

New synthetic sex attractants for the males of two endemic Iberian Procridinae species (Lepidoptera: Zygaenidae)

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Abstract

Sex attractants for *Rhagades (Wiegelia) predotae* (Naufock, 1930) and *Adscita (Tarmannita) bolivari* (Agenjo, 1937), two endemic Iberian Procridinae species (Lepidoptera: Zygaenidae), are reported for the first time. During fieldwork in Spain in 2017 and 2018, the males of *Rh. predotae* and *Rh. (Rhagades) pruni* ([Denis & Schiffermüller], 1775) were attracted to the lures with the substance “EFETOV-S-S-2” (*S*-enantiomer of 2-butyl 2-dodecenoate), while the males of *A. bolivari* reacted to “EFETOV-2” (a racemic mixture of *R*- and *S*-enantiomers). This result (together with our previous data) shows that (2*S*)-butyl 2-dodecenoate is likely to be a “generic” sex attractant for the males of the genus *Rhagades* Wallengren, 1863. We demonstrate that attractive lures may be a sensitive and efficient tool for monitoring the two rare Spanish species, viz. *Rh. predotae* and *Rh. pruni*.

KEY WORDS: Lepidoptera, Zygaenidae, Procridinae, *Rhagades predotae*, *Rhagades pruni*, *Adscita bolivari*, sex attractant, EFETOV-2, EFETOV-S-S-2, Spain.

Nuevo atrayente sintético sexual para los machos de dos especies ibéricas endémicas de Procridinae (Lepidoptera: Zygaenidae)

Resumen

Se indica por primera vez, un atrayente sexual para *Rhagades (Wiegelia) predotae* (Naufock, 1930) y *Adscita (Tarmannita) bolivari* (Agenjo, 1937), dos especies ibéricas endémicas de Procridinae (Lepidoptera: Zygaenidae). Durante el trabajo de campo en España en 2017 y 2018, los machos de *Rh. predotae* y *Rh. (Rhagades) pruni* ([Denis & Schiffermüller], 1775) fueron atraídos a los señuelos con la sustancia “EFETOV-S-S-2” (*S*-enantiómero de 2-butil 2-dodecenoato), mientras los machos de *A. bolivari* reaccionan frente a “EFETOV-2” (una mezcla racémica de *R*- and *S*-enantiómeros). Este resultado (con nuestros datos previos) indica que (2*S*)-butil 2-dodecenoato es, probablemente, un atrayente sexual para los machos del género *Rhagades* Wallengren, 1863. Demostramos que los señuelos atrayentes podrían ser una herramienta susceptible y eficiente para monitorear las dos especies singulares españolas, véase *Rh. predotae* y *Rh. pruni*.

PALABRAS CALVE: Lepidoptera, Zygaenidae, Procridinae, *Rhagades predotae*, *Rhagades pruni*, *Adscita bolivari*, atrayente sexual, EFETOV-2, EFETOV-S-S-2, España.

Introduction

The Iberian Peninsula is one of the richest European regions in animal species diversity (RAMOS *et al.*, 2001). 54% of the total species described for Europe and approximately 38% of the species assessed by the European Red List of Species are present in Spain (SÁNCHEZ *et al.*, 2013). According to the data of the International Union for Conservation of Nature (2018), the country is located in one of the 25 biodiversity hotspots in the world, thanks to its high rate of endemism.

Historically, the researchers have tended to focus on the study of sex pheromones of insect pests

rather than attractive molecules for protected and threatened species (OLEANDER *et al.*, 2015; RAZOV *et al.*, 2017; SUBCHEV *et al.*, 1998, 2009, 2012, 2013, 2016; WITZGALL *et al.*, 2010). However, pheromone-based monitoring methods can reveal rare and endangered species even with very low population density due to their sensitivity and species-selectivity. Pheromone lures and traps can detect and monitor the seasonal flight of such insects and provide a better understanding of their biology (LARSSON, 2016). Despite these obvious advantages, pheromones have been exploited for these purposes in very few cases in part because of the high cost and the difficulty of identifying them (MILLAR *et al.*, 2010; SVENSSON *et al.*, 2012). Synthetic sex attractants can successfully replace natural pheromones especially if they are expensive or unstable (EFETOV *et al.*, 2010, 2011, 2015b; SUBCHEV *et al.*, 2010; XU *et al.*, 2012). Additionally, field screening tests of sex attractants are generally simpler, more effective and less laborious than visual searches for a target species (EFETOV *et al.*, 2014b; RAY *et al.*, 2014).

Previous studies have reported the attractive properties of 2-butyl 2-dodecenoate (*R*- and *S*-enantiomers and a racemic mixture) produced in the Crimean Federal University. The species and sex specificity of the newly synthesized esters has been proved for Procrarinae moths in field observations undertaken in the Crimea, continental Russia, Austria, Greece, Italy, Tajikistan, Turkey, Japan, and some other countries (CAN CENGIZ *et al.*, 2018; EFETOV *et al.*, 2016a, 2016b, 2017, 2018; EFETOV & GORBUNOV, 2016). In 2017 and 2018, our field screening tests were carried out in Spain.

Large revisional work has been done on Palaearctic Procrarinae during the last years (EFETOV, 1992, 1996a, b, 1997a, b, 1998, 1999, 2001a, 2006, 2010; EFETOV *et al.*, 2014b; EFETOV & TARMANN, 1999, 2013a, b, 2014a, b, 2016a, b, 2017b; KEIL, 2014; MOLLET & TARMANN, 2007). Based on the recent classification, the Zygaenidae family includes five subfamilies: Inouelinae Efetov & Tarmann, 2017; Procrarinae Boisduval, 1828; Chalcosiinae Walker, 1865; Callizygaeninae Alberti, 1954; and Zygaeninae Latreille, 1809 (EFETOV, 2001b; EFETOV *et al.*, 2004, 2006, 2014a, 2015a; EFETOV & HAYASHI, 2008; EFETOV & KNYAZEV, 2014; EFETOV & SAVCHUK, 2009, 2013; EFETOV & TARMANN, 2012, 2017a; HOFMANN & TREMEWAN, 2017; KNYAZEV *et al.*, 2015a, b). The Zygaenidae fauna of Spain is represented by Procrarinae, Chalcosiinae, and Zygaeninae. Six Zygaenidae species are endemics of the Iberian Peninsula: *Rhagades (Wiegelia) predotae* (Naufock, 1930), *Adscita (Adscita) jordani* (Naufock, 1921), *A. (A.) schmidtii* (Naufock, 1933), *A. (Tarmannita) bolivari* (Agenjo, 1937), *Jordanita (Jordanita) vartianae* (Malicky, 1961) (subfamily Procrarinae), and *Zygaena (Agrumenia) ignifera* Korb, 1897 (subfamily Zygaeninae) (EFETOV, 2004; EFETOV & TARMANN, 2012).

Male specimens of Zygaenidae use chemical and visual signals for finding the females (EFETOV *et al.*, 2015b; NAZAROV & EFETOV, 1993). In the subfamily Procrarinae the majority of species has cryptic habitus and as a result chemical attraction is more important (EFETOV *et al.*, 2010, 2014c). Sex pheromones or sex attractants were unknown for any endemic Spanish species (El-SAYED, 2018; SUBCHEV, 2014). However, it was recently shown that (2*S*)-butyl 2-dodecenoate attracts the males of *Rhagades (Rhagades) pruni* ([Denis & Schiffermüller], 1775) in the Crimea and Italy (EFETOV *et al.*, 2016c, 2017), and *Rhagades (Wiegelia) amasina* (Herrich-Schäffer, 1851) in the Middle Anatolia Region of Turkey (CAN CENGIZ *et al.*, 2017; CAN *et al.*, 2018). We hypothesized that the males of the rare endemic Spanish species *Rh. predotae* also might come to lures or sticky traps with synthetic (2*S*)-butyl 2-dodecenoate. Thus, the objectives of the present work were: 1) to determine whether (2*S*)-butyl 2-dodecenoate is a sex attractant for the males of *Rh. predotae*; 2) to test 2-butyl 2-dodecenoate (the racemate and its enantiomers) as an attractant for other Spanish Procrarinae species including *Rh. pruni*, a very rare species on the Iberian Peninsula.

Materials and methods

Field experiments with synthetic sex attractants were undertaken in Spain by G. M. Tarmann in Barcelona Province from 13-20 June 2017 and by K. A. Efetov and G. M. Tarmann in Cuenca Province from 9-13 July 2018 (see Table 1 for locality information, Figs 1-6). We tested responses of Procrarinae

species to three attractants: EFETOV-2 (the racemate), EFETOV-S-2 (*R*-enantiomer), and EFETOV-S-S-2 (*S*-enantiomer) of 2 butyl 2-dodecenoate. The synthesis of the indicated ester was made according to the procedure published by EFETOV *et al.* (2014c).

Table 1.— A list of studied localities in Spain in 2017 and 2018 (Figs 1-6).

Number of locality	Description of the localities	GPS coordinates
Castilla-La Mancha, Cuenca Province		
I	vic. Huélamo, 1265 m (Fig. 1)	N 40° 15,385' / W 01° 45,920'
II	NE of Huélamo, 1228 m	N 40° 17,700' / W 01° 47,770'
III	NE of Huélamo, 1222 m (Fig. 2)	N 40° 17,706' / W 01° 47,866'
IV	NE of Huélamo, 1225 m (Figs 3-5)	N 40° 17,450' / W 01° 47,650'
V	S of Uña, 1184 m	N 40° 13,457' / W 01° 59,407'
VI	S of Tragacete, 1256 m	N 40° 20,174' / W 01° 49,570'
Catalonia, Barcelona Province		
VII	W of Alpens, 870 m (Fig. 6)	N 42° 07,767' / E 02° 05,050'

To prepare baits, rubber caps were impregnated with different types of attractants, fixed on cardboard rectangles and marked. A variety of methods were used to assess the attractiveness of the baits: Delta plastic sticky traps with lures hung on bushes or trees or cardboard rectangles with rubber caps placed on stones on the ground (Fig. 7) or attached to the hat of the researcher slowly crossing the biotope. Each method had its advantages and drawbacks. In the last two cases, the attracted specimens were collected by netting them near the rubber caps.

All specimens were determined by examination of the genitalia by K. A. Efetov and G. M. Tarmann.

Results and discussion

During our field trials in 2018 the attraction of two endemic Iberian Procridinae species to some of the exposed baits was recorded, viz. *Rh. predotae* and *A. bolivari*. In addition, the males of *Rh. pruni* were attracted in 2017. The list of attracted specimens, type of lures, and time of observation are provided below.

Rh. predotae (Naufock, 1930)

Locality III, near rubber caps with EFETOV-S-S-2, 1 ♂, 10-VII-2018, 20:38; locality III, near rubber caps with EFETOV-S-S-2, 1 ♂, 11-VII-2018, 11:30; locality III, near rubber caps with EFETOV-S-S-2, 3 ♂♂, 11-VII-2018, 20:40-20:47; locality III, near rubber caps with EFETOV-S-S-2, 4 ♂♂, 12-VII-2018, 20:20-20:30; locality IV, near rubber caps with EFETOV-S-S-2, 4 ♂♂, 13-VII-2018, 20:44-21:07.

A. bolivari (Agenjo, 1937)

Locality I, near rubber caps with EFETOV-2, 8 ♂♂, 9-VII-2018, 19:20-20:00; locality V, near trap with EFETOV-2, 1 ♂, 10-VII-2018, 14:00; locality II, near rubber caps with EFETOV-2, 5 ♂♂, 10-VII-2018, 20:00-20:20; locality III, near trap with EFETOV-2, 3 ♂♂, 11-VII-2018, 19:30-20:00; locality VI, near rubber caps with EFETOV-2, 1 ♂, 12-VII-2018, 12:30; locality IV, near rubber caps with EFETOV-2, 2 ♂♂, 13-VII-2018, 20:30.

Rh. pruni ([Denis & Schiffermüller], 1775)

Locality VII, near rubber caps with EFETOV-S-S-2, 2 ♂♂, 15-VI-2017, 11:35; locality VII, near rubber caps with EFETOV-S-S-2, 3 ♂♂, 19-VI-2017, 10:05-10:32.

Totally, 20 *A. bolivari* males were attracted to EFETOV-2, whereas 13 ♂♂ of *Rh. predotae* and 5

♂♂ of *Rh. pruni* came to lures with EFETOV-S-S-2. In the locality IV on 13th July 2018 three attractants were simultaneously placed on stones more than 10 m apart from each other. All the males of *Rh. predotae* were attracted only to lures EFETOV-S-S-2, while the males of *A. bolivari* were attracted to EFETOV-2. No specimens of any of the species approached the lure EFETOV-S-2 in this locality.

It is interesting that despite the fact that the traps with sticky layers were fixed to the branches of bushes at the altitude 1.0-1.5 m above the ground in almost all localities, we did not find any glued specimen of *Rh. predotae* and *A. bolivari* in the traps. All specimens were attracted only to rubber caps placed on the stones, fixed to the clothes or the hat of the collector.

Only male moths (Fig. 8) were captured indicating that these compounds act as a sex-specific attractants. Almost all males of *Rh. predotae* were attracted at evening twilight from 20:20-21:07 around the moment of sunset over the mountains. 18 flying males were additionally caught in locality IV without baits on 12 July also in the evening (21:00-21:35). It can thus be suggested that mating activity of this species connected with pheromone communication is at the end of the day. If we compare the time *Rh. predotae* and *A. bolivari* come to the attractants “EFETOV”, we see that *Rh. predotae* males were attracted mainly in the evening at sunset, while the males of *A. bolivari* were active also during the day.

In this study we show that EFETOV-S-S-2 ((2*S*)-butyl 2-dodecenoate) is a sex attractant for the males of *Rh. predotae*. This is a very rare species of which the biology is insufficiently studied. *Rh. predotae* is only known from several specimens kept in museum collections (NAUMANN *et al.*, 1999). The adults are not nocturnal but visual searches for this species in their habitat and inspection of host-plants have been unsuccessful. Now, the application of this attractant-based method allows us to detect *Rh. predotae*.

Previously, it was shown that *Rh. pruni* (EFETOV *et al.*, 2016c, 2017) and *Rh. amasina* (CAN CENGIZ *et al.*, 2017; CAN *et al.*, 2018) were also attracted to EFETOV-S-S-2. Based on this knowledge G. M. Tarmann used the synthetic sex attractant EFETOV-S-S-2 for rediscovering this species in Spain on the southern side of the Pyrenees in 2017. *Rh. pruni* is a very rare species on the Iberian Peninsula and occurs only in the central and eastern Pyrenees in Spain. It reaches here its westernmost distribution in the Palaearctic Region. So far, only three male specimens from three localities were known from the southern side of the Pyrenees, all situated in the Barcelona Province. All other records from Spain are from localities in Valle de Arán, situated on the northern side of the Pyrenees. Therefore, the south-eastern slopes of the Pyrenees in the Catalanian provinces of Barcelona and Lérida were carefully screened by G. M. Tarmann using a rubber lure with EFETOV-S-S-2 on cardboard that was attached to his hat. Literally hundreds of bush-groups of *Prunus spinosa*, partly mixed with *Crataegus* and *Rubus*, the well-known preferred habitats of *Rh. pruni* in Spain, France, Italy and Central Europe, were examined for a whole week, but only in one single locality was the search successful (in locality VII where five males of *Rh. pruni* had been attracted to lures with EFETOV-S-S-2 in good weather conditions with cloudless skies). In conclusion one can say that the artificial attractant EFETOV-S-S-2 was definitely responsible for this rediscovery of *Rh. pruni* in the south-eastern Pyrenees in Spain (although the species was extremely rare in 2017) after more than a third of a century (last record known is from 1980).

Our results provide further support for the hypothesis outlined in the introduction of this paper that (2*S*)-butyl 2-dodecenoate is a “generic” sex attractant for males of different species of the genus *Rhagades* Wallengren, 1863. Pheromone structure can be conservative within closely related Procridinae species of this genus. A similar situation is known also for other insects (RAY *et al.*, 2014).

Conclusion

Sex attractants for two endemic Iberian Procridinae species, viz. *Rhagades* (*Wiegelia*) *predotae* and *Adscita* (*Tarmannita*) *bolivari*, were found for the first time. *Rh. predotae* males reacted to lures with (2*S*)-butyl 2-dodecenoate, while *A. bolivari* males were attracted to the racemate of 2-butyl 2-

dodecenoate. Synthetic (2S)-butyl 2-dodecenoate is a sensitive and effective tool for monitoring *Rh. predotae* and *Rh. pruni* populations.

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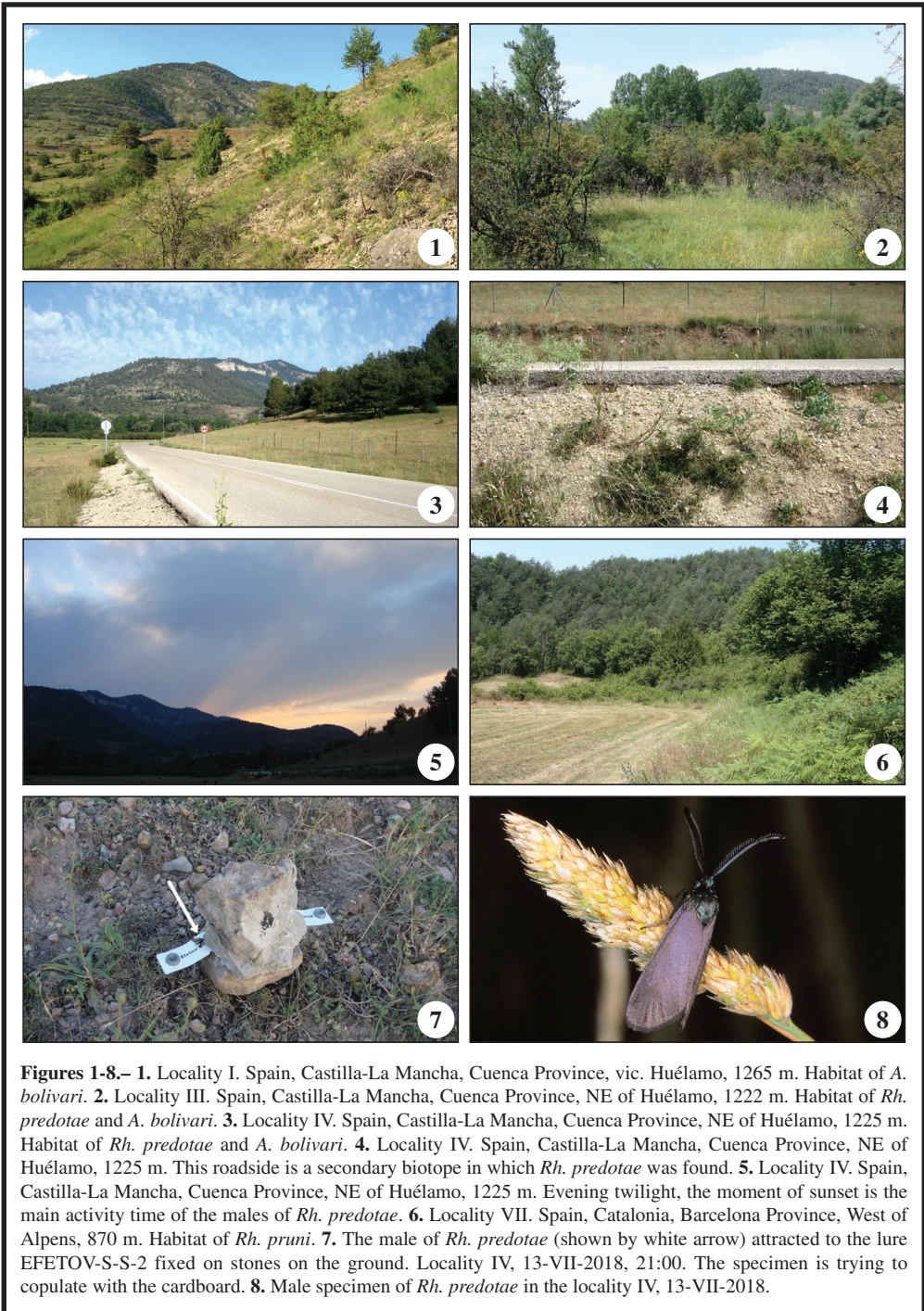
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Figures 1-8.– **1.** Locality I. Spain, Castilla-La Mancha, Cuenca Province, vic. Huélamo, 1265 m. Habitat of *A. bolivari*. **2.** Locality III. Spain, Castilla-La Mancha, Cuenca Province, NE of Huélamo, 1222 m. Habitat of *Rh. predotae* and *A. bolivari*. **3.** Locality IV. Spain, Castilla-La Mancha, Cuenca Province, NE of Huélamo, 1225 m. Habitat of *Rh. predotae* and *A. bolivari*. **4.** Locality IV. Spain, Castilla-La Mancha, Cuenca Province, NE of Huélamo, 1225 m. This roadside is a secondary biotope in which *Rh. predotae* was found. **5.** Locality IV. Spain, Castilla-La Mancha, Cuenca Province, NE of Huélamo, 1225 m. Evening twilight, the moment of sunset is the main activity time of the males of *Rh. predotae*. **6.** Locality VII. Spain, Catalonia, Barcelona Province, West of Alps, 870 m. Habitat of *Rh. pruni*. **7.** The male of *Rh. predotae* (shown by white arrow) attracted to the lure EFETOV-S-S-2 fixed on stones on the ground. Locality IV, 13-VII-2018, 21:00. The specimen is trying to copulate with the cardboard. **8.** Male specimen of *Rh. predotae* in the locality IV, 13-VII-2018.