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VASE LIFE OF *Heuchera* L. LEAVES FOLLOWING THE APPLICATION OF BENZYLADENINE

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ABSTRACT

The aim of this study was to assess vase life of leaves harvested at two dates – in the spring and summer, for 3 cultivars of *Heuchera* L. following the application of benzyladenine in spraying of maternal plants one day prior to leaf harvest. Leaves of cultivar 'Southern Comfort' were most durable, while those of cultivar 'Purple Petticoats' – least durable. Leaves harvested in summer had a longer vase life, except for leaves of cultivar 'Plum Royale'. BA (300 and 600 mg·dm^{-3}) in leaves of cultivar 'Plum Royale' improved vase life only in leaves harvested in the summer. BA (100 and 300 mg·dm^{3}) in cultivar 'Purple Petticoats' – in leaves harvested in spring, while when used at a concentration of 300 and 600 mg·dm^{-3} – in those collected in the summer. BA (100–600 mg·dm^{-3}) in cultivar 'Southern Comfort' considerably improved vase life of leaves cut in the spring and summer. BA inhibited protein degradation. In the tested cultivars in leaves, harvested in the spring and summer, treated with benzyladenine the content of saccharides was higher.

Key words: florists' green, BA, spraying, longevity, protein, saccharides

INTRODUCTION

Studies on vase life of flowers have been developing dynamically worldwide since the 1960's, while interest in vase life of florists' green is a much more recent phenomenon, sparked by its increased importance in floristry.

The ageing process in leaves, similarly as in flowers, is accelerated after they have been cut. The primary cause is the negative water balance resulted from excessive transpiration. It leads to leaf blade withering and a rapid loss of ornamental value. Moreover, proteins, sugars and chlorophyll are degraded in ageing leaves. Ageing in florists' greens may be controlled using growth regulators from the group of cytokinins and gibberellins [Skutnik et al. 2001, Pogroszewska and Woźniacki 2005, Janowska et al. 2012, 2013]. Their effectiveness depends on the species, cultivar, application method and concentration of growth regulators. Most frequently growth regulators are applied in the form of solutions in conditioning, which may last for 4–24 h in the cold room (longer conditioning) or in a room at 18–20°C (shorter conditioning). Moreover, leaf blades may be immersed for a short time in growth regulator solutions [Janowska 2012]. Among cytokinins benzyladenine is used most frequently in post-harvest treatment of leaves, while among gibberellins it is gibberellic acid.

The range of florists' green is being continuously extended. Apart from greenhouse species the commercial offer increasingly often comprises leaves and leaved shoots of plants grown outdoors. Leaves of *Heuchera* are occasionally found in floral arrangements; however, their use is very limited.

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The aim of this study was to assess vase life of leaves harvested at two dates – in the spring and summer, for 3 cultivars of *Heuchera* L. following the application of benzyladenine in spraying of maternal plants prior to leaf harvest.

MATERIAL AND METHODS

Analyses were conducted on leaves of 3 *Heuchera* L. cultivars: 'Plum Royale', 'Purple Petticoats' and 'Southern Comfort'. Leaves were harvested at two dates: spring (22.05.2014) and summer (26.07.2014). Healthy leaves with no mechanical damage were selected early in the morning from plants growing at the Department outdoor plantation and one day prior to each leaf harvest plants were sprayed with benzyladenine (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 distilled water. The leaves taken at random from the 10 plants sprayed BA at a concentrations tested. Selected leaves were fully-developed and had no damage or discolouring. After being cut the leaves were placed in containers with distilled water, which was replaced every 2 days. Post-harvest longevity was determined in the room at the temperature of 18–20°C, during a 10-hour photoperiod and under fluorescent light with quantum irradiance of 25 µmol·m^{2}·s^{1}. Relative humidity was maintained at 70%.

One treatment (date × cultivar × BA concentration) comprised 15 leaves (3 replications with 5 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. Contents of protein and sugars were also determined. After the loss of vase life the leaves were placed in the freezer. Samples (0.2 g for protein and 0.5 g for saccharide) for analysis were collected from all leaves in each treatment. The content of soluble protein was determined according to Bradford [1976]. The protein content, in three replication, was determined using bovine serum albumin as standard. Total saccharide contents were determined with the anthrone reagent [Björnesjö 1955]. The content of saccharides was read from a standard curve prepared for glucose. The final results, which were means of four replications, were expressed in mg of glucose per g of fresh weight.

The results were statistically calculated by means of three-way analysis of variance. Means were grouped with the use of Duncan test with the significance level of $\alpha = 0.05$.

RESULTS

The analyses showed a significant effect of *Heuchera* cultivars, benzyladenine concentration used in spraying maternal plants and the date of leaf harvest on vase life of leaves (tab. 1). It was found that in *Heuchera*, irrespective of BA concentration and harvest date, leaves of cultivar 'Soutern Comfort' were most durable, while the least durable leaves were produced by cultivar 'Purple Petticoats'. A positive effect of maternal plant spraying with benzyladenine on vase life of leaves was found in the analysed *Heuchera* cultivars. Irrespective of cultivar and harvest date leaves of the control plants were least durable. Following the application of BA at a concentration of 100 mg·dm^{-3} leaf durability increased on average by 32%. The most durable leaves were produced when the maternal plants were sprayed with BA at a concentration of 300 and 600 mg·dm^{-3}. In comparison to the control leaves their vase life increased by 55.8 and 59.4%, respectively. Irrespective of leaf harvest date, this dependence was evident in cultivar 'Purple Petticoats' and 'Southern Comfort'. In cultivar 'Plum Royale' leaves with a significantly improved vase life were obtained when maternal plants were sprayed with BA applied at a concentration of 600 mg·dm^{-3}. Irrespective of cultivar and BA concentration, significantly more durable leaves were produced when they were cut in the summer. A comparison of interactions showed that in cultivar 'Plum Royale' spraying of maternal plants with BA had an adverse effect on vase life of leaves collected in the spring. In that cultivar vase life improved only when leaves were harvested in the summer from maternal plants

Table 1. Effect of benzyladenine on post-harvest longevity (days) of the *Heuchera* L. leaves

Term	BA concentration (mg·dm^{-3})	Cultivar			Mean for term
		Plum Royale	Purple Petticoats	Southern Comfort	
Spring	0	89.2 d	25.5 a	36.4 a	55.1 a
	100	69.8 c	64.0 c	48.0 b	
	300	67.3 c	57.7 b	48.9 b	
	600	61.7 c	46.3 b	45.9 b	
Summer	0	62.0 c	54.0 b	67.0 c	97.2 b
	100	70.5 c	57.5 b	130.0 e	
	300	93.7 d	93.0 d	160.0 f	
	600	112.0 e	107.0 e	160.0 f	
					mean for BA concentration
Mean for concentration × cultivar	0	75.6 c	39.8 a	51.7 b	55.7 a
	100	70.2 c	60.8 b	89.0 d	73.3 b
	300	80.5 c	75.4 c	104.5 e	86.8 c
	600	86.7 d	76.7 c	103.0 e	88.8 c
Mean for cultivar		78.3 b	63.1 a	87.0 c	

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

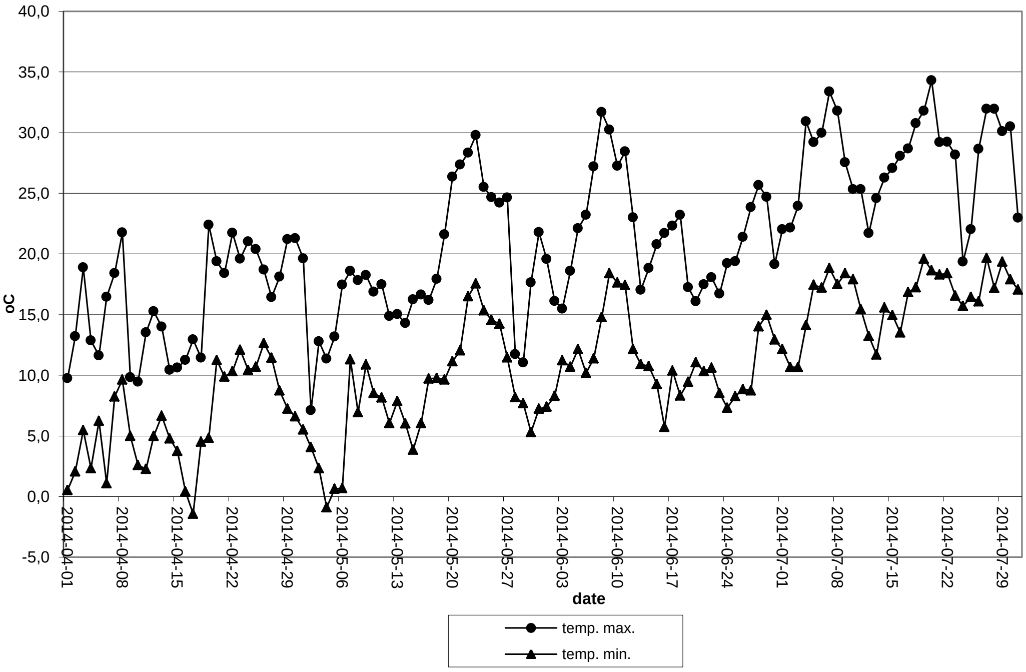

Fig. 1. Minimum and maximum temperatures in Poznań from 1.01. to 29.07 2014 (data based on measurements of the Meteorological Station of PULS, Poznań, Poland)

Table 2. Effect of benzyladenine on protein and saccharides content of the *Heuchera* L. leaves

	Term	BA concentration (mg·dm^{-3})	Cultivar			Mean for term
			Plum Royale	Purple Petticoats	Southern Comfort	
Protein content (mg·g^{-1} FW)	spring	0	24.5 f	14.2 c	10.3 b	18.7 b
		100	25.0 f	14.9 c	19.7 d	
		300	25.3 f	13.8 c	13.9 c	
		600	28.7 f	19.5 d	14.3 c	
	summer	0	19.0 d	10.1 b	14.8 c	16.0 a
		100	26.8 f	7.6 a	14.6 c	
		300	20.6 d	8.4 a	13.1 c	
		600	23.9 e	14.0 c	18.5 d	
						mean for BA concentration
	mean for concentration × cultivar	0	21.8 c	12.2 a	12.6 a	15.5 a
		100	25.9 d	11.3 a	17.2 b	18.1 b
		300	23.0 c	11.1 a	13.5 a	15.9 a
		600	26.3 d	16.8 b	16.4 a	19.8 b
	mean for cultivar		20.6 b	13.8 a	14.9 a	
Saccharides content (mg·g^{-1} FW)	spring	0	50.7 d	44.0 c	28.4 a	53.0 a
		100	58.0 e	52.2 d	35.2 b	
		300	57.8 e	60.3 f	38.6 b	
		600	57.1 e	111.3 j	42.2 c	
	summer	0	62.4 c	60.7 c	38.1 a	80.8 b
		100	73.2 d	69.7 d	48.8 b	
		300	72.0 d	79.3 e	51.5 b	
		600	72.4 d	115.3 f	59.3 c	
						mean for BA concentration
	mean for concentration × cultivar	0	62.4 c	60.7 c	38.1 a	53.7 a
		100	73.2 d	69.7 d	48.8 b	63.9 b
		300	72.0 d	79.3 e	51.5 b	67.6 b
		600	72. 4 d	115.3 f	59.3 c	82.3 c
	mean for cultivar		70.0 b	81.2 c	49.4 a	

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

sprayed with BA at a concentration of 300 and 600 mg·dm^{-3}. In cultivar 'Purple Petticoats' vase life of leaves collected in the spring was significantly better when maternal plants were sprayed with BA at a concentration of 100 and 300 mg·dm^{-3}, while in the case of those harvested in the summer it was at the application of BA at a concentration of 300 and 600 mg·dm^{-3}. In cultivar 'Southern Comfort' spraying of maternal plants with BA at the tested concentrations significantly extended vase life of leaves collected at both dates. Very durable leaves were produced in summer. Their vase life increased on by 94.0 and 138.8% in comparison to the vase life of the control leaves.

All the applied experimental factors had an effect on protein content (tab. 2). Irrespective of cultivar and BA concentration, a lower protein content was recorded in leaves harvested in the summer. Regardless of harvest date and BA concentration, significantly the greatest protein content in leaves was found in cultivar 'Plum Royale'. A comparison of interactions showed that in this cultivar BA application at a concentration of 100 and 600 mg·dm^{-3} had an advantageous effect on protein content in leaves harvested in the summer. In cultivar 'Purple Petticoats' this dependence was observed in leaves cut in the spring and summer at the application of BA at a concentration of 600 mg·dm^{-3}. In cultivar 'Southern Comfort' greater protein contents in leaves harvested in the spring were recorded in all treatments, in which BA was applied. In turn, in leaves collected in the summer a higher protein content was found only in the treatment with BA applied at a concentration of 600 mg·dm^{-3}.

A comparison of saccharides contents in leaves showed that it depended significantly on the applied experimental factors (tab. 2). Irrespective of cultivar and BA concentration, significantly higher amounts of saccharides were recorded in leaves harvested in the summer. Regardless of harvest date and BA concentration, the greatest saccharides content was found in leaves of cultivar 'Purple Petticoats', the least in leaves of cultivar 'Southern Comfort'. The application of BA at the tested concentrations had an advantageous effect on saccharides contents in leaves at both harvest dates and in all the cultivars. A comparison of interactions showed that in all the analyzed cultivars BA applied at a concentration of 100–600 mg·dm^{-3} had an advantageous effect on saccharides contents in leaves – both those harvested in the spring and those collected in the summer.

DISCUSSION

It was shown in this study leaves of cultivar 'Plum Royale' proved to be most durable, while leaves of cultivar 'Purple Petticoats' were least durable. It was also shown that leaves of the tested cultivars harvested in the summer had a longer vase life, except for leaves of cultivar 'Plum Royale'. This may be explained by the fact that the spring leaves did not reach physiological maturity. Analyses on vase life of florists' green are conducted on selected leaves, which are fully developed, free from mechanical damage and properly coloured; however, the evaluation conducted excluding physiological analyses may provide misleading results, as it was confirmed by this study. Despite the very early, warm spring, resulting in a considerable acceleration of vegetation period and a very early plant development, vase life of leaves harvested in the spring, except for leaves of cultivar 'Plum Royale', was barely satisfactory. The fluctuations of temperatures in Poznań in the first half of 2014 are presented in Figure 1. In April, the temperature exceeded 20°C, and in May – 30°C.

Efficacy of growth regulators applied to extend vase life of florists' green is closely related with the species, cultivar, concentration and applied form. This is confirmed by these experiments, which showed a positive effect of spraying maternal plants with benzyladenine on vase life of leaves in the tested *Heuchera* cultivars, while the response was dependent on the applied experimental factors. BA (300 and 600 mg·dm^{-3}) in leaves of cultivar 'Plum Royale' improved vase life only in leaves harvested in the summer. BA (100 and 300 mg·dm^{-3}) in cultivar 'Purple Petticoats' had an advantageous effect on vase life of leaves harvested in spring, while when used at a concentration of 300 and 600 mg·dm^{-3} – in those collected in the summer. BA (100–600 mg·dm^{-3}) in cultivar 'Southern Comfort' considerably improved vase life of leaves cut in the spring and summer. Results of studies conducted for over a decade indicate a highly varied response of species to applied growth regulators. As it was reported by Skutnik et al. [2004], benzyladenine is less effective than gibberellic acid in extending vase life of leaves in *Zantedeschia elliottiana* and *Z. aethiopica*. Skutnik and Rabiza-Świder [2005] showed that conditioning of *Hosta* leaves in a benzyladenine solution eliminates the adverse effects of storage. Janowska [2012] reported limited efficacy for the application of benzyladenine at a concentration of 25 and 50 mg·dm^{-3} in 24-h conditioning of *Arum italicum* leaves, since their vase life increased by as little as 2.2 to 5.4 days, whereas short-term dipping of leaf blades in this growth regulator was found to have no effect. The latest studies on the extension of vase life in florists' green indicate applicability of topolins – a new group of endogenous, aromatic cytokinins isolated from poplar at the Palacký University and Institute of Experimental Botany AS CR, the Czech Republic [Palavan-Ünsal et al. 2002]. Janowska et al. [2012] showed that *meta*-methoxytopolin and its riboside at a concentration of 25–75 mg·dm^{-3} applied in 4-h conditioning of leaves in *Zantedeschia albomaculata* 'Albomaculata' extend their post-harvest longevity. Both growth regulators applied at 25 and 50 mg·dm^{-3} in dipping of leaf blades for several seconds more effectively extended vase life of leaves in cultivar 'Albomaculata' than their 24-h conditioning. *Meta*-methoxytopolin in treatment with gibberellic acid at a concentration of 25 – 25 + 50 – 50 mg·dm^{-3} applied in soaking of leaf blades lasting for several seconds extend vase life of leaves on average by 14–24 days. In a later study Janowska et al. [2013] reported that topolins used in conditioning and soaking of leaf blades also extend vase life of leaves in *Limonium latifolium*. The greater effectiveness of dipping of leaf blades in solutions of growth regulators in comparison to conditioning is most probably caused by a lack of transport, which may delay the effects of growth regulator activity.

Proteins are important components of plant cells. They regulate life processes and are building blocks of cell structures and tissues, while they are also responsible for most biochemical reactions in living organisms. Proteolysis, i.e. the degradation of proteins, is one of the symptoms of the progressing ageing process. Senescence processes include degradation of macromolecules, including a decrease in protein level due to activation of proteases and carbohydrate losses resulting from activation of E-glucosidases [Rubinstein 2000]. Gan and Amasino [1997] reported that during the ageing process changes take place in tissues in the contents of growth regulators, 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 [1999] were of an opinion that cytokinins are responsible for the inhibition of protein and chlorophyll. These experiments showed that BA inhibited protein degradation: in leaves of cultivar 'Purple Petticoats' harvested in the spring and summer (600 mg·dm^{-3}), in cultivar 'Southern Comfort' in leaves cut in the spring (100–600 mg·dm^{-3}) and summer (600 mg·dm^{-3}), in cultivar 'Plum Royale' in leaves harvested in the summer (100 and 600 mg·dm^{-3}). In the research by Rabiza-Świder et al. [2004], leaves of *Zantedeschia aethiopica* and *Z. elliottiana* were subjected to 24-hour conditioning in solutions of benzyladenine and gibberellic acid. In both species only gibberellic acid effectively retarded the degradation of soluble proteins. The favourable effect of gibberellic acid onto the protein content in the leaves of *Zantedeschia* with colourful inflorescence spathes was also observed by Janowska and Stanecka [2011] and Janowska et al. [2012]. Similarly, in a study by Rabiza-Świder and Skutnik [2008], the conditioning of leaves of *Hosta* 'Crispula' and 'Undulata Mediovariegata' in gibberellic acid and benzyladenine retarded the degradation of soluble proteins, especially readily visible after the use of benzyladenine.

Saccharides formed in the process of photosynthesis are the primary building blocks and reserve substances in plant organisms. Intensive photosynthesis promotes accumulation of larger amounts of carbohydrates. Since soluble carbohydrates are cellular osmolytes, increase in soluble sugar content is effective in water retention and prevention of dehydration [Camejo et al. 2005]. Accumulation of soluble sugars in geranium leaves increased accumulation of starch for retention of cell turgescence. When water potential in a leaf is reduced, accumulation of sugars probably plays the main role of osmotic adjustment [Arora et al. 1998]. These investigations showed that in the tested cultivars in leaves treated with benzyladenine (100–600 mg·dm^{-3}) the content of saccharides was higher in leaves harvested in the spring and summer. The increase in the sugar content with advancement in age could be due to stimulation of amylase and other hydrolytic enzymes promoting the hydrolysis of storage reserves due to senescence [Sardoei et al. 2014]. Kozłowska et al. [2007] reported on changes in saccharides contents in leaves of *Zantedeschia elliottiana* following the application of gibberellic acid to soak root stocks depending on the phase of their development. Those authors reported that in the initial phase of vegetative development in leaf blades of plants treated with gibberellic acid the content of carbohydrates, particulary fructose and glucose, was higher than in the control plants. This content increased with the progressing leaf development, to decrease when plants entered the generative phase. During that time the total content of carbohydrates in leaves of the control plants was two times greater. Changes in saccharides contents in cut leaves of *Zantedeschia elliottiana* and *Z. aethiopica* were investigated by Skutnik et al. [2004]. Contents of reducing saccharides during the progressing ageing process increased initially, to decrease to 60–80% initial level. Conditioning of leaves in a benzyladenine solution did not inhibit this process. In contrast, gibberellic acid proved to be effective, as it delayed the degradation of saccharides in leaves of *Zantedeschia aethiopica*, while in *Z. elliottiana* it caused an increase in their contents. Downs et al. [1997] reported an advantageous effect of benzyladenine on saccharides contents in broccoli inflorescences, since it inhibited their degradation starting at the moment of their harvest. In turn, Smoleń and Sady [2009] showed an advantageous effect of benzyladenine on sugar contents in the development of radish root swelling.

CONCLUSION

1. Leaves of cultivar 'Southern Comfort' were most durable, while those of cultivar 'Purple Petticoats' – least durable.
2. Leaves harvested in summer had a longer vase life, except for leaves of cultivar 'Plum Royale'.
3. BA (300 and 600 mg·dm^{-3}) in leaves of cultivar 'Plum Royale' improved vase life only in leaves harvested in the summer.
4. BA (100 and 300 mg·dm^{3}) in cultivar 'Purple Petticoats' had an advantageous effect on vase life of leaves harvested in spring, while when used at a concentration of 300 and 600 mg·dm^{-3} – in those collected in the summer.
5. BA (100–600 mg·dm^{-3}) in cultivar 'Southern Comfort' considerably improved vase life of leaves cut in the spring and summer.
6. BA inhibited protein degradation: in leaves of cultivar 'Purple Petticoats' harvested in the spring and summer (600 mg·dm^{-3}), in cultivar 'Southern Comfort' in leaves cut in the spring (100––600 mg·dm^{-3}) and summer (600 mg·dm^{-3}), in cultivar 'Plum Royale' in leaves harvested in the summer (100 and 600 mg·dm^{-3}).
7. In the tested cultivars in leaves treated with benzyladenine (100–600 mg·dm^{-3}) the content of saccharides was higher in leaves harvested in the spring and summer.

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