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Using *Calendula officinalis* as a floral resource to enhance aphid and thrips suppression by the flower bug *Orius sauteri* (Hemiptera: Anthocoridae)

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Abstract

BACKGROUND: The flower bug *Orius sauteri* (Poppius) (Hemiptera: Anthocoridae) is widely used as a biocontrol agent against thrips and aphids infesting greenhouse vegetables in Asia. The survival and oviposition of such predators, as well as the biocontrol services they provide, may be enhanced by adding extra floral resources to the crops. In the present study we investigated the effects of the plant *Calendula officinalis* L., used as a floral resource, for promoting the control of *Myzus persicae* (Sulzer) and *Frankliniella occidentalis* (Pergande) by *O. sauteri* under laboratory and greenhouse conditions.

RESULTS: Results showed that the presence of *C. officinalis* enhanced aphid and thrips suppression via an increased *O. sauteri* population growth. The predator populations responded positively to the addition of *C. officinalis* in the system, and they also varied as a function of the temperatures tested under laboratory conditions. In a similar way, predator populations varied among seasons, with the highest densities recorded in May in the greenhouse.

CONCLUSION: *C. officinalis* can be used to increase available resources for natural enemies used in agricultural crops, notably in greenhouses. This study also provides evidence that increasing floral resources can enhance pest suppression provided by *O. sauteri*.

Keywords: Anthocoridae; floral plant; predator; biological control; companion plant

1 INTRODUCTION

Insect predators and parasitoids provide valuable ecosystem services as biological control agents of key pests that infest cultivated crops.[1–4] However, natural enemies often need extra resources, besides those provided by the crop itself. These may include additional prey,[5,6] additional food resources for adults (specifically nectar and pollen) and overwintering habitat[7–10] and hiding places/shelter.[7] Previous studies have indicated that the release of predatory or parasitic insects accompanied with the introduction of specific resource plants is one of the most effective ways for enhancing the effectiveness of biological control in agroecosystems.[11–16] These plants are classified by their function in habitat management into categories such as honey plants (floral plants), habitat plants, banker plants, trap plants, etc.,[17–20] especially for *Orius* spp.[21,22]

In the agricultural landscape, floral plant systems are designed to enhance the efficacy of biological control exhibited by natural enemies by providing an alternative source of food when prey is scarce or absent.[9,23] In Mediterranean sweet pepper greenhouses, flowering sweet alyssum *Lobularia maritima* L. and coriander *Coriandrum sativum* L. provide an effective method for enhancing native syrphid populations.[24] The buckwheat *Fagopyrum esculentum* (Moench) has a strong positive effect on longevity of the parasitoid *Necremnus artynes* (Walker) and its potential for biological control of the exotic pest *Tuta absoluta* (Kaltenbach).[25] Cage experiments conducted by Van Rijn *et al.*[26] showed that, when flowers of buckwheat are present, the hoverfly *Episyrphus balteatus* (Degeer) can strongly suppress the growth of cabbage aphid colonies living on Brussels sprouts.

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The flower bug *Orius sauteri* (Poppius) (Hemiptera: Anthocoridae) is widely used for biological control of thrips and aphid pests on greenhouse vegetables in Asia.[27–31] Food supplements enhance successful population colonisation by the flower bug owing to its small search range and self-cannibalism in its initial stage.[32] The flower bug actively visits and feeds on flowers, indicating that pollen and nectar are important resources.[33,34] A consequence of this behaviour is that the shortage of suitable flowers in manipulated agroecosystems may limit the biocontrol activity of *O. sauteri*.

No previous field studies examining the effects of floral resources on *O. sauteri* biocontrol activity have been conducted. In this study, we chose to use *Calendula officinalis* L. as a floral resource because it offers abundant nectar and pollen. Laboratory tests were conducted to estimate the influence of *C. officinalis* on *O. sauteri* population density at three constant temperatures (15, 25 and 30 °C). Following this, we assessed the effects of floral resources on *O. sauteri* population growth both under laboratory conditions and in the greenhouse. Furthermore, we also evaluated the impact on aphid *Myzus persicae* (Sulzer) and thrips *Frankliniella occidentalis* (Pergande) suppression, by releasing *O. sauteri* into greenhouse cages that contained pest-infested tomato plants both alone and in combination with flowering *C. officinalis* plants.

2 MATERIALS AND METHODS

2.1 Insects and plants

2.1.1 Insects

O. sauteri adults were netted from alfalfa *Medicago sativa* L. growing in the field of Beijing Academy of Agriculture and Forestry Sciences (Haidian district, Beijing, China) during April 2013. Newly captured flower bugs were cultured in cages (45 × 45 × 50 cm) constructed of aluminium frames and mesh nylon gauze with an initial density of 40 pairs per cage. Over 60 capsules of microencapsulated artificial diet[35] were supplied daily with several house bean, *Vicia faba* L., seedlings as oviposition substrate for *O. sauteri* reproduction.[34] Over 4000 *O. sauteri* were produced to be used in the field tests. The rearing was conducted in climatic chambers at 25 ± 1 °C, 60% RH and a 16:8 h L:D photoperiod with 1.5 W m^{-2} (L100; Suntech, Beijing, China).

2.1.2 Plants

Tomato seedlings *Solanum lycopersicum* var. Baofen-F1 (Changfeng Seed Co. Ltd, Xianyang City, Shaanxi, China) were grown in plastic trays (55 × 25 × 20 cm, ten plants per tray). They were then transplanted individually into plastic flowerpots (height 20 cm, diameter 13 cm, one plant per pot) and maintained in climatic chambers (MH-351; Sanyo, Tokyo, Japan) under environmental conditions of 27 ± 1 °C, 60–65% RH and a 14:10 h L:D photoperiod. Tomato plants for laboratory tests were used when they were approximately 30–35 cm in height with 5–7 true and fully expanded leaves (60 days).

Plants of *C. officinalis* var. Kablouna (Sinic Horticulture and Flower Co. Ltd, Beijing, China) were used as a source of floral nectar and pollen. The plants were grown using seeds collected during the previous growing season. The seedlings were started in plastic trays; when they reached 3–4 true leaves they were individually transplanted into plastic flowerpots (height 20 cm, diameter 13 cm, one plant per pot). The planted seedlings grew to 15–20 cm after 20–25 days, at which time we removed the topmost buds to ensure sufficient flower quantity. The cultured *C. officinalis* bloomed 2 weeks after we removed the buds. The plants were grown in another glass culturing greenhouse in order to maintain a constant supply of flowers throughout the experiment.

2.2 Laboratory trials

Laboratory tests were conducted to estimate the influence of *C. officinalis* on *O. sauteri* population density at three constant temperatures (15, 25 and 30 °C) using a climatic chamber (MH-351; Sanyo). Within a cage the same size as the insect rearing cages we placed four tomato plants with an average of 5–7 true leaves and one *C. officinalis* with 3–4 corollas in the centre. At the same time, we introduced 20 pairs (determined by preliminary test to ensure the best density fitting the limited space) of 2–3-day-old *O. sauteri* adults on the plants, along with abundant artificial diet microcapsules. We used an artificial diet to standardise the prey food source and better estimate the influence of the floral plant, avoiding the side effects from non-standard arthropod prey. At 20 and 40 days after release (DAR), the density of *O. sauteri* (number of nymphs and adults) was assessed. The plants were taken outside the cage, and the nymphs and adults were counted using a handheld magnifying lens. Identical cages were prepared without *C. officinalis* plants to serve as a control treatment. The treatments were replicated 15 times at each constant temperature.

2.3 Greenhouse trials

The greenhouse trials on the influence of the presence of the floral plant *C. officinalis* on *O. sauteri* population dynamics and pest suppression were conducted in the Noah Organic Farmland (NOF), located in Pinggu County, Beijing, China (40° 6′ N, 116° 59′ E). After reviewing the historical monthly average temperature records in NOF during the past 5 years, we conducted the greenhouse estimations during March (average greenhouse interior temperature 14–18 °C), May (average greenhouse interior temperature 23–28 °C) and July (average greenhouse interior temperature 30–36 °C) in 2014 in order to parallel the tested laboratory temperature regime.

Nine NOF greenhouses of the same size [55.0 m (L) × 13.0 m (W) × 6.0 m (H)] were selected for the experiments. In the first week of the month (March, May and July), we planted 2250 tomato *S. lycopersicum* cv. Baofeng -F1 (Baocheng Seeds, Shannxi, China) seedlings with 2–3 true leaves evenly as 90 lines × 25 plants in every greenhouse. Immediately after the tomato seedlings were planted, nine adjacent plants (as 3 × 3) were randomly selected as one experimental plot, and they were confined inside a fabric net cage [1.8 m (L) × 1.8 m (W) × 0.7 m (H)], constructed of an aluminium frame and 100-mesh plastic fabric mesh. Five plots were caged per greenhouse, serving as five replicates for each treatment. Then, after 1 week, 500 second- and third-instar nymphs of *M. persicae* and 400 second-instar nymphs and prepupae of the western flower thrips *F. occidentalis* (consisting mostly of second-instar nymphs with a small proportion of prepupae; the number of herbivores were determined by preliminary tests) were introduced into each cage when the tomato plants had 5–6 true leaves. The herbivores were placed on a smooth surface of white cardboard and then moved gently and evenly to the tomato leaves by using a horse tail hair brush. After an additional week, we introduced the floral plant *C. offinalis* and the predator *O. sauteri* adults as specified by the three experimental treatments.

Three treatments were compared: (1) four blooming *C. officinalis* placed into the cages, with *O. sauteri* adults released at the same time; (2) *O. sauteri* adults released alone into the cages; (3) no *C. officinalis* or *O. sauteri* added to the cages (control). Each treatment was replicated 5 times using the five caged plots in each of the three selected greenhouses. In treatments with *O. sauteri*, we released 45 pairs (determined by preliminary tests) of 2–3-day-old flower bug adults evenly on the tomato plants in each cage, employing the same method used to introduce the herbivores. We observed and recorded the total number of aphid nymphs, thrips nymphs and prepupae and flower bug adults and nymphs (in the non-control treatments) in each caged plot at 20 and 40 DAR. In order to standardise the density data, the insects were counted on the surface of all the leaves of the tomato plants in the plot by using a digital camera (D700; Nikon, Tokyo, Japan) with a microlens (Nikkor 200 mm/f4 Micro). We counted the number of insects captured by digital camera using the software ZEN 2.0 with a ZEISS stereomicroscope system. The software helped us to count the total number of insects automatically (counted as spots on the screen in a fashion similar to cells). By this method, the exact numbers of the selected life stages of all three target insects were measured. Between trials, the greenhouses were cleaned thoroughly. A total of 15 replicates per treatment were performed, using three greenhouses per treatment, each containing five plots.

2.4 Data analysis

In order to assess the influence of the presence of floral assistant plant *C. officinalis* at three different temperatures (or seasons in the greenhouse test) and two time points (20 and 40 DAR) on the population dynamics of the predator *O. sauteri* in the lab and greenhouse and on its biocontrol activity in the greenhouse, the data (densities of *O. sauteri* nymphs + adults, *M. persicae* nymphs and *F. occidentalis* nymphs) were analysed by repeated-measures ANOVA. Prior to analysis the data were tested for normality with the Kolmogorov–Smirnov test. The means were compared by Tukey's test at $P < 0.05$ level. All statistical analyses were performed using IBM SPSS 20.0.

3 RESULTS

3.1 Laboratory trials

Our results showed that the *O. sauteri* population density was significantly influenced by temperature at both 20 and 40 DAR (20 DAR: $F = 388.4$, $P < 0.01$; 40 DAR: $F = 602.4$, $P < 0.01$; both df = 2, 42) (Fig. 1). The introduction of *C. officinalis* enhanced the *O. sauteri* population significantly at each temperature and duration (20 DAR: $F_{15\,^\circ\mathrm{C}} = 36.9$, $P_{15\,^\circ\mathrm{C}} < 0.01$; $F_{25\,^\circ\mathrm{C}} = 41.7$, $P_{25\,^\circ\mathrm{C}} < 0.01$; $F_{30\,^\circ\mathrm{C}} = 47.8$, $P_{30\,^\circ\mathrm{C}} < 0.01$; 40 DAR: $F_{15\,^\circ\mathrm{C}} = 54.1$, $P_{15\,^\circ\mathrm{C}} < 0.01$; $F_{25\,^\circ\mathrm{C}} = 49.4$, $P_{25\,^\circ\mathrm{C}} < 0.01$; $F_{30\,^\circ\mathrm{C}} = 53.7$, $P_{30\,^\circ\mathrm{C}} < 0.01$; all df =1, 28 (Fig. 1). The results also showed that the population density of *O. sauteri* was significantly influenced by the interaction of temperature and presence of *C. officinalis* at both 20 and 40 DAR (20 DAR: $F = 3.6$, df = 1, $P = 0.033$; 40 DAR: $F = 25.7$, df = 1, $P < 0.01$).

3.2 Greenhouse trials

The population density of *O. sauteri* was significantly influenced by season at both 20 DAR ($F = 1233.4$, df = 2, 42, $P < 0.01$) and 40 DAR ($F = 1154.5$, df = 2, $P < 0.01$), with the highest values recorded in May (Fig. 2). The population density of *O. sauteri* was significantly higher in the treatment containing floral resource plants in all three seasons, both at 20 DAR ($F_{\mathrm{March}} = 47.6$, $P_{\mathrm{March}} < 0.01$; $F_{\mathrm{May}} = 51.7$, $P_{\mathrm{May}} < 0.01$; $F_{\mathrm{July}} = 44.7$, $P_{\mathrm{July}} < 0.01$; all df = 1, 28) and at 40 DAR ($F_{\mathrm{March}} = 75.4$, $P_{\mathrm{March}} < 0.01$; $F_{\mathrm{May}} = 53.2$, $P_{\mathrm{May}} < 0.01$; $F_{\mathrm{July}} = 41.6$, $P_{\mathrm{July}} < 0.01$; all df = 1, 28).

The control efficacy of *O. sauteri* on the aphid *M. persicae* and the thrips *F. occidentalis* showed that the densities of both pests were significantly lower in May at 20 DAR (aphid: $F = 626.4$, $P < 0.01$; thrips: $F = 513.7$, $P < 0.01$; both df = 2, 42) and 40 DAR (aphid: $F = 632.6$, $P < 0.01$; thrips: $F = 476.8$, $P < 0.01$; both df = 2, 42) following release of the predator (Figs 3 and 4). Pest population densities were also significantly lower in the treatments with both *C. officinalis* and predator *O. sauteri* in all seasons at both 20 DAR (aphid: $F_{\mathrm{March}} = 78.9$, $P_{\mathrm{March}} < 0.01$; $F_{\mathrm{May}} = 86.7$, $P_{\mathrm{May}} < 0.01$; $F_{\mathrm{July}} = 84.3$, $P_{\mathrm{July}} < 0.01$; thrips: $F_{\mathrm{March}} = 66.4$, $P_{\mathrm{March}} < 0.01$; $F_{\mathrm{May}} = 60.7$, $P_{\mathrm{May}} < 0.01$; $F_{\mathrm{July}} = 71.3$, $P_{\mathrm{July}} < 0.01$; all df = 2, 42) and 40 DAR (aphid: $F_{\mathrm{March}} = 734.2$, $P_{\mathrm{March}} < 0.01$; $F_{\mathrm{May}} = 526.8$, $P_{\mathrm{May}} < 0.01$; $F_{\mathrm{July}} = 613.4$, $P_{\mathrm{July}} < 0.01$; thrips: $F_{\mathrm{March}} = 554.1$, $P_{\mathrm{March}} < 0.01$; $F_{\mathrm{May}} = 446.2$, $P_{\mathrm{May}} < 0.01$; $F_{\mathrm{July}} = 534.1$, $P_{\mathrm{July}} < 0.01$; all df = 2, 42) (Figs 3 and 4). For both pests, the season and the presence of *C. officinalis* showed a significant interactive influence on pest population suppression at 20 DAR (aphid: $F = 396.4$, $P < 0.01$; thrips: $F = 29.3$, $P < 0.01$; both df = 2, 42) and 40 DAR (aphid: $F = 117.6$, $P < 0.01$; thrips: $F = 53.6$, $P < 0.01$; both df = 2, 42).

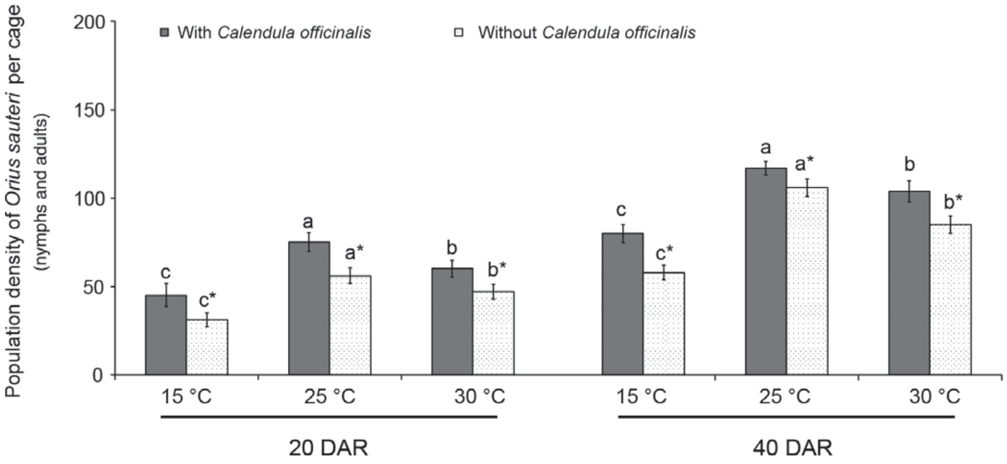

Figure 1. *O. sauteri* population density at different constant temperatures under laboratory conditions. Different letters on same-shaded columns within the same test duration indicate significant differences (Duncan's test, $P < 0.05$). Asterisks (*) on columns within the same temperature indicate significant differences between the presence and absence of companion plant (Duncan's test, $P < 0.05$).

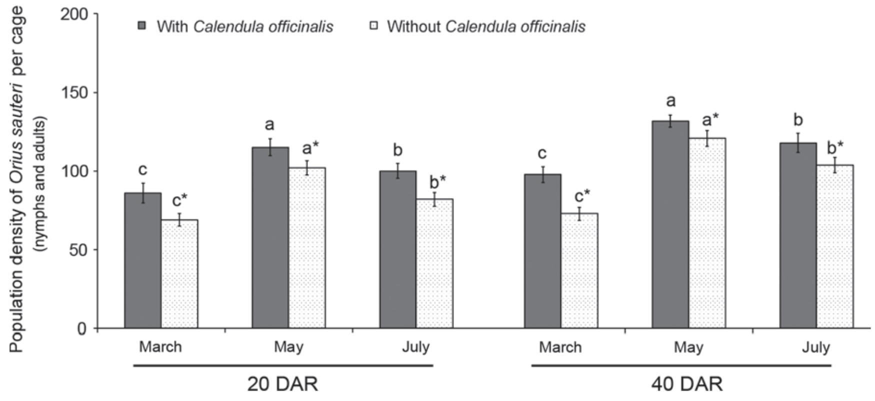

Figure 2. *O. sauteri* population density in different treatments in various seasons in greenhouse tomato. Different letters on same-shaded columns within the same observational date indicate significant differences among seasons (Duncan's test, $P < 0.05$). Asterisks (*) on columns within the same season indicate significant differences between the presence or absence of companion plant (Duncan's test, $P < 0.05$).

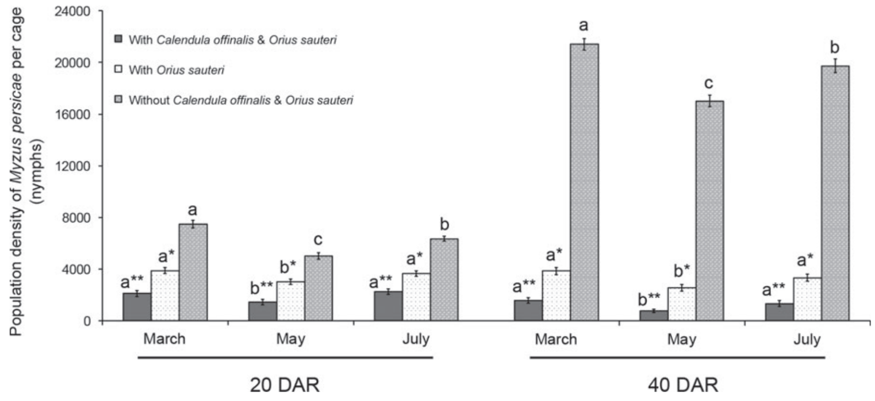

Figure 3. *M. persicae* population density in different treatments in various seasons in greenhouse tomato. Different letters on same-shaded columns within the same observational date indicate significant differences among seasons (Duncan's test, $P < 0.05$). Asterisks (*) on columns within the same season indicate significant differences among the tested treatments (Duncan's test, $P < 0.05$).

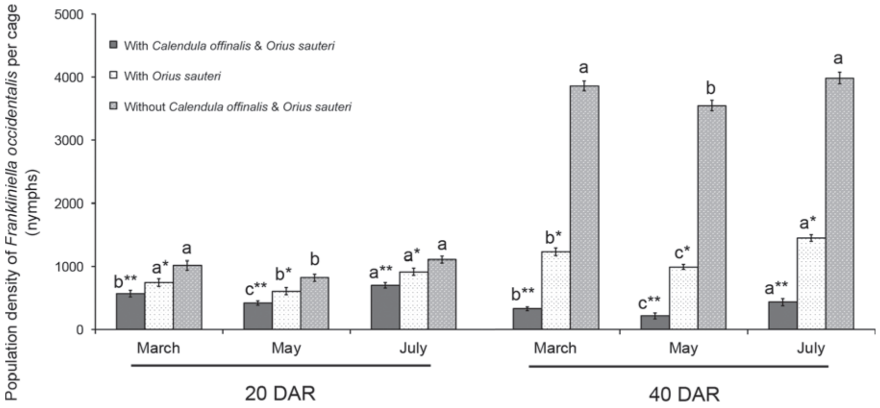

Figure 4. *F. occidentalis* population density in different treatments under various seasons in greenhouse tomato. Different letters on same-shaded columns within the same observational date indicate significant differences among seasons (Duncan's test, $P < 0.05$). Asterisks (*) on the columns within the same season indicate significant differences among the tested treatments (Duncan's test, $P < 0.05$).

4 DISCUSSION

A sharp decline in plant diversity often results in pest outbreaks in monoculture agroecosystems.[12] Indeed, lower pest population density found in multiple crop plantings is attributed to plant diversity enhancing the effect of natural enemies. The enhancement of plant diversity can provide more suitable microhabitat and more resources, including food and alternative hosts or prey for natural enemies.[1,36] An important means of conserving beneficial insects in resource-limited habitats is to meet their ecological requirements. This may be achieved in part by providing areas containing flowering plants that bloom throughout the season. In this experiment, the addition of floral resources increased *O. sauteri* population density and enhanced aphid and thrips suppression. Although the benefits of floral resources and other secondary plants that provide resources to biological control agents have been widely demonstrated in the field by previous studies,[37–40] little information was available prior to this study on the enhancement of anthocorid predators' efficacy through the use of floral plants.[41]

Our laboratory studies have demonstrated that the presence of *C. officinalis* can enhance the population growth rate of *O. sauteri* significantly at multiple temperatures. In the greenhouse, the presence of flowering plants can increase the numbers of *O. sauteri*, with consequent increases in *M. persicae* and *F. occidentalis* suppression.

Sufficient flower abundance is required to support diverse populations of insects.[42] Manipulation of structurally resource-poor habitats through the addition of flowering plants can increase beneficial insect populations in agricultural landscapes.[43] In this study, a critical step in demonstrating the role of floral resources in biological control was to show that the presence of flowering plants elevated the numbers of *O. sauteri*, consequently reducing aphid and thrips population densities. In previous studies, increasing the availability of floral resources in agricultural landscapes may have enhanced pest suppression through two potentially independent mechanisms. The presence of flowers might increase attack rates on the pest by attracting natural enemies into crop fields without any direct effects on natural enemy fitness, or it might improve components of natural enemy fitness such as longevity or fecundity.[9,36] Studies examining the effect of floral resources on the fecundity and longevity of natural enemies under field conditions have been rare,[44] particularly for predators such as flower bugs.[45] Therefore, it would be useful to evaluate the effects of floral resources on the longevity and fecundity of *O. sauteri* under field conditions.

In our research, laboratory studies showed that the strongest effect of floral resources on the numbers of *O. sauteri* occurred at a temperature of 25 °C, and the highest greenhouse population densities were recorded in May. Temperature may affect the growth, pollen production and flowering of *C. officinalis* as well as the release of volatile chemical substances influencing attractiveness to *O. sauteri*. *C. officinalis* supported *O. sauteri* population growth. However, some phanerogam pollen is not suitable to support insect natural enemy growth, and may even cause certain toxic and side effects from secondary compounds.[46] It is also possible that the longevity and reproduction of some target pests could be enhanced by the presence of floral plants.[47] Therefore, enhancing the availability of floral resources to predators such as *Orius* spp. should be done with caution. In addition, potential enhancement of predator-mediated indirect interactions between aphids and thrips (either negative or positive ones[48,49]) should be assessed for optimal implementation of floral resources in crops. Therefore, it is important to improve our understanding of the acquisition and utilisation of plant pollen and nectar by pests and their natural enemies.[50,51] Floral resource requirements can be fulfilled with a diverse assemblage of flowering plants, providing the resources necessary to support populations of predators and parasitoids throughout the season.[52,53] The present study took place under regulated conditions in the laboratory and greenhouse, which may limit the applicability of the results to the field. However, the results showed the positive influence of *C. officinalis* on predator population colonisation and development across multiple conditions. Factors in the application of floral plant resources to natural enemy pest control systems include floral resource density and distance of the floral plant from the target plant.[13,54,55] Feeding behaviour of the natural enemy and competition with insect pollinators may have effects on the efficacy of natural enemies.[56–58] In addition, *O. sauteri* was reared on artificial diet for reducing the possible impact of variation in food (prey) quality for our experiments. Therefore, various artificial diet receipts used to rear predators (as well as prey used as food) may also modulate the potential benefits gained when implementing floral resources near crops. Economic assessment of the efficiency of floral plant resources is still quite limited. Therefore, floral plant applications for production purposes require further investigation.[8,9,59,60] Tests are needed (i) to optimise the use of companion plants, (ii) to promote their optimal distribution within the crop and (iii) to evaluate potential interactions between companion plants and cultivated ones.

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