Bulletin of Entomological Research

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Adaptability of *Aphis gossypii* glover to different *Capsicum annuum* varieties

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Research Paper

Cite this article: Yang J, Bang H, Yang H, Zhao J, Farhan M, Ma X, Zhang S (2025) Adaptability of *Aphis gossypii* glover to different *Capsicum annuum* varieties. *Bulletin of Entomological Research* **115**, 287–295. https://doi.org/10.1017/S0007485325000100

Received: 16 October 2024
Revised: 27 December 2024
Accepted: 15 February 2025
First published online: 21 March 2025

Keywords:
adaptability; *Aphis gossypii* glover; biotype; *Capsicum annuum*; life table

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Abstract

Aphis gossypii Glover (Hemiptera: Aphididae) is a significant pest of *Capsicum annuum* (Solanales: Solanaceae) and exhibits intraspecific differentiation within populations. To investigate the adaptability of Hap3 and Hap17 *A. gossypii* to various *C. annuum* varieties, including 'Lvzhou101' (LZ), 'Lashen' (LA), 'Saierweilvtianjiao' (SE), 'Haimaihongri' (HM), 'Chaotianjiao' (CT), and 'Luosijiangjun' (LS), we employed life tables to analyse growth and population parameters post-feeding and conducted petri dish host choice experiments to assess the host plant preference of *A. gossypii*. Survival rates of *A. gossypii* varied significantly across *C. annuum* varieties. Notably, Hap3 and Hap17 thrived on 'LZ' but failed to establish populations on 'LA'. The net reproductive rate (R_0), average generation time (T), and intrinsic rate of increase (r_m) differed markedly between Hap3 and Hap17 across *C. annuum* varieties. Feeding on 'LZ' resulted in a significantly higher R_0 value (26.49) for Hap3 relative to other varieties. The T (7.60 days) and r_m (0.27) values for Hap3 on 'SE' were superior to those observed on other *C. annuum* varieties. These findings indicate that 'SE' is the optimal host for Hap3 growth, while 'LZ' best supports Hap17. Both haplotypes exhibited the lowest adaptability to 'LA'. Therefore, the utilisation capacity of *A. gossypii* populations on *C. annuum* demonstrates differentiation, and the resistance levels among *C. annuum* varieties to *A. gossypii* vary. This differentiation can inform targeted management strategies for aphid infestations on pepper crops.

Introduction

Capsicum annuum L., commonly known as pepper, is a widely cultivated crop that holds significant economic and nutritional value globally. As a rich source of vitamins, proteins, sugars, and pigments, *C. annuum* is a staple in many cuisines and an important agricultural commodity (Liu *et al.*, 2014). In addition to their culinary value, peppers are also cultivated for their medicinal properties, due to the presence of bioactive compounds such as capsaicinoids, carotenoids, and flavonoids, which are known for their anti-inflammatory and antioxidant effects (Deng and Wen, 2021). In China, *C. annuum* is extensively grown across various regions, contributing to both local diets and the broader agricultural economy (Zou *et al.*, 2025). However, pepper cultivation faces numerous challenges, with pest infestations, particularly from aphids, being one of the most serious threats to yield and quality.

Among the pests that damage pepper crops, the cotton aphid, *Aphis gossypii* Glover (Hemiptera: Aphididae), is of particular concern (Blackman, 2013). *A. gossypii* is a highly adaptable and damaging pest that feeds on a wide range of plants, including *C. annuum* (Gong *et al.*, 2001). The feeding behaviour of *A. gossypii* involves piercing plant cells with its stylet to extract sap, which weakens the plant, disturbs its growth, and can cause a reduction in yield and quality (Luo *et al.*, 2003). In addition, *A. gossypii* can transmit various plant viruses, further complicating pest control efforts. The damage caused by *A. gossypii* can result in significant economic losses, especially in large-scale pepper cultivation (Tang *et al.*, 2013).

Although *A. gossypii* damages *C. annuum* by feeding on its sap, resulting in symptoms like wilting, leaf curling, and stunted growth, *C. annuum* also possesses natural insecticidal compounds that help protect it from pests. These include capsaicin, phenolic acids, and flavonoids, which contribute to the plant's defence mechanisms (Deng and Wen, 2021). For instance, a study developed a chili extract-based biopesticide that significantly reduced red leaf mite populations, achieving a 94.9% killing rate at a 100-fold dilution (Meng *et al.*, 2024). This underscores the potential of capsaicin and other chili-derived compounds as natural pest control agents, supporting the role of peppers in integrated pest management. However, the specific impact of these compounds on *A. gossypii* has not been thoroughly studied.

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In addition to the chemical defences of *C. annuum*, *A. gossypii* itself exhibits considerable genetic diversity (Satar *et al.*, 2013). Traditionally, *A. gossypii* populations have been categorised into different biotypes based on host preferences. Recent molecular studies have refined this classification by identifying 57 haplotypes using C*ytb* and 16S gene regions (Zhang *et al.*, 2018). Among these, Hap1, Hap3, and Hap4 are the major haplotypes, accounting for 86% of the total *A. gossypii* population in northern China. Hap1 and Hap4 are the predominant haplotypes on cotton, while Hap3 is the most common haplotype found on cucurbit hosts. Hap3, in particular, shows a strong preference for cotton but also exhibits adaptability to other hosts, including *C. annuum*. Hap17, although less common, is another significant haplotype associated with differences in host preferences, further highlighting the genetic diversity of *A. gossypii* and its ability to infest and damage specific crops. The differentiation of these haplotypes is based on single-nucleotide polymorphisms in the complete mitochondrial genome sequences (Bao *et al.*, 2023; Ji *et al.*, 2024).

Currently, the control of *A. gossypii* on *C. annuum* relies heavily on chemical insecticides. While effective, these methods often result in resistance development, environmental pollution, and the resurgence of pest populations, representing the challenges associated with resistance, residue, and resurgence (Ma *et al.*, 2024). As a result, there is an increasing interest in exploring alternative pest management strategies that leverage the natural insecticidal properties of *C. annuum* and better understand the interactions between aphid populations and host plants. This is particularly important as natural resistance mechanisms in plants may offer more sustainable and environmentally friendly solutions to pest management.

Prior research has indicated that the adaptability of *A. gossypii* populations can vary significantly depending on the host plant. For example, different *A. gossypii* haplotypes exhibit varying performances on crops like cotton and cucumber (Li *et al.*, 2023). However, the specific adaptability of *A. gossypii* on different varieties of *C. annuum* has not been comprehensively studied. In our field surveys, we identified that Hap3 and Hap17 were the most prevalent *A. gossypii* haplotypes infesting *C. annuum* in Yangzhou University's Experimental Field. This selection was intentional, as it allows us to study the behaviour and adaptability of naturally occurring biotypes in the local environment. By focusing on these haplotypes, we aim to assess their performance on various *C. annuum* varieties. The objective of this research is to investigate how these two haplotypes respond to different pepper varieties, focusing on key factors such as survival, reproduction, and host selection. By comparing the performance of these haplotypes across different pepper varieties, this study aims to provide valuable insights into the adaptability of *A. gossypii* and contribute to the development of more effective pest management strategies for pepper cultivation.

Materials and methods

Plants and insects

The *C. annuum* varieties used in the experiment include 'Lvzhou101' (LZ), 'Lashen' (LA), 'Saierweilvtianjiao' (SE), 'Haimaihongri' (HM), 'Chaotianjiao' (CT), and 'Luosijiangjun' (LS). All varieties were first grown in an artificial climate room, then transplanted to Yangzhou University's Experimental Field (32°15′N, 119°01′E) for natural growth without chemical exposure, ensuring they were free from insect damage before use.

In 2022, *A. gossypii* specimens of the Hap3 and Hap17 biotypes were collected in Yangzhou University's Experimental. These specimens were moved to the lab and reared individually to establish populations. Haplotypes were identified through haplotype-specific sequence analysis as previously described (Zhang *et al.*, 2016). Following confirmation as Hap3 and Hap17 *A. gossypii*, these aphids were reared for several generations on healthy cotton plants under controlled conditions in an artificial light incubator (26 ± 1°C, light/dark cycle (L/D) = 14 hours light/10 hours dark, RH = 70%–80%) for future experiments.

Establishment of life tables for *A. gossypii* on *C. annuum*

The experiments were conducted in an incubator with controlled conditions: temperature of 26 ± 1°C, relative humidity of 65 ± 5%, and a light/dark cycle of 14 hours light to 10 hours dark (L:D = 14:10 hours). Different host plant leaves were affixed to petri dishes containing 1.8% agar, with fresh leaves replaced as needed.

Two haplotypes of *A. gossypii*, Hap3, and Hap17, were utilised to establish life tables on various *C. annuum* varieties. Specifically, Hap3 was established on six different pepper varieties, while Hap17 was established on three different pepper varieties, resulting in a total of nine distinct life tables. Each life table consisted of 35 replicates, requiring approximately 315 petri dishes in total for the entire experiment.

During the life table parameter measurement, G_1 generation adults were selected and transferred to the corresponding host plants. After 12 hours, the adults were removed, leaving 35 nymphs per treatment (one nymph per petri dish), corresponding to the 35 biological replicates for each life table. These G_1 generation nymphs served as the starting observation stage. The survival and offspring production of aphids in each treatment were observed and recorded every 24 hours until all initial aphids died.

Host selection behaviour experiment

Leaves from cucumbers and *C. annuum* were cut into fan-shaped pieces at a 120° angle, each less than one-third the size of a petri dish (9 cm diameter, 1.5 cm height) to facilitate controlled host plant comparisons and enable clear observation of aphid host preference. These pieces were arranged with the angled sides in contact and placed back-side-up in petri dishes containing 1.8% agar. Each behavioural choice test involved 10 *A. gossypii* per dish, with 6 biological replicates per experimental group, totalling 60 *A. gossypii* per experiment.

Before the experiments, Hap3 and Hap17 *A. gossypii* were subjected to a 2 hour starvation treatment to standardise their physiological state. After the starvation treatment, the aphids were placed in the centre of the petri dishes to allow free movement. The number of *A. gossypii* on each treated leaf area was recorded at intervals of 30 minutes, 1 hour, and 12 hours.

A total of 24 behavioural choice experiments were conducted, each comprising 6 biological replicates (6 petri dishes per experiment), resulting in the use of approximately 144 petri dishes in total for all behavioural assays.

Data analysis methods for life tables

Data analysis adheres to the methodology detailed in the referenced study (Wang, 2015).

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$$R_0 = \sum l_x m_x;$$
$$T = \sum (x l_x m_x) / \sum (l_x m_x);$$
$$r_m = \ln R_0 / T_o$$

In this study, 'x' represents the unit time period in days. 'l_x' indicates the survival rate of *A. gossypii* at day 'x', and 'm_x' denotes the average offspring produced per surviving *A. gossypii* within the time period 'x'. 'R_0' refers to the net reproductive rate, i.e. the multiplication factor of a parthenogenetic aphid population over one generation (the average number of parthenogenetic offspring produced per parthenogenetic aphid). 'T' signifies the average duration in days for one *A. gossypii* generation, while 'r_m' represents the intrinsic rate of increase. Together, these life table parameters provide a comprehensive understanding of the population dynamics of *A. gossypii*, helping to assess its growth potential and reproductive success in different environmental conditions.

Data analysis methods for host selection behaviour experiment

For each behavioural choice experiment, the selection percentage for each plant was calculated. In each biological replicate, 10 aphids were placed in a dish with leaves from 2 host plants available for choice. Aphids that did not choose either plant were excluded from the analysis. The percentage of aphids selecting the first host plant was calculated by dividing the number of aphids on the first host plant by the total number of aphids that made a choice (total aphids minus those that did not select any plant). Similarly, the percentage of aphids selecting the second host plant was calculated by dividing the number of aphids on the second host plant by the total number of aphids that made a choice. For each treatment group, the selection percentages from the six biological replicates were averaged to obtain the final data.

Statistical analysis methods for survival rates

Significant differences in life table parameters among treatments are analysed using one-way ANOVA, with Tukey's test for multiple comparisons performed in IBM SPSS Statistics 27.0. Tukey's test is used to perform pairwise comparisons of life table parameters among different haplotypes across pepper varieties after conducting one-way ANOVA. This post hoc test is appropriate for controlling Type I errors when comparing multiple groups, ensuring reliable identification of significant differences between haplotypes while accounting for the risk of false positives.

In R, Kaplan–Meier survival curves for *A. gossypii* populations on different *C. annuum* varieties are generated using the ggplot2 package. The Kaplan–Meier method is a non-parametric statistical approach suitable for analysing survival time data with censored observations. This method effectively estimates the cumulative survival probabilities of aphids under different treatments and allows us to compare survival curve differences between treatment groups. Significance analysis involves one-way ANOVA, with subsequent group comparisons conducted via the Tukey's test.

Each host selection behaviour experiment involves 60 *A. gossypii*. To calculate the selection rate, subtract the number of *A. gossypii* that do not choose from 60. Then, divide the remaining *A. gossypii* by the initial total and multiply by 100% to determine the percentage. Selection rates are visualised using stacked bar charts created with GraphPad Prism 10. Statistical significance is assessed with a chi-square test in IBM SPSS Statistics 27.0. Statistical significance of aphid selection rates among different *C. annuum* varieties were assessed using the chi-square test, as it is appropriate for evaluating associations between categorical variables and determining whether observed selection frequencies differ significantly from expected frequencies under the null hypothesis.

Results

Survival rate of *A. gossypii* on *C. annuum*

The Hap3 *A. gossypii* were transplanted onto six varieties of *C. annuum*, with their survival days ranked from highest to lowest as follows: LZ, HM, LS, SE, CT, and LA. The longest survival was observed on LZ, reaching up to 28 days, while the shortest was on LA, around 17 days (fig. 1A). The survival rate initially surged, then plateaued, while the mortality rate started low, increasing over time.

The Hap17 *A. gossypii*, originally hosted by cotton, were transplanted onto three varieties of *C. annuum*, with their survival days on the three *C. annuum* varieties ranked from highest to lowest as follows: LZ, CT, and LA. The longest survival was observed on LZ, reaching up to 25 days, while the shortest survival period on LA was approximately 12 days. The survival rate curve demonstrated an initial rapid increase followed by a slowdown, with the mortality rate starting low and subsequently increasing (fig. 1B).

To better understand how different *C. annuum* varieties influence *A. gossypii* survival, we compared Hap3 and Hap17 performance across six host plants. The survival rate of Hap17 *A. gossypii* on *C. annuum* is significantly lower than that of Hap3 *A. gossypii* (fig. 1). Ten days following the inoculation of pepper plants, the survival rates of Hap3 *A. gossypii* on six pepper varieties are: SE (77.14%), LZ (62.86%), LS (57.14%), HM (48.57%), CT (48.57%), and LA (11.43%). Conversely, at the same time point, the survival rates of Hap17 *A. gossypii* are notably lower than those of Hap3, specifically at LZ (37.14%), CT (28.5%), and LA (2.86%). By the twentieth day after inoculation onto pepper hosts, Hap3 *A. gossypii* still had a survival rate of 22.86% on LZ, whereas Hap17 *A. gossypii* demonstrated a survival rate of only 14.29% on LZ. Additionally, the survival durations on peppers also reflect that the survival rate of *A. gossypii* is significantly lower than that of Hap3 *A. gossypii*. The survival durations of Hap3 *A. gossypii* on peppers are as follows: LZ (31 days), HM (30 days), LS (29 days), SE (25 days), CT (21 days), and LA (16 days). In contrast, Hap17 *A. gossypii* can only establish populations on LA, LZ, and CT, with survival durations of 20 days for LZ and CT, and 11 days for LS, much lower than the survival durations of Hap3 *A. gossypii* on peppers.

Comparison of life table parameters for *A. gossypii* on *C. annuum*

Hap3 *A. gossypii* demonstrated superior performance on LZ and SE, with net reproductive rates (R_0) of 26.49 and 1.49, respectively, significantly higher ($P < 0.05$) than those on other hosts. In contrast, Hap17 *A. gossypii* shows a peak R_0 of 8.8 on LZ, markedly outperforming other varieties ($P < 0.05$), while the lowest R_0 value is 0.2 on LA, suggesting this host is unsuitable for both strains. Moreover, the R_0 of the Hap17 *A. gossypii* population on the host plants LZ and CT does not show significant differences ($P > 0.05$), highlighting similar adaptability between these two hosts for this strain.

In terms of fecundity, Hap3's days to oviposition (*DO*) are highest on SE and LZ, at 6.37 and 6.28 days, respectively. These values are significantly higher ($P < 0.05$) than on other varieties. Hap17's

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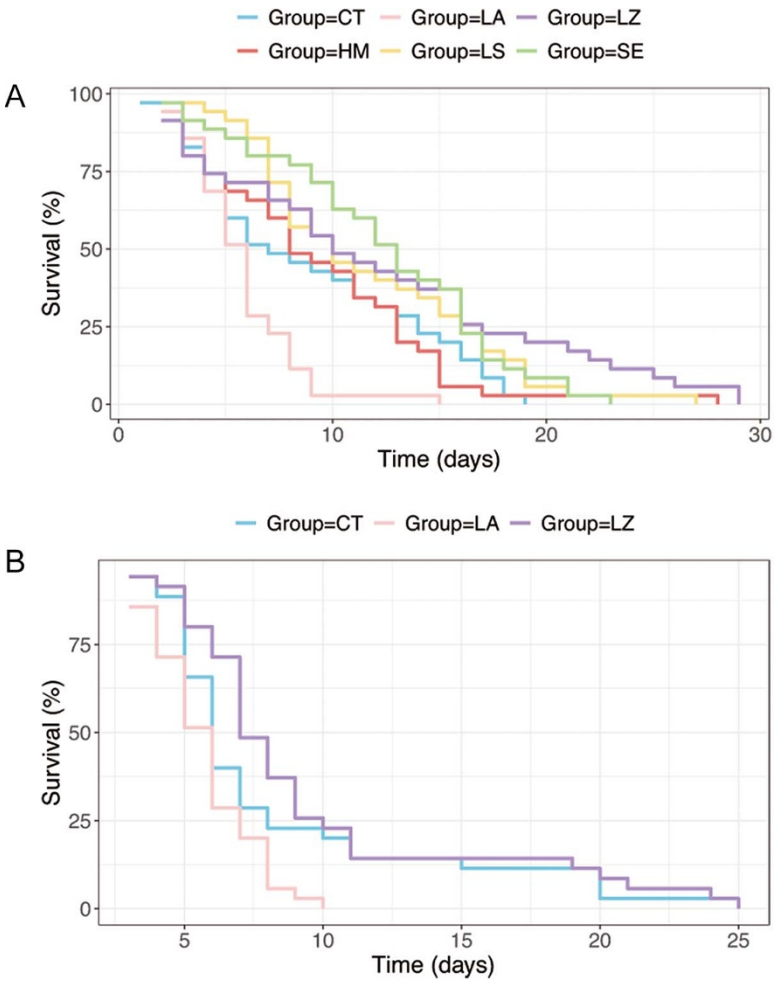


Figure 1. Changes in survival rate of Hap3 and Hap17 *A. gossypii* across different *C. annuum* varieties. (A) Kaplan–Meier survival curves of Hap3 *A. gossypii* on six *C. annuum* varieties. As determined by Tukey's test, one-way ANOVA, the survival rate of *A. gossypii* on LA is significantly different from LS, LZ, and SE (***$P < 0.0001$). Survival rates of *A. gossypii* on CT are also significantly different from LA, SE, and LZ (*$P < 0.05$), while survival rates on HM differ significantly from LA, LZ, and SE (*$P < 0.05$). However, survival rates on HM do not significantly differ from CT and LS ($P > 0.1$). Survival rates on LS do not significantly differ from CT and LZ, and between SEWLTJ ($P > 0.1$). (B) Kaplan–Meier survival curves of Hap17 *A. gossypii* on three *C. annuum* varieties. As determined by Tukey's test, one-way ANOVA, the survival rate of *A. gossypii* on LA significantly differs from that on LZ (***$P < 0.0001$). Survival rates of *A. gossypii* differ significantly between CT and LA (*$P < 0.05$). However, there is no significant difference in survival rates of *A. gossypii* between CT and LZ ($P > 0.1$).

Table 1. Life table parameters of Hap3 and Hap17 *A. gossypii* on *C. annuum* after mutual host transfer

Host plant	Net reproductive rate (R_0) (offspring individual^{-1})	Average generation time (T) (d^{-1})	Days to oviposition (DO) (d^{-1})	Intrinsic rate of increase (r_m) (d^{-1})
Hap3-LZ	(26.49 ± 4.81)a	(7.21 ± 0.89)ab	(6.28 ± 1.10)a	(0.22 ± 0.03)ab
Hap3-SE	(22.86 ± 2.94)ab	(7.60 ± 0.71)a	(6.37 ± 0.81)a	(0.27 ± 0.02)a
Hap3-LA	(1.49 ± 0.92)d	(1.09 ± 0.47)de	(0.43 ± 0.24)c	(0.03 ± 0.01)c
Hap3-HM	(10.14 ± 2.04)cd	(5.19 ± 0.79)abc	(3.31 ± 0.66)abc	(0.17 ± 0.03)bc
Hap3-CT	(12.14 ± 2.76)bcd	(4.22 ± 0.85)bcd	(3.26 ± 0.71)abc	(0.14 ± 0.03)bc
Hap3-LS	(17.20 ± 3.05)abc	(6.44 ± 0.91)ab	(4.63 ± 0.86)ab	(0.19 ± 0.03)ab
Hap17-LA	(0.20 ± 0.20)d	(0.18 ± 0.19)e	(0.09 ± 0.09)c	(0.01 ± 0.01)c
Hap17-LZ	(8.89 ± 3.26)cd	(3.02 ± 0.86)cde	(2.51 ± 0.89)bc	(0.07 ± 0.02)bc
Hap17-CT	(8.48 ± 2.91)cd	(2.71 ± 0.83)cde	(2.29 ± 0.81)bc	(0.08 ± 0.02)bc

Notes: Data in the table are mean ± standard error. Different lowercase letters in the same column indicate significant differences at the $P < 0.05$ level by the Tukey test.

peak days to oviposition (DO) is 2.51 days on LZ, considerably lower than Hap3's, with the lowest at 0.09 days on LA, versus 0.42 days for Hap3. The average generation time (T) varies significantly, with Hap3 experiencing the longest times on SE (7.60 days) and LZ (7.21 days), and the shortest on LA (1.09 days). For Hap17, the average generation time (T) ranges from 3.02 days on LZ to a mere 0.18 days on LA (table 1).

The intrinsic rate of increase (r_m) underlines these differences further, with Hap3 achieving the highest rates on SE and LZ (0.27 d^{-1} and 0.22 d^{-1}, respectively), significantly exceeding those on LA (0.03 d^{-1}, $P < 0.05$). Hap17, however, reaches its highest r_m value on SE and LZ (0.08 d^{-1}and 0.07 d^{-1}, respectively), far surpassing the rate on LA (0.01 d^{-1}). Both Hap3 and Hap17 *A. gossypii* perform poorly on LA, confirming its unsuitability as a

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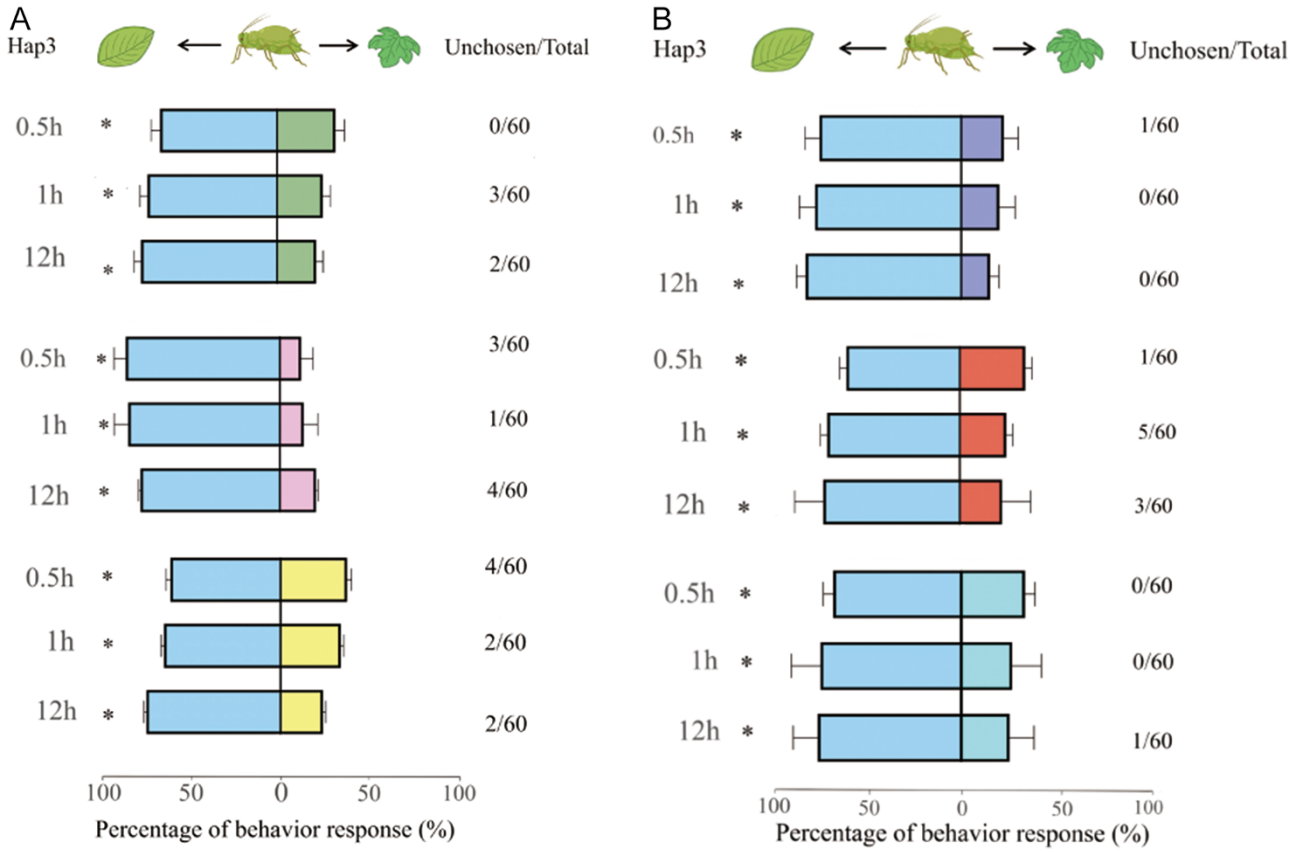


Figure 2. Behaviour selection proportion of Hap3 *A. gossypii* on cucumber and different *C. annuum* varieties. (A) Behaviour selection proportion of Hap3 *A. gossypii* on cucumber and SE, LA, LS. present cucumber (blue), present SE (green), present LS (yellow), present LA (pink). (B) Behaviour selection proportion of Hap3 *A. gossypii* on cucumber and LZ, HM, CT. present LZ (purple), present HM (red), and present CT (light blue). '*' indicates significant difference at $P < 0.05$ level by the chi-square test.

host. However, parameters are more favourable on LZ, indicating a higher susceptibility to aphid infestation. Despite this, Hap3 displays more robust life table parameters across all pepper varieties compared to Hap17 ($P < 0.05$), indicating an increased potential risk to pepper crops (table 1).

A. gossypii *host selection behaviour experiment on* C. annuum *and cucumbers*

The host selection behaviour of Hap3 and Hap17 *A. gossypii* was assessed between *C. annuum* varieties and cucumber. Significant preferences were observed in some cases, while in others, no significant differences were found. The behaviour selection of Hap3 *A. gossypii* when exposed to cucumber and different varieties of pepper demonstrated significant differences. The experimental results indicate that the selection rate of Hap3 *A. gossypii* for cucumber is significantly higher relative to any variety of pepper (fig. 2).

Hap3 *A. gossypii* were simultaneously introduced to SE, LA, LS, LZ, HM, CT leaves, and cucumber leaves. After 12 hours, the population on cucumber leaves consistently increased while the population on all other leaves decreased. Specifically, for SE, the population on SE decreased from 33% to 23%, while the cucumber population increased from 67% to 77%. For LA, the population on LA increased from 14% to 21%, while the cucumber population decreased from 86% to 79%. For LS, the population on LS decreased from 37% to 24%, while the cucumber population increased from 63% to 76% (fig. 2A). For LZ, the population on LZ decreased from 23% to 15%, while the cucumber population increased from 77% to 85%. For HM, the population on HM decreased from 36% to 23%, while the cucumber population increased from 64% to 77%. For CT, the population on CT decreased from 33% to 30%, while the cucumber population increased from 67% to 70% (fig. 2B). At intervals of 0.5 hours, 1 hour, and 12 hours, Hap3 *A. gossypii* consistently showed a significantly higher preference for cucumber leaves over all other leaves tested.

Similarly, we conducted selection behaviour experiments for Hap3 and Hap17 *A. gossypii* on different pepper varieties. The results indicated that the aphids' preference for peppers was not as pronounced as their preference when choosing between cucumber and pepper varieties. As discussed in the previous section, although the selection rate of *A. gossypii* for cucumber decreased at several time intervals, cucumber was overall significantly more attractive to *A. gossypii* than any pepper variety. However, the behavioural selection results of *A. gossypii* on different pepper varieties did not show such a consistent trend.

Further analysis of the behavioural selection experiment results shows that Hap3 *A. gossypii* significantly prefers CT and LZ pepper varieties. In the experiment with LZ and CT, the population on LZ decreased from 45% to 33%, while on CT it increased from 55% to 67%, showing significant differences at

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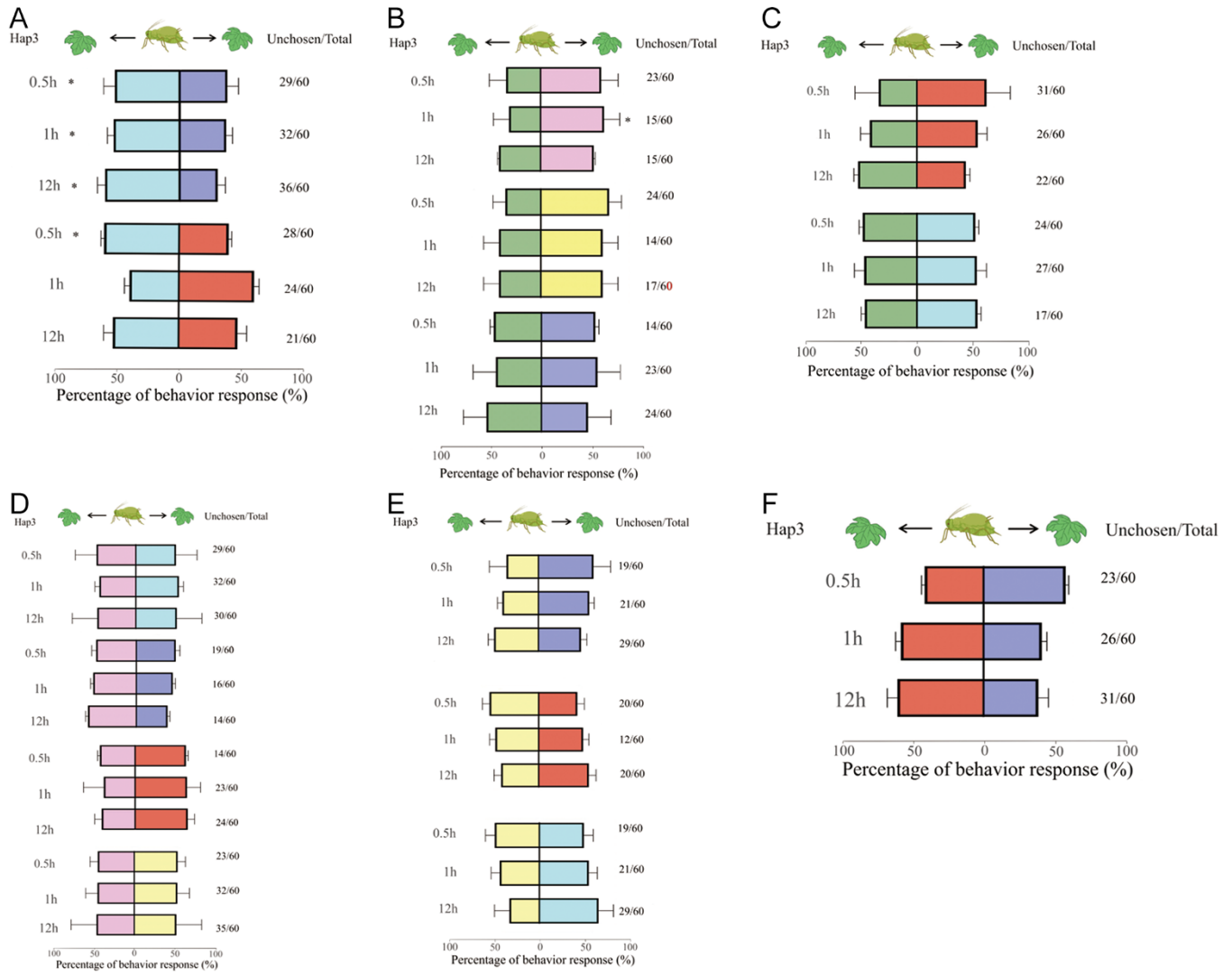


Figure 3. Behaviour selection proportion of Hap3 *A. gossypii* among different *C. annuum* varieties. (A) Behaviour selection proportion of Hap3 *A. gossypii* on CT (light blue) and LZ (purple), HM (red). (B) Behaviour selection proportion of Hap3 *A. gossypii* on SE (green) and LA (pink), LS (yellow), and LZ (purple). (C) Behaviour selection proportion of Hap3 *A. gossypii* on SE (green) and HM (red), CT (light blue). (D) Behaviour selection proportion of Hap3 *A. gossypii* on LA (pink) and CT (light blue), LZ (purple), HM (red), LS (yellow). (E) Behaviour selection proportion of Hap3 *A. gossypii* on LS (yellow) and LZ (purple), HM (red), CT (light blue). (F) Behaviour selection proportion of Hap3 *A. gossypii* on HM (red) and LZ (purple). '*' indicates a significant difference at $P < 0.05$ level by the chi-square test.

0.5, 1, and 12 hours (fig. 3A). In the experiment with HM and CT, the population on HM increased from 39% to 43%, while on CT it decreased from 61% to 57%. The initial preference was for CT within 30 minutes, but shifted to HM at the 1 hour mark (fig. 3A). When exposed to SE and LA leaves, Hap3 *A. gossypii* showed a lower preference for SE relative to LA, with a significantly higher preference for LA after 1 hour (fig. 3A). These results demonstrate Hap3 *A. gossypii*'s varying preferences for different pepper varieties, with notable preferences for CT and LA.

The behavioural selection preference of Hap17 *A. gossypii* among different pepper varieties is not distinct. Notably, Hap17 *A. gossypii* exhibited significant differences only between the CT and LZ varieties. After 12 hours of introducing Hap17 *A. gossypii* to CT and LZ, the *A. gossypii* population on LZ rose from 23% to 31%, whereas on CT, it fell from 77% to 69%. Hap17 *A. gossypii* exhibited a marked preference for CT over LZ at 0.5, 1, and 12 hours, showing a significant inclination towards CT (fig. 4).

Apart from the aforementioned significant differences, the selection of Hap3 and Hap17 *A. gossypii* for most other pepper varieties exhibited some fluctuations but did not show significant differences. Specifically, after 12 hours of treatment, the Hap3 *A. gossypii* population fluctuated on both SE (41%–46%) and LS (59%–64%). When introduced to SE and LZ, the Hap3 *A. gossypii* population slightly increased on the former (47%–52%) and decreased on the latter (53%–48%), showing no significant preference difference. Continuous increases in the Hap3 *A. gossypii* population were observed on SE (41%–55%) and decreases on HM (59%–45%). Similarly, after 12 hours, the preference ratios between SE and CT showed no significant difference. After 12 hours, Hap3 *A. gossypii* showed a similar low preference for LA and CT, with populations slightly fluctuating. The populations on LA (45%–47%) and CT (55%–53%) showed no significant difference.

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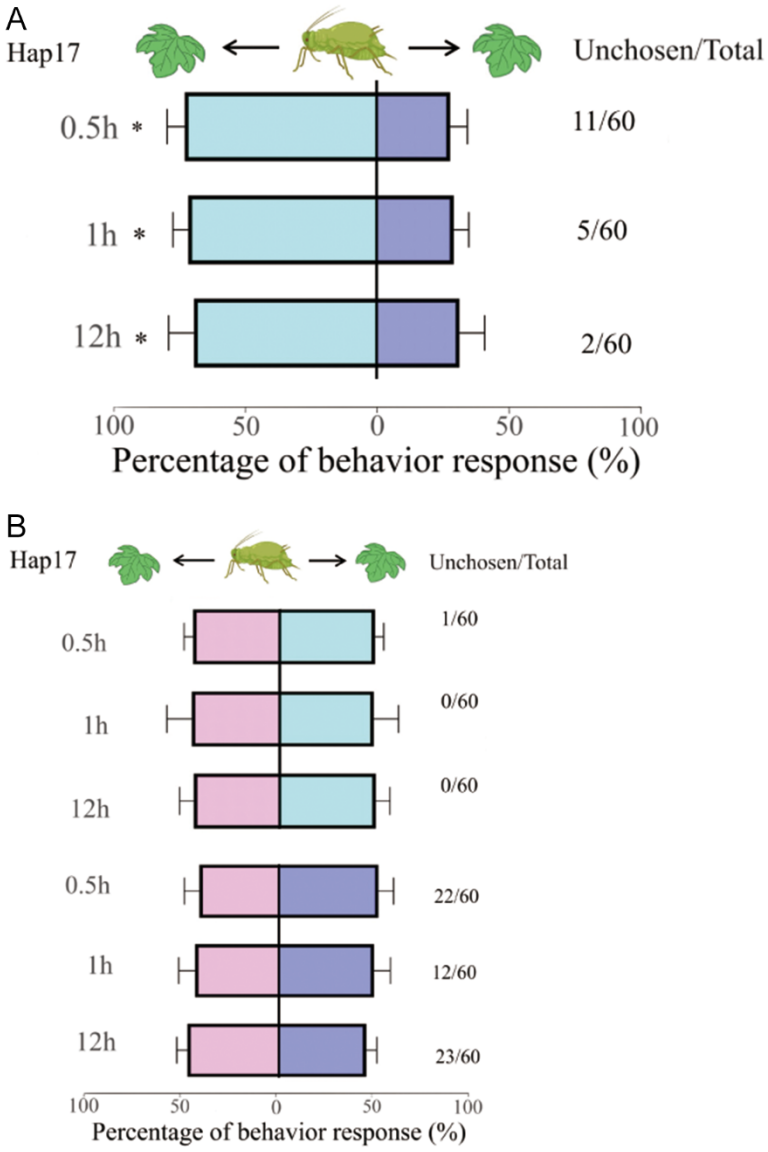


Figure 4. Behaviour selection proportion of Hap17 *A. gossypii* on cucumber and different *C. annuum* varieties. (A) Behaviour selection proportion of Hap17 *A. gossypii* on CT (light blue) and LZ (purple). (B) Behaviour selection proportion of Hap3 *A. gossypii* on LA (pink) and CT (light blue), LZ (purple). '*' indicates significant difference at $P < 0.05$ level by the chi-square test.

Introducing Hap3 *A. gossypii* to LA and LZ resulted in an increased population on LA (51%–61%) and a decreased population on LZ (49%–39%). HM consistently showed a higher preference than LA. When exposed to both LA and LS, the populations slightly changed, showing no significant difference. In experiments, the Hap3 *A. gossypii* population increased on LS (40%–51%) and decreased on LZ (60%–49%), showing no significant difference. The Hap3 *A. gossypii* population on HM increased (43%–56%), while it decreased on LS (57%–44%). Similarly, the Hap3 *A. gossypii* population decreased on LS (52%–40%) and increased on CT (48%–60%), showing no significant preference. After 12 hours, the Hap3 *A. gossypii* population decreased on LZ (from 57% to 41%), while it increased on HM (from 43% to 59%). In the experiment with Hap17 *A. gossypii* introduced to CT and LA, after 12 hours, the Hap17 *A. gossypii* population on CT slightly decreased (52%–50%) and slightly increased on LA (48%–50%), showing no significant difference. Similarly, when introduced to LZ and LA, the Hap17 *A. gossypii* population on LZ decreased (52%–46%) and increased on LA (48%–54%), with no significant preference difference.

Discussion

C. annuum is a crucial crop in agricultural production, both for its widespread consumption and its inherent resistance to various agricultural pests. This resistance is largely attributed to the diverse array of natural compounds found in peppers, which play an essential role in pest defence. For example, methyl jasmonate (MeJA) and methyl salicylate (MeSA) have been shown to inhibit the development, reproduction, and population growth of *Myzus persicae* on peppers (Fan *et al.*, 2021). In addition, the total free amino acids, soluble sugars, free proline, and vitamin C in pepper leaf tissues correlate strongly with their resistance to *Myzus persicae* (Chen, 2002). Elevated levels of chlorophyll, soluble proteins, and phenolic compounds contribute further to pest resistance (Li *et al.*, 2017). Moreover, tannin content in different pepper varieties has been shown to affect resistance to *Frankliniella occidentalis*, with resistant varieties maintaining higher tannin levels after thrips feeding (Jia *et al.*, 2018). Additionally, transgenic approaches such as stacking AtEDT1/HDG11 and Cry2Aa2 in pepper plants have significantly enhanced resistance to pests like *Prodenia litura*, with an 81.3% mortality rate in larvae (Zhu *et al.*, 2015).

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Building on these findings, we further investigated the survival, development, and behavioural preferences of Hap3 and Hap17 *A. gossypii* on various *C. annuum* varieties. The performance of both haplotypes was markedly different across the varieties, which highlights the intricate relationship between aphids and their host plants. These findings contribute to a deeper understanding of the complex interactions between *A. gossypii* and *C. annuum*, providing valuable insights for pest management strategies. This research not only deepens our knowledge of the intrinsic pest resistance mechanisms in peppers but also provides crucial guidance for breeding new pest-resistant varieties. By analysing the survival rates, developmental speeds, and behavioural preferences of Hap3 and Hap17 *A. gossypii* on various pepper varieties, we can more accurately identify the plant traits that are critical for resisting these specific pests. These findings will offer new perspectives for the development of agricultural biotechnology, aiding in the design of more effective pest management strategies.

While our experiments provide valuable insights into the survival, development, and host selection behaviour of *A. gossypii* on *C. annuum* varieties, the study has several limitations. Firstly, the number of pepper varieties examined is limited, which may constrain the applicability of our findings to other *C. annuum* cultivars. Additionally, our focus on two specific haplotypes, Hap3 and Hap17, may restrict the broader generalisation of these results to other *A. gossypii* populations with different genetic backgrounds. The results should, therefore, be considered specific to the varieties and haplotypes tested. Moreover, this study does not address the overall adaptability of *A. gossypii* across a wider range of host plants or environmental conditions. Future research incorporating a broader selection of *C. annuum* varieties and additional haplotypes would help to further elucidate the factors influencing *A. gossypii* adaptability and expand the potential applications of these findings in pest management strategies.

The differences in adaptability observed between Hap3 and Hap17 *A. gossypii* populations could be attributed to various factors related to the physical and chemical properties of the pepper varieties. One potential mechanism involves leaf texture: rougher surfaces may deter aphid feeding, while smoother leaves may facilitate sap extraction. Key physical traits, such as trichome density, vascular bundle depth, wax content, and leaf colour, may influence aphid behaviour. Increased trichome density and wax content, for example, can act as physical barriers to aphids, whereas smoother leaf surfaces may provide easier access to sap, supporting *A. gossypii* survival and reproduction (He *et al.*, 2017). Additionally, nutritional components of the host plant, such as free amino acids, sugars, and nitrogen content, may significantly influence aphid survival and reproduction. Varieties with higher concentrations of these nutrients might provide a more suitable environment for aphid development (Guo *et al.*, 2024). Furthermore, secondary metabolites such as phenolic compounds, tannins, and alkaloids are known to have insecticidal effects, influencing aphid behaviour and survival (Farhan *et al.*, 2024). For instance, higher levels of phenolic compounds and flavonoids in certain pepper varieties may act as a deterrent for aphid feeding, thus reducing their reproductive success (Zhao *et al.*, 2019). These factors, in combination, could explain why certain haplotypes perform better on specific varieties. Investigating the specific role of each of these factors in determining aphid fitness will be essential for a more comprehensive understanding of the mechanisms behind host-plant interactions and aphid adaptability.

In this study, Hap3 and Hap17 *A. gossypii* fed on different *C. annuum* varieties, reproducing on most pepper varieties. This is consistent with the findings of Wang et al., who found that the main *A. gossypii* on *C. annuum* in the Shihezi region are cotton aphids and peach aphids (Wang *et al.*, 2016), which might be related to the geographical location, crop layout, and climatic conditions of the survey area.

Understanding these interactions not only deepens our knowledge of the mechanisms underlying pest resistance in peppers but also provides crucial guidance for breeding new pest-resistant varieties. By selecting pepper varieties that exhibit lower adaptability to *A. gossypii*, we can effectively manage and control aphid populations. For instance, promoting varieties such as LA, which show poor adaptability for both Hap3 and Hap17 *A. gossypii*, in regions heavily infested by these aphids, can significantly reduce aphid-related damage. Additionally, incorporating traits such as rough leaf surfaces, which hinder aphid feeding, or higher levels of defensive secondary metabolites, can further enhance resistance (Chen *et al.*, 2011). This approach leverages the natural defences of the plants and can reduce the reliance on chemical pesticides, leading to more sustainable pest management practices.

Moreover, understanding the specific nutritional and physical traits that confer resistance can be invaluable for targeted breeding programs. By crossbreeding susceptible varieties with resistant ones, we can develop new pepper cultivars that are both high-yielding and resistant to aphids. These breeding programs can focus on enhancing traits such as increased levels of phenolic compounds, which are known to deter aphid feeding, or optimising the plant's overall health to improve its natural defence mechanisms. Integrated pest management strategies that combine the use of resistant cultivars with other control measures, such as biological control agents and cultural practices, not only help in controlling aphid populations but also contribute to the overall health and productivity of pepper crops. This holistic approach ensures that pest management is both effective and sustainable, benefiting both the environment and agricultural productivity.

Summarising the results of this study: Hap3 and Hap17 *A. gossypii* are more suited for growth and development on SE and LZ, thus the possibility of outbreaks causing damage on these two *C. annuum* is higher. Attention should be paid to the fluctuation of *A. gossypii* populations on these two *C. annuum*, with real-time monitoring of population dynamics and timely pest control measures. Additionally, it might be worth considering using *C. annuum* varieties that are less suitable for *A. gossypii* adaptation as the main crop varieties for aphid pest control.

Financial support. This research was supported by the National Natural Science Foundation of China (Grant No. 32272521).

Competing interests. No potential conflict of interest was reported by the authors.

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