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Article

Registered Procridinae (Lepidoptera: Zygaenidae) in sticky traps baited with the synthetic attractant EFETOV-2 in Bulgaria

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Abstract
The males of *Adscita* (*Adscita*) *statices drenowskii* (Alberti, 1939), *Jordanita* (*Tremewania*) *notata* (Zeller, 1847) and *Theresimima ampellophaga* (Bayle-Barelle, 1809) (Zygaenidae: Procridinae) were caught by Delta sticky traps baited with the synthetic sex attractant EFETOV-2 in Gornoslav village (Plovdiv Region, southern Bulgaria) in 2016. The attraction of these Procridinae species to EFETOV-2 (patented name of a racemic mixture of *R*- and *S*-enantiomers of butan-2-yl dodec-2-enoate) is discussed.

Key words chemical communication, attractant bait, attractant trap, butan-2-yl dodec-2-enoate, *Adscita statices*, *Jordanita notata*, *Theresimima ampellophaga*.

Introduction

Pheromones are one of the most widely used groups of signalling molecules that organisms can detect in the environment. Pheromones possess the capacity to modulate behaviour or physiological responses of conspecific individuals. The current research of the attractive molecules is mainly focused on the study of sex pheromones (which are produced naturally by organisms) and sex attractants (which have been found to be attractive to organisms in either field or laboratory experiments, but have not been found to be naturally produced by those organisms) (El-Sayed 2025).

The family Zygaenidae (Lepidoptera) is an excellent model group for ecological, zoogeographical, biochemical, karyological, genetic, morphological and taxonomic studies of the Lepidoptera (see e.g. Efetov 1996; Efetov & Tarmann 1999, 2016, 2017b; Efetov *et al*. 2006, 2011b, 2014a, b, 2015a, 2019a, c, 2020; Efetov & Savchuk 2009; Efetov & Knyazev 2014; Knyazev *et al*. 2015;

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Can *et al*. 2019; Mirić *et al*. 2024). According to the modern classification, the Zygaenidae family consists of five subfamilies: Inouelinae Efetov & Tarmann, 2017; Zygaeninae Latreille, 1809; Callizygaeninae Alberti, 1954; Chalcosiinae Walker, 1865, and Procridinae Boisduval, 1828 (Efetov & Tarmann 2012, 2017a, 2024). Sex pheromones and sex attractants of the Zygaenidae species have been an object of targeted research since the 1980s (Subchev 2014). Up to the time of publishing this work, the chemical structure of female sex pheromones and/or sex attractants was known for representatives of only two subfamilies: Procridinae and Zygaeninae (Subchev 2014; Efetov & Kucherenko 2020; Efetov *et al.* 2023; El-Sayed 2025). While esters of acetic acid and higher unsaturated alcohols possess the attractant ability for Zygaeninae species, sex pheromones and attractants of Procridinae species are esters of butan-2-ol and higher unsaturated carboxylic acids. After successful identification of components and molecular structure of female sex pheromones of several Procridinae species, viz. *Harrisina metallica* Stretch, 1885, *Theresimima ampellophaga* (Bayle-Barelle, 1809), *Illiberis* (*Primilliberis*) *rotundata* Jordan, 1907, these esters (synthetic copies of the native pheromones) alone or in various combinations were used in ecological and faunistic studies all over the world, thus leading to a discovery of sex attractants for many species from this subfamily (Efetov *et al*. 2010, 2011a, 2014b, 2015b; Subchev *et al*. 2010, 2012, 2013, 2016; Razov *et al*. 2017; Drouet *et al*. 2021).

In 2013–2016, *R*- and *S*-enantiomers of butan-2-yl dodec-2-enoate were synthesised in a racemic mixture and separately in the Crimean Federal University (hereinafter referred to as the sex attractants from the series 'EFETOV-2') (Efetov *et al*. 2014c; Efetov & Kucherenko 2021). Their attractive properties were proven for many Zygaenidae species and several non-target species of other families of the class Insecta (Efetov *et al*. 2016, 2018a, 2022, 2024, 2025; Can Cengiz *et al*. 2018; Can *et al*. 2019; Vrenozi *et al*. 2019; Drouet *et al*. 2021; Nahirnić-Beshkova *et al*. 2021; Efetov & Kucherenko 2024; Yardım *et al*. 2024). In this paper, we presented the results of our field experiments with the traps baited with butan-2-yl dodec-2-enoate (Figure 1) in Bulgaria in 2016.

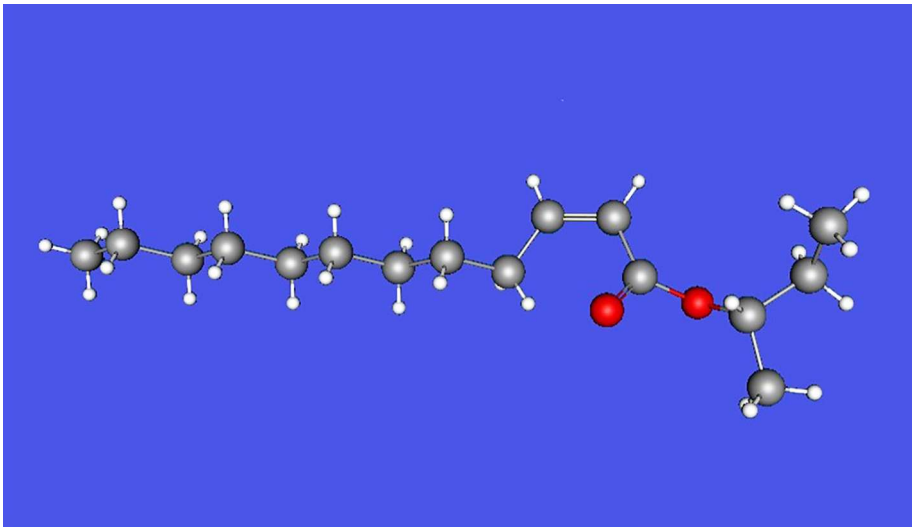

Figure 1. Structure of the synthetic sex attractant EFETOV-2 (butan-2-yl dodec-2-enoate). The model of the molecule was created by the program 'Chemistry. Virtual Laboratory' (LLC 'Virtual Spaces', Yoshkar-Ola, RU).

Materials and methods

For preparing the lure, a racemic mixture of *R*- and *S*-enantiomers of butan-2-yl dodec-2-enoate (Efetov *et al*. 2014c), patented as a synthetic sex attractant EFETOV-2 (Efetov & Kucherenko 2019), was applied onto serum vial caps of grey rubber in an amount of 100 µl. Home-made sticky Delta traps of transparent PVC foil were used for all field investigations.

Field experiments were conducted by the second author in vineyards of Gornoslav village (Asenovgrad Municipality, Plovdiv Region, southern Bulgaria). Currently the total vineyard area of the village is 90 ha where only organic management practice is applied. The control trap (without a lure) and the attractant trap baited with EFETOV-2 were set up in a private vineyard with 'Pamid' variety (0.7 ha), 41°55′55″ N, 24°57′47″ E, 480 m a.s.l. (Figure 2). They were suspended at a height of 1.0 m above

ground level. Distance between the traps was about 20 m. The monitoring period was from 10 May till 16 September 2016. Traps were inspected at least once per week (except for the period 15–28 August 2016) and the sticky layers with moths caught were collected. No replacement of the lure was carried out during the monitoring period.



Figure 2. A private vineyard with 'Pamid' variety in Gornoslav village, Asenovgrad Municipality, Plovdiv Region, southern Bulgaria.

The determination of all collected Procridinae specimens was based on the investigation of genitalia structure, morphology of antennae, and wing venation and was done by K.A. Efetov in laboratory conditions.

Results and discussion

In total, the males of three Procridinae species were attracted to the sticky trap baited with EFETOV-2, viz. *Adscita* (*Adscita*) *statices drenowskii* (Alberti, 1939), *Jordanita* (*Tremewania*) *notata* (Zeller, 1847) and *Theresimima ampellophaga* (Bayle-Barelle, 1809) (Figure 3).

According to the review of the publications available, *Theresimima ampellophaga* has following distribution: southern France, Italy (including Sicily), southeastern Austria, Slovenia, Croatia, Serbia, Montenegro, Albania, Republic of North Macedonia, Greece (mainland and islands including Rhodes and Crete), Slovakia, Hungary, Romania (Dobrogea), Bulgaria, southern Ukraine, Moldova, Russia (south of European part including southern Crimea, Northern Caucasus), Georgia, Turkey, Cyprus, Syria, Lebanon and Israel. The occurrence in Spain and Algeria is doubtful and needs confirmation (Efetov &Tarmann 2024). Recent investigations by pheromone traps baited with the main synthetic sex pheromone compound of *Theresimima ampellophaga*, (2*R*)-butyl (7*Z*)-tetradecenoate, in southern Bulgaria showed that the vine bud moth develops in two generations in years with favourable conditions

(Toshova *et al*. 2017; Mumun *et al*. 2018). The flight of the first generation of this pest is from the end of May to the second half of July. The moths of the second generation occur from the middle of August until the first half of September. During the current study, catches of *Theresimima ampellophaga* males were also registered twice, corresponding to the first (1 male) and second (1 male) flight periods of this species. It should be noted, that the lures with EFETOV-2 tested in Gornoslav were not replaced with fresh ones, unlike the lures baited with (2*R*)-butyl (7*Z*)-tetradecenoate (Toshova *et al*. 2017; Mumun *et al*. 2018), which were replaced with fresh ones after 6–8 weeks during the monitoring periods. This finding confirms our previous hypothesis (Vrenozi *et al*. 2019) that the sex attractant EFETOV-2 can remain active during the full flight of the two generations of *Theresimima ampellophaga* in regions where it is bivoltine. Based on the results obtained by attractant traps (Efetov *et al*. 2014c, 2023; Vrenozi *et al*. 2019; Nahirnić-Beshkova *et al*. 2021; Efetov & Kucherenko 2021; current study) it can be concluded that EFETOV-2 allows detecting the vine bud moth even at low population densities (e. g. in Albania and the Republic of North Macedonia).



Figure 3. *Theresimima ampellophaga* (1 ♂), *Adscita statices drenowskii* (27 ♂♂) and *Jordanita notata* (2 ♂♂) on a sticky layer of the Delta trap baited with EFETOV-2 from Gornoslav, Bulgaria (2016).

The most numerous catches were of *Adscita* (*Adscita*) *statices drenowskii* – 27 males (Figure 3). This is also consistent with our earlier observations (Can Cengiz *et al*. 2018), which showed the attractive properties of EFETOV-2 for the males of *Adscita* (*Adscita*) *statices drenowskii* in the European part of Turkey (Thrace Region). In southern Bulgaria (Gornoslav village) we tested only a sex attractant EFETOV-2 (a racemic mixture of *R*- and *S*-enantiomers of butan-2-yl dodec-2-enoate), but in Turkey this subspecies was also attracted to the traps baited with EFETOV-S-2 (*R*-enantiomer of butan-2-yl dodec-2-enoate alone). However, the number of specimens (males) was significantly higher in traps baited with EFETOV-2. It is interesting that the males of another subspecies, viz. *Adscita* (*Adscita*) *statices statices* (Linnaeus, 1758), were also mainly attracted to the lures with EFETOV-2 but not with EFETOV-S-2 during our investigations in Sweden (Efetov *et al*. 2018b). Moreover, three closely related species from the *statices*-group (see Efetov and Tarmann 2012, 2024), viz. *Adscita* (*Adscita*) *alpina* (Alberti, 1937), *Adscita* (*Adscita*) *italica italica* (Alberti, 1937) and *Adscita* (*Adscita*) *statices statices*,

reacted actively to sex attractants from the series 'EFETOV-2'. The males of *Adscita* (*Adscita*) *alpina* were attracted to the lures with EVETOV-S-2 (predominantly) and EFETOV-2 (Efetov *et al*. 2020), as well as the males of *Adscita* (*Adscita*) *statices statices* (as discussed above, Efetov & Gorbunov 2016; Efetov *et al*. 2020). The males of *Adscita* (*Adscita*) *italica italica* were attracted to the lures with EFETOV-2, EFETOV-S-2 and EFETOV-S-S-2 (*S*-enantiomer of butan-2-yl dodec-2-enoate alone). We can hypothesise that the males of the endemic of the Iberian Peninsula, *Adscita* (*Adscita*) *schmidti* (Naufock, 1933) (this species is also from the *statices*-group), might come to lures or sticky traps baited with synthetic sex attractants from the series 'EFETOV-2'. Further research should be done to establish this. A similar situation was described in Efetov *et al*. (2019b) and helped the authors to detect the rare endemic Spanish species *Rhagades* (*Wiegelia*) *predotae* (Naufock, 1930) and describe its biology, ecology and early stages (Efetov & Tarmann 2020).

Only two males of *Jordanita* (*Tremewania*) *notata* were caught in the trap baited with EFETOV-2 in Gornoslav. However, the high efficacy of the sex attractant EFETOV-2 has been already demonstrated for this species in the Crimea (Efetov *et al*. 2016; Efetov & Kucherenko 2021) where an average number of the males caught in one trap per week was 30.53 ± 9.60 (Efetov & Kucherenko 2021). The maximal saturation of one trap achieved 56 males per week. This was an almost complete saturation of the sticky layer. It is possible that the low capture rate of the *Jordanita* (*Tremewania*) *notata* males during Gornoslav fieldwork can be explained by the deficiency of the larval host-plants of this species, e. g. *Carduus* spp. and *Centaurea* spp. (all from Asteraceae), in the studied vineyard. The usual biotopes of *Jordanita* (*Tremewania*) *notata* are dry steppes and calcareous, grassy slopes of hills (Efetov 2001). It is also known that this Procridinae species is well attracted to synthetic esters of butan-2-ol and monounsaturated fatty acids: dodec-2-enoic, dodec-5-enoic, dodec-7-enoic acids (Subchev *et al*. 2010; Efetov *et al*. 2015b, 2016, 2020, 2023; Razov *et al*. 2017; Efetov & Kucherenko 2021), especially to their *R*-enantiomers. Our data matches the results observed in earlier studies.

Conclusion

The present study confirms our previous findings and contributes additional evidence that the synthetic sex attractant EFETOV-2 can be applied to detect and monitor Procridinae species.

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