

RESEARCH ARTICLE

Role of the aphid species and their feeding locations in parasitization behavior of *Aphelinus abdominalis*, a parasitoid of the lettuce aphid *Nasonovia ribisnigri*

Govinda Shrestha^{1,2*}, Henrik Skovgård², Gadi V. P. Reddy¹, Tove Steenberg², Annie Enkegaard²

1 Western Triangle Ag Research Center, Montana State University, Conrad, MT, United States of America, **2** Department of Agroecology, Science and Technology, Aarhus University, Research Centre Flakkebjerg, Forsøgsvej 1, Slagelse, Denmark

* govinda.shrestha@montana.edu



OPEN ACCESS

Citation: Shrestha G, Skovgård H, Reddy GVP, Steenberg T, Enkegaard A (2017) Role of the aphid species and their feeding locations in parasitization behavior of *Aphelinus abdominalis*, a parasitoid of the lettuce aphid *Nasonovia ribisnigri*. PLoS ONE 12(8): e0184080. <https://doi.org/10.1371/journal.pone.0184080>

Editor: Nicolas Desneux, Institut Sophia Agrobiotech, FRANCE

Received: January 15, 2017

Accepted: August 17, 2017

Published: August 30, 2017

Copyright: © 2017 Shrestha et al. This is an open access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Data Availability Statement: All relevant data are within the paper.

Funding: This study was financially supported by Aarhus University Denmark and the National Institute of Food and Agriculture, U.S. Department of Agriculture, Multistate Project W3185, The Working Group Biological Control of Pest Management Systems of Plants under Accession #231844.

Abstract

Aphid species feeding on lettuce occupy distinct feeding sites: the lettuce aphid *Nasonovia ribisnigri* prefers to feed on heart leaves, whereas the potato aphid *Macrosiphum euphorbiae* feeds only on outer leaves. The aphid parasitoid *Aphelinus abdominalis*, known to be able to regulate *M. euphorbiae* on many crops, has recently been indicated as a promising biocontrol candidate also for use against *N. ribisnigri*, a major pest of lettuce. This study therefore examined *A. abdominalis* parasitization preference between *N. ribisnigri* and *M. euphorbiae* and its ability to parasitize aphids feeding on different parts of lettuce plants. In addition, life history traits of *A. abdominalis* on these aphid species were investigated. In no-choice laboratory experiments on leaf discs and 24 h exposure, *A. abdominalis* successfully parasitized 54% and 60% of the offered *N. ribisnigri* and *M. euphorbiae*, respectively, with no significant difference. In the corresponding choice experiment, however, *A. abdominalis* had a tendency for a significantly higher preference for *M. euphorbiae* (38%) compared to *N. ribisnigri* (30%). Growth chamber experiments on whole plants demonstrated that *A. abdominalis* was able to parasitize aphids, regardless of their feeding locations on lettuce plants. However, aphid feeding behavior had a significant effect on the parasitization rate. *A. abdominalis* parasitized significantly higher percentages of *M. euphorbiae* or *N. ribisnigri* when aphids were exposed separately to parasitoids on whole lettuce plants as compared with *N. ribisnigri* exposed only on heart leaf. A significant preference of *A. abdominalis* for *M. euphorbiae* compared to *N. ribisnigri* was also observed in the growth chamber choice experiment. A high percentage of adult emergence (> 84%) and female-biased sex ratio (> 83%) were found irrespective of the aphid species.

Competing interests: The authors have declared that no competing interests exist.

Introduction

Infestation by aphids is a serious problem in the production of lettuce, *Lactuca sativa* L. (Asterales: Asteraceae), both in glasshouses and under field conditions [1]. Although several species of aphid occur on lettuce globally [2], the green peach aphid *Myzus persicae* (Sulzer), potato aphid *Macrosiphum euphorbiae* (Thomas) and lettuce aphid *Nasonovia ribisnigri* (Mosley) (Hemiptera: Aphididae) are of major importance [3, 4, 5]. Out of these, *N. ribisnigri* is the most critical due to its frequent occurrence throughout the growing season and its cryptic feeding habitat with a feeding preference for the heart leaves of lettuce [6, 7]. The other two species *M. persicae* and *M. euphorbiae* are usually considered of lesser economic importance as they only feed on outer lettuce leaves [6] and occur less frequently during the growing season [4, 5, 8].

Control strategies for *N. ribisnigri* populations on lettuce rely largely on the use of insecticides [9, 10, 11]. However, demand for alternative methods to control *N. ribisnigri* has been stimulated due to the increased risk of insecticide resistance in aphid populations [12, 13], and because of concerns related to the environment [14] and human health [15]. Potential biocontrol methods for *N. ribisnigri* comprise the use of predators, including syrphids [16, 17] and lacewings [18, 19], and fungal pathogens [20, 21]. A further potential method for *N. ribisnigri* biocontrol is the use of parasitoids [22].

Shrestha et al. [22] evaluated three commercially available parasitoid species for their potential against *N. ribisnigri* and found *Aphelinus abdominalis* (Dalman) (Hymenoptera: Aphelinidae) to be the most promising candidate. This parasitoid is believed to originate from Europe, but now occurs also in Asia and North America [23]. *A. abdominalis* has been used for biocontrol of *M. euphorbiae* in glasshouse and field crops [23, 24]. No information, however, is available regarding the parasitization preferences of *A. abdominalis* towards *N. ribisnigri* and *M. euphorbiae*, which appear simultaneously in lettuce fields. It is thus important to further evaluate the potential of *A. abdominalis* against *N. ribisnigri*, taking into account that feeding behavior may influence the degree to which an aphid species is parasitized [25, 26].

Parasitoids that attack more than one aphid species show differences in preference and performance in response to various aphid species [27, 28, 29, 30, 31, 32, 33, 34]. The preference behavior of parasitoids between aphid species or taxa is influenced by a number of factors such as 1) host quality, with better quality hosts species usually, but not always [31], being preferred over poor quality hosts [27, 28]; 2) color of aphid morph, with a greater preference for the green morphs compared to the red morphs [35, 36]; 3) aphid size, with smaller aphids usually, but not always [37, 38], being preferred over larger ones [27, 39]; and 4) aphid age, with a stronger preference for young or intermediate growth stages of aphids over old stages [40, 41]. Additional factors that influence parasitoid preference are: parasitoid age, with a greater preference for low quality hosts by short-lived than longer-lived parasitoids [42], and parasitoid egg load, with females with low egg load preferring high quality hosts compared with females with high egg loads [43].

Aphid parasitoid life-history traits such as offspring survival and offspring sex ratio are parameters commonly measured to evaluate parasitoid fitness on different host species [29, 31, 44]. Aphid parasitoids may be able to regulate the fitness of their offspring in relation to the host species they attack [27, 29, 31, 44]. Ovipositing females may allocate male and female offsprings differentially in different host species. Moreover, different host aphid species may produce changes to the survival of male and female offspring. [27, 29, 31, 44]. It is therefore important for biocontrol programs to investigate whether host aphid species influence parasitoid offspring sex ratio or survival. In addition, the capacity of parasitoids to locate hosts in their feeding sites is vital for the efficiency of a parasitoid as a biocontrol agent [25, 26]. Some

parasitoids have the capability to find and parasitize aphids feeding on concealed parts of plants [45, 46] and vice versa [46].

None of the above-mentioned aspects have been explored in relation to use of *A. abdominalis* against *N. ribisnigri* co-occurring with *M. euphorbiae*. This study therefore examined the parasitization preference of *A. abdominalis* with regard to *N. ribisnigri* and *M. euphorbiae* under laboratory conditions and its capacity to find and parasitize the two aphid species when they are feeding on different areas of the lettuce plant under growth chamber conditions. In addition, female sex ratios and successful adult emergence of *A. abdominalis* on the two aphid species were also studied to evaluate parasitoid fitness.

Materials and methods

Plants

Iceberg lettuce, *L. sativa* cv. 'Mirette' was used as a source of plant material for the laboratory and the growth chamber experiments. Seeds were sown on Jiffy-strip trays and maintained in a glasshouse at 15–18 °C, 55–70% RH and natural light conditions until three true leaves had emerged (approx. 2 weeks after seed sowing). Afterwards, plants were transplanted into 2 L pots filled with peat soil, perlite and vermiculite (mixed at 90:8:2) with a *pH* of 6–7. These plants were either utilized within 6–10 days for production of aphid cohorts, for rearing of parasitized vs. unparasitized aphids (detached leaflets, lab and growth chamber experiments) or maintained for additional three days in a glasshouse and subsequently transported to the growth chamber.

Insects

The lettuce aphid *N. ribisnigri* and the potato aphid *M. euphorbiae*, originally supplied by Dr. Gemma Hough (Warwick Crop Centre, University of Warwick, UK) and senior research scientist Lesley Smart (Department of Biological Chemistry and Crop Protection, Rothamsted Research, UK), respectively, were reared separately on iceberg lettuce plants inside the insect-proof net-covered cages (68 × 75 × 82 cm). They were maintained in a controlled environment glasshouse compartment at 22 ± 1 °C, 70 ± 5% RH and 16:8 L: D.

The parasitoid *A. abdominalis*, supplied as mummies by EWH BioProduction, Tappernøje, Denmark, were placed in plastic Petri dishes (diameter: 15 cm) and kept in a climate cabinet at 22 °C, 70 ± 5% RH and 16:8 L:D. Mummies were checked daily for adult emergence and the cohorts of adults emerging on a same day were reared until the age of three days with the technique described by Shrestha et al. [22].

Aphid cohorts

The cohorts of 2-3rd instar aphids of *N. ribisnigri* and *M. euphorbiae* were used for laboratory and growth chamber experiments since these stages of both aphid species have been reported suitable for parasitization by *A. abdominalis* [47, 48]. To obtain cohorts of the two aphid species, adults (10–12 days old) were carefully transferred from the stock culture to uninfested leaves of lettuce. The base of each leaf was wrapped with moist cotton, inserted into a 1.5 ml Eppendorf tube with demineralized water and then placed at the bottom of mesh screened Plexiglass box (17 × 11 × 3 cm) with moist filter paper. These boxes were kept in a climate cabinet at 22 °C, 70 ± 5% RH and 16:8 L: D. After 48 hours, the produced nymphs were gently transferred either to new clean leaves (Eppendorf tube and Plexiglass set up) for the laboratory experiments or to the clean plants for the growth chamber experiments. Aphids were maintained for additional two days for the nymphs to develop into 2-3rd instars at similar conditions as described above [49, 50].

Laboratory experiments: Parasitization preference and life history traits

No-choice tests. The no-choice experiments were performed to evaluate the parasitization rates and fitness of *A. abdominalis* on *N. ribisnigri* and *M. euphorbiae* by measuring parasitism events (both successful and incomplete, i.e. without mummy formation) as well as a parasitoid emergence rates and sex ratios. The experiment was performed in vented Petri dishes (diameter: 9 cm) lined with a moist filter paper. A circular lettuce leaf disc (diameter: 5 cm) was placed at the bottom of each dish. Twenty aphid individuals of 2-3rd instar, either of *N. ribisnigri* or *M. euphorbiae*, were transferred to each lettuce dish by using a fine camel hair brush. Aphids were allowed to settle on a leaf disc for one hour before the introduction of a female parasitoid.

One mated female parasitoid (4 days old) was released into each Petri dish arena containing *N. ribisnigri* or *M. euphorbiae* and left for a 24 hour parasitization period in a climate cabinet at 22 °C, 70 ± 5% RH and 16:8 L: D. The female parasitoid was subsequently removed and the number of dead and live aphids in each dish counted under a stereo microscope. The numbers of aphids dying due to host feeding by *A. abdominalis* was determined based on their shrunken appearance [51]. The live aphids of each leaf disc were transferred to two clean leaves with the petiole wrapped with moist cotton and inserted into a 1.5 ml Eppendorf tube with demineralized water. This was done to avoid degradation of the leaves. These two leaves were placed in a Plexiglass box with moist filter paper and incubated in a climate cabinet under similar conditions as described earlier. After 4–5 days, the filter paper was replaced and if necessary, a new fresh leaf was placed in the vicinity of the old leaf to allow the aphids to translocate themselves. Generally, lettuce leaves remained fresh for at least six days using this setup.

Aphids were checked at 1–2 day intervals for two weeks for appearance of mummies (successful parasitization), while only up to nine days for aphids that died without mummification (incomplete parasitization). Aphid mummies that formed in each dish were gently collected using a fine camel hair brush and transferred individually into small transparent medicine cups (diameter = 15 mm) with screened lids. Emergence of adult parasitoids was checked at 1–2 day intervals and emerged parasitoids sexed under a stereo microscope. For each treatment, 12–14 replicates were performed. For the controls, five replicates without addition of parasitoids were used for each aphid species and same procedure as above was followed.

Choice test. The choice experiment was conducted in order to assess the preference of *A. abdominalis* for parasitization with regard to *N. ribisnigri* and *M. euphorbiae*. The experimental procedures and experimental conditions were similar as described above except that cohorts of 2-3rd instar lettuce aphids and potato aphids (n = 20+20) were offered simultaneously on the same leaf disc. The lettuce aphid nymphs were introduced first and allowed to settle for 15 min prior to the releases of the potato aphid nymphs. *N. ribisnigri* nymphs are easily distinguished under a stereo microscope by their color (red) in contrast with whitish-green potato aphids. The number of replicates for treatment was 15 and the controls (replicates = 5) were performed without addition of parasitoids.

Growth chamber experiments: Aphid feeding locations

The growth chamber experiments were performed to assess whether the aphid feeding location preference on lettuce plants influences the host finding ability of *A. abdominalis* by measuring successful parasitization under no-choice and choice conditions. Lettuce plants established in the plant growth chamber were 28 days old after seeding and had five unfolded leaves (4 outer leaves and 1 heart leaf) at the time of experiment initiation. A leaf developed from the central portion of plants was denoted as heart leaf and the leaves developed from peripheral layers as outer leaves. Plants were drip irrigated daily for half an hour each morning and evening in the

growth chamber room and they were maintained at 22 °C, 70 ± 5% RH and 16:8 L: D, for the duration of the experimental period.

No-choice and choice tests. The no-choice tests consisted of three treatments: 1) *M. euphorbiae* inoculated on leaves of a lettuce plant, 2) *N. ribisnigri* inoculated on leaves of a lettuce plant and 3) *N. ribisnigri* inoculated on only the heart leaf of a lettuce plant. Fifty 1st instar aphids were inoculated on each plant in all three treatments (see section aphid cohorts), but the number of aphid individuals inoculated into each leaf of a lettuce plant varied among the treatments. In treatment 1, *M. euphorbiae* individuals were inoculated on outer leaves (4 leaves at the time of inoculation) with 12–13 individuals (totaling 50 aphid individuals) per leaf since it is known that this aphid species does not colonize the heart leaves [6]. In treatment 2, *N. ribisnigri* individuals were inoculated on all five leaves (4 outer leaves and 1 inner leaf) with 10 individuals per leaf because this aphid species is known to colonize not only the heart leaf but also on outer leaves [7]. In treatment 3, 50 *N. ribisnigri* individuals were inoculated only on the heart leaf and the outer leaves were removed one day before the aphids' introduction, as the heart leaf is the most preferred feeding site of *N. ribisnigri* on lettuce plant [7]. The removal of outer leaves in treatment 3 was done to avoid the movement of aphids to outer leaves and also to obtain the best estimate of *A. abdominalis*' parasitization on aphids situated on this leaf.

With respect to the choice test, fifty 1st instar nymphs of each aphid species (totaling 100 aphid individuals) were established simultaneously on each lettuce plant. The inoculation of *M. euphorbiae* or *N. ribisnigri* individuals was carried out in a similar fashion as in treatment 1 and 2 in the no-choice tests, respectively.

From this point forward, the experimental procedures for both choice and no-choice tests were the same. Plants established in the growth chamber were transported to the insect inoculation chamber, where the aphids were carefully inoculated on the dorsal side of the leaves by using a fine camel hair brush. Aphids were allowed to settle on the plants for 1–2 hours after which the plants were subsequently transported back into their original location in the growth chamber. Each aphid-inoculated plant was kept separately in an acrylic cylindrical insect cage (diameter = 18 cm and height = 12 cm) with 5–6 mesh screened holes (diameter = 5 cm) on the side. Forty-eight hours after inoculation, when aphids were allowed to distribute themselves on the plants and develop into 2–3 instar aphids, five female *A. abdominalis* parasitoids (mated, 4 days old) were released onto the top of the plant canopy of each aphid-inoculated plant. Parasitoids were then allowed to parasitize for 48 hours. Afterwards plants were transported to the insect inoculation chamber and carefully removed from pots in order to minimize the loss or escape of aphids.

The number of live aphids present on each plant leaf was counted and each aphid transferred onto uninfested leaves (Eppendorf tube and Plexiglass set up) and incubated in a climate cabinet at 22 °C, 70 ± 5% RH and 16:8 L: D for 2 weeks. The subsequent handling of the aphids as well as the scoring of data was conducted as described above for the laboratory experiment. There were twelve replicates (each plant = one replicate) for each treatment for the both no-choice and choice tests. The controls (replicates = 5) were performed in absence of any parasitoids.

Statistical analysis

The data were analysed in R 2.15.1 [52]. For all data, a normal quantile-quantile plot was first performed to check the normality of residuals and the equality of residual variances. A transformation (angular) was done to achieve normal distribution prior to statistical tests. Tukey contrast pairwise multiple comparisons were used to test for significant differences in means [53].

For the laboratory data set (Petri dish setup), one way analysis of variance (ANOVA) was performed to test the effect of aphid species on the percentage of successful parasitization and incomplete parasitization in the no-choice experiments and for any differences in successful parasitization or incomplete parasitization when two aphid species were offered simultaneously in the choice experiment. The percentage of successful parasitism was calculated as $(\text{Number of mummified aphids} / \text{Total numbers of aphids exposed minus host feed aphids}) \times 100$ and incomplete parasitism as $(\text{Number of corrected dead aphids without signs of mummification} / \text{Total numbers of aphids exposed minus host feed aphids}) \times 100$ [22]. Dead aphids recorded in the incomplete parasitization group [54] were corrected for control mortality [55] prior to calculation and statistical analysis.

Similarly, for the growth chamber data set, one way analysis of variance (ANOVA) was performed to examine the effect of aphid species feeding sites on successful parasitization percentage in the no-choice experiment and for any differences in successful parasitization when two aphid species were offered simultaneously in the choice experiment. The percentage of successful parasitism was calculated as $(\text{Number of mummified aphids recorded per plant} / \text{Total numbers of aphids exposed}) \times 100$.

The adult emergence and sex ratio data were found to be non-normally distributed even after the angular transformation and the non-parametric one-way analysis of variance, Kruskal-Wallis test, was therefore used to test for differences. A Mann-Whitney U-test was used as a post hoc test for multiple comparisons between the means.

Results

Laboratory experiment

Parasitization. This study showed that *A. abdominalis* has the ability to successfully parasitize two aphid species *N. ribisnigri* and *M. euphorbiae* when they were offered simultaneously or separately to a parasitoid on the same leaf discs. In no-choice situations, *A. abdominalis* successfully parasitized $54.02 \pm 5.13\%$ and $60.52 \pm 5.35\%$ of *N. ribisnigri* and *M. euphorbiae*, respectively, offered within a 24 h exposure period. There was no significant difference between in percent parasitism between the two aphid species ($df = 1, 28; F = 0.94; P = 0.34$) (Fig 1A).

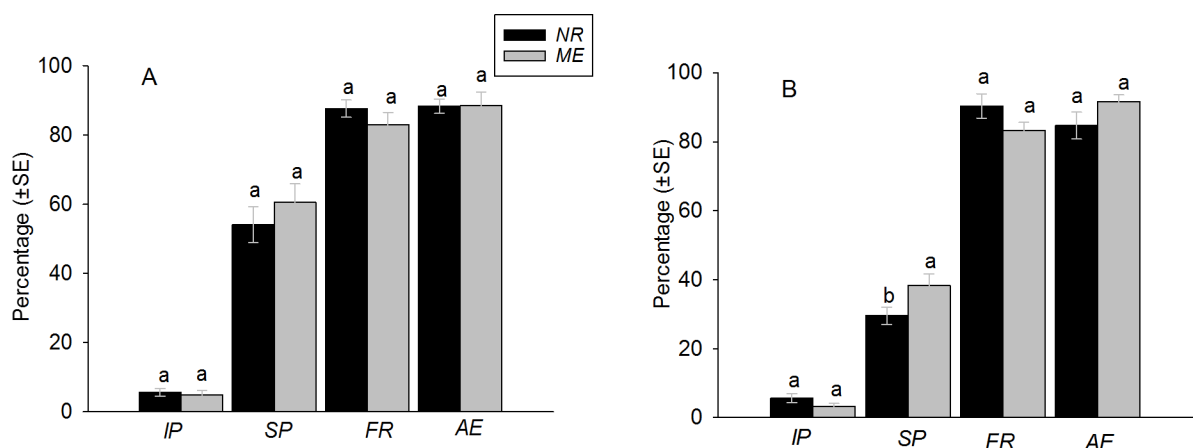


Fig 1. Percentage (mean \pm SE) of incomplete parasitization (IP), successful parasitization (SP), female sex ratio (FR) or adult emergence (AE) of *Aphelinus abdominalis* when *Nasonovia ribisnigri* (NR) and *Macrosiphum euphorbiae* (ME) were offered as hosts under no-choice (A) or choice conditions (B) in laboratory experiments. Different letters above the bars indicate significant differences in IP, SP, FR or AE (Tukey's or Mann-Whitney U-tests, $p \leq 0.05$).

<https://doi.org/10.1371/journal.pone.0184080.g001>

However, in the choice situation, there was a tendency for *A. abdominalis* successfully parasitizing more *M. euphorbiae* than *N. ribisnigri* ($df = 1, 28; F = 4.04; P = 0.05$), with parasitization of $38 \pm 3.32\%$ and $30 \pm 2.50\%$ respectively (Fig 1B). With respect to incomplete parasitization, a very low percentage (less than 6%) of aphids mortality occurred and no significant differences were detected when the two aphid species were offered simultaneously on the same leaf disc ($df = 1, 24; F = 0.01; P = 0.89$) or on separate leaf discs ($df = 1, 28; F = 0.00; P > 0.98$) (Fig 1).

Adult emergence and sex ratio. More than 84% of mummified aphids emerged and a female-biased sex ratio ($> 83\%$) was observed irrespective of aphid species (Fig 1). There was no significant difference in rates of parasitoid emergence and female sex ratio when the *N. ribisnigri* and the *M. euphorbiae* were exposed to *A. abdominalis* on separate leaf discs (parasitoid emergence: $\chi^2 = 0.04; df = 1; P = 0.85$; female sex ratio: $\chi^2 = 1.02; df = 1; P = 0.311$) or simultaneously (parasitoid emergence: $\chi^2 = 1.71; df = 1; P = 0.19$; female sex ratio: $\chi^2 = 1.65; df = 1; P = 0.20$) on the same leaf disc.

Growth chamber experiments

This study showed that *A. abdominalis* has the capacity to find and parasitize not only aphids feeding on an exposed area (outer leaves) but also on a concealed area (lettuce heart leaf). There was a significant effect of aphid feeding location on the host finding ability of *A. abdominalis* in the no-choice tests ($df = 2, 33; F = 17.46; P < 0.001$). The percentage of *M. euphorbiae* or *N. ribisnigri* parasitized by *A. abdominalis* on whole lettuce plants was significantly higher than the percentage of *N. ribisnigri* parasitized when exposed only on the heart leaves (Fig 2A). There was no significant difference in the mummification of two aphid species exposed to parasitoids on whole plants.

In the choice test, however, when lettuce plants were inoculated with *M. euphorbiae* and *N. ribisnigri* simultaneously, a significant difference was detected in the degree of successful parasitization between two aphid species ($df = 1, 22; F = 5.43; P = 0.03$). *A. abdominalis* showed a significant preference to *M. euphorbiae* over *N. ribisnigri* (Fig 2B).

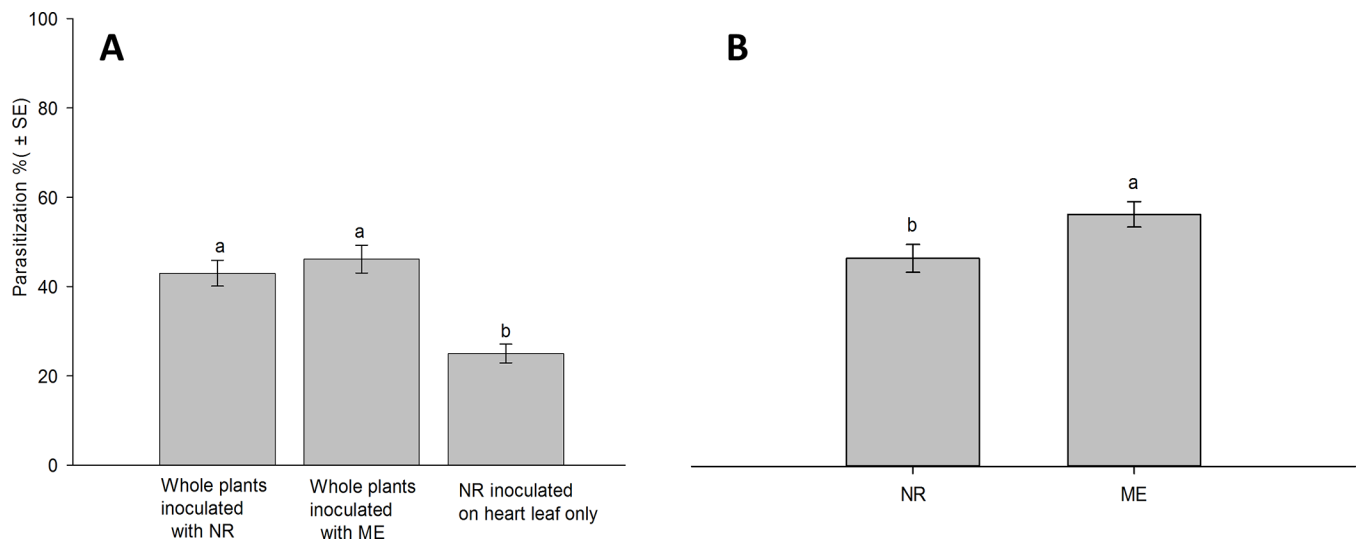


Fig 2. Percentage (mean ± SE) of successful parasitization of *Aphelinus abdominalis* when *Nasonovia ribisnigri* (NR) and *Macrosiphum euphorbiae* (ME) were offered as hosts under no-choice (A) or choice condition (B) in growth chamber experiments. Different letters above the bars indicate significant differences (Tukey's test, $p < 0.05$).

<https://doi.org/10.1371/journal.pone.0184080.g002>

Discussion

The understanding of parasitoid preference for parasitization between different aphid species, and the ability of parasitoid to find aphids feeding on different plant locations are important aspects in development of efficient biocontrol strategies against target pest populations [25, 26]. The choice experiment showed that *A. abdominalis* had a tendency for a higher successful parasitization in *M. euphorbiae* compared with *N. ribisnigri*, when they were offered simultaneously on the same leaf disc. Parasitoid preference for various aphid species have been examined previously [27, 28, 35, 36, 56]. For example, Bueno et al. [55] and Tapa-Yotto et al. [28] showed that *Lysiphlebus testaceipes* (Cresson) (Hymenoptera: Braconidae) preferred cotton aphid *Aphis gossypii* (Glover) as compared to three other aphid species: the green peach aphid, cowpea aphid *Aphis craccivora* (Koch) and mustard aphid *Lipaphis erysimi* (Kaltenbach). However, limited information exists regarding the parasitization preference of *A. abdominalis* between aphid species, except for the study by Wahab [57], who indicated that it preferred the shallot aphid *Myzus ascalonicus* (Doncaster) as compared with the ornate aphid *M. ornatus* (Laing) or mottled arum aphid *Neomyzus circumflexum* (Buckton).

Host quality [27, 28], parasitoid egg load [43], parasitoid age [42], aphid morph color [35, 36] and aphid size [27, 37] are important factors influencing parasitization preference of parasitoids between aphid species. Other factors may also mediate specialization and preferences of aphid parasitoids, including: 1) aphid phylogeny, with stronger preferences for closely related aphid species [30, 31]; 2) aphid host plant species, with greater preferences for aphids feeding on non-toxic host plants [31]; 3) the presence or absence of aphid endosymbiont bacteria, with higher preferences for non-infected aphids [33, 34].

The tendency to a higher preference of *A. abdominalis* for *M. euphorbiae* over *N. ribisnigri* found in our study is likely to be an effect of aphid size since the former species were relatively smaller in size (G. Shrestha, pers. obs.), and therefore presumably easier to handle, compared to the latter. Preference for small sized aphids was also observed for the parasitoid *Monoctonus paulensis* (Ashmead) (Hymenoptera: Braconidae) by Chau and Mackauer [27], who reported that small sized aphids have less well developed anti-parasitoid defense behaviour and therefore are easier to subdue. In addition, the green color of *M. euphorbiae* (as opposed to the red color of *N. ribisnigri*) could have played a role for the preference of *A. abdominalis* towards this aphid species [35, 36, 58]. For instance, Libbrecht et al. [36] reported that when parasitoids were given a choice, green morphs of pea aphid *Acyrtosiphon pisum* (Harris) were significantly more attacked by *Aphidius ervi* (Haliday) (Hymenoptera: Braconidae) when the neighboring colony consisted of red morphs.

The no-choice laboratory experiments showed no significant difference in degree of successful parasitization between the two aphid species; *N. ribisnigri* and *M. euphorbiae* and the parasitization percentages obtained for both aphid species are consistent with previous findings [22, 24]. This suggests that both aphid species are high quality hosts for *A. abdominalis* as it also substantiated by a high percentage of adult emergence and a strongly female-biased sex ratio being observed irrespective of aphid species. Sex ratio is important for aphid parasitoids, including *A. abdominalis*, as it affects parasitoid population growth rate and effectiveness in biocontrol [59, 60]. Parasitoids with female-biased sex ratios usually perform better in inoculative biocontrol programmes aimed at temporary establishment and reproduction in cropping systems [48, 59, 60]. Aphid parasitoid sex ratios can be influenced by a variety of host-related factors such as quality [31], age [48, 54], size [61] and species [44]. On suitable host aphid species, sex ratio in parasitoid offspring emerging from small hosts tend to be male-biased, and female-biased from intermediate or large hosts [29]. However, male-biased sex ratio in offspring from large hosts (fourth instar or adults) has been observed in some parasitoid species

[54]. Our study found that sex ratios were female-biased on aphid nymphs of intermediate ages from both *N. ribisnigri* and *M. euphorbiae*, indicating that more female parasitoids emerged from higher quality hosts. This result supports the host quality model of Charnov and Skinner [62]. Similar results have also been reported for other aphid parasitoids [27, 44].

Irrespective of the different feeding locations of the aphids, the no-choice growth chamber experiments demonstrated that *A. abdominalis* has the capacity to find and parasitize aphids feeding both on outer leaves and on the heart leaves of lettuce. No reports are available on the effect of feeding locations on parasitization behavior of *A. abdominalis*. However, our results resemble the finding of Stadler and Volki [46] who reported that other parasitoids such as *Aphidius colemani* (Viereck) (Hymenoptera: Braconidae) partitioned parasitization or searching activity for banana aphids *Pentalonia nigronervosa* (Coquerel) between open and concealed areas of the banana plants. Our results, however, also showed that *A. abdominalis* parasitized a significantly lower proportion of aphids when offered *N. ribisnigri* inoculated only on heart leaves compared to when *N. ribisnigri* were offered on whole lettuce plants. This indicates that only a proportion of lettuce aphids located on the heart leaf were accessible to the parasitoid, presumably a result of some aphid being positioned on more open part and others on the deeper and more narrow part of the heart leaf (G. Shrestha, pers. obs.). In contrast with our findings, Stadler and Volki [46] showed that the parasitoid *L. testaceipes* parasitized *P. nigronervosa* only on open areas but not on the cryptic areas of banana plants. Thus, our and these previous illustrate that parasitoid ability to find hosts feeding on cryptic locations differ between parasitoid species, probably in combination with plant species morphology.

With respect to the choice growth chamber experiment, our study showed that *A. abdominalis* preferred to parasitize *M. euphorbiae* as compared with *N. ribisnigri*. This will reduce the parasitoid's ability to regulate populations of the *N. ribisnigri* when both aphid species occur simultaneously in lettuce plants as *A. abdominalis* will probably encounter and parasitize *M. euphorbiae* feeding on outer leaves [6] more frequently than *N. ribisnigri* feeding on heart leaves [7]. This is in accordance with results from a study by Gardner and Dixon [45], which showed that the rose grain aphid *Metopolophium dirhodum* (Walker) feeding on wheat leaves were parasitized more by *Aphidius rhopalosiphii* (DeStefani-Perez) (Hymenoptera: Braconidae) than the English grain aphid *Sitobion avenae* (Fabricius) feeding on the cryptic part (ear) of the wheat.

In conclusion, the present results indicate that *A. abdominalis* has a potential for inoculative biocontrol of *N. ribisnigri* and *M. euphorbiae*. The results suggest that the use of *A. abdominalis* against *N. ribisnigri* may not be adequate on its own, but that it may be considered as an additional option to be integrated with other potential biocontrol agents (e.g., predators and fungal entomopathogens) of *N. ribisnigri* [19, 21, 63]. Further long-term field or glasshouse studies that include several potential biocontrol agents and several aphid species are therefore needed in order to further validate the potential of biocontrol agents to suppress the *N. ribisnigri* population.

Acknowledgments

The authors would like to thank technicians Steen Meier and Lars Damberg, Dept. of Agroecology, Aarhus University for their valuable assistance on lettuce production and in the designing of acrylic cylindrical insect cage, respectively. We would also like to thank Dr. Hugh A. Smith, Gulf Coast Research and Education, University of Florida for editorial and language assistance. This study was financially supported by Aarhus University Denmark and the National Institute of Food and Agriculture, U.S. Department of Agriculture, Multistate Project W3185, The Working Group Biological Control of Pest Management Systems of Plants under Accession # 231844.

Author Contributions

Conceptualization: Govinda Shrestha, Henrik Skovgård, Annie Enkegaard.

Data curation: Govinda Shrestha.

Formal analysis: Govinda Shrestha.

Funding acquisition: Govinda Shrestha, Gadi V. P. Reddy, Annie Enkegaard.

Investigation: Tove Steenberg, Annie Enkegaard.

Methodology: Govinda Shrestha, Henrik Skovgård, Annie Enkegaard.

Project administration: Annie Enkegaard.

Supervision: Annie Enkegaard.

Writing – original draft: Govinda Shrestha.

Writing – review & editing: Henrik Skovgård, Gadi V. P. Reddy, Tove Steenberg, Annie Enkegaard.

References

1. Fournier V, Brodeur J. Dose-response susceptibility of pest aphids (Homoptera: Aphididae) and their control on hydroponically grown lettuce with the entomopathogenic fungus *Verticillium lecanii*, azadirachtin, and insecticidal soap. *Environ Entomol.* 2000; 29: 568–578.
2. Blackman R, Eastop V. Aphids on the world's crops an identification and information guide. New York: Wiley and sons; 2000
3. Reinink K, Dieleman F. Survey of aphid species on lettuce. *Bulletin OILB/SROP.* 1993; 16: 56–68.
4. Parker W, Collier R, Ellis P, Mead A, Chandler D, Smyth JB, et al. Matching control options to a pest complex: the integrated pest management of aphids in sequentially-planted crops of outdoor lettuce. *Crop Prot.* 2002; 21: 235–248.
5. Åsman K. Aphid infestation in field grown lettuce and biological control with entomopathogenic fungi (Deuteromycotina: Hyphomycetes). *Biol Agric Hortic.* 2007; 25:153–173.
6. Mackenzie J, Vernon R. Sampling for distribution of the lettuce aphid, *Nasonovia ribisnigri* (Homoptera: Aphididae), in fields and within heads. *J Entomol Soc B C.* 1988; 85: 10–14.
7. Liu Y-B. Distribution and population development of *Nasonovia ribisnigri* (Homoptera: Aphididae) in iceberg lettuce. *J Econ Entomol.* 2004; 97: 883–890. PMID: [15279267](https://pubmed.ncbi.nlm.nih.gov/15279267/)
8. Fagan L, McLachlan A, Till C, Walker M. Synergy between chemical and biological control in the IPM of currant-lettuce aphid (*Nasonovia ribisnigri*) in Canterbury, New Zealand. *Bull Entomol Res.* 2010; 100: 217–223. <https://doi.org/10.1017/S0007485309990174> PMID: [19573253](https://pubmed.ncbi.nlm.nih.gov/19573253/)
9. Kift N, Mead A, Reynolds K, Sime S, Barber M, Denholm I, et al. The impact of insecticide resistance in the currant-lettuce aphid, *Nasonovia ribisnigri*, on pest management in lettuce. *Agric For Entomol.* 2004; 6: 295–309.
10. Dewar AM. Chemical control. In: van Emden HF, Harrington R, editors. *Aphids as crop pest.* CAB International, UK; 2007. p. 392–421.
11. Brück E, Elbert A, Fischer R, Krueger S, Kühnhold J, Klueken AM, et al. Movento, an innovative ambimobile insecticide for sucking insect pest control in agriculture: biological profile and field performance. *Crop Prot.* 2009; 28: 838–844.
12. Rufingier C, Schoen L, Martin C, Pasteur N. Resistance of *Nasonovia ribisnigri* (Homoptera: Aphididae) to five insecticides. *J Econ Entomol.* 1997; 90: 1445–1449.
13. Barber M, Moores G, Tatchell G, Vice W, Denholm I. Insecticide resistance in the currant-lettuce aphid, *Nasonovia ribisnigri* (Homoptera: Aphididae) in the UK. *Bull Entomol Res.* 1999; 89: 17–23.
14. Sances FV, Gaston LK, Campos R, Dusch M, Toscano NC. Multiple insecticide treatments affect harvest residues of lettuce. *J Econ Entomol.* 1993; 86: 1781–1785.
15. Koureas M, Tsakalof A, Tsatsakis A, Hadjichristodoulou C. Systematic review of biomonitoring studies to determine the association between exposure to organophosphorus and pyrethroid insecticides and human health outcomes. *Toxicol Letters.* 2012; 210: 155–168.

16. Smith HA, Chaney WE, Bensen TA. Role of syrphid larvae and other predators in suppressing aphid infestations in organic lettuce on California's Central Coast. *J Econ Entomol.* 2008; 101:1526–1532. PMID: [18950033](https://pubmed.ncbi.nlm.nih.gov/18950033/)
17. Hopper JV, Nelson EH, Daane KM, Mills NJ. Growth, development and consumption by four syrphid species associated with the lettuce aphid, *Nasonovia ribisnigri*, in California. *Biol Control.* 2011; 58: 271–276.
18. Quentin U, Hommes M, Basedow T. Studies on the biological control of aphids on lettuce in greenhouses. *J Appl Entomol.* 1995; 119: 227–232.
19. Shrestha G, Enkegaard A. The green lacewing, *Chrysoperla carnea*: preference between lettuce aphids, *Nasonovia ribisnigri*, and western flower thrips, *Frankliniella occidentalis*. *J Insect Sci.* 2013; 13: 1–10. <https://doi.org/10.1673/031.013.0101>
20. Diaz BM, Oggerin M, Lastra CCL, Rubio V, Fereres A. Characterization and virulence of *Lecanicillium lecanii* against different aphid species. *BioControl.* 2009; 54: 825–835.
21. Shrestha G, Enkegaard A, Steenberg T. Laboratory and semi-field evaluation of *Beauveria bassiana* (Ascomycota: Hypocreales) against the lettuce aphid, *Nasonovia ribisnigri* (Hemiptera: Aphididae). *Biol Control.* 2015; 85: 37–45.
22. Shrestha G, Skovgård H, Enkegaard A. Parasitization of commercially available parasitoid species against the lettuce aphid, *Nasonovia ribisnigri* (Hemiptera: Aphididae). *Environ Entomol.* 2014; 43: 1535–1541. <https://doi.org/10.1603/EN14068> PMID: [25290653](https://pubmed.ncbi.nlm.nih.gov/25290653/)
23. Van Lenteren JC. The state of commercial augmentative biological control: plenty of natural enemies, but a frustrating lack of uptake. *BioControl.* 2012; 57: 1–20.
24. Jarosik V, Honek A, Rabasse J, Lapchin L. Life-history characteristics of the aphid parasitoid *Aphelinus abdominalis* reared on *Macrosiphum euphorbiae*. *Ochr Rostl.* 1996; 32: 82–88
25. Price PW, Bouton CE, Gross P, McPherson BA, Thompson JN, Weis AE. Interactions among three trophic levels: influence of plants on interactions between insect herbivores and natural enemies. *Annu Rev Ecol Syst.* 1980; 11: 41–65.
26. Hågvar E, Hofsvang T. Aphid parasitoids (Hymenoptera, Aphidiidae): biology, host selection and use in biological control. *Biocontrol News and Information.* 1991; 12: 13–42.
27. Chau A, Mackauer M. Preference of the aphid parasitoid *Monoctonus paulensis* (Hymenoptera: Braconidae, Aphidiinae) for different aphid species: female choice and offspring survival. *BioControl.* 2001; 20: 30–38.
28. Tapa-Yotto GT, Hofsvang T, Godonou I, Tchibozi I, Sæthre M-G. Host instar suitability of *Aphis gossypii* (Hemiptera: Aphididae) for *Lysiphlebus testaceipes* (Hymenoptera: Braconidae) and parasitism effect on aphid life table. *Appl Entomol Zool.* 2013; 48: 447–453.
29. Godfray HCJ. *Parasitoids: behavioral and evolutionary ecology*: Princeton University Press; 1994.
30. Desneux N, Blahnik R, Delebecque CJ, Heimpel GE. Host phylogeny and specialisation in parasitoids. *Ecol Lett.* 2012; 15:453–60. <https://doi.org/10.1111/j.1461-0248.2012.01754.x> PMID: [22404869](https://pubmed.ncbi.nlm.nih.gov/22404869/)
31. Desneux N, Barta RJ, Hoelmer KA, Hopper KR, Heimpel GE. Multifaceted determinants of host specificity in an aphid parasitoid. *Oecologia.* 2009; 160:387–98. <https://doi.org/10.1007/s00442-009-1289-x> PMID: [19219460](https://pubmed.ncbi.nlm.nih.gov/19219460/)
32. Desneux N, Stary P, Delebecque CJ, Garipey TD, Barta RJ, Hoelmer KA, et al. Cryptic species of parasitoids attacking the soybean aphid (Hemiptera: Aphididae) in Asia: *Binodoxys communis* and *Binodoxys koreanus* (Hymenoptera: Braconidae: Aphidiinae). *Ann Entomol Soc Am* 2009; 102:925–36.
33. Desneux N, Barta RJ, Delebecque CJ, Heimpel GE. Transient host paralysis as a means of reducing self-superparasitism in koinobiont endoparasitoids. *J Insect Physiol.* 2009; 55:321–7. <https://doi.org/10.1016/j.jinsphys.2008.12.009> PMID: [19162033](https://pubmed.ncbi.nlm.nih.gov/19162033/)
34. Asplen MK, Bano N, Brady CM, Desneux N, Hopper KR, Malouines C, et al. Specialisation of bacterial endosymbionts that protect aphids from parasitoids. *Ecol Entomol.* 2014; 39:736–9.
35. Losey JE, Harmon J, Ballantyne F, Brown C. A polymorphism maintained by opposite patterns of parasitism and predation. *Nature.* 1997; 388: 269–272.
36. Libbrecht R, Gwynn D, Fellowes M. *Aphidius ervi* preferentially attacks the green morph of the pea aphid, *Acyrtosiphon pisum*. *J Insect Behav.* 2007; 20: 25–32.
37. Honek A, Jarosik V, Lapchin L, Rabasse J. Host choice and offspring sex allocation in the aphid parasitoid *Aphelinus abdominalis* (Hymenoptera: Aphelinidae). *J Agr Entomol.* 1998; 15: 201–221.
38. Mueller T, Blommers L, Mols P. Woolly apple aphid (*Eriosoma lanigerum* Hausm., Hom., Aphidae) parasitism by *Aphelinus mali* Hal. (Hym., Aphelinidae) in relation to host stage and host colony size, shape and location. *J Appl Entomol.* 1992; 114: 143–54.

39. Gerling D, Roitberg B, Mackauer M. Instar-specific defense of the pea aphid, *Acyrtosiphon pisum*: influence on oviposition success of the parasite *Aphelinus asychis* (Hymenoptera: Aphelinidae). *J Insect Behav.* 1990; 3: 501–514.
40. Perdakis DC, Lykouressis DP, Garantonakis NG, Iatrou SA. Instar preference and parasitization of *Aphis gossypii* and *Myzus persicae* (Hemiptera: Aphididae) by the parasitoid *Aphidius colemani* (Hymenoptera: Aphidiidae). *Eur J Entomol.* 2004; 101: 333–6.
41. Wyckhuys K, Stone L, Desneux N, Hoelmer K, Hopper K, Heimpel GE. Parasitism of the soybean aphid, *Aphis glycines* by *Binodoxys communis*: the role of aphid defensive behaviour and parasitoid reproductive performance. *Bull Entomol Res.* 2008; 98: 361–70. <https://doi.org/10.1017/S000748530800566X> PMID: 18294416
42. Fletcher JP, Hughes JP, Harvey IF. Life expectancy and egg load affect oviposition decisions of a solitary parasitoid. *Proc R Soc Lond B Biol Sci.* 1994; 258: 163–167.
43. Minkenberg OP, Tatar M, Rosenheim JA. Egg load as a major source of variability in insect foraging and oviposition behavior. *Oikos.* 1992; 65: 134–142.
44. Ode PJ, Hopper KR, Coll M. Oviposition vs. offspring fitness in *Aphidius colemani* parasitizing different aphid species. *Entomol Exp Appl.* 2005; 115(2):303–10.
45. Gardner SM, Dixon A. Plant structure and the foraging success of *Aphidius rhopalosiphii* (Hymenoptera: Aphidiidae). *Ecol Entomol.* 1985; 10: 171–179.
46. Stadler B, Völki W. Foraging patterns of two aphid parasitoids, *Lysiphlebus testaceipes* and *Aphidius colemani* on banana. *Entomol Exp Appl.* 1991; 58: 221–229.
47. Syngenta. *Aphelinus abdominalis*. Technical report; [cited 2016 Oct 26] Available from: <http://www.syngenta.com/global/bioline/SiteCollectionDocuments/Products/B8%20-%20Aphelinus%20abdominalis.pdf>.
48. Shrestha G, Skovgård H, Steenberg T, Enkegaard A. Preference and life history traits of *Aphelinus abdominalis* (Hymenoptera: Aphelinidae) when offered different development stages of the lettuce aphid *Nasonovia ribisnigri* (Hemiptera: Aphididae). *BioControl.* 2015; 60: 463–471.
49. Diaz BM, Fereres A. Life table and population parameters of *Nasonovia ribisnigri* (Homoptera: Aphididae) at different constant temperatures. *Environ Entomol.* 2005; 34: 527–534.
50. De Conti BF, Bueno V, Sampaio MV, Van Lenteren JC. Development and survival of *Aulacorthum solani*, *Macrosiphum euphorbiae* and *Uroleucon ambrosiae* at six temperatures. *B Insectol* 2011; 64: 63–68.
51. Röhne O. Effect of temperature and host stage on performance of *Aphelinus varipes* Förster (Hym., Aphelinidae) parasitizing the cotton aphid, *Aphis gossypii* Glover (Hom., Aphididae). *J Appl Entomol.* 2002; 126: 572–576.
52. R Development Core Team. A language and environment for statistical computing. R foundation for statistical computing. Available: <http://www.r-project.org/> (2012).
53. Hothorn T, Bretz F, Westfall P. Simultaneous inference in general parametric models. *Biom J.* 2008; 50: 346–363. <https://doi.org/10.1002/bimj.200810425> PMID: 18481363
54. Colinet H, Salin C, Boivin G, Hance T. Host age and fitness-related traits in a koinobiont aphid parasitoid. *Ecol Entomol.* 2005; 30: 473–479.
55. Abbott W. A method of computing the effectiveness of an insecticide. *J Econ Entomol.* 1925; 18: 265–267.
56. Bueno V, Carnevale A, Sampaio M. Host preference of *Lysiphlebus testaceipes* (Cresson) (Hymenoptera: Aphidiidae) for *Myzus persicae* (Sulzer) and *Aphis gossypii* Glover (Hemiptera: Aphididae). In: António OS, Ventura MA, Garcia V, Hemptinne JL, editors. *Biology, ecology and behaviour of aphidophagous insects. Aphidophaga 2002: VIII International Symposium on Ecology of Aphidophaga*; 2002 Sep 1–6; Ponta Delgada: 2002. p. 17–20.
57. Wahab W. Observations on the biology and behaviour of *Aphelinus abdominalis* Dalm. (Hym., Aphelinidae), a parasite of aphids. *J Appl Entomol.* 1985; 100: 290–296.
58. Battaglia D, Poppy G, Powell W, Romano A, Tranfaglia A, Pennacchio F. Physical and chemical cues influencing the oviposition behaviour of *Aphidius ervi*. *Ent Exp Appl.* 2000; 94: 219–227.
59. Heimpel GE, Jonathan GL. Sex ratios of commercially reared biological control agents. *Biol Control.* 2000; 19: 77–93.
60. Boivin G, Hance T, Brodeur J. Aphid parasitoids in biological control. *Can J Plant Sci.* 2012; 92:1–12.
61. Jarošík V, Holý I, Lapchin L, Havelka J. Sex ratio in the aphid parasitoid *Aphidius colemani* (Hymenoptera: Braconidae) in relation to host size. *Bull Entomol Res.* 2003; 93:255–558. <https://doi.org/10.1079/BER2003229> PMID: 12762867

62. Charnov EL, Skinner SW. Complementary approaches to the understanding of parasitoid oviposition decisions. *Environ Entomol.* 1985; 14:383–91.
63. Shrestha G, Enkegaard A, Reddy GVP, Skovgård H, Steenberg T. Susceptibility of larvae and pupae of the aphid parasitoid *Aphelinus abdominalis* (Hymenoptera: Aphelinidae) to the entomopathogenic fungus *Beauveria bassiana*. *Ann Entomol Soc Am.* 2017. 110; 121–127.