

## THE INVESTIGATION OF RARE AND PROTECTED BEETLES ASSOCIATED WITH OLD DECIDUOUS TREES, USING BAIT-WINDOW TRAPS IN LITHUANIA

SIGITAS ALGIS DAVENIS<sup>1</sup>, JOLANTA RIMŠAITĖ<sup>2</sup>, POVILAS IVINSKIS<sup>2</sup>, JURGA BŪDIENĖ<sup>3</sup>

<sup>1,2,3</sup>Nature Research Centre, Akademijos g. 2, LT-08412 Vilnius, Lithuania.

E-mails: <sup>1</sup>algis.davenis@gamtc.lt, <sup>2</sup>entlab@gmail.com, <sup>3</sup>jurga.budiene@gamtc.lt

### Introduction

Many species of beetles are trophically dependent on veteran trees (Rašomavičius, 2021). Over the recent decades, a large number of species have been facing increased risk of extinction due to intensive exploitation of natural resources, industrial growth, urbanization and increasing pollution of the environment. Rapid destruction of suitable habitats has expelled various insect species from densely populated regions of Europe (Cizek *et al.*, 2021).

These beetles' species appears to be rare over much of its Lithuania range and, although the overall population trend remains unknown, it is known that it is declining in some parts while increasing in others. There is recent evidence that it is not as rare as previously thought, in some areas at least, once appropriate trapping techniques have been applied. However, veteran trees are known to be becoming increasingly scarce across Lithuania and so a downward trend in abundance and number of localities may be expected to be occurring. In Lithuania, the cutting of mature oak woods is still running, while some old trees were lost due to the natural aging of trees as well as poor management of old trees. Until now, the investigation of veteran trees' beetle fauna in Lithuania was insufficient for a long time.

The Hermit beetle *Osmoderma barnabita*, Motschulsky, 1845 (Coleoptera: Cetoniidae) is among the endangered species in the whole of Europe (Audisio *et al.*, 2007, 2009; Sebek *et al.*, 2012). This species is linked with declining habitats – old hollow trees. Hermit beetle is the umbrella species for many invertebrates of primeval broadleaved forests with old hollow trees. It prefers both, dead-standing and living trees, and was also found on fallen trees (Oleksa *et al.*, 2003). Nevertheless, the fallen trees make up only separate fragments in the distribution range of this species.

The Golden chafer *Protaetia (Cetonischema) aeruginosa* Linnaeus, 1767 (Coleoptera: Cetoniidae) belongs to the group of obligate saproxylic invertebrates, i.e. it depends on decaying wood (Speight, 1989). There is no data on the size of the population of the Golden chafer in Lithuania. Only a few cases have been described (Pileckis, 1995; Davenis *et al.*, 2018, 2021), although this species is listed in the European Red List of Saproxylic Beetles (Nieto & Alexander, 2010).

The purpose of the research is to study the fauna of veteran tree beetles using bait-windows traps, to investigate the prevalence of saproxylic beetles (their possible populations), to determine and evaluate the composition of different attractant's components.

## Material and Methods

The investigation of beetles associated with old deciduous trees was carried out for more than the last ten years in different parts of Lithuania. The majority of data was collected during field investigations by the author Sigitas Algis Davenis (S.A.D.), and some data – by Povilas Ivinskis (P.I.). Non-destructive sampling method using the bait-window trap with sponge filling with a blend of cherry (*Prunus cerasus*) and cherry plum (*Prunus cerasifera*) wines (1:1) or eugenol was used (Fig. 1). Traps were located at 10–24 m height (eugenol in 4 m height). In every locality 5 traps were used. All the traps were checked after two days, the trapped alive beetles were released. Some beetles have been observed in their habitats.



Figure 1. Bait-window trap (P. Ivinskis construction)

After collecting the volatile organic compounds (VOCs) released from the wines, each individually and in a mixture, using a solid-phase micro extraction method (SPME), they were analyzed by gas chromatography-mass spectroscopy (GC-MS).

The collection of VOCs was carried out for 15 min by exposing the purified SPME fiber coated with a polydimethylsiloxane-divinylbenzene absorbent (PDMS/DVB, 65  $\mu\text{m}$  coating layer thickness, Supelco, Ballefonte, PA, USA) in the headspace of 100 ml Erlenmeyer flasks with 50 ml of wine at the room temperature. As soon as the collection of the volatiles was completed, the VOCs from the fiber were desorbed for 2 min in the injection liner of a gas chromatograph (GC). VOCs samplings for wines were done in triplicate.

For analysis of the headspace volatile samples, a Shimadzu GC-2010 gas chromatograph (GC) coupled with Shimadzu MS-QP 2010 Plus mass selective detector (MS) (Shimadzu, Kyoto, Japan) was employed. Separation of compounds was performed on a non-polar Rxi-5Sil MS w/Integra-Guard capillary column (30 m  $\times$  0.32 mm  $\times$  0.25  $\mu\text{m}$ ; Restek, USA) under the following conditions: initial temperature of 40°C was

maintained for 2 min, then raised up to 240°C with rate of 10°C/min and maintained isothermally for 2 min. Helium was used as a carrier gas at the flow rate of 1.5 mL/min. Electron ionization spectra were acquired at an electron energy of 70 eV; the injector, interface, and ion source temperatures were held isothermal at 250°C. Compounds were identified by comparison of their mass spectral data and retention indexes with those present in NIST version 2.0 mass spectra library (National Institute of Standards and Technology, Gaithersburg, Maryland, USA).

Taxonomical treatment and distribution follow the Fauna Europaea database (Krell, 2013) and Audisio *et al.* (2007, 2009).

#### List of localities

Locality	Administrative district	Coordinates (LAT, LONG)
Dirkliškių Miškas f.	Trakai distr.	54.67313, 25.06527
Gojaus Miškas f.	Prienai distr.	54.56157, 24.28477
Kalniškės	Vilnius distr.	54.84681, 25.17739
Punios Šilas f.	Alytus distr.	54.51355, 24.08464
Vidzgirio Miškas f.	Alytus mun.	54.37823, 24.00753

#### List of species

##### CERAMBYCIDAE

##### *Necydalis major* (Linnaeus, 1758)

Kalniškės, 29 07 2022, 1 spec. found in bait-window (blend wine) trap at height (further in the text – h) 10.0 m, on a *Quercus robur*.; Punios Šilas f., 31 07 2022, 1 spec, 01 08 2022, 1 spec. found in bait-window (blend wine) h 14.0 m, on a *Quercus robur*.

##### CETONIIDAE

##### *Osmoderma barnabita* (Motschulsky, 1845)

Dirkliškių Miškas f., 11 08 2022, 1 imago (♂), found in bait-window trap (eugenol) h 4.0 m on a *Quercus robur* (S.A.D); Gojaus Miškas f., 05 07 2022 – 04 08 2022; 4 imago (♀), found in bait-window trap (eugenol) h 4.0 m, on a *Quercus robur*; 10 imago (6 ♀, 4 ♂) found on a trunk of *Q. robur*, h 0.5–6.0 m; Punios Šilas f., 05 07 2022 – 04 08 2022; 7 imago (6 ♀, 1 ♂) found in bait-window trap (eugenol) h 4.0 m, on a *Quercus robur*; 5 imago (♀) found on a trunk of *Q. robur*, h 0.5–4.0 m.

##### *Protaetia (Cetonischema) aeruginosa* (Linnaeus, 1767) (Fig. 2)

Gojaus Miškas f., 23 06 2022 – 04 08 2022, 30 imago, 22 ♀ (73%), 8 ♂ (27%), (most detected – 27/30 06 2022, 10 spec.), found in bait-window trap (blend wine) h 10.0–24.0 m, on a *Quercus robur*, body weight  $1.595 \pm 0.233$  g (min. 1.138 g, max. 2.076 g), body length  $2.6 \pm 0.2$  cm (min. 2.3 cm, max. 3.1 cm), female's body weight  $1.606 \pm 0.243$  g (min 1.138 g, max 2.076 g), body length  $2.6 \pm 0.3$  cm (min. 2.3 cm, max. 3.1 cm), male's body weight  $1.567 \pm 0.197$  g (min. 1.152 g, max. 1.727 g), body length  $2.7 \pm 0.1$  cm (min. 2.4 cm, max. 2.8 cm); Punios Šilas f., 17 06 2022 – 04 08 2022, 20 imago, 14 ♀ (70 %), 6 ♂ (30%) (most detected – 27/30 06 2022, 14 spec.), found in bait-window trap (blend wine) h 10.0–24.0 m, on a *Quercus robur*, body weight  $1.590 \pm 0.213$  g (min. 1.158 g, max. 1.952 g), body length  $2.7 \pm 0.1$  cm (min.

2.5 cm, max. 2.8 cm), female's body weight  $1.646 \pm 0.168$  g (min. 1.424 g, max. 1.952 g), body length  $2.7 \pm 0.1$  cm (min. 2.5 cm, max. 2.8 cm), male's body weight  $1.368 \pm 0.297$  g (min. 1.158 g, max. 1.757 g), body length  $2.7 \pm 0.1$  cm (min. 2.6 cm, max. 2.7 cm) (all S.A.D.).

***Protaetia (Liocola) lugubris* (Herbst, 1786)**

Kalniškės, 12 06 2022, 1 imago, 19 07 2022, 1 imago, 16 08 2022, 2 imago, 17–27 08 2022, 2 imago (all P.I.); Dirkliškių Miškas f., 11 08 2022 – 19 08 2022, 12 imago (2 ♀, 10 ♂) found in bait-window trap (blend wine) h 10.0–24.0 m, on a *Quercus robur*, 9 larvae, hollow in the trunks of *Q. robur*, h 0.5–2.0 m; Gojaus Miškas f., 23 06 2022 – 04 08 2022, 127 imago, found in bait-window trap (wine mix) h 10.0–24.0 m, on a *Quercus robur*, (most detected – 30 06 2022, 48 spec.); Punios Šilas f., 17 06 2022 – 04 08 2022, 322 imago (sex not reported), found in bait-window trap (blend wine) h 10.0–24.0 m, on a *Quercus robur*, (most detected – 27 06 2022, 162 spec.); Vidzgirio Miškas f., 17 06 2022 – 30 06 2022, 59 imago, found in bait-window trap (blend wine) h 10.0–24.0 m, on a *Quercus robur*.



Figure 2. *Protaetia (Cetonischema) aeruginosa*, found in Punios Šilas f. 17 06 2022 (photo J. Rimšaitė)

## Discussion

According to Pileckis and Monsevičius (1997), the long-horned beetle *Necydalis major* is polyphagous in deciduous trees, but prefers old sick *Betula* trees for development. According to Rejzek & Vlasák (2000), *Necydalis major* develops in a variety of dead deciduous trees preferring *Tilia*, *Quercus*, *Salix*, *Populus*, but mainly *Alnus*. In contrast to *Necydalis ulmi* this species develops in dead trunks and branches of larger diameter (Domian *et al.*, 2010). It never develops in hollows of living trees. Larvae of *Necydalis major* are known to develop in dead trunk of common alder (*Alnus glutinosa*) attacked by the polyporus *Inonotus radiatus* (Domian *et al.*, 2010). Larvae of *Necydalis major* seem to prefer a rusty-brown substrate just under the fruitbodies of *Inonotus radiatus* but were also found in more decayed parts of the trunk, in a white substrate. This species is rarely found in Lithuania, is included in Lithuanian Red Data book and is an indicator of forest key habitats (Ivinskis *et al.*, 2018).

The Hermit beetle (*Osmoderma barnabita*) is rare and protected species in Lithuania and other European countries (Alexander *et al.*, 2010). They are included in the Bern

Convention (Annex II), EU Habitat Directive (Annexes II and IV) and the Red Data Book of Lithuania (Rašomavičius, 2021; Ivinskis, 2006, 2015) it is a species of forest key habitats (Ivinskis *et al.*, 2018). The hermit beetle is relict of broadleaf forests, its microhabitat is holes of veteran trees (Antonsson, 2002, Balčiauskas *et al.*, 2016). The main habitats are parks or forest parks, roadside trees, or alone trees (Olekša *et al.*, 2003). Suitable trees in many cases found in human living environment, because old holly tree growing in sunny places are often found in cultural landscape (Ranius & Nilsson, 1997). In recent years, hermit beetle population studies in Europe (Svensson & Larsson, 2008; Svensson *et al.*, 2008; Zauli *et al.*, 2014), Latvia (Valainis *et al.*, 2015) and Lithuania (Davenis *et al.*, 2018) have been conducted by catching individuals with bait traps using the  $\gamma$  - Decalactone as a pheromone (Vuts *et al.*, 2014) - kairomone. Using volatile attract substance - eugenol in bait traps suspended at an average height of 4.0 m on tree trunks, we managed to attract 22 imago of the Hermit beetles. During the research time the Hermit beetle came to eugenol odour, but didn't come to wine odour.

In many European countries the Golden chafer *Protaetia (Cetonischema) aeruginosa* is protected species. For example, in Poland the chafer *P. aeruginosa* is a protected species (Plewa *et al.*, 2014). It is listed as a species vulnerable to extinction (VU) (Pawłowski *et al.* 2002). This species has the status of species at risk of extinction in the near future (NT) in the European Red List of Saproxyllic Beetles (Nieto & Alexander, 2010; Mason *et al.*, 2010).

The Golden chafer *Protaetia (Cetonischema) aeruginosa* is an extremely rare species in Lithuania. According to S.Pileckis and V. Monsevičius (1995), Lithuania is at the northern edge of the species distribution area. Distribution and population abundance of golden chafer is poorly studied in Lithuania. Pileckis and Monsevičius (1995) report about one specimen caught on flowers in Alytus. Wrong identification based on one elytra belonging to this beetle was reported by V.Inokaitis in 2004 (R. Ferenc pers. comm.). In 2018 a dead beetle was found in the Punios Šilas f. (Davenis *et al.*, 2018). In 2021 S.A. Davenis in Vidzgirio Miškas f. observed and made a short film about behaviour of *Protaetia (Cetonischema) aeruginosa* (Davenis *et al.*, 2021). The species develops in hollows of the old oak trees, it depends on decaying wood (Speight, 1989). Beetles fly very high in sunny habitats and for this reason they are only accidentally observed on the ground or flowers (Tauzin, 2005a, 2005b, 2008). Usage of bait-window traps has shown that this species is not rare in suitable habitats.

The Marbled rose chafer *Protaetia (Liocola) lugubris* is included in Lithuanian Red Data Book (Rašomavičius, 2021), but new investigation with trapping of volatile compounds in wines show, that this beetle is not rare in Lithuania. The Marbled Rose Chafer frequently and abundantly came to bait-windows (blend wine) traps in the studied forests.

Traps with natural unspirited blend (cherry-plum) wine were used to attract Cetoniidae beetles in nature. Since the main attraction is by volatile compounds, one of the objectives of this study was to collect and identify as many of the wine's aromatic components as possible and to compare them with already known attractants.

The results of the analysis showed that the most abundant volatile organic compounds emitted in the headspace of wines were the saturated fatty acid esters (Fig. 3): ethyl hexanoate (pleasant pineapple smell), ethyl octanoate (fruits and flower smell), ethyl decanoate (floral and fruity flavour) and ethyl dodecanoate (waxy type odour). Ethanol was also among the most abundant compounds, accounting for up to 50% of the

total identified components in the case of the wine blend. Other well-known beetle attractants that complemented the alcohol group were 3-methyl-1-butanol (artificial banana smell) up to 10%, 1-phenylethyl alcohol (rose like smell) up to 4% (Vuts *et. al.*, 2010a, 2010b, 2019; Toth, 2012). 1-hexanol (fruity odour), which has already been mentioned amongst the golden beetle attractants, accounted for a very small proportion in the wines, together with benzyl alcohol. It should be mentioned that 3-methyl-1-butanol is frequently found among the compounds emitted by decaying wood, as well as among the microscopic fungi that live in such wood. 3-methyl eugenol, which is mentioned as an attractant in the works published by Vuts and colleagues, was not found in the single wine and blend samples that we have analysed. One of the plant-specific components, eugenol, was found in all the samples analysed, but in very small quantities and can hardly be considered as one of the main attractants. Unsaturated fatty acids such as hexanoic (fatty, cheesy, waxy, animalistic smell), octanoic and nonanoic (both smelling slightly unpleasant rancid-like) were found in small amounts, up to 1% each. The results obtained for the volatile compounds in wines suggest that the beetles are not necessarily attracted by one of these compounds, but by several of them, or by a specific composition of them, probably synergistic blend.

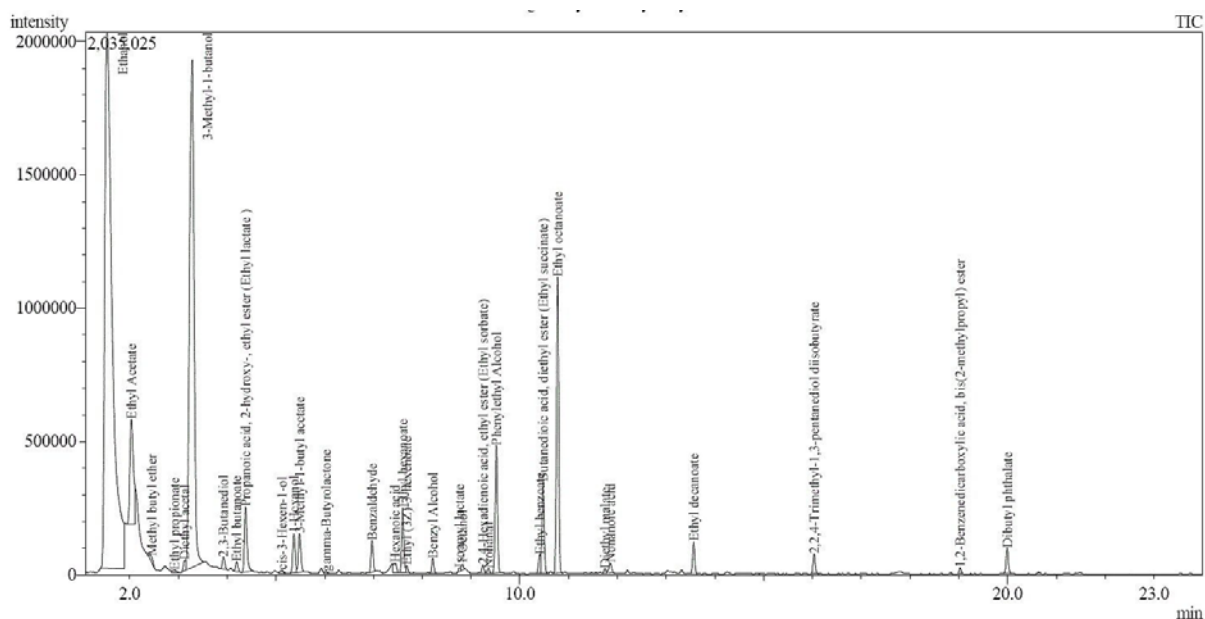


Figure 3. Representative chromatogram showing VOCs released from blended cherry-plum wine collected from the headspace using SPME

Further investigation of the volatile organic compounds produced during wine fermentation and released into the environment, which are likely to attract golden chafer into wine-filled traps, would lead to the development of one optimal combination of attractants.

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### **Retų ir saugomų vabalų, priklausančių nuo brandžių lapuočių medžių, tyrimas naudojant kvapų gaudykles**

S.A. DAVENIS, J. RIMŠAITĖ, P. IVINSKIS, J. BŪDIENĖ

#### **Santrauka**

Atliekant tyrimus ir naudojant kvapų-langines gaudykles, brandžių medžių augavietėse 2022 m. rastos saugomų vabalų rūšys, įrašytos į Lietuvos raudonąją knygą: didysis lapuotinėnkas *Necydalis major* (Cerambycidae), niūriaspalvis auksavabalis *Osmoderma barnabita*, marmurinis auksavabalis *Protaetia lugubris* (Cetoniidae) ir retas puošnūsis auksavabalis *Protaetia (Cetonischema) aeruginosa* (Cetoniidae). *Osmoderma barnabita* buvo aptiktas tiek naudojant kvapų gaudykles, tiek stebimas ant drevėtų ažuolų kamienų. *Protaetia (Cetonischema) aeruginosa* naudojant gaudykles nustatytas trijuose pietų Lietuvos miškuose; Gojaus ir Punios šilo miškuose nėra retas. Šių vabalų kūno dalys rastos senų medžių drevių trūnėsiuose. *Protaetia lugubris*, kaip rodo tyrimai, plačiai paplitęs Lietuvoje tinkamose buveinėse. Atlikti vyno fermentacijos metu susidariusių ir į aplinką sklindančių lakiųjų organinių junginių, kurie, tikėtina, vilioja auksavabalius į kempines užpildytas vynu gaudykles, tyrimai. Detalesni tyrimai leistų rasti vieną optimalų atraktyvių medžiagų derinį, kurį panaudojus būtų galima atlikti kai kurių retų vabalų populiacijos tyrimus šalyje ir kaimyninėse valstybėse.

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