

Research article

Defense reactions of the grape aphid, *Aphis illinoisensis* (Hemiptera: Aphididae) to parasitoid species *Lysiphlebus testaceipes* (Hymenoptera: Braconidae) and *Aphelinus albipodus* (Hymenoptera: Aphelinidae)

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Abstract: Developmental periods of the grapevine aphid, Aphis illinoisensis Shimer (Hemiptera: Aphididae) on the grape, Vitis vinifera L., and its two parasitoid species, Lysiphlebus testaceipes (Cresson) (exotic) (Hymenoptera: Braconidae: Aphidiinae) and Aphelinus albipodus Hayat and Fatima (indigenous) (Hymenoptera: Aphelinidae) were studied at two constant temperatures (20 and 25 \pm 2 °C), 70 \pm 5 % R.H. and a photoperiod of 14 L: 10D. The life cycle of A. illinoisensis attained 14.7 and 7.1 days at 20 and 25 °C, respectively. The developmental period was shorter as temperature increased. Developmental time from egg to adult of L. testaceipes was shorter than that of A. albipodus Hayat and Fatima at both 20 and 25 °C. Parasitized aphids were dissected daily to determine the percentage of parasitim, number of encapsulated eggs of L. testaceipes and the number of dead larvae of A. albipodus. The percentage of parasitism of aphid by L. testaceipes was 26% and 12.5% of parasitoid eggs were encapsulated, while 52% of aphids were parasitized by A. albipodus of which 32 % was dead larvae. Therefore, A. illinoisensis has a mechanism for defense against parasitoids and it should be noted that this aphid is not a suitable host for rearing either of the two tested aphid parasitoid species in the laboratory.

Keywords: Aphis illinoisensis, Aphelinus albipodus, Lysiphlebus testaceipes, developmental period, defense

Introduction

The grapevine aphid, *Aphis illinoisensis* Shimer is widespread in the USA and in Central and South America. It was introduced accidentally into Southern Turkey, Greece, Cyprus, Israel, Tunisia, Algeria, Libya and newly recorded in Egypt (El-Gantiry *et al.*, 2012). *Aphis illinoisensis* is at present a serious aphid species

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* Corresponding author, e-mail: daliaadly27@hotmail.com Received: 5 March 2014, Accepted: 22 July 2014 Published online: 31 July 2014 on grapes causing damages by feeding on leaves, shoots and stems. The aphid prefers the leaves on young terminal shoots. It feeds on the foliage and vines of grape plants, but the serious injury results from the infestation of the developing fruit clusters. Dry weather contributes to the growth of the aphid population. *Aphis illinoisensis* is also a vector of Watermelon Mosaic Virus-2. In Brazil, it transmits the virus to grapes and it is more abundant when there is no regular cutting of shoots. (Webb *et al.*, 1994; Kuniyuki *et al.*, 1995).

Lysiphlebus testaceipes (Cresson) (Hym.: Braconidae) and Aphelinus albipodus Hayat and

(Hym.: Fatima Aphelinidae) are solitary endoparasitoids of aphid species. There are few reports on association of the two species with A. illinoisensis in Colombia, Algeria and Egypt (El-Gantiry et al., 2012). Havelka et al. (2011) stated that the species, Aphidius colemani and L. testaceipes seemed to be promising bio-control agents within the framework of an ecologically friendly management in the Mediterranean area. Existence of the parasitoids is dependent on successful parasitism on their hosts. Given that parasitoids often kill their hosts as part of their lifecycle, there is intense selection pressure on the host to evolve defenses against parasitoid attack, and the parasitoid itself is selected to develop counter resistance mechanisms against these host's defenses (Kraaijeveld and Godfray, 2009).

The present work was aimed to study the development of *A. illinoisensis* and its two parasitoid species *L. testaceipes* (introduced) and *A. albipodus* (indigenous), as related to temperature and to evaluate defense reactions and suitability of the aphid to these parasitoid species as a host under laboratory conditions.

Materials and Methods

Insect colonies

Colonies of the aphid species *A. illinoisensis* and the parasitoid species *L. testaceipes* and *A. albipodus* were maintained under the laboratory conditions at 22 ± 2 °C, 70 ± 5 % R. H. and a photoperiod of L: D 14:10.

Parasitoid species

The exotic parasitoid species, *L. testaceipes*, had not been recorded in Egypt before and was obtained from Dr. P. Stary; Institute of Entomology, Academy of Science of the Czech Republic, through a personal contact and the native parasitoid species *A. albipodus*, was collected from wheat fields, Giza Governorate, Egypt, 2012.

Effect of temperature on the development of *A. illinoisensis*

The development of *A. illinoisensis* and the two aphid parasitoid species *L. testaceipes* and *A. albipodus* were studied at two constant temperatures (20 and 25 ± 2 °C), photoperiod of 14:10 (L: D) and 70 ± 5 % R. H..

Developmental time was measured by using newly deposited nymphs (40 individuals/ treatment). Each individual of the first nymphal instar of A. illinoisensis was placed on young shoots of V. vinifera. The shoots were incubated at each of the aforementioned temperatures. Nymphs in each temperature regime were monitored daily to determine molting, developmental time and mortality percentage. The grape, Vitis vinifera L. seedlings were grown in plastic pots (40 cm. in diameter and 32 cm. high) in greenhouse. The newly deposited nymphs were transferred individually to shoots and maintained by placing plastic rearing cages on young shoots. These cages were prepared from cylindrical plastic vial (4cm. diam.× 7cm height); most of its bottom and sides were cut and replaced by muslin to allow good aeration, the top of the vial was provided with a circular opening (for the insertion of young shoot). The end of the shoot was surrounded by a small piece of synthetic sponge to prevent the escape of aphid. The cage was based on a wooden pillar to prevent breakage of the shoots. When necessary, aphids were gently brushed to withdraw their proboscis, and were carefully transferred to fresh shoots by means of a camel's hair brush moistened with water.

Developmental time of L. testaceipes and A. albipodus was measured by using one hundred of 2nd and 3rd nymphal instars of A. illinoisensis that were placed on young shoots of V. vinifera. cultivated in pots (15 cm diameter) and placed in cloth rearing cages (40 x 50 x 60 cm) covered with muslin (10 replicates/treatment). In each cage, aphids were exposed to 10 newly hatched mated females of the parasitoids for four hours. Afterwards, parasitoid females were removed and the cages were placed in incubators at the selected temperatures. Fifty exposed aphids were dissected daily by a very fine needle, in a drop of Ringer's solution using a stereomicroscope to determine the developmental time of different parasitoid stages (egg, larval instars, mummy to adult and egg to adult).

Defense reactions capabilities of A. illinoisensis as host against L. testaceipes and A. albipodus

Ten mated females of each parasitoid species were provided for each 100 nymphs of A.

illinoisensis (2^{nd} and 3^{rd} instars) daily until death at 25 ± 2 °C in a small cage, containing droplets of pure honey and grape leaves the ends of which were dipped in moistened paper towels. Parasitized aphids were dissected daily to determine the number of encapsulated eggs of *L. testaceipes* and the number of dead larvae of *A. albipodus* (10 replicates/treatment).

Statistical analysis

Obtained data of the aphid and parasitoid species were statistically analyzed for means, using ANOVA and Duncan's Multiple Range Test, SPSS program (Statistical Package for the Social Sciences).

Results

Effect of temperature on the development of *A. illinoisensis*

Nymphs of A. illinoisensis grew and molted through four instars. The duration of the different nymphal instars varied significantly related to the temperature. Temperature had a significant influence on the aphid life-cycle (Table 1). The developmental period of A. illinoisensis significantly decreased, when reared at 25 °C (7.1 \pm 0.34 days), while it lasted longer at 20 $^{\circ}$ C, (14.7 \pm 1.05 days). Thus, the mean developmental period of the host aphid varied about 2.03 folds between the two temperatures. Total mortality percentage of A. illinoisensis was high (75.0%) at both temperatures (Table 1). The highest percentage of mortality was 47.50 and 50.0% in the 1st nymphal instars while the lowest was 2.5 and 5% in the 3rd nymphal instars at 20 and 25 °C, respectively. Effect of temperature on the aphid parasitoids

Mean total developmental time of the two parasitoid species, L. testaceipes and A. albipodus parasitizing A. illinoisensis (eggs, larvae, mummy to adult and egg to adult) at the two temperature levels are summarized in Table 2. Developmental time of the immature stages decreased as temperature increased in both aphid parasitoids. Developmental time from egg to adult of the parasitoid L. testaceipes was shorter than that of A. albipodus at the tested temperatures: $13.75 \pm$

0.009 and 17.9 \pm 0.13 days at 20 °C and 10.15 \pm 0.01 and 11.4 \pm 0.21 days at 25 °C, respectively.

The shortest developmental time was recorded for the eggs and the longest was in the period from mummy to adult emergence in both parasitoid species (Table 2).

Lysiphlebus testaceipes males emerged earlier than females. Males were apparently ready to mate as soon as they emerged. Males and females were easily discriminated by the posterior abdominal segment. All emerged adults of A. albipodus were females.

Defense reactions capabilities of A. illinoisensis as host for L. testaceipes and A. albipodus

As it was shown that the percentages of parasitism and adult emergence in the two parasitoid species were very low in the previous experiment, therefore an experiment was designed to find out the reason for this phenomenon.

The eggs laid by *L. testaceipes*, are spherical, surrounded with a very thin transparent and smooth chorion after 24 hours (Fig. 1 a). The egg size increases rapidly as it is deposited inside the host. The embryo is clearly visible and characterized by head and body form after 48 h., but the segmentation was not apparent inside the egg. This appears inside the egg, just before hatching (Figs. 1 b-c). Encapsulated egg had an irregular shape as haemocytes formed a multilayered envelope around it (Fig. 1d). Out of 26% of eggs laid by *L. testaceipes* 13.5% were healthy eggs and 12.5% were encapsulated eggs, almost 48% of the eggs were encapsulated.

A. albipodus has three larval instars which differ in their morphological characteristics. Alive 2nd larval instars appeared spherical in shape at thoracic and the beginning of the abdominal segments, the gut tended to be yellow (Fig. 2 a). Alive 3rd larval instar seemed to be spherical in shape. Thoracic and abdominal segments were faintly visible. The gut was vellow and tended to turn black at the end of this instar and occupied most of the body cavity (Fig. 2c). The dead larvae turned to reddish brown in color, showing dark midgut (Figs. 2 b-d). Percentage of parasitism was 52% including 32% alive 2nd and 3rd larval instars (Figs. 2 a-c) and 20% dead 2nd and 3rd larval instars, almost 38% of the larvae were dead.

Table 1 Effect of temperature on the duration (Mean \pm SE) and mortality of the nymphal instars of the grapevine aphid, *Ahis illinoisensis*, at 14 L: 10 D and 70 \pm 5 % R.H.

Developmental stages	20 (°C)		25 (°C)	
	Duration (days)	Mortality (%)	Duration (days)	Mortality%
1 st	3.1 ± 0.42^{a}	47.5	1.5 ± 0.22^{b}	50.0
2^{nd}	3.2 ± 0.22^a	15.0	2.1 ± 0.10^b	10.0
3^{rd}	4.6 ± 0.62^a	2.5	1.7 ± 0.15^{b}	5.0
4 th	3.9 ± 0.48^a	10.0	1.8 ± 0.20^b	10.0
Total	14.7 ± 1.05^a	75.0	7.1 ± 0.34^{b}	75.0

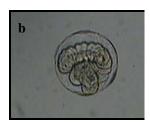
Mean with the different letters in the same row indicate significant differences at $P \le 0.05$.

Table 2 Effect of temperature on the duration in days (Mean \pm SE) of developmental stages of the two parasitoid species, *Lysiphlebus testaceipes* and *Aphelinus albipodus* on *Aphis illinoisensis* at 14 L: 10 D and 70 \pm 5 % R.H.

Developmental stages	L. testaceipes		A. albipodus	A. albipodus	
	20 (°C)	25 (°C)	20 (°C)	25 (°C)	
Egg	3.25 ± 0.01^{a}	1.65 ± 0.10^{b}	3.5 ± 0.14^{a}	1.45 ± 0.11^{b}	
Larval instar	4.20 ± 0.01^a	4.60 ± 0.11^{a}	4.8 ± 0.01^a	3.45 ± 0.11^a	
Mummy to adult	6.30 ± 0.11^{a}	3.90 ± 0.06^{b}	9.6 ± 0.12^{c}	6.50 ± 0.10^d	
Egg to adult	13.75 ± 0.01^{a}	10.15 ± 0.01^{b}	17.9 ± 0.13^{c}	11.40 ± 0.21^d	

Means in a row (for each parasitoid) followed by different letters are significantly different at P < 0.05.







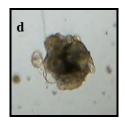
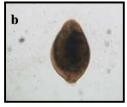
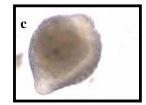


Figure 1 Living and encapsulated eggs of *Lysophlebus testaceipes*; a. b. c. Embryological development of egg < 24 h old to egg before hatching; d. Encapsulated egg.







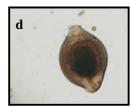


Figure 2 2nd and 3rd instars larva of *Aphelinus albipodus*; a. Alive 2nd instar larva; b. Dead 2nd instar larva; c. Alive 3rd instar larva; d. Dead 3rd instar larva.

Discussion

Temperature has a great impact on the life cycle of aphids. Higher temperature shortens life cycle, (Abdel-Rahman, 1997; EL-Gantiry et al., 1999). Results of this experiment showed that the developmental durations decreased with increasing the temperature. These results are in agreement with the findings of Cleopatra et al. (2012) who reported that A. illinoisensis developed faster on some grape cultivars as temperature increased from 17-26 °C. In the present study, high percentage of mortality occurred in 1st nymphal instar and the total percentage of mortality for A. illinoisensis was also high at both temperature levels. The high percentage of aphids' mortality may be due to the leaf morphology of the grape variety used under the experimental conditions. Various studies have shown that leaf morphology affects aphids' survival and performance (Legrand and Barbosa, 2000; Zarpas et al., 2006; Buchman and Cuddington, 2009).

The developmental time of parasitoid species decreased as temperature increased. Developmental time from egg to adult for *L. testaceipes* when parasitizing *A. illinoisensis* was 13.75 and 10.15 days at the two temperature levels, respectively, while it was 14.8, 11.3 days at 20 and 25 °C on *A. gossypii* (Rodrigues *et al.*, 2004).

In this study also, developmental time from egg to adult for *A. albipodus*, when parasitizing *A. illinoisensis* was 17.9 and 11.4 days at the two temperature levels, respectively, while it was 19, 17.5 and 17.7 days on *A. gossypii, Myzus persicae* and *Macrosiphum euphorbiae* at 18 °C, respectively (Tatsumi and Takada, 2005).

Egg encapsulation of *L. testaceipes* and dead larvae of *A. albipodus* observed in this study can be interpreted as a strategy whereby a parasitoid may find a potential host in its habitat and even select to attack it but its relationship still may not be successful if the attacked host is immune (Anjum *et al.*, 2002).

A suitable host allows all or nearly all immature stages of the parasitoid to develop

into adults, whereas marginal hosts allow only a small proportion to develop and unsuitable hosts allow no parasitoid development (Firlej *et al.*, 2007). Host suitability depends on factors such as the host immune system and host toxins (Lavine and Strand, 2002). Encapsulation has been reported as the main physiological mechanism for defense against parasitoids in insects (Godfray, 1994).

The aphid may be able to defend itself by encapsulation of eggs or larvae or with chemical toxins as an aphid physiological response to foreign objects. Encapsulation is the condition whereby homocytes form a multilayered envelope around the invading organism (Michael and Pech, 1995) and prevent its normal development.

Miller (1928) found that L. testaceipes attacked Aphis spiraecola and this host was ultimately killed but the parasitoid larvae never completed their development. Sekhar (1960), obtained oviposition by Aphidius testaceipes and *Praon aguti* in several aphid species but they emerged from only a few of the hosts. Carver and Sullivan (1988) demonstrating egg encapsulation in Myzus ascalonicus Doncaster and Aulacorthum circumflexum (Buckton), where both aphid species encapsulated eggs and young larvae of *Diaeretiella rapae*, and also in Sitobion avenae which encapsulated A. rhopalosiphi larvae. Anjum et al. (2002), reported that the parasitoid, L. ambiguus made many tapping and ovipositional attempts on Brevicoryne brassicae but did not complete development in this aphid species.

The percentage of parasitism of *L. testaceipes* was clearly low in this study and the immunity system of *A. illinoisensis* using the egg encapsulation reaction caused a considerable decrease in parasitism.

It was found here that the percentage of parasitism of *A. albipodus* on *A. illinoisensis* was low and the dead larvae caused additional decrease in parasitism. It was noticed that some of the parasitized aphids which had dead larvae continued their development and produced progeny. Physiological unsuitability of the host for immature stages of the parasitoid, *A.*

albipodus may be due to the lack of some necessary nutritional or hormonal resources or the host species that may influence the rate of development and the survival of a parasitoid (Carver and Sullivan, 1988; Godfray 1994; Pennacchio and Strand 2006; Kant et al., 2008). Some hosts sequester secondary plant metabolites parasitoid making them unsuitable for development (Ode, 2006; Behmer, 2009). In some aphid and weevil species, resistance to parasitoids can be mediated by endosymbiotic bacteria (Oliver et al., 2003 and 2005).

Previous studies reported that *L. testaceipes* and *A. albipodus* are associated with *A. illinoisensis* in some countries (El-Gantiry *et al.*, 2012; Havelka *et al.*, 2011). However, our data indicated that, due to low percentage parasitism, this host is not suitable for rearing either of these parasitoids in the laboratory. Thus, they are not recommended as biological control agents in the biological control programs against *A. illinoisensis*.

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واكنش دفاعى شته مو، (Hemiptera: Aphididae) به زنبورهاى پارازيتوييد Aphis illinoisensis (Hemiptera: Aphididae) واكنش دفاعى شته مو، (Hymenoptera: Aphelinidae) (Hymenoptera: Aphelinidae)

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