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The effect of cycloheximide and growth regulators on the senescence of cut leaves in *Hosta* sp. and *Zantedeschia aethiopica*

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ABSTRACT

The leaves of *Zantedeschia* and *Hosta* are used as florist greens in different floral arrangements. The most efficient postharvest treatment for cut foliage is the use of growth regulators, which prolong their vase life by delaying degradative changes occurring in leaves, especially proteolysis. Cycloheximide (CHI) is one of the protein synthesis inhibitors, blocking the enzymes responsible for decreasing membrane integrity, a phenomenon hastening senescence. The aim of this experiment was to evaluate the effects of CHI and benzyladenine (BA) or gibberellic acid (GA_3) on the longevity of cut foliage in hosta (*Hosta* sp.) cultivars and Ethiopian calla (*Zantedeschia aethiopica*) and to follow the changes in certain proteolytic processes occurring during senescence. Generally, 24 h conditioning with cycloheximide shortened the longevity of cut calla leaves while having no effect on hosta vase life. In ageing leaves of 'Minima Glauca' hosta and calla, the total proteolytic activity increased, including that of cysteine protease. Due to the application of BA or GA_3 in hosta and calla, respectively, this activity was limited. On the contrary, the use of CHI either did not affect the activity of cysteine protease or increased it several-fold relative to the control, in hosta and calla, respectively. Leaves treated with growth regulators had many more soluble proteins and fewer free amino acids, including free proline, than leaves from other treatments. The highest free proline level was determined in calla leaves conditioned with CHI, where it increased 18-fold relative to the initial level.

Key words: cut foliage, cysteine protease activity, free amino acids, free proline, proteolytic activity, vase life

Abbreviations:

BA – benzyladenine, CHI – cycloheximide, DTT – dithiothreitol (1,4-dithio-DL-threitol), d.w. – dry leaf weight, EDTA – ethylenediaminetetraacetic acid, GA_3 – gibberellic acid, HEPES – (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), PGRs – plant growth regulators, TCA – trichloroacetic acid

INTRODUCTION

Today, green plant elements are indispensable in floral compositions. Most plants grown for florist greens come from the tropics (palms, ferns, vine leaves or leafy shoots of tropical shrubs) and are available on the market all year long. In the temperate zone, many plants can also be grown for this purpose, either under covers or in the open

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air. The latter include perennials, especially hostas (*Hosta* sp.), whose numerous cultivars provide leaves of different shapes, sizes and colours, available for harvest during nearly the entire vegetative period (between June and October). Another popular plant producing showy foliage is calla, both Ethiopian (*Zantedeschia aethiopica* (L.) Spreng.) and interspecific hybrids (*Zantedeschia* sp.). The former provides leaves during winter and early spring while the hybrids also in summer.

When used together with long lasting flowers, cut leaves of hosta and calla usually lose their decorative values first. This is due to their unbalanced water status after detachment from the mother plant resulting in the disruption of water uptake and the endogenous hormone supply from the plant roots. Their senescence can be delayed by synthetic hormones, cytokinins or gibberellins in hosta and calla leaves, respectively. In our earlier trials the 24 h conditioning of cut leaves of *Hosta* 'Undulata Erromena' with benzyladenine prolonged their longevity 6-fold relative to the control untreated leaves (Skutnik and Rabiza-Świder 2005), while in calla a similar treatment with gibberellic acid prolonged foliage vase life 1.5-fold (Skutnik et al. 2001).

Senescence in leaves initiated by their detachment from the plant is associated with various biochemical and physiological changes. A drop in chlorophyll content is the first visual senescence symptom (Skutnik et al. 2004) and it is accompanied by protein degradation due to an increased activity of proteolytic enzymes (Rabiza-Świder et al. 2003), resulting in the accumulation of free amino acids, including free proline and ammonium (Yang and Kao 2000, Rabiza-Świder et al. 2004 a, b). Delaying proteolysis is fundamental for a delay of senescence. Hydrolysis of peptide bonds releasing amino acids is carried out by specific endo- and exoproteases. The final hydrolysis products (including ammonium) may be dangerous for cells so they are transformed into less toxic forms such as amides, which are also more easily transportable (Nooden and Guiament 1996). An application of protease inhibitors seems a logical solution when aiming to improve the longevity of cut foliage.

The aim of the experiment was to evaluate the effect of cycloheximide and growth regulators (BA and GA_3) on longevity and processes occurring in senescing cut leaves of *Hosta* and *Zantedeschia*, including proteolytic activity and soluble protein content as well as the accumulation of free amino acids including free proline.

MATERIAL AND METHODS

The leaves of *Zantedeschia aethiopica* (L.) Spreng. and *Hosta* L. ('Golden Tiara', 'Minima Glauca' and 'Undulata Erromena') were harvested from the plant collections of the Department of Ornamental Plants of the Warsaw University of Life Sciences. Mature, undamaged leaves were cut in the morning and immediately transported to the laboratory, where they were graded for uniformity and conditioned. *Hosta* leaves were treated with BA (0.1 mmol dm^{-3}) and *Zantedeschia* leaves with GA_3 (0.25 mmol dm^{-3}) for 24 hours and placed in distilled water. Similarly, pulse conditioning (24 hours) with cycloheximide for hosta (20, 50, 100, 200, 500 µmol dm^{-3}) and for Ethiopan calla (20, 50, 100, 200, 500, 750, 1000 µmol dm^{-3}) (Tab. 1) was also given for both genera under controlled conditions: temperature 20°C, relative humidity 60%, quantum irradiance of 35 µmol m^{-2} s^{-1}, under the 12 h day and 12 h night regime. Unpulsed leaves held in water were treated as respective controls. There were 10 leaves in each treatment, individually tagged and treated as separate replications. Leaf vase life was considered as terminated when approx. 30% of blade surface was yellow or wilted. These symptoms were evaluated visually.

The subsequent experiments were carried out to follow and compare the biochemical changes in leaves and consisted of three treatments: water control, leaves treated with the CHI concentration that was the most effective for vase life (for *Hosta* 'Minima Glauca' 500 µmol dm^{-3}, for *Z. aethiopica* 1000 µmol dm^{-3}) and leaves conditioned 24 h with 0.1 mmol dm^{-3} benzyladenine or 0.25 mmol dm^{-3} gibberellic acid, in hosta and calla, respectively.

Samples for biochemical analyses were collected several times during the experiments, on days: 0 (immediately after cutting off), 13, 18 and 24 for *Hosta* 'Minima Glauca' and 0, 5, 11 and 15 for *Z. aethiopica*. The above dates were related to the senescence rate of the given taxon. The blades were finely chopped, mixed and 0.5 g samples were prepared. They were kept under -80ºC until analyses.

Total proteolysis and the activity of the cysteine protease were measured as described by Zagdańska and Wiśniewski (1996). Enzymes were extracted in HEPES with 1 mmol dm^{-3} DTT and 1 mmol dm^{-3} EDTA, pH 7.5. To determine total proteolysis, the extract was incubated 3 hrs at 37°C with

citric-phosphate buffer pH 5.0 and azocasein. To determine the cysteine protease activity, 10 mmol dm^{-3} iodoacetate was added to the extract-buffer mixture, with azocasein being included after one hour of incubation. The reaction was stopped by 24% TCA. Extinction was read against a blank at 340 nm. The protease activity was calculated as a difference in readings with and without the iodoacetate. Results are given in arbitrary units which correspond to changes in absorbance of 0.01 during one hour, calculated on mg of protein in the sample as determined by the Bradford method (1976).

The soluble protein and free amino acid contents were determined according to Bradford (1976) and Rosen (1957), respectively, calculated from previously plotted standard curves, and expressed in mg of albumin bovine serum or µmol leucine, respectively, per gram of dry leaf weight (d.w.). Leaf tissue was dried at 105°C until a constant weight was achieved (Strzelecka et al. 1982). The free proline content was determined according to Bates et al. (1973) by measuring the quantity of the coloured reaction product of proline with ninhydric acid. The absorbance was read at 520 nm. The amount of proline was calculated from the previously plotted standard curve and expressed in µmol g^{-1} of d.w.

Results were statistically evaluated by ANOVA using the StatGraphics Plus program. Duncan's test at p = 0.05 was applied to assess the significant differences between the means.

RESULTS

Generally, the treatments with CHI were ineffective and some even shortened foliage longevity. Only CHI in a concentration of 500 µmol dm^{-3} prolonged the vase life of 'Minima Glauca' hosta, over 30% relative to the untreated control. Its lower concentration of 100 µmol dm^{-3} increased leaf longevity by two days in hosta 'Undulata Erromena' (Tab. 1).

During the senescence of leaves in 'Minima Glauca' hosta, the proteolytic activity, including that of cysteine protease, increased (Tab. 2). The highest activities were found in the control leaves on the third sampling date (24 days after harvest); the total proteolytic activity and that of cysteine protease increased 5- and 10-fold, respectively, as compared to the initial measurement. On the 13th day after harvest the proteolytic activity in leaves conditioned with CHI was approximately half of

Table 1. Effect of 24 h conditioning with cycloheximide on the postharvest longevity of cut leaves [days]

Treatment	*Hosta*			*Zantedeschia aethiopica*	
	Golden Tiara	Minima Glauca	Undulata Erromena	Experiment 1	Experiment 2
H_2O (control)	16.5 a*	17.4 b	6.1 a	14.4 c	9.9 b
20 µmol dm^{-3} CHI	13.0 a	11.4 a	7.8 ab	8.3 ab	-
50 µmol dm^{-3} CHI	12.4 a	17.8 b	6.8 ab	7.9 a	-
100 µmol dm^{-3} CHI	13.1 a	21.1 b	8.2 b	9.2 ab	-
200 µmol dm^{-3} CHI	14.1 a	20.4 b	7.3 ab	8.8 ab	-
500 µmol dm^{-3} CHI	13.8 a	22.9 c	7.2 ab	9.7 b	8.0 a
750 µmol dm^{-3} CHI	-**	-	-	-	9.2 ab
1000 µmol dm^{-3} CHI	-	-	-	-	10.6 b

*Values in columns marked by the same letter do not differ significantly at p = 0.05 (Duncan's test)
**Not tested

Table 2. Effect of 24 h conditioning with cycloheximide or benzyladenine on the activity of proteolytic enzymes [unit mg (protein)$^{-1}$ h^{-1}] in cut leaves of *Hosta* 'Minima Glauca'

Treatment	Total proteolytic activity on day				Mean	Cysteine protease activity on day				Mean
	0	13	18	24		0	13	18	24	
H_2O (control)	0.8 a*	6.9 bc	10.4 c	35.1 d	13.3 b	0.6 a	3.1 b	10.2 c	32.0 d	11.5 b
0.1 mmol dm^{-3} BA	0.8 a	0.9 a	1.2 a	1.9 a	1.2 a	0.6 a	0.5 a	0.5 a	1.0 ab	0.7 a
500 µmol dm^{-3} CHI	0.8 a	3.8 ab	3.6 ab	38.0 d	11.6 b	0.6 a	2.2 ab	2.3 ab	31.3 d	9.1 b
Mean	0.8 a	3.8 b	5.1 b	25.0 c		0.6 a	1.9 ab	4.3 b	21.4 c	

*Values marked by the same letter do not differ significantly at p = 0.05 (Duncan's test)

Table 3. Effect of 24 h conditioning with cycloheximide or benzyladenine on the soluble protein content [mg g^{-1} d.w.] in cut leaves of *Hosta* 'Minima Glauca'

Treatment	Soluble protein content on day				Mean
	0	13	18	24	
H_2O (control)	119.4 d*	109.9 cd	85.2 ab	75.7 a	97.6 a
0.1 mmol dm^{-3} BA	119.4 d	146.4 e	114.0 d	103.1 c	120.7 c
500 µmol dm^{-3} CHI	119.4 d	113.7 d	100.2 c	88.1 b	105.4 b
Mean	119.4 c	123.3 c	99.8 b	89.0 a	

*Explanations: see Table 2

Table 4. Effect of 24 h conditioning with cycloheximide or benzyladenine on free amino acid content [µmol (leucine) g^{-1} d.w.] and free proline content [µmol g^{-1} d.w.] in cut leaves of *Hosta* 'Minima Glauca'

Treatment	Free amino acids content on day			Mean	Free proline content on day			Mean
	0	13	18		0	13	18	
H_2O (control)	367.5 a*	673.2 b	973.7 d	671.5 b	3.2 c	4.1 e	5.6 f	4.3 c
0.1 mmol dm^{-3} BA	367.5 a	302.4 a	312.9 a	327.6 a	3.2 c	2.6 b	2.1 a	2.6 a
500 µmol dm^{-3} CHI	367.5 a	693.8 b	824.7 c	628.7 b	3.2 c	3.7 d	4.0 e	3.6 b
Mean	367.5 a	556.4 b	703.8 c		3.2 a	3.5 a	3.9 a	

*Explanations: see Table 2

that in the untreated leaves but on the 24th day it increased and attained the value determined in the control treatment. A similar tendency was observed in cysteine protease from the CHI-treated leaves. The lowest total proteolytic and cysteine protease activities were found in leaves conditioned with BA. On the 13th day of vase life they did not differ from the initial values and increased only negligibly on the next dates (18 and 24 days after harvest).

Different results were obtained for calla (Tab. 5). Here the lowest proteolytic activity was found in the control untreated leaves, where it kept increasing during vase life, up to four times relative to the initial value on the 15th day. In the GA_3 conditioned leaves the activity increased 5-fold already on the first sampling date but remained stable till the end of the experiment. A significant increase in proteolytic activity occurred in calla leaves conditioned with CHI: almost 18-fold as compared to the initial activity. Similar tendencies were observed for the activity of cysteine protease, which was the highest in the CHI-treated leaves, where it increased 12 times relative to the initial value and this increase occurred already on the 11th day of vase life.

Both the treatment and the sampling date significantly affected the soluble protein contents in hosta leaves (Tab. 3). These contents decreased during leaf senescence in all of the treatments. Only in the BA-treated leaves on the 13th day was the protein level higher than it was immediately after harvest, and it was also higher than in the control treatment and in the CHI-conditioned leaves. Similar results were obtained for calla (Tab. 6), where the GA_3 conditioned leaves were also the richest in soluble proteins. On the last sampling date (15th day), their content was only 6% lower than immediately after harvest while in the control leaves and those conditioned with CHI it fell by 50% and over 3-fold, respectively.

Table 5. Effect of 24 h conditioning with cycloheximide or gibberellic acid on the activity of proteolytic enzymes [unit mg (protein)$^{-1}$ h^{-1}] in cut leaves of *Zantedeschia aethiopica*

Treatment	Total proteolytic activity on day				Mean	Cysteine protease activity on day				Mean
	0	5	11	15		0	5	11	15	
H_2O (control)	1.5 a*	2.5 a	3.4 a	6.0 b	3.4 a	1.3 a	1.7 a	2.7 b	4.2 d	2.5 a
0.25 mmol dm^{-3} GA_3	1.5 a	7.2 bc	6.4 b	6.9 bc	5.5 b	1.3 a	1.7 a	2.6 b	2.6 b	2.1 a
1000 µmol dm^{-3} CHI	1.5 a	8.0 c	28.7 e	26.4 d	16.2 c	1.3 a	3.7 c	18.6 e	19.3 f	10.7 b
Mean	1.5 a	5.9 b	12.8 c	13.1 c		1.3 a	2.4 b	8.0 c	8.7 c	

*Explanations: see Table 2

Table 6. Effect of 24 h conditioning with cycloheximide or gibberellic acid on the soluble protein content [mg g^{-1} d.w.] in cut leaves of *Zantedeschia aethiopica*

Treatment	Soluble protein content on day				Mean
	0	5	11	15	
H_2O (control)	189.2 e*	181.0 de	146.6 c	93.0 b	152.5 b
0.25 mmol dm^{-3} GA_3	189.2 e	262.3 f	184.6 e	177.4 de	203.4 c
1000 µmol dm^{-3} CHI	189.2 e	172.1 d	54.5 a	48.8 a	116.2 a
Mean	189.2 c	205.1 d	128.6 b	106.4 a	

*Explanations: see Table 2

Table 7. Effect of 24 h conditioning with cycloheximide or gibberellic acid on the free amino acid content [µmol (leucine) g^{-1} d.w.] and free proline content [µmol g^{-1} d.w.] in cut leaves of *Zantedeschia aethiopica*

Treatment	Free amino acids content on day				Mean	Free proline content on day				Mean
	0	5	11	15		0	5	11	15	
H_2O (control)	204.4 a*	307.4 c	343.5 e	344.8 d	300.0 b	9.4 a	37.5 c	73.4 e	60.1 d	45.1 b
0.25 mmol dm^{-3} GA_3	204.4 a	312.4 c	248.2 b	229.3 ab	248.6 a	9.4 a	24.2 b	29.3 b	9.8 a	18.2 a
1000 µmol dm^{-3} CHI	204.4 a	570.2 f	663.9 g	872.8 h	577.8 c	9.4 a	137.3 f	159.2 g	199.2 h	126.3 c
Mean	204.4 a	399.7 b	455.2 bc	482.3 c		9.4 a	66.4 b	87.3 c	89.7 c	

*Explanations: see Table 2

The contents of free amino acids, free proline included, increased during the experiment in hosta leaves, both those unconditioned and conditioned with CHI (Tab. 4). Only conditioning with BA resulted in a small decrease in free proline relative to the initial value. Its content was lower than directly after harvest only in the BA-treated leaves on the 13th and 18th day.

The pattern of changes in the contents of free amino acids in calla leaves was similar (Tab. 7). The highest values were found in the CHI-conditioned leaves, where on the 15th day their content was over four times higher than directly after harvest. In CHI-treated leaves an enormous rise in free proline occurred, 15-fold and 21-fold on the 5th and 15th day, respectively, while the final increase was 6-fold in the control untreated leaves.

DISCUSSION

As flower arrangements become more attractive due to the presence of green elements, the importance of cut greens on the flower market will continue to increase. Depending on the species or cultivar, the loss of decorative value in cut plant elements may be manifested differently. Cut leaves or shoots differ in their response to growing conditions, the environmental conditions during turnover and postharvest treatments. Certain substances effectively delaying senescence in some plants may hasten the loss of decorative value in other species (Skutnik et al. 2001). To increase the postharvest longevity of cut plant material, preservatives (Pun and Ichimura 2003) and plant hormones (van Doorn et al. 1992) are used. As proteolysis is involved in plant senescence, protease inhibitors have been proposed to delay protein degradation and to improve cut flower keeping qualities (Wulster et al. 1982). Inhibitors such as puromycin, actomycin and chloramphenicol have been tested in this regard but the most studied has been cycloheximide (Cashmore 1976) as the most effective compound inhibiting the synthesis of proteolytic enzymes (McMahon 1975, Rzychoń et al. 2004). Trials on the application of CHI in order to prolong vase life have till now been conducted mostly on cut flowers, where the compound effectively delayed senescence maintaining high protein level in floral tissues. Sultan and Farooq (1997) reported an increase of longevity in cut irises. Similar observations can be found for cut daylily (*Hemerocallis hybrida*) (Lay-Yee et al. 1992), carnation (*Dianthus caryophyllus semperflorens*) (Drory et al. 1995), gladiolus (*Gladiolus hybridus*) (Yamane and Ogata 1995) and Dutch iris (*Iris hollandica*) (van Doorn et al. 1995). One can thus deduce that bud opening and flower wilting are associated with the synthesis of specific proteins. In the two hosta cultivars studied here, 'Minima Glauca' and 'Undulata Erromena', an increase in leaf longevity was obtained only after the application of a given CHI concentration: 500 µmol dm^{-3} CHI and 100 µmol dm^{-3} CHI, respectively. However, treatment with the inhibitor

did not improved leaf longevity in calla (Tab. 1) nor in cut asparagus shoots (Skutnik and Rabiza-Świder, unpublished data). The different response to CHI of particular species and cultivars may be associated with a different sensitivity to the inhibitor concentration. Gul and Tahir (2013) showed that in daffodil CHI in the concentration range between 0.01 and 0.05 mmol dm^{-3} delayed senescence, while in concentrations above 0.05 mmol dm^{-3} CHI prevented flower opening and promoted senescence.

Leaf senescence is under the control of plant hormones (Wingler et al. 1998), especially cytokinins, which effectively delay degradative processes in plant green elements used in floral arrangements (Skutnik et al. 2001, Skutnik et al. 2006, Skutnik and Rabiza-Świder 2007). Gibberellins also delay chlorophyll degradation in the leaves of certain species: Ethiopian calla (Skutnik et al. 2001), Easter lily (*Lilium longiflorum*) (Han 1997) and alstroemeria (Kappers et al. 1998). Growth regulators have been shown to be effective in delaying the senescence of *Zantedeschia* and *Hosta* leaves (Skutnik et al. 2001, Skutnik and Rabiza-Świder 2007).

The senescence-related proteolysis includes an increase in the cysteine protease activity. According to Rabiza-Świder et al. (2003), there was no expression of the cysteine protease gene in freshly harvested hosta leaves while the presence of its transcript was detectable in senescing leaves kept in water and the enzyme activity considerably increased. The conditioning of 'Minima Glauca' leaves with BA kept proteolytic activity and that of cysteine protease low. This was found earlier in hosta 'Undulata Erromena' and in calla (Rabiza-Świder et al. 2003). Similar results were obtained in broccoli (Wang et al. 2004), where the activity of cysteine protease in florets treated with kinetin (5 mg dm^{-3}) was much lower than in untreated control flower heads four days after harvest. Such results confirm the key role of cysteine protease in the senescence of cut plant material.

The level of soluble proteins in leaf cells drops due to proteolysis. This drop is especially evident in cut leaves, which lose their ability to synthesize proteins after detachment from the mother plant (Rabiza-Świder et al. 2003). The smallest decrease in protein content was observed in hosta leaves conditioned in BA while it was more pronounced in leaves treated with CHI. In both cases the protein contents remained higher than in the untreated control. Similar results were reported by Sultan and Farooq (1997) in iris where the flowers treated with the inhibitor contained more proteins than those placed directly into water. Gul and Tahir (2013) suggested that CHI maintained a high protein level in the petal issue by inhibiting the synthesis of specific proteases responsible for protein degradation. On the contrary, our calla leaves conditioned with CHI had the lowest protein level relative to other treatments. Similar results were noticed by Dar et el. (2015) in *Dianthus,* where the flowers pulsed with 0.1 mmol dm^{-3} CHI maintained a lower soluble protein content in comparison to the control.

Due to protein degradation in detached plant elements, the accumulation of free amino acids occurs, as they cannot be transported out to other plant organs. In hosta and calla leaves cytokinins (Skutnik and Rabiza-Świder 2007) and gibberellins (Rabiza-Świder et al. 2004 a), respectively, are the most effective in preventing this phenomenon. Here, the lowest free amino acid content was found in hosta leaves conditioned with BA, where on the last sampling date it was threefold lower than in the control treatment and twofold lower than in the CHI-treated leaves. In calla leaves, the highest free amino acid accumulation resulted from the CHI treatment. At the end of the experiment it was several times higher than in leaves conditioned with GA_3 and even exceeded that determined in the control untreated leaves. It is therefore evident that CHI did not delay processes typical for senescence such as protein degradation or the accumulation of free proline resulting from proteolysis, though this was expected as CHI inhibits protease activity. Gul and Tahir (2013) showed that the content of proteins decreased whereas that of amino acids increased in *Narcissus* flowers with CHI treatment, besides improving postharvest performance. Similar results were obtained in cut carnations, where the treatment of CHI resulted in an increase in the total amino acid pool (Dar et al. 2015). The question of why the vase life of cut flowers or leaves may be improved by CHI while the proteolytic activity is enhanced remains open.

During the senescence of detached plant organs, the accumulation of free proline commonly occurs as a plant response to water stress (Karolewski 1996). Yang et al. (2000) found a 17-fold increase in free proline 12 hours after the detachment of rice leaves and after four days this increase was 50-fold. Comparable high proline content was determined in calla leaves conditioned with CHI: on the 15^{th} day it was three times higher than in the control untreated

leaves and 20 times higher than immediately after harvest (Tab. 7). These results prove that senescence in calla leaves is closely associated with free proline accumulation, similarly as in rice (Yang and Kao 2000). Contrary to calla, in 'Minima Glauca' hosta the level of free proline was low and comparable to the initial amount, both for BA and CHI-treated leaves.

The above results indicate that senescence can proceed in different ways in cut flowers and leaves and that a treatment effective for cut flowers will not always give such results in detached leaves. CHI, whose efficiency in delaying senescence in cut flowers has widely been proven, probably will not be recommended for the cut foliage of calla and hosta though its positive effects on other species cannot be excluded and further studies on other plants should be continued.

CONCLUSIONS

1. The effects of cycloheximide used on the cut foliage of calla and hosta depended on the species, cultivar and inhibitor concentration.
2. The application of CHI on cut leaves delayed decreases in soluble proteins and limited the accumulation of free proline in hosta but not in calla.
3. During the senescence of cut hosta leaves the proteolytic activity, including that of cysteine protease, increased, but this increase was reduced by conditioning with BA.
4. In senescing calla leaves, the activity of proteolytic enzymes including that of cysteine protease increased, but was lower in leaves conditioned with GA_3 and those placed into distilled water than in those treated with CHI.
5. The use of growth regulators is recommended for increasing the vase life of cut leaves of hosta and calla, as they more efficiently delay senescence as compared to cycloheximide.

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AUTHOR CONTRIBUTIONS

All authors contributed equally to this study. E.S. designed and performed the experiments and wrote the paper, J.R.-Ś. designed the experiments and performed analytical measurements, A.J. collected the data and performed statistical analyses, and D.M. performed analytical measurements.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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