

# Journal of Pest Science

## Flower provision reduces intraguild predation between predators and increases aphid biocontrol in tomato --Manuscript Draft--

<b>Manuscript Number:</b>	PEST-D-21-00084R3	
<b>Full Title:</b>	Flower provision reduces intraguild predation between predators and increases aphid biocontrol in tomato	
<b>Article Type:</b>	Original Article	
<b>Keywords:</b>	Calendula officinalis; Companion plant; Habitat management; Harmonia axyridis; Myzus persicae; Propylea japonica.	
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	National Key Research and Development Program of China (2017YFD0201000; 2018YFD0200402)	Dr Su Wang
	Beijing Key Laboratory of Environmentally Friendly Management on Fruit Diseases and Pests in North China (BZ0432)	Dr Su Wang
	Key R&D Program of Jiangxi Province (202002BBF62006)	Dr Su Wang
<b>Abstract:</b>	<p>Intraguild predation (IGP) – the predation of a natural enemy species upon another one sharing a prey species – is relatively frequent in both natural and agro-ecosystems. This may reduce pest control and the establishment of predator populations during mass release of biological control agents or in multi-predator systems due to increased mortality of predators. IGP is exacerbated in isolated and space-limited systems such as greenhouses, due to reduced food resources and movement. Therefore, adding food resources as an alternative to the main prey, such as floral resources, could reduce IGP between natural enemies in these systems. In the present study we investigated the role of supplemental floral resources to help reduce intra- and interspecific IGP involving <i>Harmonia axyridis</i> and <i>Propylea japonica</i> (Coleoptera:</p>	

	<p>Coccinellidae) in laboratory conditions, and we tested its application in a greenhouse setup. We found a significant reduction in intra- and interspecific IGP in laboratory conditions when floral resources were abundant. At a greenhouse scale, abundances of both ladybird species increased when floral resources were abundant, potentially through a combination of enhanced fertility and reduced IGP. This resulted in reduced abundances of aphid pest populations on tomato crops. Our study demonstrates that companion plants in greenhouses can improve pest control in systems with multi-species biological control agent releases.</p>
<b>Response to Reviewers:</b>	<p>COMMENTS TO THE AUTHORS:</p> <p>Title: "effects of" although correct minimize the paper aim and content. In Jps we avoid starting title with empty words such as "influence of" "effects of", etc  Moreover, as suggested before, and considered the referee comments it would be better avoiding the term greenhouse, as a consequence the best title seems the following:  Flower provision reduces intraguild predation between predators and increase aphid biocontrol in tomato  ANSWER : DONE (we used the title provided above).</p> <p>L27: delete 'help'  ANSWER : DONE.</p> <p>L140-143: interrogative sentence in the introduction have never been written on the hundreds of papers I ever read. Please reword these sentences avoiding question marks  ANSWER : DONE. We have rewritten the end of the introduction to avoid interrogative sentences.</p>
<b>Additional Information:</b>	
<b>Question</b>	<b>Response</b>
<p>Please summarize the main achievement of this work, above and beyond what may have been conveyed in the manuscript title in bullet point style. In brief, the Key Message should state why the work was conducted (knowledge gap(s) as well as key question(s) and/or hypotheses tested) and highlight the main finding(s) and the conclusions derived from this study. The latter should address the wider implications of the work and the relevance for pest control. All text should be generic, seminal and understandable to non-specialists. This Key Message should also be part of your submitted manuscript and will be published in front of the Abstract.</p> <p><b>Please include 3 to 5 bullet points of maximum 95 characters, including spaces, per bullet point.</b></p>	<ul style="list-style-type: none"> <li>• Intraguild predation (IGP) is ubiquitous in natural and agroecosystems.</li> <li>• It may threaten the establishment of pests' natural enemies and destabilize pest control.</li> <li>• We tested the impact of flowers on IGP between ladybirds in laboratory and greenhouse.</li> <li>• IGP was reduced by the presence of floral resources resulting in higher larvae survival rate.</li> <li>• Reduced IGP led to higher ladybird densities and enhanced aphid pest control.</li> </ul>
Does this manuscript belong to a special issue?	No

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Oxford, June 1<sup>st</sup>, 2021

Dear Editor,

We are pleased to submit a revised version of the manuscript now entitled “**Flower provision reduces intraguild predation between predators and increases aphid biocontrol in tomato**” to *Journal of Pest Science*.

We have made all changes required as per the comments below. We hope our changes now make the manuscript acceptable for a publication in *Journal of Pest Science*. We provide below a point-by-point answer to comments.

All co-authors have all explicitly agreed with the changes in this updated version. It has not been submitted, even in part to another journal. The relevant funding sources have been duly acknowledged.

Sincerely,

Coline Jaworski.

## COMMENTS TO THE AUTHORS:

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# 1 Flower provision reduces intraguild predation between predators and increases aphid 2 biocontrol in tomato

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5

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14

## 15 Key Message

- 16 • Intraguild predation (IGP) is ubiquitous in natural and agroecosystems.
- 17 • We tested the impact of flowers on IGP between ladybirds in laboratory and greenhouse.
- 18 • IGP was reduced by the presence of floral resources resulting in higher larvae survival rate.
- 19 • Flower provision induced higher ladybird densities and enhanced pest control in greenhouses.

## 20   **Abstract**

21   Intraguild predation (IGP) – the predation of a natural enemy species upon another one sharing a prey  
22   species – is relatively frequent in both natural and agro-ecosystems. This may reduce pest control and  
23   the establishment of predator populations during mass release of biological control agents or in multi-  
24   predator systems due to increased mortality of predators. IGP is exacerbated in isolated and space-  
25   limited systems such as greenhouses, due to reduced food resources and movement. Therefore, adding  
26   food resources as an alternative to the main prey, such as floral resources, could reduce IGP between  
27   natural enemies in these systems. In the present study we investigated the role of supplemental floral  
28   resources to help reduce intra- and interspecific IGP involving *Harmonia axyridis* and *Propylea*  
29   *japonica* (Coleoptera: Coccinellidae) in laboratory conditions, and we tested its application in a  
30   greenhouse setup. We found a significant reduction in intra- and interspecific IGP in laboratory  
31   conditions when floral resources were abundant. At a greenhouse scale, abundances of both ladybird  
32   species increased when floral resources were abundant, potentially through a combination of  
33   enhanced fertility and reduced IGP. This resulted in reduced abundances of aphid pest populations on  
34   tomato crops. Our study demonstrates that companion plants in greenhouses can improve pest control  
35   in systems with multi-species biological control agent releases.

36

## 37   **Keywords:**

38   *Calendula officinalis*; Companion plant; Habitat management; *Harmonia axyridis*; *Myzus persicae* ;  
39   *Propylea japonica*.

40

## 41   **Declarations:**

42   **Funding** see acknowledgment section

43 **Conflicts of interest/Competing interests** Authors declare they have no competing interests. SW is  
44 a Subject Editor of Journal of Pest Science and was not involved in the journal's review of, or decision  
45 related to, this manuscript.

46 **Availability of data and material** Not applicable

47 **Code availability** Not applicable

48 **Authors' contributions**

49 SW, CCJ, YL and XG designed the study; CX and JW performed the experiments; CX, HDJ and CCJ  
50 analyzed the data; YL, CX, CCJ and SW wrote the manuscript. All authors read and approved the  
51 manuscript for submission.

52

53

## 1. Introduction

Intraguild predation (IGP) occurs when a predator preys on another natural enemy – the IGP prey – in addition to preying on their shared prey (Rosenheim et al. 1995; Chailleux et al. 2013; Mohammadpour et al. 2020). IGP is exacerbated when primary prey are scarce and negatively affects the development, colonization and distribution of the IGP prey species (van Veen et al. 2006). Especially, exotic predatory species have the potential to negatively affect populations of native predators, potentially disrupting pest control (Polis & Myers 1989; Polis & Holt 1992; Snyder & Evans 2006; Lamichhane et al. 2015). Despite ubiquitous cases of IGP in natural or agro-ecosystems (in absence of biological control agent release), laboratory and field studies showed that the disruption of pest control by one of the predator involved in IGP did not necessarily result in higher pest densities (Rosenheim et al. 1993; 1995; Gagnon et al. 2015; Aparicio et al. 2020; Ortiz-Martínez et al. 2020).

Yet many applications of biological control agents occur in more isolated systems, such as greenhouses, with more limited resources and arthropod movement, but studies investigating the potential for IGP to disrupt the biological pest control in such systems are scarce. In addition, applications of multiple biological control agents specialized on distinct target pests are commonly used over short periods of time and preferably at early stages of pest outbreaks in multi-pest systems (Albajes et al. 2000; Liu et al. 2012; Tan et al. 2016; Sanchez-Hernandez et al. 2021). These low-prey, multi-predator species conditions are ideal for IGP to occur among biological control agents (Chailleux et al. 2013). Thus, enhancing food provision in such systems could help reduce the intensity of IGP, and thereby avoid the disruption of biological pest control (Lu et al. 2014).

Companion plants – non-crop plants providing ecosystem services in agroecosystems – have been



77 used to provide alternative food resources and/or shelter in many conservation biological control  
78 applications (Balzan et al. 2016; Biondi et al. 2106; Gurr et al. 2017; Perovic et al. 2018; Foti et al.  
79 2019; Snyder 2019). Companion plants may also be integrated and managed at a field scale in *attract-*  
80 *and-reward* strategies (Gardarin et al. 2018; Jaworski et al. 2019; Cai et al. 2020, 2021). Flowering  
81 companion plants may attract arthropod natural enemies of crop pests via olfactory and visual floral  
82 signals and reward them with floral nectar and pollen resources (Barbosa 1998; Damien et al. 2017,  
83 2020; Chailleux et al. 2019; Wang et al. 2020). Such floral food resources may enhance natural enemy  
84 populations especially when prey density is low (Li et al. 2015; Zhao et al. 2017). Companion plants  
85 may also create habitat separation or provide micro-habitats in agroecosystems. There, arthropod  
86 natural enemies of crop pests may find shelter from their own natural enemies such as birds, or from  
87 environmental pollutants such as chemical pesticides that could negatively impact them (Bommarco  
88 & Ekbom 2000 ; Desneux et al. 2007). However, the role of companion plants in reducing IGP has  
89 been poorly documented so far.

90

91 Despite more and more abundant literature on habitat management to benefit natural enemies of crop  
92 pests in large, open fields (Albrecht et al. 2020), using similar ecological regulation remains limited  
93 in more isolated and space-limited systems, such as greenhouses (but see Li et al. 2020; Xu et al.  
94 2020). The use of companion plants providing alternative resources to biological control agents could  
95 be convenient in greenhouses, especially in multi-predator species where food resources could be  
96 limited, increasing the risk for IGP (Parolin et al. 2012; Sun & Song 2019).

97

98 Predatory ladybirds are a well-known group of very effective biological control agents and with  
99 strong colonization and spread capacity, wide food breadth and high prey consumption (Hodek et al.

100 2012; Lu et al. 2012; Thomine et al. 2020). These predatory ladybirds are top consumers in arthropod  
101 communities and play a key role in the structure and stability of food webs in natural habitats (Hodek  
102 & Michaud 2008; Ragsdale et al. 2011; Hodek et al. 2012). Many studies have shown that IGP was  
103 frequently occurring among predatory ladybirds, both among conspecific (intraspecific IGP) or  
104 between species (interspecific IGP) and especially under insufficient food supply (Fedriani et al. 2000;  
105 Ware & Majerus 2008; Pervez & Gupta 2010). IGP has been described in ladybirds between larvae,  
106 or by larvae on eggs, by adults on larvae, or between adults (Michaud 2010; Osawa 2015;  
107 Ovchinnikov et al. 2019).

108

109 Remarkably, *Harmonia axyridis* Pallas, 1773 (Coleoptera: Coccinellidae) has been widely  
110 characterized both as a very efficient biological control agent in its native territory in Asia, and as a  
111 worldwide invasive predator causing severe ecological risks in decreasing biodiversity and destroying  
112 ecological balance in introduced areas notably via IGP and resource competition (Koch & Galvan  
113 2008; Pell et al. 2008; Li et al. 2021). *Harmonia axyridis* reduces populations of ladybird species  
114 under IGP by attacking their larvae and eggs (Burgio et al. 2002; Mirande et al. 2015). Wang et al.  
115 (2012) showed in a field survey that *H. axyridis* was a dominant competitor over two other predatory  
116 ladybird species common in China, *Hippodamia variegata* Goeze, 1777 and *Propylea japonica*  
117 Thunberg, 1780 (Coleoptera: Coccinellidae): *H. axyridis* preyed on more eggs, mostly heterospecific,  
118 had the highest survival rate and the lowest rate of IGP victims. Also, *H. axyridis* had a niche breadth  
119 twice as big as the other two species, but their niche overlap with that of *H. axyridis* was extremely  
120 high. Finally, *P. japonica* is more tolerant and a more efficient biocontrol agent at higher temperature  
121 (Lei et al. 1988). For these complementary features, *H. axyridis* and *P. japonica* are often released  
122 together to control aphid populations (Gao et al. 2016), and especially in greenhouses (Vuong et al.

2001; Yang et al. 2014; Kuroda & Miura 2003). In particular, they are used against the generalist aphid species *Myzus persicae* Sulzer, 1776 (Hemiptera: Aphididae), which is a major pest in a diversity of vegetable crops including tomato crops in China (Li 2013).

Many ladybird species may feed on floral resources (Wäckers & van Rijn 2012; Hatt et al. 2019). A field study showed that *H. axyridis* used floral resources all year long even in presence of aphids (Berkvens et al. 2010). *Harmonia axyridis* used floral resources of *Vicia sativa*, *Fagopyrum esculentum*, *Coriandrum sativum* and *Calendula officinalis* in laboratory conditions (Wang et al. 2020). While *Calendula officinalis* had a negative impact on *H. axyridis* fecundity and predation activity (Wang et al. 2020), it increased the fecundity and longevity of *P. japonica* females (Jaworski et al. 2019). Besides, *C. officinalis* is commonly used in habitat management programmes in China to enhance biological pest control (Zhao et al. 2017; Jaworski et al. 2019).

In the present study, we conducted two laboratory experiments to evaluate the intraspecific IGP in *H. axyridis* and interspecific IGP between *H. axyridis* and *P. japonica*, and the impact of flowering potted plants of marigold *C. officinalis* on the intensity of IGP. In addition, we conducted a one-year greenhouse experiment to assess the practical impact of the abundance of marigold flowers on the population dynamics of a mixed community of *H. axyridis* and *P. japonica*, and on the efficacy of the biological control of the aphid pest *M. persicae* in tomato crops. We investigated the impact of the abundance of marigold flowers on (i) the intensity of intraspecific IGP in *H. axyridis*, (ii) the intensity of interspecific IGP between *H. axyridis* and *P. japonica*, and (iii) the population dynamics of the two predatory ladybirds and the regulation of aphid pest populations.

## 146 2. Materials and Methods

### 147 2.1. Ladybirds and plants

148 Experimental colonies of ladybirds were established in the insectary of the Institute of Plant and  
149 Environment Protection (IPEP), BAAFS, from live specimens collected in an alfalfa field in the  
150 campus of Weifang University of Science and Technology (GPS: E118.78, N36.89) in June 2018  
151 (*H. axyridis* : 330 adults; *P. Japonica* : 424 adults). The two species were reared in different cages  
152 with around 30-35 pairs of ladybirds per cage (35.0 cm<sup>3</sup> plastic frame covered with 40-mesh net).  
153 Fifty fresh artificial diet microcapsules similar to those used in industrial productions were supplied  
154 daily in each cage to maintain the experimental colonies, as described in Tan et al. (2015): 1.0% Ca-  
155 alginate, 1.6% chitosan and shell : core = 1 : 2, size 2.0-2.5 mm. After about five reared generations  
156 since the collection of wild specimens, five paper strips (3.0 \* 10.0 cm each) were placed in each  
157 cage as oviposition substrates to collecting fresh eggs. Each strip was removed and placed in a Petri  
158 dishes (D = 12.0 cm) after five days. Newly hatched 1<sup>st</sup> instar larvae were collected every other day  
159 and placed in a new plastic petri dish with 10 larvae per dish. The larvae were provided with 10 daily  
160 supplied fresh artificial diet microcapsules until they developed to 4<sup>th</sup> instar, after what the density  
161 was reduced to three larvae per dish. More than 5,000 4<sup>th</sup> instar larvae for each ladybird species were  
162 prepared for the laboratory and greenhouse experiments. Environmental conditions inside the  
163 insectary were automatically regulated (T = 25 ± 1 °C; RH = 60 ± 5 %; Photoperiod: 16:8 h L:D (500  
164 lux); automatic regulation systems Est100, JiangNan, Ningbo, China).

165

166 Experimental tomato *Solanum lycopersicum* cv. Beryl (Jingyan Yinong Seed Sci-tech Co., Ltd.)  
167 seedlings were grown in plastic trays (56 \* 25 \* 20 cm, 12 plants per tray). Once they reached 15cm  
168 height, we transplanted them individually in plastic flower pots (H = 25 cm, D = 15 cm, 1 plant per

169 pot). We used standard growing soil (Miracle Gro ©). All tomato plants were maintained in artificial  
170 climatic chambers (MH-351, Sanyo, Nagoya, Japan). The environmental conditions were set as  
171  $T = 27 \pm 1$  °C,  $RH = 60 \pm 5$  % RH and a 14:10 h L:D photoperiod. Over 1,000 tomato plants were  
172 prepared, and used for the laboratory and greenhouse experiments when they reached 30-35 cm height  
173 with 5-7 fully expanded true leaves.

174

175 Plants of marigold, *Calendula officinalis* Linnaeus, 1753 (Asterales: Asteraceae) var. Kablouna  
176 (SinicHorticulture and Flower Co. Ltd, Beijing, China), were bought from the Yajie flower market  
177 (Changping, Beijing) at the two true leaves stage (~10-15 cm height), and grown in plastic trays as  
178 above mentioned. They were booked specifically for our study and grown according to our  
179 specifications, and without pesticide application. Marigold is a common plant species used as an  
180 alternative floral resource for ladybirds (*P. japonica*, Jaworski et al. 2019) and easy to get. We  
181 transplanted the plants in plastic flowerpots when they reached 3-4 true leaves (H = 25 cm, D = 15  
182 cm, 1 plant per pot). About 20-25 days later, they reached around 20 cm and we removed the  
183 topmost buds of each plant; this allowed the production of more flowers by the start time of the  
184 experiments two weeks later.

185

## 186 **2.2. Laboratory experiment: impact of marigold flowers on intra- and interspecific IGP**

187 We assessed the impact of the presence of marigold flowering plants in intraspecific IGP in  
188 *H. axyridis*. Forty 4<sup>th</sup> instar larvae of *H. axyridis* were starved for 12h and then placed in a cage with  
189 three tomato plants (plastic frame covered by 40-mesh, 50.0 cm<sup>3</sup>). In the treatment cages, one  
190 marigold plant was placed simultaneously in the cage, but not in the control cages. To estimate the  
191 impact of the number of open flowers of marigold, we varied the density of flowers per cage from

one to five. We manipulated marigold plants accordingly using tinfoil paper to wrap up entire  
exceeding flowers. This led to a 5-level treatment (1 to 5 flowers), and a total of 180 cages (30 cages  
for control, and 30 cages for each treatment level). No other food was provided to ladybirds in cages  
and we recorded larvae survival after 24h. All cages were maintained simultaneously in different  
rooms of the insectary (each room :  $7 \times 4$  m) under controlled environmental conditions as above.

197

Dead larvae were further observed under a stereo microscope (Stereo V20, Zeiss, Germany) and those  
without external wounds were recorded as dead from starvation. We calculated the number of larvae  
victims of IGP as the total number of larvae introduced in each cage (40) minus the number of  
observed survivors minus the number of larvae dead from starvation.

202

Finally, to assess the impact of the abundance of marigold flowers on IGP between *H. axyridis* on  
*P. japonica*, we repeated the entire experiment above, except this time we placed 30 *H. axyridis* and  
30 *P. japonica* 4<sup>th</sup> instar larvae in each cage. We used these extreme conditions in laboratory  
experiments – high ladybird densities, and no prey provided – to increase the likelihood and intensity  
of IGP, so as to properly evaluate intra- and interspecific IGP and to assess the impact of flower  
provision on the intensity of IGP. This choice of larvae density was made based on authors experience  
who observed the occurrence of IGP at such high ladybird densities in rearing experiments.

210

### **2.3. Greenhouse experiment: impact of marigold on ladybird population dynamics and biological pest control**

To evaluate the practical effectiveness of marigold plants in enhancing ladybird populations and aphid  
pest control in systems with combined released of *H. axyridis* and *P. japonica*, we carried out a one-

215 year survey in three greenhouses in Lanhu Organic Farmland (GPS: E116.75, N39.98), Tong'zhou  
216 county, Beijing, China. All three greenhouses were the same size (length: 80 m; width: 12 m; height:  
217 6 m). We split each greenhouse into five isolated chambers using transparent plastic sheets preventing  
218 arthropod movement between chambers (each chamber: 10 × 8 m; Fig. 1). In every chamber, 80  
219 tomato plants were grown in 10 rows. All tomato plants were transplanted at the stage 8-10 true leaves,  
220 from seedlings grown in climatic chambers (see section 2.1). 48h later, we infested 3<sup>rd</sup> instar nymphs  
221 of *M. persicae* (provided by IPEP, BAAFS) on 20 randomly selected tomato plants per chamber with  
222 250 aphids each. Then we placed marigold potted plants in each chamber according to three  
223 treatments: (a) low flower density: 5 marigold plants with a total of 15 open flowers; (b) high flower  
224 density: 15 marigold plants with a total of 45 open flowers; and (c) control: no marigold plant. The  
225 position of each treatment in the five chambers of each greenhouse was fully randomized. 24h later,  
226 80 4<sup>th</sup> instar larvae of *H. axyridis* and *P. japonica* each were released evenly on 10 randomly selected  
227 tomato plants in each chamber. The aphid and ladybird densities, as well as the release strategy were  
228 chosen based on authors' experience and pilot observations. Such aphid densities simulate real  
229 greenhouse densities before aphid pest populations reach the outbreak peak. The plants chosen for  
230 ladybird larvae release were not necessarily the same as for aphid release due to random selection.

231

232 From the day we introduced ladybirds (May 6<sup>th</sup> 2019), we measured the densities of aphids and of  
233 the two species of ladybirds (including larvae from all instars and adults) by visual count on six  
234 randomly selected tomato plants per chamber (30 plants per treatment) every Monday until the last  
235 week of August 2019. Marigold plants were blooming throughout the duration of the experiment. In  
236 case one plant had stopped flowering it was immediately replaced, making sure no insect was present

on this plant. Environmental conditions inside greenhouses followed seasonal trends and temperature reached 32-34 °C in average.

## 2.4. Statistical analysis

To test the impact of intraspecific IGP on larvae survival in our laboratory experiment, we performed a generalized linear mixed model (GLMM; function ‘glmer’, library ‘lme4’; Bates et al. 2015) with a binomial distribution and the response variable implemented as a matrix whose first and second columns corresponded to the number of IGP victims versus live larvae. We implemented the number of marigold flowers (0 to 5) as a factorial fixed effect, and the insectary room identifier as a random effect, followed by an anova with a  $\chi^2$  test. Model validity was verified *a posteriori* (functions ‘simulateResiduals’ and testDispersion’, library ‘DHARMA’; Hartig 2019). To assess whether means across treatments with a different number of flowers were significantly different, we performed a *post hoc* comparison of means across treatment levels (function ‘emmeans’, library ‘emmeans’; Lenth 2019). The impact of interspecific IGP on larvae survival was assessed with the same methodology, except that we used the factorial number of marigold flowers in interaction with the species (*H. axyridis* versus *P. japonica*) as fixed effects. The significance of the interaction and fixed effects was estimated through a type-II model comparison based on a  $\chi^2$  test. A *post hoc* mean comparison across treatment levels was performed for each species independently as above (‘emmeans’: ‘specs = pairwise ~ Treatment | Species’).

To assess the impact of marigold flowers on ladybird abundances in the greenhouse experiment, we used a GLMM with a negative binomial distribution (to account for data dispersion; function ‘glmer.nb’, library ‘lme4’; Bates et al. 2015) on the number of live ladybirds per plant, with the



260 treatment (control / low, number of flowers / high number of flowers) in interaction with the species  
 261 (*H. axyridis* versus *P. japonica*) as fixed effects. Random effects were the greenhouse chamber nested  
 262 in the greenhouse to account for repeated measures through time, and the week (implemented as a  
 263 factor) to account for changes in population dynamics. The significance of the interaction between  
 264 fixed effects was assessed through a type-II model comparison based on a  $\chi^2$  test. A *post hoc* mean  
 265 comparison across treatments and for each species was performed (function 'emmeans': 'specs =  
 266 pairwise ~ Treatment | Species', library 'emmeans'). We used the same method to test the impact of  
 267 the number of flowers on aphid number per plant, except that only the treatment was used as fixed  
 268 effect. All statistics were performed using R version 3.6.2 (R Core Team 2019).

269

270

### 271 **3. Results**

#### 272 **3.1. Laboratory experiment: impact of marigold flowers on intra- and interspecific IGP**

273 The number of marigold flowers significantly reduced the number of *H. axyridis* larvae affected by  
 274 intraspecific IGP ( $\chi^2 = 225$ ,  $df = 5$ ,  $P < 0.001$ ; Fig. 2). The number of victims gradually decreased in  
 275 treatments from three to five flowers, but was not significantly different from control (no flowers)  
 276 when less than three flowers were provided. The number of larvae which survived intraspecific IGP  
 277 was more than twice as big when five flowers were present (mean  $\pm$  SE:  $31 \pm 1$ ) compared with  
 278 control (no flower;  $13 \pm 1$ ).

279

280 Similarly, the number of marigold flowers significantly reduced the number of larvae victims of IGP  
 281 when both species were present ( $\chi^2 = 297$ ,  $df = 5$ ,  $P < 0.001$ ; Fig. 3), with a 33 % higher number of  
 282 dead larvae in *P. Japonica* ( $\chi^2 = 290$ ,  $df = 5$ ,  $P < 0.001$ ; mean number  $\pm$  SE: *H. axyridis*  $14.6 \pm 0.2$ ,

283 *P. japonica*  $19.4 \pm 0.3$ ), but the interaction between these effects was not significant ( $\chi^2 = 2.05$ ,  $df = 5$ ,  
284  $P = 0.84$ ). Similar to the intraspecific experiment, the number of victims gradually decreased in  
285 treatments from three to five flowers, but was not significantly different from control (no flowers)  
286 when less than three flowers were provided. The number of larvae which survived IGP was more than  
287 twice as big when five flowers were present (mean  $\pm$  SE: *H. axyridis*  $12.4 \pm 0.3$ , *P. japonica*  $7.6 \pm 0.4$ )  
288 compared with control (no flower; *H. axyridis*  $19.2 \pm 0.5$ , *P. japonica*  $14.9 \pm 0.5$ ).

289

290 The total proportion of larvae victims of IGP was not different in the two-species experiment  
291 compared with the one-species experiment in control (mean  $\pm$  SE, no flowers: two species  $67 \pm 2$  %,   
292 one species  $67 \pm 2$  %) but it declined faster in the one-species experiment with increasing flower  
293 abundance (mean  $\pm$  SE, five flowers: two species  $43 \pm 2$  %, one species  $23 \pm 2$  %). Also, the  
294 proportion of *H. axyridis* larvae victims of total IGP (cumulated intra- and interspecific IGP) in the  
295 two-species experiment was lower than in the one-species experiment at low flower density only  
296 (mean  $\pm$  SE; no flowers: two species  $58 \pm 1$  %, one species  $67 \pm 2$  %; five flowers: two species  
297  $36 \pm 2$  %, one species:  $23 \pm 2$  %).

298

### 299 **3.2. Greenhouse experiment: impact of marigold on ladybird population dynamics and** 300 **biological pest control**

301 In our greenhouse experiment, we found that an increased number of marigold flowers per chamber  
302 resulted in a significant increase in the number of ladybirds of both species, and this was exacerbated  
303 for *P. japonica* (significant interaction between the number of flowers and the ladybird species:  $df = 2$ ,  
304  $\chi^2 = 60.7$ ,  $P < 0.001$ ; Fig. 4). The abundance of *P. japonica* was multiplied by almost three in average  
305 between the control (no flowers) and the high flower density, while the abundance of *H. axyridis* was

multiplied by roughly two between these treatments (mean number of  $\pm$  SE of ladybirds per plant: *P. japonica* control:  $4.6 \pm 0.1$ , high flower density:  $12.5 \pm 0.4$ ; *H. axyridis* control  $8.9 \pm 0.2$ , high flower density  $17.7 \pm 0.5$ ). All treatments were significantly different from each other for both species (Table 1). In absence of flowers, abundances of *P. japonica* increased before Week 1 (from 1 to  $\sim 2.5$  ladybirds per plant) but were overall stable after that. Conversely, in all other treatments and in all treatments for *H. axyridis*, abundances increased from the date of release to Week 12. After that, only *H. axyridis* abundances kept increasing in the high flower density treatment while *P. japonica* abundances remained stable in this treatment. After Week 12, abundances of both *P. japonica* and *H. axyridis* declined in the low flower density treatment, and in absence of flowers for *H. axyridis*.

An increased number of flowers per chamber also induced a significant reduction in aphid populations ( $\chi^2 = 72.2$ ,  $df = 2$ ,  $P < 0.001$ ; Fig. 5), and the three treatments were significantly different between each other (Table 1). Abundances were divided by almost two in average between control (no flower) and the high flower density treatment (Mean number  $\pm$  SE per plant: control  $163 \pm 2$ , high flower density  $92 \pm 1$ ). In all three treatments, aphid abundances declined from Week 1 to Week 6. In absence of flowers, they increased from Week 6 to Week 11 and then decreased up to Week 18, while they remained more stable in the presence of flowers from Week 6 to 14 and then declined from Weeks 14 to 18.

#### 4. Discussion

Intraguild predation – both intra- and interspecific – among biological control agents may negatively affect their population dynamics and consequently pest suppression. In the present study, we showed

that the provision of marigold flowers helped reduce the negative impacts of intra- and interspecific IGP between predatory ladybirds in a laboratory setting. The provision of flowering marigold companion plants resulted in higher ladybird abundances and improved aphid pest control in a realistic greenhouse experiment similar to practical biological control application in tomato crops.

Marigold flowers decreased both intraspecific and interspecific IGP in *H. axyridis* and *P. japonica*, with less larvae victims of IGP in the laboratory experiment. We created a food shortage in this experiment via the starvation of ladybird larvae and the absence of prey, hence conditions were ideal for a strong IGP (Polis & Myers 1989). *Harmonia axyridis* remained a dominant competitor in this system with higher survival no matter the number of marigold flowers. Total larvae mortality in the interspecific experiment was actually a combination of intraspecific and interspecific IGP. However, the lower total IGP rates in *H. axyridis* in the two-species experiment (Fig. 3) compared with the one-species experiment (Fig. 2) show that intraspecific IGP in *H. axyridis* was lower in the two-species experiment. This might be because it was compensated by high interspecific IGP by *H. axyridis* to *P. japonica*.

Interestingly, reduced IGP was found only when at least three flowers were provided in both the intraspecific and interspecific experiments. This points at a beneficial impact of marigold related to floral resources, and likely the provision of food resources (Jaworski et al. 2019; Ma et al. 2019). Adding marigold floral resources in the diet of ladybirds has been shown to have a positive impact on their development (Wolf et al. 2018; El-Kareim et al. 2019). Preliminary video records in the laboratory showed that starving ladybird larvae tended to aggregate close to the flower pistil (source of pollen) where they showed reduced aggression to congeners (Chen and Wang, unpublished data).

352 However, since the presence of a marigold plant with few flowers was not sufficient to reduce IGP, it  
353 is unlikely that marigold plants provided other services in our system, such as a refuge from IGP by  
354 plant (Gontijo 2018).

355

356 Many studies showed that companion plants may be beneficial via the provision of food resources to  
357 enhance the colonization, population growth, and efficiency of pests' natural enemies in conservation  
358 biological control (Jaworski et al. 2019; Landis et al. 2000; Li et al. 2020). The present study is to our  
359 knowledge the first demonstration of reduced negative impacts of IGP by supplying companion plants  
360 that provide alternative floral resources to biological control agents. Similar to the present study with  
361 *H. axyridis*, a previous study by our group showed that marigold flowering plants effectively  
362 enhanced the development and population growth of the predatory flower bug *Orius sauteri*  
363 (Hemiptera: Anthocoridae) in both laboratory and greenhouse settings (Zhao et al. 2017). Other  
364 companion plant species have been used to sustain the development and population growth of  
365 *H. axyridis* with the aim of enhancing pest biological control, including *Perilla frutescens* (Hatt &  
366 Osawa 2019), *Fagopyrum esculentum* and *Centaurea cyanus* (Wolf et al. 2018), and *Hibiscus*  
367 *cannabinus* (Xiu et al. 2017).

368

369 We also found a positive impact of flowering marigold companion plants on ladybird populations in  
370 our practical greenhouse biological control application. We observed that populations grew faster  
371 with increased flower density up to Week 12 and especially in *P. japonica* (Fig. 4). The flower  
372 provision may have benefit ladybird populations both via the provision of alternative resources  
373 improving fitness and reproduction and via reduced IGP. Hatt & Osawa (2019) found an increased  
374 fecundity of *H. axyridis* when fed with a mixed diet of *Ephestia kuehniella* eggs and *Perilla frutescens*

375 flowers compared with prey only, while in a previous study we found a +37 % increase in female  
376 fecundity of *P. japonica* females when fed on a mixed diet of *M. persicae* aphids with *C. officinalis*  
377 flowers compared with aphids only (Jaworski et al. 2019). In our greenhouse experiment, the  
378 *P. japonica* abundances without flowers were roughly multiplied by three in Weeks 7-8 than in Weeks  
379 1-3 (Fig. 4), while during the same period *P. japonica* abundances at high flower density were roughly  
380 multiplied by seven, i.e., more than twice as fast. Hence, only higher mortality in absence of marigold  
381 flowers – very likely via IGP – could have caused such a slower population growth.

382  
383 Evidence of IGP in mesocosms has been reported at even lower ladybird densities. Sato et al. (2003)  
384 found that 25 % of *H. axyridis* and 14 % of *P. japonica* larvae, out of nine larvae on a *Hibiscus*  
385 *syriacus* plant, died from IGP when aphid resource became rare. In mixed releases (three *H. axyridis*  
386 + three *P. japonica* + three *Coccinella septempunctata* larvae) (Coleoptera: Coccinellidae), about 20 %  
387 only of *H. axyridis* larvae died but up to 60 % of *P. japonica* larvae died. In a previous study we  
388 observed IGP between *H. axyridis*, *P. japonica* and *H. variegata* for densities of around one ladybird  
389 per plant of horsebean in greenhouse conditions (*Vicia faba*; Wang et al. 2012). Finally, Hironori &  
390 Katsuhiko (1997) reported IGP by *H. axyridis* on *C. septempunctata* at densities of less than 40  
391 ladybirds per 2 m-high *Hibiscus syriacus* trees. Despite numerous reports of IGP by *H. axyridis* in  
392 agricultural systems, we were surprised that very few studies actually reported densities at which IGP  
393 occurred in field or mesocosm systems (Koch 2003; Pell et al. 2008). However based on the studies  
394 reported here, this seems very likely that IGP was at least partly responsible for the excess mortality  
395 observed in our greenhouse experiment.

396  
397 Under the greenhouse temperatures (32-34 °C), we measured a time from ladybird releases to first

larvae of three days for *H. axyridis* and two days for *P. japonica*, while the observed development time from egg to adult was 16-17 days for *H. axyridis* and 14-15 days for *P. japonica*. Therefore, larvae were observed in the greenhouses from the first week (Fig. 4). This confirms previous studies measuring a faster developmental rate for *P. japonica* than *H. axyridis* on *M. persicae* (first instar larvae to adult at 25 °C: 11.3 days for *P. japonica*, Zhang et al. 2012; 17.1 days for *H. axyridis*; Lanzoni et al. 2004). Similar to the laboratory interspecific experiment, *H. axyridis* remained the dominant competitor over *P. japonica*, with higher abundances.

After Week 12, population growth decreased in both control and low flower density treatments for both species (Fig. 4). This could be related to the reduction in available aphid prey (Fig. 5), potentially causing both reduced fecundity and increased IGP. Note that we could not directly assess the intensity of IGP in our greenhouse experiment because of logistic limitations in observing predation on eggs and larvae at the scale of the greenhouse chambers.

By promoting the diversity of natural enemies, conservation biological control generally enhances pest control, through a higher probability for very efficient natural enemies to be present but also through the niche complementarity of diverse natural enemies (Jonsson et al. 2017). However it may simultaneously result in negative impacts and poor pest control, notably via increased IGP (Straub et al. 2008). Our study yet demonstrated that the use of flowers helped reduce both intra- and interspecific IGP (shown in laboratory experiments), and instead enhanced pest control in a greenhouse crop. We found a better, long-term suppression of aphid populations at higher marigold flower density, related to higher ladybird densities, and likely to reduced IGP. This was notably due to earlier suppression of aphid populations, otherwise increasing from Week 6 to Week 11 in absence

421 of flowers.

422

423 Companion plants may act as a buffer against IGP in agro-ecosystems and improve natural enemy  
424 coexistence in simplified crop habitat (Zhang et al. 2016). They could improve the attraction of  
425 predators by emitting plant volatiles, or provide more separated micro-habitats or alternative food  
426 resources (Song et al. 2012; Li et al. 2014; Jaworski et al. 2019). Further increasing flower density  
427 may help further decrease IGP since we found that the effect of marigold flowers depended on flower  
428 density. Also, using other species as companion plants or a mixture of species may be beneficial to  
429 both ladybird species (Mathews et al. 2016; Xiu et al. 2017). A more diverse plant community has  
430 been shown to enhance biodiversity up to the upper trophic levels, increasing species richness and  
431 evenness of predation interactions in foodwebs (Barbosa 1998). Hence, this would be worth testing  
432 the use of multi-species companion plants in greenhouse systems relying on multiple biological  
433 control agents.

434

435 In commercial greenhouses, combined releases of multiple species of natural enemies targeting the  
436 same pest species are used to improve pest control (Tan et al. 2016). Theoretically, once the nutrition  
437 supply is sufficient and stable, the coexisting predators in the same ecological niche tend to segregate  
438 spatially (Rosenheim et al. 1995; Amarasekare 2008). However, in a greenhouse available space is  
439 strongly limited because of the simplified and isolated system. Thus, we observed no spatial  
440 segregation between ladybird species in our greenhouse experiment, and effective pest suppression  
441 was observed only with the ecological support of marigold companion plants.

442

443 Our study demonstrates the potential for companion plants to improve biocontrol. Such addition of



444 floral resources could be used in systems with high risk of IGP, such as mass rearing of biological  
445 control agents, long-term storage and long-distance transportation, or inundative release of biological  
446 control agents prior to the exponential growth of pest populations. The provision of alternative floral  
447 food resources by easy to maintain companion plants would help reduce IGP and help omnivorous  
448 insect predators to overcome negative environmental conditions. Floral resources such as pollen are  
449 often integrated in the mass rearing of biological control agents such as predatory mites (Riahi et al.  
450 2016).

451  
452 We only considered one pest species, *M. persicae*, in our study. Yet, even a simplified greenhouse  
453 cropping system may be much more complex with multiple herbivorous pest and natural enemy  
454 species. The role of companion plants in reducing IGP in these more complex systems should be  
455 further investigated in the future. The use of molecular detection techniques of predator gut content  
456 (Eitzinger et al. 2019) could help quantify predation interactions and the use of companion plants as  
457 food supply by predators in complex systems. Finally, the companion plant species and diversity  
458 could be optimized to promote populations of the target natural enemies and with no benefits to pest  
459 populations.

460  
461 **5. Acknowledgments**

462 The study was funded by the project 32072479 supported by the National Natural Science Foundation  
463 of China, the National Key Research and Development Program of China (2017YFD0201000;  
464 2018YFD0200402), the Beijing Key Laboratory of Environment Friendly Management on Fruit  
465 Diseases and Pests in North China (BZ0432), and the Key R&D Program of Jiangxi Province  
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707



708 **Tables**

709

710 **Table 1.** *Post hoc* comparisons of means between levels of flower abundances (0 / 15 / 45 flowers)  
711 in the greenhouse experiment for each ladybird species (first test) and for aphids (second test).

Comparison	<i>Harmonia axyridis</i>		<i>Propylea japonica</i>		Aphids	
	Estimate ± SE	<i>P</i>	Estimate ± SE	<i>P</i>	Estimate ± SE	<i>P</i>
45 flowers – 15 flowers	0.388 ± 0.029	< 0.001 ***	0.286 ± 0.031	< 0.001 ***	-0.279 ± 0.016	< 0.001 ***
45 flowers – 0 flowers	0.689 ± 0.031	< 0.001 ***	0.942 ± 0.034	< 0.001 ***	-0.577 ± 0.016	< 0.001 ***
15 flowers – 0 flowers	0.300 ± 0.031	< 0.001 ***	0.656 ± 0.035	< 0.001 ***	-0.298 ± 0.016	< 0.001 ***

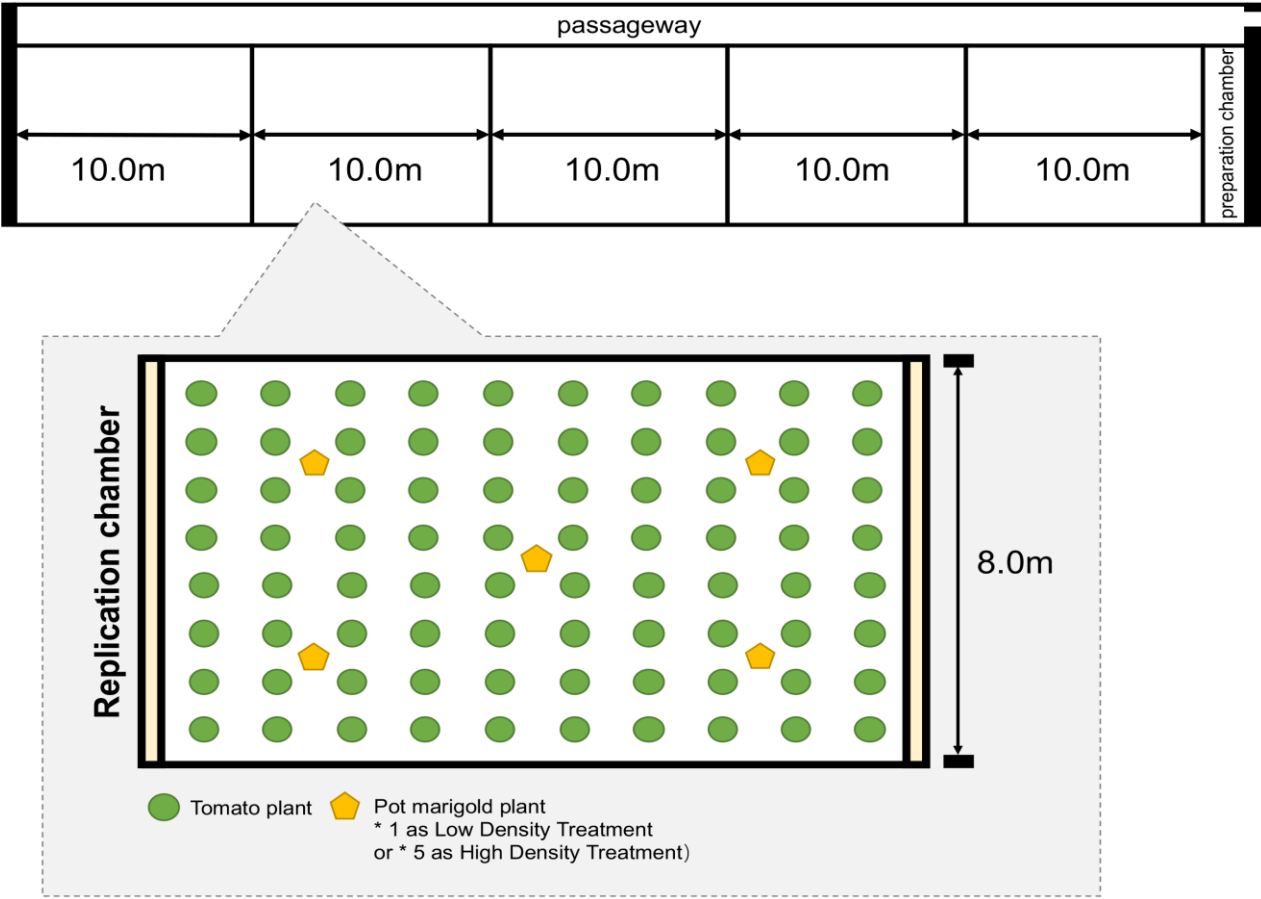
712 ‘\*\*\*’: *P* < 0.001.

713

714 **Figures**

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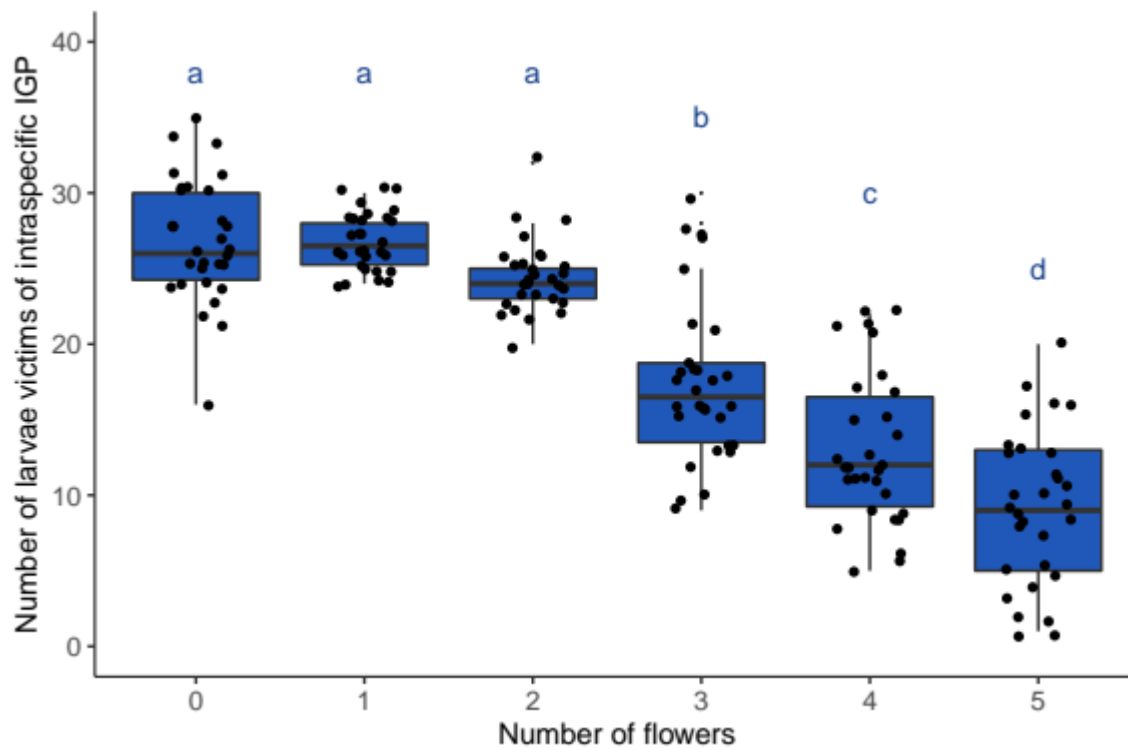
**The greenhouse**



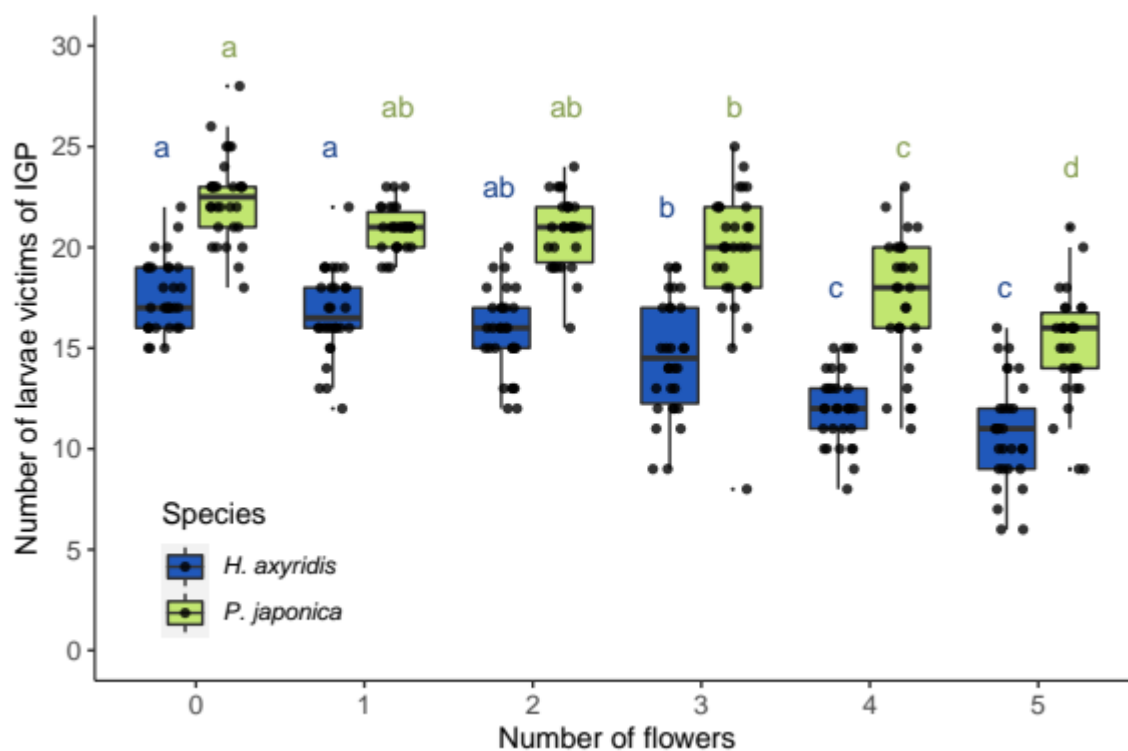
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717 **Figure 1.** Layout of one greenhouse divided into five chambers (top), and spatial arrangement of  
718 tomato plants (green) and marigold plants (orange) in one chamber (bottom). Each marigold plant  
719 had three and nine open flowers at low and high flower densities, respectively. In control chambers,  
720 no marigold plant was provided.

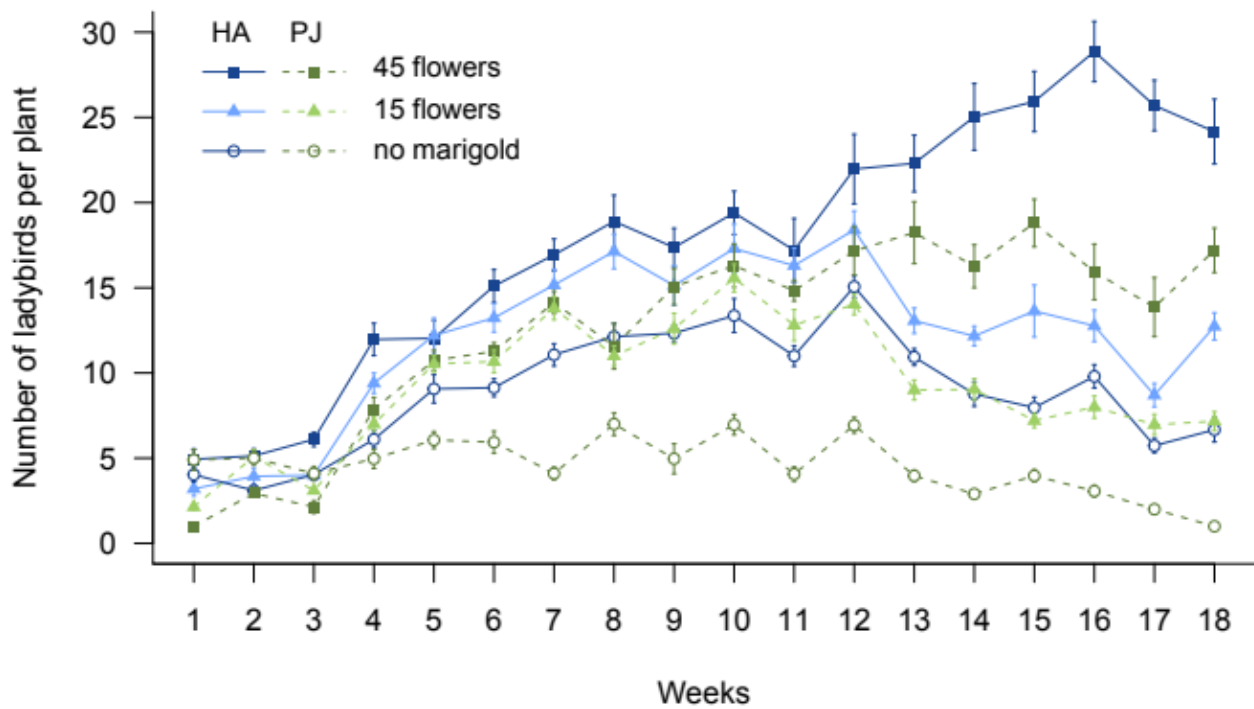
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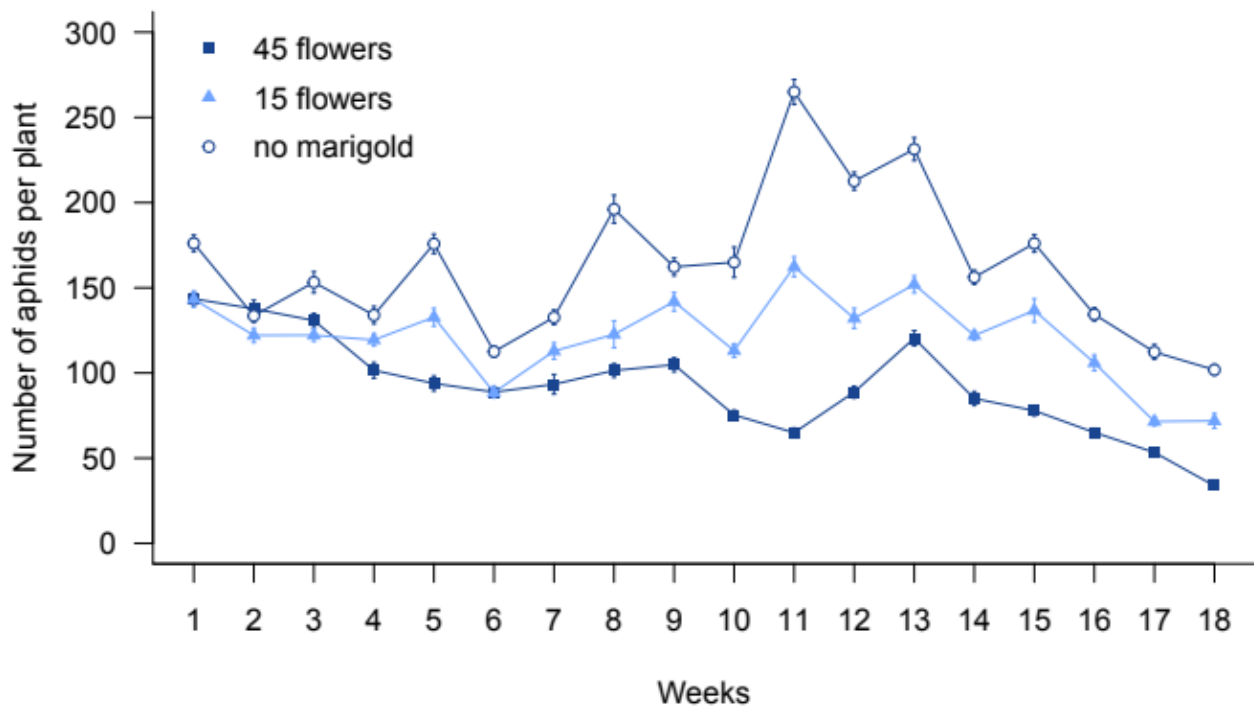
**Figure 2.** Number of *Harmonia axyridis* larvae victims of intraspecific IGP out of 40 larvae initially, as a function of the number of open flowers of the marigold companion plant (0 flowers = no plant; boxplot). Different letters indicate significant differences between the means of each group ( $P < 0.01$ ).



**Figure 3.** Number of *Harmonia axyridis* (blue) and *Propylea japonica* (green) larvae victims of IGP out of 30 larvae each initially, as a function of the number of open flowers of the marigold companion plant (0 flowers = no plant; boxplot). Different letters indicate significant differences between the means of each group in the species of the corresponding colour ( $P < 0.01$ ).



**Figure 4.** Population dynamics of *Harmonia axyridis* (HA) and *Propylea japonica* (PJ). Mean number of larvae plus adults ( $\pm$  SE) per sampled tomato plant in greenhouse chambers under various densities of marigold flowers (flower numbers are per chamber).



**Figure 5.** Population dynamics of aphids *Myzus persicae*: mean number ( $\pm$  SE) of aphids per sampled tomato plant in greenhouse chambers under various densities of marigold flowers (flower numbers are per chamber).