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New Data on the Distribution and Seasonal Flight of the Vine Bud Moth Theresimima ampellophaga (Bayle-Barelle, 1808) in Bulgaria - Investigations By Pheromone-Baited Traps

Teodora B. Toshova[1*], Daniela Y. Atanasova[2], Boyan S. Stalev[2], Ana Nahirnić[3]

1 - Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, Blvd. Tzar Osvoboditel 1, 1000 Sofia, BULGARIA

2 - Faculty of Plant Protection and Agroecology, Agricultural University, Blvd. Mendeleev 12, 4000 Plovdiv, BULGARIA

3 - National Museum of Natural History, Bulgarian Academy of Sciences, Blvd. Tzar Osvoboditel 1, 1000 Sofia, BULGARIA

* Corresponding author: teodora_toshova@yahoo.com

Abstract. The vine bud moth, *Theresimima ampellophaga* is considered as a pest species of the grapevine *Vitis vinifera* in Bulgaria. Delta sticky traps baited with the main synthetic sex pheromone compound, (2*R*)-butyl (7*Z*)-tetradecenoate, of *Th. ampellophaga* were used for detection and seasonal monitoring of this species in vineyards at four sites in Bulgaria – Lozitsa village (Municipality Nikopol) (northern Bulgaria) and Gornoslav village (Municipality Asenovgrad) in 2015 and 2016, and Plovdiv town and Krumovo village (Municipality Rodopi) (southern Bulgaria) in 2015. Catches of *Th. ampellophaga* males were recorded only in the villages of Lozitsa and Gornoslav. These are new distributional records for this species in Bulgaria. At these two sites, only one generation of *Th. ampellophaga* was established in 2015. In 2016, one generation of this species was recorded in Lozitsa and two generations – in Gornoslav. The flight period of the moths of the first generation was from the end of May (Gornoslav) – middle of June (Lozitsa) to the second half of July at both sites. The moths of the second generation in Gornoslav occur at the second half of August.

Key words: *Theresimima ampellophaga*, pheromone traps, Bulgaria, new records, seasonal flight.

Introduction

Viticulture is a traditional subsector of Bulgarian agriculture. As a result of a massive organizational restructuring and economic problems in this subsector, the total area harvested, yield and grape production has been reduced throughout the last twenty years. However, recently, despite the continuous reduction in harvested area of vines grown by the conventional method, the areas with organic grapes production grew steadily. The age structure of vineyards (many vineyards are old), selection and investment in quality grapevine varieties with high adaptation to the environmental conditions and tolerance to pests / diseases, improvement of soil fertility and application of sustainable pest control methods are important factors for viticulture development in Bulgaria (MOULTON *et al.*, 1994; DIMITROVA *et al.*, 2013; DYAKOVA *et al.*, 2014; TSVETKOV & DZHAMBAZOVA, 2014; ROYCHEVA, 2015). According to HARIZANOV *et al.* (2006), the economically important insect pests of *Vitis vinifera* L. in Bulgaria are the European grapevine moth, *Lobesia botrana*

([Denis & Schiffermüller], 1775), the vine moth, *Eupoecilia ambiguella* (Hübner, 1796), grape leafroller, *Sparganothis pilleriana* ([Denis & Schiffermüller], 1775) (Tortricidae), the vine bud moth, *Theresimima ampellophaga* (Bayle-Barelle, 1808) (Zygaenidae), species belonging to the genera *Agrotis* Ochsenheimer, 1816, *Euxoa* Hübner, 1821, *Xestia* Hübner, 1818, *Mamestra* Ochsenheimer, 1816, *Heliothis* Hübner, 1818 (Noctuidae), the cottony vine scale, *Pulvinaria vitis* (Linnaeus, 1758) (Coccidae). In different years damages caused by other insect pests like scales *Parthenolecanium corni* (Bouché, 1844) and *P. persicae* (Fabricius, 1776), the hazel leaf-roller, *Byctiscus betulae* (Linnaeus, 1758) (Rhynchitidae), the Western grape rootworm, *Bromius obscurus* (Linnaeus, 1758) (Chrysomelidae), and the tarnished plant bugs, *Lygus* Hahn, 1833 (MAF, 2017; KOSTADINOVA *et al.*, 2009) have been also reported. Recently, the spotted wing Drosophila, *Drosophila suzukii* (Matsumura, 1931) (Drosophilidae), a major invasive pest of small and stone fruits including grapes in America and Europe (ASPLEN *et al.*, 2015), was caught for the first time in a trap in 2014 in the southwestern part of Bulgaria (regions of Blagoevgrad, Kyustendil and Plovdiv) and Varna (EPPO, 2015; LAGINOVA & IVANOVA, 2015) and later established in new localities in southern Bulgaria (KARADJOVA *et al.*, 2016). In addition to this, *Scaphoideus titanus* Ball, 1932 (Cicadellidae), a vector of Flavescence dorée, was found in Varna, Veliko Tarnovo and Ruse regions (AVRAMOV *et al.*, 2011; GJONOV & SHISHINIOVA, 2014).

The vine bud moth, *Th. ampellophaga* is distributed in south eastern France, Italy, Vatican, Slovenia, Hungary, Croatia, Serbia, Albania, Macedonia, Romania, Moldova, Bulgaria, Greece (including islands), Cyprus, Ukraine (region of Odessa), South Russia, Georgia, Azerbaijan, Turkey, Cyprus, Syria, Lebanon, Israel and Algeria (TARMANN, 1998; 2003). The host plants for the larvae of this species are *V. vinifera* and *Parthenocissus* spp. In the last century, periodic and local outbreaks of *Th. ampellophaga* associated with the primary host, grapevine, were reported in several countries, Bulgaria, Italy and Hungary (REICHART & TASNADY, 1967; ANASTASOVA & GEORGIEVA, 1975; PUCCI & DOMINICI, 1986; HARIZANOV *et al.*, 1994). Currently in some regions/ countries the vine bud moth is considered as a pest and control measures were applied (CAN *et al.*, 2010; LEBEDEV, 2011; MAF, 2017), but in other ones it is relatively rare (SUBCHEV *et al.*, 2008a; NAHIRNIĆ *et al.*, 2015) and even endangered species (SHCHUROV & ZAMOTAJLOV, 2006; ZAMOTAJLOV, 2007).

Sex pheromones are an integral part of integrated pest management (IPM) programs in agriculture, particularly for monitoring abundance and distribution of insect pest populations (RODRIGUEZ-SAONA & STELINSKI, 2009). The main sex pheromone compound of *Th. ampellophaga* females, released from a gland at 3rd -5th abdominal tergites (HALLBERG & SUBCHEV, 1997), was identified as (2*R*)-butyl (7*Z*)-tetradecenoate (SUBCHEV *et al.*, 1998). Later, the synthetic sex pheromone was used for detection and monitoring of the seasonal flight of this species in several European countries and in Asiatic part of Turkey (reviewed in SUBCHEV, 2014; RAZOV *et al.*, 2017). Attraction of conspecific males to the opposite enantiomer, (2*S*)-butyl (7*Z*)-tetradecenoate, and 2-butyl 2-dodecenoate has been documented (EFETOV *et al.*, 2010; 2014).

The aim of the current study was to establish the presence and to monitor seasonal flight of *Th. ampellophaga* in four sites in Bulgaria by pheromone-baited traps.

Materials and Methods

Pheromone baits and traps: for preparing pheromone lures, the synthetic sex pheromone, (2*R*)-butyl (7*Z*)-tetradecenoate (SUBCHEV *et al.*, 1998), in a dose of 100 µg was applied onto serum bottle vials of grey rubber as a hexane solution. Home-made sticky Delta traps of transparent PVC foil were used for all field investigations.

Monitoring sites:

1. Lozitsa village (Nikopol Municipality, northern Bulgaria) is situated on Nikopol Plateau, which is representative place for the priority habitats (PEEV *et al.*, 2012) and is designated as Special Protection Area within NATURA 2000 ecological network. Twenty five years ago, the total area of cultivated vineyards of Lozitsa village was

more than 500 ha. Now the cultivated vineyards are about 10 % of this area, and the rest of plantations are abandoned or eradicated and changed into arable lands (PETKOV, pers. comm.). In 2015 and 2016, one pheromone trap in a vineyard with Mavrud variety (0.15 ha) (43°36'33.93"N; 24°59'21.45"E) and one trap in a vineyard with Muscat Ottonel variety (0.6 ha) (43°36'23.65"N; 24°58'26.67"E) were set up on 12 June, 2015 and 28 May, 2016, respectively and removed on 19 September, 2015 and 17 September, 2016, respectively. Distance between the vineyards was about 0.5-0.8 km.

2. Gornoslav village (Asenovgrad Municipality, southern Bulgaria). Currently the total vineyard area of the village is 90 ha. The pheromone traps (one in 2015 and two in 2016) were set up in a private vineyard with Pamid variety (0.7 ha) (41° 55″54.38″N; 24° 57″47.97″E). The monitoring periods were 7 June – 14 September, 2015 and 10 May – 16 September, 2016.

3. Plovdiv town (Plovdiv Municipality, southern Bulgaria). In 2015, one trap in a vineyard belonging to Agricultural University (Plovdiv) with Pleven variety (0.2 ha) (42°07″53.73″N; 24°48″19.09″E) was set up on 7 June and removed on 14 September, 2015.

4. Krumovo Village (Rodopi Municipality, southern Bulgaria). In 2015, one trap in a vineyard with Velika variety (0.5 ha) (42° 04″54.54″N; 24° 48″27.94″E) was set up on 7 June and removed on 14 September, 2015.

In all vineyards, no chemical insecticides were used in the years of our investigations, and in the vineyard in Gornoslav village only organic manage-ment practice was applied. Traps were inspected at least once per week (in exception of the period 15-28 August, 2016 in Gornoslav village) and the sticky layers with insects caught were collected and replaced with clear ones. During the monitoring periods, lures were replaced with fresh ones at 6-8 weeks. The determination of the species was done by examination of the genitalia structure of moth caught.

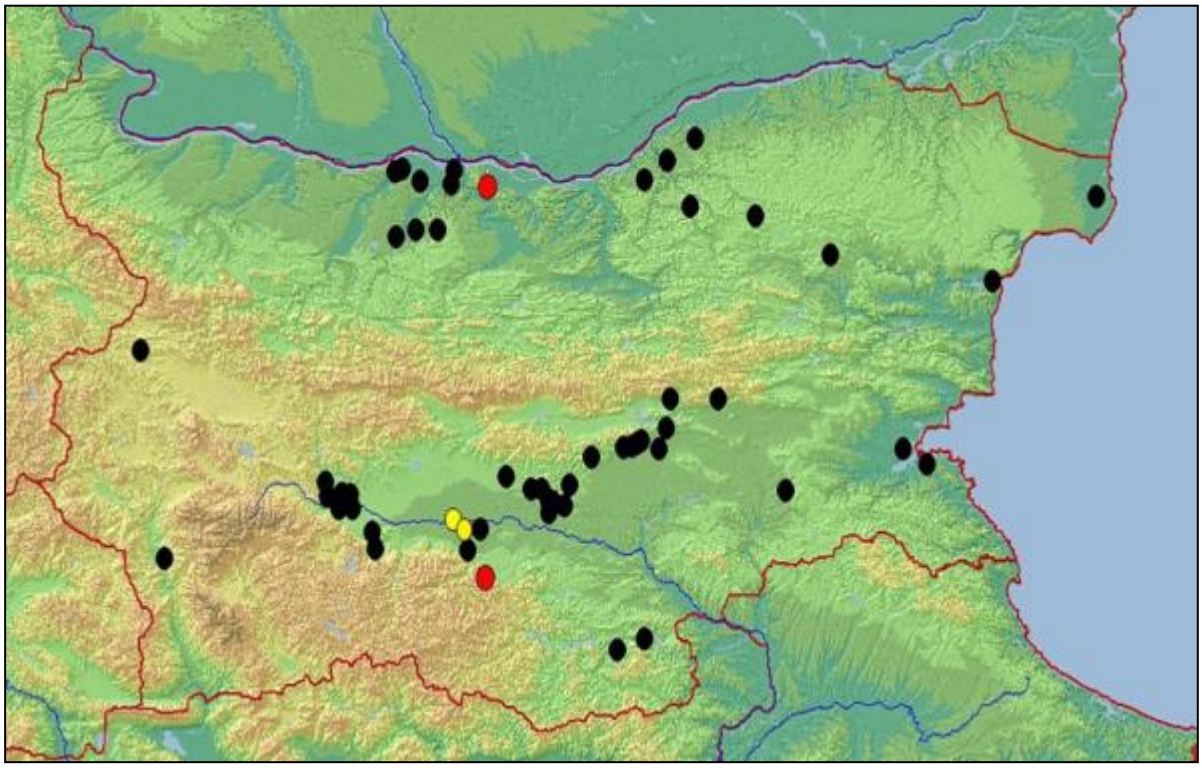

Fig. 1. Map of Bulgaria with localities (black and red dots) where *Th. ampellophaga* has been recorded so far. Black dots – published records, red dots – new records, yellow dots – absence of *Th. ampellophaga* catches during our study.

Results

Th. ampellophaga catches were recorded only in two of the four investigated sites - Lozitsa and Gornoslav. Fig. 1 represents the distribution of *Th. ampellophaga* in Bulgaria based on literature data and the results of the current study (BioOffice database).

The patterns of the seasonal flight of this species in 2015 and 2016 in the studied sites are presented on Fig. 2 and 3.

In 2015, the first catches of male moths in Lozitsa were registered at the second half of June and the last ones at the middle of July. The maximum of the flight was at the end of June – beginning of July. In Gornoslav village, *Th. ampellophaga* males were recorded at the first inspection date after the traps were set up and probably the beginning of the flight was missed; catches were recorded in the period of 7 June to 20 July. The population density of the vine bud moth in 2015 was relatively low at both sites.

In 2016, the flight of the vine bud moth in Lozitsa was from the middle of June (during the period of 12-18 June) to the middle of July (during the period of 10-16 July). In the same year, two well-expressed peaks of catches showed presence of two generations of *Th. ampellophaga* in the vineyard in Gornoslav. The flight of the first generation was in the period of the end of May to the beginning of July. Catches indicating the presence of a second generation of the pest were found in the second half of August, and the total catch of the second generation was higher than the first one (ratio 1.4: 1). Relatively higher population density of *Th. ampellophaga* was observed in Gornoslav in comparison with Lozitsa.

Discussion

In Bulgaria, *Th. ampellophaga* was firstly recorded in the region of Sliven by LEDERER (1863) and REBEL (1903). According to the "System from the Really Defined Natural Territories" proposed by HUBENOV (1997) this species was reported in all main geographic regions of Bulgaria. BURESH & LAZAROV (1956) summarized the records about the distribution of the vine bud moth in Bulgaria published after LEDERER (1863) and REBEL (1903).

After the second half of 20th century many records appeared considering the pest status of the vine bud moth in this country (ANASTASOVA & GEORGIEVA, 1975; HARIZANOV *et al.*, 1980; 1994; 2006; HARIZANOV & HARIZANOV, 1983; HARIZANOV & HARIZANOVA, 1991; HARIZANOVA, 1996; KOSTADINOVA *et al.*, 2009; MAF, 2017 - Agricultural report for 2010), and also some faunistic records (DE FREINA & WITT, 2001; BESHKOV & LANGOUROV, 2004) are available. In Bulgaria, before the current study, *Th. ampellophaga* was found by means of pheromone traps only in the southern part of the country (summarized by SUBCHEV *et al.*, 2008b). To the best of our knowledge, this species has not been reported in Lozitsa and Gornoslav, although it is known from the regions of Pleven and Plovdiv, respectively. A possible reason for absence of catches of the pest in the vineyard in Plovdiv and Krumovo in 2015 can be the fungicide application or an influence of climatic conditions or their complex effect. The intensive use of fungicides and insecticides to control the pests in the crops in the last 50 years has brought to the rapid decline of the populations of this pest and exploitation of wild and decorative vines of *Parthenocissus* spp. as larval host plants (DOMINICI & PUCCI, 1989; EFETOV, 1990; EMBACHER & TARMANN, 2002). DOMINICI & PUCCI (1989) reported that high temperatures, up to 30°C can kill first instar larvae (40%) and wind and rain can cause the death of 3rd, 4rd and 5th instar larvae.

Different factors influence the diapause mechanism and the production of an additional generation of an insect species in the same year – global warming, local weather factors, photoperiod, habitat and microhabitat, host plant, food availability, altitude and latitude, and the number of generations is different from one country to another and even in a particular country (HUNTER & MCNEIL, 1997; BRYANT *et al.*, 2002; CURTIS & ISAAC, 2015; EL IRAQUI & HMIMINA, 2016).

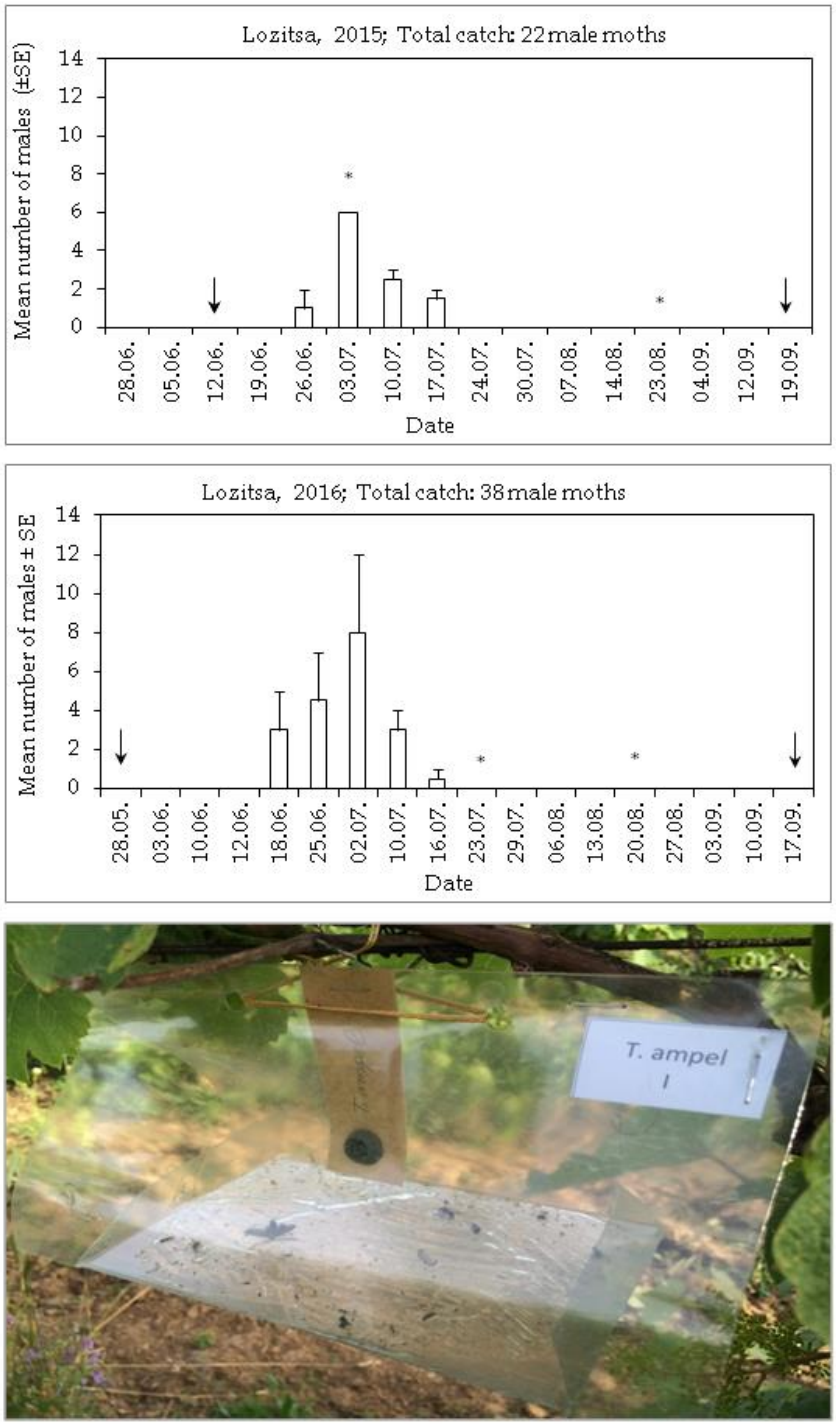

Fig. 2. Catches of *Th. ampellophaga* males in sticky traps baited with (2*R*)-butyl (7*Z*)-tetradecenoate in Lozitsa, 2015 and 2016 (two traps each year). For each year, arrows (↓) show the starting and finishing date of the investigation, and asterisks (*) the date when baits were renewed. The photograph shows a trap with a male moth captured in the period of 12-18 June, 2016.

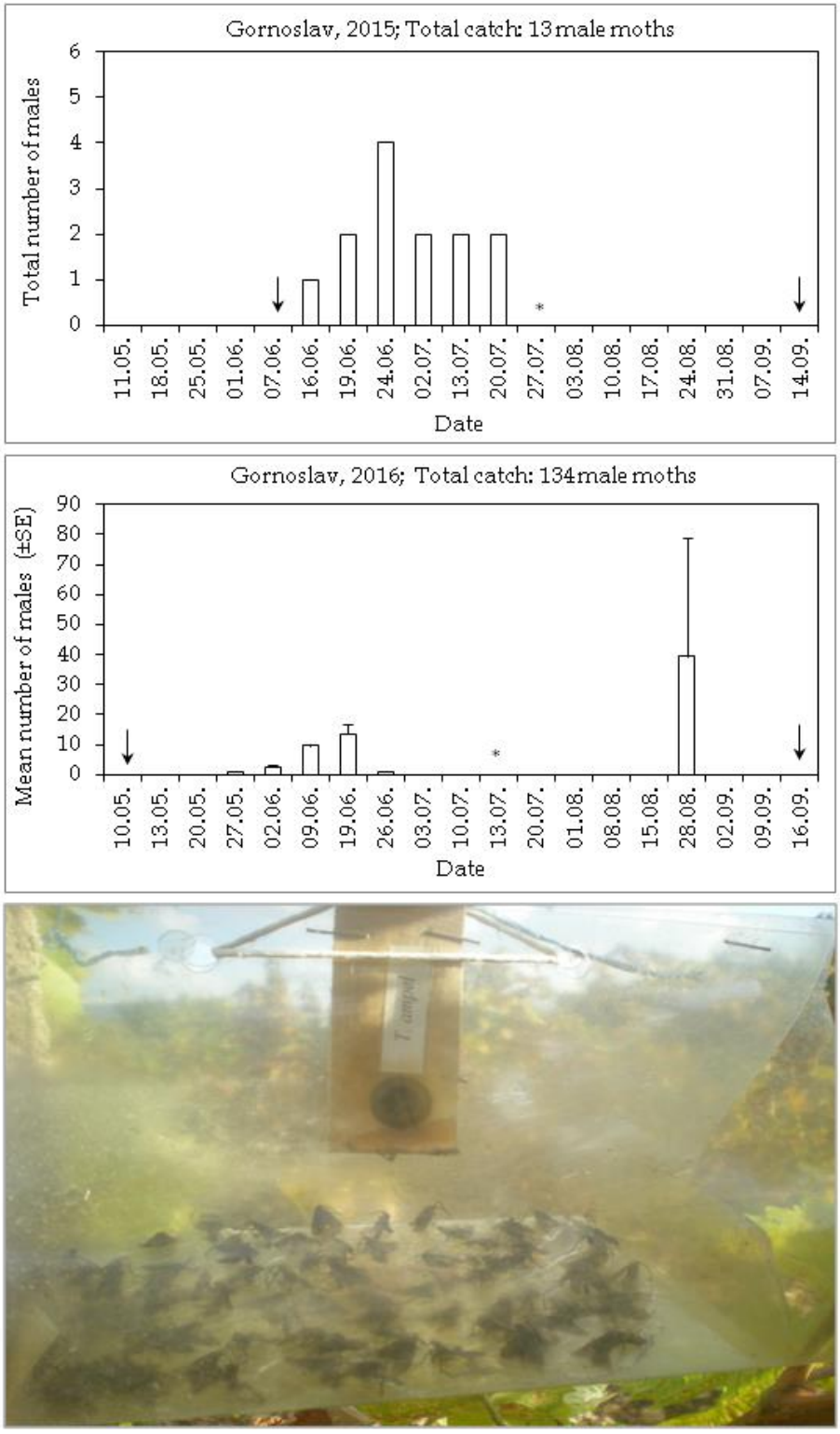

Fig. 3. Catches of *Th. ampellophaga* males in sticky traps baited with (2*R*)-butyl (7*Z*)-tetradecenoate in Gornoslav, 2015 (one trap) and 2016 (two traps). The photograph shows catches of male moths captured in the period of 15-28 August, 2016. For the legend, see Fig. 2

According to PUCCI & DOMINICI (1986) and EFETOV (2005), the vine bud moth has one generation in Central Italy and the Crimean Peninsula, respectively. Depending on climatic factors, in some years this species can develop partial second generation in South Russia (Krasnodar region), Georgia and Azerbaijan (LIPETSKAYA & RUZAEV, 1958; DEVYATKIN *et al.*, 2012). Two generations of this species were reported for the East Mediterranean countries, Syria and Lebanon (TALHOUK, 1969).

Large-scale monitoring / detection investigations by sex pheromone traps in Europe and Turkey showed that *Th. ampellophaga* develops one generation per year in Hungary (VOIGH *et al.*, 2000), Central Greece (SUBCHEV *et al.*, 2006), Romania (SUBCHEV *et al.*, 2008a), France (RYMARCZYK & DROUET, 2006; DROUET & LAMBERT, 2010), Serbia (NAHIRNIĆ *et al.*, 2015) and some localities of the eastern Mediterranean part of Turkey (CAN *et al.*, 2010) while second generation of this species was established in Rhodos Island (Greece) (SUBCHEV *et al.*, 2006), other localities of the eastern Mediterranean part of Turkey (CAN *et al.*, 2010) and Aegean Turkey (CAN CENGIZ *et al.*, 2012).

In Bulgaria, it is considered that *Th. ampellophaga* produces one full and a partial second generation per year (HARIZANOV *et al.*, 1994; HARIZANOV *et al.*, 2006). However, using enclosure field cage method for studying the life cycle of the pest in Novo selo (Ruse region) in 1974 ANASTASOVA & GEORGIEVA (1975) reported that this species is bivoltine, and the flight of the first generation moths started at the end of June with a peak of the flight – at the beginning of July (information about the flight of the second generation is not given). Using sex pheromone-baited traps TOSHOVA & SUBCHEV (2002) established only one generation of the vine bud moth in southern Bulgaria in 2000 – 2001 with flight from the beginning of June till the end of July – beginning of August.

During the current study we registered the flight of moths of one generation in northern Bulgaria (Lozitsa) in both 2015 and 2016 while in southern Bulgaria (Gornoslav) we recorded one and two flight periods in 2015 and 2016, respectively, which corresponded to the number of generations. The flight period of the first generation varies from the end of May – middle of June to the beginning – middle of July. In 2016, the early spring flight period of *Th. ampellophaga* in Gornoslav starting about three weeks earlier than the beginning of the flight in Lozitsa (20-28 May as compared to 12-18 June) favored development of a second generation in Gornoslav. In Greece (SUBCHEV *et al.*, 2006) and Turkey (CAN *et al.*, 2010), in the years and sites where the second generation of the vine bud moth was established and details are available, the flight of the second generation was from the end of July to the end of August.

Based on the results obtained by means of pheromone traps (TOSHOVA & SUBCHEV, 2002; current study) we can conclude that in Bulgaria *Th. ampellophaga* develops one generation and a second generation can occur in years with favorable conditions.

Conclusions

Th. ampellophaga was newly recorded in two sites in Bulgaria – Lozitsa (northern Bulgaria) and Gornoslav (southern Bulgaria) villages.

For first time by means of pheromone traps we registered two generations of the vine bud moth in Bulgaria.

The flight of the first generation of *Th. ampellophaga* was from the end of May - middle of June up to the second half of July at the investigated sites and years. A second generation at Gornoslav village in 2016 was registered at the second half of August.

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