassays that allowed us to determine whether alates produced were summer migrants or gynoparous.

Before experiments involving gynoparous adults (study 1), groups of 15 alates were sampled randomly and individually placed onto soybean leaves in 100-mm-diameter plastic petri dishes each containing a single buckthorn and soybean leaf of roughly equivalent size. Leaves of both species were maintained in a water pick comprised of a 0.5-ml microcentrifuge tube filled with distilled de-ionized water and capped with Paraﬁlm. Wilted or drying leaves were replaced with fresh ones as necessary throughout the trial. The petri dishes were kept in a growth chamber under fall environmental conditions. The position of each alate and the number of progeny on each leaf was recorded daily, after which the alate was placed back on the soybean leaf if necessary to assess its relative tendency to reject soybean in favor of buckthorn. After 7 d, alates were removed and all dishes placed in a growth chamber (25°C, 75% RH, and a photoperiod of 16:8 [L:D] h) until progeny could be classiﬁed as viviparous or oviparous. This procedure was repeated weekly until no viviparous offspring were found. Before experiments involving gynoparous alatoids (studies 2 and 3), 10 100-mm-diameter plastic petri dishes were set up on a weekly basis with individual soybean and buckthorn leaves as above. Four to 10 fourth-instar alatoid nymphs were randomly collected with Paraﬁlm. Wilted or drying leaves were replaced with fresh ones as necessary throughout the trial. The petri dishes were kept in a growth chamber under fall environmental conditions. The position of each alate, and the number of progeny on each leaf was recorded daily, after which the alate was placed back on the soybean leaf if necessary to assess its relative tendency to reject soybean in favor of buckthorn. After 7 d, alates were removed and all dishes placed in a growth chamber (25°C, 75% RH, and a photoperiod of 16:8 [L:D] h) until progeny could be classiﬁed as viviparous or oviparous. This procedure was repeated weekly until no viviparous offspring were found.

Before experiments involving gynoparous alatoids (studies 2 and 3), groups of 15 alates were sampled randomly and individually placed onto soybean leaves in 100-mm-diameter plastic petri dishes each containing a single buckthorn and soybean leaf of roughly equivalent size. Leaves of both species were maintained in a water pick comprised of a 0.5-ml microcentrifuge tube filled with distilled de-ionized water and capped with Paraﬁlm. Wilted or drying leaves were replaced with fresh ones as necessary throughout the trial. The petri dishes were kept in a growth chamber under fall environmental conditions. The position of each alate, and the number of progeny on each leaf was recorded daily, after which the alate was placed back on the soybean leaf if necessary to assess its relative tendency to reject soybean in favor of buckthorn. After 7 d, alates were removed and all dishes placed in a growth chamber (25°C, 75% RH, and a photoperiod of 16:8 [L:D] h) until progeny could be classiﬁed as viviparous or oviparous. This procedure was repeated weekly until no viviparous offspring were found.

**Parasitoids.** The *B. communis* strain used in this study was originally collected from *A. glycines* in soybean fields near Harbin, Heilongjiang Province, China, during 2002, and parasitoid colonies had since been maintained on *A. glycines* in the MDA/MAES Quarantine Facility. This region possesses a similar climate to our release area (St. Paul, MN, see below; Venette and Ragsdale 2004). For a more detailed account of the collection and history of this colony, refer to Wyckhuys et al. (2008). Female *B. communis* in our studies were both naive with respect to hosts and mated before conducting our assays. All parasitoids used in laboratory experiments were collected as mummies and isolated into gelatin capsules (size 0; Solaray, Inc., Park City, UT) with a drop of clover honey. Newly emerged (<24-h-old) females were paired with a similarly-aged male for 24 ± 2 h at 25°C and a photoperiod of 16:8 (L:D) h in a microcentrifuge tube of which the bottom was cut away and covered with ﬁne mesh to permit air entry. These 1-d-old females were used in all laboratory experiments.

**Study 1: Parasitism of *A. glycines* Colonies on Buckthorn. Laboratory Study.** The purpose of this study was to examine the ovipositional preference of female *B. communis* upon encountering mixed morph *A. glycines* colonies (i.e., gynoparae and their oviparous offspring) on buckthorn leaves. Such colonies mimic *A. glycines* foundress events on buckthorn during late summer–early fall, thus providing a suitable comparison to ﬁeld cage data (see below). Experiments were conducted between 7 July and 5 August 2005 at 25°C, 75% RH, and a photoperiod of 16:8 (L:D) h.

Individual buckthorn leaves were placed into 100-mm-diameter plastic petri dishes with a water pick. Each leaf then received 10 laboratory-generated gynoparae, after which aphids were allowed to settle for 1 h. Both a parasitized and nonparasitized control treatment were established in to assess levels of nonmummy aphid mortality in the presence of parasitism. In the parasitized treatment, a single, mated female *B. communis* was placed in each dish for 2 h under identical conditions to the settling period, after which the dish perimeter was sealed in Paraﬁlm to prevent parasitoid escape. Control treatments were treated identically, but without parasitoid introduction. Gynoparae were then removed and placed on a fresh buckthorn leaf in a new petri dish. Aphids were checked daily for 15 d for parasitoid mummy formation (i.e., the transformation from a seemingly healthy to a visibly parasitized aphid), and emerging adult *B. communis* were sexed and placed in 95% ethanol. Buckthorn leaves were changed periodically as necessary throughout the experiment. Hind tibia lengths (a standard measure of parasitoid body size previously used for *B. communis*; Godfray 1994, Dieckhoff and Heimpel 2010) were recorded for each adult wasp using an ocular micrometer. In addition to the experiments on buckthorn, similar trials were conducted on adult gynoparae exposed to parasitoids on soybean leaves. With the exception of the host plant, all experimental protocols were identical to those described above.

**Field cage study.** In September 2008, eight buckthorn bushes (<40 cm in height) were individually caged (Fig. 2A) within a single thicket adjacent to experimental ﬁelds on the St. Paul campus of the University of Minnesota (44° 59.334′ N, 93° 11.159′ W). All bushes were at least 7 m in from the thicket edge. Laboratory-reared gynoparae were released into each cage on two separate dates (50 gynoparae each on 6 September; 40 gynoparae each on 24 September). A single release of 20 male *A. glycines* per
cage was made on 15 September (note that male production follows gynopara production in wild A. glycines populations in North America; Ragsdale et al. 2004). All aphids were released in open 100-mm-diameter petri dishes containing a single soybean leaf. B. communis were released as male-female pairs over two staggered releases (nine pairs total between 9 and 17 September; 7 pairs total between 26 September and 3 October), with each cage receiving the same number of parasitoid pairs on a given date. Parasitoid pairs (<24 h old) were collected upon eclosion from mummies reared in greenhouse colonies under ambient daylight conditions (daytime temperatures of ≈25–30°C) and then held in and subsequently released from microcentrifuge tubes with a honey droplet. Three times during the fall (22 September, 9 October, and 25–27 October), cages were briefly opened and the number of aphids, mummies, and adult parasitoids were counted on each bush. Aphids and parasitoid mummies also were categorized by aphid morph (alate or ovipara).

To examine the overwintering potential of B. communis in the buckthorn cages, cages were modified beginning on 4 December (Fig. 2B). The following spring, on 4 April 2009, an additional "no-see-um" mesh layer (Fig. 2A) was added to each cage. On 1 May, all cages were then modified as follows: 1) a new bilayer was attached to the previously exposed portion of the cage, with a 28-cm-wide inverted white oil funnel attached to the top, and 2) a 120-ml urinalysis cup was affixed to the funnel through a hole cut in the cup’s cap. Monitoring for overwintering B. communis involved two methods. First, the contents of urinalysis cups were killed with ethanol and examined for adult wasps approximately every 3 d from 7 May to 14 July 2009. Second, a single 311-mm² pot containing two to three soybean plants infested with viviparous A. glycines was transplanted into each cage approximately every 7 d starting on 4 May 2009. Aphids from these sentinel plants were then reared over an additional 10 d under ambient sunlight and temperature (≈23°C) conditions to check for parasitoid mummy formation.

Study 2: Parasitism of Gynoparous Versus Summer Alatoid Nymphs. Between 7 February and 18 April, 2010, a series of experiments were conducted to compare the relative performance of B. communis on summer and gynoparous alatoid nymphs. Petri dish assays similar to those in study 1 were carried out, but with soybean rather than buckthorn leaves. Ten fourth-instar alatoid nymphs collected from either fall environmental condition colonies or the stock A. glycines colony at 25°C were placed on each leaf. In addition to these two treatments, a third treatment of third-instar apterous nymphs, as judged by aphid size and randomly selected in groups of 10 from the 25°C stock colony, was added as a positive control for the bioassay because third-instar A. glycines nymphs are a preferred stage for B. communis (Wyckhuys et al. 2008). Parasitoid exposure methods, control treatments, rearing conditions, and data collection were identical to those of study 1, with four exceptions. First, all gynoparae were moved to fresh buckthorn leaves in new petri dishes upon eclosion to adulthood. To control for confounds associated with handling, all summer aphids also were moved to a fresh soybean leaf and petri dish after the first molt. Second, because preliminary studies suggested that soybean leaves lose turgor pressure more rapidly than buckthorn leaves do, experimental aphids on both soybean and buckthorn leaves were moved to a fresh, appropriate leaf every three days during the course of the experiment. Third, because aphids were immature at the time of parasitoid exposure, any resultant nymphs were not counted or maintained. Finally, data collection for this trial was terminated at 13 d instead of 15 d.

Study 3: Parasitism of Third-Instar Gynoparous Nymphs. Between 5 May and 1 June, 2010, a third laboratory trial examined the rate at which parasitized third-instar gynoparous alatoids form winged mummies. Ten third-instar gynoparous alatoids were collected from fall environmental condition colonies and placed on soybean leaves in 100-mm-diameter plastic petri dishes as described above. Experimental procedures followed exactly those in study 2, with the exception that alatoids were maintained on soybean through two molts instead of one molt.
Data Analysis. Mummification rates were calculated as the number of parasitoid mummies divided by the sum of alive, dead, and mummified aphids counted at the termination of the trial (for laboratory experiments) or at the time of sampling (for field cage studies). Aphid mortality rates were calculated using the number of dead aphids at the end of the trial period, with the exception of the first study using gynoparous collected as adults. Here, because alates were of unknown age, there was concern that mortality rates at 15 d would be too high to accurately compare across treatments. Therefore, the number of dead aphids divided by the total number of aphids at the mean time to mummy formation was used as a measure of the mortality rate for both gynoparous and oviparous in this study.

Parametric statistical analyses with mummification or mortality rate as the response variable (i.e., analysis of variance [ANOVA], t-test) were performed as appropriate on arcsine square-root–transformed proportion data. If these (or any other continuous data) did not conform to the assumptions of parametric tests after transformation, then appropriate nonparametric tests (Kruskal–Wallis test, Wilcoxon rank sum test) were performed. All other continuous response data (e.g., parasitoid hind tibia length, parasitoid developmental time) were analyzed without data transformation, unless noted in the Results. All statistical analyses were performed in the JMP, version 5.1 statistical software package (SAS Institute, Cary, NC).

Results

Study 1: Parasitism of A. glycines Colonies on Buckthorn. Laboratory Study. In laboratory choice experiments, nearly all B. communis eclosed from oviparous (53 out of 54 adult wasps). This difference in host suitability also was reflected in aphid mummification rates: 61 of 248 oviparous yielded parasitoid mummies (weighted mean mummification rate of 24.6 ± 8.3%; n = 14 trials), whereas a parasitoid mummy was produced from only a single gynopara (out of 137; weighted mean mummification rate of 0.7% ± 0.8%; n = 14 trials). Similar mortality rates were observed regardless of parasitoid presence in both gynoparous (t-test: t = 1.9, df = 7, P = 0.09; back-transformed parasitized mortality of 24.8% [95% CI = 8.3–46.3%] versus unparasitized rate of 51.5% [95% CI = 26.9–75.7%]) and oviparous (t-test: t = 1.3, df = 7, P = 0.25; back-transformed parasitized mortality rate of 51.2% [95% CI = 22.5–79.5%] versus unparasitized rate of 27.8% [95% CI = 4.6–60.9%]). This suggests that high levels of premummification, parasitoid-induced mortality were not leading to underestimates of successful parasitism in our assays. B. communis took 13.8 ± 0.7 d on average (weighted mean; n = 8 trials) to develop from the egg to adult stage in first-instar oviparous, and adult parasitoids from these hosts had an average hind tibia length of 0.36 ± 0.01 mm (weighted mean; n = 8 trials). Neither of these traits varied between the sexes (t-test for parasitoid developmental time [ln transformed]; t = 1.11, df = 11, P = 0.29; t-test for parasitoid hind tibia length [weighted by number of wasps; ln transformed]; t = 0.02, df = 11, P = 0.99; n = 13). Sex ratios of parasitoids emerging from oviparous were male-biased (weighted mean sex ratio of 0.79 ± 0.06; n = 8 trials).

In the experiments on soybean leaves, adult gynoparous were parasitized at a similarly low rate (3/131 gynoparous; weighted mean mummification rate of 2.3 ± 1.7%; n = 13 trials) to those in the buckthorn trials. No difference in mortality rate was observed between the parasitized and unparasitized treatments (back-transformed mortality rates of 67.0% [95% CI = 34.4–92.2%] and 69.1% [95% CI = 33.0–95.3%], respectively; t-test: t = 0.11, df = 7, P = 0.91; n = 9 trials).

Field cage study. In total, 122 adult aphids, 1.058 oviparous (nymphs and adults), and 48 parasitoid mummies were counted on all eight buckthorn plants over all three sampling dates. Only one mummy was formed from an alate aphid. The mummification rate of oviparous did not differ across sampling dates (Kruskal–Wallis test: χ² = 0.58, df = 2, P = 0.75; n = 23) and, after pooling data for all sampling dates, the mummification rate of alates was significantly lower than that of oviparous (weighted means of 0.8 ± 0.9 and 4.6 ± 1.0%, respectively; Wilcoxon rank sum test: Z = 2.69, P = 0.007; n = 16). This result held if the analysis was restricted to the sampling date when the single alate mummy was observed (9 October counts only; Wilcoxon rank sum test: Z = 2.01, P = 0.04; n = 15). Neither the funnel trap nor sentinel plant method produced any evidence of overwintering B. communis.

Study 2: Parasitism of Gynoparous Versus Summer Alatoid Nymphs. Fifty-five parasitoid mummies were produced across the 39 trials of this experiment where parasitoids were introduced: 48 from the third-instar apterous control treatment (45 apterous mummies [out of 113 apterae; 39.8%] and three alate mummies [out of 12 total alatoids and alates; 25.0%]), four alate mummies from the fourth-instar gynoparous nymph treatment (out of 162 alatoids and alates; 2.5%), and three alate mummies from the fourth-instar summer alatoid treatment (out of 86 alatoids and alates; 3.5%). After testing for parasitoid cohort effects, significantly lower mummification rates were observed in the two fourth-instar alatoid treatments than in the third-instar control treatment (effect test within ANOVA, see Table 1). Because mummification rate data were still strongly skewed after transformation, we also performed a nonparametric analysis. Nonparametric analyses revealed a similarly significant treatment effect to the ANOVA (Kruskal–Wallis test: χ² = 8.86, df = 2, P = 0.012; data pooled over all parasitoid cohorts).

After testing for the effects of parasitoid presence, aphid morph, parasitoid cohort, and their respective interactions (whole model ANOVA: F = 2.51; df = 13, 63; P = 0.008), aphid mortality did not differ in the presence or absence of parasitoids (effect test for parasitoid presence; F = 0.54; df = 1, 63; P = 0.47). The only significant effects in the model were aphid morph (F = 5.28; df = 2, 63; P = 0.008) and an interaction
between aphid morph and parasitoid cohort ($F = 3.25$; df = 4, 63; $P = 0.02$). Here, aphids in the third-instar control (third parasitoid cohort only) and gynoparous (first parasitoid cohort only) treatments showed significantly lower mortality than gynoparous aphids in the second parasitoid cohort (data not shown).

Adult parasitoids emerged from 37 apterous mummies (82.2%), four alate mummies (57.1%), and three alatoid mummies (100%), respectively. Egg to adult parasitoid developmental time did not differ between the sexes (Wilcoxon rank sum test: $Z = 0.21$, $P = 0.84$; $n = 14$), with an overall weighted mean developmental time of 10.5 ± 0.2 d. Sex ratios for wasps emerging from third-instar apterite were near parity (weighted mean sex ratio of 0.49 ± 0.13; $n = 7$ trials). Finally, hind tibia lengths from parasitoids emerging from third-instar apterite did not differ between the sexes ($t$-test [weighted by number of wasps]: $t = 0.53$, df = 7, $P = 0.61$; $n = 9$ trials), with an overall weighted mean hind tibia length of 0.46 ± 0.01 mm.

**Study 3: Parasitism of Third-Instar Gynoparous Nymphs.** Fourteen *B. communis* mummies were observed during the third experiment (19.2% of 73 total aphids), with 13 forming from gynoparous alatoids (out of 25 alatoids that failed to reach adulthood in the parasitoid treatment; weighted mean alatoid mummification rate of 52.0 ± 8.4%; $n = 8$ trials) and a lone mummy forming from an alate gynopara (out of 48 alates in the parasitoid treatment; weighted mean alate mummification rate of 2.1 ± 2.6%; $n = 8$ trials). Alatoid aphids exposed to parasitoids were less likely to complete development than their control treatment counterparts (weighted mean complete development proportions of 0.67 ± 0.09 [parasitoid present; $n = 8$] versus 0.91 ± 0.04 [parasitoid absent; $n = 7$]; Wilcoxon rank sum test: $Z = 1.94$, $P = 0.052$). This effect, however, disappears when excluding alatoids that formed mummies from the analysis (weighted mean complete development proportions of 0.81 ± 0.06 [parasitoid present] and 0.91 ± 0.04 [parasitoid absent]; Wilcoxon rank sum test: $Z = 1.4$, $P = 0.16$; $n = 15$).

Gynoparous alate mortality did not differ between *B. communis* and control treatments (weighted mean alate mortality rates of 14.6 ± 6.4% [$n = 8$ trials] and 25.0 ± 6.7% [$n = 7$ trials], respectively; Wilcoxon rank sum test: $Z = 1.36$, $P = 0.18$). Because only three control trials yielded alatoid nymphs that failed to reach adulthood, we were unable to statistically compare mortality rates between the treatments for this aphid morph. Adult *B. communis* emerged from 10 alate mummies (76.9%) and the lone alate mummy. Of the 11 adult *B. communis* recovered during this experiment, the mean developmental time was 11.27 ± 0.19 d, and the mean adult hind tibia length was 0.42 ± 0.01 mm.

### Discussion

*A. glycines* as a Potential Overwintering Host for *B. communis*. We know of no record of *B. communis* from any *Rhamnus* species in either its native or introduced range. Our laboratory trials on nascent *A. glycines* colonies on *Rhamnus* leaves show that at least one buckthorn-specific *A. glycines* stage falls within the physiological host range of *B. communis*. On average, nearly one quarter of all first-instar oviparae encountered by female *B. communis* formed mummies, with nearly 87% of these yielding adult parasitoids. As in all of our laboratory studies, mortality rates were similar between aphids exposed to parasitoids and those in unparasitized control treatments, suggesting that mummification rate is a valid proxy measure of parasitism rate.

The presence of *B. communis* mummies in our field cages provides, to our knowledge, the first evidence of its utilization of *A. glycines* on *Rhamnus* under field conditions. Emerged mummies were found (11 out of 48; 22.9%), and at least one stinging female *B. communis* was observed 22 d after the final parasitoid release (M.K.A., unpublished data). Field cage data mirrored laboratory results with respect to the relative use of *A. glycines* morphs. *B. communis* mummification rates were higher on oviparae than on alate aphids (see Fig. 3 for a summary of all laboratory trials). Taken as a whole, these data lend support to the hypothesis of a potential overwintering relationship between *B. communis* and *A. glycines* because 1) at least one autumnal, buckthorn-specific *A. glycines* morph (the ovipara) is suitable for *B. communis* parasitism and development, and 2) successful *B. communis* reproduction was observed through late October in Minnesota buckthorn under natural temperatures and photoperiod.

---

**Table 1.** Model results for ANOVA of aphid mummification rate (response variable) and the following explanatory variables in the second laboratory experiment: aphid morph (third-instar apterous control, fourth-instar summer alatoid nymph, and fourth-instar alatoid gynoparous nymph), parasitoid cohort, and the interaction between these two variables.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Parameter estimate (mean ± SE)</th>
<th>$F$</th>
<th>df</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aphid morph</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Third instar apterous control</td>
<td>0.32 ± 0.01a</td>
<td>11.06</td>
<td>2.30</td>
<td>0.0002</td>
</tr>
<tr>
<td>Fourth instar summer alatoid</td>
<td>0.01 ± 0.01b</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fourth instar gynoparous alatoid</td>
<td>0.01 ± 0.01b</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parasitoid cohort</td>
<td>3.01</td>
<td>2.30</td>
<td>0.06</td>
<td></td>
</tr>
<tr>
<td>Aphid morph × parasitoid cohort</td>
<td>1.26</td>
<td>4.30</td>
<td>0.31</td>
<td></td>
</tr>
</tbody>
</table>

Mummification rate data were arc sine square root transformed for the analysis, but parameter estimates given here have been back-transformed. Whole model ANOVA results were significant ($F = 4.51$; df = 8, 30; $P = 0.001$). Mean values with different letters denote significance at the α = 0.05 level.
Ovipara parasitism is linked with increased diapause induction in a number of aphidine species attacking holocyclic hosts, including *Aphidius matricariae* (Haliday) and *Paun volucre* (Haliday) on *Aphis fabae* Scopoli (Polgár et al., 1991), *Pauesia unilachni* Gahan on *Schizolachnus pineti* F. (Polgár et al. 1995), and *Aphidius ervi* Haliday on pea aphid, *Acyrthosiphon pisum* (Harris) (Christiansen-Weniger and Hardie 1997). In some species, diapausing mummies form in oviparae regardless of external cues, suggesting that host morph either alone or in concert with plant quality can induce diapause (Le Ralec et al. 2010). Polgár et al. (1995) proposed an adaptive explanation for this: ovipara presence reliably portends host shortage, because they deposit eggs that are not susceptible to parasitism. Given the high eclosion rates and relatively rapid developmental times of adult parasitoids from oviparae exposed to long day conditions in our laboratory trials, however, diapause induction via host morph alone is unlikely for *B. communis* in *A. glycines*. Studies exposing parasitoids to different *A. glycines* morphs under experimentally manipulated environmental conditions are needed to further test the buckthorn overwintering hypothesis for *B. communis*, because failure to induce diapause in fall, buckthorn-specific morphs could reasonably falsify it.

*B. communis* developmental times were longer in first-instar oviparae at 25°C than in 1) third-instar apterous or alatoid nymphs in this study, or 2) first-instar apterae reared under similar conditions in a previous study (0–11 d; Wyckhuys et al. 2008). This contrasts with results from *A. ervi*, in which nondiapausing developmental time is equivalent on oviparae and viviparae (Christiansen-Weniger and Hardie 1997). Furthermore, adult parasitoid hind tibia lengths were considerably shorter in *B. communis* emerging from oviparae than from either third-instar apterous or alatoid nymphs. Finally, mean sex ratios for *B. communis* reared from first-instar oviparae were more male-biased than those obtained from the third-instar apterous treatment of the second study. Taken together, these results suggest that first-instar oviparae may be inferior hosts to other *A. glycines* stages or morphs for *B. communis* development. Because parasitoids in this study were only exposed to first-instar oviparae, it is unclear whether these differences are explained by morph-specific or age-specific variation in host quality.

Verification of spring parasitoid emergence in our field cages would have provided direct evidence of *B. communis* overwintering on *A. glycines*. We screened our cages for overwintering parasitoids, but were unable to detect *B. communis* in the following spring. Our efforts, however, were limited to eight caged plants, all of which were located in a single thicket (approximate study area of 142 m²). In addition, high mortality rates of diapausing immature parasitoids (Legrand et al. 2001, 2004) could have reduced the number of overwintering parasitoids below detection threshold. Although the point of collection of our *B. communis* strain represents a strong climatic match to our release site, nothing is known about the thermal tolerance of *B. communis*. Future field research into *B. communis* parasitism in *Rhamnus* should focus on larger scale fall releases, preferably into multiple habitats to account for mortality variation in different climates.

Potential for Dispersal of Immature *B. communis* to Buckthorn by *A. glycines*. Although immature parasitoid movement within alate aphid hosts has been well documented (Kelly 1917, Hight et al. 1972, Rauwald and Ives 2001, Feng et al. 2007, Zhang et al. 2009), it is unclear whether such movement can be used as an adaptive strategy of dispersal (i.e., phoresy; Howard 1927, Clausen 1976). Dispersal of immature *B. communis* and other parasitoids within host-alternating *A. glycines* alates could increase the likelihood of host location before overwintering when contrasted with self-directed adult movement (Zhang et al. 2009, Heimpel et al. 2010). This benefit would be more pronounced in *A. glycines* parasitoids with relatively narrow host ranges (such as *B. communis*; Desneux et al. 2009), because these would gain most from maintaining fidelity to *A. glycines* throughout the aphid life cycle.

Unlike traditionally recognized phoretic arthropods (e.g., mites [Binnis 1982, Lindquist and Moraza 2008], lice [Marshall 1981, Harbison et al. 2009], and egg parasitoids [Clausen 1976, Arakaki et al. 1996, Huigens et al. 2010]), immature aphid parasitoids cannot actively seek more mobile host stages to parasitize; rather, host choice depends solely on the ovipositional preference of the mother. As such, for *B. communis* to adaptively use buckthorn-specific alate morphs to reach *Rhamnus*, a reasonable hypothesis is that female parasitoids preferentially parasitize such morphs.
when they form. In our study, *B. communis* females at 25°C showed no difference in mummification rate between summer migrant (Fig. 3, stage C) and gynoparous (Fig. 3, stage D) fourth-instar alateoid nymphs after 2 h of exposure, with both successfully parasitized at far lower rates than third-instar apterous viviparous viviparae (Fig. 3, compare stages C and D with stage A). This suggests that, not only are fourth-instar alateoid nymphs relatively poor hosts, but *B. communis* mummification rates do not increase upon detection of gynoparous alateoids. Wyckhuys et al. (2008) reported no-choice data for *B. communis* by using whole soybean plants infested with 40 *A. glycines* each, but with 4 h of parasitoid exposure instead of 2 h. Here, mummification rates were similar between third-instar apterous, fourth-instar summer alateoid nymphs, and summer alates (~25-40%). The discrepancy between these results may be explained, in part, by longer exposure times in the 2008 study, such that *B. communis* females may have accepted greater numbers of less-favored alateoid/alate aphids (see Wyckhuys et al. 2008 for preference data).

Any potential benefits of phoretic dispersal by immature parasitoids could be offset by costs associated with parasitizing alate or alateoid aphids. First, alate or alateoid hosts could be inferior for parasitoid development, as is suggested in experiments on *A. ervi* attacking the pea aphid. Some *A. ervi* populations vary in their capacity to inhibit wing formation in alate pea aphid nymphs (Christiansen-Weniger and Hardie 2000, Demmon et al. 2004). The adaptive benefit of this trait seems to be increased body size, which is often positively correlated with parasitoid fitness (Godfray 1994). Our experiments also suggest size costs to alate parasitism: hind tibia lengths of adult parasitoids emerging from alate mummies are, on average, 0.1 mm (95% CI = 0.06 ÷ 0.15 mm) shorter than those emerging from apterous viviparous mummies (weighted mean data from trials pooled across dates, t-test: t = 5.0, df = 11, P = 0.0004; n = 13). In addition, aphids are known to exhibit defensive behaviors against parasitoid attack (Gerling et al. 1990, Håggvar and Hofsvang 1991, De Farias and Hopper 1999, Foster et al. 2007, Desneux et al. 2009). Wyckhuys et al. (2008) demonstrated that these defenses are stronger against *B. communis* in larger *A. glycines* morphs, with both summer fourth-instar alateoid and alate viviparae included in the study. Given the low mummification rates of late-stage gynoparous *A. glycines* on soybean found here (Fig. 3, stages D and E), especially in light of both the restricted search areas used and high mummification rates of apterae (Fig. 3, stage A), it may be that female *B. communis* often rejected late stage gynoparvae to avoid such costs.

Alate-mediated dispersal need not be phoretic to have implications for the biology and biological control efficacy of *B. communis* and other aphid parasitoids. Incidental parasitism during peak migratory periods, when proportions of alateoid and alate aphids are highest (Hodgson et al. 2005), could result in high levels of immature *B. communis* dispersal. Although the beneficial aspects of parasitoid transport within winged aphids have been discussed above, potential negative consequences also exist. For example, given that wind-aided alate dispersal of some species can reach hundreds of kilometers (Johnson 1969, Taylor 1977, Irwin and Thresh 1988, Riley et al. 1995), aphid-mediated movement could hamper mate-finding in the next generation, potentially leading to strong Allee effects (especially in introduced populations; Hopper and Roush 1993, Zhang et al. 2009, Heimpel et al. 2010). Our third experiment, however, suggests that, whereas mummification rates of third-instar alateoid nymphs are higher than in fourth-instar alateoid nymphs at 25°C, very few parasitized third-instar alateoids form wings (Fig. 3, stage B). Therefore, unless *B. communis* either substantially 1) lengthen developmental time relative to the host in third-instar alateoid *A. glycines* and/or 2) increase parasitism of fourth-instar alateoid or alate *A. glycines* under fall conditions, alate-mediated dispersal to buckthorn would probably be relatively rare.

In conclusion, our data suggest that soybean aphid oviparvae on buckthorn are readily susceptible to parasitism by its classical biological agent, *B. communis*. It is therefore reasonable to expect successful autumnal parasitism of *A. glycines* by *B. communis* in regions where *R. cathartica* densities are high (e.g., the north central United States). Given the low rates of winged fall aphid parasitism in *B. communis* however, it currently seems unlikely that phoretic dispersal within winged *A. glycines* represents an adaptive strategy for locating its potential overwintering host. This could have ramifications for future integrated pest management (IPM) strategies using *B. communis* against *A. glycines* in North America and may favor adopting parasitoid releases into *Rhamnus* as a possible means of facilitating overwintering by *B. communis*.

Acknowledgments

We thank Sheena Ahrar, Jonathan Dregni, Simon Lueth, David Malepsy, Jon Malepsy, and Ying Zhang for assistance in data collection and colony maintenance during the course of these experiments; Julie Martinez for illustrating the experimental summary figure; and Dave Ragsdale and Dave Voegtlin for granting their permission to use the soybean aphid life cycle diagram. This project was funded, in part, by USDA North Central IPM, the North Central Soybean Research Program, the Minnesota Soybean Research Council, and the Minnesota Agricultural Experiment Station.

References Cited


Ragdaile, D. W., B. P. McCormack, B. C. Venette, B. D. Potter, I. V. MacRae, E. W. Hodgson, M. E. O’Neal, K. D. Johnson,


Received 1 November 2010, accepted 30 May 2011.