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Pollination and pollinators of haskap (*Lonicera caerulea*)

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Abstract: The study was focused on pollination in haskap (varieties Viola and Gerda). The aim was to verify impact of free pollination on fruit harvest and to observe bee haskap pollinators. The percent fruit set was compared among four treatments: free pollination, hand-self/cross-pollination and no manipulation. The significantly highest production was found under free pollination in the both varieties. The fruit production under isolation was without statistically significant differences with except of Viola in the hand-cross-pollinated treatment. Similarly, in case of the fruit weight, the differences were highly significant in the both varieties Gerda and Viola. The significantly heaviest fruits were under free pollination in both varieties. The average fruit weight in Viola under isolation was significantly higher only in hand-cross-pollinated treatment. In Gerda, only treatment without manipulation was significantly different from hand-cross pollination. The free pollination resulted in earlier and shorter harvesting in the both varieties. Entomophilous character of haskap is proven exactly. The hand-cross-pollination was not able to maximize the fruit set. This proves that there is any unspecified impact of pollinators on effectivity of pollination in haskap. Preliminary results on haskap bee pollinator diversity suggest preference by long-tongued bees especially bumblebees. Other experiments have to be carried out to more clarify the reasons for low haskap productivity under isolation with hand-cross-pollination.

Key Words: Lonicera caerulea, haskap, hand pollination, fruit set, pollinator

INTRODUCTION

There are concerns about how to meet the growing food demand while protecting ecosystems and biodiversity (Brussaard et al. 2010). One factor how to increase the crop production in line with sustainable development is to provide crops with optimal pollination to maximize the quantity and quality of the yield (Garratt et al. 2013).

Lonicera caerulea var. *kamtschatica*, also known as haskap (Figure 1), honeysuckle or honeyberry, is fruit shrub producing edible fruits ripening extremely early – even before strawberry. Haskap is resistant to very low temperatures, plant up to -40 °C and flowers up to -8 °C (Řezníček and Salaš 2015). The recent original distribution of haskap is circumpolar (Frier et al. 2016) and becomes to be popular fruit for similar flavour as blue berries and potential health benefits (Svarcova et al. 2007).

Haskap has double-flower inflorescence forming a compact fruit with two berries (Frier et al. 2016). The fruit shape and harvest time depend on the cultivar features. Fruits ripen from the end of May till June in temperate zone (Bożek 2012).

Hascap is highly rewarding bee forage plant with attractive nectar (Bożek and Wieniarska 2006) and pollen (Bożek 2007). These features indicate demands of haskap on entomophily, therefore, cross-pollination and self-incompatibility. There were performed experiments on pollination under free and isolated conditions (Bożek 2012) confirming self-incompatibility and by different pollinators (Frier et al. 2016) confirming impact of pollinator specificity. Self-incompatibility in different haskap varieties was proved also cyto-embryologically (Boyarskikh 2017).

For comprehensively experimental examine pollination requirements it needs to be designed pollinated positive controls under isolation (Corber et al. 1991). The impact of hand-self/cross-pollination in haskap under isolation has not yet been verified, thereby, impact of free pollination/pollinators is not sufficiently assessed.

Therefore, the aim of this study was to compare percent fruit set and average weight of fruit in dependence of different pollination method also under isolation. Diversity of bee pollinators (Hymenoptera: Apiformes) was observed during blooming period of haskap.

MATERIAL AND METHODS

Experiment was carried out in spring 2018 in Žabčice (southern Moravia, Czech Republic), on the experiment farm of Mendel University where are black soils. The flat surface with average altitude 185 m and average precipitation 380–550 mm and average year temperature over 10 °C dominate in this area. Two varieties (Viola and Gerda) were selected for this experiment. Hand pollination was made by very soft painting brush. Isolation of flowers against free flying insects was achieved by special textile around branches – organdy and fruits against birds by plastic net covering whole shrubs. Rainproof marker was used to marking of flowers. The pollen from variety Tomichka and cultivar Průhonický semenáč (wild seedling) were used for pollination of varieties Viola and Gerda due to their compatibility (Boyarskikh 2017). Entomological hand-catching net was used for sampling bees pollinating haskap.

The experimental design was created according to principles by Corbet et al. (1991). Therefore, four treatments were founded: a) unlimited access of pollinators – free pollination, and three isolated treatments without pollinators b) hand-pollination by own pollen – self-pollination, c) hand-pollination by foreign pollen from compatible pollenizers – cross-pollination and d) without any manipulation.

Each variety (shrubs) and each treatment (branches) consist from three repetitions – triplets (n=3). On every branch up to one hundred flowers were included in experiments in following blooming dates: 9. 4., 11. 4., 13. 4., 15. 4., 17. 4., 19. 4. and 22. 4. 2018. Harvest of fruits was performed in these dates: 8. 5., 11. 5., 14. 5., 16. 5., 18. 5. and 21. 5. 2018. The percent fruit set was counted as proportion the number of fruit to the number of inflorescences. For every partial harvest were found number of fruits and total weight of harvested fruits. The proportion of these parameters was used to determine average weight of fruit.

Bees pollinating haskap were sampled in appropriate sunny weather and form 10 to 16 hours at site in Žabčice, Brno and Příbram na Moravě. The 20 honeybee colonies were placed in close proximity of hascap plantation in Příbram n. M.

The results were statistically analysed with a one-way ANOVA analysis and a post-hoc Tukey's test ($\alpha = 0.05$). The percent fruit set were transformed by arcsin (x) to improve normality.

Figure 1 Haskap – Lonicera caerulea:

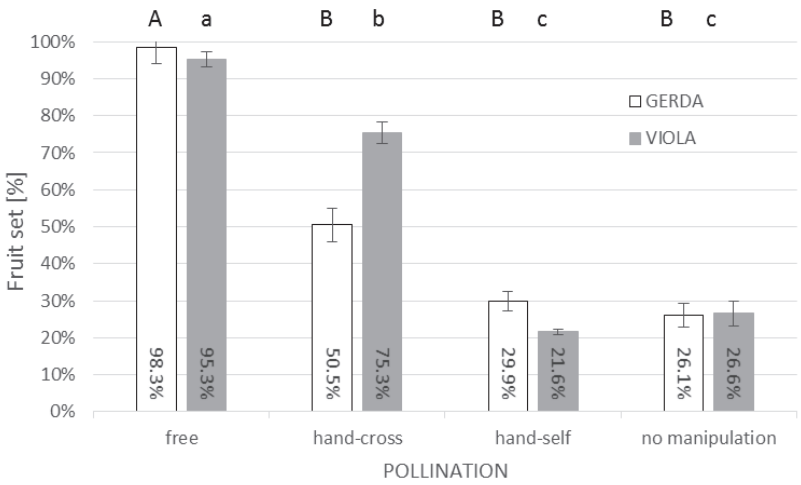
a) hand-made pollination *b) small garden bumblebee Bombus hypnorum* *c) haskap fruits*

RESULTS

Differences in the percent fruit set in dependence of the pollination method are depicted in Figure 2. The differences were highly significant in the both varieties Gerda ($F_{0.95\ (3,8)} = 41.99$; $P < 0.001$) and Viola ($F_{0.95\ (3,8)} = 34.53$; $P < 0.001$). The significantly highest production (Tukey test,

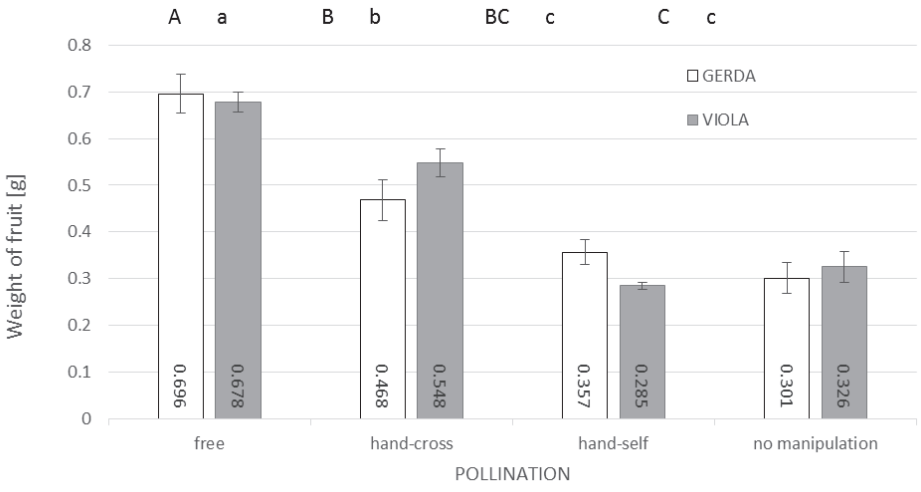
$\alpha = 0.05$) was found under free pollination in the both varieties. The fruit production under isolation was without statistically significant differences ($\alpha > 0.05$) with except of Viola in the hand-cross-pollinated treatment.

Figure 2 Percent fruit set according to variety and pollination method (ANOVA, Tukey test, $\alpha = 0.05$)



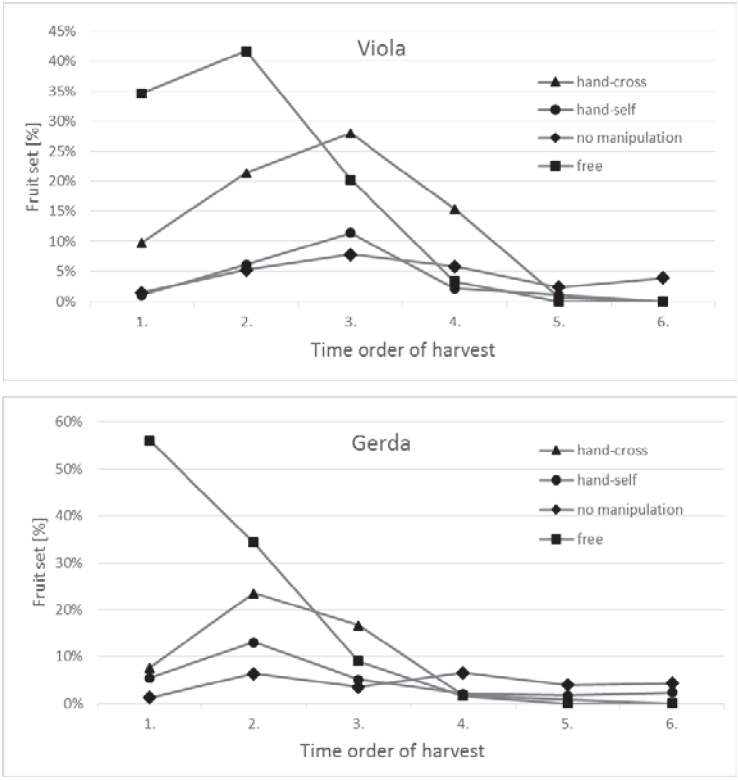
Similar but not the same results were in case of the fruit weight (Figure 3). The differences were highly significant in the both varieties Gerda ($F_{0.95\ (3,8)} = 22.12$; $P < 0.001$) and Viola ($F_{0.95\ (3,8)} = 56.39$; $P < 0.001$). The significantly heaviest fruits (Tukey test, $\alpha = 0.05$) were recorded under free pollination in both varieties. The average fruit weight in Viola under isolation was significantly higher ($\alpha > 0.05$) only in hand-cross-pollinated treatment. In Gerda, only treatment without manipulation was significantly different from hand-cross pollination.

Figure 3 Average weight of fruit according to variety and pollination method (ANOVA, Tukey test, $\alpha = 0.05$)



The free pollination resulted in earlier harvesting in the both varieties (Figure 4). The top of harvesting intensity (i.e. the highest fruit set per harvest day) was achieved clearly at first under the free pollination treatment. The harvest tops came later (about one harvest day) under isolation compared with the free pollination and at the same time compared with these three treatments. Duration of harvesting was longer about 2–3 days under isolated treatments of the pollination methods.

Figure 4 Percent fruit set in time order of harvest



There were preliminarily observed several species of bee pollinators (Table 1). Extremely high density of bumble bees was recorded just in Příbram n. M. and contrary in Žabčice and Brno where the solitary bees or honeybees were more numerous. Higher density was typical of the long-tongued bees; in bumblebees *Bombus hortorum* and in solitary bees *Anthophora plumipes*.

Table 1 Preliminary results on diversity and dominance of bee pollinators in Lonicera caerulea

Location →	**Žabčice**	**Brno**	**Příbram n. Moravě**
Bumblebees ***Bombus* spp.**	*Bombus* - *hortorum* - *pratorum* - *sylvarum* - *lapidarius* - *terrestris* [13%]	*Bombus* - *hortorum* - *pratorum* - *pascuorum* - *hypnorum* - *lapidarius* - *terrestris* - *lucorum* [9%]	*Bombus* - *hortorum* - *hypnorum* - *pratorum* - *pascuorum* [94%]
Solitary bees	*Anthophora plumipes* *Evyleus pauxillus* *Evylaeus morio* *Osmia bicornis* *Andrena bicolor* *Xylocopa* (*Xylocopa*) sp. *Eucera nigrescens* [42%]	*Anthophora plumipes* *Evylaeus morio* *Osmia cornuta* *Andrena flavipes* *Xylocopa* (*Xylocopa*) sp. [59%]	*Anthophora plumipes* *Nomada succincta* [4%]
Western honeybee ***Apis mellifera***	[45 %]	[32%]	[2%]

Legend: Names of species are listed in order of decreasing dominance. Underlined names represent eudominant species. Percentages in square brackets represent group dominance.

DISCUSSION

The significantly highest percent fruit set under free pollination and three-time lower one under isolation without manipulation confirm results by Bożek (2012). Entomophilous character of haskap is undisputable also with respect to the delayed harvesting of fruits in the treatments under isolation. The shorter harvest period due to optimal pollination conditions was described also in other crops (Williams

1985, Racys and Montviliene 2005). It is remarkable that the hand-cross-pollination was not able to maximize the fruit set in comparison with free pollination. This result indicates that there was any "pollinator" factor(s) in the free pollination maximizing fruit productivity (both fruit set and weight). Possible explanations are as follow: haskap flower needs a) higher number of visits to be thoroughly fertilized with aim to place higher amount of pollen grains on stigma (Rader et al. 2009, Frier et al. 2016) and/or b) specific pollenizer to achieve compatibility (Boyarskikh 2017). Due to these possible factors the differences in the fruit productivity between hand-cross- and hand-self-pollination treatments were statistically significant (in Viola) or insignificant (in Gerda). The minimal insignificant differences between treatments hand-self-pollinated and without manipulation could be caused by low limited level of self-compatibility. Flowers without manipulation were pollinated by an assumed internal process of self-transfer of own pollen grains inside of the same flower from anther to stigma (Frier et al. 2016). Likely, this self-fertilization process was able to maximise fruit productivity by itself, therefore, the hand-transferred pollen grains inside of the same flower by brush was redundant. It was not so in the case of hand-cross-pollination compared with hand-self-pollination where the transfer of foreign pollen increased the fruit productivity as it is discussed above. Other experiments have to be carried out to more clarify the reasons for low haskap productivity under isolation with hand-cross-pollination.

Honeybees are able to gain nectar and pollen as it was apparent from higher density at Žabčice. However, in spite of 20 colonies in close proximity haskap, extremely low honeybee dominance was recorded. These results indicate high competitive effect of long-tongued bees due to deep corolla in haskap flowers. Habitats in vicinity of Příbram n. M. are more appropriate for nesting of bumblebees. Haskap is circumpolar species. In this region the best adapted bee pollinators are just bumblebees (Kevan et al. 1993). It is possible that there is any ecological relationship "plant-pollinator". Therefore, it is probable the bumblebee populations were somewhat disadvantaged beside other bees at the sites with lower altitude and warmer and dryer conditions (i.e. Brno and Žabčice). The "*Lonicera-Bombus*" relationship can be also somewhat special as it was confirmed in the case of *Lonicera periclymenum* (Ottosen 1987). How complicated and heterogeneous relationships are between plants and pollinators is currently being discovered (Brittain et al. 2013).

CONCLUSIONS

1. Haskap harvest parameters (percent fruit set, fruit weight and term and duration of harvest) were not optimized even by hand-cross-pollination treatment in compare with free pollination. This proves that there is any unspecified impact of pollinators on effectivity of pollination process in haskap.

2. Preliminary results on haskap bee pollinator diversity suggest preference by long-tongued bees especially bumblebees.

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