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 ± 2 °C), 70 ± 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 parasitism, 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 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
Fatima (Hym.: 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.*
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. 1a). 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. 1b-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. 2a). Alive 3rd larval instar seemed to be spherical in shape. Thoracic and abdominal segments were faintly visible. The gut was yellow 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. 2a-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 ± SE) and mortality of the nymphal instars of the grapevine aphid, *Aphis illinoisensis*, at 14 L: 10 D and 70 ± 5 % R.H.

<table>
<thead>
<tr>
<th>Developmental stages</th>
<th>20 (°C)</th>
<th>25 (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Duration (days)</td>
<td>Mortality (%)</td>
</tr>
<tr>
<td>1&lt;sup&gt;st&lt;/sup&gt;</td>
<td>3.1 ± 0.42&lt;sup&gt;a&lt;/sup&gt;</td>
<td>47.5</td>
</tr>
<tr>
<td>2&lt;sup&gt;nd&lt;/sup&gt;</td>
<td>3.2 ± 0.22&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.0</td>
</tr>
<tr>
<td>3&lt;sup&gt;rd&lt;/sup&gt;</td>
<td>4.6 ± 0.62&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.5</td>
</tr>
<tr>
<td>4&lt;sup&gt;th&lt;/sup&gt;</td>
<td>3.9 ± 0.48&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.0</td>
</tr>
<tr>
<td>Total</td>
<td>14.7 ± 1.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>75.0</td>
</tr>
</tbody>
</table>

Mean with the different letters in the same row indicate significant differences at P ≤ 0.05.

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

<table>
<thead>
<tr>
<th>Developmental stages</th>
<th><em>L. testaceipes</em></th>
<th><em>A. albipodus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20 (°C)</td>
<td>25 (°C)</td>
</tr>
<tr>
<td>Egg</td>
<td>3.25 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.65 ± 0.10&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Larval instar</td>
<td>4.20 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.60 ± 0.11&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mummy to adult</td>
<td>6.30 ± 0.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.90 ± 0.06&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Egg to adult</td>
<td>13.75 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.15 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

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 *Lysiphlebus 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 making them unsuitable for parasitoid 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.

Acknowledgments

We wish to thank P. Stary (Institute of Entomology, Academy of Science of the Czech Republic) for providing us with the aphid parasitoid, L. testaceipes through a personal contact. We would like also to thank Ahmed El-Heneidy and Prof. Saber Moussa (Plant Protection Research Institute, Agricultural Research Center, Giza, Egypt) for their guidance and reviewing the manuscript.

References


 واکنش دفاعی شته مو آفیلینوس آلبیپودوس به زنبورهای پارازیت‌زده A. illinoisensis (Hemiptera: Aphididae) و لیسیلفه چوپی لیسیلفه testaceipes (Hymenoptera: Braconidae: Aphidiinae) (Hymenoptera: Aphelinidae) به Zn bonne (Hemiptera: Aphididae) روی مو Aphis illinoisensis Shimer (Hemiptera: Aphididae) و و دو زنبور پارازیتوپید آن، vinifera L. Aphelinus albipodus Hayat and Fatima (indigenes) (Hymenoptera: Braconidae: Aphidiinae) در دو دمای ثابت (۲۰ و ۲۵ درجه سلسوس) ۵ ± ۲ درصد رطوبت نسبی و دوره نوری ۱۲ ساعت تابستان و ۱۰ ساعت روشنایی مورد بررسی قرار گرفت. دوره زنده شده A. illinoisensis در دمای ۲۰ و ۲۵ درجه سلسوس به ترتیب ۷/۱ و ۷ درصد محاسبه شد. دوره شده‌پاشا A b. testaceipes Kوتاهاز از زنبور L. testaceipes Hayat and Fatima به‌منظور تعیین درصد پارازیت‌زده، تعداد تخم‌های کپسوله شده زنبو به‌صورت رونده تشريح و شمارش شدند. درصد پارازیت‌زده A. albipodus زنبو در دوره زنده ۷۴ درصد بود که از این مقدار درصد مربوط به تخم‌های کپسوله شده بود L. testaceipes در حالی که درصد پارازیت‌زده A. illinoisensis زنبو در دوره مربوط به لاروها مره بود. بنابراین می‌توان تنها گیری نمونه شده A. illinoisensis مکانیزم دفاعی در برای پارازیت‌زده‌ها و به‌نظر می‌رسد که این شته شده می‌باشد با رای برورش زنبورهای مورد بررسی در شرایط آزمایشگاهی است. واژگان کلیدی Lysiphlebus testaceipe Aphelinus albipodus Aphis illinoisensis