Bulletin of Entomological Research (2019) **109**, 612–616 doi:10.1017/S0007485318000986

Developmental duration and predation rate of the coccidophagous coccinellid *Rhyzobius lophanthae* (Blaisdell) (Coleoptera: Coccinellidae) on *Aspidiotus nerii* Bouche

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Abstract

The consumption rate, survival, and developmental duration of the coccidophagous coccinellid *Rhyzobius lophanthae* (Blaisdell) (Coleoptera: Coccinellidae), a predator of the armored scale insect, *Aspidiotus nerii* Bouche (Hemiptera: Diaspididae) were studied under laboratory conditions at two temperature regimes of 25 ± 1 and 30 ± 1°C, 50–65% RH, and 16L:8D. Developmental time (egg to adult) significantly decreased with increase in the temperature. It lasted 27.5 days at 25 ± 1°C and 21.3 days at 30 ± 1°C. The development threshold of *R. lophanthae* immature stages was 7.823°C, while the thermal constant was 472.379 degree-days. No mortality was recorded during the incubation period. The total mortality rate amongst the larval instars was with 3.33% at 25 ± 1°C and 6.77% at 30 ± 1°C. The sex ratio male to female was 1:1.06. The consumption rate significantly increased with increasing temperatures and within the larval instars. The four larval instar consumed 24.3 and 33.5 adults of *A. nerii* at 25 ± 1 and 30 ± 1°C, respectively. Overall, micro-climate temperature had a significant impact on the biological parameters of *R. lophanthae.*

Keywords: *Rhyzobius lophanthae*, Jordan, Diaspididae, armored scale insects, *Aspidiotus nerii*, developmental duration, predation rate

(Accepted 26 November 2018; First published online 8 January 2019)

Introduction

Scale insects (Hemiptera: Sternorrhyncha: Coccoidea) are sap-sucking plant pests that cause huge agricultural and horticultural losses (Erler & Tunc, 2001; Ouvrard & Kondo, 2013). Generally, they infest cultivated plants, fruit trees, ornamental shrubs, forest trees, greenhouse, and indoor plantings (Kaydan *et al*., 2012). Their eco-biological traits coupled with growing international trade of plants and plant products explain outbreaks of these insects observed on crops worldwide (Ouvrard & Kondo, 2013). The Diaspididae is one of the major scale insect families that includes many species of severs pests. They attack several economic important crops such as citrus, apple, pear, walnut, and ornamental trees all over the world (Navea & Vargas, 2012). The oleander scale, *Aspidiotus nerii* Bouche (Hemiptera, Diaspididae), is a cosmopolitan pest, known from tropical and subtropical parts of the world, particularly in the Mediterranean region. It is a polyphagous pest that has been reported associated with more than 100 plant families (Einhorn *et al*., 1998). Severe damage is caused on lemon and olive trees and ornamental and landscaping plants, such as oleander and acacia. Due to the occupation of niche on trees and armored nature, insecticides are largely ineffective (Uygun & Elekciouglu, 1998); therefore, parasitoids and predators are likely to be relatively more effective in controlling and managing such insect pests (Erler & Tunc, 2001). The predaceous coccinellids are more effective biological control agents than other organisms (Obrycki & Kring, 1998). Coccidophagous coccinellids are reported as one of the most important predator groups of diaspidid insects in the world (Smith & Cave, 2006). The predominately Australian Coccinellid predator *Rhyzobius lophanthae* is known as an

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effective natural enemy of armored scale insects. It has been introduced, inundatively released, and is commercially available in several parts of the world (Smith & Cave, 2006; Branco *et al*., 2017). It has some of the characteristic features such as specificity of prey, high fecundity and long adult longevity, no-diapause, heat resistance, good mobility, rapid population development (5–7 generations per year), and lack of parasitism and is observed to be the most important natural enemy of most armored scale species (Stathas, 2000*a*). Additionally, *R. lophanthae* was reported as a carnivore of soft scale insects. In Jordan, it was listed in the first list of Jordanian coccinellidae '*as a coccidophagous*' (Allawi, 1989). However, there is a lack of biological studies about this species in the MENA region and this study was designed and aimed to fill this gap and to assess the potential of the Jordanian *R. lophanthae* population as a native predator of armored *A. nerii* based on the developmental duration and consumption rate of the immature stages at two different temperature regimes 25 ± 1 and 30 ± 1°C. This pest is attacking many major economic crops such as citrus and olive trees in the whole country causing a significant loss.

Material and methods

The scale insect, *A. nerii* was collected from acacia (*Acacia cyanophylla* Lindley) trees in the Swaileh area 113.5 Km north of Amman (32.036969N, 35.833347E, and elev. 844 m above the sea level), and reared under controlled conditions at 27 ± 2°C, 65–70% RH, and 16L:8D (light:dark). Three host plants were used for the scale insect stock culture: butternut squash, potato tubers, and sprouts and potted 1–2 years old acacia seedlings. Crawlers of *A. nerii* were transferred by tailed hairbrush onto the first two hosts and placed in wooden cage of 54 × 40 × 33 cm. Parts of infested potato tubers and acacia leaves were laid on the new acacia seedlings. The coccinellid, *R. lophanthae* was collected from acacia trees infested with scale insects. It was reared on leaves of acacia infested with *A. nerii* and butternut squash placed in plastic cages (20 × 12 × 9 cm) in an incubator at 25 ± 1°C, 50–65% RH, and 16L:8D for three generations.

Developmental duration and mortality rate

To calculate the incubation period of eggs, two groups of eggs (50 per each) were incubated at 25 ± 1 and 30 ± 1°C, 50–65% RH, and 16L:8D. Newly hatched larvae (>1 h age) of coccinellid predator were placed individually onto leaves infested with adults of *A. nerii* that were placed on moist petri dishes (5 cm) under two temperature regimes 25 ± 1 and 30 ± 1°C, 50–65% RH, and 16 L:8D. The development threshold and thermal constant of the *R. lophanthae* immature stages were calculated based on the formula $K = D\ (T - t)$, where D = days for development at temperature T and t = the developmental threshold (Stathas, 2000*b*). Both groups of larvae were provided with fresh infested leaves daily until the pupation. The mortality rate and the developmental duration were recorded. The newly emerged adults were distinguished based on the last abdominal segments (fig. 1) (Stathas, 2001), and the sex ratio was calculated. Ten pairs of male and female were potted as couples into petri-dishes (9 cm) and provided with fresh infested leaves daily until they laid the first eggs and based on that the pre-oviposition of the adults was calculated.

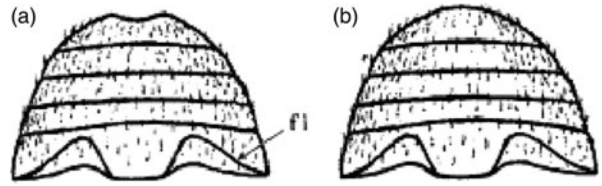

Fig. 1. Venter of abdomen of male (a) and female (b) of *Rhyzobius lophanthae*. fl, femoral line (Stathas, 2000*c*).

Consumption rate

The daily and total prey consumption rates were calculated for each larval instar. Each individual was separately reared in Petridish (9 cm) and was fed only on *A. nerii.*

Statistical analysis

A complete randomized design with one factor (two temperature regimes) was used. The analysis of variance and the least significant deference test ($P = 0.05$) in SAS (Version 9.4 for Windows; SAS Institute, Cary, NC, USA) were used to identify differences between temperature regimes.

Results

Developmental duration

The results indicated that the incubation period and developmental duration of *R. lophanthae* were significantly affected by the temperatures. The incubation period of eggs decreased significantly from 6.01 days at 30°C to 4.76 days at 25°C (table 1). The developmental time of pre- and post-embryonic stages and pre-oviposition period was longer at 25°C (27.5 days) and decreased significantly at 30°C (21.3 days). There were no significant differences between the first larval instar at 30°C (2.2 days) and third larval instar at 25°C (2.3 days). The development time of pupal stage was significantly shorter (3.5 days) at highest temperatures 30°C than this at 25°C (4.01 days). The pre-oviposition period significantly reduced from 4.71 days at 25°C to 3.23 days at 30°C. The development threshold and thermal constant of immature stages were 7.823°C and 472.379 degree-days, respectively.

Mortality rate

Survival of eggs and immature stages was very high. There was no mortality during the incubation period (table 2). The total mortality rates between the immature stages were 3.33 and 6.77% at 25 and 30°C, respectively. At the lowest temperature (25°C), the mortality was recorded during the first larval instar with 3.33%. Whereas, the mortality at the highest temperature (30°C) was recorded during the first and third larval instars with 3.33 and 3.44%, respectively.

Consumption rate

Consumption rate (numbers of consumed scale insect/different predator larval instars) for each larval instar of *R. lophanthae* at two temperatures is presented in table 3. All larval instars successfully penetrated the armored scale of the *A. nerii* adult at both temperatures and predated the whole adult. The total number *A. nerii* consumed by the

Table 1. Mean duration of pre-, post-embryogenic, and pre-oviposition stages of *Rhyzobius lophanthae reared* at (25 ± 1 and 30 ± 1°C, 50–65% RH, and 16 L: 8D). Duration of developmental stages (days) (mean ± SE).

Temperature °C	Egg stage	1st instar	2nd Instar	3rd instar	4th instar	Pre-pupa	pupa	Pre-oviposition	Total development time
25 ± 1	6.01 ± 0.01a	3.03 ± 0.04c	2.25 ± 0.04e	2.30 ± 0.04e	3.45 ± 0.043b	2.0 ± 0.044f	4.051 ± 0.05a	4.471 ± a	27.5
**n*	50	20	19	19	19	19	19	19	19
30 ± 1	4.76 ± 0.01b	2.20 ± 0.04e	1.66 ± 0.04g	1.77 ± 0.04g	2.44 ± 0.04d	1.78 ± 0.04g	3.56 ± 0.04b	3.234 ± b	21.3
**n*	50	20	18	18	18	18	18	18	18

**n* number of replicates.
Means in rows followed by different letters are significantly different using LSD test at $P < 0.05$.

Table 2. Mortality rate of immaturity stages of *Rhyzobius lophanthae* reared at (25 ± 1 and 30 ± 1°C, 50–65% RH, and 16L: 8D).

		Pre-immaturity stages						
Temperature °C	Egg stage	L1	L2	L3	L4	Pre-pupa	Pupa	Total mortality rate at all stages
25 ± 1	0.00	3.3	0.00	0.00	0.00	0.00	0.00	3.3
**n*	50	20	19	19	19	19	*n*	19
30 ± 1	0.00	3.3	0.00	3.44	0.00	0.00	0.00	6.77
**n*	50	20	19	19	18	18	18	18

**n* number of replicates.

Table 3. Consumption rate (mean numbers of scale ±SE/day) by larval instars of *Rhyzobius lophanthae* at 25 ± 1 and 30 ± 1°C, 50–65% RH, and 16 L: 8D.

Temperature °C.	Larval instars				Total
	L1	L2	L3	L4	
25 ± 1	1.72 ± 0. 27f	2.40 ± 0.29ef	4.34 ± 0.28d	15.84 ± 0.27b	24.3 ± 00
**n*	25	22	22	22	22
30 ± 1	2.55 ± 0.25e	3.72 ± 0.29d	6.00 ± 0.29c	21.21 ± 0.28a	33.48 ± 00
**n*	27	22	21	21	21
Differences	0.83	1.32	1.66	5.37	9.13

**n* number of replicates.
Means in rows followed by different letters are significantly different using LSD test at $P < 0.05$.

coccinellid predator from the first larval instar to the end of fourth larval instar ranging from 24.3 at 25°C and 33.5 at 30°C. Mean number of consumed *A. nerii* increased evidently from the first larval instar to the last instar. At the two temperatures studied, the fourth larval instar was more voracious and significantly different from the other instars. Mean number consumed by fourth instar exceeded 60% of the total consumption.

Discussion

The Cooccinellid *R. lophanthea* has been introduced, released and proven as bio-control agent for wide spectrum of armored scale insects worldwide (Thorson, 2009). Many studies (Stathas, 2000*a*, 2000*b*, 2000*c*, 2001; Stathas *et al*., 2002; Thorson, 2009) provided eco-biological information about this predator under laboratory and open field conditions. All these studies concluded that this predator reduced the population of all studied diaspidids, such as *A. nerii*, *Chrysomphalus aonidum* and *Aonidiella aurantii*. The current study aimed to test the ability of local *R. lophanthae* to consume and develop on the adult of *A. nerii*. It successfully developed and survived and the study confirmed the effect of temperatures on the predation capacity and all biological parameters of immature stages. The development threshold and thermal constant reported in this study were 7.823°C and 472.379 degree-days, respectively. Stathas (2000*b*) reported that the development threshold of *R. lophanthae* immature stages range from 7.6 to 9.3 and the thermal constant was 443.5 degree-days. The temperature appears to alter biotic potential of the predators, which corroborates with earlier work (Stathas, 2000*b*). Hodek (1973) and Hodek & Honek (1996) confirmed that the quality of prey influences significantly the duration of the developmental period and consumption rate of the instars of coccinellid, and other factors (i.e., host plant and its stage) affect the biological characteristics, such as prey consumption, adult longevity, and fecundity. Şimşek *et al*. (2016) reported the effects of armored species on the developmental times of immature stages of *R. lophanthae*. It seems that the developmental duration of coccidophagous coccinelllids is highly associated with the prey species. Such finding is confirmed by Uygun & Elekciouglu (1998), where the developmental duration performed of coccinellid predator *Chilocorus bipustulatus* significantly varied with three studied armored scale insects: *A. nerii*, *A. aurantii*, and *Pseudaulacapsis pentagona*. The survival of *R. lophanthea* was very high at low temperatures (25 and 30°C) and low mortalities have been reported. Stathas (2001) reported the same results with *A. nerii* but the mortality rates have been increased with other coccid species. The mortality rate obtained in this study and in previous study shows that the oleander scale is more preferable or a more suitable prey for *R. lophanthae*. The voracity and the effectiveness of coccinellids varied greatly in relation to species and developmental stage of coccinellid (Isikber & Copland, 2001). Branco *et al*. (2017) pointed that the disapidid *Chrysomphalus dictyospermi* is an essential prey for *R. lophanthae* and give better performance comparing with the other prey species that have been used in previous studies. In the present study, all larval stages showed good voracity capacity and it was very clear that the temperatures and the age of larvae influenced the consumption rate. It was significantly increased with the increasing temperatures as well as with aging. The highest consumption rate was recorded by the fourth larval instars, which appeared in the largest size and performed fastest mobility compared with the first three larval instars. Low number of preys consumed by first and second instars compared with other more developed predatory stages may be due to their small size, slow movement, and longer prey handling time (Barbar *et al*., 2016). This study provides good information on the performance of *R. lophanthae* fed *A. nerii*. We believe the results and real observations from the field will be fruitful to consider the use of these common and widespread coccinellid as a biological control agent and the efficient breeding of these predator rearing programs. However, further research on their life table parameters, and functional and numerical responses, optimal release strategies, as well as the efficiency with other kinds of preys under various environment, i.e., fruit trees, open crops fields, plastic houses, nurseries, are needed for a better understanding of the role of these predators in the suppression of scale insects.

Acknowledgement

The author warmly thank the reviewer/s for their efforts, fruitful comments and improvements. Special thanks for the Bulletin of Entomological Research and willingness to publish this work.

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