Predation efficiency of the green lacewings *Chrysoperla agilis* and *C. mutata* against aphids and mealybugs in sweet pepper

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Abstract

Chrysoperla species include well-known predators of aphids and other soft-bodied arthropods. As such, they are considered important biological control agents of herbivorous pests in agroecosystems where many of green lacewings species occur. Despite the high number of species of the genus *Chrysoperla*, only a few have been assessed for the predation efficiency of their larvae against pests infesting plants, and even fewer are currently marketed for use in biocontrol practice. Difficulties in species identification within the *Chrysoperla carnea* complex species in particular has been related to varying success of commercial *C. carnea* s.l. releases in the field. In this study, we assessed the ability of two *Chrysoperla* species, *Chrysoperla agilis* a member of the *carnea* cryptic species group, and *Chrysoperla mutata* of the *pudica* group to consume aphid and mealybug individuals and suppress their populations in sweet pepper plants. We found that third-instar larvae of both species were able to consume a high number of aphids (approximately 120 nymphs per larva) and mealybugs (approximately 105 nymphs per larva) within 24 h. Furthermore, the release of second-instar larvae of both *C. agilis* and *C. mutata* was shown to be remarkably efficient in suppressing the pest populations in long-term greenhouse experiments. Aphid populations were suppressed by approximately 98% and mealybugs by 78% as compared to control plants. Our results highlight the predation efficiency and the biocontrol potential of two widespread *Chrysoperla* species for their use in pest control.

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Introduction

The family Chrysopidae includes approximately 1200 species. The larvae of all species are voracious predators and feed on key pests of crops such as aphids, mealybugs, lepidopteran and coleopteran eggs, thrips and spider mites (Canard *et al.*, 1984; McEwen *et al.*, 2001). In addition, chrysopids of certain genera (e.g. *Chrysopa*) are voracious during the adult stage as well (Brooks and Barnard, 1990). Chrysopids are widespread in several habitats including agroecosystems and forests (McEwen *et al.*, 2001). Particularly, because of the occurrence of several species in agricultural systems and due to the voracity of certain species, green lacewings of the family Chrysopidae are considered important biological control agents of key pests (Tauber *et al.*, 2000; Pappas *et al.*, 2011). Despite the fact that many chrysopids are well known for their high predation efficiency against several pests, only a few species are currently commercially available, with *Chrysoperla carnea* sensu lato being the most widely used in biological control (Pappas *et al.*, 2011). Concretely, among the 20 described species of the genus *Chrysoperla*, 'Chrysoperla carnea' is the only species marketed by the European biocontrol industry for aphid control. Yet, a number of other *Chrysoperla* species may prove more efficient/suitable than the commercial *C. carnea* in controlling different pests under specific conditions.

Our previous work on the European species of the *C. carnea* complex has revealed their potential as biological control agents for use in central and southern Europe (Pappas *et al.*, 2013; Athanasiadis *et al.*, 2021). These lacewings are reproductively isolated species that can be distinguished by the low-frequency species-unique courtship songs they produce by vibrating their abdomen on the substrate before mating (Brooks, 1994; Henry *et al.*, 2001; Noh and Henry, 2010; Henry *et al.*, 2013). Among the five European species of the *C. carnea* group, *Chrysoperla agilis* has been recorded in southern Europe, southwestern Asia to northern Iran and central Alaska (Henry *et al.*, 2003; Henry *et al.*, 2011). *C. agilis* is able to survive and reproduce in a wide range of temperature regimes and larvae prey upon factitious foods such as *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae) eggs in the lab (Pappas *et al.*, 2013; Athanasiadis *et al.*, 2021). These findings, along with its wide distribution and

habitat range and occurrence in agricultural crops (Henry *et al.*, 2003; Henry *et al.*, 2011), render *C. agilis* a promising candidate for mass-rearing and use in augmentative biological control.

Chrysoperla mutata (McLachlan) is not a member of the *carnea* complex but of the *pudica* group (Brooks, 1994; Duelli, 2001). With a distribution ranging from Africa to the Mediterranean Europe and Asia, and lately in Russia and Tenerife (Thierry *et al.*, 2004; Canard and Thierry, 2013; Duelli *et al.*, 2019; Makarkin and Shchurov, 2019) it is considered to be able to survive and reproduce in areas with dry and hot summers as well as mild winters (Szentkirályi, 2001). As with the *carnea* cryptic species, the biology and ecology of *C. mutata* as well as its predation efficiency against crop pests remain largely unexplored (Canard and Thierry, 2013).

The two lacewings show overlapping distribution in southern Europe, and in addition, *C. agilis* can be found in northern habitats as well. Moreover, our experience suggests that the rearing of *C. mutata* in large numbers is feasible due to its low occurrence of cannibalism as well as the wide prey/food range. Given the need to increase biocontrol solutions against key pests of crops and the potential of both *C. agilis* and *C. mutata* for use in augmentative biocontrol, we here evaluated the predation efficiency and ability of both species to suppress the populations of the green peach aphid *Myzus persicae* and the cotton mealybug *Phenacoccus solenopsis* on sweet pepper plants. Both pest species cause serious damage to many crops including greenhouse sweet pepper (van der Ent *et al.*, 2017; Pekas *et al.*, 2020; Bragard *et al.*, 2021). The results will be useful to assess the use of *C. agilis* and *C. mutata* in applied biocontrol programmes.

Materials and methods

Laboratory rearing of green lacewings

The laboratory colonies of *C. agilis* and *C. mutata* were maintained at 25 + 1°C and 16:8 LD. The colony of *C. mutata* was established with adults collected in the area of Chalkidiki (Northern Greece), whereas *C. agilis* was collected in Crete (Southern Greece). The larvae of both species were fed on frozen *E. kuehniella* eggs and adults were maintained in cylindrical plastic cages (35 cm in height × Ø25 cm), as described by Pappas *et al.* (2007). Briefly, adults were reared in groups of females and males in cages and fed with a protein-based liquid food (a volumetric mixture of honey, sugar, yeast hydrolysate and water, 1:1:1:1) applied daily on the top of the mesh covering each cage. Lacewing identification was performed by song analysis and morphological traits by Prof. Charles S. Henry (University of Connecticut).

Experimental green lacewing larvae

For the experiments, different instars of lacewing larvae were used. For this purpose, eggs were collected at 24 h intervals from the lacewing colonies and maintained individually in Petri dishes until hatching. Afterwards, the larvae were reared until the desired instar (second or third, depending on the experiment, see below) with *ad libitum* access to *E. kuehniella* eggs.

Herbivores used as prey in the experiments

Two different prey species were tested in the experiments: (a) the aphid *M. persicae* that originated from tobacco in the area of Komotini (Northern Greece) and the mealybug *P. solenopsis* that was collected from sweet pepper plants in the area of Crete. Both species were reared on potted sweet pepper (*Capsicum annuum* L.) plants under laboratory conditions (25 ± 2°C, 65 ± 5% RH, 16:8 LD).

Plants used in the experiments

Pepper (*C. annuum* L., cv P13) seedlings were transplanted in plastic pots (0.5 litres) filled with peat (Klasmann-Deilmann GmbH). Plants were maintained in a growth chamber (25 ± 2°C, 65 ± 5% RH and 16:8 LD) and were watered and fertilized once per week (N-P-K, 10-10-10, $1 \, \mathrm{g \, l^{-1}}$). For the experiments, we used plants 4-weeks after transplantation (d28).

Predation efficiency against aphids and mealybugs

Prey consumption by *C. agilis* and *C. mutata* when provided aphids or mealybugs was assessed in the laboratory and the greenhouse.

Laboratory trials

In the laboratory, daily prey consumption by third-instar larvae of *C. agilis* and *C. mutata* was assessed in Petri dish assays at 25 ± 1°C, 16:8 LD. The larvae were placed individually in Petri dishes (Ø5 cm) without food for 24 h. Afterwards, each lacewing larva was offered 200 (third–fourth instar) aphid (*M. persicae*) nymphs or 150 newly hatched mealybug (*P. solenopsis*) nymphs. Prey consumption was recorded after 24 h. Each treatment included 15 replicates (lacewing larvae).

Greenhouse experiments

In the greenhouse, 60 potted pepper plants were infested with either 100 aphid (third–fourth instar) nymphs or one mealybug female carrying an egg-sac (d28). The plants were placed individually in insect-proof cages ($60 \times 33 \times 33 \, \mathrm{cm}^3$) (25 ± 1°C, 16:8 LD). Forty plants were assigned to the predator treatment (i.e. 20 repetitions per predator). For this, ten lacewing larvae from each lacewing species (second instar) were transferred onto each plant, both 3 days after the aphid infestation (d31) and again 1 week later (i.e. 20 lacewing larvae were released per plant in total). Lacewing larvae had been fed with *E. kuehniella* eggs until they reached the second larval instar. Another 20 plants were infested with aphids without predators being released (control treatment). Aphid numbers per plant were assessed 1 week after the second release (d42) both in the predator and control plants (fig. 1a).

For the mealybug experiments, 60 plants were infested with one mealybug female carrying an egg-sac (d0). Forty plants were assigned to the predator treatment, where 15 lacewing larvae (second instar) of each lacewing species were transferred on each plant after 1 (d35), 2 (d42) and 3 weeks (d49) (i.e. 45 larvae per plant in total). Lacewing larvae had been fed with *E. kuehniella* eggs until the second larval instar. Another 20 plants were infested with mealybug without predators being released (control treatment). Mealybug numbers per plant were assessed 1 week after the last predator release (d56), both in the predator and control plants (fig. 1b).

Lacewing individuals (i.e. emerging adults and live lacewing pupae) inside each cage were also recorded per plant (insect cage).
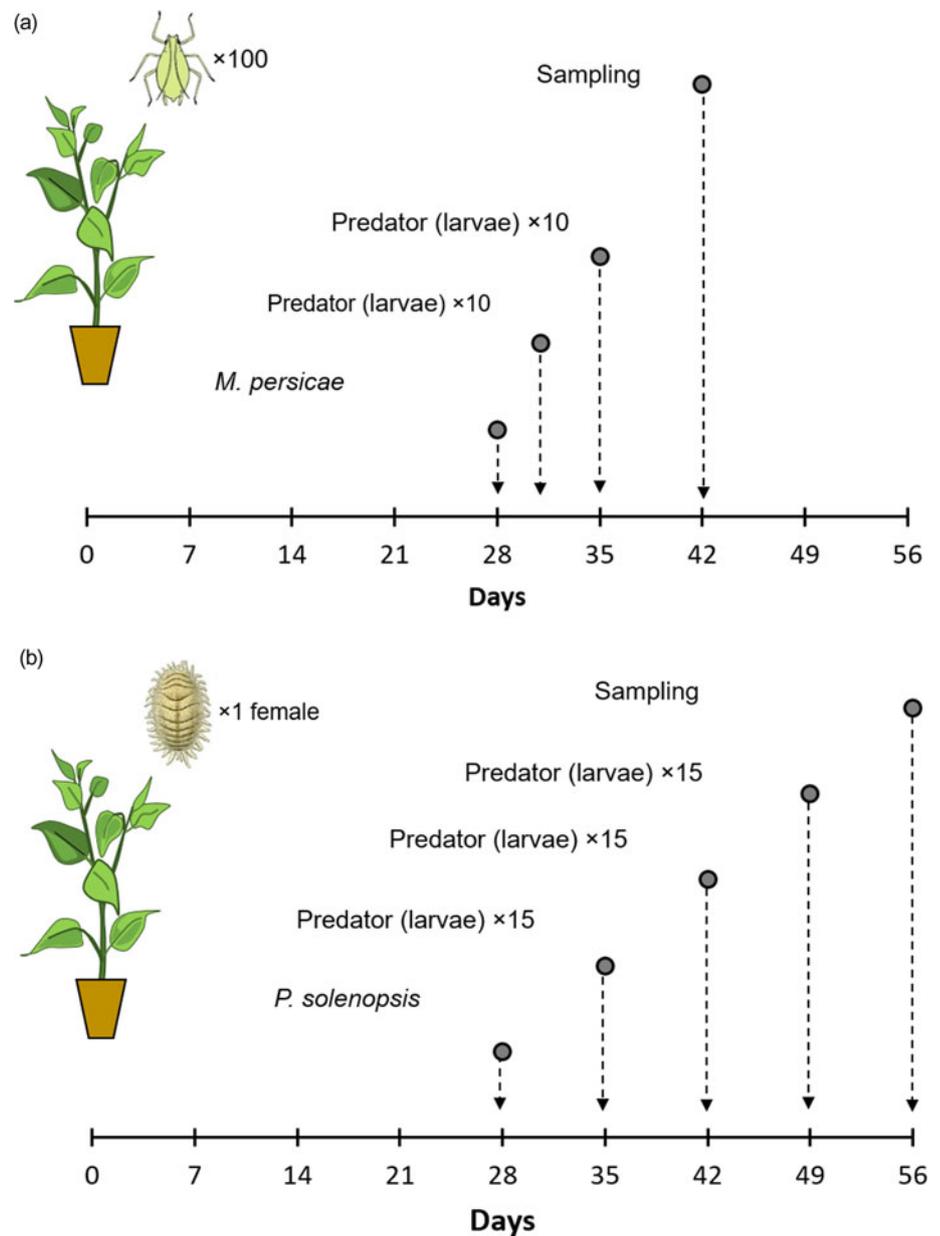
Figure 1. Experimental set-up used to evaluate the predation efficiency of larvae of *C. mutata* and *C. agilis* on pepper plants in insect cages infested by (i) *M. persicae* or (ii) *P. solenopsis*. Pest suppression was assessed 11 or 21 days after lacewing (second-instar larvae) initial release on the plants for aphids and mealybugs, respectively.

Data analysis

To compare daily prey consumption between the different lacewing species a *t*-test was used. In the greenhouse experiment, failing to meet the requirements for parametric analysis, means were compared with the non-parametric Kruskal–Wallis test, followed by pairwise comparisons with Dunn's test. Statistics were performed using SPSS (2020).

Results

Short-term prey consumption

Prey consumption by third-instar lacewing larva in 24 h was 104.3 ± 4.4 and 103.5 ± 3.6 mealybug individuals for *C. agilis* and *C. mutata*, respectively, and 116.0 ± 4.9 and 118.2 ± 4.9 aphid individuals for *C. agilis* and *C. mutata*, respectively (fig. 2). No significant differences were recorded in the mean numbers of mealybugs ($t = 0.153$; $df = 28$; $P = 0.88$; fig. 2a) or

aphids ($F = 0.316$; $df = 28$; $P = 0.754$; fig. 2b) consumed by *C. agilis* and *C. mutata*.

Pest suppression in the greenhouse

Aphid population levels were significantly affected by the release of the lacewing larvae ($\chi^2 = 49.64$; $df = 2$; $P < 0.001$). Mean number of aphids per plant was 4146.2 ± 151.3 individuals in control plants and decreased to 48.9 ± 5.5 and 11.4 ± 2.3 aphids after releasing *C. mutata* and *C. agilis* larvae, respectively (fig. 3a). Out of the initial number of released lacewings (20 larvae in total per plant), a low number of lacewing individuals per species (3.0 ± 1.3 and 2.6 ± 1.2 pupae per cage for *C. mutata* and *C. agilis*, respectively) was recorded in each cage for both species ($U = 162.0$; $Z = -1.067$; $P = 0.286$; fig. 3b).

The level of mealybug infestation was significantly affected by the release of the lacewing larvae as well ($\chi^2 = 39.403$; $df = 2$; $P < 0.001$). The mean number of mealybugs per plant was
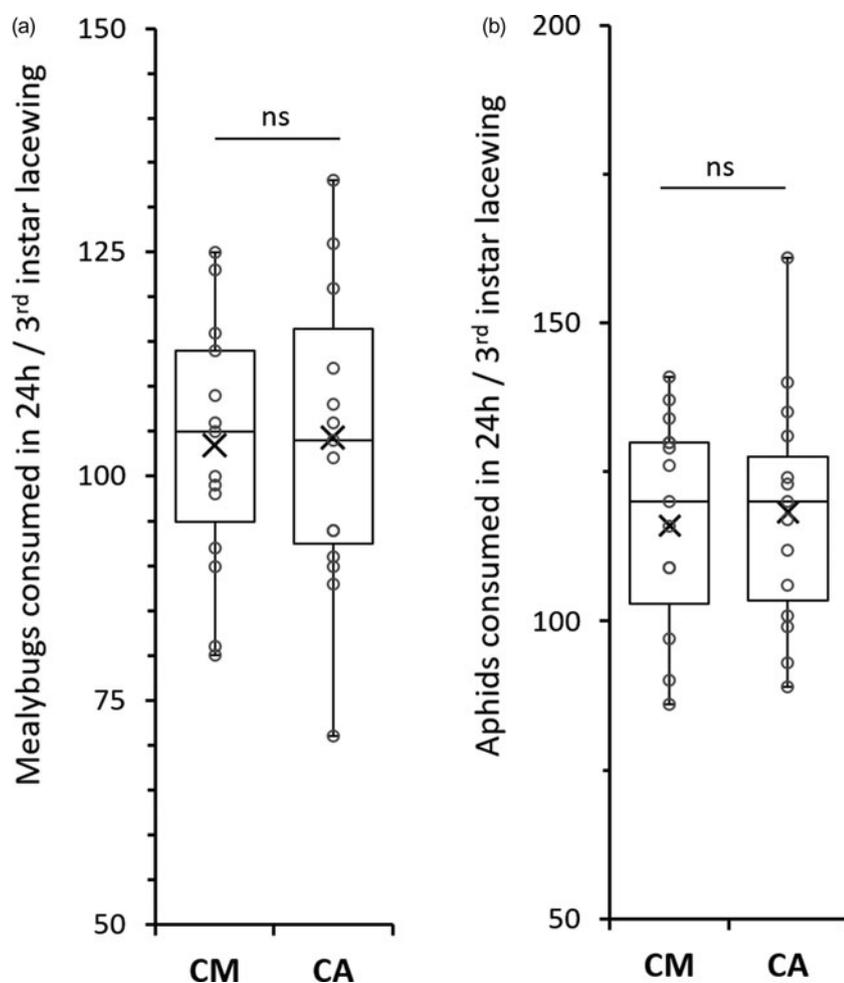
Figure 2. Mean number of (a) mealybugs and (b) aphids consumed by *C. mutata* (CM) and *C. agilis* (CA) third-instar lacewing larvae in 24 h in laboratory Petri dish assays (25 ± 1°C, 16:8 LD). ns: *P* > 0.05.

808.4 ± 55.3 individuals in control plants, which decreased to 208.5 ± 26.7 and 201.2 ± 25.5 mealybugs after releasing *C. mutata* and *C. agilis* larvae, respectively (fig. 4a). Out of the initial number of released lacewings (45 larvae in total per plant), the number of emerging lacewing adults and live pupae was 7.8 ± 0.7 and 3.2 ± 0.3 for *C. mutata* and *C. agilis* respectively (*U* = 29.5; *Z* = −4.655; *P* < 0.001; fig. 4b).

Discussion

In the present study, we assessed the ability of two green lacewing species of the genus *Chrysoperla* to consume individuals of two key pests of vegetable crops, the green peach aphid *M. persicae* and the mealybug *P. solenopsis*. Furthermore, we compared the two lacewings for their efficacy in suppressing the populations of these two pests when infesting pepper plants in the greenhouse. We found that both lacewings can similarly consume individuals of the green peach aphid as well as mealybugs. In addition, both were shown to be efficient in suppressing aphid and mealybug populations in the greenhouse, relative to a predator-free control.

Short-term consumption of aphids and mealybugs was similar between the two lacewing species. Both *C. agilis* and *C. mutata* third-instar larvae were shown to be able to consume a relatively high number of aphids (approximately 120 nymphs per larva) within 24 h. These results confirm the high voracity of both lacewing species and confirm what is already known about the predation efficiency of late instar larvae of *Chrysoperla* species (Canard

and Principi, 1984; Pappas *et al.*, 2011). Notably, both lacewings were also capable of consuming a high number of mealybug individuals, which can be an additional asset of these chrysopid species, which are largely seen as aphidophagous predators. Other *Chrysoperla* species have previously been recorded as natural enemies of different mealybug species in agroecosystems (McEwen *et al.*, 2001). However, the suitability of mealybugs to support the development and survival of lacewings should be further explored. For example, the citrus mealybug *Planococcus citri* was found to be suboptimal as prey for *Chrysoperla lucasina*, another European species of the *carnea* group (Messelink *et al.*, 2016). Similarly, different aphid species may differentially affect the life-history traits of chrysopids, as was shown for example in the case of *Pseudomallada prasinus* and *Chrysoperla sinica* (Pappas *et al.*, 2007; Khuhro *et al.*, 2012).

On the other hand, the (long-term) pest suppression in the greenhouse was found to be remarkably efficient for both lacewing species. Aphid populations were suppressed, with *C. agilis* being slightly more efficient than *C. mutata* with approximately 10 vs. 50 aphids found alive per plant, respectively as compared to the control (approximately 4000 aphids per plant). With regard to the suppression of mealybug populations in the greenhouse, both *C. agilis* and *C. mutata* larvae resulted in a reduced mealybug population growth, relative to the predator-free control, with no significant differences between the two lacewings. Hence, our results highlight the potential of both lacewing species as aphid and mealybug predators. At this point, it is important to consider

Figure 3. Mean number of (a) aphids 14 days (d42) after plant infestation with 100 aphid (third–fourth instar) nymphs followed by the release of *C. mutata* (CM) or *C. agilis* (CA) second-instar larvae (ten larvae were released per species twice on each plant at d31 and d35, i.e. 20 larvae in total) as compared to control (CON) plants (no lacewing release), and (b) live individuals (i.e. emerging lacewing adults and live pupae) per lacewing species in each cage, Kruskal–Wallis multiple comparison analysis with a Dunn's post-hoc test. ns, not significant, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.
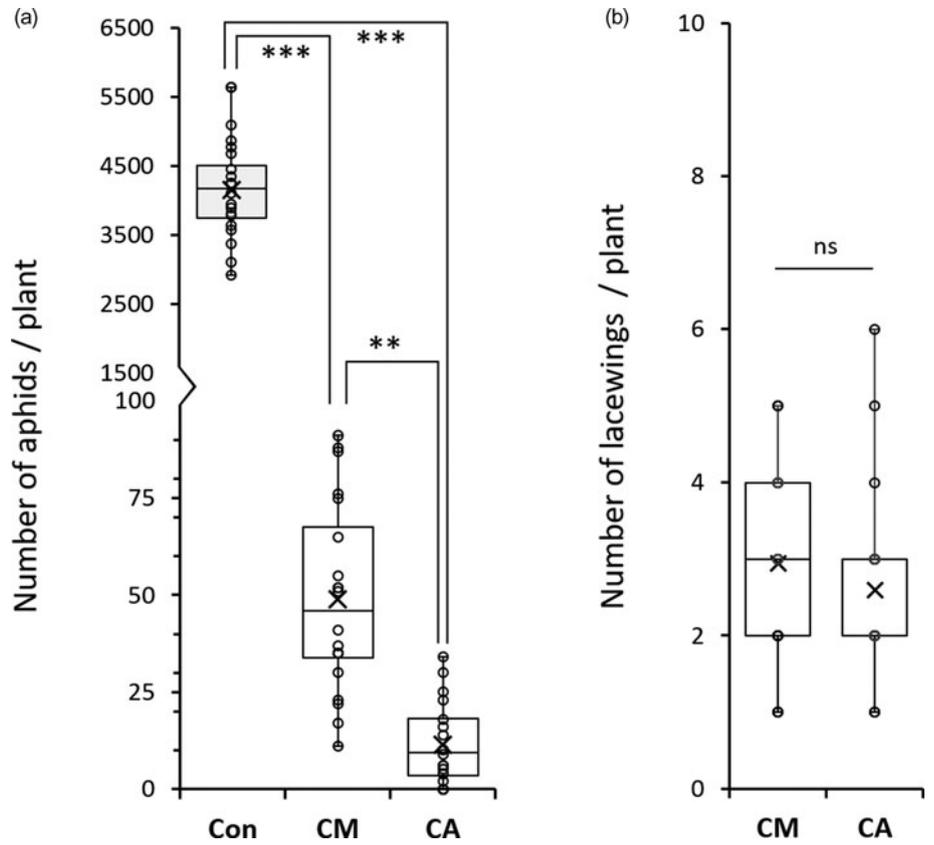
Figure 4. Mean number of (a) mealybugs 28 days (d56) after plant infestation with one mealybug female carrying an egg-sac followed by the release of *C. mutata* (CM) or *C. agilis* (CA) second-instar larvae (per species, 15 larvae released thrice on each plant at d35, d42 and d49, i.e. 45 larvae in total) as compared to control (Con) plants (no lacewing release), and (b) live individuals (i.e. emerging lacewing adults and live pupae) per lacewing species in each cage, Kruskal–Wallis multiple comparison analysis with a Dunn's post-hoc test. ns, not significant, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.
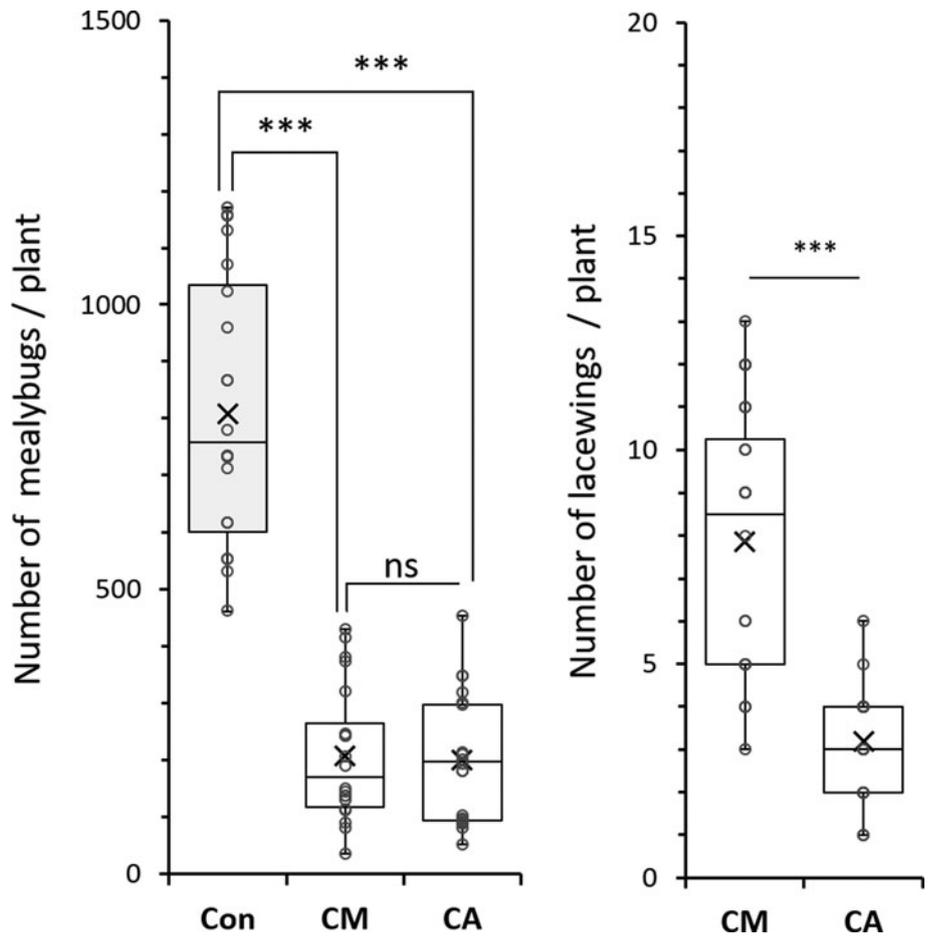
that pepper plants used in our experiments were infested with a quite high number of pest individuals (100 third–fourth-instar aphid nymphs or one mealybug female carrying an egg-sac) suggesting that an earlier release at lower pest infestations could have resulted in a more pronounced reduction or even the complete pest elimination. Furthermore, it is also important to acknowledge that the releases of the predators took place in cages that may have facilitated the encounters and eventually consumption of the prey. More experiments under more realistic field conditions are needed to better evaluate the potential of *C. agilis* and *C. mutata* to control aphids and mealybugs in sweet pepper.

The reduction in aphid populations achieved by *C. agilis* larvae was more pronounced when compared to *C. mutata*. This can be explained by species-specific differences in the predation potential of each instar or the developmental rates of the two lacewings. While no studies have assessed the life-history traits of *C. mutata* so far, second-instar larvae of *C. agilis* require approximately 11 days to complete development when fed with *E. kuehniella* eggs at 27°C (Pappas *et al.*, 2013) suggesting that most of the released larvae (20 larvae per plant) may have pupated by the recording day. On the other hand, in the case of the mealybugs experiment that ended 2 weeks later, possibly most of the larvae released in the first two time points (in total 30 larvae per plant in d35 and d42) of both *C. agilis* and *C. mutata* may have pupated/reached adulthood. Nevertheless, a significantly higher number of *C. mutata* as compared to *C. agilis* individuals was recorded on mealybug-infested plants (approx. eight vs. three individuals) 3 weeks after the initial release of second-instar larvae, whereas this difference was not seen 11 days after the release of second-instar larvae on aphid-infested plants. Further experiments are required to verify these differences and assess the population growth of the two lacewings on plants infested by each pest.

Despite the wide distribution of *C. agilis* and *C. mutata*, relatively little is known about their feeding habits, life-history traits and ability to suppress pest populations. Both species show a preference for Mediterranean climates (Szentkirályi, 2001; Henry *et al.*, 2003) and under these conditions are capable of consuming aphid and mealybug individuals and suppressing their populations. Hence, both species can be promising biological control agents in warmer and dry parts of their distribution range, where other species of the -carnea group are not common (Tauber *et al.*, 2000; Henry *et al.*, 2003; Canard and Thierry, 2013; Pappas *et al.*, 2013; Duelli *et al.*, 2019; Athanasiadis *et al.*, 2021). With regard to their feeding habits, *C. agilis* was previously shown to prey on *E. kuehniella* eggs (Pappas *et al.*, 2013), whereas there is only limited information on *C. mutata* larval ability to consume aphids such as *Lipaphis erysimi* and the spider mite *Tetranychus turkestani* (Zhang, 2003; Abdulhay, 2021). Furthermore, we show in the present study that *C. mutata* can be reared on *E. kuehniella* eggs, while preying on *M. persicae* and *P. solenopsis* nymphs.

Previous studies suggest that *C. agilis* and *C. mutata* could be mass-reared for biocontrol purposes by adopting the methods and techniques currently used for *C. carnea* s.l. because of the ability of both lacewings to develop and reproduce on factitious foods such as *E. kuehniella* eggs and their adults' non-predatory diet of sugar-rich liquids (Pappas *et al.*, 2011; Athanasiadis *et al.*, 2021). Currently, *C. carnea* s.l. is the main chrysopid species used in biological control, whereas difficulties in the identification of the different species of the carnea group have been related to varying biocontrol success in the field (Tauber *et al.*, 2000; Pappas *et al.*, 2011; Henry *et al.*, 2013). Here, we revealed the biocontrol potential of *C. agilis* which is distinguishable from the other cryptic species by the distinct mating signals of adults (Henry *et al.*, 2003; Noh and Henry, 2010). Also, *C. mutata* produces different mating signals and bears distinct morphological traits from *C. pudica* (Duelli *et al.*, 2019). The predation efficiency of *C. carnea* s.l. has been studied against different aphid and mealybug species (e.g. Hagley, 1989; Atlihan *et al.*, 2004; El-Sahn and Gaber, 2012; Shrestha and Enkegaard, 2013; Jessie *et al.*, 2015; Saljoqi *et al.*, 2016). To date, no study had assessed its biocontrol efficiency against *M. persicae* or *P. solenopsis* in pepper by *C. agilis* and *C. mutata*. Considering the preference of the latter for warmer and drier habitats (Canard and Thierry, 2013), comparative studies with the commercial *C. carnea* s.l. could reveal advantages of *C. agilis* and *C. mutata* in aphid and mealybug biocontrol under warm and dry conditions.

In conclusion, both *C. agilis* and *C. mutata* are predators with high potential in biological pest control. The larvae of both were shown capable of suppressing *M. persicae* as well as *P. solenopsis* populations on pepper plants for the time period of our experiments, with *C. agilis* being more efficient against aphids than *C. mutata*. Further research focusing on the life-history traits of both chrysopids under different conditions coupled with long-term greenhouse experiments with varying densities of prey and released larvae is needed to clarify the role of *C. agilis* and *C. mutata* under realistic conditions.

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