



Article An Efficient Breeding Method for *Eupeodes corollae* (Diptera: Syrphidae), a Pollinator and Insect Natural Enemy in Facility-Horticulture Crops

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Abstract: Facility horticulture plays a crucial role in modern agriculture by utilizing the environment efficiently and ensuring food supply. The hoverfly Eupeodes corollae (Fabricius) (Diptera: Syrphidae) performs a dual ecological function in facility agriculture as larvae prey on aphids and adults pollinate, but it is not widely applied in agriculture due to the lack of a large-scale breeding technology. In this study, we investigated the effects of different factors on the development and reproduction of the prey (i.e., aphids), eggs, larvae, pupae and adults of E. corollae, and determined its propagation techniques. We transferred five pairs of newly emerging E. corollae adults and 40 broad bean plantlets infested with Aphis craccivora Koch to an insect cage. Aphid-infested broad bean seedlings were replaced each day to encourage E. corollae reproduction. Following hatching, we fed the E. corollae larvae with mixed populations of Myzus persicae (Sulzer), Megour japonica Matsumura and A. craccivora in insect boxes, and covered E. corollae pupae with fresh broad bean leaves. Based on the experiment results, an average female E. corollae produced 584.9 eggs. Survival, pupation and emergence rates were 91.1%, 100% and 96.3%, respectively. In conclusion, each pair of E. corollae adults produced 391 adult offspring (58.8% females). This research supplies an optimized mass-breeding technique for commercial production of the hoverfly, which will be helpful to promote its application in the production of green fruits and vegetables.

Keywords: Eupeodes corollae; intensive breeding; horticultural crop; aphid control; pollination

1. Introduction

Facility agriculture is a specialized agricultural-production system that utilizes specific equipment and techniques to regulate temperature, humidity and light. This approach plays an important role in global food security and the economy [1,2]. The systems of facility agriculture are semi-enclosed, providing a safe environment for crops but excluding pollinators as well. Therefore, mechanical vibrations, hand pollination or synthetic hormones are used to promote fruit setting in facility agriculture [3,4]. Synthetic hormones (i.e., plant-growth regulators) such as forchlorfenuron and 4-chlorophenoxyacetic acid are widely used in facilities due to their high fruiting rate and ease of operation, but they are susceptible to weather change [5]. Insect-pollinated crops produce high yields and quality while benefiting the environment [6-8]. Traditionally, various species of bees provide the bulk of the pollinating services in the world. Social bees appeared about 30 million years ago, in the Oligocene era of the Tertiary [9], and in recent years European honeybees (Apis mellifera L.) and bumblebees (Bombus spp.) have been introduced into facility agriculture to ensure fruit set in horticultural crops [8]. Before the appearance of the bees, however, other insects such as beetles, butterflies and Diptera (including hoverflies) were the main pollinators, and today hoverflies are getting increasing attention. Just like bees, hoverflies possess color vision [10] and use their eyes to detect suitable plants to collect pollen from



Citation: Li, H.; Wu, K. An Efficient Breeding Method for *Eupeodes corollae* (Diptera: Syrphidae), a Pollinator and Insect Natural Enemy in Facility-Horticulture Crops. *Horticulturae* **2023**, *9*, 664. https://doi.org/10.3390/ horticulturae9060664

Academic Editors: Filipe Madeira, Ramon Albajes, Massimo Pugliese and Elisabete Figueiredo

Received: 14 May 2023 Revised: 1 June 2023 Accepted: 2 June 2023 Published: 4 June 2023



Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). and to deposit their eggs on [11]. However, many small farmers in China still use synthetic hormones or hand pollinate to set fruit in their crops to save money.

Aside from pollination, pest control is another important aspect of facility agriculture. Suitable temperatures and host plants that grow well in facility agriculture cause pest proliferation, and small pests such as aphids, thrips, whiteflies and leaf mites adversely affect crop yields [12,13]. Aphids are common pests in horticultural crops, reducing yields through direct feeding and virus transmission, and they are commonly controlled with insecticides. However, insecticide use has resulted in pesticide resistance, environmental pollution, food safety concerns and a decline in pollinator populations [14,15]. Biological control is an environmentally friendly alternative to insecticide-based approaches, such as the parasitic wasps which have been introduced to control aphids in facility agriculture [16,17]. Introducing pollinators and natural enemies simultaneously is a vital direction in facility-agricultural-crop management [18]. For example, bumblebee pollinators carrying *Beauveria bassiana* (Balsamo) were introduced to pollinate sweet pepper crops and control thrip populations [19]. However, it is possible that the simultaneous deployment of insect pollinators and biological-control agents will adversely affect crop yields, such as high pest loads, i.e., low levels of biological control, resulting in enhanced pollinator benefits for oilseed-rape seed yields [20]. Due to the variable impact of pollinators' and natural-enemies' simultaneous use on crop productivity, as well as the additional costs involved, this practice has only received marginal attention in greenhouse agriculture.

The hoverfly *Eupeodes corollae* (Fabricius) (Diptera: Syrphidae) has the dual ecological functions of pollination and biological control [21], and is distributed in Europe, North Africa and North Asia [22–24]. The adults feed on pollen and nectar and deposit eggs on aphid-infected plants [25–27], providing pollination services for a variety of horticultural crops in facility agriculture, such as tomatoes, strawberries and melons [28–30]. The larvae prey on many species of aphids [31,32], decreasing aphid populations in sweet peppers, lettuces and strawberries [33–35] and increasing the yield and quality of crops [30]. Therefore, developing large-scale breeding techniques for *E. corollae* serves both economic and ecological purposes.

In recent years, researchers have studied the effects on the development and reproduction of hoverflies from aphids, host plants, pollen, etc., and they have already mastered indoor-breeding methods for hoverflies. These are as follows: (A) provide fresh pollen, honey and plant hosts infested with aphid populations for hoverfly adults to consume and lay eggs on; (B) provide enough aphids for larvae to feed on; and (C) preserve pupae until they emerge. This method allows *E. corollae* to be bred for multiple generations [31,36]. As well as pollen and honey, hoverfly fecundity is also affected by the aphid species used, their density and the host-plant species [37–41]. Hoverfly adults produce no or few eggs on plants with no aphids, or a moderate number of hoverfly eggs [38,42,43], and different numbers of eggs on different kinds of plants infested with the same aphid species [39]. Breeding hoverflies on a large scale poses several problems: (a) Adults produce insufficient eggs. They require a large amount of food, such as fresh pollen, which is difficult to obtain in large quantities and expensive. (b) Hoverfly eggs hatch at a low rate. Hoverfly larvae feed on aphids and self-mutilate to consume unhatched eggs when food is scarce. In terms of larval survival and development, different types and numbers of aphids have different effects on hoverfly larvae [44,45]. (c) Hoverfly pupae emerge at a low rate. The pupae will shrivel and liquefy due to humidity.

Based on the above, this experiment examined the propagation method for *E. corollae*, and specifically the effects of different feeding methods on the development of the prey (i.e., aphids), eggs, larvae, pupae and adults of *E. corollae*. It studied the reproduction of hoverflies when using different species and densities of aphids, as well as the hatching rates with the different aphid species, the larval development and survival in different rearing containers, and the emergence of pupae when covered with various substances. We determined an efficient breeding method for *E. corollae* after analyzing the development

and reproductive parameters of hoverflies when using different treatments, providing guidance for commercial hoverfly production.

2. Materials and Methods

2.1. Insect Material

In June 2018, *Eupeodes corollae* (Diptera: Syrphidae) adults were collected by sweep netting from wheat fields at the Langfang Experiment Station of the Chinese Academy of Agricultural Sciences (CAAS) (39.53° N, 116.70° E, 18 m elevation) in Hebei Province, China. Every 5 pairs of *E. corollae* adults (male and female) were placed in an insect cage ($0.5 \times 0.3 \times 0.4$ m; 120 mesh; Beijing Luhebang Technology Development Co., Ltd., Beijing, China). Each colony was provided with 10% honey-water solution and mixed pollen (3 commercial rape pollen: 1 maize pollen, by weight) in two dishes (9 cm diam × 1.5 cm height), along with 40 broad bean plantlets infested with *Megoura japonica* Matsumura aphids. A total of 40 soybean plantlets infested with *Me. japonica* (approximately 8000 individuals) were cut and added to the cage daily to feed the *E. corollae* larvae. Upon pupation and emergence, the hoverfly adults were transferred to another cage for further raising. Three separate aphid populations of *Me. japonica*, *Aphis craccivora* Koch and *Myzus persicae* (Sulzer) were established on broad bean plantlets. Aphid-culture rooms were maintained at 20 ± 1 °C, 20–40% RH and 16h L:8h D. Hoverflies were kept in climate-controlled rooms maintained at 25 ± 1 °C, 30–70% RH and 16h L:8h D.

All the insects mentioned in this paper have been retained at the Langfang Experiment Station of the Chinese Academy of Agricultural Sciences (CAAS) in Hebei Province, China, and the voucher specimen labels are Ec-20180605, Me-20171010, My-20171011 and Ac-20171012. The insect species were identified by the authors using two books [46,47].

2.2. Method of Aphid Culture

The mixed-aphid populations (*Me. japonica, A. craccivora* and *My. persicae*) were maintained on broad bean seedlings grown in the laboratory and changed every 7 days. We planted 20 broad bean seedlings in each basin (10 cm diam \times 10 cm height), and we cut and placed the broad bean seedlings (20 cm) infested with mixed aphids on the newly planted seedlings at heights of 2 cm, 4 cm and 6 cm. The total number of aphids on each seedling was recorded after 7 days. There were 20 seedlings in each treatment and each treatment was replicated 10 times. Aphid populations were maintained in climate-controlled rooms at 20 ± 1 °C, 20–40% RH and 16h L:8h D.

2.3. Method of E. corollae Culture

2.3.1. E. corollae Adult Culture

The reproduction of *E. corollae* depends on adult density, and on aphid density and species, as shown in the figure below (Figure 1).

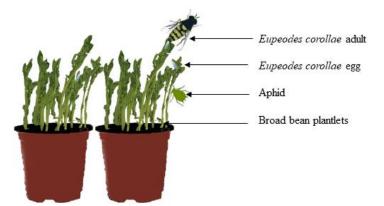


Figure 1. Reproduction of *E. corollae* adults.

Aphid Supply Method

As described in Section 2.1, one pair of *E. corollae* adults (<24 h) was reared in a cage containing 10% honey water, mixed pollen and 40 broad bean plantlets (20 cm height) infested with *Me. japonica*. Bean seedlings served as an oviposition substrate, and were removed from rearing cages to collect and record the eggs. Hoverflies were provided with different aphid densities during the breeding period by replacing broad bean seedlings every day, every 2 days and every 4 days. Broad bean seedlings were not replaced in the control treatment. We measured hoverfly-larvae numbers and calculated egg-hatching rates after all aphids on the seedlings were eaten. The hoverflies were raised in a climate chamber at 25 ± 1 °C, 30-70% RH and 16 h L:8 h D. There were 30 replicates of each treatment.

E. corollae Adult Density Treatment

Different numbers of recently emerged (<24 h) *E. corollae* adults were reared in a cage containing 10% honey water, mixed pollen and 40 broad bean plantlets infested with *Me. Japonica*, as described in Section 2.1. More specifically, the numbers of *E. corollae* adults released in each cage were 1 pair, 5 pairs, 10 pairs and 20 pairs, respectively. We changed bean seedlings in cages daily, counted the number of hoverfly eggs and larvae, and calculated egg-hatching rates after all aphids on the seedlings were eaten. The laboratory conditions were 25 ± 1 °C, 30–70% RH and 16 h L:8 h D, and each treatment was repeated 10 times.

Aphid Species Treatment

We transferred 5 pairs of newly emerged *E. corollae* adults (<24 h) into a cage containing honey water and mixed pollen, as described in Section 2.1, and 40 broad bean seedlings infested with either *Me. japonica*, *A. craccivora*, *My. persicae*, or a mixed population of the 3 aphid species (total of 4 different treatments). Aphid-infested broad bean seedlings were replaced daily in another cage to record the number of hoverfly eggs. We counted the hoverfly larvae and calculated egg-hatching rates after all aphids on the seedlings were eaten. The laboratory conditions were 25 ± 1 °C, 30–70% RH and 16h L:8h D, and each treatment was repeated 10 times.

2.3.2. Method of E. corollae Larvae Culture

Eupeodes corollae larvae feed on eggs in the absence of aphids or in limited space. In this section, we examine how the aphid species used and different containers affect hoverfly larval development and self-injury.

Aphid Species Treatment

We transferred 5 pairs of newly emerged *E. corollae* adults (<24 h) into a rearing cage containing honey water and mixed pollen, as described in Section 2.1, and 40 broad bean seedlings infested with either Me. japonica, A. craccivora, My. persicae, or a mixed population of the 3 aphid species (total of 4 different treatments). During the 3rd, 4th and 5th days of hoverfly breeding, the leaves on which *E. corollae* laid eggs were randomly cut and placed into a dish (3.5 cm in diam \times 1 cm height, egg/Petri dish). We fed new larvae of E. corollae with the aphid species originating from where the eggs were located (one of the 4 treatments), adding 80 aphids per dish daily until pupation. The development and survival of hoverfly larvae were observed at 8:00 and 20:00 every day. Each treatment was repeated 90 times. After pupation and emergence, the numbers of hoverfly pupae and adults were recorded for all 4 treatments. Then, we moved 1 pair of newly emerged hoverfly adults (which were developed from larvae feeding on either Me. japonica, A. craccivora, *My. persicae,* or the mixed aphids) to a cage containing 10% honey water, mixed pollen and 40 broad bean plantlets infested with *Me. Japonica*, as described in Section 2.1. The seedlings were changed daily, and the total oviposition of hoverflies was observed and recorded. The laboratory conditions were 25 ± 1 °C, 30-70% RH and 16 h L:8 h D, and each treatment was repeated 15 times.

Rearing Containers

As described in Section 2.1, five pairs of *E. corollae* adults were reared in a cage containing 10% honey water, mixed pollen and 40 broad bean plantlets infested with *Me. japonica*. During the 3rd, 4th and 5th days of hoverfly breeding, all the leaves on which *E. corollae* laid eggs were cut off and placed in another cage or box. Insect cage: 60 broad bean seedlings infested with mixed aphids were cut and added to the insect cage to feed the hoverfly larvae at 8:00 daily. Insect box: 60 broad bean seedlings infested with mixed aphids were cut and added to the insect cage core, 1.5 cm aperture mesh) to feed the hoverfly larvae at 8:00 daily. Hoverfly larvae were observed daily until pupation, and pupae were weighed on the third day. The larval survival rate, pupation rate and emergence rate were calculated. The laboratory conditions were 25 ± 1 °C, 30–70% RH and 16h L:8h D, and each treatment was repeated 5 times.

Larval survival rate = (No. of mature larvae/No. of tested larvae) \times 100%;

Pupation rate = (No. of pupae/No. of mature larvae) \times 100%;

Emergence rate = (No. of adults/No. of pupae) \times 100%.

2.3.3. Preservation Method for E. corollae Pupae

Considering the impact of humidity on hoverfly pupae emergence, 4 substances with different water content were used to cover the pupae for their emergence. We placed 200 *E. corollae* pupae (<24 h) in Petri dishes (9 cm diam \times 1.5 cm height) covered with vermiculite, nutritive soil, foam balls or fresh broad bean leaves (1 cm thick) in insect cages. Pupae were not covered with any material in the control group. We calculated the emergence rate after pupa emergence. Each treatment was repeated 10 times. Laboratory conditions were the same as in Section 2.1.

The factors affecting *E. corollae* intensive breeding are shown in the figure below (Figure 2):

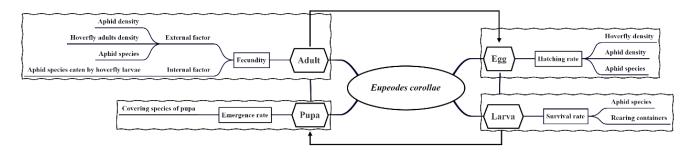


Figure 2. Breeding process of *E. corollae*.

2.4. Statistical Analysis

The number of eggs and larvae of *E. corollae*, their hatching rates, emergence rates and different instar durations of larvae fed on different aphid species were analyzed by one-way analysis of variance (ANOVA). Larval survival rate, pupation rate and pupae weight of hoverfly larvae reared in box versus cage were analyzed by Student's t-test. Prior to analysis, all data were checked for normality and heteroscedasticity. Where necessary, data were transformed to meet normality assumptions. Statistical analysis was conducted by SPSS 21.0 (IBM, Armink, NY, USA) and images were plotted with SigmaPlot 14.0 (Systat Software, Inc., Hamburg, Germany).

3. Results

3.1. Optimized Breeding Technique for Aphid Populations

Broad bean seedlings displayed varying degrees of damage when inoculated with the mixed aphids (*Me. japonica, A. craccivora* and *My. persicae*) at different heights (Figure 3). Broad bean seedlings inoculated with aphids at 2 cm (Figure 3A) and 4 cm (Figure 3B) exhibited a higher number of aphids after 7 days than seedlings inoculated with aphids at 6 cm (Figure 3C). When inoculated with aphids at 2 cm, a broad bean seedling's aphids gathered at the top of the leaves, causing the leaves to curl and negatively affecting the seedling's growth (Figure 3a), while those inoculated at 4 cm (Figure 3b) and 6 cm (Figure 3c) displayed fewer aphids and normal leaf development.

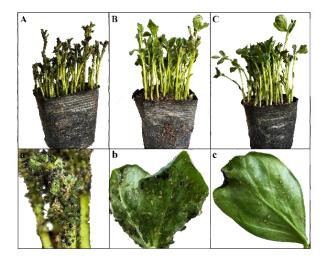


Figure 3. Broad bean seedlings inoculated with mixed aphids at different heights for 7 days. (**A**–**C**) represent broad bean seedlings inoculated with mixed aphids at 2 cm, 4 cm and 6 cm for 7 days; (**a**–**c**) are the broad bean leaves of (**A**–**C**), respectively. The mixed aphids consisted of *Me. japonica*, *A. craccivora* and *My. persicae*.

Aphid numbers were lowest on seedlings inoculated at 6 cm after 7 days of inoculation (69.1), and were markedly lower than for those inoculated at 2 cm (231.4) and 4 cm (217.3) ($F_{2,597} = 358.169$, p = 0.000)(Figure 4).

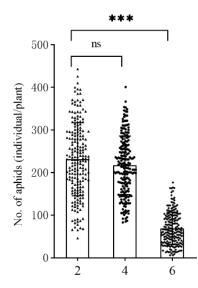


Figure 4. The number of aphids on broad bean seedlings inoculated with aphids at different heights for 7 days. Note: ns indicates no significant difference between data by Student's *t*-test (p > 0.05); *** indicates significant difference between data by Student's *t*-test (p < 0.001).

3.2. Optimized Breeding Technique for E. corollae

3.2.1. Reproduction of *E. corollae* Reared Using Different Methods Reproduction of *E. corollae* Using Different Aphid Densities

The fecundity of *E. corollae* increased with aphid density (i.e., replacement frequency of broad bean seedlings) (Table 1). In this experiment, a single *E. corollae* had the greatest fecundity when the broad bean seedlings were replaced daily. The total number of eggs, the hatching rate and the number of surviving larvae under this treatment were 584.9, 95.6% and 549.3, respectively, markedly higher than at other replacement frequencies of broad bean seedlings (Table 1) (total spawning number: $F_{3,116} = 0.58$, p = 0.000; egg-hatching rate: $F_{3,116} = 0.14$, p = 0.000; number of hoverfly larvae: $F_{3,116} = 0.44$, p = 0.000).

Table 1. Reproduction of E. corollae using different aphid densities.

Replacement Frequency of Broad Bean Seedlings (d)	Total Spawn Number/Hoverflies	Egg-Hatching Rate (%)	Number of Hoverfly Larvae/Adults
1	$584.9 \pm 22.92 \text{ A}$	$95.6\pm6.52~\mathrm{A}$	$549.3 \pm 17.65 \; { m A}$
2	$450.0\pm16.23~\mathrm{B}$	$84.2\pm5.11~\mathrm{B}$	$301.0\pm15.26~\mathrm{B}$
4	$283.5\pm23.43\mathrm{C}$	$77.6\pm3.20~\mathrm{C}$	$198.6\pm12.72~\mathrm{C}$
No replacement	$168.2\pm26.20~\text{D}$	$43.5\pm4.50~\mathrm{D}$	$73.2\pm4.55~\mathrm{D}$

Note: The data in the table are means \pm SE, and different capital letters indicate significant differences between data in the same column by one-way ANOVA (p < 0.05, Tukey).

Reproduction of E. corollae with Different Adult Numbers

With increasing hoverfly adult population numbers, the number of eggs produced by every *E. corollae* decreased (Figure 5). There were 35.5 and 35.6 eggs laid per female adult when 1 pair or 5 pairs of hoverflies, respectively, were housed in the cage during the breeding period, which was significantly higher than the number of eggs laid by 10 pairs (28.9) and 20 pairs (22.6) ($F_{3,36} = 5.102$, p = 0.003).

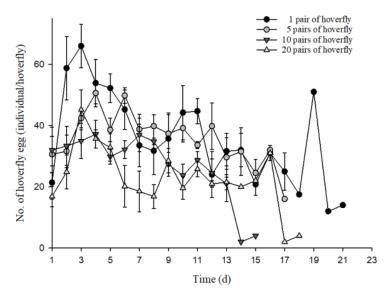


Figure 5. Number of eggs (means \pm SE) laid by *E. corollae* daily with different numbers of adults housed in cages.

Additionally, hoverflies' lifespans decreased as the number of adults in the cage increased (Figure 6). The life expectancies of 1 pair and 5 pairs of *E. corollae* adults kept in cages were 17.3 d and 16.8 d, respectively, which were longer than those for 10 pairs (15.4 d) and 20 pairs (14.2 d) ($F_{3,36} = 5.007$, p = 0.004).

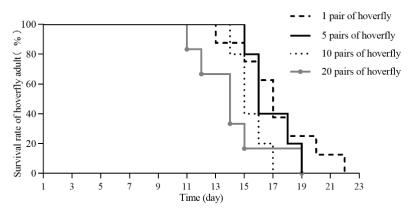


Figure 6. Survival rate of *E. corollae* adults with different numbers of adults housed in cage.

The fecundity of *E. corollae* declined as the number of adults in the cage increased (Table 2). When 5 pairs and 1 pair of hoverflies were in the cage, the number of eggs laid by a single hoverfly was 546.4 and 584.9 individuals, respectively, which was significantly higher than that for 10 pairs (424.8) and 20 pairs (303.7) ($F_{3,36} = 36.41$, p = 0.000) (Table 2). The egg-hatching rates of *E. corollae* were 95.6% and 94.5% when there were 1 and 5 pairs of hoverfly adults housed in the cage, respectively, markedly higher than those for 10 and 20 pairs ($F_{3,36} = 4.120$, p = 0.000). When there were 5 pairs of *E. corollae* adults in the cage, 489.5 larvae survived in the offspring per hoverfly, which is comparable to the offspring for 1 pair of hoverflies in the cage (549.3), and markedly higher than for 10 pairs (231.6) and 20 pairs (96.4) ($F_{3,36} = 17.21$, p = 0.000) (Table 2).

Number of Hoverfly Adults	Total Spawn Number/Hoverflies	Egg Hatching Rate (%)	Number of Hoverfly Larvae/Adults
1 pair	$584.9 \pm 22.92 \; \mathrm{A}$	$95.6\pm6.52~\mathrm{A}$	$549.3 \pm 17.65 \; {\rm A}$
5 pairs	$546.4\pm19.40~\mathrm{A}$	$94.5\pm3.52~\mathrm{A}$	$489.5 \pm 20.33 \; { m A}$
10 pairs	$424.8\pm19.43~\mathrm{B}$	$58.3\pm5.47~\mathrm{B}$	$231.6\pm16.52~\mathrm{B}$
20 pairs	$303.7\pm20.32~\mathrm{C}$	$33.4\pm6.20~\mathrm{C}$	$96.4\pm6.87\mathrm{C}$

Table 2. Reproduction of *E. corollae* with different numbers of adults housed in cage.

Note: The data in the table are means \pm SE, and different capital letters indicate significant differences between data in the same column by one-way ANOVA (p < 0.05, Tukey).

Reproduction of E. corollae Using Different Aphid Species

The fecundity of *E. corollae* was highest when using *A. craccivora* compared with *Me. japonica*, followed by *My. persicae* and the mixed-aphid populations (Table 3). The number of eggs laid by every *E. corollae* female when using the *A. craccivora* populations was highest (546.4), higher than those for *My. persicae* (289.5) and *Me. japonica* (483.2) and the mixed-aphid population (425.9) ($F_{3,36} = 76.370$, p = 0.000). The egg hatching rate was 94.5%, and the number of surviving larvae was 489.5 under this treatment, which was significantly higher than that for the other two aphid species and the mixed-aphid population (egg hatching rate: $F_{3,36} = 12.036$, p = 0.000; number of surviving larvae: $F_{3,36} = 53.2$, p = 0.000).

Table 3. Reproduction of E. corollae using different aphid species.

Aphid Species	Total Spawn Number/hoverflies	Egg-Hatching Rate (%)	Number of Hoverfly Larvae/Adults
Me. japonica	$483.2\pm12.2~\mathrm{B}$	$58.3\pm6.41~\mathrm{C}$	$367.9\pm15.72~\mathrm{B}$
A. craccivora	$546.4\pm19.40~\mathrm{A}$	$94.5\pm3.52~\mathrm{A}$	$489.5 \pm 20.33 \; \text{A}$
My. persicae	$289.5\pm23.2~\mathrm{C}$	$81.3\pm4.86~\mathrm{B}$	$206.6 \pm 26.42 \text{ C}$
Mixed aphids	$425.9\pm16.8~\mathrm{C}$	$78.3\pm5.43~\mathrm{B}$	$316.4\pm15.20~\mathrm{B}$

Note: The data in the table are means \pm SE, and different capital letters indicate significant differences between data in the same column by one-way ANOVA (p < 0.05, Tukey).

The larval stage of *E. corollae* fed on *My. persicae* was 6.0 d, much shorter than for those fed on *Me. japonica* (6.6 d), *A. craccivora* (7.6 d) or the mixed aphids (6.8 d) ($F_{3,116} = 15.962$, p = 0.000) (1st instar: t = -7.539, df = 53.783, p = 0.000; 2nd instar: t = -2.115, df = 58, p = 0.039; 3rd instar: t = -4.292, df = 58, p = 0.000; larval stage: t = -7.180, df = 58, p = 0.000) (Figure 7).

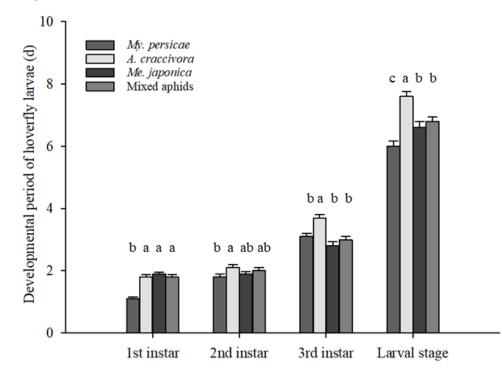


Figure 7. Larval development time of various *E. corollae* instars feeding on different aphid species. Note: Different lowercase letters indicate significant differences between treatments at the same age by one-way ANOVA (p < 0.05, Tukey).

The larval survival rate, pupation rate and emergence rate of *E. corollae* varied significantly depending on the aphid species used (Table 4). Survival, pupation and emergence rates of *E. corollae* larvae fed on the mixed-aphid populations were all higher than 90% (larval survival rate: $F_{3,8} = 10.227$, p = 0.000; pupation rate: $F_{3,8} = 7.512$, p = 0.01; emergence rate: $F_{3,8} = 11.892$, p = 0.003). In addition, 65.6%, 77.8%, 42.2% and 87.7% of *E. corollae* larvae fed on *My. persicae*, *A. craccivora*, *Me. japonica* and mixed aphids, respectively, developed into adults.

Table 4. Development of *E. corollae* larvae fed on different aphid species.

	Aphid Species			
	My. persicae	A. craccivora	Me. japonica	Mixed Aphids
Larval survival rate (%)	$94.4\pm2.33~\mathrm{A}$	$87.8\pm4.93~\mathrm{A}$	$63.3\pm6.94\mathrm{C}$	$91.1\pm1.10~\mathrm{A}$
Pupation rate (%)	$83.5\pm1.41~\mathrm{C}$	$92.2\pm2.35~\mathrm{B}$	$90.1\pm4.14~\mathrm{B}$	$100\pm0.00~\mathrm{A}$
Emergence rate (%)	$83.2\pm2.12~\text{B}$	$95.5\pm2.63~\mathrm{A}$	$74.9\pm3.26\ \mathrm{C}$	$96.3\pm3.70~\mathrm{A}$

Note: The data in the table are means \pm SE, and different capital letters indicate significant differences between data in the same column by one-way ANOVA (p < 0.05, Tukey).

The aphid species which *E. corollae* larvae feed on significantly influence the hoverfly's fecundity (Figure 8). Mature *E. corollae* females laid 563.4 eggs when their larvae fed on *Me. japonica*, which is similar to the number when they areg reared on mixed aphids (550.4) but

higher than when they are given access only to *My. persicae* (390.6) or *A. craccivora* (360.2) ($F_{3,56} = 32.7$, p = 0.000).

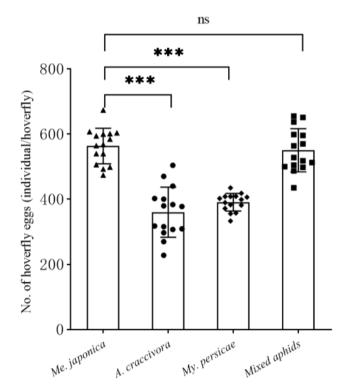


Figure 8. Number of eggs (means \pm SE) laid by *E. corollae* whose larvae feed on different aphid species. Note: ns indicates no significant difference between data by Student's *t*-test (p > 0.05); *** indicates significant difference between data by Student's *t*-test (p < 0.001). Triangles, dots, rhombus, and squares represent the number of eggs laid by hoverflies whose larvae feed on class *Me. japonica*, *A. craccivora*, *My. persicae* and Mixed aphids (each dot represents the number of eggs laid by one female hoverfly).

Development of E. corollae Larvae Reared in Box versus Cage

Different rearing containers affected the larval development of *E. corollae* (Table 5). Of the larvae reared in a box, 79.9% survived, and 95.6% of the survivors pupated and they had an average pupal weight of 30.1 mg. These values were significantly higher than those for larvae reared in a cage (49.3% survived; 72.3% pupated and average pupal weight was 25.4 mg) (larval survival rate: t = 14.941, df = 30, p = 0.000; pupation rate: t = 21.164, df = 19, p = 0.000; pupae weight: t = 3.851, df = 30, p = 0.001).

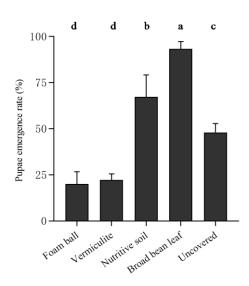
Table 5. Development of *E. corollae* larvae reared in box versus cage.

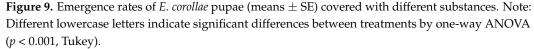
Larval Rearing Method	Larval Survival Rate (%)	Pupation Rate (%)	Pupae Weight (mg)
Insect box	$79.9\pm3.42~\mathrm{A}$	$95.6\pm3.25~\mathrm{A}$	$30.1\pm5.42~\mathrm{A}$
Insect cage	$49.3\pm6.85~\mathrm{B}$	$72.3\pm4.32~\text{B}$	$25.4\pm1.84~\mathrm{B}$

Note: The data in the table are means \pm SE, and different capital letters indicate significant differences between data in the same column by Student's *t*-test (*p* < 0.05).

3.2.3. Emergence of E. corollae Pupae When Covered with Different Substances

Covering them significantly affected the pupae emergence of *E. corollae* ($F_{4,45} = 278.431$, p = 0.000). The emergence rate of the pupae covered with broad bean leaves was 93.3%, significantly higher than that for vermiculite (22.2%), nutritive soil (67.3%), foam balls (20%) or no mulch (48%) (Figure 9).





4. Discussion

Facility horticulture regulates crop fruiting with synthetic hormones, which requires considerable manpower and pollutes the environment with chemical residues [5]. Pollinators such as bumblebees provide efficient pollination services in horticulture facilities, but are easily weakened by chemical pesticides used for pest management [6,19]. The joint-use technique of combining pollinators and natural enemies in facility agriculture is not mature, and the input costs are high. *Eupeodes corollae* are beneficial in horticulture facilities as pollinators and biocontrol agents, both decreasing aphid populations and increasing seed retention and yield, making it worthwhile to explore an intensive breeding technology for them [28,30,35].

Commercial insect production emphasizes improving insect fertility and reducing mortality and breeding costs. In *E. corollae* adult culture, the study of the "aphid supply method" (i.e., the replacement frequency of broad bean seedlings infested with aphids) had a different objective from the "*E. corollae* adult density treatment" in the cage. *Eupeodes corollae* continue to lay eggs in aphid colonies during the breeding period. A lower frequency of replacement of broad bean seedlings in cages would result in hoverfly larvae overlapping and self-injury increasing. To minimize material and manpower costs, this experiment initially investigated the effect of aphid density (i.e., replacement frequency of broad bean seedlings was determined, the experiment continued to study hoverfly reproduction with various adult numbers in the cages to maximize hoverfly reproduction without any restriction by aphid numbers, maximizing hoverfly population growth and minimizing economic expenditure.

The aphid species used affects *E. corollae*'s growth, development and reproduction [32,48]. Due to the lack of mature artificial food for *E. corollae*, many host plants were grown to feed aphids and thus hoverflies. Selecting and identifying host-plant species that are easy to grow indoors, have a short growth cycle and can support aphid proliferation is a crucial step to solve hoverfly-breeding problems. We selected broad bean seedlings as hosts based on their ease of reproduction and low cost. *Eupeodes corollae* lay eggs in wheat-aphid colonies and their larvae significantly reduce wheat-aphid populations through predation (such as *Rhopalosiphum padi* (L.) and *Sitobion avenae* (Fabricius)) [22,49]. As wheat seeds can be hydroponically grown indoors, quickly, easily and in a cost-effective manner, it is advantageous to propagate hoverflies and feed aphids from indoor wheat seedlings.

Aphid populations affect hoverfly fecundity [37]. Hoverflies rarely lay eggs in aphid colonies already having enough eggs, to prevent their offspring from self-injury due to

lack of food [50]. Consequently, large-scale hoverfly breeding requires a large number of aphids and host plants, which in turn require a large amount of space and are costly. There is no doubt that artificial feeds are an effective method of breeding natural enemies on a large scale. Researchers have shown that *Episyrphus balteatus* (De Geer) larvae can become adults by feeding on artificial feed containing bee pupae, but adults cannot reproduce normally [51,52]. Providing young hoverfly larvae with aphids and older larvae with artificial diets is a more rational way to accurately regulate hoverflies' propagation.

For hoverfly applications, studies suggest that hoverfly adults should be used during the spawning period to facilitate precise positioning for biocontrol and pollination purposes, but the transportation cost is high. In this experiment, pupae were used as the product form and their emergence was observed when covered with different substances. Temperature and humidity affect pupa emergence [53,54]. Hoverfly pupae overwinter in the shallow soil layer of fertile soil and emerge from the moist and loose soil created by plant roots. We conducted the study indoors, and pupae placement should be adapted to the ambient temperature and humidity of the application site. Studies have shown that the introduction of parasitic wasps in potted plants can significantly reduce the aphid population in greenhouses [17]. As a consequence, the introduction of eggs, larvae and pupae from potted plants at the same time may facilitate the stable colonization of hoverflies at application sites and allow them to perform their pollination and biological control functions to the fullest possible extent.

Natural-enemy insects should not only be utilized for indoor propagation and outdoor release, but should also be utilized for wild population conservation in a given area [55–59]. To increase the number of wild hoverflies, honey plants were planted in protected areas to provide nutrition for adults [33], functional plants were preserved for larvae development [60–63] and sufficient weeds were retained to provide overwintering sites for hoverflies. In addition, changing the planting pattern, i.e., expanding the single farm ecosystem into a more rational farmland-landscape ecosystem, and enhancing pollinator populations and natural enemies, are important directions for agriculture's future development [64–66].

5. Conclusions

Hoverflies' fecundity is affected by the aphid species that the larvae feed on and those present on the plant where the adults lay eggs. Their fecundity when using *A. craccivora* populations was highest, compared with *Me. japonica*, *My. persicae* or mixed-aphid populations. The survival, pupation and emergence rates of *E. corollae* larvae fed on mixed-aphid populations were all higher than for those fed on the three single-aphid-species populations. In addition, the *E. corollae* obtained higher fecundity when their larvae fed on *Me. japonica* or mixed aphids.

We integrated the breeding methods for the prey (aphids), larvae, pupae and adults and established indoor-propagation techniques for *E. corollae*. Five pairs of *E. corollae* adults were placed in a cage with 40 broad bean seedlings infested with *A. craccivora* that were replaced daily. The larvae were fed a mixture of aphids (*A. craccivora*, *My. persicae* and *Me. japonica*) and covered with fresh broad bean leaves after pupation. In summary, each pair of *E. corollae* adults produced 391 adult offspring. This study will assist with the commercial production and application of *E. corollae*.

Author Contributions: H.L.: Investigation, formal analysis and writing—original draft. K.W.: Funding acquisition, conceptualization, supervision, resources and writing—review and editing. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by the Lingman Modern Agriculture Project (NT2021003), the Key R&D Program of Shandong Province, China (2020CXGC010802) and the National Modern Agricultural Industry Technology System Construction Fund of China (CARS-02).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest.

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