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ORIGINAL RESEARCH PAPER

# Post-harvest longevity of ×*Heucherella* L. leaves after the application of benzyladenine sprayed on maternal plants

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## Abstract
Analyses were conducted on leaves of two ×*Heucherella* L. cultivars: 'Solar Power' and 'Kimono'. Leaves were harvested at two dates: spring (2014-05-22) and summer (2014-07-26). Prior to each leaf harvest day, the maternal plants were sprayed with benzyladenine (BA) at a concentration of 100, 300 and 600 mg $dm^{-3}$. After being cut, the leaves were placed in containers with distilled water. In the cultivar 'Solar Power', BA at a concentration of 100–600 mg $dm^{-3}$ had an advantageous effect on post-harvest longevity of leaves harvested in summer, improving it by 31.7–98.3%, while in the cultivar 'Kimono' BA at the above-mentioned concentrations improved post-harvest longevity of leaves harvested both in spring and summer by almost 2 months. BA at a concentration of 100–600 mg $dm^{-3}$ inhibited proteolysis in leaves of the tested ×*Heucherella* cultivars, except for leaves of the cultivars 'Solar Power' and 'Kimono' harvested in summer from plants sprayed with BA at a concentration of 100 mg $dm^{-3}$. In these leaves, the protein level was comparable to that in the control leaves. In leaves treated with BA at a concentration of 100–600 mg $dm^{-3}$, the content of saccharides was higher than in the control plants harvested in the spring and summer.

## Keywords
florists' greens; BA; longevity; protein; saccharides

## Introduction

Interest in florists' greens, presently a necessary element of cut flower arrangements, has been increasing with the development of the art of floral design. However, interest in the previously very popular phylloclades of *Asparagus* has been decreasing from year to year. Nowadays, the primary materials used in bouquet arrangements are leaves and shoots of greenhouse plants, both produced in Poland and imported. The growing role of leaves of plants grown outdoors, such as *Hosta* [1], *Limonium latifolium* [2], and *Arum italicum* [3], etc., is stressed. Analyses on vase life of florists' greens are conducted on selected leaves, which are fully developed, free from mechanical damage and properly colored; however, evaluation excluding physiological analyses may provide misleading results.

The process of ageing in florists' greens may be regulated using growth regulators from the group of cytokinins and gibberellins [2,4–6]. Their effectiveness depends on the species, cultivar, method of application, and the concentration of applied regulators. Growth regulators impact on post-harvest longevity and chlorophyll, protein and saccharide content. Most frequently, growth regulators are used in the form of conditioning solutions. Another possibility is short-term immersion of leaf blades in growth regulator solutions [3].

In this study, post-harvest longevity of leaves was analyzed in two ×*Heucherella* L. cultivars following the application of benzyladenine (BA) sprayed on maternal plants prior to leaf harvest.

## Material and methods

Analyses were conducted on leaves of two ×*Heucherella* L. cultivars: 'Solar Power' and 'Kimono'. Leaves were harvested at two dates: spring (2014-05-22) and summer (2014-07-26). Healthy leaves with no mechanical damage were selected early in the morning from plants growing at the Department's outdoor plantation and prior to each leaf harvest day the maternal plants were sprayed with BA at a concentration of 100, 300, and 600 mg $dm^{-3}$. Plants from which leaves were collected for the control treatment were sprayed with water. After being cut, the leaves were placed in containers with distilled water, which was replaced every 2 days. Post-harvest longevity was determined at a room temperature of 18–20°C, during a 10-hour photoperiod and under fluorescent light with a quantum irradiance of 25 μmol $m^{-2}$ $s^{-1}$. Relative humidity was maintained at 70%.

One treatment (date × BA concentration) comprised 15 leaves (three replications with five leaves each).

Post-harvest longevity was determined in days. Loss of decorative value was defined as the moment in which 30% of leaf surface turned yellow or were wilted. The contents of protein and sugars were also determined.

The content of soluble protein was determined according to Bradford [7]. Weighed portions (0.2 g) crushed in a mortar with 2 mL of Coomassie Brilliant Blue G-250 (CBB) solution in 85% orthophosphoric acid were added to 100 μL of a diluted extract, with the extraction in a phosphate-potassium buffer (pH 7.0). After 10 minutes absorbance was measured at a wavelength of 595 nm. The protein content was determined from a curve plotted for albumin.

Total saccharide contents were determined with the anthrone reagent [8]. Weighed portions (0.5 g) were crushed in a mortar with 5 $cm^3$ distilled water added and the homogenate was centrifuged for 20 minutes. Obtained supernatant (1 $cm^3$) was added to 2 $cm^3$ of a cooled anthrone reagent (0.02% in concentrated $H_2SO_4$) and then while being slowly mixed, the content of the test tubes was heated in a water bath at 90°C for 14 minutes. After the tubes were cooled, the absorbance of the solutions was measured with a spectrophotometer at a wavelength of 620 nm. The content of saccharides was read from a standard curve prepared for glucose. The results are expressed in mg of glucose per g of fresh weight ($N = 4$ replications).

The results were statistically evaluated by means of two-way analysis of variance. A post hoc comparison of the means was tested by Duncan's test at the significance level $\alpha = 0.05$.

## Results

The concentration of benzyladenine applied in sprays on maternal plants and the date of leaf harvest were found to have a significant effect on post-harvest longevity of leaves in ×*Heucherella* cultivars (Tab. 1). In *Heucherella* 'Solar Power', irrespective of BA concentration, significantly more durable leaves were observed when they were cut in summer. Post-harvest longevity of summer-cut leaves was three times longer in comparison to spring-cut leaves. After a comparison of interactions, it was found that post-harvest longevity of the control leaves cut in summer was almost two-fold longer in comparison with leaves cut in spring. The tested concentrations of BA (100, 300, and 600 mg $dm^{-3}$) had a significant effect on post-harvest longevity only in summer-cut leaves. Longevity of summer-cut leaves increased by 31.1%, 57.7%, and 46.9%, respectively. In the cultivar 'Kimono', irrespective of BA concentration, leaf longevity was significantly longer for summer-cut leaves. A comparison of interactions showed that in the summer harvest the control leaves had a significantly longer vase life.

**Tab. 1** Post-harvest longevity (days) of ×*Heucherella* leaves after application of benzyladenine.

| BA concentration (mg dm$^{-3}$) | Harvest date | |
|---|---|---|
| | 2014-05-22 | 2014-07-26 |
| 'Solar Power' | | |
| 0 | 26.3 a | 64.0 b |
| 100 | 24.6 a | 84.3 c |
| 300 | 24.7 a | 101.0 d |
| 600 | 26.5 a | 94.0 d |
| Mean | 25.5 A | 85.8 B |
| 'Kimono' | | |
| 0 | 80.7 c | 100.2 d |
| 100 | 94.9 d | 160.0 e |
| 300 | 111.1 d | 160.0 e |
| 600 | 160.0 e | 100.3 d |
| Mean | 111.7 A | 130.1 B |

Means followed by the same letter do not differ significantly at $\alpha = 0.05$.

Post-harvest longevity of control leaves harvested in summer was by 19.5 days longer than that of leaves harvested in spring. BA at the tested concentrations had an advantageous effect on leaf longevity, irrespective of harvest dates. For spring-cut leaves, the longevity increased with increasing concentrations of BA. In the case of summer-cut leaves, post-harvest longevity increased when maternal plants were sprayed with BA at a concentration of 100–300 mg mg$^{-3}$. In comparison to the control, 2-month longer decorative value of the leaves was obtained.

The protein content in leaves of the investigated ×*Heucherella* cultivars was significantly influenced both by the harvest date and the concentration of BA (Tab. 2). Irrespective of BA concentration, the mean protein content was higher in leaves harvested in spring ('Solar Power') and in summer ('Kimono'). Irrespective of harvest date, BA at the tested concentrations inhibited proteolysis in aging leaves of both ×*Heucherella* cultivars. In both cultivars analyzed, BA inhibited protein degradation at a concentration of 100–600 mg dm$^{-3}$ in spring-harvested leaves, and at a concentration of 300–600 mg dm$^{-3}$ in summer-harvested leaves.

Sugar contents in leaves of the ×*Heucherella* cultivars depended significantly both on the date of leaf harvest and BA concentration (Tab. 2). Irrespective of BA concentration, in both cultivars a significantly higher sugar content was recorded in summer harvested leaves. Irrespective of harvest date, BA applied at the tested concentrations had a beneficial effect on sugar content in leaves for both cultivars. For both cultivars, the sugar content was significantly higher in post-harvest leaves when maternal plants were sprayed with BA at the tested concentrations compared to control plants. This effect was particularly evident in leaves cut in summer.

## Discussion

Post-harvest longevity of leaves in the ×*Heucherella* cultivars depended both on the cultivar, date of harvest and BA concentration applied to maternal plants. A better post-harvest effect in leaf longevity was recorded for the cultivar 'Kimono'. Considering the harvest date, the leaves of both cultivars harvested in summer exhibited a longer post-harvest effect compared to those collected in spring. The possible explanation may be related to the fact that spring-harvest leaves did not reach physiological maturity. Our experiment showed that BA at a concentration of 100–600 mg dm$^{-3}$ applied by spraying ×*Heucherella* maternal plants extended post-harvest longevity of leaves of the 'Solar Power' cultivar harvested in summer and leaves of the cultivar 'Kimono' harvested in spring and summer.

Growth regulators from the groups of cytokinins and gibberellins are being more frequently used in studies on post-harvest longevity of florists' greens. Their efficacy is dependent on the species, method of application and the growth regulator concentration. Typically, florists' greens are conditioned in water solutions of growth regulators. This may last from 4 to 24 h in facilities at room temperature (18–20°C) or in a cold room, in accordance with the principle that the longer the conditioning, the lower the temperature. A positive effect of conditioning in growth regulator solutions was shown, e.g., in *Zantedeschia* with colored spathes [6,9–11], *Zantedeschia aethiopica* [9,12], and *Arum italicum* [3]. Growth regulator solutions may also be used in short-term immersion of leaf blades. Thus, application of gibberellic acid extends post-harvest longevity in leaves of *Arum italicum* [3], while application of *meta*-methoxytopolin and its riboside – in leaves of *Zantedeschia albomaculata* 'Albomaculata' [6] and *Limonium latifolium* [2].

**Tab. 2** Protein and saccharide content (mg $g^{-1}$ FW) in leaves of ×*Heucherella* after application of benzyladenine.

| BA concentration (mg $dm^{-3}$) | Harvest date | | Mean |
|---|---|---|---|
| | 2014-05-22 | 2014-07-26 | |
| Protein content | | | |
| 'Solar Power' | | | |
| 0 | 15.1 a | 16.5 a | 15.8 a |
| 100 | 22.1 b | 15.7 a | 18.9 b |
| 300 | 23.0 b | 20.4 b | 21.7 c |
| 600 | 24.0 b | 19.4 b | 21.7 c |
| Mean | 21.1 B | 18.0 A | |
| 'Kimono' | | | |
| 0 | 9.3 a | 16.2 c | 12.8 a |
| 100 | 14.7 b | 15.8 c | 15.3 b |
| 300 | 12.9 b | 22.6 d | 17.8 c |
| 600 | 22.0 d | 28.4 e | 25.2 d |
| Mean | 17.2 A | 20.8 B | |
| Saccharide content | | | |
| 'Solar Power' | | | |
| 0 | 25.6 a | 60.8 c | 43.2 a |
| 100 | 60.2 c | 70.8 d | 65.5 c |
| 300 | 50.6 b | 70.2 d | 60.4 b |
| 600 | 57.8 c | 100.4 e | 79.1 d |
| Mean | 48.6 A | 75.6 B | |
| 'Kimono' | | | |
| 0 | 52. 2 a | 80.4 c | 66.3 a |
| 100 | 66.2 b | 90.1 d | 78.2 b |
| 300 | 80.8 c | 94.3 d | 87.6 c |
| 600 | 80.2 c | 101.6 e | 90.9 c |
| Mean | 69.9 A | 91.6 B | |

Means followed by the same letter do not differ significantly at α = 0.05.

The experiments showed that BA at a concentration of 100–600 mg $dm^{-3}$ inhibited proteolysis in leaves of the tested ×*Heucherella* cultivars, except for leaves of 'Solar Power' harvested in summer from plants sprayed with BA at a concentration of 100 mg $mg^{-3}$, in which the protein level was comparable to that in the control leaves. Gan and Amasino [13] reported that during the ageing process, the contents of growth regulators are changeable in plant tissues, being inhibitors of ageing. These compounds are, e.g., cytokinins and gibberellins. Their contents decrease and the levels of regulators accelerating ageing increase. The latter compounds include ethylene, abscisic and jasmonic acids. Brault and Maldiney [14] were of an opinion that cytokinins are responsible for the inhibition of protein and chlorophyll degradation in leaves, which is particularly evident in fragments cut off from maternal plants. Cytokinins applied in studies on post-harvest longevity of florists' greens frequently exhibit greater efficacy than gibberellins. In a study by Rabiza-Świder et al. [15], leaves of *Zantedeschia aethiopica* and *Z. elliottiana* were subjected to 24-h conditioning in solutions of benzyladenine and gibberellic acid. In both species, only gibberellic acid effectively delayed the degradation of soluble proteins. The standard nutrient solution used in the extension of vase life of cut flowers accelerated proteolysis in leaves of *Zantedeschia aethiopica*, although it did not have an equally adverse effect on leaves of *Z. elliottiana*. The reduction in soluble protein content was accompanied by the accumulation of free amino acids. Similarly, in a study by Rabiza-Świder and Skutnik [1] conditioning of leaves of *Hosta* 'Crispula' and 'Undulata Mediovariegata' in gibberellic acid and benzyladenine delayed the degradation of soluble proteins, which was particularly evident after the application of benzyladenine. In turn, proteolysis was accelerated when *Hosta* leaves were placed in a nutrient solution typically used for cut flowers.

Apart from gibberellic acid and benzyladenine, other cytokinin regulators are also used to extend vase life of florists' greens. Janowska et al. [6] investigated the potential extension of vase life in leaves of *Zantedeschia albomaculata* 'Albomaculata' using topolins – aromatic cytokinins isolated from poplar, which had previously been used scarcely in vitro experiments [16,17]. The authors also showed that *meta*-methoxytopolin and its riboside influence post-harvest longevity and quality of leaves in the

cultivar 'Albomaculata'. *Meta*-methoxytopolin and its riboside at a concentration of 25–75 mg $dm^{-3}$ applied in 4-h conditioning of leaves extended their post-harvest longevity, at the same time inhibiting protein degradation. In their later studies, Janowska et al. [2] reported that topolins used in conditioning and immersion of leaf blades also extended post-harvest longevity in leaves of *Limonium latifolium*. The greater efficacy of leaf blade immersion in growth regulator solutions in comparison to conditioning is most probably caused by the absence of transport, which may delay the effectiveness of the action of growth regulators.

Sugars formed in the process of photosynthesis are the primary building and reserve materials in plant organisms. Intensive photosynthesis promotes the accumulation of greater amounts of carbohydrates. In the tested cultivars in leaves treated with benzyladenine at a concentration of 100–600 mg $dm^{-3}$, the content of saccharides was higher than in the control plants harvested in the spring and summer. In the available literature, we may find only scarce information on changes in sugar contents in ornamental plants as a result of the application of growth regulators. Kozłowska et al. [18] reported on changes of sugar contents in leaves of *Zantedeschia elliottiana* following the application of gibberellic acid in soaking of rhizomes depending on the development phase. In the initial phase of vegetative development in leaf blades of plants treated with gibberellic acid, the authors reported higher carbohydrate contents, particularly fructose and glucose, in comparison to the control plants. These contents increased with leaf development; however, they decreased when plants entered into the generative phase. At that time, the total content of carbohydrates in leaves of the control plants was two-fold greater. Changes in sugar contents in cut leaves of *Zantedeschia aehtiopica* and *Z. elliottiana* were investigated [9]. The content of reducing sugars in the course of the ageing process increased in the beginning, to subsequently fall to 60–80% of the initial contents. Conditioning of leaves in benzyladenine solution did not inhibit this process. In contrast, gibberellic acid proved to be effective, as it delayed the degradation of sugars in leaves of *Zantedeschia aethiopica*, while in *Z. elliottiana* it caused an increase in their contents.

## Conclusions

- In ×*Heucherella* 'Solar Power', BA at a concentration of 100–600 mg $dm^{-3}$ had an advantageous effect on post-harvest longevity of leaves harvested in summer, improving it by 31.7–98.3%. In the cultivar 'Kimono', BA at the concentration of 100–600 mg $dm^{-3}$ improved post-harvest longevity of leaves harvested both in spring and summer by almost 2 months.
- BA at a concentration of 100–600 mg $dm^{-3}$ inhibited proteolysis in leaves of the ×*Heucherella* cultivars. The exception was summer-harvested leaves of 'Solar Power' and 'Kimono' sprayed with BA at a concentration of 100 mg $dm^{-3}$, in which protein content was comparable to that in the control leaves.
- In both tested cultivars, the content of saccharides was higher in leaves treated with BA at a concentration of 100–600 mg $dm^{-3}$ than in the control plants harvested in the spring and summer.

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## Opryskiwania roślin matecznych benzyloadeniną a pozbiorcza trwałość liści ×*Heucherella* L.

### Streszczenie

Do badań wykorzystano liście dwóch odmian ×*Heucherella* L.: 'Solar Power' i 'Kimono'. Liście pobrano w dwóch terminach: wiosną (22.05.2014) i latem (26.07.2014). Rośliny mateczne, w dniu poprzedzającym każdy termin zbioru liści, opryskano benzyloadeniną (BA) o stężeniu 100, 300 i 600 mg $dm^{-3}$. Po ścięciu liście umieszczono w pojemnikach z wodą destylowaną. U odmiany 'Solar Power' BA o stężeniu 100–600 mg $dm^{-3}$ korzystnie wpłynęła na pozbiorczą trwałość liści pozyskiwanych latem poprawiając ją o 31.7–98.3%, a u odmiany 'Kimono' BA w podanych stężeniach przedłużyła pozbiorczą trwałość liści pozyskanych zarówno wiosną jak i latem o prawie 2 miesiące. BA o stężeniu 100–600 mg $dm^{-3}$ hamowała proteolizę i rozkład cukrów w liściach badanych odmian ×*Heucherella*, za wyjątkiem ścinanych latem liści odmian 'Solar Power' i 'Kimono' z roślin opryskanych BA o stężeniu 100 mg $mg^{-3}$, u których poziom białka był porównywalny do oznaczonego w liściach kontrolnych.