THE EFFECT OF SILICON ON NECTAR AND POLLEN PRODUCTION IN Hosta Tratt. ‘Krossa Regal’

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Abstract. Recently, the importance of silicon (Si) has been demonstrated for many plant physiological processes. The recognized function of Si is to help plants to overcome multiple biotic and abiotic stresses, e.g. disease or pests, low temperature, water deficit, salinity or heavy metals. Silicon advantageously impact on plant development and may improve the quality of ornamental plants. Hosta is an ornamental perennial, that flowers can serve as a source of nectar and pollen for floral visitors. The effect of artificial silicon (Si) supply on flowering, nectar production and pollen traits in Hosta Tratt. ‘Krossa Regal’ was studied. A foliar spraying of 0, 120, 180 and 240 mg Si dm$^{-3}$ was applied with water solutions of Actisil Hydro Plus, containing silicon in the form of H$_4$SiO$_4$. Silicon supply in concentration of 180 and 240 mg Si dm$^{-3}$ affected the morphometric traits of the flower (perianth width and floral tube length), but not the number of flowers produced per inflorescence. The silicon supply in concentration of 180 and 240 mg Si dm$^{-3}$ resulted in the increase of nectar production and in sugars excess in floral nectar. Treatments in concentration of 180 and 240 mg Si dm$^{-3}$ positively influence both pollen production and pollen viability. Presumably, most of these results can be explained through the effect of the Si on metabolism enhancement, e.g. by water balance regulation and increase in photosynthetic efficiency.

Key words: Actisil Hydro Plus, foliar fertilizer, flowering, nectar, pollen

INTRODUCTION

Multiple exogenous and endogenous factors have long been known to affect distinctive stages of plant growth and development. Particularly, environmental variables such as temperature, light intensity, the availability of water and essential minerals are decisive. The nutrients (macro- and microelements) are involved in proper biochemical, physiological and biological functioning of integrated plant system [Szymańska 2012].
However, little is known how fertilization, commonly applied in horticultural flowering plants influences nectar and pollen production and their quality. It has been found that soil fertilization affects the quality of floral nectar, e.g. fertilization with nitrogen caused the increase in concentration of amino acids in the nectar from *Agrostemma githago* (corncockle) [Gardener and Gillman 2001] and had a positive effect on the pollen performance of *Cucurbita pepo* (pumpkin) [Lau and Stephenson 1993]. The ambiguous effects of fertilization on nectar production are reported by Petanidou et al. [1999].

Knowledge of the links between nutrient availability and floral characters is a first step towards understanding the direct and indirect effects of nutrients on plant function. Recently, the importance of silicon (Si) has been demonstrated for many plant physiological processes [Ma and Takahashi 2002, Kamenidou et al. 2008, 2010, Wróblewska and Dębicz 2011]. The recognized function of Si is to help plants to overcome multiple biotic and abiotic stresses, e.g. disease or pests, low temperature, water deficit, salinity or heavy metals [Mateos-Naranjo et al. 2013]. Under abiotic stressed conditions, silicon supports the efficient water utilization by improvement of osmoregulation and impact on the water status by reduction in water loss during transpiration [Ma and Takahashi 2002, Kaya et al. 2006, Kazemi et al. 2012, Rubinowska et al. 2014]. It has also been recognized that silicon highly contributes to photosynthesis efficiency, causes the inhibition of reactive oxygen status (ROS) [Shen et al. 2010], partakes in adequate supply of nutrients [Sacala 2009] and accelerates the accumulation of various organic and inorganic osmolytes like proline (Pro), glycinebetaine (GB), or others [Ahmad and Haddad 2011]. Most horticultural plants are non-Si accumulators, however the number of reports have shown beneficial effects of Si application on the quality of crops [Kamenidou et al. 2008, 2009], leading to the increase of attention in Si fertilization in both agricultural and horticultural production [Adamiak and Hetman 2006, Wróblewska and Dębicz 2011, Rubinowska et al. 2014].

Silicon (Si) is a component of Actisil Hydro Plus – a liquid fertilizer proper for both soil and foliar fertilization. The Actisil Hydro Plus is a source of easily digestible silicon, present in a form of orthosilicic acid (H₄SiO₄). After foliar spraying, the silicon diffuses through cuticle and epidermis and is stored in cell walls of mesophyll parenchyma [Marschner et al. 1990].

Here, we investigated whether or not foliar application of Si from Actisil Hydro Plus fertilizer impact on the flowering as well as the nectar and pollen production. A model plant was *Hosta ‘Krossa Regal’* – a species with a large flowers (adequate for nectar and pollen harvest). In particular, we examined the impact of Si concentration on the number of flowers, the flower morphometrics, nectar production and pollen traits.

**MATERIAL AND METHODS**

*Experiment design.* The observations were conducted in 2012–2013. *Hosta Tratt.* ‘Krossa Regal’ was cultivated in experimental field of the Department of Ornamental Plants of the University of Life Sciences in Lublin, Poland (51°11’ N, 22°28’ E). The plants were grown on separated plots for each treatment (6 × 3 = 18 plants per treatment). We chose Actisil Hydro Plus, foliar fertiliser, popular on Polish market. The
plants were sprayed with water solutions of Actisil Hydro Plus, containing 0.6% of silicon in the form of H₄SiO₄. The concentrations of Actisil 0.2%, 0.3%, and 0.4% (treatments 1, 2, 3, respectively), being an equivalent of 120, 180 and 240 mg Si·dm⁻³ were applied. The foliar spray of distilled water (= no mineral fertilizer treatment) was a control (treatment 0). Plants were treated six times during the growing season in weekly intervals, starting from May 9th in 2012, and from May 21st in 2013, always in the morning hours (between 7.00–8.00).

The collection of samples for morphometric measurements and for nectar and pollen analyses was carried out in late morning (9.30–11.00), at full flowering phase [Denisow 2009b]. To minimize the impact of flower position within the inflorescence on the analysed criteria only the flowers from the mid raceme parts were harvested for analyses. Safeguarded samples were immediately transported to the laboratory.

**Morphometric measurements.** Evaluation of flower number per inflorescence was assessed for 20–30 inflorescences per treatment. The following phenotypic traits at randomly selected flowers were determined in the stage just after opening (1) narrow tube length (Nl) – measured as the distance from the tube base to the base of broad tube; (2) broad tube length (Bl) – the distance from the broad tube base to the top of broad tube lobs; (3) narrow tube width (Nw) – quantified in the middle of tube length; (4) broad tube width (Bw) – quantified in the middle of tube length (fig. 1). The total number of flowers measures was n = 105 in 2012, and n = 111 in 2013. The morphometric measurements were performed using a digital calliper (± 0.1 mm of error).

**Nectar production.** Nectar was collected from flowers three times during full bloom phase. The pipette method was applied to collect the nectar [Jabłoński 2002]. Prior to nectar collecting, flowers were covered by the tulle isolators to exclude insect visitors. In each replication, 5–6 samples were collected (one flower per inflorescence); a single sample contained nectar from 5–8 flowers. Sugar concentration (in %) was measured with an Abbe refractometer. Nectar volume (in mg) and sugar concentration were used to calculate the total sugar mass in each sample. Relevant calculations allowed determination of the amount of sugars produced per flower (in mg).
**Pollen production.** The mass of pollen production was established using the ether method [Denisow 2009a]. Mature buds were collected and unopened anthers were separated from filaments and inserted in weighed glass containers (n = 60 anthers, in four replications per treatment). The glass containers were placed into a dryer (ELCON CL 65) at ca. 33°C. After the anthers opening, the pollen was rinsed from anthers once with pure ether (1–2 ml) and then 2–3 times with 70% ethanol (2–8 ml). The accuracy of the pollen rinsing was checked using a binocular. The mass of produced pollen was calculated per flower.

**Pollen viability.** Pollen viability was estimated using the standard acetocarmine test [Denisow 2006]. Fresh pollen was collected from randomly chosen flowers. In this test, pollen grains with cytoplasm stained red were considered as viable and with cytoplasm remaining transparent as nonviable. The viability of the pollen grains was calculated in 3 repeats (n = 3 × 100 per treatment) and expressed as percentage of the stained grains in total analysed. These observations were conducted using Nikon Eclipse E–200 LM at a magnification of 40 × 10.

**Statistical analysis.** The results are presented as mean values and standard deviations (SD). ANOVAs procedures, to discover the difference for the traits studied among treatments and among years of study, were applied. *Post hoc* comparison of means was tested by the HSD Tukey test [Stanisz 2007]. The Pearson’s correlation coefficient (r) was applied to measure the strength of the relationship between chosen traits (morphological floral traits or number of flowers per inflorescence, nectar and pollen production). The level of statistical significance to measure the differences between means for all analyses was at \( p = 0.05 \). Statistica software version 6 was applied for these analyses (Statsoft, Krakow).

**RESULTS**

**Flower characteristics.** Flower of *Hosta* ‘Krossa Regal’ is actinomorphic, trimertic. No year effect was found for the phenotypic traits of the flower (Tukey test: \( F_{1.218} = 42.23, p = 0.142 \) for Nl; \( F_{1.216} = 37.35, p = 0.231 \) for Nw; \( F_{1.218} = 52.65, p = 0.442 \) for Bl; \( F_{1.216} = 38.75, p = 0.093 \) for Bw). The foliar application of Si significantly affected morphometric traits of the flower, except for the treatment with 0.2% of Actisil concentration (= 120 mg Si·dm⁻³) (tab. 1).

The raceme inflorescence comprises 12–32 flowers. No treatment effect on the number of flowers per inflorescence was found (fig. 1; Tukey test: \( F_{3.84} = 22.83, p = 0.248 \) in 2012; \( F_{3.116} = 32.85, p = 0.142 \) in 2013), however the mean number of flowers differed between study years and more flowers was noted in the inflorescences in 2012.

**Nectar production.** Nectar in *Hosta* ‘Krossa Regal’ is secreted by a septal nectary situated between the base of the ovary and the stamens of the inner circle. Flowers produced nectar (extreme values: 4.1–11.9 mg/flower) of changeable concentration (extreme values: 18.6–35.3%). It was noted that both year and treatment affected nectar quantity and quality. The higher volume of nectar per flower was produced in 2012 compared to 2013. Except for fertilization with 0.2% concentration of Actisil, the production of nectar in both years was significantly higher on fertilized than on unfertilized

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plots ($F_{3,186} = 2.38, p = 0.022$), the same trend was recorded for the sugar concentration (%) in nectar ($F_{3,186} = 3.16, p = 0.031$) and the mass of sugars (mg) in nectar. The mean sugar mass was between 1.16 and 2.9 mg per flower.

Table 1. Morphometrics of the flower of *Hosta* ‘Krossa Regal’ cultivated on plots with different treatments of foliage fertilization of Actisil. Means for 2012–2013 are given

<table>
<thead>
<tr>
<th>Treatment (flowers)</th>
<th>No. of samples</th>
<th>narrow-tube length (Nl) mean ±Sd</th>
<th>narrow-tubewidth (Nw) mean ±Sd</th>
<th>broad-tubelength (Bl) mean ±Sd</th>
<th>broad-tube width (Bw) mean ±Sd</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – control</td>
<td>53</td>
<td>9.23 ±2.37</td>
<td>0.22 ±0.12</td>
<td>24.21 ±2.47</td>
<td>16.20 ±1.89</td>
</tr>
<tr>
<td>2 – 0.2%</td>
<td>54</td>
<td>9.68 ±3.02</td>
<td>0.26 ±0.21</td>
<td>25.32 ±4.21</td>
<td>15.80 ±3.74</td>
</tr>
<tr>
<td>3 – 0.3%</td>
<td>53</td>
<td>11.26 ±4.85</td>
<td>0.41 ±0.31</td>
<td>28.60 ±3.61</td>
<td>17.10 ±4.71</td>
</tr>
<tr>
<td>4 – 0.4%</td>
<td>56</td>
<td>13.42 ±3.43</td>
<td>0.36 ±0.27</td>
<td>28.20 ±2.78</td>
<td>17.90 ±5.11</td>
</tr>
</tbody>
</table>

ANOVAs procedures were performed separately for each analyzed feature. Values followed by the same small letters are not statistically significant among treatments within the flower trait at $P < 0.05$, based on HSD Tukey test.

Table 2. The effect of some of the studied flower traits on nectar and pollen production in *Hosta* ‘Krossa Regal’ (for all the treatments and years of study). The Pearson’s correlation coefficient ($r$) are given. Significant correlation are in bold

<table>
<thead>
<tr>
<th>Type of correlation</th>
<th>$r$</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Correlation between mass of nectar per flower and:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. flower size</td>
<td>0.266</td>
<td>0.124</td>
</tr>
<tr>
<td>b. number of flowers per inflorescence</td>
<td>-0.124</td>
<td>0.051</td>
</tr>
<tr>
<td>2. Correlation between mass of nectar sugars per flower and:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. flower size</td>
<td>-0.176</td>
<td>0.053</td>
</tr>
<tr>
<td>b. number of flowers per inflorescence</td>
<td>-0.279</td>
<td>0.039</td>
</tr>
<tr>
<td>3. Correlation between pollen mass per flower and:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. flower size</td>
<td>-0.399</td>
<td>0.083</td>
</tr>
<tr>
<td>b. number of flowers per inflorescence</td>
<td>-0.234</td>
<td>0.041</td>
</tr>
</tbody>
</table>

**Pollen quality and quantity.** The year effect was found for the pollen mass produced in flowers (fig. 2). Almost 2-fold more pollen was produced in 2013. In both years, the reatment effect was significant for the pollen mass produced in flowers ($F_{3,148} = 31.52, p = 0.043$ in 2012; $F_{3,344} = 22.05, p = 0.038$ in 2013); the considerable increase was noted after application of 180 or 240 mg Si·dm$^{-3}$. In addition, there was significant impact of treatment on the pollen viability and pronounced effect was noted on plants fertilized with 180 or 240 mg Si·dm$^{-3}$. The same trend was documented in both years.
Fig. 2. Nectar production per flower of Hosta ‘Krossa Regal’ cultivated on plots with different treatments of foliage fertilization of Actisil in the years 2012 and 2013: 0 – control, 1 – 0.2% = 120 mg Si × dm⁻³, 2 – 0.3% = 180 mg Si × dm⁻³, 3 – 0.4% = 240 mg Si × dm⁻³. ANOVAs procedures were performed separately for each analyzed feature. Means with the same small letter do not differ significantly between treatments, and means with the same capital letter do not differ significantly between years at $p < 0.05$, based on HSD Tukey test.
The effect of silicon on nectar and pollen production in Hosta Tratt. ‘Krossa Regal’

DISCUSSION

The results concerning the Si effect on nectar and pollen production have no equivalent in literature reports. In Hosta ‘Krossa Regal’, the exogenous application of Si in foliar fertilizer affected floral traits (flower size, nectar and pollen production). A pronounced effect of Si on flower size and the amount of floral reward was observed after an addition of 180 or 240 mg Si dm\(^{-3}\). That is consistent with Si concentration that substantially impacted on the increase of green pigments (chlorophylls a and b) in the leaves of Polygonatum multiflorum, also monocotyledonous plant [Rubinowska et al. 2014]. Although, the silicon is not classified as an element essential for growth and development, the scientific reports are agreeable that Si is beneficial to many metabolic
processes, i.e. suppresses the chlorophyll degradation [Shen et. al. 2010] or encourages the photosynthetic apparatus by promoting the chlorophyll contents, as well as positively impact on the water balance [Mateos-Naranjo et al. 2013, Rubinowska et al. 2014], which altogether results in the increase of the photosynthetic efficiency [Silva et. al. 2012]. Photosynthetic activity of leaves which supplies flower structures with organic compounds (primarily carbohydrates) most probably implicated the nectar and pollen results, as primarily carbohydrates are being utilized to flower growth and/or production of floral reward. The production of nectar and development of pollen grains involve considerable expenditure of energy, e.g. nectar sugar production requiring as much as 37% of that produced daily by photosynthesis [Pacini and Nepi 2007]. Pollen grain development is also an energy-consuming process, and a lot of energy is required for carbohydrates of cell walls and variety of cytoplasm compounds, i.e. amino acids, protein, starch, sterols, lipids, vitamins [Cruden 2000]. Therefore, apart from pollen production, the Si addition was advantageous for pollen quality (measured as pollen viability). Similar trend, the increase of pollen viability related to the increases of Si concentration was reported for *Lycopersicon esculentum* (tomato) [Miyake and Takahashi 1978] and *Cucumis sativus* (cucumber) [Miyake and Takahashi 1983]. Moreover, pollen cytoplasm is short-lived and very sensitive to changes in external factors, i.e. temperature, draught [Denisow 2009a, 2011, Denisow et. al. 2014]. Therefore, the beneficial effects of Si application on pollen viability is presumable related to Si impact on both the photosynthetic efficiency and water balance [Ma and Takahashi 2002]. The beneficial impact of silicon on osmoregulation and effects of *water impoundment* was confirmed by different authors [Kaya et al. 2006, Kazemi et. al. 2012, Rubinowska et. al. 2014].

Likewise, the association of Si in the development of cell wall components should be considered, while analysing the favourable impact of Si application on pollen production. It is accepted that Si is incorporated in the synthesis of cell wall constituents and controls polymerization of smaller molecules during formation of cellulose and more complex polysaccharides (hemicelluloses, pectins), as well as long chain fatty acids, phenylpropanoids, phenolics and traces of carotenoids [Currie and Perry 2007]. These compounds are present in a highly complex matrix of 2 layers of pollen grains wall.

The beneficial effect of Si supplementation on flower or inflorescence phenotypic character has been earlier documented for several ornamental plant species, e.g. *Helianthus annus* (sunflower), *Zinnia* (zinnia), *Gerbera* (gerbera) [Kamenidou et al. 2008, 2009, 2010]. In particular, the changes in perianth width and tube length are important for flower function, e.g. may influence flower-insect interaction by changes of attraction for pollinators. For example, according to Suzuki et al. [2002], in *H. sieboldiana* both the perianth width and length are important for efficiency of pollination and more effective pollen deposition by pollinators was in flowers with wider perianth.

In *Hosta ‘Krossa Regal’*, the addition of Si did not affect the number of flowers per inflorescence. On contrary, the increase in the number of buds and flowers was observed for many ornamental plants – *Verbena* ( vervain), *Portulaca* (moss rose), *Xerochrysum bracteatum* (strawflowers), *Osteospermum ecklonis* (african daisy), *Gaura lindheimeri* (gaura) after application of silicon from Actisil [Dębicz and Wróblewska...
The effect of silicon on nectar and pollen production in Hosta Tratt. ‘Krossa Regal’

2011, Wróblewska and Dębic 2011]. In above mentioned study the positive effects were recorded already after application of low dosages – 60 and 120 mg Si·dm⁻³. In general, beneficial impact of Si application on bud formation and flower development is related to various accompaniment factors (e.g. the species, type of soil substrate, the period and frequency of treatments, other nutrients availability). For example, it is accepted that Si effects in plants are direct or indirect and may be due to the availability of calcium (Ca) [Kamenidou et al. 2010] or weather conditions [Ross 1982, Hermans et al. 2007]. It is also hypothesized that the effect of Si on blooming is becoming more marked if plants are under stress conditions [Savvas et al. 2002, Sacała 2009]. These concepts presumably may explain no effects of Si application on the number of flowers noted in our study.

The trade-offs theory may explain the results concerning the correlations obtained in our experiment between nectar or pollen traits and the number of flowers per inflorescence. Plants are known to allocate resources between flower parts and nectar or pollen rewards [Stpiczyńska and Nepi 2006, Denisow 2011].

In summary, to understand the Si involvement in the biology of nectar and pollen, further explorations under controlled conditions investigating the concurrent effects of various factors, i.e. soil fertility and soil water content, air moisture, temperature could be useful.

CONCLUSIONS

1. Hosta ‘Krossa Regal’ sprayed with 180 mg and 240 mg Si × dm⁻³ from Actisil Hydro Plus produced flowers considerably greater in size (both the perianth width and tube length).
2. Silicon supplementation with Actisil Hydro Plus did not affect the number of flowers developed per inflorescence, regardless of the dosage.
3. Incorporation of 180 and 240 mg Si·dm⁻³ were the most beneficial treatments for nectar production, sugar mass in nectar, pollen production and pollen viability.

REFERENCES


B. Denisow, E. Pogroszewska, H. Laskowska

cukru w nektarze. Traktowanie roślin krzemem w ilości 180 i 240 mg Si dm⁻³ korzystnie wpłynęło na masę produkowanego pyłku oraz jego żywotność. Rezultaty te mogą być tłumaczone dodatnim wpływem krzemu na intensywność metabolizmu, np. poprzez regulację bilansu wodnego i zwiększenie wydajności fotosyntezy.

Słowa kluczowe: Actisil Hydro Plus, nawożenie dolistne, kwitnienie, nektarowanie, pyłek

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