Larval Parasitoids of *Lobesia botrana* (Denis and Schiffermüller, 1775) (Lepidoptera: Tortricidae) in Orumieh Vineyards

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**ABSTRACT**

Grape berry moth, *Lobesia botrana* (Denis and Schiff.) is a destructive pest of grapevine in Orumieh (Northwest, Iran) vineyards. It is mainly controled through application of broad-spectrum insecticides, which can adversely affect vineyard ecosystem and consequently human health. Since a first step in setting up an IPM program is to assess the biological control agents within the ecosystem, so in this research work the larval parasitoid complex of Grape Berry Moth (GBM) on vine was identified, and its natural potential in controlling the pest compared for different generations and in different locations in Orumieh during 2004-2006. Adults of the larval parasitoids of GBM were recorded from laboratory-reared larvae, which had been collected on damaged inflorescences of the first generation and berries of the second and the third generations. Rate (%) of parasitism on *L. botrana* larvae was estimated as the number of parasitoids over the total count of parasitoids and moths. Six larval parasitoids of the host were found: *Enytus apostata* Gravenhorst, 1829; *Pristomerus vulnerator* (Panzer, 1799); *Temelucha* sp. (Hymenoptera: Ichneumonidae), *Nemorilla maculosa* (Meigen, 1824) (Diptera: Tachinidae), *Habrobracon hebetor* (Say) and *Bracon* sp. (Hymenoptera: Braconidae). Total parasitism varied from 1 to 16.8 %, with an average of 7.7% as revealed through the present study. A comparison of the means of the larval parasitism rate showed a significant difference at 5% level between different generations and localities. The highest rate of parasitism occurred in the first generation in all localities, as well as in years.

**Keywords**: Braconidae, Grape berry moth, Ichneumonidae, Iran, Orumieh, Parasitoid, Tachinidae.

**INTRODUCTION**

Grape berry moth, *Lobesia botrana* is one of the economically important pests in vineyards of Europe, southern Russia, Japan, the Middle East, Near East and Northern as well as West Africa (Venette et al., 2003). It is considered as a key pest of grapes in all vinegrowing parts of Iran as well (Gharib, 1960; Rezvani, 1981; Saber et al., 1998). The pest causes direct damage to grapes by penetration flowers and berries and indirect damage by favoring the growth of such rot fungi as *Botrytis cinerea*. During the first generation, each larvae builds a shelter called ‘glomerula’, and feeds on flowers and buds, but the second and third generation larvae feed on unripe and ripening berries (Bovey, 1966; Thiéry and Moreau, 2005). GBM larvae complete five instars, and the total developmental time from egg hatching to pupation is about 5-6 weeks under spring climatical conditions. Biological control is a possible future strategy against grape berry moths (Roehrich and Boller, 1991). Among the biological control agents of grape berry moth, the egg parasitoids specially Trichogrammatids attracted more attention for mass rearing and releasing. They have reduced

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the grape berry moth populations from 20 to 80%, depending upon trials and sites (Remund, 1990; Castaneda-Samayoa et al., 1993; Barnay, 1999; El-Wakeil et al., 2009).

Several larval or pupal parasitoids may naturally control the population of *L. botrana* in European vineyards (Coscola, 1982; Belcari and Raspi, 1989; Marchesini and Della Monta, 1994; Thiéry et al., 2001; Thiéry and Xuéreb, 2003; Xuéreb and Thiéry, 2006), but only few studies have evaluated their importance in controlling grape tortricids (Schmid, 1978; Coscola, 1997; Perez Moreno et al., 2000; Thiéry et al., 2001). There are few reports on the occurrence of grape berry moth parasitoids from Iran. Egtedar (1996) has reported 20-25% mortality of *L. botrana* larvae by an Ichneumonid wasp in Fars Province. Egg parasitism of *L. botrana* by *Trichogramma ingricum* Sorokina, 1984 was reported from Orumieh, Iran (Ebrahimi and Akbarzadeh, 2008). Soudi and Shojaii (2006) have announced the parasitation of grape berry moth pupae by *Enytus apostata* Gravenhorst in Shahryar and Takestan regions of Iran.

There are more than 20,000 ha of vineyards in Orumieh, Iran, and *L. botrana* is considered as its key pest. There are three generations to the pest per year and all the three are controlled by use of effective pesticides mainly belonging to organophosphate group. It is fundamental that the first step in setting up an IPM program be the assessment of the biological control agents within the local ecosystem. Therefore, in this study the larval parasitoid complex of Grape Berry Moth on vine was determined, and its natural potential for pest control evaluated for different generations and in vine growing regions of Orumieh. This work presents a study on the species’ composition and impact of the larval parasitoid complex against *L. botrana* in Orumieh as well as on the species’ dynamics.

**MATERIALS AND METHODS**

Larvae of *L. botrana* were collected from unprayed vineyards to survey the larval parasitoids of the pest during 2004-2006. These samples were collected from five main grape growing sites of Orumieh including Goushchii (50 km to north from Orumieh along the Orumieh Lake coast), Kahriz (Horticultural Research Station), Bakshlouchayi (20 km toward the East from the Orumieh city), Nazlouchayi (10 km toward the West from the city) and Barandouzchayi (10 km toward the South west from the city). In all the selected vineyards, trees were reared in the traditional system and with at least 20 years old. *Vitis vinifera* cv. Bidakheh Sefid, as the prevalently grown cultivar, was taken for sampling. For the first generation, glomerulae occupied by *L. botrana* larvae were randomly selected from different vineyards and collected twice by hand in 10-15 day intervals from flight peaks which were determined by pheromone traps. Larvae of the second and third generations of *L. botrana*, which feed on the berries, were collected by randomly incising the damaged berries. In each sample a minimum number of 50 larvae was collected. The larvae in each sample were nursed using florescences or grape berries (depending upon the generation of the pest) in separate rearing containers at room conditions of 23±2°C and 60±5 RH. They were checked daily for emergence of moths and parasitoids. Following a completion of the emergence of the moths and parasitoids, percent parasitization of *L. botrana* larvae was estimated as the number of parasitoids over the total count of parasitoids and moths. Emerged parasitoids were counted and, then separated into orders and families. They were identified by related specialists from different entomological institutes in and out of Iran (Museum fuer Naturkunde der Humboldt-Universitaet zu Berlin, Germany; Universitaet Wuerzburg Biozentrum, Zoologie3, Germany and Iranian Research Institute of Plant Protection Tehran, Iran). In order to compare mean of parasitism rates among generations and locations, Mstc software was used after transforming the means in to $\sqrt{p}$.
RESULTS

The Identified Parasitoid Species

Six hymenopteran and dipteran larval parasitoids of *L. botrana* were recorded from Orumieh vineyards (Table I). They belong to two orders and three families as follows: *Enytus apostata* Gravenhorst 1982, *Pristomerus vulnerator* (Panzer, 1799), *Temelucha* sp. (Hymenoptera: Ichneumonidae), *Habrobracon hebetor* (Say), *Bracon* sp. (Hymenoptera: Braconidae) and *Nemorilla maculosa* (Meigen) (Diptera, Tachinidae).

*Enytus apostata, Pristomerus vulnerator, Temelucha sp., Habrobracon hebetor* and *Nemorilla maculosa* were registered as new records of GBM parasitic wasp from Orumieh, Iran. The relative mean abundances of all Ichneumonids, Braconids and Tachinids in the parasitoid complex of *L. botrana* larvae were recorded as 70%, 16% and 14%, respectively. During all the three years of study, the Ichneumonid *Enytus apostata*, forming 55.3% of the parasitoid population, was the most conspicuous parasitoid among the identified larval parasitoids of *L. botrana*. It was the prevalent species, with its parasitic activity remarkably considerable during all the three generation periods of the pest. *Pristomerus vulnerator*, forming 17% of the Ichneumonids and 11.9% of the total parasitoids, was considered as an important agent with regard to its activity during the growing season and on all three generations of *L. botrana*. The Tachinid *Nemorilla maculosa* was the only dipteran species, which constituted 14% of the parasitoid abundance. It was recorded only from the first generation larvae of the pest. The remaining parasitoids occurred in relatively low numbers and did not play much an important role as biological control agents.

Parasitism Rate

The parasitism rates of *L. botrana* larvae for the respective years of 2005 and 2006, at different locations and for the three different generations are shown in Figures 1 and 2.

The mean total parasitism rate of *L. botrana* larvae in all the studied regions of Orumieh during 2005-2006 was 7.7 percent. In 2005, the parasitism rate of the pest larvae in different localities of Orumieh varied from a minimum of 3% up to a maximum of 16.8% with a mean of 8.5%. In 2006, it varied from a minimum of 1% up to a

### Table 1. The identified parasitoid species of *L. botrana* and their abundance in Orumieh vineyards.

<table>
<thead>
<tr>
<th>Species</th>
<th>Family</th>
<th>Parasitoids’ abundance (Percentage) 2005</th>
<th>Parasitoids’ abundance (Percentage) 2006</th>
<th>Mean of parasitoids abundance (Percentage)</th>
<th>Relative abundance of parasitoids</th>
<th>Emerged generation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ichneumonidae</td>
<td></td>
<td>67</td>
<td>73</td>
<td>70</td>
<td>55.3</td>
<td>1, 2, 3</td>
</tr>
<tr>
<td><em>Enytus apostata</em></td>
<td></td>
<td>82</td>
<td>76</td>
<td>79</td>
<td>11.9</td>
<td>1, 2, 3</td>
</tr>
<tr>
<td><em>Pristomerus vulnerator</em></td>
<td></td>
<td>16</td>
<td>18</td>
<td>17</td>
<td>2.8</td>
<td>2</td>
</tr>
<tr>
<td><strong>Temelucha sp.</strong></td>
<td></td>
<td>2</td>
<td>6</td>
<td>4</td>
<td>14.6</td>
<td>1</td>
</tr>
<tr>
<td>Braconidae</td>
<td></td>
<td>20</td>
<td>12</td>
<td>16</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Habrobracon hebetor</em></td>
<td></td>
<td>88</td>
<td>94</td>
<td>91</td>
<td>14.6</td>
<td>2, 3</td>
</tr>
<tr>
<td>Bracon sp.</td>
<td></td>
<td>12</td>
<td>6</td>
<td>9</td>
<td>1.4</td>
<td>1, 3</td>
</tr>
<tr>
<td>Tachinidae</td>
<td></td>
<td>13</td>
<td>15</td>
<td>14</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Nemorilla maculosa</em></td>
<td></td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>14</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

* Newly recorded as parasitoid of the host.

** Genus or species, as new records for Iran’s insect fauna.
maximum of 14% with a mean of 6.8%.

**Impact of the Parasitoids**

The means of parasitism rate in different locations and for different generations in two years of the study period were analyzed, the results for which are shown in Table 2. The results reveal that there are significant differences in larval parasitism rates as regards between locations and generations and at 1% level of probability.

According to the results presented in Table 2, the parasitism rate varied between locations and generations. The data are illustrated in Figure 1 and Figure 2.

**Figure 1.** Larval parasitism rate (%) of *L. botrana* in Orumieh vineyards (2005).

**Figure 2.** Larval parasitism rate (%) of *L. botrana* in Orumieh vineyards (2006).
Table 2. Analysis of variance table.

<table>
<thead>
<tr>
<th>Value</th>
<th>Source</th>
<th>Degree of Freedom</th>
<th>Sum of Squares</th>
<th>Prob.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Year</td>
<td>1</td>
<td>0.943</td>
<td>0.0368</td>
<td></td>
</tr>
<tr>
<td>Location</td>
<td>4</td>
<td>10.566</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>Generation</td>
<td>2</td>
<td>2.661</td>
<td>0.0061</td>
<td></td>
</tr>
<tr>
<td>L×G</td>
<td>8</td>
<td>1.201</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Error</td>
<td>14</td>
<td>2.479</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>29</td>
<td>17.850</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Coefficient of Variation: 15.8%

Table 3. Mean (Percent) of larval parasitism at different locations during 2005-2006, Orumieh vineyards.

<table>
<thead>
<tr>
<th>Location</th>
<th>Kahriz</th>
<th>Goushchi</th>
<th>Baranduzchayi</th>
<th>Nazloochayi</th>
<th>Bakeshloochayi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (Percent) of parasitism</td>
<td>7.8 b</td>
<td>8.7 ab</td>
<td>2.8 c</td>
<td>11.9 a</td>
<td>7.2 b</td>
</tr>
</tbody>
</table>

Means followed by the same letter within a row are not significantly different.
Data analyzed using a √p transformation
P< 0.05.

3, the highest larval parasitism occurred in Nazloochayi with 11.9%, with significant differences being observed between Nazloochayi and Goushchayi regions, and Baranduzchayi, which had only 2.8% of parasitism at the 5% level. The remaining regions including Kahriz and Bakeshloochayi stood between these two groups.

Results concerning comparison of the means of larval parasitism rates for different generations indicated that there were significant differences among generations at a 5% level (Table 4). The first generation with a rate of 9% significantly differed from the second and third generations at a 5% level of probability.

Table 4. Mean (percent) of larval parasitism at different generations during 2005-2006, Orumieh vineyards.

<table>
<thead>
<tr>
<th>Generation</th>
<th>first</th>
<th>Second</th>
<th>Third</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (Percent) of parasitism</td>
<td>9.9 a</td>
<td>7.8 b</td>
<td>6.0 b</td>
</tr>
</tbody>
</table>

Means followed by the same letter within a row are not significantly different.
Data analyzed using a √p transformation.
P< 0.05

DISCUSSION

In Orumieh, L. botrana fulfills three generations per year. Enytus apostata was found to be the most common parasitoid of L. botrana with a 55.3 percent of total larval parasitism. This occurred in all the studied regions and for all the three generations. There had not been any evidence of parasitism of L. botrana larvae by this Ichneumonid wasp reported in Iran although Soudi and Shojaii (2006) have reported the parasitation of Grape Berry Moth pupae by E. apostata Gravenhorst in Shahryar and Takestan regions of Iran.

Pristomerus vulnerator parasitized up to 12% of L. botrana larvae and is recorded as a new parasitoid of L. botrana. Although known as a parasitoid of codling moth, Cydia pomonella L., larvae in Iran (as reported by Radjabi (1986)), L. botrana is also mentioned as a new host of the parasitoid. The Ichneumonid wasp Temelucha sp. was found in low densities in Orumieh vineyards. It may not have a major
role in biological control, but it is mentioned as a new genus for Iranian insect fauna from Orumieh. The Braconid wasps of *Habrobracon hebetor* (Say) and *Bracon* sp. are known as parasitoids of many lepidopterous larvae and this study certified their occurrence on Grape Berry Moth larvae as well. The Tachinid fly *Nemorilla maculosa* (Meigen) is the only Dipterean parasitoid detected in Orumieh vineyards on this pest. The fly has been known as the larval parasitoid of *Hyponomeuta malinellus* Zell. and *H. padellus* L. in Iran (Radjabi, 1986), with the Grape Berry Moth larvae introduced as a new host to it. None of the introduced larval parasitoids of Grape Berry Moth and *Xanthandrus comtus* (Harr.) was able to destroy all of the first generation larvae (Belcari and Raspi, 1989) and for *Phytomyza nigrina* larval parasitism being reported from France (Thiéry et al., 2006), they formed only up to 15% of the parasitoid abundance and were effectively acting only on the first generation larvae of Grape Berry Moth in Orumieh vineyards. None of the introduced larval parasitoids of Grape Berry Moth from Orumieh had been registered among European and American *L. botrana* parasitoids (Moleas, 1979; Nuzzaci and Triggiani, 1982; Belcari and Raspi, 1989; Marchesini and Della Montà, 1994; Thierè et al., 2006), so they could be considered as new parasitoids of the Grape Berry Moth. According to the present study, the Grape Berry Moth larval parasitism could reach up to 16.8%, which is not sufficient enough for damage control, but along with other larval parasitoids, there is a considerable complex of natural enemies of *L. botrana* in Orumieh vineyards including egg parasitoids of: *Trichogramma embryophagum* (Hartig) and *T. inricum* Sorokina, 1984 Which are able to cause up to 40% egg parasitism on first generation of the pest (Akbarzadeh Shoukat and Ebrahimi, 2008). Pupae parasitoids (Akbarzadeh et al., 2008) along with other different groups of predators (unpublished) effective at different life stages of *L. botrana*, make the performance of biological control to be promising, specially at the first generation of the pest.

**ACKNOWLEDGEMENTS**

I am grateful to ProF. Klaus Horstmann, Universitaet Wuerzburg, Biozentrum, Zoologie 3 Am Hubland, Germany, for identifying the species of Ichneumonidae, Dr. Joachim Ziegler, Curator of Diptera and Siphonaptera, Museum fuer Naturkunde der Humboldt-Universitaet zu Berlin, Germany, for identifying the Tachinidae. The author is also indebted to Dr. Ebrahim Ebrahimi and Dr. Ashkan Masnadi Yazdinejad, Iranian Research Institute of Plant Protection, Tehran, Iran for identifying the species Ichneumonidae and Braconidae. I also thank Mrs. Carola Lora for critical reading and revision of the English version of the manuscript.

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Larval Prasitoids of Grape Berry Moth

پارازیتوئیدهای لارو شب پره خوشه‌های انگور

*Lobesia botrana* (Denis and Schiffermüller, 1775) (Lepidoptera: Tortricidae)

در تاکستان‌های اروپه

غ. اکبرزاده شوکت

چکیده

شب پره خوشه‌های انگور در *Lobesia botrana* (Denis & Schiffermüller) در شمال غرب ایران است. مبارزه با این آفت عمده‌ای با استفاده از جنگلهای طبیعی و سمی انجام می‌شود که می‌تواند باعث عوارض و زثر بر سلامت انسان و اکوسیستم تاکستان‌ها گردد. این آفت جزو اولین گام در برقراری یک برنامه مدیریت تلفیقی شناسایی و ارزیابی عوامل مبارزه بیولوژیک در هر اکوسیستم است. لذا در این تحقیق پارازیتوئیدهای مرحله لاروی شب پره خوشه‌های انگور طی سال‌های ۱۳۸۳ تا ۱۳۸۵ شناسایی گردیدند. پتانسیل طبیعی آنها در کنترل آفت در نسل‌ها و مناطق مختلف تاکستان‌های اروپه در دو سال آخیر مقایسه گردید. حشرات کامل پارازیتوئیدهای لارو از پرورش آزمایشگاهی لاروی در سه تغذیه از طبقه کل در سه نسل و همان نتایج و رشد آنها به لارو مدل قابل استفاده بود. نسل‌های دوم و سوم استحصال شدند. درصد پارازیتوئید از نسبت تعداد پارازیتوئیدهای به مواد طبیعی و شب پره‌های محاسبه شد. گونه پارازیتوئید لارو به شرح زیر بودند:

- *Enytus apostata* Gravenhorst, 1829; *Pristomerus vulnerator* (Panzer, 1799); *Temelucha sp.* (Hymenoptera: Ichneumonidae), *Nemorilla maculosa* (Meigen, 1824) (Diptera: Tachinidae), *Habrobracon hebetor* (Say) and *Bracon sp.* (Hymenoptera: Braconidae).

بر اساس این پژوهش میزان پارازیتوئید از ۱ به ۱/۸ درصد با میانگین ۰/۵ درصد متغیر بود. مقایسه میانگینی در صد پارازیتوئید لارو نشان داد که اختلاف معنی‌داری در سطح ۵ درصد در بین مناطق و نسل‌های مختلف آفت وجود دارد. بیشترین میزان پارازیتوئید نسل اول تمام مناطق و سالهای یبرسی مشاهده شد.
