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Effects of different sucrose concentrations on some parameters of the life cycle in two wild *Drosophila* species

Original Article

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Abstract:

The fruit flies collected from the wild can be easily cultured in laboratory for research purposes. On that occasion many similar recipes for cornmeal-based feeding media can be used for *Drosophila* cultivation. However, in these recipes concentrations of ingredients may differ what might imply a need to choose the recipe which is the most appropriate for culturing of wild *Drosophila* species. The aim of this study was to check how two wild species, *Drosophila melanogaster* and *Drosophila suzukii* respond to different sucrose concentration in feeding media by monitoring of life cycle key parameters. The total number of oviposited eggs, formed pupae and eclosed adults as well as dynamics of pupae formation and adult eclosion were recorded. The results showed that the sucrose concentrations of 160 gL^{-1} caused significantly lower number of formed pupae and eclosed adults in *D. suzukii* than at concentrations of 40 gL^{-1} and 80 gL^{-1}. Prolonged and stretched dynamics of pupation and eclosion were recorded in *D. melanogaster* only at concentration of 160 gL^{-1}. All tested *Drosophila* showed no differences in life cycle parameters between sucrose concentration of 40 gL^{-1} and 80 gL^{-1}. Presented results can be helpful in deciding which cornmeal-based feeding media recipe to choose for cultivation of mentioned wild *Drosophila* species.

Key words:

Fruit fly, *Drosophila melanogaster*, *Drosophila suzukii*, feeding media, life cycle, sucrose concentration

Apstract:

Efekat različitih koncentracija šećera na neke parametre životnog ciklusa kod dve divlje vrste *Drosophila*

Voćne mušice, prikupljene u prirodi, mogu se jednostavno gajiti u laboratoriji za potrebe različitih istraživanja. Za tu svrhu se može upotrebiti jedan od mnogih recepata sličnih sastojaka za pravljenje hranljive podloge, bazirane na kukuruznom grizu za gajenje drozofila. Međutim, u tim receptima koncentracije sastojaka mogu da variraju što može da podrazumeva potrebu da se odabere recept koji najviše odgovara za gajenje divljih vrsta drozofila. Cilj ovog istraživanja je da se praćenjem ključnih parametara životnog ciklusa proveri kako dve divlje vrste *Drosophila melanogaster* i *D. suzukii* reaguju na različite koncentracije šećera u hranljivoj podlozi. Praćeni su ukupan broj položenih jaja, ulutkanih i eklodiranih imaga kao i dinamike ulutkavanja i eklodiranja. Rezultati su pokazali da koncentracija šećera od 160 gL^{-1} izaziva značajno manje ulutkavanja i eklodiranja kod *D. suzukii* nego što je to pri koncentracijama od 40 gL^{-1} i 80 gL^{-1}. Produžena i rastegnuta dinamika ulutkavanja i izleganja su zabeležene kod *D. melanogaster* samo pri koncentraciji od 160 gL^{-1}. Kod svih testiranih drozofila nisu detektovane značajne razlike u praćenim parametrima životnog ciklusa između tretmana sa koncentracijama šećera od 40 gL^{-1} i 80 gL^{-1}. Prikazani rezultati mogu biti od koristi prilikom odabira hranljivog medijuma baziranog na kukuruznom grizu za gajenje pomenutih divljih vrsta drozofila.

Ključne reči:

Voćna mušica, *Drosophila melanogaster, Drosophila suzukii,* hranljiva podloga, životni ciklus, koncentracija šećera

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Introduction

The common fruit fly, *Drosophila melanogaster* Meigen is a famous model organism that has very wide implementation in biological sciences, primarily in genetic researches. There are many stock centers in the world that provide an array of different *Drosophila* stocks intended for experimental purposes. Nevertheless, for some research purposes, there is a need for *Drosophila* species that are obtained from the wild. On that occasion, the flies are caught and transferred into a laboratory ambient for further experiments. For instance, species from the genus *Drosophila* caught in the wild are very often processed in this way, for example *D. melanogaster* (Anagnostou et al., 2010; Lin et al., 2014; Gao et al., 2018). Spotted wing drosophila, *Drosophila suzukii* (Matsumura) is a very serious pest that has been extensively explored in laboratory conditions (Lin et al., 2014; Tochen et al., 2014; Silva-Soares et al., 2017; Fellous & Xuéreb, 2017; Schlesener et al., 2017). Since this species is introduced worldwide, it has been already recognized as a real threat on production of certain soft fruits that are of economic importance (De Ros et al., 2013; Benito et al., 2016; Mazzi et al., 2017). Also, the presence of this fruit pest fly is confirmed for the first time in Serbia in 2014 by Toševski et al. (2014). Thus, good laboratory practice is to provide optimal conditions including appropriate feeding media for such species to be transferred from the wild to a laboratory to obtain correct results from conducted experiments.

Apart from laboratory *Drosophila* stocks, it is well-known that wild *Drosophila* species can be successfully cultured on cornmeal-based feeding media, even *D. suzukii* (Schlesener et al. 2017). There are many available recipes that are relevant for *Drosophila* culturing, for example at the site of Bloomington Drosophila Stock Center (https://bdsc.indiana.edu) or in papers (Lewis, 1960; Schlesener et al. 2017; Jovanović et al., 2018; Cvetković et al., 2020). However, different recipes for cornmeal-based feeding media imply different amounts of some ingredients such as carbohydrates and yeast. In addition, numerous articles are dealing with the influence of dietary yeast on *D. melanogaster* (Anagnostou et al., 2010) and in *D. suzukii* on life-history traits (Bellutti et al., 2018) or protein and carbohydrate ratio on larvae in *D. suzukii* (Silva-Soares et al., 2017) and dietary protein and carbohydrates balance in *D. melanogaster* (Lee, 2015). These studies suggested that carbohydrates and proteins play very important role in *Drosophila* lifespan so the special attention should be paid on the concentration of these two ingredients in the feeding media. According to Lushchak et al., (2014) the influence of dietary carbohydrate type and dosage is still poorly understood. In addition, we believe that among different recipes, the most appropriate recipe should be selected for wild *Drosophila* species that are transferred from natural to laboratory conditions because they cannot be adapted to artificial feeding media to the same extent as *Drosophila* laboratory stocks. Here, we ran a study to rapidly screen the degree of tolerance of the two wild species on three different sucrose concentrations in cornmeal-based feeding media at standard laboratory conditions by monitoring of the life cycle parameters. We believe that this will help to depict which cornmeal-based feeding media, in relation to sucrose concentration, is the most suitable for laboratory cultivation of wild *D. melanogaster* and *D. suzukii* collected in Southeastern Serbia. Also, the study should contribute to overall knowledge about influence of dietary sucrose on the life cycle of two *Drosophila* species.

Material and methods

Culturing conditions and types of feeding media

The fruit flies were cultured at standard laboratory conditions which implies 60% of relative humidity, 12h/12h of the day/night light regime and constant ambient temperature at about 25±1 °C. Standard feeding media based on cornmeal was used for culturing of all flies from this experiment. Particularly, agar (6 gL^{-1}), cornmeal (96 gL^{-1}), sucrose (80 gL^{-1}) and yeast (20 gL^{-1}) prepared by cooking in dH_2O with subsequent addition of fungicide as explained in our previous studies (Cvetković et al., 2015; Jovanović et al., 2016).

For purposes of the experiment three different feeding media were prepared: All previously mentioned components were used in the same concentrations, except for the sucrose concentration that varied. Medium with sucrose concentration of 40 gL^{-1} – labeled as half, medium with sucrose concentration of 80 gL^{-1} – labeled as normal and medium with sucrose concentration of 160 gL^{-1} – labeled as double, were used.

Fruit flies

The wild *D. melanogaster* specimens were caught in the City of Niš, near the Faculty of Sciences and Mathematics (43°18'32.5"N; 21°55'22.9"E) in traps using grape as bait, during the September of 2018., leg. V. J. Cvetković. The adult flies were immediately transferred on the standard feeding media for culturing. For the starter generation, *D. suzukii* adults were incubated from larvae from the infested raspberries collected in private raspberry orchard near Lebane town (Southeastern Serbia),

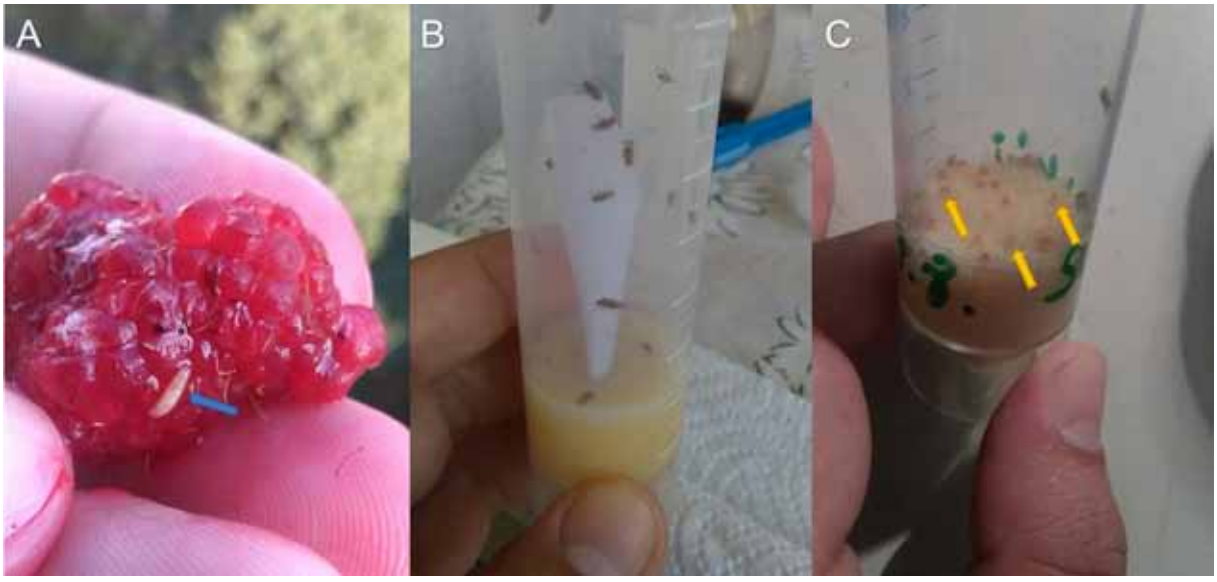

Fig. 1. *Drosophila suzukii* - the starter generation. A – larvae (showed with blue arrow) from infested raspberry. B – eclosed adults, incubated from infested raspberries, transferred on standard fresh feeding media for culturing. C – pupae (showed with yellow arrows) within the feeding media, the first laboratory generation of captured *D. suzukii*. Photos by V. J. Cvetković

42°55'56.1"N; 21°45'21.6"E, during the August of 2018., leg. S. S. Stanković. Immediately after eclosion they were transferred onto fresh standard media for culturing (**Fig. 1**). Captured fruit fly species were determined by V. Žikić and S. S. Stanković. In order to establish stock, the fruit flies from the wild were cultured on standard (normal) feeding media (see *Culturing conditions and types of feeding media*) for a dozen generations during 4 months. The referent laboratory strain of wild-type *D. melanogaster* Oregon-R-C strain (Bloomington Drosophila Stock center at Indiana University, USA), was used in this study as referent line because this strain is adapted on artificial cornmeal-based feeding media that was used in this experiment.

Experimental protocol

The whole experiment was performed at standard laboratory conditions (as explained in *Culturing conditions and types of feeding media*). For the *D. melanogaster* – referent line and two wild species flies, three different types of vials were prepared, ten of each, that contained half, normal and double concentration of sucrose in experimental feeding media. From referent laboratory stock culture (*D. melanogaster*) and wild flies established stocks (*D. melanogaster* and *D. suzukii*), pairs of young flies at the same age (5-7 days old) were chosen for further analyses, similarly as it was done in our previous research (Jovanović et al., 2018). In total, ten pairs were transferred into ten vials in each type of experimental feeding media to mate and lay eggs for two days. Afterwards, the adult flies were discarded and the oviposited eggs were counted immediately. Each day, number of formed pupae and subsequently eclosed adults was recorded.

Statistical analysis

The statistical analysis was performed within each of the three *Drosophila* group. The comparisons were performed by the following parameters: 1) the total numbers of oviposited eggs, 2) the total numbers of formed pupae and 3) the total numbers of eclosed adults, for each of the three sucrose concentrations.

The percentage of pupation (percentage of formed pupae from hatched and survived larvae) and eclosion (percentage of eclosed adults from formed pupae) were calculated as it was described by Kinjo et al. (2014). The obtained values for the same parameter (percentage of pupation or eclosion) in feeding media with different sucrose concentration were compared within each of the *Drosophila* group.

Statistically significant differences were determined with the Kruskall-Wallis ANOVA test for $p<0.05$. Further, post hoc Mann-Whitney test was used to determine significant differences between any of two compared values. Statistical analysis was conducted by using software package SPSS (SPSS Inc., Chicago, IL, USA).

Results

Statistical analysis of the life cycle parameters i.e. the total number of oviposited eggs, formed pupae and eclosed adults are presented in **Tab. 1**. The presented mean values and standard deviations were obtained based on 10 replicates for each strain, *D.*

Table 1. Life cycle parameters in *D. melanogaster*-referent line, wild *D. melanogaster* and *D. suzukii* cultured on feeding media with different sucrose concentrations. Mean values are presented with ± standard deviation.

Species	Sucrose concentration	Oviposited eggs	Formed pupae	Eclosed adults
D. melanogaster	half	57.63 ±19.04	30.13 ±11.42	29.63 ±11.61
	normal	71.5 ±21.19	41.1 ±14.49	40.7 ±14.24
	double	65.87 ±29.43	37.88 ±11.51	36.38 ±11.36
D. melanogaster referent line	half	71.22 ±24.54	62.22 ±20.28	59.11 ±17.88
	normal	75.38 * ±20.70	70.38 * ±16.49	69.63 * ±16.55
	double	55.4* ±19.98	50.1 * ±18.54	48.00 * ±17.51
D. suzukii	half	11.5 ±8.22	8.6† ±5.68	8.1† ±5.5
	normal	12.2 ±6.05	8.8‡ ±2.3	8.5‡ ±3.2
	double	7.1 ±5.09	2.9 †‡ ±3.6	2.7 †‡ ±3.3

-Values marked with the same symbol * are significantly different within *D. melanogaster* – referent line
-Values marked with the same symbol † and/or ‡ are significantly different within *D. suzukii* group.
-Significant differences were considered for $p<0.05$

melanogaster-referent line, wild *D. melanogaster* and *D. suzukii*. All calculations were done for each of three feeding media types.

The only significant difference in percentage of pupation between normal and double concentration were recorded in *D. suzukii* (**Fig. 2**).

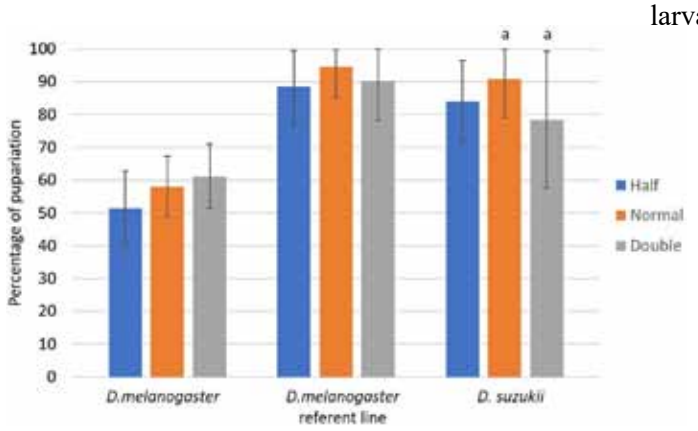

Fig. 2. Percentage of pupation in *D. melanogaster, D. melanogaster*-referent line and *D. suzukii* cultured on feeding media with half, normal and double concentration of sucrose. The lower case letter "a" marked the two values that are significantly different. Differences were determined using Kruskall-Wallis ANOVA and post hoc Mann-Whitney test. Statistical significance was considered for p<0.05

The only significant differences in percentage of eclosion were observed between normal and double group within wild *D. melanogaster* and normal compared to half and double within *D. melanogaster* – referent line (**Fig. 3**).

In *D. melanogaster* – referent line, the peak of larval pupation was recorded on the fourth day, fifth and the seventh day in half, normal and double sucrose concentration, respectively (**Fig. 4**). The peak of adult eclosion was recorded at the fifth day in half and normal and at the eighth day in double sucrose concentration (**Fig. 4**). In addition, the peaks were higher and the pupation, as well as eclosion, lasted shorter in groups with half and normal sucrose concentration than in the group with double of the sucrose concentration.

In the wild *D. melanogaster*, the peak of larval pupation was recorded on the fourth day in normal and double and the eighth day in half concentration of sucrose (**Fig. 5**). The peak of adult eclosion was recorded also on the fourth day in half and normal and on the eighth day in a double concentration of sucrose (**Fig. 5**). In addition, the peaks were higher and the pupation, as well as eclosion,

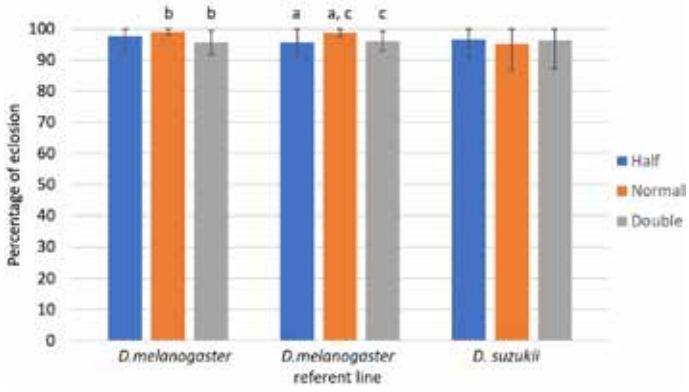

Fig. 3. Percentage of eclosion in *D. melanogaster, D. melanogaster*-referent line and *D. suzukii* cultured on medium with half, normal and double concentration of sucrose. The same lower cases letter (a, b or c) marked the two values that are significantly different. Differences were determined using Kruskall-Wallis ANOVA and post hoc Mann-Whitney test. Statistical significance was considered for $p<0.05$.

lasted shorter in groups with half and normal sucrose concentration than in double of the sucrose concentration.

In *D. suzukii* line, the peak of larval pupation was recorded on the third, fourth and the seventh day in half, normal and double sucrose concentration, respectively (**Fig. 6**). The peak of adult eclosion was recorded on the fourth, fifth and the eighth day in half, normal and double concentration of sucrose, respectively (**Fig. 6**). Additionaly, the peaks were higher in groups with half and normal sucrose concentration than in double of the sucrose concentration but the duration of the pupation and eclosion was mostly the same in all three groups.

Discussion

All three parameters of the life cycle – the total number of oviposited eggs, formed pupae and

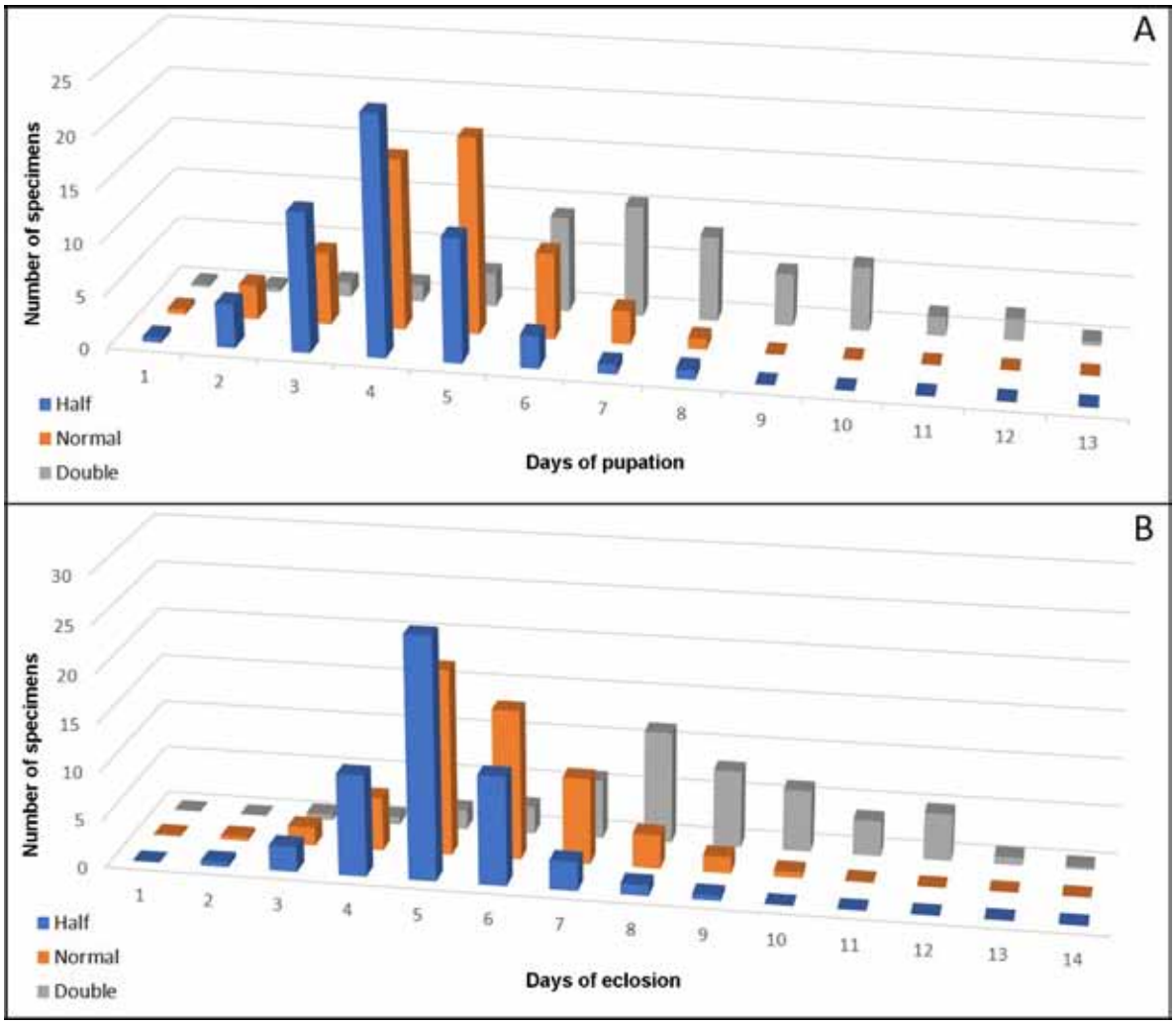

Fig. 4. Dynamics of pupation and eclosion in *D. melanogaster* – referent line. A – Dynamics of larval pupation and B – Dynamics of adult eclosion. Half, normal and double refers to sucrose concentration in feeding media.

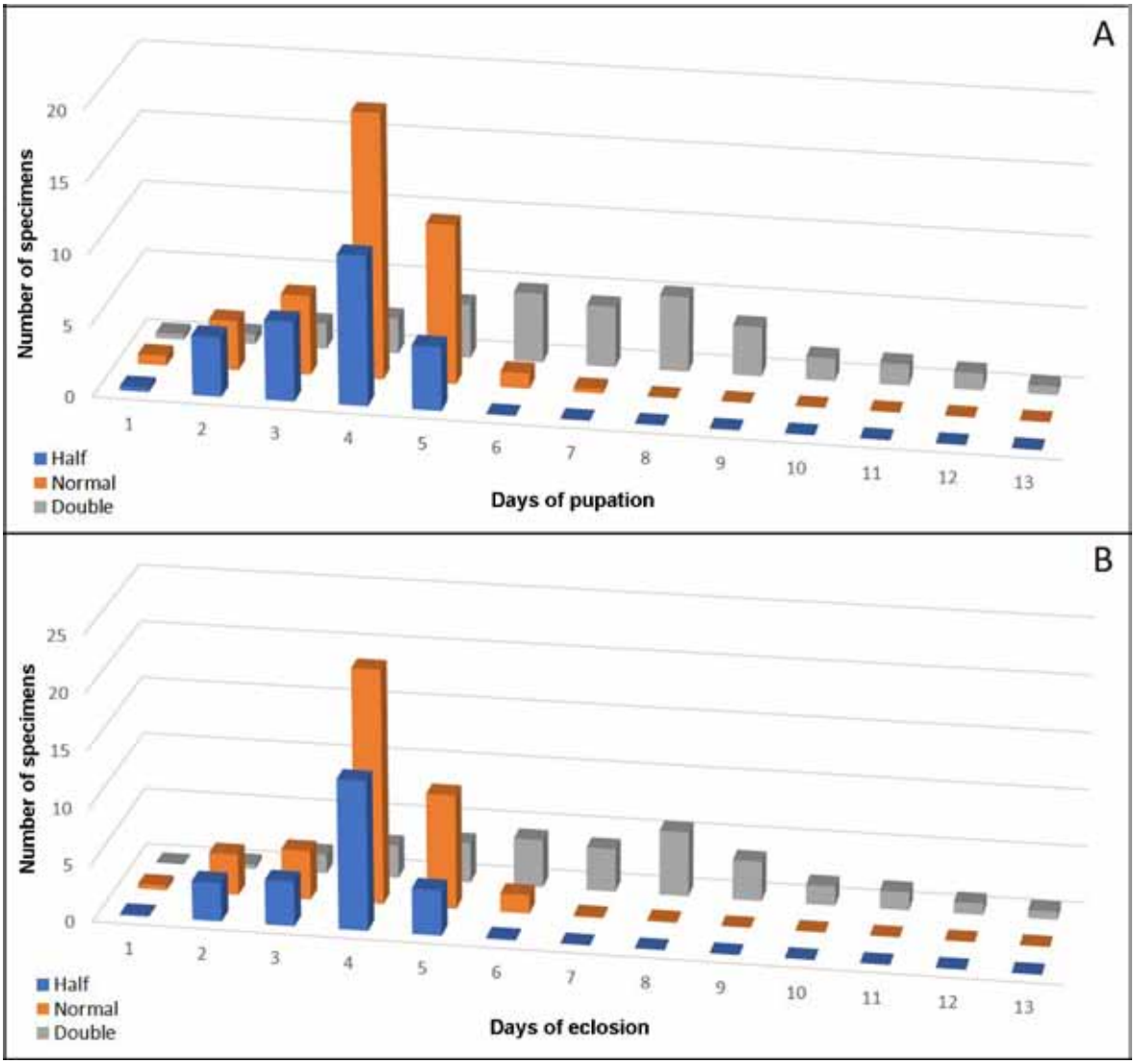

Fig. 5. Dynamics of pupation and eclosion in wild *D. melanogaster.* A – Dynamics of larval pupation and B – Dynamics of adult eclosion. Half, normal and double refers to sucrose concentration in feeding media.

eclosed adults were not significantly different in the wild *D. melanogaster* cultivated on feeding media with different sucrose concentrations. According to these results, the wild *D. melanogaster* strain used in this experiment were tolerant to variation of sucrose concentration in feeding media at standard ambient conditions. In *D. suzukii*, at double sucrose concentration, the total number of formed pupae and eclosed adults were significantly lower ($p<0.05$) than in half and normal sucrose concentrations. However, the total number of oviposited eggs was not significantly different between groups with different sucrose concentrations. In some way, the higher sucrose concentrations disrupted incubation of the eggs and/or development of the hatched larvae until the pupation stage. Also, in the *D. melanogaster*-referent line significantly lower ($p<0.05$) numbers of oviposited eggs, formed pupae and eclosed adults were observed in double than in normal sucrose concentration. A possible explanation is that the higher sucrose concentration in feeding media probably stimulates the increase of the products of fermentation. The higher levels of the fermentation products (ethanol, carbon dioxide etc.) may have a negative impact on larval hatching, development as well as pupation. Also, it might have a negative effect on the number of oviposited eggs that was significantly lower in double concentration in *D. melanogaster* – referent line. This hypothesis is waiting to be confirmed or rejected in further experiments because this time we did not measure the levels of fermentation products in our experiment.

A lower percentage of pupation were recorded in all three types of feeding media in the wild *D.*

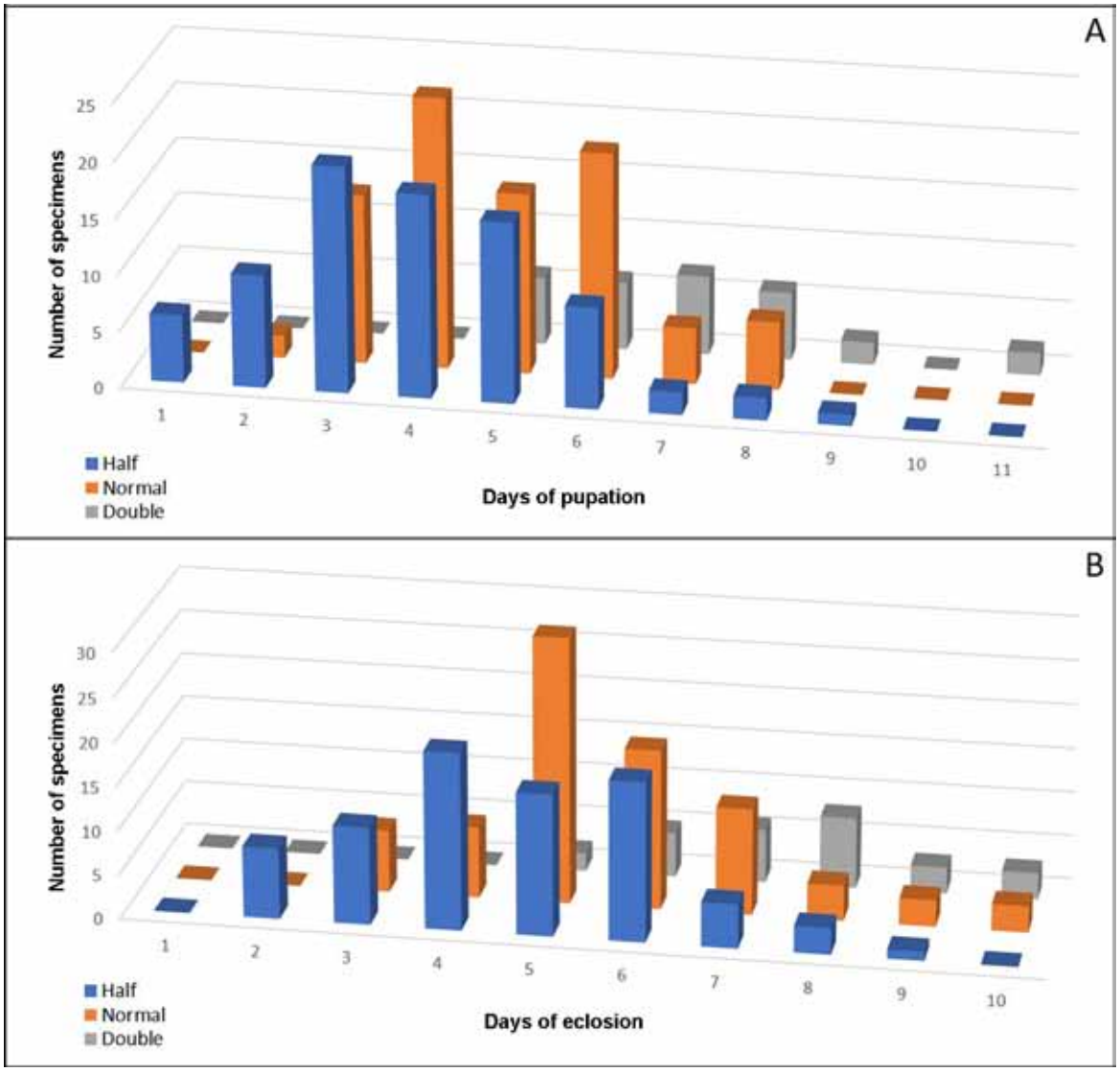

Fig. 6. Dynamics of pupation and eclosion in wild *D. suzukii*. A – Dynamics of larval pupation and B – Dynamics of adult eclosion. Half, normal and double refers to sucrose concentration in feeding media.

melanogaster while in the *D. melanogaster* – referent line was higher. This means that the laboratory *Drosophila* strain is well adapted on applied cornmeal feeding media, as it was expected. The percentage of pupation values in *D. suzukii* were about 90% in average. As we were not able to count the hatched larvae, the much lower percentage of pupation could mean that many of the eggs were not fertilized or/and that many larvae have died before pupation in wild *D. melanogaster*. Thus, these results indicate that standard laboratory conditions and applied feeding media (for medium details see section: *Culturing conditions and types of feeding media*) influenced pupation in wild *D. melanogaster*. Besides previous observations, in wild *D. melanogaster* percentage of pupations were not significantly different in all three sucrose concentrations, as in the *D. melanogaster* – referent line, while in *D. suzukii* percentage of pupation was significantly lower in double concentration compare to normal sucrose concentration. We propose one possible explanation for such a result. During pupation, larvae of *D. melanogaster* mostly went out of the feeding media and formed pupae on the walls of the vials where they were cultivated. In *D. suzukii*, pupae are formed mostly within the feeding media. Thus, the explained effects of high sucrose concentrations in feeding media and the consequent presence of higher levels of fermentation products like ethanol and carbon dioxide might affect the pupation process in this case by affecting the larvae hatching or/and eggs incubation or/and larvae survival. As we did not count the number of hatched larvae, so we cannot state at which state the detrimental effect occurred,

this observation remains to be tested in forthcoming analysis. Gao et al. (2018) showed that both adults and larvae of *D. melanogaster* were more tolerant of environmental ethanol than *D. suzukii* (Gao et al., 2018). This is consistent with our observation about the influence of possible higher levels of ethanol in double sucrose concentrated media. In addition, it has long been known that *D. melanogaster* was more tolerant on alcohol compared to other *Drosophila* species (McKenzie & Parsons, 1972).

On the other hand, a minor difference in the percentage of eclosion in both wild *Drosophila* species as in the *D. melanogaster* – referent line in any type of the tested feeding media. This could mean that the process of incubation from pupal to the adult stage was not affected in the same percentage as the pupation process by different sucrose concentrations in any experimental group since pupal mortality was very low in all *Drosophila* groups. Only significant differences showed that the percentage of eclosion was significantly affected by double sucrose concentration comparing with normal within the wild *D. melanogaster* group. Also, within *D. melanogaster* – referent line, in double and half concentration percentage of eclosion was significantly affected compared to normal concentration (the percentages of eclosion were above 95%). However, these differences were minimal in both *D. melanogaster* groups. Interestingly there were no significant differences between the percentage of eclosed flies in different feeding media within *D. suzukii* group, so the different sucrose concentrations did not affect *D. suzukii* at the pupal stage.

It was already stated that in the wild *D. melanogaster* there were no significant differences for the total number of formed pupae and eclosed adults at different sucrose concentrations. But the differences were observed at dynamics of pupation and eclosion. The peaks for the dynamic of these parameters in half and standard concentration was on the fourth day while in double was about the eighth day. The pupation and eclosion were very prolonged and stretched at double concentration compared with the half and standard within the wild *D. melanogaster*. Analyzing these parameters in the *D. melanogaster* – referent line group and *D. suzukii* group, a similar pattern could be observed in the double sucrose concentration. Nevertheless, in *D. suzukii* the other two groups, half and normal, also express prolonged pupation and eclosion without pronounced peaks unlike both *D. melanogaster* groups. Thus, due to its detrimental effect in all groups the double sucrose concentration in feeding media caused prolonged and extended pupation and eclosion. The reason might be the same, explained in previous paragraph, the detrimental effect of the higher level of fermentation products. On the other hand, half concentration did not cause any visible effect on dynamics of pupation and eclosion in any *Drosophila* group. Overall, our findings is in accordance with the conclusion of Fellous & Xuéreb (2017) that high sugar concentrations are detrimental for development of the *D. suzukii*. Also, Lushchak et al., (2014) stated that sucrose, commonly used for *Drosophila* laboratory food, shorten lifespan and contribute to lower egg-laying capability in *D. melanogaster*. According to the results and previous observations, the detrimental effects, especially of the highest applied sucrose concentration are obvious in *Drosophila.* Even more, *D. suzukii* was more sensitive to higher sucrose concentration than *D. melanogaster*.

There are many available cornmeal-based feeding media recipes that can be applied for laboratory culturing of *Drosophila* species, but the concentrations of ingredients may vary in different recipes. Despite differences in ingredients, many of them can be used for *Drosophila* culturing. For example, some fly food recipes are available at the site of Bloomington Drosophila Stock Center from Indiana University in USA (https://bdsc.indiana.edu). Schlesener et al. (2017) reported modified cornmeal-yeast-glucose-agar medium that are suitable for *D. suzukii* rearing. The composition of standard feeding media that we use in this experiment were not the same but it is quite similar as in Schlesener et al. (2017). We found that one of the differences between the feeding media that we used from the mentioned one is in yeast concentration. Particularly, we used almost twice smaller concentration of yeast, as a protein source, what in addition to the highest applied sucrose concentration also might influenced development of the *D. suzukii* in our experiment. However, we did not vary yeast concentration in this experiment because it was not the subject of this research and in all treatment media was added in same amount according to one of the recipe (see section: *Culturing conditions and types of feeding media*) that we use for laboratory *Drosophila* stocks culturing. Also, Fellous & Xuéreb (2017) reported that intermediate protein concentration is optimal for *D. suzukii* development. These observations indicate that protein concentration in feeding media should be taken into consideration when choosing appropriate feeding media for wild *D. suzukii* and we believe also for *D. melanogaster* because it is known that dietary balance of proteins and carbohydrates concentrations is important because its influence on *Drosophila* lifespan (Lushchak et al., 2014; Lee, 2015).

Conclusion

According to our findings the sucrose concentration of 160 gL^{-1} in the feeding media caused significantly lower number of formed puape and eclosed adults in *D. suzukii* than at concentrations of 40 gL^{-1} and 80 gL^{-1}. In all tested *Drosophila* there were no significant differences in analyzed life cycle parameters between sucrose concentrations of 40 gL^{-1} and 80 gL^{-1}. Moreover, there were no significant differences between any of three tested sucrose concentrations for numbers of laid eggs, pupation and eclosion in wild *D. melanogaster*. The dynamics of pupation and eclosion were prolonged and stretched in wild *D. melanogaster* at 160 gL^{-1} sucrose concentration while in *D. suzukii* was mostly the same as in 40 gL^{-1} and 80 gL^{-1}. We believe that the presented results might be helpful in deciding which cornmeal-based feeding media, in relation to the sucrose concentration, should be selected for the cultivation of mentioned *Drosophila* species that originating from the wild. Also, the obtained results represent contribution to the overall knowledge of the influence of dietary sucrose on lifecycle of two *Drosophila* species.

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