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The effect of growth regulators on stomatal aperture in senescing cut leaves of *Zantedeschia aethiopica* Spr. and *Hosta* Tratt. 'Undulata Erromena'

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ABSTRACT

This paper reports the results of observations of the stomatal aperture in senescing cut leaves of *Zantedeschia aethiopica* Spr. and *Hosta* 'Undulata Erromena', two species used as the florists' green. Their display life after harvest can be extended by the application of growth regulators, therefore the detached leaves of *Zantedeschia* and *Hosta* were dipped in aqueous solutions containing 1 mmol dm^{-3} (= 346 mg dm^{-3}) GA_3 or 1 mmol dm^{-3} (= 225 mg dm^{-3}) BA, respectively, before being placed into vases with distilled water. Leaves not treated with growth regulators and placed directly into water or a preservative containing 8-hydroxyquinoline citrate (8-HQC 200 mg dm^{-3}) and sucrose (S 20 g dm^{-3}) served as controls. The degree of stomata opening was determined by a modified replica method of Poskuta and Karpowiczowa (1971). During leaf senescence of both species stomatal closure was observed in all treatments. The preservative shortened the leaf vase life and hastened the closing of stomata which were less open by 12% (*Zantedeschia*) and 18% (*Hosta*) relative to water controls. Hormones – GA_3 in *Zantedeschia* and BA in *Hosta* – delayed leaf senescence and enhanced stomata opening by 21% and 44%, respectively.

Abbreviations:
BA – benzyladenine
GA_3 – gibberellic acid
8-HQC – 8-hydroxyquinoline citrate
S – sucrose

INTRODUCTION

Detached leaves of *Zantedeschia aethiopica* Spr. and *Hosta* 'Undulata Erromena' are widely used as the florists' green. Their display life can be extended by the application of growth regulators however the response of detached leaves is different in both species. In *Z. aethiopica* gibberellic acid delays senescence while in *Hosta* benzyladenine is most effective in extending leaf longevity (Skutnik et al. 2001, Skutnik and Łukaszewska 2001). The standard preservative solution (8-HQC + 2% S) used to prolong vase life of cut flowers is generally ineffective for the florists' green. Moreover, it sometimes enhances senescence of cut leaves including *Zantedeschia* and *Hosta* (Skutnik et al. 1999, Skutnik et al. 2004a).

In this paper we report the effect of growth regulators and standard preservative solution on stomatal aperture in senescing cut leaves of *Z. aethiopica* and *Hosta* 'Undulata Erromena'. Stomata occupy a central position in the pathway for the transport CO_2 and O_2 and stomatal conductance is the main mechanism by which plants control gas exchange and leaf temperature (Jones 1998). Phytohormones affect stomata with ABA inducing stomatal closure (Zhang et al. 2001) while cytokinins promote stomatal opening (Rulcová and Pospíšilová 2001). We analyzed stomatal aperture in the leaves of *Zantedeschia* and *Hosta* treated with GA_3 and BA, respectively, and in the leaves held in the standard preservative solution.

MATERIAL AND METHODS

Plants of *Zantedeschia aethiopica* Spr. were grown in the greenhouses and *Hosta* 'Undulata Erromena' in the perennials collection of the Department of Ornamental Plants of the Warsaw Agricultural University, Warsaw, Poland.

Mature, healthy, undamaged leaves were harvested in the morning, in winter and summer months for *Zantedeschia* and *Hosta*, respectively, and graded for uniformity. Detached leaves of *Zantedeschia* and *Hosta* were dipped in aqueous solutions containing 1 mmol dm^{-3} (= 346 mg dm^{-3}) GA_3 or 1 mmol dm^{-3} (= 225 mg dm^{-3}) BA, respectively, and placed in vases with distilled water. These treatments had been shown to be effective in prolonging vase life of both species (Skutnik and Łukaszewska 2001). Leaves not treated with growth regulators and placed directly into water or a preservative containing 8-hydroxyquinoline citrate (8-HQC 200 mg dm^{-3}) and sucrose (S 20 g dm^{-3}) served as controls. The vases were placed under controlled conditions: temperature 20ºC, relative humidity 60%, 12 hrs photoperiod with light intensity of 35 µmol m^{-2} s^{-1} PAR. In *Zantedeschia aethiopica* measurements were taken on 5 dates during 22 days of the experiment while in *Hosta* measurements were made 4 times during 19 days of vase life.

The degree of stomata opening was determined by a modified replica method of Poskuta and Karpowiczowa (1971). To make replicas of stomata silicon thin layers of polymer, THIFLEX served as negatives and to make positives, transparent nail polish was used. Replicas were made between 1 and 3 PM when stomata remained widely open as it was found in preliminary studies when stomatal aperture was measured every hour during 12 hours of day light. There were 6 leaves in each combination giving 90 readings for each data point. The observations and measurements were done on OLYMPUS IX70 microscope. The results were subjected to ANOVA 2 and the means compared using the Duncan's test at $p = 0.05$

RESULTS

The initial value of stomata aperture differed in *Zantedeschia* and *Hosta* leaves (Tables 1 and 2). It was 9.38 µm and 4.55 µm, respectively. The stomatal aperture in *Zantedeschia* leaves placed into water increased during the first 24 hrs after harvest comparing to freshly cut leaves, stayed stable for a week and then decreased, reaching 54% of the initial value on the last day of the observations. A different reaction was observed in leaves of *Hosta*, where stomatal aperture sharply decreased to 62% already on the next day after harvest and then kept slightly increasing to 78% of the initial value on the 19^{th} day of vase life. In both species the preservative solution caused steady stomatal closure. On the last day of the experiment the stomatal width decreased to 71%

and 53%, in *Zantedeschia* and *Hosta*, respectively, as compared to the aperture observed in the freshly harvested leaves (Tables 1 and 2). Both growth regulators, effective in inhibiting leaf senescence: GA_3 for *Zantedeschia* (Fig. 1) and BA for *Hosta* (Fig. 2) kept stomata open in both species (Figs 3 and 4). Although a steady decrease in stomatal aperture was noted during the senescence of *Zantedeschia aethiopica* leaves and there was no difference in aperture in *Hosta* leaves from day 1 to 19, only growth regulators kept stomata wide open and on day 1 in *Hosta* and 1 and 14 in *Zantedeschia* stomata apertures were larger than in fresh cut leaves. Finally, stomata aperture was higher about 21% in *Zantedeschia* and 44% in *Hosta* cut leaves treated with GA_3 and BA, respectively, as compared to water control.

Table 1. The effect of GA_3 and standard preservative on stomata aperture [μm] in senescing cut leaves of *Zantedeschia aethiopica* (1.00-3.00 PM); Stomata aperture in fresh cut leaves: 9.38 μm

Treatment	Stomata aperture on day [μm]				Mean for treatment
	1	7	14	22	
H_2O	10.69	10.28	9.27	5.11	8.84 b*
8-HQC + 2% S	9.67	7.82	7.12	6.64	7.81 a
GA_3 1 mmol dm^{-3}	11.37	9.02	12.25	10.31	10.74 c
Mean for a term	10.58 c	9.04 b	9.55 b	7.35 a	

*Means followed by the same letter do not differ significantly at p = 0.05; to compare the means within the table: $LSD_{0.05}$ = 1.136

Table 2. The effect of BA and standard preservative on stomata aperture [μm] in senescing cut leaves of *Hosta* 'Undulata Erromena' (1.00-3.00 PM); Stomata aperture in fresh cut leaves: 4.55 μm

Treatment	Stomata aperture [μm] on day:			Mean for treatment
	1	6	19	
H_2O	2.80	3.25	3.55	3.20 b*
8-HQC + 2% S	3.15	2.25	2.42	2.61 a
BA 1 mmol dm^{-3}	5.12	4.03	4.64	4.60 c
Mean for a term	3.69 a	3.18 a	3.54 a	

*Means followed by the same letter do not differ significantly at p = 0.05; to compare the means within the table: $LSD_{0.05}$ = 0.534

Fig. 1. The effect of gibberellic acid on senescence of cut leaves of *Zantedeschia aethiopica*. From left: H_2O, 1 mmol (GA_3) dm^{-3} – dipping

Fig. 2. The effect of benzyladenine on senescence of cut leaves of Hosta 'Undulata Erromena'. From left: H_2O, 1 mmol (BA) dm^{-3} – dipping

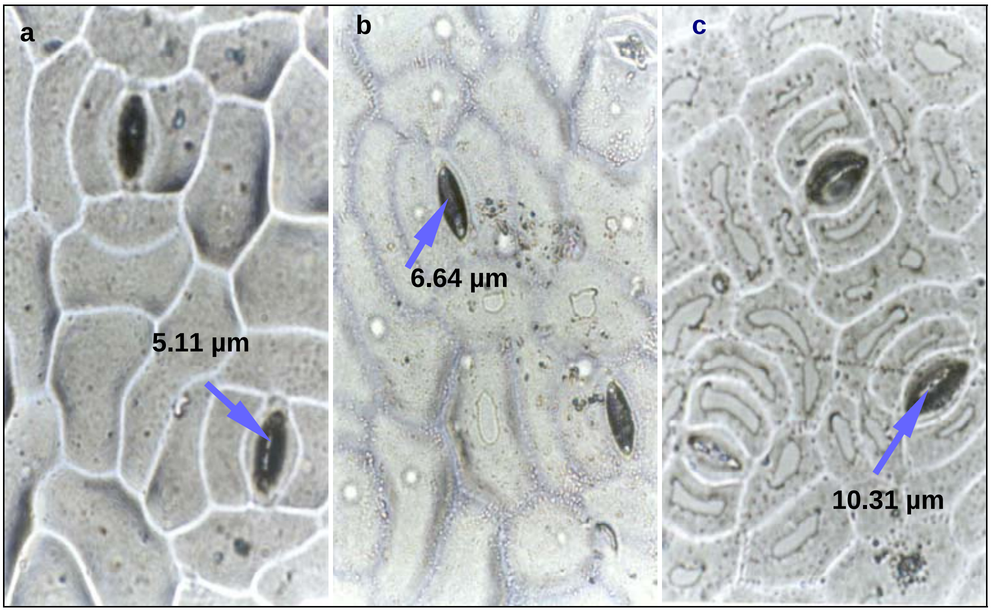

Fig. 3. Replics of the lower epidermis with stomata of *Zantedeschia aethiopica*: a – water control, b – standard preservative, c – gibberellic acid. Replics were made on day 22, 3:30 PM. Zoom 400x

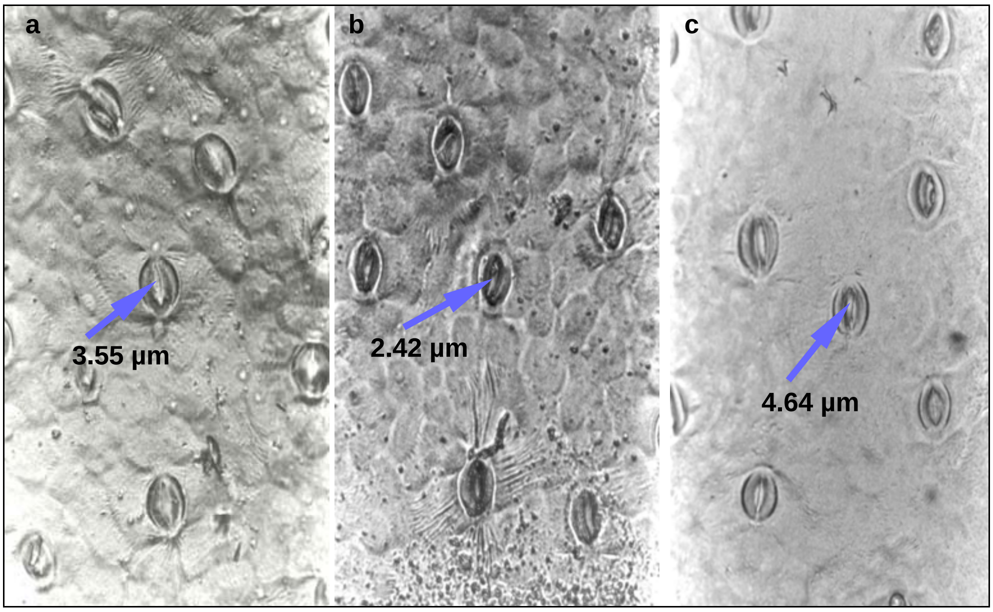

Fig. 4. Replics of the lower epidermis with stomata of *Hosta* 'Undulata Erromena': a – water control, b – standard preservative, c – benzyladenine. Replics were made on day 19, 1:30 PM. Zoom 400x

DISCUSSION

Zantedeschia and *Hosta* 'Undulata Erromena' are grown for the florists' green. Their postharvest longevity can be extended by the postharvest treatments with growth regulators. GA_3, the most effective for *Zantedeschia aethiopica*, inhibits leaf senescence by delaying chlorophyll loss and soluble protein degradation (Skutnik et al. 2004a, Skutnik et al. 2004b, Rabiza-Świder and Skutnik 2004). The same beneficial effects in *Hosta* were noted after conditioning the detached leaves with benzyladenine solution by Skutnik and co-workers (1999).

The other phenomenon characteristic for the leaf senescence can be stomatal aperture. Stomata occupy a central position in the pathway for the transport of water vapour, carbon dioxide, and oxygen. While CO_2 is taken up by diffusion into the leaf, water vapour is lost at the same time. The regulation of stomatal conductance is the main mechanism by which plants control gas exchange and leaf temperature (Jones 1998). It is known that stomata closing induces senescence (Thimann and Satler 1979) while treatments allowing stomata to stay open delay this process which was proved for *Hibiscus* cuttings where open stomata enabled escape of internal ethylene from leaf tissues (Kirk et al. 1986). There is, however, little or no evidence for the implication of ethylene in the senescence of leaves. In higher plants, most tissues produce more or less ethylene depending on their age and environmental conditions. Enhanced biosynthesis of this gaseous plant growth regulator is induced by extreme temperatures, wounding and mechanical and chemical factors, but in some species there is no a suggestive parallel between ethylene biosynthesis and senescence. Although in detached leaves of *Brassica*, *Capsicum* and *Ricinus* exposed to stress, continuous increase in ethylene content was observed (Aharoni 1978), decrease in ethylene production was observed in *Gerbera jamesonii* (Olivella et al. 1998). Neither was stomatal closure regulated by ethylene in *Rosmarinus officinalis* exposed to drought (Munné-Bosch et al. 2002). It is another hormone, ABA, which induces closing of stomata in many species (Zhang et al. 2001, Pospíšilová 2003a) but it was neither included nor determined in the leaves in these experiments.

Cytokinins are often considered ABA antagonists in many processes including the regulation of stomatal opening, but the effects are species specific and depend on cytokinin type, concentration and method of application (Pospíšilová 2003b). Promotion of stomatal opening induced by application of cytokinin (BA) in *Hosta* 'Undulata Erromena' was in accordance with the results of Rulcová and Pospíšilová (2001).

The effects of gibberellin application on stomatal aperture are less known, but in some species their promoting effect on stomata opening was observed, for example in water-stressed leaves of lettuce (Aharoni et al. 1977) and *Commelina benghalensis* (Santakumari and Fletcher 1987). The same effect was observed in cut leaves of *Zantedeschia aethiopica*, where an increase in stomatal aperture 24 hrs after harvest was noted.

On the other hand, the standard preservative solution (8-HQC + 2% S) caused closing of stomata in both species what could be anticipated as both sucrose and 8-HQC are 'antitranspirants' (De Stigter 1981). They are often included in the commercial preservatives for cut flowers to diminish transpirational losses and maintain flower turgidity (Łukaszewska and Skutnik 2003). Paradoxically, their effect on leaves was mostly reported as negative (Skutnik et al. 2001) what was confirmed here in *Hosta* and *Zantedeschia*. The preservative hastened leaf senescence not only by accelerating chlorophyll degradation and proteolysis as shown earlier (Skutnik et al. 2004a, Skutnik et al. 2004b) but also by decreasing stomatal aperture.

What was observed in these experiments is that respective treatments with growth regulators which delayed senescence of both species under study allowed the stomata to stay more open as compared to the water control while the preservative application shortened leaf longevity and promoted stomata closure. However, we cannot state that a wider stomatal aperture is associated with an extended longevity of detached leaves of *Hosta* or *Zantedeschia* as such a relationship was not confirmed in our earlier work on the effect of light on leaf senescence in both species. Under red light which delayed chlorophyll degradation and prolonged vase life of *Zantedeschia* leaves stomata were more closed than in white or blue light while in *Hosta* the blue light delayed senescence without affecting stomata opening (Rabiza-Świder and Skutnik 2004). Neither a relation between the initial stomatal aperture in freshly harvested leaves and leaf vase life could be observed in these experiments: the width of stoma in *Zantedeschia* was twice as big as in *Hosta* while the longevity of both species was comparable. In the extremely long lasting leaves of the iron leaf (*Aspidistra elatior*) the stoma width was 5.39 μm, i.e. much less than in *Z. aethiopica* (unpublished).

Though a degree of stomatal aperture seems to be related to leaf senescence a nature of this relation in the two species under study remains unclear. Further studies on ethylene production, gas exchange and stomata movement will be carried out in hope to get more insight into the problem.

CONCLUSIONS

1. The initial value of stomata aperture is not related to postharvest longevity of cut leaves of *Zantedeschia aethiopica* Spr. and *Hosta* Tratt. 'Undulata Erromena'.
2. GA_3 for *Zantedeschia* and BA for *Hosta* delay leaf senescence and keep stomata opened.

3. The preservative solution accelerates senescence of cut leaves of *Zantedeschia* and *Hosta* and decreases stomatal aperture.

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WPŁYW REGULATORÓW WZROSTU NA STOPIEŃ ROZWARCIA APARATÓW SZPARKOWYCH W CIĘTYCH LIŚCIACH *ZANTEDESCHIA AETHIOPICA* SPR. I *HOSTA* TRATT. 'UNDULATA ERROMENA'

Streszczenie: W doświadczeniach analizowano stopień rozwarcia szparek w trakcie starzenia się ciętych liści *Zantedeschia aethiopica* Spr. i *Hosta* 'Undulata Erromena', dwóch gatunków stosowanych jako zieleń cięta. Ich pozbiorcza trwałość może być zwiększona przez zastosowanie regulatorów wzrostu, dlatego też liście obu gatunków zamaczano odpowiednio w roztworze 1 mmol dm^{-3} GA_3 lub BA, po czym umieszczano je w wodzie destylowanej. Liście nie traktowane regulatorami wzrostu i wstawione wprost do wody destylowanej lub pożywki zawierającej cytrynian 8-hydroksychinoliny (8-HQC 200 mg dm^{-3}) stanowiły kontrolę. Stopień rozwarcia aparatów szparkowych określono zmodyfikowaną metodą replik (Poskuta i Karpowiczowa 1971). W trakcie starzenia się liści obu gatunków we wszystkich kombinacjach obserwowano przymykanie aparatów szparkowych. Pożywka skróciła trwałość liści oraz zmniejszyła stopień rozwarcia szparek o 12% (*Zantedeschia)* i 18% (*Hosta*) w porównaniu do liści umieszczonych w wodzie. Regulatory wzrostu – GA_3 dla *Zantedeschia* i BA dla *Hosta* – spowolniły proces starzenia, a stopień rozwarcia szparek był odpowiednio o 21% i 44% wyższy, niż u liści stojących w wodzie.

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