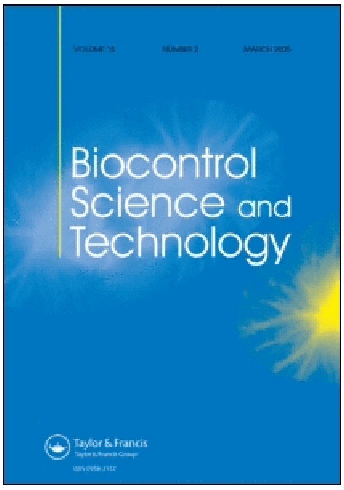

Biocontrol Science and Technology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713409232

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Online Publication Date: 01 November 2004

To cite this Article Mehrnejad, M. R. and Jalali, M. A.(2004)'Life history parameters of the coccinellid beetle, Oenopia conglobata contaminata, an important predator of the common pistachio psylla, Agonoscena pistaciae (Hemiptera: Psylloidea)',Biocontrol Science and Technology,14:7,701 — 711

To link to this Article: DOI: 10.1080/09583150410001682377

URL: http://dx.doi.org/10.1080/09583150410001682377

Biocontrol Science and Technology (November 2004), Vol. 14, No. 7, 701–711

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Life History Parameters of the Coccinellid Beetle, *Oenopia conglobata contaminata*, an Important Predator of the Common Pistachio Psylla, *Agonoscena pistaciae* (Hemiptera: Psylloidea)

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(*Received 23 January 2003; returned 11 April 2003; accepted 14 January 2004*)

The predatory beetle, Oenopia conglobata contaminata, is *associated with the common pistachio psylla,* Agonoscena pistaciae*, the major pistachio pest in Iran. Successful development and reproduction on both* A. pistaciae *and* Aphis gossypii *(the major weed aphid in the pistachio orchards) indicated they were suitable prey for* O. conglobata contaminata. *Under ample prey supply, larval development on* A. pistaciae *was shorter and mortality was lower compared to those reared on* A. gossypii. *Furthermore, this ladybird attacks and destroys a large number of psyllid nymphs during the whole of its larval period (620 $\pm$17 fourth stage nymphs) and also in its adult stage (191 $\pm$7.5 4^{th} stage nymphs daily). The optimum temperature for development was 30°C, the theoretical threshold for development was 13°C and thermal requirements from egg to adult was estimated as 196 degree-days (°D). Fecundity for the first 21 days of adult life was 387 and 355 eggs when females fed on* A. pistaciae *and* A. gossypii *nymphs, respectively. The intrinsic rate of increase (r_m) at 27.5°C was 0.19 and 0.18 when ladybirds were fed on psyllid and aphid nymphs, respectively. In a laboratory choice experiment, the adult ladybirds showed a strong preference for* A. pistaciae *compared to* A. gossypii. *This was in agreement with our field observation that* O. conglobata contaminata *is almost always found on psyllid colonies, even when aphids were plentiful on weeds in the pistachio orchards. The influence of the predator on psyllid seasonal population under natural condition was not studied in this investigation and this subject must be measured in subsequent trials.*

Keywords: Agonoscena pistaciae*,* Aphis gossypii*, pistachio tree, development, food consumption, intrinsic rate of increase, prey preference, reproduction, biological control, predator, psylla*

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ISSN 0958-3157 (print)/ISSN 1360-0478 (online)/04/070701-11

DOI: 10.1080/09583150410001682377

INTRODUCTION

The common pistachio psylla, *Agonoscena pistaciae* Burckhardt and Lauterer (Hemiptera: Psylloidea), is now the most destructive insect pest of cultivated pistachio trees (*Pistacia vera* Linnaeus) in Iran (Mehrnejad, 1998, 2001). This pest occurs throughout the pistachio plantation region of the country and causes severe reductions in pistachio yields. This pest is controlled almost exclusively by pesticides; however, environmental contamination and resistance by *A. pistaciae* to insecticides (Mehrnejad, 1998) has lead to considerable efforts to understand the potential of biocontrol agents for the common pistachio psylla control. Although several species of predacious arthropods attack the pistachio psyllid, their impact is currently insufficient for pest control. Coccinellids have been widely used in biological control for over a century and are considered to be important natural enemies of pest species, especially whitefly, aphids, mealy bugs, scales and mites (Obrycki & Kring, 1998). Recent investigations on the bionomics of the coccinellid beetles associated with the common pistachio psylla (Jalali, 2001; Mehrnejad, 2002) showed that several ladybird species such as *Adalia bipunctata* (Linnaeus), *Coccinella septempunctata* (Linnaeus), *Coccinella undecimpunctata aegyptica* (Reiche), *Exochomus nigripennis* (Erichson), *Hippodamia variegata* (Goeze) and *Oenopia* (= *Synharmonia*) *conglobata contaminata* (Menetries) attack *A. pistaciae*. Among the psyllophagous coccinellids, *O. conglobata contaminata* was one of the most abundant predatory beetles in the pistachio orchards of Rafsanjan (the main pistachio plantation area of Iran). These ladybirds were highly active during spring and autumn on pistachio trees, but they were very scarce during July, August and early September. The present investigation is believed to be the first study on *O. conglobata contaminata* as a predator of the common pistachio psylla. This study provides information for understanding the primary potential of a native predator of the common pistachio psylla. Obtaining knowledge about this species and other psyllid biocontrol agents will help extension officers to reduce chemical applications against the psyllid.

This paper describes the relationship between the predatory ladybird, *O. conglobata contaminata*, and its prey, the common pistachio psylla (the target prey), as well as an alternative prey, the cotton aphid *Aphis gossypii* Glover (the most abundant aphid species on weeds in pistachio orchards). The temperature threshold for development and thermal requirement for preimaginal development, food consumption and prey preference were determined under controlled conditions. Also, the reproductive responses and r_m of this ladybird on psyllid and aphid nymphs were quantified.

MATERIALS AND METHODS

All laboratory trials were conducted under controlled conditions (27.5 $\pm$ 0.5°C, 55 $\pm$ 5% RH and 16:8 L:D) unless stated otherwise. The ladybird larvae and adults were fed either third and fourth instars pistachio psyllid or third instar cotton aphids. Prey were presented in a leaf-disc cage made of a plastic Petri-dish (52 mm in diameter) with a 20-mm diameter hole in the middle of the lid, which was covered by a piece of fine net (2-mm mesh) to provide ventilation. Agar medium (8^{-1} g L) was used as a source of moisture for the plant leaf. A young and fully developed pistachio leaf or weed leaf (for psyllids and aphids, respectively) was cut to the same size as the dish and left lower side down on the 3-mm thick agar medium covered by a filter paper. Pistachio leaves were collected from bearing pistachio trees *Pistacia vera* Linnaeus (cultivars Kallah-quchi and Ohadi), and the weed leaves (*Chenopodium album* Linnaeus) were collected in the pistachio orchards. Eight leaf-disc cages and two small glass jars containing a saturated 'Magnesium Nitrate' salt solution (for maintaining relative humidity 55 $\pm$ 5%) were placed in a plastic box inside an incubator. Lighting in the incubators was provided by white fluorescent lamps with light intensity of about 13 W m^2 in the cabinet area. The nymphs of pistachio psyllid and cotton aphid were regularly collected from pistachio orchards and used as food source for ladybirds in the experiments.

Initial Culture

A laboratory colony was established with field-collected adult *O. conglobata contaminata* from the pistachio orchards of Rafsanjan. The colony was maintained in ventilated plastic boxes (25 × 17 × 10 cm) and the ladybirds were fed psyllid nymphs at $27 \pm 1^{\circ}$C and 16L:8D. Fresh pistachio leaves infested by psyllid nymphs were offered to ladybirds every morning and the old leaves were removed. Five generations were completed before tests were initiated. Adult ladybirds from the colony served as the parent stock for all rearing experiments.

Development

For each temperature three mated adult females were released into a leaf-disc cage separately and were fed psyllid nymphs. The cages were checked at 12-h intervals and the ladybirds moved to new cages. Eggs were kept and checked three times a day to determine the egg incubation period and rate of mortality. To study the developmental period of larvae, a newly hatched larva was introduced into a leaf disc cage containing sufficient third and fourth stage psyllid nymphs. The cages were checked once a day and the larvae moved to new cages. Also the larva's moulting status or mortality was recorded until pupation, and pupae were checked until adult eclosion. On the day of emergence the adults were weighed and the sexes determined by observations of mating. This experiment was conducted at seven constant temperatures (17.5, 20, 22.5, 25, 30, 32.5 and 35 all $\pm 0.5^{\circ}$C).

The rate of development from egg to adult was plotted against temperature to determine the thermal constant (degree day developmental time) for *O. conglobata contaminata* (Campbell et al., 1974; Jervis & Copland, 1996). The rate of development was plotted as the reciprocal of developmental time (days). Third-order polynomial regression curves gave the best fit regression line to the data on developmental rates for constant temperature. Temperatures in the mid-range of the polynomial curve (linear area) were selected and used to fit a linear regression. The equation of linear regression ($y = a + bT$) was then used to predict the T_l and K, where y is rate of development and T is temperature. The theoretical lower threshold for development (T_l) was calculated from the formula $T_l = -a/b$, and the thermal constant (K) was also obtained using the formula $K = 1/b$. K is the reciprocal of the slope b of the regression line (Campbell *et al*., 1974). The mean for each temperature, lower threshold and constant temperature were all calculated from the pooled data of male and female developmental time from the egg to adult eclosion.

Reproduction

This experiment was carried out at three constant temperatures (22.5, 25 and $30 \pm 0.5^{\circ}$C) to examine the reproductive capacity of *O. conglobata contaminata* for 21 days when fed psyllid nymphs. Newly emerged and similar sized females were selected and introduced into a leaf-disc cage containing about 250 psyllid nymphs (third and fourth stages). Each day the numbers of eggs laid including the remains of cannibalised eggs were counted and the ladybirds carefully transferred to the new Petri dishes. The females were kept with a male during the first week, but thereafter a male was introduced to each female at 3-day intervals for about 3 h. The pre-oviposition period was determined by recording the number of days from eclosion to the initiation of egg laying. The numbers of female used as replication were 6, 5, and 11 for the above temperatures, respectively.

Influence of Prey Species on Development and Reproduction

The development and reproduction potential of the ladybird was studied by rearing 68 newly hatched larvae singly on psyllids and aphids separately throughout the preimaginal and imaginal periods at $27.5 \pm 0.5^{\circ}$C. The eclosed adults were weighed and the sexes determined. Similar sized females were selected and male–female pairs were enclosed in leaf-disc cages. Adults were fed the same diet as during the preimaginal period (27 and 26 replications were made on psyllids and aphids, respectively). The number of eggs laid including the remains of cannibalised eggs over 24 h was counted and ladybirds were moved to new cages. During the first week, a male was caged with each female, but thereafter a male was introduced to each female at 3-day intervals for about 3 h.

Intrinsic Rate of Increase (r_m)

Data obtained from these development and reproduction experiments (development time, mortality, sex ratio and fecundity) were compiled into a life table for the first 21 days of adult life under controlled conditions. The parameters were calculated by QBASIC program (Jervis & Copland, 1996). From this, the parameter 'intrinsic rate of natural increase (r_m)' (Birch, 1948) was determined.

Prey Preference

Prey preference for pistachio psyllid or cotton aphid was studied in a choice experiment. A second colony of *O. conglobata contaminata* was established on the cotton aphid and reared for five generations. Then two groups of newly hatched ladybird larvae were collected from the two different colonies (ladybirds reared on psyllids and ladybirds reared on aphids) and reared to adult, one on *A. pistaciae* and another on *A. gossypii* separately. After adult emergence from the pupae, 23 females from each group were fed the same prey for 2 days, and then deprived of food for 12 h prior to the experiment. Afterward, adult ladybirds were released individually into a ventilated Petri dish containing 20 psyllid nymphs (fourth stage) and 20 aphid nymphs (third stage). The two species of prey were similar sized. After 2 h the survivors of each prey species were counted. Earlier trials had shown that either 20 psyllid nymphs (fourth stage) or 20 aphid nymphs (third stage) would be sufficient to provide ample food for 2 h for an ovipositing female.

Chesson (1978) preference models were used for quantifying ladybird preference for psyllid and aphid nymphs. Chesson (1978) measure of preference for psyllid, α_1, was calculated using the following equation:

$$\alpha_1 = (r_1/n_1)/[(r_1/n_1) + (r_2/n_2)]$$

where r_1 is the proportion of attacked psyllid nymphs, n_1 is the proportion of total psyllid nymphs, r_2 is the proportion of attacked aphid nymphs, n_2 is the proportion of total aphid nymphs. Preference for aphid, $\alpha_2 = 1 - \alpha_1$.

Prey Consumption

Prey consumption of *O. conglobata contaminata* feeding on psyllid nymphs (fourth stage) for both immature and mature stages was determined at two temperatures (27.5 and 30 ± 0.5°C). The number of psyllids attacked (killed) was counted once a day and the larvae moved to new cages with a constant number of prey until pupation. For the two temperatures, 12 and nine replications were made, respectively. Also the number of prey attacked by ovipositing females over 24 h were counted and recorded (five and six females were tested at the two temperatures, respectively).

Data Analysis

Data were analysed using MINITAB program. Analysis of variance (ANOVA) was used to test the differences in all experiments. The LSD-tests at 0.05% procedure were used for pair wise comparisons among means.

RESULTS

Development

The rate of development of *O. conglobata contaminata* was influenced by temperature. The mean developmental time decreased with an increase in temperature from 17.5 to 30°C and then increased. The developmental period was the shortest at 30°C (Table 1). The theoretical lower threshold for development was 13°C. At 32.5°C, the rate of development declined suggesting that the upper temperature threshold was being approached. A temperature of 35°C was found to be lethal; no eggs hatched and the larvae suffered 100% mortality (Table 1). The thermal constant (in degree-days centigrade above the threshold) of this species was estimated to be 196.1°D (Figure 1). Total mortality of the immature life stages increased with increasing temperature; however, mortality drastically increased at temperatures above 30°C. Female weight was significantly lower at 32.5°C than at all other temperatures (Table 1).

Reproduction

The fecundity of *O. conglobata contaminata* improved as the temperature increased from 22.5 to 27.5°C; however, the number of eggs laid at 25, 27.5 and 30°C was not significantly different (Table 1).

Influence of Prey Species on Development and Reproduction

Ladybird larvae reared on psyllid nymphs at 27.5°C developed in 8.1 days, compared with 9.6 days on aphid nymphs. Furthermore, the total mortality of immature ladybird larvae fed psyllid nymphs (15%) was lower than when reared on aphid nymphs (22.7%). Adult beetle weight did not differ significantly when fed on psyllids or aphids (Table 2). The pre-oviposition period for females feeding on psyllids and for those feeding on aphids was similar, on average 5.7 and 5.8 days, respectively. The total number of eggs laid (fecundity) during the first 21 days of adult life did not differ significantly among females on the two different diets (Table 2).

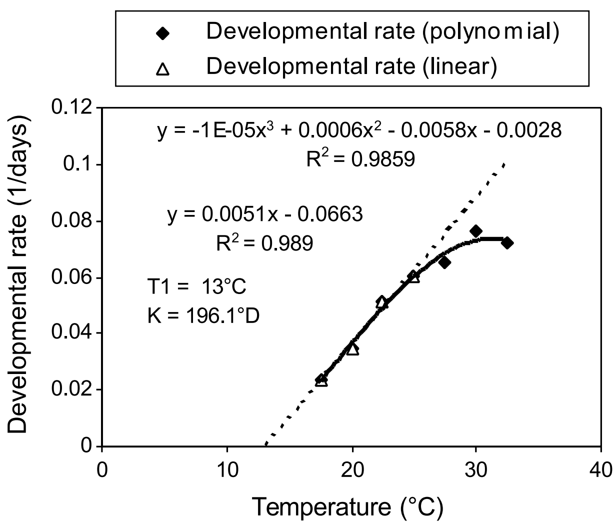

FIGURE 1. Relationship between temperature and rate of development of *O. conglobata contaminata* from egg to adult at constant temperatures. Rates were computed as reciprocals of developmental periods in days (the sample sizes (n) are stated in Table 1).

TABLE 1. Effect of temperature on development, mortality, adult weight and reproduction of *O. conglobata contaminata* fed on psyllid nymphs (constant temperature ± 0.5°C, 55 ± 5% RH and 16:8 L:D)

	Development (day ± SE)				Mortality (%)				Weight (mg ± SE)		
Temperature (±0.5°C)	Egg	Larva	Pupa	Total	Egg	Larva	Pupa	Total	Male	Female	Fecundity (egg ± SE)*
17.5	7.1 ± 0.07a (80)	22.6 ± 0.18a (59)	13.0 ± 0.09a (52)	42.7	0	3.3	5.4	8.7	6.74 ± 0.1a (19)	8.75 ± 0.1a (32)	
20	5. ± 0.03b (76)	15.3 ± 0.24b (29)	8.15 ± 0.05b (29)	28.5	0	6.5	2.2	8.7	7.1 ± 0.15a (14)	8.4 ± 0.02ab (14)	
22.5	3.4 ± 0.02c (105)	10.5 ± 0.13c (38)	5.5 ± 0.11c (18)	19.4	0.9	2.6	5.3	8.8	5.75 ± 0.25bc (4)	7.25 ± 0.16d (8)	229 ± 16.9b (6)
25	2.9 ± 0.03d (87)	8.3 ± 0.07d (34)	5.3 ± 0.1cd (15)	16.5	1	2.9	11.8	15.7	6 ± 0.10b (5)	7.57 ± 0.2cd (7)	310 ± 16.6a (5)
27.5**	2.4 ± 0.9e (68)	8.1 ± 0.09d (65)	4.9 ± 0.09d (60)	15.4	3	4.3	7.7	15	5.86 ± 0.1bc (33)	7.8 ± 0.2c (27)	387 ± 35a (27)
30	2.5 ± 0.04e (55)	6.1 ± 0.18e (17)	4.5 ± 0.11e (21)	13.1	1.8	6.5	8.7	17	6.5 ± 0.27ab (8)	8.15 ± 0.1b (13)	361 ± 18a (11)
32.5	2.0 ± 0.04f (48)	8.0 ± 0.09d (34)	3.9 ± 0.12f (23)	13.9	7.7	10.8	30.3	48.8	5.2 ± 0.37c (5)	6.57 ± 0.13e (7)	
35	–	–	–	–	100	100	–				
P value	0.001	0.001	0.001						0.001	0.001	0.001

Means in each column followed by the same letter are not significantly different in one way ANOVA, using LSD-tests at $P = 0.05$. The figures in parentheses indicate the number of replicates.

*Fecundity was examined for the first 21 days of the adult's life.

**Data for 27.5 ± 0.5°C came from the study on prey species versus development and reproduction of *O. conglobata contaminata*.

TABLE 2. Development, mortality, adult weight, preoviposition period, fecundity and r_m of *O. conglobata contaminata* reared on *A. pistaciae* and *A. gossypii* as preimaginal and imaginal diets (27.5±0.5°C, 55±5% RH and 16:8 L:D)

	Development (day±SE)				Mortality (%)				Weight (mg±SE)				
Prey	Egg	Larva	Pupa	Total	Egg	Larva	Pupa	Total	Male	Female	Pre-oviposition (day±SE)	Fecundity (egg±SE)*	r_m
Agonoscena pistaciae	2.4±0.9 (68)	8.09±0.09b (65)	4.9±0.09 (60)	15.45	3 (70)	4.35 (68)	7.7 (65)	15.05	5.86±0.1 (33)	7.8±0.2 (27)	5.71±0.2 (27)	387±35 (27)	0.19
Aphis gossypii	2.56±0.05 (68)	9.55±0.2a (58)	4.5±0.15 (55)	16.6	3 (70)	15.7 (68)	4 (58)	22.7	5.4±0.15 (29)	7.4±0.13 (26)	5.8±0.12 (26)	355±18 (26)	0.18
P value	0.064	0.001	0.055						0.1	0.1	0.1	0.67	

Means in each column followed by the same letter are not significantly different in one way ANOVA, using LSD-tests at $P=0.05$. The figures in parentheses indicate the number of replicates.

*Fecundity was examined for the first 21 days of the adult's life.

Intrinsic Rate of Increase (r_m)

The r_m values were similar when the ladybirds were fed either psyllids (0.19) or aphids (0.18) (Table 2).

Prey Preference

The coefficients for host preference resulting from the laboratory experiments are showed in Table 3. The results indicate that both ladybird groups (reared on *A. pistaciae* or on *A. gossypii*) preferred psyllid nymphs. No defensive behaviour by either prey species was observed.

Prey Consumption

This predator killed 620 fourth stage psyllid nymphs during the whole larval period and also 191 fourth stage psyllid nymphs daily by ovipositing females at 27.5°C. The number of psyllids consumed by ladybirds at 27.5 and 30°C was not significantly different (Table 4).

DISCUSSION

Oenopia conglobata contaminata preys on several aphid species, e.g. Russian wheat aphid, *Diuraphis noxia* (Kurdjumov) in North-America (Gordon & Vandenberg, 1991; Michels *et al*., 1997), different aphid species of pome and stone fruit trees (Erkin, 1983; Radjabi, 1989), and *A. gossypii* in the cotton field of Turkmenia (Alekseev & Niyazov, 1975). Furthermore, several species of coccinellids including *O. conglobata* were reared for control of the pear psylla, *Cacopsylla pyricola* Forster, in Washington (Fye, 1981). Although the coccinellid beetle, *O. conglobata contaminata* was known as an aphidophagous predator, our records clearly show that this species is a good psyllophagous ladybird as well.

The ladybird developed successfully over a wide temperature range 17.5–32.5°C, and development was rapid between 25 and 32.5°C. The optimum temperatures for development, lower and upper development threshold for the psyllid *A. pistaciae* are 30, 10.7 and 32.5°C, respectively (Mehrnejad, 1998). The suitable temperature for rapid development for *O. conglobata contaminata* was 30°C and the upper extreme 32.5°C, indicating that these parameters coincide with those of its psyllid host. However, the lower threshold for the ladybird (13°C) is higher than for the psyllid (10.7°C). We predict that in cooler temperatures in early spring psyllid populations will develop before predator populations. This might allow the pest to escape control especially when the ladybird population is low. However, the thermal constant for this ladybird (196°D) was found to be lower than for its psyllid prey (238°D) (Mehrnejad, 1998). In comparison with data reported by Mehrnejad (1998) this ladybird developed faster than its psyllid prey particularly at temperatures between 27.5 and 32.5°C. The shorter generation time of this species may be considered an advantageous characteristic for utilising the predator as a biological control agent.

TABLE 3. Preference coefficient of adult ladybird, *O. conglobata contaminata*, for the common pistachio psylla and cotton aphid nymphs. Chesson (1978) preference model was used ($n = 23$)

	Coefficient (±SE)		
Prey for larvae	Psyllids	Aphids	*P* value
Psyllid nymphs	0.79±0.014 a	0.21±0.014 b	0.001
Aphid nymphs	0.68±0.015 a	0.32±0.015 b	0.001

Means in each row followed by a different letter are significantly different. Data analysed using one-way ANOVA (LSD-tests at $P = 0.05$).

TABLE 4. Consumption by *O. conglobata contaminata* of psyllid nymphs (fourth stages) at two temperatures

Temperature ($\pm 0.5°C$)	No psyllid nymphs ($\pm$SE) fed on during larval period	No psyllid nymphs ($\pm$SE) fed on by ladybird adult (daily)
27.5	619.8 ± 16.6 (12)	191.2 ± 7.5 (5)
30	543.6 ± 37.5 (9)	199 ± 11.9 (6)
P value	0.057	0.61

The figures in parentheses indicate the number of individuals examined. Data analysed using one-way ANOVA (LSD-tests at $P = 0.05$).

Prey species had a significant influence on ladybird developmental time, but ladybird weight was not affected by prey species. Several factors influence the weight and size of coccinellids; as Smith (1966) stated, the weight and size of coccinellid adults varies with species, sex and feeding. In this regard, Honek (1993) and Jervis and Copland (1996) suggested that fecundity in many predator and parasitoid insects varies with the body size of the female. However, Leather (1988) showed that large individuals were not always more fecund than small ones. In contrast, Smith (1965) stated that longevity and fecundity are reduced in small individuals of Coccinellidae.

The tested diets did not affect the number of eggs laid by this ladybird. Based on results reported by Mehrnejad (1998), the r_m of *O. conglobata contaminata* (0.19) was less than the r_m value of its prey, the common pistachio psylla (for its whole life span), $r_m = 0.22$ at 27.5°C. In this regard, Huffaker *et al*. (1977) suggested that it is a common error to conclude that a natural enemy having a lower r_m than its host will be a poor biological control agent. But Van Lenteren and Woets (1988) suggested that natural enemies might be effective for biological control when, among other criteria, the intrinsic rate of increase of the natural enemy is equal to or larger than the intrinsic rate of increase of the pest. In this study, the reproductive capacity of the ladybird was investigated only for the first 21 days of its adult life, whereas for its prey this parameter was investigated for the whole life span, so it is not possible to come to a definitive conclusion about the reproductive efficiency of this predator against its prey. More investigations are needed to study and determine the demographic parameters of *O. conglobata contaminata* at various constant and cycling temperatures and in response to different host densities as well.

Results of the laboratory choice experiment showed the ability of ladybird to select a preferred prey, as the ladybird significantly preferred to attack psyllid nymphs before aphids. Also the preimaginal diet did not influence the adult's behaviour for host selection. Although a little significant difference was found in performance on psyllids and aphids, both prey species are suitable for the ladybird *O. conglobata contaminata*. Based on these results, it might be concluded that there is an association between preference and performance in *O. conglobata contaminata* with respect to two prey species. It is suggested that *A. gossypii* could be utilized by this ladybird as an alternative prey during periods of low availability of psyllid *A. pistaciae* on pistachio trees. In this regard, Kalushkov and Hodek (2001) stated that suitable prey not visited by the predator in the field can be successfully used in laboratory cultures producing predators for releases in biocontrol or integrated pest management.

We observed that when there was adequate food (psyllid) on pistachio trees, this ladybird showed a tendency to feed, and especially to lay their eggs on pistachio trees, although herbaceous plants contaminated with *A. gossypii* were available in the pistachio orchards at the same time. The coccinellid beetle *Oenopia* (= *Synharmonia*) *conglobata* is a stenotopic ladybird and deciduous trees are the preferred habitat for this insect (Hodek, 1973). Furthermore, Blackman (1967) stated that the distribution of adult coccinellids will depend partly on the location and abundance of food and partly on their specific habitat preferences. The prey and habitat preference by *O. conglobata contaminata* may often keep it on the pistachio trees contaminated by psyllid. Hodek (1967) wrote that the usual criterion for considering a species typical for a particular habitat is that the species would breed there. In conclusion, *O. conglobata contaminata* could be valuable as a biocontrol agent for the common pistachio psylla and at this stage conservation might be the right strategy for improving the efficiency of this species and other natural enemies of the psyllid in the field. However, more research needs to be undertaken on this ladybird, particularly under natural conditions, to fully realize its potential in controlling common pistachio psylla under commercial production conditions.

ACKNOWLEDGEMENTS

We thank Dr Ivo Kovar, National Museum, Prague, for identification of ladybird, Dr S.H. Hodjat, the University of Shahid Chamran, Ahvaz, Iran for identification of aphids, Drs I. Hodek and J. Obrycki for their helpful comments on an earlier version of the manuscript, Dr M.R. Shaw for a critical reading of the manuscript, to R. Mirzaei and A. Hadg-Abdollahi for assistance. The Pistachio Research Institute of Iran supported this study.

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