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Harmonia axyridis (Boyer de Fonscolombe) (Hemiptera: Coccoidea) as a potential biological control agent of the invasive soft scale, *Sphaerolecanium prunastri* (Boyer de Fonscolombe) (Hemiptera: Coccoidea) in native wild apricot forests

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Abstract

Background The globose scale (GS), *Sphaerolecanium prunastri* (Boyer de Fonscolombe) (Hemiptera: Coccoidea), has invaded wild apricot forests in their native range in Central Eurasia threatening the ancestral germplasm resource. Biological control efficacy of the harlequin ladybird, *Harmonia axyridis* (Pallas, 1773) (Coleoptera: Coccinellidae) against the globose scale was assessed in laboratory and field experiments.

Results In the laboratory, *Harmonia axyridis* has a high feeding capacity on GS with numbers consumed daily increasing with temperature (15, 20, 25, 30, 35 °C), reaching an upper asymptote of 160–200 scales per day. In field cage experiments, efficacy of biological control (EBC) against first instar (49–99%) and second instar nymphs (20–80%) increased with GS density. When ants were present, control efficiency was reduced by 10–15%. In open-field experiments without cages, EBC was comparatively lower regardless of duration and how *H. axyridis* were released whether as adults, eggs cards or a mixture of adults and eggs cards.

Conclusions In the long term, biological control with this ladybird predator could be considered as part of an IPM program package that includes banning or delaying mowing grass and understory plants in the forests that offer pollen and nectar for natural enemies.

Keywords Soft scale, *Sphaerolecanium prunastri*, *Harmonia axyridis*, Ant attendance, Biological control, Efficacy

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Background

Invasive species are a major cause of crop loss and deforestation, adversely affecting food security, biodiversity and ecological resilience (Early et al. 2016). In China alone annual economic losses due to alien invasive species is more than USD \$18.9 billion (Wan and Yang 2016) and a minimum of US\$70.0 billion per year globally (Bradshaw et al. 2016). This negative effect of invasive species is increasing with global trade and international tourism, climatic change, and changing agricultural practices (Lu et al. 2022).

One of the dominant pathways for invasions is related to the horticulture industry and nursery trade, with more than one third of species associated with these plants introduced outside of their original geographical ranges (Hulme et al. 2018). Various scale insects have been widely dispersed and threaten woody plants such as fruit and ornamental trees in orchards and cities, as well as in forests and conservation parks (Dilley et al. 2020).

How to mitigate the widespread dispersal and damage caused by invasive species presents challenges for governments, scientists, entrepreneurs, and the public. Biological control, the use of parasitoids and predators, is a common approach to manage scale pests in various agricultural systems (Wyckhuys et al. 2018). The efficacy of biological control (EBC) is influenced by multiple factors including quality and abundance of scale insect pest, the searching capacity and rate of food consumption by natural enemies, the specific species involved, interference due to ants attending scales and weather (Abd-Rabou 2011). Predatory coccinellids are effective bio-control agents for curbing scale insect pests (Symondson et al. 2002). Classic success stories include the release of *Hyperaspis pantherina* Fürsch against ensign scale (*Orthezia insignis* Browne) in many parts of the world (Booth et al. 1995). Coccidophagous ladybirds are more likely to be effective against scales than aphidophagous ladybirds (Sloggett 2021). In practice less work has been undertaken on coccidophagous ladybird beetles against scale insect pests particularly in forest systems (Camacho et al. 2018).

The globose scale (GS), *Sphaerolecanium prunas-tri* (Boyer de Fonscolombe) (Hemiptera: Coccoidea), is principally an insect pest of *Prunus*, but can be found on other stone fruit trees throughout the Holarctic region (Wang et al. 2021). This insect has invaded and established in the whole area where wild apricot, *Prunus armeniaca* is native and grows in forests in the mountain ecosystem in Xinjiang, China since 2017, and poses a threat to the key germplasm resource of these ancestors of domesticated apricots (Wang et al. 2021). GS is univoltine and overwinters as second instars (Wang et al. 2021). The critical damage window is from May or Mid-June,

and GS mostly infest 1–3-year-old branches leading to shorter deformed branches, crown loss and a decline of photosynthesis eventually killing trees if left unmanaged (Wang et al. 2021). Suppression of GS in remote mountain areas is challenging as one is trading off environmental security against the large costs of management. Generally, for sustainable management of pests, biological control is considered a priority in remote mountain forests where it is hard to conduct complex tactics such as pruning and targeted spraying of insecticides.

Harmonia axyridis (Pallas, 1773) (Coleoptera: Coccinellidae), occurs naturally in Xinjiang, feeding on GS in the field on occasions; however, its effectiveness was unknown. A series of experiments in the laboratory and field (2019–2021) were conducted to answer the following questions: how effectively does *H. axyridis* feed on different stages of *S. prunas-tri*? As temperatures can be limiting in high-latitude, high altitude areas we investigated how temperature influences the efficacy of biological control (EBC). These two questions were addressed in the laboratory and can somewhat give an indication of predation potential performance in field. Thus, we undertook both cage and absence cage in open-field experiments, the latter using releases of the predator to assess the control efficacy against GS in the apricot forest.

Methods

In all experiments, adult ladybird beetles and eggs were purchased from Beijing KUOYE Company (Beijing, China). Experiments were conducted at the field experimental station (82° 48' 12" N, 43° 12' 30" E, Gongliu country, Xinjiang, China) located in the wild apricot forest.

Experiment 1: feeding capacity of adult ladybird beetle against different instars of globose scale in the laboratory

Climate chambers (Hengchuang, Guangzhou, PYX-300Q-C) were used to understand the effect of temperature on feeding capacity of the ladybird beetles during 2019. Temperature regimes as 15, 20, 25, 30, 35 °C were set and one outdoor treatment (average temperature 25 °C, range 18–35 °C). In mid-June, the 1st instar nymphs hatched and attached on the tree bark of younger 1–3-year-old branches. Apricot branch terminals (50 cm in length), infested by with large numbers of first instars nymph of GS, were obtained from the field. The numbers of 1st instar nymphs on 5-cm sections of branches were counted and excess nymphs removed to establish starting densities of 100, 200, 300, 400, and 500 individuals per section. Each branch section was placed into a plastic cup (200 ml), and then one adult female of *H. axyridis* was released and covered with gauze. For each temperature condition, six replications of each density were applied.

After 24 h, the remaining GS were counted to assess the number eaten.

To understand the feeding capacity on different stages of GS, a similar experiment at 25 °C as above was conducted with first or second instar of GS nymphs at 100, 200, 300, 400 and 500 individuals per section of branchlet in a non-choice experiment. Branches with nymphs at the appropriate stage were collected, cut into sections and thinned to the initial density treatment. Branch sections with either 1st or 2nd instar nymphs were placed separately in plastic cups and then one adult *H. axyridis* was added. The number of GS nymphs was counted after 24 h to estimate the feeding efficiency of the ladybird beetles. Based on field observations, ladybird beetles do not readily eat GS adults due to their hard helmets. To test this observation, initial densities of 4, 8, 12, 16 and 20 GS adults/branchlet were used to assess the feeding capacity of ladybird beetles on adults using the same experimental protocols for early instars of GS.

Experiment 2: cage experiment in field apricots

This experiment was carried out at the end of April in 2021 (peak time for the second instar GS) and mid-June (first instars as crawler) in the apricot forest in Gongliu, Xinjiang, China (82° 48' 52" N, 43° 13' 16" E). *Lasius niger* (Hymenoptera: Formicidae), a dominate ant species locally, was used to investigate the effect of attendant ants on the biological control effectiveness of beetles.

Three factors were considered in this field experiment. Initial density of GS was 50, 150 and 300 as first (or second) instar of GS nymphs on the terminal branches. The choice of GS initial densities was based on our field observations over 3 years (Wang 2021). The presence (one female in one cage) or absence of ladybird beetles was the second factor, and the presence or absence of ant attendance was the third. An ant was contained and access by field ants prevented by the fine mesh net covering all treatment branches (100 meshes). Mesh net excluding was regarded as a cage. There were nine treatments in this cage experiment (Table 1). Each treatment had 20 trees as replicates with a branch caged at each of the cardinal points; one branch each in north, south, east and west. In total, 180 trees were observed. The ladybird beetles were starved for 24 h before being used in the experiment, and all treated branches were covered by a mesh net, labeled and the number of GS on labeled branches counted after 1, 3, 5 and 7 days.

Experiment 3: predator release in apricot forests

In 2021, four flood relief channels (10–15 m in depth, 20–30 in width and more than 100 m in length) were selected as our release sites which had the same slope and similar landform. Each channel site was separated by

Table 1 Different treatments for cage experiments in the apricot fields

Treatments	Initial density of <i>S. prunastri</i>		
	50	150	300
CK	50-0	150-0	300-0
1	50-H	150-H	300-H
2	50-H-A	150-H-A	300-H-A

"50-0" means 50 GS in cage, "50-H" means 50 GS with one female Ladybird beetle in cage, and "50-H-A" means 50 GS with one female Ladybird beetle and one ant in cage

at least 1 km and dominated by wild apricot trees (more than 80% of trees, at a density at 450–600/ha) and a small percentage of shrubs (less than 5%). Three channels were designated as release plots: one for adult ladybird beetles, one for ladybird adults plus egg cards, and the other for egg cards only. The fourth channel was used as a control plot without release of commercial bio-control beetle adults or eggs. In each plot, five trees and counted all GS 3 days before release of ladybirds, and 3, 7 and 14 days after releasing ladybirds. In each tree, counted GS on four branches (50 cm length from the branch terminals) located at cardinal points (north, south, east and west) were sampled and averaged these as to estimate GS population size.

The experiment was conducted twice, once in spring and the other in summer when GS was in different stages. The first was at the end of April when GS second instars nymphs were terminating diapause and starting to feed. 5000 adult ladybird beetles were released in the first channel (adult predator plot), 300 egg cards (each card with 20 eggs) in the second channel (egg-card plot) and 3000 adult ladybird beetles and 300 egg cards in the third channel (adult-egg cards plot). For the second experiment, first instars nymphs (crawlers) were targeted. On 10th June, 2500 adult ladybirds were released in first channel (predator plot), 500 egg cards (each card with 20 eggs) in second channel (egg-card plots) and 2500 adult ladybirds plus 500 egg cards in the third channel (adult-egg cards plot).

Data analysis

Holling's type II equation (Holling 1959) was used to model the functional response: $N_a = aTN_0/(1 + aT_hN_0)$, where N_a is the daily predation amount and N_0 is the initial number of prey; T_h is the handling time of *H. axyridis* preying on GS, a is the successful attack rate, T is the exposure time which in this case was one day. Searching efficiency, S : $S = a/(1 + aT_hN)$ was calculated. Model fitting was performed using Origin version 7.5 for Windows (OriginLab Corporation, USA).

In order to describe the feeding capacity of ladybird, the efficacy of biological control (EBC) in apricot forests was estimated using the Henderson–Tilton formula (Henderson and Tilton 1955):

$$\text{EBC\%} = \left(1 - \frac{N_{C0} * N_{T1}}{N_{C1} * N_{T0}}\right) * 100 \quad (1)$$

N_{C0} is the abundance of GS in the control plot before treatment; N_{C1} the abundance of GS in control plot after treatment; N_{T0} the abundance of GS in each release plot before predator release; N_{T1} the abundance of GS in each plot after predator release.

EBC was estimated for each treatment and survey days. Nonparametric Kruskal–Wallis test (SPSS, IBM Company, Version 20) was used for comparison of difference between patterns of predator release on each survey day in cage experiments. For open-field experiments, the EBC of each treatment was square root transformed to conform to normality and variance homogeneity. Then Two-way ANOVA (SPSS, IBM Company, Version 20) was employed for comparison of difference in EBC between *H. axyridis* release treatments and days (as fixed factors). Multiple comparisons were made using the LSD (least significant difference) test.

Results

Effect of temperature on feeding capacity of adult ladybird beetle against first instars of globose scale in the laboratory

Functional response of *H. axyridis* preying on first instar of GS was described well by the Holling's type II equation within the range of 15–35 °C and field condition (Fig. 1 and Table 2). The number of scale insects consumed daily by *H. axyridis* increased gradually until reaching an upper asymptote of 158,160, 258, 143, 205 and 354 scales/predator at 15, 20, 25, 30, 35 °C and in field condition (average 25 °C), respectively (Fig. 1). Predation capacity was highest at 25 °C (constant temperature) and under fluctuating field temperature with a mean of 25 °C.

Feeding capacity of adult ladybird beetle against different instars of globose scale in the laboratory

Functional response of *H. axyridis* preying on first and second instars of GS was well described by the Holling type II equation at 25 °C in laboratory but not when exposed to adult scales ($R^2=0.052$, $\chi^2=60.7$). Predation capacity (a'/Th) was 395 in first instar scale and 177 in second instar scale, respectively (Table 3). Given second instars scale nymphs are twice as large *H. axyridis* consumes a similar amount. Adult ladybirds were not able to readily feed on scale adults.

Efficacy of adult ladybird beetle against early (1–2) instars of globose scale in cage field experiment

The density of prey (GS as 1st instars) influenced the efficacy of biological control (EBC) in all treatments (Fig. 2a). At the lowest density of GS (50), EBC increased from 48% on the first day to 99.6% after 7 days without ants but was decreased by 0.3–13% with ants present. At the moderate density (150), EBC increased from 33% after the first day to 96% after 7 days without ants, but was reduced by 14–23% with ants present. At the highest density (300), EBC increased from 22 to 68% from day 1 to 7 without ants but was 11 to 58% with ants (Fig. 2a).

Consumption of ladybird beetles of second instar globose scales was lower compared to that of first instar at all densities (Fig. 2b). At the low GS density (50), EBC increased from 20% from first day to 81% after 7 days without ants, and 12–63% with ants. At moderate density (150), EBC increased from 12% after one day to 66% after 7 days without ants, 8 to 40% with ants. At the high initial density (300), EBC increased from 18 to 48% with time without ants, with ants EBC was 8–34%.

Efficacy of biological control in the open field

Ladybird beetles (*H. axyridis*) were released in June, the peak time of first instar scales in the open field. Release patterns were not effective in controlling 1st instar GS (Table 4). The EBC was low regardless of what was released; EBC was –3.17 (adults), 1.59 (eggs) and 5.16% (adults and eggs) against first instar scales 14 days after predators were release (Table 5).

There was more effective control by released predators in the spring for second instar GS (overwinter generation). There was a significant interaction effect of release type and time since release on control of 2nd instar (Table 4). The combined use of adults and egg cards was the most effective. The combined release pattern of adult and egg cards had a significantly higher EBC effect, 35% after 14 days than the independent release mode. The EBC was 27% for adults alone and 18% for eggs alone after two weeks, respectively (Table 5).

Discussion

Predation capacity is typically assessed in controlled laboratory experiments, but these can be misleading. In all our laboratory experiments, this coccinellid species showed good feeding efficacy on GS at various temperature regimes from 15 to 35 °C, as well as ambient condition in the open field (Fig. 1, Table 2). Moreover, *H. axyridis* readily fed and showed high feeding efficacy when offered nymphs but not adults of GS (Table 3). Average temperatures in wild apricot forests in Xinjiang in summer are around 25 °C and predation rates

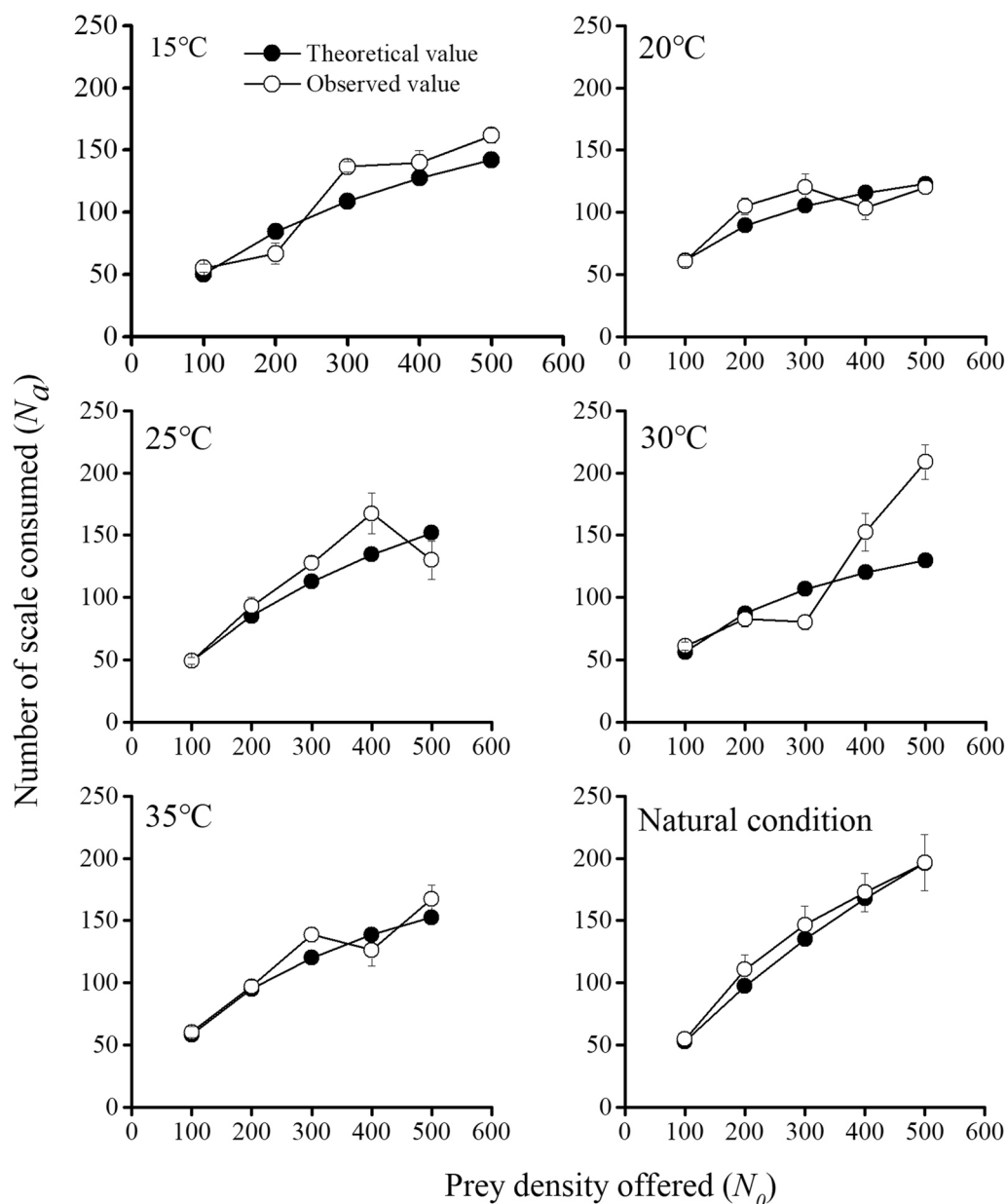


Fig. 1 Fitted curves for Holling II functional response of ladybird adult (*Harmonia axyridis*) feeding on 1st-instar nymphs of *Sphaerolecanium prunastri* at different temperatures

were maximal under these conditions. Additionally, higher EBC in cage experiments in the field supported *H. axyridis* as a potential biological control agent. The laboratory and caged field experiments taken on their own suggested this predator would be effective in field releases. Moreover, the ladybird fed more on 1st than 2nd nymphs in laboratory and open field with cage (Table 3 and Fig. 2). Open-field experiments showed very different outcomes.

Although the EBC was high in laboratory and in the field cage experiment it was lower in the open field without cages regardless of what stage was released (lady-beetle eggs, adults or both). The highest EBC, 26% using adults and 35% using eggs and adults, was achieved after 14 days when targeting second instar nymphs. This was the reverse of no-choice laboratory experiments where first instars were readily consumed. This coccinellid is highly variable (Kenis et al. 2008) and has been recorded

Table 2 Holling II functional response parameter values and mathematical models for *Harmonia axyridis* adults feeding on 1st-instar nymphs of *Sphaerolecanium prunastri* at different temperature regimes

Temperature (°C)	Holling type II equation	Attacking efficiency a'	Handling time Th (d)	Predation capacity a'/Th	Maximum predation number/day	χ^2	R^2 values
15	$Na=0.6221 N/(1+0.0024 N)$	0.7879	0.0050	158	200	6.23	0.90
20	$Na=0.9756 N/(1+0.0059 N)$	0.9756	0.0061	160	164	6.26	0.89
25	$Na=0.5242 N/(1+0.0013 N_0)$	0.5242	0.0030	207	395	3.86	0.95
30	$Na=0.7698 N/(1+0.0041 N)$	0.7698	0.0054	143	186	7.39	0.93
35	$Na=0.7591 N/(1+0.0028 N)$	0.7591	0.0037	206	271	1.18	0.99
Field condition	$Na=0.5769 N/(1+0.0016 N)$	0.5769	0.0016	354	614	3.03	0.99

*Temperature in open field was about 25 °C

Table 3 Holling II functional response parameter values and mathematical models for *Harmonia axyridis* adults feeding on different stages of *Sphaerolecanium prunastri* at 25 °C

Scale stage	Holling type II equation	Attacking efficiency a'	Handling time Th (d)	Predation capacity a'/Th	Maximum predation number/day	χ^2	R^2 values
1st instar	$Na=0.5242 N/(1+0.0013 N_0)$	0.5242	0.0030	207	395.26	3.86	0.95
2nd instar	$Na=0.3619 N/(1+0.020 N)$	0.3619	0.0057	64	176.78	7.00	0.95
Adult	$Na=5.1538 N/(1+0.2724 N_0)$	5.1538	0.0529	0.27	18.92	60.70	0.052

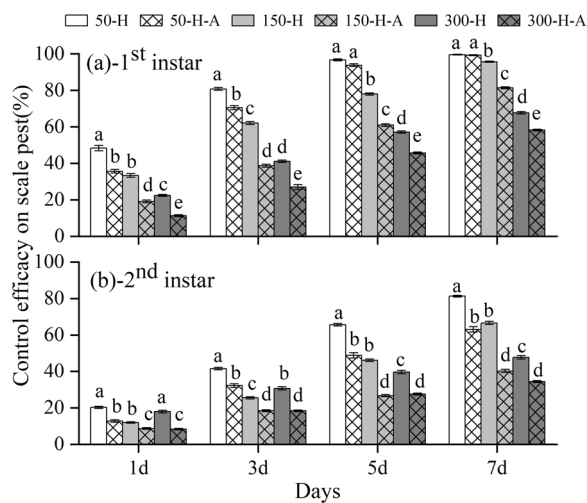


Fig. 2 Control efficiency of *Harmonia axyridis* adults feeding on 1st instar nymphs (a) and 2nd instar overwintering nymphs (b) of *Sphaerolecanium prunastri* at three initial densities (50, 100, 300) with and without ants after 1, 3, 5 and 7 days

feeding on various prey including psyllids (Michaud 2002), coccids (Hodek et al. 2012), spider mites (Lucas et al. 1997) and lepidopteran eggs (Hermann et al. 2019), but likely prefers to feed on aphids (Canovai et al. 2019). So, despite apparent considerable polyphagy as to what food is accepted when there is no choice, coccinellids are

very specific (Hodek et al. 2012). In addition, they can be very selective even among closely related prey items (Soares et al. 2004).

A higher feeding efficacy of ladybirds occurred against 2nd nymph in spring which may reflect the absence of alternative preys such as aphids at this time of year. The low control efficacy on 1st in mid-summer occurred when larger numbers of aphids can be found in various plants and may explain why ladybirds preferred 2nd instars over 1st nymph in open field (Table 5) the reverse of what occurred in the laboratory and field experiment with cage- higher feeding on 1st nymph (Table 3 and Fig. 2). At the start of summer, most scales are in the adult stage and we would expect even less predation by this lady beetle (Table 3). In the autumn, 2nd nymphs are covered under a thick wax layer which this scale secretes before going into diapause. This coating will likely prevent feeding by this ladybird on scale in autumn. Hence, the appropriate window for bio-control with *H. axyridis* should be in spring when scale pests are in 2nd nymph after overwintering and the waxy covering has fallen off. The 2nd nymph in this period is exposed in the branches and easily detected by ladybird.

In the open field, adult ladybirds can move freely and feed on other foods such as various aphids in grasses and shrubs. Further, the disturbances of ants can mitigate the effect of biological control on scale pests, as we found in our cage experiments. Attendant ants reduced biological

Table 4 Interaction of control effects of *Harmonia axyridis* after 3, 7 and 14 days against 1st-instar and 2nd-instar nymphs of *Sphaerolecanium prunastri*

Trait	Source	SS	df	MS	F	P
1st instar	Release pattern	2191.300	2	1095.650	2.225	0.111
	Days	6784.419	2	3392.210	6.890	0.001
	Release pattern × Days	337.502	4	84.375	0.171	0.953
	Residual	106,351.881	216	492.370	Null	Null
2nd instar	Release pattern	86.334	2	43.167	28.936	0.000
	Days	120.644	2	60.322	40.435	0.000
	Release pattern × Days	81.228	4	20.307	13.612	0.000
	Residual	322.232	216	1.492	Null	Null

Table 5 Field control effects of *Harmonia axyridis* after 3, 7 and 14 days against 1st-instar and 2nd-instar nymphs of *Sphaerolecanium prunastri* in Gongliu, Xinjiang in 2021

Scale stage	Release pattern	3 days (%)	7 days (%)	14 days (%)
1st instar	Adult	9.31 ± 1.47	-0.38 ± 4.84	-3.17 ± 5.65
	Egg cards	10.84 ± 1.81	1.51 ± 6.11	1.59 ± 5.8
	Adult and egg cards	19.16 ± 1.40	4.08 ± 4.75	5.16 ± 4.57
2nd instar	Adult	11.38 ± 0.80	18.21 ± 1.99	26.92 ± 1.81
	Egg cards	1.89 ± 0.34	7.53 ± 0.79	17.52 ± 1.22
	Adult and egg cards	12.07 ± 1.63	27.49 ± 2.29	34.98 ± 2.35

The data were the mean ± SE

control effectiveness of ladybird beetles by more than 80% in *Saissetia oleae* (Dao et al. 2014) and of parasitoids with a 22–90% reduction in parasitization for California red scale (Martinez-Ferrer et al. 2002). Moreover, excluding ants was associated with increased abundance and richness of natural enemies and led to more than 80% reduction in magnolia scale densities (Vanek and Potter 2010). Therefore, ant attendance and prey preference of ladybirds can combine to interfere the bio-control in open forests.

Although not the sought after “silver-bullet” *H. axyridis* could offer general biological control services for different pests in wild apricot forests, including aphids, scales and psyllids, particularly as it occurs naturally. This ladybird beetle could reduce the seasonal abundance of pests such as GS regardless of its low EBC. By reducing the initial density in early summer, it may ameliorate damage to young branches in mid-summer. The biological control service of *H. axyridis* needs to be considered as one component in the long-term management of scale in wild apricot forests. This ladybird occurs naturally in these forests, and it has been extensively used as a biological control agent in other parts of the world, although

it is now considered an invasive (Kenis et al. 2008). We see two ways of increasing predation either by augmentative releases and/ or by improving conditions for these beetles. Our fairly small-scale releases produced local effects and further experiments and cost benefit analysis are needed. In addition, to enhance the biological control against GS, banning or delaying the cutting of grasses in wild apricot forest can conserve the flowering plants offering pollen and nectar for *H. axyridis* and other natural enemies, especially at the end of June when the scale insect pest population peaks. Again, field experiments are needed to move beyond theory, limited and misleading laboratory experiments and better inform practice.

Conclusion

This study showed that *H. axyridis* has a high feeding capacity on GS (*Sphaerolecanium prunastri*) at various temperature regimes from 15 to 35 °C in laboratory. EBC was high in laboratory as well as within cages in open field, but lower in open field without cages due to ant disruption or ladybird mobility and feeding on alternative and likely preferred foods. In long term, biological control with *H. axyridis* should be considered as part of an IPM program. Banning or at least delaying mowing understory grass in the forests could increase pollen and nectar for natural enemies and population could be augmented by releasing adults at least in the late spring. However, field experiments are needed to test the effectiveness of such approaches.

Abbreviations

GS	The globose scale
EBC	Efficacy of biological control
IPM	Integrated pest management
ANOVA	Analysis of variance
a'/Th	Predation capacity

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Author contributions

GZG and ZZL conceived and designed the experiments. BDD and YLW performed the experiments. GZG, BDD and PZ analyzed the data. GZG, PZ and ZZL wrote the manuscript main text. MA, MPA and ZZL revised the manuscript. All authors approved the manuscript for submission.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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