



Sugar and pollen supply enhances aphid control by hoverflies in strawberry

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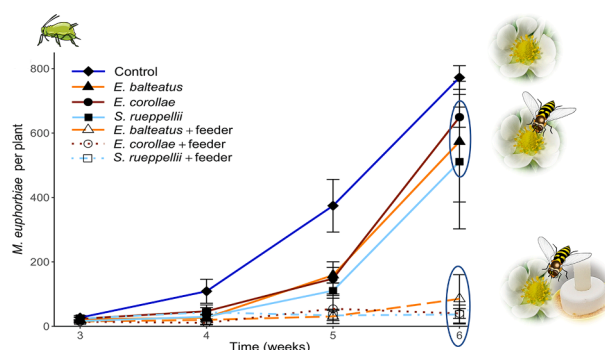
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HIGHLIGHTS

- All hoverfly species tested effectively controlled potato aphids in an experimental strawberry greenhouse.
- Strawberry flowers alone did not support preventive hoverfly releases.
- Addition of sugars and pollen strongly enhanced the performance of all hoverflies species.
- Food supplementation is essential for the hoverflies in a preventative aphid control strategy.

GRAPHICAL ABSTRACT



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ABSTRACT

Larvae of many hoverfly species (Diptera: Syrphidae) are efficient aphid predators, while the adults feed on pollen and nectar. Strawberry crops can be infested by several aphid species, such as the strawberry aphid *Chaetosiphon fragaefolii* (Cockerell) and the potato aphid *Macrosiphum euphorbiae* (Thomas), both of which can cause economic damage. Protected cultivation of strawberry seems to be ideal for introducing biological control agents that depend on flower resources, but the nectar and pollen provided by the crop may not be the most suitable for all flower-dependent insects. In this study, we evaluated the biocontrol potential of hoverflies released preventively or curatively against the potato aphid in strawberry in two separate greenhouse experiments and the effect of adding sugars and pollen on their control efficacy.

In the curative experiment, *Eupeodes corollae* (Fabricius) and *Sphaerophoria rueppellii* (Wiedemann) were released and supported with flowering plants of *Fagopyrum esculentum* Moench (buckwheat). Both species reduced the aphid numbers significantly compared to the control treatment.

In the preventive experiment, we tested three syrphid species, *Episyrphus balteatus* (De Geer), *S. rueppellii*, and *E. corollae*, in the presence and absence of additional food sources (sugar solution and pollen). All three syrphid species reduced the aphid population to a similar extent, but only in the presence of additional food sources. This study shows that hoverflies perform better in strawberry crops when adults have access to different supplementary food sources.

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1. Introduction

The application of biological pest control is well adopted in greenhouse crops worldwide (Pilkington et al., 2010; van Lenteren et al., 2018). However, the monocultures of greenhouse crops are in many cases not an ideal environment for natural enemies to establish populations due to a lack of shelters, suitable oviposition sites, prey or plant-provided food sources (Messelink et al., 2014). Increasing awareness of these impediments led to a range of studies into measures to support natural enemy populations, such as the application of factitious prey, supplemental food, banker plants, or artificial shelters (Frank, 2010; Huang et al., 2011; Messelink et al., 2014; Pijnakker et al., 2020; Pérez-Rodríguez and Messelink, 2023). An important group of natural enemies that needs support in their establishment are the aphidophagous predators such as lacewings, hoverflies, and ladybugs. Adults of these natural enemies require supplemental food or can survive longer in the presence of supplemental food sources (Riddick, 2009). Adult hoverflies are strictly dependent on sources of sugar and pollen, needing sugars (nectar) for survival and pollen for egg maturation (Hickman et al., 1995; Pinheiro et al., 2015). Providing these supplementary foods can be an effective strategy to enhance long-term and preventive establishment (Wäckers et al., 2005). Supporting the establishment and efficacy of these aphidophagous predators can be an interesting approach to improve the biological control strategies of aphids, which have become one of the most challenging pests to control in greenhouse crops (Riddick, 2017; Hullé et al., 2020).

The cultivated strawberry *Fragaria × ananassa* Duchesne (Rosaceae) is a soft fruit crop that is increasingly cultivated in greenhouses in Northern Europe year-round. Growers have adopted integrated pest management (IPM) practices against the main pests. Effective biocontrol strategies have been developed against spider mites and broad mites (Gilstrap and Friese, 1985; Tuovinen, 2000; Cross et al., 2001; Trandem, 2001; Kim and Park, 2006), and thrips (Coll et al., 2007; Sampson, 2014; Clymans et al., 2017; Vervoort et al., 2017). However, growers are still struggling with whiteflies (Trandem, 2001) and aphids (Riddick, 2017; Hullé et al., 2020) infestations. Several species of aphids may occur and cause economic damage to strawberries. The most common species are the green peach aphid *Myzus persicae* (Sulzer), the strawberry aphid *Chaetosiphon fragaefolii* (Cockerell), the cotton aphid *Aphis gossypii* Glover and the potato aphid *Macrosiphum euphorbiae* Thomas (Rondon et al., 2005; Cédola and Greco, 2010; Bernardi et al., 2013). Aphids can cause damage at high population densities mainly by the honeydew they produce, leading to the subsequent growth of sooty mold fungi that affects plant growth and fruit quality. In addition, *C. fragaefolii* is an important vector of virus diseases such as the Strawberry mild yellow-edge virus (SMYEV) and Strawberry Crinkle Virus (SCV), which severely weaken plants (Krczal, 1982; Sylvester, 1987; Cross et al., 2001; Lavandero et al., 2012; Martin and Tzanetakis, 2013; Dughetti et al., 2017; Whittaker, 2022). *Macrosiphum euphorbiae* is a particularly challenging pest to control with biological control agents due to its strong antipredator-dropping behavior (Humphreys et al., 2021).

Hoverfly larvae (Diptera: Syrphidae) are known to be important predators of aphids, whiteflies, thrips, and mealybugs (Ankersmit et al., 1986; Rojo et al., 2003; Bugg et al., 2008; Dunn et al., 2020; Rodríguez-Gasol et al., 2020). Research on aphidophagous hoverflies as biological control agents in Northern Europe was so far mainly focused on *Episyrphus balteatus* (De Geer) (Chambers and Adams, 1986; Tenhumberg, 1995; Jansen, 2000;), which is the most common spontaneously occurring species in crops (Reemer et al., 2009). The abundance of aphidophagous hoverflies in the Mediterranean region has been studied by Pineda and Marcos-García (2008). In their survey, three hoverfly species were the most abundant: *Sphaerophoria rueppellii* (Wiedemann), *Episyrphus balteatus*, and *Eupeodes corollae* (Fabricius), and the potential of these species as aphid biocontrol agents has been increasingly studied in recent years (Rojo et al., 1996; Amorós-Jiménez et al., 2012, 2014; Pekas et al., 2020; Moerkens et al., 2021; van Oystaeyen et al., 2022).

In strawberries, the use of hoverflies in augmentative aphid control is relatively unexplored, and so far finds only limited use (Moerkens et al., 2021). One of the obstacles may be the scarcity of suitable food sources for adults. Adult hoverflies rely on protein and saccharides obtained through feeding on pollen and nectar to sustain reproduction, as adults are not carnivorous (Pinheiro et al., 2015). The quality and quantity of these food sources are critical for the successful establishment and residence of hoverflies in crops (Amorós-Jiménez et al., 2014). Studies in sweet pepper have shown that adding flowering-companion plants to an already flowering pepper crop strongly enhances the abundance of hoverflies (Pineda and Marcos-García, 2008). Furthermore, hoverfly adults may act as pollinators in strawberry (Hodgkiss et al., 2018; van Oystaeyen et al., 2022; Li et al., 2023), thus providing an important additional ecosystem service to the crop.

In this study, we first evaluated the biocontrol potential of the hoverflies *S. rueppellii* and *E. corollae* released curatively on strawberry plants infested with the potato aphid *M. euphorbiae* and supplemented with flowering buckwheat plants. Then, we assessed their preventive biocontrol potential in a trial where hoverflies *S. rueppellii*, *E. corollae*, and *E. balteatus* were released before aphid infestation, in the presence or absence of pollen and sugars as additional food sources that are known to enhance the establishment of hoverflies (Laubertie et al., 2012; Wäckers and van Rijn, 2012; van Rijn et al., 2013; Pinheiro et al., 2015; van Rijn and Wäckers, 2016; Bellefeuille et al., 2021). Providing sugars and pollen might be an easy way to support natural enemies and less complex and possibly cheaper than providing banker plants or flowering plants. Moreover, compared to the use of flowering plants, it can reduce the risk of promoting flower thrips, which are important pests in greenhouse crops.

2. Materials and methods

2.1. Plants and insects

Pesticide-free everbearing strawberry plants (cv. Favori) were obtained from a propagation company (van der Avoird Trayplant BV, Bavel, the Netherlands) and were used in all experiments and insect rearings. Plants arrived in frozen mini trays. After three days of defrosting, plants were planted in 100 % cocopeat (Lensli substrates BV, Bleiswijk, the Netherlands).

Hoverflies were supplied as pupae by biocontrol companies [Biobest Group NV (Westerloo, Belgium) for *E. corollae* and *S. rueppellii*; Biopol B. V. (Stellendam, the Netherlands) for *E. balteatus*]. All pupae were incubated for 3–4 days until adult emergence in meshed insect cages (40 × 40 × 40 cm, Verman del, Hulst, the Netherlands) in a climate chamber (25 °C and RH of 70 %). Newly emerged hoverflies (<8h old) were used in all experiments.

Macrosiphum euphorbiae colonies were initiated from individuals collected from roses from a commercial greenhouse in the area of Bleiswijk (the Netherlands) and maintained on strawberry plants, in a climate cell at 22 °C and RH 70 %. Mixed stages of aphids were transferred to strawberry leaf discs (6 cm diameter) with a fine hairbrush and later introduced to strawberry plants in the experiments.

2.2. Curative greenhouse experiment

The experiment was conducted from July 5th until August 23rd, 2021 in an experimental greenhouse (144 m²) compartment of Wageningen University and Research, BU Greenhouse Horticulture and Flower Bulbs in Bleiswijk, the Netherlands. The compartment was equipped with fourteen tables of 9 m² each, eight of which were used for the experiment. On each table, two cages of 1.5 × 1 × 1 m (l × d × h) made of an aluminum frame and fine insect gauze (mesh size 0.22 × 0.31 mm) were placed, and drippers to provide nutrients and water were installed with six drippers per cage. The cages had a double zipper opening on the long side for accessing the plants and no bottom to allow

the excess water to leak into the table gutters. Inside the cages, four plastic crates 30 × 40 cm were placed upside down (two double-stacked crates in each cage). On this elevation a 16 L plastic container (100 × 17 × 14 cm, Bato Plastics BV, Zevenbergen, the Netherlands) was placed, carrying six strawberry plants in cocopeat. The plants were sixteen weeks old, full-grown, and in production.

On the 5th of July, open flowers and fruits were removed from all strawberry plants to achieve uniformity, and a pot with 4 four-weeks-old flowering buckwheat plants (*Fagopyrum esculentum* L.) cultivated from seeds was placed alongside the container with strawberries. The buckwheat plants were placed on the table surface and connected to the same nutrient and water drippers as the strawberries. Buckwheat plants were also added in the control treatment with aphids, as *M. euphorbiae* can feed on this plant as well. Three of the six plants in each cage were marked with bamboo sticks and fifteen individuals (mixed stages) of potato aphids were introduced on the 8th of July on each marked plant. Two weeks after the introduction of aphids, two couples of freshly emerged adults were released in the cages. Introductions of hoverflies were repeated twice, two and four weeks later, for a total of 3 introductions (21st of July, 4th and 18th of August). In the second and third week of the experiment, slow-release sachets with the predatory mite *Neoseiulus cucumeris* (Oudemans), cards of the parasitoid *Encarsia formosa* (Gahan), and predatory mite *Phytoseiulus persimilis* (Athias-Henriot) were introduced to the plants preventively against pests commonly occurring in strawberries: i.e. against thrips, whiteflies, and spider mites, respectively. All biocontrol products were supplied by Biobest NV and were applied at an equal rate in all cages.

The experiment was performed in four replicates with the following treatments: (i) control (only aphids), (ii) *S. rueppellii*, and (iii) *E. corollae*. Unfortunately, due to extreme weather conditions before the experiment, the commercial production line of *E. balteatus* collapsed and was not available, thus excluded from the curative experiment. Populations of aphids were assessed *in situ* on each of the three marked plants weekly starting two weeks after their introduction and before the introduction of the syrphids (week 3). The number of syrphid eggs was counted weekly on each of the three marked plants, starting in week 4, while hoverfly adults were counted on a per-cage basis. Hoverfly larvae are nocturnal predators (Rotheray, 1986), and we thus excluded them from the assessments of both greenhouse experiments as diurnal countings greatly underestimate their numbers. Throughout the experiment, temperature and relative humidity were registered every 5 min with a climate recorder (Hoogendoorn Growth Management). The average measured temperature in the greenhouse was 20.1 °C (range 14.6–30.2 °C) and the average relative humidity was 76 % (range 45–99 %). The climate conditions were in line with commercial cropping conditions.

2.3. Preventive greenhouse experiment with feeders

The second greenhouse trial was conducted from May 23rd until June 27th in 2022 in the same greenhouse compartment as described above. Forty mesh cages (75 × 75 × 115 cm, BugDorm-2400 Insect Rearing Tent, MegaView Science Co., Taichung, Taiwan) were distributed on four tables. On the bottom of each cage a piece of cloth 70 × 70 cm was placed to prevent the accumulation of water that could lead to insects drowning. Strawberry plants were planted in groups of 3 in 8 L plastic containers (50 × 17 × 14 cm, Beekenkamp Verpakkingen BV, Maasdijk, the Netherlands), filled with cocopeat. Plants were then placed on the elevation of a plastic crate inside the cages, and predatory mites *N. cucumeris* in sachets (one sachet per cage) and *E. formosa* on cards (one card per cage) were released preventatively to prevent pest outbreak. Throughout the experiment, plants were continuously flowering and fruits were harvested when ripe.

Feeders to provide additional food for the hoverflies were provided

by Biobest and had been constructed from 30 ml plastic bottles (9 cm high and 3 cm Ø) closed with a lid (1.5 cm high, 3 cm Ø) with an opening in the middle. In the opening (0.5 cm Ø) a wick (8 cm long) was placed in a way that it was protruding 2 cm above the lid surface to allow hoverflies access to the liquid inside. The bottles were filled with Biogluc® obtained from Biobest Group NV. Biogluc® is a sugar solution used to feed bumblebees inside commercial hives and contains the sugars fructose (37.5 %), glucose (34.5 %), sucrose (25 %), maltose (2 %) and oligosaccharides (1 %) (Wäckers et al., 2017). Around the bottleneck, a hollow ring of yellow plastic (3 cm diameter, 1 cm width, 1 cm height) was placed and filled with a commercial mix of polyfloral honeybee-collected pollen which had been gamma irradiated (PAD, Biobest Group NV). Each feeder was taped to the upper side of a bamboo stick of 20 cm, and was positioned at about 10 cm above the plants next to the middle-marked plant. Biogluc® was not replenished during the period of the experiment, while pollen was refilled weekly. In the control treatment, no feeder was offered as it would not be relevant for the population growth of aphids.

Two couples of hoverflies were introduced twice on the 24th and 31st of May, one week and three days before the aphid introduction, respectively. Fifteen individuals of (mixed stages) *M. euphorbiae* were introduced in the centre of each of the three plants in the cage on the 3rd of June, for a total of 45 aphids per cage. The numbers of aphids and hoverfly eggs were assessed weekly on a single marked plant in each cage, while adult hoverflies were on a per-cage basis, starting on the 7th of June (week 3). The trial was set up with four replicates and the following treatments: (i) control (only aphids), (ii) *E. balteatus*, (iii) *E. balteatus* + feeder (iii), (iv) *S. rueppellii*, (v) *S. rueppellii* + feeder, (vi) *E. corollae*, and (vii) *E. corollae* + feeder. The average measured temperature in the greenhouse was 18.7 °C (range 9.8–32 °C) and an average RH of 64 % (range 31–99 %).

2.4. Statistical analysis

Hoverfly larvae are nocturnal predators (Rotheray, 1986), and we thus excluded them from the analyses of both greenhouse experiments as diurnal countings greatly underestimate their numbers. All data from the greenhouse experiments were analyzed by fitting Generalized Linear Mixed Models (GLMM). In the curative experiment, 3 plants per cage were assessed for the number of aphids and hoverfly eggs. Thus time, cage, and plant within each cage were included as random effects, while predator species (including no predator) were included as the fixed effect. When analyzing the total number of hoverfly adults per cage (i.e. for a total of 6 plants), only time and cage were included as random effects.

In the preventive experiment, one plant per cage was assessed for the number of aphids and hoverfly eggs, while hoverfly adults were assessed per cage. First, to analyze the population build-up of aphids, a one-way GLMM including treatment (with 7 levels) as the explanatory variable was fitted. Then, to analyze the number of syrphid eggs and adults, two-way GLMMs were fitted, including predator species, the presence/absence of the feeder, and their interaction as the fixed explanatory variables. In all models, time and cage were included as random effects.

Error distributions for all models were initially set to Poisson for count data and their fit was assessed through residual diagnostics for hierarchical regression models. When overdispersion was found, the distribution was changed to negative binomial and reassessed. Post-hoc tests using Tukey's HSD to separate means were performed on significant main effects and when the interaction between main effects was not significant. All statistical analyses were performed using the statistical software R 4.0.2 (R Core Team, 2021). We further used the 'glmmTMB' package to fit GLMMs (Brooks et al., 2017), the 'DHARMa' package to perform residual diagnostics (Hartig, 2022), and the 'emmeans' package to perform posthoc comparisons (Lenth, 2021).

3. Results

3.1. Greenhouse experiment curative control

Aphid densities on strawberry plants were reduced to low levels by both *E. corollae* and *S. rueppellii* and differed significantly from the control treatment ($\chi^2 = 25.141$, $df = 2$, $P < 0.001$) (Fig. 1A). The aphid density on the control plants reached a peak on the 6th week of the experiment and then gradually decreased due to plant defoliation. No significant difference through time was found on the number of adult hoverflies ($\chi^2 = 2.718$, $df = 1$, $P = 0.099$). (Fig. 1B). Furthermore, no difference was found in the total number of eggs between the two predators ($\chi^2 = 0.026$, $df = 1$, $P = 0.872$) (Fig. 1C).

3.2. Greenhouse experiment preventive control

Aphid population densities were significantly affected by the different treatments ($\chi^2 = 42.046$, $df = 6$, $P < 0.001$). Significantly lower populations of aphids compared to the control were found in all treatments where hoverflies were provided with feeders (Fig. 2A).

The addition of feeders significantly increased the total number of hoverfly adults ($\chi^2 = 37.369$, $df = 1$, $P < 0.001$) (Fig. 2B). Numbers of adult hoverflies significantly differed among the different species ($\chi^2 = 9.664$, $df = 2$, $P = 0.008$), with *E. corollae* developing to lower numbers than *E. balteatus* (Fig. 2B). The interaction between predator species and feeder was not significant ($\chi^2 = 3.849$, $df = 2$, $P = 0.146$), showing that all three hoverfly species benefited equally from the additional food sources. The total number of syrphid eggs was higher in the presence of feeders ($\chi^2 = 12.065$, $df = 1$, $P < 0.001$) (Fig. 2C). However, no differences in egg deposition were found for the different syrphid species ($\chi^2 = 1.16$, $df = 2$, $P = 0.56$), while the interaction between predator species and feeder was also not significant ($\chi^2 = 1.537$, $df = 2$, $P = 0.464$).

4. Discussion

In our research, we assessed the ability of three commercial species of aphidophagous hoverflies to control potato aphids in strawberries, and we tested whether the provision of additional food sources could improve their efficacy. Our experiments showed that the three hoverfly species reduced potato aphid infestation significantly, both when used curatively together with flowering buckwheat (only for two species) or preventively in combination with sugars and pollen as a supplemental food source. Moreover, we show that the pollen and nectar of the strawberry cultivar used in this study were not suitable enough to achieve effective aphid control with the hoverflies tested. Adding nectar and pollen to the strawberry plants significantly enhanced aphid control and increased the reproduction rate of the hoverflies compared to treatments without these supplemental food sources. With the fluctuating flower numbers in strawberries at the beginning and end of the growing period, hoverflies may lack suitable food sources to be optimally effective. Our study shows that adding sugars and pollen to the crop can support adult hoverflies and reduce the cost of repeated introductions. Supplementation in the form of nectar plants or alternative prey showed already great potential to support the establishment and enhance the efficacy of natural enemies in greenhouse crops (Lundgren et al., 2009; Messelink et al., 2014; Leman and Messelink, 2015; Damien et al., 2020; Li et al., 2021; Zuma et al., 2023;). Access to plant-provided resources can have a strong effect on the life history parameters of beneficial insects and can play an essential role in their functioning (Wäckers et al., 2005). For example, providing floral food sources is beneficial for *E. balteatus*, as it improves its longevity and fertility (Laubertie et al., 2012). Van Rijn et al. (2013) showed that hoverflies can strongly suppress the growth of cabbage aphid colonies on Brussels sprouts when provided with suitable flowers. Both studies show that buckwheat flowers (*Fagopyrum esculentum*) provide both nectar and pollen, prolonging adult longevity and

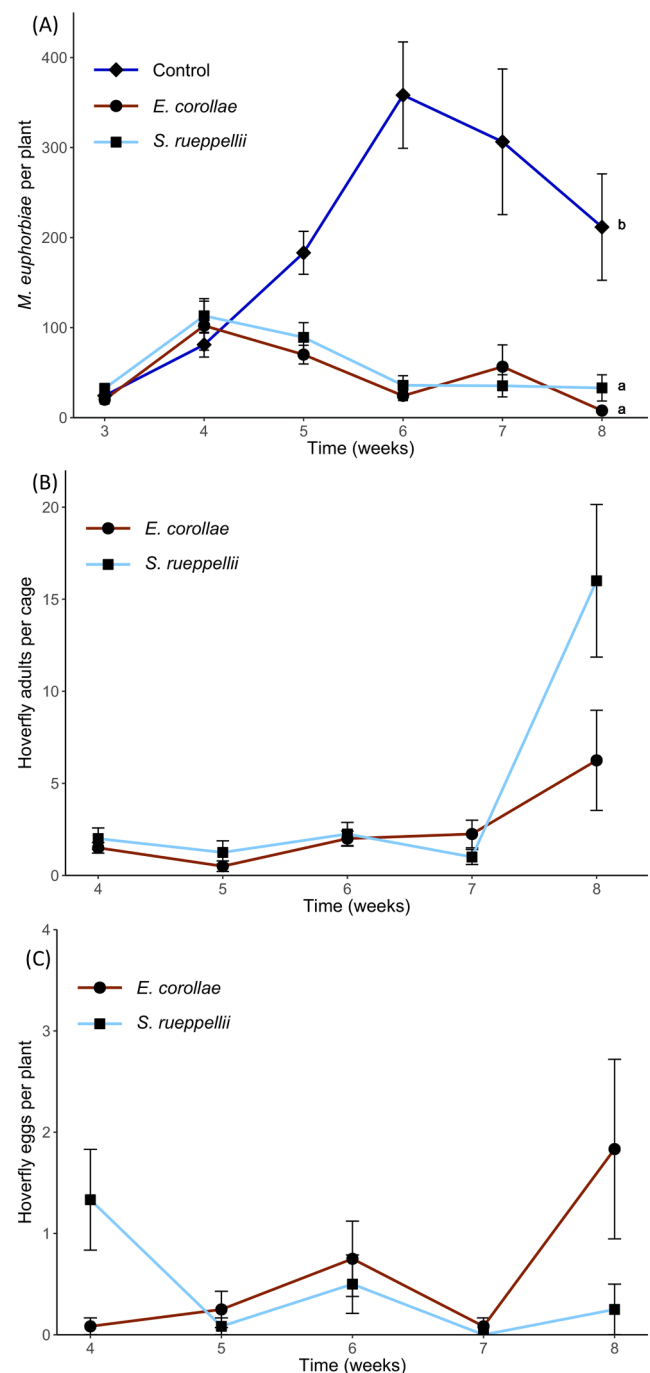


Fig. 1. Population dynamics in the curative greenhouse experiment of (A) *Macrosiphum euphorbiae* on strawberry plants in the absence or presence of the hoverfly predators, (B) hoverfly adults, and (C) hoverfly eggs. Plants were infested with 15 aphids each in week 1, and two couples of hoverflies were introduced in the cages in week 3 and after the first assessment of aphid populations, and repeated in week 5 and week 7. The average number ($\pm$ SE) of individuals is shown through time. Different lowercase letters indicate significant differences among treatments through time (GLMM, Tukey's HSD, $P < 0.05$).

also support egg production.

In the curative experiment, where adult hoverflies had unlimited access to pollen and nectar from strawberry and buckwheat flowers, both *E. corollae* and *S. rueppellii* controlled the pest within three weeks after introduction, reducing the population of aphids by more than 90 %. In the control treatment, the plants were highly infested and after week

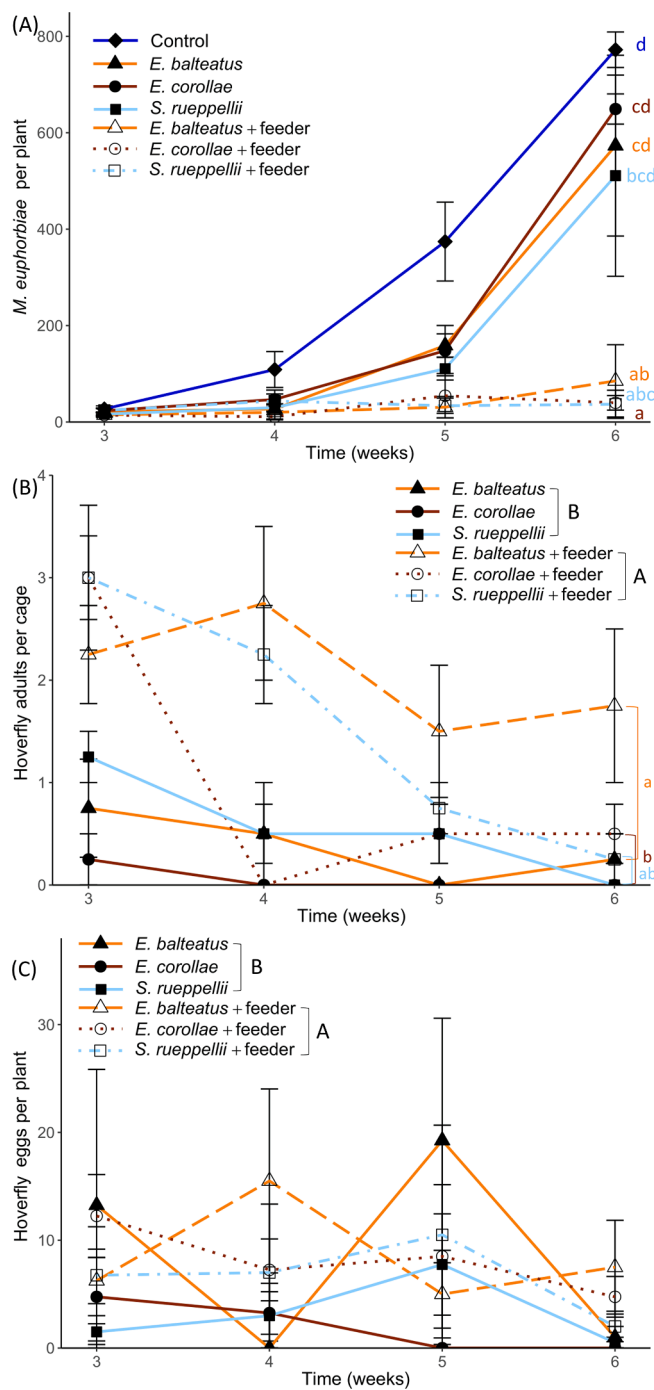


Fig. 2. Population dynamics in the preventive greenhouse experiment of (A) *Macrosiphum euphorbiae* on strawberry plants in the absence or presence of the hoverfly predators and feeders, (B) hoverfly adults, and (C) hoverfly eggs. Two hoverfly couples were introduced in each cage in weeks 1 and 2. Three days after the last hoverfly introduction 15 individuals of *M. euphorbiae* were released on the strawberry plants (for a total of 45 aphids per cage). Feeders consisted of a sugar solution and pollen. The average number ($\pm$ SE) of individuals is shown through time. Different lowercase letters indicate significant differences among treatments in the population of aphids through time (A) and the population of hoverfly adults (B) (GLMM, Tukey's HSD, $P < 0.05$). Differences in uppercase letters in the legends (B and C) indicate overall significant differences between treatments with and without feeders.

6 their population started dropping due to the poor condition of the plants. The voracity of the hoverflies has already been evaluated in detail in previous studies. Amorós-Jiménez et al. (2012) found that a single *S. rueppellii* larva can consume up to 220 aphids during its developmental time. *Eupeodes corollae* consumes 1000 individuals of *Aphis craccivora* Koch (Pu et al., 2019), 485 nymphs of *M. persicae* (Lillo et al., 2021), and 392 individuals of *Aphis pomi* de Geer (Jalilian et al., 2016) during its juvenile development. The high voracity of both hoverfly species and the tolerance of eggs of *E. corollae* to extreme temperatures (Benestad, 1970) makes them valuable candidates to include in biological control strategies of aphids.

The number of *S. rueppellii* eggs increased in the last week of our experiment along with a growing number of adult hoverflies, but did not differ significantly over time from the number of eggs laid by *E. corollae*. These results differ from the findings of (Moerkens et al., 2021) and (van Oystaeyen et al., 2022), where *S. rueppellii* laid significantly more eggs than *E. corollae*. The higher number of *S. rueppellii* adults found in the last week of the trial compared to the *E. corollae* treatment is in line with previous research (Moerkens et al., 2021; van Oystaeyen et al., 2022). This may be due to the higher juvenile mortality during the development from egg to adult for *E. corollae* compared to *S. rueppellii* (Moerkens et al., 2021), while in another laboratory study, only 50 % of the eggs of *E. corollae* hatched successfully (Lillo et al., 2021). Furthermore, high larval voracity may negatively affect population development, as larval survival of *E. corollae* can be reduced by limited prey availability (Rojo et al., 1996).

In commercial protected growing systems growers are sometimes reluctant to use additional plants to provide floral resources, as they require separate maintenance. Adding factitious or supplementary food sources instead of flowering plants to supply pollen and nectar, can be an easier to adopt strategy for growers. The benefits of adding these sources to flowering strawberry plants for hoverflies were tested in the preventive experiment. In this trial, aphid populations increased drastically in the control treatment without hoverflies, whereas in all hoverfly treatments, aphid numbers showed a decreased growth by the fourth week of the experiment, although this reduction was significant only when additional food sources were offered. The delayed effects of the hoverflies can be explained by the time needed for oviposition and egg hatching. Furthermore, the limited aphid consumption rates of the first larval stages may explain why the effect of hoverflies was only visible from the fourth week onwards. This delayed effect of hoverflies on aphid populations highlights the importance of preventive releases. The limited effect of hoverflies on aphid population growth in the treatments without feeders may have been due to the reduced longevity and fecundity of adult hoverflies present. The lifespan of the hoverflies exposed only to the strawberry flowers was shorter and oviposition was lower, allowing the aphids to increase exponentially. The longer lifespan of the adults in the treatment with sugar and pollen supplementation had a major negative effect on the growth of the aphid population. For example, Jalilian et al. (2016) showed that *E. corollae* adult longevity increases from 3.5 days when not fed to 9.6 days when fed with 10 % honey solution. Van Rijn et al. (2013) showed that the presence of sucrose or buckwheat flowers strongly increases the lifespan of *E. balteatus* in a situation without aphids (fivefold) and even more so (tenfold) when a high aphid population and flowers are present and the hoverflies have access to both flowers and honeydew. Pinheiro et al. (2015) showed that *E. balteatus* feeding on black scale honeydew only slightly improved longevity compared to water, but significant improvements were recorded in individuals fed with other sugar sources. This is in agreement with earlier reports showing the suboptimal quality of honeydew as a sugar source (Wäckers et al., 2008). In our experiment, the number of hoverfly eggs in the treatments where additional food sources were provided was higher compared to treatments without additional food sources. These findings are in agreement with previous research

showing that pollen is an essential food source for egg maturation of hoverflies (Hickman et al., 1995) and that they cannot produce eggs when fed honeydew alone (Lundgren, 2009).

In conclusion, we show that all three hoverfly species are capable of successfully controlling the potato aphid in strawberries while being supported by additional food sources in both preventive and curative releases. In the preventive experiment, adults provided with supplementary food sources lived longer and laid more eggs, thus responding quicker to the rapidly growing aphid populations, and were eventually able to completely control the infestation. Our study also suggests that strawberry flowers may only have a limited nutritional value for hoverflies and that extra food is needed to meet their needs and perform optimally. Supplementing food is also a way to reduce the costs of repeated introductions in the crops and is essential for the use of hoverflies as a preventive aphid control strategy. Further research with non-caged plants in greenhouses is now necessary to evaluate the effectiveness and economic viability of augmentative hoverfly releases in strawberry.

CRedit authorship contribution statement

Ada Leman: Conceptualization, Methodology, Investigation, Funding acquisition, Writing – original draft. **Angelos Mouratidis:** Data curation, Formal analysis, Writing – review & editing. **Juliette Pijnakker:** Investigation, Writing – review & editing. **Kyra Vervoorn:** Investigation. **Felix Wäckers:** Writing – review & editing. **Gerben J. Messelink:** Writing – review & editing.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: J. Pijnakker and F. Wäckers work for Biobest Group N.V., which produces and supplies commercially the hoverflies *Eupeodes corollae* and *Sphaerophoria rueppellii*, and the sugar solution Bioglucl® used in this study.

Data availability

The dataset that supports this study can be accessed at Mendeley Data: <https://doi.org/10.17632/9vf4vfrhfd.1>.

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