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Effect of temperature on predation by *Harmonia axyridis* (Pall.) (Coleoptera: Coccinellidae) on the walnut aphids *Chromaphis juglandicola* Kalt. and *Panaphis juglandis* (Goeze)

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Abstract

Background: The walnut aphid species *Chromaphis juglandicola* Kalt. and *Panaphis juglandis* (Goeze) are destructive insect pests. *Harmonia axyridis* (Pall.) (Coleoptera: Coccinellidae) is the main predatory insect with a wide geographical distribution. The feeding behavior of the predator against the two different aphid species might influence bio-control efficacy in walnut orchards.

Main body: Functional response of *H. axyridis* to various densities of the two aphid species was examined under temperatures ranging from 15 to 30 °C. The results showed that functional responses of *H. axyridis* towards *C. juglandicola* or *P. juglandis* fitted well with the Holling-II equation within the range of 15–30 °C. A greater biomass of aphids was consumed when the temperature increased from 15 to 30 °C. The predation efficacy of *H. axyridis* against *C. juglandicola* was greater than against *P. juglandis*, and the searching efficiency of *H. axyridis* against *C. juglandicola* was more effective than against *P. juglandis*. Moreover, predation rates against both aphid species decreased with increasing the *H. axyridis* density.

Conclusion: This study showed that *H. axyridis* was an effective predator against the two walnut aphids. Increasing temperature (15–30 °C) increased prey consumption. Interference between individuals from increasing predator density had a negative impact on predation rate against the two aphid species.

Keywords: Walnut aphid, Biological control, Functional response, Temperature, Predator interference

Background

The small walnut aphid, *Chromaphis juglandicola* Kaltenbach and the large walnut aphid, *Panaphis juglandis* Goeze (Hemiptera: Aphididae), are distributed mainly in temperate regions and are destructive pests in walnut orchards in Xinjiang Uygur Autonomous Region, north-western China (Xing et al. 2018). *Chromaphis juglandicola* lives on the undersides of the leaves of walnut trees.

Panaphis juglandis typically feeds along the midrib of the upper surface of the leaves. These two species are not usually found together on the same tree in the Kashmir valley, India (Wani and Ahmad 2014a, b), while both species commonly coexist at the same orchard in the Yili River Valley in Xinjiang Uygur Autonomous Region (Xing et al. 2018).

Coccinellids (lady beetles) are the most important and dominant predatory species regulating populations of the walnut aphid *C. juglandicola* (Sluss 1967). *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae) is the main Coccinellid species that feeds on walnut aphids in Yili

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River Valley (Xing et al. 2018). *Harmonia axyridis* was introduced to North America (Gordon 1985) and Europe (Katsoyannos et al. 1997, Iperti and Bertand 2001) as a biological control agent. Knowledge about the capacity of a predator to kill target prey is essential to developing comprehensive strategies for its release as a biological control agent (Symondson et al. 2002).

Temperature is one of the main abiotic factors influencing the potential of natural enemies to suppress pests (Zamani et al. 2006; Sarnevesht et al. 2018; Su et al. 2018; Lin et al. 2019, Luhring et al. 2019), but its effects may differ between different predator species. Little is known about the functional response of *H. axyridis* towards the two walnut aphid species above at different temperatures, although this information is requisite to establishing an effective biological control strategy using *H. axyridis*. The walnut aphids are becoming a more serious problem as the development of walnut orchards intensifies in China. The objective of this study was to determine the effect of temperature on the functional response of *H. axyridis* against *C. juglandicola* and *P. juglandis* of different densities and at different temperatures.

Materials and methods

Sources of *H. axyridis*, *C. juglandicola*, and *P. juglandis*

The adult lady beetles of *H. axyridis* and the two aphid species, *C. juglandicola* and *P. juglandis*, used in this study were collected from fresh leaves of walnut trees in Yili(N43°14'53", E82°49'40"), Xinjiang, China. Aphid colonies were maintained on young walnut plants in a controlled-climate room held at 25 ± 1 °C, 60 ± 10% RH, and under a photoperiod of 14:10 h (L:D).

Functional response: predation by *H. axyridis* on aphids at various densities and four temperatures

The functional response (number of aphids consumed per day) of *H. axyridis* was determined by offering varying densities of 2nd/3rd instar nymphs of each aphid species separately on a walnut leaf disc placed in a Petri dish (90 mm by 18 mm) lined on the base with moist filter paper. Prey densities were 80, 160, 240, 320, 400, and 600 aphid nymphs of *C. juglandicola* and 60, 120, 180, 240, 300, 360 aphid nymphs of *P. juglandis*. After adding the prey, one adult of *H. axyridis*, starved for 24 h, was added to and confined in the Petri dish. The Petri dishes were kept in environmental chambers under four different constant temperatures: 15, 20, 25, and 30 ± 1 °C, with 60 ± 10% RH and a light: dark photoperiod of 14:10 h. The number of aphids consumed by the *H. axyridis* was recorded after 24 h. Each aphid density and aphid species was replicated six times for each temperature, simultaneously.

Intraspecific interference within *H. axyridis* whilst feeding on aphids

To test how intraspecific interference affected predation by *H. axyridis* on walnut aphids, one, two, three, four, or five adult(s) of *H. axyridis* lady beetles were starved for 24 h and then added to Petri dishes (as above) containing 600 2nd/3rd instar nymphs of each aphid species separately. The number of aphids consumed was recorded after 24 h. Experiments were conducted under 25 ± 1 °C, 60 ± 10% RH and a photoperiod of 14:10 h (L:D). Six replicate experiments using each *H. axyridis* density were undertaken.

Data analysis

The Holling's type II equation (Holling 1959, 2003) was used to model the functional response of *H. axyridis* preying on aphids:

$$N_a = aTN_0 / (1 + aT_hN_0) \quad (1)$$

where N_a is the number of aphids consumed per day and N_0 is the initial number of prey (aphids); T_h is the handling time of *H. axyridis* preying on aphids, a is the successful attack rate, and T is the time of the experiment, which in this case was one day.

The values of a and T_h were found by linearly regressing $1/N_a$ against $1/N_0$. The resultant y -intercept is the initial estimate of T_h and the reciprocal of the regression coefficient (slope) is an estimate of a (Livdahl and Stiven 1983). We used equation (1) $N_a = aTN_0 / (1 + aT_hN_0)$, when N_0 was infinitely great. The equation, $N_a/N_0 = T / T_h$, estimated maximum predation number.

The searching efficiency was calculated as

$$S = a / (1 + aT_hN) \quad (2)$$

where S is searching efficiency, a is the successful attack rate, T_h is the handling time and N is the number of prey.

The average predation rate was calculated using Watt's (1959) equation:

$$A = QP^{-m} \quad (3)$$

where A is the average predation rate, P is predator density of 1, 2, 3, 4, or 5 *H. axyridis* adults, m is the coefficient of interference, and Q is the seeking constant. The values of Q and m were found by power-exponential regressing A and P .

Model fitting was performed using Origin version 7.5 for Windows.

Results and discussion

The functional response of *H. axyridis* lady beetles preying on *C. juglandicola* and *P. juglandis* was described well by the Holling's type II equation within the range of

15–30°C (Fig. 1). The number of aphids consumed daily by *H. axyridis* increased gradually until reaching the upper asymptote of 219, 239, 256, 278 aphids/predator for *C. juglandicola* and 99, 129, 156, 177 aphids/predator for *P. juglandis* at 15, 20, 25, and 30°C, respectively (Fig. 1). This result is in accordance with the results of Lee and Kang (2004), Atlihan et al. (2010) and Islam et al. (2020).

The predation of *H. axyridis* on *C. juglandicola* and *P. juglandis* was different within the range of 15–30 °C (Fig. 1 and Table 1). The attacking efficiency of *H. axyridis* was 0.7944 to 1.0862 on *C. juglandicola* and 0.5798 to 0.8468 on *P. juglandis* within the range of 15–30 °C. The predation capacity (a/T_h) and the maximum daily predation ($1/T_h$) of *H. axyridis* on *C. juglandicola* was higher than on *P. juglandis*. Moreover, the handling time (T_h) of *H. axyridis* was 0.0031 to 0.0019 days on *C. juglandicola* and 0.0075 to 0.0029 days on *P. juglandis* within the range of 15–30 °C. This might be because the body size of *P. juglandis* is larger than *C. juglandicola*, and *H. axyridis* need much more time to handle and digest accordingly.

Temperature significantly influenced the predation capacity of *H. axyridis* to walnut aphids (Fig. 1 and Table 1). The predation capacity of *H. axyridis* and the maximum daily number of walnut aphids eaten (predation number) increased as the temperature increased from 15 to 30 °C. This agrees with the finding of Schwarz and Frank (2019) that *H. axyridis*, as well as three other lady beetle species, consumed more aphid biomass under increasing temperatures. Hong et al. (2013) also found that the predation capacity of

Micraspis discolor Fabricius (Coleoptera: Coccinellidae) feeding on *Brevicoryne brassicae* L. (Hemiptera: Aphididae) was higher at 30.7 °C than at 23.5 °C. The handling time by *H. axyridis* of walnut aphids was shortened as the temperature increased, which is in accordance with the finding of Jalali et al. (2010) who reported that the handling time of *Adalia bipunctata* L. (Coleoptera: Coccinellidae) to *Myzus persicae* Sulzer (Hemiptera: Aphididae) significantly decreased as the temperature increased from 19 to 27 °C.

The searching efficiency (S) by *H. axyridis* for walnut aphids decreased as the prey density increased within the range of 15–30°C, and increased as the temperature increased from 15 to 30 °C (Fig. 2). *Harmonia axyridis* spent more time undertaking non-searching activities at low temperatures (15 °C), whereas more time searching occurred at higher temperatures. This may be attributed to temperature-related changes in the metabolism and activity of the predator (McCoull et al. 1998). The searching efficiency by *H. axyridis* for *C. juglandicola* was higher than for *P. juglandis* under each temperature regime. *Chromaphis juglandicola* and *P. juglandis* live on the undersides and the upper surface of the leaves in the field, respectively. Feng et al. (2018) found that prey distribution could affect *H. axyridis* foraging under relative high prey densities. Spatial heterogeneity also play a role in predator–prey systems (Hauzy et al. 2010). The small size of arenas used in laboratory predation experiments may be not representative of the natural searching efficiency of a predator (Murdoch, 1983). Nevertheless, our study has value as a first step in evaluating *H. axyridis* as a biological control agent of walnut aphids.

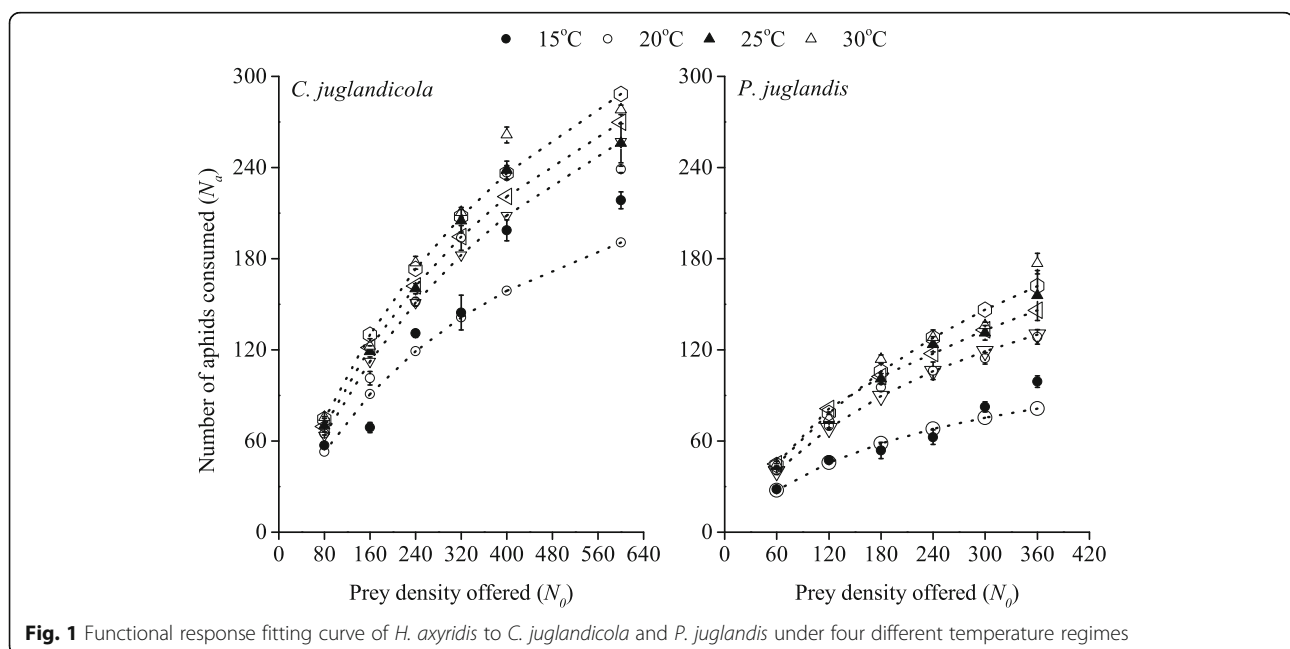


Table 1 Functional response of *H. axyridis* on *C. juglandicola* and *P. juglandis* under four different temperature regimes

Aphids	Temp. (°C)	Attacking efficiency (a)	Handling time T_h (d)	Predation capacity a/T_h	Maximum predation number $1/T_h$	Holling's type II equation	R^2
<i>C. juglandicola</i>	15	0.7944	0.0031	256.2604	322.5806	$Na = 0.7944N/(1 + 0.0025N)$	0.8756
	20	0.9169	0.0021	434.5543	473.9336	$Na = 0.9169N/(1 + 0.0019N)$	0.9821
	25	1.0158	0.0020	495.5050	487.8049	$Na = 1.0158N/(1 + 0.0021N)$	0.9964
	30	1.0862	0.0019	565.7481	520.8333	$Na = 1.0862N/(1 + 0.0021N)$	0.9923
<i>P. juglandis</i>	15	0.5798	0.0075	77.2065	133.1558	$Na = 0.5798N/(1 + 0.0044N)$	0.9641
	20	0.8034	0.0042	189.4811	235.8491	$Na = 0.8034N/(1 + 0.0034N)$	0.9969
	25	0.8386	0.0035	236.8973	282.4859	$Na = 0.8386N/(1 + 0.0030N)$	0.9978
	30	0.8468	0.0029	293.0194	346.0208	$Na = 0.8468N/(1 + 0.0024N)$	0.9923

The rates of predation by *H. axyridis* on *C. juglandicola* were 42.7, 28.7, 19.4, 17.5, and 17.0%; and on *P. juglandis* 24.9, 22.5, 18.6, 17.5, and 14.8% at the predator densities of one, two, three, four and five, respectively. These results indicated intraspecific interference and a negative impact on predation when more than one predator was present (Fig. 3). Feng et al. (2019) found that interference between *H. axyridis* conspecifics might alter their foraging patterns. The coefficient of predator interference was 0.6124 when feeding on *C. juglandicola*, and 0.3134 when feeding on *P. juglandis*, which showed that interference between predators had a greater negative effect

against *C. juglandicola* than against *P. juglandis*. This might be because *C. juglandicola* is more mobile than *P. juglandis*.

Conclusion

This study showed the potential of using *H. axyridis* lady beetles as biological control agents against two walnut aphid species, *C. juglandicola* and *P. juglandis*, under temperatures of 15–30 °C. Further studies to determine the effect of inter- and intraspecies interference of predatory species should be conducted in the field.

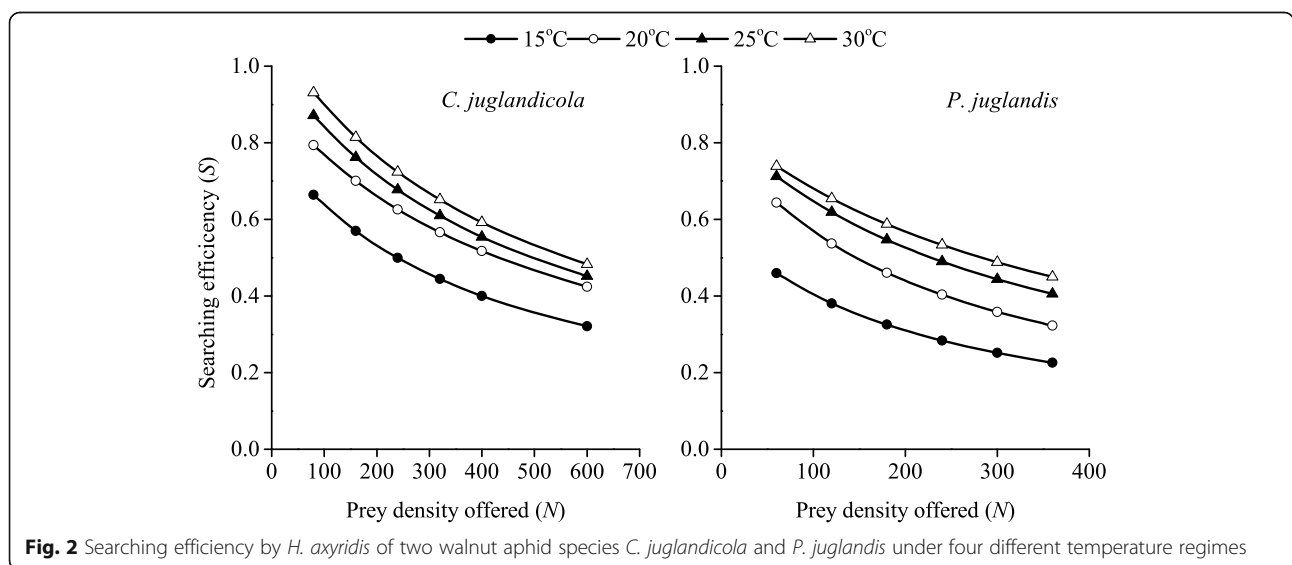


Fig. 2 Searching efficiency by *H. axyridis* of two walnut aphid species *C. juglandicola* and *P. juglandis* under four different temperature regimes

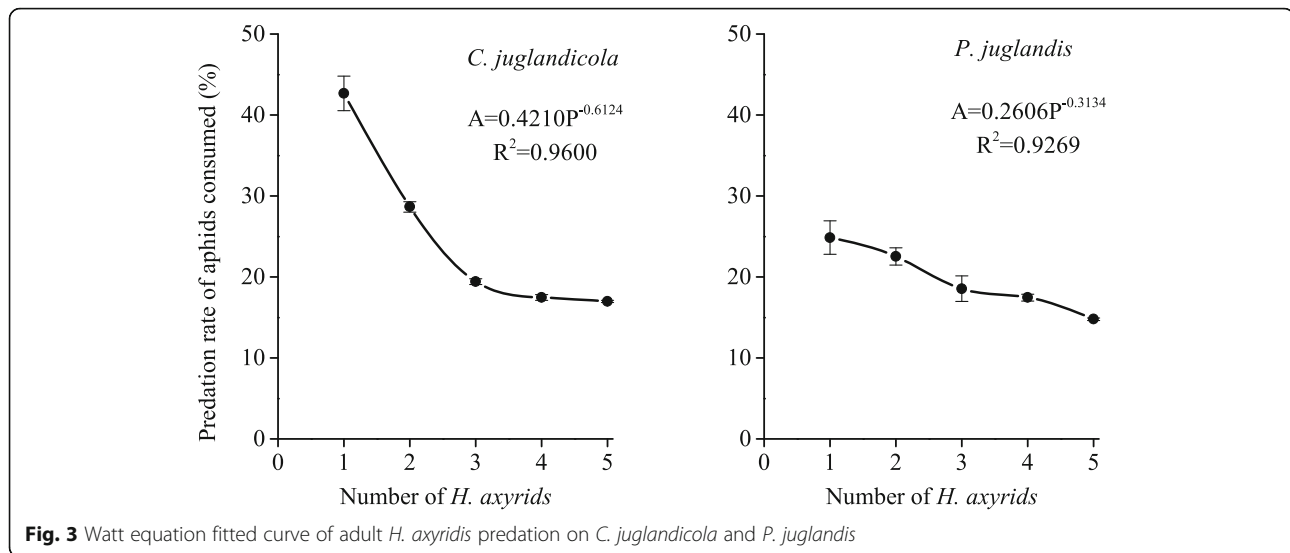


Fig. 3 Watt equation fitted curve of adult *H. axyridis* predation on *C. juglandicola* and *P. juglandis*

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Authors' contributions

GZG and ZZL conceived and designed the experiments. SQL and YLW performed the experiments. GZG and SQL analyzed the data. GZG and ZZL wrote the manuscript main text. LKF 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.

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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