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THE EFFECT OF PENTAKEEP®-V APPLICATION ON FLOWER TRAITS AND NECTAR PRODUCTION IN *Hosta* TRATT. 'KROSSA REGAL'

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Abstract. In modern horticulture the use of biostimulators for obtaining the maximum yields and qualities of the crops is favoured. Pentakeep®-V, the fertilizer containing 5-aminolevulinic acid (ALA) is currently recommended. The ALA is recognized as biostimulator of different physiological processes. It increases the photosynthetic efficiency, impact on the nitrogen metabolism – increases activity of nitrate reductase, decreases the content of nitrates. The flowering, nectar production and sugar content by flower position and after foliar application of Pentakeep®-V in *Hosta* Tratt. 'Krossa Regal' were examined in 2012–2013. The plants were sprayed with water solutions of Pentakeep®-V in concentrations: – 0 (control – distilled water), 0.02, 0.04, and 0.06%. Nectar was extracted from 24-hs flowers, using pipette method. Nectar amount and concentration was strongly influenced by floral position in the raceme, and fertilizer application. The total amount of nectar produced per flower declined along the inflorescence, starting from the bottom positioned. Pentakeep®-V fertilizer, depending on concentrations had a variety of effects on flower traits and nectar production. The application of 0.06% of Pentakeep®-V increased the number of flowers per inflorescence, however only in one year of the study. Pentakeep®-V in 0.02 and 0.04% concentrations increased the flower size, with respect to the perianth width as well as improved nectar production. The 0.06% of Pentakeep®-V application suppressed both the flower size and nectar production. These results might be explained by the stimulation of chlorophyll synthesis and CO_2 absorption in lower dosages of ALA and reduced photosynthetic efficiency with excess dosages of ALA.

Key words: 5-aminolevulinic acid, foliar fertilizer, flowering, nectar

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INTRODUCTION

Nectar is a sugar-rich liquid produced in nectaries located usually at the base of the flower. It is a food resource for various insect groups [Denisow 2005], therefore nectar secretion is one of the most important issue for plant-insect relationship and pollination process [Denisow 2002]. It is also accepted that the amount of nectar produced by plants influences the number of colonies of bees that could be keep in the vicinity of the crops and therefore good nectar-yielding plants are required by beekeepers [Biesmeijer et al. 2006, Zych and Jakubiec 2006] as well as nectar resources are fundamental to sustaining wild pollinator populations [Denisow and Wrzesień 2007]. In recent years, a large number of anatomical, physiological, and microchemical studies were made to reveal factors that impact on nectar quantity and quality [rewived in Nepi 2007, Pacini and Nepi 2007]. The influence of latitude, temperature, rainfall or atmospheric humidity on nectar secretion have been documented [rewived in Nicolson and Thornburg 2007]. Although floral nectar plays central ecological, evolutionary and economic functions, relatively little is known if we can control and/or stimulate nectar secretion. Faced with global ‘pollination crisis’, the improvement of nectar secretion in plants might be useful for increase of fruit and seed set [Kremen and Ricketts 2000, Viik et al. 2012].

Diverse macro- and microelements are required for flowering and can impact on bud formation, time of flowering, anthesis, flower traits or reproduction [Burkle and Irwin 2009a, b, 2010]. The type and amount of fertilizer to use in a given situation have been the topic of study for many years [Baude et al. 2011]. In the pioneer study, Shuel [1957] indicated that nectar production require: ‘a level of nitrogen low enough to avoid excessive vegetative growth, a level of phosphorus sufficient to promote good flowering, and a level of potassium which is neither low enough to limit growth severely nor high enough to reduce flower production’. However, plant species may differently response to nutrient addition. Mineral fertilization is known to stimulate the number of flowers as well as nectar production in the perennial plant *Ipomopsis aggregata* [Burkle and Irwin 2009a]. On the contrary, the nectar production of *Vaccinium macrocarpon* was unaffected by fertilizer application [Cane and Schiffhauer 1997].

Currently, the ideas of sustainable development are implemented to agriculture. The concept relates to different environmental-friendly activity and require the use of substances which are safe both for humans and environment [Dhargalkar and Pereira 2005]. In modern agriculture and horticulture the use of biostimulators for obtaining the maximum yields and qualities of the crops is recommended [Anonymous 2013, Calvo et al. 2014]. Biostimulators can change a number of physiological and biochemical processes in plant cell metabolism [Hotta et al. 1997, Yaronskaya et al. 2006, Tilly-Mándy et al. 2010]. Positive effects of biostimulators on plants were reported [Smoleń and Sady 2010, Smoleń et al. 2010, Wróblewska and Dębicz 2011]. One of exogenous plant biostimulants is 5-aminolevulinic acid (ALA), present in Pentakeep® fertilizers. ALA is currently tested to improve medical procedures, e.g. to reduce the risk of complications during diagnosos of brain tumor [von Campe et al. 2012]. ALA is also becoming popular in the agriculture and horticulture sectors for its unique ability to promote the growth and quality of plants without harming living organisms or contaminating soil [Akram 2013].

5-aminolevulinic acid (ALA) is a precursor in the porphyrin synthesis pathway that in plants leads to chlorophyll synthesis, the photosynthetic activity [Memon et al. 2009], impacts on the nitrogen metabolism – increases activity of nitrate reductase [Mishra and Srivastava 1983], decreases the content of nitrates [Tanaka et al. 2005]. Consequently, ALA has an advantageous impact on the process of blooming in ornamental plants [Yoshida et al. 2005, Nowak 2006] and on the yield of vegetable plants [Yoshida et al. 2006]. The Pentakeep®, an ALA based fertilizers, is having a significant impact on the steadily expanding horticultural market in Europe. The Pentakeep® fertilizers are also gaining popularity in Poland, as the country's home gardening market has grown in recent years [Smoleń et al. 2010, Jarosz 2012].

The aim of the study was to find out if foliar application of Pentakeep®-V, impact on the flowering as well as the nectar production. In addition, the impact of flower position within inflorescence on the nectar production was examined and the relationship between the weather factors and nectar production were established. The experiment was conducted on *Hosta* Tratt. 'Krossa Regal', the plant with large flowers sufficient for nectar extracting.

MATERIAL AND METHODS

Location and experiment design. The observations were conducted in 2012–2013. *Hosta* Tratt. 'Krossa Regal' (Asparagaceae) 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 plots in a randomized complete block design (6 × 3 = 18 plants per treatment). The plants were sprayed with water solutions of Pentakeep®-V-foliar fertilizer produced by Cosmo Seiwa Agriculture Co., Ltd., Japan. Pentakeep®-V was applied in concentrations 0.02, 0.04, 0.06%. The foliar spray of distilled water (= no mineral fertilizer treatment) was a control (treatment 0). Pentakeep®-V contains (in gravimetric percent): N – 9.5% (N-NO_3 – 3.8%, N-NH_4 – 5.7%), MgO – 5.7%, B – 0.14%, Cu – 0.02%, Fe-DTPA – 0.6%, Mn – 0.23%, Mo – 0.02%, Zn – 0.16%, and 5-aminolevulinic acid (ALA) in concentration not declared by the producer .

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).

Flowering. The number of flowers per inflorescence (n = 15 inflorescences per treatment) and flower size were analysed. For flower size, the perianth length and width using digital calliper to the nearest 0.01 mm, at randomly selected flowers (n = 20–25 per treatment) were measured. The quantifications were performed in the stage just after opening for (1) narrow tube length (Nl) – 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) – in the middle of tube length; (4) broad tube width (Bw) – in the middle of tube length (fig. 1). The total number of measurements was n = 74 in 2012, and n = 95 in 2013.

Nectar production. The collection of samples was carried out in late morning (9.30–11.00) [Denisow 2009]. The samples were immediately transported to the laboratory. 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. We collected nectar produced in the preceding 24 hr, as the peak of nectar secretion was found between 20 and 24 hr of flower life-span in our pilot study. The nectar was separately harvested from the flowers from different regions of an inflorescence, i.e. from bottom (2–6), mid (10–14), and apical (18–23) flowers. 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 production (in mg) and sugar concentration were used to calculate the total sugar mass in each sample. Relevant calculations allowed to determine the amount of sugars produced per flower (in mg).

Weather conditions. The average air temperatures during the period of blooming of *Hosta* 'Krossa Regal' was 20.7°C in 2012 and 18.6°C in 2013, and were higher than long-term average (17.8°C). Total precipitation was approx. 25% lower than normal in 2012, while was on average level in 2013. The air temperature and relative humidity (RH) data for particular days of nectar collecting were used for measure the interdependence between weather conditions and nectar traits.

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, among years of study, and among flower positions were applied. *Post hoc* comparison of means was tested by the HSD Tukey test [Stanisz 2007]. The Pearson's correlation coefficient (r) was used to measure the strength of the relationship between nectar traits and meteorological data (air temperature and relative humidity (RH). 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

An inflorescence of *Hosta* 'Krossa Regal' is raceme – an unbranched, indeterminate type (fig. 1A). Nutrients addition affected floral and nectar characters. Pentakeep®-V application at the 0.06% concentration increased the number of flowers per inflorescence, however only in 2012 (fig. 2). Plants fertilized with 0.02 and 0.04% of Pentakeep®-V produced flowers with approx. 8% wider corollas than control plants (tab. 1, $F_{2.68} = 5.2$, $P = 0.0078$).

The nectar glands are situated in the septal region between adjacent carpels (fig. 1 B). Nectar secretion starts at the bud stage and ceases parallel with the wilting of the tepals.

Flowers produced nectar (extreme values: 3.6–12.7 mg/flower) of changeable concentration (extreme values: 17.5–37.8%). The mean sugar mass was between 1.1 and 2.4 mg per flower. On average, a single flower secreted 5.25 ±6.64 mg of nectar and produced 1.5 ±0.94 mg sugars (means and SDs calculated across years, treatments and floral positions).

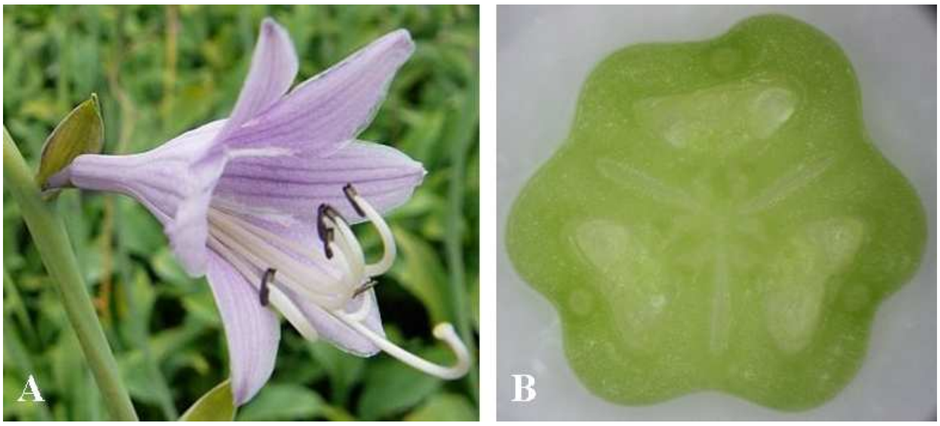

Fig. 1. A – Flower and B – cross section at half height of the 3-carpellate ovary and septal nectaries in *Hosta* 'Krossa Regal'

The treatment significantly impacted on the nectar production ($F_{3.53}$ = 3.31, P = 0.039) (fig. 2). Application of Pentakeep®-V in 0.02 and 0.04% concentrations showed 10–30% promotive effects over the control in the amount of nectar produced per flower. The same trend was recorded both for the sugar concentration in nectar ($F_{3.53}$ = 3.16, P = 0.004) and the mass of sugars (mg) in nectar ($F_{3.53}$= 5.61, P = 0.031).

There was an effect of year ($F_{1.53}$ = 7.74, P = 0.007) on nectar volume produced in flowers (fig. 2). The amount of nectar was approx. 50% higher in flowers in 2012 compared to 2013. At a mean air temperature below 22°C and a relative humidity between 60–80%, the nectar production was the highest after application of 0.02 and 0.04% of Pentakeep®-V (tab. 2). At air temperature above 27°C together with a relative humidity between 35–55% the nectar production was increased after Pentakeep®-V application in 0.04 and 0.06% concentrations. The same trend was recorded for the sugar concentration (%) in nectar and the mass of sugars (mg) in nectar. Generally, the negative correlation was noted between the air temperature and the amount of nectar production in flowers (tab. 3), while positive effect of relative humidity on the mass of nectar was found (calculated across years, treatments and floral positions).

The flowers of raceme inflorescence of the *Hosta* 'Krossa Regal', do not open simultaneously, the first to open are the bottom positioned flowers, followed by mid- and apical ones. Regardless the applied concentration of Pentakeep®-V, the amount of nectar produced per flower ($F_{2.53}$ = 6.28, P = 0.003) and the mass of produced sugars ($F_{2.53}$ = 4.98, P = 0.012) varied depending on flower position in the inflorescence (fig. 3). On average, the mass of produced nectar was the highest in the bottom positioned flowers. As compared with bottom positioned flowers, nectar amount at mid-positioned was reduced by 10–20%, and at apical positioned was reduced by 20–40%. The reduction in the mass of sugars in nectar between bottom positioned flowers and apical positioned flowers was 15–20%.

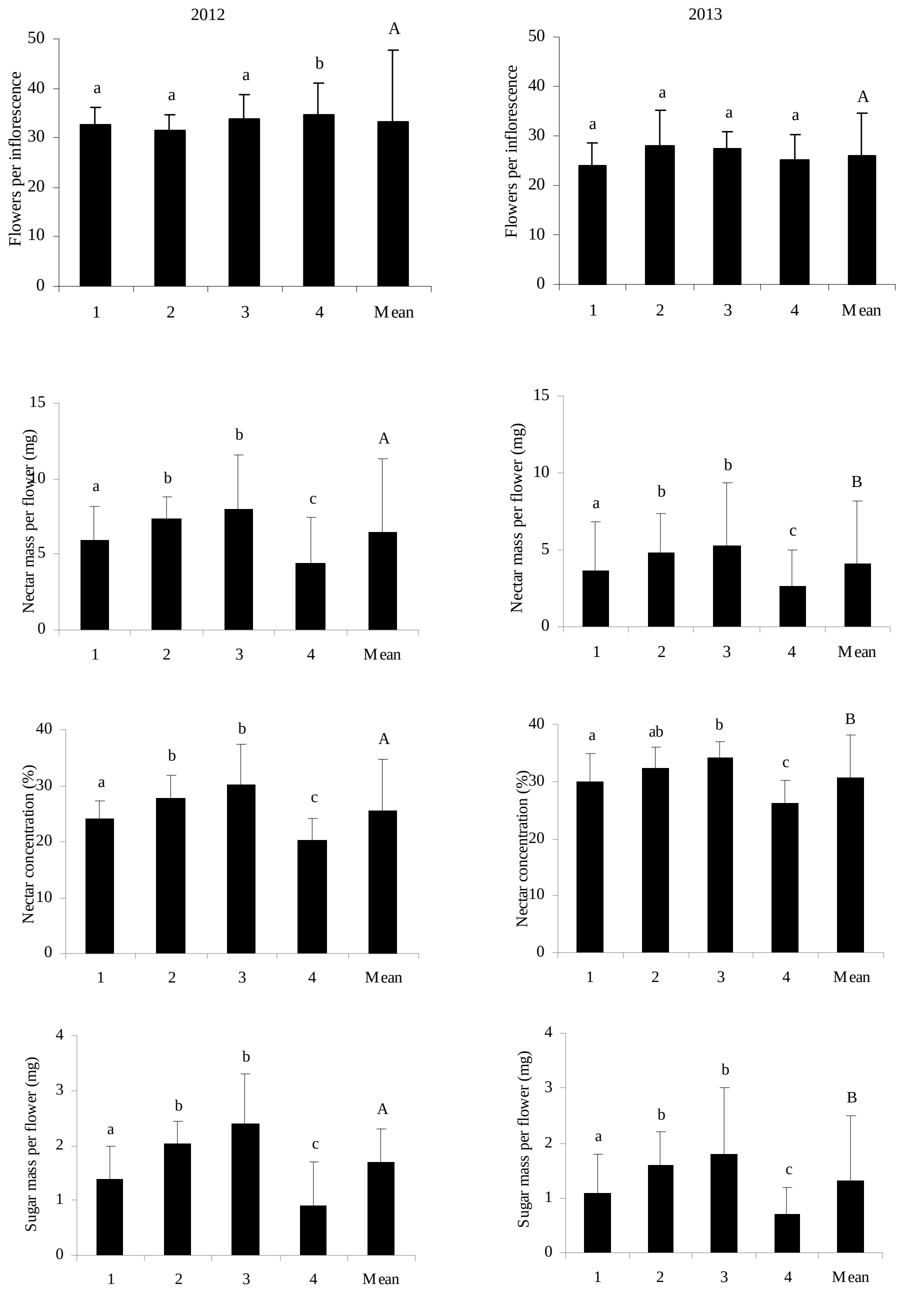

Fig. 2. Flowering, nectar production and sugar mass per flower of *Hosta* 'Krossa Regal' after application of Pentakeep-V: 1 – control, 2 – 0.02%, 3 – 0.04%, 4 – 0.06%. Means with the same small letter do not differ significantly between treatments, and means with the same capital letter do not differ significantly between years at P < 0.05, based on HSD Tukey test

Table 1. Morphometrics of the flower of *Hosta* 'Krossa Regal' cultivated on plots with different treatments of Pentakeep®-V fertilization. Means for 2012–2013 are given

Treatment	No. of samples (flowers)	Flower trait			
		narrow-tube length (mm) Nl	narrow-tube width (mm) Nw	broad-tube length (mm) Bl	broad -tube width (mm) Bw
		mean ±Sd	mean ±Sd	mean ±Sd	mean ±Sd
1 – control	42	9.12a ±2.07	0.20a ±0.12	23.16a ±2.17	15.90a ±1.69
2 – 0.02%	46	9.61a ±3.52	0.23a ±0.21	24.02a ±3.21	17.20b ±2.04
3 – 0.04%	43	9.44a ±4.15	0.28a ±0.31	23.09a ±3.61	17.10b ±2.71
4 – 0.06%	40	8.72a ±3.43	0.31a ±0.27	22.20a ±2.78	16.02c ±0.96

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's test

Table 2. The weather parameters impact on nectar production in *Hosta* 'Krossa Regal' after application of Pentakeep®-V. Means for 2012–2013 are given

Treatment	Nectar mass per flower (mg)		Sugar concentration (%)		Sugar mass per flower (mg)	
Pentakeep-V concentration	< 22°C * RH 60–80%	> 27°C RH 35–55%	< 22°C RH 60–80%	> 27°C RH 35–55%	< 22°C RH 60–80%	> 27°C RH 35–55%
1 – control	6.3a	3.8a	18.4a	25.1a	1.2a	0.9a
2 – 0.02%	11.2b	4.6a	19.3a	24.6a	2.2a	1.1a
3 – 0.04%	12.5b	11.1b	22.1a	33.3b	2.8a	3.7b
4 – 0.06%	8.2a	12.7b	19.1a	36.0b	1.6a	4.6b
Mean	9.55A	8.05B	19.7A	29.7B	1.1A	2.4B

* RH relative humidity. ANOVAs procedures were performed separately for each analyzed feature.
Values followed by the same small letters are not statistically significant among treatments within the columns, while values followed by the same capital letters are not statistically significant between the collumns at $P < 0.05$, based on HSD Tukey test

Table 3. The weather parameters effect on nectar production in *Hosta* 'Krossa Regal' (for all the treatments and years of study). The Pearson's correlation coefficients (r) are given. Significant correlations are in bold

Type of correlation		r	P
Correlation between mass of nectar per flower and	air temperature	- 0.301	0.028
	relative humidity RH	0.421	0.039
Correlation between sugar concentration in nectar	air temperature	0.372	0.041
	relative humidity RH	0.161	0.054
Correlation between mass of nectar sugars per flower and	air temperature	0.416	0.026
	relative humidity RH	- 0.148	0.074

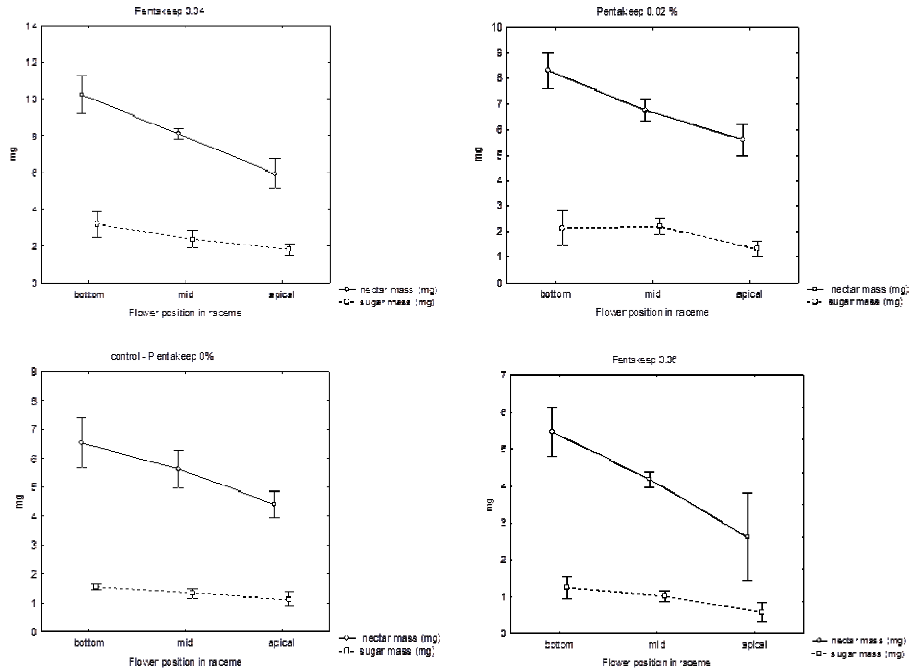

Fig. 3. The effect of flower position within raceme inflorescence on the nectar amount and mass of sugars produced per flower in *Hosta* 'Krossa Regal' after application of Pentakeep®-V (means from the years of study)

DISCUSSION

In this study we showed that Pentakeep®-V fertilizer, depending on concentrations, had a variety of effects on flower traits and nectar production in the *Hosta* 'Krossa Regal'.

In our study the application of foliar fertilization increased the number of flowers per inflorescence, however only in one year of the study and only with 0.06% of Pentakeep®-V concentration. A poor synchronization between fertilizer application and the number of flowers might be related to different factors, e.g. the plant-life history which define the time of bud formation [Hermans et al. 2006] together with meteorological conditions [Campbell and Halama 1993] may play a role in determining this response. In perennial plants, the resources available to flowering may come from stored organs and/or current foliar photosynthesis [Harper 1977]. In *Chrysanthemum* × *grandiflorum* the increase in the number of flowers was found after treatment with 0.05% concentration of Pentakeep® [Nowak 2006]. Ongoing research showed that plant species respond differently to nutrients [Burkle and Irwin 2009a, 2010] and biostimulators [Wróblewska and Dębicz 2011].

An appropriate concentration of Pentakeep®-V increased the flower size, in respect to the perianth width, as well as nectar production. It is well known that ALA, present in Pentakeep®-V stimulated chlorophyll synthesis, and by that way impact on the photosynthetic CO_2 absorption [Hotta et al. 1997]. These might explain our results that the flower size, nectar production and sugar mass in nectar were improved after 0.02 and 0.04% of Pentakeep®-V treatments. Although, no information are available on how ALA impact on nectar traits, the pronounced effects of exogenous ALA application on growth and yield of various plants were reported, e.g. in barley, potato, corn, wheat [Hotta et al. 1997, Lou et al. 1998]. The advantageous effects obtained were linked to increase in photosynthetic efficiency. It is accepted that production of nectar, a sugar-rich liquid, is associated with high energy expenditure. According to Pacini and Nepi [2007], nectar sugar production requires as much as 37% of energy produced daily by photosynthetic activity of leaves. Likewise, the promotive effect on nectar production was documented on plants treated with other biostimulator, Actisil Hydro Plus [Denisow et al., in press].

Interestingly, the application of 0.06% of Pentakeep®-V suppressed both the flower size and nectar production. Hotta et al. [1997] observed that excess dosages of ALA hinder plant growth due to reduced photosynthetic efficiency. Presumably, the decrease in photosynthetic efficiency impaired the flower size and the amount of secreted nectar.

Differences in weather conditions between study year can explain the variation in nectar characteristics observed between growing seasons. Generally, nectar volume and sugar concentration is influenced by external factors affecting photosynthesis, i.e. light, temperature, evaporation, transpiration [Pacini and Nepi 2007]. Moreover, changes in relative humidity, or even wind speed have been documented to change nectar characteristics [Nicolson and Thornburg 2007]. Therefore nectar traits may vary greatly during the day, during the season or between growing seasons. In our study, the higher nectar parameters observed in the year with 25% of rainfall deficite (in relation to normal long-term level) and during the days with hot and dry weather (>25^{o}C, RH 35–55%) may indicate the positive effects of exogenous application of 5-aminolevulinic acid (ALA), present in Pentakeep®-V fertilizer. Presumably, ALA alleviated the inhibitions of photosynthesis and by that way improved the nectar parameters under dry weather conditions. Few reports highlighted that spraying with ALA results with better plant functioning, particularly under suboptimal conditions [Hotta et al. 1997, Lou et al. 1998]. In the context of climate warming, Pentakeep®-V fertilizer, containing ALA biostymulator, is expected to support indirectly adequate pollination. In particular, the improvement of nectar production and sugar content in nectar could modify resource availability for pollinators and their activity.

The opening sequence of *Hosta* 'Krossa Regal' flowers starts with the lower positioned and continues with mid- and then with apical ones. The total amount of nectar produced per flower was related to flower position in the raceme and declined along the inflorescence. The intra-inflorescence variation in floral traits are typical for raceme inflorescences [Campbell 1989] and may relate the floral longevity or the number of developed seeds [Campbell and Halama 1993]. Previous studies on nectar production have also reported that in acropetaly blooming inflorescences upper flowers produce less nectar [Denisow 2005]. Presumably, the phenomenon of nectar distribution within inflorescence, with apical flowers producing less nectar, is connected with resource limitation [Stephenson 1981]. According to 'resource limitation hypothesis', the lower flowers in racemes, after being pollinated receive the most resources for seed and fruit development. Consequently, upper flowers are under considerable limitation in available resources and therefore their floral traits and function are impaired [Campbell and Halama 1993, Denisow 2002]. Reduced floral function (number of pollen grains and/or ovules) from the base towards the apex within racemes of *Hosta ventricosa* [Cao et al. 2011] and *H. rectifolia* [Cao et al. 2007] were evidenced.

In conclusion, the present study established that nectar secretion in *Hosta* 'Krossa Regal' is strongly influenced by fertilizer application, floral position in the raceme and weather conditions. Pentakeep®-V fertilizer acts directly on flower size and on nectar secretion. It is well documented that flower size and nectar production contribute to plant-pollinator interactions. Flower size, the amount of nectar production as well as sugar concentration may influence pollinator behaviour because these are traits important for pollinator attraction [e.g.: Devlin et al. 1992]. However, to uprate nectar production in the field, further studies under controlled conditions are needed.

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WPŁYW NAWOZU PENTAKEEP®-V NA KWITNIENIE I NEKTAROWANIE FUNKII (*Hosta* TRATT.) 'KROSSA REGAL'

Streszczenie. W nowoczesnym ogrodnictwie wskazane jest stosowanie biostymulatorów w celu uzyskania dużych i dobrej jakości plonów. Aktualnie polecany jest nawóz Pentakeep®-V zawierający kwas 5-aminolewulinowy (ALA). ALA jest uznawany za biostymulator różnych procesów fizjologicznych, m.in. zwiększa on wydajność fotosyntezy, wpływa na metabolizm azotu – zwiększa aktywność reduktazy azotanowej, zmniejsza zawartość azotanów. W latach 2012–2013, wykorzystując roślinę modelową *Hosta* Tratt. 'Krossa Regal', badano kwitnienie, produkcję nektaru i masę wydzielanych cukrów w zależności od położenia kwiatów w kwiatostanie i po zastosowaniu nawozu Pentakeep®-V w formie dolistnej. Rośliny zostały opryskane wodnym roztworem Pentakeep®-V w stężeniach: 0 (kontrola – woda destylowana), 0,02; 0,04 i 0,06%. Nektar pobierano z kwiatów w 24. godzinie ich życia, stosując metodę pipetową. Ilość produkowanego nektaru oraz koncentracja cukrów istotnie zależały od położenia kwiatu w gronie i stosowanej dawki nawożenia Pentakeep®-V. Całkowita ilość nektaru wyprodukowana przez pojedynczy kwiat była istotnie niższa w kwiatach położonych wyżej w gronie. Zastosowanie nawozu Pentakeep®-V w stężeniu 0,06% zwiększyło liczbę kwiatów w kwiatostanie, ale tylko w jednym roku badań. Pentakeep®-V w stężeniu 0,02 i 0,04% wpłynął korzystnie na wymiary kwiatów w odniesieniu do szerokości okwiatu oraz powodował wzrost produkcji nektaru. Natomiast Pentakeep®-V w stężeniu 0,06% spowodował wytworzenie kwiatów o mniejszych wymiarach oraz ograniczył produkcję nektaru. Otrzymane wyniki można uzasadnić stymulacją syntezy chlorofilu i absorpcji CO_2 przy mniejszych dawkach ALA i zredukowaną wydajnością fotosyntezy przy większej dawce ALA.

Słowa kluczowe: kwas 5-aminolewulinowy, nawożenie dolistne, kwitnienie, nektar

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