

Article

The Vase Life of the Leaves of Selected Perennial Species after the Application of Growth Regulators

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Abstract: The aim of the study was to assess the post-harvest life of the leaves of *Hemerocallis × hybrida* ‘Agata’, *Limonium latifolium*, and *Heuchera hybrida* ‘Chocolate Ruffles’ after the application of growth regulators from the group of gibberellins (GAs) and cytokinins (CKs), ionic liquids (2-hydroxyethyl)dimethylethylammonium gibberellinate [Chol][Gib] and acetylcholine gibberellinate [Gib][Ach], as well as quaternary ammonium salts with the gibberellinate anion (1-ethyl quinine gibberellinate [Q-C₂][Gib]) and 1-dodecyl acetylcholine gibberellinate [Q-C₁₂][Gib]). The leaves were conditioned for 4 h in aqueous solutions of benzyladenine (BA), *meta*-methoxytopolin (MemT) and its riboside (MemTR), gibberellic acid (GA₃), [Q-C₂][Gib], [Gib][Ach], [Chol][Gib], and [Q-C₁₂][Gib] at concentrations of 50 and 100 mg·dm⁻³. Conditioning of *Hemerocallis × hybrida* ‘Agata’ with MemT and [Chol][Gib] at both concentrations, [Q-C₂][Gib] (100 mg·dm⁻³) and [Gib][Ach] (50 mg·dm⁻³), extended the vase life of the leaves by 7–9 days. The application of [Gib][Ach] (50 and 100 mg·dm⁻³) and [Q-C₁₂][Gib] (100 mg·dm⁻³) resulted in the longest vase life of the leaves of *Limonium latifolium*. Conditioning of the leaves of *Heuchera hybrida* ‘Chocolate Ruffles’ with BA, MemT, and MemTR (50 and 100 mg·dm⁻³) extended their vase life by 9.5–51.3 days. BA at a concentration of 100 mg·dm⁻³ was the most effective. MemT (50 mg·dm⁻³), MemTR (100 mg·dm⁻³), [Q-C₂][Gib] (100 mg·dm⁻³), [Gib][Ach] (100 mg·dm⁻³), and [Chol][Gib] (50 mg·dm⁻³) inhibited the degradation of proteins in the leaves of *Hemerocallis × hybrida* ‘Agata’; [Chol][Gib] (50 and 100 mg·dm⁻³)—in the leaves of *Limonium latifolium*; all the conditioners except for BA—in the leaves of *Heuchera hybrida* ‘Chocolate Ruffles’. GA₃, MemTR, [Gib][Ach], [Q-C₁₂][Gib] at both concentrations, [Q-C₂][Gib], and [Chol][Gib] (50 mg·dm⁻³) inhibited the degradation of chlorophyll in the leaves of *Hemerocallis × hybrida* ‘Agata’. All conditioners except for [Gib][Ach] and [Q-C₁₂][Gib] inhibited chlorophyll degradation in the leaves of *Limonium latifolium*. All conditioners except for MemT and MemTR (50 mg·dm⁻³) inhibited chlorophyll degradation in the leaves of *Heuchera hybrida* ‘Chocolate Ruffles’. [Chol][Gib] (50 mg·dm⁻³) was the most effective.

Keywords: florists’ greens; cytokinins; gibberellic acid; gibberellinates; postharvest longevity



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1. Introduction

The development of the art of arranging cut flowers increased the interest in florists’ greens, which is now an indispensable element of floral arrangements. The interest in *Asparagus* phylloclades, which used to be very popular, is gradually declining. The leaves and shoots of greenhouse plants are the most important elements of modern bouquets. The significance of the leaves of field plants is increasing. The post-harvest life of the vegetative organs of the species grown for florists’ greens is genetically determined. The leaves, leafy shoots, and phylloclades of *Anthurium cultorum* [1], *Asparagus falcatius*, and species of the

Ruscus sp. genus [2] are long-lived after cutting. However, the vase life of the leaves of most species grown for florists' greens is not satisfactory, as was observed by Janowska [3] in the study on *Arum italicum*, as well as by Janowska and Stanecka [4] and by Janowska et al. [5] in their studies on *Zantedeschia* cultivars with colorful spathes.

The vase life of florists' greens is greatly influenced by the cultivation conditions. The conditions of transporting florists' greens and their supply on markets are also important. Unfortunately, these organs are usually transported in unsuitable vehicles, without access to water. Then, they are sold on markets, where they are offered in bunches without access to water again. Moreover, florists' greens are not supplied with growth regulators, which play a very important role in the ageing process [6].

Chlorophyll and proteins break down in senescent leaves, whereas photosynthesis is slower [6]. The permeability of membranes in senescent plant cells changes and their integrity is lost [7]. As a result of the imbalance between the amount of water taken up and transpired, cells lose their turgor [8,9]. Proteolysis accelerates in senescent plant tissues and there is increased activity of enzymes involved in the catabolism of membrane lipids [10]. It is believed that one of the causes of cell ageing is the uncontrolled formation of free radicals, which leads to the autoxidation of cell components [11].

The ageing process is controlled by growth regulators, which have different effects. Cytokinins (CKs) and gibberellins (GAs) are considered ageing inhibitors. During the ageing process, their content in plant tissues decreases, whereas the level of growth regulators such as ethylene, salicylic acid (SA), brassinosteroids (BR), abscisic acid (ABA), and jasmonic acid (JA) increases and the ageing process accelerates [12]. Therefore, it is desirable to reverse the harmful effects of regulators signaling the ageing process [13] and/or disrupt the developmental memory [14].

GAs are synthesized in active growing tissues, i.e., shoot apices, young leaves, and flowers [15]. They are transported through the conducting tissues: xylem and phloem [16]. GAs, which can be found in various plants, have a broad spectrum of activity. They eliminate the hereditary stunting of plants [17], stimulate shoot elongation and flower development [18], interrupt deep dormancy [19], stimulate seed germination [20], accelerate or delay flowering [21], influence the growth of seedless fruits, extend the life of flowers and leaves of various species of ornamental plants [18], and affect the content of chlorophyll and proteins [4,5]. The best-known and most frequently used GA is gibberellic acid (GA₃) [17]. In the case of florists' greens, GA₃ is usually used as an aqueous solution for 4–24 h conditioning. Short conditioning is carried out at the temperature of 18–20 °C, longer conditioning—at the temperature of 5 °C. Its effectiveness in extending the vase life of florists' greens has been demonstrated in species cultivated in the open field [3,8] and in protected environments [4,5,8]. GAs are obtained through the submerged or solid-state fermentation of *Gibberella fujikuroi* or *Fusarium moniliforme* (*F. fujikuroi*) fungi. Nitrogen-fixing bacteria such as *Azospirillum* spp. are also used for the production of GA₃. Researchers discovered that a large amount of GA₃ could be produced by using *F. moniliforme* (*F. fujikuroi*) on pomace (oil waste) from *Jatropha curcas* seeds [22]. GA₃ is produced on a large scale through the fermentation of the *G. fujikuroi* fungus [23]. However, due to the fact that this process is long, GA₃ is not soluble in water, and degrades at high temperature, researchers are conducting investigations to invent new methods of GA₃ acquisition to reduce the time of its production [24]. Scientists from the Faculty of Chemical Technology, Poznań University of Technology, Poland, conducted research and obtained quaternary ammonium salts with selected organic cations and gibberellinate anions. Salts with choline ([Chol][Gib]) and acetylcholine (ACh) ([Gib][ACh]) cations were obtained through metathesis with GA₃ potassium salt in methanol (CH₃OH). Salts with the 1-ethyl quinine ([Q-C₂][Gib]) cation and 1-dodecyl quinine ([Q-C₁₂][Gib]) cation were obtained in a two-stage reaction. At the first stage, the bromide anion was exchanged for a hydroxyl one by means of an ion-exchange resin. It was followed by a neutralization reaction with GA₃. The application of both methods enabled the exchange of the halide anion for the gibberellinate anion. The reactions were characterized by high efficiency of 97–99%. At

a temperature of 25 °C, [Chol][Gib] and [Gib][Ach] are glassy solids and belong to the group of ionic liquids. The melting point of [Q-C₂][Gib] and [Q-C₁₂][Gib] is outside the tested range and these are quaternary ammonium salts. The transformation of the active substance (choline or ACh) into quaternary ammonium salts made it possible to obtain more effective growth regulators characterized by better bioactivity. The synthesis methods developed are rapid and highly efficient. The solubility of the obtained salts depends on the structure of the cation, with most compounds being soluble in polar solvents (water, methanol (CH₃OH), DMSO). Additionally, the obtained salts are chemically stable at higher temperatures [24].

CKs are adenine derivatives. They stimulate cell division, seed germination [25], and, above all, they inhibit the ageing of plants, cut flowers, and florists' greens [26–28]. They are mainly produced by the roots, from which they are transported to the aerial parts of the plant [25]. CKs play a key role in various phases of plant growth and development. The basic molecular mechanisms of their biosynthesis and signal transduction have been explained recently [29]. Benzyladenine (BA), which is a synthetic CK, is commonly used in floriculture [30]. BA is primarily used as a growth regulator responsible for the in vitro propagation of ornamental plants. In recent years, it has also been applied to plants growing in vivo [15].

According to the latest research, topolins (Ts) can also extend the life of florists' greens. They are a group of endogenous, aromatic CKs isolated from poplars at the Palacký University and the Institute of Experimental Botany in the Czech Republic. Ts are benzylaminopurine (BAP) derivatives. They have an ortho- or meta-hydroxyl group in the benzene ring [31]. Experiments have shown that MemT and its riboside (MemTR) extend the post-harvest life of the leaves of *Zantedeschia albomaculata* 'Albomaculata' [5] and *Limonium latifolium* [32].

The effectiveness of growth regulators used to extend the life of florists' greens is closely related to the plant species and cultivar as well as the concentration and form of application. According to Skutnik et al. [33], BA cannot extend the life of the leaves of *Zantedeschia aethiopica* and *Z. elliotiana* as effectively as GA₃. According to Janowska [34], the use of BA for 24-hour conditioning of the leaves of *Arum italicum* has poor effects, because their life can be extended only by 2.2–5.4 days, whereas a short-term soaking of the leaves in this regulator is completely ineffective. The researcher observed a very positive effect of GA₃, because the post-harvest life of the leaves of *Arum italicum* was extended for about 2 weeks by both their 24-hour conditioning and their short-term soaking in the solution of this growth regulator.

The aim of the study was to assess the post-harvest life of the leaves of *Hemerocallis × hybrida* 'Agata', *Limonium latifolium*, and *Heuchera hybrida* 'Chocolate Ruffles' after the application of growth regulators from the group of GAs and CKs, ionic liquids ([Chol][Gib] and [Gib][Ach]), and quaternary ammonium salts with the gibberellinate anion ([Q-C₂][Gib]) and [Q-C₁₂][Gib].

2. Materials and Methods

Leaves of *Hemerocallis × hybrida* Hort. 'Agata', *Limonium latifolium*/Sm./Kuntze, and *Heuchera hybrida* L. 'Chocolate Ruffles' belonging to the collection of soil plants growing at the Department of Ornamental Plants, Dendrology, and Pomology were cut early in the morning on 18 May 2020. Healthy leaves without mechanical damage were cut from the middle part of the rosette. They were conditioned in aqueous solutions of BA, MemT and MemTR, GA₃, [Q-C₂][Gib], [Gib][Ach], [Chol][Gib], and [Q-C₁₂][Gib] concentrated at 50 and 100 mg·dm⁻³ for 4 h at a room temperature of 18–20 °C (Figure 1). The characteristics of gibberellinates are described in Table 1. The concentration for the ionic salts was determined relative to the percentage of GA₃ anion content. The growth regulators were dissolved in a few (4–5) drops of 96% ethyl alcohol (C₂H₅OH). Then, water was added to obtain the appropriate concentration. After conditioning, the leaves were placed in distilled water.

The leaves which were placed in distilled water from the beginning of the experiment were used as control samples.



Figure 1. Leaves of *Limonium latifolium*, *Heuchera hybrida* 'Chocolate Ruffles', and *Hemerocallis × hybrida* 'Agata' after conditioning.

Table 1. Ionic liquids and quaternary ammonium salts with the gibberellate anion. T_m , melting point; T_g , glass transition temperature; $T_{onset5\%}$, decomposition temperature of 5% sample; $T_{onset50\%}$, decomposition temperature of 50% sample.

Ionic Liquids/Quaternary Ammonium Salts with the Gibberellinate Anion	Concentration of GA ₃ Anion (%)	T_m (°C)	T_g (°C)	$T_{onset5\%}$ (°C)	$T_{onset50\%}$ (°C)
[Chol] [Gib]	77.0	98	78	170	236
[Gib] [Ach]	70.0	97	110	180	252
[Q-C ₂] [Gib]	49.4	99	-	175	285
[Q-C ₁₂] [Gib]	41.2	99	-	219	315

The vase life of the leaves was determined at a room temperature of 18–20 °C, 12-hour photoperiod, and fluorescent light with a quantum radiation intensity of 25 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. The relative humidity was maintained at 70%. The water was changed twice a week during the experiment.

There were 24 treatments within one species in the experiment. There were three replications, each with three leaves. There were nine leaves in each treatment (conditioning solution \times concentration).

The vase life of the leaves was expressed as the number of days until they lost their ornamental value, i.e., the moment when 30% of the leaf blade surface dried, yellowed, and/or wilted. At the end of the experiment, the leaf content of chlorophyll *a* + *b* and proteins was measured.

The content of chlorophyll *a* + *b* was measured with the method invented by Hiscox and Israelstam [35], which enabled the extraction of pigments with dimethyl sulphoxide (DMSO) without tissue maceration. Aliquots (100 mg) were treated with 5 cm³ of DMSO and incubated in a water bath at 65 °C for 60 min. The level of the pigments in the obtained extract was measured spectrophotometrically at the appropriate wavelength. The

absorbance of the extract was measured at 663 nm for chlorophyll *a* and at 645 nm for chlorophyll *b*. Arnon's [36] formulas were used to calculate the content of the pigments, which was expressed as $\text{mg}\cdot\text{g}^{-1}$ fresh weight. The results were expressed as the sum of chlorophyll *a* and *b*.

The protein content was measured with the Bradford method [37]. The procedure for determination of protein concentration utilizes the phenomenon of formation of a pigment-protein complex, which shifts the wavelength corresponding to the maximum absorption of the pigment from 465 to 595 nm. The absorbance value is proportional to the protein concentration. In this study, Coomassie Brilliant Blue G-250 (CBB) (2 cm^3) dissolved in 85% orthophosphoric acid was added to 50 μL of the extract. After 10 min, the absorbance was measured at a wavelength of 595 nm. The curve prepared for albumin was used to determine the protein content.

The results of the experiment were analyzed with two-way analysis of variance. The means were grouped with Duncan's test at a significance level $\alpha = 0.05$. The results were processed with the Statistica 13.3 software.

3. Results

3.1. Vase Life and Quality of *Hemerocallis* \times *hybrida* 'Agata' Leaves

3.1.1. Leaf Vase Life

The comparison of the results showed that the vase life of the *Hemerocallis* \times *hybrida* 'Agata' leaves depended significantly on both the type of conditioner and its concentration (Table 2). The comparison of the effects showed that the vase life of the leaves was significantly the longest after the application of MemT at both concentrations, [Q-C₂][Gib] at a concentration of $100\text{ mg}\cdot\text{dm}^{-3}$, [Gib][Ach] at a concentration of $50\text{ mg}\cdot\text{dm}^{-3}$, and [Chol][Gib] at concentrations of 50 and $100\text{ mg}\cdot\text{dm}^{-3}$. The vase life of the leaves in these treatments was 26.5–32.6% longer than that of the control leaves. The vase life of the leaves treated with MemTR and [Q-C₁₂][Gib] at concentrations of 50 and $100\text{ mg}\cdot\text{dm}^{-3}$ and [Gib][Ach] at a concentration of $100\text{ mg}\cdot\text{dm}^{-3}$ was shorter, but it was still longer than that of the control leaves. The vase life of the leaves in the other treatments was comparable to that of the control leaves.

Table 2. Vase life (days) of leaves, protein, and chlorophyll *a* + *b* content ($\text{mg}\cdot\text{g}^{-1}$ F.W.) in leaves of *Hemerocallis* \times *hybrida* 'Agata' depending on conditioner type and concentration. Means followed by the same letter column-wise and row-wise do not differ significantly at $\alpha = 0.05$.

Conditioner	Concentration ($\text{mg}\cdot\text{dm}^{-3}$)		
	0	50	100
Vase life (days)			
BA	26.4 a	25.0 a	25.7 a
MemT	26.4 a	33.8 c	34.9 c
MemTR	26.4 a	30.0 b	31.6 b
GA ₃	26.4 a	25.4 a	27.1 a
[Q-C ₂][Gib]	26.4 a	24.8 a	35.4 c
[Gib][Ach]	26.4 a	33.4 c	31.9 b
[Chol][Gib]	26.4 a	34.3 c	35.0 c
[Q-C ₁₂][Gib]	26.4 a	30.1 b	31.8 b
Protein content ($\text{mg}\cdot\text{g}^{-1}$ F.W.)			
BA	3.50 a	3.25 a	3.56 a
MemT	3.50 a	4.93 b	4.37 ab
MemTR	3.50 a	4.18 ab	4.87 b
GA ₃	3.50 a	3.81 a	4.25 ab
[Q-C ₂][Gib]	3.50 a	4.25 ab	4.68 b
[Gib][Ach]	3.50 a	5.06 c	4.62 b
[Chol][Gib]	3.50 a	4.81 b	4.00 ab
[Q-C ₁₂][Gib]	3.50 a	3.68 a	3.98 ab

Table 2. Cont.

Conditioner	Concentration (mg·dm ⁻³)		
	0	50	100
	Chlorophyll a + b content (mg·g ⁻¹ F.W.)		
BA	0.12 a	0.11 a	0.25 d
MemT	0.12 a	0.12 a	0.12 a
MemTR	0.12 a	0.29 e	0.36 f
GA ₃	0.12 a	0.41 g	0.24 d
[Q-C ₂][Gib]	0.12 a	0.35 f	0.12 a
[Gib][Ach]	0.12 a	0.21 c	0.31 e
[Chol][Gib]	0.12 a	0.37 f	0.13 a
[Q-C ₁₂][Gib]	0.12 a	0.18 b	0.25 d

3.1.2. Leaf Protein Content

The comparison of the results showed that the leaf protein content depended significantly on both the type of conditioner and its concentration (Table 2). The comparison of the effects showed that the conditioning of the leaves with [Gib][Ach] at a concentration of 50 mg·dm⁻³ resulted in the highest protein content. It was 44.6% higher than in the control leaves. The leaves conditioned with MemT at a concentration of 50 mg·dm⁻³, MemTR at a concentration of 100 mg·dm⁻³, [Q-C₂][Gib] at a concentration of 100 mg·dm⁻³, [Gib][Ach] at a concentration of 100 mg·dm⁻³, and [Chol][Gib] at a concentration of 50 mg·dm⁻³ had a lower protein content, but it was still significantly higher than in the control leaves. The leaf protein content in the other treatments was comparable to that of the control leaves.

3.1.3. Leaf Chlorophyll a + b Content

The leaf chlorophyll content depended significantly on both the type of conditioner and its concentration (Table 2). The lowest chlorophyll content were found in the control leaves as well as in the leaves conditioned with BA at a concentration of 50 mg·dm⁻³, MemT concentrated at 50 and 100 mg·dm⁻³, [Q-C₂][Gib], and [Chol][Gib] at a concentration of 100 mg·dm⁻³. By contrast, the highest chlorophyll content was found in the leaves conditioned with GA₃ at a concentration of 50 mg·dm⁻³. Their chlorophyll content was 241.67% higher than in the control leaves. The content of chlorophyll was lower in the other treatments, but it was still significantly higher than in the control leaves.

3.2. Vase Life and Quality of *Limonium latifolium* Leaves

3.2.1. Leaf Vase Life

The comparison of the results showed that the vase life of the *Limonium latifolium* leaves depended on the type of conditioner only (Table 3). The comparison of the effects showed that the vase life of the leaves was significantly extended only in three treatments, i.e., [Gib][Ach] at concentrations of 50 and 100 mg·dm⁻³ (extension by 22% and 29.3%, respectively) and [Q-C₁₂][Gib] at a concentration of 100 mg·dm⁻³ (extension by 22%).

Table 3. Vase life (days) of leaves, protein, and chlorophyll *a + b* content ($\text{mg}\cdot\text{g}^{-1}$ F.W.) in leaves of *Limonium latifolium* depending on conditioner type and concentration. Means followed by the same letter column-wise and row-wise do not differ significantly at $\alpha = 0.05$.

Conditioner	Concentration ($\text{mg}\cdot\text{dm}^{-3}$)		
	0	50	100
Vase life (days)			
BA	15.0 a	16.8 a	15.6 a
MemT	15.0 a	15.6 a	16.1 a
MemTR	15.0 a	14.3 a	17.1 ab
GA ₃	15.0 a	15.6 a	15.4 a
[Q-C ₂][Gib]	15.0 a	15.1 a	13.9 a
[Gib][Ach]	15.0 a	18.3 b	19.4 b
[Chol][Gib]	15.0 a	15.7 a	16.1 a
[Q-C ₁₂][Gib]	15.0 a	16.4 a	18.3 b
Protein content ($\text{mg}\cdot\text{g}^{-1}$ F.W.)			
BA	17.50 a	18.12 ab	18.93 b
MemT	17.50 a	18.37 b	17.12 a
MemTR	17.50 a	17.93 ab	18.43 b
GA ₃	17.50 a	19.81 c	17.66 a
[Q-C ₂][Gib]	17.50 a	20.56 c	20.31 c
[Gib][Ach]	17.50 a	21.06 cd	18.87 b
[Chol][Gib]	17.50 a	21.62 d	20.87 d
[Q-C ₁₂][Gib]	17.50 a	20.00 c	20.25 c
Chlorophyll <i>a + b</i> content ($\text{mg}\cdot\text{g}^{-1}$ F.W.)			
BA	1.24 b	1.80 f	1.90 g
MemT	1.24 b	1.87 fg	1.97 g
MemTR	1.24 b	1.60 e	1.54 e
GA ₃	1.24 b	1.31 c	2.23 h
[Q-C ₂][Gib]	1.24 b	1.80 f	1.78 f
[Gib][Ach]	1.24 b	1.31 c	1.00 a
[Chol][Gib]	1.24 b	1.42 d	1.70 f
[Q-C ₁₂][Gib]	1.24 b	1.50 e	1.00 a

3.2.2. Leaf Protein Content

The comparison of the results showed that the leaf protein content depended significantly on both the type of conditioner and its concentration (Table 3). The comparison of the effects showed that the protein content was significantly the lowest in the control leaves and those conditioned with MemT and GA₃ at a concentration of 100 $\text{mg}\cdot\text{dm}^{-3}$. The highest protein content was found in the leaves conditioned with [Chol][Gib] concentrated at 50 and 100 $\text{mg}\cdot\text{dm}^{-3}$, where it increased by 23.5% and 19.2%, respectively. The increase in the leaf protein content in the other treatments ranged from 2.5% to 20.6%.

3.2.3. Leaf Chlorophyll *a + b* Content

The comparison of the effects showed that the chlorophyll content was significantly the lowest in the leaves conditioned with [Gib][Ach] and [Q-C₁₂][Gib] at a concentration of 100 $\text{mg}\cdot\text{dm}^{-3}$. The highest chlorophyll content was found in the leaves conditioned with GA₃ at a concentration of 100 $\text{mg}\cdot\text{dm}^{-3}$. Their chlorophyll content was 79.8% higher than in the control leaves. The chlorophyll content in the other treatments was higher than in the control leaves, which means that the regulators inhibited the degradation of this pigment (Table 3).

3.3. Vase Life and Quality of *Heuchera hybrida* 'Chocolate Ruffles' Leaves

3.3.1. Leaf Vase Life

The comparison of the results showed that the vase life of *Heuchera hybrida* 'Chocolate Ruffles' leaves depended significantly on both the type of conditioner and its concentration (Table 4). The comparison of the effects showed that the vase life of the leaves was significantly the longest after the application of BA at a concentration of 100 mg·dm⁻³. The vase life of these leaves was 111.3% longer than that of the control leaves. Apart from that, the vase life of the leaves in the treatments treated with MemT and MemTR was also significantly extended—by 20.6–68.3%. The vase life of the leaves conditioned with GA₃, [Q-C₂][Gib], [Gib][Ach], [Chol][Gib], and [Q-C₁₂][Gib] at both concentrations was 37.1–66.2% shorter than that of the control leaves.

Table 4. Vase life (days) of leaves, protein, and chlorophyll *a* + *b* content (mg·g⁻¹ F.W.) in leaves of *Heuchera hybrida* 'Chocolate Ruffles' depending on conditioner type and concentration. Means followed by the same letter for column-wise and row-wise do not differ significantly at $\alpha = 0.05$.

Conditioner	Concentration (mg·dm ⁻³)		
	0	50	100
Vase life			
BA	46.1 d	65.0 f	97.4 h
MemT	46.1 d	77.6 g	57.4 e
MemTR	46.1 d	55.6 e	73.1 g
GA ₃	46.1 d	21.3 ab	16.9 a
[Q-C ₂][Gib]	46.1 d	25.8 b	22.4 ab
[Gib][Ach]	46.1 d	17.9 ab	18.1 ab
[Chol][Gib]	46.1 d	19.8 ab	21.6 ab
[Q-C ₁₂][Gib]	46.1 d	29.0 c	15.6 a
Protein content (mg·g ⁻¹ F.W.)			
BA	3.25 a	3.43 a	3.50 a
MemT	3.25 a	4.50 ab	5.00 b
MemTR	3.25 a	3.93 ab	5.12 b
GA ₃	3.25 a	12.62 f	11.06 df
[Q-C ₂][Gib]	3.25 a	40.75 h	11.43 df
[Gib][Ach]	3.25 a	10.87 d	16.43 g
[Chol][Gib]	3.25 a	12.75 f	10.31 d
[Q-C ₁₂][Gib]	3.25 a	8.18 c	10.18 d
Chlorophyll <i>a</i> + <i>b</i> content (mg·g ⁻¹ F.W.)			
BA	0.43 a	0.84 b	0.58 b
MemT	0.43 a	0.37 a	0.64 b
MemTR	0.43 a	0.45 a	0.65 b
GA ₃	0.43 a	1.82 f	1.52 e
[Q-C ₂][Gib]	0.43 a	1.80 f	1.83 f
[Gib][Ach]	0.43 a	1.30 d	1.50 e
[Chol][Gib]	0.43 a	2.03 g	1.56 e
[Q-C ₁₂][Gib]	0.43 a	1.14 c	1.36 d

The comparison of the results showed that the leaf protein content depended significantly on both the type of growth regulator and its concentration (Table 4). The comparison of the effects showed that the conditioning of the leaves with [Q-C₂][Gib] at a concentration of 50 mg·dm⁻³ resulted in the highest significant protein content. It was 1153.8% higher than in the control leaves. The lowest protein content was noted in the control treatment and in the leaves conditioned with BA at both concentrations. The leaf protein content in other treatments was significantly lower, but it was still higher than the protein content in the control leaves. The increase in the protein content ranged from 53% to 405.5%.

3.3.2. Leaf Chlorophyll a + b Content

The comparison of the results showed that the leaf chlorophyll content depended significantly on both the type of growth regulator and its concentration (Table 4). The comparison of the effects showed that the chlorophyll content was significantly the highest in the leaves conditioned with [Chol][Gib] at a concentration of $50 \text{ mg}\cdot\text{dm}^{-3}$. Their chlorophyll content was 372.1% higher than that of the control leaves. The lowest chlorophyll content was found in the control leaves as well as those conditioned with MemT and MemTR at a concentration of $50 \text{ mg}\cdot\text{dm}^{-3}$. The content of chlorophyll in the other treatments was 34.9–325.6% higher than in the control leaves.

4. Discussion

The beginnings of research on the adjustment of the post-harvest life of plants date back to the 1960s. At that time, researchers were interested in using CKs to extend the post-harvest life of vegetables. They observed that these growth regulators effectively extended the post-harvest life of celery, endive, and lettuce. Later, they applied CKs to extend the life of cut flowers and florists' greens [4]. Cut flowers and florists' greens are delicate and short-lived products. Their life ranges from less than ten days to a dozen or so depending on the genus, species, and cultivar, as researchers observed. In our study, the vase life of leaves of *Hemerocallis* × *hybrida* 'Agata' was 26.4 days, *Limonium latifolium*—15 days, and *Heuchera hybrida* 'Chocolate Ruffles'—46.1 days. The vase life of leaves of these species was extended by conditioning them with the growth regulators. The effectiveness depended on the type of conditioner and its concentration. The four-hour conditioning of *Hemerocallis* × *hybrida* 'Agata' leaves with MemT and [Chol][Gib] at both concentrations, [Q-C₂][Gib] at a concentration of $100 \text{ mg}\cdot\text{dm}^{-3}$, and [Gib][Ach] at a concentration of $50 \text{ mg}\cdot\text{dm}^{-3}$ resulted in the best vase life extension effect. The vase life of the leaves in these treatments was about 8 days longer than that of the control leaves. The vase life of the leaves conditioned with MemTR and [Q-C₁₂][Gib] at concentrations of 50 and $100 \text{ mg}\cdot\text{dm}^{-3}$ and [Gib][Ach] at a concentration of $100 \text{ mg}\cdot\text{dm}^{-3}$ was slightly shorter, but it was still longer than that of the control leaves. The available scientific publications do not provide information on the influence of growth regulators on the post-harvest life of the leaves of species of the *Hemerocallis* genus, but Gulzar et al. [38] observed that CKs extended the vase life of *Hemerocallis fulva* flowers. The authors found that spraying partially open *Hemerocallis fulva* flowers with cycloheximide (CHI) and transferring them into benzylaminopurine (BAP) or kinetin delayed their senescence and maintained the quality of their flowers, which had a higher water content. The researchers also observed that the treatments reduced ion leakage, and thereby membrane integrity was maintained. These treatments not only effectively slowed down the degradation of proteins when flowers opened and aged, but they also maintained the respiratory pool of sugars in the tissues of the perianth. Ammonium salts with the gibberellinate anion are new growth regulators. Therefore, there has not been much information known about their effects so far. Szymaniak et al. [24] observed that when these salts were applied at a concentration of $100 \text{ mg}\cdot\text{dm}^{-3}$ to *Convallaria majalis* leaves, they significantly improved the vase life. The only exception was the salt containing the 1-ethyl quinine cation, which exhibited higher activity at a concentration of $50 \text{ mg}\cdot\text{dm}^{-3}$. This effect may have been caused by the unique interactions of the quinine cation, which was not blocked by the presence of a long alkyl chain.

In our study, the treatment of *Limonium latifolium* with [Gib][Ach] at concentrations of 50 and $100 \text{ mg}\cdot\text{dm}^{-3}$, as well as [Q-C₁₂][Gib] at a concentration of $100 \text{ mg}\cdot\text{dm}^{-3}$, resulted in the longest vase life of the leaves. The other regulators were completely ineffective. The results of our research did not confirm the findings of earlier studies. Janowska and Schroeter-Zakrzewska [39] were the first to observe that GAs and CKs effectively extended the vase life of *Limonium latifolium* leaves. According to Janowska et al. [32], GA₃, BA, and Ts extended the vase life of the leaves of this species.

Our study showed that the conditioning of *Heuchera hybrida* 'Chocolate Ruffles' leaves with BA at a concentration of $100 \text{ mg}\cdot\text{dm}^{-3}$ resulted in their longest vase life, which was as

much as 111% longer than that of the control leaves. BA at the lower concentration was also effective, but it extended the vase life of the leaves only by 41%. The conditioning of the leaves of this *Heuchera* cultivar with Ts at concentrations of 50 and 100 mg·dm⁻³ extended their vase life by 68.3% and 20.6%, respectively. On the other hand, GA₃ and ammonium salts with the gibberellate anion significantly shortened the leaf vase life. The results of our experiment confirmed the thesis that the effectiveness of growth regulators depends on the plant species to which they are applied, their type, and concentration. Therefore, the final results do not always meet our expectations. Janowska et al. [40] observed that the spraying of *Heuchera* leaves with BA at concentrations of 300 and 600 mg·dm⁻³ extended their vase life by 55.8% and 59.4%, respectively.

Proteins and chlorophyll become degraded in the cells of senescent leaves. It is a natural but unfavorable process. Therefore, all post-harvest treatments are aimed at stopping this phenomenon. Proteins are important components of plant cells. They regulate life processes, are the building blocks of cellular structures and tissues, and they are responsible for the majority of biochemical reactions which occur in living organisms. Proteolysis, i.e., protein degradation, is a symptom of progressive senescence. Ageing causes the degradation of macromolecules. Protein levels decrease as a result of protease activation, whereas the activation of E-glucosidases causes the loss of carbohydrates [41]. According to Gan and Amasino [42], the senescence process causes changes in the tissue content of GAs and CKs, which are ageing inhibitors. In our study the growth regulators inhibited, to different extents, the breakdown of protein in the leaves of the plant species under analysis. The conditioning of *Hemerocallis* × *hybrida* “Agata” with MemT at a concentration of 50 mg·dm⁻³, MemT at a concentration of 100 mg·dm⁻³, [Q-C₂][Gib] and [Gib][Ach] at a concentration of 100 mg·dm⁻³, and [Chol][Gib] at a concentration of 50 mg·dm⁻³ inhibited the degradation of proteins in the leaves. This effect was observed in *Limonium latifolium* when the leaves of the plant were conditioned with [Chol][Gib] at concentrations of 50 and 100 mg·dm⁻³. All conditioners except for BA inhibited the decomposition of protein in the leaves of *Heuchera hybrida* ‘Chocolate Ruffles’. The effect was particularly noticeable after the treatment with [Q-C₂][Gib]. The positive effect of growth regulators from the GAs and CKs groups on the leaf protein content was confirmed by research on the vase life of florists’ greens. Janowska et al. [40] observed that when BA was applied at an appropriate concentration, it inhibited the degradation of proteins in the leaves of *Heuchera* ‘Purple Petticoats’ harvested in spring and summer, in the leaves of the ‘Southern Comfort’ cultivar harvested in spring and in summer, and in the leaves of the ‘Plum Royale’ cultivar harvested in summer. Janowska and Stanecka [4] and Janowska et al. [5] observed the positive influence of GA₃ on the content of protein in the leaves of *Zantedeschia* with colorful spathes.

Our study showed that the growth regulators had positive influence on the leaf chlorophyll content. Their effectiveness depended on the type of the growth regulator, its concentration, and the plant species. The treatment of *Hemerocallis* × *hybrida* ‘Agata’ with GA₃, MemTR, [Gib][Ach], and [Q-C₁₂][Gib] at both concentrations, as well as [Q-C₂][Gib] and [Chol][Gib] at a concentration of 50 mg·dm⁻³, inhibited the degradation of chlorophyll in the leaves. All the conditioners, except for [Gib][Ach] and [Q-C₁₂][Gib], inhibited the degradation of chlorophyll in *Limonium latifolium*, but GA₃ at a concentration of 100 mg·dm⁻³ was the most effective. As far as *Heuchera hybrida* ‘Chocolate Ruffles’ is concerned, the degradation of chlorophyll was not inhibited only by Ts at a concentration of 50 mg·dm⁻³. By contrast, chlorophyll degradation in this cultivar was particularly effectively inhibited by [Chol][Gib] at a concentration of 50 mg·dm⁻³. Interestingly, GA₃ and ionic liquids had an unfavorable effect on postharvest leaf longevity of ‘Chocolate Ruffles’ cultivar, and yet they inhibited chlorophyll degradation. The results obtained require further research to find the reasons for this phenomenon. Perhaps the answer is to be found in a different chlorophyll degradation process, as seen, for example, in stay-green plants (SGR). Thomas and Ougham [43] report on the existence of functional SGR phenotypes in *Arabidopsis thaliana*, which are characterized by a significant delay in the

initiation (SGR type A mutants) or prolongation (SGR type B mutants) of the aging process, of which the breakdown of chlorophyll is only one of the important elements. According to Nakajima et al. [44], the persistence of green pigments in the leaves of mutants is related to the delayed detachment of light harvesting complex of photosystem II (LHCII), which slows down the conversion of chlorophyll *b* to chlorophyll *a* and reduces substrate availability for pheophorbide *a* oxygenase (PaO). Studies conducted by researchers around the world show how important it is to select the right type and concentration of the growth regulator to obtain the best effect in specific plant species. Janowska et al. [45] observed that only GA₃ at a concentration of 50 mg·dm⁻³ inhibited the degradation of chlorophyll in *Alchemilla mollis*, whereas CKs–BA, MemT, and its riboside proved to be ineffective in this species. As early as the 1990s, studies on *Lilium* sp. and *Alstroemeria* sp. showed the high efficiency of GAs as leaf yellowing inhibitors. The slowing down of leaf-yellowing in these species also extended the life of their cut flowers [46,47]. This effect was confirmed in later studies by Taheri-Shiva et al. [48] and Yeat et al. [49]. Janowska and Schroeter-Zakrzewska [39] also observed the positive effect of GA₃ on the content of chlorophyll in the ageing leaves of *Limonium latifolium*. Janowska and Stanecka [4] observed a similar effect in *Zantedeschia* with colorful spathes. Moreover, these authors found that the degradation of chlorophyll in *Zantedeschia* leaves could also be effectively inhibited by BA and a mixture of BA with GA₃. Khan et al. [50] observed that GA₃ concentrated at 50 and 100 ppm increased the chlorophyll content in *Cymbopogon martinii* leaves by 14.5% and 62%, respectively. The treatment of *Abelmoschus esculentus* leaves with GA₃ at a concentration of 60 ppm increased their chlorophyll content [51]. According to Szymaniak et al. [24], GA₃ solutions and gibberellinates inhibit chlorophyll degradation in *Convallaria majalis* leaves. Salts with 1-alkyl quinine cations are as effective as GA₃.

5. Conclusions

The vase life of *Hemerocallis* × *hybrida* ‘Agata’ leaves was 26.4 days, *Limonium latifolium*—15 days, and *Heuchera* × *hybrida* ‘Chocolate Ruffles’—46.1 days. Conditioning of *Hemerocallis* × *hybrida* ‘Agata’ with MemT and [Chol][Gib] at both concentrations, [Q-C₂][Gib] (100 mg·dm⁻³) and [Gib][Ach] (50 mg·dm⁻³), extended the vase life of the leaves by 7–9 days. The application of [Gib][Ach] (50 and 100 mg·dm⁻³) and [Q-C₁₂][Gib] (100 mg·dm⁻³) resulted in the longest vase life of the leaves of *Limonium latifolium*. Conditioning of the leaves of *Heuchera hybrida* ‘Chocolate Ruffles’ with BA, MemT, and MemTR (50 and 100 mg·dm⁻³) extended their vase life by 9.5–51.3 days. BA at a concentration of 100 mg·dm⁻³ was the most effective. MemT (50 mg·dm⁻³), MemTR (100 mg·dm⁻³), [Q-C₂][Gib] (100 mg·dm⁻³), [Gib][Ach] (100 mg·dm⁻³), and [Chol][Gib] (50 mg·dm⁻³) inhibited the degradation of proteins in the leaves of *Hemerocallis* × *hybrida* ‘Agata’; [Chol][Gib] (50 and 100 mg·dm⁻³)—in the leaves of *Limonium latifolium*; the all conditioners, except for BA—in the leaves of *Heuchera hybrida* ‘Chocolate Ruffles’. GA₃, MemTR, [Gib][Ach], [Q-C₁₂][Gib] at both concentrations, [Q-C₂][Gib], and [Chol][Gib] (50 mg·dm⁻³) inhibited the degradation of chlorophyll in the leaves of *Hemerocallis* × *hybrida* ‘Agata’. All conditioners except for [Gib][Ach] and [Q-C₁₂][Gib] inhibited chlorophyll degradation in the leaves of *Limonium latifolium*. All conditioners except for MemT and MemTR (50 mg·dm⁻³) inhibited chlorophyll degradation in the leaves of *Heuchera hybrida* ‘Chocolate Ruffles’. [Chol][Gib] (50 mg·dm⁻³) was the most effective.

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