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The first well-documented record of the vine bud moth *Theresimima ampellophaga* (Bayle-Barelle, 1808) in Albania established by field screening of sex pheromone and sex attractant traps (Lepidoptera: Zygaenidae, Procridinae)

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Abstract

The vine bud moth, *Theresimima ampellophaga* (Bayle-Barelle, 1808), has been known as a pest on grapevine (*Vitis vinifera*) since ancient Roman times. Despite the fact that the vineyards are widely distributed in Albania, there are no clearly documented data about the presence of this species in this country. Delta sticky traps baited with the synthetic sex pheromone of *Th. ampellophaga*, (2*R*)-butyl (7*Z*)-tetradecenoate or the sex attractant EFETOV-2 (the racemic mixture of (2*R*)-butyl 2-dodecenoate and (2*S*)-butyl 2-dodecenoate) were used in eight vineyards in Albania for possible detection of the occurrence of the vine bud moth. As a result, this species was recorded for the first time in Albania in four vineyards of three different municipalities: Fier (Apolloni), Konispol (Qafë Botë and Xarrë), and Vlorë (Panaja). The results showed a low population density of *Th. ampellophaga* in the southern and southwestern part of the country. Male moths were caught from the end of June - beginning of July and at the beginning of September indicating the presence of two generations of *Th. ampellophaga* in Albania.

KEY WORDS: Lepidoptera, Zygaenidae, Procridinae, vine pest, *Theresimima*, *Vitis vinifera*, sex pheromone, EFETOV-2, Albania.

El primer registro bien documentado de la zigena de la vid *Theresimima ampellophaga* (Bayle-Barelle, 1808) en Albania establecido por la revisión de campo entre las feromonas sexuales y las trampas atrayentes sexuales (Lepidoptera: Zygaenidae, Procridinae)

Resumen

La zigena de la vid, *Theresimima ampellophaga* (Bayle-Barelle, 1808), desde los tiempos de la Antigua Roma, ha sido conocida como plaga de la vid (*Vitis vinifera*). A pesar que los viñedos están ampliamente distribuidos en Albania, no hay datos, evidentemente documentados, sobre la presencia de este especie en el país. Trampas adhesivas cebadas con la feromona sintética sexual de *Th. ampellophaga*, (2*R*)-butil (7*Z*)-tetradodecanoato o el atrayente sexual EFETOV-2 (la mezcla racémica de (2*R*)-butil 2-dodecanoato y (2*S*)-butil 2-dodecanoato) fueron usados en ocho viñedos en Albania para detectar la posible presencia de la polilla de la vid. Por consiguiente, esta especie fue registrada en Albania, por primera vez, en cuatro viñedos de tres diferentes municipalidades: Fier (Apolloni), Konispol (Qafë Botë and Xarrë) y Vlorë (Panaja). Los resultados muestran la baja densidad de poblaciones de *Th. ampellophaga* en el sur y sudoeste del país. Los machos fueron capturados a finales de junio-comienzos de julio y desde comienzo de septiembre indicando la presencia de dos generaciones de *Th. ampellophaga* en Albania.

PALABRAS CLAVE: Lepidoptera, Zygaenidae, Procrinae, plaga de la vid, *Theresimima*, *Vitis vinifera*, feromona sexual, EFETOV-2, Albania.

Introduction

The vine bud moth, *Theresimima ampelophaga* (Bayle-Barelle, 1808), (Lepidoptera, Zygaenidae) has been known as a pest on *Vitis vinifera* L. (Vitaceae) since ancient Roman times (COSTA, 1857; ISSEKUTZ, 1957a, b). This species has a Western Palaearctic Ponto-Mediterranean distribution and it occurs in many southern countries of Europe and western Asia (EFETOV, 2001, 2004; TARMANN, 2003). Nowadays it has pest status on grapevine in some eastern European countries and regions e.g. Bulgaria (HARIZANOV *et al.*, 2006), Hungary (VOIGT *et al.*, 2000), Crimea (EFETOV, 2005), and in some regions of Turkey (CAN *et al.*, 2010). The presence of *Th. ampelophaga* in Albania was mentioned only in summarizing comments of the global distribution of this species (REBEL & ZERNY, 1934; ISSEKUTZ, 1957a, b; TARMANN, 1998). All mentioned authors have reported this species only based on the fact that it is present in the neighbouring countries, Greece and North Macedonia. However, there are no records in Albania based on original material.

Sex pheromones (chemical(s) identified in the pheromone gland of one of the sexes) and sex attractants (chemicals for which there is no such information) are frequently used in agriculture, horticulture, viticulture and forestry for biological pest control and as an important part of an integrated pest management (WITZGALL *et al.*, 2010; SUBCHEV, 2014). The main compound of the sex pheromone of *Th. ampelophaga* females, which is released from a gland situated between 3rd and 5th segment of the abdomen (HALLBERG & SUBCHEV, 1997), has been identified as (2*R*)-butyl (7*Z*)-tetradecenoate (SUBCHEV *et al.*, 1998). The synthetic form of this compound has high biological activity and it has been used in traps for detection and monitoring of the seasonal activity of this species in Bosnia and Herzegovina (Karalija, Nahirnić, Toshova, personal communications), Bulgaria (TOSHOVA & SUBCHEV, 2002; SUBCHEV *et al.*, 2008b; TOSHOVA *et al.*, 2017; MUMUN *et al.*, 2018), Croatia (RAZOV *et al.*, 2017), France (RYMARCZYK & DROUET, 2006; DROUET & LAMBERT, 2010), Greece (SUBCHEV *et al.*, 2006; TARMANN *et al.*, 2019), Hungary (VOIGT *et al.*, 2000), Italy (SUBCHEV *et al.*, 2008b), Romania (SUBCHEV *et al.*, 2008a), Crimea (EFETOV, 2001; SUBCHEV *et al.*, 2008b), Serbia (NAHIRNIĆ *et al.*, 2015) and Turkey (CAN *et al.*, 2010; CAN-CENGİZ *et al.*, 2012). Recently, 2-butyl 2-dodecenoate which has a different position of the double bond in comparison with the main pheromone compound of *Th. ampelophaga* was synthesized from 2-butanol and dodecenoic acid in the Crimean Federal University (EFETOV *et al.*, 2014b). The racemic mixture of (2*R*)-butyl 2-dodecenoate and (2*S*)-butyl 2-dodecenoate and (2*R*)-butyl 2-dodecenoate alone were named “EFETOV-2” and “EFETOV-S-2” respectively. Their attractiveness for the males of *Th. ampelophaga* was estimated in commercial and abandoned vineyards in different countries. It was shown that EFETOV-2 was attractive for the males of *Th. ampelophaga* in the Crimea (EFETOV *et al.*, 2014b) and North Macedonia (MICEVSKI *et al.*, 2018), while EFETOV-S-2 attracted the males of this species in Turkey (Thrace region) (CAN CENGİZ *et al.*, 2018). Moreover, these substances were also attractive for the males of some other Zygaenidae species (EFETOV *et al.*, 2014b, 2016, 2018). It is known that the sex pheromone of one species can be active at the same time as the sex attractant for other species (EFETOV *et al.*, 2010, 2011, 2014a, 2015; SUBCHEV *et al.*, 2010, 2012, 2013, 2016). However, these chemicals can attract different species of one subgenus, but not of another subgenus of the same genus. For example, in the genus *Illiberis* Walker, 1854, sex pheromones and attractants are known for the subgenus *Primilliberis* Alberti, 1954 (SUBCHEV *et al.*, 2012, 2013, 2016; EFETOV *et al.*, 2018), but not for the subgenus *Alterasvenia* Alberti, 1971. These two subgenera have also good morphological differences (EFETOV, 1996; EFETOV & TARMANN, 2013, 2014). Furthermore, sex pheromones and attractants can be successfully used for the detection and monitoring of low-density populations of harmful species or species of conservation interest (OLEANDER *et al.*, 2015). The objectives of the present

research were to establish a possible occurrence of the vine bud moth *Th. ampelophaga* in Albania, and to obtain information about its seasonal flight in this country.

Materials and methods

STUDY AREAS

Vineyards selected for this study were located in southern and southeastern Albania. In all vineyards, except the one at Grapsh, a broad spectrum pyrethroid insecticide Decis® 2.5 EC (Bayer Crop science Inc.) with an active compound deltamethrin was used twice per year (after bud emerging and after grape ripening).

The study areas are presented on Fig. 1:

1. Devoll municipality - Sul village: 40°32'44"N, 20°55'00"E, 1024 m a. s. l. Grape varieties are Vranac, Manakuq and Prokupac.
2. Dropull municipality - Grapsh village: 39°57'29.7"N, 20°14'55.1"E, 223 m a. s. l. Grape varieties are Shesh i Zi and Merlot.
3. Fier municipality - Apolloni ancient city: 40°43'45"N, 19°28'48"E, 35 m a. s. l. The vineyard is located in the hills close to the old ancient city of Apolloni, part of the village Pojan. Grape variety is a French hybrid.
4. Kolonjë municipality - Cerckë village: 40°08'15.5"N, 20°35'54.7"E, 732 m a. s. l. The vineyard is located in the western sunny side of a hill in an area known as Kroj i Ri, part of the former municipality Qendër Leskovik. Grape variety is Pinot Gris.
5. Konispol municipality - Qafë Botë custom: 39°39'56.1"N, 20°10'01"E, 160 m a. s. l. The vineyard is located on the side of the main street at the mountain pass, in the most southern part of Albania, close to the city of Konispol and to the border with Greece. Grape variety is Shesh i Zi.
6. Konispol municipality - Xarrë village: 39°43'58.8"N, 20°03'52.4"E, 9 m a. s. l. Grape varieties are Shesh i Bardhë and Riesling.
7. Patos municipality - Zharrëz village: 40°42'27.5"N, 19°38'46.1"E, 31 m a. s. l. Grape variety is Rhoditis.
8. Vlorë municipality - Panaja village: 40°32'12.5"N, 19°28'21.4"E, 14 m a. s. l. Grape varieties are Shesh i Bardhë and Shesh i Zi.

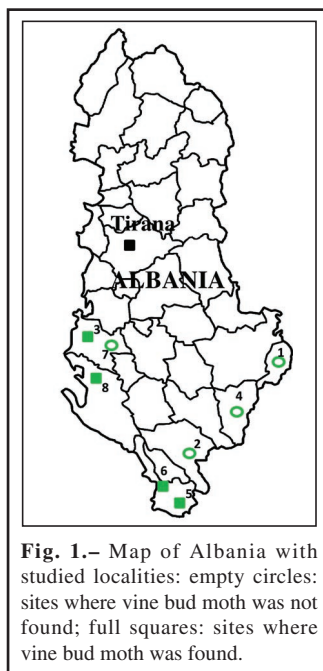


Fig. 1.– Map of Albania with studied localities: empty circles: sites where vine bud moth was not found; full squares: sites where vine bud moth was found.

LURES

For preparing lures, synthetic sex pheromone (2*R*)-butyl (7*Z*)-tetradecenoate, synthesized in the Institute of Organic Chemistry, Hamburg (Germany) (SUBCHEV *et al.*, 1998), was applied onto serum bottle caps of grey rubber in a dose of 100 µg as a hexane solution.

For our field work, we also used the rubber caps impregnated with 50 µl of the sex attractant EFETOV-2 (the racemic mixture of (2*R*)-butyl 2-dodecenoate and (2*S*)-butyl 2-dodecenoate), which was produced in the Crimean Federal University, Simferopol (Crimea) (EFETOV *et al.*, 2014b).

The lures were wrapped singly in aluminium foil and stored in a freezer up to the date of field work.

TRAPS AND THEIR PLACEMENT IN THE VINEYARDS

The lures were fixed in Delta sticky traps with cardboard holders. The Delta type traps were

prepared by a PVC foil. At the base of each trap a removable PVC sheet (15 cm x 10 cm) covered with insect glue (Chemstop Ecofix®, Slovakia) was placed. The traps were hung on the grape branches, 1 m above the ground (Fig. 2). The distance between the traps in a locality was at least 10 m. Placement date was in the period of 22-25-V-2017 for all the vineyards, except for Apolloni on 01-VI-2017, Panaja and Zharrëz on 26-27-VII-2017; meanwhile the removal date was on 30-IX-2017, except for the vineyards of Cerckë on 30-V-2017, Grapsh and Sul on 24-25-VII-2017.

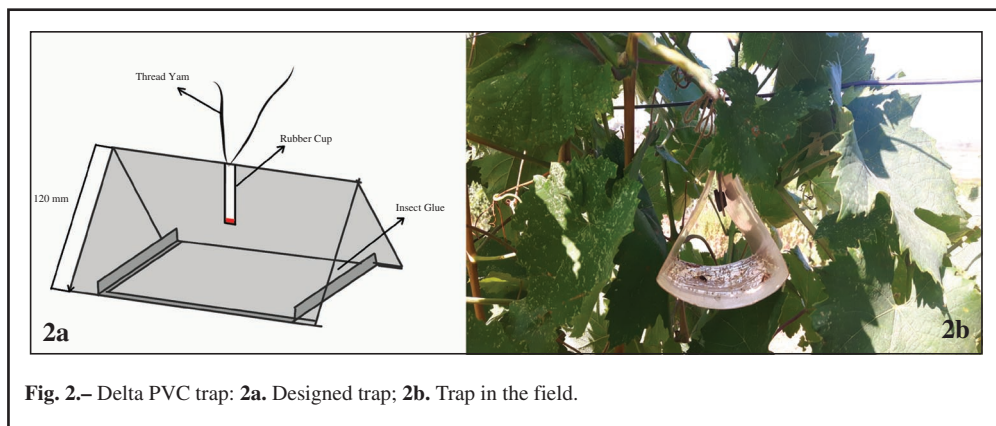


Fig. 2.– Delta PVC trap: **2a.** Designed trap; **2b.** Trap in the field.

Four traps were used for each vineyard, two traps baited with sex pheromone, one trap baited with sex attractant and one unbaited (control) trap (Table 1), which in total makes 20 traps in 8 vineyards (with different varieties of *V. vinifera*). Traps were inspected and sticky inserts with insects captured were collected and replaced with new sticky sheets weekly. The determination of the species was done by examination of the genitalia structure of each moth caught. The collected reference material was stored at the Museum of Natural Sciences, Tirana, Albania.

Table 1.– Total catches of *Theresimima ampellophaga* in the study localities in Albania.

Locality	Treatment	Number of traps	Placement	Periods with catches of <i>Th. ampellophaga</i>		
				24-30-VI-2017	01-06-VII-2017	01-06-IX-2017
Qafë Botë	Sex pheromone	2	24-V-2017	7 ♂♂	-	-
	EFETOV-2	1	24-V-2017	-	-	-
	Control	1	24-V-2017	-	-	-
Xarrë	Sex pheromone	2	24-V-2017	1 ♂	-	-
	EFETOV-2	1	24-V-2017	-	-	-
	Control	1	24-V-2017	-	-	-
Apolloni	Sex pheromone	2	01-VI-2017	-	1 ♂	-
	EFETOV-2	1	01-VI-2017	-	-	-
	Control	1	01-VI-2017	-	-	-
Panaja	Sex pheromone	2	27-VII-2017	-	-	-
	EFETOV-2	1	27-VII-2017	-	-	2 ♂♂
	Control	1	27-VII-2017	-	-	-

Results

Th. ampellophaga was recorded in Albania for the first time, namely in the south-western part of the country. The species was found in four of eight vineyards where the traps were placed - Apolloni,

Qafë Botë, Xarrë, and Panaja (Figs 3, 4). A total number of 11 specimens were captured: nine male moths in the pheromone traps and two males in the traps baited with the sex attractant EFETOV-2. Only one generation of the vine bud moth was registered in Fier (Apolloni) and Konispol (Xarrë - one moth and Qafë Botë - seven moths). Catches in Vlorë (Panaja - two moths) in September indicated a presence of the second generation. The highest number of male moths caught was registered in Qafë Botë.

These results indicate that the vine bud moth can develop two generations in Albania, the 1st one with catches at from the end of June to early July and the 2nd in early September (Table 1).

Discussion

The sex pheromone and sex attractant EFETOV-2 were applied for the first time in the vineyards of southern Albania to detect the presence of *Th. ampelophaga*. The results of this study confirm the presence of this species in Albania. The data obtained are comparable with those known from similar studies by pheromone traps in the Mediterranean region (CAN *et al.*, 2010). Based on results obtained using sex pheromone traps in previous research, the flight period of *Th. ampelophaga* lasted 3-5 weeks, as the beginning of the flight of the first generation was different in different countries based on the meteorologic and local conditions. Earlier catches in the year than those obtained in the recent study were registered in central Greece and Lefkada island (SUBCHEV *et al.*, 2006), where the flight period started at the end of May. In some countries the period of flight was from the middle of June to early July - Serbia (NAHIRNIC *et al.*, 2015), Bulgaria (TOSHOVA & SUBCHEV, 2002; SUBCHEV *et al.*, 2008b; MUMUN *et al.*, 2018), Romania (SUBCHEV *et al.*, 2008a), and Crimea (EFETOV *et al.*, 2014b). Using pheromone traps, two generations of the vine bud moth were reported in Rhodes Island (Greece) (SUBCHEV *et al.*, 2006), Halkidiki (Greece) (TARMANN *et al.*, 2019), southern Turkey (CAN *et al.*, 2010), and in southern Bulgaria in some years (TOSHOVA *et al.*, 2017; MUMUN *et al.*, 2018). In Rhodes, the flight of the second generation started from the end of July and ended in late August while in the mediterranean part of Turkey and Bulgaria – from early August to early September. This species has two generations also in Israel (the second generation has been confirmed by two specimens from Jerusalem, 18-VIII-1949, leg. J. H. Brair, in coll. Museum Witt, Munich), Lebanon (TALHOUK, 1969; LARSEN, 1980) and in the Caucasus (EFETOV, 2005).

SUBCHEV *et al.* (2004) reported that the sex pheromone of *Th. ampelophaga* applied at a dose of 100 µg on rubber septa has a useful field life at least 40 days, that is long enough for monitoring throughout one full flight period of the vine bud moth (in the regions where the species has only one generation) without the need for replacing dispensers. The lures tested during the current study were not replaced with fresh ones. EFETOV-2 attractant had longer activity in the field - at least three months which explained the catches of males of the target species only in a trap baited with EFETOV-2 (with high amount of the racemic mixture applied) in early September 2017. Previous studies showed that the sex attractant did not attract *Th. ampelophaga* when it was tested in the same vineyard with the active sex pheromone lures (Toshova, personal communication). Based on the longer duration of activity of EFETOV-2 lure we can hypothesize that it can remain active during the flight of the two generations of this species in regions where it is bivoltine. Additional studies are needed to confirm this hypothesis.

Based on the results of this study, the vineyards with autochthon Albanian grape varieties Shesh i Bardhë (white) and Shesh i Zi (black) respectively in Panaja (Shesh i Bardhë and Shesh i Zi), Qafë Botë (Shesh i Zi) and Xarrë (Shesh i Bardhë and Riesling), resulted in catches of *Th. ampelophaga*. These varieties are the most important ones for wine production in Albania as they have a high alcohol percentage, and in the vineyards with fewer fertilizers the quality of the wine is higher based to the sugar-acidity ratio (ZIGORI & ZIGORI, 2000). Meanwhile we had positive results in the vineyard in Apolloni with a grape variety - French hybrid (a crossing between *V. vinifera* and another *Vitis* species), which has a good resistance against grape phylloxera, fungus and other diseases and abiotic stresses, leading to the usage of a limited application of pesticides (BARRETT, 1956).

Conclusions

Th. ampellophaga has been recorded for the first time in Albania in three south-western municipalities: Fier (Apolloni), Vlorë (Panaja) and Konispol (Qafë Botë and Xarrë). The results showed low population density of the vine bud moth in these localities. The catches were registered at the end of June - beginning of July and at the beginning of September indicating the presence of two generations of this species in Albania.

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Figures 3-4.– 3. Traps with catches in 1) Qafë Botë and 2) Panaja. 4. Distribution map of *Theresimima ampellophaga* in the Southern Balkan. This figure summarizes all known published records of the vine bud moth in the Southern Balkans, found in the current BioOffice database.