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Results of the use of synthetic sex attractant lures for Zygaenidae in south-eastern France (Lepidoptera: Zygaenidae)

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Abstract

The sex attractant 2-butyl 2-dodecenoate (EFETOV-2), (2*R*)-butyl (7Z)-dodecenoate (R12) (a component of *Illiberis rotundata* sex pheromone) and a mixture with its opposite enantiomer (2*S*)-butyl (7Z)-dodecenoate (R12+S12, 1:1), were tested over a period of two years for studying Zygaenidae fauna in south-eastern France. As a result, eight Zygaenidae species were attracted in the vicinity of the lures, one of them in quantities never previously recorded by other methods in France.
KEY WORDS: Lepidoptera, Zygaenidae, sex attractants, *Rhagades pruni*, 2-butyl 2-dodecenoate, (2*R*)-butyl (7Z)-dodecenoate, (2*S*)-butyl (7Z)-dodecenoate, France.

Résultats de l'usage d'attractants sexuels pour l'étude des Zygaenidae, dans le sud-est de la France (Lepidoptera: Zygaenidae)

Résumé

L'attractant sexuel 2-butyl 2-dodecenoate (EFETOV-2), (2*R*)-butyl (7Z)-dodecenoate (R12) (un composé des phéromones sexuelles de *Illiberis rotundata*) et un mélange avec son énantiomère opposé (2*S*)-butyl (7Z)-dodecenoate (R12+S12, 1:1), ont été testés durant deux années pour étudier la faune des Zygaenidae du sud-est de la France. Ces appâts ont attirés 8 espèces de Zygaenidae et pour l'une d'entre elle, dans des quantités jamais observées avec d'autres méthodes en France.
MOTS CLES: Lepidoptera, Zygaenidae, attractants sexuels, *Rhagades pruni*, 2-butyl 2-dodecenoate, (2*R*)-butyl (7Z)-dodecenoate, (2*S*)-butyl (7Z)-dodecenoate, France.

Resultados del uso de los señuelos del atrayente sexual para Zygaenidae en el sudeste de Francia (Lepidoptera: Zygaenidae)

Resumen

El atrayente sexual 2-butil 2-dodecanoato (EFETOV-2), (2*R*)-butil (7Z)-dodecanoato (R12) (un componente de la feromona sexual de *Illiberis rotundata*), y una mezcla con su enantiómero opuesto (2*S*)-butil (7Z)-dodecanoato (R12+S12, 1:1), fueron testados por un período de dos años para estudiar la fauna de Zygaenidae del sudeste de Francia. Como el resultado ocho especies de Zygaenidae fueron atraídas en las proximidades de los señuelos, una de ellas en cantidades antes nunca registradas, por otros métodos en Francia.
PALABRAS CLAVE: Lepidoptera, Zygaenidae, atrayente sexual, *Rhagades pruni*, 2-butil 2-dodecanoate, (2*R*)-butil (7Z)-dodecanoate, (2*S*)-butil (7Z)-dodecanoate, Francia.

Introduction

Over last two decades, new sex pheromones and sex attractants of the Zygaenidae were discovered (SUBCHEV *et al*., 1998, 2009; EFETOV *et al*., 2014c; EFETOV & KUCHERENKO, 2020; SUBCHEV, 2014). Their application allowed to obtain new knowledge on the distribution, ecology and phenology of many species of this family in different countries (SUBCHEV *et al*., 2004, 2006, 2008a, 2008b, 2010, 2012, 2013, 2016; CAN *et al.* 2010, 2019; EFETOV *et al*., 2010, 2011, 2012, 2014b, 2015, 2016, 2018, 2019; NAHIRNIĆ *et al*., 2015; RAZOV *et al*., 2017, CAN CENGIZ *et al*., 2018; TOSHOVA *et al*., 2017; VRENOZI *et al*., 2019, 2020).

According to the contemporary systematics the family Zygaenidae is represented by five subfamilies (EFETOV, 1999; EFETOV & TARMANN, 2013, 2014a, 2017; EFETOV *et al*., 2014a), three of which are present in France: Chalcosiinae, Procridinae and Zygaeninae, the last subfamily being most numerous (RYMARCZYCK, 2007; DROUET & LAMBERT, 2010; EFETOV & TARMANN, 2014b; DROUET, 2016). Several species of French foresters (Procridinae) are not observed attracted to flowers, or rarely have been seen sitting on them, during the day. Locating adults, therefore, is very difficult so that their regional distribution and local abundance are poorly known. Such species often do not appear during surveys conducted to evaluate conservation measures in protected territories. This situation applies also to some species of burnet moths (Zygaeninae), which are not on the wing during the hottest part of the day, or which are difficult to be detected with low indigenous populations.

Consequently, a combination of methods is necessary to obtain a clearer picture of the species spectrum of Zygaenidae in certain region and relative abundance of the target species. Most common methods are searching by eye for larvae and adults on host plants, or by beating convenient bushes. Resorting to live virgin females is very efficient but not easy to carry out, since it is limited to the calling period, and by the short life of captive females. The use of synthetic sex pheromones or sex attractants gives possibilities for faunistic investigations and the results obtained could enrich our knowledge of Zygaenidae distribution. Recently, a comprehensive review of the known sex pheromones and attractants of Zygaenidae species had been published (SUBCHEV, 2014). Later synthesis, discovering of the attractive properties of 2-butyl 2-dodecenoate (EFETOV *et al*., 2014c) and intensive field testing of the enantiomer molecules and their racemic mixture in different countries resulted of increased knowledge about distribution and relative abundance of several Procridinae species in the studied regions (EFETOV *et al*., 2016, 2018, 2019, 2020).

The purpose of this study was to establish occurrence and distribution of Zygaenidae species attracted by the synthetic sex attractant and sex pheromone lures in south-eastern France.

Materials and methods

In 2017 and 2018, the first author used the sex attractant EFETOV-2 (racemic mixture of enantiomers of 2-butyl 2-dodecenoate), produced by the method described in EFETOV *et al*. (2014c). For preparing lures, rubber caps mounted on cardboard holders were impregnated with 50 µl of the racemic mixture and wrapped singly in aluminium foil pieces. During the experiments, the static method of using lures without trap was followed (EFETOV *et al*., 2011; TARMANN *et al*., 2019). The lure wrapped in a piece of aluminum foil was stored in a freezer before the use in the field. At the selected locality, the lure was exposed for 10-15 minutes. It was fixed to a stem or a branch at a maximum height of 1 meter from the ground, taking care of the wind direction when there was one. The occurrence of Zygaenidae species in the biotope were checked by visual observation before and during the trial. The Procridinae males attracted to the lure were netted and captured. The netted *Zygaena* were identified, counted, kept in a net and then released after the trial. The trials had been carried out from May to July 2017 during the afternoon, in places where target species where flying or supposed to exist. Some results obtained in 2016 with a lure containing the blend EFETOV-2, using the same protocol are added.

In 2018, (2*R*)-butyl (7*Z*) dodecenoate) (R12), the sex pheromone component of *Illiberis rotundata* Jordan (SUBCHEV *et al*., 2009) and the mixture of this compound and its opposite enantiomer, (2*S*)-butyl (7*Z*) dodecenoate) (S12), (R12 + S12; ratio 1:1) were also tested in the field. For preparing the lures, the pure compounds were dissolved in hexane and applied at a dose of 100 µg onto rubber vial caps of serum bottles mounted on cardboard holders. The lures were prepared at the Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences. The lure with R12+S12 was tested using the static method while the lure with R12 alone was tested by Delta sticky trap of transparent PVC foil.

A total of 31 trials were carried out using the static method, 20 with EFETOV-2, and 11 with R12+S12.

All trials took place in France, in the departement of Hautes-Alpes, except for those in Venterol, Curbans and Uvernet-Fours, which are located in the department of Alpes-de-Haute-Provence and that at Quet-en-Beaumont, located in the department of Isère. Details about localities and periods of observations are present in Table 1, with a map of localities on Fig. 1.

Table 1.– Trials with EFETOV-2 and R12+S12 using the static method of exposure of attractant lure in south-eastern France in 2016-2018.

Commune, locality	Altidude, m a.s.l.	Date	Time of observation		Lure	Family	Attracted species	Nb ex
			Beginning	End				
Théus, Maruvert	750	09-V-2017	15h15	15h30	EFETOV-2	Psychidae	*Ptilocephala albida*	2
Chorges, Rivay	1000	09-VI-2017	16h30	16h40	EFETOV-2		nul	0
Chorges, Rivay	1000	09-VI-2017	21h26	21h30	EFETOV-2	Zygaenidae	*Adscita statices*	2
La Cluse, Bauchière	1250	15-VI-2016	15h10	15h20	EFETOV-2	Zygaenidae	*Adscita statices*	2
Cervières, Le Laus	1800	05-VII-2017	16h40	16h55	EFETOV-2		nul	0
Venterol, Serre Barbière	750	10-VII-2017	17h40	17h50	EFETOV-2	Zygaenidae	*Zygaena transalpina*	11
Savournon, Torrent du Béal	880	13-VII-2017	18h00	18h15	EFETOV-2	Zygaenidae	*Zygaena transalpina*	10
Montmaur, Col de Gaspardon	1450	14-VII-2015	15h00	15h15	EFETOV-2	Zygaenidae	nul	0
Saint-Michel-de-Chaillol, Sellaret, ravin du Renc	1950	18-VII-2017	17h05	17h25	EFETOV-2	Zygaenidae	*Adscita statices*	3
Saint-Michel-de-Chaillol, Sellaret, ravin du Renc	1950	18-VII-2017	17h05	17h25	EFETOV-2	Zygaenidae	*Zygaena transalpina*	1
Val Buëch-Méouge, Chemin du Roc de l'Aigle	950	09-VI-2018	15h10	15h25	EFETOV-2		nul	0
Val Buëch-Méouge, Les Autarets	550	09-VI-2018	17h55	18h10	EFETOV-2	Zygaenidae	*Adscita mannii*	1
Curbans, Les Deux Riou	640	16-VI-2018	15h45	16h00	EFETOV-2	Zygaenidae	*Zygaena viciae*	1
Eourres, Col d'Araud	900	17-VI-2018	15h50	16h05	EFETOV-2	Zygaenidae	*Adscita mannii*	1
Gap, Col de Gleize	1390	19-VI-2018	14h55	15h10	EFETOV-2		nul	0
La Piarre, Le Vissac	1060	22-VI-2018	13h40	13h55	EFETOV-2		nul	0
La Piarre, Le Vissac	1060	22-VI-2018	15h40	16h05	EFETOV-2	Zygaenidae	*Adscita mannii*	1
Sigottier, La Montagne	1090	22-VI-2018	18h30	18h45	EFETOV-2		nul	0
Eourres, Col d'Araud	890	28-VI-2018	17h20	17h35	EFETOV-2	Zygaenidae	*Adscita mannii*	11
Eourres, Col d'Araud	890	28-VI-2018	17h20	17h35	EFETOV-2	Zygaenidae	*Zygaena loti*	1
Théus, Maruvert	760	29-VI-2018	16h22	16h37	EFETOV-2	Zygaenidae	*Adscita mannii*	1
Théus, Maruvert	760	29-VI-2018	16h22	16h37	EFETOV-2	Zygaenidae	*Jordanita subsolana*	1
Curbans, Les Deux Riou	670	29-VI-2018	17h25	17h40	EFETOV-2	Zygaenidae	*Adscita mannii*	10
Curbans, Les Deux Riou	670	29-VI-2018	18h25	18h40	R12+S12	Zygaenidae	*Adscita mannii*	2
La Beaume, Ruisseau de Chauranne	970	30-VI-2018	17h45	18h00	R12+S12	Zygaenidae	*Adscita mannii*	4

La Beaume, Ruisseau de Chauranne	970	30-VI-2018	17h45	18h00	R12+S12	Zygaenidae	*Zygaena transalpina*	2
Quet-en-Beaumont, Gros Bois	980	02-VII-2018	16h00	16h10	R12+S12	Zygaenidae	nul	0
Saint-Michel-en-Beaumont, Combe des Ferrands	1180	02-VII-2018	17h35	17h50	R12+S12	Zygaenidae	nul	0
Uvernet-Fours, Col d'Allos-ouest	2260	08-VII-2018	14h40	15h00	R12+S12	Zygaenidae	*Adscita geryon*	1
Venterol, Pont de l'Archidiacre	620	10-VII-2018	17h45	18h00	R12+S12	Zygaenidae	nul	0
Val Buëch-Méouge, Les Charles	800	11-VII-2018	15h20	15h35	R12+S12	Zygaenidae	*Adscita mannii*	8
Val Buëch-Méouge, Chabottes	800	11-VII-2018	16h15	16h30	R12+S12	Zygaenidae	*Adscita mannii*	4
Barcillonnette, Le Viarar	570	18-VII-2018	16h30	16h45	R12+S12	Zygaenidae	*Zygaena transalpina*	1
Chorges, Rivay	1000	30-VII-2018	17h15	17h30	R12+S12	Zygaenidae	*Adscita mannii*	2
Embrun, Mont Guillaume	2530	31-VII-2018	13h25	13h40	R12+S12	Zygaenidae	*Adscita geryon*	3

Results

Totally five species of Procridinae and three species of Zygaeninae were attracted to the lures tested during the study (Tables 1-2). They belong to the genera *Adscita* Retzius, 1783 (three species), *Jordanita* Verity, 1946 (one species), *Rhagades* Wallengren, 1863 (one species), and *Zygaena* Fabricius, 1775 (three species). EFETOV-2 lures attracted males of the following species: *Adscita statices* (Linnaeus, 1758) (three localities), *A. mannii* (Lederer, 1853) (six localities), *J. subsolana* (Staudinger, 1862) (one locality), *Zygaena transalpina* (Esper, 1780) (three localities), *Z. loti* ([Denis & Schiffermüller], 1775) (one locality), and *Z. viciae* ([Denis & Schiffermüller], 1775) (one locality) (Table 1). The lures with the mixture R12+S12 showed behavioural activity for *A. mannii* (five localities), *A. geryon* (Hübner, 1813) (two localities) and *Z. transalpina* (two localities). The most frequently observed species during the study were *A. mannii* and *Z. transalpina*.

Traps baited with R12 captured males of *Rhagades pruni* ([Denis & Schiffermüller], 1775), in two localities at the end of June - middle of July, 2018 (Table 2, Fig. 2).

Table 2.– Trials with the sex pheromone compound R12 by means of Delta sticky trap in south-eastern France in 2018 (one trap per locality).

Commune, locality	Altidude m a.s.l.	Period	Lure	Family	Attracted species	Nb ex
Val Buëch-Méouge, Chabottes	800	11-VII to 18-VII-2018	R12	Zygaenidae	*Rhagades pruni*	9
Remollon, Gouitrouse	760	29-VI to 10-VII-2018	R12	Zygaenidae	*Rhagades pruni*	9
Lardier-et-Valença, La Citadelle	585	21-VII to 02-VIII-2018	R12	Tischeriidae	*Tischeria ekebladella*	1

Eleven trials were unsuccessful and no Zygaenidae specimens were attracted. One of the observations, on 14-VII-2015, from 15:00 to 15:15, gave no result in a biotope where *A. geryon*, *J. subsolana*, *J. globulariae* (Hübner, 1793), and eight *Zygaena* species (*Zygaena carniolica* (Scopoli, 1763), *Z. hilaris* Ochsenheimer, 1808, *Z. loti, Z. filipendulae* (Linnaeus, 1758), *Z. lonicerae* (Scheven, 1777), *Z. transalpina, Z. viciae,* and *Z. romeo* Duponchel, 1835, were observed on the wing. In addition to the target species, the lure with EFETOV-2 was attractive to two males of *Ptilocephala albida* (Esper, 1786) (Psychidae) in Théus, Maruvert in May 2017 (Table 1 and Fig. 3). A single specimen of *Tischeria ekebladella* (Bjerkander, 1795) (Tischeriidae) was captured in a trap baited with R12 (Table 2).

Discussion

During the investigation period, eight Zygaenidae species were attracted out of the 34 potential species in the area (DROUET, 2016; BENCE & RICHAUD, 2020). EFETOV-2 attracted wider spectrum of species (six species) while the mixture R12+S12 attracted four species. The most frequent response to the sex attractant was by *A. mannii* - it was attracted during 11 observation periods to two types of lures - EFETOV-2 and R12+S12.

Attractiveness of a lure with EFETOV-2 to males of *A. statices* (EFETOV & GORBUNOV, 2016; CAN CENGIZ *et al*., 2018), *A. mannii* (EFETOV *et al*., 2020) and *J. subsolana* (EFETOV *et al*., 2016; CAN CENGIZ *et al*., 2018) have been published from several countries.

R. pruni was recorded by trapping method in traps baited with R12. It is an interesting result, because we do not have a clear idea about the density of *R. pruni* in the region. Earlier it was recorded in France mainly by beating blackthorn *Prunus spinosa* L. and *Crataegus* sp. in the spring, with rarely more than one caterpillar per locality (BENCE & RICHAUD, 2020). R12 or the mixture R12+S12 were attractive to *R. pruni* in Crimea and Hungary (SUBCHEV *et al*., 2010)

Z. transalpina has been the most attracted *Zygaena* species and the number of specimens is a testimony of the efficiency of the EFETOV-2 sex attractant. Some specimens were attracted also to R12+S12. Attraction of *Zygaena* species to esters of long-chain unsaturated fatty acids was observed for the first time.

Trials were carried out only during the afternoon. Nevertheless, the results show the peak of flight activity for *A. mannii* males, to be in the afternoon, between 16:00 to 18:00. This is consistent with the results of visual observations of responses of males of this species made by G. TARMANN, in Pordenone, Friuli, Italy to the sex attractant EFETOV-S-2 (EFETOV *et al*., 2020).

As far as we know, no sex attractant is known for the species *P. albida* (EL-SAYED, 2020). Although few males of this species were observed attracted to the lure baited with EFETOV-2, this is not surprising taking into account the similarity in pheromone chemistry and sex pheromone glands in the Zygaenidae subfamily Procridinae and Psychidae (see SUBCHEV *et al.*, 2000). In one of the Delta traps, although without result regarding Zygaenidae, *T. ekebladella* male was captured. The larvae of this species mine the leaves of *Quercus* sp. Since the trap was hung on a *Quercus* branch and, without a control trap, this capture could have been purely accidental. MOLNÁR *et al*. (2012) identified the sex pheromone of *T. ekebladella* females as a two-component mixture of (3*Z*,6*Z*,9*Z*)-tricosa-3,6,9-triene and (3*Z*,6*Z*,9*Z*,19*Z*)-tricosa-3,6,9,19-tetraene but only the tetraene attracted males in the field.

Our results reinforce our knowledge of the distribution of the Zygaenidae in the study area, enabling the discovery of species in places, where they were previously unknown. The methods applied in the current study are less time consuming and can be managed easily in locations of interest to assess the presence of a particular species and its abundance.

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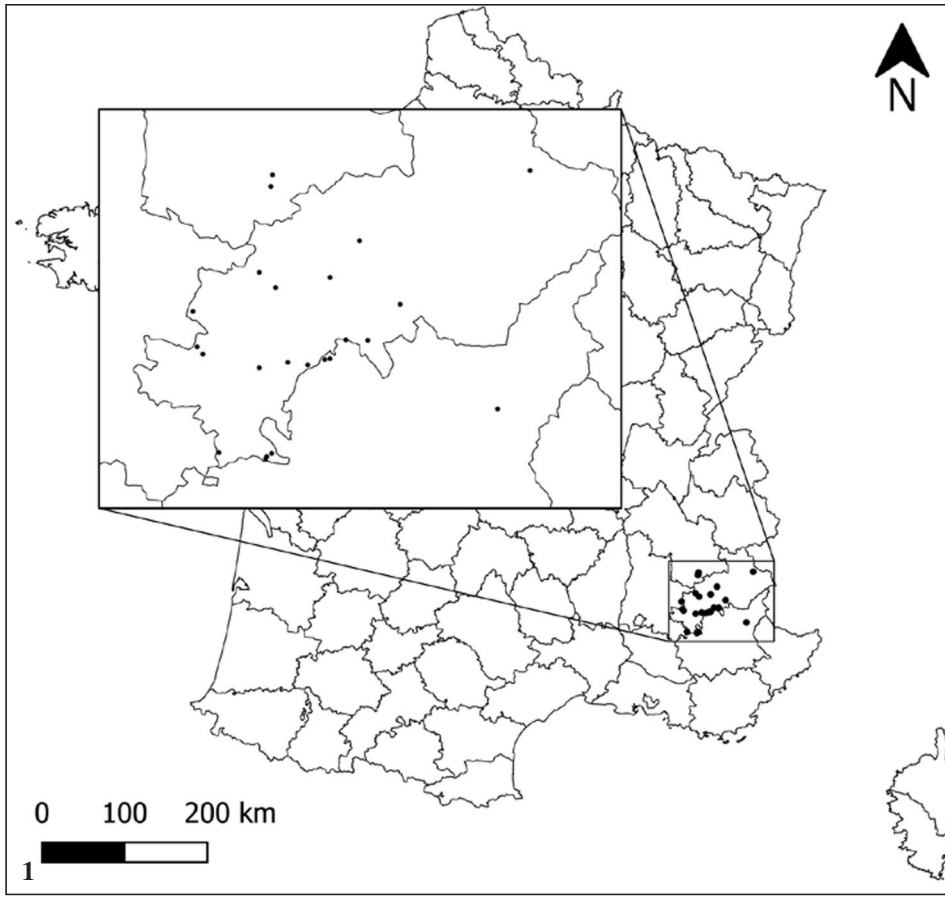

Figs 1-3.– 1. Map of France showing the area covered by the survey. One dot corresponds to a surveyed commune. **2.** Catches of *Rh. pruni* in a Delta trap baited with R12, Hautes-Alpes, Remollon, 10-VII-2018, photo E. Drouet. **3.** *P. albida*, Hautes-Alpes, Théus, Maruvert, 750 m, 09-V-2017, which was attracted to EFETOV-2 lure, photo Jean Raillot.