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Target and non-target Lepidoptera species captured in semiochemical-baited traps for Zygaenidae in two NATURA 2000 protected areas in Bulgaria (Insecta: Lepidoptera)

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Abstract

Semiochemical lures known to attract Zygaenidae species were screened by means of sticky Delta traps and CSALOMON® VARL funnel traps in two Natura 2000 protected areas in Bulgaria - Nikopolsko plato (near Lozitsa village) (northern central Bulgaria) and Lozenska planina Mts, in 2015-2018 and 2023, respectively. As a result, *Rhagades* (*Rhagades*) *pruni*, *Theresimima ampellophaga*, *Zygaena* (*Agrumenia*) *carniolica* and *Z.* (*Zygaena*) *filipendulae* were recorded in the vineyards near Lozitsa vill. while *Adscita* (*Eurasiterna*) *geryon*, *A.* (*Tarmannita*) *mannii*, *Jordanita* (*Tremewania*) *notata* and *Rh.* (*Rh.*) *pruni* were registered in Lozenska planina Mts. *Rhagades* (*Rh.*) *pruni* was newly recorded in northern central Bulgaria. In addition to Zygaenidae species, a total of 62 non-target species belonging to following Lepidoptera families: Coleophoridae, Crambidae, Erebidae, Gelechiidae, Geometridae, Noctuidae, Nolidae, Nymphalidae, Oecophoridae, Pieridae, Plutelliidae, Pyralidae, Tortricidae and Yponomeutidae, were recorded. Sex attractants for one Coleophoridae, one Cambidae, one Geñechiidae and two Noctuidae species were established for the first time. Two *Augasma* species are new records for the fauna of the Balkan Peninsula.
Keywords: Insecta, Lepidoptera, new attractants, new distributional records, Balkan Peninsula.

Especies de Lepidoptera objetivo y no objetivo, capturadas en trampas con cebo semioquímico para Zygaenidae en dos espacios protegidos NATURA 2000 de Bulgaria (Insecta: Lepidoptera)

Resumen

Se seleccionaron semioquímicos conocidos por atraer a especies de Zygaenidae mediante trampas Delta adhesivas y trampas de embudo CSALOMON® VARL en dos zonas protegidas Natura 2000 en Bulgaria: el plato de Nikopolsko (cerca del pueblo de Lozitsa), en el centro-norte de Bulgaria, y los montes Lozenska planina, en 2015-2018 y 2023, respectivamente. Como resultado, se registraron *Rhagades* (*Rhagades*) *pruni*, *Theresimima ampellophaga*, *Zygaena* (*Agrumenia*) *carniolica* y *Z.* (*Zygaena*) *filipendulae* en los viñedos cercanos a la aldea de Lozitsa, mientras que *Adscita* (*Eurasiterna*) *geryon*, *A.* (*Tarmannita*) *mannii*, *Jordanita* (*Tremewania*) *notata* y *Rh.* (*Rh.*) *pruni* se registraron en Lozenska planina Mts. *Rhagades* (*Rh.*) *pruni* se registró por primera vez en el centro-norte de Bulgaria. Además de las especies de Zygaenidae, se registraron un total de 62 especies no objetivo pertenecientes a las siguientes familias de Lepidoptera: Coleophoridae, Crambidae, Erebidae, Gelechiidae, Geometridae, Noctuidae, Nolidae, Nymphalidae, Oecophoridae, Pieridae, Plutelliidae, Pyralidae, Tortricidae e Yponomeutidae. Se establecieron por primera vez atrayentes sexuales para una especie de Coleophoridae una de Crambidae, una de Gelechiidae y dos de Noctuidae. Dos especies

de *Augasma* son nuevos registros para la fauna de la península balcánica.
Palabras clave: Insecta, Lepidoptera, nuevos atrayentes, nuevos registros de distribución, península Balcánica.

Introduction

The earliest publication reporting a synthetic chemical attractant of a zygaenid species was the study of Benz & von Salis (1973), who reported attraction of *Zygaena transalpina* Esper, 1780, males to compounds in a field test organized for the Larch bud moth *Zeiraphera griseana* (Hübner, 1799) (= *Z. diniana* (Guenée, 1845)). Focused studies on zygaenid sex attractants started with the investigations of Decamps et al. (1981), Renou & Decamps (1982), Priesner et al. (1984) and Renou & Zagatti (1984). Over the last fifty years sex attractants for species belonging to the following genera have been published: *Acoloithus*, *Adscita*, *Doratopteryx*, *Epizygaenella*, *Goazrea*, *Harrisina*, *Illiberis*, *Jordanita*, *Neoalbertia*, *Neoilliberis*, *Neoprocris*, *Pyromorpha*, *Reissita*, *Rhagades*, *Staphylinochrous*, *Theresimima*, *Triprocris*, *Zygaena* and *Zygaenoprocris* (Efetov et al. 2011, 2023; Efetov & Kucherenko, 2020; El-Sayed, 2025; Subchev, 2014; Yardım et al. 2025). However, sex pheromones are known for a few species in Zygaenidae. Two pests of *Vitis vinifera* L., viz. *Harrisina brillians* Barnes & McDunnough, 1910, and *Theresimima ampellophaga* (Bayle-Barelle, 1809), use the enantiomers of 2-butyl-(7*Z*)-tetradecenoate in their intraspecific chemical communication systems, (2*S*)-butyl (7*Z*)-tetradecenoate (S-7-14) (Myerson et al. 1982; Soderstrom et al. 1985) and a mixture of (2*R*)-butyl (7*Z*)-tetradecenoate (main pheromone compound) (R-7-14) and S-7-14 (minor pheromone compound) (Subchev et al. 1998, erratum - 1999), respectively. Subchev et al. (2009) identified (2*R*)-butyl (7*Z*)-dodecenoate (R-7-12) and (2*R*)-butyl (9*Z*)-tetradecenoate (R-9-14) as sex pheromone compounds of *Illiberis* (*Primilliberis*) *rotundata* Jordan, 1907 females. Zagrobelny et al. (2015) analysed volatile emissions from males and females of the six-spot burnet moth, *Zygaena* (*Zygaena*) *filipendulae* (Linnaeus, 1758), which resulted in the identification of novel putative pheromones for this species. Thus, sex pheromone components exactly established for only three species of Zygaenidae from the subfamily Procridinae. All of them are esters of butan-2-ol and higher unsaturated carboxylic acids. Similar esters are known as sex attractants for much more Zygaenidae species from two subfamilies, viz. Procridinae and Zygaeninae. In particular, the enantiomers of 2-butyl 2-dodecenoate are sex attractants from the series 'EFETOV-2' (patented name), which are active for the males of almost 30 Zygaenidae species from two subfamilies noted above (Can et al. 2019; Can Cengiz et al. 2018; Drouet et al. 2021; Efetov et al. 2016, 2018, 2019, 2020, 2024, 2025; Efetov & Kucherenko 2021; Yardım et al. 2024).

The aim of the current study was to investigate the occurrence and diversity of forester and burnet moths (Zygaenidae) in an agricultural land in northern Bulgaria and in a mountain habitat in western part of the country; both study places fall in Natura 2000 protected areas. The paper presents also data about other lepidopteran species captured during the study including the first records of two *Augasma* species in Bulgaria and new sex attractants for five lepidopteran species.

Materials and methods

Study sites

The experiments were carried out in two regions - Nikopolsko plato situated in northern central Bulgaria and Lozenska Mts situated in southwest Bulgaria in areas included within NATURA 2000 ecological network under Directive 92/43 / EEC for the Conservation of Natural Habitats and Wild Flora and Fauna, NATURA 2000 sites BG0000274 and BG0000165, respectively.

Investigations were performed over a three-year period (2015-2018) in vineyards in Nikopolsko plato near Lozitsa village (Nikopol Municipality, Pleven Province). The wine grape varieties were 'Muskat Ottonel' and 'Mavrud'. The area is characterized by vineyards of different type of management - well managed, neglected or abandoned vineyards (Toshova et al. 2017), arable lands and perennial meadows. The last two types of vineyards are habitats, which also offer suitable host-plants of numerous insect species including pests.

In 2023, the tests with attractant lures were performed in Lozenska planina Mts at the base of Lalina mogila peak, where the habitat is characterized by mixed deciduous forest and meadows. The dendroflora in the studied sites is represented by *Quercus cerris* L., *Quercus frainetto* Ten., *Ulmus minor* Mill., *Fagus sylvatica* L., *Corylus avellana* L., *Carpinus orientalis* Mill., *Pyrus pyraster* (L.) Burgsd., *Prunus spinosa* L., *Cornus mas* L., *Juniperus communis* L. and *Rosa* sp.

Traps

For Procridinae tests home-made transparent Delta traps with replaceable sticky inserts were used while for Zygaeninae tests the traps were CSALOMON® VARL funnel traps (Plant Protection Institute, Centre for Agricultural Research, HUN-REN, Budapest; www.csalomontraps.com). Delta sticky inserts were with a size 10 cm x 16 cm. A piece (1 × 1 cm) of a household anti-moth insecticide strip (Chemotox®, Slouth, UK; active ingredient 15% dichlorvos) was placed into the collection container of the VARL funnel traps to kill captured insects. The traps used in the experiments are shown on Figure 1.

Lures

Lures for Procridinae

For preparing the lures, the synthetic pheromone compounds, R-7-14 and R-7-12 and the enantiomer of the last compound, S-7-12, in a dose of 100 μg were applied singly onto serum vial caps of grey rubber as a hexane solution. The two-component lure of R-7-14 and R-7-12 (tested in Lozitsa vill. in 2017 and Lozenska planina Mts in 2023) was in a ratio 100:100 (R-7-14 + R-7-12). The lures were prepared at the Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences (IBER-BAS). The sex attractant EFE-TOV-2, the rubber caps were impregnated with 100 μl of the racemic mixture of (2*R*)-butyl 2-dodecenoate and (2*S*)-butyl 2-dodecenoate, which was prepared in the Crimean Federal University, Simferopol (Crimea) (Efetov et al. 2014).

Lures for Zygaeninae

The synthetic compounds used were (*Z*)-5-dodecenyl acetate (Z5-12Ac), (*Z*)-7-dodecenyl acetate (Z7-12Ac), (*Z*)-7-tetracenyl acetate (Z7-14Ac), (*Z*)-9-dodecenyl acetate (Z9-12Ac), (*Z*)-9-tetradecenyl acetate (Z9-14Ac), (*Z*)-11-hexadecenyl acetate (Z11-16Ac), and (*Z*)-8-dodecenyl acetate (Z8-12Ac). They were purchased from Sigma-Aldrich® or obtained from Pherobank (Netherlands) and their purity was > 96%.

The following one-compound lures and mixtures were prepared at IBER-BAS by the method described above: Z5-12Ac, Z7-12Ac, Z9-14Ac, Z7-12Ac + Z9-14Ac (100:100) and Z7-12Ac + Z9-14Ac + Z8-12Ac (100:100:100).

The lures for *Zygaena* (*Mesembrinus*) *purpuralis* (Brünnich, 1763) (Z_pur) and *Z.* (*Agrumenia*) *viciae* ([Denis & Schiffermüller], 1775) (Z_vic) were prepared by the Ecology Research Group, Canterbury Christ Church University, England (UK). The lure preparation and composition for *Z.* (*M.*) *purpuralis* and *Z.* (*A.*) *viciae* were described in Can et al. (2019) and Vrenozi et al. (2020) and contained Z7-12Ac + Z7-14Ac + Z9-14Ac (100:10:100) and Z5-12Ac + Z7-12Ac + Z9-12Ac + Z9-14Ac + Z11-16Ac (10:100:1:10:5) for Z_pur and Z_vic, respectively.

Dispensers were wrapped singly in pieces of aluminum foil and were stored at -30ºC until use.

Field Experiments

In seven field experiments we evaluated the attractiveness of lures for Procridinae and Zygaeninae in two sites in Bulgaria in the period of 2015-2018 and 2023. One to five lures were tested within each experiment. Two replications/blocks of the treatments were deployed at each site, and the traps in a replicate were spaced 10-15 m apart along the field. Distance between the vineyards in Lozitsa vill. was about 0.800 km while the distance between localities in Lozenska planina Mts - about 0.500 km. For unbaited controls, traps without any lure were also used in the most of the experiments. Lures last for different lengths of time. Previous experience obtained in many years in different countries showed that the traps baited with the lures for *Th. ampellophaga* and also lures containing R-7-12, S-7-12 and their blend do not attract any other lepidopteran species, and they are active at least six - seven weeks (Subchev et al. 2004; Toshova et al., unpublished data) while EFETOV-2 lures are active not less than six months (Efetov et al. 2022, 2024; Vrenozi et al. 2019). Other lures tested were replaced every four weeks. Details about the experiments are provided in Table 1.

Traps were checked at approximately weekly intervals and only in some single cases for a longer period, however not exceeding four weeks (August, 2016 and 9-VI - 21-VII-2018, Lozitsa vill.). Trap positions within each trap block were rotated at each inspection date. In tests where sticky traps were used at each sample interval, the sticky layers were replaced with new bottoms. The collected moths were taken to the laboratory for species identification. For accurate identification of Microlepidoptera species captured into the sticky traps, insects were kept in glass vials with xylenes purriss., ≥ 96.0% (Sigma-Aldrich®) for 48 hours to remove the sticky material from the insect's body. After any residual sticky materials were dissolved the specimens were placed on filter paper to dry. The procedure of cleaning of insects was done under a fume hood in the laboratory. The identification of specimens caught during the field tests was based on external morphological characters and genitalia examination. The species were identified using the following identification keys: Anikin & Sinichkina (2018), Danilevsky & Kuznetzov (1968), Falkovitsh (1986), Razowski (2003), and without identification guides for the "Macrolepidoptera" species.

Statistical analysis

A minimum of 30 specimens of a non-target species per year was the criterion chosen for performing the statistical analysis. For a robust analysis we included in each analysis only data that had a sufficient number of replicates (n ≥ 10 replicates). The data were tested for normal distribution using the Kolmogorov-Smirnov test. Since these conditions were not met, a Kruskal-Wallis test was used as nonparametric test to determine the significance of treatments. When the Kruskal-Wallis test indicated significant differences between treatment means, subsequent pairwise Mann-Whitney U test was used to detect the significant differences between the tested variants. All statistical analyses were performed in STATISTICA v.7.0 (StatSoft, Tulsa, OK, USA). The significant difference was set at $P < 0.05$.

Results

Target species

A total of 230 males of the following seven Zygaenidae species were captured during the study. All Zygaenidae were captured in the sticky traps. *Rhagades* (*Rhagades*) *pruni* ([Denis & Schiffermüller], 1775), *Th. ampellophaga*, *Zygaena* (*Agrumenia*) *carniolica* (Scopoli, 1763) and *Z.* (*Z.*) *filipendulae* were recorded in the vineyards near Lozitsa vill. while *Adscita* (*Eurasiterna*) *geryon* (Hübner, 1813), *A.* (*Tarmannita*) *mannii* (Lederer, 1853), *Jordanita* (*Tremewania*) *notata* (Zeller, 1847) and *Rh.* (*Rh.*) *pruni* were registered in Lozenska planina Mts (Tables 2-3). In Lozitsa vill., the most numerous species were *Th. ampellophaga* and *Z.* (*Z.*) *filipendulae* captured in the traps baited with R-7-14 + R-7-12 and mainly Z_vic, respectively. In 2017, one flight peak of the males of the vine bud moth occurred with catches from the middle of June to the middle of July. In the same year, *Z.* (*Z.*) *filipendulae* catches were registered during the whole period of the investigation from the middle of June to the end of August - beginning of September. In Lozenska planina Mts, *A.* (*T.*) *mannii* was the most abundant species followed by *J.* (*T.*) *notata* captured in the traps baited with S-7-12 and R-7-12 (alone or in combination with the opposite enantiomer), respectively. In Lozitsa vill., catches of the males of *Rh.* (*Rh.*) *pruni* were recorded during the period of 12-26-VI-2015 (males attracted to R-7-12 and S-7-12) and 3-12-VI-2016 (single males attracted to R-7-12 and EFETOV-2 lures, respectively) while in Lozenska planina Mts - 15-28-VII-2023 (single male attracted to R-7-12).

Non-target species

In addition to the Zygaenidae species, traps tested captured a wide range of species from different Lepidoptera families - Coleophoridae, Crambidae, Erebidae, Gelechiidae, Geometridae, Noctuidae, Nolidae, Nymphalidae, Oecophoridae, Pieridae, Plutelliidae, Pyralidae, Tortricidae and Yponomeutidae, and some of them were caught in high numbers. Catches of the individual species are shown in Tables 2-3.

In Lozitsa vill., the most numerous non-target species was *Augasma aeratella* (Zeller, 1839) (Coleophoridae) followed by *Autographa gamma* (Linnaeus, 1758) (Noctuidae) and *Evergestis aenealis* ([Denis & Schiffermüller], 1775) (Crambidae) (Table 2). The trap catches in the experiments performed in 2015 and 2016 indicated that the highest mean catches of *A. aeratella* males were found in the traps baited with Z7-12Ac alone or in combination with Z9-14Ac (100:100) and no significant difference between catches was observed despite the trap type was different in the two years (Table 4). The flight of the adults was at least five months from the end of April - beginning of May to the end of September. In 2015, when Delta sticky traps were used similar results were obtained with *A. gamma* males. However, in the next year when lures were tested in combination with VARL funnel traps Z7-12Ac attracted significantly more males compared with the two-component mixture.

Evergestis aenealis ([Denis & Schiffermüller], 1775) males were captured in the traps baited with the two-component mixture of Z7-12Ac and Z9-14Ac in Lozitsa vill. and Lozenska planina Mts, and a single male was recorded also in the traps baited with Z9-14Ac in Lozitsa vill. in 2016. The period of flight activity differed between the study sites and years. In the vineyards in Lozitsa vill. in 2015, catches were registered in the period of 19-VI - 20-IX, and two peaks of flight were observed: 1) the end of June - beginning of July and the second half of August. In 2016 in the same area, the flight activity began earlier - at the end of April - beginning of May while the last catch was recorded at the end of September. In Lozenska planina Mts, *E. aenealis* males were captured during the whole monitoring period - from the beginning of June to the middle of July.

In Lozitsa vill., where EFETOV-2 lures were tested *Udea ferrugalis* (Hübner, 1796) (Crambidae) males were attracted. Catches were recorded in the period of 3-25-VI-2016 and 9-VI - 5-VIII-2018.

Pammene gallicolana (Lienig & Zeller, 1846) was caught in the traps baited with the triple mixture of Z7-12Ac + Z9-14Ac + Z8-12Ac in unusually high number of males in June 2023 in Lozenska planina Mts Bulgaria.

In addition to the lepidopteran species, a single male specimen of *Tilloidea unifasciata* (Fabricius, 1787) (Coleoptera: Cleridae) was registered in Lozitsa vill. in the trap baited with EFETOV-2 lure during the period of 25-VI - 2-VII-2015.

Discussion

Target species

The Zygaenidae species recorded during the study are widely distributed species in Bulgaria in exception of *Rh.* (*Rh.*) *pruni*. *Rhagades* (*Rh.*) *pruni* is newly recorded in the region of northern central Bulgaria. In the whole northern part of Bulgaria, on the north of Stara planina Mts, *Rh.* (*Rh.*) *pruni* has been recorded only in Sveshtari vill. (Razgrad Province) in 1923 (Buresh & Tuleshkov, 1943). There are only two more historical records in the Danube plain, Bucharest and Comana, originating from 1916 (Popescu-Gorj, 1964). In the wider area of Sofia, only one record has been known so far. Subchev et al. (2010) recorded *Rh.* (*Rh.*) *pruni* in Botanical garden of the Bulgarian Academy of Sciences in Sofia in 2005. Our record from Lozenska planina Mts confirms its presence in this region. Both of new records fill in considerable wide gaps in distribution of this species (Figure 2). *Prunus spinosa* L., *Rosa sp.* and *Rubus fruticosus* L., which are host-plants for the caterpillars of *Rh.* (*Rh.*) *pruni* were observed in vineyards in Lozitsa vill. and the studied habitats in Lozenska planina Mts (Toshova, personal observations). *Zygaena* (*A.*) *carniolica* and *Z.* (*Z.*) *filipendulae* are common species in Bulgaria and neighbouring countries, however there are no published records after 1923 for *Z.* (*A.*) *carniolica* and after 1982 for *Z.* (*Z.*) *filipendulae* for the wider area of Danube Plain in Bulgaria and Romania.

Our results confirm the data obtained earlier that S-7-12 (alone or in mixtures) attracts *A.* (*Eur.*) *geryon* and *A.* (*T.*) *mannii* while R-7-12 (alone or in mixtures) is an attractant for *J.* (*T.*) *notata* (Drouet et al. 2021; Efetov et al. 2015; Subchev et al. 2010; Vrenozi et al. 2020). Attraction of *Rh.* (*Rh.*) *pruni* males to R-7-12 alone or in mixtures has been reported from Bulgaria, Crimea, Hungary and France (Drouet et al. 2021; Subchev et al. 2010). It was also reported that the males of this species were attracted to EFETOV-2 in the Crimea (Efetov et al. 2016) and its *S*-enantiomer, EFETOV-S-S-2, in Spain (Efetov et al. 2019; Efetov &

Kucherenko, 2021). The five-component lure Z_vic (Z5-12Ac + Z7-12Ac + Z9-12Ac + Z9-14Ac + Z11-16Ac in a ratio 10:100:1:10:5) attracted for the first time the male specimens of *Z.* (*Z.*) *filipendulae*. Single specimens of *Z.* (*Z.*) *filipendulae* and *Z.* (*A.*) *carniolica* were captured in the traps baited with Z7-12Ac + Z9-14Ac (100:100) in Lozitsa vill. in July, 2015. The last two compounds have been reported in the attractive mixtures for these species with Z7-12Ac as a dominant component in these mixtures (El-Sayed, 2025). It seems the addition of the sex pheromone component of *I.* (*P.*) *rotundata* (R-7-12) to the main sex pheromone compound of *Th. ampellophaga* females (100:100) does not influence the activity of the latter compound. In 2017, male specimens recorded in the traps with the two-component mixture in Lozitsa vill. belongs to the first generation of this species.

All zygaenid species were captured in the baited sticky Delta traps in the two localities - Lozitsa vill. and Lozenska planina Mts. Knodel & Agnello (1990) reported that sticky traps caught higher number of moths than various funnel traps. When comparing different trap designs baited with sex pheromone including VARL funnel traps for effectiveness in capturing of *Th. ampellophaga* males Subchev et al. (2004) reported that there was no statistically significant difference between the catches of males in all types of traps, although numerically somewhat more moths were caught in sticky Delta traps than in VARL funnel traps.

Non-target species

Augasma nitens Amsel, 1935 and *A. uljanovi* Anikin, 2017

Augasma nitens and *A. uljanovi* are new records for the Bulgarian fauna and Balkan Peninsula. *Augasma nitens* was described by Amsel (1935) from Palestine (Jéricho) by a moth caught in the first half of August, 1930, and no other localities have been reported. Male genital apparatus was presented by Toll (1962). Female and developmental stages are unknown. In our study, *A. nitens* was captured in the traps baited with Z7-12Ac + Z9-14Ac at the second half of July, in the period of 17-14-VII-2015 in Lozitsa vill. *Augasma uljanovi* was described by Anikin (2017) from Uljanovsk region in Russia. Recently it was recorded in Volgograd and Saratov regions in Russia (Anikin & Khapugin, 2024) and in the vicinity of Mingechaur in Azerbaijan (Anikin et al. 2025). The moths of the type series of *A. uljanovi* have emerged from galls of *Atraphaxis frutescens* (L.) Eversm. (Polygonaceae) (Anikin, 2017). *Atraphaxis frutescens* is native to European Russia, Siberia, Kazakhstan, Turkmenistan, Uzbekistan, Mongolia and China (Abilkassymova et al. 2024).

Augasma aeratella (Zeller, 1839)

The results of the present study confirmed literature data on Z7-12Ac as being a sex attractant of *A. aeratella* males (Tóth et al. 1992). Although a high number of males was found also in the traps baited with the mixture with Z9-14Ac (100:100) in Nikopolsko plato in 2015-2016 we consider that Z7-12Ac was responsible for the attraction. The larva of *A. aeratella* lives on *Polygonum aviculare* Linnaeus, 1753 and *Persicaria lapathifolia* (L.) Delarbre 1800 (Toll, 1962) from October to April and the species fly from May to August (Fazekas & Schreurs, 2010). However, we recorded catches of males in the period from the end of April - beginning of May to the end of September, 2016. Kulikov & Naumkin (2004) reported *A. aeratella* as a pest of buckwheat, *Fagopyrum esculentum* Moench (Polygonaceae).

It is worth mentioning that some dry *Augasma* specimens captured in the sticky traps were sent for molecular analysis. Unfortunately probably the rapid degradation of DNA in the insects has rendered them unsuitable for genetic analyses. Later Butterwort et al. (2022) presented a DNA extraction method designed for the detection of insects from sticky traps.

Tuberculia albitarsella Zeller, 1849

During screening test in Pancharevo (Sofia) Subchev et al. (1990) reported two-component sex attractant, Z5-10Ac + Z7-12Ac, for *T. albitarsella* males as captures were registered in various ratios of the two compounds (100:100, 100:10 and 10:100). However, the single compounds were inactive in their study. We recorded a single male of this species in a VARL funnel trap baited with Z7-12Ac in Lozitsa vill.

Evergestis aenealis ([Denis & Schiffermüller], 1775)

Tóth et al. (1994) reported that the mixture of Z7-12Ac + Z9-14Ac is an attractant for *E. aenealis* in Hungary. In the current study, the same mixture was proved to be attractive to *E. aenealis* males in Lozitsa vill. (2015-2016) and Lozenska planina Mts (2023). In Bulgaria, this species is widely distributed from the sea level to above 2000 m (Ganev, 1986; Plant, 2016). The species develops two generations per year in Bulgaria but in some years (Plant, 2016). Our results from Lozitsa vill., where monitoring periods were longer showed that *E. aenealis* had two generations. The flight of the first generation was from the end of August - beginning of May to the beginning of July, and catches of the males from the second generation were recorded from the second half of July to the middle of September.

Udea ferrugalis (Hübner, 1796)

Udea lutealis (Hübner, [1809]), is the only species of the genus *Udea* with known sex attractant (Z11-16Ac) (El-Sayed 2025). However, during our study with the exception of *Rh.* (*Rh.*) *pruni* the rusty dot pearl moth, *U. ferrugalis* was the only species attracted to EFETOV-2 lures in Lozitsa vill. in 2016 and 2018. This species is a common and widespread species in Bulgaria. *Udea ferrugalis* is polyphagous attacking numerous plant species (Slamka, 2013). It is considered as a pest of greenhouse plants in Bulgaria (Loginova, 1992).

Bryotropha tachyptilella (Rebel, 1916)

This species was described by Rebel (1916) by two males captured by Petar Chorbadziev from Burgas, Bulgaria in June 1910. Additional data about distribution of this species in Bulgaria has been provided by Karsholt & Rutten (2005): Arkutino (Black sea coast), Sliven, Sandanski, all sites in southern Bulgaria. The attraction of males of *B. tachyptilella* to the two-component blend of Z7-12Ac and Z9-14Ac in Lozitsa vineyards was documented for the first time. According to the literature, Z9-14Ac alone or in mixtures with other compounds attracts *B. similis* (Stainton, 1854) (Roelofs & Comeau, 1969), *B. galbanella* (Zeller, 1839), *B. terrella* ([Denis & Schiffermüller], 1775) (Mozūraitis et al. 1998) and *B. senectella* (Zeller, 1839) (Kovaleva et al. 1975). From the other hand, Arn et al. (1974) reported that *B. terrella* males have been attracted by the opposite isomer, *trans*-9-tetradecenyl acetate. According to Kovács & Kovács (2022), *B. tachyptilella* has two generations, adults from the first generation fly in May and June and the moths from the second generation – from the beginning of August to the middle of September. We recorded catches in August-September, which correspond to the adults of the second generation. There is no information available on the host-plant of *B. tachyptilella* but *Bryotropha* larvae are known to feed on mosses and some species on grasses (Karsholt & Rutten, 2005).

Chloantha hyperici ([Denis & Schiffermüller], 1775)

Captures of *Ch. hyperici* males in the traps baited with Z7-12Ac in 2015 and 2016 in Lozitsa vill. to the traps baited with Z7-12Ac is an indication that this alcohol acts as a sex attractant of the males of this species. No reports about sex attractants have been published for this species. *Ch. hyperici* occurs in two or partially three generations between March and September. Larva feeds on *Hypericum* (Hypericaceae). This species is widely distributed in Bulgaria (Beshkov et al. 1999; Kostova et al. 2019; Zlatkov, 2007).

Axylia putris (Linnaeus, 1761)

Our results confirmed the attraction of the males of *A. putris*, to Z9-14Ac already reported for several European and Asian countries (El-Sayed, 2025). The flight period of male moths in Lozitsa vill. (April-July) overlaps that reported by Zlatkov (2007) (April - August). Host-plants for the larvae of this species are variety of plants belonging to Fabaceae, Poaceae, Polygonaceae, Rubiaceae, Urticaceae, etc. (Agius, 2023). Molinari et al. (1996) reported *A. putris* as a pest of peach orchards in Italy.

Hoplodrina respersa ([Denis & Schiffermüller], 1775)

No sex attractant has been previously described for *H. respersa* and our results showed behavioral activity of three lures containing Z9-14Ac. The attraction of males of this species to the three-component lure tested in Lozenska planina Mts, Z7-12Ac + Z9-14Ac + Z8-12Ac, should be confirmed.

Pammene gallicolana (Lienig & Zeller, 1846)

Pammene gallicolana is a species associated with wasp galls of oak (Kemal & Koçak, 2010). Our results confirm the importance of Z8-12Ac in attraction of *P. gallicolana* males (Olenici et al. 2007; Rotundo et al. 1991; Sziráki, 1978). Velcheva (2000) reported relatively low number of males of this species captured in the traps baited with Z8-12Ac in combination with the geometrical isomer, *trans*-8-dodecenyl acetate, and the single Z8-12Ac in Sofia (Pancharevo), Bulgaria. Males of several *Pammene* species including *P. albuginana* (Guenée, 1845) respond to the commercially available sex attractants containing Z8-12Ac (Jakubíková et al. 2016; Mayer & McLaughlin, 1991).

Autographa gamma (Linnaeus, 1785) and *Macdunnoughia confusa* (Stephens, 1850) males were attracted most strongly by the single-component lure of Z7-12Ac. This compound has been previously identified in the sex pheromone mixtures of the conspecific females. From the other hand the importance of Z9-14Ac in the intraspecific communication of *Noctua janthina* ([Denis & Schiffermüller], 1775) and *Panemeria tenebrata* (Scopoli, 1763) has been documented (El-Sayed 2025). Our data confirmed the attraction of the turnip moth *Agrotis segetum* ([Denis & Schiffermüller], 1775) to the binary mixture of Z7–12Ac and Z9-14Ac in Bulgaria (Subchev et al. 1986).

We confirmed the attraction of the checkered beetle *Tilloidea unifasciata* to EFETOV-2, which was reported by Efetov & Kucherenko (2024) in five sites in the Crimea in 2013-2015.

Protected area effectiveness depends on both the quantity (e.g., coverage) and quality (e.g., representativeness, abundance, good condition, high species richness) (Cooke et al. 2023). Our results highlight the role of two protected areas in Bulgaria for lepidopteran diversity studied by sex pheromone/ attractant traps.

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Conflict of Interest

The authors declare that there is no known financial interest or personal relationship that could have influenced the work presented in this article.

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Figure 1. Traps in the study sites: **A.** Delta sticky traps and **B.** VARL funnel traps in Lozitsa vill. **C.** Delta sticky traps and **D.** VARL funnel traps in Lozenska planina Mts.

Figure 2. Distribution of *Rhagades* (*Rhagades*) *pruni* in Bulgaria and surrounding countries. Grey dots - published records before 1974, black dots - published records after 2000, red dots - new records, 1 -Lozenska planina Mts, 2 - Nikopolsko plato.

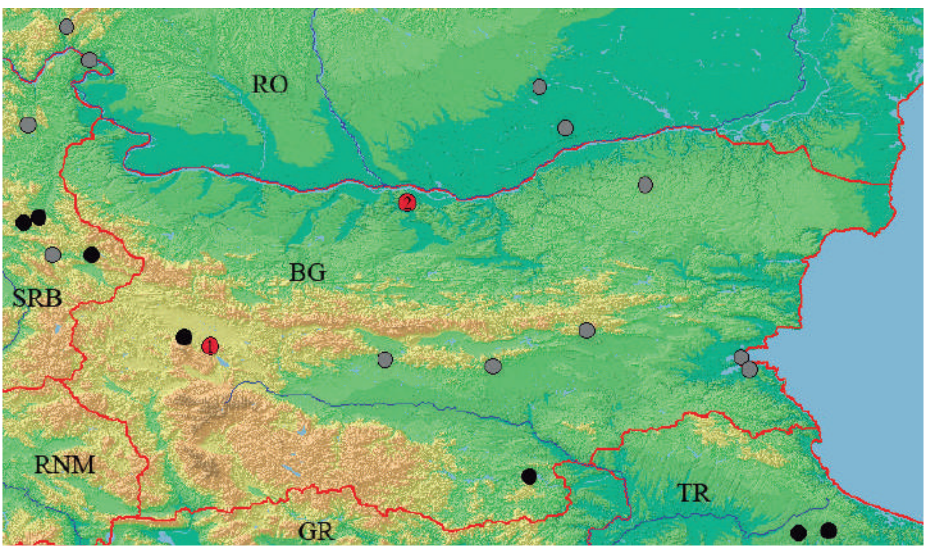

Table 1. Details about the experiments near Lozitsa vill. (2015-2018) and Lozenska planina Mts (2023): site, habitat type, geographic coordinates of the localities, duration of the experiment (period), date of lure replacement, lure tested and trap type.

Experiment Nº	Site, habitat	Coordinates, altitude, m a.s.l.	Period start - end	Date of lure replacement	Lure/ treatments	Trap type
Exp. 1	Lozitsa vill., vineyard, arable land	**locality 1** 43.60656°N, 24.97407°E, 239 m **locality 2** 43.60942°N, 24.98929°E, 228 m	12-VI-2015-07-X-2015	03-VII-2015 30-VII-2015 12-IX-2015	R-7-12 S-7-12 Z5-12Ac Z7-12Ac Z7-12Ac + Z9-14Ac Unbaited	Delta sticky trap
Exp. 2	Lozitsa vill., vineyard, arable land	**locality 1** 43.60656°N, 24.97407°E, 239 m **locality 2** 43.60942°N, 24.98929°E, 228 m	28-V-2016-29-VII-2016	02-VII-2016 (except EFETOV-2 lures, which work all season)	R-7-12 S-7-12 EFETOV-2 Unbaited	Delta sticky trap
Exp. 3	Lozitsa vill., vineyard, arable land	**locality 1** 43.60656°N, 24.97407°E, 239 m **locality 2** 43.60942°N, 24.98929°E, 228 m	17-IV-2016-25-IX-2016	24-V-2016 25-VI-2016 30-VII-2016 03-IX-2016	Z7-12Ac Z9-14Ac Z7-12Ac + Z9-14Ac Unbaited	VARL funnel traps
Exp. 4	Lozitsa vill., vineyard, arable land	**locality 1** 43.60942°N, 24.98929°E, 218 m **locality 2** 43.59872°N, 24.97404°E, 239 m	16-VI-2017-16-IX-2017	06-VIII-2017 (R-7-14+R-7-12) 20-VII-2017 20-VIII-2017 (*Zygaena* lures)	R-7-14 + R-7-12 Z_pur Z_vic	Delta traps
Exp. 5	Lozitsa vill., vineyard, arable land	**locality 1** 43.60942°N, 24.98929°E, 218 m **locality 2** 43.59872°N, 24.97404°E, 239 m	06-VI-2018-05-VIII-2018	no replacement	EFETOV-2 Unbaited	Delta traps
Exp. 6	Lozenska planina Mts, S Lalina mogila, mixed forest, meadow	**locality 1** 42.57772°N, 23.49367°E, 1034 m **locality 2** 42.57564°N, 23.49673°E, 1012 m	02-VI-2023-28-VII-2023	15-VII-2023	R-7-12 S-7-12 R-7-12 + S-7-12	Delta traps
Exp. 7	Lozenska planina Mts, S Lalina mogila, mixed forest, meadow	**locality 1** 42.58006°N, 23.49298°E, 1079 m **locality 2** 42.57564°N, 23.49673°E, 1012 m	02-VI-2023-15-VII-2023	no replacement	Z7-12Ac Z9-14Ac Z7-12Ac+Z9-14Ac Z7-12Ac+Z9-14Ac+ Z8-12Ac Unbaited	VARL funnel traps

Table 2. Catches of lepidopteran species in baited and control (unbaited) traps, Lozitsa village.

Family/Species	Number of moths caught (period of collection: months and year); Lure	Sum
Coleophoridae		
Augasma aeratella (Zeller, 1839)	539 ♂ (VI-IX-2015); Z7-12Ac (402) and Z7-12Ac+Z9-14Ac (137) 154 ♂ (IV-IX-2016); Z7-12Ac (83), Z7-12Ac+Z9-14Ac (70) and Z9-14Ac (1)	693
Augasma nitens Amsel, 1935	1 ♂ (VII-2015); Z7-12Ac+Z9-14Ac	1
**Augasma uljanovi* Anikin, 2017	5 ♂ (VI-VII-2016); Z7-12Ac	5
Tuberculia albitarsella Zeller, 1849	1 ♂ (IV-V-2016); Z7-12Ac	1
Crambidae		
Evergestis aenalis ([Denis & Schiffermüller], 1775)	74 ♂ (VI-IX-2015); Z7-12Ac+Z9-14Ac 34 ♂ (V-IX-2016); Z7-12Ac+Z9-14Ac (33) and Z7-12Ac (1)	108
Platytes cerusella ([Denis & Schiffermüller], 1775)	1 ♂ (VI-2016); Z7-12Ac+Z9-14Ac	1
**Udea ferrugalis* (Hübner, 1796)	14 ♂ (VI-2016); EFETOV-2 5 ♂ (VII-VIII-2018), EFETOV-2	19
Erebidae		
Dysgonia sp.	1 ♂ (VII-VIII-2018); EFETOV-2	1
Lygephila craccae ([Denis & Schiffermüller], 1775)	2 ♂ (IX-2015); Z5-12Ac (1) and Z7-12Ac (1)	2
Gelechiidae		
Anarsia lineatella Zeller, 1839	1 ♂, 1 ♀ (VII-2015); control	2
Bryotropha senectella (Zeller, 1839)	2 ♂ (VII-2016); control	2
**Bryotropha tachyptilella* (Rebel, 1916)	30 ♂, 2 ♀ (VIII-IX-2015); Z7-12Ac+Z9-14Ac 1 ♂ (IX-2016); Z7-12Ac+Z9-14Ac	33
Dichomeris alacella (Zeller, 1839)	3 ♂ (3♀) (VII-2015); R-7-12	3
Helcystogramma triannulella (Herrich-Schäffer, 1854)	1 ♂ (VIII-IX-2016); R-7-14	1
Geometridae		
Ematurga atomaria (Linnaeus, 1758)	1 ♂ (VI-2015); R-7-14 1 ♂ (VI-2016); Z7-12Ac+Z9-14Ac	2
Scopula sp.	1 ♂ (VII-2015); Z7-12Ac	1
Noctuidae		
Agrotis exclamationis (Linnaeus, 1758)	1 ♂ (V-2016); Z7-12Ac+Z9-14Ac	1

Agrotis segetum ([Denis & Schiffermüller], 1775)	63 ♂ (VII-X-2015); Z7-12Ac+Z9-14Ac (33); Z7-12Ac (23) and Z5-12Ac (7) 15 ♂ (IV-IX-2016); Z7-12Ac+Z9-14Ac (14) and Z7-12Ac (1)	78
Autographa gamma (Linnaeus, 1758)	33 ♂ (VII-IX-2015); Z7-12Ac (16) and Z7-12Ac+Z9-14Ac (17) 178 ♂ (V-IX-2016); Z7-12Ac (177) and Z7-12Ac+Z9-14Ac (1)	211
Axylia putris (Linnaeus, 1761)	43 ♂ (IV-VII-2016); Z9-14Ac	43
Caradrina flavirena Guenée, 1852	1 ♂ (IV-2016); Z7-12Ac	1
Charanyca trigrammica (Hufnagel, 1766)	1 ♂, 1 ♀ (V-2016); control	2
**Chloantha hyperici* ([Denis & Schiffermüller], 1775)	4 ♂ (VII-IX-2015); Z7-12Ac 4 ♂ (VI-2016); Z7-12Ac	8
Helicoverpa armigera (Hübner, [1808])	1 ♂ (VII-2016); Z7-12Ac	1
Macdunnoughia confusa (Stephens, 1850)	2 ♂ (VII-2015); Z7-12Ac (1) and Z7-12Ac+Z9-14Ac (1) 42 ♂ (IV-VI-2016); Z7-12Ac (33) and Z7-12Ac+Z9-14Ac (9)	44
Mesapamea secalis (Linnaeus, 1758)	2 ♂ (VIII-2015); control (1) and Z5-12Ac (1)	2
Mythimna albipuncta ([Denis & Schiffermüller], 1775)	2 ♂ (VIII-2015); Z5-12Ac (1) 1 ♂ (IX-2016); control	3
Mythimna congrua (Hübner, [1817])	1 ♂ (IX-2015); Z7-12Ac+Z9-14Ac	1
Mythimna ferrago (Fabricius, 1787)	3 ♂ (VIII-2015); Z5-12Ac (2) and Z7-12Ac (1) 1 ♂ (VIII-2016); Z7-12Ac	4
Mythimna vitellina (Hübner, [1808])	2 ♂ (IX-2016); control	2
Noctua janthina ([Denis & Schiffermüller], 1775)	1 ♂ (IX-2016); Z9-14Ac	1
Noctua pronuba (Linnaeus, 1758)	1 ♂ (IX-X-2015); control	
Oligia latruncula ([Denis & Schiffermüller], 1775)	1 ♂ (V-2016); control	1
Panemeria tenebrata (Scopoli, 1763)	1 ♂ (IV-2016); Z9-14Ac	1
Tyta luctuosa ([Denis & Schiffermüller], 1775)	1 ♂ (VIII-2016); Z7-12Ac+Z9-14Ac	2
Xestia xanthographa ([Denis & Schiffermüller], 1775)	4 ♂ (IX-2015); Z5-12Ac (2), Z7-12Ac (1) and R-7-12 (1)	4
Nolidae		
Nola cicatricalis (Treitschke, 1835)	1 ♂ (IX-2015); R-7-14	1
Pieridae		
Gonepteryx rhamni (Linnaeus, 1758)	6 ♂ (IV-2016); Z7-12Ac (5) and Z9-14Ac (1)	6
Plutelliidae		
Plutella xylostella (Linnaeus, 1758)	1 ♀ (VII-2015); R-7-12 2 ♀ (VII-2016); control	3
Pyralidae		
Oncocera semirubella (Scopoli, 1763)	1 ♂ (VIII-2015); Z5-12Ac	1

Tortricidae		
Cochylis hybridella (Hübner, [1813])	1 ♂ (IX-2016); Z7-12Ac	1
Epinotia abbreviana (Fabricius, 1794)	3 ♂ (V-2016); Z7-12Ac	3
Hedya pruniana (Hübner, [1799])	2 ♀ (VI-2016); control	2
Neosphaleroptera nubilana (Hübner, [1799])	2 ♂, 2 ♀ (V-2016); Z7-12Ac+Z9-14Ac	4
Yponomeutidae		
Paraswammerdamia albicapitella (Scharfenberg, 1805)	1 ♀ (IX-2015); Z7-12Ac+Z9-14Ac	1
Zygaenidae		
Rhagades pruni ([Denis & Schiffermüller], 1775)	10 ♂ (VI-2015); R-7-12 (9) and S-7-12 (1) 2 ♂ (VI-2016); R-7-12 (1); EFETOV-2 (1)	12
Theresimima ampellophaga (Bayle-Barelle, 1809)	68 ♂ (VI-VII-2017); R-7-14+R-7-12	68
Zygaena carniolica (Scopoli, 1763)	1 ♂ (VII-2015); Z7-12Ac+Z9-14Ac	1
Zygaena filipendulae (Linnaeus, 1758)	2 ♂ (VII-2015); Z7-12Ac+Z9-14Ac 47 ♂ (VI-IX-2017); Z_vic**	49

*species for which new attractants were found for the first time
**new attractant for the species with known attractants

Table 3. Catches of lepidopteran species in baited and control (unbaited) traps, Lozenska planina Mts.

Species	Number of moths caught (period of collection: months and year); Lure	Sum
Crambidae		
Evergestis aenealis ([Denis & Schiffermüller], 1775)	37 ♂ (VI-VII-2023); Z7-12Ac + Z9-14Ac	37
Erebidae		
Euclidia glyphica (Linnaeus, 1758)	1 ♀, 1 ♂ (VI-VII-2023); Z7-12Ac (1 ♀) and R-7-12 (1 ♂)	2
Geometridae		
Chiasmia clathrata (Linnaeus, 1758)	2 ♂ (VI-2023); S-7-12	2
Gandaritis pyraliata ([Denis & Schiffermüller], 1775)	1 ♂ (VII-2023); R-7-12	1
Heliomata glarearia ([Denis & Schiffermüller], 1775)	1 ♂ (VII-2023); S-7-12	1
Horisme corticata (Treitschke, 1835)	2 ♂ (VI-VII-2023); control (1) and Z7-12Ac + Z9-14Ac + Z8-12Ac (1)	2
Noctuidae		
Autographa gamma (Linnaeus, 1758)	4 ♂ (VI-2023); Z7-12Ac	4

Cucullia umbratica (Linnaeus, 1758)	1 ♂ (VI-2023); Z7-12Ac + Z9-14Ac	1
**Hoplodrina respersa* ([Denis & Schiffermüller], 1775)	13 ♂ (VII-2023); Z7-12Ac + Z9-14Ac + Z8-12Ac (9); Z9-14Ac (2) and Z7-12Ac + Z9-14Ac (2)	13
Sideridis reticulata (Goeze, 1781)	1 ♂ (VII-2023); Z7-12Ac + Z9-14Ac + Z8-12Ac	1
Nymphalidae		
Coenonympha glycerion (Borkhausen, 1788)	1 ♀ (VI-2023); Z7-12Ac	1
Coenonympha leander (Esper, [1784])	1 ♂ (VI-2023); S-7-12	1
Brintesia circe (Fabricius, 1775)	1 ♂ (VII-2023); R-7-12 + S-7-12	1
Oecophoridae		
Borkhausenia unitella (Hübner, [1801])	1 ♀ (VII-2023); Z7-12Ac + Z9-14A + Z8-12Ac	1
Tortricidae		1
Pammene gallicolana (Lienig & Zeller, 1846)	745 ♂ (VI-2023); Z7-12Ac + Z9-14A + Z8-12Ac** (743) and Z9-14Ac (2)	745
Endothenia gentianaeana (Hübner, [1799])	1 ♂ (VI-2023); Z9-14Ac	1
Grapholita tenebrosana (Duponchel, 1842)	2 ♂ (VI-2023); Z7-12Ac + Z9-14A + Z8-12Ac	2
Pammene albuginana (Guenée, 1845)	4 ♂ (VI-2023); Z7-12Ac + Z9-14A + Z8-12Ac	4
Zygaenidae		
Adscita geryon (Hübner, [1813])	2 ♂ (VII-2023); S-7-12	2
Adscita mannii (Lederer, 1853)	71 ♂ (VI-VII-2023); S-7-12 (48) and R-7-12 + S-7-12 (23)	71
Jordanita notata (Zeller, 1847)	26 ♂ (VI-VII-2023); R-7-12 (20) and R-7-12 + S-7-12 (6)	26
Rhagades pruni ([Denis & Schiffermüller], 1775)	1 ♂ (VII-2023); R-7-12	1

*species for which new attractants were found for the first time
**new attractant for the species with known attractants

Table 4. Results of screening of Z5-12Ac, Z7-12Ac, Z9-14Ac and a mixture of Z7-12Ac and Z9-14Ac (100:100), Lozitsa vill., 12- VI – 7-X-2015, Delta sticky traps, two replications; 25-IV – 25-IX-2016, VARL funnel traps, two replications and Lozenska Planina Mts, 2-VI – 15-VII-2023, VARL funnel traps, two replications. Means captures marked with different letter within a column are significantly different (Kruskal-Wallis test followed by Mann-Whitney U test, $P < 0.05$).

Compound Dose, μg per lure			**Mean number of male moths captured (± SE) in the respective year**											
Z5-12Ac	Z7-12Ac	Z9-14Ac	*A. aeratella*		*E. aenealis*			*B. bachyptilella*	*A. segetum*		*A. gamma*		*A. putris*	*M. confusa*
			2015	2016	2015	2016	2023	2015	2015	2016	2015	2016	2016	2016
0	0	0	0.00b	0.00b	0.00b	0.00b	0.00b	0.25 ± 0.24	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b
100	0	0	0.00b	-	0.00b	-	-	0.00	0.58 ± 0.31b	-	0.08 ± 0.08b	-	-	-
0	100	0	40.20 ± 13.37a	3.86 ± 0.98a	0.00b	0.08 ± 0.08b	0.00b	0.00	1.92 ± 1.16ab	0.06 ± 0.06b	1.33 ± 0.51a	8.05 ± 1.91a	0.00b	4.13 ± 2.59a
0	0	100	-	0.05 ± 0.05b	-	0.00b	0.00b	-	-	0.00b	-	0.00b	3.58 ± 2.08a	0.00b
0	100	100	13.70 ± 4.91a	3.18 ± 1.16a	5.29 ± 1.46a	2.75 ± 0.91a	3.70 ± 1.36a	7.25 ± 3.77	2.75 ± 1.35a	0.78 ± 0.24a	1.42 ± 1.42a	0.05 ± 0.05b	0.00b	1.13 ± 0.88ab

- Lure not tested