

*ISSN 1302-0250*



***Journal  
of the Entomological Research  
Society***

-----  
***Volume: 19***

***Part: 1***

***2017***

# **JOURNAL OF THE ENTOMOLOGICAL RESEARCH SOCIETY**

Published by the Gazi Entomological Research Society

## **Editor (in Chief)**

Abdullah Hasbenli

## **Managing Editor**

Zekiye Suludere

## **Associate Editor**

Selami Candan

## **Review Editors**

Damla Amutkan

Dođan Erhan Ersoy

Nurcan Özyurt Koçakođlu

## **Subscription information**

Published by GERS in single volumes three times (March, July, November) per year. The Journal is distributed to members only. Non-members are able to obtain the journal upon giving a donation to GERS.

One issue price:	Annual subscription price (3 issues, about 300 pp.)
5 ₺ (Turkey)	20 ₺ (Turkey)
US\$ 20.00	US\$ 60.00

Papers in *J. Entomol. Res. Soc.* are indexed and abstracted in Biological Abstract, Zoological Record, Entomology Abstracts, CAB Abstracts, Field Crop Abstracts, Organic Research Database, Wheat, Barley and Triticale Abstracts, Review of Medical and Veterinary Entomology, Veterinary Bulletin, Review of Agricultural Entomology, Forestry Abstracts, Agroforestry Abstracts, EBSCO Databases, Scopus and in the Science Citation Index Expanded.

Publication date: April 05, 2017

© 2017 by Gazi Entomological Research Society

Printed by Hassoy Offset

Tel: +90 3123415994 [www.hassoy.com.tr](http://www.hassoy.com.tr)

## Weevils (Coleoptera: Curculionoidea) of Taleghan Region (North of Iran) with Reporting of Ten New Records for Iran

Ehsan SANAEI<sup>1, 2</sup>

Marjan SEIEDY<sup>2\*</sup>

<sup>1</sup>School of Biological Sciences, The University of Queensland, Brisbane, AUSTRALIA.

<sup>2\*</sup>(Corresponding author) School of Biology and Center of Excellence in Phylogeny of Living Organisms, College of Science, University of Tehran, IRAN  
e-mails: ehsansanai@gmail.com, mseyyedi@ut.ac.ir

### ABSTRACT

The faunistic knowledge of Iranian weevils is still relatively low, especially in comparison with the vastness and rich environment of this country. During the years 2014-2015, a faunistic study on superfamily Curculionoidea was done in Taleghan Region. This region is located on the southern slope of the Alborz Mountains and irrigated by several rivers. Taleghan Region is known mainly for its green landscape which is covered mostly by rangelands, grasslands, but also agricultural farms and fruit gardens. Recently, several human activities have shaped into the Taleghan landscapes and then it resulted in a dramatic reduction of vegetation. In our study a total of 51 species of weevils were confirmed for this region and 10 of them (*Hemitrichapion pavidum* (Germar, 1817), *Protapion laevicolle* (Kirby, 1811), *Pseudoprotapion elegantulum* (Germar, 1818), *P. astragali astragali* (Paykull, 1800), *Mecinus labilis* (Herbst, 1795), *Mogulones asperifoliarum* (Gyllenhal, 1813), *Tychius astragali* Becker, 1862, *T. callidus* Caldara, 1990, *T. tridentinus* Penecke, 1922, and *T. trivialis* Boheman, 1843) have been recorded in Iran for the first time. In addition, a brief discussion is given for several species considered as rare weevils or interesting records for Iranian fauna. Finally, the environmental factors with influence effect into the weevil community in Taleghan Region are discussed.

Key words: Alborz Mountains, Brentidae, Curculionidea, Taleghan Region, faunistic study, grasslands, Iran, new records, rangelands.

### INTRODUCTION

The superfamily Curculionoidea includes 62000 species (Oberprieler *et al.*, 2007). With regard to species number, into the superfamily Curculionoidea, the main species diversity is assigned to families Brentidae and especially Curculionidae (Löbl and Smetana, 2011). The family Curculionidae, commonly called weevils, is one of the numerous family in Coleoptera (Alonso-Zarazaga, M. A., Lyal, C. H., , 1999; Mckenna *et al.*, 2015). According to their diversity and huge numbers of species, there is a permanent debate about the internal classification (Marvaldi *et al.*, 2002; Oberprieler *et al.*, 2007; Jordal *et al.*, 2014).

Iran is a vast country with huge numbers of varieties in landscapes and weather conditions (Zehzad *et al.*, 2002). This rich environmental condition led to the high diversity of flora of Iran Plateau (Ghahreman and Attar, 1999) that affects the species

richness of herbivorous insects, like weevils. In the latest Iranian checklist, Legalov *et al.* (2010) catalogued 711 species of Curculionoidea for Iran fauna. Unfortunately, the number of weevils that has been reported from Iran is not compatible with the vastness and biodiversity of this Country. Our today's perception of Curculionoidea in Iran is indebted to the efforts of Modares Aval (1997) and Broumand (1998). However, during the last decade, some dispersal faunistic studies have been done in order to increase the local knowledge of weevil diversity in Iran (Ghahari *et al.*, 2009, 2010; Modarres Awal and Hosseinpour Jajarm, 2010; Sadeghi *et al.*, 2010; Ghahari and Legalov, 2011; Ghahari and Arzanov, 2012; Ghahari and Colonnelli, 2012; Sanaei *et al.*, 2015). Respect to the specialists' attention to the Iran fauna, the number of new confirmed Iranian endemic weevil species is still growing up (Gültekin and Podlussany, 2012; Borovec, 2014; Gültekin and Shahreyary-Nejad, 2015). It is obvious that certain number of species is still undetected and it means that the Iran fauna will remain as a big potential for future faunistic studies.

To continue the faunistic surveys in unstudied areas of Iran, we investigated for weevils in the Taleghan Region (in Alborz Province). The Taleghan Region includes Taleghan city and 76 villages and it is located at 140 km from northwest of Tehran (Monavari *et al.*, 2013). Taleghan Region is bordered by the roads of the Karaj city from east, the Alamout Mountain from west, and the Alborz Mountains from north. This region consists of the area of almost 955.7 km<sup>2</sup> (Monavari *et al.*, 2013) and the altitude in this area varies from 1500 to 3500 meters (Kiyani, 2013). This area is boosted by several rivers. The watershed of Taleghan Region is situated in the Sefidroud basin (Guest *et al.*, 2006). This region is semi humid and it is affected mostly by the presence of the Alborz Mountains and Taleghan Dam (Kiyani, 2013). The average annual precipitation is 455 mm, maximal temperature is +35°C and minimal temperature is -24°C in a year (Siroosi *et al.*, 2013). The vegetation of Taleghan varies from agricultural fields and fruit gardens to rangelands, grasslands and wetlands. These green areas make the Taleghan Region a suitable place for attracting many tourists (Kiyani, 2013). However, the tourist attraction has also made several damages for environment health such as water pollution (Kiyani, 2013). Moreover, recent soil erosion and sheep overgrazing led to the sudden turn of green lands into the bare lands.

The main aim of this faunistic study was to identify the weevil species from Taleghan Region to understand the weevil community in this region and also to investigate for the new possible records or even species in Iran. Determining factors for weevil diversity in Taleghan Region and possible treats for weevil habitat are discussed.

## **MATERIALS AND METHODS**

During 2014-2015, several field activities were performed in Taleghan Region (Fig. 1). A total of 10 localities in this region were visited on different dates (Table 1). The selected sites were usually closed to the some streams (Fig. 2). We collected weevils mainly in the open grasslands, rangelands and wetlands by sweeping. In the wetlands, we also looked under stones and plant debris. In addition, we sampled also on a few trees in the area by umbrella beat traps. A total of 50 wet pitfall traps

*Weevils (Coleoptera: Curculionoidea) of Taleghan Region*

filled with chloroform were placed in different sites (Glinak, Taleghan Dam, Hasanjun, Nazizan and Jazinan) and controlled after 24-48 hours. After the field activity, all weevil specimens were mounted on cart points in the laboratory. In order to simplify the identification progress, the male genitalia was dissected. The specimens were identified by keys from the following publications: (Hoffman, 1958; Monaco, 1970; Dieckmann, 1980; 1988; Alonso-Zarazaga, 1990; Caldara, 1990, 1993; Morris, 1990; Sobhian *et al.*, 1992; Wanat, 1995; 1997; El-Akkad; 1998; Pelletier, 1999; Colonnelli, 2004; Meleshko and Korotyaev, 2005; Bahr *et al.* 2006; Bayer *et al.*, 2007; Gültekin, 2006; Friedman and Freidberg, 2007; Yunakov and Korotyaev, 2008; Skuhrovec, 2009; Velázquez de Castro, 2009, 2011; Balalaikins *et al.*, 2010; Stüben *et al.*, 2010; Gültekin, and Podlussany, 2012; Skuhrovec *et al.*, 2012, 2014; Stüben *et al.*, 2013, 2014) and then confirmed by specialist in some group of weevils (see Acknowledgements). For list of species, we followed the classification of Löbl and Smetana (2011; 2013), which is based on main work done by Alonso-Zarazaga and Lyal (1999). However, according to Oberprieler *et al.* (2007) and Bouchard *et al.* (2011) and also recent molecular phylogenetic analysis based on full mitochondrial genome of weevils (Gillett *et al.*, 2014), we used the family Brentidae instead of Apionidae here.

Table 1. Coordination, elevation and date of collecting for each station.

Station	Coordination	Elevation	Date of collecting
Taleghan Dam	N: 27°11'45.4" E: 50°38'2.5"	1715 m	30.8.2014 10.4.2015 8.8.2015
Gelinak	N: 36°10'42.6" E: 50°51'12.8"	1315 m	9.5.2015 31.8.2014
Hasanjun	N: 36°12'8.7" E: 50°45'32.8"	1889 m	31.8.2014
Jazinan	N: 36°12'41.2" E: 50°47'1.6"	2023 m	10.5.2015
Khasban	N: 36°11'37" E: 50°46'57.7"	1939 m	10.5.2015
Naviz	N: 36°12'12.8" E: 50°51'47.9"	2120 m	9.5.2015 13.7.2015
Karkabud	N: 36°13'5.9" E: 50°51'12"	2215 m	1.9.2014
Kuein	N: 36°11'35.9" E: 50°52'5.3"	1930 m	1.9.2014
Dizan	N: 36°12'12.8" E: 50°51'47.9"	2120 m	11.5.2015
Askan	N: 36°10'12.3" E: 51°01'30.4"	1841 m	3.9.2014

The Iranian distributions of species were mostly adopted from the following publications: (Caldara, 1990; Wanat, 1995; Modarres Awal, 1997; Borumand, 1998; Pelletier, 1999; Colonnelli, 2004; Legalov *et al.*, 2010; Ghahari and Legalov, 2011; Löbl and Smetana, 2011; Ghahari and Colonnelli, 2012; Gültekin and Podlussany, 2012; Löbl and Smetana, 2013; Sanaei *et al.*, 2015). The specimens were kept in Zoological Museum, University of Tehran (ZUTC) and also personal collection of the first author.

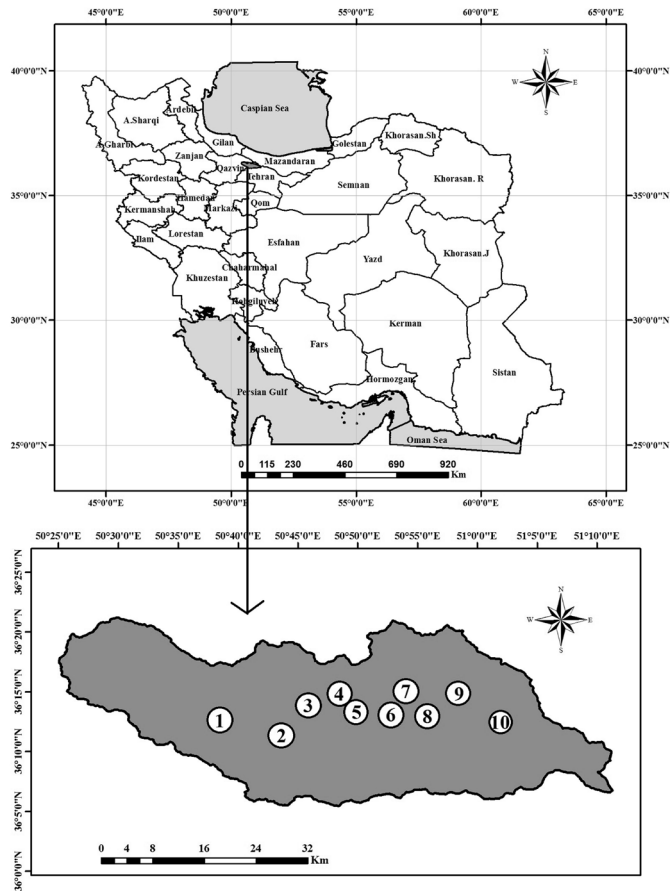


Fig. 1. The locality of Taleghan region in Iran and the localities of each station in Taleghan, 1: Taleghan Dam, 2: Gelinak, 3: Hasanjun, 4: Jazinan, 5: Khasban, 6: Naviz, 7: Karkabud, 8: Kuein, 9: Dizan, 10: Askan.

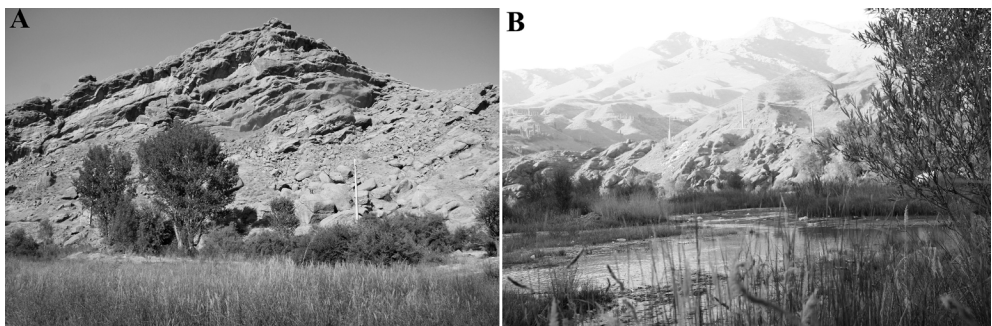


Fig. 2. Two selected different habitats in Taleghan. A. Open grasslands in Jazinan, B. River basin of Taleghan Dam.

## Weevils (Coleoptera: Curculionoidea) of Taleghan Region

Knowledge about the Palaearctic distribution of each species follows (Löbl and Smetana, 2011; 2013). In the addition, the family name of host plant for each species was given.

## RESULTS

A total of 538 weevil specimens, which has been identified into 51 species from four families, nine subfamilies, 24 tribes and 29 genera were collected. The majority of the specimens were collected by the sweeping method. Only *Sphenophorus parumpunctatus* Gyllenhal, 1838 and *Larinus scolymi* (Olivier, 1807) were collected individually under the stones. We have no observation of any weevil specimen by using umbrella beat or even pitfall traps. *Epirhynchites auratus* (Scopoli, 1763) was the only species which was collected on trees. The list of species is arranged in Table 2.

## DISCUSSION

In this study, 10 species from 51 identified species were recorded for the Iran fauna for the first time (Table 2). All these new records belong to tiny weevils with less than 5 mm length: Brentidae: *Hemitrichapion pavidum* (Germar, 1817), *Protapion laevicolle* (Kirby, 1811), *Pseudoprotapion elegantulum* (Germar, 1818), *P. astragali astragali* (Paykull, 1800); Curculionidae: *Mecinus labilis* (Herbst, 1795), *Mogulones asperifoliarum* (Gyllenhal, 1813), *Tychius astragali* Becker, 1862, *T. callidus* Caldara, 1990, *T. tridentinus* Penecke, 1922, *T. trivialis* Boheman, 1843. More attention to the Iranian fauna of mentioned groups may result to the loads of scientific achievements.

*Araxia mucronata* Khnzorian, 1957: The first description of *A. mucronata* was done on specimens from Armenia (Khnzorian, 1957). Yunakov and Korotyayev (2008) reported this species also from Turkey, Transcaucasia and Turkmenistan. According to the mentioned report, the presence of this species in Iran was expected. Davidian and Gültekin (2015) reported a specimen of *A. mucronata* from Tehran collected in 1961. The new record from Taleghan fills the gap between its distributions in the northern part of the Middle East.

*Wittmerrella viridisetosa* Pesarini, 1973: This is the only species of genus *Wittmerrella* recorded by Pesarini (1973) from Mazandaran (North of Iran). There was no other record until a recent report from Turkey (Yunakov and Klass, 2012). However, this species was recently collected from 3 different localities in Taleghan Region with relatively high abundance only in females. The males of this species were never described. Our specimens in this study were also females. As Yunakov and Klass (2012) suggested, this species can be closely related to genus *Polydrusus*. We suggest this species may have a parthenogenesis life cycle as some of *Polydrusus* species (Kajtoch and Lachowska-Cierlik, 2009). In addition, *W. viridisetosa* is a common species in Taleghan Region, therefore, it is assumed that the range of its distribution should be more wide than only our current recorded localities. The host plant of *W. viridisetosa* is still unknown. However, all specimens were collected by sweeping grasslands with domination of *Hypericum perforatum* (Hypericaceae). It is suggested that Hypericaceae might be a potential host plant of this species.

Table 2. List of all collected species with additional information.

Species name	Subfamily	Iran distribution	World distribution	Examined materials	Host plant
<i>Epirhynchites auratus</i> (Scopoli, 1763)	Rhynchitinae	Well distributed	W/C Palaearctic	3♂♂, Gelinak 31.8.2014.	Rosaceae
<i>Apion frumentarium</i> (Linnaeus, 1758)	Apioninae	Well distributed	W/C Palaearctic	2♂♂ Karkabud 1.9.2014, 1♀ Gelinak 31.8.2014, 1♀ Hasanjun 31.8.2014	Polygonaceae
<i>Protapion trifolii</i> (Linnaeus, 1768)	Apioninae	Well distributed	W/C Palaearctic	14♂♂ 18♀♀ Kuein 21.9.2014, 3♀♀ Gelinak 9.5.2015, 12♀♀ 8♂♂ Jazinan 10.5.2015	Fabaceae
<i>Protapion filirostre</i> (Kirby, 1808)	Apioninae	Tehran	W Palaearctic & Russia	1♀ Gelinak 9.5.2015	Fabaceae
<i>Protapion laevicolle</i> (Kirby, 1811)	Apioninae	<b>New Record</b>	W Palaearctic	2♀♀ 1♂ Dizan 11.5.2015	Fabaceae
<i>Protapion varipes</i> (Germar, 1817)	Apioninae	NW	W Palaearctic	2♂♂ Askan 3.9.2014	Fabaceae
<i>Ceratopion scalptumcaviceps</i> (Desbrochers des Loges, 1870)	Apioninae	Well distributed	E Europe & C Palaearctic	3♂♂ 2♀♀ Taleghan Dam 30.8.2014, 5♂♂ 2♀♀ Gelinak 31.8.2014	Asteraceae
<i>Ceratopion basicorne</i> (Illiger, 1807)	Apioninae	E Azarbaijan, Isfahan	W Palaearctic	2♂♂ 4♀♀ Gelinak 31.8.2014	Asteraceae
<i>Hemitrichapion pavidum</i> (Germar, 1817)	Apioninae	<b>New Record</b>	W Palaearctic	6♂♂ 2♀♀, Gelinak 9.5.2015	Fabaceae
<i>Hemitrichapion reflexum</i> (Germar, 1833)	Apioninae	Ardabil	W Palaearctic	7♂♂ 8♀♀ Naviz 13.7.2015, 3♂♂ Taleghan Dam 8.8.2015	Fabaceae
<i>Holotrichapion pullum</i> (Gyllenhal, 1833)	Apioninae	E Azarbaijan, Khorasan	W Palaearctic	1♀ Naviz 9.5.2015, 2♂♂ Taleghan Dam 8.VIII.2015	Fabaceae
<i>Oxystoma ochropus</i> (Germar, 1818)	Apioninae	Azarbaijan, Isfahan	W Palaearctic	2♂♂ Kuein 1.9.2014, 5♂♂ Naviz 13.7.2015	Fabaceae
<i>Pseudoprotapion elegantulum</i> (Germar, 1818)	Apioninae	<b>New Record</b>	W Palaearctic	2♂♂ Gelinak 31.8.2014, 1♂ 1♀ Taleghan Dam 10.4.2015	Fabaceae
<i>Pseudoprotapion astragali astragali</i> (Paykull, 1800)	Apioninae	<b>New Record</b>	W/C Palaearctic.	2♀♀ Dizan 11.5.2015	Fabaceae
<i>Rhopalapion longirostre</i> (Olivier, 1807)	Apioninae	Well distributed	Holarctic	1♀ Taleghan Dam 10.4.2015, 1♀ Naviz 13.7.2015	Malvaceae
<i>Isochnopterapion loti</i> (Kirby, 1808)	Apioninae	E Azarbaijan	W Palaearctic	2♀ Naviz 9.5.2015.	Fabaceae
<i>Squamapion latesquamatum</i> Wanat, 1997	Apioninae	Lorestan, Tehran	Asia Minor	3♀ 1♂ Kuein 1.9.2014	Unknown
<i>Sphenophorus parumpunctatus</i> Gyllenhal, 1838	Rhynchophorinae	Khuzestan	W Palaearctic	1♂ Taleghan Dam 10.4.2015, 11♂♂ 7♀♀ Taleghan Dam 8.8.2015	Fabaceae
<i>Sitona cylindricollis</i> Fahraeus, 1840	Entiminae	Well distributed	Holarctic	10♂♂ 8♀♀ Gelinak 8.31.8.2014, 3♂♂ Dizan 11.5.2015, 4♂♂ 6♀♀ Jazinan 10.5.2015.	Fabaceae
<i>Sitona humeralis</i> Stephens, 1831	Entiminae	Well distributed	Holarctic	15♂♂ 23♀♀ Dizan 31.8.2014, 11♂♂ 12♀♀ Naviz 9.5.2015, 5♂♂ Jazinan 10.5.2015	Fabaceae
<i>Sitona concavostris</i> Hochhuth, 1851	Entiminae	Well distributed	W/C Palaearctic.	3♂♂ 4♀♀ Gelinak 31.8.2014, 4♂♂ 2♀♀ Gelinak 9.5.2015, 9♂♂ 1♀ Khasban 10.5.2015	Fabaceae
<i>Sitona longulus</i> Gyllenhal, 1834	Entiminae	Well distributed	W/C Palaearctic.	6♂♂ 10♀♀ Gelinak 31.8.2014	Fabaceae
<i>Sitona macularius</i> (Marsham, 1802)	Entiminae	Well distributed	W/C Palaearctic.	5♂♂ Kuein 1.9.2014	Fabaceae
<i>Araxi amuronata</i> Khnzorian, 1957	Entiminae	Tehran	N Middle East	5♀♀ Kuein 1.9.2014, 3♀♀ Khasban 10.5.2015, 8♀♀ Naviz 13.7.2015	Unknown
<i>Polydrusus pilifer</i> Hochhuth, 1847	Entiminae	Well distributed	W/C Palaearctic.	2♀♀ Karkabud 1.9.2014, 21♀♀ Askan 1.9.2014, 15♀♀ Khasban 10.5.2015, 7♀♀ Naviz 9.5.2015, 11♀♀ Naviz 13.7.2015	Rosaceae
<i>Wittmerrella viridisetos</i> Pesarini, 1973	Entiminae	Mazandaran	Iran & Turkey	1♀ Askan 1.9.2014, 4♀♀ Naviz 9.5.2015, 9♀♀ Gelinak 9.5.2015	Unknown
<i>Chloebius immeritus</i> Boheman, 1834	Entiminae	Ghazvin, Tehran	Palaearctic	1♀ Hasanjun 31.8.2014	Fabaceae
<i>Pholicodes fausti</i> (Reitter, 1890)	Entiminae	Azarbaijan	Middle East	2♀♀ Jazinan 10.5.2015	Unknown
<i>Strophomorpus porcellus</i> (Scjoenherr, 1832)	Entiminae	Tehran	W/C Palaearctic	2♀♀ Askan 3.9.2014, 1♀♀ Gelinak 9.5.2015	Polyphagous

In distribution columns: E=East, W=Western, C=Central and N=North.

## Weevils (Coleoptera: Curculionoidea) of Taleghan Region

Table 2. Continued.

Species name	Subfamily	Iran distribution	World distribution	Examined materials	Host plant
<i>Hypera postica</i> (Gyllenhal, 1813)	Hyperinae	Well distributed	Holarctic	2♂♂ 4♀♀ Askan 9.2014, 4♂♂ 6♀♀ Naviz 9.5.2015, 5♂♂ 11♀♀ Gelinak 9.5.2015, 8♂♂ 8♀♀ Jazinan 10.5.2015, 2♀♀ Khasban 10.5.2015, 12♂♂ 17♀♀ Dizan 11.5.2015	Fabaceae
<i>Hypera farinosa</i> (Boheman, 1840)	Hyperinae	NW	E Europe & C Palaeartic	1♀ Taleghan Dam 10.4.2015	Fabaceae
<i>Lixus ascanii</i> Linnaeus 1767	Lixinae	Golestan	W Palaeartic	1♀ Hasanjun 31.8.2014	Brassicaceae
<i>Lixus pulverulentus</i> Scopoli 1763	Lixinae	Well distributed	W/C Palaeartic	1♂ Jazinan 10.5.2015	Malvaceae
<i>Lixus recurvus</i> Olivier, 1807	Lixinae	Golestan	Caucasus, Iran, Turkey	2♀♀ Karkabud 1.9.2014	Malvaceae
<i>Larinus scolymi</i> (Olivier, 1807)	Lixinae	Well distributed	W Palaeartic	1♂ Taleghan Dam 8.8.2015	Asteraceae
<i>Larinus iranicus</i> Gültekin and Podlussány, 2012	Lixinae	NW	Iran	1♂ Gelinak 31.8.2014	Unknown
<i>Bangastermus orientalis</i> (Capiomont, 1873)	Lixinae	Well distributed	W Palaeartic	3♂♂ Naviz 9.5.2015, 5♂♂ 2♀♀ Dizan 11.5.2015	Asteraceae
<i>Malvaevora timida</i> (Rossi, 1792)	Baridinae	Well distributed	W/C Palaeartic	1♀ Taleghan Dam 10.4.2015	Malvaceae
<i>Cionus olivieri</i> Rosenhauer, 1838	Curculioninae	Well distributed	W/C Palaeartic	1♀ Kuein 1.9.2014	Scrophulariaceae
<i>Mecinus labilis</i> (Herbst, 1795)	Curculioninae	<b>New Record</b>	W Palaeartic	1♂ Karkabod 1.9.2014 1♂ Dizan 11.5.2015	Plantaginaceae
<i>Tychius aureolus</i> Kiesenwetter, 1851	Curculioninae	Well distributed	W/C Palaeartic	2♂♂ 6♀♀ Dizan 30.8.2014, 1♂ Naviz 9.5.2015	Fabaceae
<i>Tychius pictrostris</i> (Fabricius, 1787)	Curculioninae	Golestan, Lorestan	Holarctic	2♂♂ Naviz 9.5.2015	Fabaceae
<i>Tychius tridentinus</i> Penecke, 1922	Curculioninae	<b>New Record</b>	W/C Palaeartic	1♂ Taleghan Dam 10.8.2014	Fabaceae
<i>Tychius astragali</i> Becker, 1862	Curculioninae	<b>New Record</b>	N America, E Europe	3♂ Taleghan Dam 10.4.2015	Fabaceae
<i>Tychius trivialis</i> Boheman, 1843	Curculioninae	<b>New Record</b>	W Palaeartic	1♂ Naviz 9.5.2015	Fabaceae
<i>Tychius callidus</i> Caldara, 1990	Curculioninae	<b>New Record</b>	Armenia and Macedonia	1♂ Kuein 1.9.2014	Fabaceae
<i>Tychius hiekei</i> Caldara, 1990	Curculioninae	Mazandaran, Sistan	Armenia and Iran	2♂♂ Jazinan 10.5.2015, 1♀ Taleghan Dam 10.4.2015	Fabaceae
<i>Ceutorhynchus chalybaeus</i> Germar, 1824	Ceutorhynchinae	Mazandaran	W/C Palaeartic	2♂♂ Khasban 10.5.2015, 12♂ Gelinak 9.5.2015	Brassicaceae
<i>Ceutorhynchus hirtulus</i> Germar, 1824	Ceutorhynchinae	Khorasan	W Palaeartic	2♂♂ Khasban 10.5.2015, 12♂ Gelinak 9.5.2015	Brassicaceae
<i>Ceutorhynchus sulcicollis</i> (Paykull, 1800)	Ceutorhynchinae	Mazandaran	W Palaeartic	1♀♀ Taleghan Dam 10.4.2015	Brassicaceae
<i>Mogulones asperifoliarum</i> (Gyllenhal, 1813)	Ceutorhynchinae	<b>New Record</b>	W Palaeartic	1♂ Gelinak 9.5.2015, 1♂ Taleghan Dam 10.4.2015	Brassicaceae

In distribution columns: E=East, W=Western, C=Central and N=North.

*Strophomorphus porcellus* (Scjoenherr, 1832): The only Iranian record of this species from Tehran was published by Pelletier (1999). In this study, we confirmed Taleghan Region as another locality for this species in Iran.

*Lixus ascanii* Linnaeus, 1767: This species was recorded from Golestan Province (Ghahari and Colonnelli, 2012). According to the Golestan record (NE Iran) our new record from Taleghan Region and also its wide distribution in Europe and the Middle East, this species may be distributed from West to East of Iran.

*Larinus scolymi* (Olivier, 1807): This species is considered as a common species in Western Palaeartic. There was no precise direct report of *L. scolymi* from Iran, however, taxon *L. flavescens* Germar, 1824 (recently the synonym of *L. scolymi*) has been already recorded from Iran (Borumand, 1998; Nematollahi, 2010). In our study this species was found surprisingly only in lapidicolous habitat.

*Larinus iranicus* Gültekin and Podlussány, 2012: This endemic species of Iran was recently described by Gültekin & Podlussány (2012). The localities of paratypes were

limited to the East of Iran (East Azarbayjan, Hamadan and West Azarbayjan). Our new record from Taleghan Region has extended the known distribution area of this species.

*Tychius hiekei* Caldara, 1990: According to the geographic data of the paratypes of *T. hiekei* Caldara (1990) mentioned one locality in Armenia and two localities in Iran (Mazandaran, Sistan and Baluchestan). After 25 years, this is the first record for this species. According to these data, the distribution range of this species should be extended from the North to the South East of Iran (near to Pakistan border).

*Ceutorhynchus hirtulus* (Germar, 1824): This species was reported by Colonnelli (2004) for the first time from Khorasan (East of Iran). According to the new record of this species from North of Iran (Taleghan Region), our information about the distribution of this species is quietly extended.

*Ceutorhynchus sulcicollis* (Paykull, 1800): Several records (Colonnelli, 2004; Barari and Alziar, 2008; Barari and Serri, 2010) confirmed this species only in Mazandaran province. However *C. sulcicollis* is known as a pest of oilseed rape and a common species in Europe (Grantija *et al.*, 2011). The abundance of grape gardens in Mazandaran province is one of the reasons of over attention to *C. sulcicollis* in Mazandaran. Due to an economic importance of this pest, the Iranian geographic distribution of *C. sulcicollis* should be studied by further detailed research.

The species richness of plants and vegetation is the key factor for weevil diversity in each region. Several river basins and the high quantity and quality of underground waters led to the vast green area in most of the parts of Taleghan Region (Moghaddam *et al.*, 2013). Moghaddam *et al.* (2013) detected 547 water springs in just a limited area of Taleghan Region. In the addition, the average of solar radiation in Taleghan is relatively high (4.5 kWh/m<sup>2</sup>) (Shiroudi and Taklimi, 2011). High solar radiation, huge source of available water and also high annual precipitation (Kiyani, 2013) are responsible for growth of variety of plants especially in rangelands that affect weevil fauna as well.

The most frequent plant families in rangelands of Taleghan Region are following: Gramineae, Hypericaceae, Compositae, Labiatae and Fabaceae (Fahimipoor *et al.*, 2010). According to the diversity of plant species in rangelands and grasslands, the high diversity of Apioninae species is expected in this region. In the current study, we confirmed 16 species of Apioninae and four of them were recorded in Iran for the first time. Legalov *et al.* (2010) listed 70 Apioninae for the Iran fauna. In the comparison of the area of Iran (1,623,779 km<sup>2</sup>) (Zehzad *et al.*, 2002) and Taleghan (955.7 km<sup>2</sup>), the finding of 16 Apioninae species in such small area could be considered as a high number. However, it is suggested that a total number of known Apioninae species in Iran is not compatible to environment richness and vastness of Iran and more investigation should be done.

It seems that the weevil fauna of the grasslands and rangelands of Taleghan are also affected by presence of many agricultural fields. For instance, *Hypera postica* (Gyllenhal, 1813), *Tychius aureolus* Kiesenwetter, 1851 and several species of genus *Sitona* that are found in our current study, are the most known and also frequent pests of alfalfa in Iran (Sanaei *et al.*, 2015). The alfalfa is cultivated for feeding cattle in many

## *Weevils (Coleoptera: Curculionoidea) of Taleghan Region*

villages in Taleghan Region. The weevil community in Taleghan Region can be affected also by other factors. During last decades, it was observed significant degradation of the vegetation in this region. Between years 1987 to 2007, more than 90% of the agricultural fields and 30% of rangelands of limited studied area of Taleghan Region changed turned to bare lands (Kiyani, 2013). Taleghan Dam (Fig. 2) is an important source of drinking water and create also electric power not only for Taleghan but also for Tehran (Capital of Iran) (Kazemi *et al.*, 2012). The coast of Taleghan Dam is an attractive location for tourists and this is the main source of environmental waste and pollution (Kazemi *et al.*, 2012; Kiyani, 2013). The volume of human mediate waste plus overgrazing of livestock are the main reasons for sudden change of vegetation in Taleghan Region (Kiyani, 2013). These environmental problems may have an unrecoverable damage to the fauna, especially the herbivorous species like weevils. In order to decrease the future extinction of several species, the environmental management in Taleghan Region is highly recommended.

## ACKNOWLEDGMENT

We appreciate the help of specialists for confirming our identifications, Karel Schön and Carlo Giusto in Apionidae, Fabio Talamelli and Gabriel Alziar in Lixini, Roman Borovec and Nicolai Yunakov in part of Entiminae, Antonio J. Velázquez de Castro in *Sitona*, Roberto Caldara in *Tychius* and Mecinini and Jiri Kratky and finally Enzo Colonnelli in Ceutorhynchinae. The authors are in debt to Milosz A. Mazur for reviewing the early version of the manuscript and Luca Bartolozzi for providing several European specimens in order to do a comparative study and an accurate identification. Many thanks Also to Davood Davoodi Moghadam for preparing Taleghan GIS map.

## REFERENCES

- Alonso-Zarazaga, M. A., 1990, Revision of the supraspecific taxa in the Palaearctic Apionidae Schoenherr, 1823 (Coleoptera, Curculionoidea). 2. Subfamily Apioninae Schoenherr, 1823: introduction, keys and descriptions. *Graellsia*, 46: 19-156.
- Alonso-Zarazaga, M. A., Lyal, C. H., 1999, *A World Catalogue of Families and Genera of Curculionoidea (Insecta: Coleoptera): (excepting Scolytidae and Platypodidae)*. Entomopraxis, Barcelona, 315.
- Balalaikins, M., Tamutis, V., Ferenca, R., 2010, New data on two similar species of *Cionus* Clairville & Schellenberg, 1798 (Coleoptera, Curculionidae) from Lithuania. *Acta Zoologica Lituanica*, 20(4): 225-228.
- Bahr, F., Bayer, Ch., Behne, L., Sprick, P., Stüben, P. E., 2006, Digital-Weevil-Determination for Curculionoidea of Western Palaearctic. *Transalpina: Sitona (Entiminae: Sitonini)*. Snudebiller, 7: 14-20.
- Barari, H., Alziar, G., 2008, Rearing and identification of three new stem-borer weevils of oilseed rape from Mazadaran province. 18th Iranian Plant Protection Congress, 24-27 August 2008, Bu-Ali Sina University, Hamedan, Iran University, 90.
- Barari, H., Serri, S., 2010, Investigation on leaf-feeder and stem-borer beetles of oilseed rape in Mazandaran province. 19th Iranian Plant Protection Congress, 31 July- 1 August 2010, Iranian Research Institute of Plant Protection, Tehran, Iran, 603.
- Bayer, C., Winkelmann, H., Bahr F., 2007, *Ergebnisse einer faunistischen Studie auf der Insel Rhodos. Erster Beitrag zur Fauna von Griechenland (Coleoptera, Curculionoidea)*. Curculio-Institute, Mönchengladbach, Germany, Weevil News, 37: 10

- Borovec, R., 2014, A new species of *Gyratogaster* from Iran (Coleoptera: Curculionidae: Entiminae: Cyphicerini). *Klapalekiana*, 50: 45-49.
- Borumand, H., 1998, Insects of Iran: *The list of Coleoptera in the insect collection of Plant Pests & Diseases Research Institute. Coleoptera (XXIV): Curculionoidea: Fam. (Anthribidae, Attelabidae, Brentidae, Apionidae, Curculionidae, Scolytidae, Platypodidae)*. Plant Pests & Diseases Research Institute, Insect Taxonomy Research Department, Tehran, Iran, 162: 166-171.
- Bouchard, P., Bousquet, Y., Davies, A. E., Alonso-Zarazaga, M. A., Lawrence, J. F., Lyal, C. H. C., Newton, A. F., Reid, C. A. M., Schmitt, M., Slipinski, S. A., Smith, A. B. T., 2011, Family-group names in Coleoptera (Insecta). *ZooKeys*, 88: 1-972.
- Caldara, R., 1990, Revisione tassonomica delle specie paleartiche del genere *Tychius* Germar (Coleoptera Curculionidae). *Memorie della Società Italiana di Scienze Naturali e del Museo Civico di Storia Naturale di Milano*, 25(3): 53-218.
- Caldara, R., 1993, Aggiunte alla revisione delle specie paleartiche del genere *Tychius* (Coleoptera Curculionidae). *Atti della Società italiana di Scienze naturali e del Museo civico di Storia naturale di Milano*, 134(1): 123-130.
- Colonnelli, E., 2004, *Catalogue of Ceutorhynchinae of the world, with a key to genera. (Insecta: Coleoptera: Curculionidae)*. Argania editio, Barcelona, 124.
- Davidian, G., Gültekin, L., 2015, Distribution ranges of weevils of the genera *Araxia* Khnzorian and *Ptochomorphus* F. *Solari* (Coleoptera, Curculionidae: Entiminae) with disjunction in the Kura-Aras Lowland. *Entomological Review*, 95(1): 49-53.
- Dieckmann, L., 1988, Beiträge zur Insektenfauna der DDR.: Curculionidae (Curculioninae: Ellescini, Acalyptini, Tychiini, Anthonomini, Curculionini). Akademie-Verlag. *Beiträge zur Entomologie*, 38(2): 365-468.
- Dieckmann, L., 1980, Revision der *Lixus ascanii*-Gruppe (Coleoptera, Curculionidae). *Reichenbachia*, 18(29): 203-212.
- El-Akkad, M. K., 1998, Key to the genera and species of tribe Lixini of Egypt (Coleoptera: Curculionidae: Cleoninae). *Annals of Agricultural Science, Moshtohor*, 36(1): 663-674.
- Fahimipoor, E., Chahouki, M. Z., Jafari, M., Goldansaz, M., Mohhebi, Z., 2010, Investigation of Plant Diversity in Middle Rangelands of Taleghan by Using BIO-DAP. *Journal of Range Science*, 1(1): 47-51.
- Friedman, A. L. L., Freidberg, A., 2007, The Apionidae of Israel and the Sinai Peninsula (Coleoptera: Curculionoidea). *Israel Journal of Entomology*, 37: 55-180.
- Ghahari, H., Arzanov, Y., 2012, Curculionidae (Coleoptera: Curculionoidea) from Lorestan province, western Iran. *Archives of Biological Sciences*, 64(1): 359-364.
- Ghahari, H., Arzanov, Y., Legalov, A. A., Tabari, M., Ostovan, H., 2010, Weevils (Coleoptera: Curculionidae) from Iranian rice fields and surrounding grasslands. *Munis Entomology & Zoology*, 5(1): 163-169.
- Ghahari, H., Colonnelli, E., 2012, Curculionoidea from Golestan province, northern Iran (Coleoptera). *Fragmenta Entomologica*, 44(1): 101-161.
- Ghahari, H., Legalov, A., 2011, Contribution to the knowledge of Curculionidae (Coleoptera) from Kurdistan province (western Iran). *Amurian Zoological Journal*, 3(4): 345-348.
- Ghahari, H., Legalov, A. A., Arzanov, Y., 2009, An annotated list of the weevils (Coleoptera: Curculionidae) from the Arasbaran biosphere reserve and vicinity, northwestern Iran. *Baltic Journal of Coleopterology*, 9(2): 177-182.
- Ghahreman, A., Attar, F., 1999, *Biodiversity of Plant Species in Iran, The Vegetation of Iran, Plant Species, Red Data of Iran, Endemic Species, Rare Species, Species Threatened by Extinction*. Central Herbarium of Tehran University, Tehran, Iran, 1: 1176.
- Gillett, C. P., Crampton-Platt, A., Timmermans, M. J., Jordal, B., Emerson, B. C., Vogler, A. P., 2014, Bulk de novo mitogenome assembly from pooled total DNA elucidates the phylogeny of weevils (Coleoptera: Curculionoidea). *Molecular Biology and Evolution*, 31(8): 2223-2237.
- Grantiņa, I., Apenīte, I., Turka, I., 2011, Commonly found species of *Ceutorhynchus* (Coleoptera: Curculionidae) on the oilseed rape in Latvia. *Acta Biologica Universitatis Daugavpiliensis*, 11(2): 260-264.

*Weevils (Coleoptera: Curculionoidea) of Taleghan Region*

- Guest, B., Axen, G. J., Lam, P. S., Hassanzadeh, J., 2006, Late Cenozoic shortening in the west-central Alborz Mountains, northern Iran, by combined conjugate strike-slip and thin-skinned deformation. *Geosphere*, 2(1): 35-52.
- Gültekin, L., 2006, On some Lixinae types of J C Fabricius (Coleoptera, Curculionidae). *Fragmenta entomologica*, 38(1): 111-133.
- Gültekin, E., Podlussany, A., 2012, Two new species of *Larinus* from Iran (Coleoptera: Curculionidae: Lixinae). *Acta Entomologica Musei Nationalis Pragae*, 52: 245-258.
- Gültekin, L., Shahreyary-Nejad, S., 2015, A new trehala-constructing *Larinus* Dejean (Coleoptera: Curculionidae) from Iran. *Zoology in the Middle East*, 61(3): 246-251.
- Hoffman, A., 1958, *Faune de France 62, Coleopteres, Curculionides (troisieme partie)*. Federation Française des societes de sciences naturelles-Librairie de la Faculte des sciences, Paris, France, 1209-1839.
- Jordal, B. H., Smith, S. M., Cognato, A. I., 2014, Classification of weevils as a data-driven science: leaving opinion behind. *ZooKeys*, 439: 1-18
- Kajtoch, Ł., Lachowska-Cierlik, D., 2009, Genetic constitution of parthenogenetic form of *Polydrusus inustus* (Coleoptera: Curculionidae) hints of hybrid origin and recombinations. *Folia biologica*, 57(3-4): 149-156.
- Kazemi, Y., Salajegheh, A., Mahdavi, M., Rostami, N., Abbassi, M., 2012, The relation of bed and suspended loads in central Alborz Rivers, Iran. *Elixir Agriculture*, 42: 6120-6123.
- Khnzorian, S. M., 1957, Novye vidy zhestkokrylyh iz Armyanskoi SSR i Nakhichevanskoi ASSR. *Zoologicheskyyi sbornik*, 10: 153-183.
- Kiyani, V., 2013, Management ecosystem by Assessment of plant covers changes (Case study: Taleghan Township). *International journal of Advanced Biological and Biomedical Research*, 1(2): 161-170.
- Legalov, A., Ghahari, H., Arzanov, Y. G., 2010, Annotated catalogue of Curculionid-beetles (Coleoptera: Anthribidae, Rhynchitidae, Attelabidae, Brentidae, Brachyceridae, Dryophthoridae and Curculionidae) of Iran. *Amurian Zoological Journal*, 2(3): 191-244.
- Löbl, I., Smetana, A., 2011, *Catalogue of Palaearctic Coleoptera, Curculionoidea I*. Apollo Books, Stentrup, 7: 373.
- Löbl, I., Smetana, A., 2013, *Catalogue of Palaearctic Coleoptera, Curculionoidea II*. Brill, Leiden, Netherlands, 8: 700.
- Marvaldi, A. E., Sequeira, A. S., O'Brien, C. W., Farrell, B. D., 2002, Molecular and morphological phylogenetics of weevils (Coleoptera, Curculionoidea): do niche shifts accompany diversification? *Systematic Biology*, 51(5): 761-785.
- Mckenna, D. D., Wild, A. L., Kanda, K., Bellamy, C. L., Beutel, R. G., Caterino, M. S., Farnum, C. W., Hawks, D. C., Ivie, M. A., Jameson, M. L. and Leschen, R. A., 2015, The beetle tree of life reveals that Coleoptera survived end Permian mass extinction to diversify during the Cretaceous terrestrial revolution. *Systematic Entomology*, 40(4), 835-880.
- Meleshko, J. E., Korotyaev, B. A., 2005, Two New Species of the Weevil Genus *Polydrusus* Germar (Coleoptera: Curculionidae: Entiminae) from Southern Iran. Contributions to Systematics and Biology of Beetles: Papers Celebrating the 80th Birthday of IK Lopatin, Sofia, Moscow, *Pensoft Publishers. Series Faunistica*. 43: 289-297.
- Modarres Awal, M., 1997, *List of Agricultural Pests and Their Natural Enemies in Iran*. Ferdowsi University Press, Mashhad, Iran, 429.
- Modarres Awal, M., Hosseinpour Jajarm, F., 2010, A contribution to the snout beetles fauna of Khorasan province in Iran (Coleoptera: Curculionidae). *Munis Entomology & Zoology*, 5(2): 623-626.
- Moghaddam, D. D., Rezaei, M., Pourghasemi, H. R., Pourtaghie, Z., Pradhan, B., 2013., Groundwater spring potential mapping using bivariate statistical model and GIS in the Taleghan watershed, Iran. *Arabian Journal of Geosciences*, 8(2): 913-929.
- Monaco, R., 1970, Studies on Coleoptera Curculionidae. III. *Strophomorphus porcellus* Sch. *Entomologia*, 6: 105-144.

- Monavari, S. M., Khorasani, N., Mirsaed, S. G., 2013, Delphi-based strategic planning for tourism management - a case study. *Polish Journal of Environmental Studies*, 22(2): 465-473.
- Morris, M. G., 1990, Orthocerous weevils. Coleoptera: Curculionoidea (Nemonychidae, Anthribidae, Urodontidae, Attelabidae and Apionidae), *Handbooks for the identification of British insects*. Royal Entomological Society, London, UK, 108.
- Nematollahi, M. R., 2010, Insect community structure in safflower fields of Esfahan province. In 19th Iranian Plant Protection Congress, 31 July - 3 Aug 2010 Iranian Research Institute of Plant Protection, Tehran, Iran. 260 pp.
- Oberprieler, R. G., Marvaldi, A. E., Anderson, R. S., 2007, Weevils, weevils, weevils everywhere. *Zootaxa*, 1668: 491-520.
- Pelletier, J., 1999, Révision du genre *Strophomorphus* Seidlitz, 1867 (Coleoptera, Curculionidae). *Zoosystema*, 21(4): 681-750.
- Pesarini, C., 1973, Nuove specie di curculionidi raccolte in turchia ed in persia dal dott. W. Wittmer e descrizione di un nuovo genere. *Estratto dal Bollettino della Società Entomologica Italiana*, 105: 80-85.
- Sadeghi, H., Eshraghi, S., Behne, L., 2010, A contribution to the fauna of weevils. (Coleoptera: Curculionidae) associated with sugar beet fields in North-East of Iran. *Munis Entomology & Zoology*, 5(2): 753-757.
- Sanaei, E., Seiedi, M., Velázquez de Castro, A. J., 2015, Distribution of weevils (Coleoptera: Curculionidae) in alfalfa fields of Iran's northern provinces with a new record for the country. *Zoology and Ecology*, 25: 1-7.
- Shiroudi, A., Taklimi, S. R. H., 2011, *Demonstration project of the solar hydrogen energy system located on Taleghan-Iran: Technical-economic assessments*. In: Moshfegh, P., (Eds.). World Renewable Energy Congress. Linköping University Electronic Press, Linköping, Sweden, 1158-1165.
- Siroosi, H., Heshmati, G., Mahini, A., Naseri, H., 2013, An Investigation of Management Effects on Range Functionality (Case Study: Karkaboud and Kouin, Taleghan). *Pakistan Journal of Biological Sciences*, 16(5): 239-244.
- Skuhrovec, J., 2009, *Digital-Weevil-Determination for Curculionoidea of West Palaearctic. Transalpina: Hypera/Limobius/Metadonus (Hyperinae: Hyperini)*. In: Snudebiller 10: Studies on taxonomy, biology and ecology of Curculionoidea. Curculio-Institute, Mönchengladbach, Germany, 39-47.
- Skuhrovec, J., Schön, K., Stejskal, R., Gosik, R., Kresl, P., Trnka, F., 2012, *Digital-Weevil-Determination for Curculionoidea of West Palaearctic. Rhynchitidae and Attelabidae*. In: Snudebiller 13: Studies on taxonomy, biology and ecology of Curculionoidea, Curculio-Institute, Mönchengladbach, Germany, 138-161.
- Skuhrovec, J., Stejskal, R., Trnka, F., Gosik, R., 2014, *Digital-Weevil-Determination for Curculionoidea of West Palaearctic. Cleonini (Lixinae)*. In: Snudebiller. Studies on taxonomy, biology and ecology of Curculionoidea, Curculio-Institute, Mönchengladbach, Germany, 15(227): 18.
- Sobhian, R., Campobasso, G., Dunn, P. H., 1992, Contribution to the biology of *Bangasternus orientalis* Capiomont (Col., Curculionidae). *Journal of Applied Entomology*, 113(1-5): 93-102.
- Stüben, P. E., Behne, L., Grebennikov, V. V., 2010, *Rüsselkäfer-Gesiebe-Exkursion des Curculio-Instituts im Frühjahr 2009 nach Marokko - unter besonderer Berücksichtigung der Cryptorhynchinae-Fauna. (Coleoptera: Curculionoidea)* In: Snudebiller 11, Studies on taxonomy, biology and ecology of Curculionoidea. Curculio-Institute, Mönchengladbach, Germany, 155: 54-79
- Stüben P. E., Müller G., Krátky J., Bayer, Ch., Behne, L., Sprick, P., 2013, *Digital Weevil-Determination for Curculionoidea of West Palaearctic: Transalpina: Ceutorhynchinae (Ceuthorhynchini: Amalorrhynchus, Drupenatus, Poophagus, Coeliodes, Pseudocoeliodes, Coeliodinus, Eucoeliodes, Neoxyonyx, Thamiocolus, Micrelus, Zacladus, Phrydiuchus, Stenocarus, Nedyus, Ceutorhynchus: Marklissus)*. In: Snudebiller 14, Studies on taxonomy, biology and ecology of Curculionoidea. Curculio-Institute, Mönchengladbach, Germany, 2(210): 23 pp
- Stüben P. E., Bayer Ch., Müller G., Müller U., Krátky J., Behne L., Sprick P., 2014, *Digital-Weevil-Determination for Curculionoidea of the West Palaearctic: Transalpina: Ceutorhynchinae. (Ceutorhynchini: Calosirus, Ceutorhynchus (black species), Coeliastes, Prisistus, Sirocalodes,*

*Weevils (Coleoptera: Curculionoidea) of Taleghan Region*

- Trichosirocalus*), In: Snudebiller 16, Studies on taxonomy, biology and ecology of Curculionoidea. Curculio-Institute, Mönchengladbach, Germany, 4(234): 27 pp
- Velázquez de Castro, A. J., 2009, Sitonini del Norte de África (Coleoptera: Curculionidae: Entiminae). *Boletín de la Sociedad Entomológica Aragonesa*, 45: 73-89.
- Velázquez de Castro, A. J., Friedman, A. L. L., Borovec, R., 2011, Sitonini (Curculionidae: Entiminae) of Israel. *Israel Journal of Entomology*, 40: 71-108.
- Wanat, M., 1995, Systematics and phylogeny of the tribe Ceratapiini (Coleoptera: Curculionoidea: Apionidae). *International Journal of Invertebrate Taxonomy Suppl.* 3: 1-406.
- Wanat, M., 1997., New and little known Squamapion species [Coleoptera: Apionidae] from western Palaearctic. *Annales zoologici*, 47(1): 275-295.
- Yunakov, N. N., Klass, K. D., 2012, Contribution to the taxonomy and nomenclature of Entiminae from the Palaearctic region and South Africa. *Beiträge Entomology*, 62(2): 427-446.
- Yunakov, N. N., Korotyaev, B. A., 2008, On the systematics of the weevil genus *Araxia* Khnzorian with descriptions of new taxa from Transcaucasia, Turkey, and Turkmenistan (Coleoptera: Curculionidae: Entiminae). *Insect Systematics & Evolution*, 39(1): 39-54.
- Zehzad, B., Kiabi, B. H., Madjnoonian, H., 2002, The natural areas and landscape of Iran: an overview. *Zoology in the Middle East*, 26(1): 7-10.

*Received: December 28, 2015*

*Accepted: January 23, 2017*



## **New Data of Ground Beetles (Coleoptera: Carabidae) in Tunisia**

Samir GHANNEM<sup>1\*</sup>

Moncef BOUMAIZA<sup>1</sup>

<sup>1</sup>Laboratory of Environment Bio-monitoring (L.B.E), Faculty of Sciences of Bizerte, University of Carthage, Zarzouna 7021, TUNISIA,  
Corresponding author's e-mail: ghan\_samir@yahoo.fr

### **ABSTRACT**

A faunistic survey was carried out on ground beetles (Insecta, Carabidae) over the course of 2012-2013 in four localities from Northern Tunisia. Insects were collected mostly by hand and pitfall traps. The habitat preferences of ground beetle species are also discussed. In total 65 species belonging to 45 genera, 24 tribes, and nine subfamilies were found, whose six species are new records for Tunisia and seven species are endemic to northern Africa. The present study gives new faunistic data and better knowledge on the biogeography of Tunisia.

*Key words:* Carabidae, taxonomy, new records, endemic, distribution, Tunisia.

### **INTRODUCTION**

The family Carabidae, or ground beetles, currently includes about 40,000 (Thiele, 1977) to 50.000 species (Desender, 1987) but some authors consider it could be about 60,000 species (Gaston, 1991). Carabidae are the most abundant beetle families (Dajoz, 2002). They are located in all terrestrial habitats and represent a major part of the invertebrate predator for the soil fauna (Arndt *et al.*, 2011). Their evolutionary differentiation took place during the Quaternary ice ages (Palestrini *et al.*, 2012). Studies on the distribution and survival of many Carabidae species in different habitats serve to consolidate our level of knowledge of the environmental and climate change (Kerr *et al.*, 2007; Vaibhao *et al.*, 2013). They are one of the three richest families of extant beetles (Lorenz, 2005) and are important bioindicators for assessment the effect of anthropogenic impact on the terrestrial ecosystem (den Boer, 1977; Butovsky, 1997; Butovsky *et al.*, 1999; Brandmayr *et al.*, 2005; Lagisz and Laskowski, 2008; Schirmel *et al.*, 2015; Ghannem *et al.*, 2016; Simon *et al.*, 2016). However, as they are predators of terrestrial invertebrates and, at the same time can be part of the diet of vertebrates (amphibians, reptiles, birds) they can play a key role in clarifying the route of toxic elements in food chain (Butovsky, 2011). These animals have been used in studies of several terrestrial habitats (grassland, forest, agro-ecosystems, and even roadside) in the assessment of human impacts on terrestrial ecosystems (Andrews and Cooke, 1984; Beyer *et al.*, 1985; Butovsky, 1994; Jelaska *et al.*, 2007; Purchart and Kula, 2007). This group of invertebrates is frequently used for ecotoxicological

analysis for the reason of its strong ability to be found in all types of terrestrial environments (Conti *et al.*, 2017). Carabidae beetles consume a wide assortment of soil arthropods (caterpillars, wireworms, maggots, ants, aphids and slugs), are also feeding on seeds during its development. They considered as one of the regulating agents of weed populations (Liebman and Gallandt, 1997). One of the most daunting tasks challenging an aspiring entomologist is the identification of species within a family as diverse as carabids (Choate, 2001). This family is characterized by a very wide adaptive success on the multiple ecological conditions encountered on the scale of the globe. According to Moret (2003) and based on their great diversity, Carabidae are a suitable taxonomic group for ecological and biogeographical studies. Their high endemism, the inability to fly of most of Carabidae species and their restricted geographic distribution is a key element to define areas of endemism (Moret, 2000). In addition, they are easy to sample using several collecting techniques. They can be found under stones or in the middle of the pillow vegetation. At night, when they leave to hunt, it will be easy to collect them using a flashlight or using pitfall traps. Species checklists are effective tools in the domain of natural science. They serve to consolidate our level of knowledge and at the same time indicate areas in need of further survey. The fauna of Carabidae of Tunisia is rather unknown in comparison to Europe as well as other zoogeographical areas. Only a few studies cover the topic of ground beetles in Tunisia. Good examples are so far Bedel (1895), Peyerimhoff (1909) and Normand (1933), recent work leading to the discovery of new species (Gueorguiev, 2012; Queinnec and Ollivier, 2012; Ghannem *et al.*, 2015), and that prompted us to make a new contribution and update the list of ground beetles in Tunisia.

The aim of our study was to analyze spatial patterns in the species richness of carabids in order to achieve a better knowledge of the ground beetles of Tunisia.

## **MATERIAL AND METHODS**

### **Study area**

The materials for this study were collected from various habitats between the years 2012 and 2013. The study areas (four sites) are located in Northern Tunisia. GPS coordinates altitudes and morphodynamic characteristics of the study areas are listed in Table 1.

### **Sampling procedure**

The specimens were sampled by pitfall traps (diameter 10 cm, height 12 cm). We used 10 containers per locality, separated about 5m and buried in a way that the microhabitat do not look like modified to facilitate the insect catch. Each pot was partially filled with acetic acid diluted at 30% to kill and preserve samples. Sampling was biweekly in March, April, May and June, and the beetles were collected, separated, and identified. Additional specimens were collected directly by hand while walking through the area of study. Dates, locations, and number of specimens were recorded. Beetles were stored in the collection of the first author in the Faculty of Sciences of

*New Data of Ground Beetles (Coleoptera: Carabidae)*

Bizerte, University of Carthage, Tunisia. The biological materials were identified using literature carry out by specialists: Bedel (1895), Antoine (1955-1957-1959-1961-1962), and by comparison with the collection of Peyerimhoff at the National Museum of Natural History, Paris, France (NHMP) and later confirmed by some experts (see Acknowledgements).

The new records are marked in the list by an asterisk (\*).

In general, for practical reasons we suggest in our study using the highest classification suggested by Bouchard *et al.* (2011) and for the genus level and species names we have followed the Palearctic catalogue (Löbl and Smetana, 2003).

Table 1. Main characteristics of study areas.

Site	Geographical coordinates	Altitude, m a.s.l.	Habitat
Ras Inja	37°20' 23.55" N 9°44' 54.05" E	14	It is located in northern Tunisia, only 8 km to the south-east of the city of Bizerte, near to the sea. The site is characterized by forest plantations ( <i>Acacia Cyclops</i> A. Cunn. ex G. Don, 1832, <i>Acacia cyanophylla</i> Lindl, 1839, <i>Pinus pinaster</i> Aiton, 1789, <i>Pinus pinea</i> Linnaeus, 1753, <i>Pinus halepensis</i> Mill. 1768 and <i>Eucalyptus</i> sp. Hook, 1844). Existing and especially shrubs and herbaceous vegetation, found mostly Juniper, whether Phoenician, or oxycedre ( <i>Juniperus Phoenicia</i> Linnaeus, 1753, <i>Juniperus oxycedrus</i> Linnaeus, 1753, <i>Ephedra fragilis</i> Moris, 1828 and <i>Matthiola tricuspidata</i> R.Br. 1812.)
Sidi Nsir	36°53'671"N 009°26'648" E	218	Located in the northern part of Tunisia, breeding ground wheat ( <i>Triticum aestivum</i> Linnaeus, 1753) on the main road Beja-Mateur, crossed by a permanent watercourse. This environment is characterized by forest plantations of Acacia and Eucalyptus, including <i>Eucalyptus</i> sp. Hook, 1844 and <i>Acacia Cyclops</i> A.Cunn.ex G.Don, 1832.
Ain Draham	36°47'17.14"N 8°40'47.72" E	800	This region is the most humid area of Tunisia for holding the national rainfall record with 1,534 millimeters of precipitation per year. Plantation: Forest cork oak or oak Zen (we usually at Tribeted to <i>Quercus suber</i> Linnaeus, 1753 and <i>Quercus mirbecki</i> Durieu ex Bory, 1846). Without limestone soils, is presented with characteristic soils leached mull. In the litter including forming a continuous sheets cork oak and oak litter Zen, is light textured topsoil, blackish, heavily penetrated by the roots, with a richness organic substances declining with depth
Cap Serrat	37°14'25"N 9°12'33.5"E	108	This region is characterized by an alternation of sandy areas and steep areas. Ecosystem Cap Serrat is belonging to the field of Montado vegetation cork oak. They are in the form of forests, matorral of varying height and grassy erme (thermo-Mediterranean Cork Oak Landscapes floor includes bass altitude where the lentisk is abundant and Montado his matorral and ermes used for grazing livestock farmers).At the level of interdunal depressions evolve hydromorphic gley soils and permanent water shallow. The vegetation is characterized by <i>Cutandia maritime</i> Barbey, 1885, <i>Crucianella maritime</i> Linnaeus, 1753

## RESULTS

The investigated area gives data on 65 species from 45 genera, 24 tribes, and nine subfamilies found in North Tunisia. The registered taxa are listed below. All registered species belong to the following subfamilies: Nebriinae (with two tribe, two genera, and two species), Cicindelinae (with one tribe, two genera, and two species), Carabinae (one tribe, one genera, and one species), Omophroninae (one tribe, one genera, and one species), Scaritinae (with three tribe, four genera, and five species), Siagoninae (with one tribes, one genera, and one species), Trechinae (with three tribe, four genera, and 12 species), Brachininae (with one tribe, one genera, and six

species) and Harpalinae (with 10 tribes, 28 genera, and 35 species). The species and subspecies that belonged to subfamilies and tribes are presented in the following list.

### **Subfamily Nebriinae Laporte, 1834**

#### **Tribe Nebriini Laporte, 1834**

#### **Genus *Nebria* Latreille, 1802**

#### ***Nebria (Nebria) andalusia variabilis* Lucas, 1842**

Material examined. Sidi Nsir: 2♂♂, 1♀; 11. 04. 2012.

Collection circumstances: The specimen was found underneath stone and leaves.

Geographical distribution: North Africa: Tunisia, Algeria, Morocco. Europe: Italy, Portugal, Spain; (Löbl and Smetana, 2003).

#### **Tribe Notiophilini Motschulsky, 1850**

#### **Genus *Notiophilus* Duméril, 1806**

#### ***Notiophilus geminatus* Dejean, 1831**

Material examined. Ras Injla: 2♀♀, 31. 06. 2012; Ain Draham: 1♂♂, 2♀♀; 23. 03. 2013.

Collection circumstances: Species collected in a fresh environment, between roots, under wood, stones and dead leaves. He lives in a variety of environments. Although the number of places where it was detected, it reflects the diversity of the conditions mentioned because they are captured at the same time in zones wet and in others sunny and dry, which suggests that it is a species with a big capacity of adaptation.

Geographical distribution. North Africa: Tunisia, Algeria, Morocco, Canary Island. Europe: France, Greece, Italy, Spain. Asia: Syria (Bedel, 1895; Antoine, 1955; Löbl and Smetana, 2003).

### **Subfamily Cicindelinae Latreille, 1802**

#### **Tribe Cicindelini Latreille, 1802**

#### **Genus *Cicindela* Linnaeus, 1758**

#### ***Cicindela (str.) campestris* Linnaeus, 1758**

Material examined. Ain Draham: 2♂♂, 1♀, 04. 05. 2012.

Collection circumstances: The species was collected, especially on shrubs in soil free of limestone.

Geographical distribution: North Africa: Tunisia, Algeria, Morocco. Europe: Italy, Spain, France, Portugal (Bedel, 1895; Antoine, 1955; Löbl and Smetana, 2003). The range of this species is an example of chorotype Palearctic (Serrano, 2003).

*New Data of Ground Beetles (Coleoptera: Carabidae)*

## **Genus *Lophyra* Motschulsky, 1859**

### ***Lophyra flexuosa* (Fabricius, 1787)**

Material examined: Ras Injla, 7♂♂, 5♀♀, 31. 06. 2012; Cap Serrat, 7♂♂, 6♀♀, 31.06.2012.

Collection circumstances: They were generally collected on sandy grounds and dunes, near the sea and lakes, sometimes along the river banks.

Geographical distribution: North Africa: Tunisia, Algeria, Egypt, Libya, Morocco. Europe: France, Italy, Portugal, Spain, Switzerland. Asia: Syria (Bedel, 1895; Antoine, 1955; Löbl and Smetana, 2003).

## **Subfamily Carabinae Latreille, 1802**

### **Tribe Carabini Latreille, 1802**

## **Genus *Macrothorax* Desmaret, 1850**

### ***Macrothorax morbillosus* (Fabricius, 1792)**

Material examined. Ras Injla, 1♂♂, 3♀♀, 31.06.2012; Sidi Nsir, 4♂♂, 3♀♀, 11.04.2012.

Collection circumstances: These species were collected under stones, in the woods and in snail shells.

Geographical distribution: All of North Africa. Europe: Spain, Italy (Sicily, Sardinia), France (Bedel, 1895; Antoine, 1955; Machard, 1997; Löbl and Smetana, 2003).

## **Subfamily Omophroninae Bonelli, 1810**

### **Tribe Omophronini Bonelli, 1810**

## **Genus *Omophron* Latreille, 1802**

### ***Omophron limbatum* Fabricius, 1776**

Material examined: Cap Serrat, 2♂♂, 3♀♀, 22.04.2013; Ras Injla, 2♂♂, 1♀♀; 13.05.2013.

Collection circumstances: Species captured in sandy soil near water course.

Geographical distribution: North Africa: Tunisia, Algeria. Europe: Spain, France, Italy (incl Corsica and Sicily.), Montenegro, Serbia, Slovenia, Albania, Bosnia and Herzegovina, Bulgaria, Croatia, Greece, Turkey (incl Crete) (Löbl and Smetana, 2003; Valainis, 2009).

## **Subfamily Scaritinae Bonelli, 1810**

### **Tribe Clivinini Rafinesque, 1815**

## **Genus *Clivina* Latreille, 1802**

***Clivina ypsilon* Dejean and Boisduval, 1829**

Material examined. Cap Serrat, 2♂♂, 3♀♀, 22.03.2013; Ras Injla, 1♂; 12.04.2013.

Collection circumstances: Hidden under stones and plant residues in a wet and coastal zone.

Geographical distribution: North Africa: Tunisia, Algeria, Morocco (Löbl and Smetana, 2003). Western Mediterranean from Sicily to Spain, Central Europe (Kapla, 2004).

**Tribe Dyschiriini Kolbe, 1880****Genus *Dyschirius* Bonelli, 1810*****Dyschirius numidicus* Putzeys, 1846**

Material examined. Cap Serrat, 2♂♂, 1♀, 11.06.2012, Ras Injla, 1♂, 3♀♀, 29.06.2012.

Collection circumstances: Collected in the sand of the coast.

Geographical distribution: North Africa: Tunisia, South Algeria, Morocco, Egypt (Antoine, 1955). The western Mediterranean, on the coast of the Mediterranean Sea from Spain, France and Italy (Fedorenko, 1996).

**Tribe Scaritini Bonelli, 1810****Subtribe Scaritina Bonelli, 1810****Genus *Distichus* Motschoulsky, 1857*****Distichus planus* (Bonelli, 1813)**

Material examined: Cap Serrat, 7♂♂, 8♀♀; 22.04.2013; Ras Injla, 12♂♂, 11♀♀; 13.05.2013.

Collection circumstances: Wide spread species on sandy beaches.

Geographical distribution: North Africa: Tunisia, Egypt, Morocco. Europe: Spain, Italy, Corsica (Löbl and Smetana, 2003).

**Genus *Scarites* Fabricius, 1775*****Scarites (Parallelomorphus) laevigatus* Fabricius, 1792**

Material examined. Ras Injla, 9♂♂, 13♀♀, 21.06. 2012; Cap Serrat, 5♂♂, 2♀♀, 23.03. 2013.

Collection circumstances: Halophiles insects living on the coastal beaches, especially at the mouths of rivers and on the wet sand.

Geographical distribution: North Africa: Tunisia, Algeria, Egypt, Morocco (Löbl and Smetana, 2003). Europe: Spain, Italy, Greece, Slovenia, Portugal. It is an element and Mediterranean coasts of the Black Sea (Magistretti, 1965).

***Scarites (Scallophorites) striatus* Dejean, 1825**

Material examined. Ras Injla, 4♂♂, 3♀♀, 31.06. 2012.

*New Data of Ground Beetles (Coleoptera: Carabidae)*

Collection circumstances: Species collected on the sand.

Geographical distribution: North Africa: Tunisia, Algeria, Libya, Egypt. Asia: Saudi Arabia (Löbl and Smetana, 2003).

**Subfamily Siagoninae Bonelli, 1813**

**Tribe Siagonini Bonelli, 1813**

**Genus *Siagona* Latreille, 1804**

***Siagona rufipes* Fabricius, 1792**

Material examined. Sidi Nsir, 1♂, 11. 04. 2012.

Collection circumstances: Species collected in a clay ground under a stone.

Geographical distribution: Endemic to North Africa, mainly distributed in Tunisia, Algeria, Morocco (Antoine, 1955; Löbl and Smetana, 2003).

**Subfamily Trechinae Bonelli, 1810**

**Tribe Bembidiini Stephens, 1827**

**Subtribe Bembidiina Stephens, 1827**

**Genus *Asaphidion* Gozis, 1886**

***Asaphidion stierlini* (Heyden, 1880)**

Material examined: Ain Draham, 2♂♂, 4♀♀, 04.05. 2012.

Collection circumstances: Species collected in edges of running water.

Geographical distribution: North Africa: Tunisia, Morocco. Europe: Austria, Belgium, France, Great Britain, Germany, Greece, Italy, Netherlands, Spain (Löbl and Smetana, 2003).

**Genus *Bembidion* Latreille, 1802**

***Bembidion quadrimaculatum quadrimaculatum* Linnaeus, 1761**

Material examined: Ras Injla, 1♂, 2♀♀, 04.05. 2012; Cap Serrat, 2♀♀, 24.06. 2012.

Collection circumstances: Species found at the edge of puddles.

Geographical distribution: North Africa: Tunisia, Algeria, Morocco. Europe: Italy (Sardinia) (Bonavita and Taglianti, 2005)

**\**Bembidion (Testedium) bipunctatum* (Linnaeus, 1761)**

Material examined: Ain Draham, 3♂♂, 5♀♀, 05.05. 2013.

Collection circumstances: Species collected along streams.

Geographical distribution: North Africa: Algeria, Morocco. Europe: Italy, Spain, France, Great Britain, Germany. Asia: Turkey (Bedel, 1895; Antoine, 1955; Löbl and Smetana, 2003).

**\**Bembidion (Actedium) kuesteri* Schaum, 1845**

Material examined: Ain Draham, 2♂♂, 4♀♀, 22.05.2013; Cap Serrat, 3♀♀, 12.03.2013.

Collection circumstances: Mediterranean coast, and at the mouths of rivers.

Geographical distribution: North Africa: Algeria, Morocco. Europe: Italy (Sardegna), France (Bedel, 1895; Antoine, 1955; Löbl and Smetana, 2003).

***Bembidion (Nepha) callosum* Küster, 1847**

Material examined: Ain Draham, 2♂♂, 5♀♀, 04.05. 2012; Cap Serrat, 2♂♂, 1♀, 4.06.2012.

Collection circumstances: Species found in wet soil near a stream.

Geographical distribution: North Africa: Tunisia, Algeria, Morocco (Antoine, 1955; Löbl and Smetana, 2003).

***Bembidion (Nepha) genei* (Küster, 1847)**

Material examined: Ain Draham, 2♂♂, 1♀, 04.05.2012; Cap Serrat, 1♂, 3♀♀, 14.06. 2012.

Collection circumstances: The species was found at the edge of ponds, rivers and streams.

Geographical distribution: North Africa: Tunisia, Algeria, Morocco. Europe: France, Greece, Italy, Portugal, Spain, Italy (Sardinia) (Bonavita and Taglianti, 2005).

***Bembidion (Phyla) tethys* (Netolitzky, 1926)**

Material examined: Ras Injla, 3♀♀, 21.VI. 2012; Cap Serrat, 3♂♂, 4♀♀, 14.VI. 2012.

Collection circumstances: The specimen was found at the edge of the water.

Geographical distribution: North Africa: Algeria, Libya, Morocco, Tunisia. Europe: Bosnia and Herzegovina, Croatia, France, Italy, Malta, Portugal, Spain, Yugoslavia (Serbia, Montenegro); Asia: Turkey (Löbl and Smetana, 2003).

***Bembidion (Ocyturanus) dudichi* (Csiki, 1928)**

Material examined: Ain Draham, 11♂♂, 9♀♀, 04.05. 2012.

Collection circumstances: These species were collected in streams of sand edge.

Geographical distribution. North Africa: Algeria, Morocco, Tunisia. Europe: Spain, France, Portugal (Antoine, 1955; Löbl and Smetana, 2003).

***Bembidion (Ocyturanus) praeustum* Dejean, 1831**

Material examined: Ain Draham, 2♀♀, 04.05. 2012.

Collection circumstances: Species harvested at the edge of a stream in sandy soil.

*New Data of Ground Beetles (Coleoptera: Carabidae)*

Geographical distribution. North Africa: Tunisia, Egypt, Libya. Europe: continental Italy and Sicily (Neri *et al.*, 2010).

***Bembidion (Neja) cirtense* Netolitzky, 1914**

Material examined: Ain Draham, 3♀♀, 04.05. 2012.

Collection circumstances: Located at the edge of rivers and streams.

Geographical distribution: Endemic species for North Africa, distributed in Tunisia and Algeria (Löbl and Smetana, 2003).

**Subtribe Tachyina Motschulsky, 1862**

**Genus *Sphaerotachys* Müller, 1926**

***Sphaerotachys haemorrhoidalis* (Ponza, 1805)**

Material examined: Cap Serrat, 1♂, 4.VI.2012.

Collection circumstances: Species found in zones of sand on the banks of running water and under stones in a wet salty ground.

Geographical distribution: North Africa: Tunisia, Morocco, Canary Island, Egypt. Europe: Spain, Italy, France (Löbl and Smetana, 2003).

**Tribe Pogonini Laporte, 1834**

**Genus *Pogonus* Dejean, 1821**

***Pogonus (str.) littoralis* Duftschmid, 1812**

Material examined: Sidi Nsir, 2♂♂, 3♀♀, 11.04 2012; Ras Injla, 1♂, 2♀♀, 11.05. 2012.

Collection circumstances: It was found under a stone near a permanent stream near an agricultural field.

Geographical distribution: North Africa: Tunisia, Algeria, Morocco. Europe: Belgium, Bulgaria, France, Greece, Italy, Moldova, Netherlands, Romania, Spain, the European territory of the South, Ukraine; Asia: Syria, Turkey (Löbl and Smetana, 2003).

**Tribe Trechini Bonelli, 1810**

**Subtribe Trechina Bonelli, 1810**

**Genus *Trechus* Clairville, 1806**

***Trechus obtusus* Erichson, 1837**

Material examined: Ain Draham, 2♂♂, 1♀, 04. 05. 2012.

Collection circumstances: Species hygrophile collected under dead leaves.

Geographical distribution: North Africa: Tunisia, Algeria, Morocco. Europe. Spain, Italy, France (Zaballos, 1993; Löbl and Smetana, 2003).

**Subfamily Brachininae Bonelli, 1810****Tribe Brachinini Bonelli, 1810****Subtribe Brachinina Bonelli, 1810****Genus *Brachinus* Weber, 1801*****Brachinus* (str.) *efflans* Dejean and Boisduval, 1829**

Material examined: Sidi Nsir: 1♂, 2♀♀, 08.III.2012.

Collection circumstances: The species were found under a stone in a wet field, near a farm field and a permanent water course.

Comments on classification: The former citations of *B. efflans* Dejean and Boisduval from Tunisia are included as a variety of *Brachinus crepitans* L. (Bedel, 1895) from Haïdra and under the synonym *Brachinus etslans* Dejean.

Geographical distribution: Mediterranean element (Ruiz-tapiador and Zaballos, 2001), Bulgaria, Italy, Portugal, Spain, Syria, Western Morocco, from Casa Blanca to Tangier and the Middle Atlas (Machard, 1997), Algeria, Tunisia (Löbl and Smetana, 2003).

***Brachinus* (str.) *crepitans* Linnaeus, 1758**

Material examined: Sidi Nsir, 2♂♂, 1♀, 08.03.2012.

Collection circumstances: Species found under vegetation debris.

Geographical distribution: North Africa, Tunisia, Algeria, Morocco. Middle and southern Europe. Asia: Syria (Bedel, 1895).

**\**Brachinus* (*Brachinus*) *psophia* Serville, 1821**

Material examined: Cap Serrat, 1♂, 3♀♀, 13.03.2013; Ras Injla, 2♂♂, 4♀♀, 06.04.2013.

Collection circumstances: Species collected at the edges of the river, especially near their mouths.

Geographical distribution: North Africa: Algeria, Morocco. Southern Europe: Spain, Italy, France, Portugal (Löbl and Smetana, 2003).

***Brachinus* (*Brachynolomus*) *immaculicornis* Dejean, 1825**

Material examined: Cap Serrat, 1♂, 2♀♀, 08.03.2012.

Collection circumstances: Species collected under a stone in a clay-humus ground.

Geographical distribution: North Africa: Tunisia, Algeria, Morocco. Europe: Spain, Italy, France (Löbl and Smetana, 2003).

***Brachinus* (*Brachynoaptinus*) *mauretanicus* Bedel, 1914**

Material examined: Cap Serrat, 2♂♂, 3♀♀, 23.03.05.2013; Ain Draham, 1♂, 2♀♀, 21.02.2013.

*New Data of Ground Beetles (Coleoptera: Carabidae)*

Collection circumstances. Species found under stones and plant debris in wet clay soil.

Geographical distribution. Endemic to North Africa. Morocco, Tunisia (Bedel, 1895, Antoine, 1955).

***Brachinus (Cnecostolus) humeralis* Ahrens, 1812**

Material examined: Ain Draham, 2♂♂, 1♀, 26.02.2013.

Collection circumstances: Species found under vegetation debris and under stone.

Geographical distribution: North Africa: Algeria, Morocco, Tunisia. Europe: Spain, Italy, France (Bedel, 1895; Antoine, 1955; Löbl and Smetana, 2003).

**Subfamily Harpalinae Bonelli, 1810**

**Tribe Chlaeniini Brullé, 1834**

**Subtribe Chlaeniina Brullé, 1834**

**Genus *Chlaenites* Motschoulsky, 1860**

***Chlaenites spoliatus* (Rossi, 1792)**

Material examined: Sidi Nsir, 2♂♂, 3♀♀, 24.02.2012.

Collection circumstances: These species have been found in flooded terrain under plant debris.

Geographical distribution: North Africa: Tunisia, Algeria, Morocco, Canary Island, Libya, Egypt. All moderate and Southern Europe and Central Asia (Antoine, 1955; Machard, 1997; Löbl and Smetana, 2003).

**Genus *Chlaenius* Bonelli, 1810**

***Chlaenius velutinus* ssp. *auricollis* Géné, 1839**

Material examined: Sidi Nsir, 2♂♂, 1♀, 11. 04.2012.

Collection circumstances: These species were collected under stones and roots of vegetables.

Geographical distribution: North Africa: Tunisia, Algeria, Morocco, Mauritania. Europe: Spain, Italy (Sicily and Sardinia), France, Portugal, Greece, Germany (Antoine, 1955; Machard, 1997; Serrano, 2003; Löbl and Smetana, 2003).

**Genus *Trichochlaenius* Seidlitz, 1887**

***Trichochlaenius chrysocephalus* (Rossi, 1790)**

Material examined: Sidi Nsir, 3♂♂, 1♀, 26.II.2012.

Collection circumstances: Species collected in the edges of stagnant waters, under

stones. It is a species of meso-hygrophilous behavior. In the study area we sometimes notice a gregarious behavior and are frequently captured by living together with diverse species of *Brachinus* Weber, a similar behavior marked by Therond (1975), who emphasizes that in winter this tendency is more marked.

Geographical distribution. North Africa: Tunisia, Algeria, Morocco. Europe: Spain, Italy, Portugal, Greece, France (Antoine, 1955; Löbl and Smetana, 2003).

***Trichochlaenius aeratus varvasi* Laporte, 1834**

Material examined: Sidi Nsir, 2♂♂, 5♀♀, 11.04.2012.

Collection circumstances: These species were collected under stones in a wet clay soil.

Geographical distribution: Endemic to North Africa: Tunisia, Algeria, Morocco (Serrano, 2003).

**Tribe Dryptini Bonelli, 1810**

**Genus *Drypta* Latreille, 1796**

***Drypta dentata* (Rossi, 1790)**

Material examined: Ain Draham, 1♀, 12. 02. 2013.

Collection circumstances: This species is collected under plant debris in wet ground.

Geographical distribution: North Africa: Tunisia, Algeria, Morocco, Egypt (Bedel, 1895; Antoine, 1955; Löbl and Smetana, 2003).

**Tribe Graphipterini Latreille, 1802**

**Genus *Graphipterus* Latreille, 1802**

***Graphipterus serrator* Forsskal, 1775**

Material examined: Ras Injla 2♂♂, 5♀♀, 31.06.2012.

Collection circumstances: These species were collected on the coast around the turfs and captured around small knolls of sand. Insect xerophile, short sunlight and ran stridulates.

Geographical distribution: North Africa: Tunisia, Algeria, Morocco, Egypt, Libya, Mauritania (Bedel, 1895; Antoine, 1955; Machard, 1997).

**Tribe Harpalini Bonelli, 1810**

**Subtribe Anisodactylina Lacordaire, 1854**

**Genus *Scybalicus* Schaum, 1862**

***Scybalicus oblongiusculus* Dejean, 1829**

Material examined: Sidi Nsir, 2♀♀, 11.03. 2012.

*New Data of Ground Beetles (Coleoptera: Carabidae)*

Collection circumstances. The species was found buried in wet ground.

Geographical distribution. North Africa: Tunisia, Algeria, Morocco. Western Europe (Gibraltar to England); Italy (Sicily) (Bedel, 1895; Machard, 1997; Löbl and Smetana, 2003).

**Subtribe Harpalina Bonelli, 1810****Genus *Carterus* Dejean, 1829*****Carterus* (str.) *rotundicollis* Rambur, 1837**

Material examined: Sidi Nsir, 1♂♂, 3♀♀, 11. IV. 2012.

Collection circumstances: The specimen was collected under stones in wet clayey soils.

Geographical distribution: North Africa: Tunisia, Algeria, Morocco. Europe: Spain, Italy, Portugal (Bedel, 1895; Antoine, 1955; Machard, 1997).

***Carterus* (str.) *interceptus* Dejean**

Material examined: Sidi Nsir, 2♂♂, 11. IV. 2012.

Collection circumstances: Species found in wet clay soil under stones.

Geographical distribution. North Africa: Tunisia, Algeria, Morocco. Europe: Spain, Portugal (Bedel, 1895 ; Löbl and Smetana, 2003).

**Genus *Graniger* Motschoulsky, 1864*****Graniger cordicollis* Serville, 1821**

Material examined. Sidi Nsir, 2♀♀, 11. 04. 2012.

Collection circumstances: This species is collected under a stone in a wet ground.

Geographical distribution: North Africa: Algeria, Morocco, Tunisia. Europe: Spain, Armenia, Bulgaria, Croatia, France, Greece, Italy, Malta, Portugal (Löbl and Smetana, 2003).

**Genus *Harpalus* Latreille, 1802*****Harpalus* (*Pseudophonus*) *rufipes* (De Geer, 1774)**

Material examined: Sidi Nsir, 2♂♂, 3♀♀, 14. 04. 2012.

Collection circumstances: Species collected under stones in wet ground and under pieces of wood.

Geographical distribution: North Africa: Tunisia, Algeria Morocco. Europe: France, Germany, Portugal, Spain (Löbl and Smetana, 2003).

***Harpalus* (str.) *distinguendus* (Duftschmid, 1812)**

Material examined: Ain Draham, 2♂♂, 27. 05. 2012.

Collection circumstances: These species were collected under stones and vegetable debris.

Geographical distribution: North Africa: Tunisia, Algeria, Morocco. Middle Europe, Caucasus, Asia Minor (Antoine, 1955; Löbl and Smetana, 2003; Wrase, 2009).

### **Genus *Parophonus* Ganglbauer, 1892**

#### ***Parophonus hispanus* Rambur, 1838**

Material examined: Sidi Nsir, 4♂♂, 1♀♀, 11. 04. 2012.

Collection circumstances: The specimen was found on sandy soil between the debris of vegetation.

Geographical distribution: North Africa: Algeria, Morocco, Tunisia. Europe: Italy, Spain (Sciaky, 1992; Zaballos and Jeanne, 1994).

#### ***Parophonus hespericus* Jeanne, 1985**

Material examined: Sidi Nsir, 2♂♂, 3♀♀, 11. 04. 2012.

Collection circumstances: These species have been collected under stones and plant debris, mostly in colonization with *P. hispanus* Rambur, 1838

Geographical distribution: North Africa and Italy (Sciaky, 1992); Spain (Jeanne and Zaballos, 1986).

### **Subtribe *Stenolophina* Kirby, 1837**

#### **Genus *Stenolophus* Dejean, 1821**

#### ***Stenolophus (Stenolophus) teutonius* (Schrank, 1781)**

Material examined. Sidi Nsir, 2♂♂, 11. 04. 2012.

Collection circumstances. These species were recorded under stones and debris in a humid place.

Geographical distribution. North Africa: Tunisia; Algeria, Canary Islands, Egypt, Libya, Morocco. Europe: Serbia (Curcic and Stojanovic, 2011), Spain, Malta, Macedonia, Moldova, Poland, Portugal, Romania, Slovakia, Slovenia, Sweden, Turkey, Ukraine, Yugoslavia. Asia: Syria, Turkey (Bedel, 1895; Antoine, 1955; Machard, 1997; Löbl and Smetana, 2003).

#### **\**Stenolophus (Stenolophus) mixtus* (Herbst, 1784)**

Material examined. Sidi Nsir, 1♂, 4♀♀, 02.05. 2013.

Collection circumstances. Marshy coastal places.

Geographical distribution. North Africa: Algeria, Morocco. Europe: Italy, Spain, Malta, Macedonia, Moldova, Poland, Portugal, Romania, Slovakia, Slovenia, Sweden, France, Ukraine (Bedel, 1895; Antoine, 1955; Machard, 1997; Löbl and Smetana, 2003).

*New Data of Ground Beetles (Coleoptera: Carabidae)*

**Tribe Lebiini Bonelli, 1810**

**Subtribe Apenina Ball, 1983**

**Genus *Platytarus* Fairmaire, 1850**

***Platytarus bufo* (Fabricius, 1801)**

Material examined: Sidi Nsir, 1♂, 2♀♀, 17. 01. 2013.

Collection circumstances: The species was found under a stone.

Geographical distribution. North Africa: Tunisia, Algeria, Morocco. Europe: Spain, Italy, Portugal (Löbl and Smetana, 2003).

**Subtribe Cymindidina Laporte, 1834**

**Genus *Cymindis* Latreille, 1806**

***Cymindis setifensis* Lucas, 1842**

Material examined: Sidi Nsir, 3♀♀, 11. 04. 2012; Ras Injla, 1♂♂, 1♀♀, 13. 06. 2013.

Collection circumstances: The species was found under a small stone.

Geographical distribution: *C. sitifensis* Lucas, 1842 is appropriate to the North of Africa and spread, under diverse forms, in all the Barbary Coast and in the islands Madeira, Salvages and the Canary Islands (Bedel, 1895; Ghannem *et al.*, 2014).

**Subtribe Dromiusina Bonelli, 1810**

**Genus *Apristus* Chaudoir, 1846**

***Apristus striatipennis* Lucas, 1846**

Material examined: Ain Draham, 2 ♀♀ 21.03. 2012.

Collection circumstances: Species collected in the gravel, at the edge of running waters.

Geographical distribution: North Africa: Tunisia, Morocco, Algeria, Egypt (Löbl and Smetana, 2003; Machard, 1997). It is an endemic element for West Africa.

**Genus *Microlestes* Schmidt-Goebel, 1846**

***Microlestes corticalis* (Dufour, 1820)**

Material examined: Ain Draham, 3♂♂, 4♀♀, 08.03.2012; Cap Serrat, 2♂♂, 3♀♀, 18.03.2012.

Collection circumstances: Under dead leaves and plants bass in an argilo-sandy ground.

Geographical distribution: North Africa: Tunisia, Morocco, Algeria, Mauritania, Canary Island, Egypt. Europe: Spain, Italy, France, Germany, Greece, Portugal. Central Asia (Antoine, 1955; Machard, 1997; Löbl and Smetana, 2003).

**Subtribe Lebiina Bonelli, 1810****Genus *Lamprias* Bonelli, 1810*****Lamprias fulvicollis* Fabricius, 1792**

Material examined: Cap Serrat, 1♂♂, 4♀♀, 08.03.2012; Ain Draham, 2♂♂, 1♀, 23.05.2012.

Collection circumstances: The species was recolted under the tree of bark.

Geographical distribution: North Africa: Tunisia, Algeria, Morocco. Western Europe (Bedel, 1895; Antoine, 1955; Machard, 1997; Löbl and Smetana, 2003).

**Tribe Licinini Bonelli, 1810****Subtribe Licinina Bonelli, 1810****Genus *Licinus* Latreille, 1802*****Licinus punctatulus* Fabricius, 1972**

Material examined: Cap Serrat, 6♂♂, 4♀♀, 14.06. 2012; Ras Injla, 3♂♂, 1♀, 31. 06.2012.

Collection circumstances: These species were collected under stones, pieces of wood and fragments of vegetables, usually in an arid field.

Geographical distribution: North Africa: Tunisia, Algeria, Morocco. Europe: Spain, France: (Antoine, 1955; Machard, 1997; Löbl and Smetana, 2003)

**Tribe Platynini Bonelli, 1810****Genus *Olisthopus* Dejean, 1828*****Olisthopus fuscatus* Dejean, 1828**

Material examined: Ain Draham, 1♂, 2♀♀, 26.05.2013.

Collection circumstances: Specimens were collected under stones and fragments of vegetables in a dry ground.

Geographical distribution: North Africa: Tunisia, Algeria, Morocco. Europe: Spain, Italy, Portugal, France (Antoine, 1955; Machard, 1997; Löbl and Smetana, 2003).

**Genus *Agonum* Bonelli, 1810*****Agonum* (str.) *nigrum* Dejean, 1828**

Material examined: Ras Injla, 1♂, 3♀♀, 26. 06. 2012; Cap Serrat, 2♂♂, 1♀, 21. 05. 2012.

Collection circumstances: The species was collected under a stone in a marshy ground.

Geographical distribution: North Africa: Tunisia, Morocco. Southern and Western Europe (Antoine, 1955; Machard, 1997; Löbl and Smetana, 2003).

*New Data of Ground Beetles (Coleoptera: Carabidae)*

**Tribe Pterostichini Bonelli, 1810**

**Subtribe Pterostichina Bonelli, 1810**

**Genus *Poecilus* Bonelli, 1810**

***Poecilus* (str.) *tyrrhenicus* Csiki, 1930**

Material examined: Sidi Nsir, 2♂♂, 1♀, 22. 05. 2012.

Collection circumstances: The species were collected under stones and fragments of vegetables.

Geographical distribution. North Africa: Tunisia, Morocco, Algeria. Europe: Spain (Andalusia) (Antoine, 1955; Machard, 1997; Löbl and Smetana, 2003).

**Genus *Orthomus* Chaudoir, 1838**

***Orthomus rubicundus* Coquerel, 1859**

Material examined: Sidi Nsir, 1♂, 2♀♀, 11. 04. 2012.

Collection circumstances: It was found underneath stone surrounded with debris of plants.

Geographical distribution: Endemic for North Africa, mainly found in northern Algeria, northern Tunisia (Mateu, 1954).

Wrong locality: "Sicilia" (Wrase and Jeanne, 2005).

**Genus *Percus* Bonelli, 1810**

***Percus* (str.) *lineatus* Solier, 1835**

Material examined: Ras Injla, 3♂♂, 2♀♀, 31. 06. 2012; Sidi Nsir, 4♂♂, 3♀♀, 11. 04. 2012.

Collection circumstances: They were collected underneath stone and wood.

Geographical distribution: North Africa: Tunisia, Algeria. Europe: Italy (Sicily) (Löbl and Smetana, 2003).

**Tribe Sphodrini Laporte, 1834**

**Subtribe Calathina Laporte, 1834**

**Genus *Calathus* Bonelli, 1810**

***Calathus* (*Bedelinus*) *circumseptus* Germar, 1824**

Material examined: Ain Draham, 2♂♂, 1♀, 4.05. 2012; Ras Injla, 2♂♂, 3♀♀, 26. 06. 2012.

Collection circumstances: The specimen was collected under feet of trees, in wet ground.

Geographical distribution: North Africa: Algeria, Morocco. Europe: Albania, Croatia, France, Greece, Italy, Portugal, Spain (Löbl and Smetana, 2003; Serrano *et al.*, 2003).

***Calathus (Neocalathus) mollis* Marsham, 1802**

Material examined: Ras Injla, 5♂♂, 4♀♀, 26. 06. 2012.

Collection circumstances: They were found underneath feet of tree, in sandy soil.

Geographical distribution: North Africa: Tunisia, Morocco. Europe: Azerbaijan, Armenia, Belgium, Bulgaria, Bosnia Herzegovina, Croatia, Denmark, Estonia, France, Great Britain, Germany, Georgia, Greece, Ireland, Italy, Latvia, Lithuania, Macedonia, Norway, Poland, Portugal, Slovenia, Spain, Sweden, Ukraine, Yugoslavia; Asia: Turkmenistan, Turkey (Bedel, 1895; Antoine, 1955; Machard, 1997; Serrano *et al.*, 2003; Löbl and Smetana, 2003).

**Subtribe Sphodrina Laporte, 1834**

**Genus *Laemostenus* Bonelli, 1810**

***Laemostenus (Pristonychus) algerinus* Gory, 1833**

Material examined: Ras Injla, 2♂♂, 1♀, 26. 06. 2012.

Collection circumstances: The specimen was collected underneath feet of trees.

Geographical distribution: North Africa: Algeria, Morocco, Tunisia. Europe: Croatia, France, Italy, Spain (Bedel, 1895; Antoine, 1955; Machard, 1997; Löbl and Smetana, 2003).

**Subtribe Synuchina Lindroth, 1956**

**Genus *Platyderus* Stephens, 1828**

***Platyderus ruficollis* Marsham, 1802**

Material examined: Sidi Nsir: 2♂♂, 4♀♀, 17.01. 2013.

Collection circumstances. The species were collected under stones and plants.

Geographical distribution: North Africa: Tunisia, Morocco, Algeria. Europe: Western Europe (Bedel, 1895; Antoine, 1955; Machard, 1997)

**Tribe Zabrinini Bonelli, 1810**

**Subtribe Amarina Zimmermann, 1832**

**Genus *Zabrus* Clairville, 1806**

**\**Zabrus (Aulacozabrus) distinctus* Lucas, 1842**

Material examined: Ain Draham, 2♂♂, 3♀♀, 23.04. 2012.

Collection circumstances: Species collected in high places, buried in the sand, and under stones.

*New Data of Ground Beetles (Coleoptera: Carabidae)*

Geographical distribution: Endemic to North Africa: Algeria, Morocco (Bedel, 1895; Antoine, 1955; Machard, 1997; Löbl and Smetana, 2003).

**Genus *Amara* Bonelli, 1810**

***Amara* (str.) *subconvexa* Putzeys, 1865**

Material examined: Ras Injla, 2♂♂, 1♀; 26.06.2012; Ain Draham, 2♂♂, 3♀♀; 23.04. 2012.

Collection circumstances: The specimen was collected by had underneath dead leaves.

Geographical distribution. North Africa: Tunisia, Algeria, Morocco. Europe: Spain, Italy (Sardinia, Sicily), France, Portugal (Bedel, 1895; Antoine, 1955; Machard, 1997; Löbl and Smetana, 2003).

***Amara* (str.) *aenea* Degeer, 1774**

Material examined: RAs Injla, 2♂♂, 3♀♀; 26. 06. 2012; Sidi Nsir, 4♂♂, 2♀♀; 11. 04. 2012; Ain Draham, 3♀♀; 04. 05. 2012.

Collection circumstances: Species collected underneath pieces of wood, and vegetation debris.

*A. aenea* De Geer, 1774 present a marked tendency heliophilic and thermophilic which manifested, in both in its preferences in the dry land, exposed to the sun and open, and both for its diurnal behavior (Barndt, 1982; Desender and Alderweireldt, 1990; Kegel, 1990), upon reaching the maximal activity with the maximum temperature.

Geographical distribution. North Africa: Tunisia, Algeria, Egypt, Libya, Morocco. Europe: Belgium, Bosnia Herzegovina, Bulgaria, Croatia, Denmark, France, Great Britain, Germany, Georgia, Greece, Hungary, Ireland, Italy, Luxembourg, Malta, Macedonia, Moldavia, Poland, Portugal, Spain, Sweden, Switzerland, Turkey, Ukraine (Bedel, 1895; Antoine, 1955; Machard, 1997; Löbl and Smetana, 2003). This is a Palearctic element. The species is mentioned in Canada (Spence, 1990).

**Tribe Zuphiini Bonelli, 1810**

**Subtribe Zuphiina Bonelli, 1810**

**Genus *Zuphium* Latreille, 1806**

***Zuphium* (str.) *olens* Rossi, 1790**

Material examined: Sidi Nsir, 1♂, 3♀♀; 04. 05. 2012.

Collection circumstances: The species were collected marshy edge of rivers.

Geographical distribution: North Africa: Tunisia, Morocco, Algeria. Europe: Spain, Italy, Portugal, France, Germany, Greece (Bedel, 1895; Machard, 1997; Löbl and Smetana, 2003).

## DISCUSSION

For more than 82 years, Normand (1933) remained the principal source of data on the Tunisian beetle fauna, while progressively becoming outdated. A new, revised and updated list of the carabid species is provided here, recognizing 65 species.

This list does not claim to be complete but provides an updated listing. Further, continuous observations will be necessary to complete the species list. For the 65 species, we found 45 genera, 24 tribes, and nine subfamilies of Carabids from Northern Tunisia. Five species are new records: *Bembidion (Testedium) bipunctatum* (Linne, 1761), *Bembidion (Actedium) kuesteri* Schaum, 1845, *Brachinus (Brachinus) psophia* Serville, 1821, *Stenolophus (Stenolophus) mixtus* (Herbst, 1784) and *Zabrus (Aulacozabrus) distinctus* Lucas, 1842. Additionally, six species endemic to northern Africa: *Siagona rufipes* Fabricius, 1792, *Bembidion (Ocyturanus) praeustum* Dejean, 1831, *Brachinus (Brachynoptinus) mauretanicus* Bedel, 1914, *Apristus striatipennis* Lucas, 1846, *Orthomus rubicundus* Coquerel, 1859 and *Zabrus (Aulacozabrus) distinctus* Lucas, 1842.

In conclusion, it could be noted that new records increase the strength of carabid fauna of Tunisia. More effort must be made to get more information about the spatio-temporal distribution of carabid species in all ecosystems of the country to help to identify and locate endemic species, rare or endangered species for conservation. We hope that this work will be a basis for future taxonomical and ecological investigations of the Tunisian Carabids.

## ACKNOWLEDGEMENTS

We are grateful to Mr. Olegario del Junco (Jerez de la Frontera, Spain) and Dr. Ildefonso Ruiz-Tapiador Aparicio (Universidad Politécnica de Madrid, Spain), who helped us for the identification of Tunisian material.

## REFERENCES

- Andrews, S. M., Cooke, J. A., 1984, *Cadmium within a contaminated grassland ecosystem established on a metalliferous mine waste*. In: Osborn, D. (Eds), *Metals in animals*. Symposium N 12, Institute of Terrestrial Ecology. Abbots Ripton, 11-15.
- Antoine, M., 1955, Coléoptères Carabiques du Maroc (1ère partie). *Mémoires de la Société des Sciences Naturelles et Physiques du Maroc, Nouvelle série, Zoologie*, 1: 1-176.
- Antoine, M., 1957, Coléoptères Carabiques du Maroc (deuxième partie). *Mémoires de la Société des Sciences Naturelles et Physiques du Maroc, Nouvelle série, Zoologie*, 3: 179-314.
- Antoine, M., 1959, Coléoptères carabiques du Maroc (troisième partie). *Mémoires de la société des Sciences Naturelles et Physiques du Maroc*, 6: 315-465.
- Antoine, M., 1961, Coléoptères Carabiques du Maroc (4ème partie). *Mémoires de la Société des Sciences Naturelles et Physiques du Maroc, Nouvelle série, Zoologie*, 8: 467-537.
- Antoine, M., 1962, Coléoptères Carabiques du Maroc (5ème partie). *Mémoires de la Société des Sciences Naturelles et Physiques du Maroc, Nouvelle série, Zoologie*, 9: 535-692.
- Arndt, E., Schnitter, P., Sfenthourakis, S., Wrase, D. W., 2011, *Ground Beetles (Carabidae) of Greece*. Pensoft publishers, 394 pp.

*New Data of Ground Beetles (Coleoptera: Carabidae)*

- Barndt, D., 1982, Untersuchung der diurnalen und saisonalen Aktivität von Käfer mit einer neu entwickelten Elektro-Bodenfalle. *Entomologische Blätter*, 78: 81-97.
- Bedel, L., 1895, *Catalogue raisonné des Coléoptères du nord de l'Afrique (Maroc, Algérie, Tunisie et Tripolitaine) avec notes sur la Faune des tiers Canaries et de Madère, Première partie*, Paris. Société Entomologique de France, 320 pp.
- Beyer, W. N., Pattee, O. H., Sileo, L., Hoffman, D. J., Mulhern, B. M., 1985, Metal contamination in wildlife living near two zinc smelters. *Environ. Pollut.* 38: 63-86.
- Bonavita, P., Taglianti, A., 2005, Le Alpi orientali come zona di transizione nel popolamento dei bembidini (Coleoptera, Carabidae). Biogeografia delle Alpi e Prealpi centro-orientali. *Biogeographia, Lavori della Società italiana di Biogeografia*, 26: 203-228.
- Bouchard, P., Bousquet, Y., Davies, A., Alonso-Zarazaga, M., Lawrence, J., Lyal, C., Newton, A., Reid, C., Schmitt, M., Slipinski, A., Smith, A., 2011, Family-group names in Coleoptera (Insecta). *ZooKeys*, 88: 972pp.
- Brandmayr, P., Zetto, T., Pizzolotto, R., 2005, *I Coleotteri Carabidi per la valutazione ambientale e la conservazione della biodiversità*. APAT, Manuali e Linee Guida, 240 pp.
- Butovsky, R. O., 1994, Motorway impact on structure of carabid community (Coleoptera, Carabidae) in agroecosystems using biomass. *Russian. Journal of Ecology*, 6: 90-93.
- Butovsky, R. O., 1997, Heavy metals and carabids (Coleoptera, Carabidae). *Agrohimija*, 11: 78-86.
- Butovsky, R. O., 2011, Heavy metals in carabids (Coleoptera, Carabidae). *ZooKeys*, 100: 215-222.
- Butovsky, R. O., Verhoef, S. C., Zaitsev, A. S., van Straalen, N. M., 1999, *Heavy metals in different invertebrate groups as related to soil contamination*. In: Butovsky, R. O., Van Straalen, N. M. (Eds.), *Pollution-induced Changes in Soil Invertebrate Food webs*. Vrije Universiteit, Amsterdam, 117-129.
- Choate, P. M., 2001, Manual for the identification of Ground beetles (Coleoptera: Carabidae) (including tiger beetles) of Florida. *Department Entomology and Nematology University of Florida*, 19pp.
- Conti, E., Dattilo, S., Costa, G., Puglisi, C., 2017, The ground beetle *Parallelomorphus laevigatus* is a potential indicator of trace metal contamination on the eastern coast of Sicily. *Ecotoxicology and Environmental Safety*, 135: 183-190.
- Curcic, S. B., Stojanović, D. V., 2011, New data on the Carabid beetles (Coleoptera: Carabidae) of Mt. Fruška Gora (Northern Serbia). *Acta Entomologica Serbica*, 16: 45-59.
- Dajoz, R., 2002, *Les Coléoptères Carabidés et Ténébrionidés: écologie et biologie*. Lavoisier, Paris, 522 pp.
- den Boer, P. J., 1977, Dispersal power and survival. Misc. Pap. Landbouw. *Wagen*. 14: 1-190.
- Desender, K., 1987, Ground beetles (Col., Carabidae) new or confirmed for the belgian fauna. *Bulletin et Annales de la Société royale belge d'Entomologie*, 123: 334-336
- Desender, K. Y., Alderweireldt, M., 1990, Yearly and seasonal variation of carabid diel activity in pastures and cultivated fields. *Revue d'Ecologie et de Biologie du Sol*, 27: 423-433.
- Fedorenko, D., 1996, *Reclassification of world Dyschirini, with a revision of the Palearctic Fauna (Coleoptera, Carabidae)*. Pensoft. Sofia, Moscow and St. Petersburg, 224 pp.
- Gaston, K. J., 1991, The magnitude of global insect species richness. *Conservation Biology*, 5: 283-296.
- Ghannem, S., Khalloufi, N., Boumaiza, M., 2014, Primera contribución al conocimiento de los insectos del Parque Nacional Bou Hedma de Túnez. *Revista gaditana d'Entomología*, 1: 203-210.
- Ghannem, S., Khazri, A., Sellami, B., Boumaiza, M., 2016, Assessment of heavy metal contamination in soil and *Chlaenius (Chlaeniellus) olivieri* (Coleoptera, Carabidae) in the vicinity of a textile factory near Ras Jbel (Bizerte, Tunisia). *Environmental Earth Sciences*, 75(5): 1-10.
- Ghannem, S., Perez-Gonzalez, S., Zaballos, J.P., Boumaiza, M., 2015, New records of Carabidae (Insecta: Coleoptera) from Tunisia. *Arquivos Entomológicos*, 14: 37-41.
- Gueorguiev, B., 2012, *Laemostenus (Sphodroides) tiouirii*, a new troglophile beetle from Tunisia (Coleoptera: Carabidae). *Historia naturalis bulgarica*, 20: 69-74.

- Jeanne, C., Zaballos, J. P., 1986, Catalogue des coléoptères carabiques de la Péninsule Ibérique. *Société Linnéenne de Bordeaux*.
- Jelaska, L. S., Blanusa, M., Durbesic, P., Jelaska, S. D., 2007, Heavy metal concentrations in ground beetles, leaf litter, and soil of a forest ecosystem. *Ecotoxicology and Environmental Safety*, 66: 74.
- Kapla, A., 2004, *Clivina ypsilon* Dejean, 1829, in Slovenia (Coleoptera: Carabidae). *Acta entomologica slovenica*, (1318-1998): 166-167.
- Kegel, B., 1990, *Diurnal activity of Carabid Beetles living on arable land*. In: Stork, N. *Intercepted*. Andover. England, 424 pp.
- Kerr, J. T., Kharouba, H. M., Currie, D. J., 2007, The macroecological contribution to global change solutions. *Science*, 316: 1581-1584.
- Lagisz, M., Laskowski, R., 2008, Evidence for between-generation effects in carabids exposed to heavy metals pollution. *Ecotoxicology*, 17: 59-66.
- Liebman, M., Gallandt, E. R. 1997, Many little hammers: ecological management of crop-weed interactions. *Ecology in agriculture*, 291-343.
- Lorenz, W., 2005, *Systematic list of extant ground beetles of the world. (Coleoptera "Geadephaga": Trachypachidae and Carabidae incl. Paussinae, Cicindelinae, Rhysodinae)*. 2nd Edition. Published by the author, Tutzing, 530 pp.
- Löbl, I., Smetana, A., 2003, *Catalogue of Palaearctic Coleoptera, Vol. 1. Archostemata - Myxophaga Adephaga*. Stenstrup, Apollo Books, 819 pp.
- Machard, P., 1997, *Catalogue des Coléoptères Carabiques du Maroc*. Machard, P. (autoed.), Molineuf, 54 pp.
- Magistretti, M., 1965, *Coleoptera Cicindelidae, Carabidae. Fauna D'Italia. Calderini*. Bolonia, 3: 512.
- Mateu, J., 1954, Notas sobre los *Orthomus* Chaudoir, Eos Madrid. *Revista Espanola de Entomologia*, 30: 353-361.
- Moret, P., 2000, Le genre *Pelmatellus* Bates dans l'étage montagnard des Andes équatoriales (Coleoptera: Carabidae: Harpalini). *Nouvelle Revue d'Entomologie*, 17: 215-232.
- Moret, P., 2003, Identification key to the genera of Carabidae (Coleoptera) of the paramos of Ecuador and southern Colombia. *Revista Colombiana de Entomologia*, 29: 185-190.
- Neri, P., Bonavita, P., Vigna Taglianti, A., Gudenzi, I., 2010, Note tassonomiche e nomenclatoriali (2° contributo) su alcuni Bembidiina della Fauna Italiana (Insecta Coleoptera Carabidae). *Quaderno di Studi e Notizie di Storia Naturale della Romagna*, 29: 119-133.
- Normand, H., 1933, Contribution au catalogue des Coléoptères de la Tunisie. *Bulletin de la Société d'histoire naturelle d'Afrique du Nord*, 24: 149-168.
- Palestrini, C., Roggero, A., Hernandez, Nova, L. K., Giachino P. M., Rolando, A., 2012, On the evolution of shape and size divergence in *Nebria* (*Nebriola*) ground beetles (Coleoptera, Carabidae). *Systematics and Biodiversity*, 10: 147-157.
- Peyerimhoff, P., 1909, Nouveaux coléoptères du Nord-Africain (10e. note). *Bulletin de la Société entomologique de France*, 277-279.
- Purchart, L., Kula, E., 2007, Content of heavy metals in bodies of field ground beetles (Coleoptera, Carabidae) with respect to selected ecological factor. *Polish Journal of Ecology* 55:305-314.
- Queinnec, E., Ollivier, E., 2012, Deux nouveaux *Trechus* de Tunisie (Coleoptera Carabidae Trechini) et discussion sur le complexe *fulvus* » en Afrique du Nord. *Bulletin mensuel de la Société Linnaeusienne de Lyon*, 82: 2-14.
- Ruiz-Tapiador, I., Zaballos, J. P., 2001, The Caraboidea (Coleoptera) of the Montes of Toledo (Central Spain). *Boletín de la Sociedad Entomológica Aragonesa*, 29: 11-31.
- Schirmel, J., Mantrilla-Conteras, J., Gauger, D., Blindow, I., 2015, Carabid beetles as indicators for shrub encroachment in dry grasslands. *Ecol. Indic.* 49: 76-82.
- Sciaky, R., 1992, Revisione dei Selenophorina paleartici occidentali (Coleoptera: Carabidae: Harpalinae). *Bollettino di Zoologia Agraria e di Bachicoltura*, 24: 37-65.

*New Data of Ground Beetles (Coleoptera: Carabidae)*

- Serrano, J., 2003, *Catálogo de los Carabidae (Coleoptera) de la Península Ibérica, Monografías S.E.A., vol. 9.* Sociedad Entomológica Aragonesa, Zaragoza, 130 pp.
- Serrano, J., Lencina, J. L., Andujar, A., 2003, Distribution patterns of Iberian Carabidae (Insecta, Coleoptera). *Graellsia*, 59: 129-153.
- Simon, E., Harangi, S., Baranyai, E., Braun, M., Fabian, I., Miszer, S., Nagy, L., Tothmeresz, B., 2016, Distribution of toxic elements between biotic and abiotic components of terrestrial ecosystem along an urbanization gradient: soil, leaf litter and ground beetles. *Ecological Indicators*. 60: 258-264.
- Spence, J., 1990, Success of European Carabid species in Western Canada: Preadaptation for synanthropy. En: The role of ground beetles in ecological and environmental studies. *Stork. Intercepted. Andover, England*, 8:129-141.
- Therond, J., 1975, Catalogue des Coléptères de la Camargue et du Gard. 1<sup>ère</sup> partie. *Société d'étude des sciences naturelles*, 10: 1595.
- Thiele, H. U., 1977, Carabid Beetles in their environments. Springer Verlag. Berlin. Heidelberg. New York.10: 372
- Vaibhao, G., Varsha, S., Vishwanath, D., 2013, Ground beetles (Coleoptera: Carabidae) of Melghat Tiger Reserve, Central India. *Journal on New Biological Reports*, 2(2): 173176.
- Valainis, U., 2009, A review of genus *Omopron* Latreille, 1802 (Coleoptera: Carabidae) Mediterranean fauna and distribution. *Acta Biologica Universitatis Daugavpiliensis*, 9(1): 63-72.
- Wrase, D. W., 2009, New or interesting records of carabid beetles from Europe, Madeira, northern Africa, Turkey, from the Near East, Iran, Iraq, Kuwait, and Pakistan, with nomenclatorial and taxonomic notes (Coleoptera, Carabidae, Bembidiini, Brachinini, Cyclosomini, Elaphrini, Harpalini, Lebiini, Nebriini, Platynini, Pterostichini, Scaritini, Sphodrini, Zabryni). *Linzer Biologische Beiträge*, 41: 901-935.
- Wrase, D. W., Jeanne, C., 2005, Synopsis of the *Orthomus rubicundus* group with description of two new species and a new subspecies from Morocco and Algeria (Coleoptera: Carabidae: Pterostichini). *Linzer Biologische Beiträge*. 37(1): 875-898.
- Zaballos, J. P., 1993, Los carábidos (Col. Caraboidea) de la Sierra de Gredos (España Central). *Eos*, 69: 83-99.
- Zaballos, J. P., Jeanne, C., 1994, *Nuevo Catálogo de los Carábidos (Coleoptera) de la Península Ibérica. Monografías S.E.A. 1.* Sociedad Entomológica Aragonesa Zaragoza, 159 pp.

Received: December 03, 2015

Accepted: February 06, 2017



## The Effect of Plant Quality on Survival of *Lymantria dispar* L. (Lepidoptera: Lymantriidae) Larvae Infected by Nucleopolyhedrovirus

Oğuzhan YANAR<sup>1\*</sup> Sibel GÖMEÇ<sup>2</sup> Elif Fatma TOPKARA<sup>1</sup>  
İsmail DEMİR<sup>3</sup> Zihni DEMİRBAĞ<sup>3</sup>

<sup>1</sup>Ondokuz Mayıs University, Faculty of Arts and Sciences, Department of Biology, 55139 Samsun, TURKEY

<sup>2</sup>Department of Plant Protection, College of Agriculture, University of Ankara, Ankara, TURKEY

<sup>3</sup>Karadeniz Technical University, Faculty of Sciences, Department of Biology, 61080, Trabzon, TURKEY

e-mails: \*oyanar46@gmail.com, cavdar.sibel@gmail.com, topkaraelif@hotmail.com, idemir@ktu.edu.tr, zihni@ktu.edu.tr

### ABSTRACT

In this study the influence of plant protein and secondary compounds on the survival rate of *Lymantria dispar* L. which was infected with *Lymantria dispar* nucleopolyhedrovirus (LdNPV), was investigated by using four different plant species, *Elaeagnus rhamnoides* (L.) A. Nelson, *Quercus cerris* L. 1753, *Corylus maxima* Mill. and *Crataegus monogyna* Jacq. The highest survival rate has been observed on the larvae feeding on the *E. rhamnoides* (L.) A. Nelson that had the highest protein rate. On the other hand, the highest mortality rate was recorded on the larvae which fed on the *C. monogyna* Jacq. containing the lowest protein rate. We have also discovered that the survival rate was related to gallotannin amounts. The survival rates of the infected larvae which fed on *E. rhamnoides* (L.) A. Nelson with the highest gallotannin amount were higher than the other diets. Our results showed that the survival rates in the larvae infected with *Lymantria dispar* nucleopolyhedrovirus were positively related to the proanthocyanidin (condensed tannin) and total phenolic content of foods.

*Key words:* *Lymantria dispar*, nucleopolyhedrovirus, secondary compounds, insect survival, mortality.

### INTRODUCTION

Various microbial factors can cause natural infections in insects (Tanada and Kaya, 1993; Boucias and Pendland, 1998; Yılmaz *et al.*, 2009; Charles *et al.*, 2000; Gökçe *et al.*, 2010; Sevim *et al.*, 2010; Tanyeli *et al.*, 2010; Danişmazoğlu *et al.*, 2012). Insect viruses can be considered as the most effective natural factor that causes insects to get sick and die (Hunter-Fujita *et al.*, 1998; İnce *et al.*, 2008; Demir *et al.*, 2009, 2013). The infection which is caused by pathogens negatively affects the survival rate and reproductive output of the hosts (Moore, 2002).

Plant diet can affect the interactions between insects and their pathogens (Duffey *et al.*, 1995). The food consumption in which the quality has changed as a consequence of either previous or current plant defoliation by herbivores is highly related with a

change in insect fitness (Larsson, 2002; Howe and Schaller, 2008). There are some studies that put forward the positive (Felton and Duffey, 1990; Hoover *et al.*, 1998a; Martemyanov *et al.*, 2006) and negative (Cook *et al.*, 2003) effects between the content of secondary compounds in an insect's diet and its resistance to pathogens. One of these secondary compounds is tannin. Tannins are known as polyphenolic compounds which are able to bind to proteins and decrease the activity of many enzymes (Swain, 1979). Tannins can be categorized into two main groups: condensed tannins (proanthocyanidins) and hydrolysable tannins (including ellagitannins and gallotannins). Tannins are capable of inhibiting a great number of microorganisms such as viruses, bacteria, and fungi. By this way, the organisms are protected against the effects of pathogens (Swain, 1979). Tannins, probably, provide this protection by binding to the microbial proteins (Cadman, 1960).

The host is able to struggle against and withstand infection and this is highly related to the host's nutritional state (Chandra, 1996; Keating *et al.*, 1988; Coop and Kyriazakis, 2001). Protein is considered as the most important substrate for producing immunological components which are used to resist viral infections (Swain and Hillis, 1959; Trudeau *et al.*, 2001; Keating *et al.*, 1990).

The previous studies have proved that phenolic compounds of diets frequently restrain the viral infection (Felton *et al.*, 1987; Keating *et al.*, 1990; Hoover *et al.*, 1998b), yet it is revealed that this doesn't always show proper efficiency. Therefore, this study makes a search of how protein and tannins in plants have an effect on *L. dispar* larvae, which are exposed to viral infection, and it checks whether total phenolic amount in plants has any effects on the larvae or not.

## MATERIALS AND METHODS

### Virus and plants

*Lymantria dispar* nucleopolyhedrovirus (LdNPV) which is used in this study was isolated from field collected *L. dispar* larvae, in Bafra, Turkey, in May, 2012. After detecting the baculovirus infection under light microscope from dead insects, occlusion bodies (OBs) were purified according to the procedure described by O'Reilly *et al.* (1992). Viral propagation was performed in healthy *L. dispar* larvae in the laboratory. The larvae which were placed in plastic dishes, fed with a few leaves contaminated with OBs isolated from the infected larvae, and maintained at 25°C to develop infection. OBs from newly infected larvae were purified and stored at -20°C.

In this study, four plant species belonging different families, *Elaeagnus rhamnoides* L. (Fam.: Elaeagnaceae), *Quercus cerris* L. (Fam.: Fagaceae), *Corylus maxima* Mill. (Fam.: Betulaceae) and *Crataegus monogyna* Jacq. (Fam.: Rosaceae) were used. All plant samples were collected daily and the larvae fed on them.

### Obtaining larvae

The eggs of *L. dispar* were collected from the Cernek Lake area, which is within the borders of Kızılırmak Delta in Bafra, Samsun. To provide the disinfection, the eggs

### *The Effect of Plant Quality on Survival of Lymantria dispar L.*

were treated with 10% of sodium hypochlorite. Then, they were washed with pure water and put into the refrigerator at 5°C. After six months, the eggs were taken out of the refrigerator and put into the climate cabin. This was adjusted to the temperature of 22°C and 70% of humidity during a period of 16 hours of light and 8 hours of dark. The larvae that came out of the eggs were put into the plastic containers (sized 5×10×2 cm) for each food group and they were fed with four plants that were indicated in the study until the 4<sup>th</sup> instar.

#### **Feeding experiment**

The larvae of the 4<sup>th</sup> instar were put into the plastic containers (sized 5×10×2 cm) which contained 30 larvae in each experimental group. Fifteen of these were control groups and others were infected by virus, all of which were put into each food group in the experiment. This process was carried out with 30 larvae and repeated 3 times in each experiment group. During the feeding experiment, as there were four plants, 360 larvae in total were put into the containers. The plastic containers had six holes so that the larvae could get air.

The control group and the larvae, which were infected by virus, were fed for 10 days in different incubators having the same temperature and humidity. During the feeding experiments fresh leaves of each plant were given each day and the remaining was dried in incubator. The survivor larvae which were both in control and infected groups were fed until they became pupae.

#### **Drying and grinding leaves**

In order to determine the amount of total phenolics, protein, gallic acid and proanthocyanidin, the leaf samples were taken from the plants which the larvae were fed. Then, they were wrapped inside the aluminum foil and were dried for two months under laboratory conditions, and then for 5 days in incubator at 50°C. After the dried leaves were taken out and ground, they were kept in nylon bags.

#### **Plant analysis**

The protein content of the leaf samples were measured by semi-micro Kjeldahl method with Kjeltac Auto 1030 analyzer (Tecator, Sweden). The method which was used to determine gallic acid contents of the leaf samples was described by Bate-Smith (1977). Proanthocyanidin contents of the leaf samples were determined spectrophotometrically by a method which was described by Bate-Smith (1975). The total phenolic contents of the leaf samples were determined by a method originally used by Swain and Hillis (1959). The nitrogen content of each sample which was obtained by Kjeldahl method was multiplied with 6.25 to calculate the total protein content of the plant sample (Monk, 1987).

#### **Infection of larvae with LdNPV**

In order to infect the larvae with virus, concentration of LdNPV was adjusted to 10<sup>5</sup> OB/ml by using a Neubauer haemocytometer. Each of the plant samples taken from

the four plants used in feeding process was treated with 1 ml of virus suspension. After the surface sterilization of each leaf in control group was carried out with 50% of ethyl alcohol, they were treated with 1 ml of pure/distilled water and put into the containers for the experiment.

### **Determination of pupal protein contents**

Pupae were left to constant mass in incubator at 50°C, weighed to the nearest 0.1 mg and lipid-extracted in three, 24-hour changes of chloroform before being re-dried and re-weighed. Nitrogen content of the lipid-free pupae was measured by semi-micro Kjeldahl method with Kjeltac Auto 1030 analyzer (Tecator, Sweden). The nitrogen content of each sample obtained by Kjeldahl method was multiplied with 6.25 to calculate the total protein content of the pupae (Monk, 1987).

### **Statistical analyses**

The comparison of the amounts of protein, gallotannin, proanthocyanidin and total phenolic in plants was made by using ANOVA Duncan Test. According to four different plants; Kaplan-Meier Survival Analysis Test was applied to determine the relationship between the survival rates of the larvae which were infected by the virus and the larvae of the control group. The total food consumption amounts of the larvae in the control group and the larvae which were infected by virus were compared by paired-T test. In this comparison, the data of the survivor larvae were used. In order to compare the effect of the amounts of protein, gallotannin, proanthocyanidin and total phenolic in plants on survival larvae Cox-Regression analysis test was used.

## **RESULTS**

### **Chemical composition of the leaf samples**

The total protein contents of the leaf samples were 15.1% in *E. rhamnoides*, 10.0% in *Q. cerris*, 10.9% in *C. maxima* and 8.3% in *C. monogyna*. While the highest total protein content was obtained from the leaves of *E. rhamnoides*, *C. monogyna* leaves contained the lowest protein content. A significant difference within the protein content of host plant species was found (Table 1).

The gallotannin contents of the plant samples observed in the present study were 5.2% for *E. rhamnoides*, 2.6% for *Q. cerris*, 4.3% for *C. maxima* and 1.8% for *C. monogyna* (Table 1). These results put forward that *E. rhamnoides* had much higher gallotannin content in its leaves than that of others. There were important differences in the proanthocyanidin contents of the leaf samples (Table 1). The proanthocyanidin contents of the leaves of *E. rhamnoides*, *Q. cerris*, *C. maxima* and *C. monogyna* were 3.9%, 7.6%, 11.5% and 7.2% respectively. The total phenolic contents of the leaves of *E. rhamnoides*, *Q. cerris*, *C. maxima* and *C. monogyna* were 10.6%, 7.9%, 9.9% and 6.2%, respectively. Results from statistical data analysis revealed that the host plant species had a significant difference in their total phenolic content (Table 1).

## The Effect of Plant Quality on Survival of *Lymantria dispar* L.

Table 1. The total protein, gallotannin, total phenolic and proanthocyanidin contents of the leaf samples.

Tests	Plants	N	Mean	SE	*Significant groups	ANOVA	
						F	P
Total Protein (%)	<i>Elaeagnus rhamnoides</i>	10	15,1	0,06	a	1577,5	< 0,001
	<i>Quercus cerris</i>	10	10,0	0,09	b		
	<i>Corylus maxima</i>	10	10,9	0,08	c		
	<i>Crataegus monogyna</i>	10	8,3	0,07	d		
Gallotannin (%)	<i>Elaeagnus rhamnoides</i>	10	5,2	0,04	a	1508	< 0,001
	<i>Quercus cerris</i>	10	2,6	0,04	b		
	<i>Corylus maxima</i>	10	4,3	0,04	c		
	<i>Crataegus monogyna</i>	10	1,8	0,03	d		
Proanthocyanidin (%)	<i>Elaeagnus rhamnoides</i>	10	3,9	0,03	a	11542,5	< 0,001
	<i>Quercus cerris</i>	10	7,6	0,04	b		
	<i>Corylus maxima</i>	10	11,5	0,02	c		
	<i>Crataegus monogyna</i>	10	7,2	0,03	d		
Total phenolic (%)	<i>Elaeagnus rhamnoides</i>	10	10,1	0,03	a	3208,7	< 0,001
	<i>Quercus cerris</i>	10	7,9	0,03	b		
	<i>Corylus maxima</i>	10	9,9	0,04	c		
	<i>Crataegus monogyna</i>	10	6,2	0,03	d		

\*Different letters stand for significantly different group means ( $p < 0.05$ ). (The groups named as a, b, c and d have statistically significant means according to Duncan's Multiple Range Test).

### Consumption amounts of larvae in regard to plants

A decrease in total food consumption amounts in the larvae infected by LdNPV was found when it was compared to control groups. In all four plants, there was a significant difference between the larvae in control groups and the larvae infected by virus in terms of total food consumption. Moreover, the highest consumption amount in control groups and virus-infected groups was obtained from the larvae feeding on *E. rhamnoides*. The comparison of the total food consumption amounts of virus-infected larvae with the ones in control group in regard to plants is shown in Table 2.

### The survival rates of infected larvae in regard to plant

The larvae in control groups and the larvae infected by the virus had been compared by through Log-Rank test and it was found that there was a significant difference ( $df=1$ ,  $P<0,001$ ). The survival rates were checked with Kaplan-Maier Test and it was found that the survival rate of virus-infected larvae was 65% while the survival rate of the control groups was 90%. The possibility of cumulative survival is shown in Fig. 1. The survival rates of virus-infected larvae and those in control groups in regard to plants are shown in Fig. 2. The survival rates of the larvae feeding on *E. rhamnoides*, which were both virus-infected and were in control groups, were higher than in other food groups.

Table 2. The comparison of the consumption amount of virus-infected larvae with those in control group in regard to plants.

Plants	N	Groups	Mean $\pm$ SE	t	P
<i>Elaeagnus rhamnoides</i>	27	Infected	395.3 $\pm$ 1.6	- 30.0	< 0,001
	45	Control	440,8 $\pm$ 0,7		
<i>Quercus cerris</i>	12	Infected	302.7 $\pm$ 5.2	- 45.7	< 0,001
	39	Control	414.3 $\pm$ 0.8		
<i>Corylus maxima</i>	12	Infected	265.8 $\pm$ 2.3	- 64.1	< 0,001
	42	Control	391,3 $\pm$ 5,2		
<i>Crataegus monogyna</i>	9	Infected	297.0 $\pm$ 2.1	- 54.4	< 0,001
	36	Control	412.5 $\pm$ 1.0		

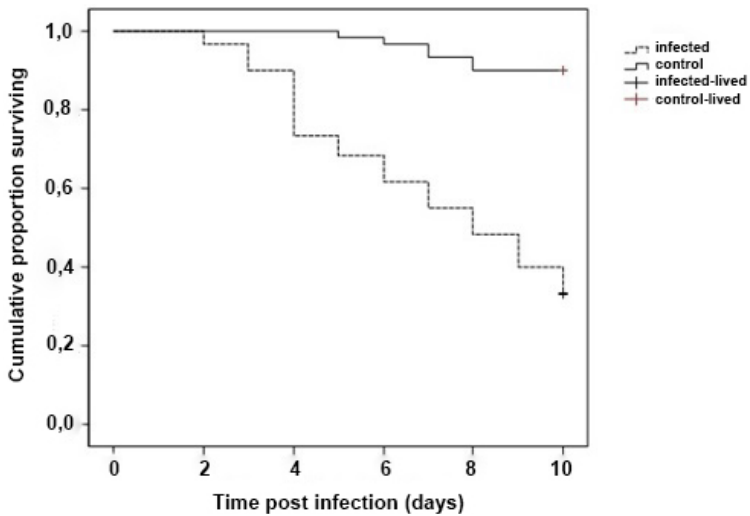


Fig.1. The possibility of cumulative survival of virus-infected larvae and larvae in control group.

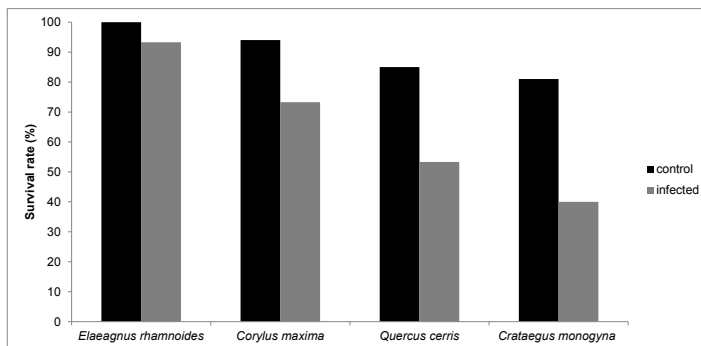


Fig. 2. The survival rates of virus-infected larvae and those in control group in regard to food.

### The Effect of Plant Quality on Survival of *Lymantria dispar* L.

The highest survival rate, 93.3%, was observed in larvae feeding on *E. rhamnoides*. The lowest survival rate, 40%, was found in larvae feeding on *C. monogyna*. While the survival rate was 53.3% in the larvae feeding on *Q. cerris*, it was 73.3% in those feeding on *C. maxima*. The most extraordinary result observed in virus-infected larvae was that some larvae in all food groups were pupae. Four of the larvae feeding on *E. rhamnoides*, *Q. cerris* and *C. maxima* and three of the larvae feeding on *C. monogyna* became pupae (Table 3).

Table 3. The survival rates of virus-infected larvae.

Plants	N	Died larvae	Alived		Percent of alive (%)
			Larvae	Pupated	
<i>Elaeagnus rhamnoides</i>	45	3	30	12	93.3
<i>Quercus cerris</i>	45	21	12	12	53.3
<i>Corylus maxima</i>	45	12	21	12	73.3
<i>Crataegus monogyna</i>	45	27	9	9	40.0
Total	180	63	117		65.0

It was found that there was a difference between the survival rates of virus-infected larvae in regard to plants. The survival rate of the larvae feeding on *E. rhamnoides* was different from that of the larvae feeding on *Q. cerris* and *C. monogyna* (Table 4,  $P < 0.05$ ). The survival rate of the larvae feeding on *C. maxima* was different in critical value from that of the larvae feeding on *C. monogyna* (Table 4,  $P = 0.087$ ).

Table 4. The comparison of the survival rates of the larvae feeding on the plants infected by virus in regard to plants with Log Rank test.

Plants	<i>Elaeagnus rhamnoides</i>		<i>Quercus cerris</i>		<i>Corylus maxima</i>		<i>Crataegus monogyna</i>	
	Chi-Square	P	Chi-Square	P	Chi-Square	P	Chi-Square	P
<i>Elaeagnus rhamnoides</i>			6,285	,012	2,102	,147	9,009	,003
<i>Quercus cerris</i>	6,285	,012			1,359	,244	,263	,608
<i>Corylus maxima</i>	2,102	,147	1,359	,244			2,921	,087
<i>Crataegus monogyna</i>	9,009	,003	,263	,608	2,921	,087		

### Pupae analysis

The larvae of the control group which were fed on four species of plants had much more pupal protein amount when compared to infected-larvae. The highest pupal protein amount among larvae, which were both in control groups and were infected, has been observed in the larvae feeding on *E. rhamnoides*. The protein amount of the larvae feeding on *C. monogyna* was the lowest among the larvae which were both in control groups and were infected. The protein amount of the infected-pupae was 9-17% less than control groups ( $P < 0.05$ ) (Fig. 3).

### The effect of protein and secondary compounds on the survival of infected larvae

Cox-Regression analysis results have shown that total protein, proanthocyanidin and total phenolic contents have a positive effect on the survival of the infected larvae.

However, virus infection increased the mortality 12 fold. The results of the analysis are shown in Table 5.

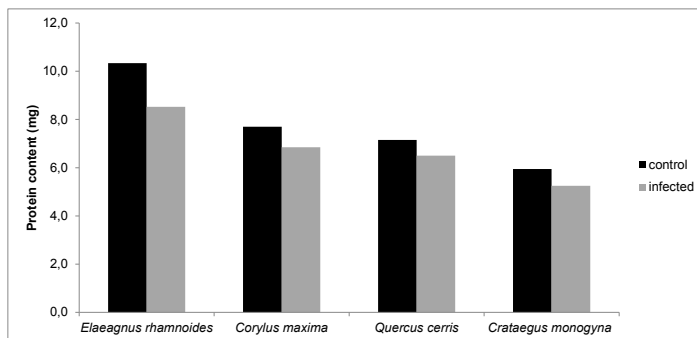


Fig. 3. The protein content of virus-infected larvae and those in control group in regard to food.

Table 5. The comparison of the effect of virus infection, protein and secondary substances on survival by Cox-Regression analysis.

	B	SE	Wald	df	Sig.	Exp (B)
Infection with virus	2,503	,865	8,372	1	,004	12,225
Protein	-,939	,319	8,664	1	,003	,391
Phenolic	-,177	,026	46,030	1	,000	,838
Gallotannin	-,302	,057	27,987	1	,000	,740
Proanthocyanidin	-,466	,155	9,064	1	,003	,628

B: Coefficient of regression, SE: Standard error, Wald: Significance of the regression coefficients, df: Degree of freedom, Sig.: Significant, Exp (B): Hazard proportion.

## DISCUSSION

The results showed that the protein amount in the plant on which the insects infected with the virus feed had a positive effect on its survival. The highest survival rate in the larvae infected by LdNPV has been obtained from the larvae feeding on *E. rhamnoides* containing the highest protein amount. The highest mortality has been found in the larvae feeding on *C. monogyna* containing the lowest protein amount. Protein is a vital substance to produce the immunological components used in resisting to viral infections (Washburn *et al.*, 1996; Trudeau *et al.*, 2001). The results show that when the protein amount in the plant which the larvae infected by LdNPV feed and survival rate were increased. This result showed that protein had a strong important role in *L. dispar*'s immune system. Moreover, the protein amounts of the pupae of the infected larvae in all food groups are less than the pupae of their own control groups.

Host's ability to defend and to stand out against infection is related to its diet (Chandra, 1996; Lochmiller and Deerenberg, 2000; Coop and Kyriazakis, 2001).

*The Effect of Plant Quality on Survival of Lymantria dispar L.*

Lee *et al.* (2006) have put forward that the larvae feeding on artificial food containing low protein-high carbohydrate amount lose performance three fold more than food containing high protein-low carbohydrate amount. It has been found that the survival rate is lower in the ones feeding on artificial food containing low protein-carbohydrate amount. The fact that when a virus-infected insect is fed on food containing more protein, the survival rate increases have been shown in the previous studies of the field (Thompson and Redak, 2000; Lee *et al.*, 2002; Simpson *et al.*, 2004).

The most interesting result of this study is that some of the larvae infected by virus became pupae in all food groups. The previous studies in the field have put forward that the development period of the larvae infected by NPV prolongs either indirectly by resisting the infection or directly by lethal virus infection (Rothman and Myers, 1996; Cooper *et al.*, 2003; Cory and Myers, 2003). The results of this study emphasize that *L. dispar* larvae infected by NPV reduce the development period.

In this study, the maximum deaths of the larvae infected by virus occurred on the second day. These deaths were observed in the larvae feeding especially on *C. monogyna* containing low protein amount. Previous studies have emphasized that the resistance to NPV, encapsulation, melanization with phenoloxidase, and the process of dismissing viruses out of haemolymph happened 2-3 days after the infection and the protein was especially chosen (Washburn *et al.*, 1996; Trudeau *et al.*, 2001; Lee *et al.*, 2006). The results of this study have shown that when the protein amount decreases, the pathogen resistance stated above also decreases.

Biologically activated phytochemicals can be bound to the structure (Occlusion Body) in midgut of the larvae and can reduce the infection of virus on host insect (Felton and Duffey, 1990). This interaction can be regulated by digestion methods of the host insect (Glare *et al.*, 2003). The effect of gallotannin on survival rate has importance in critical value in the larvae infected by the virus. In a previous study, it was found that when the host plant was treated by the virus which is consumed by the larvae; the mortality changed with the effect of virus and this effect was related to amount of hydrolysable tannin (polyphenol) (Keating *et al.*, 1988).

This study has pointed out that in the virus-infected larvae; the increase in total phenolic amount has a positive effect on the survival rate. It was stated in a previous study that the chemicals obtained from a plant were bound to midgut or they changed the host's physiology by effecting normal immune system sufficiently, or such chemicals gave starting signals for stages that would decrease number of cells that are vulnerable to infection (Lee *et al.*, 2006). The results of this study show that there is a positive correlation between total phenolic amount of the diet consumed and the survival rate.

Washburn *et al.* (1998) have found out that when *Trichoplusia ni* and *Heliothis virescens* larvae infected by *Autographa californica* nucleopolyhedrovirus fed on artificial food free from secondary compounds, the mortality rate increased. The results of this study show that there is a positive correlation between the survival rate of the larvae and condensed tannins.

Even though the viruses as entomopathogens have a negative effect on the survival of insects, the resistance of herbivores against entomopathogens can differ

depending on the plants they feed on. Secondary compounds that are regarded as defensive substances against herbivores seem to change the negative effects of entomopathogens. Moreover, peritrophic membrane in insects has an important role in changing these effects. Although this study focuses on protein and the effect of secondary compounds in the plant on the resistance of insects against entomopathogens, it can be seen that this relation is complicated. It will be better to pay attention to the secondary compounds in the plants along with protein and the digestive system of the host.

## ACKNOWLEDGEMENTS

This study was supported by the Ondokuz Mayıs University Research Foundation (PYO.FEN.1904.11.027).

## REFERENCES

- Bate-Smith, E. C., 1975, Phytochemistry of proanthocyanidins. *Phytochemistry*, 14(4): 1107-1113.
- Bate-Smith, E. C., 1977, Astringent tannins of *Acer* species. *Phytochemistry*, 16(9), 1421-1426.
- Boucias, D. G., Pendland, J. C., 1998, *Principles of Insect Pathology*. Kluwer Academic Publishers, Boston, 537 p.
- Cadman, C. H., 1960, *Inhibition of plant virus infection by tannins*. In: Pridham, J. B. (Ed.). Phenolics in Plants in Health and Disease. Oxford and London, Pergamon Press, 101-105 pp.
- Chandra, R. K., 1996, Nutrition, immunity and infection: from basic knowledge of dietary manipulation of immune responses to practical application of ameliorating suffering and improving survival. *Proceedings of the National Academy of Sciences*, 93(25): 14304-14307.
- Charles, J. F., Delecluse, A., Nielsen-LeRoux, C., 2000, *Entomopathogenic Bacteria: From Laboratory to Field Application*. 1st edn. Springer, Dordrecht, 524 p.
- Cook, S. P., Webb, R. E., Podgwaite, J. D., Reardon, R. C., 2003, Increased mortality of gypsy moth *Lymantria dispar* (L.) (Lepidoptera: Lymantriidae) exposed to gypsy moth nuclear polyhedrosis virus in combination with the phenolic glycoside salicin. *Journal of Economic Entomology*, 96(6): 1662-1667.
- Coop, R. L., Kyriazakis, I., 2001, Influence of host nutrition on the development and consequences of nematode parasitism in ruminants. *Trends in Parasitology*, 17(7): 325-330.
- Cooper, D., Cory, J. S., Myers, J. H., 2003, Hierarchical spatial structure of genetically variable nucleopolyhedroviruses infecting cyclic populations of western tent caterpillars. *Molecular Ecology*, 12(4): 881-890.
- Cory, J. S., Myers, J. H., 2003, The ecology and evolution of insect baculoviruses. *Annual Review of Ecology, Evolution, and Systematics*, 34: 239-272.
- Danışmazoğlu, M., Demir, İ., Sevim, A., Demirbağ, Z., Nalçacıoğlu, R., 2012, An investigation on the bacterial flora of *Agriotes lineatus* (Coleoptera: Elateridae) and pathogenicity of the flora members. *Crop Protection*, 40: 1-7.
- Demir, İ., Gürel, N., Nalçacıoğlu, R., Demirbağ, Z., 2009, Comparative susceptibilities of six insect cell lines to infection by *Malacosoma neustria* nucleopolyhedrovirus (ManeNPV). *Turkish Journal of Biology*, 33(4): 259-273.
- Demir, İ., Nalçacıoğlu, R., Gholizad, L. M., Demirbağ, Z., 2013, Characterization of a new isolate of *Malacosoma neustria* nucleopolyhedrovirus (ManeNPV) from Turkey. *Turkish Journal of Biology*, 37(4): 385-391.

*The Effect of Plant Quality on Survival of Lymantria dispar L.*

- Duffey, S. S., Hoover, K., Bonning, B., Hammock, B. D., 1995, The impact of host plant on the efficacy of baculoviruses. *Reviews in Pesticide Toxicology*, 3: 137-275.
- Felton, G. W., Duffey, S. S., 1990, Inactivation of a baculovirus by quinones formed in insect-damaged plant tissue. *Journal of Chemical Ecology*, 16: 1211-1236. 16(4): 1221-1236.
- Felton, G. W., Duffey, S. S., Vail, P. V., Kaya, H. K., Manning, J., 1987, Interaction of nuclear polyhedrosis virus with catechols: Potential incompatibility for host-plant resistance against noctuid larvae. *Journal of Chemical Ecology*, 13(4): 947-957.
- Glare, T. R., Walsh, P. J., Kay, M., Barlow, N. D., 2003, Strategies for the eradication or control of gypsy moth in New Zealand. *Report for the Forest Health Research Collaborative of New Zealand*, 30-31.
- Gökçe, C., Sevim, A., Demirbag, Z., Demir, İ., 2010, Isolation, characterization and pathogenicity of bacteria from *Rhynchites bacchus* (Coleoptera: Rhynchitidae). *Biocontrol Science and Technology*, 20(9): 973-982.
- Hoover, K., Kishida, K. T., Digiorgio, L. A., Workman, J., Alaniz, S. A., Hammock, B. D., Duffey, S. S., 1998a, Inhibition of baculoviral disease by plant-mediated peroxidase activity and free radical generation. *Journal of Chemical Ecology*, 24(12): 1949-2001.
- Hoover, K., Stout, M. J., Alaniz, S. A., Hammock, B. D., Duffey, S. S., 1998b, Influence of induced plant defenses in cotton and tomato on the efficacy of baculoviruses on noctuid larvae. *Journal of Chemical Ecology*, 24(2): 253-271.
- Howe, G. A., Schaller, A., 2008, *Direct defenses in plants and their induction by wounding and insect herbivores*. In: Schaller, A. (Ed.). *Induced Plant Resistance to Herbivory*. New York, USA, Springer, 7-29 pp.
- Hunter-Fujita, F. R., Entwistle, P. F., Evans, H. F., Crook, N. E., 1998, *Insect Viruses and Pest Management*. John Wiley and Sons, Chichester, England, 620 pp.
- Ince, İ. A., Kati, H., Yılmaz, H., Demir, İ., Demirbağ, Z., 2008, Isolation and identification of bacteria from *Thaumetopoea pityocampa* Den. and Schiff. (Lep., Thaumetopoeidae) and determination of their biocontrol potential. *World Journal of Microbiology and Biotechnology*, 24(12): 3005-3015.
- Keating, S. T., Hunter, M. D., Schultz, J. C., 1990, Leaf phenolic inhibition of gypsy moth nuclear polyhedrosis virus: role of polyhedral inclusion body aggregation. *Journal of Chemical Ecology*, 16(5): 1445-1457.
- Keating, S. T., Yendol, W. G., Schultz, J. C., 1988, Relationship between susceptibility of gypsy moth larvae (Lepidoptera: Lymantriidae) to a baculovirus and host plant foliage constituents. *Environmental Entomology*, 17: 942-958. 17(6), 952-958.
- Larsson, S., 2002, *Resistance in trees to insects-an overview of mechanisms and interactions*. In: Wagner, M. R., Clancy, K. M., Lieutier, F., Paine, T. D. (Eds.). *Mechanisms and Deployment of Resistance in Trees to Insects*. Dordrecht, The Netherlands, Kluwer Academic Publishers, 1-29 pp.
- Lee, K. P., Behmer, S. T., Simpson, S. J., Raubenheimer, D., 2002, A geometric analysis of nutrient regulation in the generalist caterpillar *Spodoptera littoralis* (Boisduval). *Journal of Insect Physiology*, 48(6): 655-665.
- Lee, K. P., Cory, J. S., Wilson, K., Raubenheimer, D., Simpson, S. J., 2006, Flexible diet choice offsets protein costs of pathogen resistance in a caterpillar. *Proceedings of the Royal Society of London B: Biological Sciences*, 273: 823-829.
- Lochmiller, R. L., Deerenberg, C., 2000, Trade-offs in evolutionary immunology: just what is the cost of immunity? *Oikos*, 88(1): 87-98.
- Martemyanov, V. V., Bakhvalov, S. A., Dubovskiy, I. M., Glupov, V. V., Salakhutdinov, N. F., Tolstikov, G. A., 2006, Effect of tannic acid on the development and resistance of the gypsy moth *Lymantria dispar* L. to viral infection. *In Doklady Biochemistry and Biophysics*, 409(1): 219-222.
- Monk, C. D., 1987, Sclerophylly in *Quercus virginiana* Mill. *Castanea*, 52(4): 256-261.
- Moore, J., 2002, *Parasites and the Behavior of Animals*. Oxford University Press, Oxford, UK, 315 pp.

- O'Reilly, D. R., Miller, L. K., Luckow, V. A., 1992, Baculovirus Expression Vectors: A Laboratory Manual. Oxford University Press. New York, USA. 347 pp.
- Rothman, L. D., Myers, J. H., 1996, Debilitating effects of viral diseases on host Lepidoptera. *Journal of Invertebrate Pathology*, 67(1): 1-10.
- Sevim, A., Demir, İ., Demirbağ, Z., 2010, Molecular characterization and virulence of *Beauveria* spp. from the pine processionary moth, *Thaumetopoea pityocampa* (Lepidoptera: Thaumetopoeidae). *Mycopathologia*, 170(4): 269-277.
- Simpson, S. J., Sibly, R. M., Lee, K. P., Behmer, S. T., Raubenheimer, D., 2004, Optimal foraging when regulating intake of multiple nutrients. *Animal Behaviour*, 68(6): 1299-1311.
- Swain, T., 1979, *Tannins and lignins*. In: Rosenthal, G. A., Janzen, D. H. (Eds.). *Herbivores: Their Interaction with Secondary Plant Metabolites*. Academic Press, New York, 657-682.
- Swain, T., Hillis, W. E., 1959, The phenolic constituents of *Prunus domestica*. I.-The quantitative analysis of phenolic constituents. *Journal of the Science of Food and Agriculture*, 10(1): 63-68.
- Tanada, Y., Kaya, H. K., 1993, *Insect Pathology*. Academic Press, California, USA, 633 pp.
- Tanyeli, E., Sevim, A., Demirbağ, Z., Eroğlu, M., Demir, İ., 2010, Isolation and virulence of entomopathogenic fungi against the great spruce bark beetle, *Dendroctonus micans* (Kugelann) (Coleoptera: Scolytidae). *Biocontrol Science and Technology*, 20(7): 695-701.
- Thompson, S. N., Redak, R. A., 2000, Interactions of dietary protein and carbohydrate determine blood sugar level and regulate nutrient selection in the insect *Manduca sexta* L. *Biochimica et Biophysica Acta (BBA)-General Subjects*, 1523(1): 91-102.
- Trudeau, D., Washburn, J. O., Volkman, L. E., 2001, Central role of hemocytes in *Autographa californica* M nucleopolyhedrovirus pathogenesis in *Heliothis virescens* and *Helicoverpa zea*. *Journal of Virology*, 75(2): 996-1003.
- Washburn, J. O., Kirkpatrick, B. A., Volkman, L. E., 1996, Insect protection against viruses. *Nature*, 383: 767.
- Washburn, J. O., Kirkpatrick, B. A., Haas-Stapleton, E., Volkman, L. E., 1998, Evidence that the stilbene-derived optical brightener M2R enhances *Autographa californica* M nucleopolyhedrovirus infection of *Trichoplusia ni* and *Heliothis virescens* by preventing sloughing of infected midgut epithelial cells. *Biological Control*, 11(1): 58-69.
- Yılmaz, H., Waeyenberge, L., Demir, İ., Moens, M., Demirbağ, Z., 2009, A new entomopathogenic nematode species for Turkey, *Heterorhabditis megidis* Poinar, Jackson & Klein 1987 (Rhabditida: Heterorhabditidae). *Turkish journal of Agriculture and Forestry*, 33(4): 385-391.

Received: December 18, 2015

Accepted: August 02, 2016

## Effect of Vermicompost on Life History of *Hippodamia variegata* Preying on *Aphis gossypii* Glover

Taiebeh ALIZAMANI<sup>1</sup> Jabraeil RAZMJOU<sup>1\*</sup> Bahram NASERI<sup>1</sup>  
Mahdi HASSANPOUR<sup>1</sup> Anvar ASADI<sup>2</sup> Christopher KERR<sup>3</sup>

<sup>1</sup>Plant Protection Department, Faculty of Agriculture and Natural Resources, University of Mohaghegh Ardabili, Ardabil, IRAN

<sup>2</sup>Agriculture and Natural Resources Center, Moghan, Ardabil Province, IRAN

<sup>3</sup>Entomology and Nematology Department, University of Florida, FL, USA

e-mails: alizamanitaiebeh@yahoo.com, \*razmjou@uma.ac.ir, bnaseri@uma.ac.ir, hassanpour@uma.ac.ir, ishbuga@ufl.edu

### ABSTRACT

The variegated ladybird beetle, *Hippodamia variegata* (Goeze), with worldwide distribution is among the most successful predators of aphids. In this study, the life history of *H. variegata* feeding on *Aphis gossypii* Glover as prey was evaluated on cucumber plants treated with different vermicompost concentrations (0, 15 and 30%) in a growth chamber at  $25 \pm 2^\circ$  C,  $60 \pm 10\%$  RH, and 16:8 (L: D) h. The results showed that demographic parameters of *H. variegata* were affected by vermicompost treatments. Duration of total developmental time of larvae and pupal period of *H. variegata* on the tested vermicompost concentrations were significantly different. No significant differences were found for the adult longevity, life span, fecundity, and oviposition period of *H. variegata* when fed on aphids reared on vermicompost-treated cucumber plants. Based on the results, the differences of intrinsic rate of natural increase ( $r_m$ ), finite rate of increase ( $\lambda$ ), net reproductive rate ( $R_0$ ) and doubling time (DT) of *H. variegata* among different vermicompost treatments were significant; The values of  $r_m$  and  $R_0$  increased as the percentage of vermicompost increased from 0 to 30%. It seems that some population growth parameters of *H. variegata* were positively affected by high rates of vermicompost. Our results showed that the addition of vermicompost to the growing medium of cucumber plants could positively affect some of the demographic parameters of *H. variegata* which would make it as a suitable predator of *A. gossypii* in vermicompost-treated plants.

**Key words:** Organic fertilizer, population growth, *Hippodamia variegata*.

### INTRODUCTION

The greenhouse cucumber is threatened by outbreaks of a variety of pests due to the ideal environmental conditions encountered under greenhouse production. Sucking pests, especially aphids, are the most important pests limiting the production of cucumber. Among aphids, the melon aphid, *Aphis gossypii* Glover (Hom., Aphididae) is the most serious pest (Ebert and Cartwright, 1997; Blackman and Eastop, 2000). It is a polyphagous aphid that attacks a broad range of crops such as cotton, okra, potato, eggplant and cucurbits (Eastop, 1983; van Steenis and El-Khawass, 1995;

Stoetzel *et al.*, 1996; Blackman and Eastop, 2000; Razmjou *et al.*, 2006). The aphid produces large populations on the host plants and causes direct and indirect damage to them. Direct damage of aphids by feeding on the phloem sap causes the leaves to curl. Furthermore, a heavy infestation may result in the buildup of large amounts of honeydew secretions on the leaves which increases sooty mold on the host plants. The mold limits available light to the leaves and impairs plant photosynthesis. Indirect damage is via transmission of more than 50 plant viruses to the host plants (Ebert and Cartwright, 1997; Blackman and Eastop, 2000).

Greenhouse cucumber producers mostly use insecticides to suppress the melon aphid population, but the application of chemicals on the crop may result in the development of resistance to the insecticides which would lead to the need for more frequent application of pesticides to obtain similar rates of control (Hardee, 1993; Barber *et al.*, 1999; Foster *et al.*, 2002). For decreasing the harmful effects of insecticides multiple control tactics such as cultural, physical, and biological control methods are used in combination with insecticides in integrated pest management (IPM) programs. To produce high quality yields, fertilizers are being applied into the growing medium of plants. Organic fertilizers, due to their improvement of soil structure and by providing essential nutrients for plant growth, are being extensively used in agriculture as a cultural method. Vermicompost is an organic fertilizer that has been shown to increase plant growth (Atiyeh *et al.*, 2000; Chaoui *et al.*, 2002; Arancon *et al.*, 2004, 2006; Razmjou *et al.*, 2011). Vermicompost is produced through interaction between earthworms and microorganisms under a non-thermophilic process. This organic amendment increases biodiversity of the soil which is necessary for maintaining soil health and the activity of microorganisms (Zink and Allen, 1998; Arancon *et al.*, 2005).

Several species of Coccinellidae are used in both fields and greenhouses as biological control agents. The variegated ladybird beetle, *Hippodamia variegata* (Goeze) (Coleoptera: Coccinellidae), is a polyphagous and cosmopolitan coccinellid species (Krafsur *et al.*, 1996; Wheeler and Stoops, 1996). Both adults and larvae of this species are voracious predators of aphids, mealybugs, scales, whiteflies, cicadellids, curculionid larvae as well as mites (Singh *et al.*, 1991; Sadeghi and Esmaili, 1992; Obrycki and Kring, 1998). Feeding on 12 different aphid species (Franzmann, 2002) *H. variegata* has been reported as an effective natural enemy of aphids on various host plants in different countries, for example: on pepper in Bulgaria (Natskova, 1973), maize in Ukraine (Gumovskaya, 1985), shrubs in Italy (Nicoli *et al.*, 1995), grain in India (Hammed *et al.*, 1975) and cotton in Turkmenistan (Belicova and Kosaeve, 1985). It has also been shown to control melon aphid infestations on related host plants (Kavallieratos *et al.*, 2002).

Most reports in the literature indicate that vermicompost can decrease pest populations through bottom-up effects (Razmjou *et al.*, 2011, 2012). For example, Arancon *et al.* (2006) found that vermicompost can negatively affect establishment of arthropod pest populations such as two spotted spider mites, mealy bugs and aphids on the host plants. Edwards *et al.* (2010) reported similar results for cucumber beetles and tobacco hornworms. Furthermore, it has been reported that adding fertilizers into

### *Effect of Vermicompost on H. variegata Population*

the soil may affect the strength of top-down effects by the natural enemies (Hosseini *et al.*, 2010; Veromann *et al.*, 2013). For example, Pope *et al.* (2012) reported that *Brassica oleracea* L. plants grown with different fertilizer treatments had significant differences in the percentage of *Brevicoryne brassicae* L. parasitized by *Diaeretiella rapae* (M'Intosh). Fox *et al.* (1996) reported that the parasitoid *Diadegma insulare* Cress preferred diamondback moths, *Plutella xylostella* (L.), on fertilized plants compared to those on unfertilized plants. Since there are few reports (Duchovskiene *et al.*, 2012) on the effect of vermicompost on the third trophic level, we tried to evaluate the life table and population growth parameters of *H. variegata* preying on *A. gossypii* to test the hypothesis that these parameters are influenced by the application of vermicompost into the growing medium of cucumber plants.

## **MATERIALS AND METHODS**

This study was conducted during 2012 in the greenhouse and laboratory of Plant Protection Department, Faculty of Agriculture and Natural Resources, University of Mohaghegh Ardabili, Ardabil, Iran. The cow manure vermicompost used in this research was obtained from Anoshe Aaraab Co. Ltd., Tehran, Iran.

### **Plant rearing**

Cucumber seeds, *C. sativus* cv. Royal, were planted in plastic pots (16 cm diameter × 12.5 cm height) filled with field soil mixed with three different rates of vermicompost (0 (as a control), 15 and 30%). The plants were kept in a greenhouse at 19-28 °C, 40-50% RH, and the natural photoperiod before using in experiments. Cucumber plants at the six- to eight- leaf stages were used for experiments.

### **Insect culture**

The melon aphid population used in this experiment was collected from infested cucumber plants in greenhouses of Moghan region, Ardabil province in spring of 2012. The aphid colony was separately reared on cucumber seedlings grown at the mentioned vermicompost rates for about 7-8 generations prior to experiments. Every week, some apterous aphids were transferred from infested plants to non-infested young seedlings of cucumber to maintain the aphid colony. Adults of *H. variegata* were collected by sweep-netting from the wheat fields near the city of Ardabil, Iran. Males and females of *H. variegata* were paired and kept in separate plastic Petri dishes (9 cm diameter and 1.5 cm height) lined with wet filter papers (Wu *et al.*, 2010). A hole, 2 cm in diameter, was cut in the lid of each Petri dish and covered with a fine mesh cloth for ventilation. *H. variegata* was daily fed with different life stages of *A. gossypii* on cucumber leaf disks. The eggs produced by females were collected and transferred to new Petri dishes every day. The larvae of *H. variegata* were individually reared in the mentioned Petri dishes and fed with *A. gossypii*. The insect rearing was conducted in a growth chamber at 25 ± 2°C, 60 ± 10% RH, and a photoperiod of 16:8h (L: D).

## Experiments

To study the effects of feeding on aphids reared at different vermicompost concentrations on the survival and pre-adult period of *H. variegata*, egg clusters less than 24 h old were selected and held in Petri dishes (at above-mentioned conditions) inside a growth chamber at  $25 \pm 2^\circ\text{C}$ ,  $60 \pm 10\%$  RH, and 16L: 8D. The hatch rate and incubation period were recorded daily. The newly hatched larvae of *H. variegata* were individually transferred to Petri dishes by a fine brush and fed daily on *A. gossypii*. The aphids were daily provided for larvae on cucumber leaf discs (2 cm in diameter) grown at different vermicompost rates until pupation. The larvae were monitored daily for molting, survivorship and duration of the larval and pupal periods.

Newly emerged adults of *H. variegata* were paired and placed in individual Petri dishes containing aphids infested leaf discs for subsequent mating and oviposition. The Petri dishes were checked daily for possible egg clutches. During the experiments numbers of eggs laid by each female were recorded and removed from Petri dishes. Observations continued until the death of last adult. By monitoring the Petri dishes, pre-oviposition period, oviposition period, post-oviposition period, adult longevity and fecundity of *H. variegata* were recorded.

## Statistical analysis

All data of survivorship, duration of immature stages, oviposition period, pre-and post- oviposition period, adult longevity and fecundity of *H. variegata* were evaluated for each treatment by analysis of variance (ANOVA) using the MINITAB-16 statistical software (Minitab Inc., 2010). When differences among treatments were significant, comparison among means were determined using *Tukey's* test at  $P = 0.05$ . Differences in population growth parameters including intrinsic rate of increase ( $r_m$ ), net reproductive rate ( $R_0$ ), mean generation time ( $T$ ), doubling time ( $DT$ ), and finite rate of increase ( $\lambda$ ) values and their standard errors were tested by Jackknife procedure (Maia et al., 2000) using the SAS system software v 6.12 (SAS Institute, 1989). Their mean values were compared by *Tukey's* test at  $P = 0.05$ .

## RESULTS

### Development of *H. variegata*

Effects of feeding on aphids reared on cucumber plants amended with different vermicompost ratios on the development of *H. variegata* are listed in Tables 1 and 2. Based on the results, there were no significant differences for the egg incubation period ( $F = 1.03$ ;  $df = 2, 168$ ;  $P = 0.359$ ), the duration of first ( $F = 0.73$ ;  $df = 2, 138$ ;  $P = 0.484$ ), second ( $F = 2.01$ ;  $df = 2, 138$ ;  $P = 0.138$ ), third ( $F = 1.39$ ;  $df = 2, 138$ ;  $P = 0.252$ ) larval instars and immature stages ( $F = 0.13$ ;  $df = 2, 138$ ;  $P = 0.878$ ) of *H. variegata* on various treatments. However, the duration of fourth instar larvae of *H. variegata* significantly differed on the tested treatments ( $F = 13.18$ ;  $df = 2, 138$ ;  $P = 0.01$ ). The shortest value of this parameter was recorded for the *H. variegata* when feeding on aphids reared at 30% vermicompost treatment (Table 1). Eggs from 30%

### Effect of Vermicompost on *H. variegata* Population

vermicompost treatment had higher hatch rate (64%) than those at control (51%) or 15% (60%) vermicompost concentrations. Furthermore, significant differences were observed for the total developmental time of larvae ( $F = 8.20$ ;  $df = 2, 138$ ;  $P = 0.01$ ) and pupal period ( $F = 8.91$ ;  $df = 2, 138$ ;  $P = 0.01$ ) of *H. variegata* feeding on aphids reared on the tested vermicompost-treated plants. Total developmental time of larvae was longest at control and 15% vermicompost rates and shortest at 30% vermicompost rate. The same trend was observed for pupal period of *H. variegata* (Table 1).

Table 1. Development of immature stages of *H. variegata* feeding on *A. gossypii* reared on cucumber plants amended with three vermicompost concentrations.

Parameter	Vermicompost 0%	Vermicompost 15%	Vermicompost 30%
Egg incubation	2.48±0.50a	2.51±0.54a	2.61±0.49a
First instar	1.63±0.63a	1.56±0.54a	1.71±0.74a
Second instar	1.59±0.57a	1.85±0.65a	1.79±0.45a
Third instar	1.85±0.36a	1.77±0.52a	1.67±0.56a
Fourth instar	2.07±0.38a	1.92±0.40a	1.55±0.61b
Total developmental time of larva	7.15±0.53a	7.10±0.59a	6.71±0.63b
Pupal period	3.78±0.42a	3.81±0.44a	4.18±0.63b
Immature stages	13.48±0.85a	13.44±0.71a	13.51±0.84a
Survival rate%	41	57.14	59

Differences among different vermicompost concentrations were determined by Tukey's test. In columns, mean values followed by the same letter are not significantly different ( $P > 0.05$ ).

In the current study, no significant differences were observed for the female longevity ( $F = 2.03$ ;  $df = 2, 33$ ;  $P = 0.148$ ), life span ( $F = 1.71$ ;  $df = 2, 33$ ;  $P = 0.196$ ) and fecundity ( $F = 1.81$ ;  $df = 2, 33$ ;  $P = 0.179$ ) of *H. variegata* feeding on aphids treated at vermicompost amended plants (Table 2). Furthermore, although pre-oviposition ( $F = 0.4$ ;  $df = 2, 33$ ;  $P = 0.671$ ), oviposition ( $F = 0.38$ ;  $df = 2, 33$ ;  $P = 0.684$ ), and post-oviposition period ( $F = 3.18$ ;  $df = 2, 33$ ;  $P = 0.055$ ) of *H. variegata* did not significantly differ on the tested treatments, increasing the dosage of vermicompost in the soil resulted in decreasing of the pre-oviposition and post-oviposition periods of *H. variegata* (Table 2).

### Population growth parameters

According to the data, population growth parameters of *H. variegata* were significantly affected when feeding on aphids reared at cucumber plants amended with various vermicompost rates. The intrinsic rate of natural increase ( $r_m$ ) ( $F = 20.07$ ;  $df = 2, 33$ ;  $P = 0.01$ ), finite rate of increase ( $\lambda$ ) ( $F = 18.10$ ;  $df = 2, 33$ ;  $P = 0.01$ ), and net reproductive rate ( $R_0$ ) ( $F = 9.05$ ;  $df = 2, 33$ ;  $P = 0.001$ ) of *H. variegata* were significantly increased by raising the vermicompost concentration from 0 (control) to 30%. Furthermore, *H. variegata* had significantly shorter doubling time ( $DT$ ) when fed on aphids reared on plants that had received 15 and 30% vermicompost ( $F = 18.30$ ;  $df = 2, 33$ ;  $P = 0.01$ ). The mean generation time ( $T$ ) of *H. variegata* was not significantly influenced by vermicompost rates ( $F = 3.09$ ;  $df = 2, 33$ ;  $P = 0.059$ ) (Table 3).

Table 2. Longevity, hatching rate (%), life span and fecundity (Mean±SD) of *H. variegata* feeding on *A. gossypii* reared on cucumber plants amended with three vermicompost concentrations.

Parameter	Vermicompost 0%	Vermicompost 15%	Vermicompost 30%
Female longevity	49.90±5.507a	43.30±10.531a	44.39±7.148a
Pre-oviposition	7.90±6.887a	7.00±1.528a	6.47±1.450a
Oviposition	30.60±7.090a	28.84±4.580a	30.61±5.801a
Post-oviposition	11.40±3.596a	7.61±5.606a	7.30±2.750a
Fecundity	188.40±71.37a	238.69±69.10a	236.85±69.18a
Life span	62.60±5.481a	56.84±10.605a	57.15±6.866a
Hatching rate%	51	60	64

Differences among different vermicompost concentrations were determined by Tukey's test. Means followed by the same letters in columns are not significantly different ( $P > 0.05$ ).

Table 3. Population growth parameters and sex ratio (%) of *H. variegata* feeding on *A. gossypii* reared on cucumber plants amended with three vermicompost concentrations.

Parameter	Vermicompost 0%	Vermicompost 15%	Vermicompost 30%
$R_0$	39.40±14.94a	63.15±17.70b	71.28±20.82b
$r_m$	0.120±0.01a	0.138±0.01b	0.152±0.01c
T	30.59±01.82a	30.01±3.18a	28.01±2.63a
DT	5.76±0.66a	5.015±0.40b	4.56±0.34b
$\lambda$	1.128±0.02a	1.147±0.01b	1.164±0.01c
Sex ratio%	51.85	45.83	51.51

Differences among vermicompost treatments were determined by Tukey's test, based on Jackknife method. Within columns, means followed by different letters are significantly different ( $P > 0.05$ ).

## DISCUSSION

Host plants are known to be one of the important bottom-up factors which could influence the top-down ones (i.e., predators and parasitoids) (Schädler *et al.*, 2010). Changing the nutritional qualities of host plants by addition of fertilizers to the soil may affect the quality of herbivores. Insects can usually distinguish high quality host plants from those of low quality. For example, herbivores generally have better performance on well fertilized host plants (Wang *et al.*, 2006) and parasitoids lay more eggs on plants with high leaf nitrogen contents (Fox *et al.*, 1990).

Based on the obtained data, both the total developmental time and the pupal period of *H. variegata* fed on *A. gossypii* were significantly affected by the vermicompost treatments. The total developmental time of *H. variegata* at 30% vermicompost treatment was  $6.71 \pm 0.63$  d, which was shorter than those developing at control and 15% vermicompost treatments. In this study, no significant difference was found for the life span of *H. variegata*. However, it had shorter life span on vermicompost-treated cucumber plants compared to non-treated ones (i.e., control). According to Kindlmann and Dixon (1999), short-lived species are able to complete more generations and

### Effect of Vermicompost on *H. variegata* Population

predators with shorter developmental times can deplete the prey more quickly than a species with a relatively long developmental time. Thus, in our study *H. variegata* could produce more generations when feeding on the melon aphid populations reared on plants with higher vermicompost rates compared to the control plants.

Raising the amount of vermicompost from 0 to 30% resulted in increasing of the intrinsic rate of natural increase ( $r_m$ ) and the net reproductive rate ( $R_o$ ) of *H. variegata* confirming the importance of plant quality on the predator. The highest  $r_m$  value at the 30% vermicompost treatment indicates that *H. variegata* has a greater reproductive potential at this treatment than the other treatments. The finite rate of increase ( $\lambda$ ) of *H. variegata* showed a similar trend being significantly highest at 30% vermicompost treatment. Interestingly, the intrinsic rate of natural increase reported in our study ( $0.152 \text{ day}^{-1}$ ) for *H. variegata*, is comparable with the value reported by Davoodi Dehkordi *et al.* (2013) for the same species feeding on *A. gossypii* on *Chrysanthemum indicum* Kitan ( $0.155 \text{ day}^{-1}$ ). However, the value for intrinsic rate of natural increase in our study is different from the one reported ( $0.114 \text{ day}^{-1}$ ) by Lanzoni *et al.* (2004) when *H. variegata* fed on *Myzus persicae* Sulzer. The difference could be related to the different prey species and plant materials.

In the present study, the doubling time ( $DT$ ) was significantly affected by vermicompost rates. The shortest values of  $DT$  were obtained when *H. variegata* fed on aphids reared on high rates of vermicompost (15 and 30%). As a result, *H. variegata* needed less time to double its size on these treatments. Other studies have shown that modifying the quality of host plants through soil fertility managements affects the natural enemies. For example, Fallahpour *et al.* (2015) reported that fertilization of canola significantly affected the performance of the gall midge, *Aphidoletes aphidimyza* Rondani, as a predator for the mustard aphid, *Lipaphis erysimi* (Kaltenbach). According to the findings of Suryawana and Reyes (2007), the level of parasitism by different parasitoids on pea leafminer (*Liriomyza huidobrensis* Blanchard) was greater on vermicompost treatments in a potato field. Similar observations were made by Ponti *et al.* (2007), who reported that in compost-fertilized broccoli the parasitism rates of *Brevicoryne brassicae* L. by *Diaeretiella rapae* M'Intosh significantly increased. Furthermore, in cow manure amended white cabbage coccinellids settled better in aphid colonies compared to synthetically fertilized plants (Duchovskiene *et al.*, 2012). It seems that biological traits of the third trophic level are influenced by changing the uptake of special elements by plants via fertilization and, for the case of our study, it could be attributed to the slow and continuous uptake of mineral nutrients from vermicompost-amended substrate.

Our results showed that, despite the fact that some life history parameters of *H. variegata* were not significantly influenced by vermicompost rates, other parameters like intrinsic rate of natural increase were significantly affected by this organic fertilizer. An important result however, is that *H. variegata* does not seem to be negatively affected by vermicompost when different concentrations were used. This study offered opportunities for better understanding the relationship between organic fertilization and the predator demographic potential which would be useful for both organic growers

and pest management programmers. However, further investigation on the relationship between the application of vermicompost and the predation capacity of *H. variegata* under natural conditions are recommended to obtain optimal biological control.

## ACKNOWLEDGMENTS

The authors would like to thank Mr. S. Foroutani for providing the cucumber seeds. This work was financially supported by the University of Mohaghegh Ardabili, Ardabil, Iran.

## REFERENCES

- Arancon, N. Q., Edwards, C. A., Bierman, P., Welch, C., Metzger, J. D., 2004, Influence of vermicompost on field strawberries: part 1. Effect on growth and yields. *Bioresource Technology*, 93: 145-153.
- Arancon, N. Q., Edwards, C. A., Bierman, P., 2006, Influences of vermicomposts on field strawberries: part 2. Effects on soil microbiological and chemical properties. *Bioresource Technology*, 97: 831-840.
- Arancon, N. Q., Galvis, P. A., Edwards, C. A., 2005, Suppression of insect pest populations and damage to plants by vermicomposts. *Bioresource Technology*, 96: 1137-1142.
- Atiyeh, R. M., Subler, S., Edwards, C. A., Bachman, G., Metzger, J. D., Shuster, W., 2000, Effects of vermicomposts and composts on plant growth in horticultural container media and soil. *Pedobiologia*, 47: 741-744.
- Barber, M. D., Moores, G. D., Tatchell, G. M., Vice, W. E., Denholm, I., 1999, Insecticide resistance in the current lettuce aphid, *Nasonovia ribisnigri* (Hemiptera: Aphididae) in the UK. *Bulletin of Entomological Research*, 89: 17-23.
- Belicova, E. V., Kosaev, E. M., 1985, The biology of the most important species of Coccinellidae and their role in controlling aphids in a cotton-lucerne rotation. *Biologiches Nauk*, 5: 61-63 (in Russian).
- Blackman, R. L., Eastop, V. F., 2000, Aphids on the world's crops: an identification and information guide. Wiley, London, 476.
- Chaoui, H., Edwards, C. A., Brickner, A., Lee, S. S., Arancon, N. Q., 2002, Suppression of the plant diseases, *Pythium* (damping-off), *Rhizoctonia* (root rot) and *Verticillium* (wilt) by vermicomposts. Proceedings of an international conference held at the Brighton Hilton Metropole Hotel, Brighton, UK. 18-21 November. 1-2.
- Davoodi Dehkordi, S., Sahragard, A., Hajizadeh, J., 2013, The effect of prey density on life table parameters of *Hippodamia variegata* (Coleoptera: Coccinellidae) fed on *Aphis gossypii* (Hemiptera: Aphididae) under laboratory conditions. ISRN. *Entomology*, 2013, Article ID 281476, 7 pages, <http://dx.doi.org/10.1155/2013/281476>.
- Duchovskiene, L., Surviliene, E., Valiūškaite, A., Karkleliene, R., 2012, Effects of organic and conventional fertilization on the occurrence of *Brevicoryne brassicae* L. and its natural enemies in white cabbage. *Acta Agriculturae Scandinavica, Section B - Soil & Plant Science*, 62:16-22.
- Eastop, V. F., 1983, *The biology of the principal aphid virus vector*. In: Plumb, R. T., Thresh, J. M. (Eds.). *Plant Virus Epidemiology*. Blackwell Scientific Publication, 115-132.
- Ebert, T. A., Cartwright, B., 1997, Biology and ecology of *Aphis gossypii* Glover (Homoptera: Aphididae). *Southwestern Entomologist*, 22: 116-153.
- Edwards, C. A., Arancon, N. Q., Vasko-Bennett, M., Askar, A., Keeney, G., 2010, Effect of aqueous extracts from vermicomposts on attacks by cucumber beetles (*Acalymna vittatum*) (Fabr.) on cucumbers and tobacco hornworm (*Manduca sexta*) (L.) on tomatoes. *Pedobiologia*, 53: 141-148.
- Fallahpour, F., Ghorbani, R., Nassiri Mahallati, M., Hosseini, M., 2015, Interaction of different nitrogen fertilization regimes of canola with mustard aphid (*Lipaphis erysimi* Kalt.) and the predatory gall midge (*Aphidoletes aphidimyza* Rondani). *Biological Control of Pests and Plant Diseases*, 4: 1-12.

### *Effect of Vermicompost on H. variegata Population*

- Foster, S. P., Harrington, R., Dewar, A. M., Denholm, I., Devonshir, A. L., 2002, Temporal and spatial dynamics of insecticide resistance in *Myzus persicae* (Hemiptera: Aphididae). *Pest Management Science*, 58: 895-907.
- Fox, L. R., Kester, K. M., Eisenbach, J., 1996, Direct and indirect responses of parasitoids to plants: sex ratio, plant quality and herbivore diet breadth. *Entomologia Experimentalis et Applicata*, 80: 289-292.
- Fox, L. R., Letourneau, D. K., Eisenbach, J., Nouhuys, S. V., 1990, Parasitism rates and sex ratios of a parasitoid wasp: effects of herbivore and plant quality. *Oecologia*, 83: 414-419.
- Franzmann, B. A., 2002, *Hippodamia variegata* (Goeze) (Coleoptera: Coccinellidae), a predacious ladybird new in Australia. *Australian Journal of Entomology*, 41: 375-377.
- Gumovskaya, G. N., 1985, The coccinellid fauna. *Zashchita Rastenii*, 11:43 (in Russian).
- Hammed, S. F., Sud, V. K., Kashyap, N. P., 1975, *Adonia variegata* (Goeze) (Coleoptera: Coccinellidae), an important predator of the Indian grain aphid *Macrosiphum (Sitobion) miscanthi* Tak. in Kulu Valley (Himachal Pradesh). *Indian Journal of Entomology*, 37: 209-210.
- Hardee, D. D., 1993, Resistance in aphids and whiteflies: principle and keys to management, In Proceedings, Beltwide Cotton Production Research Conferences. National Cotton Council of America, Memphis, Tennessee, 20-23 pp.
- Hosseini, M., Ashouri, A., Enkegaard, A., Weisser, W. W., Goldansaz, S. H., Nassiri Mahalati, M., Sarraf Moayeri, H. R., 2010, Plant quality effects on intraguild predation between *Orius laevigatus* and *Aphidoletes aphidimyza*. *Entomologia Experimentalis et Applicata*, 135: 208-216.
- Kavallieratos, N. G., Stathas, G. J., Athanassiou, C. G., Papadoulis, G. T., 2002, *Dittrichia viscosa* and *Rubusulmifolius* as reservoirs of aphid parasitoids (Hymenoptera: Aphelinidae) and the role of certain coccinellid species. *Phytoparasitica*, 30:231-242.
- Kindlmann, P., Dixon, A. F. G., 1999, Generation time ratios-determinants of prey abundance in insect predator-prey interactions. *Biological Control*, 16: 133–138.
- Krafsur, E.S., Obrycki, J.J., Nariboli, P., 1996, Gene flow in colonizing *Hippodamia variegata* ladybird beetle populations. *Journal of Heredity*, 87: 41-47.
- Lanzoni, A., Accineli, G., Bazzocchi, G., Burgio, G., 2004, Biological traits and life table of the exotic *Harmonia axyridis* compared with *Hippodamia variegata*, and *Adalia bipunctata* (Coleoptera: Coccinellidae). *Journal of Applied Entomology*, 128: 298-306.
- Maia, A. H. N., Luiz, A. J. B., Campanhola, C., 2000, Statistical influence on associated fertility life table parameters using jackknife technique, computational aspects. *Journal of Economic Entomology*, 93: 511-518.
- Minitab Inc., 2010, Minitab Version 16, Philadelphia, PA, USA.
- Natskova, V., 1973, The effect of aphid predators on the abundance of aphids on peppers. *Rastitelna Zashchita*, 21: 20-22 (in Bulgarian).
- Nicoli, G., Limonta, L., Gavazzuti, C., Pozzati, M., 1995, The role of hedges in the agroecosystem. Initial studies on the coccinellid predators of aphids. *Informatore Fitopatologico*, 45: 7-8 (in Italian).
- Obrycki, J. J., Kring, T. J., 1998. Predaceous Coccinellidae in biological control. *Annual Review of Entomology*, 43: 295-321.
- Ponti, L., Altieri, M. A. Gutierrez, A. P., 2007, Effects of crop diversification levels and fertilization regimes on abundance of *Brevicoryne brassicae* (L.) and its parasitization by *Diaeretiella rapae* (M'Intosh) in broccoli. *Agricultural and Forest Entomology*, 9: 209-214.
- Pope, T. W., Girling, R. D., Staley, J. T., Trigodet, B., Wright, D. J., Leather, S. R., van Emden, H. F., Poppy, G. M., 2012, Effects of organic and conventional fertilizer treatments on host selection by the aphid parasitoid *Diaeretiella rapae*. *Journal of Applied Entomology*, 136: 445-455.
- Razmjou, J., Moharrampour, S., Fathipour, Y., Mirhoseini, S. Z., 2006, Effect of cotton cultivar on performance of *Aphis gossypii* (Homoptera: Aphididae) in Iran. *Journal of Economic Entomology*, 99: 1820-1825.
- Razmjou, J., Mohammadi, M., Hassanpour, M., 2011, Effect of vermicompost and cucumber cultivar on population growth attributes of the melon aphid (Hemiptera: Aphididae). *Journal of Economic Entomology*, 104: 1379-1383.

- Razmjou, J., Vorburger, C., Mohammadi, M., Hassanpour, M., 2012, Influence of vermicompost and cucumber cultivar on population growth of *Aphis gossypii* Glover. *Journal of Applied Entomology*, 136: 568-575.
- Sadeghi, E., Esmaili, M., 1992, Preying habits and hibernation site of *Coccinella septempunctata* L., *Hippodamia (Adonia) variegata* (Goeze), *Psyllobora vigintiduopunctata* L. in Karaj. *Journal of Entomological Society of Iran*, 11: 5-8.
- SAS Institute, 1989, SAS software version 8.02. SAS Institute, Cary, NC.
- Schädler, M., Brandl, R., Kempel, A., 2010, Host plant genotype determines bottom-up effects in an aphid-parasitoid-predator system. *Entomologia Experimentalis et Applicata*, 135: 162-169.
- Singh, T. V. K., Singh, K. M., Singh, R. N., 1991, Influence of intercropping: III. Natural enemy complex in groundnut. *Indian Journal of Entomology*, 53: 333-368.
- Stoetzel, M. B., Miller, G. L., O'Brien, P. J., Graves, J. B., 1996, Aphids (Homoptera: Aphididae) colonizing cotton in the United States. *Florida Entomologist*, 79: 193-205.
- Suryawana, I. B. G., Reyes, S. G., 2007, The influence of cultural practice on population of pea leafminer (*Liriomyza huidobrensis*) and its parasitoids in potato. *Indonesian Journal of Agricultural Science*, 7: 35-42.
- van Steenis, M. J., El-Khawass, K. A. M. H., 1995, Life history of *Aphis gossypii* on cucumber: influence of temperature, host plant and parasitism. *Entomologia Experimentalis et Applicata*, 76: 121-131.
- Veromann, E., Toome, M., Kännaste, A., Kaasik, R., Copolovici, L., Flink, J., Kovács, G., Narits, L., Luik, A., Niinemets, Ü., 2013, Effects of nitrogen fertilization on insect pests, their parasitoids, plant diseases and volatile organic compounds in *Brassica napus*. *Crop Protection*, 43: 79-88.
- Wang, J. J., Tsai, J. H., Broschat, T. K., 2006, Effect of nitrogen fertilization of corn on the development, survivorship, fecundity and body weight of *Peregrinus maidis* (Hom., Delphacidae). *Journal of Applied Entomology*, 130: 20-25.
- Wheeler, A. G. Jr., Stoops, C. A., 1996, Status and spread of the Palaearctic lady beetles *Hippodamia variegata* and *Propylea quatuordecimpunctata* (Coleoptera: Coccinellidae) in Pennsylvania, 1993-1995. *Entomological News*, 107: 291-298.
- Wu, X. H., Zhou, X. R., Pang, B. P., 2010, Influence of five host plants of *Aphis gossypii* Glover on some population parameters of *Hippodamia variegata* (Goeze). *Journal of Pest Science*, 83: 77-83.
- Zink, T. A., Allen, M. F., 1998, The effect of organic amendments on the restoration of a disturbed coastal sage scrub habitat. *Restoration Ecology*, 6: 52-58.

Received: December 29, 2015

Accepted: July 20, 2016

## Invasive Fruit Flies (Diptera: Drosophilidae) Meet in a Biodiversity Hotspot

Carla REGO<sup>1\*</sup>

António Franquinho AGUIAR<sup>2</sup>

Délia CRAVO<sup>2</sup>

Mário BOIEIRO<sup>1</sup>

<sup>1</sup>Centre for Ecology, Evolution and Environmental Changes (cE3c), Azorean Biodiversity Group and Department of Agrarian Sciences, University of Azores, Angra do Heroísmo, Azores, PORTUGAL

<sup>2</sup>Laboratório de Qualidade Agrícola, Camacha, Madeira, PORTUGAL  
e-mails: \*crego@fc.ul.pt, antonioaguair@gov-madeira.pt, deliacravo@gov-madeira.pt, mboieiro@fc.ul.pt

### ABSTRACT

Oceanic islands' natural ecosystems worldwide are severely threatened by invasive species. Here we discuss the recent finding of three exotic drosophilids in Madeira archipelago - *Acletoxenus formosus* (Loew, 1864), *Drosophila suzukii* (Matsumura, 1931) and *Zaprionus indianus* (Gupta, 1970). *Drosophila suzukii* and *Z. indianus* are invasive species responsible for severe economic losses in fruit production worldwide and became the dominant drosophilids in several invaded areas menacing native species. We found that these exotic species are relatively widespread in Madeira but, at present, seem to be restricted to human disturbed environments. Finally, we stress the need to define a monitoring program in the short-term to determine population spread and environmental damages inflicted by the two invasive drosophilids, in order to implement a sustainable and effective control management strategy.

**Key words:** Biological invasions, *Drosophila suzukii*, invasive species, island biodiversity, Madeira archipelago, *Zaprionus indianus*.

### INTRODUCTION

Oceanic islands are known to contribute disproportionately to their area for Global biodiversity and by harbouring unique evolutionary lineages and emblematic plants and animals (Grant, 1998; Whittaker and Fernández-Palacios, 2007). Nevertheless, many of these organisms are particularly vulnerable to human-mediated changes in their habitats due to their narrow range size, low abundance and habitat specificity (Paulay, 1994). In fact, the present biodiversity crisis is nowhere else more evident than in island ecosystems, where major extinctions occurred following habitat destruction and invasive species introductions (Sax and Gaines, 2008).

Some insect species are among the worst invasive species worldwide being responsible for huge economic losses and severe environmental impacts (Lowe *et al.*, 2000). During the last decades many drosophilid species have spread around the world mostly aided by human transport of goods (Westphal *et al.*, 2008; Hulme, 2009).

Some of those species, like *Drosophila suzukii* and *Zaprionus indianus*, proved to be serious economic threats, being responsible for heavy losses in various flesh fruit cultures in many countries (van der Linde *et al.*, 2006; Cini *et al.*, 2012). For instance, the economic losses caused by *D. suzukii* to fruit production in USA are estimated to cost over 500 million dollars each year (Bolda *et al.*, 2010) and, in Brazil, *Z. indianus* severely impacted fig production leading to annual losses on the order of 50% (Tidon *et al.*, 2003). Furthermore, associated with the economic impacts in colonized regions, these two invasive drosophilids also have significant ecological impacts on native ecosystems that start to become better understood (Galego and Carareto, 2005).

Some oceanic island ecosystems, like Madeira, are vulnerable to the negative effects of invasive pest species due to the particular structure of local economies (often specialized in the production of few goods) and to the high levels of island endemism, where rare endemics may be threatened by the invaders (Reaser *et al.*, 2007). Madeiran native drosophilids, particularly the endemic *D. madeirensis*, may be negatively impacted by invasive drosophilids. This endemic species seems to be strictly associated to the native laurel forest (Laurisilva), has seldom been recorded and is not abundant (Monclús, 1984). Furthermore, the study of life-history traits of *D. madeirensis* indicates that it may be particularly vulnerable to competition from invasive congeners since this species has a poor performance when compared with a closely related species with a wide distribution (Rego *et al.*, 2007).

The aim of our study is to provide information on the recent invasion of Madeira archipelago by three drosophilid species *Acletoxenus formosus* (Loew, 1864), *Drosophila suzukii* (Matsumura, 1931) and *Zaprionus indianus* (Gupta, 1970) and evaluate its potential impact on local economy and on native species.

## MATERIAL AND METHODS

Madeira archipelago is located on the Atlantic Ocean, nearly 800 km away from Continental Europe (Portugal). The archipelago is composed by three groups of islands: Madeira Island, Desertas Islands and Porto Santo and its surrounding islets. In Madeira Island there is a variety of native habitat-types from coastal xeric vegetation to altitudinal meadows, but Laurisilva, the native laurel forest, is the most emblematic one, being classified as World Natural Heritage Site due to its outstanding natural value (IUCN, 1999).

Sampling took place in selected sites from different habitat-types throughout Madeira and Porto Santo. Several complementary sampling techniques were used to collect the drosophilid species reported in this study, namely: Ad-hoc direct sampling, Moericke and yellow colour traps. Moericke and colour traps were set in several areas of Madeira, in different habitat-types, as part of an early warning system to detect the presence of exotic species. Ad-hoc direct sampling was applied mostly in flesh fruit cultures throughout Madeira, because the presence of predictable food resources (mature and rotten fruits) allows the attraction of drosophilids.

The specimens collected in this study are deposited in the entomological collection of the Laboratório de Qualidade Agrícola (ICLAM) in Camacha (Madeira, Portugal). The

### *Invasive Fruit Flies (Diptera: Drosophilidae) Meet in a Biodiversity Hotspot*

study of specimens from previous collections housed in the ICLAM was important by making available information concerning species distribution, thus providing insights on invasion date and dispersal pathways.

In order to evaluate and predict the environmental impact of the three introduced drosophilid species in Madeira archipelago, we applied the Generic Impact Scoring System adapted to the IUCN criteria and categories (GISS-IUCN) (Blackburn *et al.*, 2014), taking in consideration the available information on species biology and colonization history.

## RESULTS

In this study we report for the first time the finding of two exotic drosophilid species *A. formosus* and *D. suzukii* in Madeira and confirm the occurrence of the invasive *Z. indianus* in this archipelago (Figs. 1A, 1B, 1C).

The recent finding of the invasive *D. suzukii* in Madeira confirmed the worst expectations of its rapid spread worldwide benefiting from human-aided long distance dispersal (Cini *et al.*, 2012). The species was recorded from distanced locations within Madeira (Fig. 2, Table 1) suggesting the occurrence of multiple infestations following its first introduction. Nonetheless, at present *D. suzukii* seems to be restricted to human disturbed areas since all specimens were collected in vineyards. Further, with the exception of Arco de São Jorge (in north Madeira), this invasive species was not abundant in the invaded areas. At present there is no single record of this species from native habitats despite the occurrence of potential host plants (e.g. *Rubus*, *Sorbus* and *Vaccinium* species).

Another exotic drosophilid, *A. formosus*, is also reported for the first time to Madeira archipelago after being sampled from both Madeira and Porto Santo islands. This species with a remarkable colour pattern was always found in low abundance in man-made habitats, mostly in urban gardens (Fig. 2, Table 1). The larvae of *A. formosus* are predaceous and all the adult specimens were collected near their whitefly prey (e.g. *Trialeurodes vaporariorum*). The study of specimens housed in the entomological collection of ICLAM provided additional information on the ecological associations of *A. formosus* (three whitefly species were recorded as hosts: *Aleyrodes proletella*, *Siphoninus phillyreae* and *Trialeurodes vaporariorum*) and on its introduction history in Madeira (collection specimens date back to mid- and late 90s).

The invasive *Z. indianus* was recorded in several fruit plantations (e.g. banana, grape, mango, pineapple) from sites mostly located in southwestern Madeira (Fig. 2, Table 1). This drosophilid was relatively abundant at Serra de Água (in the center of Madeira) while in most of the other locations only a few specimens were sampled. Population genetic studies on the invasive *Z. indianus* had previously reported this species to Madeira, but no mention was made to species abundance and geographic distribution in this island (Nardon *et al.*, 2005; Yassin *et al.*, 2008). The study of drosophilids housed in the ICLAM allowed the finding of specimens dating back from 1992, a decade before the first reports of the presence of this invasive species in Madeira.

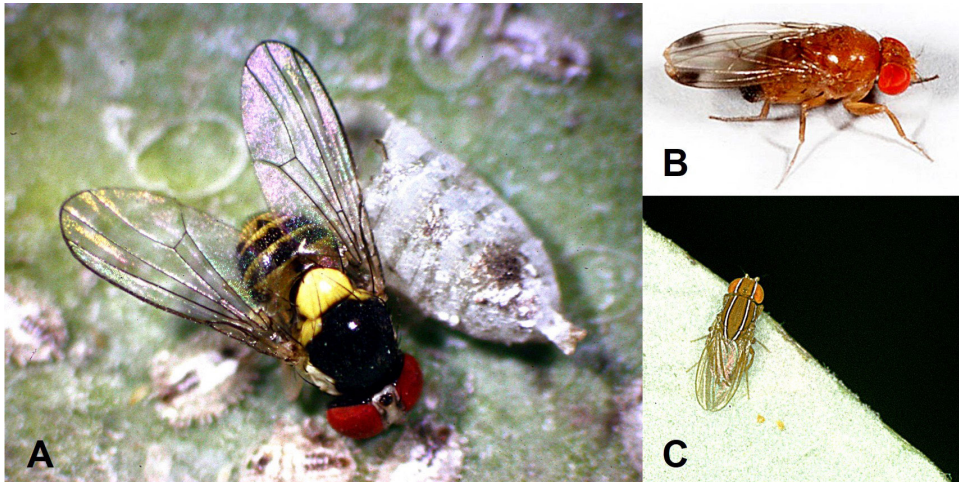


Fig. 1. Drosophilids recently found in Madeira archipelago. A: *Acletoxenus formosus* associated with a colony of the ash whitefly *Siphoninus phillyreae* on pomegranate (photo by AMF Aguiar); B: *Drosophila suzukii* (photo by M Hauser); C: *Zaprionus indianus* (photo by AMF Aguiar).

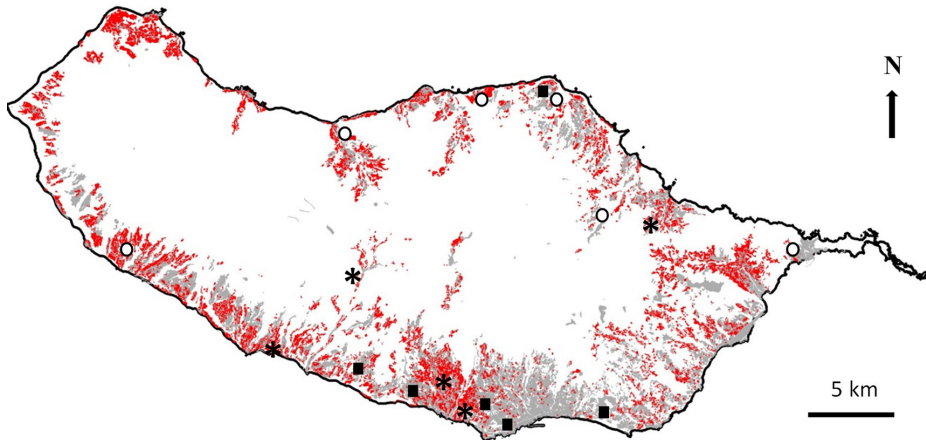


Fig. 2. Distribution of the exotic drosophilids in Madeira Island: *A. formosus* (in squares), *D. suzukii* (in circles) and *Z. indianus* (in asterisks). The geographic distribution of urban areas (in grey) and fruit plantations (in red) are also presented.

The assessment of the ecological impact of the three alien drosophilid species using the GISS-IUCN protocol (Blackburn *et al.*, 2014) allowed us to classify *D. suzukii* and *Z. indianus* as having a moderate impact on natural communities since the decline of population densities in some native species is expected. On the other hand, *A. formosus* may have a minor impact on natural communities since this species seems to be restricted to human disturbed areas in coastal lowlands, not presenting for now a threat to native potential prey (endemic whiteflies).

*Invasive Fruit Flies (Diptera: Drosophilidae) Meet in a Biodiversity Hotspot*

Table 1. Records of exotic drosophilids in Madeira with information on sampling dates, locations and techniques applied. The geographical coordinates (in degrees, minutes and seconds) of each sampling site are indicated together with the altitude (in meters) and habitat-type. Site names are listed alphabetically for each species.

Species	Site	Habitat	Lat. (N)	Long. (W)	Altitude	Date	N	Technique
<i>A. formosus</i>	Amparo	Urban garden	32°38'45.67"	16°56'39.88"	160	2.ix.1999	1♂	Direct sampling
	Caldeira	Strawberry plantation	32°39'25.42"	16°59'47.54"	312	12.xi.1998	1♂	Direct sampling
	Campanário	Agricultural land	32°40'12.79"	17°1'44.22"	325	6.x.1994	2♂,2♀	Direct sampling
	Farrobo	Cabbage plantation	32°50'2.40"	16°54'29.34"	270	1.viii.2012	1♂,4♀	Direct sampling
	Ribeiro Salgado	Urban garden	33°2'52.84"	16°21'6.95"	15	22-26.vii.2013	1♂,2♀	Direct sampling
	São Gonçalo	Urban garden	32°38'53.05"	16°52'35.69"	238	9.viii.2006	1♂,2♀	Direct sampling
	São Martinho	Urban garden	32°38'21.16"	16°55'50.70"	64	6.vii-2006	1♂,1♀	Direct sampling
<i>D. suzukii</i>	Arco de São Jorge (M)	Vineyard	32°49'29.62"	16°57'14.44"	134	1.x.2014	11♂,4♀	Colour traps
	Canical	Vineyard	32°44'17.66"	16°44'37.73"	58	25.ix.2014	1♂	Colour traps
	Estreito da Calheta (M)	Vineyard	32°44'5.66"	17°11'11.83"	335	13.x.2014	1♀	Colour traps
	Faial	Vineyard	32°45'32.26"	16°52'27.84"	328	25.ix.2014	4♂,1♀	Colour traps
	São Jorge	Vineyard	32°49'38.93"	16°54'09.94"	256	25.ix.2014	1♂	Colour traps
	São Vicente	Vineyard	32°47'56.44"	17°02'28.15"	90	1-15.x.2014	2♂,3♀	Colour traps
<i>Z. indianus</i>	Câmara de Lobos	Pineapple plantation	32°39'21.17"	16°58'51.96"	148	15.ix.1997	1♂,1♀	Direct sampling
	Ponta do Sol	Banana plantation	32°40'50.65"	17°05'15.79"	30	1992/1993	3♂,6♀	Moericke trap
	Porto da Cruz	Vineyard	32°45'59.36"	16°50'8.09"	233	13.ix.2001	4♂,1♀	Direct sampling
	Quebradas	Mango groove	32°38'51.90"	16°57'44.39"	114	18.xi.1998	1♂,2♀	Direct sampling
	Serra de Água	Agricultural land	32°43'18.00"	17°02'03.79"	400	1992/1993	24♂,20♀	Moericke trap

## DISCUSSION

During the last few centuries human-mediated disturbance has led to the homogenization of island biotas worldwide as a result of species introductions and the extinction of endemic life forms (Olden, 2006; Florencio *et al.*, 2013). The recent discovery of two invasive drosophilid species in Madeira archipelago is a matter of concern since both species have potential negative impacts on the economy and on the environment. The worldwide invasion of *D. suzukii* is proving to be a serious issue due to its severe economic impacts on fruit production and the difficulties in managing this fast spreading pest species (Cini *et al.*, 2012). In Madeira, *D. suzukii* is already widespread in the island, but seems to be associated to vineyards where it can be found in low to moderate abundance. This species was most probably introduced in Madeira following the importation of contaminated fruits or plants from mainland Portugal or Spain, which are the main sources of commercial traffic to the archipelago. In Madeira, *D. suzukii* benefited from human-assisted dispersal to spread rapidly throughout the island. The great improvements on Madeira road network made during the last decade, including the construction of many road tunnels, eased considerably the transportation of people and goods to remote areas of the island and also contributed to a significant increase in traffic. Consequently, exotic species, like *D. suzukii*, can be accidentally

transported jointly with commodities to distant locations soon after their arrival on the island. The invasion of Madeira by *D. suzukii* seems to be quite recent since these are the first records of its occurrence after being detected by an early warning system for introduced species managed by the regional agriculture services. The lack of reports on increased economic losses in fruit production during previous years also seems to support our assumptions of a quite recent arrival of *D. suzukii* in Madeira. A study carried out in Trentino (Italy) showed that following the introduction of *D. suzukii*, soft fruit production economic losses have escalated from 0.5 million euros in 2010 to over three million euros in 2011, not considering the management costs of control strategies and societal impacts (De Ros *et al.*, 2013). The impacts of *D. suzukii* on the environment are still poorly understood, but the finding of oviposition on wild hosts (Grassi *et al.*, 2009) clearly highlights a potential negative role on plant reproduction since fruit dispersers generally tend to avoid damaged fruits (García *et al.*, 1999). Since a considerable number of Madeira endemic plants potentially threatened by *D. suzukii* are dispersed by frugivorous birds (e.g. *Rubus grandifolius*, *R. vahlilii*, *Sambucus lanceolata*, *Sorbus maderensis*, *Vaccinium padifolium*), a precautionary principle should be carried out by extending population monitoring of this invasive species to natural areas.

The other invasive drosophilid reported from Madeira, *Z. indianus*, was also recorded from several low-altitude locations (0-250 m a.s.l.) in north and south Madeira associated to a variety of flesh fruit cultures (banana, grape, mango, pineapple). In Tenerife (Canary Islands), *Z. indianus* was found to be abundant at lower altitudes in urban and agricultural ecosystems, less abundant in a well preserved coastal xeric vegetation environment and absent from both the laurel and pine native forests (Báez and Ortega, 1980, 1981). Thus, in the two Macaronesian archipelagos, *Z. indianus* is mainly distributed throughout the milder coastal lowlands where fruit cultures provide an abundant and stable food resource. During the last decades, this African species has undergone a considerable range expansion having colonized Asia, Europe, South and North America (e.g. Vilela, 1999; Yassin and Abou-Youssef, 2004; van der Linde *et al.*, 2006; Carles-Tolrá, 2009). This invasive species is known to have negative impacts on native insects, particularly on other drosophilids. According to Galego and Carareto (2005), the larval residues of *Z. indianus* interfere with the viability and development of other drosophilids and may lead to a decrease of their abundance over time. In fact, in many invaded areas *Z. indianus* became the dominant species accounting in some cases for 90% of all the drosophilids collected (Tidon *et al.*, 2003). Thus, if we take in consideration the early colonization of fruits by *Z. indianus*, its local high abundance and the negative effects on the viability of native species, we may conclude that its overall impact on native communities can be quite severe (Tidon *et al.*, 2003; Leão and Tidon, 2004; Galego and Carareto, 2005).

The exotic *Acletoxenus formosus* was found associated with colonies of their whitefly prey in cultivated areas from Madeira and Porto Santo. The larvae of *A. formosus* are predators and may have a significant impact on the populations of their prey (Arzone, 1998). Amongst the potential prey of *A. formosus* in Madeira, there

*Invasive Fruit Flies (Diptera: Drosophilidae) Meet in a Biodiversity Hotspot*

are two species of concern: The endemic whitefly species *Bemisia lauracea* (Martin, Aguiar and Pita, 1996) and *Pealius madeirensis* (Martin, Aguiar and Pita, 1996) which are relatively rare and restricted to Laurisilva (Martin *et al.*, 1996). The present lack of records of *A. formosus* from Madeira native habitats make us believe that this species could be confined to urban and agricultural environments, not posing for now a threat to the Madeiran Laurisilva endemic whitefly species, but further studies are needed to confirm this.

The two invasive drosophilid species *D. suzukii* and *Z. indianus* share some characteristics that contribute for their ecological success: both species are very fecund, have a wide host range, show adaptive flexibility and broad physiological tolerance, and have high dispersal capability by benefiting from human assisted transportation (Leão and Tidon, 2004; Cini *et al.*, 2012). Furthermore, the finding of niche shift capability by *Z. indianus* considerably challenges the accurate prediction of its potential distribution in introduced areas through conventional ecological modelling (da Mata *et al.*, 2010), which may in turn compromise the effectiveness of population control actions.

According with our evaluation using the GISS-IUCN impact assessment protocol (Blackburn *et al.*, 2014), *D. suzukii* and *Z. indianus* were both classified as having a moderate impact on natural communities in Madeira since the decline of population densities in some native species could be expected. However, there is some uncertainty associated to these classifications due to the difficulties in evaluating the role of natural ecosystems' resilience to drosophilid species introductions. If these alien species are able to colonize Madeira Laurisilva their ecological impact will be much more severe and some narrow endemics may be in peril. At this stage, the development of a monitoring scheme to assess the distribution and abundance of *D. suzukii* and *Z. indianus* in Madeira is mandatory. The monitoring of invasive species should be performed not only on cultivated areas but also in native habitats, particularly Laurisilva, coupled with a thorough evaluation of their economic impacts in fruit production and potential ecological repercussions. Furthermore, it is also critical to identify the origin and pathways of invasion in order to prevent recurrent reintroductions. These steps are crucial for the comprehension of the invasion process of *D. suzukii* and *Z. indianus* in Madeira archipelago and for the establishment of efficient and effective integrated management strategies.

## ACKNOWLEDGMENTS

The authors wish to thank to Direcção Regional do Ordenamento do Território e Ambiente for allowing the use of geographic information, Enésima Mendonça for providing the maps and Martin Hauser for permission to use his photo of *D. suzukii*. CR and MB were supported by Fundação para a Ciência e a Tecnologia grants SFRH/BPD/91357/2012 and SFRH/BPD/86215/2012, respectively.

## REFERENCES

- Arzone, A., 1998, *Acletoxenus formosus* a predator of *Trialeurodes vaporariorum*. *Bollettino di Zoologia Agraria e di Bachicoltura*, 30: 55-60.
- Báez, M., Ortega, G., 1980, Notas taxonómicas y ecológicas sobre el género *Zaprionus* (Diptera, Drosophilidae) en las islas Canarias. *Bulletin de l'Institut Scientifique*, 4: 87-94.
- Báez, M., Ortega, G., 1981, Estudio preliminar sobre la dinámica de las poblaciones de drosophilidos en la isla de Tenerife (Diptera, Drosophilidae). *Vieraea*, 11: 77-96.
- Blackburn, T. M., Essl, F., Evans, T., Hulme, P. E., Jeschke, J. M., Kühn, I., Kumschick, S., Marková, Z., Mrugała, A., Nentwig, W., Pergl, J., Pyšek, P., Rabitsch, W., Ricciardi, A., Richardson, D. M., Sendek, A., Vilà, M., Wilson, J. R. U., Winter, M., Genovesi, P., Bacher, S., 2014, A unified classification of alien species based on the magnitude of their environmental impacts. *Plos Biology*, 12(5): 1-11.
- Bolda, M., Goodhue, R. E., Zalom, F. G., 2010, Spotted wing drosophila: potential economic impact of a newly established pest. *Agricultural and Resources Economic Update*, 13: 5-8.
- Carles-Tolrà, M., 2009, *Zaprionus indianus* Gupta: género y especie nuevos para la Península Ibérica (Diptera: Drosophilidae). *Boletín de la Sociedad Entomológica Aragonesa*, 45: 316.
- Cini, A., Loriatti, C., Anfora, G., 2012, A review of the invasion of *Drosophila suzukii* in Europe and a draft research agenda for integrated pest management. *Bulletin of Insectology*, 65: 149-160.
- da Mata, R. A., Tidon, R., Côrtes, L. G., De Marco Jr, P., Diniz-Filho, J. A. F., 2010, Invasive and flexible: niche shift in the drosophilid *Zaprionus indianus* (Insecta, Diptera). *Biological Invasions*, 12: 1231-1241.
- De Ros, G., Anfora, G., Grassi, A., Loriatti, C., 2013, The potential economic impact of *Drosophila suzukii* on small fruits production in Trentino (Italy). *International Organisation for Biological and Integrated Control West Palaearctic Regional Section Bulletin*, 91: 317-321.
- Florencio, M., Cardoso, P., Lobo, J. M., Azevedo, E. B., Borges, P. A. V., 2013, Arthropod assemblage homogenization in oceanic islands: the role of indigenous and exotic species under landscape disturbance. *Diversity and Distributions*, 19: 1450-1460.
- Galego, L. G. C., Carareto, C. M. A., 2005, Intraspecific and interspecific pre-adult competition on the neotropical region colonizer *Zaprionus indianus* (Diptera: Drosophilidae) under laboratory conditions. *Bragantia*, 64: 257-262.
- García, D., Zamora, R., Gómez, J. M., Hódar, J. A., 1999, Bird rejection of unhealthy fruits reinforces the mutualism between juniper and its avian dispersers. *Oikos*, 84: 536-544.
- Grant, P. R. (ed.), 1998, *Evolution on Islands*. Oxford University Press, Oxford, UK, 352.
- Grassi, A., Palmieri, L., Giongo, L., 2009, *Drosophila (Sophophora) suzukii* (Matsumura) Nuovo fitofago per i piccoli frutti in Trentino. *Terra Trentina*, 10: 19-23.
- Hulme, P. E., 2009, Trade, transport and trouble: managing invasive species pathways in an era of globalisation. *Journal of Applied Ecology*, 46: 10-18.
- IUCN, 1999, The Laurisilva of Madeira (Portugal). In: Bureau of the World Heritage Committee. IUCN Evaluation of Nominations of Natural and Mixed Properties to the World Heritage List. The 23rd ordinary session of the World Heritage Committee, Paris, France, 106-111.
- Leão, B. F. D., Tidon, R., 2004, Newly invading species exploiting native host-plants: the case of the African *Zaprionus indianus* (Gupta) in the Brazilian Cerrado (Diptera, Drosophilidae). *Annales de la Société Entomologique de France (N.S.)*, 40: 285-290.
- Lowe, S., Browne, M., Boudjelas, S., De Poorter, M., 2000, *100 of the World's worst invasive Alien species. A selection from the Global Invasive Species Database*. 1st edn. The Invasive Species Specialist Group, Species Survival Commission, IUCN, New Zealand, 12.
- Martin, J. H., Franquinho Aguiar, A. M., Pita, M. T., 1996, Aleyrodidae of Madeira: descriptions of three new species, with notes on a pan-Mediterranean species of *Aleurotrachelus*. *Journal of Natural History*, 30: 113-125.

*Invasive Fruit Flies (Diptera: Drosophilidae) Meet in a Biodiversity Hotspot*

- Monclús, M., 1984, Drosophilidae of Madeira, with the description of *Drosophila madeirensis* n. sp.. *Zeitschrift fuer zoologische Systematik und Evolutionsforschung*, 22: 94-103.
- Nardon, C., Deceliere, G., Loevenbruck, C., Weiss, M., Vieira, C., Biémont, C., 2005, Is genome size influenced by colonization of new environments in dipteran species? *Molecular Ecology*, 14: 869-878.
- Olden, J. D., 2006, Biotic homogenization: a new research agenda for conservation biogeography. *Journal of Biogeography*, 33: 2027-2039.
- Paulay, G., 1994, Biodiversity on oceanic islands: its origin and extinction. *American Zoologist*, 34:134-144.
- Reaser, J. K., Meyerson, L. A., Cronk, Q., De Poorter, M., Eldrege, L. G., Green, E., Kairo, M., Latasi, P., Mack, R. N., Mauremootoo, J., O'Dowd, D., Orapa, W., Sastroutomo, S., Saunders, A., Shine, C., Thrainsson, S., Vaiutu, L., 2007, Ecological and socioeconomic impacts of invasive alien species in island ecosystems. *Environmental Conservation*, 34: 98-111.
- Rego, C., Rose, M. R., Matos, M., 2007, Do species converge during adaptation? A case study in *Drosophila*. *Physiological and Biochemical Zoology*, 80: 347-357.
- Sax, D. F., Gaines, S. D., 2008, Species invasions and extinction: The future of native biodiversity on islands. *Proceedings of the National Academy of Sciences*, 105: 11490-11497.
- Tidon, R., Leite, D. F., Leão, B. F. D., 2003, Impact of the colonisation of *Zaprionus* (Diptera, Drosophilidae) in different ecosystems of the Neotropical Region: 2 years after the invasion. *Biological Conservation*, 112: 299-305.
- van der Linde, K., Steck, G. J., Hibbard, K., Birdsley, J. S., Alonso, L. M., Houle, D., 2006, First records of *Zaprionus indianus* (Diptera, Drosophilidae), a pest species on commercial fruits from Panama and the United States of America. *Florida Entomologist*, 89: 402-403.
- Vilela, C. R., 1999, Is *Zaprionus indianus* Gupta, 1970 (Diptera, Drosophilidae) currently colonizing the Neotropical region? *Drosophila Information Service*, 82: 37-39.
- Westphal, M. I., Browne, M., Mackinnon, K., Noble, I., 2008, The link between international trade and the global distribution of invasive alien species. *Biological Invasions*, 10: 391-398.
- Whittaker, R. J., Fernández-Palácios, J. M., 2007, *Island Biogeography: Ecology, Evolution and Conservation*. 2nd edn. Oxford University Press, Oxford, UK, 416.
- Yassin, A., Abou-Youssef, A. Y., 2004, A new front for a global invasive drosophilid: the colonization of the Northern-Western desert of Egypt by *Zaprionus indianus* Gupta, 1970. *Drosophila Information Service*, 87: 67-68.
- Yassin, A., Capy, P., Madi-Ravazzi, L., Ogereau, D., David, J. R., 2008, DNA barcode discovers two cryptic species and two geographical radiations in the invasive drosophilid *Zaprionus indianus*. *Molecular Ecology Resources*, 8: 491-501.

Received: January 14, 2016

Accepted: March 29, 2016



## Effect of Seed Proteinaceous Extracts from Two Wheat Cultivars against *Phthorimaea operculella* (Lepidoptera: Gelechiidae) Digestive $\alpha$ -amylase and Protease Activities

Soheyla FATEHI<sup>1</sup>

Reza FARSHBAF POUR ABAD<sup>1\*</sup>

Ali Reza BANDANI<sup>1</sup>

Mehdi DASTRANJ<sup>2</sup>

<sup>1</sup>Department of Plant Protection, Faculty of Agriculture, University of Tabriz, Tabriz, IRAN

<sup>2</sup>Department of Plant Protection, College of Agricultural and Natural Resources, University of Tehran, Karaj, IRAN

e-mails: soheylafatehi67@gmail.com, \*rfpourabad@yahoo.com, abandani@ut.ac.ir, m.dastranj@ut.ac.ir

### ABSTRACT

The potato tuberworm, *Phthorimaea operculella* (Zeller) is a worldwide pest of solanaceous crops. Larvae feed inside the galleries in foliage, stems and tubers which makes chemical control unsuccessful, and other control methods should be applied. In recent years many plants have received genes that encoding toxic proteins as a strategy to resist against insect pests. *P. operculella* is a solanaceous-specific pest and it has no evolutionary background with cereals. Therefore the goal of current study was to investigate the effect of wheat (*Triticum aestivum* L. cv. MV17 and cv. Azar) seed proteinaceous extract against digestive  $\alpha$ -amylase and protease activities of potato tuberworm and was also to determinate the optimal pH of these enzymes using 1% starch and 2% azocasein as substrate, respectively. The optimum pH of  $\alpha$ -amylase and protease activities was found to be highly alkaline. Enzyme inhibition assays showed that amylase activity was significantly affected by extracts from MV17 and Azar at different pH values and maximum effect was observed at pH 9. The inhibitory effect of MV17 and Azar extracts on protease activity did not markedly vary between pH 8 and 11 and among pH 8, 9, and 11, respectively. Inhibition manner of various concentrations (1.5, 0.75, 0.375, 0.187, and 0.093 (mg protein/ml) of extracts were dose-dependent. Maximum inhibitory effect occurred at the highest concentration and the minimum was at the lowest concentration. In polyacrylamide gel assay, both enzymes, without inhibitors showed two isozymes. At highest concentration of extracts, both bands disappeared or their intensity decreased. So, these proteins can be tested in producing resistant potato crops against potato tuberworm.

*Key words:*  $\alpha$ -amylase, protease, wheat, potato tuberworm.

### INTRODUCTION

The potato tuberworm, is a universal pest of solanaceous crops and especially damages to potatoes. Although it is mainly a pest of potato, it can also be found in other solanaceous plants. It is one of the most important pests of potato in many temperate and tropical regions of the world. The larvae mine leaves, stems, and petioles causing irregular galleries and cave tunnels through tubers. Foliar damage to the potato crop usually does not result in considerable yield losses but infested tubers especially in

non-refrigerated systems may have reduced marketability. Several approaches are available for the development of an integrated pest management system for potato tuberworm. Since the larvae feed inside the tubers and they are not exposed to the insecticides, pesticide application in order to control this insect was not successful (Rondon, 2010). Wide use of pesticides has caused malefic effects to human health and environment and the occurrence of resistance in the phytophagous insect-pests against the pesticides has led to focus on enzymes inhibitors encoded in transgenic plants as an alternative strategy to control the insect pests, because insect pests rely on their digestive enzymes such as  $\alpha$ -amylases and proteases to keep on feeding on host plants (Gatehouse *et al.*, 1999; Franco *et al.*, 2002). Cereals and legumes seeds are the rich sources of digestive enzyme inhibitors (Franco *et al.*, 2002). So, it is recommendable to characterize digestive enzymes as well as to do *in vitro* and *in vivo* bioassay with plant proteinaceous inhibitors in order to achieve a control strategy based on digestive enzyme inhibitors (Harrison and Bonning, 2010). So, the goal of current study was to investigate the effect of seed proteinaceous extracts from wheat cv. MV17 and cv. Azar on the amyolytic and proteolytic activities of the potato tuberworm.

## **MATERIAL AND METHODS**

### **Rearing of insect**

A population of potato tuberworm was taken from the insect physiology laboratory (University of Mohaghegh Ardabili) and maintained on potato tubers (*Solanum tuberosum* L. cv. Agria) located in plastic containers in the incubator set at  $30 \pm 1^\circ\text{C}$  and 55% RH.

### **Preparation of enzyme extract**

Insect enzyme extraction was done based on procedures described by Mehrabadi *et al.* (2012). Fifth instar larvae of potato tuberworm was used for enzyme extraction, because the most feeding occurs in this instar. The larvae were randomly selected, cold-immobilized on ice for 10 minutes and carefully dissected in distilled water under stereomicroscope (Nikon WD<sup>®</sup>). Guts were separated and grounded in 1.5 ml of phosphate buffer at pH 7 and homogenized with homogenizer (Ultra Turrax T8<sup>®</sup>). The 1.5 ml homogenates from preparations were centrifuged at 13,000 rpm for 30 min at  $4^\circ\text{C}$ . The supernatants were transferred to a new tube and stored at  $-20^\circ\text{C}$  for further use as an enzyme source.

### **Determination of optimum pH of $\alpha$ -amylase and protease activity**

The optimal pH of  $\alpha$ -amylase and protease activities were determined using different pH values; 8, 9, 10, 11, and 12 of universal buffer (Hosseinkhani and Nemat-Gorgani, 2003) containing Glycin (0.02 M), 2-morpholinoethansulphonic acid (0.02 M) and succinate (0.02 M). To determine the optimal pH of  $\alpha$ -amylase activity, 10  $\mu\text{l}$  of enzyme extract was dissolved in 65  $\mu\text{l}$  universal buffer at distinct pH, then 25  $\mu\text{l}$  of 1% starch solution as substrate was added to the reaction and the mixture was

### *Effect of Seed Proteinaceous Extracts*

incubated at 40°C for about 30 min. Then the reaction was stopped by adding 100 µl DNS (dinitrosalicylic acid) according to (Bernfeld, 1955) and heated in boiling water for 10 min. Then absorbance of reaction mixture was read at 540 nm by using ELISA reader. To determine the optimal pH of protease activity, 10 µl enzyme extract was incubated with 50µl of 2% azo-casein as substrate in 40 µl of distinct pH at 45°C for about 60 min. According to Saadati *et al.* (2011), the reaction was stopped by adding 100 µl TCA (trichloroacetic acid) and kept in refrigerator at 4°C for about 30 min, followed by centrifugation at 13,000 rpm for about 20 min to precipitate non-hydrolysed substrate. Finally the absorbance of reaction mixtures was measured at 405 nm.

### **Seed protein extraction procedure**

Seeds of MV17 cultivar were supplied by Seed and Plant Improvement Institute, Karaj, Iran and seeds of Azar were obtained from Agricultural Jihad Organization of Saghez, Kurdistan, Iran. According to Baker (1987) and Melo *et al.* (1999), seeds were milled completely and then 30 grams of grinded seeds from each plant separately was mixed with 100 ml of 0.1M NaCl and stirred for 90 min, then the mixture was centrifuged at 8,000 rpm for about 30 min at 4°C. The pellet was discarded and proteins were concentrated using a saturation of 70% ammonium sulfate followed by centrifugation the mixture at the same condition. The pellet was dissolved in ice-cold Tris-HCl buffer (0.02 M and pH 7.0) and dialyzed against the same buffer for about 20 h. Then this dialyzed solution was transferred to 1.5 ml tubes and placed at 70°C for about 15 min in order to inactivate the enzymes within the seeds. Finally, the 1.5 ml tubes were centrifuged at the same condition and the supernatants were transferred to other tubes and they were frozen at -20°C as inhibitor source for enzyme inhibition assays.

### **The effect of pH on inhibitory activity of seed extracts**

In-vitro assay of the effect of different pH on inhibitory activity of seed extracts from MV17 and Azar on α-amylase and protease activities was assayed. At 55 µl of given pH value of universal buffer (8, 9, 10, 11, and 12), 10 µl enzyme extract was pre-incubated with 10 µl of each seed extracts solution at 40°C for 15 min for amylase inhibition assay and for 60 min at 45°C for inhibition assay of protease. Then 1% starch solution as α-amylase substrate and 2% azocasein as protease substrate were added to each enzyme mixture. Appropriate blanks were included in the experiments, too. The inhibition percentage of α-amylase and protease (%) was calculated according to Mehrabadi *et al.* (2011):

$$\%I_{\alpha\text{-amylase}} = 100 * ((\Delta A_{540} \text{ Control} - \Delta A_{540} \text{ Experiment}) / \Delta A_{540} \text{ Control})$$

$$\%I_{\text{protease}} = 100 * ((\Delta A_{405} \text{ Control} - \Delta A_{405} \text{ Experiment}) / \Delta A_{405} \text{ Control})$$

### **The effect of different concentrations of seed extracts on enzymes activity**

The effect of seed proteinaceous extracts on α-amylase and protease activities was determined as described by Mehrabadi *et al.* (2010). Various concentrations including 1.5, 0.75, 0.375, 0.187, and 0.093 mg/ml protein of seed extracts were prepared by diluting the most dens extract (1.5 mg/ml protein). Then 10 µl of enzyme extract at

defined pH (pH 9 for  $\alpha$ -amylase and pH 11 for protease) was pre-incubated with each of above-mentioned concentrations. Thereafter, specific substrate of both enzymes was added to the mixtures and the continuation of assay was done as described in the previous section.

All assays were performed with three replicates using 20 samples per replicates.

### **Semi-denaturing native-page**

Electrophoretic detection of amylolytic and proteolytic activity was done basically according to the procedures described by Laemmli (1970) and Walker *et al.* (1998). Amylolytic activity were detected using 10% (w/v) polyacrylamide gel co-polymerized with 0.5% starch according to Mehrabadi and Bandani (2010) and 4% for stacking gel with 10% SDS (Gel electrophoresis apparatus includes a gel cassette filled with polyacrylamide gel and placed in a tank filled with electrode buffer and an electrical field is applied via the power supply to the rear. The negative terminal is at the top end (cathode), so proteins migrate downward to the anode. Electrophoresis was conducted at a voltage of 70V at 4°C until the blue dye reached the bottom of the gel. Then, the gel was rinsed with distilled water and washed by 1% (v/v) Triton X-100 buffer for about 30 min followed by incubation in Tris-base buffer (pH 9.0) containing 2 mM  $\text{CaCl}_2$  and 10 mM NaCl for about 2 h. Finally, the gel was treated with a solution of 1.3%  $\text{I}_2$  and 3% KI to stop the reaction and stain the un-reacted starch background. Proteolytic activity was detected using 10% (w/v) polyacrylamide gel co-polymerized with 1% gelatin. Electrophoresis was conducted at a voltage of 70V at 4°C until the blue dye reached the bottom of the gel. Then, the gel was rinsed with distilled water and washed by 2.5% (v/v) Triton X-100 buffer for about 60 min followed by incubation in Tris-base buffer (pH 11) for about 24 h. Finally, the gel was treated with staining buffer as described by Hosseininaveh *et al.* (2007) containing 50% (v/v) methanol, 10% (v/v) acetic acid, and 0.25% (w/v) Coomassie blue R-250 to stain the un-reacted gelatin background for about 24h and finally was treated with destain buffer containing 10% (v/v) methanol and 5% (v/v) acetic acid for about 4 h.

Zones of  $\alpha$ -amylase and protease activities appeared at light bands against dark background.

### **Protein determination**

Protein concentration of enzyme extracted from insect gut and proteinaceous extract of seeds was measured according to the method of Bradford (1976), using bovine serum albumin (Bio-Rad, Munchen, Germany) as a standard.

### **Material supply**

Azocasein, bovine serum albumin (BSA), succinic acid disodium salt, and ammonium persulfate for electrophoresis (APS) were supplied by Sigma (St Louis, MO, USA). Tris, phosphate buffer solution (pH 7.0), 2-hydroxy-3,5-dinitrosalicylic acid (DNS), potassium sodium tartrate tetrahydrate, starch soluble, trichloroacetic acid (TCA), sodium hydroxide, ammonium sulfate, acrylamide, N,N'-methylene

### Effect of Seed Proteinaceous Extracts

diacrylamide, dodecyl sulfate sodium salt (SDS), 2-morpholinoethanesulfonic acid (MES), sodium chloride, calcium chloride, phosphoric acid, glycerol, potassium iodide, iodine, coomassie brilliant blue G 250, bromophenol blue, and N,N,N',N'-tetramethyl ethylenediamine (Temed) were purchased from Merck (Darmstadt, Germany). Methanol was from Arman Sina (Tehran, I.R.I); glycine from Scharlau (Barcelona, Spain) and Triton X-100 from Applichem (GmbH in Darmstadt, Germany). Spectrophotometric measurements were made using ELISA reader, BioTek® (Winooski, VT), ELx800.

### Statistical analysis

Data was analyzed using MSTAT-C software by Single factor ANOVA followed by mean comparison with Tukey's Honestly Significant Difference Test (HSD). The error bars in the figures represent SE (Standard Error).

## RESULTS

### Optimum pH of $\alpha$ -amylase and protease activity

Some features of  $\alpha$ -amylase and protease enzymes in the potato tuberworm gut were determined in introductory experiments. We found that the optimal pH of  $\alpha$ -amylase (Fig. 1a) and protease (Fig. 1b) was in the alkaline range with a peak at about pH 9 and 11, respectively. The  $\alpha$ -amylase and protease activity level in optimal pH was 0.024319 and 0.130000 U/min/mg protein, respectively.

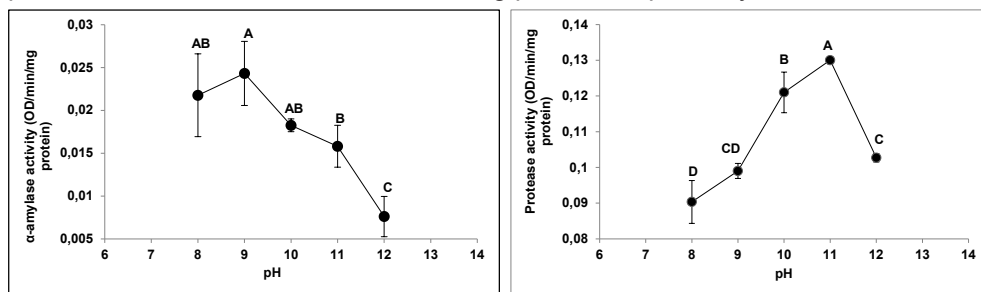


Fig. 1. The effect of pH on  $\alpha$ -amylase (a) and protease activities (b) of potato tuberworm. Means followed by the same letters are not significantly different by Tukey's test ( $P < 0.05$ ).

### The effect of pH on the inhibitory activity of seed proteinaceous extracts

Inhibitory activity of seed extracts on  $\alpha$ -amylase and protease activities was evaluated in different pH values to study the importance of pH factor in insect midgut. The effect of extracts from MV17 and Azar on  $\alpha$ -amylase differed at several pH values and the highest inhibition percentage of  $\alpha$ -amylase by both extracts was observed at pH 9 (Fig. 2a). Whereas the inhibition of protease activity by extract from MV17 did not significantly differ between pH 8 and 11 and by extract from Azar did not markedly vary among 8, 9, and 11 pH values (Fig. 2b).

## The effect of different concentrations of seed extracts on the $\alpha$ -amylase and protease activity

Preliminary assay to estimate the protein concentration of seed extracts was done to start the enzyme inhibition assays. Both extracts showed dose dependent style of inhibition. Various concentrations; 1.5, 0.75, 0.375, 0.187, and 0.093 (mg protein/ml) of extract from MV17 inhibited  $\alpha$ -amylase activity by 59.72, 45.44, 34.88, 23.21, and 12.89% and of extract from Azar decreased the enzyme activity by 50.63, 38.78, 27.16, 25.53, and 17.62% (Fig. 3a). Protease activity was also reduced by extract from MV17 by 42.46, 33.73, 23.77, 20.46, and 16.56% and by extract from Azar by 49.34, 35.22, 20.29, 12.82, and 4.88%, (Fig. 3b).

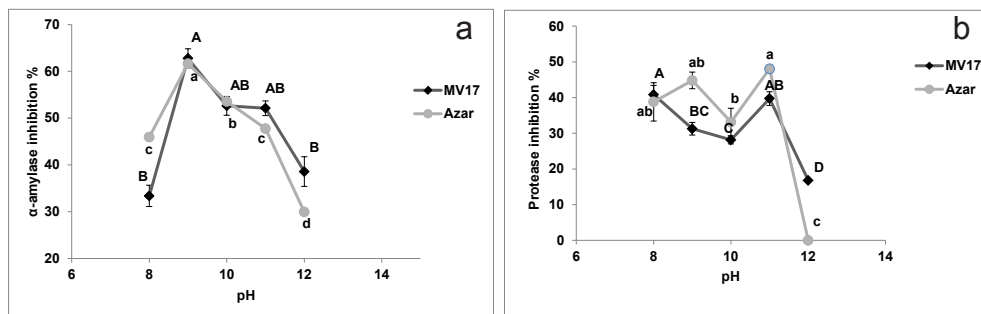


Fig. 2. The effect of pH on inhibition of potato tuberworm  $\alpha$ -amylase (a) and protease (b) by extracts from MV17 and Azar. Means followed by the same letters are not significantly different by Tukey's test ( $P < 0.05$ ).

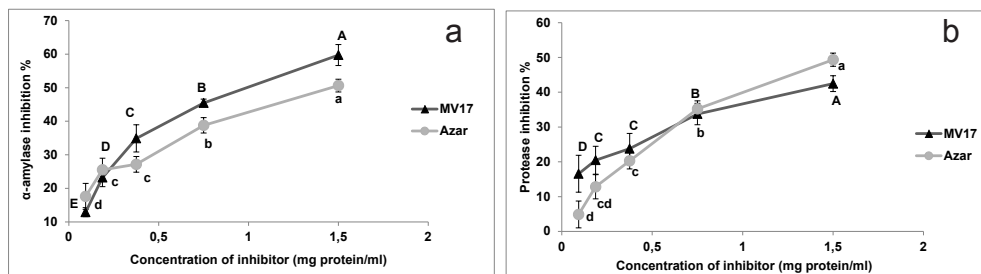


Fig. 3. Inhibition of potato tuberworm  $\alpha$ -amylase (a) and protease (b) by different concentrations of MV17 and Azar. Means followed by the same letters are not significantly different by Tukey's test ( $P < 0.05$ ).

## Gel assays of the effect of seed proteinaceous extracts on the $\alpha$ -amylase and protease activity

Gel assays showed that there were two major isozymes of  $\alpha$ -amylase and protease enzymes in the larval gut. When different concentrations of seed extracts were used, the intensity of bands was differed in both cases. At lowest concentration of seed extracts (0.093 mg/ml protein) both bands were faint. At the highest concentration of extract from MV17 both bands of  $\alpha$ -amylase (Fig. 4a) and protease (Fig. 5a) disappeared. Whereas the highest concentration of extract from Azar only decreased

### Effect of Seed Proteinaceous Extracts

the intensity of bands of both enzymes (Figs. 4b and 5b).

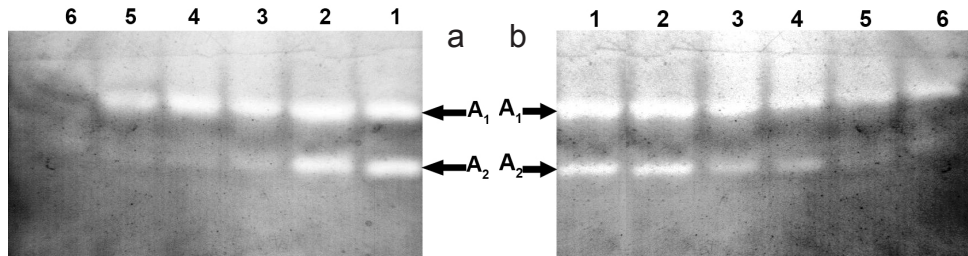


Fig. 4. In gel inhibition assay of the effect of different concentrations of plant extracts on the  $\alpha$ -amylase of potato tuberworm using 0.5% starch as substrate. Lane numbers are as follow: (1) enzyme extract with no inhibitor, (2) 0.093 mg/ml, (3) 0.187 mg/ml, (4) 0.375 mg/ml, (5) 0.75 mg/ml, (6) 1.5 mg/ml protein of extract from MV17 (a) and Azar (b). A1:  $\alpha$ -amylase first isozyme, A2:  $\alpha$ -amylase second isozyme.

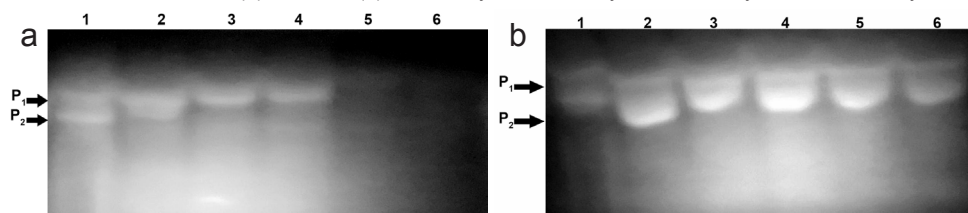


Fig. 5. In gel inhibition assay of the effect of different concentrations of plant extracts on the protease of potato tuberworm using 1% gelatin. Lane numbers are as follow: (1) enzyme extract with no inhibitor, (2) 0.093 mg/ml, (3) 0.187 mg/ml, (4) 0.375 mg/ml, (5) 0.75 mg/ml, (6) 1.5 mg/ml protein of extract from MV17 (a) and Azar (b). P1: Protease first isozyme, P2: Protease second isozyme.

## DISCUSSION

The potato is one of the most important food crops along with rice, wheat, and maize all over the world (Ross, 1986; Douches *et al.*, 2004). Unfortunately, severe losses may occur in storage, especially in developing countries where low income farmers cannot afford refrigerated storages (Rondon, 2010).

In this study, the digestive  $\alpha$ -amylase and protease enzymes of potato tuberworm were characterized for the first time and also the effect of extracts from two wheat varieties was tested on enzymes activities. Many of Lepidopteran insects live on a polysaccharide-rich diet and require digestive  $\alpha$ -amylase to break down and utilize the starch in their food sources. These amylases play a very important role in starch digestion and in insect survival (Borzoui *et al.*, 2013).

Plant seeds are known to contain a diversity of enzyme inhibitors that are thought to be involved in defense mechanisms against herbivores. Among these proteins, the  $\alpha$ -amylase and proteinase inhibitors are found in several legume and cereal species. There are many examples where amylase inhibitors, especially those isolated from cereals, inhibit amylases from insect guts (Valencia-Jiménez *et al.*, 2008). The role and mechanism of action for most of these inhibitors are being studied in detail and their respective genes isolated. These genes have been used for the construction

of transgenic plants to be incorporated in integrated pest management programs (Lawrence and Koundal, 2002).

The acidity of the contents of digestive tract is the main factor affecting the digestive enzymes (Terra and Ferreira, 1994). It has been reported that high levels of acidity in the phytophagous lepidopteran gut is to deal with high levels of tannins in their foods. These materials at low acidity join the insect enzymes and resulting in reduced digestion performance. The digestive tract of Lepidopteran insects such as mediterranean flour-moth *Anagasta kuehniella* Zeller, *Plodia interpunctella* Zeller, and many other Lepidoptera is extremely alkaline (Baker, 1989; Sivakumar *et al.*, 2006; Amorim *et al.*, 2008; Pytelkova *et al.*, 2009). In the present study, the results of pH assays were in consistent with previous experiments. As we observed, potato tuberworm  $\alpha$ -amylase and protease exhibited elevated activity at alkaline pH 9 and 11, respectively. Also, it was found that pH factor affected the inhibition of  $\alpha$ -amylase and protease activity of potato tuberworm by seed extracts. An inhibitor which reduces enzyme activity at the optimum pH of enzyme activity, can be used in producing transgenic plants against insect pests. The highest inhibition of  $\alpha$ -amylase by extract from MV17 and Azar was observed at pH 9 (the optimum pH of  $\alpha$ -amylase activity). Whereas the inhibition of protease by the extracts was not significantly affected by pH factor. There are many other reports confirming that inhibition manner of digestive  $\alpha$ -amylase and protease by seed proteinaceous extracts is pH dependent e.g., the inhibitory effect of wheat seed extract on digestive  $\alpha$ -amylase of *Plutella xylostella* L. (Borzoei *et al.*, 2013), and inhibition of proteolytic and amylolytic activity of *Tenebrio molitor* L. by plant proteinaceous seed extracts (Dastranj *et al.*, 2013).

The effect of seed proteinaceous extracts also was dose dependent. A gradual increase in the amount of the enzyme inhibition was observed along with a gradual increase in the amount of the seed extracts concentrations. In line with these data, Mehrabadi *et al.* (2010) found that the effect of triticale seed extract on the  $\alpha$ -amylase activity of the Sunn pest (*Eurygaster integriceps* Puton) was also dose dependent. They found that lowest concentration of triticale seed extract (0.25 mg) inhibited about 10% of enzyme activity, while the highest dose of seed extract (1.5 mg) caused 80% inhibition of enzyme activity.

## CONCLUSIONS

Since the insects such as potato tuberworm are substantially dependent on  $\alpha$ -amylases to their survival and digestion the starch as the main and the most necessary material in their food, amylase could be a good target for insect control through  $\alpha$ -amylase inhibitors (Franco *et al.*, 2002; Svensson *et al.*, 2004; Sivakumar *et al.*, 2006). The current results showed that potato tuberworm  $\alpha$ -amylases are more sensitive than protease in terms of the inhibition by tested proteinaceous seed extracts. The plants possess several mechanisms against herbivore insects. One of these mechanisms are proteinaceous inhibitors which block the active site of digestive enzymes and consequently prevent their usual function. In plant-pest co-evolution pathway, the insect pests have overcome to defense mechanism of host plants

### *Effect of Seed Proteinaceous Extracts*

such as proteinaceous enzyme inhibitors and have been able to keep on feeding on host plants. Hence in the strategy of transgenic plant production against insect pests, proteins of non-host plants should be encoded in transgenic plants which the insect pests have no evolutionary background with these proteins. Finally, since MV17 and Azar could affect the insect digestive enzymes specially  $\alpha$ -amylase, these seed extracts can be tested to produce resistant potato crops against potato tuberworm.

## ACKNOWLEDGMENTS

This project was funded by a grant from University of Tabriz which is greatly appreciated.

## REFERENCES

- Amorim, T. M. L., Macedo, L. L. P., Uchoa, A. F., Oliveira, A. S., Pitanga, J. C. M., Macedo, F. P., Sales, M. P., Santos, E. A., 2008, Proteolytic digestive enzymes and peritrophic membranes during the development of *Plodia interpunctella* (Lepidoptera: Piralidae): Targets for the action of Soybean Trypsin Inhibitor (SBTI) and Chitin-Binding Vicilin (EvV). *Journal of Agricultural and Food Chemistry*, 56: 7738-7745.
- Baker, J. E., 1987, Purification of isoamylases from the rice weevil, *Sitophilus orizae* L. by HPLC and their interaction with partially purified amylase inhibitor from wheat. *Insect Biochemistry*, 17: 37-44.
- Baker, J. E., 1989, Interaction of partially-purified amylases from larvar *Anagasta kuehniella* Zeller (Lep: Pyralidae) with amylase inhibitors from wheat. *Companies of Biochemistry and Physiology*, 2: 239-246.
- Bernfeld, P., 1955, Amylase,  $\alpha$  and  $\beta$ . *Method Enzymology*, 1: 149-154.
- Borzoei, E., Bandani, A. R., Dastranj, M., Belbasi., M., 2013, Effect of proteinaceous extract of wheat seeds on  $\alpha$ -amylase activity of diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae). *Signpost Open Access Journal of Entomological Studies*, 2: 1-10.
- Borzoui, E., Bandani, A. R., Goldansaz. S. H., 2013, Effects of cereal seed protinaceous extracts on  $\alpha$ -amylase and proteinase activity of salivary glands of Carob moth, *Ectomyelois ceratoniae* (Lepidoptera: pyralidae). *Journal of Crop Protection*, 2(3): 285-296.
- Bradford, M. A., 1976, Rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. *Analytical Biochemistry*, 72(1-2): 248-254.
- Dastranj, M., Bandani, A. R., Mehrabadi, M., 2013, Age-specific digestion of *Tenebrio molitor* (Coleoptera: Tenebrionidae) and inhibition of proteolytic and amyolytic activity by plant proteinaceous seed extracts. *Journal of Asia-Pacific Entomology*, 16(3): 309-315.
- Douches, D. S., Pett, W., Santos, F., Coombs, J., Grafius, E., Metry, E. A. W. L., El-Din, T. N., Madkour. M., 2004, Field and storage testing Bt potatoes for resistance to *P. operculella* (Lepidoptera: Gelechiidae). *Journal of Economic Entomology*, 97(4): 1425-1431.
- Franco, O. L., Regden, D. J., Melo, F. R., Grossi-de-Sa, M. F., 2002, Plant alpha amylase inhibitors and their interaction with insect alpha amylases. Structure, function and potential for crop protection. *European Journal of Biochemistry*, 269(2): 397-412.
- Gatehouse, A. M. R., Norton, E., Davison, G. M., Babbe, S. M., Newell, C. A., Gatehouse. J. A., 1999, Digestive proteolytic activity in larvae of tomato moth, *Acanobia oleracea*: effects of plant protease inhibitors in vitro and in vivo. *Journal of Insect Physiology*, 45(6): 545-558.
- Harrison, R. L., Bonning., B. C. 2010, Proteases as insecticidal agents. *Toxins*, 2(5): 935-953.
- Hosseininaveh, V., Bandani, A. R., Azmayesshfar, P., Hosseinkhani, S., Kazzazi. M., 2007, Digestive proteolytic and amyolytic activities in *Trogoderma granarium* Everts (Dermestidae: Coleoptera). *Journal of Stored Products Research*, 43(4): 515-522.

- Hosseinkhani, S., Nemat-Gorgani, M., 2003, Partial unfolding of carbonic anhydrase provides a method for its immobilization on hydrophobic adsorbents and protects it against irreversible thermoinactivation. *Enzyme and Microbial Technology*, 33(2): 179-184.
- Laemmli, U. K., 1970, Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, 227: 680-685.
- Lawrence, P. K., Koundal, K. R., 2002, Plant Protease Inhibitors in Control of Phytophagous Insects. *Electronic Journal of Biotechnology*, 5(1): 93-109.
- Mehrabadi, M., Bandani, A. R., Kwon, O., 2011, Biochemical characterization of digestive  $\alpha$ -D-glucosidase and  $\beta$ -D-glucosidase from labial glands and midgut of wheat bug *Eurygaster maura* (Hemiptera: Scutelleridae). *Journal of Entomological Research Society*, 41(3): 81-87.
- Mehrabadi, M., Bandani, A. R., Mehrabadi, R., Alizadeh, H., 2012, Inhibitory activity of proteinaceous  $\alpha$ -amylase inhibitors from Triticale seeds against *Eurygaster integriceps* salivary  $\alpha$ -amylases: Interaction of the inhibitors and the insect digestive enzymes. *Pesticide Biochemistry and Physiology*, 102(3): 220-228.
- Mehrabadi, M., Bandani, A. R., Saadati, F., 2010, Inhibition of Sunn pest, *Eurygaster integriceps*,  $\alpha$ -amylases by  $\alpha$ -amylase inhibitors (T- $\alpha$ AI) from Triticale. *Journal of Insect Science*, 10: 179-191.
- Mehrabadi, M., Bandani, A. R., 2010, New approach toward  $\alpha$ -amylase electrophoresis and isoamylase detection. *Munis Entomology and Zoology*, 5: 1085-1087.
- Melo, F. R., Sales, M. P., Pereira, L. S., Bloch, C., Franco, O. L., Ary, M. B., 1999,  $\alpha$ -amylase inhibitors from cowpea seeds. *Protein and Peptide Letter*, 6: 385-390.
- Pytelková, J., Hubert, J., Lepšík, M., Šobotník, J., Šindelka, R., Křížková, I., Horn, M., Mareš, M., 2009, Digestive  $\alpha$ -amylases of the flour moth *Ephestia kuehniella* adaptation to alkaline environment and plant inhibitors. *The Federation of European Biochemical Societies*, 276: 3531-3546.
- Rondon, S. I., 2010, The potato tuberworm: A literature review of its biology, ecology, and control. *American Journal of Potato Research*, 87(2): 149-166.
- Ross, H., 1986, *Potato breeding and perspectives*. Paul Parey Scientific Pub, Verlag Paul Parey, Berlin, 132.
- Saadati, F., Bandani, A. R., Moslemi, A., 2011, Effect of plant seeds protein extract on the Sunn pest, *Eurygaster integriceps* Puton, growth and development and its gut serine protease activity. *African Journal of Biotechnology*, 10(55): 11502-11510.
- Sivakumar, S., Mohan, M. Franco, O. L., Thayumanavan, B., 2006, Inhibition of insect pest  $\alpha$ -amylases by little and finger millet inhibitors. *Pesticide Biochemistry and Physiology*, 85(3): 155-160.
- Svensson, B., Fukuda, K., Nielsen, P. K., Bonsager, B. C., 2004, Proteinaceous  $\alpha$ -amylase inhibitors. *Biochimica et Biophysica Acta-Proteins and Proteomics*, 1696(2): 145-156.
- Terra, W. R., Ferreira, C., 1994, Insect digestive enzymes: properties, compartmentalization and function. *Companies of Biochemistry and Physiology*, 109(1): 1-62.
- Valencia-Jiménez, A., Arboleda Valencia, J. W., Grossi De Sa, M. F., 2008, Activity of  $\alpha$ -amylase inhibitors from *Phaseolus coccineus* on digestive  $\alpha$ -amylases of the coffee berry borer. *Journal of Agricultural and Food Chemistry*, 56: 2315-2320.
- Walker, A. J., Ford, L., Majerus, M. E. N., Geoghegan, I. E., Birch, N., Gatehouse, J. A., Gatehouse, A. M. R., 1998, Characterization of the midgut digestive proteinase activity of the two spot ladybird (*Adalia bipunctata* L.) and its sensitivity to proteinase inhibitors. *Insect Biochemistry and Molecular Biology*, 28(3): 173-180.
- Zeller, P. C., 1873, Beitrage Zur Kenntniss der nordamericanishchen Nachtfolter, besonders der Microlepidopteran. *Verhandlungen der Zoologisch-Botanischen Gesellschaft in Wien*, 23; 262-263.

## A Practical Molecular Diagnostic Tool of the Date Moth *Ectomyelois ceratoniae* (Lepidoptera: Pyralidae) in Tunisia

Sawsen SEDGHIANI<sup>1</sup> Faten RABOUDI<sup>2</sup> Dhia BOUKTILA<sup>1,3\*</sup>  
Hanem MAKNI<sup>1,2</sup> Mohamed MAKNI<sup>1</sup>

<sup>1</sup>Unité de Recherche Génomique des Insectes Ravageurs des Cultures d'intérêt agronomique (UR11ES10), Faculté des Sciences de Tunis, Université de Tunis El Manar, TUNISIA

<sup>2</sup>Institut Supérieur de l'Animation pour la Jeunesse et la Culture de Bir El Bey (ISAJC), Université de Tunis, TUNISIA

<sup>3</sup>Institut Supérieur de Biotechnologie de Béja (ISBB), Université de Jendouba, TUNISIA  
e-mails: sedghianisawsen@yahoo.fr, faten.raboudi@isajc.rnu.tn,

\*dhia\_bouktila2000@yahoo.fr, hanem.makni@isajc.rnu.tn, md.makni@fst.rnu.tn

### ABSTRACT

In Tunisia, date palms are infested by various insect pests, especially Pyralids, whose caterpillars feed inside dates, spoiling their quality. As a critical pre-requisite towards controlling these pests, their accurate taxonomic diagnosis must be applied at molecular level, independently from pest life stages. In the present study, we report the molecular diagnosis of the Pyralid moth *Ectomyelois ceratoniae* at the larvae stage, based on the Cytochrome Oxidase I (COI) gene sequence. We have sequenced a 710 base pairs fragment of the mitochondrial COI gene from 10 Tunisian *E. ceratoniae* specimens. The analysis of intra-specific diversity, using additional public sequences originating from Africa (GenBank: KP083440.1, KP083444.1, KP083442.1, KP083441.1 and JF748065.1) and Australia (GenBank: KF405701.1, KF40073.1 and KF397550.1) provided evidence of population differentiation over geographic scales, as the representatives of each continent (i.e. Africa or Oceania) formed a homogeneous cluster in phylogenetic analysis. Besides, we have used a quick method, based on Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP), to distinguish between *E. ceratoniae* and two other Pyralidae moths commonly found in dates following harvest, namely *Plodia interpunctella* and *Anagasta kuehniella*. This study describes a rapid and efficient molecular diagnostic tool for highly-damaging Pyralidae species. It represents a significant contribution that will impact future pest control strategies of date moths in Tunisia.

**Key words:** Date palm, Pyralidae, *Ectomyelois ceratoniae*, cytochrome oxidase I, DNA barcoding, PCR-RFLP.

### INTRODUCTION

The date palm, *Phoenix dactylifera* L., is a strict dioecious evergreen tree capable of living over 100 productive years. It is one of the oldest domesticated trees, today playing important socio-economic roles in the Middle-East and North Africa (Mahmoudi *et al.*, 2008). It is thought that the earliest cultivation of *P. dactylifera* dates back to 3,700 BC in the area between the Euphrates and the Nile rivers, namely in what is now southern Iraq (Al-Mssallem *et al.*, 2013). Date palm was, historically, introduced by humans to northern India, North Africa, and southern Spain. As a part of North Africa,

Tunisia lends date palms a prominent place in its national economy, with a national production totaling 225,000 T, in 2014/2015 (Tunisian Ministry of Agriculture-Fruit inter professional group, 2015). In spite of their importance, date palms are susceptible to attacks by several pathogens and pests. Especially during harvest or storage, the fruit faces a high rate of infestation by moths belonging to the family of Pyralidae, such as the raisin moth *Cadra figulilella* (Gregson), The Indian mealmoth, *Plodia interpunctella* (Hübner), the Mediterranean flour moth, *Anagasta kuehniella* (Zeller), the almond moth, *Ephestia cautella* (Walker) and the carob moth *Ectomyelois ceratoniae* (Zeller). In Tunisia, *E. ceratoniae* has been considered as the most damaging pest of dates and the main constraint to dates export (Dhouibi, 1989; Jarraya, 2003).

Taxonomic identification of pests is an essential issue in integrated pest management approaches. However, classical, morphology-based identification methods are sometimes unable to detect differences between specimens, especially those with deteriorate morphology or immature stages as eggs, pupae and larvae. The identification of these juvenile forms, which may have no distinguishable features, often needs to be delayed till maturity. Thus, the morphological approach is time-consuming, and requires a high expertise. Alternatively, molecular approaches, based on enzymatic amplification, digestion and/or sequencing of key genes could be of great help. The mitochondrial gene cytochrome oxidase subunit I (COI) has been nominated by Hebert *et al.* (2003a,b; 2004) as a standard gene for the identification of cryptic animal species. This gene has been used for resolving several insect complexes, such as *Bactrocera* sp. (Mezghani-Khemakhem *et al.*, 2012), *Mayetiola* sp. (Bouktila *et al.*, 2006), *Culex* sp. (Hemmerter *et al.*, 2009) and *Anopheles* sp. (Gutiérrez *et al.*, 2010). COI has several advantages; for example, it is devoid of introns and contains few insertions and deletions (Mardulyn and Whitfield, 1999). In addition, its evolution is rapid enough to discriminate not only related species but also phylogeographic groups within the same species (Cox and Hebert, 2001; Hebert *et al.*, 2003b). The aim of the DNA barcoding method is to assign unidentified specimens to a given taxon, at specific or infra-specific level, based on the COI gene sequence. Various studies have corroborated the success of this method (Hebert *et al.*, 2003a; Hebert *et al.*, 2004; Ward *et al.*, 2005; Hogg and Hebert, 2005; Tsao and Yeh, 2008; Wilson *et al.*, 2013). Furthermore, Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) was successfully applied to distinguish between several insects of cryptic species (Raboudi *et al.*, 2005; McKern and Szalanski, 2007; Garrick *et al.*, 2015). Taking this into consideration, the purpose of our present study was to identify and characterize larval specimens of *E. ceratoniae*, collected from date palms in different regions in Tunisia, based on COI gene sequencing and PCR-RFLP.

## MATERIAL AND METHODS

### Insect samples

Pyralidae larvae were collected from five oases located in southern Tunisia, in spring 2013. Geographic location and number of samples are given in Table 1. Specimens were stored at -20 °C until DNA extraction.

## A Practical Molecular Diagnostic Tool

Table 1. Sampling and public sequence data.

Samples from Tunisian oases		
Insect species	Samples labels	Sampling site
<i>Ectomyelois ceratoniae</i>	K1, K2, K3, K4	Kebili (33° 42' N; 8° 58' E)
	T1, T2	Tozeur (33° 55' N; 8° 08' E)
	N10	Nefta (33° 52' N; 7° 53' E)
	G1, G2	Gabes (33° 53' N; 10° 07' E)
	D1	Douz (33° 27' N; 9° 01' E)
Public sequences		
Insect species	Accession numbers in Genbank or BOLDSYSTEMS databases	Geographical origin
<i>Ectomyelois ceratoniae</i>	GenBank : KP083440.1	South Africa
	GenBank : KP083444.1	South Africa
	GenBank : KP083442.1	South Africa
	GenBank : KP083441.1	South Africa
	GenBank : JF748065.1	South Africa
	GenBank : KF405701.1	Western Australia
	GenBank : KF40073.1	Queensland (Australia)
	GenBank : KF397550.1	Queensland (Australia)
<i>Plodia interpunctella</i>	GenBank : KF492033.1	Maryland (USA)
	GenBank : KM572075.1	Austria
	GenBank : KF399544.1	Australia
	GenBank : GU096543.1	Ontario, Ottawa (Canada)
	GenBank : GU096544.1	Ontario, Ottawa (Canada)
<i>Anagasta kuehniella</i>	GenBank : GU828613.1	No data available
	BOLDSYSTEMS : MSWEB061-15	Ontario, Guelph (Canada)
	BOLDSYSTEMS : MSWEB064-15	Ontario, Guelph (Canada)
	BOLDSYSTEMS : MSWEB063-15	Ontario, Guelph (Canada)
	BOLDSYSTEMS : MSWEB062-15	Ontario, Guelph (Canada)

### COI gene amplification and sequencing

DNA was extracted from larvae, using Cetyl Trimethylammonium Bromide (CTAB) method (Doyle and Doyle, 1987). Extracted DNA was resuspended in 30 µL of sterile Milli-Q water. Polymerase Chain reactions were conducted with primers LCO1490: 5'-GGT-CAA-CAA-ATC-ATA-AAG-ATA-TTG-G-3' and HCO2198: 5'-TAA-ACT-TCA-GGG-TGA-CCA-AAA-AAT-CA-3' (Folmer et al., 1994). These primers amplified a 710 base pairs fragment in the 5' end of the mtDNA cytochrome oxidase subunit I (COI). Each reaction, performed in 25µL volume, used 50ng of DNA template, 0.1 unit of Taq polymerase (Promega), 200µM MgCl<sub>2</sub>, 0.1 mM of each primer and 0.2 mM dNTPs. PCR conditions consisted of an initial denaturation at 94°C for 5

minutes, followed by 35 cycles each consisting of 3 steps: denaturation (94°C, 1 min), annealing (54 °C, 1 min) and extension (72°C, 1 min); and a final extension at 72°C for 5 min. PCR products were separated by 1% agarose gel and stained with ethidium bromide. The appropriate bands were purified using Quik Gel Extraction (Invitrogen) before being sequenced by an automated DNA sequencing system.

### PCR-RFLP assay

Fifteen (15) µL of the COI gene PCR product of each larva were incubated in a total reaction volume of 20 µL with 5 U of the endonuclease *DraI* and 10X digestion buffer (Promega) at 37 °C for 4 hours. The digested products were visualized on 2% agarose gel, stained with ethidium bromide and photographed under ultraviolet light.

### Bioinformatic analyses of sequences

In order to characterize *E. ceratoniae* specimens, at intra-species level, based on COI gene sequence, we have enriched the genetic COI gene pool, by 8 additional public sequences, corresponding to partial sequences of cytochrome oxidase subunit I (COI) gene of *E. ceratoniae*. Five of these sequences were from South Africa, while three originated from Australia (Table 1). The COI gene sequences obtained through DNA amplification and those retrieved from databases were aligned using ClustalW algorithm implanted in BioEdit 7.2.5 (Hall, 1999). The method reported by Elsasser *et al.* (2009) and Shokralla *et al.* (2011), based on Kimura 2-parameter (K2P) model of base substitution (Kimura, 1980), was used for phylogeny reconstruction and for generating a Neighbor Joining (NJ) phylogenetic tree by MEGA software version 6 (Tamura *et al.*, 2013), with a bootstrap analysis of 1000 replications. Besides, in an attempt to identify discriminatory restriction sites between *E. ceratoniae* and other Pyralidae species, we have analyzed restriction polymorphisms, using NEBcutter v.2.0 software (Vincze *et al.*, 2003), and three consensus sequences: (a) a consensus of *E. ceratoniae* COI gene sequences that was developed based on the combined 10 local sequences and the eight public accessions of this species, shown in Table 1, and (b) two consensus sequences of each *P. interpunctella* and *A. kuehniella* COI gene that were generated from public accessions of these species, shown in Table 1.

## RESULTS

### Correlation between genetic diversity and geographical distribution

The 710 bp fragment of the COI mitochondrial gene was amplified and sequenced for each sample (n=10). After checking and manual editing, the total length of DNA sequence used from each individual was adjusted to 604 bp. A Blast search in Genbank (Benson *et al.*, 2013) and BOLDSYSTEMS (Ratnasingham and Hebert, 2013) databases showed that all specimens belonged to *E. ceratoniae* species, with BOLDSYSTEMS compatibilities ranging between 99.83% and 100%, indicating a high “DNA barcode” sequence similarity between the studied Tunisian specimens and their most similar conspecific *E. ceratoniae* ones. As expected in insect mitochondrial DNA,

### A Practical Molecular Diagnostic Tool

these sequences contained a high AT percentage (Crozier and Crozier, 1993). No insertions, deletions, or stop codons were observed in the sequences, indicating the absence of nuclear copies of mitochondrial origin (NUMTs) (Bensasson *et al.*, 2001; Song *et al.*, 2008). The alignment at the nucleotide level showed that COI sequences were either identical or highly similar. Moreover, the translation of these sequences revealed identical amino acid sequences (data not shown).

To examine the extent of intra-specific divergence, 8 additional sequences of *E. ceratoniae* COI were downloaded from Genbank and BOLDSYSTEMS database. The pair-wise sequence comparison across *E. ceratoniae* individuals showed that genetic distances ranged from 0.0% to 4.1%, with an average of 1.2%. These values of intra-specific divergence, were influenced by the presence of two groups showing 3.9% divergence: The first, included specimens from Tunisia and South Africa (African continent), while the second was made of specimens originating from Queensland and Western Australia (Oceania). Each group was homogeneous, with low mean divergence rates of 0.4% and 0.2% for the first and second groups, respectively. Indeed, when specimens from Oceania were excluded, the overall intra-specific divergence dropped significantly from 1.2% to 0.4%. Because of their deep divergence, sequences from Queensland and Western Australia formed a distinct cluster in Neighbor-joining tree (Fig. 1).

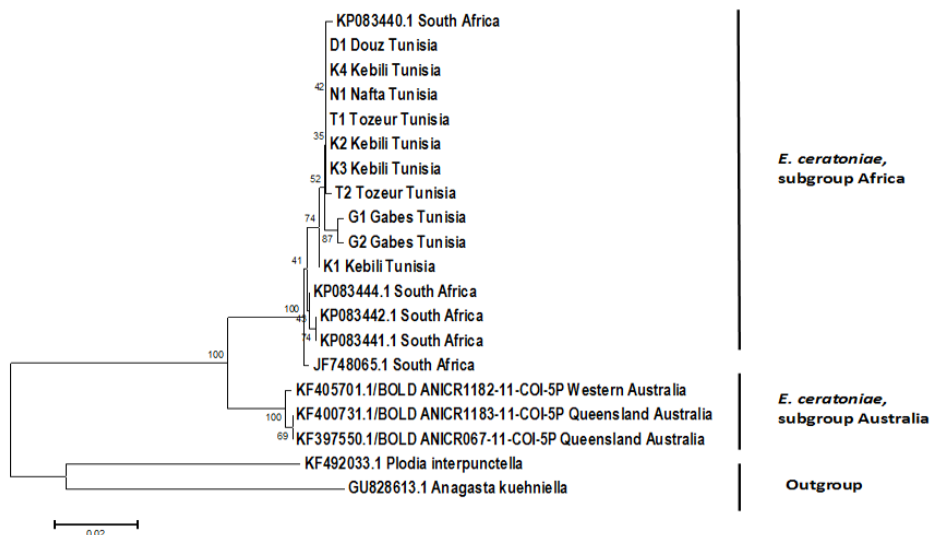


Fig. 1. Neighbor-joining phylogenetic tree, based on a 604 bp COI gene fragment, showing genetic relationships among *Ectomyelois ceratoniae* specimens from Tunisia, South Africa and Australia. Lepidoptera species *Plodia interpunctella* and *Anagasta kuehniella* were used as outgroup. The numbers above branches refer to the bootstrap values based on 1000 replications.

### COI-PCR-RFLP: an efficient tool for distinguishing between *E. ceratoniae* and the complex *P. interpunctella* and *A. kuehniella*

The COI sequences of *E. ceratoniae*, *P. interpunctella* and *A. kuehniella* were

examined for their recognition site of restriction enzyme using the virtual sequence digestion program NEBcutter. *Dral* was chosen because of its discriminatory potential. It yielded, *in silico*, diagnostic RFLPs that discriminated easily between *E. ceratoniae*, on one hand, and *P. interpunctella* and *A. kuehniella*, on the other hand. Indeed, *Dral* endonuclease acted specifically at a diagnostic restriction site at position 570 in *E. ceratoniae*, but not in *P. interpunctella* and *A. kuehniella* (Fig. 2). The visualized PCR-RFLP patterns, obtained in wet lab experimentation for 20 Tunisian specimens of *E. ceratoniae*, were similar to those predicted *in silico*, showing a standard profile with a 570 bp and a 140 bp DNA fragments on agarose gel (Fig. 3), confirming that all specimens belong to *E. ceratoniae* species.

## DISCUSSION AND CONCLUSIONS

Our results are in agreement with previous studies reporting that the levels of intra-specific variation rarely exceed 2% in Lepidoptera (Hebert *et al.*, 2003b; 2009; Hajibabaei *et al.*, 2006; Hausmann *et al.*, 2011). The K2P distances calculated between samples from the two continents were higher (1.2% mean intra-specific divergence) compared with those calculated from the representatives of a single continent (0.4% and 0.2% for Africa and Oceania, respectively). This fact pleads in favor of a population differentiation over geographic scales. Likewise, several entomological studies reported that geography and genetic distances were co-dependent (Bergsten *et al.*, 2012; Bouktila *et al.*, 2012). Similarly, Čandek and Kuntner (2014) found that increased geographical sampling affects slightly the intra-specific distance variation but did not hamper the performance of the DNA barcodes as a tool for species identification. This variation may be the result of isolation by distance (Wright, 1943) or distance decay (Nekola and White, 1999).

Although DNA sequencing based on cytochrome oxidase I (COI) gene is an effective and reliable tool for insect identification, the use of this method could be time-consuming and/or expensive. For this reason, we suggest that a combination of COI genotyping and PCR-RFLP will be a rapid diagnostic tool between Pyralid moths, as this method was previously applied to several insect pests (Raboudi *et al.*, 2005; McKern and Szalanski, 2007; Valenzuela *et al.*, 2007; Mezghani-Khemakhem *et al.*, 2012). We have shown that the endonuclease *Dral* has a specific cleavage site in *E. ceratoniae* COI gene sequences, facilitating the distinction between this species and other Pyralids, namely *P. interpunctella* and *A. kuehniella*. Results reported, in this paper, will facilitate species identification, not only by specialized entomologists, but also non-specialists who are not familiarized with the morphology of Pyralidae.

## ACKNOWLEDGEMENTS

This work was supported by the Tunisian Ministry of Higher Education and Scientific Research. The authors greatly acknowledge all the industrial staff of 'Boudjebel SA VACPA' Company for specimen supply.

## A Practical Molecular Diagnostic Tool

```

          10      20      30      40      50
Cons E.ceratoniae  ATCTGGTATA GTAGGAACAT CTTTAACTCT TCTAATTCGA GCAGAAATAG
Cons A.kuehniella  .A.A.A...T...T...CC.C...T...AT...T...T...
Cons P.interpunctella .G.C..A...A.T...AT...AT...

          60      70      80      90     100
Cons E.ceratoniae  GTACACCTGG TTCTTTAATT GGAGATGATC AAATTTATAA TACTATTGTA
Cons A.kuehniella  .A.T...A.A...T...
Cons P.interpunctella .A..T...A...T...

          110     120     130     140     150
Cons E.ceratoniae  ACGGACATG CTTTTATTAT AATTTTTTTT ATAGTAAATGC CTATTATAAT
Cons A.kuehniella  .T.T...T...G...A...
Cons P.interpunctella .TA.T...T...A...A...

          160     170     180     190     200
Cons E.ceratoniae  TGGAGGATTC GGTAATTGAT TAGTTCCTTT AATATTAGGA GCCCCTGATA
Cons A.kuehniella  ..G...T..A...C...T...
Cons P.interpunctella .....G..T..A...T...

          210     220     230     240     250
Cons E.ceratoniae  TAGCTTTCCG CCGAATAAAT AATATAAGAT TTTGACTTTT ACCCCCATCA
Cons A.kuehniella  .....T...C...T...T...T...
Cons P.interpunctella .....T...T...T...T...

          260     270     280     290     300
Cons E.ceratoniae  TTACTCTTT TAATTTCAAG AAGAATTGTA GAAATGGTG CAGGAACTGG
Cons A.kuehniella  .....TCT...T...A...T...
Cons P.interpunctella .....C...T...G...

          310     320     330     340     350
Cons E.ceratoniae  ATGAACTGTG TACCCCCAC TTTCATCTAA TATCGCCAT AGTGAAGCT
Cons A.kuehniella  G...A..T..T...C...A...T...T...G...A...
Cons P.interpunctella G...A..T..T...C...T...T...G...C...T...

          360     370     380     390     400
Cons E.ceratoniae  CTGTAGATTT AGCTATTTT TCTTACATT TAGCTGGAAT TTCTTCTATT
Cons A.kuehniella  ...T..C...C...CC.T...T...A...
Cons P.interpunctella ...T...C...CC.T...G..T...C..C...

          410     420     430     440     450
Cons E.ceratoniae  TTAGGAGCTA TTAATTTTAT TACAACATTT ATTAATATAA AACTTAATGG
Cons A.kuehniella  ...G..A...C...T...T...T...A...
Cons P.interpunctella C.T..T..C...T..A...T...T...A...

          460     470     480     490     500
Cons E.ceratoniae  TCTATCATTG GATCAAATAC CTTTATTGCT CTGAGCTGTT GGTATTACCG
Cons A.kuehniella  AA..AT...C...T...A...A...T...
Cons P.interpunctella AA..AT...T...T...T...

          510     520     530     540     550
Cons E.ceratoniae  CACTTTTATT ACTTCTTCT TTACTCTGAT TAGCAGGAGC TATTACTATA
Cons A.kuehniella  .TT.A..C..T..C..A...A..T...T...
Cons P.interpunctella .TT.AC.C...T..A..A.C.T...T...T...

          560     570     580     590     600
Cons E.ceratoniae  TTATTAACAG ATCGAAATTT AAATACTTCT TTTTTCGACC CTGCAGGAGG
Cons A.kuehniella  C.T...T...C..T...C..C...T...T...
Cons P.interpunctella C.T...T...C..T...C...C...T...T...

          ....
Cons E.ceratoniae  AGGA
Cons A.kuehniella  ....
Cons P.interpunctella T...

```

Fig. 2. Comparison of a 604 bp COI gene portion between *Ectomyelois ceratoniae*, *Anagasta kuehniella* and *Plodia interpunctella*. The red frame refers to the differential *DraI* restriction site.

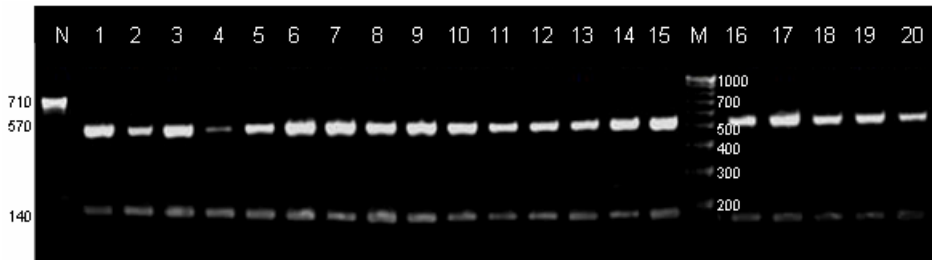


Fig. 3. Banding profiles of the COI gene obtained from 20 *E. ceratoniae* individuals, after digestion with *DraI*. N: native (undigested) 710 bp fragment of the COI gene; M: Molecular weight marker (1000 bp).

## REFERENCES

- Al-Mssallem, I. S., Hu, S., Zhang, X., Lin, Q., Liu, W., Tan, J., Yu, X., Liu, J., Pan, L. *et al.*, 2013, Genome sequence of the date palm *Phoenix dactylifera* L. *Nature Communications*, 4 (2274): 1-9.
- Bensasson, D., Zhang, D. X., Hartl, D. L., Hewitt, G. M., 2001, Mitochondrial pseudogenes: evolution's misplaced witnesses. *Trends in Ecology and Evolution*, 16: 314-321.
- Benson, D. A., Cavanaugh, M., Clark, K., Karsch-Mizrachi, I., Lipman, D. J., Ostell, J., Sayers, E. W., 2013, GenBank. *Nucleic Acids Research*, 41(Database issue): 36-42.
- Bergsten, J., Bilton, D. T., Fujisawa, T., Elliott, M., Monaghan, M. T., Balke, M., Hendrich, L., Geijer, J., Herrmann, J., Foster, G. N., Ribera, I., Nilsson, A. N., Barraclough, T. G., Vogler, A. P., 2012, The effect of geographical scale of sampling on DNA barcoding. *Systematic Biology*, 61: 851-869.
- Bouktila, D., Mezghani, M., Marrakchi, M., Makni, H., 2006, Genetic variation and relatedness in Tunisian wheat midges of the genus *Mayetiola* (Diptera: Cecidomyiidae), inferred from biological and molecular data. *Acta Entomologica Sinica*, 49: 822-828.
- Bouktila, D., Kharrat, S., Mezghani-Khemakhem, M., Jerraya, A., Makni, M., 2012, Genetic diversity in different populations of citrus leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) in Tunisia, assessed by RAPD-PCR. *Journal of Crop Protection*, 1: 189-199.
- Čandek, K., Kuntner, M., 2014, DNA barcoding gap: reliable species identification over morphological and geographical scales. *Molecular Ecology Resources*, 15: 268-277.
- Cox, A. J., Hebert, P. D. N., 2001, Colonization, extinction and phylogeographic patterning in a freshwater crustacean. *Molecular Ecology*, 10: 371-386.
- Crozier, R. H., Crozier, Y. C., 1993, The mitochondrial genome of the honeybee *Apis mellifera*: complete sequence and genome organization. *Genetics*, 133: 97-117.
- Dhouibi, M. H., 1989, *Biologie et Écologie d'Ectomyelois ceratoniae* Zeller (Lepidoptera- Pyralidae) Dans Deux Biotopes Différents au sud de la Tunisie et Recherche de Méthodes Alternatives de Lutte. PhD Thesis, University of Paris VI, France, 176.
- Doyle, J. J., Doyle, J. L., 1987, A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Photochemical Bulletin*, 19: 11-15.
- Elsasser, S. C., Floyd, R., Hebert, P. D. N., Schulte-Hostedde, A. I., 2009, Species identification of North American guinea worms (Nematoda: Dracunculus) with DNA barcoding. *Molecular Ecology Resources*, 9: 707-712.
- Folmer, O., Black, M., Hoeh, W., Lutz, R., Vrijenhoek R., 1994, DNA primers for amplification of mitochondrial cytochrome C oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, 3: 294-299.

## A Practical Molecular Diagnostic Tool

- Garrick, R. C., Collins, B. D., Yi, R. N., Dyer, R. J., Hyseni, C., 2015, Identification of Eastern United States *Reticulitermes* termite species via PCR-RFLP, assessed using training and test data. *Insects*, 6: 524-537.
- Gutiérrez, L. A., Orrego, L. M., Gómez, G. F., López, A., Luckhart, S., Conn, J. E., Correa, M. M., 2010, A new mtDNA COI gene lineage closely related to *Anopheles janconnae* of the Albitarsis complex in the Caribbean region of Colombia. *Memórias do Instituto Oswaldo Cruz*, 105:1019-1025.
- Hajibabaei, M., Janzen, D. H., Burns, J. M., Hallwachs, W., Hebert, P. D. N., 2006, DNA barcodes distinguish species of tropical Lepidoptera. *Proceedings of the National Academy of Science of the United States of America*, 103: 968-971.
- Hall, T. A., 1999, BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, 41: 95-98.
- Hausmann, A., Haszprunar, G., Hebert P. D. N., 2011, DNA Barcoding the Geometrid fauna of Bavaria (Lepidoptera): Successes, surprises, and questions. *Plos One*, 6: e17134.
- Hebert, P. D. N., Cywinska A., Ball, S. L., De Waard, J. R., 2003a, Biological identification through DNA barcodes. *Proceedings of the Royal Society, Biological Sciences*, 270: 313-321.
- Hebert, P. D. N., Ratnasingham, S., De Waard, J. R., 2003b, Barcoding animal life: Cytochrome C oxidase subunit I divergences among closely related species. *Proceeding of the Royal Society, Biological Sciences*, 270: 896-899.
- Hebert, P. D., Penton, E. H., Burns, J. M., Janzen, D. H., Hallwachs W., 2004, Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astraptes fulgerator*. *Proceedings of the National Academy of Sciences of the United States of America*, 101: 14812-14817.
- Hebert, P. D. N., Dewaard, J. R., Landry, J. F., 2009, DNA barcodes for 1/1000 of the animal kingdom. *Biology letters*, 6: 359-362.
- Hemmerter, S., Slapeta, J., Beebe, N. W., 2009, Resolving genetic diversity in Australasian *Culex* mosquitoes: incongruence between the mitochondrial cytochrome c oxidase I and nuclear acetylcholine esterase 2. *Molecular and Phylogenetic Evolution*, 50: 317-25.
- Hogg, I. D., Hebert, P. D. N., 2005, Biological identifications of springtails (Hexapoda: Collembola) from the Canadian arctic, using mitochondrial barcodes. *Canadian Journal of Zoology*, 82: 749-754.
- Jarraya, A., 2003, *Principaux nuisibles des Plantes Cultivées et des Denrées Stockées en Afrique du Nord: Leur Biologie, Leurs Ennemis Naturels, Leurs Dégâts et Leur Contrôle*. Maghreb Editions, Tunis, Tunisia.
- Kimura, M., 1980, A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution*, 16: 111-120.
- Mahmoudi, H., Hosseininia, G., Azadi, H., Fatemi, M., 2008, Enhancing date palm processing, marketing and pest control through organic culture. *Journal of Organic Systems*, 3: 29-39.
- Mardulyn, P., Whitfield, J. B., 1999, Phylogenetic signal in the COI, 16S, and 28S genes for inferring relationships among genera of *Microgastrinae* (Hymenoptera; Braconidae): Evidence of a high diversification rate in this group of parasitoids. *Molecular Phylogenetics and Evolution*, 12: 282-294.
- McKern, J. A., Szalanski, A. L., 2007, Molecular diagnostics of economically important clearwing moths (Lepidoptera: Sesiidae). *Florida Entomologist*, 90: 475-479.
- Mezghani-Khemakhem, M., Ben Lazahr, W., Bouktila, D., Ben Slimen, H., Makni, H., Makni, M., 2012, A rapid diagnostic technique of *Bactrocera cucurbitae* and *Bactrocera zonata* (Diptera: Tephritidae) for quarantine application. *Pest Management Science*, 69: 744-746.
- Nekola, J. C., White, P. S., 1999, The distance decay of similarity in biogeography and ecology. *Journal of Biogeography*, 26: 867-878
- Raboudi, F., Mezghani, M., Makni, H., Marrakchi, M., Rouault, J. D., Makni, M., 2005, Aphid species identification using cuticular hydrocarbons and cytochrome b gene sequences. *Journal of Applied Entomology*, 129: 75-80.
- Ratnasingham, S., Hebert, P. D. N., 2013, A DNA-based registry for all animal species: The barcode index number (BIN) system. *Plos One*, 8: e66213.

- Shokralla, S., Zhou, X., Janzen, D. H., Hallwachs, W., Landry, J. F., Jacobus, L. M., Hajibabaei, M., 2011, Pyrosequencing for mini-barcoding of fresh and old museum specimens. *Plos One*, 6: e21252. doi:10.1371/journal.pone.0021252.
- Song, H., Buhay, J. E., Whiting, M. F., Crandall, K. A., 2008, Many species in one: DNA barcoding overestimates the number of species when nuclear mitochondrial pseudogenes are coamplified, *Proceedings of the National Academy of Sciences of the United State of America*, 105: 13486-13491.
- Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S., 2013, MEGA 6: Molecular Evolutionary Genetics Analysis Version 6.0. *Molecular Biology and Evolution*, 30: 2725-2729.
- Tsao, W. C., Yeh, W. B., 2008, DNA-based discrimination of subspecies of swallowtail butterflies (Lepidoptera: Papilioninae) from Taiwan. *Zoological Studies*, 47: 633-643.
- Tunisian Ministry of Agriculture-Fruit inter professional group, 2015, [http://gifruits.com/?page\\_id=2311&lang=fr](http://gifruits.com/?page_id=2311&lang=fr) (12.02.2015).
- Valenzuela, I., Hoffmann, A. A., Malipatil, B. B., Ridland, P. M., Weeks, A. R., 2007, Identification of aphid species (Hemiptera: Aphididae: Aphidinae) using a rapid polymerase chain reaction restriction fragment length polymorphism method based on the cytochrome oxidase subunit I gene. *Australian Journal of Entomology*, 46: 305-312.
- Vincze, T., Posfai, J., Roberts, R. J., 2003, NEBcutter: a program to cleave DNA with restriction enzymes. *Nucleic Acids Research*, 31: 3688-3691.
- Ward, R. D., Zemlak, T. S., Innes, B. H., Last, P. R., Hebert, P. D. N., 2005, DNA barcoding Australia's fish species. *Philosophical Transactions of the Royal Society, Biological Sciences*, 360: 1847-1857.
- Wilson, J. J., Sing, K. W., Sofian-Azirun, M., 2013, Building a DNA Barcode Reference Library for the True Butterflies (Lepidoptera) of Peninsula Malaysia: What about the Subspecies? *Plos One*, 8: e79969.
- Wright, S., 1943, Isolation by distance. *Genetics*, 31: 114-138.

Received: March 09, 2016

Accepted: November 02, 2016

## The First Stages of Xylobiont Entomofauna Succession on European Silver Fir Logging Residues in Relation to Utilisation Method and Microenvironmental Conditions

Magdalena KACPRZYK<sup>1\*</sup>

Piotr BILAŃSKI<sup>2</sup>

<sup>1,2</sup>Department of Forest Protection, Entomology and Forest Climatology,  
University of Agriculture in Krakow, 31-425 Al. 29 Listopada 46, Kraków; POLAND  
e-mails: \*m.kacprzyk@ur.krakow.pl, rlbilans@cyf-kr.edu.pl

### ABSTRACT

The effect of post-cleaning Silver fir logging residues treatment method on the occurrence of early arriving xylobiont insects was studied in 2013 in two stands of fresh upland broadleaved forest and fresh mountain broadleaved forest habitats. At each study site, branches and tree tops were arranged in four piles and scattered disorderly in two plots. It was demonstrated that fine woody debris of fir, regardless of the disposal variant and habitat conditions prevailing at the place of leaving the material, were the breeding and the feeding base for *Pityophthorus pityographus* (Ratzeburg, 1837), *Pityokteines vorontzowi* (Jakobson 1895), *P. curvidens* (Germar, 1824) and *Cryphalus piceae* (Ratzeburg, 1837) (Coleoptera: Scolytinae). It was noted that the attractiveness of fir branches and tree tops for phytophagous insects increased proportionally to the stage of decomposition of logging residues. The influence of forest habitat and the method of fir slash utilisation, as well as microenvironmental conditions, prevailing in the material surroundings, on the severity of branch and tree top infestation by insects was not confirmed. It was, however, proved that fine woody debris of fir was the place of the occurrence of saprophages and natural enemies of bark beetles, i.e. predators, parasites and parasitoids. The most abundant entomophagous insect species was *Phloeopora corticalis* (Gravenhorst, 1802) (Coleoptera: Staphylinidae), whereas saproxylobiont entomofauna was represented mostly by insects from the order Diptera and family Mycetophilidae.

*Key words:* *Abies alba*, fine woody debris, Coleoptera, beneficial insects, forest habitat, slash management

### INTRODUCTION

Fine woody debris such as branches and tree tops, left in the forest after logging, are the habitat for numerous xylobiont insects species, including both cambio- xylophages harmful to the stability of stands (Grégoire *et al.*, 2004; Starzyk *et al.*, 2008a; Kacprzyk, 2012), entomophagous insects as beneficial in pest management (Kenis *et al.*, 2004; Hilszczański *et al.*, 2005; Johansson *et al.*, 2007) and saprophagous insects actively accelerating the process of organic matter circulation (Gunnarsson *et al.*, 2004; Lindhe and Lindelöw, 2004; Jonsell, 2008b; Zeniauskas and Gedminas, 2010).

Silver fir (*Abies alba* Mill.) is, next to European beech (*Fagus sylvatica* L.), one of the main forest-forming species in the Carpathians (Pawlaczyk *et al.*, 2005). In the Polish Carpathians the share of fir stands is estimated at ca. 20% but, according to

Jaworski and Pach (2014), having regard to the progressive climate change and loss of pure spruce stands, with simultaneous high production and adaptability of fir to different environmental conditions (Tinner *et al.*, 2013), the importance of this species in the considered region will increase in the future.

Silver fir stands, followed by spruce (*Picea abies* L. Karst.) stands are among the mountain forests most severely exposed to outbreaks of cambio- xylophagous insects although, as emphasised by Brauns (1975), the number of different groups of bark beetles is twice smaller in fir than in Scots pine (*Pinus sylvestris* L.) and Norway spruce. So far, little is known about the impact of disposal method of post-harvesting branches and tree tops of fir on the quantitative and qualitative diversity of occurring insects. The results obtained by Starzyk *et al.* (2008a) indicate, that in the case of accumulation of large number of cut-off or broken branches and tree tops in the stands, the material must be removed from the forest before swarming of most dangerous secondary insect pests, and in a situation where this is not possible it is advisable to leave the residues loosely on the surface in direct sunlight, to allow quick drying out of cambium and phloem. On the other hand, Ząbecki and Kacprzyk (2007) did not confirm that random scattering of spruce branches and tree tops on surfaces directly exposed to sun has the limiting impact on the development of bark beetles. In contrast, according to these authors, on surfaces with high (at least 0.5 m height) undergrowth vegetation in weakened Norway spruce stands this method can promote the colonisation of logging residues by cambio- xylophagous insect pests to much greater extent than their piling. Therefore, consideration of local environmental conditions seems reasonable when selecting the method of fine woody debris treatment. The effect of Silver fir logging residues (LR) treatment method on attractiveness for early successional xylobiont entomofauna was going to be examined. Additionally in this study the site conditions in the place of fir LR storage impact on the species insects composition were investigated. The following hypotheses were tested: 1) The spreading fir branches and tree tops on the forest floor reduces the attractiveness of the material for the cambio- xylophagous insects, (2) The frequency and infestation density of the fir LR by early colonizing insect species are determined by the physiological state of the material, which is associated with a forest habitat in the place of its storage and a utilisation method.

## MATERIAL AND METHODS

The study was conducted in south-eastern Poland, in two, over 100 years old, Silver fir stands growing in areas of the Low Bieszczady Mountains (experimental site 1: 49°51'62" N; 22°31'91" E) and the Central Beskidian Piedmont (experimental site 2: 49°44'60" N; 22°29'77" E). The stand in the study site No. 1 overgrew the area at an altitude of 355 m above sea level, in the fresh upland broadleaved forest (FUBF) habitat. The second forest stand (study site No. 2) was located at a height of 445 m above sea level, in a fresh mountain broadleaved forest (FMBF) habitat. The local soils are derived from different parent material, i.e. siltstones for experimental site No.

### *The First Stages of Xylobiont Entomofauna*

1 and sandstones for experimental site No. 2. The soils were classified as Epidystric Cambisol and Hyperdystric Cambisol, respectively. Soils derived from different types of parent material had different texture. In the first research area it was silt, while in the second research area the soils were sandy loam. Mull type of humus was noted in both experimental sites.

Both stands were situated on the slopes of northern exposure. The health condition of test stands was good, as in the period of 2008-2013 neither dead lying trees infested by bark beetles nor severe drought were observed.

In the first half of May fir branches and tree tops left after logging conducted in the period of March-April, uninfested earlier by insects, were partially stored in 1.50 m-high piles, whereas the remaining LR were scattered disorderly on the forest floor over an area of 20x100 m in both sites. In total, four piles and 2 plots with material loosely scattered were established at each study site.

Also at each study site, both the material collected in piles and loosely scattered was located under partial cover of trees.

The minimum distance between the piles was 10 m, while the plots with material scattered disorderly were situated at a distance of minimum 30 m away from the piles. Entomological analyses were performed from early June to late September, at 2 week intervals. Each time, 15 branches and tree tops, taken randomly from the piles of three separate layers (5 pieces for each layer): external (depth of about 30 cm, measured from the top to the centre of the pile), middle (31-60 cm measured from the top to the interior of the pile) and bottom (at a depth of 60 cm, measured from the top to the interior of the pile) were inspected. At the same time, 15 pieces of material left loosely on the study plot were analysed. In total, 540 pieces of fir branches and tree tops, including 270 pieces for each of the LR disposal method were examined. The average dimensions of the analysed LR reached the length of 1.70 m and 7.15 cm for diameter measured at the half length of the material.

Physiological condition of each tree top and branch was assessed in relation to 4 characteristics (Table 1). Identification of insects and calculating the number of galleries were performed after debarking the entire length of the branch growing directly from the trunk. To determine the xylobiont insects associated with pioneer cambio-phages colonising LR, 20 cm long pieces (N=60) from each layer of the pile and plot with material scattered disorderly were collected at random in the first half of December 2013. Then, the samples were taken to the laboratory where they were kept in plastic cylinders secured from the top with a fine-mesh curtain, for a period of five months, until hatching of insects. Thermal conditions prevailing in the storage material fluctuated around 20°C and 60% for relative air humidity. For insects whose species identification was not possible, lower taxon was assigned.

The prevalence of phloemophages on the analysed fir LR was characterised by the frequency expressed as the percentage of the analysed material colonised by a given species and by the infestation density, expressed as the number of exit holes or galleries of a given insect species per bark area. The Simpson diversity index (D)

was used to compare the xylobiont entomofauna diversity (Simpson 1949) and the species richness (S) was given as the number of species per sample.

Table 1. Parameters and classification determining the physiological condition of the analysed Silver fir branches and tree tops.

Parameter	Degree	Criteria for evaluation
Bark decomposition (BDC)	1	bark fresh and strongly adherent to the wood
	2	bark dry and strongly adherent to the wood
	3	dry and loose bark
	4	moist and loose bark
Bark coverage (BC)	1	bark cover of 100%
	2	bark cover above 75% up to 100%
	3	bark cover above 50% up to 75%
	4	bark cover from 25% to 50%
	5	bark cover below 25%
Presence of needles (NP)	1	green needles
	2	yellow needles
	3	brown needles
	4	no needles
Phloem and cambium decomposition (PCDC)	1	phloem living and undecomposed
	2	phloem partly dead, partly decomposed
	3	phloem dead and completely decomposed

The relationships between the species of phloemophages and the physiological condition of the material as well as the study site habitat condition were presented using correspondence analysis (CA). The frequency of insects was statistically compared ( $P \leq 0.05$ ) between LR treatment method and their location inside the pile using the chi-square test. The infestation density of fir LR by the most frequent species of bark beetles, taking into consideration the variant of the material treatment and position within the pile was examined using the Mann–Whitney U and Kruskal–Wallis tests. To demonstrate the effect of microenvironmental conditions on branch and tree top infestation density by the selected species of bark beetles, the general linear model (GLM) was used with study site localisation, variant of LR treatment and the physiological condition of the material as qualitative factors and temperature and relative humidity as continuous predictor variables. Kruskal–Wallis test was used to express the preferences of bark beetles for the physiological condition of fir LR. Statistical analyses were performed in STATISTICA software, Version 10.0 (StatSoft, Inc., Tulsa USA).

## RESULTS

During the period of June-September 2013, fir branches and tree tops at the study sites were colonised by 4 species of bark beetles (Coleoptera) from the family Curculionidae, i.e. *Pityophthorus pityographus* (Ratzeburg, 1837), *Pityokteines vorontzowi* (Jakobson, 1895), *Cryphalus piceae* (Ratzeburg, 1837) and *Pityokteines curvidens* (Germar, 1824). Regardless of study site, the highest prevalence on the analysed material was observed for *P. pityographus*, that prefers branches and tree tops scattered disorderly on the forest floor rather than collected in piles (FUBF:  $\chi^2=9.968$ ,  $df=1$ ,  $p<0.05$ ; FMBF:  $\chi^2=19.377$ ,  $df=1$ ,  $p<0.05$ ) (Table 2). On the other hand, the presence of *P. pityographus* inside the piles differs between the tested areas. Nevertheless, the location of the material within the piles did not have significant influence on the occurrence of bark beetles (Table 3) ( $\chi^2=3.082$ ,  $df=2$ ,  $p>0.05$ ). *P. vorontzowi*, in contrast to *P. pityographus* was more prevalent on the material collected in piles, than in the one scattered loosely on the study plot (Table 2) (FUBF:  $\chi^2=12.007$ ,  $df=1$ ,  $p<0.05$ ; FMBF:  $\chi^2=34.020$ ,  $df=1$ ,  $p<0.05$ ). The frequency index of *P. vorontzowi* reached the highest values in the lower pile layer, but the location of branches and tree tops inside the piles, similarly as in the case of *P. pityographus* did not influence significantly their attractiveness for this insect species in either of the study sites (Table 3) (FUBF:  $\chi^2=2.997$ ,  $df=2$ ,  $p>0.05$ ; FMBF:  $\chi^2=3.173$ ,  $df=2$ ,  $p>0.05$ ). The frequency of *C. piceae* and *P. curvidens* did not exceed 3% (Table 2).

Table 2. The percentage of Silver fir branch and tree top colonisation by phloemophages and the mean infestation density\* by the most frequently occurring insect species (indiv.dm<sup>-2</sup>) (in parentheses), with regard to the utilisation method.

Forest habitat	Species	Logging residues treatment method	
		Pile	Scattered disorderly
Fresh upland broadleaved forest	<i>P. pityographus</i>	52.59 (0.35)	73.33 (0.34)
	<i>P. vorontzowi</i>	39.25 (0.32)	18.51 (0.23)
	<i>P. curvidens</i>	2.96	0.74
	<i>C. piceae</i>	2.22	0.00
No. of analyzed branches		135	135
Fresh mountain broadleaved forest	<i>P. pityographus</i>	48.88 (0.46)	75.55 (0.34)
	<i>P. vorontzowi</i>	41.48 (0.37)	10.37 (0.17)
	<i>P. curvidens</i>	2.22	0.00
	<i>C. piceae</i>	0.00	0.74
No. of analyzed branches		135	135

\*Explanation: the mean values of infestation density indices were calculated for the cases where the presence of bark beetles on branches and tree tops was confirmed.

The correspondence analysis showed similar affinity of bark beetles to the LR in different physiological condition. The insects colonised mostly branches in the initiation phase (2 degree) of bark, cambium and phloem decomposition. However,

*C. piceae*, *P. vorontzowi* and *P. pityographus* were related to residues collected in piles, whereas *P. curvidens* was mostly associated with branches scattered loosely on the forest floor (Fig. 1).

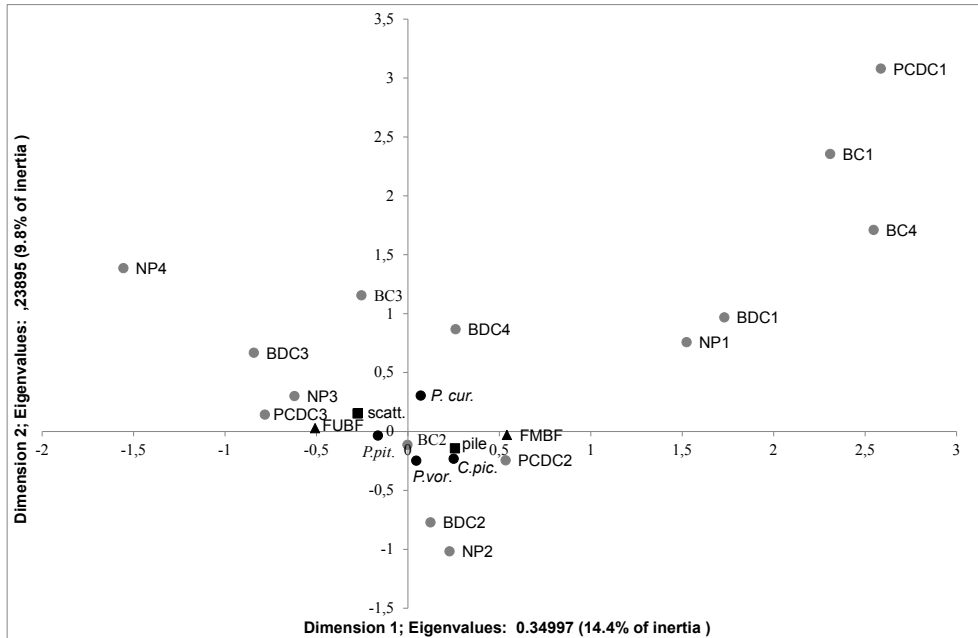


Fig. 1. Correspondence analysis (CA) showing the relationship between the prevalence of bark beetles and physiological condition of LR, their utilisation method and forest habitats. Species of bark beetles (*P. vor.*-*Pityokteines vorontzowi*, *P. cur.*-*Pityokteines curvidens*, *C. pic.*-*Cryphalus piceae*, *P. pit.*-*Pityophthorus pityographus*) are represented by black points, forest habitats-by black triangles, LR treatment method by black squares, physiological condition of the material (BDC-bark decomposition, BC-bark coverage, NP-presence of needles, PCDC-phloem and cambium decomposition) by grey points

It was observed that the mean LR infestation density by *P. pityographus* and *P. vorontzowi* was higher when the material was arranged into the piles rather than randomly spread over the study plot. However, only in the case of *P. vorontzowi* colonising fir LR in FMBF habitat the method of material disposal was a factor which significantly influenced the infestation density (Mann - Whitney U test:  $Z=-2.533$ ,  $p<0.05$ ) (Table 2).

There was a significant increase in LR infestation by bark beetles in deeper pile layers (Table 3) (Kruskall-Wallis test: *P. pityographus*, FMBF:  $H(2, N=56)=18.852$ ,  $p=0001$ ; *P. vorontzowi*, FUBF:  $H(2, N=72)=6.505$ ,  $p=03870$ ; FMBF:  $H(2, N=67)=21.445$ ,  $p=0000$ ).

It was demonstrated that the forest habitat, LR utilisation method and microenvironmental conditions had no impact on the infestation density by bark beetles. However, there was a significant correlation with physiological conditions of fir LR (Table 4).

### The First Stages of Xylobiont Entomofauna

Table 3. The percentage of Silver fir branch and tree top colonisation by phloemophages and the mean infestation density\* by the most frequently occurring insect species (ndiv.dm<sup>-2</sup>) (in parentheses), with regard to the position of logging residues inside the pile.

Forest habitat	Insect species	Pile layer		
		External	Middle	Bottom
Fresh upland broadleaved forest	<i>P. pityographus</i>	60.00 (0.27)	46.67 (0.34)	51.11 (0.45)
	<i>P. vorontzovi</i>	31.11 (0.18)	37.77 (0.40)	46.66 (0.69)
	<i>P. curvidens</i>	2.22	0.00	4.44
	<i>C. piceae</i>	4.40	4.40	0.00
No. of analyzed branches		45	45	45
Fresh mountain broadleaved forest	<i>P. pityographus</i>	42.22 (0.21)	46.67 (0.33)	60.00 (0.39)
	<i>P. vorontzovi</i>	26.67 (0.15)	46.67 (0.33)	48.00 (0.55)
	<i>P. curvidens</i>	0.00	0.00	0.00
	<i>C. piceae</i>	1.22	0.00	0.00
No. of analyzed branches		45	45	45

\*Explanation: the mean values of infestation density indices were calculated for the cases where the presence of bark beetles on branches and tree tops was confirmed.

Table 4. The results of multivariate analysis of variance based on the general linear model (GLM) for the infestation density of Silver fir fine woody debris by bark beetles, with regard to the forest habitat, LR treatment method and their physiological condition.

Effect	<i>P. pityographus</i>		<i>P. vorontzovi</i>		Total insects	
	F	<i>p</i>	F	<i>p</i>	F	<i>p</i>
Forest habitat	0.19	0.6604	0.24	0.6252	0.28	0.5989
BDC	4.96	0.0021	3.50	0.0197	18.16	0.0000
PCDC	3.58	0.0285	0.69	0.4993	11.22	0.0000
Forest habitat x LR treatment method	0.33	0.5638	0.68	0.4097	0.02	0.9657
Forest habitat x LR treatment method x BDC	0.75	0.5240	2.84	0.0152	2.54	0.0275
Forest habitat x LR treatment method x PCDC	0.69	0.5564	3.95	0.0036	5.40	0.0003
Forest habitat x Temperature	0.66	0.4181	2.77	0.0968	3.66	0.0562
Forest habitat x Temperature x Humidity	0.93	0.3358	1.29	0.2572	3.03	0.0824

The more advanced the process of LR bark and phloem decomposition, the higher infestation density of material by bark beetles was detected (Fig. 2).

A laboratory analysis of the LR fragments coming from piles and plots with material scattered disorderly showed the presence of cambiohages such as the six-toothed spruce bark beetle (*Pityogenes chalcographus* L., 1761) (Coleoptera, Scolytinae) and xylophages represented by the greater horntail wasp (*Urocerus gigas* L., 1758) (Hymenoptera, Siricidae) (both male and female). The occurrence of saprophagous insects of the order Diptera and the family Mycetophilidae, being

the most numerous group of early-arriving xylobiont entomofauna on the analysed fir LR, was also confirmed. Among the insect predators, the bark-gnawing beetles (*Nemosoma elongatum* L., 1761) (Coleoptera, Trogossitidae) and typical bugs such as *Scoloposcelis pulchella* (Zetterstedt, 1838) (Hemiptera, Anthocoridae) were recorded. The parasites of bark beetles were represented by insects of the family Braconidae (Hymenoptera), whereas on LR collected in piles in FUBF parasitoid the Ibalid wasp (*Ibalia leucospoides* (Hochenwarth, 1785) (Hymenoptera, Ibalidae) was observed (Table 5).

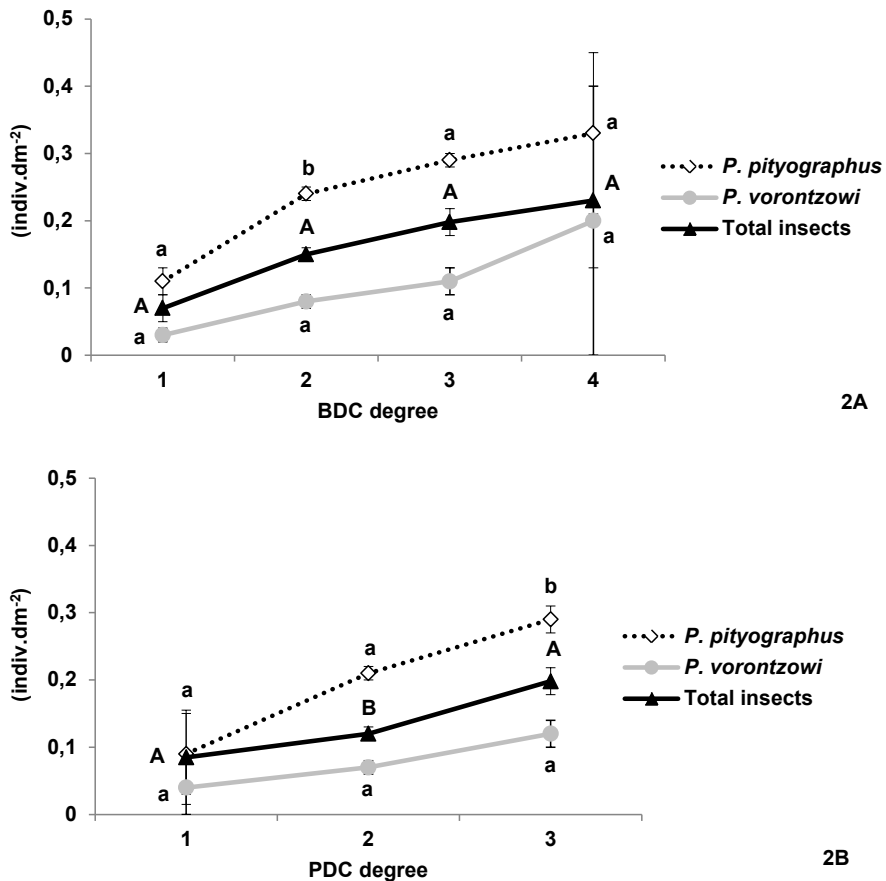


Fig. 2. Infestation density (mean  $\pm$ SE) of Silver fir logging residues by the most numerous bark beetle species and total insects in different phloem, cambium (A) and bark decomposition (B) degrees. For each species, the values indicated by the same letter are not significantly different at  $p \leq 0.05$ .

A laboratory analysis of the LR fragments coming from piles and plots with material scattered disorderly showed the presence of cambiohages such as the six-toothed spruce bark beetle (*Pityogenes chalcographus* L., 1761) (Coleoptera,

### The First Stages of Xylobiont Entomofauna

Scolytinae) and xylophages represented by the greater horntail wasp (*Urocerus gigas* L., 1758) (Hymenoptera, Siricidae) (both male and female). The occurrence of saprophagous insects of the order Diptera and the family Mycetophilidae, being the most numerous group of early-arriving xylobiont entomofauna on the analysed fir LR, was also confirmed. Among the insect predators, the bark-gnawing beetles (*Nemosoma elongatum* L., 1761) (Coleoptera, Trogossitidae) and typical bugs such as *Scoloposcelis pulchella* (Zetterstedt, 1838) (Hemiptera, Anthocoridae) were recorded.

Table 5. List and percentage of collected individuals of bark beetles and accompanying insects in Silver fir fine woody debris in the study plots in south-eastern Poland.

Taxonomic group	Fresh upland broadleaved forest		Fresh mountain broadleaved forest	
	sattered disorderly	collected in piles	sattered disorderly	collected in piles
<b>Diptera</b>				
Diptera spp.	7.84	61.11	20.00	12.50
Mycetophilidae	62.74	-	-	-
<i>Crypturgus hispidulus</i> Thom.	-	-	-	1.25
<b>Hymenoptera</b>				
Hymenoptera spp.	-	-	6.15	1.25
Braconidae	-	-	5.56	-
<i>Phloeopora corticalis</i> Grav.	-	-	9.23	-
<i>Ibalia leucospoides</i> Hoch.	-	5.56	-	-
<i>Urocerus gigas</i> L.	-	11.11	-	-
<b>Hemiptera</b>				
Anthocoridae spp.	3.92	5.56	-	-
<i>Cryptolestes ferrugineus</i> Steph.	1.96	-	-	-
<i>Scoloposcelis pulchella</i> Zett.	-	5.56	-	-
<i>Zeteotomus brevicornis</i> Erich.	-	-	-	1.25
<b>Coleoptera</b>				
<i>Nemosoma elongatum</i> L.	-	5.56	-	1.25
<i>Pityokteines vorontzowi</i> (Jacobs.) <sup>*</sup>	15.68	-	63.08	77.50
<i>Pityogenes chalcographus</i> L. <sup>*</sup>	-	-	1.54	-
<i>Pityophthorus pityographus</i> Ratz. <sup>*</sup>	7.84	-	-	-
Total insects	51	18	65	80
Species richness (S)	6	6	6	6
The Simpson diversity index (D)	0.58	0.63	0.56	0.38

\*Phloemophagous insect species infesting logging residues. Explanation: Frequency of insect occurrence expressed as the number of a given insect species per total number of insects collected x100

The parasites of bark beetles were represented by insects of the family Braconidae (Hymenoptera), whereas on LR collected in piles in FUBF parasitoid the Ibalid wasp (*Ibalia leucospoides* Hochenwarth, 1785) (Hymenoptera, Ibalidae) was observed (Table 5).

The similar saproxylic insect diversity (D) was observed in the case of LR scattered disorderly on the study plots, regardless of the forest habitat. In the case of branches and tree tops collected in piles, the difference between the index values was high (39.7%) in favour of LR left in FUBF habitat. No impact of forest habitat and LR disposal method on xylobiont entomofauna species richness (S) was proved (Table 5).

## DISCUSSION AND CONCLUSIONS

The positive influence of post-harvesting LR on biodiversity of forest ecosystems in terms of soil enrichment in nutrients, preventing soil erosion and the creation of places for the development and shelter for many animal groups was demonstrated by numerous authors (Benson *et al.*, 1980; Bengtsson *et al.*, 1997; Proe *et al.*, 2001; Ecke *et al.*, 2002; Gunnarsson *et al.*, 2004; Jonsell, 2008a; Fossetøl and Sverdrup-Thygeson, 2009; Briedis *et al.*, 2011). Brin *et al.* (2011) shown that the presence of both large and small size fragments of residues left in the forest after logging operations, inhabited by various species of saproxylic insects, is an important biodiversity-increasing factor. On the other hand during considerable accumulation of breeding material in the form of branches and tree tops in the forest, insect pests may pose a threat to the vitality of the surrounding stands (Grijpma and Schuring, 1984; Hedgren *et al.*, 2003). Cambio-xylophages infesting LR following the breeding success on the studied material can colonise the tops of weakened and/or even healthy trees, playing an important role in the process of forest decline (Starzyk, 1996; Grodzki, 2004, 2010; Kacprzyk, 2014).

Based on the conducted entomological analysis of post-harvesting fir branches, 4 species of bark beetles were reported regardless of the forest habitat and the method of material disposal. The species identified on the analysed material are common cambiophagous insect pests of coniferous stands in the Carpathians, colonising the tops of trees (Starzyk *et al.*, 2008b). The observed lack of differences in the species composition of insects occurring on fir branches and tree tops between the applied disposal variants is quite unexpected. In stands with increased prevalence of cambio-xylophagous insects the colonisation is observed in the material of different physiological condition (Kacprzyk, 2014; Kacprzyk and Bednarz, 2015), whereas in healthy fir stands, with no bark beetles pressure, such as in the study sites, larger selection in choosing places for settlement by insects should be expected. Due to the absence of available breeding and feeding base for insects in the forest, the quality of dried LR becomes crucial for the bark beetles. However, all cambiophages identified on the fir branches and tree tops are thermophilic species with similar thermal and humidity requirements. We suggest that this, to some extent, blurs the differences in the species composition of bark beetles between the treatment methods of LR,

### *The First Stages of Xylobiont Entomofauna*

that could have been observed in bark beetles characterised by different ecology of occurrence. Similar preferences of different species of bark beetles in relation to the physiological condition of the analysed material seem to also confirm it. At the same time, no differences were observed in the insect species composition colonising fir LR between forest habitats. It may indicate that the position above the sea level, the species composition of trees, geological structure and soil subtype are of secondary importance for early arriving entomofauna, especially for bark beetles characterised by high ecological plasticity in the material selection, as identified in the case of cambio-phagous insects of fir LR.

The conducted study indicates that leaving fir branches and tree tops loosely on the forest floor was more favourable to the prevalence of *P. pityographus*, as the most numerous insect colonising fir LR, than collecting the material in piles. The opposite relationship was observed for the second most abundant species on the analysed LR, i.e. *P. vorontzowi*. Interestingly, the arrangement of the material in the pile does not have significant effect on the bark beetles frequency, while the location of branches and tree tops within the piles have a significant impact on the infestation density of LR by *P. vorontzowi* and *P. pityographus*. For both insect species an increased density of galleries in deeper layers of piles was recorded. This is contrary to the results obtained by Kacprzyk (2014), who proved that spruce branches deposited in the upper layers of piles are more attractive for *P. pityographus* than those located in the bottom layers.

For the most frequently observed insect species the mean number of galleries does not exceed one per 1 dm<sup>2</sup> of bark area. Such low value of this index can probably confirm good health condition and vigour of trees in the study sites, where the current population of *P. pityographus* and *P. vorontzowi* does not pose a threat to the stability of the examined stands.. Moreover, low infestation density of fir branches and tree tops by bark beetles may result from the material location under partial cover of trees. A negative correlation between infestation density of spruce tops by cambio-xylophagous insects and stocking index of stands was proved by Röker (1986) and Kula *et al.* (2011). Also Kacprzyk (2014) observed that the variability of thermal conditions related to the location and the utilisation method of spruce LR significantly affects the infestation density of the material colonised by *P. chalcographus*, *P. pityographus* and *Dryocoetes autographus* (Ratzeburg, 1837) (Coleoptera: Scolytinae). In the presented experiment the effect of utilisation method and microenvironmental conditions on the infestation density of branches and tree tops by cambium-feeding insects was not significant. However, strong positive correlation between the density of bark beetle galleries on LR and advancement in the process of the material phloem and cambium decomposition was proved. It may result from the fact that the colonisation of the LR by insects increases with time and with progressing decomposition of the material.

No effect of utilisation method and site conditions in the place of fir LR storage on xylobiont entomofauna species richness was found. Nevertheless branches and tree tops collected in piles were characterized by a slightly higher share of beneficial entomophagous insects, in relation to the second LR treatment method. Moreover, entomophagous insects were more numerous on material left in FUBF forest site,

than in FMBF forest site. The presence of saprophages, predators and parasitoids, beneficial to the environmental biodiversity, was proved. Saprophagous insects play an important role in forest ecosystems, as their adults and larvae - by the fragmentation of organic material-contribute to its decay and enrich the soil humus layer with valuable nutrients (Bańkowska, 1981; Soszyński *et al.*, 2000). Predatory insects and parasitoids are important in biological control of insect pests (Johansson *et al.*, 2007; Stojanović and Marković, 2007). In our study the group of beneficial insects was mostly represented by saprophagous insects from order Diptera and the family Mycetophilidae. The share of natural enemies of insect pests was relatively small and did not exceed 15%.

Grodzki (2010) emphasises, that there is still no detailed data on entomophagous organisms as regulators of the prevalence of Silver fir cambiophagous insect pests. Therefore, it seems to be necessary for conducted such experiments in a wider range by taking into account other environment conditions and longer period of leaving LR in the forest.

In conclusion, the low degree and colonisation intensity of fine woody debris of fir by cambiophagous insects coupled with frequent occurrence of saprophagous insect species, as well as parasites and predators, indicate that the fir branches and tree tops left after cuttings on the forest floor is desirable for the biodiversity protection and does not threat the stability of fir stands in the study sites.

## ACKNOWLEDGEMENTS

The authors would like to thank the management staff of the Lesko Forest District for enabling the study and Msc. Katarzyna Misiura for helping in the field works. The research was financed by the Polish Ministry of Science and Higher Education by the statutory research mechanism.

## REFERENCES

- Bańkowska, R., 1981, Bzygowate (Syrphidae, Diptera) (Hoverfly fly (Syrphidae, Diptera)). *Fragmenta Faunistica*, 26(25): 407-420 (in Polish).
- Bengtsson, J., Persson, T., Lundkvist, H., 1997, Long-term effects of logging residue addition and removal on macroarthropods and enchytraeids. *Journal of Applied Ecology*, 34, 1014-1022.
- Brauns, A., 1975, *Owady Leśne. Występowanie Na Tle Drzewostanów I Siedlisk* (Insects in Forests. Occurrence of Insects In Relation to Forest Habitats). PWRiL, Warszawa, Poland, 624 (in Polish).
- Brin, A., Bouget, C., Brustel, H., Jactel, H., 2011, Diameter of downed woody debris does matter for saproxylic beetle assemblages in temperate oak and pine forests. *Journal of Insect Conservation*, 15(5): 653-669.
- Benson, R. E., Schleiter, J. A., 1980, *Logging residues in principal forest types of the Northern Rocky Mountains*. USDA Forest Service Research Paper INT. 260. Ogden, UT: U.S. Department of Agriculture, Forest Service, Intermountain Forest and Range Experiment Station, 14.
- Briedis, J. I., Wilson, J. S., Benjamin, J. G., Wagner, R. G., 2011, Logging residues volumes and characteristics following integrated roundwood and energy-Wood whole-Tree harvesting in central maine. *Northern Journal of Applied Forestry*, 28(2): 66-71.

### The First Stages of Xylobiont Entomofauna

- Ecke, F., Löfgren, O., Sörlin, D., 2002, Population dynamics of small mammals in relation to forest age and structural habitat factors in northern Sweden. *Journal of Applied Ecology*, 39(5): 781-792.
- Fossetøl, K. O., Sverdrup-Thygeson, A., 2009, Saproxylic beetles in high stumps and residual downed wood on clear-cuts and in forest edges. *Scandinavian Journal of Forest Research*, 24(5): 403-416.
- Grégoire, J. C., Evans, H. F., 2004, *Damage and control of BAWBILT organisms an overview*. In: Lieutier, F., Day, K. R., Battisti, A., Grégoire, J. C., Evans, H. F. (Eds.). *Bark and wood boring insects in living trees in Europe: A synthesis*. Dordrecht, Boston and London, Kluwer Academic Publishers, 19-37.
- Grijpma, P., Schuring, W. 1984, De ontwikkeling van debastkevers *Ips typographus*, *Ips cembrae* en *Pityogenes chalcographus* in niet-marktwaardig dunningshout (Development of the bark beetles *Ips typographus*, *Ips cembrae* and *Pityogenes chalcographus* in non-marketable thinning material). *Nederlands Bosbouw Tijdschrift*, 56:159-164.
- Grodzki, W., 2004, Zagrożenia górskich drzewostanów świerkowych w zachodniej części Beskidów ze strony szkodników owadzych (Threat to Norway spruce stands of insect pests in the western part of Beskid Mountains). *Leśne Prace Badawcze*, 2:35-47 (in Polish).
- Grodzki, W., 2010, Stan zdrowotny i zagrożenie jodły *Abies alba* i świerka *Picea abies* w Pienińskim Parku Narodowym (The health condition and threat to Silver fir and Norway spruce stands in Pieniński National Park). *Pieniny-Przyroda i Człowiek*, 11: 55-67 (in Polish).
- Gunnarsson, B., Nittérus, K., Wirdeñäs, P., 2004, Effect of logging residue removal on ground-active beetles in temperate forests. *Forest Ecology and Management*, 201(2): 229-239.
- Gutowski, J. M., 2006, Saproksyliczne chrząszcze (Saproxylic beetles). *Kosmos. Problemy Nauk Biologicznych*, 270: 53-73 (in Polish).
- Hedgren, P. O., Weslien, J., Schroeder, L. M., 2003, Risk of attack by the bark beetle *Pityogenes chalcographus* (L.) on living trees close to colonized felled spruce trees. *Scandinavian Journal of Forest Research*, 18(1): 39-44.
- Hilszczański, J., Gibb, H., Hjältén, J., Atlegrim, O., Johansson, T., Pettersson, R. B., Ball, J. P., and Danell, K., 2005, Parasitoids (Hymenoptera, Ichneumonoidea) of saproxylic beetles are affected by forest successional stage and dead wood characteristics in boreal spruce forest. *Biological Conservation*, 126(4): 456-464.
- Jaworski, A., Pach, M., 2014, A comparison of lower montane natural forest (*Abies*, *Fagus*, *Picea*) in Oszaśt Reserve and spruce monocultures in the Żywiecki Beskid and Śląski Beskid. *Forest Research Papers*, 75(1): 13-23.
- Johansson, T., Gibb, H., Hjältén, J., Hilszczański, J., Alinvi, O., Ball, J. P., Danell, K., 2007, The effects of substrate manipulations and forest management on predators of saproxylic beetles. *Forest Ecology and Management*, 242(2): 518-529.
- Jonsell, M., 2008a, *The effects of forest biomass for energy*. In: Röser, D., Asikainen, A., Raulund Rasmussen, K., Stupak, I. (Eds.). *Sustainable use of forest biomass for energy managing forest ecosystems. A Synthesis with Focus on the Baltic and Nordic Region*, Springer, Netherlands, 129-154.
- Jonsell, M., 2008b, Saproxylic beetle species in logging residues: which are they and which residues do they use? *Norwegian Journal of Entomology*, 55(1): 109-122.
- Kacprzyk, M., 2012, Feeding habits of *Pityogenes chalcographus* (L.) (Coleoptera: Scolytinae) on Norway spruce (*Picea abies*) L. (Karst.) logging residues in wind-damaged stands in southern Poland. *International Journal of Pest Management*, 58(2): 121-130.
- Kacprzyk, M., 2014, Wpływ warunków mikrośrodowiskowych na zasiedlenie przez entomofaunę kambio-ksylofagiczną gałęzi świerkowych pozostawianych w drzewostanach po cięciach gospodarczych (Effect of microsite conditions on colonization of cambio-xylophagous insects on Norway spruce branches left after the silvicultural treatments). *Sylvan*, 158(10): 761-768 (in Polish with English summary).
- Kacprzyk, M., Bednarz, B., 2015, The occurrence of bark beetles on Norway spruce branches left after cuttings in managed stands in relation to the branch transpiration area. *Journal of Forest Research*, 20(1):143-150.

- Kenis, M., Wermelinger, B., Grégoire, J. C., 2004, Research on parasitoids and predators of Scolytidae - A Review. In: Lieutier, F. et al. (Eds.). Bark and Wood Boring Insects in Living Trees in Europe, a Synthesis. Springer, Netherlands, 237-290.
- Kula, E., Kajfosz, R., Polívka, J., 2011, Cambioxylophagous fauna developing on logging residues of blue spruce (*Picea pungens* Engelmann). *Journal of Forest Science*, 57(1): 24-33.
- Lindhe, A., Lindelöw, Å., 2004, Cut high stumps of spruce, birch, aspen and oak as breeding substrates for saproxylic beetles. *Forest Ecology and Management*, 203(1): 1-20.
- Pawlaczyk, E. M., Grzebyta, J., Bobowicz, M. A., Korczyk, A. F., 2005, Individual differentiation of *Abies alba* Mill. population from the Tisovik Reserve. Variability expressed in morphology and anatomy of needles. *Acta Biologica Cracoviensia, Series Botanica*, 47(2): 137-144.
- Proe, M. F., Griffiths, J., McKay, H. M., 2001, Effect of whole-tree harvesting on microclimate during establishment of second rotation forestry. *Agricultural and Forest Meteorology*, 110(2), 141-154.
- Röker, F., 1986, Kupferstecherbefall nach Durchforstung. *Allgemeine Forst Zeitschrift*, 14: 328.
- Simpson, E. H., 1949, Measurement of diversity. *Nature*, 163: 688.
- Soszyński, B., Palaczyk, A., Krzemiński, W., 2000, Zagrożenia i perspektywy ochrony móchówek (Diptera) w Polsce (Threats and protection perspective for flies (Diptera) in Poland). *Wiadomości Entomologiczne*, 18(2):165-176 (in Polish).
- Starzyk, J. R., 1996, Bionomics, ecology and economic importance of the fir weevil *Pissodes piceae* (Col. Curculionidae) in mountain forests. *Journal of Applied Entomology*, 120(1-5): 65-75.
- Starzyk, J. R., Bilecka, K., Purgal, M., Rotman, K., 2008a, Cambio and xylophagous insects infesting Scots pine (*Pinus sylvestris* L.) cut off tree-tops and branches left in the forest after thinnings and final cuttings. *Acta Scientiarum Polonorum, Silvarum Colendarum Ratio et Industria Lignaria* 1(7): 59-74.
- Starzyk, J. R., Gajewski, J., Habel, K., 2008b, Owady kambio- i ksylofagiczne zasiedlające odcięte wierzchołki i gałęzie jodłowe pozostające po różnego rodzaju cięciach w drzewostanie [Cambio- and xylophagous insects infesting European fir (*Abies alba* Mill.) cut off tree-tops and branches left in the forest after cuttings]. *Acta Agraria et Silvicultura. Series Silvestris*, 46: 3-19 (in Polish with English summary).
- Stojanović, A., Marković, C., 2007, The hymenopteran parasitoids of some elm bark beetles in Serbia. *Phytoparasitica*, 33(3): 239-243.
- Tinner, W., Colombaroli, D., Heiri, O., Henne, P. D., Steinacher, M., Untenecker, J., Vescovi, E., Allen, J. R. M., Carraro, G., Conedera, M., Joos, F., Lotter, A. F., Luterbacher, J., Samartin, S., Valsecchi, V., 2013, The past ecology of *Abies alba* provides new perspectives on future responses of silver fir forests to global warming. *Ecological Monographs*, 83(4): 419-439.
- Ząbecki, W., Kacprzyk, M., 2007, A potentiality of using spruce branches left in the forest after incidental cuttings to attract *Pityogenes chalcographus* (L.). *Beskydy*, 20: 184-192.
- Zeniauskas, R., Gedminas, A., 2010, Insects infesting Norway spruce (*Picea abies* Karst.) branches in clear-cuts and adjacent stands. *Baltic Forestry*, 16(1): 93-101.

Received: April 08, 2016

Accepted: December 27, 2016

## The Effects of 5-Aza-2'-deoxycytidine on Total Lipid and Fatty Acid Composition of *Apanteles galleriae* Wilkinson (Hymenoptera: Braconidae) and on Its Parasitized Host

Olga SAK

Department of Biology, Faculty of Science-Literature, Balikesir University, Balikesir,10145, TURKEY e-mail: altun@balikesir.edu.tr

### ABSTRACT

I investigated the changes in total lipid, total fatty acids, and fatty acid composition of endoparasitoid *Apanteles galleriae* Wilkinson (Hymenoptera: Braconidae) reared on *Achroia grisella* Fabr. (Lepidoptera: Pyralidae) that was exposed to various doses of 5-Aza-2'-deoxycytidine (5-Aza-dC), an epigenetically-effective agent. The combined effects of parasitism and 5-Aza-dC treatment on the total lipid and fatty acids of host larvae were also examined. 5-Aza-dC caused insignificant fluctuations in total lipid and fatty acid ratios of *A. galleriae* and parasitized host larvae (PHL). Total lipid and fatty acids reached considerably the highest values in PHL and the lowest ratios in parasitoid larvae for all of the control and experimental groups. All stages of *A. galleriae* and PHL contained the 14-24 carbon fatty acids. Palmitic acid was the most abundant fatty acid for PHL and parasitoid pupae in each testing group, while oleic acid for parasitoid larvae. 5-Aza-dC treatment caused significant changes in linolenic acid ratio for parasitoid pupa and in lignoceric acid ratio for PHL, while the percent of different fatty acid classes did not differ significantly for stages/PHL. However, eicosenoic acid could not be detected in both insect species at doses >0.1 mg/ml. There were significant differences among individual fatty acids and fatty acid classes (SFAs, UFAs, and PUFAs) for all stages/PHL. Besides, UFAs constituted the major proportion of fatty acids, whereas PUFAs were the smallest for all stages/PHL regardless of 5-Aza-dC treatment. 5-Aza-dC-induced changes for *A. galleriae*-*A. grisella* system seem to be considerable but the effect is reversible according to the result of fatty acid classes.

*Key words:* *Apanteles galleriae*, *Achroia grisella*, 5-Aza-dC, lipid, fatty acids.

### INTRODUCTION

Living in a natural balance, organisms give similar reactions to similar conditions even though they have dissimilar genetic structures. For instance, frequent usage of chemicals such as medicals and pesticides has carcinogenic, teratogenic, and mutagenic effects on all living organisms. As being the first demethylating agents, 5-Aza-2'-deoxycytidine (5-Aza-dC) and 5-azacytidine (5-AzaC) also negatively affect DNA methylation by similar metabolic pathways (Osgood and Seward, 1989; Schauenstein *et al.*, 1991; Prakash and Kumar, 1997; Lantry *et al.*, 1999; Doerksen *et al.*, 2000; Sato *et al.*, 2003). Chemically synthesized initially in 1964 as a chemotherapeutic drug (Piskala and Sorm, 1964), 5-Aza-dC has a wide range of antimetabolic activities against cancer cells (Wijermans *et al.*, 2000; Christman, 2002;

Stresemann *et al.*, 2006; Gurion *et al.*, 2010). However, the influence of a chemical on an organism largely depends on the dose and the application method used. In this respect, it was shown that 5-Aza-dC and 5-AzaC could induce mutagenicity and cancer *in vivo* (Carr *et al.*, 1984; Jackson-Grusby *et al.*, 1997; Lantry *et al.*, 1999) and may be cytotoxic (Stresemann *et al.*, 2006). Hence, it becomes so important to determine the degree of toxic effects of these chemicals for all living organisms.

Investigating the effects of substances such as 5-Aza-dC on invertebrates as well as vertebrates will help us to have a better understanding the potential impact ways of toxic materials in all organisms as they descended from a common origin. However, there is little information about the extension of the toxicological effects of a cytosine analog, 5-Aza-dC especially on insects (Uçkan *et al.*, 2007; Amarasinghe *et al.*, 2014; Alvarado *et al.*, 2015). Besides, almost nothing is known about the effects of chemicals such as 5-Aza-dC on parasitoid species (Uçkan *et al.*, 2007; Pegoraro *et al.*, 2015). *Apanteles galleriae* Wilkinson (Hymenoptera: Braconidae) is a koinobiont, solitary, larval endoparasitoid of several lepidopterans, including the pyralid wax moths, *Galleria mellonella* L., *Achroia grisella* Fabr., *A. innotata* Walker, and *Vitula edmandsae* (Packard) (Shimamori, 1987; Watanabe, 1987; Whitfield *et al.*, 2001). Some data have been obtained previously on the changes of the lipid and the fatty acid ratio and composition during the development of the parasitoid, *A. galleriae* (Nurullahoğlu *et al.*, 2004; Uçkan *et al.*, 2009). The effects of 5-Aza-dC on the biological parameters of larval endoparasitoid *A. galleriae* have also been determined along the effect of this chemical on egg-adult development time of its host species, *A. grisella* (Uçkan *et al.*, 2007). 5-Aza-dC treatment increased time that is required to complete parasitoid immature development especially at 0.5 mg/ml dose. Adult longevity and size and the fecundity of parasitoid species were reduced by 5-Aza-dC exposure. The sex ratio of adults was in favor of males in 5-Aza-dC treatments. Moreover, exposure to 5-Aza-dC slightly increased the immature development of *A. grisella* and rarely caused some morphological disorders in the host such as reduced body size and curved-wings (Uçkan *et al.*, 2007). Therefore, I have developed our investigations on how this chemical affects lipid metabolism of the insects mentioned above in a host-parasitoid interaction. For the first time, the effects of 5-Aza-dC applied to host diet on total lipid, total fatty acids, and fatty acid composition were shown in *A. galleriae* and parasitized host larvae (PHL).

## MATERIALS AND METHODS

### Insects and bioassay

Laboratory colonies of the host, *A. grisella* and endoparasitoid, *A. galleriae* were established from adults that were collected from the honeycombs maintained from several beehives located in the vicinity of Rize, Turkey. Insect cultures and experimental groups were held in two different rooms at  $25\pm 1^\circ\text{C}$ ,  $60 \pm 5\%$  RH, and a photoperiod of 12:12h (L:D). The details of the method for cultivating both host and parasitoid species were presented in the articles of Uçkan and Gülel (2000) and Uçkan

### *The Effects of 5-Aza-2'-deoxycytidine*

and Ergin (2003). Honey solution with 30% (wt:v) was used to feed adult parasitoids. Host colony was maintained with a diet described by Bronskill (1961) and modified by Sak *et al.* (2006).

Experimental procedure was designed according to Uçkan *et al.* (2007). Briefly, 5-Aza-dC (EEC no.219-089-4, Sigma-Aldrich, St. Louis, MO) solutions prepared in distilled water were used in all bioassays as water source of host diet. Early instars of *A. grisella* were exposed to four different doses (0.1, 0.5, 0.75, and 1.0 mg/ml) of 5-Aza-dC to evaluate the effects of the chemicals on lipid and fatty acid characteristics of stages/PHL (defining larvae and pupae of *A. galleriae* and PHL).

#### **Lipid extraction**

Lipid analyses were conducted with the stages of *A. galleriae* and parasitized 14- to 16-d-old *A. grisella* larvae (PHL) in which the third (last) instar parasitoids were collected. I selected random samples of 35 third instars and 25 pupae of *A. galleriae* and 35 PHL, which were obtained from 5-Aza-dC treated and chemical-free host individuals for analyzing. Total lipid and fatty acid extractions were repeated three times at different times for stages/PHL with the same number given above. Total wet weight of each group was calculated, and insects were kept in 3 ml chloroform-methanol (2:1 vol:vol) at -20 °C until extractions. The lipid fractions in stages/PHL were extracted and total lipid and fatty acid contents were determined using the method described by Folch *et al.* (1957). Total lipid and fatty acid contents as a percentage of wet weight were calculated. Thereafter, lipid extracts were brought to 3 ml with chloroform-methanol (2:1 vol:vol) and kept at -20°C until fatty acid analysis.

#### **Fatty acid analysis-gas chromatography**

Fatty acid analysis was conducted at TUBITAK-BUTAL Research Centre (Bursa, Turkey) by using the direct methylation method. Briefly, solvents (chloroform-methanol) of lipid extracts were dried under N<sub>2</sub>. The residue was dissolved with 1 ml methanol, mixed with 3 ml HCl, and kept in water bath for 60 min at 95°C. Then 88% NaCl was added to the mixture and vortex-mixed. The esterified and methylated fatty acids were analyzed with a Perkin Elmer AutoSystem XL gas chromatograph equipped with a hydrogen flame ionization detector (FID). A fused silica capillary column (SP 2560; 100 m x 0.25 mm id, 0.20 µm film; Supelco, Supelco Park, PA) was used to separate the fatty acid samples. The FID parameters were optimized as follows: 260°C for detector temperature, 35 ml/min flow rate for hydrogen, and 450 ml/min flow rate for air. The column oven condition was scheduled as 120°C for 5 min, 4°C increase/min up to 240°C, and 240°C for 25 min. The helium flow rate in column was 1 ml/min. Identification of fatty acid methyl esters was achieved by comparison of their retention times with those of standards attained from Supelco. The percentage of each peak area for each fatty acid was calculated for each of the tested group.

#### **Statistical analysis**

Data for total lipid, total fatty acids, individual fatty acids, and fatty acid classes were subjected to one-way analysis of variance (ANOVA) to determine the main effects of

5-Aza-dC on each stage and PHL. The relationship between total lipid and stages/PHL, total fatty acids and stages/PHL, and the differences among the levels of individual fatty acids or fatty acid classes in all stages/PHL were also compared with one-way ANOVA for each control and 5-Aza-dC treated groups. Differences were separated by Tukey's honestly significant post hoc test (HSD) according to homogeneity of variances (SPSS Inc. 1999). An arcsine square-root transformation was performed on percentage values before analyses but untransformed means were presented. Results were considered statistically significant when  $P < 0.05$ .

## RESULTS

### Total lipid and fatty acids

The ratio of total lipid and fatty acids as percentages of wet weight for different stages of *A. galleriae* and PHL are presented in Figs. 1 and 2. The lipid values of PHL slightly increased on exposure to 5-Aza-dC while the fatty acid ratios tended to decrease in general. The dose, 0.75 mg/ml had the highest lipid value about 19% in PHL. 5-Aza-dC treatment caused both increases and decreases at the percent lipid and fatty acid of stages when compared to the untreated group. The metabolite contents did not differ significantly between controls and 5-Aza-dC treated groups in PHL (lipid ratio:  $F = 1.385$ ;  $df = 4, 10$ ;  $P > 0.05$ , fatty acid ratio:  $F = 2.214$ ;  $df = 4, 10$ ;  $P > 0.05$ ), parasitoid larvae (lipid ratio:  $F = 2.038$ ;  $df = 4, 10$ ;  $P > 0.05$ , fatty acid ratio:  $F = 1.313$ ;  $df = 4, 10$ ;  $P > 0.05$ ), and parasitoid pupae (fatty acid ratio:  $F = 1.930$ ;  $df = 4, 10$ ;  $P > 0.05$ ). However, there was a significant decrease only at 0.5 mg/ml according to 0.1 mg/ml in term of lipid values of parasitoid pupae ( $F = 4.100$ ;  $df = 4, 10$ ;  $P < 0.05$ ).

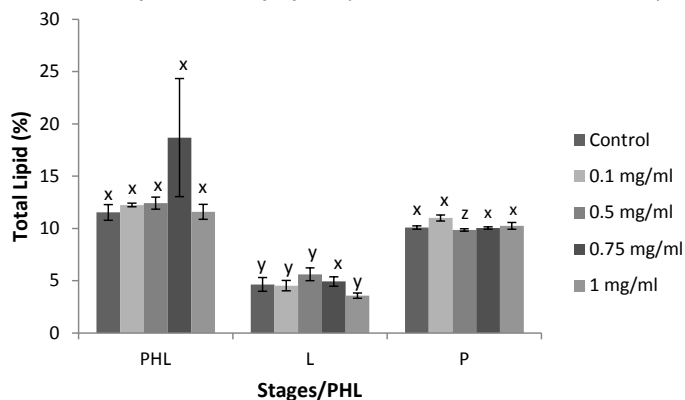


Fig. 1. 5-Aza-dC-related changes in total lipids of *A. galleriae* and *A. grisella*. PHL, parasitized host larva; L, parasitoid larva; P, parasitoid pupa. Stages/PHL (x-z) labeled with different letters are significantly different (Tukey's HSD test,  $P < 0.05$ ).

When stages and PHL were compared in terms of lipid and fatty acid ratios (Table 1), I found that both lipid and fatty acids of *A. galleriae* larvae were considerably lower than PHL (Figs. 1 and 2). However, the differences were only insignificant for lipids at 0.75 mg/ml and for fatty acids at 0.1 and 1 mg/ml 5-Aza-dC doses. Besides, the percent

### The Effects of 5-Aza-2'-deoxycytidine

of lipid and fatty acids significantly increased from larval to pupal stage especially at 1 mg/ml dose. The increase rate was not statistically important for lipids at 0.75 mg/ml and for fatty acids at 0.1 mg/ml (Figs. 1 and 2). Another observation was slightly decreasing of lipid and fatty acid ratios in parasitoid pupa according to PHL, which was only significant at 0.5 mg/ml. The only exception of this tendency was significantly higher fatty acid value of parasitoid pupa according to PHL at 1 mg/ml (Fig. 2).

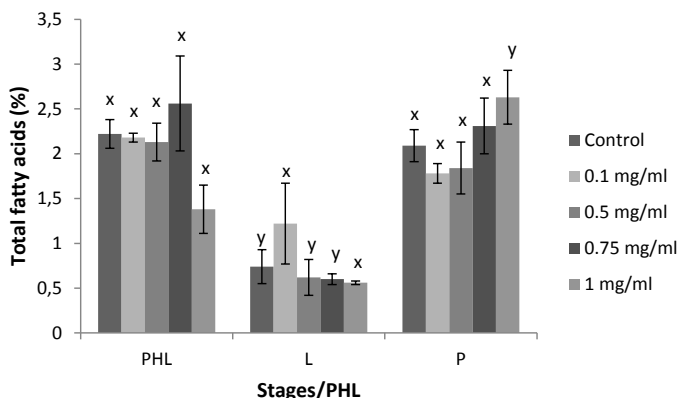


Fig. 2. 5-Aza-dC-related changes in total fatty acids of *A. galleriae* and *A. grisella*. PHL, parasitized host larva; L, parasitoid larva; P, parasitoid pupa. Stages/PHL (x-y) labeled with different letters are significantly different (Tukey's HSD test,  $P < 0.05$ ).

Table 1. ANOVAs of the differences among stages/PHL for each testing group in terms of total lipid and fatty acid ratios.

	Statistics	Control	0.1 mg/ml	0.5 mg/ml	0.75 mg/ml	1 mg/ml
Lipid	F	38.877	144.677	49.930	4.520	82.551
	df	2, 6	2, 6	2, 6	2, 6	2, 6
	P	0.000	0.000	0.000	0.063	0.000
Fatty Acid	F	21.033	3.255	11.333	9.049	20.340
	df	2, 6	2, 6	2, 6	2, 6	2, 6
	P	0.002	0.110	0.009	0.015	0.002

### Fatty acid composition

A total number of nine, sixteen, and twenty different fatty acids were identified in parasitoid larvae, pupae, and PHL, respectively. For stages/PHL,  $C_{14:0}$ ,  $C_{22:1}$ ,  $C_{22:2}$ , and  $C_{24:1}$  fatty acids were evaluated statistically but were not shown in the tables since the ratios of these fatty acids were almost zero. Moreover, I could not detect  $C_{14:1}$ ,  $C_{15:0}$ ,  $C_{15:1}$ ,  $C_{17:1}$ ,  $C_{18:3}$ ,  $C_{20:2}$ ,  $C_{20:3}$ ,  $C_{20:4}$ ,  $C_{21:0}$ ,  $C_{22:6}$ , and  $C_{23:0}$  fatty acids for parasitoid larvae and  $C_{14:1}$ ,  $C_{15:1}$ ,  $C_{20:3}$ , and  $C_{21:0}$  fatty acids for parasitoid pupae (Table 2-4). I found that all stages of *A. galleriae* and PHL contained the 14-24 carbon fatty acids.

Six fatty acids dominated in the composition in PHL and parasitoid pupae while five in parasitoid larvae. These fatty acids differed in order of plentitude in stages/PHL and were palmitic (C<sub>16:0</sub>), palmitoleic (C<sub>16:1</sub>), stearic (C<sub>18:0</sub>), oleic (C<sub>18:1</sub>), linoleic (C<sub>18:2</sub>), eicosenoic (C<sub>20:1</sub>), and arachidic (C<sub>20:0</sub>) acids. Palmitic acid was considerably the most abundant fatty acid for PHL (except for 0.5 mg/ml) and parasitoid pupae in all control and experimental groups, but oleic acid was significantly the highest one for parasitoid larvae (Table 2-4).

There were significant fluctuations among the levels of individual fatty acids in all stages/PHL (Table 2-5). However, 5-Aza-dC treatment caused significant changes only in the ratio of linolenic acid (C<sub>18:3</sub>) and lignoceric acid (C<sub>24:0</sub>) for parasitoid pupa and PHL, respectively (Table 2, 4). Besides, eicosenoic acid slightly decreased at 0.1 mg/ml dose and could not be detected at higher doses of 5-Aza-dC in PHL. Similarly, 5-Aza-dC also caused a prominent reduction in the ratio of eicosenoic acid in larval and pupal stages of parasitoid species (Table 3, 4). Moreover, C<sub>20:1</sub> was the fifth most dominant fatty acid in controls of both PHL and parasitoid pupae. However, arachidic acid arose to the fifth abundant fatty acid level at all doses of 5-Aza-dC in place of eicosenoic acid in PHL and parasitoid pupae (Table 2, 4). Tables 2-4 also show the fatty acid pattern of stages/PHL. There were significant differences between fatty acid classes (SFAs, UFAs, and PUFAs) (except for PHL at 1.0 mg/ml) (Table 2-4, 6) but the percent of different fatty acid classes did not differ significantly with respect to 5-Aza-dC application in all stages/PHL (Table 2-4, 7). Furthermore, UFAs constituted the major proportion of fatty acids in all stages/PHL except for 1 mg/ml in PHL, whereas PUFAs were the smallest, regardless of 5-Aza-dC treatment.

## CONCLUSIONS AND DISCUSSION

Parasitoid species alter the intermediary metabolism of their hosts and parasitization induces metabolic alterations in the fat body of hosts (Thompson, 1993; Salvador and Consoli, 2008). However, the regulation is species and host tissue-specific among parasitoid species (Thompson, 1993; Rivers and Denlinger, 1994; Bischof and Ortel, 1996) and do not cause a selective reduction or elevation of the lipid or fatty acid levels in the host body at any time (Thompson, 1982; Nurullahođlu *et al.*, 2004; Uđkan *et al.*, 2009). In the *A. galleriae*-*A. grisella* (Nurullahođlu *et al.*, 2004) and *A. galleriae*-*G. mellonella* (Uđkan *et al.*, 2009) systems parasitization did not cause a significant change in total lipid and fatty acids of host species. Hence, I decided to examine only the PHL for 5-Aza-dC-dependent effects on lipid and fatty acid ratios of host insect, *A. grisella* in the current paper. When all stages/PHL were evaluated collectively, the most noticeable changes (but insignificant) were an increase rate of 62% at 0.75 mg/ml for lipid values and a reduction ratio of 38% at 1 mg/ml for fatty acid values compared to controls in PHL. Besides, lipid and fatty acid ratios were sorted according to the abundance as PHL>parasitoid pupa>parasitoid larva in all groups except for 1 mg/ml dose being as parasitoid pupa>PHL>parasitoid larva. At this point, higher doses of 5-Aza-dC seem to have an adverse effect on PHL in terms of total lipid and fatty acid ratios. Significant decreasing of lignoceric acid (C<sub>24:0</sub>) ratio at 0.75 and

### The Effects of 5-Aza-2'-deoxycytidine

1 mg/ml 5-Aza-dC doses according to control and 0.1 mg/ml groups and destruction of eicosenoic acid ( $C_{20:1}$ ) at doses  $>0.1$  mg/ml support this assumption for PHL.

Table 2. 5-Aza-dC-related changes in fatty acid composition (%) of parasitized host larva <sup>a,b</sup>.

Fatty acids	Control	0.1 mg/ml	0.5 mg/ml	0.75 mg/ml	1 mg/ml
$C_{14:1}$	0.02 ± 0.02w a	---	0.13 ± 0.13w a	---	---
$C_{15:0}$	0.06 ± 0.03w a	0.04 ± 0.02w a	---	---	0.02 ± 0.02w a
$C_{15:1}$	---	0.02 ± 0.02w a	---	---	---
$C_{16:0}$	25.53 ± 0.51w b	25.02 ± 0.07w b	25.37 ± 0.55w b	27.17 ± 0.73w a	28.43 ± 1.44w b
$C_{16:1}$	21.99 ± 1.51w b	22.17 ± 0.59w b	21.31 ± 1.40w c	23.00 ± 1.17w b	15.54 ± 7.80w c
$C_{17:1}$	2.33 ± 1.23w a	2.73 ± 0.09w ac	---	---	---
$C_{18:0}$	2.34 ± 0.29w a	2.25 ± 0.18w ac	2.45 ± 0.13w a	2.28 ± 0.07w c	2.52 ± 0.40w a
$C_{18:1}$	14.09 ± 0.31w c	14.40 ± 0.21w d	15.55 ± 0.79w d	14.41 ± 0.63w d	16.54 ± 2.05w c
$C_{18:2}$	24.82 ± 1.89w b	24.69 ± 0.83w b	25.98 ± 1.00w b	24.76 ± 0.60w b	28.14 ± 3.53w b
$C_{18:3}$	0.88 ± 0.13w a	0.93 ± 0.18w ac	1.12 ± 0.28w a	0.88 ± 0.18w ce	0.60 ± 0.31w a
$C_{20:0}$	2.19 ± 2.08w a	2.15 ± 2.11w ac	6.49 ± 0.44w e	6.15 ± 0.45w f	7.12 ± 0.53w ac
$C_{20:1}$	4.04 ± 2.04w a	3.75 ± 1.88w c	---	---	---
$C_{20:3}$	---	---	---	---	0.21 ± 0.21w a
$C_{20:4}$	0.47 ± 0.03w a	0.61 ± 0.09w ac	0.76 ± 0.35w a	0.71 ± 0.38w ce	0.25 ± 0.13w a
$C_{21:0}$	---	---	---	0.03 ± 0.03w e	0.03 ± 0.02w a
$C_{22:0}$	0.44 ± 0.06w a	0.43 ± 0.01w ac	0.12 ± 0.12w a	0.28 ± 0.14w e	0.12 ± 0.12w a
$C_{22:6}$	---	---	0.16 ± 0.16w a	0.09 ± 0.09w e	0.08 ± 0.08w a
$C_{23:0}$	0.26 ± 0.14w a	0.22 ± 0.11w a	---	---	---
$C_{24:0}$	0.55 ± 0.08w a	0.58 ± 0.11w ac	0.23 ± 0.13w a	0.08 ± 0.08w e	---
$C_{20:2}$	---	---	0.33 ± 0.17w a	0.16 ± 0.16w e	0.40 ± 0.21w a
SFAs <sup>c</sup>	31.36 ± 2.71w ab	30.69 ± 2.13w a	34.65 ± 0.54w ab	35.99 ± 0.70w a	38.24 ± 2.33w a
UFAs	42.47 ± 4.21w b	43.08 ± 2.68w b	37.00 ± 1.92w b	37.41 ± 0.77w a	32.08 ± 5.81w a
PUFAs	26.17 ± 1.98w a	26.23 ± 0.57w a	28.35 ± 1.57w a	26.60 ± 1.10w b	29.68 ± 3.48w a

<sup>a</sup>Data are means ± SE of three replicates using 35 parasitized host larva per replicate.

<sup>b</sup>Means in the same horizontal row and group (fatty acids or fatty acid classes) followed by the same letter (w-x) and means in the same vertical column and group followed by the same letter (a-f) are not significantly different from each other (Tukey HSD testi,  $P>0.05$ ).

<sup>c</sup>SFAs, saturated fatty acids; UFAs, unsaturated fatty acids; and PUFAs, polyunsaturated fatty acids.

The lipid and fatty acid content of insects' show inter- and intraspecific variation (Downer, 1985) and defining the differences among insect species and different developmental stages contribute to the literature about insect physiology because of various metabolic functions (Ogg and Stanley-Samuelson, 1992; Ogg *et al.*, 1993; Çakmak *et al.*, 2007). Although PHL and parasitoid larvae had almost the same lipid

ratio, total fatty acids of parasitoid larvae were significantly higher than PHL according to our previous data (Nurullahoğlu *et al.*, 2004; Uçkan *et al.*, 2009). In contrast, I found in this study that both lipid and fatty acid values of *A. galleriae* larvae were considerably lower than PHL in control groups. The reason for this difference might be attributed to the use of different nutrients for host feeding, which was synthetic diet here versus natural blackened comb in our former studies (Nurullahoğlu *et al.*, 2004; Uçkan *et al.*, 2009). Considering all the results up to now, the significant difference in total fatty acids between the hosts and the parasitoid may also be related to variances among insect species. Another observation in this study was expressive increasing in lipid and fatty acid ratios during the pupal stage relative to the larval stage especially at 1 mg/ml dose.

Table 3. 5-Aza-dC-related changes in fatty acid composition (%) of *A. galleriae* larvae <sup>a,b</sup>.

Fatty acids	Control	0.1 mg/ml	0.5 mg/ml	0.75 mg/ml	1 mg/ml
C <sub>14:1</sub>	---	---	---	---	---
C <sub>15:0</sub>	---	---	---	---	---
C <sub>15:1</sub>	---	---	---	---	---
C <sub>16:0</sub>	12.95 ± 6.50w ab	16.26 ± 2.42w a	11.65 ± 4.51w a	20.67 ± 0.85w a	17.99 ± 0.43w a
C <sub>16:1</sub>	15.77 ± 0.84w a	11.02 ± 2.75w ab	7.73 ± 4.96w a	19.79 ± 2.04w a	16.60 ± 1.10w a
C <sub>17:1</sub>	---	---	---	---	---
C <sub>18:0</sub>	5.92 ± 1.15w abc	5.38 ± 1.02w bcd	2.62 ± 1.84w a	5.31 ± 0.60w b	5.47 ± 0.58w b
C <sub>18:1</sub>	59.12 ± 5.67w d	54.28 ± 6.11w e	72.29 ± 10.95wb	46.94 ± 0.56w c	53.58 ± 2.95w c
C <sub>18:2</sub>	4.77 ± 1.79w bc	9.26 ± 1.08w abc	4.45 ± 3.69w a	6.48 ± 2.50w b	6.04 ± 2.76w b
C <sub>18:3</sub>	---	---	---	---	---
C <sub>20:0</sub>	0.20 ± 0.20w c	0.19 ± 0.19w d	0.54 ± 0.54w a	---	0.31 ± 0.31w d
C <sub>20:1</sub>	---	0.65 ± 0.65w cd	---	---	---
C <sub>20:3</sub>	---	---	---	---	---
C <sub>20:4</sub>	---	---	---	---	---
C <sub>21:0</sub>	---	---	---	---	---
C <sub>22:0</sub>	0.84 ± 0.84w c	0.18 ± 0.18w d	---	0.16 ± 0.16w d	---
C <sub>22:6</sub>	---	---	---	---	---
C <sub>23:0</sub>	---	---	---	---	---
C <sub>24:0</sub>	0.43 ± 0.43w c	2.79 ± 2.79w bcd	0.71 ± 0.71w a	0.65 ± 0.65w d	---
C <sub>20:2</sub>	---	---	---	---	---
SFAs <sup>c</sup>	20.34 ± 4.34w a	24.80 ± 5.52w a	15.53 ± 6.07w a	26.79 ± 1.03w a	23.78 ± 0.39w a
UFAs	74.89 ± 6.13w b	65.95 ± 4.52w b	80.03 ± 7.10w b	66.73 ± 2.36w b	70.18 ± 2.37w b
PUFAs	4.77 ± 1.79w a	9.26 ± 1.08w a	4.45 ± 3.69w a	6.48 ± 2.50w c	6.04 ± 2.76w c

<sup>a</sup>Data are means ± SE of three replicates using 35 third instars per replicate.

<sup>b</sup>Means in the same horizontal row and group (fatty acids or fatty acid classes) followed by the same letter (w) and means in the same vertical column and group followed by the same letter (a-e) are not significantly different from each other (Tukey HSD testi, P>0.05).

<sup>c</sup>SFAs, saturated fatty acids; UFAs, unsaturated fatty acids; and PUFAs, polyunsaturated fatty acids.

### The Effects of 5-Aza-2'-deoxycytidine

Table 4. 5-Aza-dC-related changes in fatty acid composition (%) of *A. galleriae* pupae <sup>a,b</sup>.

Fatty acids	Control	0.1 mg/ml	0.5 mg/ml	0.75 mg/ml	1 mg/ml
C <sub>14:1</sub>	---	---	---	---	---
C <sub>15:0</sub>	---	---	---	0.04 ± 0.04w a	0.01 ± 0.01w a
C <sub>15:1</sub>	---	---	---	---	---
C <sub>16:0</sub>	25.42 ± 0.73w a	26.33 ± 0.30w a	26.75 ± 0.37w a	26.32 ± 0.52w b	27.22 ± 0.27w b
C <sub>16:1</sub>	22.30 ± 1.00w ab	21.77 ± 0.65w b	20.78 ± 1.38w b	22.52 ± 1.09w c	20.39 ± 0.43w c
C <sub>17:1</sub>	2.47 ± 1.25w c	2.14 ± 1.08w cd	---	---	---
C <sub>18:0</sub>	2.79 ± 0.29w c	2.46 ± 0.14w cd	3.08 ± 0.34w c	3.31 ± 0.22w d	3.19 ± 0.15w d
C <sub>18:1</sub>	21.12 ± 0.33w b	21.30 ± 0.29w b	21.97 ± 0.50w b	21.93 ± 0.48w c	22.16 ± 0.12w e
C <sub>18:2</sub>	20.37 ± 1.26w b	21.40 ± 1.00w b	21.22 ± 0.40w b	19.35 ± 1.42w e	21.50 ± 0.56w e
C <sub>18:3</sub>	0.03 ± 0.03wx c	0.02 ± 0.02w c	0.15 ± 0.01y d	0.12 ± 0.01xy a	0.11 ± 0.00wxy a
C <sub>20:0</sub>	1.67 ± 1.64w c	3.11 ± 1.56w d	5.50 ± 0.44w e	5.96 ± 0.91w f	5.16 ± 0.43w f
C <sub>20:1</sub>	3.55 ± 1.81w c	1.33 ± 1.33w cd	---	---	---
C <sub>20:3</sub>	---	---	---	---	---
C <sub>20:4</sub>	0.02 ± 0.02w c	0.09 ± 0.06w c	0.22 ± 0.09w d	0.14 ± 0.10w a	0.05 ± 0.01w a
C <sub>21:0</sub>	---	---	---	---	---
C <sub>22:0</sub>	0.21 ± 0.02w c	0.05 ± 0.05w c	0.16 ± 0.08w d	0.22 ± 0.02w a	0.06 ± 0.06w a
C <sub>22:6</sub>	---	---	---	0.08 ± 0.08w a	---
C <sub>23:0</sub>	0.05 ± 0.02w c	---	---	---	---
C <sub>24:0</sub>	---	---	0.06 ± 0.06w d	---	---
C <sub>20:2</sub>	---	---	0.10 ± 0.10w d	---	0.15 ± 0.08w a
SFAs <sup>c</sup>	30.14 ± 2.43w a	31.95 ± 1.74w a	35.56 ± 0.87w a	35.85 ± 0.78w a	35.64 ± 0.23w a
UFAs	49.44 ± 3.65w b	46.53 ± 1.47w b	42.75 ± 0.90w b	44.45 ± 0.61w b	42.55 ± 0.56w b
PUFAs	20.42 ± 1.30w a	21.52 ± 1.02w c	21.69 ± 0.32w c	19.69 ± 1.38w c	21.81 ± 0.53w c

<sup>a</sup>Data are means ± SE of three replicates using 25 pupae per replicate.

<sup>b</sup>Means in the same horizontal row and group (fatty acids or fatty acid classes) followed by the same letter (w-y) and means in the same vertical column and group followed by the same letter (a-f) are not significantly different from each other (Tukey HSD test, P>0.05).

<sup>c</sup>SFAs, saturated fatty acids; UFAs, unsaturated fatty acids; and PUFAs, polyunsaturated fatty acids.

Referring to Figs. 1 and 2, the reason of this accumulation for lipid and fatty acid levels are seen to be different. Observable reduction in the percent of lipids at 1 mg/ml dose for larvae compared to the control, while almost unchanged values for pupae indicate that 5-Aza-dC affects the lipid levels of larvae much more than pupae at this dose. Unlike lipids, total fatty acid values of pupae increased visibly at 1 mg/ml dose according to the control. However, larvae had almost constant levels of fatty acids for the same dose compared to pupae. In this case, it is plausible to assume that pupae were more sensible than larvae.

Significant increasing of linolenic acid (C<sub>18:3</sub>) ratio at 0.5 mg/ml dose with respect to control and 0.1 mg/ml groups and destruction of eicosenoic acid (C<sub>20:1</sub>) at doses

>0.1 mg/ml support this assumption for parasitoid pupa. It is showed in a number of studies that the changes in lipid and fatty acid milieu are closely correlated with the physiological needs of insects and are influenced by environmental factors such as pesticides and other chemicals (Stanley-Samuelson *et al.*, 1988; Ogg and Stanley-Samuelson, 1992; Sak *et al.*, 2006).

Table 5. ANNOVAs of the differences among individual fatty acids for each testing group in *A. galleria* and parasitized host larva (PHL).

Stages/PHL	Statistics	Control	0.1 mg/ml	0.5 mg/ml	0.75 mg/ml	1 mg/ml
Parasitoid Larvae	F	46.040	51.793	28.744	227.274	179.365
	df	23, 48	23, 48	23, 48	23, 48	23, 48
	P	0.000	0.000	0.000	0.000	0.000
Parasitoid Pupae	F	156.255	250.982	641.544	381.268	2256.853
	df	23, 48	23, 48	23, 48	23, 48	23, 48
	P	0.000	0.000	0.000	0.000	0.000
PHL	F	100.140	175.941	393.183	575.438	22.849
	df	23, 48	23, 48	23, 48	23, 48	23, 48
	P	0.000	0.000	0.000	0.000	0.000

Table 6. ANNOVAs of the differences among fatty acid classes for each testing group in *A. galleria* and parasitized host larva (PHL).

Stages/PHL	Statistics	Control	0.1 mg/ml	0.5 mg/ml	0.75 mg/ml	1.0 mg/ml
Parasitoid Larvae	F	68.150	49.365	49.525	218.460	246.233
	df	2, 6	2, 6	2, 6	2, 6	2, 6
	P	0.000	0.000	0.000	0.000	0.000
Parasitoid Pupae	F	31.263	75.943	206.014	163.919	517.057
	df	2, 6	2, 6	2, 6	2, 6	2, 6
	P	0.001	0.000	0.000	0.000	0.000
PHL	F	7.175	19.007	9.311	45.188	1.141
	df	2, 6	2, 6	2, 6	2, 6	2, 6
	P	0.026	0.003	0.014	0.000	0.380

Table 7. ANNOVAs of 5-Aza-dC-related changes for fatty acid classes in *A. galleria* and parasitized host larva (PHL).

Stages/PHL	Fatty acid classes	F	df	P
Parasitoid Larvae	SFAs	1.119	4, 10	0.400
	UFAs	1.457	4, 10	0.286
	PUFAs	0.571	4, 10	0.690
Parasitoid Pupae	SFAs	3.325	4, 10	0.056
	UFAs	2.451	4, 10	0.114
	PUFAs	0.855	4, 10	0.522
PHL	SFAs	2.768	4, 10	0.087
	UFAs	1.617	4, 10	0.245
	PUFAs	0.602	4, 10	0.670

### *The Effects of 5-Aza-2'-deoxycytidine*

*A. galleriae* and PHL lacked some of fatty acids including especially those of 20 carbons. Ogg *et al.* (1993) and Uscian *et al.* (1992) stated that  $C_{20}$  components could be missed while analyzing lipid samples on gas chromatography with flame ionization detection. Therefore, using FID could be the reason for the absence of these fatty acids in this study. Although I could not observe here, we had found  $C_{14:0}$ ,  $C_{14:1}$ ,  $C_{15:0}$ ,  $C_{18:3}$ ,  $C_{20:2}$ ,  $C_{21:0}$ ,  $C_{22:1}$ , and  $C_{22:2}$  fatty acids in our previous studies (Nurullahoğlu *et al.*, 2004; Uçkan *et al.*, 2009) for both host and parasitoid species. The most important reason for these differences may be the use of synthetic diet to feed the host species in this study, unlike our previous studies using natural blackened comb for nourishing (Nurullahoğlu *et al.*, 2004; Uçkan *et al.*, 2009). Fatty acid composition of an insect can reveal metabolic pathways and the differences in insect feeding can cause a different distribution in the fatty acid content. Since the life of parasitoids depend on host species, their fatty acid composition could be affected by the feeding conditions of host indirectly. It seems that our data show fourteen different fatty acids for PHL in all experimental groups but seven for parasitoid larvae are agree with this assumption. It is known that accumulation or consumption of fatty acids could be examined in the stress conditions (Hoch *et al.*, 2002). On the other hand, re-occurrence of certain fatty acids in the pupal stage suggests that the adverse effect of 5-Aza-dC on the larval stage seems to be compensated.

The fatty acids have a variety of function such as sources of energy in the form of triacylglycerols and are structural components of membranes in insects (Downer, 1985). The distribution of fatty acids alters greatly based on different factors such as nutrition and development (Ogg and Stanley-Samuelson, 1992; Bozkuş, 2003; Çakmak *et al.*, 2007). We had shown before that each developmental stage of *A. galleriae* had a different fatty acid distribution (Nurullahoğlu *et al.*, 2004; Uçkan *et al.*, 2009). The results of this study support this statement because of the differences of larval and pupal fatty acids. There are also a lot of studies showing insects having different fatty acids in different stages and sexes (Ogg and Stanley-Samuelson, 1992; Bashan *et al.*, 2002; Bozkuş, 2003; Çakmak *et al.*, 2007). Having various functions, the rate of fatty acids would be expected to fluctuate according to physiological needs of an insect. In earlier studies we have found that all stages and sexes of *A. galleriae* and PHL contained 10-24 carbon fatty acids (Nurullahoğlu *et al.*, 2004; Uçkan *et al.*, 2009). Similarly, I detected 14-24 carbon fatty acids for same stages in this study. It is known that carbon number within this range is characteristic for parasitic hymenopteran species (Thompson and Barlow, 1974). Our early results (Nurullahoğlu *et al.*, 2004; Uçkan *et al.*, 2009) and current findings, which were showing the major proportion of fatty acids was palmitic, palmitoleic, stearic, oleic, and linoleic acids, were also in agreement with those reported for other parasitoid species (Bracken and Barlow, 1967; Thompson and Barlow, 1974). All these data support the generalization about insects that the major fatty acid component is usually  $C_{16}$  and  $C_{18}$  saturated and unsaturated fatty acids (Candy and Kilby, 1975). I found that the most abundant fatty acid of PHL was palmitic acid ( $C_{16:0}$ ) in all control and experimental groups (except for 0.5 mg/ml dose). In contrast, palmitoleic acid ( $C_{16:1}$ ) was the major fatty acid in abundance in *A.*

*grisella* larvae (both parasitized and nonparasitized) according to our previous data (Nurullohoğlu *et al.*, 2004). Using a synthetic diet instead of natural blackened comb for feeding the host insect might be the reason of this difference. Moreover, oleic acid ( $C_{18:1}$ ) was considerably the most abundant fatty acid in all stages/sexes of *A. galleriae* even if the parasitoid was grown in different host insects (Nurullohoğlu *et al.*, 2004; Uçkan *et al.*, 2009). In this study, I also showed that oleic acid constituted the major proportion of fatty acids in the larval stage of *A. galleriae* unlike to pupal stage containing palmitic acid at the highest rate. This difference between larva and pupa may result from the use of 5-Aza-dC and synthetic nutrients simultaneously. More definitive conclusions on this subject can be obtained by the use of natural blackened comb and synthetic diet at the same experimental design.

The host-parasitoid interaction is an ideal model for studying how chemicals affect the life of an organism depending on another one. Aside from the effects of development, it is known that fatty acid compositions of whole insect change in response to the change in dietary conditions (Stanley-Samuelson *et al.*, 1988). Certain of the parasitoid wasps appear to match their fatty acid profiles almost the same of the host (Nurullohoğlu *et al.*, 2004; Uçkan *et al.*, 2009). Thus, chemicals could cause changes in the metabolite composition of the parasitoid species indirectly through host physiology (Sak *et al.*, 2006). Eicosenoic acid ( $C_{20:1}$ ), one of the six dominant fatty acid determined in PHL constituted above 4% of the fatty acid composition in control groups. However, the ratio of this acid fell below 4% at 0.1 mg/ml dose and could not be detected at higher doses of 5-Aza-dC. Similarly, eicosenoic acid did not be observed in both control and experimental groups except for 0.1 mg/ml doses for parasitoid larvae feeding with host sources. Thus, the results showed that 5-Aza-dC caused a prominent reduction in the ratio of eicosenoic acid in the larval stage of parasitoid species. Moreover, arachidic acid ( $C_{20:0}$ ) arose to the fifth most dominant fatty acid level at all doses of 5-Aza-dC instead of eicosenoic acid in both PHL and parasitoid pupae. At the same time, the ratio of eicosenoic acid down to a very low value, such as 1%, at 0.1 mg/ml dose in pupae and could not be detected at higher doses of 5-Aza-dC as in PHL. It seems that the negative effects of 5-Aza-dC also continued in the later developmental stages of *A. galleriae*. The situation of reduction and then total destruction of eicosenoic acid may be attributed to the 5-Aza-dC-induced adverse effect in diet quality resulting in an intervention of adequate food supply from the host (Uçkan and Ergin, 2002; Uçkan *et al.*, 2007). The changes in the value of eicosenoic acid mentioned above and the considerable rising of arachidic acid in PHL and parasitoid pupae may indicate that fatty acid composition could be rearranged during different developmental stages of an insect as a result of chemical-induced stress. It is known that insect lipids and fatty acids are affected by a number of neuroendocrinological, physiological and environmental influences, which are closely interrelated (Downer, 1985) and insects are able to modify their fatty acid compositions to suit the physiological requirements for overcoming the stress conditions.

Unsaturated fatty acids constituted the major proportion of fatty acids except for one case, whereas PUFAs were the smallest in all stages/PHL regardless of 5-Aza-dC

### *The Effects of 5-Aza-2'-deoxycytidine*

treatment. Our results are fully consistent with our previous study (Nurullahoğlu *et al.*, 2004) concerning fatty acid classes. On the other hand, SFAs were the most dominant fatty acids in parasitoid larva, pupa, and PHL in *A. galleriae*-*G. mellonella* (Uçkan *et al.*, 2009) system. This difference can be attributed to the use of different insect species as a host and may be related to variance among insect species. Unlike to single fatty acids, 5-Aza-dC did not cause significant changes in the percent of different fatty acid classes for all stages/PHL. In the matter of lipid metabolism at this point, it is not possible to state that 5-Aza-dC causing irreversible adverse effect on insects examined here. Poirier *et al.* (2014) demonstrated that 5-AzaC selectively and strongly reduced the expression of key genes that regulate lipid metabolism unlike 5-Aza-dC. Thus, insignificant changes in the ratio of total lipid, total fatty acids, and fatty acid classes might be resulted from inefficiency of 5-Aza-dC on special genes controlling the lipid metabolism. When evaluating the issue from another angle, the crucial changes in the ratio of linolenic, lignoceric, eicosenoic, and arachidic acids indicate the effectiveness of 5-Aza-dC on lipid metabolism of *A. galleriae* and PHL. However, insignificant differences in fatty acid classes bring to mind that these insects may be changing the synthesis and the degradation pathways in metabolism in order to protect the total fatty acid composition in body content. The author is currently evaluating the effect of 5-Aza-dC on the lipid and fatty acids of *A. galleriae* adults and on the biological parameters of *A. grisella*. The findings of how 5-Aza-dC affects the lipid and fatty acid content of parasitoid adults will make this assumption more reasonable. Besides, considering the effects of the drug on DNA, the results obtained from this study requires a more global perspective of metabolism eliminating the harmful effects of 5-Aza-dC in *A. galleriae* and *A. grisella*; thus it is necessary to integrate the possible effects of 5-Aza-dC on protein, carbohydrate, and also other substrate levels and to recognize how one metabolite impinges upon another.

## ACKNOWLEDGMENTS

This research was supported by a grant (2010-109T004) from The Scientific and Technological Research Council of Turkey (TÜBİTAK). I would like to thank Bahar BUDAK to help in the experimental studies. May she rest in peace.

## REFERENCES

- Alvarado, S., Rajakumar, R., Abouheif, E., Szyf, M., 2015, Epigenetic variation in the *Egfr* gene generates quantitative variation in a complex trait in ants. *Nature Communications*, 6: 6513, doi: 10.1038/ncomms7513.
- Amarasinghe, H. E., Clayton, C. I., Mallon, E. B., 2014, Methylation and worker reproduction in the bumble-bee (*Bombus terrestris*). *Proceedings of the Royal Society B*, 281: 20132502. <http://dx.doi.org/10.1098/rspb.2013.2502>
- Bashan, M., Akbas, H., Yurdakoc, K., 2002, Phospholipid and triacylglycerol fatty acid composition of major life stages of sunn pest, *Eurygaster integriceps* (Heteroptera: Scutelleridae). *Comparative Biochemistry and Physiology*, 132B: 375-380.

- Bischof, C., Ortel, J., 1996, The effects of parasitism by *Glyptapanteles liparidis* (Braconidae: Hymenoptera) on the hemolymph and total body composition of gypsy moth larvae (*Lymantria dispar*, Lymantriidae: Lepidoptera). *Parasitology Research*, 82(8): 687-692.
- Bozkuş, K., 2003, Phospholipid and triacylglycerol fatty acid compositions from various development stages of *Melanogryllus desertus* Pall. (Orthoptera: Gryllidae). *Turkish Journal of Biology*, 27(2): 73-78.
- Bracken, G. K, Barlow, J. S., 1967, Fatty acid composition of *Exeristes comstockii* (Cress) reared on different hosts. *Canadian Journal of Zoology*, 45(1): 57-61.
- Bronskill, J. F., 1961, A cage to simplify the rearing of the greater wax moth, *Galleria mellonella* (Pyrilidae). *Journal of Lepidopteran Society*, 15(2): 102-104.
- Cakmak, O., Bashan, M., Satar, A., 2007, Total lipid and fatty acid compositions of *Lertha sheppardi* (Neuroptera: Nymphoptera) during its main life stages. *Biologia*, 62(6): 774-780.
- Candy, D. J., Kilby, B. A., 1975., *Insect Biochemistry and Function*. Chapman and Hall, London, United Kingdom, 314.
- Carr, B. I., Garrett-Reilly, J., Smith, S. S., Winberg, C., Riggs, A. D., 1984, The tumorigenicity of 5-azacytidine in the male Fischer rat. *Carcinogenesis*, 5(12): 1583-1590.
- Christman, J. K., 2002, 5-Azacytidine and 5-aza-2'-deoxycytidine as inhibitors of DNA methylation: mechanistic studies and their implications for cancer therapy. *Oncogene*, 21(35): 5483-5495.
- Doerksen, T., Benoit, G., Trasler, J. M., 2000, Deoxyribonucleic acid hypomethylation of male germ cells by mitotic and meiotic exposure to 5-azacytidine is associated with altered testicular histology. *Endocrinology*, 141(9): 3235-3244.
- Downer, R. G. H., 1985, *Lipid metabolism*. In: Kerkut, G. A., Gilbert, L. I. (Eds.). *Comprehensive Insect Physiology, Biochemistry and Pharmacology*. Pergamon Press, Oxford, United Kingdom, 77-113.
- Folch, J., Lees, M., Sloane-Stanley, G. H., 1957, A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry*, 226(1): 497-509.
- Gurion, R., Vidal, L., Gafter-Gvili, A., Belnik, Y., Yeshurun, M., Raanani, P., Shpilberg, O., 2010, 5-azacytidine prolongs overall survival in patients with myelodysplastic syndrome-a systematic review and meta-analysis. *Haematologica*, 95(2): 303-310.
- Hoch, G., Schafellner, C., Henn, M. W., Schopf, A., 2002, Alterations in carbohydrate and fatty acid levels of *Lymantria dispar* larvae caused by a microsporidian infection and potential adverse effects on a co-occurring endoparasitoid, *Glyptapanteles liparidis*. *Archives of Insect Biochemistry and Physiology*, 50(3): 109-120.
- Jackson-Grusby, L., Laird, P. W., Magge, S. N., Moeller, B. J., Jaenisch, R., 1997, Mutagenicity of 5-Aza-2'-deoxycytidine is mediated by the mammalian DNA methyltransferase. *Proceedings of the National Academy of Sciences of the United States of America*, 94(9): 4681-4685.
- Lantry, L. E., Zhang, Z., Crist, K. A., Wang, Y., Kelloff, G. J., Lubet, R. A., You, M., 1999, 5-Aza-2'-deoxycytidine is chemopreventive in a 4-(methyl-nitrosamino)-1-(3-pyridyl)-1-butanone-induced primary mouse lung tumor model. *Carcinogenesis*, 20(2): 343-346.
- Nurullahoğlu, Z. Ü., Uçkan, F., Sak, O., Ergin, E., 2004, Total lipid and fatty acid composition of *Apanteles galleriae* and its parasitized host. *Annals of the Entomological Society of America*, 97(5): 1000-1006.
- Ogg, C. L., Meinke, L. J., Howard, R. W., Stanley-Samuels, D. W., 1993, Phospholipid and triacylglycerol fatty acid compositions of five species of *Diabrotica* (Insecta: Coleoptera: Chrysomelidae). *Comparative Biochemistry and Physiology*, 105(1): 69-77.
- Ogg, C. L., Stanley-Samuels, D. W., 1992, Phospholipid and triacylglycerol fatty acid compositions of the major life stages and selected tissues of the tobacco hornworm *Manduca sexta*. *Comparative Biochemistry and Physiology*, 101(3): 345-351.
- Osgood, C. J., Seward, S. M., 1989, 5-azacytidine induces sex chromosome loss and interchange in immature germ cells of *Drosophila mei-9* males. *Environmental and Molecular Mutagenesis*, 14(3): 135-145.

### The Effects of 5-Aza-2'-deoxycytidine

- Pegoraro, M., Bafna, A., Davies, N. J., Shuker, D. M., Tauber, E., 2016, DNA methylation changes induced by long and short photoperiods in *Nasonia*. *Genome Research*, 26(2): 203-210. doi:10.1101/gr.196204.115
- Piskala, A., Sorm, F., 1964, Nucleic acids components and their analogues. LI. Synthesis of 1-glycosyl derivatives of 5-azauracil and 5-azacytosine. *Collection of Czechoslovak Chemical Communications*, 29(9): 2060-2076.
- Poirier, S., Samami, S., Mamarbachi, M., Demers, A., Chang, T. Y., Vance, D. E., Hatch, G. M., Mayer, G., 2014, The epigenetic drug 5-azacytidine interferes with cholesterol and lipid metabolism. *The Journal of Biological Chemistry*, 289(27): 18736-18751.
- Prakash, A. P., Kumar, P. P., 1997, Inhibition of shoot induction by 5-azacytidine and 5-Aza-2'-deoxycytidine in *Petunia* involves DNA hypomethylation. *Plant Cell Reports*, 16(10): 719-724.
- Rivers, D. B., Denlinger, D. L., 1994, Redirection of metabolism in the flesh fly, *Sarcophaga bullata*, following envenomation by the ectoparasitoid *Nasonia vitripennis* and correlation of metabolic effects with the diapause status of the host. *Journal of Insect Physiology*, 40(3): 207-215.
- Sak, O., Uçkan, F., Ergin, E., 2006, Effects of cypermethrin on total body weight, glycogen, protein, and lipid contents of *Pimpla turionellae* (L.) (Hymenoptera: Ichneumonidae). *Belgian Journal of Zoology*, 136(1): 53-58.
- Salvador, G., Cônsoli, F. L., 2008, Changes in the hemolymph and fat body metabolites of *Diatraea saccharalis* (Fabricius) (Lepidoptera: Crambidae) parasitized by *Cotesia flavipes* (Cameron) (Hymenoptera: Braconidae). *Biological Control*, 45(1): 103-110.
- Sato, N., Maehara, N., Su, G. H., Goggins, M., 2003, Effects of 5-Aza-2'-deoxycytidine on matrix metalloproteinase expression and pancreatic cancer cell invasiveness. *Journal of the National Cancer Institute*, 95(4): 327-330.
- Schauenstein, K., Csordas, A., Krömer, G., Dietrich, H., Wick, G., 1991, In-vivo treatment with 5-azacytidine causes degeneration of central lymphatic organs and induces autoimmune disease in the chicken. *International Journal of Experimental Pathology*, 72(3): 311-318.
- Shimamori, K., 1987, On the biology of *Apanteles galleriae*, a parasite of the two species of wax moths. *Honeybee Science*, 8(3): 107-112.
- Spss, I. N. C., 1999, SPSS Base 10.0 for Windows User's Guide, Chicago IL: SPSS Inc.
- Stanley-Samuelson, D. W., Jurenka, R. A., Cripps, C., Blomquist, G. J., DeRenobles, M., 1988, Fatty acids in insects: composition, metabolism, and biological significance. *Archives of Insect Biochemistry and Physiology*, 9(1): 1-33.
- Stresemann, C., Brueckner, B., Musch, T., Stopper, H., Lyko, F., 2006, Functional diversity of DNA methyltransferase inhibitors in human cancer cell lines. *Cancer Research*, 66(5): 2794-2800.
- Thompson, S. N., 1982, Effect of the insect parasite, *Hyposoter exiguae*, on the total body glycogen and lipid levels of its host, *Trichoplusia ni*. *Comparative Biochemistry and Physiology*, 72(2): 233-237.
- Thompson, S. N., 1993, *Redirection of host metabolism and effects on parasite nutrition*. In: Beckage, N. E., Thompson, S. N., Federici, B. A. (Eds.). *Parasites and Pathogens of Insects*. Academic Press, New York, 125-144.
- Thompson, S. N., Barlow, J. S., 1974, The fatty acid composition of parasitic hymenoptera and its possible biological significance. *Annals of the Entomological Society of America*, 67(4): 627-632.
- Uçkan, F., Ergin, E., 2002, Effect of host diet on the immature developmental time, fecundity, sex ratio, adult longevity, and size of *Apanteles galleriae* (Hymenoptera: Braconidae). *Environmental Entomology*, 31(1): 168-171.
- Uçkan, F., Ergin, E., 2003, Temperature and food source effects on adult longevity of *Apanteles galleriae* Wilkinson (Hymenoptera: Braconidae). *Environmental Entomology*, 32(3): 441-446.
- Uçkan, F., Gülel, A., 2000, Effects of host species on some biological characteristics of *Apanteles galleriae* Wilkinson (Hymenoptera: Braconidae). *Turkish Journal of Zoology*, 24(Ek): 105-114.

- Uçkan, F., Hepçorman Şengül, Ş., Sak, O., Korkmaz, M., 2007, Effects of 5-Aza-2'-deoxycytidine on Biological Parameters of Larval Endoparasitoid *Apanteles galleriae* (Hymenoptera: Braconidae) and on Its Host *Achoria grisella* (Lepidoptera: Pyralidae). *Annals of the Entomological Society of America*, 100(2): 265-269.
- Uçkan, F., Nurullahoğlu, Z. Ü., Sak, O., Öztürk, R., 2009, *Apanteles galleriae* Wilkinson (Hymenoptera: Braconidae) ve Parazitlenmiş Konağı *Galleria mellonella* L. (Lepidoptera: Pyralidae)'nin Toplam Lipit ve Yağ Asidi Bileşimleri. *Biyoloji Bilimleri Araştırma Dergisi*, 2: 89-95.
- Uscian, J. M., Miller, J. S., Howard, R. W., Stanley-Samuels, D. W., 1992, Arachidonic and eicosapentaenoic acids in tissue lipids of two species of predacious insects, *Cicindela circumpecta* and *Asilis* sp. *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry*, 103(4): 833-838.
- Watanabe, C., 1987, Occurrence of *Apanteles galleriae* (Hymenoptera: Braconidae), a parasite of wax moth in Japan. *Kontyû*, 55(1): 165-168.
- Whitfield, J. B., Cameron, S. A., Ramirez, S. R., Roesch, K., Messinger, S., Taylor, O. M., Cole, D., 2001, Review of the *Apanteles* species (Hymenoptera: Braconidae) attacking Lepidoptera in *Bombus* (*Fervidobombus*) (Hymenoptera: Apidae) colonies in the new world, with description of a new species from South Africa. *Annals of the Entomological Society of America*, 94(6): 851-857.
- Wijermans, P., Lübbert, M., Verhoef, G., Bosly, A., Ravoet, C., Andre, M., Ferrant, A., 2000, Low-dose 5-Aza-2'-deoxycytidine, a DNA hypomethylating agent, for the treatment of high-risk myelodysplastic syndrome: a multicenter phase II study in elderly patients. *Journal of Clinical Oncology*, 18(5): 956-962.

Received: May 05, 2016

Accepted: December 07, 2016

## Larvicidal and Pupicidal Potential of *Aframomum melegueta* K. Schum Extracts Against Mosquito, *Anopheles* Species

Kayode David ILEKE<sup>1</sup>      Jacobs Mobolade ADESINA<sup>2,3\*</sup>  
Oladoyin Ganiyat OKUNOLA<sup>1</sup>

<sup>1</sup>Department of Environmental Biology and Fisheries, Adekunle Ajasin University, PMB 001, Akungba Akoko, Ondo State, NIGERIA

<sup>2</sup>Insect Chemical Ecology Laboratory, Institute of Bioresources and Sustainable Development, Takyelpat, Imphal, 795001, Manipur, INDIA

<sup>3</sup>Department of Crop, Soil and Pest Management Technology, Rufus Giwa Polytechnic, P. M. B. 1019, Owo, Ondo State, NIGERIA  
e-mail: \*mobolade72@gmail.com

### ABSTRACT

The use of synthetic insecticides to control vector mosquitoes has caused physiological resistance and adverse environmental effects in addition to high operational cost. The use of herbal products is one of the best alternatives for mosquito control. The present study was to screen the larvicidal and pupicidal potential of *Aframomum melegueta* leaf and seed extracts against larvae and pupae of *Anopheles* species in the laboratory at ambient temperature of  $28\pm 2^{\circ}\text{C}$  and relative humidity of  $75\pm 5\%$ . The extracts of the plant parts were extracted with methanol and they were prepared at concentrations of 0.1, 0.2, 0.3, 0.4 and 0.5. The larvae and pupae of *Anopheles* species were exposed to these concentrations of the plant extracts for 24 hours and mortality was recorded. At all levels of concentrations, larval and pupal mortalities of this insect increased with increase in the concentrations irrespective of the type of plant parts extract used. However, seed extract of *A. melegueta* showed more insecticidal effect on both the larvae and pupae of *Anopheles* species at rate 0.1% but its effect was not significantly ( $p>0.05$ ) different from extract of *A. melegueta* at 0.2%, 0.3%, 0.4% to 0.5% concentrations. It was observed that the larvae of *Anopheles* species were more susceptible to the extracts of the plant parts tested. All the plant parts extracts used in this work showed high effectiveness to larval and pupal mortalities and could therefore be used to reduce occurrence of malaria incidence. Their adoption might prove to be an effective and eco-friendly herbal insecticides in ensuring maximum safety in any ecological condition.

*Key words:* *Aframomum melegueta*, *Anopheles*, concentrations, larvicidal, pupicidal.

### INTRODUCTION

Malaria remains one of the most prevalent diseases in the tropical world and Nigeria alone accounts for over 40 percent of the estimated total malaria death globally. Mosquito-borne diseases have an economic impact, including loss in commercial and labour outputs, particularly in countries with tropical and subtropical climates (Srinivasan *et al.*, 2014).

*Anopheles stephensi* Liston is one of the primary vectors of malaria in many tropical and subtropical countries. Mosquitoes in the larval stage are attractive targets for pesticides because mosquitoes breed in water, and thus, it is easy to deal with them in this habitat. An obvious method for the control of mosquito borne diseases is the use of insecticides, and many synthetic agents have been developed and employed in the field with considerable success. The use of conventional pesticides in the water sources for mosquito control introduces many risks to people and the environment. It has also provoked undesirable effects, including toxicity to non-target organisms, and fostered environmental and human health concerns (Muthusamy *et al.*, 2014). These problems have necessitated the need for search and development of alternative strategies using eco-friendly, environmentally safe, biodegradable and low cost natural products.

In recent years, natural products of plant origin with insecticidal properties have been tried in the recent pest control of variety of insect pests and vectors around the world.

*Aframomum melegueta* (Zingiberaceae) K. Schum., known as Alligator pepper, is an herbaceous tropical perennial West Africa spice plant which imparts a pungent peppery flavor with hints of citrus and used mainly as food, in brewing, and in both veterinary and traditional medicine (Igwe *et al.*, 1999). It is believed to have purgative and hemostatic properties and also to be very effective against schistosomiasis (Alaje *et al.*, 2014). Various authors had reported the potential of *A. melegueta* for the management of stored products' insect pests (Ofuya, 1990; Adedire and Lajide, 1999; Adeyemo *et al.*, 2014; Onekutu *et al.*, 2015; Adesina *et al.*, 2015). However, there appears to be dearth of information on the empirical utilization of *A. melegueta* for the control of malaria vector.

Therefore this study was conceived to determine the insecticidal effect of *A. melegueta* leaf and seed extracts on larvae and pupae of *Anopheles* mosquito as potential bio-insecticide in the integrated management of malaria vectors.

## **MATERIALS AND METHODS**

### **Collection and rearing of mosquito larva and pupa**

Mosquitoes' baits in opaque coloured shallow containers with a large surface area were established in the Hatchery Laboratory, Department of Environmental Biology and Fisheries, Adekunle Ajasin University Akungba Akoko, Ondo State, Nigeria. The opaque coloured container was filled with rain water in order to mimic mosquito's natural breeding environment and also to attract adult mosquitoes for oviposition. Small quantity of industrial yeast was sprinkled on the water surface and allowed to decompose slowly to nourish the developing larva. Wild mosquitoes were allowed to freely visit the bait and to lay eggs. Thereafter, the containers bearing mosquitoes larvae and pupae were transferred to the laboratory, identified and maintained at temperature of  $28 \pm 2$  °C and  $75 \pm 5$  % relative humidity.

## *Larvicidal and Pupicidal Potential of Aframomum melegueta*

### **Collection of plant materials and preparation**

Fresh leaves and seeds of *A. melegueta* were obtained free of pesticides from Kowode market in Ikare Akoko, Ondo State, Nigeria and authenticated at the Department of Plant Science and Biotechnology, Adekunle Ajasin University, Akungba Akoko, Ondo State. The plant materials were rinsed in clean water to remove dirt and air dried in a well ventilated laboratory and ground into very fine powder using an electric blender. The powder was further sieved to pass through 1mm<sup>2</sup> perforations, and packed in plastic containers with tight lids and then stored in a refrigerator at 4 °C prior to use.

Methanol extracts of *A. melegueta* were carried out using cold extraction method. About 150g of *A. melegueta* leaf and seed powders were soaked separately in an extraction bottle containing 99.5% methanol for 72 hours. The mixture was stirred occasionally with a glass rod and the resulting mixture was filtered using a double layer of Whatman No. 1 filter paper and the solvent was evaporated using a rotary evaporator at 30 to 40 °C with rotary speed of 3 to 6 rpm (Udo, 2011). The resulting materials were air dried in order to remove traces of solvents. The crude extracts were kept in a labeled amber specimen bottle and preserved in the refrigerator till further use.

### **Effect of *A. melegueta* extracts on larvae and pupae of *Anopheles* mosquito**

Larvicidal and pupacidal activities of the plant extracts were carried out at different concentrations by preparing the required stock solutions following the standard procedure (WHO, 1996). The desired concentrations were achieved by adding 1.0 µg of the crude extract from leaves of as well as seeds to 100 ml of distilled water. From this, five concentrations of 0.1, 0.2, 0.3, 0.4 and 0.5% of the plant extracts were prepared. The treatments were separately added to 2.5l of water inside a bowl and yeast powder was added in order to provide source of food for the introduced larvae. Twenty larvae and pupae of *Anopheles* species were separately introduced into the treated water and untreated water was set as control. Each treatment was replicated three times. Mortality was observed over 24 hours after the introduction of larvae and pupae to notice recovery; a recovery time of 5 min was allowed. The larval mortality in treatments was corrected for the controls. Larvae and pupae were counted as dead when they were not coming to the surface for respiration and were insensitive to probe.

### **Phytochemical screening of *A. melegueta* leaves and seeds**

Qualitative phytochemical constituents screening was carried out on the methanol extracts using standard laboratory procedures as described by Harborne (1973); Trease and Evans (1985); Sofowora (1993).

### **Analysis of data**

Data collected were subjected to analysis of variance (ANOVA) using statistical package for social sciences (SPSS) 16.0 software (SPSS, inc., 2007) and significant treatment means were separated using the new Duncan's Multiple Range Test at 5% probability level.

## RESULTS

### Effects of *A. melegueta* on *Anopheles* species

Table 1 presented the mean percentage mortality of larvae and pupae of *Anopheles* species at 24 hours after exposure to different concentration levels of *A. melegueta*. The result indicated that mortality of *Anopheles* specie larvae and pupae is directly proportional to increase in the *A. melegueta* products concentration levels.

At 0.1% *A. melegueta* leaves extract caused 40% larval mortality and 35.37% pupal mortality while the seed extract caused 65.37% larvae mortality and 50% pupae mortality and at 0.2% leaves extracts of *A. melegueta* caused 70% larval mortality of mosquito and 60% pupal mortality while the seed extracts recorded 80% larval and 70% pupal mortality respectively. The developmental stages of *Anopheles* species exposed to 0.3% *A. melegueta* leaves extracts caused 90% larval mortality and 85.37% pupal mortality while the seed extracts caused 100% larval mortality and 95.37% pupal mortality; while the plant products evoked 100% mortalities on both larvae and pupae when exposed to 0.4% *A. melegueta* (Table 1), no mortality was observed in the control treatment.

Treatment means showed that the leaves and seeds extracts had significant difference on the larval and pupal mortalities compared to the control when applied 0.1% concentration and non-significant treatment means was observed on the developmental stages of *Anopheles* species exposed to both leaves and seeds extracts at 0.2-0.4% when compared statistically (Table 1).

### Phytochemicals screening of *A. melegueta*

Table 2 presented the result of the phytochemical screening of leaves and seeds of *A. melegueta* methanol extracts. The phytochemicals of *A. melegueta* products revealed the presence of Alkaloids, Saponins, Tannins, Flavonoids and Cardiac glycosides and these may be responsible for the high insecticidal property for the control of mosquitoes.

Table 1. Percentage mortality of *Anopheles* species at 24 hours post treatment

Extract	Developmental stages							
	Larvae (0.1%)	Pupae (0.1%)	Larvae (0.2%)	Pupae (0.2%)	Larvae (0.3%)	Pupae (0.3%)	Larvae (0.4%)	Pupae (0.4%)
Leaf	40.00±0.9 <sup>a</sup>	35.37±0.3 <sup>b</sup>	70.00±0.9 <sup>b</sup>	60.00±0.9 <sup>b</sup>	90.00±0.93 <sup>b</sup>	85.37±0.33 <sup>b</sup>	100.00±0.00 <sup>b</sup>	100.00±0.00 <sup>b</sup>
Seed	65.37±0.3 <sup>b</sup>	50.00±0.3 <sup>c</sup>	80.00±0.9 <sup>b</sup>	70.00±0.9 <sup>b</sup>	100.00±0.0 <sup>b</sup>	95.37±0.33 <sup>b</sup>	100.00±0.00 <sup>b</sup>	100.00±0.00 <sup>b</sup>
Control	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>

Each value is a mean ± standard error of three replicates. Mean followed by the same letter along the column are not significantly different ( $p>0.05$ ) using Duncan's Multiple Range Test.

## DISCUSSION

Adverse environmental effects and the need to maintain a sustainable environment have created the need for environmental-safe, degradable and target specific

### Larvicidal and Pupicidal Potential of *Aframomum melegueta*

insecticides (Ezeonu *et al.*, 2001). Extracts or essential oils from plants may be alternative sources of malaria vectors' control agents, since they constitute a rich source of bioactive compounds that are biodegradable into non-toxic products and are potentially suitable for use in control of mosquito larvae (Amer and Mehlhorn, 2006).

Table 2. Phytochemicals in methanol and aqueous extract of *A. melegueta* leaf and seeds.

Phytochemicals	Methanol extract of <i>A. melegueta</i> leaf	Methanol extract of <i>A. melegueta</i> seed
Alkaloids	+	+
Saponins	+	+
Tannins	+	+
Phlobatanins	-	-
Anthraquinines	-	-
Flavonoids	+	+
Cardiac glycosides	+	+

KEY: - Absent, + Present

The results from this study showed that the extracts of *A. melegueta* evoked high mortality of developmental stages of *Anopheles* mosquito compared to control. This confirmed earlier studies that reported the insecticidal activities of the plant against stored products' insect infestation management (Ofuya, 1990; Adedire and Lajide, 1999; Adeyemo *et al.*, 2014; Onekutu *et al.*, 2015; Adesina *et al.*, 2015). Also the result from this investigation are in accordance with the findings of Raji and Akinkulore (2010); Akinkulore *et al.*, (2011); Ghosh *et al.* (2012); Yousaf and Zuharah, (2015); Ileke *et al.*, (2014); Dinesh *et al.*, (2015) who reported the toxicity of some indigenous plants extracts on developmental stages of mosquitoes. However, at all the levels of concentration, larvae of *Anopheles* mosquito were more susceptible to the plant extracts than pupae. Also the seed extract exerted higher larvicidal activity compared to its effects on the pupal stage. This suggests that the bioactive constituents of the plant materials may be more available in the seeds which may be responsible for the higher mortality observed throughout the period of exposure (Ashamo and Akinnawonu, 2012; Ileke *et al.*, 2014).

The active constituents in the plant materials appear to be responsible for their insecticidal properties against *Anopheles* mosquito (Ileke *et al.*, 2014). Botanical source of insecticides have been noted to have a considerable effect on the normal respiration of insects as many of them have a knack to block the respiratory organ, i.e. spiracles of insect. Therefore, since the larvae of this insect depend solely on their spiracles for breathing, blockage of the spiracles by these plant extracts could lead to asphyxiation and subsequent death of the larvae (Akinkulore *et al.*, 2011; Ileke *et al.*, 2014; Ileke and Olotuah, 2012; Ileke and Oni, 2011; Ogungbite *et al.*, 2014). Therefore, the ability of the tested plant to exert high mortality could be linked to the phytochemical constituents present in the plant parts tested.

The high larval mortality recorded in comparison to pupae could also be related to the larval active feeding activity since pupal stage is a resting (inactive) stage of the insect developmental cycle and do not feed. In this study, the larvicidal and pupacidal

effects of *A. melegueta* leaf and seed extracts on *Anopheles* could be as a result of respiratory, contact and stomach poisoning. These findings support the result of Ileke *et al.*, 2014; Al-Dakhil and Morsy, 1999; Amusan and Okorie, 2002; Nathan *et al.*, 2005; as well as Akinkulore *et al.*, 2011 in which plant extracts were found effective against mosquito larvae and pupae. Ileke and Ogunbite (2015) opined that phytochemicals present in most plants can disrupt growth and reduce larval survival as well as disruption of life cycle of insects.

## CONCLUSION

The study revealed the effectiveness of *A. melegueta* extracts in the reduction of the larval and pupal stages of *Anopheles* mosquito and therefore *A. melegueta* based compounds can play an important role in the interruption of the transmission of mosquito-borne diseases at the individual, as well as at the community level in ensuring maximum safety in any ecological condition.

## REFERENCES

- Adedire, C. O., Lajide, L., 1999, Toxicity and oviposition deterrence of some plants extracts on cowpea storage bruchid, *Callosobruchus maculatus* (Fabricius). *Journal of Plant Disease and Protection*, 106: 647-653.
- Adesina, J. M., Jose, A. R., Rajashekar, Y., Afolabi, L. A., 2015, Entomotoxicity of *Xylopia aethiopia* and *Aframomum melegueta* in suppressing oviposition and adult emergence of *Callosobruchus maculatus* (Fabricius) (Coleoptera: Chrysomelidae) infesting stored cowpea seeds. *Jordan Journal of Biological Sciences*, 8(4): 263-268.
- Adeyemo, A. C., Ashamo, M. O., Odeyemi, O. O., 2014, *Aframomum melegueta*: A potential botanical pesticide against *Sitotroga cerealella* infestation on two paddy varieties. *Archives of Phytopathology and Plant Protection*, 47(15): 1841-1851.
- Akinkulore, R. O., Adedire, C. O., Odeyemi, O. O., Raji, J., Owoeye, J. A., 2011, Bioefficacy of extracts of some indigenous Nigerian plants on the developmental stages of mosquito (*Anopheles gambiae*). *Jordan Journal of Biological Science*, 4(4): 237-242.
- Alaje, D. O., Owolabi, K. T., Olakunle, T. P., Oluoti, O. J., Adetuberu, I. A., 2014, Nutritional, minerals and phytochemicals composition of *Garcinia cola* (Bitter cola) and *Aframomum melegueta* (Alligator pepper). *Journal of Environmental Science Toxicology and Food Technology*, 8: 86-91.
- Al-Dakhil, M. A., Morsy, T. A., 1999, The larvicidal activities of the peel oils of three citrus fruits against *C. pipiens*. *Journal of Egypt Social and Parasitology*, 29: 347-352.
- Amer, A., Mehlhorn, H., 2006, Larvicidal effects of various essential oils against *Aedes*, *Anopheles*, and *Culex* larvae (Diptera, Culicidae). *Parasitology Research*, 99: 466-472.
- Amusan, A. A. S., Okorie, T. G., 2002, The use of piper fruits oil as protectant of dried fish against *Dermestes maculatus*. *Global Journal of Pure and Applied Science*, 8(2): 197-201.
- Ashamo, M. O., Akinnawonu, O., 2012, Insecticidal efficacy of some plant powders and extracts against the Angoumois grain moth, *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae). *Archives of Phytopathology and Plant Protection*, 45(9):1051-1058.
- Dinesh, D. S., Kumari, S., Pandit, V., Kumari N., Kumar, P., Hassan, F., Kumar, V., Das, P., 2015, Insecticidal effect of plant extracts on *Phlebotomus argentipes* (Diptera: Psychodidae) in Bihar, India. *Indian Journal of Medical Research*, 142(Supplementary S1): 95-100.
- Ezeonu, F. C., Chidume, G. I., Udedi, S. C., 2001, Insecticidal properties of volatile extracts of orange peels. *Bioresource Technology*, 76: 273-274.

### Larvicidal and Pupicidal Potential of *Aframomum melegueta*

- Ghosh, A., Chowdhury, N., Chandra, G., 2012, Plant extracts as potential mosquito larvicides. *Indian Journal of Medical Research*, 135: 581-598.
- Harborne, J. B., 1973, *Phytochemical Methods: A Guide to Modern Technique of Plant Analysis*. Chapman and Hall, London, 279.
- Igwe, E. A., Emeruwa, L. C., Modie, J. A., 1999, Ocular toxicity of *Aframomium melegueta* (Alligator pepper) on healthy Igbo of Nigeria. *Journal of Ethnopharmacology*, 65: 203-206.
- Ileke, K. D., Oluotuah, O. F., 2012, Bioactivity of *Anacardium occidentale* (L) and *Allium sativum* (L) powders and oils extracts against cowpea bruchid, *Callosobruchus maculatus* (Fab). (Coleoptera: Chrysomelidae). *International Journal of Biology*, 4(1): 23-28.
- Ileke, K. D., Afolabi, O. J., Ogungbite, O. C., Olagunju, J. O., Akanbi, O. M., 2014, Mosquitocidal activity of *Anacardium occidentale*, *Aframomum melegueta*, *Garcinia kola* and *Citrus sinensis* against the developmental stages of mosquito, *Anopheles gambiae* Giles. *Journal of Mosquito Research*, 4(3): 21-26.
- Ileke, K. D., Ogungbite, O. C., 2015, *Alstonia boonei* De Wild oil extract in the management of mosquito (*Anopheles gambiae*) a vector of malaria disease. *Journal of Coastal Life Medicine*, 3(7): 557-563.
- Ileke, K. D., Oni, M. O., 2011, Toxicity of some plant powders to maize weevil, *Sitophilus zeamais* (Coleoptera: Curculionidae) on stored wheat grains, *African Journal of Agricultural Research*, 6(13): 3043-3048.
- Muthusamy, R., Ramkumar, G., Karthi, S., Shivakumar, M. S., 2014, Biochemical mechanisms of insecticide resistance in field population of Dengue vector *Aedes aegypti* (Diptera: Culicidae). *International Journal of Mosquito Research*, 1(2): 1-4.
- Nathan, S. S., Kalaivani, K., Murugan, K., 2005, Effects of neem limonoids on the malaria vector *Anopheles stephensi* Liston (Diptera: Culicidae), *Acta Tropica*, 96: 47-55.
- Ofuya, T. I., 1990, Oviposition deterrence and ovicidal properties of some plant powders against *Callosobruchus maculatus* in stored cowpea (*Vigna unguiculata*) seed during storage. *The Journal of Agricultural Science*, 115(3): 343-345.
- Ogungbite, O. C., Ileke, K. D., Akinneye, J. O., 2014, Bio-pesticide treated jute bags: potential alternative method of application of botanical insecticides against *Rhyzopertha dominica* (Fabricius) infesting stored wheat. *Molecular Entomology*, 5(4): 30-36.
- Onekutu, A., Nwosu, L. C., Nnolim, N. C., 2015, Effect of seed powder of three pepper species on the bionomics of cowpea bruchid, *Callosobruchus maculatus* Fabricius. *International Journal of Science Research*, 5: 1-5.
- Raji, J. I., Akinkurolere, R. O., 2010, The toxicity of some indigenous plant extracts on the developmental stages of Mosquito (*Anopheles gambiae*). *Nigerian Bioscientist*, 1-5.
- Sofowora, A., 1993, *Screening plants for bioactive agents*. Medicinal Plants and Traditional Medicinal in Africa. 2nd. ed., Spectrum Books Ltd, Sunshine House, Ibadan, Nigeria, 134-156.
- SPSS Inc., 2007, Statistical package for social sciences, Statistics 17.0 Brief Guide, SPSS, Inc., Chicago, IL., 181-185.
- Srinivasan, R., Natarajan, D., Karthi, S., Shivakumar, M. S., 2014, Chemical composition and larvicidal activity of *Elaeagnus indica* Servett. (Elaeagnaceae) plant leaf extracts against dengue and malaria vectors. *International Journal of Mosquito Research*, 1(4): 66-71.
- Trease, G. E., Evans, W. C., 2002, *Pharmacognosy*. 15th edn. Saunders Publishers, London, 42-44, 221-229, 246-249, 304-306, 331-332, 391-393.
- Udo, I. O., 2011, Potentials of *Zanthoxylum xanthoxyloides* as a model, In: Dr. Farzana Perveen (Eds.). *Insecticides-Advances in Integrated Pest Management*, 367-390.
- WHO, 1996, *World Health Organization. Instruction for Determining the Susceptibility and Resistance of Mosquito Larvae to Insecticides*, Mimeographed document. Geneva, Switzerland WHO. WHO/VBC/75.583.
- Yousaf, A., Zuharah, W. F. 2015, Lethal response of the dengue vectors to the plant extracts from family Anacardiaceae. *Asian Pacific Journal of Tropical Biomedicine*, 5(10): 812-818.

## AUTHOR GUIDELINES

*Journal of the Entomological Research Society (J. Entomol. Res. Soc.)* accepts and publishes original research articles in the all field of entomology. The journal publishes regular research papers, review articles. Short, timely reports may be submitted as short communications. The Editors first evaluate all manuscripts. At this stage insufficiently original, have serious scientific flaws, have poor grammar or English language, or are outside the aims and scope of the journal papers will be rejected. Those that meet the minimum criteria are passed on to at least 2 experts for review. Authors of manuscripts rejected at this stage will usually be informed at most 1 months of receipt. Authors should suggest (jersturkey@gmail.com) four reviewer name, address and e-mail address about manuscript subject to examine the manuscript. At most two of them will be in your country and others will be different countries. Two reviewers are selected from these or editor assign another reviewers. A final decision to accept or reject the manuscript will be sent to the author along with any recommendations made by reviewers. Editor's Decision is final Reviewers advise the editors, who are responsible for the final decision to accept or reject the article. Manuscript must be written in Arial with 12 type size with double spacing in Word for Windows. Please do not make paragraph in the text. Manuscripts generally should not exceed 30 pages.

**Heading:** The title of the paper should be informative, but preferably not exceed twenty words. Just under the heading, give title, full name(s) of author(s) (surname(s) in capital letters), with full address and e-mails of each on a separate line.

**Abstract:** An abstract provided at the beginning of the paper will indicate the main aspects of the subject, to be followed by 5-7 key words.

**Text:** The standard order of sections for original papers is as follows: Introduction, Material and Methods, Results, Conclusions and Discussion, Acknowledgements, References. The scientific names (e.g. genus- and species-group names) are to be italicized. For faunistic research follow this order, Distribution:..., Material examined:..., Host plant:....

Example:

*Sphex oxianus* Gussakovskij, 1928

Distribution: Central and South West Asia, Afghanistan, Iran, Israel, Turkey (Bohart and Menke, 1976; Menke and Pulawski, 2000; Kazenas, 2001), Turkey: Artvin (De Beaumont, 1967).

Material examined: Ankara, Altındağ, Çubuk Dam Lake, 900 m, 29. 07. 1998, 1 ♂; Kalecik, 600 m, 24. 06. 2001, 2 ♀♀.

Host plant: *Echinophora* sp.

Please use ♀, ♂ symbols. Please write upper genus categories with capital letters.

**Illustrations:** Illustrations, graphs, their caption or legends should form separate, self-explanatory unit. Abbreviations in the legends should be explained but if there are too many, they should be included into a separate list. The original drawing and photographs should be not more than twice as large as when printed. Morphological illustrations should be including a scale bar. Photographs and electron micrographs must be in JPEG file format (300 dpi). Drawings (black and white type) must be in TIFF format and their size no more than 10 MB. **Graphs should be in excel file format.** Color figures pay charge. Tables should include headings and explanations, and should be numbered consecutively. Their approximate position in the text should be indicated in the margin. **Legends and title of the graphs and**

**tables must be in Arial with 12 type size. Please do not embed the figures, graphs and table into the text, send them as supplementary files.** In the text attribution to the figures should be given in parenthesis and must be abbreviate like this; (Fig.1).

**References:** In the text, reference to the literature should conform to the "name- and- date" system, e.g. (Lyneborg, 1983); Beirne and Young (1953); Edwards (1894-1896); Gayuboet *al.* (2003).

Titles of papers published in languages other than the major ones (English, German, French, Spanish, Portuguese, Turkish) should be an English translation (in parentheses) with an explanatory note at end, e.g. (in Russian). The list references will be given at the end of the article and listed alphabetically, according to the following examples, all periodicals must be **unabbreviated and italicized**.

#### **Journal Article**

Beirne, B. P., Young, D. A., 1953, The North American species of *Cicadula* (Homoptera, Cicadellidae). *Canadian Entomologist*, 85: 215-226.

#### **Book Chapter**

Kirejtshuk, A. G., 1992, *Evolution of mode of life as the basis for division of the beetles into groups of high taxonomic rank*. In: Zunino, M., Bellés, X., Blas, M. (Eds.). *Advances in Coleopterology*. European Association of Coleopterology, Barcelona, 249-261.

#### **Book**

Steinmann, H., Zombori, L., 1985, *An Atlas of Insect Morphology*. 2nd edn. Akadémiai Kiadó, Budapest, Hungary, 253.

#### **URLs**

Geller-Grimm, F., 2007, Database Asilidae: Catalog of Species. <http://www.geller-grimm.de/catalog/species.htm> (10.03.2010).

Nomenclature must be in absolute agreement with current **ICZN** rules. The only acceptable type concepts are: holotype, paratype etc. The following abbreviations should be adopted: gen. n., sp. n., stat. n. and comb. n.

**10 free reprints** are supplied per contribution: An additional number may be ordered at the prices quoted on the order form sent to the corresponding author.

*Journal of the Entomological Research Society* uses the Open Journal Systems (OJS) platform, which will enable the journal to accept submissions online. For submitting a manuscript please go to web page <http://www.entomol.org> and register as author and submit your manuscript online.

Manuscripts send by e-mail will be not accepted.

**URL:** <http://www.entomol.org>

**e-mails:** [jersturkey@gmail.com](mailto:jersturkey@gmail.com); [entomol@gazi.edu.tr](mailto:entomol@gazi.edu.tr)

**Address:** Journal of the Entomological Research Society, P.box.110 Bahcelievler P.Isl.Mud. 06502, Ankara/TURKEY

## CONTENTS

SANAEI, E., SEIEDY, M., Weevils (Coleoptera: Curculionoidea) of Taleghan Region (North of Iran) with Reporting of Ten New Records for The Iran.....	1
GHANNEM, S., BOUMAIZA, M., New Data of Ground Beetles (Coleoptera: Carabidae) in Tunisia .....	15
YANAR, O., GÖMEÇ, S., TOPKARA, E. F., DEMİR, İ. DEMİRBAĞ, Z., The Effect of Plant Quality on Survival of <i>Lymantria dispar</i> L. (Lepidoptera: Lymantriidae) Larvae Infected by Nucleopolyhedrovirus....	39
ALIZAMANI, T., RAZMJOU, J., NASERI, B., HASSANPOUR, M., ASADI, A., KERR, C., Effect of Vermicompost on Life History of <i>Hippodamia variegata</i> Preying on <i>Aphis gossypii</i> Glover.....	51
REGO, C., AGUIAR, A. F., CRAVO, D., BOIEIRO, M., Invasive Fruit Flies (Diptera: Drosophilidae) Meet in a Biodiversity Hotspot .....	61
FATEHI, S., FARSHBAF POUR ABAD, R., BANDANI, A. R., DASTRANJ, M., Effect of Seed Proteinaceous Extracts from Two Wheat Cultivars against <i>Phthorimaea operculella</i> (Lepidoptera: Gelechiidae) Digestive $\alpha$ -amylase and Protease Activities.....	71
SEDGHIANI, S., RABOUDI, F., BOUKTILA, D., Hanem MAKNI, H., MAKNI, M., A Practical Molecular Diagnostic Tool of the Date Moth <i>Ectomyelois ceratoniae</i> (Lepidoptera: Pyralidae) in Tunisia.....	81
KACPRZYK, M., BILAŃSKI, P., The First Stages of Xylobiont Entomofauna Succession on European Silver Fir Logging Residues in Relation to Utilisation Method and Microenvironmental Conditions.....	91
SAK, O., The Effects of 5-Aza-2'-deoxycytidine on Total Lipid and Fatty Acid Composition of <i>Apanteles galleriae</i> Wilkinson (Hymenoptera: Braconidae) and on Its Parasitized Host.....	105
ILEKE, K. D., ADESINA J. M., OKUNOLA, O. G., Larvicidal and Pupicidal Potential of <i>Aframomum melegueta</i> K. Schum Extracts Against Mosquito, Anopheles Species.....	121