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# Chemoecological peculiarities of *Rhagoletis batava* Hering (Diptera, Tephritidae)

**SUMMARY OF DOCTORAL DISSERTATION**

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VALSTYBINIS MOKSLINIŲ TYRIMŲ INSTITUTAS
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# *Rhagoletis batava* Hering (Diptera, Tephritidae) chemoekologijos bruožai

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Ekologija ir aplinkotyra N 012

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# INTRODUCTION

## Relevance of the research

The genus *Rhagoletis* belongs to the family Tephritidae, order Diptera. Tephritidae contain about 500 genera with about 4,000 species; of these, 65 species belong to the genus *Rhagoletis* (White, Elson-Harris, 1992). Most Tephritidae, including *Rhagoletis*, are fruit and vegetable pests common in holarctic and neotropic regions (Boller, Prokopy, 1976). Their larvae are mostly stenophagous, feeding on the soft parts of ripening fruits of several related plant species (*e.g.*, Mohamadzade Namin, Rasoulian, 2009). Larvae-damaged fruits lose their shape, colour, nutritional and economical value. Because of stenophagy *Rhagoletis* species began to develop not only on native host-plants of no economic importance, but also on the cultivated alien plants, especially those that are grown in large areas and densities and are related to native host-plants (Boller, Prokopy, 1976). For example, larvae of *Rhagoletis pomonella* Walsh is developing normally on hawthorn *Crataegus* sp. berries, but also have adapted to develop in apple *Malus pumila* Mill fruits (*e.g.*, Feder *et al.*, 1993). Cherry fly, *Rhagoletis cerasi* L., larvae can develop in at least six cherry species, *Prunus* sp., and honeysuckle, *Lonicera* sp., fruits of different economical value.

There are 4 species of the genus *Rhagoletis* found in Lithuania: *R. cerasi*; *Rhagoletis alternata* Fall., feeding on thorns (*Rosa* sp.); *Rhagoletis meigenii* Loew, feeding on barberry (*Berberis vulgaris* L.) and *Rhagoletis batava* Hering, feeding on sea buckthorn (*Hippophae rhamnoides* L.) (Pakalniškis *et al*., 2000; Stalažs, 2014a; Lutovinovas, 2015). The latter plant is particularly important for its tolerance of poor soil and high demand of the fruits (berries)in food, medical and cosmetic industries (Li, Schroeder, 1996; Small *et al*., 2002; Ruan *et al*., 2007; Bal *et al*., 2011; Kaur *et al*., 2017).

Although *R. batava* is not on the list of *Rhagoletis* species causing the highest economic losses globally, it is a potential candidate for it.

*Rhagoletis batava* species was described in Europe (Hering, 1958), but it is believed a new subspecies of *R. batava obscuriosa* Kol. occured in Siberia (Kolomiec, 1970; Stalažs, Balaikins, 2017). This subspecies causes huge damages of buckthorn berries and can destroy from 87% up to 100% of total berry yield (Shalkevich *et al*., 2015; Shamanskaya, 2015). It should be noted that even with minor damages berries contaminate the rest of the crop. In the last decade, *R. batava* (most probably *R. b. obscuriosa*) has reached the Baltic countries (Stalažs, 2014a; b) and has continued to spread to Western and Southern Europe (Stalažs, Balaikins, 2017).

In order to improve the capabilities of *R. batava* monitoring and control, it is necessary to learn more about its biology and ecology, including chemical interactions by means of volatile organic compounds. Most promising compounds are those which can modify insect behaviour and acting interspecificaly and intraspecificaly. Such compounds include pheromones responsible for the success of finding a mating partner, and allelochemicals involved in food source searching or searching for fruit for suitable offspring development. Application of volatile organic compounds (VOCs) effecting behaviour could significantly contribute to successful and environmentally friendly pest control.

## The aim of the research

The aim of this study was to reveal sea buckthorn fruit fly, *Rhagoletis batava* Hering, interspecific and intraspecific interactions by volatile organic compounds.

## Tasks of the present research

1. To establish the influence of trap colour, type and host-plant's (sea buckthorn, *Hippophae rhamnoides* L.) gender on the catches of *R. batava* in the plantation.
2. To determine dynamics of seasonal flight of *R. batava* adults, which allow to predict the start of occurrence of the pest adults in plantations.
3. To determine volatile organic compounds of sea buckthorn berries, which cause olfactory responses of *R. batava* adults.
4. To determine *R. batava* olfactorily active volatile organic compounds released into the environment by yeasts related to the sea buckthorn berries: *Hanseniaspora uvarum*, *Metschnikowia pulcherrima* and *Pichia kudriavzevii*, and to evaluate the effect of synthetic analogues of the volatiles to the fruit fly adult behaviour.
5. To determine daily rhythm of *R. batava* adults mating.
6. To reveal pheromone – *R. batava* adult-produced volatile organic compound(s) which is (are) important in intraspecific interactions.

## Scientific novelty of the study

- ✓ Seasonal dynamics of *R. batava* adults flying in Lithuania was established.
- ✓ The most attractive colour as visual stimulus for *R. batava* was revealed.
- ✓ The prediction of the start of *R. batava* adults seasonal flight following the sum of active average daily temperatures of Lithuanian climatic conditions was estimated.

✓ New insect species, *Phygadeuon wiesmanni* Sachtleben (Hymenoptera), was recorded for Lithuanian fauna.

✓ For the first time there were established 20 volatile organic compounds in the sea buckthorn berries that are olfactorily active for the sea buckthorn fly, *R. batava*.

✓ For the first time it was shown that another group of organisms, the yeasts on sea buckthorn berries, interact between *R. batava* flies and their host-plant.

✓ For the first time it was found the emissions of yeasts on the sea buckthorn berries to contain 10 volatile organic compounds, which are perceived by *R. batava* adults and evoke behavioural sesponses.

✓ For the first time sexual dimorphism of *R. batava* adult behavioural responses to olfactory stimuli was detected.

✓ For the first time aggregation pheromone of *R. batava* species and for the whole *Rhagoletis* genus was identified, it will be used in *R. batava* pheromone traps.

## Scientific and practical significance

The research results provide new data on interactions of insects from *Rhagoletis* genus with host-plant fruits, and yeasts associated with the fruits, as well as on *R. batava* intraspecific interactions by volatile organic compounds. The investigation revealed some new ecological features of the pest and perspective natural compounds suitable for applications:

✓ in electrophysiological investigation of olfactory receptors of insects;

✓ for search of infochemicals of the genus *Rhagoletis*;

✓ for research on multitrophic interactions by means of volatile organic compounds;

✓ for establishment of seasonal start of the *R. batava* adult occurrence in a plantation;

✓ for development and optimization of tools for *Rhagoletis* pest monitoring and environmentally friendly control.

## Defended statements

- ✓ In sea buckthorn plantation, yellow traps hanged in feminine plants are the most effective for *R. batava* catching.
- ✓ *Rhagoletis batava* can smell at least 20 volatile organic compounds of sea buckthorn berries.
- ✓ *Rhagoletis batava* can smell at least 10 volatile organic compounds released by yeasts associated with sea buckthorn berries.
- ✓ *Rhagoletis batava* aggregation pheromone is (-)-δ-heptalactone.

## Approbation of results

Dissertation results were presented at: "Pheromones and Other Semio-Chemicals in Integrated Production" (Jerusalem, Israel, 2015), "Young scientists for advanced agriculture" (Vilnius, Lithuania, 2017), the 33$^{th}$, 34$^{th}$ and 35$^{th}$ ISCE Annual Meetings (Kyoto, Japan, 2017; Hungary, Budapest, 2018; Atlanta, USA, 2019). Results of the research were published in 8 publications: 2 scientific papers and 6 conference abstracts.

## Structure of dissertation

This dissertation contains the following chapters: Introduction, Literature Review, Materials and Methods, Results and discussion, Conclusions, References (176 sources), List of Publications where the dissertation material was published. The dissertation covers 87 pages; it contains 3 tables and 33 figures. The text of the dissertation is presented in Lithuanian with the abstract in English.

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## LITERATURE REVIEW

This chapter reviews the life cycle, and chemical ecology of fruit flies from genus *Rhagoletis*. It also provides overview of sea buckthorn as well as basic biological and ecological features of fruit associated yeasts.

## MATERIALS AND METHODS

**Experimental site.** Field tests were carried out in an organic sea buckthorn plantation located in the vicinity of Stacijava village (N lat. 55.253383, E long. 25.439736), Molėtai district, Lithuania.

**Colour traps.** Flat sticky traps of rectangular shape (10 cm wide and 25 cm long) were made of clear double PVC film (Fellowes, China), 300 μm thickness. Colour paper was inserted between the two film layers to provide colour for a trap. White, blue, black, green, red, yellow and transparent (no colour) traps were made. For white trap paper Universal (Navigator, Portugal) 80 g $m^{-2}$ was used, for blue, black, green, red and yellow paper Image Coloration (Antalis, Latvia) 80 g $m^{-2}$ was used. The manufacturer indicated these colours as clear blue, black, meadow green, scarlet and lemon yellow. Both surfaces of the traps were covered by sticky glue (PestiFix, Estonia) developed to fix insects in sticky traps.

The traps were hung in blocks. Six blocks, each containing a full set of all colour traps, were used in total. The distance between the traps was approximately from 1.5 to 2 m, the distance between the blocks was not less than 30 m. Three blocks were hung on masculine plants only (no berries) and three blocks on feminine plants (berries were present). Trapping was carried out in July 2016. Catches were counted and traps were rotated regularly with 3 to 4 days' intervals.

**Trap type.** Two types of traps were used, both containing yellow surface: flat sticky and McPhail (Pherobank, the Netherlands) traps of cone shape with transparent upper and yellow bottom parts. The latter traps are recommended for trapping fruit flies (Pherobank catalogue,

https://www.pherobank.com/catalog-item/fruitfly-and-wasp-trap-mcphail-inclcage-33202.html). To fix the insects trapped, 1% NaCl water solution was added into the bottom vial.

The traps of both types were hung on masculine plants only. The traps were arranged in three groups, each containing a couple of both type traps. The distance between the traps was not less than 2 m, between the groups not less than 20 m. The traps were inspected and rotated regularly with 3 to 4 days' intervals. Trapped fruit flies were counted and identified as *Rhagoletis batava* Hering, 1938 (Korneyev *et al*., 2017). Sticky traps were replaced when the surface was contaminated; water solution was either added or replaced in McPhail traps. Trapping was carried out in July and August 2016. Catches were counted 11 times with 3 to 4 days' intervals.

**Flight season.** To establish flight season, McPhail traps were used. Data were collected from June to August 2016, 2017, 2018 and 2019. Three traps were hung on masculine plants in 2016, 2017 and five on feminine plants in 2018, 2019. The distance between the traps was at least 20 m. The catches were recorded with 3 to 4 days' intervals.

**Mating period.** To establish mating period, a single 50 m long transect was chosen in buckthorn plantation. All mating *R. batava* couples we recorded while walking through the distance in 15 min. The couples were recorded both on shrubs and grasses. Observations and records were started at 9 AM and lasted until 8 PM with 1-hour intervals during 7 days in June and July 2015, with 3 to 4 days' intervals. There was no rainfall during this period and average hourly temperature fluctuated from 18°C to 24°C during the observations (data from Lithuanian Meteorological Service, Molėtai station, located at N lat. 55.235230, E long. 25.416350).

**Insects.** Sea buckthorn flies used in laboratory studies, were collected in spring months as puparia in soil under sea buckthorn shrubs with damaged berries located in organic sea buckthorn plantation (experimental site). Each puparium was separately placed in 14 mL glass vial containing wet 3 $cm^2$ filter paper inside and corked

by foam stoppers. Vials were placed in a climate chamber “Fitotron” (Weiss Gallenkamp, UK) under 20-24 °C, 16L:8D (light:dark) photoperiod and 65-75% relative humidity. Two times a week 2-3 drops of water were added on a filter paper to maintain high humidity inside a vial. Emerged adults were kept in the same vials in walk-in climate room under 18-20 °C, natural day light photoperiod, 50-60% relative humidity and fed on 10% sugar solution in water. The sex of flies was identified based on the presence or absence of ovipositor.

Hymenopteran parasitoids emerged from some *R. batava* puparia. Morphologically they were studied by Martin Schwarz (Austria). Morphological identification was confirmed by application of molecular methods (Eduardas Budrys, Nature Research Centre).

**Extract preparation.** Berries extracts were prepared by hydrodistillation: 30 g of berries were homogenized and soaked in 250 mL of water for 1 h, and boiled in a Clevenger type apparatus for 2 h. Volatiles were collected into 2 mL of a mixture of hexane and diethyl ether (2:1). The extracts were dried over $MgSO_4$, filtrated and stored in a freezer at −18 °C until gas chromatography–electroantennogram detection (GC-EAD) and gas chromatography–mass spectrometry (GC-MS) analyses.

**Sampling of yeast-produced and *R. batava*-produced volatiles**. The solid phase micro-extraction (SPME) technique (Ebert *et al*., 2017) was used to sample the headspace of sea buckthorn related yeasts species: *Hanseniaspora uvarum* (strain SB-18-31; 99,85% compare to culture NT-52 in GenBank), *Metschnikowia pulcherrima* (strain SB-18-34; 100% compare to culture CBS:9701 in GenBank) and *Pichia kudriavzevii* (strain SB-16-15; 100% compare to culture B-WHX-12-19 in GenBank) yeast. The yeasts were obtained from Laboratory of Genetics, Nature Research Centre. The yeasts were cultivated in polystyrene Petri dishes (Ø 55 mm x 14 mm) poured with 14 mL of YPD-agar for 2 days at 25 °C. As control, YPD-agar plates were used for sampling background odours. Before each collection period, the routine purification of SPME fibres coated with

polydimethylsiloxane-divinylbenzene polymer (DVB/PDMS, 65 μm coating layer thickness, (Supelco, Pennsylvania, USA), was conducted at 240 ºC for about 10 min in a GC injector. Afterwards, the needle of SPME syringe was pierced through a small hole made in a wall of a Petri dish just above the yeast culture; the fibre was pushed out from the needle and exposed to the headspace for 60 min at room temperature. After sampling was finished, the fibre was transferred to the injection port of gas chromatograph and volatiles were thermally desorbed from the fibre during 2 min.

The same technique was used to sample the headspace of the *R. batava* adults. For SPME, from 8 to 20 adult fruit flies (either males or females) were placed in 50-mL glass vial covered in aluminium foil and exposed to the fiber for 120 min.

**Gas chromatography–electroantennogram detection and identification of volatiles.** GC-EAD (gas chromatograph Clarus 500, PerkinElmer, Waltham, MA, USA) was used to identify EAD-active compound(s) in the headspace of *R. batava* flies as well as to test EAD-activity of synthetic chemical(s) (Blažytė-Čereškienė *et al*., 2016).

The GC effluent was divided by a splitter in two equal parts allowing a simultaneous flame ionization and EAD detection of the separated volatile compounds. One part of the column effluent was directed to the flame ionization detector (FID). A nitrogen make-up gas at the flow rate of 5 mL $min^{-1}$ was used to enhance FID performance. Another part of the effluent was introduced into a purified and humidified air stream flowing at 0.5 m/s through a glass tube over antenna preparation. The flies used in GC-EAD analyses were not chilled or anesthetized prior to use. A glass capillary electrodes were filled with 0.9 % NaCl saline (Ilsanta, Lithuania) and grounded via a silver wire. Indifferent electrode was inserted in the severed head of the fly. Recording electrode, connected to a high-impedance DC amplifier (IDAC-4, Synthech, the Netherlands) with automatic baseline drift compensation, was brought into contact with

the distal end of the fly antenna. The antennal and the FID signals were recorded simultaneously, stored and analysed using software GcEad V. 4.4 (Synthech, the Netherlands). Each EAD test was replicated five times, each insect preparation was used once only.

For berries and yeasts VOC analysis GC was equipped with a DB-Wax capillary column (30 m x 0.25 mm x 0.25 μm; Agilent Technologies, Santa Clara, CA, USA). The GC injector and the detector temperatures were set at 240 °C. The oven temperature was maintained isothermally at 40 °C for 1 min, afterwards it was raised to 200 °C at a rate of 5 °C $min^{-1}$, then increased to 240 °C at a rate of 10 °C $min^{-1}$, and then maintained isothermally for 11 min. Hydrogen at the flow rate 1.5 mL $min^{-1}$ was used as a carrier gas.

The same DB-Wax capillary column was used for VOC analysis of *R. batava*. The injector and the detector temperatures were set at 240°C. The oven temperature was maintained isothermally at 40 °C for 1 min, afterwards it was raised to 240°C at a rate of 10°C$min^{-1}$, and then maintained isothermally for 13 min. Hydrogen at the flow rate 1.5 mL $min^{-1}$ was used as a carrier gas.

After identification of EAD-active chemical and revealing potential presence of enantiomers, column RtR-bDEXsm (30 m × 0.25 mm × 0.25 μm, Restek Corporation, Bellefonte, PA, USA) was used instead of previous one. In this case, the injector and the detector temperatures were set at 230°C. The oven initial temperature was 90 °C, then programmed to 200 °C at a rate of 3 °C/min.

**EAG dose-response.** Using the same electrophysiological recording setup and the same antennal preparation technique, electroantennogram (EAG) dose–responses of *R. batava* males and females to the synthetic δ-heptalactone were recorded.

The compound was tested at doses of $10^{-5}$, $10^{-4}$, $10^{-3}$, and $10^{-2}$ μg following application of 10 μL solution on a piece of filtered paper (5 × 45 mm) (Whatman®1, England) and placed to a Pasteur pipette. Hexane solutions were prepared by 10-step dilution of previous one. The four doses were tested in the ascending order. A solvent blank (10

μL of hexane after evaporation) was tested as a control stimulus both at the beginning and the end of stimulation. The peak voltage amplitude was recorded during the puff delivery of each stimulus as an antennal response. Each stimulation was followed by a minimum of a 1 min purge period of filtered air to ensure recovery of antennal receptors. The EAG response (R) to EAD-active compound dose was calculated according to the formula:

$R=RA-(RC_1+RC_2)/2$, where RA was EAG response to the EAD-active compound, and $RC_1$ and $RC_2$ were EAG responses to the first and the second control stimuli.

**Gas chromatography-mass spectrometry analysis.** Gas chromatograph Shimadzu GC-2010 coupled with Shimadzu MS-QP 2010 Plus mass selective detector (Shimadzu, Japan) was used. EAD-active compound present in the *R. batava* flies headspace was analysed by the GC equipped with Stabil-wax column (30m × 0.25 mm × 0.25 μm, Restek Corporation, Bellefonte, PA, USA). For analysis of synthetic enantiomers as well as those present in the headspace RtR-bDEXsm column (30 m × 0.25 mm × 0.25 μm, Restek Corporation, Bellefonte, PA, USA) was used. The GC operated under the same conditions as described above (subchapter *Gas chromatography–electroantennogram detection*), except that helium was used as the carrier gas at the flow rate 1.5 mL/min. Electron ionization spectra were acquired at an electron energy of 70 eV, and the interface and ion source temperatures were hold isothermal at 250 ºC. The headspace EAD-active compound was identified by comparison of its mass spectral data with that available from NIST (version 2.0, National Institute of Standards and Technology, USA) and retention index with that of the synthetic standard compound.

Synthetic standards were obtained from Sigma-Aldrich (USA), Alfa Aesar (USA) and Carl Roth (Germany). Enantiomers of δ-heptalactone were synthesized by Stefan Schulz (Germany). Purity of all synthetic compounds were not less than 92%.

**Olfactometer assay.** A Y-tube olfactometer (14.5 cm main tube, 10 cm arms, 130° branching angle, 0.9 cm inner diameter) was used to test preference of flies to synthetic samples versus control (Hare, 2000). Four 18 W tube type lamps (T8/840, Colourlux plus, NARVA, Germany) covered with white, mat, plastic shield (65 cm length, 42 cm width) at a distance of 23 cm were placed in front of the Y tube of the olfactometer. Positive phototaxis is characteristic for sea buckthorn flies and the light stimulated the insects to move towards the light source. The Y tube was held at a 10º angle upward from horizontal on a holder. The arms of the olfactometer were connected to separate glass tubes that contained the stimulus versus control. Clean air was pushed at a rate of 0.5 L $min^{-1}$ through each arm using a clean air delivery system CADS-4CPP (Sigma Scientific LLC, Micanopy, FL, USA).

Bioassays were conducted to test attraction of the artificial yeast blend and (-)-δ-heptalactone against a control. The blend was comprised of ethyl acetate, ethyl propionate, 3-methylbutyl acetate, 3-methylbutyl propionate, 3-methylbutanol, ethyl hexanoate, ethyl octanoate, phenylethyl acetate and 2-phenyl ethanol at the ratio 42:2:30:1:8:1:4:5:7. This ratio was selected based on relative amounts of EAD-active headspace volatiles released by *P. kudriavzevii*. The blend was tested at 1 μL $mL^{-1}$ and 0.1 μL $mL^{-1}$ concentrations by dispensing 10 μL of the prepared solution onto a filter paper strip (5×40 mm). After 0.5 min of solvent evaporation, the filter paper strip was placed in the glass tube connected to one arm of the olfactometer. The same size filter paper was treated with 10 μL of hexane and after solvent evaporation was placed in the other arm serving as control. To test attraction of (-)-δ-heptalactone against a hexane control, the compound was presented at 0.09 μL $mL^{-1}$, 0.05 μL $mL^{-1}$ and 0.01 μL $mL^{-1}$ concentrations by dispensing 10 μL of each solution onto a filter paper strip.

The olfactometer was dismantled and the glassware was cleaned with hexane after each test, soaked overnight in distilled water, and

dried for 2 h in an oven by rising the oven temperature to 200 °C. Silicone parts of the Y-tube olfactometer were cleaned with hexane, soaked overnight in distilled water, and air dried or replaced between the tests. Male and female adults between 2 and 5-day-old were used in the experiment. Flies were allowed feeding on the sucrose solution until used in olfactometer bioassays. Single fly was released into the Y olfactometer at the end of the main tube. Pre-choice duration, i.e. the time within which a fly must have reached the branch point, was 15 min. A fly was considered to have made a choice when the fly reached the distal end of the glass tube containing the stimulus or a solvent control irrespectively whether the fly switched arms or not before reaching the odour source. If the fly did not choose an arm within 15 min, it was considered as not making choice. The positions of the two Y-tube arms were periodically reversed. All insects were observed individually and used in a bioassay only once. In mixture test out of 106 flies tested 11 specimens (all of them were females) failed to make a choice (10.4 %). In (-)-δ-heptalactone test out of 133 flies tested 11 specimens (8 females and 3 males) failed to make a choice (8.3 %). The tests were carried out at 25 ± 2 °C, 60 % RH, between 10.00 and 17.00 h local time.

**Field assay.** Organic sea buckthorn plantation in Stacijava village, Molėtai district, Lithuania was chosen for field assay. Traps McPhail (Pherobank, the Netherlands) and red rubber dispensers with inner diameter 7.1 mm (5x11, Pherobank, the Netherlands) were used. Dispensers were loaded with 25 µL of (-)-δ-heptalactone and 50 µL of hexane solvent. For control dispensers were loaded with the same amount of the solvent. For 20 min solvent was allowed to evaporate. Following evaporation each dispenser was covered with another one of the same type. This allowed to reduce emissions of the test compound. Dispensers were put into 6 traps and after three days period those were placed in the plantation at a distance of 4 m between test and control trap at least, and at a distance between such couples

approximately 50 m. Field assay was started from 30 July and lasted to 20 August, 2019.

**Statistical analysis.** All catches were counted and the number of insects trapped per day was calculated. Catches in traps were transformed using (x + 0.5)1/2 and evaluated by Duncan's analysis of variance (ANOVA, Post-hoc) test (Bewick *et al*., 2004).

Flight and mating dynamics was evaluated by comparing statistical difference between recorded values of neighbouring data points by means of Fisher LSD (ANOVA, Post-hoc) test (Čekanavičius, Murauskas, 2008).

EAG amplitudes of *R. batava* antennae of both sexes were compared using a nonparametric Mann-Whitney U (Čekanavičius, Murauskas, 2008) test.

Kruskal-Wallis test for more than two groups (Čekanavičius, Murauskas, 2008) was used to compare antennal responses to EAD-active compound doses.

All this statistical analysis was performed using Statistica 6.0 software (StatSoft, Inc., USA).

In olfactometric assay the total number of flies that made a choice was analysed with a G-test (Sokal, Rohlf, 1995). This test was performed using MS Office Professional Excel 2016 (Microsoft, JAV).

Generalised linear mixed model (GLMM), logistic regression (glmer) with binomial distribution of dependent variable (fly choice: 0, 1) was used to determine whether the effects of independent variables such as the treatment (factorial: control and stimulus), and sex (factorial: male and female) were significant. Afterwards, we evaluated the effect of treatment on fly choice in each fly sex (male or female) and for each stimulus concentration separately. The effect of the stimulus concentration has only been evaluated in the female flies. In all models, experimental replication was treated as a random variable. Statistical evaluations were carried out with program R,

version 3.5.1 and RStudio version 1.1.463 (R Core Team, 2017; RStudio Team, 2018)

Statistically significant difference was established when $P \leq 0,05$.

The sum of the effective temperatures of the *R. batava* adult flying start was calculated using the formula $\Sigma(t\text{-}10\ °C)$, t – average of daily air temperature ≥10 °C.

## RESULTS AND DISCUSSION

### *Rhagoletis batava* catches in traps of different colours

Catches in sticky traps of different colour revealed differences in their attractiveness to *R. batava* fruit flies. Captures differed statistically significantly (Duncan's test) in the three groups of colour traps tested. The group of blue, red, black and transparent traps was the least attractive (Duncan's test, $P<0.05$). Approximately 2.4–5.3 times more attractive were green and white traps. Yellow traps with most abundant captures were the most attractive (Duncan's test, $P<0.05$). The latter traps were approximately 4.3–7.8 times more attractive compared to the first group (Fig. 1). The results obtained in the present study which demonstrated the highest attractiveness of yellow traps agree with those obtained for other fruit flies from genus *Rhagoletis*, which were the most attracted to yellow traps (Agee *et al*., 1982; Yee, 2011).

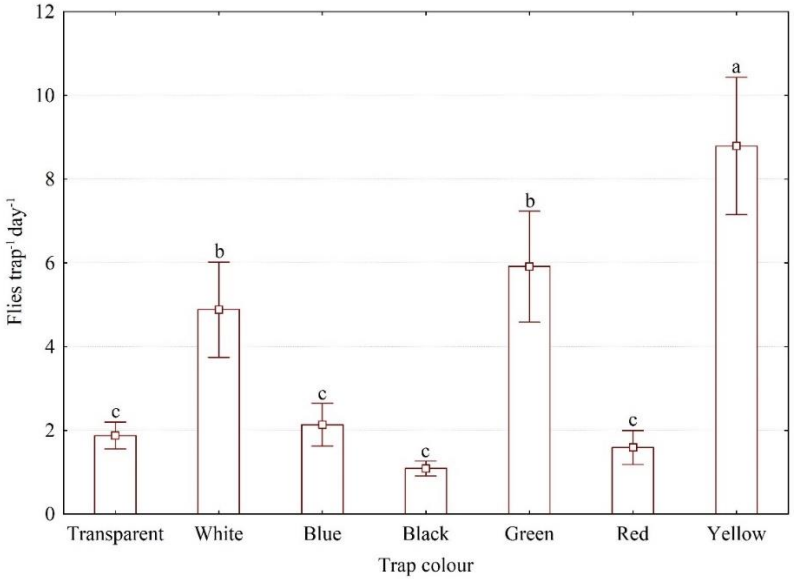


Figure 1. Catches of *Rhagoletis batava* flies by traps of different colour. Different letters indicate statistically significant difference (Duncan's test, $P<0.05$); vertical bars indicate SE of the mean.

Catches of *Rhagoletis batava* by traps of different type

Effectiveness. On sticky traps, average catches were $7.2 \pm 1.2$ flies per day, and in McPhail traps those were $2.1 \pm 0.4$ (Fig. 2). The difference was statistically significant (Duncan's test, $P<0.001$). Thus, the effectiveness of sticky traps was approximately 2.9 times higher compared to that of McPhail traps. Our experience in maintaining both sticky and McPhail traps under field conditions suggests that the effectiveness of sticky traps changes in time as their surface is covered with dust, small parts of plants and insects. Sticky area decreases as well as visibility of the colour from a distance (Navarro-Llopis, Vacas, 2014). Thus, in the long run high effectiveness decreases to minimal, if traps are checked and replaced quite rarely. Maintenance of high effectiveness leads to increased service costs. Trapping ability of

McPhail traps remains nearly the same for a long time. It allows the user to count insects and refill water (solution) within the traps with longer intervals.

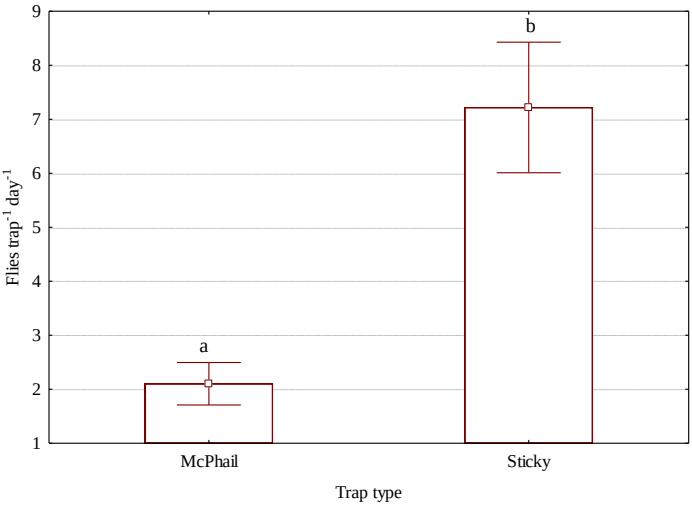


Figure 2. Catches of *Rhagoletis batava* flies by different type traps. Different letters indicate statistically significant difference (Duncan's test, P<0.001); vertical bars indicate standard error of the mean.

*Rhagoletis batava* catches on buckthorns of different gender

Catches by sticky traps placed on feminine and masculine buckthorn plants differed nearly twice and the difference was statistically significant (Duncan's test, P<0.001). The catches on feminine plants exceeded those on masculine ones (Fig. 3). This might be the result of unequal distribution of larvae and cocoons, thus emerging adults. *Rhagoletis* adults are relatively good flyers capable of covering distances up to 500 meters (*e.g.*, Daniel, Grunder, 2012), and their distribution within plantation could become approximately even soon after emergence. However, this was not the case. Thus, we assume that attractiveness of sea buckthorn plants of different genders differs for

adult *R. batava* fruit flies. The reasons for these differences remain to be investigated in the future.

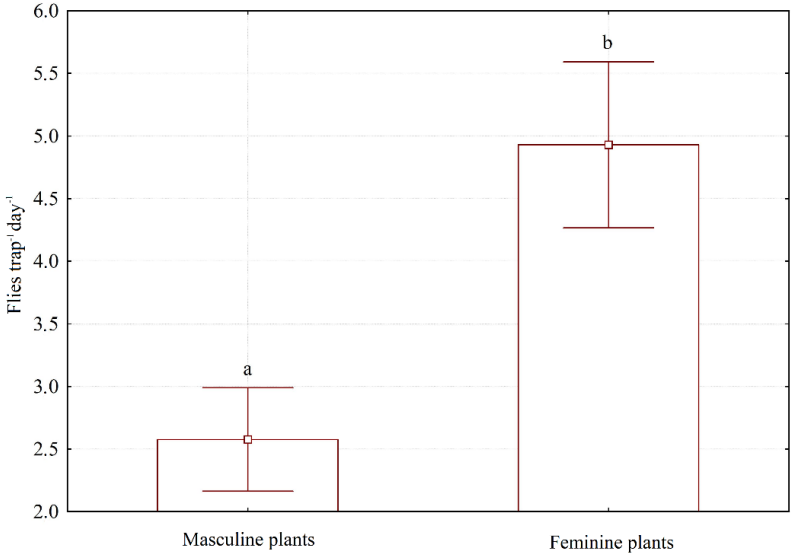


Figure 3. Catches of *Rhagoletis batava* flies on sticky traps hung on masculine and feminine sea buckthorn plants. Different letters indicate statistically significant difference (Duncan's test, P<0.001); vertical bars indicate standard error of the mean.

## Seasonal flight dynamics

In 2016 from 30$^{th}$ June to 8th August, 82 *R. batava* flies trap$^{-1}$ were recorded with a statistically significant peak (Fisher LSD test, P<0.05) from 14$^{th}$ to 18$^{th}$ of July (Fig. 4). In 2017, trapping was started on 11$^{th}$ July and stopped on 22$^{nd}$ August; however, no flies were recorded until 21$^{st}$ July. In total, 26.3 fruit flies trap$^{-1}$ were captured on average. Flight peak was not clearly expressed and based on statistics (Fisher LSD test, P<0.05) lasted from 28$^{th}$ July to 18$^{th}$ August. In 2018, from 26$^{th}$ June to 9$^{th}$ July, 71 *R. batava* flies trap$^{-1}$ were recorded with a statistically significant (Fisher LSD test, P<0.05) peak from 20$^{th}$ to 24$^{th}$

of July. In 2019, trapping was started on 21st June and stopped on 23th August. In total, 143.8 flies trap$^{-1}$ were captured on average. Flight peak (Fisher LSD test, $P<0.05$) was on July 12th-30th.

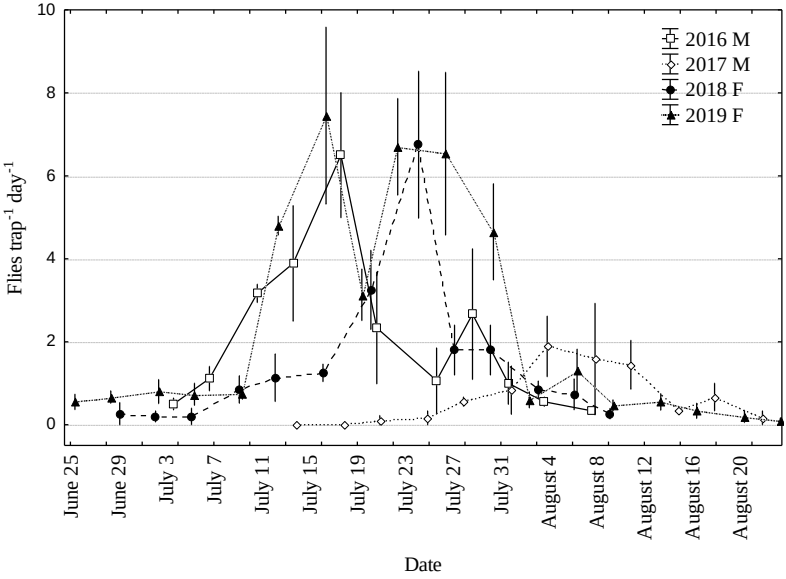


Figure 4. Seasonal dynamics of *Rhagoletis batava* catches. Vertical bars indicate standard error of the mean; M – traps hung on masculine plants, F – on feminine plants.

It should be noted that the abundance of *R. batava* population during four successive years fluctuated greatly, and based on the seasonal catches differed approximately more than five times. In Lithuania, seasonal period of adult flight of the species lasted almost 2 months. The flight started at the beginning of July and lasted until the first ten-day period of August in 2016, and from the second ten-day period of July until the end of August in 2017. In 2018, flight dynamics was similar to that in 2016. Flight lasted from the end of June until the first ten-day period of August. In 2019 Flight lasted from the end of June until the end of August.

The summarised results of the four experimental years suggest that the flight period of *R. batava* adults lasted from the very end of June to end of August. Flight peak was reached between the middle to the end of July in Lithuanian population. Such annual fluctuations of flight period were most likely caused by the differences in temperature conditions, but other weather conditions can be important too. The total duration of *R. batava* flight period established in the present study, is close to that recorded in Germany (Toth *et al*., 2016) and differs from that reported in Mongolia and Russia (Siberia, Asian part), where flight period starts 2-3 weeks earlier (Shamanskaya, 2015; Zhao *et al*., 2017).

## Adult occurrence

Based on *R. batava* adult flying dynamics in sea buckthorn plantation, the temperature when adult fruit flies occur was estimated. In 2016, *R. batava* began to fly when total active air temperature reached 349.6 °C. In 2017, it was 353.2 °C; 396.6 °C in 2018, and 411.4 °C in 2019. The results can be compared to those obtained in Russia, where the active flight of *R. batava* started at 252.1 °C and 319 °C, in 2016 and 2017 respectively (Zeynalov, 2018). The temperatures were lower compare to those recorded in Lithuania.

Summarizing our data obtained during four years period, *R. batava* adults appear in summer when the sum of daily active air temperatures reaches 377.7 ± 15.5 °C on average in Lithuania. This parameter can be applied in future to predict the start of *R. batava* flight. The predictability of pest occurence is important both for effective monitoring or control.

## Adult emergence

The insects originated from wild populations and have spent part of their development cycle in their natural environment. *Rhagoletis batava* puparia were incubated at the laboratory under controlled

conditions. The male to female ratio was analysed in the natural *R. batava* population. Between 2016 and 2018, a total of 1789 *R. batava* puparia were incubated. Out of the 29.2% of puparia, none developed (they were lifeless or underwent prolonged diapause). Out of the remaining 70.8%, males, females and their parasitoids emerged (Fig. 5).

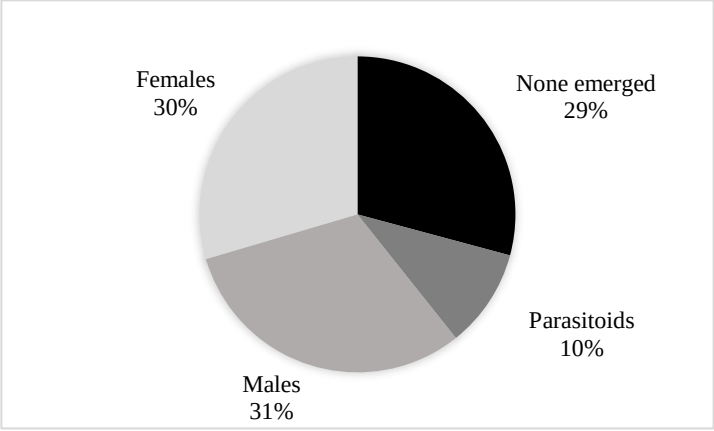


Figure 5. Adults emerged of *Rhagoletis batava* puparia.

Data collected during a three-year period showed hatchling ratio of male to female close to 1:1. Parasitoids killed at least 10.1% of individuals that reached the puparium stage, which is close to the 10% rule (Lindeman, 1991). These parasitoids were identified as *Phygadeuon wiesmanni* Sachtleben, 1934 (Hymenoptera) and its sibling species. *Phygadeuon wiesmanni* is a generalist parasitoid laying eggs in the puparia of various of *Rhagoletis* species (Daniel, 2015). Five species of the *Phygadeuon* genus are known in Lithuania, but not *P. wiesmanni* (Jonaitis, 1991; Ivinskis *et al*., 2004). Therefore, this species was for the first time discovered in Lithuania and is new to the country's fauna list.

*Rhagoletis batava* and host-plant berries: chemoecological interaction

A total of 20 *R. batava* olfactorily active VOCs were detected in the sea buckthorn berries (Fig. 6, Table 1). Most of the olfactorily active compounds in berry extracts consisted of esters of unbranched and branched alcohols as well as unbranched and branched acids. The number of olfactorily active VOCs varied in berries in accordance to their level of ripening. 17 biologically active VOCs were found in green berry extract and 19 in ripe berry extract.

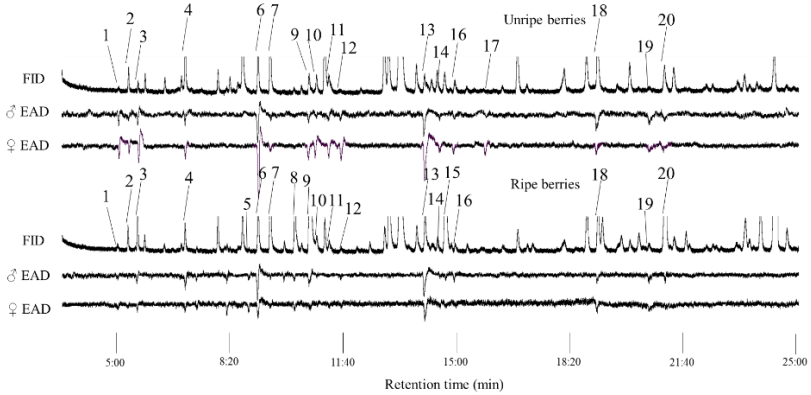


Figure 6. Flame-ionization (FID) and electroantennogram detector (EAD) recordings of antennal responses of *Rhagoletis batava* male and female fruit flies to VOCs of unripe and ripe sea buckthorn berries. Names of the compounds indicated by numbers are presented in Table 1.

Table 1. Sea buckthorn berries produced volatiles and their electroantennographic activity to *Rhagoletis batava* flies.

| No | RI | Compound | EAD | | | |
|---|---|---|---|---|---|---|
| | | | Unripe | | Ripe | |
| | | | ♂ | ♀ | ♂ | ♀ |
| 1 | 1045 | Ethyl butanoate | 4 (4) | 4 (2) | 3 (2) | 3 (3) |
| 2 | 1062 | Ethyl 2-methylbutanoate | 4 (3) | 4 (1) | 3 (2) | 3 (2) |
| 3 | 1077 | Ethyl 3-methylbutanoate | 4 (4) | 4 (2) | 3 (2) | 3 (3) |
| 4 | 1144 | (*Z*)-3-hexenal | 4 (3) | 4 (4) | 3 (2) | 3 (2) |
| 5 | 1223 | Ethyl 3-methyl-2-butenoate | | | 3 (3) | |
| 6 | 1235 | Ethyl hexanoate | 4 (4) | 4 (4) | 3 (3) | 3 (3) |
| 7 | 1249 | *Z*-*β*-ocimene | 4 (3) | 4 (3) | 3 (2) | 3 (3) |
| 8 | 1278 | 3-Methylbutyl 2-methylbutanoate | | | 3 (3) | |
| 9 | 1295 | 3-Methylbutyl 3-methylbutanoate | 4 (4) | 4 (4) | 3 (3) | 3 (3) |
| 10 | 1301 | Unknown | 4 (3) | 4 (3) | 3 (2) | 3 (3) |
| 11 | 1317 | Propyl hexanoate | 4 (3) | 4 (3) | | 3 (2) |
| 12 | 1332 | Ethyl heptanoate | 4 (4) | 4 (3) | 3 (2) | 3 (2) |
| 13 | 1431 | Ethyl octanoate | 4 (4) | 4 (4) | 3 (3) | 3 (2) |
| 14 | 1450 | 1-Octen-3-ol | 4 (3) | 4 (3) | 3 (3) | 3 (1) |
| 15 | 1456 | 3-Methylbutyl hexanoate | | | 3 (3) | 3 (2) |
| 16 | 1467 | Unknown | 4 (3) | 4 (4) | 3 (2) | 3 (1) |
| 17 | 1489 | Unknown | 4 (2) | 4 (4) | | |
| 18 | 1646 | Ethyl benzoate | 4 (4) | 4 (4) | 3 (2) | 3 (1) |
| 19 | 1713 | Unknown | 4 (4) | 4 (4) | 3 (3) | 3 (1) |
| 20 | 1736 | (*E*,*E*)-*α*-farnesene | 4 (3) | 4 (4) | 3 (2) | |

Values in the columns headed as EAD represents how many replicates were tested and values in the brackets indicates the number of antennae which responded to the compound.

Comparison of EAD-active host-plant berry compounds between *R. batava* and other species of the genus *Rhagoletis* shows that 5 to 12 EAD-active compounds were found in different species of host-plant fruits of *R. pomonella* and *Rhagoletis zephyria* Snow. Meanwhile, the number of compounds detectable in *R. batava* was higher both in unripe and berries. However, these differences should be counted as preliminary only due to methodological differences in compound collection (Fein *et al*., 1982; Zhang *et al*., 1999; Nojima *et al*., 2003a;

2003b; Olsson *et al*., 2009; Cha *et al*., 2011a; 2011b; 2012a; 2012b; 2017; Powell *et al*., 2012). There are olfactorily similarities between these 3 species of the genus *Rhagoletis*: one compound (1-octen-3-ol, peak 14 in Fiure. 6) was common for the three species, and 5 compounds were common for *R. batava* and *R. pomonella* (those were 3-methylbutyl-2-methyl butanoate, peak 8 in Fig. 6; propyl hexanoate, peak 11 in Fig. 6; ethyl heptanoate, peak 12 in Fig. 6; 3-methylbutyl hexanoate, peak 15 in Fig. 6, and 1-octen-3-ol,) (Fein *et al*., 1982; Zhang *et al*., 1999; Nojima *et al*., 2003a; 2003b; Olsson *et al*., 2009; Cha *et al*. al., 2011a; 2011b; 2012a; 2012b; 2017; Powell *et al*., 2012).

Electroantennographic activity of yeast-produced volatiles

In the DC-EAD assay of *Hanseniaspora uvarum* (n=6) VOCs, 8 compounds were found to be olfactory active to *R. batava* males and females. Only 4 EAD-active compounds were present in emissions of *Metschnikowia pulcherrima* (n=10). GC-EAD analysis of *P. kudriavzevii* headspace collections (n=10) showed that antennae of *R. batava* flies were able to detect 10 volatile compounds in total (Fig. 7, Table 2). This is the highest number of VOCs among the three yeast species tested and composition covers that present in emissions of in *H. uvarum* and *M. pulcherrima.* The interaction of *R. batava* and *P. kudriavzevii* by VOCs has been tested in more detail.

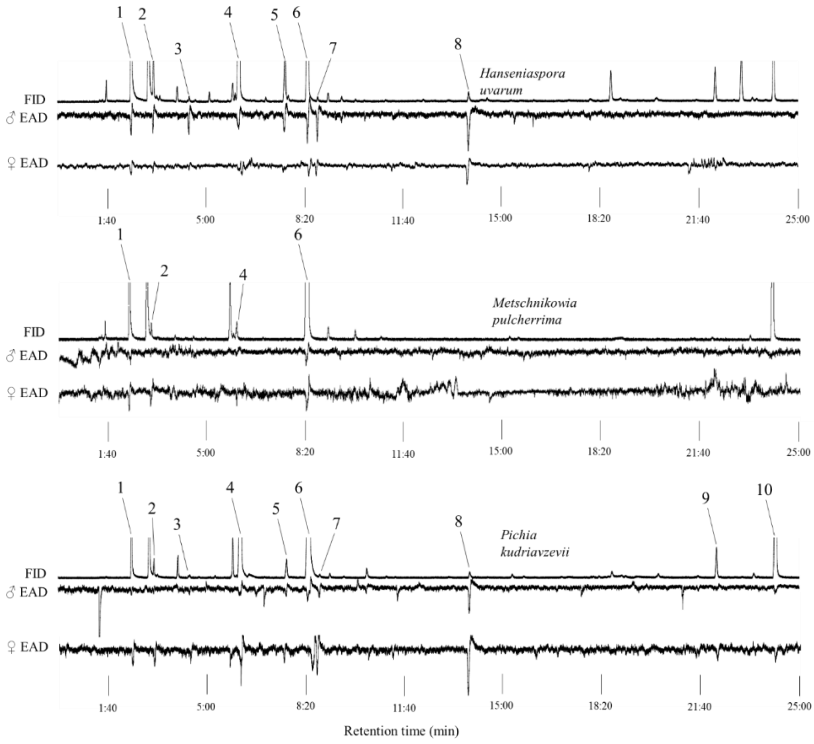


Figure 7. Flame-ionization (FID) and electroantennogram detector (EAD) recordings of antennal responses of *Rhagoletis batava* male and female flies to volatiles produced by the three yeast species. Name of the compounds indicated by numbers are presented in Table 2

Table 2. Volatiles produced by three species of yeast and their electroantennographic activity towards *Rhagoletis batava* fruit flies.

| No | RI | Compound | Yeast spacies | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | *Hanseniaspora uvarum* | | *Metschnikowia pulcherrima* | | *Pichia kudriavzevii* | |
| | | | EAD | | EAD | | EAD | |
| | | | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ |
| 1 | 898 | Ethyl acetate | 3 (3) | 3 (3) | 5 (5) | 5 (5) | 5 (4) | 5 (4) |
| 2 | 915 | Ethyl propionate | 3 (3) | 3 (3) | 5 (4) | 5 (4) | 5 (5) | 5 (5) |
| 3 | 1013 | Ethyl butanoate | 3 (3) | 3 (2) | | | 5 (5) | 5 (4) |
| 4 | 1105 | 3-Methylbutyl acetate | 3 (3) | 3 (3) | 5 (2) | 5 (5) | 5 (4) | 5 (5) |
| 5 | 1176 | 3-Methylbutyl propionate | 3 (3) | 3 (3) | | | 5 (4) | 5 (5) |
| 6 | 1213 | 3-Methylbutan-1-ol | 3 (3) | 3 (1) | 5 (5) | 5 (5) | 5 (5) | 5 (5) |
| 7 | 1224 | Ethyl hexanoate | 3 (3) | 3 (3) | | | 5 (5) | 5 (5) |
| 8 | 1430 | Ethyl octanoate | 3 (3) | 3 (3) | | | 5 (5) | 5 (5) |
| 9 | 1795 | 2-Phenylethyl acetate | | | | | 5 (2) | 5 (4) |
| 10 | 1894 | 2-Phenyl ethanol | | | | | 5 (4) | 5 (3) |

Values in the columns headed as EAD represents how many replicates were tested and values in the brackets indicates the number of antennae which responded to the compound.

## Behavioural responses of *Rhagoletis batava* fruit flies to the blend of electroantennographic active compounds

Evaluation of statistical data by GLMM revealed that in the two-choice experiments, *R. batava* males were significantly more attracted to the mixture of nine synthetic compounds, analogous to EAD-active volatiles released by *P. kudriavzevii* yeasts compare to the control (P=0.001). However, female fruit flies showed no preference to the same mixture presented at 1 μL $mL^{-1}$ (P=0.758) but were attracted to the mixture at the concentration 0.1 μL $mL^{-1}$ (P=0.026) (Fig. 8).

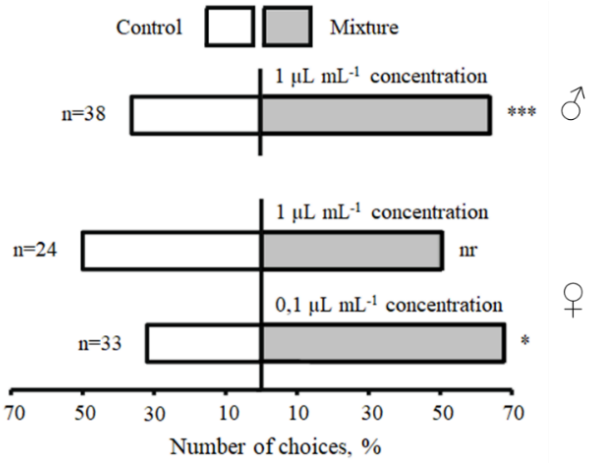


Figure 8. Preference of *Rhagoletis batava* males and females to the mixture of 9 synthetic compounds versus control. The synthetic compounds were analogous to EAD-active volatiles released by *Pichia kudriavzevii* yeasts. *** P<0.001; * P<0.05, ns – not significant, based on generalised linear mixed model, logistic regression with binomial distribution of dependent variable.

Females were attracted to the lower concentration of the blend compare with males most probably due to the differences in responses to olfactory cues eliciting search for oviposition site compare to that mediating food source finding. *Pichia kudriavzevii* were characterised as well-fermenting yeasts (Nyanga *et al*., 2013; Sharma *et al*., 2012) and were more abundant on damaged than on intact grape berries (Nemcova *et al*. 2015). We assume that *P. kudriavzevii* yeast produced odours could indicate to flies easier access to berry interior and higher nutrient quality due to presence of yeasts for adult feeding and less suitability for larval development. Odour-mediated interactions between yeasts and insect are complex and diverse ranging from attractive to repellent depending on whether mutualistic or harmful yeasts inhabited a substrate.

There is growing experimental evidence that yeast volatiles can be successfully used in integrated pest management programmes (Hamby, Becher, 2016; Holighaus, Rolhfs, 2016; Mori *et al*., 2017). Our present work revealed that the blend composed of nine EAD active yeast volatiles attracted *R. batava* flies under laboratory conditions providing background for further optimisation of the blend and development of semiochemical based trap for monitoring and controlling this pest in *H. rhamnoides* orchards.

## Diurnal rhythm of mating

It is useful to know the time of the most active mating when searching for a pheromone, i.e. timing of potential pheromone release. For this reason, diurnal mating rhythm of *R. batava* was analysed.

Mating of *R. batava* flies was recorded during the day time from 9 AM till 8 PM. In total, there were counted 1847 pairs in copula, most of them within time interval from 10 AM to 7 PM with no clear peak (Fisher LSD test, $P<0.05$) in mating activity (Fig. 9). Significantly fewer mating couples were counted at 9 AM and at 8 PM. The air temperature within the range from 18°C to 24°C (Fig. 9) had no clearly pronounced effect on the mating activity. Comparison of our data with those previously obtained in Mongolian *R. batava* population (Zhao *et al*., 2017) reveals both similarities and differences. The total duration of *R. batava* daily mating period in Mongolia (Zhao *et al*., 2017) corresponded to that recorded for the flies of Lithuanian population. However, in Mongolian *R. batava* population there were recorded two peaks of daily mating activity, which does not agree with our findings. Unfortunately, the authors of the mentioned paper made no statistical analysis. Thus, we conclude that daily mating period of *R. batava* flies is long and lasts during all light period of a day with no clear peak. The peak on any specific day might appear under special weather conditions.

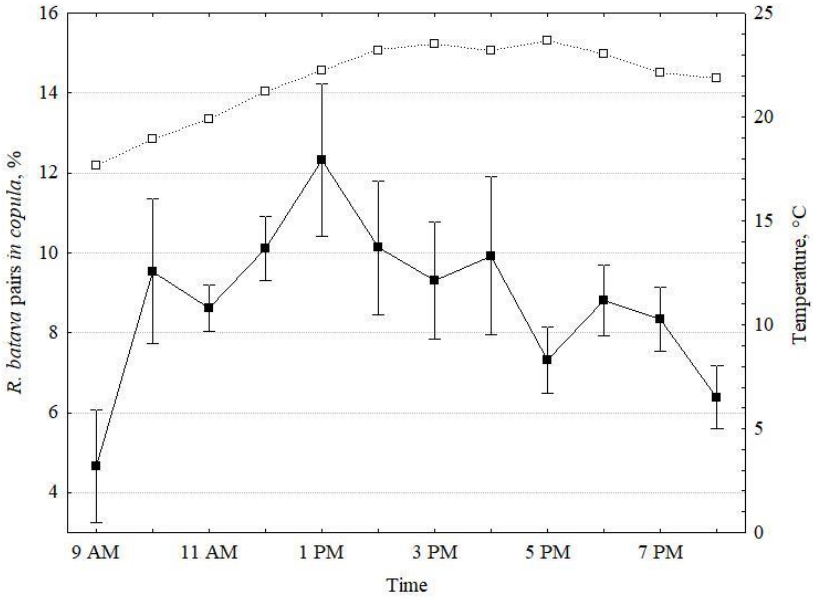


Figure 9. Diurnal rhythm of *Rhagoletis batava* mating. Vertical bars indicate standard error of the mean; black square indicates couples; white square indicates air temperature.

## Male produced EAD-active compound

Couplet GC-EAD analysis of headspace volatiles collected from live adult either males or females of *R. batava* showed that antennae of both sexes responded strongly to the single compound in the headspace of males (Fig. 10). The antennae of males did not respond to any compound in the female headspace. GC-MS analyses of volatiles collected from *R. batava* males demonstrated that EAD-active compound retention time and mass spectrum matched those of the synthetic standard of δ-heptalactone.

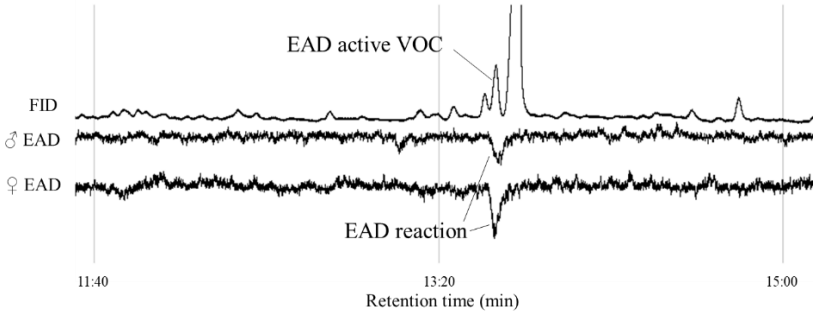


Figure 10 Gas chromatography–electroantennogram detection response of male and female of *Rhagoletis batava* to headspace volatiles of *R. batava* males.

EAD-active enantiomer of δ-heptalactone

Couplet GC-EAD analysis of synthetic enantiomers of δ-heptalactone revealed strong response of *R. batava* fruit flies of both sexes to the single enantiomer only. Anosmia to (+)-δ-heptalactone was found and high sensitivity to (-)-δ-heptalactone recorded (Fig. 11).

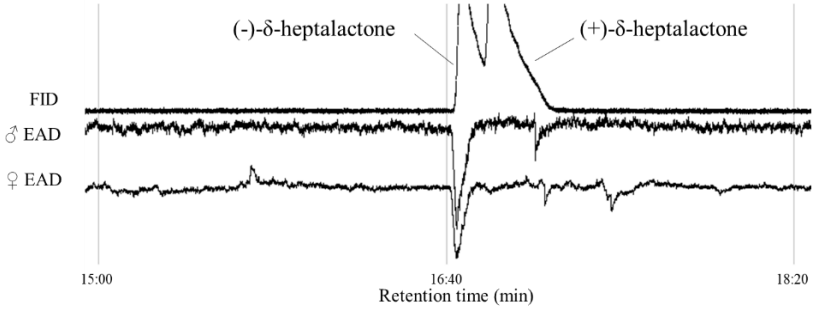


Figure 11. Gas chromatography–electroantennogram detection response of male and female of *Rhagoletis batava* to synthetic enantiomers of δ-heptalactone.

Dose response

The sensitivity of males and females to the lowest dose tested was different, with males being more sensitive than females (Mann-Whitney U test, P=0.037). At higher doses, no differences in EAG responses between sexes was recorded (Mann-Whitney U test, P>0.05) (Fig. 12).

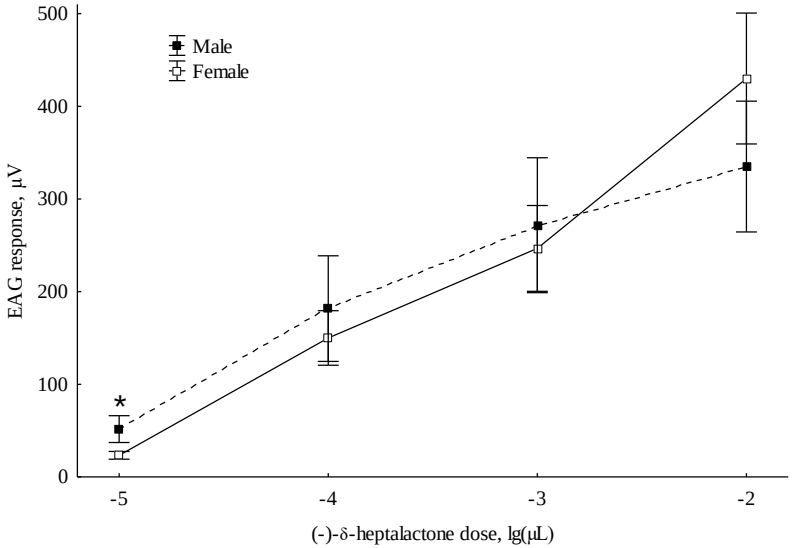


Figure 12. Electroantennogram responses (mean amplitude ± SE, mV) of *Rhagoletis batava* male and female to different doses ($1\times10^{-5}$ to $1\times10^{-2}$ µL) of synthetic (-)-δ-heptalactone. Asterisk denotes significant differences in EAG responses between sexes (Mann-Whitney U Test, P=0.037).

Behavioural test in olfactometer

In the two-choice experiments, *R. batava* female flies showed no preference to (-)-δ-heptalactone at 0.01 µL $mL^{-1}$ (G-test, P=0.039) and 0.05 µL $mL^{-1}$ (G-test, P=0.115) concentrations, but were attracted to

the compound at 0.09 µL $mL^{-1}$ concentration (G-test, P=0.015). Males showed a significant attraction to 0.01 µL $mL^{-1}$ (G-test, P=0.009) and 0.05 µL $mL^{-1}$ (G-test, P<0.001) concentrations of (-)-δ-heptalactone, but exhibited non-significant response to 0.09 µL $mL^{-1}$ concentration (G- test, P=0.808) (Fig. 13).

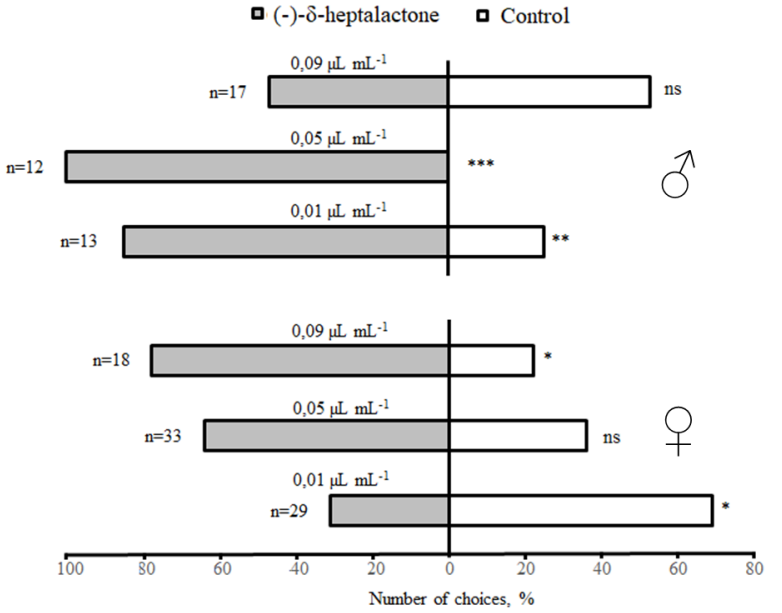


Figure 13. *Rhagoletis batava* male and female choices to different concentrations of (-)-δ-heptalactone versus hexane control in a Y-tube olfactometer. *** P<0.001; * P<0.05, n.s. – not significant (G-test).

Behavioural test under field conditions

Both males and females of *R. batava* were captured in traps loaded with (-)-δ-heptalactone. The average number of insects 2.5 times exceeded that in control traps (Fig. 14). The difference was statistically significant (Duncan's test, P=0.018). As the traps contained the fruit flies of both sexes, it is concluded that (-)-δ-heptalactone functioned as aggregation pheromone.

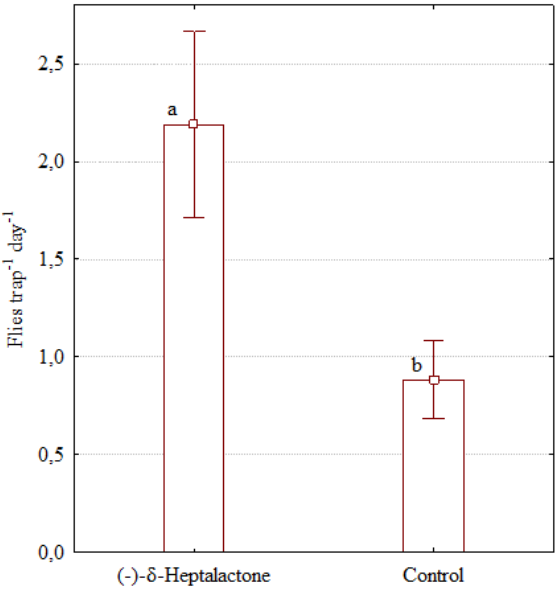


Figure 14. Field catches of *Rhagoletis batava* in McPhail traps. Different letters indicate statistically significant difference (Duncan's test, P=0.018); intervals mark SE.

## CONCLUSIONS

1. The catches of *Rhagoletis batava* Hering (Diptera) by traps depended on their colour, construction and host-plant gender: the most attractive was the yellow colour, which attracted 1.5 to 7.8 times more adult fruit flies compare to the other 6 colours tested; flat sticky traps were approximately 3 times more effective than McPhail design traps; catches on feminine host-plants were 2 times higher than those in masculine plants.

2. The flying dynamics of adults of *R. batava*: lasted about 8 weeks (from the end of June to the end of August), peak activity lasted about 12 days on average (from middle of July to early August).

3. *Rhagoletis batava* adults emerged from overwintering pupae when the sum of effective temperatures (average daily temperature above +10 $^{o}$C) reaches 377.7 ± 5.5 °C under Lithuanian climate conditions.

4. *Rhagoletis batava* adults perceived 20 host-plant (sea-buckthorn, *Hippophae rhamnoides*) berry volatile organic compounds at least: 17 from unripe and 19 from ripe berries.

5. *Rhagoletis batava* adults were able to smell volatile organic compounds released by the yeast species related to sea buckthorn berries: 8 compounds released by *Hanseniaspora uvarum*, 4 by *Metschnikowia pulcherrima*, and 10 by *Pichia kudriavzevii*.

6. The mixture of 9 volatile organic compounds (ethyl acetate, ethyl propionate, 3-methylbutyl acetate, 3-methylbutyl propionate, 3-methyl butanol, ethyl hexanoate, ethyl octanoate, 2-phenylethyl acetate and 2-phenyl ethanol) released by sea buckthorn berries-related yeasts was attractive to *R. batava* adults.

7. *Rhagoletis batava* mates during day light period without clearly pronounced peak.

8. *Rhagoletis batava* males release (-)-δ-heptalactone, which evokes responses in olfactory receptors and attracts conspecifics of both sexes, i.e. play role as aggregation pheromone.

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## LIST OF PUBLICATION ON THE DISSERTATION TOPIC

1. **Aleknavičius D.**, Būda V. 2019. Trapping peculiarities, flight and mating dynamics of sea buckthorn fruit fly (*Rhagoletis batava*) in Lithuania. Zemdirbyste-Agriculture, 106, 1: 81-86.
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## ABSTRACTS OF CONFERENCE REPORTS

Būda V., Baužienė V., **Aleknavičius D.**, Butkienė R. 2015. Sea buckthorn volatiles involved in host plant choice by *Rhagoletis batava* females. Pheromones and Other Semio-Chemicals in Integrated Production. Jerusalem, Israel, November 8-13: 47.

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fly interaction mediated by yeast? The 35th annual meeting of the International Society of Chemical Ecology. Atlanta, USA, June 2-6: poster presentations, 15.

Mozūraitis R. **Aleknavičius D.**, Radžiutė S., Blažytė-Čereškienė L., Servienė E., Būda V. 2019. Effect of the volatiles released by yeast related to sea buckthorn *Hippophae rhamnoides* berries on behavior of *Rhagoletis batava* flies. The 35th annual meeting of the International Society of Chemical Ecology. Atlanta, USA, June 2-6: poster presentations, 66.

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