SENESCENCE OF CUT LEAVES OF *Zantedeschia aethiopica* AND Z. elliottiana. PART III. THE REDUCING SUGARS CONTENT

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Abstract. Cut leaves of *Zantedeschia aethiopica* and *Z. elliottiana* are widely used as the florists’ green. Over the years we studied the parameters related to postharvest quality of leaves in the two above species. Here the effect of plant hormones known to delay leaf senescence (benzyladenine and gibberellic acid) and of the standard preservative solution (8-HQC + 2% sucrose) on the reducing sugars contents is presented. BA (0.1 mmol·dm$^{-3}$) and GA$_3$ (0.25 mmol·dm$^{-3}$) were applied as 24 h pulse treatments. Pulsed and unpulsed leaves were kept either in water or in the preservative. In both species contents of reducing sugars during their senescence in vases initially rose and then dropped to 60–80% of the initial levels. Pretreatment with BA did not counteract a decrease in reducing sugar contents while in the GA$_3$-treated leaves sugar loss was prevented in *Z. aethiopica* and a 20% increase occurred in *Z. elliottiana*. Placing leaves in the sugar containing solution provoked a dramatic loss of reducing sugars in *Z. aethiopica* (to 12% of the initial level) while in *Z. elliottiana* this loss was less pronounced (52% of the initial value). Cytokinin did not mitigate the negative effect of the preservative on reducing sugar losses while GA$_3$ was more effective in this respect. Results of analyses do not support hypothesis that sugar depletion might be a cause of senescence of detached leaves in the two *Zantedeschia* species.

Key words: reducing sugars, cut leaves, florists’ green, gibberellic acid, benzyladenine, *Zantedeschia aethiopica*, *Zantedeschia elliottiana*

INTRODUCTION

Major changes that take place during senescence of detached plant organs include color change and weight loss [Finger et al. 1999 b], proteolysis [Huffaker 1990], amino acids [Peeters and Van Laere 1992] and changes in sugar contents [Pramanik et al. 1999].
Sugars modulate a range of vital processes during plant growth and development such as leaf differentiation, floral transition and senescence [León and Sheen 2003], as well as the response to stress [Ashraf and Harris 2004]. They serve as substrates in carbon and energy metabolism and have important hormone-like functions as primary messengers in signal transduction [Sheen et al. 1999]. Sugar production through photosynthesis is the most fundamental activity in plant life. The processes of sugar production, transport, consumption, and storage are dynamic and linked to cellular physiology, environmental conditions and developmental stages. Sugar levels can serve plant as control mechanisms to integrate the external environmental conditions including such an abiotic stresses as water stress in cut leaves, with developmental programs directed by plant hormones [Sheen et al. 1999]. Low sugar status enhances photosynthesis, reserve mobilization, and export, whereas abundant presence of sugars promotes growth and carbohydrate storage [Koch 1996]. Thimann et al. [1977] suggested that sugar starvation is the direct cause of leaf senescence but the opposite may well be true – an increase rather than a decrease in sugar levels induces leaf senescence. An accumulation of sugars at the onset of senescence was noted in the leaves of Nicotiana tabacum [Masclaux et al. 2000] and Arabidopsis thaliana, so decreased sugar concentrations are unlikely to be the primary factor triggering leaf senescence [Yoshida 2003]. In detached leaves of Avena sativa, an accumulation of reducing sugars is higher than in attached leaves [Thimann et al. 1974].

In this paper we report on the effect of plant hormones (benzyladenine and gibberellic acid) and of the standard preservative solution (8-HQC + 2% S) used to prolong longevity of cut flowers, on the reducing sugars content during senescence of cut leaves of Zantedeschia aethiopica Spr. and Z. elliottiana Engl., both plants widely used as florists' green. Our preliminary investigations showed that reducing sugars constitute a major pool of carbohydrates in leaves of the two species under study.

MATERIAL AND METHODS

Plants of Z. aethiopica and Z. elliottiana were grown in the greenhouses of the Department of Ornamental Plants of the Warsaw Agricultural University. Mature, healthy and undamaged leaves were cut in the morning (in April 2002 for Z. aethiopica and in August 2002 for Z. elliottiana), graded for uniformity, treated with growth regulators (BA or GA3) and placed in vases with distilled water or a preservative solution containing 8-hydroxyquinoline citrate (8-HQC 200 mg·dm⁻³) and sucrose (S 20 g·dm⁻³), in controlled conditions: temperature 20°C, relative humidity 60%, 12 hrs photoperiod with light intensity of 35 µmol·m⁻²·s⁻¹ PAR. There were 20 leaves in each treatment. Growth regulators were applied as pulse conditioning: leaves were placed in aqueous solutions containing 0.25 mmol·dm⁻³ GA3 or 0.10 mmol·dm⁻³ BA. Leaves untreated with growth regulators and placed directly into distilled water or the preservative served as controls.

In the longer-lived Z. aethiopica, reducing sugars contents were measured on the 12th, 19th and 25th days of the experiment. In the short-lasting Z. elliottiana, the measurements were taken on the 6th and 10th days. For a more detailed picture of changes in
the reducing sugars contents during leaf senescence a separate experiment was conducted with leaves held in distilled water and with samples for measurements collected at shorter intervals: in *Z. aethiopica* eight measurements were made during the 19 days of experiment, while in *Z. elliottiana* – six measurements were taken during the 13 days. Leaves of *Z. aethiopica* were cut in April 2001 and *Z. elliottiana* – in July 2002.

Tissue from three leaves was pooled on each sampling date. Three samples were weighted for the analyses of reducing sugars content, and three replications of each extract were made, creating nine readings per data point. At the same time, three samples were tested for dry weight – the material was dried at 105°C until a constant weight was achieved.

The reducing sugars contents were determined according to Somogyi in Nelson’s modification [1944], calculated from the previously plotted standard curves, and expressed in mg of glucose per gram of dry leaf weight (DW). The results were statistically evaluated with ANOVA 1 and ANOVA 2 and the means were compared using Duncan’s test at probability level $P = 95\%$.

**RESULTS AND DISCUSSION**

**Changes in the reducing sugars content in senescing cut leaves of *Z. aethiopica* and *Z. elliottiana* kept in water**

The two species tested differed in their initial reducing sugars contents in cut leaves – it was 73.3 and 52.2 mg (glucose)$\cdot$g$^{-1}$ DW for *Z. aethiopica* and *Z. elliottiana* respectively (fig. 1 and 2), but changes in the sugars content during senescence were similar in the two species. In *Z. aethiopica*, a persistent increase was noted by the 8th day after harvest, followed by a gradual decrease to the level of 64% of the initial sugar content at the end of the experiment (fig. 1). In *Z. elliottiana*, an increase by day 6 and a subsequent sugar loss were observed, but more than 80% of the initial sugars content was still present at the end of the experiment (fig. 2).
The same issue has been studied in harvested broccoli, a highly perishable green vegetable, and completely different results were noted [Pramanik et. al. 2004]. During the five days of storage no marked changes occurred in the glucose and fructose contents. However, Finger and co-workers [1999 b] noted that the content of reducing sugars in broccoli during 24 hours of storage decreased by about 70%. In cut flowers, degradation of polysaccharides, proteins, lipids, and nucleic acids results in mobilization of sugars and nitrogenous compounds well before visible symptoms of senescence appear [Van Doorn 2004]. In our earlier experiments we have found that in cut leaves of Hosta plantaginea, reducing sugars content remained stable by day 11 after harvest although during the same period the chlorophyll content dropped to 68 % of the initial value [Skutnik et al. 2003].

The effect of a preservative (8-HQC + 2% S) on the reducing sugars content in senescing cut leaves of Z. aethiopica and Z. elliottiana

In Z. aethiopica leaves treated with the sugar-containing preservative almost one half of the initial content of reducing sugars was lost by the 12th day of the experiment while in control leaves a significant increase was noted (tab. 1). In contrast, in Z. elliottiana, the solution of 8-HQC + 2% sucrose had no such dramatic effect: on day 6, 91% of the initial reducing sugars content was still present. On the last day of the experiment, the reducing sugars content was much higher in the sugar-fed leaves of Z. elliottiana (48% of the initial level) than in Z. aethiopica (12%).

A standard preservative solution, containing sucrose and 200 mg·dm⁻³ citrate or sulphate of hydroxyquinoline (8-HQC or 8-HQS), often prolongs the vase life of many species of cut flowers. Sucrose supplements the carbohydrate supply while 8-HQC is a bactericide and acidifying agent that inhibits vascular occlusion in the cut flowers stems [Gilman and Steponkus 1972]. Also, a pulse treatment with sucrose [Finger et al. 1999 a], germicides with sucrose [Liao et al. 2000] or a continuous treatment with sucrose [Yakimova et al. 1997] or the germicide alone [Ichimura et al. 2002] extends cut

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**Fig. 2. The reducing sugars content in senescing cut leaves of Zantedeschia elliottiana Engl. placed into distilled water.**


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flowers longevity. Sucrose is not usually used alone because without germicides it promotes bacterial proliferation, which shortens vase life.

Table 1. The reducing sugar content in senescing cut leaves of *Zantedeschia aethiopica* Spr. treated with BA and GA$_3$, placed into distilled water or preservative. Initial value: 85.0 mg (glucose)·g$^{-1}$ DW (100%)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Reducing sugars content [mg (glucose)·g$^{-1}$ DW] on day:</th>
<th>Mean for a treatment</th>
<th>12.</th>
<th>19.</th>
<th>25.</th>
<th>6.</th>
<th>10.</th>
<th>19.</th>
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<tbody>
<tr>
<td>H$_2$O</td>
<td>100.3 (118%) 81.6 (96%) 76.3 (90%) 86.0 d$^1$</td>
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<tr>
<td>8-HQC + 2%S</td>
<td>46.5 (55%) 47.4 (56%) 10.4 (12%) 34.7 a</td>
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<tr>
<td>BA 0.1; 24h → H$_2$O</td>
<td>68.3 (80%) 65.1 (77%) 60.7 (71%) 64.7 c</td>
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<tr>
<td>BA 0.1; 24h → 8-HQC + 2%S</td>
<td>97.3 (114%) 56.0 (66%) 22.9 (27%) 58.7 b</td>
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<tr>
<td>GA$_3$ 0.25; 24h → H$_2$O</td>
<td>134.7 (158%) 89.7 (105%) 86.8 (102%) 103.7 e</td>
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<tr>
<td>GA$_3$ 0.25; 24h → 8-HQC + 2%S</td>
<td>102.1 (120%) 86.3 (101%) 65.4 (77%) 84.6 d</td>
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<tr>
<td>Mean for a term LSD$_{0.05}$ = 1.73</td>
<td>91.5 c 71.0 b 53.7 a</td>
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<tr>
<td>Średnia dla terminu NIR$_{0.05}$ = 9,72</td>
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1 Means followed by the same letter do not differ significantly at $\alpha = 0.05$ (Duncan’s test); to compare the means within the table: LSD$_{0.05}$ = 4.24.

Table 2. The reducing sugar content in senescing cut leaves of *Zantedeschia elliottiana* Engl. treated with BA and GA$_3$, placed into distilled water or preservative. Initial value: 52.3 mg (glucose)·g$^{-1}$ DW (100%)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Reducing sugars content [mg (glucose)·g$^{-1}$ DW] on day:</th>
<th>Mean for a treatment</th>
<th>6.</th>
<th>10.</th>
<th>6.</th>
<th>10.</th>
<th>6.</th>
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<tbody>
<tr>
<td>H$_2$O</td>
<td>59.8 (114%) 22.1 (42%) 40.8 c$^1$</td>
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<tr>
<td>8-HQC + 2%S</td>
<td>47.4 (91%) 25.3 (48%) 36.3 b</td>
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<tr>
<td>BA 0.1; 24h → H$_2$O</td>
<td>38.1 (73%) 35.5 (68%) 36.8 b</td>
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<tr>
<td>BA 0.1; 24h → 8-HQC + 2%S</td>
<td>34.7 (66%) 13.4 (26%) 24.1 a</td>
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<tr>
<td>GA$_3$ 0.25; 24h → H$_2$O</td>
<td>47.9 (92%) 62.9 (120%) 55.4 d</td>
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<tr>
<td>GA$_3$ 0.25; 24h → 8-HQC + 2%S</td>
<td>57.3 (110%) 65.9 (126%) 61.6 e</td>
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<tr>
<td>Mean for a term LSD$_{0.05}$ = 3.77</td>
<td>47.5 b 37.5 a</td>
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<tr>
<td>Średnia dla terminu NIR$_{0.05}$ = 9,72</td>
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1 Explanations as in table 1, to compare the means within the table: LSD$_{0.05}$ = 9.24.

1 Objaśnienia jak w tabeli 1, dla porównania wartości wewnątrz tabeli: NIR$_{0.05}$ = 9,24.
Response to exogenous sugars may at times be cultivar- or species-specific [Ichimura et al. 2002]. In leaves, sugar treatment often accelerates senescence [Gan and Amasino 1997; Wingler et al. 1998 and references therein]. Chlorophyll breakdown and proteolysis in leaf segments of Avena sativa treated with a sugar solution were promoted under light though delayed in the darkness [Luna and Trippi 1986]. In Hosta plantaginea and Z. aethiopica the standard preservative solution containing sucrose caused a significant decrease in chlorophyll content relative to control leaves [Skutnik et al. 2003] while in Z. elliottiana the pigment loss was significantly smaller. As shown before [Skutnik et al. 2003] the effect of the preservative on certain parameters of leaf senescence is less deleterious in Z. elliottiana than in Z. aethiopica. The former species has shorter vase life related probably to lower chlorophyll content as its leaves are white maculated. Having less chlorophyll its leaves contain less sugars so the exogenous sucrose from the preservative solution might serve as an additional respiratory substrate sustaining leaf vase life and retarding senescence-related hydrolyses.

The effect of BA and GA3 on the reducing sugars content in cut leaves of two Zantedeschia species

The effects of BA on the senescence of cut leaves of the two tested Zantedeschia species were similar. In both cases, decreases in sugars contents by the first dates of analyzes were noted: reducing sugars dropped to 80% and 73% of the initial values for Z. aethiopica and Z. elliottiana, respectively (tab. 1 and 2). Progressive decrease by the last day of the experiment was observed in Z. aethiopica (tab. 1) while in contrast, in Z. elliottiana there was no further significant change in sugar content on day 10 (tab. 2).

Pretreatment with 0.10 mmol·dm\(^{-3}\) BA caused an increase in sugars content in Z. aethiopica leaves placed into the preservative solution on day 12, similar to that observed in leaves placed in distilled water. However, in subsequent measurement days, a dramatic reduction in sugars contents took place in the leaves from this treatment with only 27% of the initial content present at the end of experiment (day 25). In the control leaves, 90% of reducing sugars remained (tab. 1). In Z. elliottiana, a reduction in the reducing sugars contents was observed in the BA-treated leaves kept in the preservative. At the end of the experiment the sugar contents was 26% of the initial value; quite similar to that of Z. aethiopica. In the latter species, cytokinin somewhat mitigated the negative effect of the preservative while in Z. elliottiana a reverse response to BA was noted in the sugar-fed leaves.

The GA3 pretreated leaves of Z. aethiopica accumulated reducing sugars at the beginning of the experiment (12\(^{th}\) day – 158% of the initial level) but later, a decrease in sugars contents was observed. On Day 25 it was comparable to the initial level (tab. 1). A pretreatment with GA3 of leaves placed subsequently into the preservative solution caused similar changes in the sugar content but with a lower increase at the beginning of the experiment, and with a more notable decrease at the end.

In the Z. elliottiana leaves pretreated with gibberellic acid and kept in water, the sugar content remained stable by day 6, and then increased to 120% of the initial level. The GA3-pretreated leaves placed into the preservative responded similarly.

Plant senescence, similarly to other developmental processes, is controlled by hormones. Generally, cytokinins delay the senescence of leaves [Chen et al. 1997, McCabe...
et al. 2001] and flowers [Paull and Chantrachit 2001, Chang et al. 2003]. In few cases, gibberellins were also effective in delaying leaf senescence [Skutnik et al. 2001]. Our earlier experiments have shown that gibberellic acid prolonged vase life of the Z. aethiopica leaves by retarding chlorophyll loss and reducing the electrical conductivity of leaf cell sap [Skutnik et al. 2001]. Also in Z. elliottiana leaves, beneficial effect of GA$_3$ treatment on chlorophyll content during senescence was noted [Skutnik et al. 2004]. In the two species under study it was GA$_3$ which more effectively counteracted the deleterious action of the sugar-containing preservative on protein and chlorophyll contents. As shown here, plant hormones also had an effect on sugars during leaf senescence but it was the gibberellic acid, and not benzyladenine, which delayed losses of reducing sugars in both Zantedeschia species under study. Reducing sugars preserved by GA$_3$ may serve as substrates for respiration, preventing hydrolysis of other compounds (such as proteins) [Rabiza-Swider et al. 2004] thus prolonging the vase life of cut leaves. Paradoxically, sucrose, the major form of translocated sugars in plants, when added to the preservative solution provoked losses of reducing sugars which could not always be counteracted by the hormones. Altogether, the results of this work do support the hypothesis that sugar depletion is the cause of leaf senescence – at least not in the two Zantedeschia species in this study.

**CONCLUSIONS**

1. Reducing sugars contents in senesced detached leaves of Zantedeschia aethiopica and Z. elliottiana fall below the initial levels determined immediately after harvest.
2. Placing leaves of the two species into the preservative solution (8HQC + sucrose) results in massive losses of reducing sugars.
3. Pulse treatment with GA$_3$ after harvest prevents a loss of reducing sugars in leaves of Z. aethiopica and results in their accumulation above an initial level in Z. elliottiana, both in the water- and preservative-held leaves while BA is completely ineffective in this respect.

**REFERENCES**


Senescence of cut leaves of Zantedeschia aethiopica and Z. elliottiana. Part III....


STARZENIE CIĘTYCH ŁIŚCI Zantedeschia aethiopica I Z. elliottiana

CZĘŚĆ III. ZAWARTOŚĆ CUKRÓW REDUKUJĄCYCH

Streszczenie. Zantedeschia aethiopica i Zantedeschia elliottiana znajdują szerokie zastosowanie jako ziele cięta. W doświadczeniach analizowano wpływ regulatorów wzrostu, skutecznych w opóźnianiu starzenia ciętych liści (benzyloadeniny i kwasu giberelinowego) oraz pożywki standardowej (8-HQC + 2% sacharozy) na zawartość cukrów redukujących. BA (0,1 mmol dm$^{-3}$) i GA3 (0,25 mmol dm$^{-3}$) aplikowano w formie 24-godzinnego kondycjonowania. T trattowane i nietraktowane regulatorami wzrostu liście wstawione zostały do wody lub pożywki standardowej. W przypadku obu gatunków zawartość cukrów redukujących w trakcie ich starzenia się początkowo rosła, po czym spadała do poziomu 60-80% zawartości początkowej. Kondycjonowanie liści Z. aethiopica w roztworze BA nie zapobiegało spadkowi ich zawartości, podczas gdy skutecznym w opóźnieniu tego procesu okazało się traktowanie kwasem giberelinowym a co więcej – w przypadku Z. elliottiana odnotowano 20% wzrost. Pożywka standardowa spowodowała gwałtowny spadek zawartości cukrów rozpuszczalnych w ciętych liściach Z. aethiopica (do poziomu 12% zawartości początkowej), podczas gdy w liściach Z. elliottiana odnotowano spadek jedynie do poziomu 52% zawartości początkowej. Cytokinina nie złagodziła negatywnego wpływu pożywki standardowej na zawartość cukrów rozpuszczalnych, skuteczna natomiast okazał się GA3.

Słowa kluczowe: cukry redukujące, ziele cięta, kwas giberelinowy, benzyloadenina, Zantedeschia aethiopica, Zantedeschia elliottiana.

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