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Laboratory evaluation of functional responses of predator *Anthocoris nemoralis* (Hemiptera: Anthocoridae) to *Cacopsylla pyri* (Hemiptera: Psyllidae), at two temperatures

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Abstract

Biological control constitutes an effective approach for managing pest populations in horticulture, providing an alternative to chemical pesticide reliance. To ensure successful integration of predators into biological control programs, a precise assessment of their functional response to prey density variations under controlled laboratory conditions is essential. This study aimed to investigate the functional response of 5th stage nymph, male and female of *Anthocoris nemoralis* predator to pear psylla *Cacopsylla pyri* 4th and 5th nymphs on two temperatures (15 and 27 °C). Logistic regression followed by nonlinear least squares regression were used to determine the type of functional response of predator and to estimate the attack rate (a) and the handling time (T_h). Our results exhibited a type II functional response for all experiments. There was a significant difference between attack rate of same stage of predator due to raising temperatures (a positive relationship), whereas no significant differences were observed for handling times (a negative relationship). 5th stage nymph showed the lowest attack rate and the highest handling time compared to other stages (0. 010, 0. 028 for a and 1. 846, 1. 658 for T_h at 15 and 27 °C, respectively), whereas females had the highest attack rate and the shortest handling time at all experiments (0. 035, 0. 086 for a and 1. 347, 1. 658 at 15 and 27 °C, respectively), anyway there was no significant difference between parameters of 5th stage nymph and male. Female had the highest maximum theoretical attack rate (20. 924 and 30. 341 nymph/day) at 15 and 27 °C, respectively. Our findings demonstrate that adult female of *A. nemoralis* can be used as optimal biological agents for controlling *C. pyri*.

Keywords: Functional response, temperature, *Anthocoris nemoralis*, pear psylla, *Cacopsylla pyri*.

Introduction

The pear psylla, *Cacopsylla pyri* (Linne) (Hemiptera: Psyllidae), is one of the most serious economic pest of pear trees *Pyrus communis* Linnaeus, (Rosaceae) worldwide (Nin *et al*, 2012) [54], which may cause heavy economical losses to most pear growing regions (Tešanović and Spasić, 2018) [75]. This pest can damage pear trees in two main ways classified as direct damages including weakening the plant by subtraction of nutrients; and indirect damages like production of large amounts of honeydew which stimulates the black sooty mold (Montanari *et al.*, 2015) [47], and the possible transmission of pear decline disease (*Candidatus* Phytoplasma pyri); which decreases pear vegetative and fruit growth eventually leading to the death of trees (Süle *et al.*, 2007) [73]. For the beginning of laying egg by overwintered individuals a temperature over 10 °C for two successive days is required, as the first eggs are laid directly on wood, typically near of unopened bud bases (Nin *et al.*, 2012) [54], *C. pyri* develops 5-7 generations per year (Nin *et al.*, 2012) [54], and it has five development nymphal stages (Tešanović and Spasić, 2018) [75]. Throughout years and because of excessive use of chemical control, pear psylla has developed resistance to many commonly used insecticides (Emami, 2008; Civolani, 2012) [14, 7], consequently, there has been growing interest in adopting Integrated Pest Management (IPM) strategies of pear psylla,

with particular emphasis on enhancing populations of natural enemy populations against this pest (Shaw *et al.*, 2021) [69]. Several species of generalist predators commonly attack *C. pyri* like Coccinellidae, Chrysopidae, Araneae, Miridae and Anthocoridae (Daugherty *et al.*, 2007) [8]. Anthocorid bugs (Anthocoridae) are among the most important predators of pear psylla, with *Anthocoris nemoralis* (Fabricius) (Hemiptera: Anthocoridae) being a particularly effective biological control agent of pear psylla, its natural range includes Europe and the Mediterranean region (Krndija *et al.*, 2024) [39]. The adult overwinters beneath tree bark and other sheltered places, emerging when prey availability and weather conditions become favorable (Sigsgaard, 2010) [70], then usually peaks during mid to late summer, helping to suppress *C. pyri* populations (Nagy *et al.*, 2008) [51]. This predator has five nymphal instars, and both mature and immature stages feed on eggs as well as nymphs of pear psylla, and effectively reduce their numbers (Sigsgaard *et al.*, 2006; Emami, 2010) [71, 15], an individual anthocorid predator can consume approximately 5,000 pear psylla eggs during its lifetime (Yanik and Ugur, 2004) [83], showing equal feeding efficiency on both eggs and nymphs per unit biomass (Sigsgaard, 2010) [70]. In different ecological conditions, *A. nemoralis* might give three up to six generations/year being affected to temperature (Oudeh *et al.*, 2013) [54]. Researches showed that anthocorids can be

released artificially into orchards as a biological control agents, to suppress pear psylla populations effectively (Gajski and Pekár, 2021), adult anthocorids as well as nymphs are able to bring about 30-50% reductions or more in pear psylla numbers in pear orchards (Sigsgaard *et al.*, 2006) ^[71], also augmentative releases of the immature stage of *A. nemoralis* in olive orchards resulted in significant reductions in psylla densities (Gharbi, 2021) ^[20].

The functional response is the basic mechanism that reveals different aspects of prey-predator behavior, explains their coevolutionary interactions, and can determine the efficiency of a predator in regulating prey populations (Holling, 1965; Martinou *et al.*, 2010) ^[30, 44]. According to Solomon (1949) ^[72] and Holling (1959) ^[32], the number of prey consumed per predator as a function of different prey densities is known as the functional response. The functional response of a predator is determined by two important parameters: attack rate (a) which conducts the ability to find a prey, and the handling (T_h) which represents how fast a prey is consumed including discovering of a prey, killing, capturing, eating and digesting it (Holling, 1966) ^[31]. Three main functional response types are observed in predator-prey systems, a type I functional response shows a linear relationship between consumption rate and prey density, a type II functional response shows a hyperbolic relationship between consumption rate and prey density, that eventually reaches the predator's satiation level and remains constant at high densities and type III functional response which exhibits a sigmoid relationship between the number of prey consumed and the number of prey offered, as at low prey densities the predator consumption increases slowly, then accelerates at intermediate densities, and finally plateaus at high densities (Holling, 1959) ^[32].

Various abiotic and biotic factors can affect the functional response type and its parameters: attack rate (a) and handling time (T_h), such as host plant type (Emami *et al.*, 2014; Jafarian *et al.*, 2022) ^[12, 33], prey type and size (Donnelly and Phillips, 2001 ^[11]; Kalinoski and Delong, 2016 ^[37]; Hassanzadeh-Avval *et al.* 2019) ^[24], different life stages of predator or prey (Farhadi *et al.*, 2010 ^[17]; Shahpouri *et al.*, 2019 ^[68]; Hassanzadeh-Avval *et al.* 2019 ^[24], Dehkordi *et al.*, 2023) ^[9], sex of predator (Emami *et al.*, 2014 ^[12]; Hassanzadeh-Avval *et al.* 2019 ^[24]; Reeves *et al.*, 2023) ^[57], prey distribution (Feng *et al.*, 2018) ^[18], temperature (Gitonga *et al.*, 2012; adbouni, 2017 ^[43]; Hassanzadeh-Avval *et al.* 2019 ^[24]; Hassanpour *et al.*, 2020 ^[23]; Reeves *et al.*, 2023) ^[57], spatial complexity (Alexander, 2012) ^[2], presence of alternative prey (Murdoch, 1969; Wei and Walde, 1997) ^[50, 82], and arena size (Sabaghi *et al.*, 2011 ^[61]; Uiterwaal and Delong, 2018) ^[61].

Psylla mentioned as a key pest of pears in the climatic conditions of regions where spring and summer are dry and rainfall is rare, like The Middle East and near by. In Iran, Emami (2016) ^[13] mentioned *Cacopsylla pyricola* as an important pest of commercial pear in all pear-growing regions, also Esmaeily *et al.*, (2022) ^[16] mentioned that *Cacopsylla permixta* and *Cacopsylla bidens* are major pests of pears, also Latifian, and Abdollahi (2025) ^[40] mentioned *C. pyri* as an important pest of pear orchards In Syria, *C. pyri* recorded to infest pear orchards in north-west Damascus (Saour *et al.*, 2010) ^[62], also Almatni and

Alabdulla (2003) ^[4] mentioned pear psylla *C. bidens* in as a main pest of pear in south of Syria (Alswaidaa), Oudeh *et al.*, (2015) also mentioned pear psylla *C. pyricola* in pear orchards in the middle of Syria (Homs). In Turkey, Serce *et al.* (2006) ^[65] and Sertkaya *et al.* (2008) ^[66] mentioned *C. pyri* as a vector of pear decline diseases (PD) in the region of Turkey. In Iraq, despite projections that indicate sustained growth, with production expected to rise to 14,580 metric tons by 2026 for pear (a 12. 7% annual increase from 13,550 metric tons in 2025 (ReportLinker, 2025) ^[58], highlighting pear's escalating economic significance production of pear reached 10613. 27 metric tons in 2023, with a yield of 7068. 4 kg/h harvested from 1502 hectares (FAOSTAT, 2023) ^[19], research on pear insect pests particularly *C. pyri* still limited, underscoring the urgent need for focused studies on this damaging pest to support sustainable production of pear.

To date experiments of studying functional response for mature and immature stages of predator *A. nemoralis* at multiple temperatures, still scarce, so the present research was designed to observe the type of functional response of 5th stage nymphs, male and female predator *A. nemoralis* at two different temperatures (15 and 27 °C), The temperature 15 °C was selected as it is close to the thermal threshold for oviposition initiation in *C. pyri*, and 27°C represents an optimal developmental temperature for both pest and predator populations.

Materials and Methods

Collection and rearing of predator and prey

The experiment was conducted in 2023, different nymphal instars and adults of pear psylla and Anthocorid were collected from pear orchards in Babel governorate, pear psylla nymphs were hand-collected carefully using a fine-haired brush, to prevent injury to vulnerable nymph stage. For adult sampling, *A. nemoralis* and *C. pyri* individuals were captured via beat tray methodology: a foam-covered stick was used to sharply tap pear tree branches, while a white collection tray (230 × 450 mm) positioned beneath intercepted dislodged insects, besides collecting infested plant material (shoots with psyllids), and place them in ventilated cages, and harvest emerging predators, Adult *C. pyri* were maintained in transparent aerated plastic containers (10 cm diameter, 18 cm height) with 4 pear shoots (9 cm in length) damped with a wet cotton wool to prevent dehydration. Individuals were kept in an environmentally controlled growth chamber at 23 °C and 65% RH. Adult and nymphal stages of *C. pyri* were morphologically identified using the standardized psyllid key (Agroscience, 2022) ^[1]. Anthocorid individuals were maintained in an aerated plastic pots, and fed on *C. pyri* nymphs daily. The collected bugs were identified using the taxonomic keys of Linnavuori and Hosseini (2000) ^[42] and Hassanzadeh and Modarres (2010) ^[22] and adults were allowed to mate as they were kept in one container at 23 °C in the controlled chamber.

Sex determination of anthocorids was conducted via light microscopic examination of genitalia. Individuals with inconclusive morphology were subsequently dissected to detect male-specific parameres (Hassanzadeh-Avval *et al.* 2020), or female copulatory tubes (Bu, 2007) ^[6].

Functional Response Experiments

Trials were conducted in a 10 cm petri dish, the petri dish lid fitted with a ventilation hole covered with fine gauze. fresh pear leaf discs (30 mm) were placed abaxial side up on a moistened filter paper to preserve leaf discs (Montserrat *et al.*, 2000) [48]. Different densities of 4th and 5th *C. pyri* nymphs (5, 10, 20, 40 and 80)/petri dish were used, as prey densities were established based on preliminary dose-response assays conducted prior to the main experiment, besides preys were depleted during all trials. An adult predator (3 days old) or newly emerged 5th stage nymph was transferred to each petri dish after a 24-hours starvation period, finally the petri dishes were secured using a paraffin film to avoid escaping of *C. pyri* nymphs (Emami *et al.*, 2014) [12].

After 24 hours, the predators were removed from the petri dishes and the numbers of consumed nymphs were recorded. The trials were performed at 15 and 27 °C, 65% RH, and a photoperiod of 16:8 h (L: D) in an environmentally controlled chamber. Ten replicates were used for 5th stage nymph, male and female of predator for each prey density at two tested temperatures, with 300 total observations. To check the natural survival rate of nymph stage psylla, ten replicates were conducted at each density in the absence of predator. In our current study we referred to different stages and sexes of *A. nemoralis* predator as stages.

Data Analyses

Functional Response Experiments

This experiment involved two main steps: model Selection and parameters estimation. The type of the functional response for each stage and temperature was determined using a logistic regression analyse (Juliano 2001) [35]. Data were fitted to (Eq. 1) that describes the relationship between the proportion of consumed prey N_a/N_0 as a function of initial prey density N_0 .

$$\frac{N_a}{N_0} = \frac{\text{EXP}(P_0 + P_1 N_0 + P_2 N_0^2 + P_3 N_0^3)}{1 + \text{EXP}(P_0 + P_1 N_0 + P_2 N_0^2 + P_3 N_0^3)} \quad (\text{Eq. 1})$$

where N_a is the number of consumed prey, N_0 is the initial number of preys, P_0 is the intercept, P_1 is the linear parameter, P_2 is the quadratic parameter, and P_3 is the cubic parameter. The parameters were calculated, using the method of maximum likelihood (PROC CATMOD, SAS Institute, 2004) [64]. If the linear term does not differ from 0, it indicates a type I functional response that is density-independent. Suppose the linear parameter is significantly negative ($P_1 < 0$), in that case, the proportion of prey consumed by the predator declines monotonically as the initial prey density increases, indicating a type II functional response. In contrast, a significant positive linear parameter ($P_1 > 0$) with a negative quadratic parameter ($P_2 < 0$) demonstrates a type III functional response (Juliano, 2001) [35].

In the second step of the analysis, because the experiments allowed prey depletion (nymphs were not replaced when consumed), we used Rogers random equation (Eq. 2) (Rogers, 1972) [60] for functional response Type II and the integral Hassell equation (Eq. 3) (Hassell, 1978) [26] for functional response type III to fit data, using a nonlinear least squares regression (PROC NLIN; SAS Institute Inc.

2004) [64] as these equations are applicable to non-replacement experiments.

$$N_a = N_0 [1 - \exp(a(T_h N_a - T))] \quad (\text{Eq. 2})$$

N_a is the consumed number of prey (pear psylla nymphs) by predator individual, N_0 is the initial prey density, a is the attack rate, T_h is the handling time and T is the total time in hours for the experiment (24 h).

$$N_a = N_0 \frac{1 - \exp[(d + b N_0)(T_h N_a - T)]}{(1 + c N_0)} \quad (\text{Eq. 3})$$

where b , c , and d are constants from the function that relates the attack coefficient (a) and N_0 in the type III functional response. The attack coefficient (Eq 4) (Hassell 1978) [26].

$$a = \frac{d + b N_0}{1 + c N_0} \quad (\text{Eq. 4})$$

Functional response models (Types II and Type III) were statistically compared using Okuyama's (2013) [53] AIC-based framework (Eq. 5), with optimal model selection determined by minimum AIC values

$$\text{AIC} = n \log(\text{MSE}) + 2q \quad (\text{Eq. 5})$$

n is the sample size, MSE is mean square error and q is number of parameters

To compare predator functional response parameters (attack rate and handling time) for *A. nemoralis* stages on two temperatures, we fitted the data to Juliano (2001) [35] equation (Eq. 6).

$$N_a = N_0 \{1 - \exp[a + D_a(j)(T_h + D_{Th}(j)N_e - T)]\} \quad (\text{Eq. 6})$$

Where, j , is an indicator variable takes value 0 for data set 1 and value 1 for data set 2. The parameters, D_a and, D_{Th} , estimate the differences between the two data sets being compared for the parameters a and, T_h values, respectively. In other words, a and T_h are the estimates of the parameters for data set 1, and $a + D_a$ and, $T_h + D_{Th}$, are the estimates of the parameters for dataset 2. We can assess the significant difference in parameters for the two sets as 95% confidence intervals included 0 (the difference was non-significant) or not (the difference was significant) (Juliano, 2001) [35], nonlinear least square regressions were again used to obtain parameter estimates.

For each prey density we calculated total handling time (Eq. 7), search time (Eq. 8), attack rate (Eq. 9), and search efficiency (Eq. 10) (Hassell, 2000; Rocha and Redaelli, 2004) [27, 59].

$$T_{h\text{total}} = T_h \times N_a \quad (\text{Eq. 7})$$

$$T_s = T - T_{h\text{total}} \quad (\text{Eq. 8})$$

$$a = N_a / (N_0 \times T_s) \quad (\text{Eq. 9})$$

$$E = N_a / N_0 \quad (\text{Eq. 10})$$

Statistical analysis was performed using SAS software, the difference between numbers of prey consumed depending on initial density at the two tested temperatures was tested using a KruskalWallis test, as data were non-normally distributed. The curves of the number of consumed prey by *A. nemoralis* different stages to different densities of *C. pyri* nymphs were plotted by Excel software.

Results

Functional Response Experiments

Functional response curves of 5th stage nymph, male and female of *A. nemoralis* to the various densities of *C. Pyri* nymphs are presented in (Figs. 1 and 2). The female curve

was the highest which indicate that female was more voracious than other stages. The number of consumed nymphs by the predator increased with increasing prey density and temperature (Figs. 1 and 2). The proportion of the prey killed by 5th stage nymph, male and female of *A. nemoralis* declined with increasing prey density for the two tested temperatures (Fig. 3 and 4). Parameter estimates from the logistic regressions equation (Eq. 1) showed a significantly negative linear coefficients ($P1$) for all tested stages (5th stage nymph, male and female) (Table 1) on the two tested temperatures, which all suggested a Type II functional response. No mortality was observed during the experiments in the control.

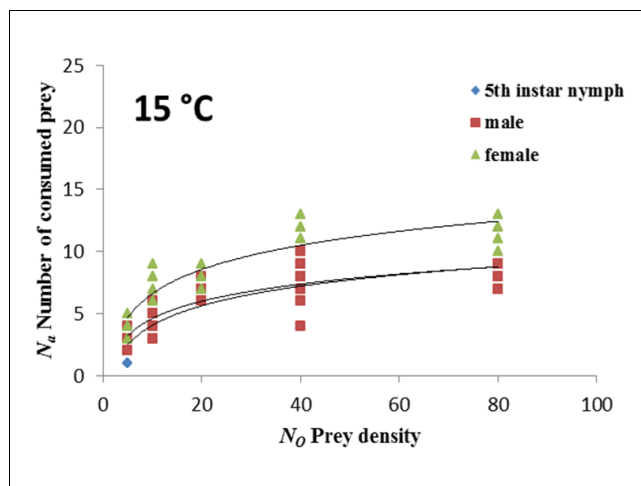


Fig 1: Mean number of *C. Pyri* 4th and 5th stage nymphs preyed upon by 5th nymph stage, males and females of *A. nemoralis* in 24 h as a function of prey nymph density at 15 °C

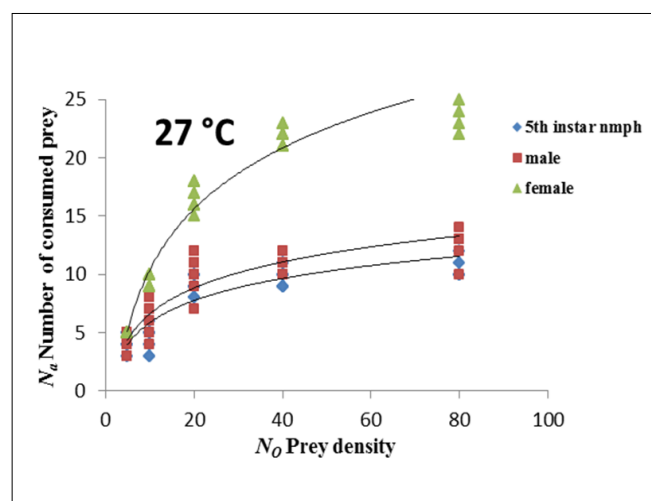


Fig 2: Mean number of *C. Pyri* 4th and 5th stage nymphs preyed upon by 5th nymph stage, males and females of *A. nemoralis* in 24 h as a function of prey nymph density at 27 °C

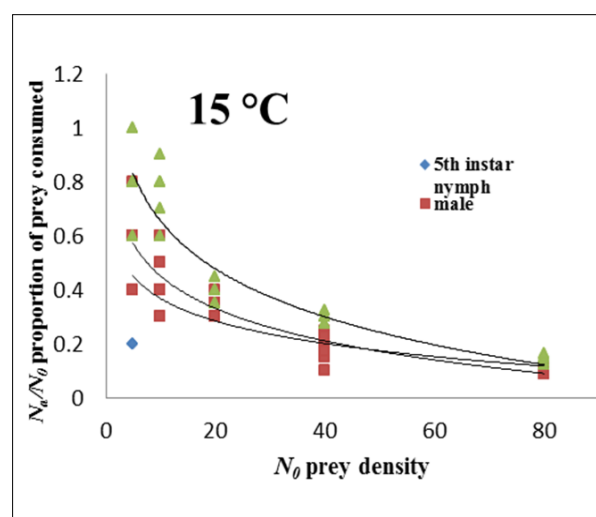


Fig 3: The proportion of the prey consumed by 5th stage nymph, male and female of *A. nemoralis* at 15 °C

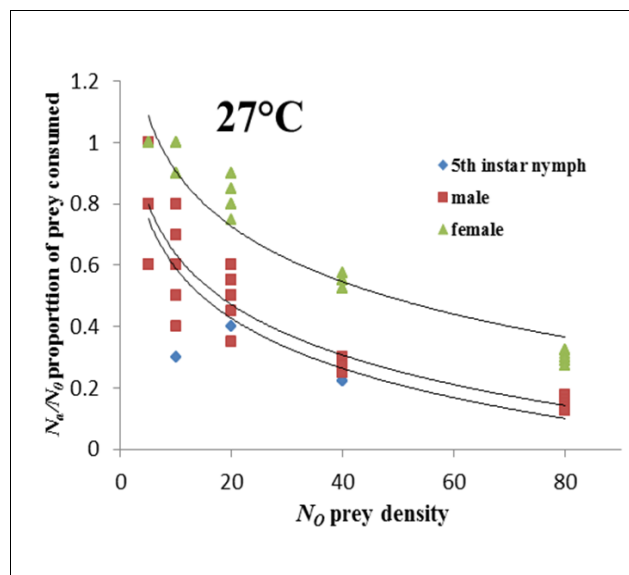


Fig 4: The proportion of the prey consumed by 5th stage nymph, male and female of *A. nemoralis* at 27 °C

Table 1: Maximum likelihood estimates of linear coefficient from logistic regression of proportion of prey consumed as a function of initial prey densities by 5th stage nymph, male and female *A. nemoralis* on two different temperatures (15 and 27 °C)

Temperature	Stage	Estimate	S. E	P value
15	L ₅	-0.009	0.018	0.0023
	M	-0.050	0.017	<0.0001
	F	-0.050	0.015	0.0072
27	L ₅	-0.100	0.020	0.0032
	M	-0.035	0.016	<0.0001
	F	-0.001	0.003	<0.0001

According to Akaike Information Criterion (AIC) we demonstrated that type II model fitted data better than type III model for all *A. nemoralis* stages on temperatures studied, as it had the smaller value of AIC (Table 2).

Table 3: Maximum likelihood estimates (MLE) of attack rate (a) and handling time (T_h) and their standard errors (SE) for 5th stage nymph, male and female *A. nemoralis* at two different temperatures (15 and 27 °C)

Temp (°C)	Stage	Parameter	Estimate	S. E	P value	95% Confidence intervals		T/T_h	a/T_h
						Lower	Upper		
15	L ₅	a	0.010	0.004	<0.0001	0.005	0.018	13.001	0.005
		T_h	1.846	0.294	<0.0001	1.654	2.337		
27	L ₅	a	0.028	0.006	<0.0001	0.016	0.040	14.475	0.016
		T_h	1.658	0.141	<0.0001	1.374	1.942		
15	M	a	0.015	0.004	<0.0001	0.003	0.025	14.345	0.008
		T_h	1.673	0.614	<0.0001	1.449	2.008		
27	M	a	0.030	0.006	<0.0001	0.012	0.043	18.140	0.022
		T_h	1.323	0.197	<0.0001	1.123	1.818		
15	F	a	0.035	0.006	<0.0001	0.026	0.038	20.924	0.030
		T_h	1.147	0.192	<0.0001	0.861	1.434		
27	F	a	0.086	0.013	<0.0001	0.061	0.112	30.341	0.108
		T_h	0.791	0.033	<0.0001	0.425	1.098		

The 95% confidence intervals, estimated by (Eq. 5), for D_a and D_{T_h} comparisons between 5th stage nymph and male included 0 in all experiments (Table 4), which indicated that no significant differences of attack rate and handling time was found between these two stages (the 95% confidence intervals for a and T_h overlapped between 5th stage nymph and male in all experiments). Whereas the 95% confidence

Table 2: Akaike information criterion (AIC) for the two functional response models for each *A. nemoralis* developmental stage.

Temperature	<i>A. nemoralis</i> stage	Type II	Type III
15 °C	5 th stage nymph	388.173	490.15
	Male	592.05	601.44
	female	2037.7	2549.45
27 °C	5 th stage nymph	491.54	599.94
	Male	605.54	737.43
	female	2230.34	2605.23

Values of attack rate (a) and handling times (T_h) estimated by (Eq. 2) showed that females of *A. nemoralis* had the highest attack rate and the shortest handling time in all experiments: (0.035, 0.086 for a ; 1.147, 0.791 for T_h at 15 and 27 °C, respectively), whereas 5th stage nymph had the lowest attack rate and longest handling time in all experiments (0.010, 0.028 for a ; 1.846, 1.658 for T_h at 15 and 27 °C, respectively) (Table 3). Our results demonstrated that attack rate had a positive relationship with temperature as it increased for all predator stages by temperature increasing. On the other hand, handling time had a negative relationship with temperature for all predator stages (Table 3). Female had the highest maximum theoretical predation rate to pear psylla nymphs (T/T_h) 20.924 and 30.341 at 15 and 27 °C, respectively, whereas 5th stage nymph had the minimum theoretical preparation rate, 13.001 and 14.475 nymph/day at 15 and 27 °C, respectively. The predatory efficiency of *A. nemoralis* can be accurately objectified by the functional response ratio ($FRR = a/T_h$) (Wang *et al.*, 2023) ^[41], according to our results female showed the highest FRR, which had a positive relationship with temperature increasing (0.030, 0.108 for 15 and 27 °C, respectively), while 5th stage nymph showed the lowest FRR at all temperatures (0.005 and 0.016 at 15 and 27 °C, respectively).

intervals for D_a and D_{T_h} comparisons between female and other stages (5th stage nymph and male) in all experiments didn't include 0, (Table 4), which indicated a significant differences in attack rate and handling time between female and other stages (the 95% confidence intervals for a and T_h didn't overlap between female and other stages; 5th stage nymph and male; in all experiments).

Table 4: Comparisons of 5th stage nymph, male and female *A. nemoralis* at two different temperature treatments (15 and 27 °C)

Comparison	Coefficients	Estimate	S. E	95% Confidence intervals		P value
				Lower	Upper	
15 L5 /15 M	<i>Da</i>	0. 001	0. 001	-0. 002	0. 004	0. 231
	<i>D_{Th}</i>	0. 002	0. 009	-0. 016	0. 020	0. 143
15 L5 /15 F	<i>Da</i>	0. 010	0. 002	0. 007	0. 013	0. 0007
	<i>D_{Th}</i>	0. 006	0. 009	0. 003	0. 025	0. 0001
15 F/15 M	<i>Da</i>	0. 009	0. 002	0. 001	0. 013	0. 0002
	<i>D_{Th}</i>	0. 004	0. 010	0. 001	0. 017	0. 0003
27 L5/27 M	<i>Da</i>	0. 004	0. 002	-0. 001	0. 008	0. 921
	<i>D_{Th}</i>	-0. 0002	0. 010	-0. 020	0. 020	0. 233
27 L5 /27 F	<i>Da</i>	0. 039	0. 003	0. 033	0. 045	0. 0002
	<i>D_{Th}</i>	0. 009	0. 008	0. 004	0. 020	0. 0002
27 F/27 M	<i>Da</i>	0. 035	0. 004	0. 028	0. 042	0. 0003
	<i>D_{Th}</i>	0. 006	0. 009	0. 004	0. 015	0. 0008

Raising in temperature had a significant impact on attack rate (*a*) of the same stage or sex of *A. nemoralis* as the 95% confidence intervals didn't contain 0, on the other hand it

didn't have any significant effect on handling time *T_h* of the same stage or sex of *A. nemoralis* as the 95% confidence intervals contained 0 (table 5).

Table 5: Comparisons of two different temperature treatments (15 and 27 °C), for 5th stage nymph, male and female *A. nemoralis*

Temp (°C)	Coefficients	Estimate	S. E	95% Confidence intervals		P value
				Lower	Upper	
15 L5/27 L5	<i>Da</i>	0. 007	0. 001	0. 004	0. 009	0. 026
	<i>D_{Th}</i>	0. 003	0. 009	-0. 015	0. 021	0. 213
15 M/27 M	<i>Da</i>	0. 009	0. 002	0. 005	0. 013	0. 032
	<i>D_{Th}</i>	0. 001	0. 011	-0. 021	0. 022	0. 432
15 F/27 F	<i>Da</i>	0. 035	0. 003	0. 029	0. 042	0. 013
	<i>D_{Th}</i>	0. 001	0. 008	-0. 015	0. 016	0. 534

The results showed a significant difference between the number of prey consumed on different prey densities on all predator stages in all experiments, (Kruskal Wallis: $\chi^2=186.562$, $df=4$, $p<0.001$). With an average of 3.7 ± 1.14 , 12.46 ± 2.72 nymphs consumed over 24 h at the lowest (5) and highest (80) densities, respectively. However, no significant differences were observed between the three densities 20, 40 and 80 for 5th stage nymph and male, also no significant differences were observed between the two densities 40 and 80 for suggesting that the satiation point for prey consumption had been reached (Reeves *et al.*, 2023) [57].

There was a significant difference in number of nymphs consumed depending on predator stage (Kruskal Wallis: $\chi^2=43.094$, $df=2$, $p<0.001$), with an average of 9.3 ± 2.34 nymphs consumed by 5th stage nymph, an average of 10.25 ± 3.52 consumed by males and an average of 17.85 ± 2.46 nymphs consumed by females at the highest density (80).

Also, the number of nymphs consumed depending on temperature differed significantly for 5th stage nymph (Kruskal Wallis: $\chi^2=14.041$, $df=1$, $p<0.001$), males (Kruskal Wallis: $\chi^2=16.951$, $df=1$, $p<0.001$), and females (Kruskal Wallis: $\chi^2=19.258$, $df=1$, $p<0.001$) with an average of 9.3 ± 0.34 nymphs killed at 15 °C and 15.63 ± 1.14 nymphs at 27 °C, for the highest prey densities (80).

The total handling time (*T_h*) for 5th stage nymph, male and females of *A. nemoralis* increased with increasing prey densities and temperature increasing, but was the least for female at all densities. However, the total searching time (*T_s*), the attack rates (*a*) and searching efficiency (*E*) for 5th stage nymph, male and females of *A. nemoralis* showed an inverse relationship with both prey densities and temperature, with highest values for female at all densities (table 6).

Table 6: Functional response parameters of 5th stage nymph, male and female of *A. nemoralis* to different densities of *C. pyri* at two different temperature treatments (15 and 27 °C)

Temp. °C	Prey density	Total handling time (<i>T_h</i>)			Total search time (<i>T_s</i>)			Attack rate (<i>a</i>)			Search efficiency (<i>E</i>)		
		5 th stage nymph	male	female	5 th stage nymph	male	female	5 th stage nymph	male	female	5 th stage nymph	male	female
15	5	5.88	5.85	4.59	18.12	18.15	19.41	8.45	11.11	15.53	0.42	0.58	0.80
	10	8.20	8.36	7.72	15.8	15.64	16.28	6.55	7.32	11.61	0.39	0.44	0.76
	20	11.81	11.38	8.83	12.19	12.62	15.17	3.90	4.29	5.84	0.32	0.34	0.39
	40	14.55	13.85	13.03	9.45	10.15	10.97	1.86	2.14	3.09	0.20	0.20	0.30
	80	14.77	13.55	13.31	9.23	10.45	10.69	0.92	1.06	1.55	0.10	0.10	0.15
27	5	6.96	5.29	3.96	17.04	18.71	20.04	14.31	14.97	20.05	0.84	0.80	1.00
	10	7.63	8.20	6.51	16.37	15.8	17.49	7.53	9.79	15.66	0.46	0.62	0.95
	20	15.09	13.63	11.21	8.91	10.37	12.79	4.06	5.34	9.01	0.46	0.52	0.84
	40	17.25	17.29	14.24	6.75	6.71	9.76	1.90	2.62	3.68	0.25	0.27	0.55
	80	19.57	18.14	16.06	4.43	5.86	7.94	0.85	1.20	1.49	0.13	0.15	0.30

Discussion

Functional Response of predator *A. nemoralis* to *C. pyri*

According to the results of our research, all tested stages of *A. nemoralis* (5th stage nymph, male and female) showed an increasing predation rate with increasing prey density, and a declining proportion of prey consumed by increasing prey density, which indicated a type II functional response (Holling, 1959) ^[32] for all temperature experiments when feeding on 4th and 5th stage nymphs of *C. pyri*. Several authors reported a type II functional response for some species of anthocorids including *Xylocoris flavipes* to three store insect pests (Rahman *et al.*, 2009) ^[56], *Orius sauteri* on *Thrips palmi* (Hemerik and Yano, 2010) ^[28], *Orius aalbidipenni* on barely aphid *Sipha maydis* (Moghaddam *et al.*, 2012) ^[46], *A. nemoralis* on *C. pyri*; (Reeves *et al.*, 2023) ^[57], adult predator *A. minki* to 4th stage nymph of prey *Psyllopsis repens* (Hassanzadeh-Avval *et al.* 2019) ^[24], *A. nemoralis* on *C. pyricola* (Emami *et al.*, 2014) ^[12], *A. minki* pistaciae on *Agonoscena pistaciae* (Kheradmand *et al.*, 2017) ^[38] and *Orius vicinus* on *Bactericera cockerelli* (Tran *et al.* 2012) ^[76], so we can say that *A. nemoralis* has potential in suppressing *C. pyri* populations in pear orchards, as predators that show type II functional response can be used in inundative biological control programs that aim at direct pest suppression (Van Lenteren, 2012) ^[79].

In contrast, Anthocorid predators have occasionally exhibited type III functional responses in some cases like adult predator of *A. minki* to 2nd stage nymphs of prey *P. repens* (Hassanzadeh-Avval *et al.*, 2019) ^[24] and adult female of predator *Hyperaspis popoli* to the 1st instar of *Phenacoccus solenopsis* (Seyfollahi *et al.*, 2019) ^[67], and this typically occur when feeding on cryptic, relatively small and immobile prey (Hassell, 1978) ^[26] as predation rate accelerates at intermediate prey densities due to learning or switching behavior.

Type II functional responses may lead to instability in predator-prey systems, as high predation pressure at low prey densities may lead prey population to collapse. In contrast, Type III functional responses typically stabilize predator-prey systems through two mechanisms: providing a prey refuge at low densities (due to low predator search efficiency), and density-dependent switching to alternative prey, coupled with improved foraging efficiency at high prey densities (Varshney *et al.*, 2018) ^[78]. Thus, Type II functional response predators like *A. nemoralis* are often more effective at controlling low-density pest populations, idealizing them for augmentative biological control, particularly at high predator-prey ratios.

Theoretical maximum predation rate (T/T_h) is the upper asymptote of the functional response curve and represents the high potential of a predator to eat its prey during a period of 24 h (Jafari *et al.*, 2012) ^[34], and in our findings female showed the highest T/T_h along with the highest temperature (27 °C) which indicated that it was more voracious than other stages, and more efficient at high temperature, this is likely due to nutritional requirements for reproduction (Li *et al.*, 2024), that was in agreement with Hassanpour *et al.* (2020) ^[23] who found that the highest maximum predation rate (T/T_h) showed by females followed by males of *Orius laevigatus* feeding on *A. gossypii* happened at 30 °C compared to lower temperatures tested.

The estimation of FRR (a/T_h) can be useful for determining

the possibility of the optimal ratio between predator and target pest for inoculative release (Wang *et al.*, 2019) ^[81], and according to our results the estimated a/T_h showed that female of *A. nemoralis* had an effective predatory potential for the control of *C. pyri* at high temperature, and 5th stage nymph had the least efficiency for the control of *C. pyri* at all temperatures, which indicate that FRR influenced by temperature, stage and sex predator.

Functional Response and stage of *A. nemoralis*

Attack rate and handling time are important indicators to the voracity of stages of *A. nemoralis* predator. Our results showed significant differences in the estimated attack (a) rates across the mature and immature stages of *A. nemoralis*, finding that female had the highest attack rate, which indicated that attack rate was affected by predator stage and sex. The larger body size of females and their metabolic costs associated with producing eggs makes a high foraging requirement of females consequently a higher prey attack and consuming compared than to other stages (Hodek and Honek 1996) ^[29]. The attack rate determines how steeply the functional response curve rises with increasing prey density (Dehkordi *et al.*, 2023) ^[9]. Thus, according to our findings, the steepness changed among different developmental stages of *A. nemoralis*, while female had the highest capacity to respond to prey densities, the 5th stage nymph and male had almost similar capacities.

The handling time (T_h) is a good indicator of the predation rate since it exhibits the total accumulative time spent in catching, subduing, killing, and digesting the prey (Veeravel and Baskaran, 1997) ^[80], our results indicated that the 5th stage nymph of *A. nemoralis* spent more time handling and consuming *C. pyri* nymphs than adult males and females, while the handling time of female was the shortest among other stages, in other word, the handling time decreased as the predator age increased, but with no significant differences between 5th stage nymph and male.

According to our results we can suggest that female was the most effective stage of predator *A. nemoralis* to *C. pyri* nymph, as natural enemies with high attack rate (a) and low handling time (T_h) are considered to be the most effective biocontrol agents (Bayoumy, 2011; Tazerouni *et al.*, 2016) ^[5, 74]. Also we can say that adult male of *A. nemoralis* was more favourable as a biocontrol agent compared to 5th nymph stage, as it had a slightly higher attack rate and shorter handling time.

Our results was in agreement with Reeves *et al.* (2023) ^[57] who found that female of *A. nemoralis* predator had a significantly higher attack rate and a significantly lower handling time compared to male at three different temperatures (18, 21 and 23 °C) when feeding on *C. pyri*. Also our results was similar to Hassanzadeh-Avval *et al.* (2019) ^[24] who mentioned that attack rates of nymph and adult of *A. minki* predators were significantly different but no differences on handling times were observed when feeding on *P. repens*.

Rocha and Redaelli (2004) ^[59] found that handling time of *Cosmoclopius nigroannulatus* on *Spartocera dentiventris* was higher in males than females; also Bayoumy (2011) ^[5] found that the functional response of 4th instar larvae and adult females of *Nephus includens* to *Aphis gossypii* differed in handling time, which was in agreement to our results.

In some cases, predators may appear a short handling time

(T_h) with a low attack rate (a) similarly to findings by Moradi *et al.* (2020) who studied functional response of different stages of predator *Symnus syriacus* to *Aphis gossypii*, and found an adverse relationship between a and T_h , which may be due to partial consumption, especially at high prey densities (Hodek and Honek, 1996; Aljetlawi *et al.*, 2004) ^[29, 31].

Functional response and temperature

Our results showed that all temperature experiments exhibited a type II functional response. For all tested stages attack rate had increased significantly as temperature increased, indicating that temperature clearly influenced the magnitude of predator response to prey, this may be attributed to temperature-related changes in the metabolism and activity of predator and prey (McCoull *et al.* 1998) ^[45]. Reeves *et al.*, 2023 ^[57] revealed that no significant difference was noticed in attack rate of male and female *A. nemoralis* due to temperature, and demonstrated that this non significant difference might be due to small intervals among tested temperatures (i. e 18, 21 and 23 °C), while our result was in agreement with other authors who studied temperature effect on functional response with clear intervals between tested temperatures like Hassanzadeh-Avval *et al.* (2019) ^[24] who found a significant differences in attack rate between the 15 and 30 °C treatments used with the anthocorid *A. mink*, Hassanpour *et al.* (2020) ^[23] who studied the functional responses of *Orius laevigatus* at 20, 25 and 30 °C and found that attack rate raised significantly of female anthocorids as temperature raised from 30 °C to 20 °C and DingXu *et al.* (2007) who find that temperature had a significant effects on the predation and functional response of the predator *Scolothrips takahashii* when fed on *Tetranychus viennensis*,

On the others hand, handling time had decreased as temperature increased, which suggested that predator across developmental stages exhibited prolonged non-searching behavior at reduced temperatures like resting, while elevated temperatures triggered more intensive searching and feeding activities, indicating that high temperature had a positive effect on the foraging behavior of predator. Our findings was in agreement with Hassanzadeh-Avval *et al.* (2019) ^[24] who revealed that temperature rising had an adverse effect to handling time, while it had a positive effect on attack rate.

The negative relationship between temperature and handling time might be due to the increasing rate of metabolism which led to a reducing in digestion time (Sentis *et al.*, 2013; Robertson and Hammill 2021).

Conclusions

This study provides valuable insights into the functional response of *A. nemoralis* predator to pear psylla *C. pyri*, contributing to the understanding of more effective pest management strategies in pear-growing regions. With Pear Psylla posing an escalating threat to pear orchards, these findings are critical for mitigating its impact on pear production in Iraq and beyond. To build upon these findings, we propose conducting field studies on the predator's functional response towards the target prey. This will yield more comprehensive results reflecting the influence of local climatic conditions on predator-prey dynamics. Furthermore, studying numerical response and

life tables for *A. nemoralis* on this specific prey and for *C. pyri* itself is recommended. Crucially, linking the pest's life table parameters with locally cultivated pear cultivars would help identify varieties exhibiting heightened susceptibility to infestation, as indicated by elevated values in key life table parameters. This integrated approach will significantly enhance the practical application of biological control measures against *C. pyri* in Iraq.

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