

ISSN 1392-3196 / e-ISSN 2335-8947

Zemdirbyste-Agriculture, vol. 106, No. 1 (2019), p. 81–86

DOI 10.13080/z-a.2019.106.011

Trapping peculiarities, flight and mating dynamics of sea buckhorn fruit fly (*Rhagoletis batava*) in Lithuania

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Abstract

The sea buckhorn fruit fly *Rhagoletis batava* Hering, 1938 is the most important pest of sea buckthorn (*Hippophae rhamnoides* L.) and causes big losses of berry yield. Recently, the fly has been rapidly spreading in European buckthorn plantations. The objective of the research was to establish seasonal flight period and diurnal mating period of *R. batava* as well as to compare the efficiency of traps differing in colour and type for trapping fruit flies under field conditions. In Lithuania, annual flight period of *R. batava* lasts approximately six weeks, it starts at the end of June and ceases in August with a peak from the middle of July till the middle of August on average. Both beginning of the flight period and peak activity may vary by about 20 days between years. Mating period of sea buckhorn fruit flies lasts during all light period of a day without a clearly pronounced peak. Among the 7 colours of traps tested, the most attractive was yellow, in which catches were 1.5–7.8 times more abundant compared to the traps of the other colours. The ratio of *R. batava* females caught by McPhail traps was higher compared to that in sticky traps. Location of traps on feminine bushes increases captures of sea buckthorn fruit flies by approximately 50% compared to the traps located on masculine bushes.

Key words: daily mating rhythm, flight season, fruit fly, pest monitoring, trap colour, trap efficacy.

Introduction

Sea buckthorn (*Hippophae rhamnoides* L.) is a highly valuable plant because it does not need fertile soils and its berries are especially in demand in food, medicine and cosmetic industry (Li, Schroeder, 1996; Ruan et al., 2007; Bal et al., 2011). Fruit fly *Rhagoletis batava* (Diptera: Tephritidae) has become the most important pest of sea buckthorn recently. Although most species of genus *Rhagoletis* are stenophagous (e.g., Mohamadzade Namin, Rasouljan, 2009), as is known, sea buckthorn fruit flies are monophagous and lay eggs exceptionally into berries of sea buckthorn (e.g., Korneyev et al., 2017). Hatched larvae feed on berry flesh and severely damage berries. Those lose their appearance, colour and nutritional as well as economic value. In Siberia, Russia a subspecies *R. batava obscuriosa* Kol. has occurred, which is capable of causing especially severe damage (review in: Stalažs, Balalaikins, 2017) and destroying up to 87–100% of the berry crop (Shalkevich et al., 2015; Shamanskaya, 2015), thus causing huge economic losses.

During the last decades, *R. batava* has reached Baltic countries (Stalažs, 2014 a; b), and has been spreading towards western and southern Europe (Stalažs, Balalaikins, 2017). To improve the efficiency of monitoring and control of the pest, it is necessary to get a better understanding of its biology and ecology, including flight period and mating activity. As special

traps are commonly used for insect pest monitoring, the data on trap type (including colour) suitable for *R. batava* trapping are needed. Although flies of many species from genus *Rhagoletis* were attracted by yellow colour (Agee et al., 1982; Yee, 2011; 2012; 2013; Yee, Goughnour, 2011; Toth et al., 2014), this was not the case for *R. batava*, when field testing was carried out using sticky “cloak” (PAL and PALz) type traps (Toth et al., 2016). It still remains to be investigated whether this behavioural phenomenon of *R. batava* species is an exception within the genus *Rhagoletis*.

The main objective of the present study was to establish seasonal flight period and diurnal mating period of fruit flies *R. batava* as well as to compare the efficiency of traps differing in colour and type for catching fruit flies in sea buckthorn plantations.

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. Sea buckthorn (*Hippophae rhamnoides* L.) berries had not been harvested yet.

Please use the following format when citing the article:

Aleknavičius D., Būda V. 2019. Trapping peculiarities, flight and mating dynamics of sea buckhorn fruit fly (*Rhagoletis batava*) in Lithuania. Zemdirbyste-Agriculture, 106 (1): 81–86. DOI 10.13080/z-a.2019.106.011

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 (Fig. 1A) and McPhail (Pherobank, the Netherlands) traps (Fig. 1B) 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-incl-cage-33202.html>). To fix the insects trapped, 1% NaCl water solution was added into the bottom vial.



Figure 1. Types of traps: flat sticky (A) and McPhail (B)

The traps of both types were hung on masculine bushes 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. Sex of trapped *R. batava* (Diptera: Tephritidae) flies was identified based on the presence or absence of ovipositor. 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 (Fig. 1B). Data were collected from June to August 2016, 2017 and 2018. Three traps were hung on masculine bushes in 2016, 2017 and five on feminine bushes in 2018. 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 bushes 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).

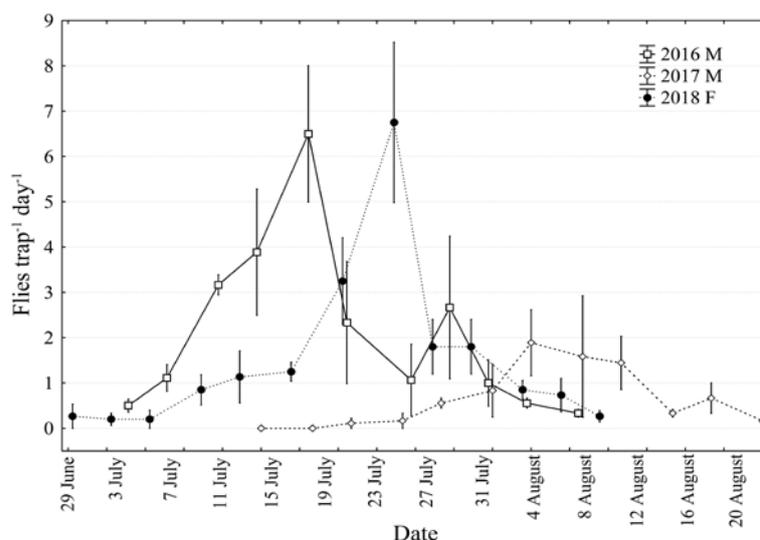
Statistical analysis. All catches were counted and the number of insects trapped per day was calculated. The data were statistically analysed with the software *Statistica*, version 8.0 (StatSoft Inc., USA). Results of catches on buckthorns of different gender were evaluated by Mann-Witney *U* test (Čekanavičius, Murauskas, 2008). The same test was applied for statistical evaluation of catches in traps of different design. Catches in traps of different colours were transformed using $(x + 0.5)^{1/2}$ and evaluated by Duncan's analysis of variance (*ANOVA*, Post-hoc) test (Bewick et al., 2004). Statistical significance of difference in ratio of males to females trapped was evaluated by Wilcoxon Matched Pairs test. 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). Statistically significant difference was established when *p* was equal or less than 0.05.

Results and discussion

Seasonal flight dynamics. In 2016 from 30th June to 8th August, 82 *R. batava* flies trap^{-1} were recorded with a statistically significant peak from 14th to 18th of July (Fig. 2). In 2017, trapping was started on 11th July and stopped on 22nd August; however, no flies were recorded until 21st July. In total, 26.3 fruit flies trap^{-1} were captured on average. Flight peak was not clearly expressed and based on statistics lasted from 28th July to 18th August. In 2018, from 26th June to 9th July, 71 *R. batava* flies trap^{-1} were recorded with a statistically significant peak from 20th to 24th of July.

It should be noted that the abundance of *R. batava* population during three successive years fluctuated greatly, and based on the seasonal catches differed approximately more than three times.

In Lithuania, seasonal period of adult flight of the species lasted a bit more than one month. 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. The summarised results of the three experimental years suggest that the flight period



Note. Vertical bars indicate standard error of the mean; M – traps hung on masculine bushes, F – on feminine bushes.

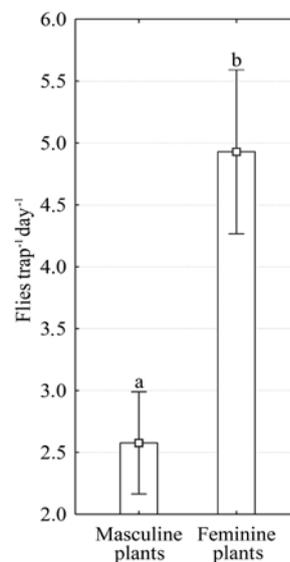
Figure 2. Seasonal dynamics of *Rhagoletis batava* catches

of *R. batava* adults lasted from the very end of June to August. Flight peak was reached between the middle of July and middle of August in the Lithuanian population. Such annual fluctuations of flight period were most likely caused by the differences in the weather conditions.

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).

***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 ($Z = -3.972, p < 0.001$). 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.

***R. 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 in the three groups of colour traps tested. The group of blue, red, black and transparent traps was the least attractive. Approximately 2.4–5.3 times more attractive were green and white traps. Yellow traps with most abundant captures were the most attractive. The latter traps were approximately 4.3–7.8 times more attractive compared to the first group (Fig. 4). 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). Besides, it



Note. Different letters indicate statistically significant difference; vertical bars indicate standard error of the mean.

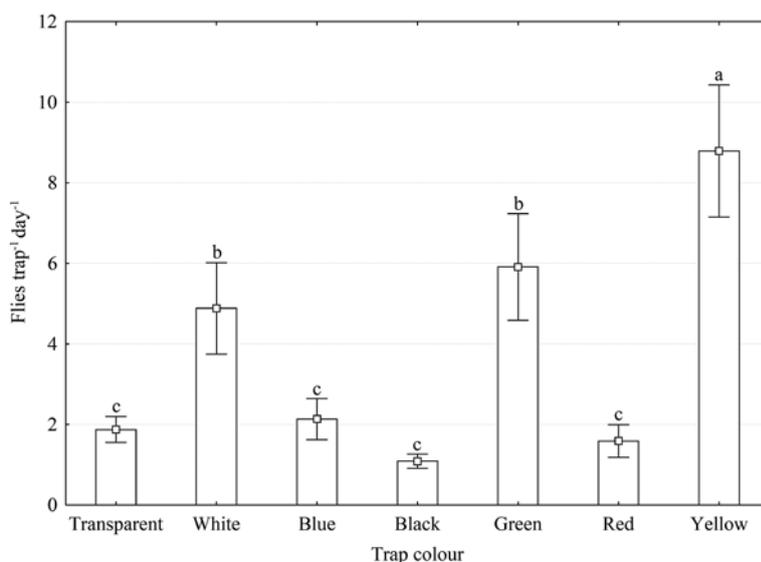
Figure 3. Catches of *Rhagoletis batava* flies on sticky traps hung on masculine and feminine sea buckthorn plants

should be noted that white and green colours, which were less attractive to *R. batava* compared to yellow, contain component of yellow colour within their spectrum. In our opinion, this might explain their relatively higher effectiveness compared to that of the rest (but not yellow) of the colour traps tested, which were significantly less attractive.

Thus, yellow traps are the most suitable for *R. batava* trapping.

Catches of *R. batava* by traps of different type.

Effectiveness. On sticky traps, average catches were 7.2 ± 1.2 flies per day, and in McPhail traps those were 2.1 ± 0.4 (Fig. 5). The difference was statistically significant ($Z = -3.779, p < 0.001$). Thus, the effectiveness of sticky traps was approximately 2.9 times higher compared to that of McPhail traps. Our experience in maintaining



Note. Different letters indicate statistically significant difference; vertical bars indicate standard error of the mean.

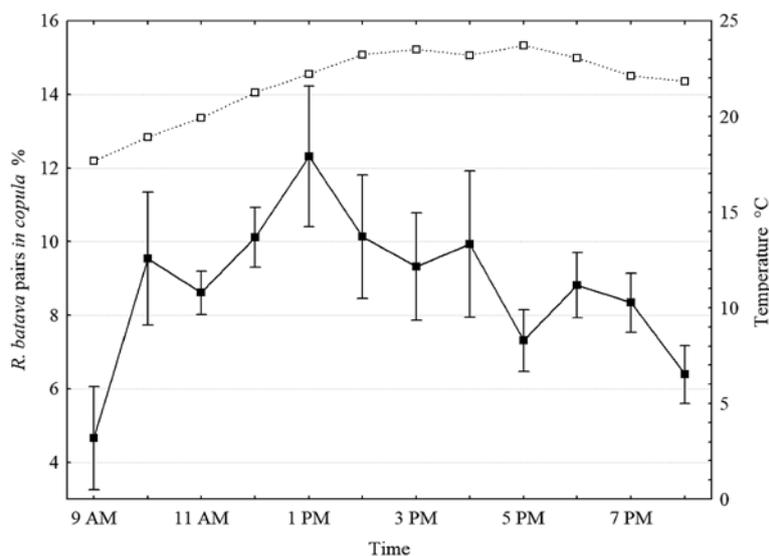
Figure 4. Catches of *Rhagoletis batava* flies by traps of different colour

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.

Sex ratio. Attractiveness of sticky and McPhail traps to *R. batava* flies differed between the sexes. The proportion of females was higher in McPhail traps compared to that in sticky ones. Among the 296 of *R. batava* flies trapped by McPhail traps 129 were males and 167 females (the difference was significant, $Z = 2.257$,

$p = 0.02$), while the catches in sticky traps contained 369 males and 357 females ($Z = 0.281$, $p = 0.78$). Thus the ratio of males to females was approximately equal to 1:1.3 and 1:1, respectively. Higher proportion of females in catches is important as females cause damage and ensure spreading of the pest towards new territories and newly established plantations. The notable drawback of McPhail trap is the price per unit which is much higher compared to that of sticky trap.

Diurnal rhythm of mating. Mating of *R. batava* flies was recorded during all light period of a day 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 in mating activity (Fig. 5). Significantly fewer mating couples were counted at 9 AM and at 8 PM. The middle of the active mating period was around 1 PM. The air temperature within the range from 18°C to 24°C (Fig. 5) had no clearly pronounced



Note. Vertical bars indicate standard error of the mean; black square indicates couples; white square indicates air temperature.

Figure 5. Diurnal rhythm of *Rhagoletis batava* mating

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.

Conclusions

1. Annual flight period of fruit flies *Rhagoletis batava* lasts approximately six weeks. It starts at the very end of June and ceases in August with a peak from the middle of July till the middle of August on average. Both beginning of the flight period and peak activity may vary by about 20 days between years.

2. Traps deployed on feminine sea buckthorn bushes capture approximately 50% more *R. batava* flies compared to those deployed on masculine ones.

3. Trap colour is important for *R. batava* fruit flies. The most attractive is yellow with catches 1.5–7.8 times more abundant compared to the 6 other colours tested.

4. The proportion of *R. batava* females caught by McPhail traps is higher compared to that caught by sticky traps.

5. Mating of *R. batava* fruit flies lasts during all light period of a day without a clearly pronounced peak.

Acknowledgements

The authors would like to thank Mr. Povilas Šukys for kind permission to carry out field research in his sea buckthorn plantation. The research was co-funded from the EU Structural Funds, project No. 09.3.3-LMT-K-712-01-0099.

Received 27 07 2018

Accepted 09 11 2018

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ISSN 1392-3196 / e-ISSN 2335-8947

Zemdirbyste-Agriculture, vol. 106, No. 1 (2019), p. 81–86

DOI 10.13080/z-a.2019.106.011

Dygliuotojo šaltalankio musės (*Rhagoletis batava*) gaudymo gaudyklėmis ypatumai, skraidymo ir poravimosi dinamika Lietuvoje

D. Aleknavičius, V. Būda

Gamtos tyrimų centras

Santrauka

Šaltalankinė musė (*Rhagoletis batava* Hering) yra žalingiausias dygliuotojo šaltalankio (*Hippophae rhamnoides* L.) uogų kenkėjas. Dėl šio kenkėjo šaltalankių augintojai patiria didžiulius derliaus nuostolius. Pastaruoju metu Europoje ši musė sparčiai plinta. Tyrimo tikslas – lauko sąlygomis nustatyti *R. batava* musių sezoninę skraidymo dinamiką, poravimosi aktyvumo paros ritmą ir gaudyklių spalvos bei konstrukcijos įtaką jų sugavimui. Tyrimo metu nustatyta, kad *R. batava* musėms svarbi gaudyklės spalva – jų daugiausia jų sugauta geltonos spalvos gaudyklėmis. Jomis šio kenkėjo pagauta 1,5–7,8 karto daugiau nei kitų testuotų spalvų gaudyklėmis. Lietuvoje *R. batava* skraidymo laikotarpis prasideda birželio pabaigoje ir baigiasi rugpjūčio mėnesį, jo trukmė – maždaug 6 savaitės. Didžiausias skraidymo aktyvumas trunka nuo liepos vidurio iki rugpjūčio vidurio. Įvairiais metais skraidymo laikotarpis, pradžia ir didžiausias skraidymo aktyvumas gali svyruoti ne mažiau nei 20 dienų intervalu. *R. batava* poruojasi visu šviesiuoju dienos metu, be ryškaus aktyviausio poravimosi laikotarpio. Tarp „McPhail“ gaudyklėmis pagautų *R. batava* musių patelių dalis yra didesnė nei tarp pagautų lipniomis gaudyklėmis. Gaudyklėmis *R. batava* musių šaltalankio moteriškuose krūmuose pagunama maždaug 50 % daugiau nei vyriškuose.

Reikšminiai žodžiai: gaudyklės efektyvumas, gaudyklės spalva, kenkėjo stebėjimas, poravimosi paros ritmas, skraidymo trukmė, vaisinė musė.