POLISH SOCIETY FOR HORTICULTURAL SCIENCE

Folia Hort. 28/2 (2016): 137-142

DOI: 10.1515/fhort-2016-0016

FOLIA HORTICULTURAE

Published by the Polish Society
for Horticultural Science since 1989

ORIGINAL ARTICLE | Open access | http://www.foliahort.ogr.ur.krakow.pl

The effect of growth regulators on post-harvest *Alchemilla mollis* (Bauser) Rothm. leaf longevity

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ABSTRACT

Leaves of *Alchemilla mollis* (Bauser) Rothm. were the subject of the study. The leaves were harvested early in the morning from the department's ornamental plant collection. Selected leaves were fully-developed and showed no signs of damage or discolouring. Gibberellic acid (GA_3), benzyladenine (BA), *meta*-methoxytopolin (MemT) and its riboside (MemTR) at concentrations of 25, 50 and 75 mg dm^{-3} were applied in the form of solutions to four-hour leaf-conditioning in the room at a temperature of 18-20°C. After conditioning, the leaves were placed in distilled water. Leaves put into distilled water immediately after cutting served as the control. The post-harvest longevity of leaves of *Alchemilla mollis* was 7.2-11.8 days. The conditioning of leaves in gibberellic acid solutions at concentrations of 25-50 mg dm^{-3}, benzyladenine at concentrations of 25 mg dm^{-3} and *meta*-methoxytopolin and its riboside at concentrations of 75 mg dm^{-3} extended the post-harvest longevity of leaves by 10.1-81.9%. The conditioning of leaves in gibberellic acid at a concentration of 50 mg dm^{-3} inhibited the degradation of chlorophyll, as indicated by the highest SPAD index values.

Key words: BA, florists' greens, GA_3, MemT, MemTR quality, vase life

INTRODUCTION

With the development of the art of cut flower arrangement, there has been a growing interest in florists' greens, which is an indispensable element of floral arrangements today. The popularity of *Asparagus* shoots, once extremely fashionable, has been falling from year to year. Today's bouquets contain leaves and shoots of greenhouse plants from both domestic production and those imported from abroad. The leaves of perennial plants that are gaining in importance include *Hosta*, *Bergenia*, *Dictamnus*, *Paeonia*, *Heuchera* and many others. With fuel and energy prices growing, this is economically sound (Janowska and Schroeter-Zakrzewska 2010). The range of florists' greens grown outdoors might be supplemented with the large, round in outline and with a scalloped, serrated margin, greyish green leaves of *Alchemilla mollis*. The inflorescences of this species have been used for floral arrangement.

The post-harvest longevity of vegetative organs in species grown for florists' greens is genetically determined. In senescent leaves the process of photosynthesis is slower and chlorophyll and proteins are degraded (Hayden 2003). Species whose leaves, leaved shoots or phylloclades exhibit

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greatly extended post-harvest longevity after cutting include *Anthurium cultorum* (Hansen et al. 1991), *Asparagus falcatus* (Stamps et al. 2005) and species from the genus *Ruscus* sp. (Pacifici et al. 2013). However, the post-harvest longevity of leaves is far from satisfactory in most species grown for florists' greens. The leaves of *Arum italicum* wilt after 13.9 days (Janowska 2010); the leaves of cultivars of *Zantedeschia* with colourful spathes wilt after 6.9-15.7 days (Janowska and Stanecka 2011, Janowska et al. 2012).

Cultivation conditions have also been observed to have a considerable effect on post-harvest longevity – the greater the extent to which they satisfy the requirements of individual species, the more prolonged their longevity (Hayden 2003). The conditions under which florists' greens are transported and sold in wholesale also play a considerable role in this respect. Unfortunately, they are typically transported dry, frequently in vehicles not adapted to such a purpose, while in wholesale flower exchanges, florists' greens are offered in bunches of flowers, which are not kept in water. Under such conditions water stress, initiated at the moment that the leaves and leaved branches are cut off the maternal plants, becomes increasingly severe. Moreover, florists' greens are not supplemented with growth regulators, which play a very important role in the process of their senescence (Hayden 2003). The treatment of leaves with cytokinins and gibberellins delays the senescence process, since they inhibit the degradation of chlorophyll and proteins. In the case of cytokinins it is assumed that the sites on which these regulators are applied will become metabolically active and assimilates and biologically active compounds will target them.

The aim of this study was to assess the post-harvest leaf longevity of *Alchemilla mollis* following the application of growth regulators from the group of gibberellins and cytokinins in four-hour conditioning.

MATERIAL AND METHODS

The experiment was conducted at the Department of Ornamental Plants of the Poznan University of Life Sciences from June to July 2013 and from May to June 2014. Leaves of *Alchemilla mollis* (Bauser) Rothm. were the subject of the study. The leaves were harvested early in the morning from the department's ornamental plant collection. The selected leaves were fully developed and showed no signs of damage or discolouring.

Gibberellic acid (GA_3), benzyladenine (BA), *meta*-methoxytopolin (MemT) and its riboside (MemTR) at concentrations of 25, 50 and 75 mg dm^{-3} were applied in the form of solutions to four-hour leaf-conditioning in the room at a temperature of 18-20°C. After conditioning the leaves were placed in distilled water. Leaves put into distilled water immediately after cutting served as the control.

One treatment (growth regulator × concentration – in 2013 and 2014) comprised 15 leaves, five in three replications.

Post-harvest longevity was determined in the room at a temperature of 18-20ºC, 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%.

The post-harvest longevity was determined in days. The loss of decorative value was determined as the moment when 30% of the leaf surface turned yellow or was wilted. Moreover, the index of leaf greenness (SPAD) was determined using an N-Tester apparatus.

The results were statistically calculated by means of the two-factor analysis of variance. Means were grouped using the Duncan test with a significance level of $p = 0.05$.

RESULTS AND DISCUSSION

It was found that the post-harvest longevity of *Alchemilla* leaves was significantly dependent only on the concentration of growth regulators during both 2013 and 2014 (Tab. 1). Irrespective of the concentration of growth regulator applied in leaf conditioning in both years of the analysis, leaves with the shortest vase life were those of the control. Following the application of growth regulators at the adopted concentrations, leaf longevity increased by 18.6%, 22.9% and 23.7% (2013) and 36.1% and 38.9% (2014), respectively. Comparing the interaction, it was found that more leaves with longer longevity were obtained in 2013 when they were conditioned using GA_3 at a concentration of 25-50 mg dm^{-3}, BA at a concentration of 25 and 75 mg dm^{-3} and MemT and MemTR at a concentration of 25-75 mg dm^{-3}. In 2014, more leaves with longer longevity were obtained after the application of GA_3 and BA at a concentration of 25-50 mg dm^{-3} and MemT and MemTR at a concentration of 75 mg dm^{-3}, respectively.

Studies on the post-harvest longevity of florists' greens were initiated earlier than 2000. This was connected with the increased importance of

Table 1. Post-harvest longevity (days) of *Alchemilla mollis* leaves after the application of growth regulators

Year	Concentration (mg dm^{-3})	Growth regulator				Mean
		GA_3	BA	MemT	MemTR	
2013	0	11.8 a*	11.8 a	11.8 a	11.8 a	11.8 A
	25	15.4 c	13.0 b	14.1 b	13.3 b	14.0 B
	50	16.1 c	12.1 ab	13.9 b	15.8 c	14.5 B
	75	12.1 a	14.1 b	14.7 b	17.3 c	14.6 B
	Mean	13.9 A	12.8 A	13.6 A	14.6 A	
2014	0	7.2 a	7.2 a	7.2 a	7.2 a	7.2 A
	25	12.3 b	13.1 b	6.1 a	7.5 a	9.8 B
	50	12.1 b	12.3 b	7.0 a	8.4 a	10.0 B
	75	8.9 a	8.1 a	12.0 b	10.9 b	10.0 B
	Mean	10.1 AB	10.2 AB	8.1 A	8.5 A	

*Means followed by the same letter for each year do not differ significantly at $p = 0.05$

florists' greens added to floral arrangements. Due to the fact that the senescence process in florists' greens differs from that of flowers, in most cases the substances typically applied for flowers may not be used in florists' greens (Skutnik et al. 2001). Studies conducted worldwide focus on the application of growth regulators in the post-harvest handling of florists' greens. It was shown that their effectiveness depends on the species, cultivar, concentration and application method. In this study, the leaves of *Alchemilla mollis* positively responded to the tested growth regulators from the group of cytokinins and gibberellins. However, not all of the applied concentrations of growth regulators had an advantageous effect on the post-harvest longevity of leaves. The conducted analysis showed that individual species most frequently responded positively to one specific growth regulator. For example, the efficacy of gibberellic acid was indicated by a study by Janowska and Schroeter-Zakrzewska (2010) and Janowska et al. (2013). Those authors showed that GA_3 extends the post-harvest longevity of *Limonium latifolium* leaves. The leaves of *Arum italicum* also responded to gibberellic acid (Janowska and Schroeter-Zakrzewska 2008, Janowska 2010). In studies conducted by Janowska and Jerzy (2003), gibberellic acid had a favourable influence on the cut leaves of *Zantedeschia* with colourful spathes. In the cultivars 'Florex Gold' and 'Black Magic', leaves conditioned in gibberellic acid at a concentration of 300 mg dm^{-3} maintained their decorative values for the longest period of time. A comparable leaf longevity was recorded in the cultivar 'Florex Gold' placed in water after being conditioned in GA_3 at a concentration of 200 mg dm^{-3}. In turn, the results of studies conducted by Skutnik et al. (2001) indicate the high efficacy of gibberellic acid in the extension of the post-harvest longevity of *Zantedeschia aethiopica* leaves.

This study shows that the application of BA at concentrations of 25 and 75 mg dm^{-3} in 2013 and BA at a concentration of 25-50 mg dm^{-3} in 2014 had an advantageous effect on the post-harvest longevity of *Alchemilla mollis* leaves (Tab. 1). A similar reaction was observed in leaves of *Limonium latifolium* (Janowska et al. 2013) and *Arum italicum* (Janowska and Schroeter-Zakrzewska 2008).

Topolins are a new group of endogenous, aromatic cytokinins isolated from poplars at the Palacky University Olomouc and at the Institute of Experimental Botany in the Czech Republic. Topolins are derivatives of benzylaminopurine. In their benzene ring there is a hydroxyl group in the ortho- or meta- position. In the very few studies conducted so far, topolins have been used only in order to assess their usefulness in *in vitro* cultures. It has been determined in standard biological tests that these substances strongly prevent leaf ageing (Palavan-Űnsal et al. 2002, Podwyszyńska et al. 2012). In studies on the post-harvest longevity of florists' greens, topolins have been used relatively recently. Results published by Janowska et al. (2012 and 2013) showed that topolins may also be used *in vivo* in order to extend the vase life of florists' greens. Janowska et al. (2012) showed that topolins (MemT and MemTR) at a concentration of 25-75 mg dm^{-3} have an advantageous effect on the post-harvest longevity of leaves in *Zantedeschia albomaculata* 'Albomaculata'. These cytokinins applied in the four-hour conditioning of *Limonium latifolium* leaves proved to be equally effective, prolonging their vase life by 4.0-4.9 days (Janowska

Table 2. Index of leaf greenness (SPAD) of Alchemilla mollis leaves after the application of growth regulators

Year	Concentration ($mg\ dm^{-3}$)	Growth regulator				Mean
		GA_3	BA	MemT	MemTR	
2013	0	36.2 c*	36.2 c	36.2 c	36.2 c	36.2 B
	25	37.9 c	33.2 b	31.0 b	36.2 c	34.6 A
	50	39.7 d	32.2 b	35.0 c	26.8 a	33.4 A
	75	36.0 c	28.9 b	30.6 b	29.9 b	31.4 A
	Mean	37.5 B	32.6 A	33.2 A	32.3 A	
2014	0	34.8 a	34.8 a	34.8 a	34.8 a	34.8 A
	25	35.4 ab	36.0 ab	33.9 a	33.9 a	34.8 A
	50	36.8 b	33.6 a	34.1 a	34.5 a	34.8 A
	75	36.0 ab	33.2 a	35.6 ab	36.1 ab	35.2 A
	Mean	35.8 A	34.4 A	34.6 A	34.8 A	

*Explanations: see Table 1

et al. 2013). In this study, topolins also improved the vase life of *Alchemilla mollis* leaves, although an advantageous effect of these growth regulators in 2014 was found only when MemT and MemTR were applied at a concentration of 75 mg dm^{-3}. During 2013 the application of MemT and MemTR at all tested concentrations had an advantageous effect on the post-harvest longevity of *Alchemilla mollis* leaves, while for MemTR it was at 50-75 mg dm^{-3}, which confirms a varying response of different species not only to applied growth regulators, but also to their concentrations.

When assessing the post-harvest longevity of florists' greens, chlorophyll content or the correlated SPAD index (Pacewicz and Gregorczyk 2009) were investigated. These parameters make it possible to assess to what extent growth regulators used in experiments by inhibiting chlorophyll degradation influence the quality of green plant organs. As a result of chlorophyll loss by leaves, which is a natural process in the progressing senescence, gradually yellowing leaves reduce the decorative value of floral arrangements or flowers with leaved shoots.

In this study, a significant effect of growth regulators and concentration on the SPAD index was shown in *Alchemilla mollis* leaves only during 2013 (Tab. 2). Irrespective of their concentration, the highest SPAD index was recorded in leaves conditioned in gibberellic acid solutions. Leaves conditioned in cytokinin solutions had similar values of the SPAD index. Irrespective of the growth regulators, the highest index of leaf greenness was recorded in the control. When comparing interactions, the highest SPAD index was recorded in those leaves that were conditioned in gibberellic acid applied at a concentration of 50 mg dm^{-3}, while it was lowest in leaves conditioned in MemTR at a concentration of 50 mg dm^{-3}. In the other treatments the SPAD index was similar, comparable to that of the control, except in the treatments that used BA at a tested concentration, MemT at a concentration of 25 and 50 mg dm^{-3} and MemTR at a concentration of 75 mg dm^{-3}. The high efficacy of gibberellins in the inhibition of leaf yellowing in *Lilium* spp. and *Alstroemeria* spp., which extended the post-harvest longevity of flowers of these species, had been reported earlier (Dai and Paull 1991, Hicklenton 1991, Han 1995), which was confirmed by later studies by Taheri-Shiva et al. (2014) and Yeat et al. (2012). Gibberellins applied to florists' greens of many species inhibit senescence, thus limiting the degradation of chlorophyll. This was confirmed in a study by Janowska and Schroeter-Zakrzewska (2010), who used gibberellic acid to condition leaves of *Limonium latifolium*. Skutnik et al. (2004) reported the inhibition of chlorophyll degradation following the application of gibberellic acid in leaves of *Zantedeschia elliottiana* and *Z. aethiopica*. The advantageous effect of GA_3 on chlorophyll content in *Zantedeschia* leaves with colourful spathes was also confirmed in a study by Janowska and Stanecka (2011). Those authors also showed that chlorophyll degradation in *Zantedeschia* leaves may also be effectively inhibited by benzyladenine and a mixture of benzyladenine and gibberellic acid. Janowska and Śmigielska (2010) showed the advantageous effect of benzyladenine on the SPAD index in leaves on shoots of *Hypericum* 'Magical Beauty'. In turn, Skutnik et al. (2006) observed a similar effect in *Asparagus setaceus.* Scarce studies published to date on the post-harvest longevity of florists' greens after the application of topolins have shown that in *Limonium latifolium* only *meta*-methoxytopolin had an advantageous effect on the SPAD index of leaves (Janowska et al. 2013), while topolins inhibited only protein degradation in *Zantedeschia albomaculata* 'Albomaculata' leaves, having no effect on the chlorophyll content. In turn, Podwyszyńska et al. (2012) reported that in *Cotinus coggygria* 'Royal Purple' propagated *in vitro* following medium supplementation with MemT and MemTR, the chlorophyll content in the leaves was 60% higher in comparison to the leaves of the control plants.

CONCLUSIONS

1. The post-harvest longevity of leaves of *Alchemilla mollis* was 7.2-11.8 days.
2. The conditioning of leaves in gibberellic acid solutions at concentrations of 25-50 mg dm^{-3}, benzyladenine at concentrations of 25 mg dm^{-3}, meta-methoxytopolin and its riboside at concentrations of 75 mg dm^{-3} extended the post-harvest longevity of leaves by 10.1-81.9%.
3. Leaves conditioned in gibberellic acid at a concentration of 50 mg dm^{-3} had higher SPAD index values.

FUNDING

This study was financially supported by the Ministry of Sciences and Higher Education as part of the research potential of the Department of Ornamental Plants and the Department of Plant Physiology at the Poznan University of Life Sciences.

AUTHOR CONTRIBUTIONS

The following declaration about author contributions to the research have been made: B.J. designed the research; B.J., P.J., A.A., D.N. and A.K. conducted the research; R.A. made data analyses; B.J. wrote the paper.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

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Received October 28, 2015; accepted May 17, 2016