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ORIGINAL CONTRIBUTION

Combining lacewings and parasitoids for biological control of foxglove aphids in sweet pepper

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Keywords
Aphidius ervi, *Aulacorthum solani*, dropping behaviour, intraguild predation, *Micromus variegatus*, predator facilitation

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Abstract

The role of natural enemy diversity in biological pest control has been debated in many studies, and understanding how interactions amongst predators and parasitoids affect herbivore populations is crucial for pest management. In this study, we assessed the individual and combined use of two species of natural enemies, the parasitoid *Aphidius ervi* Haliday, and the predatory brown lacewing *Micromus variegatus* (Fabricius), on their shared prey, the foxglove aphid, *Aulacorthum solani* (Kaltenbach), on sweet pepper. We hypothesized that the presence of intraguild predation (IGP) and predator facilitation (through induced aphid dropping behaviour) might have both negative and positive effects on aphid control, respectively. Our greenhouse trial showed that overall, the greatest suppression of aphids occurred in the treatment with both the parasitoid and the lacewing. While the combination of lacewings and parasitoids significantly increased aphid control compared to the use of parasitoids alone, the effect was not significantly different to the treatment with only predators, although there was a clear trend of enhanced suppression. Thus, the combined effects of both species of natural enemies were between additive and non-additive, suggesting that the combination is neither positive nor negative for aphid control. High levels of IGP, as proven in the laboratory, were probably compensated for by the strong aphid suppression provided by the lacewings, whether or not supplemented with some level of predator facilitation. For aphid management over a longer time scale, it might still be useful to combine lacewings and parasitoids to ensure stable and resilient aphid control.

Introduction

The role of natural enemy diversity in biological pest control has been debated in many studies (Cardinale et al. 2003; Casula et al. 2006; Straub et al. 2008; Letourneau et al. 2009; Tylianakis and Romo 2010). Although the majority of studies show greater herbivore suppression with increased natural enemy richness (Letourneau et al. 2009), it is also true that some interactions amongst natural enemies can disrupt biological control and reduce herbivore suppression (Rosenheim et al. 1995; Finke and Denno 2004; Messelink et al. 2011). Natural enemies that are engaged in predatory interactions between each other are in most cases also competing for the same prey, which is called intraguild predation (IGP) (Polis et al. 1989). IGP can affect both the likelihood of these natural enemies being able to coexist and their ability to suppress prey populations (Rosenheim et al. 1995). Despite this, these potential negative effects are often weakened by habitat complexity (Janssen et al. 2007) or outweighed by positive effects, such as a strong pest suppression by the intraguild predator (Messelink and Janssen 2014; Gontijo et al. 2015).

Natural enemy interference can also result in non-additive effects (Ferguson and Stiling 1996) that

neither positively or negatively influence biological control programmes. Additive effects occur when species of natural enemies are complementary, for example when they attack different life stages of the pest (Calvo et al. 2009), or when they attack the same pest but on different parts of the plant (Onzo et al. 2004; Gable et al. 2012). Some interactions between natural enemy species can even be synergistic, that is when the combined effect of both natural enemy species on populations of a specific pest is greater than the sum of their individual effects. This can occur when one natural enemy alters the behaviour or feeding niche of prey making them more susceptible to attack by another natural enemy, a mechanism called 'predator facilitation' (Losey and Denno 1998a; Sih et al. 1998). Thus, the effects of multiple species of natural enemies on pest populations can be larger or smaller than the effect of each enemy species on its own. Assemblages of natural enemies are often engaged in a mixture of several direct and indirect interactions amongst species with both negative and positive effects on biological control (Janssen et al. 1998; Messelink et al. 2012b; Roubinet et al. 2015). These complexities make it difficult to predict whether some potentially negative interactions might be outweighed by positive interactions, and is why more multispecies trials are needed to evaluate these interactions, particularly under field conditions.

In this study, we assessed the individual and combined effects on aphids of two species of natural enemies that have the potential to be involved in both positive and negative interactions with each other. Our study system consisted of the foxglove aphid, *Aulacorthum solani* (Kaltenbach) (Hemiptera: Aphididae), which is a major pest of both vegetable and ornamental crops in greenhouses (Sanchez et al. 2010; Jandricic et al. 2014) and two species of natural enemies. The two species of natural enemies we evaluated were the parasitoid, *Aphidius ervi* Haliday (Hymenoptera: Braconidae), and the predatory brown lacewing, *Micromus variegatus* (Fabricius) (Neuroptera: Hemerobiidae). *Aphidius ervi* is commonly augmented for control of *A. solani* in commercial greenhouses (Blümel 2004). However, aphid control by parasitoids is often disrupted by hyperparasitoids (Boivin et al. 2012) and additional species of natural enemies are usually needed to control aphids. *Micromus variegatus* is relatively new and has only been evaluated experimentally on a small scale, but it might be an interesting candidate to use in combination with parasitoids (van Schelt et al. 2005; Messelink et al. 2012a). How well these two species might control aphids if used together is unknown. As most lacewing larvae are generalist predators, feeding on all kinds of prey (Canard 2001), they have the potential to prey on aphids parasitized by *A. ervi*. According to theory (Holt and Polis 1997), this interaction could disrupt aphid control if *A. ervi* is a more effective control agent of aphids than *M. variegatus*. In contrast to this negative interaction, there is also potential for the two species to work together synergistically via predator facilitation. Earlier studies show that *A. solani* readily falls from plants (dropping behaviour) in response to the presence of predators or parasitoids (Lommen et al. 2008; Gillespie and Acheampong 2012). Aphids that fall from the plant could be an easy target for *M. variegatus* because it forages preferentially on the lower parts of plants (Szentkirályi 2001). However, the positive effects of predator facilitation may be counteracted by the negative effects of IGP, resulting in negative or additive effects, rather than synergistic effects, on the control of aphids. In this study, we first verified whether IGP occurred and to what extent when *M. variegatus* was provided with a choice between *A. ervi*-parasitized and unparasitized aphids. Secondly, we determined to what extent both natural enemies induced dropping behaviour in *A. solani*. Finally, the individual and combined effects of *A. ervi* and *M. variegatus* on aphid suppression were evaluated in a greenhouse trial on sweet pepper. We hypothesized that the presence of IGP and predator facilitation (through induced aphid dropping behaviour) might have both negative and positive effects on aphid control, respectively.

Material and Methods

Plants and insect rearing

Twelve-week-old and insecticide-free sweet pepper plants (*Capsicum annuum* L. cv. Spider) were purchased from a commercial producer (the Grow Group, Naaldwijk, the Netherlands). Peach-potato aphids, *M. persicae* (Sulzer) (red phenotype), were reared on these sweet pepper plants in one greenhouse compartment. *Aulacorthum solani* were reared in plastic boxes (5 cm × 6 cm) on a sweet pepper leaf disc that was embedded, abaxial side uppermost, in water agar (1%). The boxes were placed upside-down, thus ensuring that the abaxial side of the leaf discs (where the aphids feed) faced downwards as they would on intact plants. Ventilation was possible through a hole in the lid covered with insect gauze (mesh size 80 μm) and because the boxes were placed on a wire gridwall shelf in the climate chamber. The parasitoids *A. ervi* were purchased from Koppert Biological Systems (Berkel en Rodenrijs, the Netherlands) as 'mummies'

(pupae) within parasitized pea aphids, *Acyrthosiphon pisum* (Harris). The mummies were placed in plastic boxes (5 cm × 6 cm) until adults emerged when a drop of diluted honey was added as food. Adults were maintained in these boxes for at least 1 day to ensure that all females had mated prior to use in experiments. The culture of *M. variegatus* was originally purchased from Applied Bio-Nomics Ltd., Victoria, British Columbia, Canada (although this Palaearctic species is also endemic in Europe (Klimaszewski et al. 2009)), and thereafter reared in plastic boxes (26 cm × 26.5 cm) and fed *M. persicae* supplemented with sterilized eggs of *Ephestia kuehniella* Zeller (purchased from Koppert Biological Systems) and diluted honey on paper towels. Buckwheat hulls were added to provide shelter for larvae, thus reducing cannibalism. Adult lacewings were put in separate boxes provided with jute ropes as oviposition substrates. Ropes with eggs were collected from these boxes to start new cultures. Insect rearing and the IGP study were carried out in growth chambers maintained at 25°C, 70% RH and a 16:8 L/D regime; the other experiments were performed under greenhouse conditions, and the average climatic conditions were recorded. All experiments were performed at Wageningen UR Greenhouse Horticulture, the Netherlands.

IGP study

The purpose of this study was to determine whether *M. variegatus* showed any feeding preference when given a choice of equal numbers of unparasitized aphids and parasitized aphids. Two types of parasitized aphids were evaluated: young parasitized aphids (5 days after oviposition) and old parasitized aphids (mummies, 8–10 days after oviposition).

To obtain parasitized aphids, individual mated female *A. ervi* (2 days old) were placed inside boxes (5 cm × 6 cm) each containing 40–60 fourth and fifth nymphal instars of *A. solani* on sweet pepper leaf discs (set up as described previously). After 1 h, the female parasitoids were removed and the boxes of parasitized *A. solani* incubated for either 5 days to produce young parasitized aphids (n = 15), or 8–10 days to produce parasitized aphids that had developed in to mummies (n = 11). The boxes were established at different times to ensure that aphids at different stages of parasitism were available on the same day for the experiment. Only 20 young parasitized aphids or mummies were left in each box (all other individuals were removed with a needle) and 20 unparasitized aphids were added to the same box to provide the prey choice necessary. 1 h later (to allow the aphids to settle), one 24-h-starved adult female *M. variegatus* (10–12 days old) was placed into each box and allowed to forage for 24 h after which time it was removed and the number of undamaged mummies, undamaged parasitized aphids and unparasitized aphids that remained, were counted. Young parasitized aphids were distinguishable from unparasitized aphids because the parasitoid larva inside the aphid was visible under a binocular microscope (40×). The prey preference of *M. variegatus* was analysed using a paired *t*-test. Data (number of individuals of each prey type eaten) were $\sqrt{n+0.5}$ transformed prior to analysis in order to create normal distribution and/or homogeneity of variance in the data set (Sokal and Rohlf 1987). All analyses were performed in the software package R version 3.2.1 (R Foundation for Statistical Computing, Vienna, Austria; http://www.R-project.org).

Dropping behaviour study

The effects of both the parasitoid *A. ervi* and the predator *M. variegatus* on the dropping behaviour of *A. solani* were studied on sweet pepper plants, in experimental cages under greenhouse conditions. The experiment was conducted in June under natural light conditions. The treatments were as follows: A) control (without natural enemies), B) one 2-day-old mated female *A. ervi* and C) one 24-h-starved female *M. variegatus,* and there were four replicate cages per treatment.

Each replicate cage (60 × 60 × 90 cm, mesh size 500 μm) contained one potted sweet pepper plant (approximately 50 cm high) with 10–12 leaves which was infested with approximately 30 mixed-instar *A. solani* aphids. After 1 week, the majority of aphids were removed using a brush to leave a standardized mixed-instar population of 100 aphids on the upper leaves and no aphids on the basal leaves. Subsequently, the corresponding natural enemy was introduced into each cage of each treatment and allowed to forage for 150 min. After this time, all natural enemies were removed and the number of aphids on the upper leaves and on the basal leaves was counted, respectively. After a further 24 h, the number of aphids on the basal leaves was counted again to determine how many aphids had reinfested the plants. The proportion of aphids dropping, re-infesting and missing and/or dead were analysed using one-way ANOVA. Proportions were arcsin square-root-transformed before analysis to reduce heterogeneity of variances. Differences amongst treatments were tested using Tukey HSD method ($P < 0.05$). All analyses were performed in the software package R version 3.2.1.

Population dynamics experiment

This experiment was conducted in a greenhouse during autumn–winter to evaluate the individual and combined effects of *A. ervi* and *M. variegatus* on populations of *A. solani* on a sweet pepper crop. Sweet pepper plants, cv Spider, were grown individually in peat in 10-litre pots in a greenhouse compartment (24 m^2). For the experiment, each potted plant was placed into the centre of an open-topped plastic box (50 cm × 60 cm × 15 cm) on a 10 cm layer of peat. Nutrients for the plants were provided through drip irrigation, and holes were drilled in the base of each box for water drainage. A 1 cm layer of buckwheat hulls was placed on top of the peat to provide shelter for any subsequently introduced lacewing larvae that might drop from plants. Plants were each infested with 200 mixed-instar *A. solani* when it had reached ca. 1 m high. This was achieved by introducing small pieces of sweet pepper leaf each bearing 10–20 aphids; the leaf pieces were placed randomly on to leaves in the upper 50 cm of the plant. Each boxed sweet pepper plant was subsequently enclosed in a mesh cage (60 × 60 × 180 cm) made of fine gauze (mesh size 500 μm) representing an experimental unit.

Each experimental unit was distributed within the greenhouse using a randomized block design, and 1 week after aphid infestation, natural enemies were added to achieve each of the following treatments: A) untreated (control), B) two mated female *A. ervi* (1–2 days old) introduced to the mesh cage, C) four female *M. variegatus* (1 week old) introduced to the mesh cage and D) two female *A. ervi* + four female *M. variegatus* (same ages as treatments B and C) introduced to the mesh cage. There were five replicates of each treatment, and thus, 20 experimental units were used in total. One replicate of the treatment with only parasitoids was omitted due to a contaminating infestation of *M. persicae*. Densities of aphids, lacewings and parasitized aphids (mummies) were monitored weekly for four consecutive weeks by counting the total number of each species (*A. solani* (all instars combined); mummies (including empty cocoons) of *A. ervi*; eggs, larvae and adults of *M. variegatus*) on 20 randomly selected leaves per experimental unit: ten leaves in the upper part of the plants and ten leaves in the lower part of the plant.

Temperature and relative humidity in the greenhouse compartment were recorded every 5 min using a climate recorder (Hoogendoorn Growth Management, Vlaardingen, the Netherlands) throughout the experiment. The average temperature and relative humidity during the experiment (from the time aphids were introduced) was 19.1°C (range 17.9–25.4°C) and 66% (range 35%–89%), respectively. The plants were illuminated (5000 lux of artificial light) for 12 h each day.

Differences in population dynamics of aphids, parasitized aphids (mummies) and lacewings amongst treatments were analysed over time using generalized linear mixed models (GLMM) with a Poisson error distribution of the data. Treatment was the fixed factor and time was included as a random factor to correct for pseudo-replication (Bolker et al. 2009). Two-sided *t*-probabilities were calculated to test pairwise differences between at the 5% level. Differences in ratios between aphids in the upper and lower leaves amongst treatments and over time were also analysed with GLMM, but with a binomial distribution of the data. All analyses were performed in the software package Genstat, 18th edition.

Results

IGP study

Adult *M. variegatus* showed a significant preference for feeding on young parasitized aphids over unparasitized aphids ($t = -2.97$; d.f. = 14; $P = 0.01$), although the preference was not particularly strong and they commonly fed on both prey (fig. 1a). An opposite and much stronger effect was observed in the trial with older parasitized aphids, that is mummies: adult *M. variegatus* consumed significantly more unparasitized aphids than mummies ($t = 7.84$; d.f. = 10; $P < 0.001$). Despite this, *M. variegatus* still fed on both prey items, consuming a mean of 9.45 ± 0.9 unparasitized aphids and 4.18 ± 0.6 mummies (fig. 1b).

Dropping behaviour study

There was a significant effect of treatment on the percentage of aphids dropping from the plant ($F = 91.01$; d.f. = 2,33; $P = <0.001$); significantly more aphids dropped from the plant in the presence of *A. ervi* compared with the presence of *M. variegatus* (fig. 2). The percentage of aphids that dropped in the absence of natural enemies was practically zero (fig. 2). Of all the aphids that dropped in the presence of natural enemies, about half successfully re-infested the plant within 24 h, whereas 80% of the few aphids that dropped in the control treatment re-infested the plants (fig. 2).

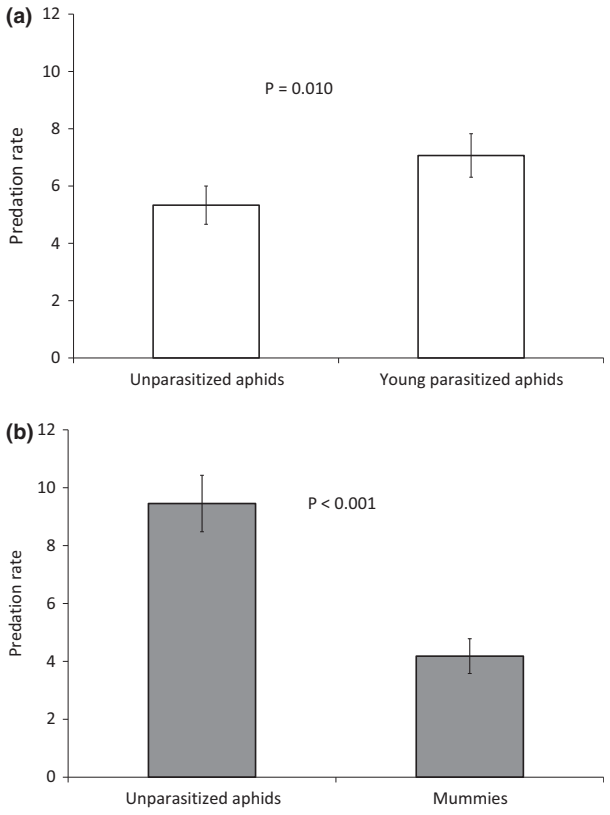

Fig. 1 Predation rates of *Micromus variegatus* females in two-choice experiments with unparasitized aphids of *Aulacorthum solani* and the same aphid species parasitized by *Aphidius ervi*: (a) unparasitized aphids vs. young parasitized aphids (5 days after parasitization) and (b) unparasitized aphids vs. mummies (10 days after parasitization). Data shown are the mean (±SE) numbers of prey consumed by *M. variegatus* females in 24 h. The P-value indicates the significance of differences between treatments.

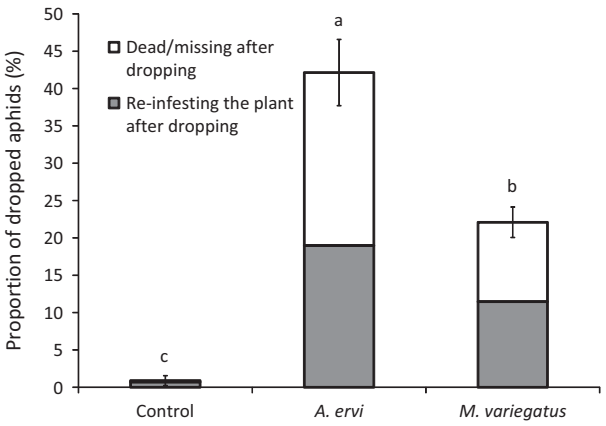

Fig. 2 Effect of natural enemy species on the proportion of the *Aulacorthum solani* population (100 mixed instars) dropping from sweet pepper plants when exposed for 150 min to the natural enemies. The parasitoid *Aphidius ervi* was compared with adult females of the lacewing predator *Micromus variegatus* and a control treatment without natural enemies. Data shown are the mean (±SE) percentages of aphids that dropped per treatment and the percentage of aphids that dropped and successfully re-infested the plants or were dead/missing after 24 h. SE bars are for the total proportion of aphids that dropped. Different letters above the bars indicate significant differences amongst treatments for the total proportion of aphids that dropped (Tukey's HSD test, $P < 0.05$).

Population dynamics experiment

There was a significant effect of treatment on the densities of aphids ($F = 10.63$; d.f. = 3,69; $P = <0.001$). None of the treatments controlled the aphids entirely, but the greatest reduction in rate of increase was achieved in the treatment where both *A. ervi* and *M. variegatus* were present (fig. 3a). The combination of *A. ervi* and *M. variegatus* resulted in a significantly slower increase in the aphid population compared with the treatment with *A. ervi* alone, but the combination did not significantly differ from the treatment with the *M. variegatus* alone (fig. 3a). No statistical differences were found in lacewing densities between the treatment with *M. variegatus* alone and in combination with *A. ervi* both for eggs ($F = 1.81$; d.f. = 1,38; $P = 0.186$), larvae ($F = 0.2$; d.f. = 1,38; $P = 0.655$), adults ($F = 0.59$; d.f. = 1,38; $P = 0.449$) or the sum of all stages ($F = 1.33$; d.f. = 1,38; $P = 0.256$) (fig. 3b). However, significantly lower densities of mummies were found in the treatment with both natural enemies compared with *A. ervi* alone ($F = 7.25$; d.f. = 1,31; $P = 0.011$; fig. 3c).

The proportion of aphids in the upper plant parts over time was significantly different amongst treatments ($F = 3.31$; d.f. = 3,69; $P = 0.025$). Over time, these proportions were highest in the treatment with lacewings alone (fig. 4). Densities of parasitized aphids were in general two times higher on the upper plant parts compared with the lower plant parts, but differences were not significant ($F = 2.45$; d.f. = 1,65; $P = 0.122$), neither was the interaction plant part × treatment ($F = 1.35$; d.f. = 1,65; $P = 0.249$). Lacewing eggs were found in significantly higher densities in the upper plant parts than in the lower plant parts ($F = 5.24$; d.f. = 1,76; $P = 0.024$), but this unequal distribution was not found for larvae ($F = 0$; d.f. = 1,73; $P = 0.955$) or adults ($F = 0.66$; d.f. = 1,73; $P = 0.421$).

Discussion

The greatest suppression of aphids in the greenhouse population study was achieved in the treatment with both the parasitoid *A. ervi* and the lacewing

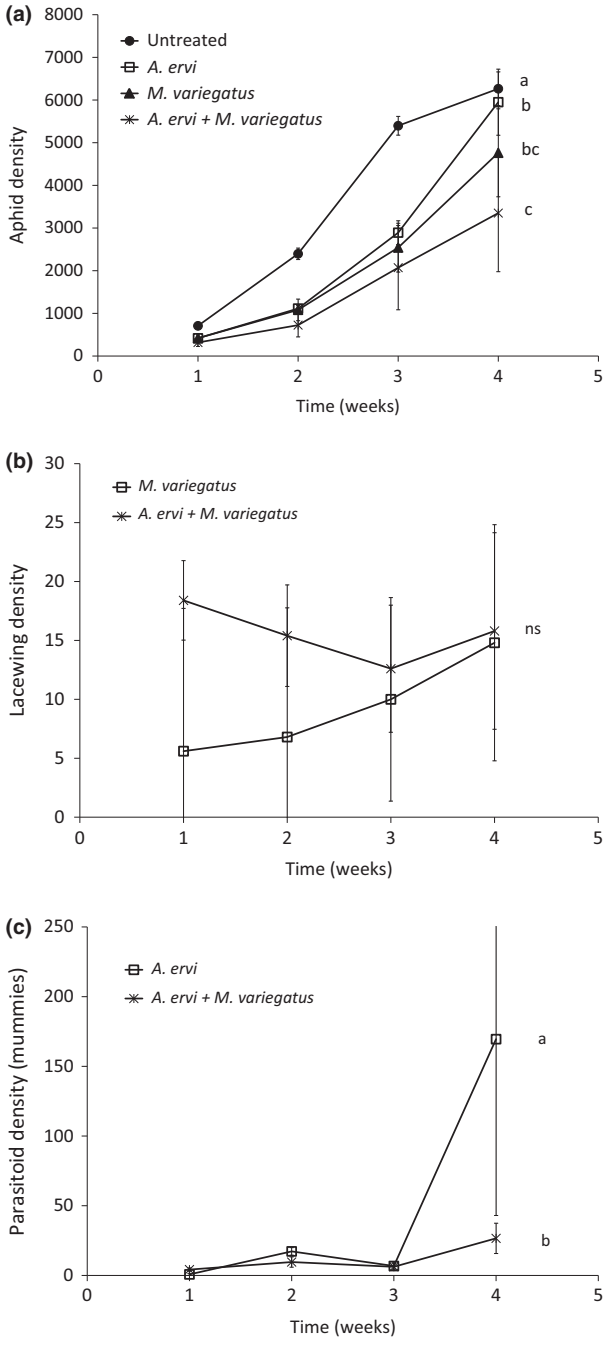

Fig. 3 Population dynamics of (a) the aphid *Aulacorthum solani* in treatments with no natural enemies (untreated), with parasitoids (*Aphidius ervi*), with predators (*Micromus variegatus*) and with both species of natural enemies (*A. ervi* + *M. variegatus*), (b) the lacewing *M. variegatus* in the absence or presence of the parasitoid *A. ervi* and (c) the parasitoid *A ervi* in the absence or presence of the predator *M. variegatus*. Data shown are the mean (±SE) densities of (a) all stages of aphids, (b) all stages of lacewings and (c) parasitized aphids (mummies) per 20 leaves. Mummies represent closed and empty (those where parasitoids already emerged) mummies. Different letters indicate significant differences over time ($P < 0.05$).

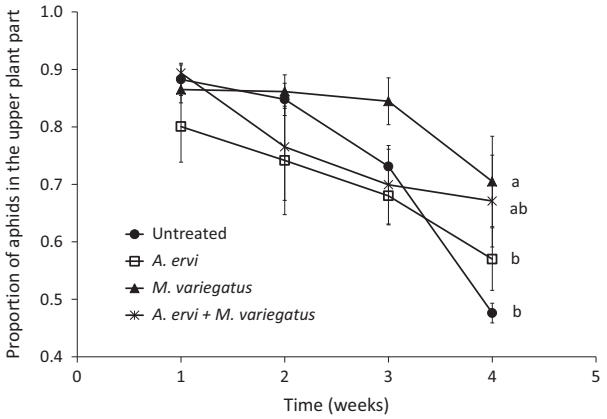

Fig. 4 Proportion of *Aulacorthum solani* aphids in the upper leaves in treatments with no natural enemies (untreated), with parasitoids (*Aphidius ervi*), with predators (*Micromus variegatus*) and with both species of natural enemies (*A. ervi* + *M. variegatus*). Data shown are the mean (±SE) fractions of aphids based on densities of 10 upper and 10 lower leaves. Different letters indicate significant differences over time ($P < 0.05$).

M. variegatus. The combination of lacewings to parasitoids significantly enhanced the impact on aphids, but the effect of the combination was not significantly different to the treatment with only predators, although there was a clear trend of enhanced suppression. Thus, the combined effects of both species of natural enemies are between additive and non-additive, suggesting that the combination is neither positive nor negative for aphid control. In other words, the same results might have been achieved by doubling the release densities of either the parasitoids or the lacewings alone. The non-additive effect of parasitoids added to lacewings might be as a result of the generally poor performance of the parasitoids in this experiment. This was unexpected, because other experiments show this parasitoid to be very effective against *A. solani* (Gillespie and Acheampong 2012); also the dropping behaviour experiment showed that even one single female parasitoid could be very aggressive and induce a high proportion of aphids to drop. It is possible that this poor performance of the parasitoids in the population experiment might be as a result of the reduced light conditions during autumn–winter, as activity of parasitoids was found to be related to day length for a related species (Abe et al. 2014).

We hypothesized that both positive and negative interactions between lacewings and parasitoids might be involved when they are used together for controlling aphids. Indeed, we confirmed the occurrence of IGP in the laboratory, which can potentially disrupt

biological control. Adult lacewings consumed parasitized aphids and even preferred to consume young parasitized aphids over unparasitized aphids. Similar results were reported by Meyhöfer and Klug (2002), who found that parasitized aphids were caught significantly more frequently than unparasitized aphids by *Coccinella septempunctata* L. and *Chrysoperla carnea* (Stephens). However, when we compared relative consumption of parasitoid mummies and unparasitized aphids, the lacewings clearly preferred the unparasitized aphids, thus reducing IGP. The preference for unparasitized aphids over mummies has been reported for several species of predators (Colfer and Rosenheim 2001; Meyhöfer and Klug 2002). Mummies are inferior prey for predators because they are lower in nutritional value, and it takes the predator longer to handle mummies compared with unparasitized aphids (Takizawa et al. 2000). Despite this, IGP is likely to occur under greenhouse conditions and could potentially reduce the efficacy of the parasitoids. Indeed, there were significantly fewer mummies in the treatments with both parasitoids and lacewings compared with the treatment with only parasitoids in our greenhouse trial, indicating the occurrence of IGP. However, this negative effect of IGP did not affect the results of biological control. Combining predators with parasitoids clearly enhanced the overall suppression of aphids. It could be that IGP effects were weak because of the relatively high prey densities. Also, in this particular experiment, the predators were more effective than the parasitoids; thus, the negative effects of IGP would, in this case, be compensated for by the strong regulatory effect of the intraguild predator (lacewing in our case) on the shared prey (Holt and Polis 1997). Our results are in agreement with other studies that also demonstrated that high levels of IGP do not necessarily disrupt biological control of aphids because of the partial preference of the intraguild predator for unparasitized aphids and/or the high levels of predation by the intraguild predator of the shared prey (Colfer and Rosenheim 2001; Snyder and Ives 2003; Costamagna et al. 2007). Lacewings could still have the potential to disrupt aphid control by parasitoids in cases where the parasitoids are highly effective. This study provides no evidence for that.

The negative effects of IGP might also be compensated for by other positive effects, such as predator facilitation. We hypothesized that this might occur when the parasitoids induced dropping of the aphids and thereby facilitated predation by lacewing larvae on the soil or in the lower plant parts. The dropping behaviour experiment confirmed that the aphids responded strongly to the parasitoids with a dropping escape response, which is in agreement with the study of Gillespie and Acheampong (2012). Almost 50% of the aphids dropped when exposed for only 150 min to a single female parasitoid. The aphids also dropped in response to the lacewings, but to a much lesser extent (22%), confirming that this escape response differed depending on the species of natural enemy (Losey and Denno 1998b), which might be caused by differences in foraging rates and mobility (Brodsky and Barlow 1986). If the natural enemy has a fast searching rate (i.e. has a relatively high foraging rate, like parasitoids), the best option for the aphid is to drop from the plant, but if the natural enemy is slow moving (i.e. has a low foraging rate), walking away is a more common and successful way to escape (Brodsky and Barlow 1986). Between 45% and 52% of the aphids that dropped in the presence of a natural enemy were capable of re-infesting the sweet pepper plant after 24 h. The rest of the missing aphids probably died through predator consumption, parasitoid attack or because they were unable to relocate the plant after dropping to the ground. So, the presence of both natural enemies, but mainly the parasitoid, caused dropping behaviour in aphids, which might make the aphids more susceptible to attack by predators in the lower plant parts or on the soil surface. However, as we did not observe a synergistic effect of the combined parasitoid–predator treatment, our study does not provide evidence for this predator facilitation. If it was occurring, it might have been compensated for by the negative effects of IGP, or perhaps it was just not a very strong effect. Moreover, we did not find clear niche partitioning between the two species of natural enemies. Both species were found in both the upper and lower plant parts, although most mummies were found in the upper plant parts and there may have been unrecorded lacewing larvae present on the soil surface. Interestingly, the treatment with only lacewings showed the highest proportion of aphids in the upper plant parts compared with the other treatments. This indicates that there was more predation activity in the lower plant part than in the upper plant parts, which may have been caused by unrecorded lacewing larvae during daytime. Thus, it could be that predator facilitation on the soil surface occurred, but if so, it was unlikely to play a major role.

In summary, the combination of the parasitoid *A. ervi* and the brown lacewing *M. variegatus* resulted in neither positive nor negative effects on the overall control of the aphid, *A. solani*, in sweet pepper. It seems that the observed negative effects of IGP were

compensated for by the strong aphid suppression provided by the lacewings whether or not supplemented with some level of predator facilitation as a result of aphid dropping in response to parasitoid attack. For aphid management over a longer time scale, it might still be useful to combine lacewings and parasitoids to ensure resilient aphid control. Although aphid parasitoids are important and widely used natural enemies for aphid management in greenhouse crops, they are also vulnerable for hyperparasitoids (Boivin et al. 2012). Natural enemy diversity might also be important to achieve better results during climatic fluctuations, such as low temperatures during spring, or heat waves in summer. For example, it was found that different species of aphid parasitoids can differ in vulnerability for heat waves (Gillespie et al. 2012). More studies are needed to evaluate these effects on long-term aphid control strategies with multiple species of natural enemies; the influence of the presence of other pests and natural enemies must also be evaluated to design more pest-resilient cropping systems. The results of this study show the importance of understanding the various types of interactions than can occur between predators and parasitoids when assessing their value for biological control, especially in greenhouse systems where biodiversity can be managed.

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