Natural flightless morphs of the ladybird beetle *Adalia bipunctata* improve biological control of aphids on single plants

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ABSTRACT

The challenge of using ladybird beetles for biological control of insect pests such as aphids is that the adult beetles tend to fly away from the host plants. Therefore, flightless ladybirds might improve biocontrol. There are several artificial ways to obtain flightless beetles, but it may be preferable to use natural variation in flight ability. We investigated, for the first time, biocontrol by inundative augmentation of natural flightless morphs of the ladybird beetle *Adalia bipunctata*. Microcosm experiments using single leaves with one of three species of aphid revealed no differences in consumption behavior between flightless and winged beetles. Monitoring for 48 h of single, caged pepper plants infested with aphids of *Myzus persicae nicotianae* or *Aulacorthum solani* showed that flightless beetles had a longer residence time on the plants than winged beetles. This only translated into significantly better biocontrol of *M. persicae*. Despite their difference in residence time, both beetle morphs reduced the population growth of *A. solani*. This is probably explained by the tendency of *A. solani* to drop from the plant upon disturbance, and we predict that flightless beetles may outperform winged ones in the long term. Overall, our results provide a proof of principle that natural flightless *A. bipunctata* can improve biocontrol of aphids by ladybird beetles. However, we recognize that the effect of biocontrol will vary with the species of aphid used and that further examination in long term and large scale experiments is required.

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1. Introduction

Predatory ladybird beetles have a long history in biological control of aphid infestations. Early augmentative biocontrol attempts made use of adult beetles that had been collected from overwintering sites or that were laboratory-reared (Majerus, 1994; Obrycki and Kring, 1998). However, even in greenhouses, adult beetles tend to fly away from the crop soon after release and are, therefore, not reliable biocontrol agents (Hämäläinen, 1977; Minoretti and Weisser, 2000). One way to increase the residence time of ladybird beetles in the crop and obtain reduction in aphid numbers is the release of non-flying juvenile stages (e.g. Ferran et al., 1996; Kehrli and Wyss, 2001). However, young beetle larvae are less efficient than adults because they consume fewer aphids per day and once developed into adults they are prone to migrate if aphid densities are low (Hodek et al., 1993). Alternatively, flightless beetles can be used to prolong the residence time of adults (Ignoffo et al., 1977; Ferran et al., 1998) and might, therefore, improve biocontrol of aphids.

To obtain flightless morphs, various researchers have surgically removed wings of ladybird beetles (Ignoffo et al., 1977; Olszak, 1986). Others have applied mutagenesis followed by artificial selection on wing malformations or the ability to fly in the Asian species *Harmonia axyridis* Pallas (Ferran et al., 1998; Tourniaire et al., 1999). In contrast to such artificial techniques to create flightless ladybird beetles, it could be beneficial to utilize natural variation in flight ability. Examples of species of ladybird exhibiting such variation include *Rhyzobius litura* Fabricius, where variation in flight ability results from variation in wing folding (Hammond, 1985), *Adalia bipunctata* (L.), with dimorphism for wing reduction (Marples et al., 1993), and *H. axyridis*, with quantitative variation in flight ability not associated with any changes in the wings (Tourniaire et al., 2000). In the latter species, flightless strains have successfully been obtained by artificial selection for reduced flight ability (Tourniaire et al., 2000; Seko et al., 2008).

To assess the potential of these flightless beetles to improve biocontrol of aphids, comparison to the performance of winged conspecifics is essential. So far, this comparison has only been made for selected flightless strains of *H. axyridis* by Seko et al. (2008). They have shown that, in small open fields, flightless *H. axyridis* have a longer residence time on the crop and are more

effective in suppressing aphid numbers. So, these selected flightless beetles have potential as biocontrol agents. However, the use of predatory ladybirds is restricted to their native distribution because of the ecological risks of releasing non-native species (Koch, 2003; Evans, 2004; Majerus et al., 2006; Van Lenteren et al., 2008). Though *H. axyridis* has recently spread from its native area in Asia to parts of Europe and North America, where it is now abundant (Koch, 2003; Brown et al., 2008), its use is at best debatable in these areas, and it is prohibited in several European countries. Therefore, natural flightless morphs of the two-spot ladybird beetle, *A. bipunctata*, are suitable candidates for biocontrol where this species is native to parts of Europe, North America, and north and central Asia. Flightless *A. bipunctata* that lack the distal part of both pairs of wings are occasionally encountered in the wild. Since this trait is controlled genetically (Marples et al., 1993), one can easily rear pure-breeding populations from field-collected flightless individuals. In addition, the species can be reared all year round in the laboratory (Hämäläinen, 1976). Hence, the use of flightless *A. bipunctata* for biological control might save production costs while it does not involve risks connected to introducing genetic modified organisms (GMOs) or non-native species.

Though Seko et al. (2008) obtained positive results for the application of flightless *H. axyridis* in biocontrol, those results cannot be directly extrapolated to flightless *A. bipunctata* because the nature of the flightlessness is different in the two species. Whereas the strain of *H. axyridis* has been selected for individuals with intact wings but reduced flight ability, natural flightless morphs of *A. bipunctata* have reduced wings which prevent flight. The genetics underlying different types of flight inability may each have different pleiotropic effects on traits that affect their suitability for biocontrol. For example, flightless *H. axyridis* that were selected for wing malformations after a chemical mutagenic treatment had 9-fold higher mortality, fecundity was reduced to a third and egg hatch rate was reduced to a quarter (Ferran et al., 1998) whereas natural flightless *H. axyridis* that were selected for normal morphology but flight inability showed no differences with wild-type beetles in these traits (Tourniaire et al., 2000). Another strain of flightless *H. axyridis* that was selected for flight inability after a chemical mutagenic treatment needed more time to search and ingest prey than wild-type beetles (Tourniaire et al., 1999). Therefore, it is necessary to specifically test the efficacy of natural flightless *A. bipunctata* in biocontrol.

Here we report on the ability of natural flightless adult *A. bipunctata* to reduce aphid pests compared to their winged conspecifics. The experiments were designed to test indoor biocontrol by inundative augmentation, where the released individuals are intended to control the pest species for a short period after release. In contrast, inoculative augmentation aims at establishing a self-sustaining population, where the progeny of the released individuals should control the pest over a longer period. However, there is little empirical and theoretical support for the success of ladybird beetles in inoculative augmentation (Obrycki and Kring, 1998; Dixon, 2000). From laboratory experiments it is known that longevity and life-time reproduction of flightless *A. bipunctata* are reduced to about a third of that of wild-types (Ueno et al., 2004), but it is not known if foraging traits of flightless morphs are altered. Reduced longevity might diminish the benefits of the expected prolonged residence time of flightless beetles in inundative augmentation, whereas altered foraging traits may either enhance or hamper biocontrol. In contrast, reproduction traits do not affect the success of inundative control. We first compare the beetles' dispersal behavior and their effect on aphid populations in a biocontrol experiment using single, caged pepper plants and two species of aphid. We then compare the food consumption of the two morphs of the ladybird beetle using three species of aphid in a microcosm experiment. Finally, we discuss the potential of flightless *A. bipunctata* for inundative biocontrol of different aphid species.

2. Material and methods

2.1. Ladybird beetles

Two *A. bipunctata* found in the wild in Utrecht, The Netherlands, and bearing alleles for the flightless trait, were outcrossed to over a hundred wild-types from the same locality to obtain a laboratory population of flightless (−/− genotype for the trait) and winged ladybird beetles (+/− genotype) between 2000 and 2004. New wild-types from the same locality were collected to establish a pure winged laboratory stock (+/+ genotype) in 2005. The populations were reared on *Ephestia kuehniella* Zeller eggs and flower pollen ad libitum and were kept at a temperature of 20.5 (±1) °C and in a 16L:8D light regime. This temperature is higher than outdoor temperatures because the experiments focus on indoor application of ladybird beetles. Only female adult ladybird beetles were selected for use in the experiments, because they require more nutrients and spend more time on foraging than males (Hodek, 1973).

2.2. Aphids

For the biocontrol experiment, we used two species that are well known pests in greenhouses and are reported to be suitable prey for *A. bipunctata*: *Aulacorthum solani* (Kaltenbach) (Mills, 1981) and *Myzus persicae* (Sulzer) var. *nicotianae* (Blackman, 1965). They were kept on leaves of sweet pepper (*Capsicum annuum* L. cv. Lesley) maintained on solid 1% agar solution in the bottom of Petri dishes (75 mm Ø × 30 mm) which were covered with mesh net and inverted. We used a temperature of 23.5 (±1) °C and a 16L:8D light regime. For the consumption experiment we also used *Rhodobium porosum* (Sanderson), another pest species, which is not known as a suitable prey species. These aphids were reared in the same way but on strawberry leaves (*Fragaria x ananassa* Duchesne cv. Elsanta).

2.3. Biocontrol experiment

After eclosion from the pupae, female ladybirds were individually transferred to Petri dishes (75 mm Ø × 30 mm) containing pepper leaves, where they were allowed to feed ad lib on the target species of aphid at a temperature of 23.5 (±1) °C. In this way we familiarized them with consuming aphids to prevent immediate dispersal when released on aphid-infested plants in the experiment. To minimize variation in the phenotype only virgins 2–17 days old with an approximate body length of 6 mm (corresponding to the mean size) and without any elytral tissue were used. Experiments were performed in nine cages (2.65 × 0.75 × 1.25 m) made of fine mesh net and with a solid bottom, and kept in a greenhouse with minimum temperatures of 22.5 °C and 21.5 °C in day and night, respectively. A single pepper plant (*C. annuum* cv. Lesley) with a height of 65–80 cm was placed on the bottom of each cage such that it was not in contact with the cage. At least six leaves of each plant were infested with in total 15–50 aphids of one of the species of aphid. Aphids were allowed to settle and reproduce for one to three days and then counted. The plants were then randomly allocated to one of three treatments: flightless ladybird, winged ladybird (+/− for the trait) or control (no ladybird). In the ladybird treatments, a single beetle was placed on top of the lowest leaf of the plant. During the next 48 h, its location (on/off the plant) was monitored at 11 time points during daytime. It was then removed from the cage and the aphids remaining on the plants counted. For *M. persicae*, each treatment was replicated 12 times,

divided over six consecutive series of two replicates each. After that, 10 replicates divided over four series of two or three replicates were obtained for *A. solani*.

2.4. Consumption experiment

Microcosm experiments were performed to prevent dispersion of predators and prey, and to minimize the effect of searching ability of the predators. We used Petri dishes with sweet pepper (randomly allocated to *A. solani* and *M. persicae*) or strawberry leaves (for *R. porosum*) as described above. The experimental conditions were a temperature of 20 (±1) °C and 16L:8D. Aphids were allowed to settle in the dishes for one day, after which their number was reduced to 10 per dish. Dishes were then randomly allocated to one of three treatments: flightless ladybird, winged ladybird (+/+ for the trait) or control (no ladybird). In the ladybird treatments, a 24-h starved virgin female of age 8–12 days was introduced into the dish. Starvation was applied because the ladybirds had been reared on *Ephestia* eggs before use in the experiment and though it has been shown that they do consume aphids after switching to an aphid diet (Ferran et al., 1996), their appetite for aphids might have decreased. One day later all remaining aphids were counted. Each combination of treatment and aphid species consisted of 15 replicates.

2.5. Statistical analysis

All data were analyzed using R.2.6.1. (R Development Core Team, 2007). To analyze the position of the ladybird beetles in the biocontrol experiment, we fitted a GLM with ladybird morph as factor and a binomial error distribution for each time point. We then removed the ladybird morph factor from the model and compared the reduced model with the original one by a Chi-square test on the residual deviances to detect effects of ladybird morph. When a beetle could not be detected, it was regarded as a missing value for that time point.

We used an Analysis of Variance (ANOVA) on aphid population growth to analyze the effect of treatments on aphid numbers in the biocontrol experiment. Aphid population growth was calculated as final count of aphids 48 h after ladybird release over initial count of aphids at time zero. We applied an ANOVA for each species of aphid separately, since the experiments on the two species were performed consecutively. We first fitted a full factorial model with treatment and series as fixed effects and then stepwise deleted factors from this model until the minimal adequate was found, using Chi-square tests on the residual deviances to compare models. One series consisting of two replicates was excluded from the analysis of *A. solani*, because aphid population growth in the control treatments of this series was below one, indicating unfavorable conditions.

To test the relationship between aphid population growth and ladybird residence time, we fitted a linear regression model. We used the total minimum residence time of ladybirds on the plants in daylight as deduced from cases where ladybirds were registered to be on the plant. When a data point was missing in between consistent observations on presence or absence, ladybirds were assumed to not have changed position in between, since this would require the unlikely scenario of two movements in a short period. Since ladybirds cease foraging in the dark (Hodek and Honk, 1996), residence time on the plant at night (8 h in that time of the year) was subtracted from the total residence time. Consistent with the previously described analysis on aphid population growth, two replicates of *A. solani* were excluded.

The consumption experiment was analyzed by a full factorial Generalized Linear Model (GLM) with treatment and aphid species as fixed factors. To adjust the error structure as closely as possible

to the distribution of the data and to avoid overdispersion, we specified a quasi error structure with a square root link function and a variance equal to the quadratic mean. This was supported by a Box–Cox Normality plot which finds the maximum likelihood power transformation of the response variable in a regression model by the Box–Cox method. It showed that a power transformation of 0.5 (corresponding to a square root transformation) would be optimal for our data. We excluded three outliers from the analysis (one in each treatment of *A. solani*), since model checking revealed they strongly affected the results. We investigated differences between treatments by pooling two of the treatments successively and testing whether the new model was significantly different from the full model by an *F* test on the deviances. Because 15% of the data did not fit the assumed error structure, we re-analyzed the same data with non-parametric statistics in SPSS 15.0 (Kruskal-Wallis for overall treatment effect, followed by pair-wise Mann Whitney *U* tests for differences between treatments where a Bonferroni correction was applied to adjust α). The results of both analyses were in agreement. To test differences in consumption of different species of aphid, the ladybird treatments were pooled and analyzed separately in the same way, using *y* + 1 as dependent variable. Similarly the control treatments were analyzed separately to test differences in intrinsic population growth between the different species of aphids.

3. Results

3.1. Biocontrol experiment

With both species of aphid, winged beetles tended to leave the plants earlier than flightless beetles, and most of them did so within 4 h after release (Fig. 1). Only two hours after release, flightless ladybird beetles were significantly more often found on the plant than winged beetles (*M. persicae*: $p < 0.01$; *A. solani*: $p < 0.05$). This distribution persisted during the remainder of the experiment, but was no longer significant after 30 h for plants with *A. solani* ($p = 0.053$). The latter can be explained by some winged ladybirds that returned to the plants with *A. solani* (Fig. 1).

Fig. 2 shows the effect of treatments on the growth of the aphid populations. There was no significant effect of series on aphid population growth in either species of aphid (*M. persicae* $p = 0.60$; *A. solani* $p = 0.53$), so per species all series were pooled for further analysis. The control treatments demonstrate that both species of aphid reproduced readily on the plants (mean aphid population growth $\gg 1$ in Fig. 2). Population growth was lowest when flightless ladybirds were released (mean aphid growth ± SE for flightless, winged and control treatments in *M. persicae*: 1.406 ± 0.166, 2.985 ± 0.287, and 2.433 ± 0.179, respectively; in *A. solani*: 1.339 ± 0.434, 1.526 ± 0.242, and 3.226 ± 0.362, respectively). The difference of flightless beetles with the control was highly significant for both species of aphid (both $p < 0.001$), but flightless beetles only reduced aphid numbers significantly more than winged beetles with *M. persicae* (*M. persicae* $p < 0.001$; *A. solani* $p = 0.709$). This occurred because winged beetles did not produce any reduction in population growth of *M. persicae* ($p = 0.073$), whereas they did with *A. solani* ($p < 0.010$). Overall, only a few ladybird beetles were able to decrease the absolute number of aphids on the plants (those cases below $y = 1$ in Fig. 3) but in no cases were all aphids removed.

The minimum residence time of the ladybird beetles on the plants in daylight was significantly negatively related to aphid population growth and explained about a third of the variation in their numbers for both species, suggesting a causal relationship (*M. persicae*: $F_{1,22} = 13.440$, $p < 0.010$, $R^2 = 0.379$; *A. solani* $F_{1,14} = 6.478$, $p < 0.050$, $R^2 = 0.316$) (Fig. 3).
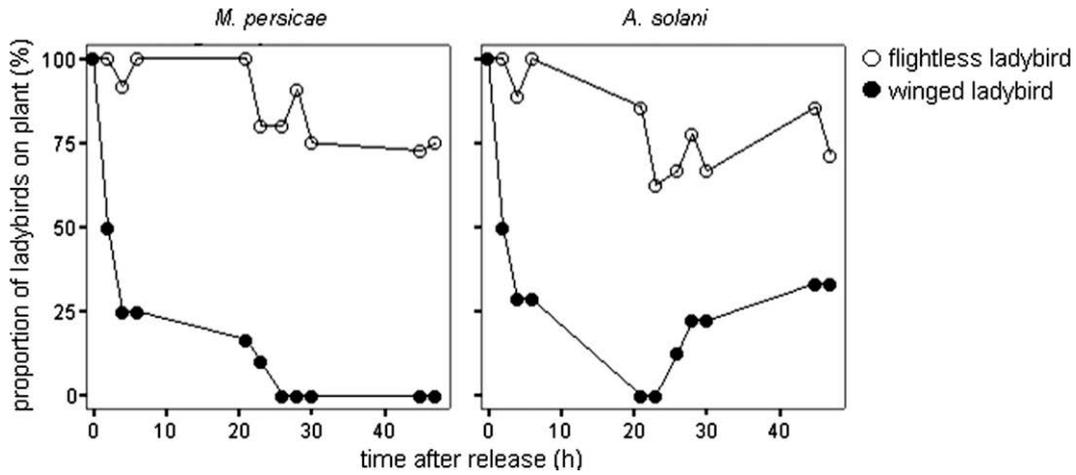
Fig. 1. Location of flightless and winged beetles after release on single, caged plants infested with either *M. persicae* (left, *N* = 8–12 plants) or *A. solani* (right, *N* = 6–10).
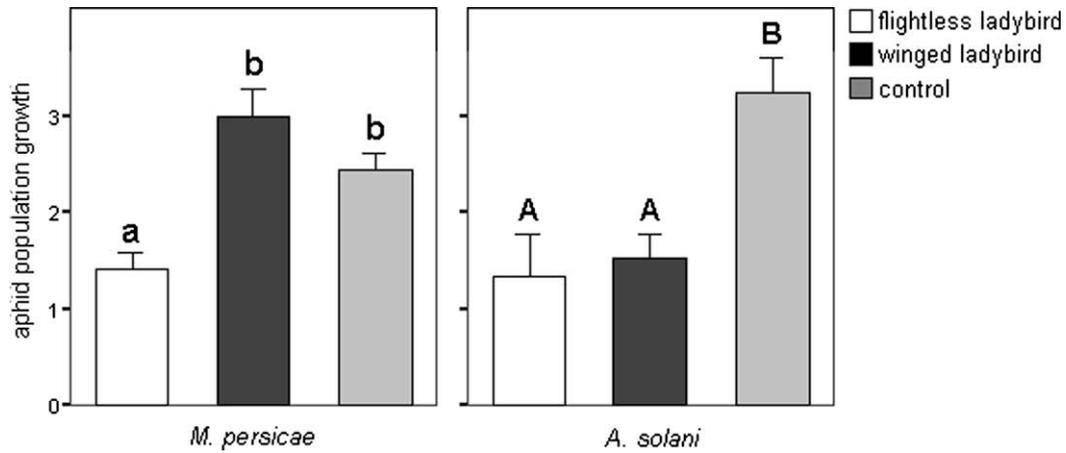

Fig. 2. Aphid population growth, calculated as final/initial count, on a pepper plant infested with *M. persicae* (left panel, *N* = 12) or *A. solani* (right panel, *N* = 8) 48 hours after the release of flightless or winged ladybirds or the control treatment (no ladybirds). Bars represent mean values; error bars represent standard errors of the mean. Identical letters above bars indicate no significant differences between means (*p* > 0.05).
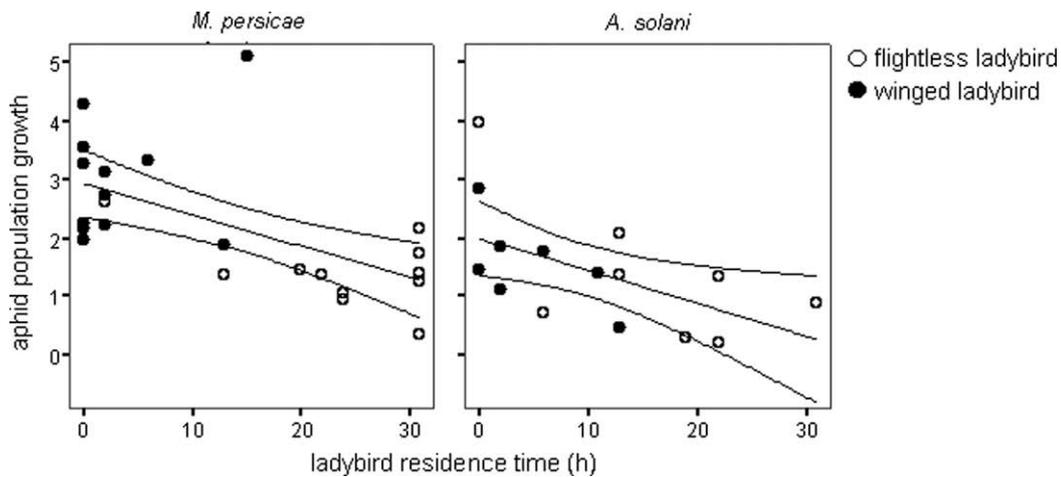

Fig. 3. The relationship between aphid population growth and ladybird residence time on pepper plants infested with *M. persicae* (left panel, *N* = 24) or *A. solani* (right panel, *N* = 16). Symbols indicate whether flightless or winged ladybirds were released. Lines represent the overall regression lines in between the 95% confidence interval lines. Regression lines are significant (*M. persicae*: *p* < 0.01; *A. solani*: *p* < 0.05) and explain about a third of the variation in both species of aphid.
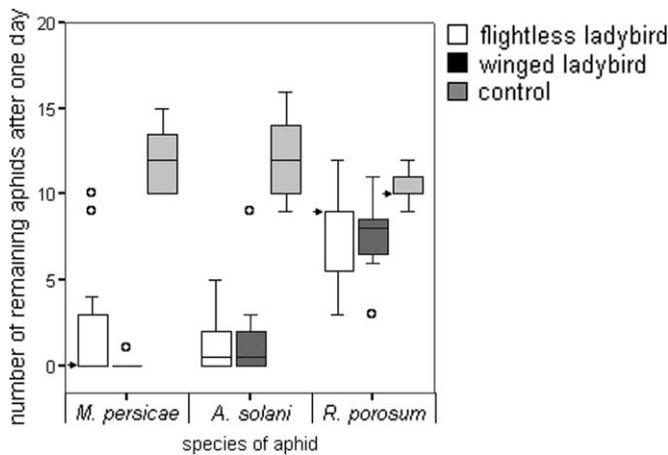
Fig. 4. Number of remaining aphids in a Petri dish one day after a flightless ladybird beetle, a winged ladybird or no beetles (control) were introduced in a dish with 10 aphids of one of three species ($N = 15$ for *M. persicae* and *R. porosum*, $N = 14$ for *A. solani*). Boxes represent interquartile ranges (IQR), covering 50% of all data points, lines in boxes or arrows indicate median values, whiskers show the range of values that fall within 1.5 IQR of the box, and circles represent data that fall outside this range. There were no significant differences between the two ladybird treatments ($p > 0.05$), and both ladybird treatments significantly reduced the number of aphids compared to the controls ($p < 0.001$). Fewer *R. porosum* were consumed than *A. solani* or *M. persicae* ($p < 0.001$), and *R. porosum* grew slower than both of the two other species of aphid ($p < 0.01$).

3.2. Consumption experiment

The consumption experiment showed a significant interaction between treatment and species of aphid ($F_4 = 3.390$, $p < 0.050$). Nevertheless, both ladybird beetle morphs consistently reduced the number of aphids from all three species within 24 h when offered on a leaf in a Petri dish (Fig. 4) ($F_3 > 7.190$ for each ladybird morph, $p < 0.001$). There was no significant difference between the numbers of aphids eaten by winged and flightless beetles ($F_3 = 0.516$, $p = 0.672$).

Comparing the consumption of the different aphid species, there was no difference between *M. persicae* and *A. solani* (median of remaining aphids of *M. persicae* = 0; median *A. solani* = 0.5; $F_1 = 0.025$, $p = 0.875$) but the ladybird beetles ate higher numbers of those species than of *R. porosum* (median *R. porosum* = 8; $F_1 = 44.271$, $p < 0.001$). The control treatments show that populations of *M. persicae* and *A. solani* grew at the same pace (median aphid number *M. persicae* and *A. solani* = 12; $F_1 = 0.050$, $p = 0.825$), but faster than those of *R. porosum* (median *R. porosum* = 10; $F_1 = 8.408$, $p < 0.010$).

4. Discussion

4.1. Proof of principle

The results of the biocontrol experiment show that flightless beetles stay longer on single, aphid-infested plants than winged beetles regardless of the species of aphid. They consistently reduced aphid population growth compared to the controls for both species, and were more successful in control of *M. persicae* than winged beetles, because winged beetles did not have any effect on population growth of this species. The effective control of *M. persicae* can be explained by the longer residence time of the flightless beetles on the plants, which is inversely related to aphid growth.

In contrast, flightless beetles were not significantly more successful than winged beetles in controlling *A. solani* because both ladybird morphs reduced population growth of this species. This

can probably be explained by a tendency of *A. solani* to drop from the plant upon disturbance as defense behavior, in contrast to *M. persicae* that does not tend to drop (unpublished results). Dropping behavior is a well known escape response of aphids that can be elicited by the presence of a predator or parasitoid. Ladybird beetles are among the species of predator that cause highest displacement by dropping (Losey and Denno, 1998; Day et al., 2006). This behavior increases mortality risk of the aphid from other sources (Dill et al., 1990) and has been shown to contribute to the reduction of aphid population growth in field experiments (Nelson et al., 2004). As ladybird beetles show negative geotropism and climb to the top of the plant to take off for flight (Bänsch, 1966; Hodek, 1973), the winged ladybird beetles in our experiment are likely to have induced dropping behavior before they left the plants. In addition, in our cages some winged ladybirds returned to the plants after they had left them, whereas this is unlikely in an agricultural cropping system. Therefore, the experimental results for *A. solani* might be conservative. Residence time of the ladybirds was also significantly inversely related to aphid growth of *A. solani*, suggesting that a longer residence time does contribute to aphid removal. Due to the short duration of the experiment, this contribution was probably small compared to the effect of the presence of ladybirds after release. However, in the long term, flightless beetles may well outperform winged beetles regardless of the aphids' tendency to dropping behavior, because their longer residence time is likely to increase aphid displacement by both consumption and dropping behavior.

Together, these results form the proof of principle that flightless *A. bipunctata* indeed stay longer on the plants and, as a consequence, might improve biocontrol by reducing aphid population growth.

This result was also found for flightless *H. axyridis* released on experimental outdoor fields of eggplants infested with the cotton aphid, *Aphis gossypii* (Seko et al., 2008). These ladybirds had been artificially selected for reduced flight ability without wing malformations, in contrast to the flightless *A. bipunctata* which are flightless as a result of wing reduction. Though the cause of flightlessness is different for the two species, the results with respect to residence time and biocontrol is similar. Apparently, possible negative pleiotropic effects of the genes causing flightlessness are outweighed by the positive effects of longer residence time. The similarity in the results of two different species of ladybirds, together with the relationship between residence time and reduction of aphids demonstrated in this paper, strengthens the idea that flightless beetles can improve the efficacy of ladybirds in control of aphids.

4.2. Properties of predator and prey species affect success of biocontrol

Besides predator dispersal behavior, the predator's ability to remove prey from the host plants is another important factor determining the effectiveness of biocontrol. Many properties of both predator and prey affect this ability, such as searching efficiency and consumption behavior of the ladybirds, and growth rate and defense mechanisms (including the previously discussed escape behavior) of the aphids. Therefore, we compared the consumption behavior of flightless and winged ladybird beetles using one of three species of aphid in microcosms. The control treatments without beetles allowed us to examine aphid population growth rates.

The results of the consumption experiment show no significant differences in feeding behavior between flightless and winged beetles (homozygote recessive ($-/-$) and dominant ($+/+$) for the trait, respectively). Therefore, we also expect winged beetles that are heterozygote ($+/-$) for the trait, as used in the biocontrol experiment, to behave similarly. Consequently, we assume that consumption behavior did not contribute to the differences between

flightless and winged beetles treatments observed in the biocontrol experiment. However, experiments with larger numbers of aphids may reveal differences in consumption and we cannot exclude differences in other aspects of foraging behavior, such as searching efficiency. For example, Tourniaire et al. (1999) showed that their strain of artificial flightless *Harmonia* was slower in encountering and ingesting aphids on plants than wild-type conspecifics.

In contrast, we have observed differences between the prey species. First, though *A. bipunctata* is known to accept a wide range of prey species (Hodek, 1973), the number of aphids consumed by the ladybirds when offered in a Petri dish showed variation: *M. persicae* and *A. solani* were consumed at substantially higher rates than *R. porosum*. By restricting space and time, we minimized the effect of searching ability of the ladybird beetles, and of population growth and defense behavior of the aphids. Therefore, observed differences are likely to represent the variation in the ladybirds' readiness to eat various species of aphid. Consequently, we predict the effectiveness of flightless *A. bipunctata* in controlling *R. porosum* to be minimal. However, we cannot rule out potential effects of the host plant, which was different for *R. porosum*. As flightless beetles behaved similarly to wild-types in their consumption behavior, existing literature on feeding habits of wild-type *A. bipunctata* can be consulted to predict the success of biocontrol of a particular species of aphid by flightless morphs. Secondly, the species of aphids varied in population growth rate, which becomes relevant to biocontrol if flightless ladybirds do not remove all aphids from the plants immediately (Minoretti and Weisser, 2000). Together, these results highlight the potential for wide variation in the effectiveness of biocontrol of different species of aphid by flightless ladybird beetles.

4.3. Optimizing flightless beetles for use in biocontrol

Although flightless ladybird beetles reduced aphid population growth significantly in the biocontrol experiment, the effectiveness of biocontrol may be optimized through further investigation. For example, none of the beetles was able to eradicate the aphid population on the plant. These results are in line with Minoretti and Weisser (2000), who showed that individual *Coccinella septempunctata* beetles did not remove all individuals of small pea aphid colonies (10–30 aphids) from bean plants before they left the plants (which was within two hours after release). In a long term study with low densities of the cotton aphid on outdoor eggplants, flightless *H. axyridis* were able to keep aphids at the initial densities, but also never eradicated aphid populations (Seko et al., 2008). In contrast, Olszak (1986) reported that two manually de-winged adults of *A. bipunctata* succeeded in removing small colonies (10 or 20 aphids) of *Aphis pomi* from single, caged apple trees in seven days, but the predators were replenished when they escaped. Theoretical work predicts that ladybird females will not consume aphid colonies completely at the place where they oviposit, in order to guarantee food availability for their offspring (Dixon, 2000). We should monitor over longer periods to examine whether absolute aphid numbers would decrease when flightless ladybirds are given more time. In addition, it is known that the predation pattern of ladybird beetles is scale-dependent (Ives et al., 1993) and, therefore, experiments should be conducted at larger scales resembling agricultural settings, such as greenhouses. Further, we would have to apply various predator densities to determine the optimum in aphid reduction. Since there was substantial variation among individual ladybird beetles, it might be better to introduce more than one beetle at every release spot. Finally, the flightless phenotype may be improved by artificial selection on traits important for biocontrol. In addition, we should also investigate how large scale releases of this morph would alter

allele frequencies of natural *A. bipunctata* populations, even if flightless *A. bipunctata* represent a natural phenotype.

4.4. Conclusion

Our experiments have demonstrated the proof of principle that naturally flightless morphs of *A. bipunctata* remain longer on aphid-infested plants and, as a consequence, can reduce aphid numbers more than their winged conspecifics. This longer residence time does not translate into more effective biocontrol in the short term when the particular species of aphid involved has a high tendency for dropping behavior, but we predict positive results in the long term. In line with previous experiments and theory, the flightless beetles were not able to eradicate the aphids. Nevertheless, they kept aphid numbers close to their initial densities. We have shown that flightless ladybirds were not different from wild-types with respect to their feeding behavior, suggesting that knowledge on the prey range of wild-types can be applied to the flightless morphs. In addition, we have pointed out that there are many differences between aphid species that can affect the success of biocontrol, that is, their defense mechanisms, their population growth rates, and the readiness of the ladybirds to eat them. Hence, whilst flightless ladybirds may improve biocontrol, further research is needed to examine whether the reported effects will prevail in larger, realistic agricultural settings and for longer periods with different species of aphid.

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