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ORIGINAL ARTICLE

A life cycle study of *Coccinella algerica* Kovar, 1977 (Coleoptera, Coccinellidae): Census of a new larval stage in this lady beetle from Béni-Douala area (Tizi-Ouzou)

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Abstract

The objective of biological control is to reduce chemical treatments on crops. To reduce aphid attacks with the use lady beetles is a positive, respectful alternative since it can maintain an ecological balance. In order to achieve this objective, the Algerian seven-spotted lady beetle (*Coccinella algerica*) was bred under laboratory conditions, and biological parameters of this species were studied. The study, conducted from April to May, showed that temperature and relative humidity greatly affected the incubation time of *C. algerica* eggs. Egg fertility was very high and reached up to 100%. The present work highlighted that the developmental cycle of this lady beetle from the Beni-Douala area (Tizi-Ouzou) passes through five larval stages. The fifth instar larva was recorded for the first time. Indeed, all studies carried out to date have identified only four larval stages in this species and have never mentioned the existence of L5, meaning that this result is original.

Keywords: *Aphis fabae*, auxiliary, *Coccinella algerica,* incubation time, larva L5, life cycle

Introduction

The fight against crop pests in Algeria involves the use of chemicals. Their abusive and uncontrolled use leads gradually to genetic, environmental and health problems (Guesmi-Jouini *et al.* 2011). The large-scale use of aphicide products has led to the selection of resistant aphid populations (Harmel *et al.* 2008). Furthermore, pesticides are recognized as being harmful to human health and the environment (Dedryver *et al.* 2010). Excessive and intensive chemical control has demonstrated their inability to eradicate pests. These latter circumvent in various ways this obstacle by their multiplication. Intensive chemical control has revealed other adverse effects on crops, such as the induction of outbreaks of the target pest or the multiplication of pests judged to be of low risk by the destruction of the secondary fauna (Milaire 1986). Phytosanitary problems find a quick and often toxic solution that disregard natural equilibrium.

Researchers are currently attempting to develop the application of biological control, such as the use of lady beetles which constitute an entomophagous group likely to play an important role in reducing aphids and scaling down insect populations (Saharaoui and Gourreau 1998). Among them, *C. algerica* is a species with an important role in biological control. Benoufella-Kitous (2015) reported that the seven-spotted lady beetle is among the first of the auxiliaries to arrive in fields and exploit the first aphid outbreaks; it has a high predatory impact on the aphid *Aphis fabae*. The research of Ben Halima-Kamel and Ben Hamouda (2005) show the efficiency of *C. algerica* in the fight against *Aphis gossypii* in protected culture. An adult of this lady beetle consumes up to 1,069 individuals of *A. gossypii* in 11 days under natural conditions.

According to Ben Halima-Kamel *et al.* (2011), this auxiliary consumes 20 aphid species belonging to four subfamilies: Aphidinae, Calaptidinae, Chaitophorinae and Lachininae. It is considered to be one of the most widespread aphidophagous insects (Saharaoui *et al.* 2001). Therefore, this lady beetle can be very useful in reducing aphid populations.

In this context, the objective of our study was to identify some aspects of this aphidophagous lady beetle which is widespread in Kabylia, by breeding it in a laboratory and to characterize prevention strategies in order to improve the levels of suppression of these harmful species by this auxiliary. Lady beetles are a good model for approaching the principle of biological control and integrated pest management against aphids. This is especially true since there is limited knowledge about the biological and ecological parameters underlying this aphidophagous species in Kabylia.

Among the research carried out on this lady beetle in Algeria, we find that of Saharaoui and Gourreau (2000), Saharaoui *et al.* (2001) and Benoufella-Kitous (2015).

Materials and Methods

The biological material consisted of *C. algerica* adults harvested from bean plants *Vicia faba*. Samples were taken during 2 months, between the beginning of April and the end of May by using the method of visual control. Considering the very active behavior and the height capacity of the lady beetle dispersion, surveys were carried out at 6:00 a.m. by direct observation of the foliage. All the adults of *C. algerica* were delicately collected, put on Petri dishes and taken to the laboratory for mass rearing. The bean culture was found on a private farm based in the Beni-Douala commune (36°37'N, 4°4'E) at an altitude of 800 m and 17 km from the center of Tizi-Ouzou province (Northern Algeria) and 55 km from the Mediterranean Sea. This region is part of the Mediterranean sub-humid ecological region with mild winters, an annual average temperature of 16°C and a precipitation average of 1,006 mm per year.

Raising lady beetles

The lady beetles were raised in the Laboratory of Plant Production, Improvement and Protection based in the Biological and Agricultural Sciences Faculty of Mouloud Mammeri University – Tizi-Ouzou (Algeria).

Harvested individuals were put in a square plastic box (19 × 19 cm) provided with an opening covered with a white screen made of white mosquito netting with fine mesh to ensure optimum ventilation. Accordion-shaped paper was installed in the breeding box to increase the egg-laying area. In addition, fragments of stems and tender bean leaves (*Vicia faba)* were placed in the box to increase the lifespan of aphid populations which involves different stages of development. To avoid cannibalism, *C. algerica* adults were fed daily black bean aphid (*Aphis fabae* Scopoli, 1763) harvested from fields in Beni-Douala. Each pair was placed on a plastic Petri dish, and monitored to calculate average mating length. Once mating was complete, the male was returned to the breeding box, while the female remained on the Petri dish.

Breeding boxes and Petri dishes were cleaned and dried daily to prevent the formation of mold due to excess moisture.

Breeding was carried out under laboratory conditions. Minimum, maximum and average temperatures and humidity were recorded on a daily basis.

Determination of egg incubation time and female fertility

The study of this parameter consisted of a follow-up of eggs laid by 30 *C. algerica* females from mass rearing. The eggs laid by each female were recovered and put separately on a Petri dish with a label giving the laying date and number of eggs laid. Hatching date as well as the number of hatched eggs were recorded later.

Average duration of *Coccinella algerica* development cycle

Once *C. algerica* eggs had hatched, 30 first instar larvae were collected with a soft brush and placed individually on Petri dishes and covered with mosquito nets. Daily, larvae were fed aphids *A. fabae* (approximately 20 for the first two stages, and around 100 for the last stages). Each day, the possible appearance of moulting, indicating a change of larval stage, was noted.

Results

After observing the time from the laying of the eggs until their hatching, the incubation time of *C. algerica* eggs fed black bean aphids averaged 3 to 4 days at an average temperature of 24.29°C and a relative humidity of 47.37% (Table 1). Egg incubation time was inversely proportional to the temperature. In fact, it should be noted that the higher the temperature, the shorter the incubation period. Regarding the relative humidity, the more it increased, the longer the incubation period.

From the calculation of egg fertility which was obtained by the percentage of eggs hatched relative to the number of eggs laid, egg fertility was very high. It

Table 1. Egg incubation duration and fertility of *Coccinella algerica* fed on black bean aphid under laboratory conditions

Repetitions	No. of eggs laid	No. of hatched eggs	Fertility [%]	Incubation time [days]	Mean temperature [°C]	Average relative humidity [%]
1	50	42	84.00	05	21.88	50.14
2	54	44	81.48	05	21.73	48.77
3	50	37	74.00	04	21.81	53.43
4	57	50	87.72	04	21.72	45.00
5	30	25	83.33	04	21.85	47.51
6	40	36	90.00	04	21.85	47.51
7	45	41	91.11	05	21.98	49.70
8	40	35	87.50	05	21.70	48.66
9	34	30	88.24	04	22.42	49.87
10	51	45	88.24	04	22.42	49.87
11	26	26	100.00	04	22.42	49.87
12	32	32	100.00	05	21.87	51.93
13	44	42	95.45	05	22.84	55.71
14	52	41	78.85	04	23.02	55.75
15	42	41	97.62	03	23.04	55.50
16	155	125	80.65	02	24.71	44.70
17	31	29	93.55	03	24.87	43.14
18	37	36	97.30	02	24.87	43.14
19	35	28	80.00	02	24.92	43.90
20	76	63	82.89	02	24.92	43.90
21	51	43	84.31	02	24.92	43.90
22	45	41	91.11	02	24.92	43.90
23	72	57	79.17	02	24.92	43.90
24	69	27	39.13	02	25.06	45.00
25	37	37	100.00	03	25.50	44.20
26	50	48	96.00	03	25.50	44.20
27	43	20	46.51	03	25.50	44.20
28	105	97	92.38	03	25.50	44.20
29	38	38	100.00	03	25.35	44.75
30	77	73	94.81	03	25.35	44.75

reached a rate of 100% for average temperatures between 21.87 and 25.5°C and relative humidity between 44.2 and 51.93%.

Coccinella algerica development cycle lasted an average of 25 days at an average temperature of 22.82°C and an average relative humidity of 48.29% (Table 2).

The mean mating time of *C. algerica* was calculated by direct and careful observation of males and females during mating (from the beginning to the end of the mating of the two adults). This averaged 37 min. The number of eggs varied from 26 to 105 eggs per female. A total of 1,568 eggs was recovered from 30 egg-laying. Eggs hatched after a mean 4 day incubation period, and gave 1,329 first instars. From these, larval development began and went through five larval

Table 2. Average development cycle length of *Coccinella algerica*

Developmental instars	Mean development time [days]	Mean temperature [°C]	Average relative humidity [%]
Pre-oviposition	3	21.81	49.23
Incubation	4	21.72	44.70
Larva 1 (L1)	2	21.41	49.50
Larva 2 (L2)	2	21.10	57.33
Larva 3 (L3)	2.7	22.08	57.05
Larva 4 (L4)	3	22.50	46.37
Larva 5 (L5)	3.3	24.38	47.42
Prepupa	1	24.69	41.75
Nymph	4	25.03	41.29

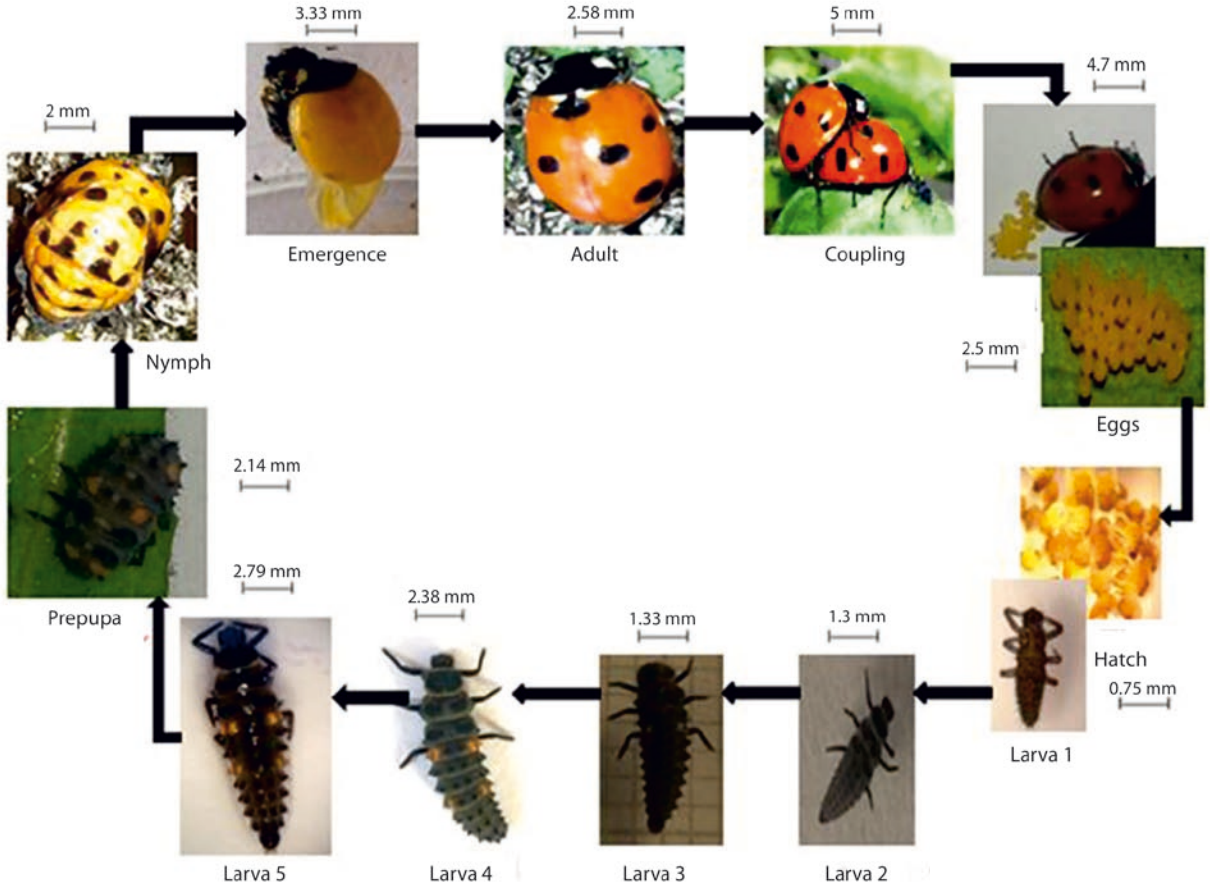

Fig. 1. *Coccinella algerica* life cycle

instars (L1, L2, L3, L4 and L5). Larval development averaged 14 days. At the end of its development, the last stage larvae stopped to pupate. After 4 days, the adults emerged (Fig. 1).

Discussion

From our achieved results, it appeared that egg size for a seven-spotted lady beetle varied from one female to another. These results confirm those of previous studies. Indeed, Saharaoui *et al.* (2001) reported that a *C. algerica* female fed *A. fabae* laid 39 to 875 eggs under field conditions. Average daily fertility ranged from 1.34 to 38.04 eggs. The average number of eggs produced per day varied from 00.6 to 2.09 eggs. According to these authors, diet is a factor which very likely modifies the reproduction rate of lady beetles. This difference seems to be under the control of female physiology. The study conducted by Rahmouni *et al.* (2017) confirms that *C. algerica* egg-laying varies from female to female. Under laboratory conditions 64 to 123 eggs are laid per female at temperatures varying between 25 and 30°C, while under greenhouse conditions, egg-laying varies from 42 to 59 eggs/female at temperatures varying between 24.53 and 27.42°C. These authors state that temperature and diet affect *C. algerica* reproduction. Egg-laying duration, mating and egg size vary from one species to another among lady beetles, and is often determined by climate and trophic conditions (Saharaoui 1987).

Our observations indicate that temperature and relative humidity seem to be factors likely to modify the incubation period of *C. algerica* eggs. This period is longer as the temperature decreases and humidity rises. Ongagna *et al.* (1993) reported that very low incubation times are obtained for the Harlequin Lady Beetle *Harmonia axyridis* at an average temperature of 23.3°C. The study of Benoufella-Kitous (2015) shows that the incubation times of *C. algerica* eggs fed *A. fabae* vary between 3 days at an average temperature of 24°C and a relative air humidity of 73.5%, and 5 days at an average temperature of 20.8°C and a relative air humidity of 68.3%. Saharaoui *et al.* (2001) reported that under field conditions, the incubation time of *C. algerica* eggs varied from 5.20 to 7.88 days at average temperatures of 24.53 and 27.42°C, respectively.

Under controlled conditions, incubation time was 8 days at 20°C; 3.7 days at 25°C and 2 days at 30°C with a relative humidity of 65 to 75%. Incubation period is therefore influenced by temperature and relative

humidity. Rahmouni *et al.* (2017) noted that the incubation period of *C. algerica* eggs was essentially identical under laboratory and greenhouse conditions, i.e. 3 to 5 days, respectively, at average temperatures of 27°C and 30°C. Schanderi *et al.* (1985) found incubation periods of 9.2 days at 15°C, 14.4 days at 20°C and 3.4 days at 25°C for *H. axyridis*. According to Iperti (1964), incubation time of lady beetle eggs varied from 2 to 7 days, irregardless of the species. For Iperti and Brun (1978), this period averaged 5 days. Legemble (2009) stated that embryogenesis only requires 2 to 7 days for all species and seasons. Insects are ectothermic and therefore have very little ability to regulate their body, therefore ambient temperature determines all biological activity of an insect (Brodeur *et al.* 2013).

Fertility rates recorded in this study were very high for the majority of *C. algerica* females. These rates varied according to temperature. It reached a rate of 100% for average temperatures between 21.87 and 25.5°C and relative humidity between 44.2 and 51.93%. According to Benoufella-Kitous (2015), the fertility rate of seven-spotted lady beetles is greater than 45.5% and reaches 100% for average temperatures ranging from 21.2 to 24.3°C. Ongagna *et al.* (1993) reported that mean temperatures below 12°C did not allow hatching of *H. axyridis* eggs. According to Brodeur *et al.* (2013), survival depends on the temperature of individuals, development and reproduction. The same authors note that no complete development is observed in lady beetles at 12 and 36°C. Their lower temperature limit is between 12 and 16°C. The upper thermal threshold is between 33 and 36°C.

In this study, larval development of *C. algerica* coming from the Beni-Douala area (Tizi-Ouzou) passed through five larval instars. This is an original result, as it is different from all previous research results done on lady beetles in general and on the Algerian seven-spotted lady beetle (*C. algerica*) in particular. Earlier it was asserted that the larval development of lady beetles passes only through four larval instars. As a matter of fact, Saharaoui (1998) felt that the development cycle of lady beetles included four instars separated from the adult stage by a pupal stage. Likewise, Benoufella-Kitous (2015) reported four larval instars for the *C. algerica*. Rahmouni *et al.* (2017) observed that *C. algerica* larvae moulted three times, therefore, there were four larval stages with different development durations.

The results of this work show that *C. algerica* development cycle lasts an average of 25 days. The mean larval and nymphal development time was 18 days at 23.03°C. According to Rahmouni *et al.* (2017), the average development duration of *C. algerica* larval state under greenhouse conditions was 18 days at temperatures that varied between 20 and 27°C and a relative humidity of 65 to 70%. Under controlled conditions (laboratory), this time was 11 days for temperatures ranging from 25 to 30°C with a relative humidity of 65 to 75%. It was at the larval stages (especially L2 and L3) that the differences in development time were the most pronounced.

These same authors add that the values may vary according to climatic parameters and available dietary intake. Lady beetle larvae are very voracious, especially those of the 3rd and 4th instar larvae to ensure their growth. According to Saharaoui and Gourreau (1998), the developmental duration of instars varies from one species to another among lady beetles. These authors report that the duration of each instar is often related to climate and trophic conditions. Early larval instars have shorter durations than late ones. Saharaoui *et al.* (2001) noted that under field conditions, the duration of *C. algerica* larval and nymphal growth was 29.36 days and 17.68 days for an average temperature of 22.75 and 28.75°C, respectively, and a photoperiod of 12 to 13 h. However, under controlled conditions, the mean duration of larval and nymphal states was 25.58 days, 13.55 days and 7.96 days, at temperatures of 20, 25 and 30°C, respectively, and a relative humidity of 65 to 75%.

The results of Benoufella-Kitous (2015) show that *A. fabae* provide good trophic conditions for the seven-spotted lady beetle (*C. algerica*) which has a development time of 26.3 days at 22.47°C when fed this aphid species. The average development time of larval and nymphal states is 14.92 days. On the other hand, a diet based on *Aphis citricola* seems to prolong the cycle of this lady beetle development up to 30.53 days at an average temperature of 22.54°C. Saharaoui *et al.* (2001) argue that the average duration of larval development and pupae of *C. algerica* fed black bean aphids, is 19.48 days at 20°C, 12.19 days at 25°C and 6.76 days at 30°C.

On the other hand, a diet based on *A. citricola* seems to prolong the development cycle of this lady beetle which reaches 30.53 days at an average temperature of 22.54°C. Saharaoui *et al.* (2001) argue that the average duration of larval and pupal development of *C. algerica,* fed black bean aphids, is 19.48 days at 20°C, 12.19 days at 25°C and 6.76 days at 30°C. According to Saharaoui and Gourreau (2000) *A. fabae* is one of *C. algerica* favorite prey species to reproduce. This lady beetle consumes 20 aphidian species belonging to four subfamilies, among which the Aphidinae are the most represented with 16 species divided between two tribes and 14 genera, such as: *Acyrthosiphon pisum, Aphis fabae*, *Aphis gossypii*, *Aphis craccivora*, *Brachycaudus cardui, Macrosiphum euphorbiae*, *Rhopalosiphum maidis* and *Rhopalosiphum insertum* (Ben Halima-Kamel *et al.* 2011).

To date, research on the biology of aphidophagous lady beetles has shown that food quantity and quality is an essential element for the development of these

predators, as well as abiotic factors that also affect their natural behavior (Ferran and Larroque 1979). The cycle duration, which for most lady beetles is about 1 month, depends on climate conditions (temperature, relative humidity and photoperiod) and the abundance of food (Iperti 1986).

According to Schaub *et al*. (2010), the development time from egg to adult is relatively short and lasts less than a month. It depends on the species, climate and food. Similarly, Roy *et al*. (2010) note that the development duration of most aphidophagous lady beetles is highly dependent on temperature. When climate and food conditions are favorable, the egg to adult cycle is achieved after a period of 35 to 40 days.

Conclusions

The results concerning the biology of *C. algerica* are interesting, but remain fragmentary and depend on several factors (temperature, relative humidity, prey, possible positive or negative interactions). More extensive studies in the Beni-Douala region are required in order to acquire detailed knowledge on the development of this regional species as well as to study other parameters to obtain better values and protect this auxiliary in biological control programs.

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