

Biological control of *Aphis spiraecola* in apples using an insectary plant that attracts and sustains predators

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ARTICLE INFO

Keywords:

Insectary plant
Predatory natural enemy
Spirea aphid
Biological control
Rubidium marking

ABSTRACT

Habitat management, such as adding insectary plants to agroecosystems, is a useful technique for biological control. It can improve pest control through natural enemy augmentation and conservation. We evaluated use of the insectary plant *Cnidium monnieri* (L.) Cuss. to increase the diversity and population density of natural enemies, promote the movement of natural enemies, and improve suppression of the spirea aphid (*Aphis spiraecola* Patch). An apple orchard experiment included *C. monnieri* as the treatment and the absence of *C. monnieri* as the control. The results suggested that *C. monnieri* can foster predatory natural enemies, such as *Propylaea japonica*, *Harmonia axyridis*, *Hippodamia variegata*, *Chrysoperla sinica*, and *Episyrphus balteata*. Density of the predators on the apple trees in the treatment was significantly higher than in the control. Density of spirea aphids on the apple trees in the treatment was significantly lower than in the control. Rubidium (Rb) was used as a tracking marker transferred along the food chain of *C. monnieri* (4.08 µg/ml) – celery aphid (*Semiaphis heraclei*) (0.46 µg/ml) – lady beetle (0.51 µg/ml) in a laboratory experiment. The highest Rb content in a lady beetle (0.62 µg/ml) was found 3 days after marking. Combined with field sampling and Rb marking, the results showed that 24.2% and 42.7% of *H. axyridis* on the apple trees moved from the insectary plant *C. monnieri* in 2018 and 2019, and that 53.2% and 48.4% of *C. sinica* on the apple trees transferred from *C. monnieri* in 2018 and 2019. The insectary plant *C. monnieri* attracts and fosters predatory natural enemies, which then move to the apple trees and suppress spirea aphids. These findings illustrate an effective method for enhancing biological control of aphids in apple orchards.

1. Introduction

Conservation biological control (CBC) includes various management practices that can protect natural enemy populations in agroecosystems, enhance their fitness and increase their impact on pest populations

(Jaworski et al., 2019; Shields et al., 2019; Thomine et al., 2020a). Habitat management is one CBC practice, which can create a more suitable ecological infrastructure and enhance the natural enemy populations (Porcel et al., 2016; Gurr et al., 2017; Thomine et al., 2020b). This is often achieved by introducing a non-crop plant species within, or

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<https://doi.org/10.1016/j.biocontrol.2021.104532>

Received 9 June 2020; Received in revised form 28 December 2020; Accepted 31 December 2020

Available online 8 January 2021

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near, the crop (Parolin et al., 2012; Kishinevsky et al., 2017). The insectary plants provide benefits to the natural enemies, including shelter, nectar, alternative prey/hosts, and pollen, and also facilitate contact between the natural enemies and their target prey which are pests on the crop plants. (Naranjo et al., 2015; Gurr et al., 2017; Karp et al., 2018; Moore et al., 2019; Damien et al., 2020; Wang et al., 2020). Intercropping of insectary plants with main crops can increase biodiversity and CBC (Li et al., 2015; Zhao et al., 2017). Intensive cropping systems, such as orchards, often have low plant species diversity, which limits the pest control efficacy of natural enemies (Piffner et al., 2019). Intercropping of orchards with insectary plants has become an important technique for CBC.

Apple (*Malus domestica* Borkh.) orchards are perennial crop systems with large areas of intensive planting. Apple orchards generally lack the biodiversity that would be optimal for beneficial arthropods (Simon et al., 2010; Piffner et al., 2019). Intercropping of other plants within apple orchards has contributed to a more diverse environment, provides nutritional benefits for the beneficial arthropods and often improves the biological control (Herz et al., 2019). For example, the addition of sweet alyssum flowers (*Lobularia maritima*) bolstered natural enemies (e.g., syrphid flies) and improved biological control of the woolly apple aphids (*Eriosoma lanigerum*) in an apple orchard (Gontijo et al., 2013). Flower strips of *Centaurea cyanus*, *Silene vulgaris*, *Silene latifolia alba* and *Achillea millefolium* increased natural enemy populations and improved the biological control of *Dysaphis plantaginea* in apple orchards (Albert et al., 2017). Complex assemblages of natural enemies have more promise for sustainable biological control than single species (Herz et al., 2019). The combination of a parasitoid and a generalist predator guild provided better suppression of the woolly apple aphid (Gontijo et al., 2015). Planting insectary plants in apple orchards can leverage the favorable characteristics of specific plant species (Hooks and Johnson, 2003). It can also change the composition of plant communities (Stephens et al., 1998) to regulate the interactions between herbivores and natural enemies and result in more effective biological control (Bostanian et al., 2004; Spellman et al., 2006; Brown et al., 2008). Insectary plants can increase the populations of natural enemies in orchards, but few studies have documented the movement of natural enemies between insectary plants and trees.

Ecologically based research on biological control requires understanding of the population dynamics of natural enemies and their dispersal into the crops from other habitat patches (Woodcock et al., 2010; Skirvin et al., 2011). Selecting an appropriate technique for marking and tracking is a critical step for investigating the movement of natural enemies. Rubidium (Rb) is an element rarely found naturally, in high concentrations so there is a low background level in all of the plants and soils (Kobelt et al., 2009). Rb is easily absorbed by plants and can be transferred through the food chain to herbivorous pests (primary consumers) and natural enemies (secondary consumers) (Berry et al., 1972; Muratori et al., 2005; Armes et al., 2011). Rb marking is important in tracing insect behaviors, such as feeding, transfer and diffusion, habitat selection and mate competition (Graham et al., 1978; Nowatzki et al., 2003; Mackinnon et al., 2016). For example, Villegas et al. (2013) used Rb to determine whether coccinellid beetles moved between crop edges and within alfalfa. Madeira and Pons (2016) measured the movement of *Calathus fuscipes*, *Poecilus cupreus*, *Bembidion lampros* and *Pseudophonus rufipes* between adjacent alfalfa and maize fields using Rb marking.

The spirea aphid, *Aphis spiraeicola* Patch (Hemiptera: Aphididae), is a major pest of apples (Song et al., 2017; Hullé et al., 2020). These aphids on apple trees generally begin to appear on late-April, and the population peaks of spirea aphids appeared on mid-June in the north part of China (Song et al., 2013). The spirea aphid reduces overall tree vigor by piercing leaves, sucking phloem sap and deforming plant tissues (e.g., shoot twisting and leaf rolling) (Dedryver et al., 2010). It reduces the apple yield by affecting fruit size and shape and causing premature fruit drop (Rimbaud et al., 2015; Rousselin et al., 2017; Santos et al., 2018). Control of spirea aphids mainly depends on chemical pesticides (Simon

et al., 2010). The pesticides used in commercial apple growing have seriously affected the environment and reduced orchard biodiversity (Baumgärtner and Bieri, 2006). Biological control needs to be used as an alternative to insecticides for control of the spirea aphid in apple orchards.

Enhancing natural enemies for management of pests in the apple orchards offers opportunities for biological control and it may reduce, or even eliminate, pesticide use and associated negative side effects (Desneux et al., 2007; Zhou et al., 2014; Cahenzli et al., 2017). Insectary plants are proposed for introduction into apple orchards to maintain and enhance natural enemies. *Cnidium monnieri* (L.) Cuss. is an annual herb (Apiaceae) that has a medicinal value and is distributed in most parts of China. *C. monnieri* usually blooms from May to July and can maintain a number of predatory natural enemies of pest insects (Yang et al., 2018). *C. monnieri* attracts a large number of predatory natural enemies during its flowering period. In addition, there are many celery aphids (*Semiaphis heraclei*) on *C. monnieri*, which are not harmful to apple trees. The celery aphids in *Lonicera macranthoides* begin to appear on early April, and the peak occurrence period in mid- to late-May in Chongqing City, China (Zhang et al., 2012). Therefore, *C. monnieri* has features that are favorable for an insectary plant. However, there is little information describing increased populations of natural enemies and decreased populations of pest insects using insectary plants in apple orchards.

In this study, we tested the possibility that *C. monnieri* could increase the density of natural enemies and improve suppression of the spirea aphid. Experiments in apple orchards with the insectary plant as a treatment and without the insectary plant as a control were conducted in the northern part of China. *C. monnieri* was planted between rows of apple trees to attract the main predators of the spirea aphid. A Rb marking method was used to track movement of the predators between *C. monnieri* and apple trees. The main objectives of this study were to (1) determine species and population abundance of predators and pests on *C. monnieri*, (2) determine population dynamics of spirea aphids and predators on apple trees, (3) verify the shift and change of rubidium in the food chain of the insectary plant – celery aphid – lady beetle, and (4) verify the movement of the predators between apple trees and *C. monnieri*. The answers to these questions may provide a model system for the use of insectary plants in apple orchards to enhance pest control.

2. Materials and methods

2.1. Study area

The study was conducted in an apple orchard (37°43'34.5" N, 120°55'16.7" E) in Yantai, Shandong Province, China. The study region is a major apple producing area in China, with a warm temperate monsoon continental climate, mean annual temperature of 11.7 °C and mean annual rainfall of 664 mm. Apple trees in the orchard were planted at a row spacing of 4 m × 6 m in 2014, and the area was 0.67 ha. The test orchard has been managed using organic farming practices since 2015 and the pest management was dependent on biological and physical control measures with no pesticides.

2.2. Experiment design

The study was conducted from May to July in 2018 and 2019. The experiments were managed on treatment plots with *C. monnieri* and control plots without *C. monnieri*. There were three replicates per treatment (total of six plots), which were arranged in alternate design (Fig. S1). Each plot size was 480 m² (24 m × 20 m) with approximately 12 m (east – west, down row) and 12 m (north – south, across row) buffers. Seeds of *C. monnieri* were sown in treatment areas on 23 September in 2017 and 21 September in 2018 between rows of trees, and final thinning out was done on 10 April in 2018 and 5 April in 2019 at a spacing of 0.3 m × 0.5 m. All of the plots were weeded once a month during the growing season.

2.3. Insect population survey

To assess the population density of aphids and predatory natural enemies, a survey was conducted every 15 days in 2018 and every 10 days in 2019 on each replicate plot in the morning (7:00–11:00 am) during the study from May to July in 2018 and 2019. For the insect survey on apple trees, 12 trees in the middle of each plot were used as survey objects, and an annual branch was selected in the east, south, west, and north directions of each tree for sampling. The population of predators (adults and larvae) at the top 50 cm of each branch, and the population of spirea aphids at the top 10 cm of each branch, including leaves and shoots were counted. For insect surveys on *C. monnieri*, three points in each plot which measured 1 m² (1 m × 1 m) were randomly selected, and we measured the population of aphids and predators (adults and larvae) on the plants. The date of each survey, field plot number, tree number and branch number were recorded, and the number of aphids and predators (adults and larvae) on each surveyed date was recorded to estimate their abundance and diversity.

2.4. Design of rubidium marking experiment and sampling

Larvae and adults of *Harmonia axyridis* were collected from farmlands and orchards near the experimental fields. They were placed in mesh cages (25 cm × 25 cm × 30 cm) in the laboratory and held at approximately 25 °C, 60% relative humidity (RH) and a 14:10 h L:D photoperiod. *H. axyridis* colonies were fed with pea aphids

(*Acyrtosiphon pisum* Harris), reared on broad bean (*Vicia faba* L.) seed in the laboratory. For all of the marking trials 4–10 days adults were used. The celery aphids were collected from the insectary plants of *C. monnieri* and the colonies were fed with *C. monnieri* plants in the laboratory.

Three *C. monnieri* plants were planted in a pot (25 cm diam. × 16.5 cm high) for each replicate. Each pot was covered with a zippered gauze cage (65 cm × 65 cm × 120 cm) in the laboratory and held at approximately 25 °C, 60% relative humidity (RH) and a 14:10-h L:D photoperiod. When the plants were approximately 30 cm tall, 300 celery aphids (2- or 3-instar) were placed on each plant. When the plants reached the flowering stage, three experiments were conducted: (1) A spraying treatment in which 200 ml of 2000 µg/ml rubidium chloride (RbCl) solution was evenly sprayed on each plant, (2) An irrigation treatment in which 200 ml of 2000 µg/ml RbCl solution was irrigated to each plant, and (3) a control in which 200 ml of water was evenly sprayed on each plant. Each treatment had five replicates. Two hours after application of treatment, 30 adults of *H. axyridis* which had been starved for 48 h were placed into each gauze cage. After 48 h, *H. axyridis* adults were removed from the plants in the gauze cages, placed in the insect boxes and fed with unmarked celery aphids.

Plant samples were taken at 0.5 (12 h), 3, 6, 9, 12 and 15 days after spraying and irrigation with the RbCl solution. Three leaves and three flowers of the plants were randomly selected from each pot, placed separately in envelopes and then oven dried at 60 °C for 12 h. The plant samples were preserved in a Ziplock bag. The 150 celery aphids of uniform size were randomly selected from each gauze cage, placed in a

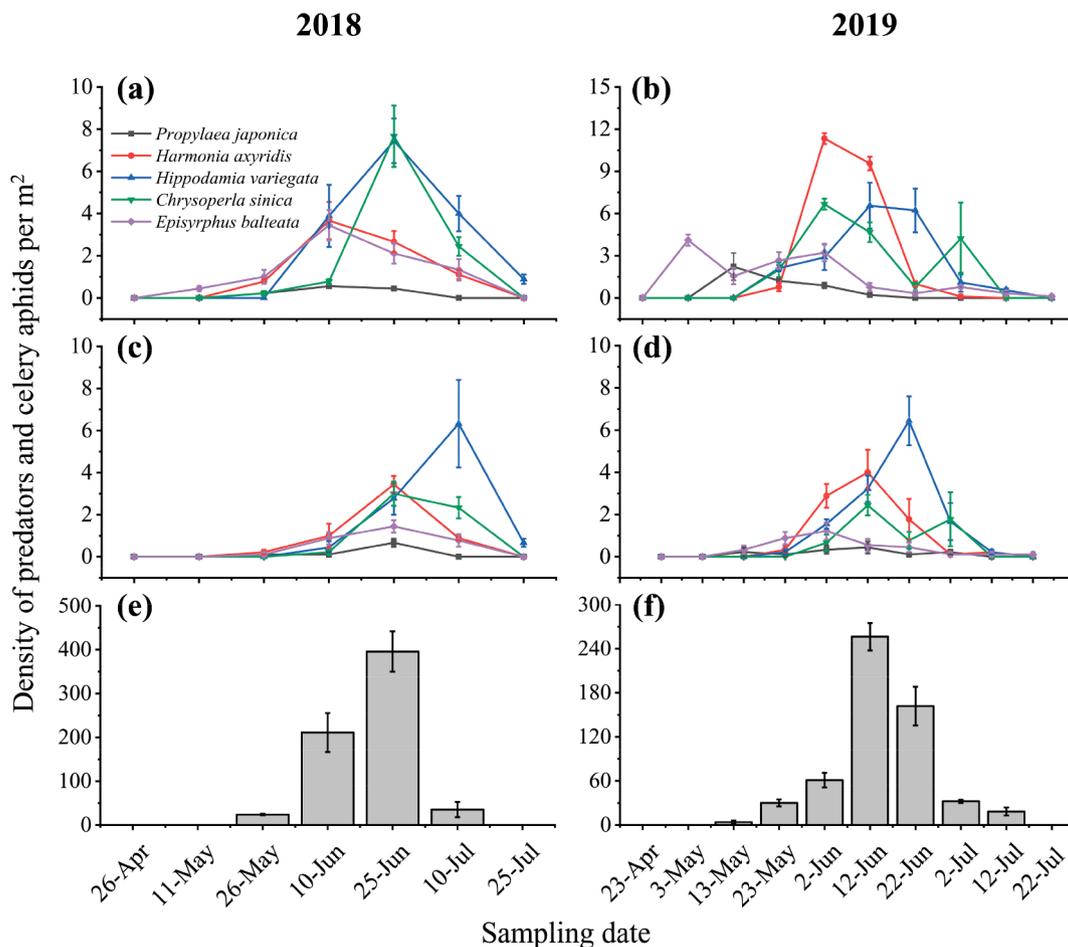


Fig. 1. The dynamics of predatory natural enemies and celery aphids on the insectary plants *C. monnieri*. Density of adult predators on the insectary plants in 2018 (a) and 2019 (b). Density of larva predators on the insectary plants in 2018 (c) and 2019 (d). Density of celery aphids on the insectary plants in 2018 (e) and 2019 (f). Predatory natural enemies include *Propylaea japonica*, *Harmonia axyridis*, *Hippodamia variegata*, *Chrysoperla sinica*, and *Episyrphus balteata*. Error bars indicate the standard error (SE).

Table 1

Results for the repeated measures ANOVA regarding the effects of treatment, time and treatment \times time interaction on the population density of spirea aphids.

Year	Effect	F	df	P
2018	Treatment	10.106	1, 4	0.034
	Time	76.307	6, 24	<0.001
	Treatment \times Time	7.576	6, 24	<0.001
2019	Treatment	24.982	1, 4	0.007
	Time	273.876	9, 36	<0.001
	Treatment \times Time	22.775	9, 36	<0.001

1.5 ml centrifuge tube in groups of 50, and then stored in a freezer at $-20\text{ }^{\circ}\text{C}$. Three adults of *H. axyridis* were randomly selected from each gauze cage at 0.5 days and also randomly selected from the insect boxes at 3, 6, 9, 12 and 15 days. They were singly placed in a 1.5 ml centrifuge tube and stored in a freezer at $-20\text{ }^{\circ}\text{C}$.

2.5. Field recording transfer of predators

Field sampling of the Rb marking experiment was conducted in June 2018 and June 2019. Adults of *H. axyridis* and *C. sinica* were sampled from insectary plants and apple trees in the treatment plots before the experiment began to determine the background Rb content of the predators. About 20 adults of *H. axyridis* and 20 adults of *C. sinica* on plants and trees were collected for each plot in 12 June 2018 and 7 June 2019, respectively. When the plants of *C. monnieri* were in the flowering stage, 2000 $\mu\text{g/ml}$ RbCl solution was sprayed evenly on the plants with a sprayer in the treatment in 13 June 2018 and 8 June 2019. Samples were taken three days after spraying. About 20 adults of *H. axyridis* and 20 adults of *C. sinica* on plants and trees were collected for each plot in 16 June 2018 and 11 June 2019, respectively. They were individually placed into 1.5 ml centrifuge tubes and stored in a freezer at $-20\text{ }^{\circ}\text{C}$. This experiment had three replicates.

2.6. Rubidium detection process

The Rb detection methods used were modified from previous studies (Kobelt et al., 2009; Villegas et al., 2013). For this experiment, 0.01 g of dried leaves or flowers were weighed and then placed in a mortar. The celery aphids, *H. axyridis* adults, and *C. sinica* adults were placed in a 20 ml crucible and carbonized on an alcohol burner. The leaves, flowers and charred insects were placed in a digestion tank. 4 ml of 65% nitric acid (HNO_3) and 0.5 ml of 30% hydrogen peroxide (H_2O_2) were added and the mixture was placed in a microwave digestion apparatus (MDS-6G, Shanghai, China) for digestion. The digestion temperature and time

were set to $130\text{ }^{\circ}\text{C}$ for 10 min, $150\text{ }^{\circ}\text{C}$ for 5 min and $180\text{ }^{\circ}\text{C}$ for 15 min. When the temperature in the tank had cooled down to room temperature, the digestion tank was removed and opened. The liquid in the tank was then dispensed into to a 5 ml volumetric flask and diluted to 5 ml using deionized water. The Rb content was determined by an atomic absorption spectrophotometer (TAS-990, Beijing, China). A RbCl standard of 1000 $\mu\text{g/ml}$ was diluted with deionized water drops to prepare standard solutions of 1, 2, 3 and 4 $\mu\text{g/ml}$. A blank sample (deionized water) was also prepared. Then the standard curve was made by preparing the standard samples of different concentrations. Disposable filters were used to transfer the processed samples from volumetric flasks to 10 ml vials successively, and capillary tubes were used to prepare the blank samples and the test samples successively. After the data curve of samples were stabilized, the measurement was started. The readings on each sample were repeated three times.

2.7. Statistical analysis

All of the descriptive statistics (means and standard errors) and tests of differences were conducted using SPSS software (SPSS 22.0). Origin software (Origin 2018) was used to construct the graphs. In the field experiments, the mean number of predatory natural enemies and celery aphids per square meter at different sampling dates on *C. monnieri*, the mean number of spirea aphids per 10 cm branch and predators per 50 cm branch at different sampling dates on apple trees were determined. Each mean was calculated by averaging the number of predators and aphids on *C. monnieri* and the apple trees from each replicate. We determined the effects of the two treatments (with and without insectary plants) on the densities of spirea aphids and predators. Differences in the densities of spirea aphid and predator on apple trees between treatment and control plots were analyzed with an ANOVA, and the effects of group (treatment and control), sampling date (time), and their interaction (group \times time) on the densities of spirea aphid and predator were tested with repeated measures. In the Rb marking experiments, Rb content was the average value of flowers, leaves, aphids, and beetles at different sampling dates. The differences in the Rb content of leaves, flowers, celery aphids, and lady beetles between spray treatment and irrigation treatment were analyzed on the sampling dates with independent-sample T test.

3. Results

3.1. Species and abundance of predators and celery aphids on insectary plants

During the field surveys, five species of predators were associated

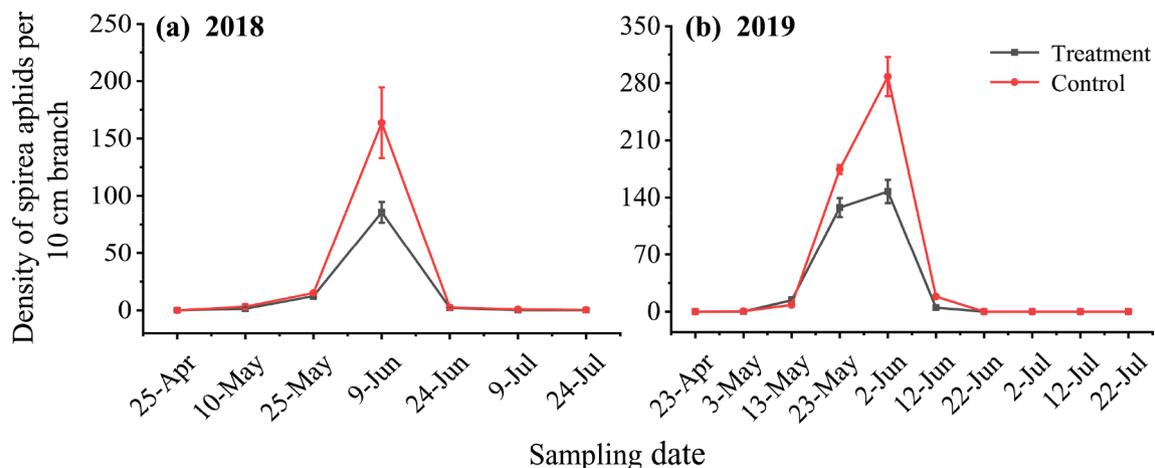


Fig. 2. The dynamics spirea aphids on the apple trees. Density of spirea aphids on the apple trees in 2018 (a) and 2019 (b). Error bars indicate the standard error (SE). Treatment: the insectary plants *C. monnieri* was planted in apple orchard. Control: without *C. monnieri* in apple orchard.

with the insectary plants: *Propylaea japonica*, *Harmonia axyridis*, *Hippodamia variegata*, *Chrysoperla sinica*, and *Episyrphus balteata*. A total of 631 predators (403 adults and 228 larvae) in 2018 and 1013 predators (711 adults and 302 larvae) in 2019 were collected from *C. monnieri*. Of these predator groups, adults of *H. variegata* was the most abundant (22.8%), followed by adults of *C. sinica* (15.7%) and larvae of *H. variegata* (14.4%) in 2018; adults of *H. axyridis* was the most abundant (20.2%), followed by adults of *H. variegata* (17.3%) and *C. sinica* (16.3%) in 2019. Adults of *E. balteata* appeared first on *C. monnieri*, the peak occurrence period of adults of *P. japonica*, *H. axyridis*, and *E. balteata* were 10 June, and adults of *H. variegata* and *C. sinica* were 25 June in 2018 (Fig. 1a); the peak occurrence period of adults of *E. balteata* was 3 May, adults of *P. japonica* was 13 May, adults of *H. axyridis*, and *C. sinica* were 2 June, and adults of *H. variegata* was 12 June in 2019 (Fig. 1b). The peak occurrence period of larvae of *P. japonica*, *H. axyridis*, *C. sinica*, and *E. balteata* were 25 June, and *H. variegata* was 10 July in 2018 (Fig. 1c); the peak occurrence period of larvae of *E. balteata* was 2 June, larvae of *P. japonica*, *H. axyridis*, and *C. sinica* were 12 June, and larvae of *E. balteata* was 22 June in 2019 (Fig. 1d). These predators maintained high abundance on *C. monnieri* during its flowering period (from May to July) and the peak occurrence period of most predators was during June. Celery aphid densities increased over time on *C. monnieri* before 25 June in 2018 and 13 June in 2019, and then gradually decreased (Fig. 1e, f).

3.2. Population dynamics of spirea aphids on apple trees

Spirea aphid densities in the treatment areas throughout the sampling period (from late-May to late-July) were significantly lower than in the control areas in 2018 and 2019 (Table 1). Spirea aphid densities increased over time in the treatment and control areas before 9 June 2018 and 2 June 2019, and then gradually decreased (Fig. 2). The time had a significant effect on the density of spirea aphid (Table 1), and the group and time also had an interaction effect (Table 1).

3.3. Population dynamics of predators on apple trees

During the field surveys, three species of predators were associated with the apple trees: *H. axyridis*, *C. sinica*, and *E. balteata*. The adults and larvae of *H. axyridis*, *C. sinica*, and *E. balteata* densities throughout the sampling period in the treatment areas were significantly higher than the control, the time had significant effect on the density of all predators (adults and larvae), and the group and time also had interaction effect (Table 2). All predator densities increased over time in the treatment and control areas, and then gradually decreased (Fig. 3). The peak occurrence period of adults and larvae of *H. axyridis* in the treatment and control areas were 9 June in 2018 (Fig. 3a, c). The peak occurrence period of adults and larvae of *C. sinica* in the treatment areas were 24 June, and the control areas were 9 June in 2018 (Fig. 3e, g). The peak occurrence period of adults and larvae of *E. balteata* in the treatment areas and the larvae of *E. balteata* in the control areas were 9 June, and the adults of *E. balteata* in the control areas were 25 May in 2018 (Fig. 3i, k). The peak occurrence period of adults of *H. axyridis* in the treatment and control areas were 2 June and 12 June in 2019 (Fig. 3b, d). The peak occurrence period of adults and larvae of *C. sinica* in the treatment and control areas were 12 June in 2019 (Fig. 3f, h). The peak occurrence period of adults and larvae of *E. balteata* in the treatment areas were 23 May, and the control areas were 2 June in 2019 (Fig. 3j, l).

3.4. Shift and change of rubidium to the food chain of insectary plant – celery aphid – lady beetle

Rb was detected in the leaves, flowers, celery aphids, and lady beetles in the spray and irrigation treatments after 0.5 days, but not in the control (Fig. 4a, b). Therefore, both the spray and irrigation treatments

transferred Rb in the food chain consisting of insectary plant - celery aphid - lady beetle. The Rb content of leaves and flowers decreased with time. It slowly decreased after 9 days in the spray treatment. The Rb content of leaves and flowers initially increased with time, and then decreased slowly after 9 days in the irrigation treatment. No Rb was detected in the control (Fig. 5a, b). The Rb content of leaves in the spray treatment was significantly higher than the irrigation treatment before

Table 2
Results for the repeated measures ANOVA regarding the effects of treatment, time and treatment × time interaction on the population density of predators.

Species	Stage	Year	Effect	F	df	P
<i>Harmonia axyridis</i>	Adult	2018	Treatment	8.790	1, 4	0.041
			Time	12.686	6, 24	<0.001
			Treatment × Time	3.379	6, 24	0.015
		2019	Treatment	22.459	1, 4	0.009
			Time	29.101	9, 36	<0.001
			Treatment × Time	6.209	9, 36	<0.001
	Larva	2018	Treatment	12.048	1, 4	0.026
			Time	60.296	6, 24	<0.001
			Treatment × Time	5.179	6, 24	0.002
		2019	Treatment	74.655	1, 4	0.001
			Time	203.543	9, 36	<0.001
			Treatment × Time	46.121	9, 36	<0.001
<i>Chrysoperla sinica</i>	Adult	2018	Treatment	72.982	1, 4	0.001
			Time	24.965	6, 24	<0.001
			Treatment × Time	15.685	6, 24	<0.001
		2019	Treatment	8.610	1, 4	0.043
			Time	16.115	9, 36	<0.001
			Treatment × Time	4.493	9, 36	0.001
	Larva	2018	Treatment	17.646	1, 4	0.014
			Time	8.486	6, 24	<0.001
			Treatment × Time	7.614	6, 24	<0.001
		2019	Treatment	13.043	1, 4	0.023
			Time	16.971	9, 36	<0.001
			Treatment × Time	4.394	9, 36	0.001
<i>Episyrphus balteata</i>	Adult	2018	Treatment	9.290	1, 4	0.038
			Time	15.561	6, 24	<0.001
			Treatment × Time	4.658	6, 24	0.003
		2019	Treatment	28.538	1, 4	0.006
			Time	18.480	9, 36	<0.001
			Treatment × Time	2.589	9, 36	0.021
	Larva	2018	Treatment	7.784	1, 4	0.049
			Time	21.837	6, 24	<0.001
			Treatment × Time	5.655	6, 24	0.001
		2019	Treatment	76.003	1, 4	0.001
			Time	19.918	9, 36	<0.001
			Treatment × Time	2.963	9, 36	0.010

6 days but there was no significant difference after 6 days (Fig. 5a). The Rb content of flowers in the spray treatment was significantly higher than in the irrigation treatment before 3 days, but there was no significant difference after 3 days (Fig. 5b). The Rb content of celery aphids decreased with time in the spray treatment. The Rb content of celery aphids initially increased with time, and then decreased slowly after 3 days in the irrigation treatment; no Rb was detected in the control (Fig. 5c). The Rb content of celery aphids in the spray treatment was significantly higher than in the irrigation treatment before 6 days, and there was no significant difference after 6 days (Fig. 5c). The Rb content was not similar between two treatments but its concentration over time changed is similar pattern. The Rb initially increased with time, and then decreased after 3 days. No Rb was detected in the control (Fig. 5d). The Rb content of lady beetles in the spray treatment was significantly higher than in the irrigation treatment (Fig. 5d). In general, the Rb content of the spray treatment was higher than the irrigation treatment.

In particular, the Rb content of lady beetles were highest in the spray treatment after 3 days (Fig. 5d).

3.5. Movement of predators between apple trees and insectary plants

Rb was not detected, in 2018 or 2019, in *H. axyridis* and *C. sinica* adults before field marking. This indicated that Rb was not present in these areas. After marking, the Rb detection rates of *H. axyridis* and *C. sinica* on the insectary plant *C. monnieri* were $56.8\% \pm 4.8\%$ ($n = 21, 17, 25$) and $55.2\% \pm 3.2\%$ ($n = 18, 26, 22$); and the Rb levels of *H. axyridis* and *C. sinica* on apple trees were $24.2\% \pm 5.4\%$ ($n = 19, 22, 22$) and $53.2\% \pm 5.8\%$ ($n = 20, 19, 24$) in 2018 (Fig. 6a, b). The Rb detection rates of *H. axyridis* and *C. sinica* on *C. monnieri* were $63.9\% \pm 3.9\%$ ($n = 16, 16, 21$) and $61.0\% \pm 3.2\%$ ($n = 23, 27, 24$), and that of *H. axyridis* and *C. sinica* on apple trees were $42.7\% \pm 2.4\%$ ($n = 16, 21, 13$) and $48.4\% \pm 2.1\%$ ($n = 25, 23, 20$) in 2019 (Fig. 6a, b).

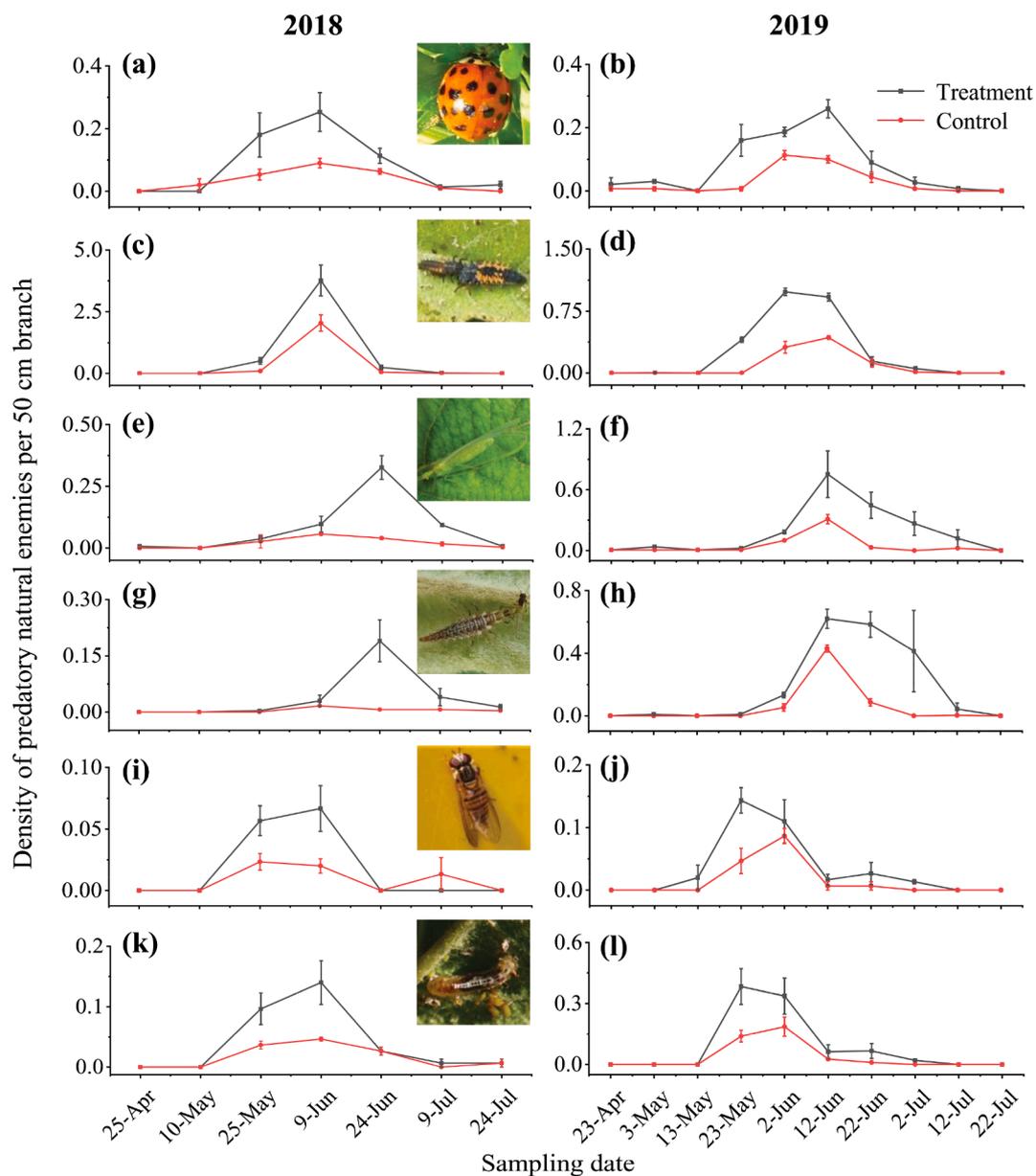


Fig. 3. The dynamics predatory natural enemies on the apple trees. Density of adult *H. axyridis* on the apple trees in 2018 (a) and 2019 (b); density of larva *H. axyridis* on the apple trees in 2018 (c) and 2019 (d); density of adult *C. sinica* on the apple trees in 2018 (e) and 2019 (f); density of larva *C. sinica* on the apple trees in 2018 (g) and 2019 (h); density of adult *E. balteata* on the apple trees in 2018 (i) and 2019 (j); density of larva *E. balteata* on the apple trees in 2018 (k) and 2019 (l). Error bars indicate the standard error (SE). Treatment: the insectary plants *C. monnieri* was planted in apple orchard. Control: without *C. monnieri* in apple orchard.

4. Discussion

Using a combination of field investigation and marker technology, we confirmed the beneficial effects of the insectary plant *C. monnieri* for sustaining predatory natural enemies. We quantitatively evaluated movement of the main predators from *C. monnieri* to apple trees and demonstrated that planting *C. monnieri* in apple orchards resulted in the decreased abundance of spirea aphids. These data support the use of *C. monnieri* for the conservation of insect predators and the biological control of spirea aphids on apples.

4.1. Ability of insectary plants to sustain predatory natural enemies

Appropriate insectary plants should be screened for their flowering periods and attractiveness to natural enemies and pests (Hogg et al., 2011). Insectary plant strips can supply pollen, nectar, and alternative prey as food resources for the natural enemies (Amaral et al., 2013). Addition of insectary plants to agroecosystems could bolster natural enemy populations and improve the biological control of agricultural pests (Gontijo et al., 2013). In this study, the main aphid predators (Coccinellidae, Chrysopidae, and Syrphidae) gathered on the insectary plant *C. monnieri*. These predators were few in the pre-growth period of *C. monnieri*, but the numbers of these predators increased during its flowering period. From the age composition of the predators, five species of predators had more adults than larvae, and the adults of predators appear earlier than the larvae. This demonstrates that insectary plants *C. monnieri* could attract predators, and predators could lay eggs and reproduce on insectary plants. A previous study showed that *C. monnieri*, during flowering, could release the volatiles 1,2-diethylbenzene and p-diethylbenzene to attract *H. axyridis* (Cai et al., 2020). The predators could locate the host by searching for volatiles of *C. monnieri* and feed on pollen and nectar from *C. monnieri*. Many predators feed on floral resources, and this improves their nutritional intake, longevity, activity, and fertility (Gurr and Nicol, 2000; Patt et al., 2003; Venzon et al., 2006; Gurr et al., 2017). In this study, the flowering period of *C. monnieri* is from May to July, when there are large numbers of predators and some celery aphids on *C. monnieri*. The celery aphids do not harm apple trees, and they provide an alternative prey for predators on *C. monnieri*. This is beneficial to the development and reproduction of the predators. The celery aphids occurred at the same time as *C. monnieri* flowers, which provided the predators with pollen, nectar, and alternative prey. They can be sown in spring, summer, and fall, and their easy emergence and rapid growth habits of *C. monnieri*, match well with local apple growers' desire for an insectary plant. *C. monnieri* appears to be a useful insectary plant in apple orchards for CBC.

4.2. Biological control of spirea aphids on apple trees by predators on insectary plants

Orchards are perennial crop systems that have a space for establishing and maintaining functional biodiversity that aids in pest control (Simon et al., 2010). Intercropping with insectary plants in orchards is one of the approaches to enhance biodiversity and promote natural enemies to control pests. The insectary plant, *C. monnieri*, was planted between rows of an apple orchard in northern China and used to increase the population densities of predatory natural enemies for biological control of spirea aphids on the apple trees. Our results showed that *C. monnieri* significantly increased predator population densities and significantly decreased the population of spirea aphids on the apples. Similarly, planting sweet alyssum in the apple orchard can attract syrphid flies and suppress the woolly apple aphid on the apple trees (Gontijo et al., 2013). In our studies, the adult and larval of predator populations in the treatment plots were higher than those in the control plots. This indicates that planting insectary plants in orchard can not only increase the population of adults, but also increase the population of larvae, and these predators were able to effectively control spirea

aphids. Coccinellidae, Chrysopidae, and Syrphidae have many generalist predators with high population control potential for aphids and other small arthropods (Stewart et al., 2002; Brown, 2011; Gontijo et al., 2015). However, their capacity to control aphids is often considered to be limited (Latham and Mills, 2010). Aphids often cannot be controlled by predators because the numerical response of predators is not sufficiently rapid to overcome aphid population growth (Brown, 2011). Therefore, the early seasonal arrival of predators is an important prerequisite for control of aphid populations (Harwood et al., 2007; Brown, 2011). In our study areas, the adults of predator appeared on *C. monnieri* in early-May, and these predators had a relatively high population density during the flowering period of the insectary plants. The peak occurrence period of spirea aphids on apple trees was from late-May to early-June, when many predators (adults and larvae) had gathered on insectary plants. Some adults of predator then moved from *C. monnieri* to the apple trees, they could lay eggs and reproduce on the apple trees, which significantly increased the population of predators (adults and larvae) on the apple trees. Therefore, the predators that arrived early on *C. monnieri* would then move to the apple trees to control the spirea aphids. Although our results are encouraging, there are some limitations

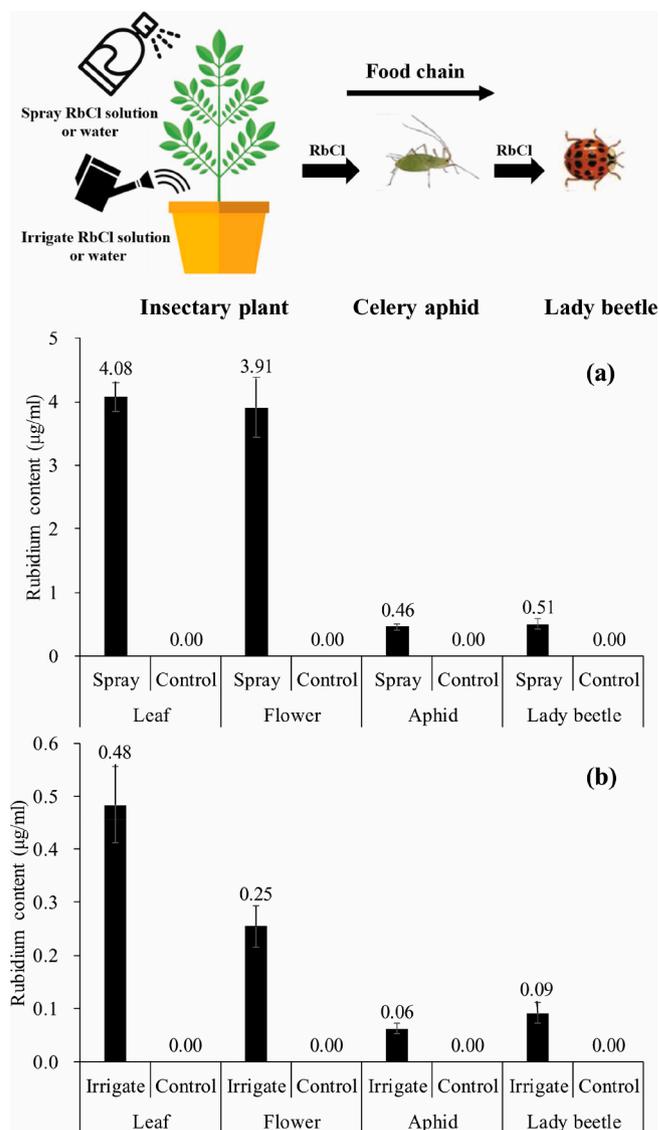


Fig. 4. The transfer of rubidium in food chain after 0.5 days. The content of rubidium in the leaf and flower of *C. monnieri*, celery aphid, and lady beetle *H. axyridis* in spray treatment (a) and irrigation treatment (b). Error bars indicate the standard error (SE).

to our study. The field investigation covered less than three months (from late April to late July), although these times of the year when spirea aphid populations are usually highest. Thus, it remains unclear whether *C. monnieri* plantings could encourage a long season improvement in spirea aphid suppression.

4.3. Shift and change of rubidium to the food chain

The Rb marking method is a simple and effective method for insect marking, and it has been used to track insect migration and dispersal (Mackinnon et al., 2016; Madeira and Pons, 2016). Rb is an element which has chemical properties similar to potassium, and evenly distributed across the earth's surface and rarely found in high concentrations, so there is a low background level in the environment (Kobelt et al., 2009). Before to our laboratory experiment, Rb content of insectary plants and lady beetles in the background were determined, and the result were not detected. Rb can enter plants via leaf or root absorption but most soils will bind Rb so it is preferable to use foliar application, and the small amount of Rb does not have a significant effect on the insect's biology or behavior (Kobelt et al., 2009). In our Rb marking experiment, RbCl solution spraying or irrigating on *C. monnieri*, the Rb can be transferred by feeding relationship, so that *C. monnieri* can be marked rubidium, herbivorous pests can be marked by feeding plants, and predators can also obtain mark by preying on pests or feeding on pollen and nectar. Rb content of lady beetles in the spray treatment was significantly higher than in the irrigation treatment, and therefore, we chose spray treatment in field trials.

4.4. Movement of predators from insectary plants to apple trees

The movement of insects in agricultural ecosystems is associated with their abundance and distribution in time and space (Mazzi and Dorn, 2012). In CBC of pests in crop fields using insectary plants, the natural enemies can readily move back and forth between the insectary plants and crops, and do not aggregate and remain on the insectary plants (Gurr et al., 2017). Most studies on the movement of natural enemies are conducted by investigating their population changes in the field. This qualitatively describes the movement of natural enemies, but cannot quantitatively evaluate their movements. A few studies have used marking methods to quantitatively evaluate the movement of natural enemies. For example, Ouyang et al. (2012) used a stable carbon isotope method to quantitatively evaluate the movement of predators *P. japonica* between cotton and maize fields. In our study, both the insectary plant *C. monnieri* and apple tree were C_3 plants, and predators movements could not be tracked using a stable carbon isotope. Gontijo et al. (2013) used egg-white protein as a marker to document movement of natural enemies between sweet alyssum and apple trees, but they only tracked the movement of syrphids. In this study, we used rubidium marking to track the movement of major predators between insectary plants and apple trees. On apple trees the predators were mainly *H. axyridis* (adults and larvae), *C. sinica* (adults and larvae), and *E. balteata* (larvae). Adults of *E. balteata* were rarely found on the apple trees because they are flower-visiting insects. Therefore, we sampled adults of *H. axyridis* and *C. sinica* in the field and measured the Rb content. Our results confirmed that some predators on the trees had moved from *C. monnieri*. This quantitatively evaluated the movement of

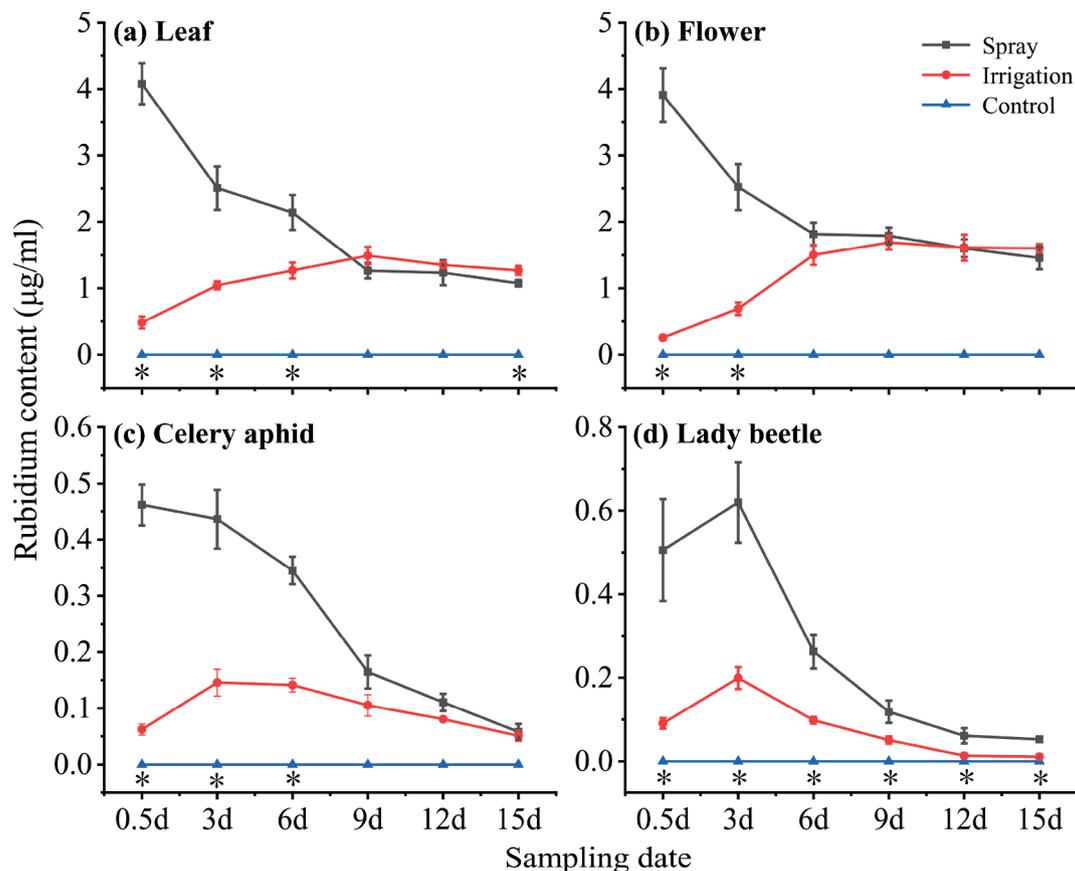


Fig. 5. The change of rubidium in food chain. The content of rubidium in the leaf (a) and flower (b) of *C. monnieri*, celery aphid (c) and lady beetle *H. axyridis* (d) at different sampling dates in spray treatment, irrigation treatment and control. Error bars indicate the standard error (SE). *Significant differences between rubidium content of spray and irrigation treatment at $P < 0.05$.

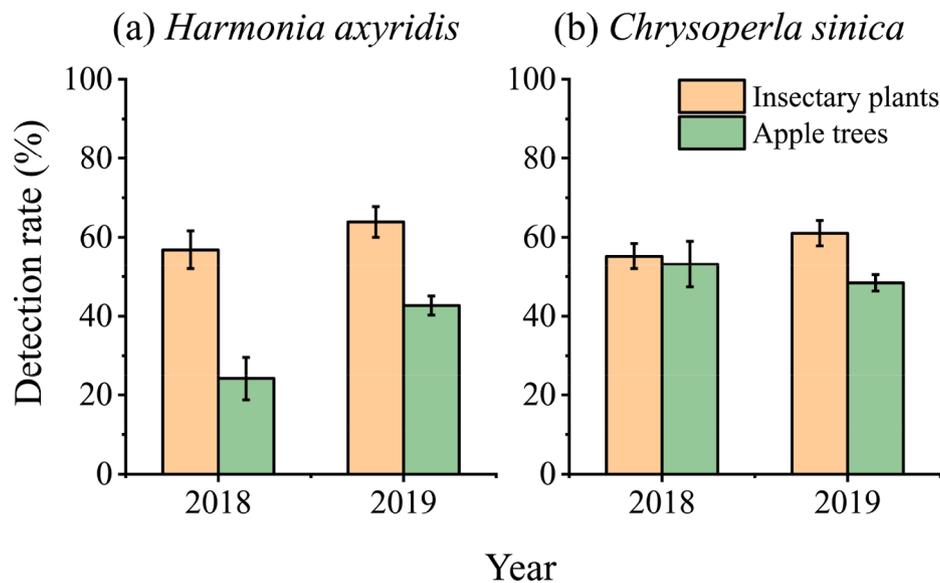


Fig. 6. The movement of predators from insectary plants to apple trees. The detection rate of *H. axyridis* (a) and *C. sinica* (b). Error bars indicate the standard error (SE).

predators *H. axyridis* and *C. sinica* and provided useful information for conservation biological control of pests using insectary plants in orchards. Even so, in our field trials, we measured the movement of predators only on trees in the insectary plant plots, and not throughout the whole orchard. Even so, the insectary plant *C. monnieri* may have benefits at scales greater than we examined, although this remains to be tested, future work should investigate the movement distance of predators between insectary plants and apple trees.

5. Conclusions

The insectary plant *C. monnieri* can attract predatory natural enemies, such as *P. japonica*, *H. axyridis*, *H. variegata*, *C. sinica*, and *E. balteata*, especially during its flowering period. It provides food resources, such as pollen, nectar, and alternative prey, for these natural enemies. *C. monnieri* was planted in an apple orchard, and it attracted natural enemies that provided biological control of spirea aphids on the apple trees. We found that the density of predators on apple trees in treatment areas intercropped with *C. monnieri* was significantly higher than in control areas, while the density of spirea aphids on apple trees in treatment areas was significantly reduced compared with control areas. Rb marking analysis showed that predators moved from *C. monnieri* to apple trees to feed on spirea aphids. Planting insectary plants, such as *C. monnieri*, can increase the diversity and populations of predatory natural enemies in orchard ecosystems. The predators from insectary plants can move to the apple trees for the biological control of aphid pests. These results suggest that intercropping the insectary plant *C. monnieri* is an environmentally friendly biological control measure that can be used to suppress the spirea aphid in apple orchards and reduce the use of chemical pesticides. In future studies, additional species of insectary plants and natural enemies should be studied for the biological control of pests of apple and pests of other orchard trees.

CRedit authorship contribution statement

Zhiping Cai: Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing - original draft. **Fang Ouyang:** Data curation, Formal analysis, Supervision, Writing - original draft. **Jing Chen:** Data curation, Formal analysis. **Quanfeng Yang:** Investigation. **Nicolas Desneux:** Conceptualization, Methodology. **Yunli Xiao:** Conceptualization, Project administration, Supervision,

Writing - review & editing. **Jianping Zhang:** Conceptualization, Methodology, Supervision, Writing - review & editing. **Feng Ge:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing - review & editing.

Acknowledgements

We thank all undergraduate internship students from Shandong Agricultural University and Hunan University of Humanities, Science and Technology for their help in the field. This project was supported by the National Key Research and Development Plan of China (2017YFD0200400), and the State Key Laboratory of Integrated Management of Pest Insects and Rodents (Grant No. ChineseIPM1805). We thank LetPub (www.letpub.com) for its linguistic assistance during the preparation of this manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.biocontrol.2021.104532>.

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