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Effect of light and gibberellic acid on photosynthesis during leaf senescence of alstroemeria cut flowers

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The functioning of the photosynthetic apparatus during leaf senescence was investigated in alstroemeria cut flowers by a combination of gas-exchange measurements and analysis of in vivo chlorophyll fluorescence. Chlorophyll loss in leaves of alstroemeria cut flowers is delayed by light and by a treatment of the cut flowers with gibberellic acid (GA_3). The maximal photosynthesis of the leaves was approximately 6 μmol CO_2 m^{-2} s^{-1} at 1 350 μmol m^{-2} s^{-1} (PAR) which is relatively low for intact C_3 leaves. Qualitatively the gas-exchange rates followed the decline in chlorophyll content for the various treatments, i.e. light and GA_3-treatment delayed the decline in photosynthetic rates. However, when chlorophyll loss could not yet be observed in the leaves, photosynthetic rates were already strongly decreased. In vivo fluorescence measurements revealed that the decrease in CO_2 uptake is (partly) due to a decreased electron flow through photosystem II. Furthermore, analysis of the fluorescence data showed a high nonphotochemical quenching under all experimental conditions, indicating that the consumption of reducing power in the Calvin cycle is very low. The chlorophyll, remaining after 9 days incubation of leaves with GA_3 in the dark should be considered as a 'cosmetic' pigment without any function in the supply of assimilates to the flowers.

Key words – Alstroemeria (*Alstroemeria pelegrina*), chlorophyll, fluorescence, gibberellic acid, photosynthesis, senescence.

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Introduction

A large number of metabolic changes occur during leaf senescence (for review: Thomas and Stoddart 1980), e.g., increases in the activity of proteases (Thimann 1980), glyoxysomal enzymes (Gut and Matile 1988), nucleases (Blank and McKeon 1989) and enzymes of chlorophyll catabolism (Thomas et al. 1989, Schellenberg et al. 1990, Matile et al. 1992, Nock et al. 1992). As a consequence of this increase in enzymatic activities, the contents of protein, RNA and chlorophyll strongly decrease during senescence. Light and phytohormones both influence leaf senescence. The effect of light on leaf senescence has been proposed to act on photosynthesis (Goldthwaite 1988) and/or a photomorphogenic effect involving phytochrome (e.g. Okada et al. 1992). Phytohormones like cytokinins, and in some systems gibberellins, delay the loss of chlorophyll whereas ethylene and abscisic acid enhance the rate of chlorophyll loss (Thimann 1980). Additionally, jasmonic acid can induce senescence of leaves (e.g. Chou and Kao 1992) and in a number of systems it has been shown that polyamines delay foliar senescence (Evans and Malmberg 1989).

Loss of chlorophyll in leaves of alstroemeria cut flowers is strongly delayed by GA_3 (Dai and Paull 1991, Hicklenton 1991, van Doorn et al. 1992, Jordi et al. 1993) and white light (Jordi et al. 1993). Recently it was demonstrated that the sink strength of the developing buds is not the cause of chlorophyll loss of the leaves. In addition, GA_3 did not delay loss of chlorophyll by delaying

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export of nutrients from the leaves to the buds (Jordi et al. 1993). The loss of chlorophyll may affect the photosynthetic activity. In the present paper, the processes involved in photosynthetic degradation have been measured by a combination of gas-exchange measurements and in vivo chlorophyll fluorescence. Gas-exchange measurements give information on the net CO_2 uptake and stomatal control whereas fluorescence signals, analysed according to Genty et al. (1989) provide a measure of the electron transport rate through photosystem II. This analysis gives the calculated yield of electron transport through photosystem II and the resulting synthesis of reducing power and ATP. The electron transport rate through photosystem II has been shown to be equivalent to the efficiency of CO_2 assimilation, provided that the systems are in a steady-state condition and at low O_2 concentrations. A second parameter is the nonphotochemical quenching, which stands for a decrease of fluorescence due to an increased probability of energy dissipation as heat. This process is enhanced when abundant reducing power is available in the chloroplast, for instance under sink-limiting conditions. In that case the efficiency of photosynthesis is down-regulated by the dissipation of heat to prevent formation of free radicals. The concomitant decrease of fluorescence is denoted as nonphotochemical quenching.

In the present study, we report on the effect of light and gibberellic acid on both the amount of chlorophyll and the processes underlying photosynthesis, and the assimilate supply to the flowers during leaf senescence of alstroemeria cut flowers.

Abbreviations – F_0, dark-adapted basic fluorescence; F_m, dark-adapted maximal fluorescence; F'_m light-adapted maximal fluorescence; F_v, variable fluorescence; GA_3, gibberellic acid; Rubisco, ribulose 1,5-bisphosphate carboxylase/oxygenase.

Materials and methods

Plant material

Cut flowers of alstroemeria (*Alstroemeria pelegrina* L. cv. Westland) were obtained from a commercial nursery at a developmental stage in which all buds were closed but petals of the first bud were already coloured red. The stem of alstroemeria is cymose and contains 3 or 4 florets per cyme. A floret contains a peduncle and 2 or 3 flower buds. The cut flowering stem contains 5 upper leaves in the whorl. The other leaves were removed. Leaves of alstroemeria are twisted, facing the light with the anatomically lower surface. The measurements were performed with leaves in that position. The flowers were cut at 60 cm from the top of the inflorescence, placed in demineralized H_2O or 10 μM GA_3 and kept in a climate room at 20°C, 65% relative humidity and a 16-h photoperiod at an irradiance of 115 μmol m^{-2} s^{-1}, warm white fluorescent light (Osram L38W/31) or permanent darkness. Photosynthesis and fluorescence were measured on the upper leaves with an ADC portable photosynthesis measuring device (Analytical Development Co., Hoddesdon, UK) and a PAM fluorometer (PAM 101 Chlorophyll Fluorometer, H. Walz, Effeltrich, Germany), respectively.

Gibberellic acid

The commercial GA_3 product (Sigma G-3250) contains contaminations of GA_1 (Dr E. Knegt, personal communication). Control experiments in which the commercial GA_3 was purified by reversed phase HPLC demonstrated that the delay of chlorophyll loss ascribed to GA_3 was not caused by contaminations in the commercial product (data not shown).

Chlorophyll

Chlorophyll contents of the upper leaves were determined both after extraction with dimethylformamide (Inskeep and Bloom 1985) and by using the chlorophyll meter SPAD-502 (Minolta).

Photosynthesis

Photosynthesis measurements, i.e. analysis of CO_2 and H_2O vapour, were carried out with a portable leaf chamber analyzer (ADC). Young fully unfolded whorl leaves were measured. Air was obtained from a gas cylinder to ensure constant composition (78% N_2, 20% O_2, 33 Pa CO_2). The anatomically lower side of the leaf, which is the actual upward side, was illuminated by a Philips EFN $A_{1/230}$ halide lamp. Long wave irradiance was filtered by a short wavelength band pass filter (Schott 115). Different irradiance levels were obtained by fractional filtering with neutral grey filters. Average conditions within the chamber were: temperature 22.6°C, vapour pressure deficit 0.57 kPa and CO_2 concentration 33 Pa. Rates of photosynthesis were calculated from the flow rates and the measured concentrations of CO_2 and vapour in the ingoing and outgoing air stream and the flow rate of the stream by the procedure described by von Caemmerer and Farquhar (1981).

Fluorescence

At intervals of 24 h, cut flowers were illuminated (115 μmol m^{-2} s^{-1}) for 45–60 min (light adaptation) to allow the stomata to open, after which photosynthesis was measured. After a subsequent 30-min dark adaptation, the leaves were clamped in a small cuvette and flushed with humidified air (98% N_2, 2% O_2, 33 Pa CO_2). For all leaves, the minimal (F_0) and the maximal fluorescence F_m were measured with the PAM 101 Chlorophyll Fluorometer according to Schreiber et al. (1986). F_m was determined during a saturating light pulse with a duration of 0.7 s. From F_0 and F_m, the efficiency of the open photosystem II reaction centres was estimated:

$$\Phi_{pc} = (F_m - F_0)/F_m$$

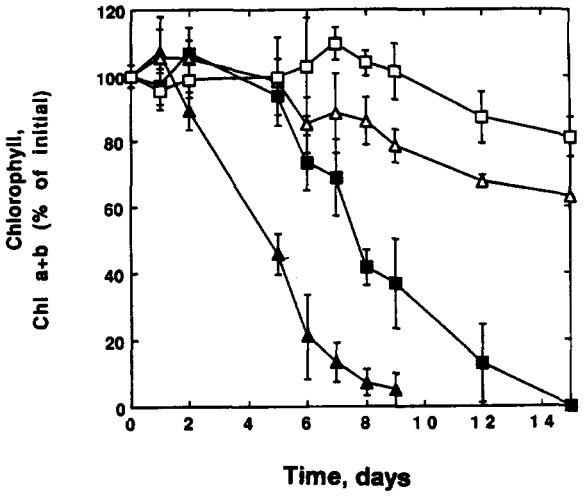

Fig. 1. Chlorophyll $a+b$ content of leaves of control (triangles) and 10 μM GA_3-treated (squares) cut flowers under light (open symbols) or dark (closed symbols) conditions. Each data point represents the mean of leaves from 4 cut flowers. The bars represent ±SE.

The efficiency of electron flow through photosystem II (yield), at different light intensities, was measured under steady-state conditions according to Genty et al. (1989). Light was produced by quartz halogen lamps passed through a heat reflecting filter. Light intensities were comparable with those of the gas exchange measurements. The yield was determined as the ratio of the steady state fluorescence during illumination and the maximal fluorescence during a saturating light pulse: yield = F_v/F'_m.

Statistics

Cut flowers were subjected to 4 different treatments (dark versus light and demineralized water versus GA_3 in the holding solution) in 4 replicates. Samples were taken at the start of the experiment (day 0) and at 1, 2, 5, 6, 7, 8 and 9 days after the start of the experiment. Four flowers per treatment were measured yielding 16 independent measurements per day. Analysis of variance using the statistics package Genstat (Rothamsted Experimental Station) provided the standard errors of differences.

Results and discussion

Chlorophyll content

The absolute amounts of chlorophyll $a+b$ of the leaves varied from approximately 0.05 to 1.3 mg g^{-1} fresh weight. Under these experimental conditions the arbitrary units determined with the chlorophyll meter SPAD-502 showed a near linear relation with the absolute amounts of chlorophyll in the leaves measured after extraction with dimethylformamide (data not shown). The correlation between the absolute amounts of chlorophyll $a+b$ (Y) and the arbitrary units of the chlorophyll meter SPAD-502 (X) can be adequately described by the equation: $Y = 3.217 \times 0.01\,X$ ($r^2 = 0.948$).

The average chlorophyll $a+b$ content in the leaves, on a fresh weight basis, at the start of the experiment, was approximately 1.218 (SE = 0.044) mg Chl g^{-1} fresh weight. During the first two days, the amount of chlorophyll remained approximately constant (Fig. 1). After 5 days in the dark, it strongly decreased in the absence of GA_3. In agreement with previous observations (Jordi et al. 1993) both GA_3 and/or white light strongly delayed chlorophyll loss in the leaves. After 5 days of GA_3 and/or white light treatment, the levels of chlorophyll $a+b$ in leaves from cut flowers were similar to the initial content. The effects of GA_3 and light appear to be additive. The ratio of absorption at 647 nm and 664.5 nm of the chlorophyll extractions remained constant during the entire experiment, demonstrating that Chl a and b levels decreased at the same rate.

Photosynthesis

Light response curves of alstroemeria leaves at different times after the start of the treatments are shown in Fig. 2A–D. At the start of the experiment the leaves of the cut flowers exhibited a typical light response curve with a relatively low maximal photosynthesis rate of approximately 6 μmol CO_2 m^{-2} s^{-1} at 1350 μmol m^{-2} s^{-1} PAR (Fig. 2A). The reference response of intact healthy C_3-

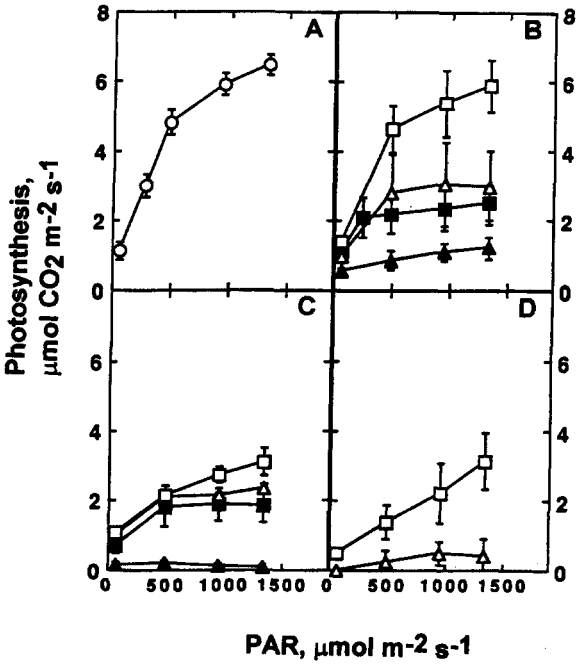

Fig. 2. Light response curves of leaves at the start of the experiment (A), after 5 days (B), 7 days (C) and 9 days (D) of control (triangles) and 10 μM GA_3-treated (squares) cut flowers under light (open symbols) or dark (closed symbols) conditions. Each data point represents the mean of leaves from 4 cut flowers. The bars represent ±SE.

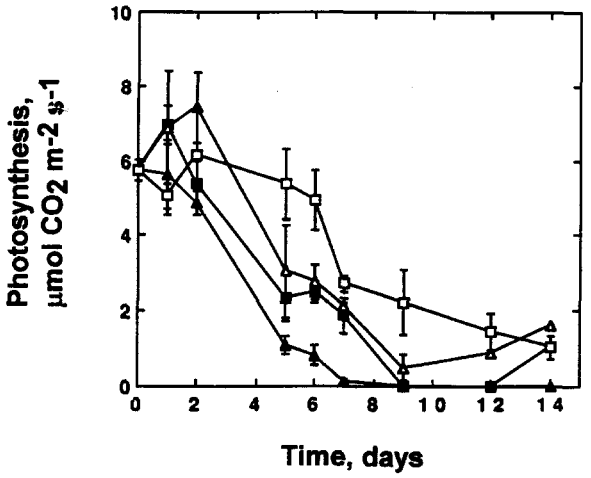

Fig. 3. Photosynthetic activities of leaves at a light intensity of 950 µmol m^{-2} s^{-1} (PAR) of control (triangles) and 10 µ*M* GA_3-treated (squares) cut flowers under light (open symbols) or dark (closed symbols) conditions. Each data point represents the mean of leaves from 4 cut flowers. The bars represent ±SE.

plants would be 15–25 µmol CO_2 m^{-2} s^{-1}. After 5 days, it was evident that the photosynthesis rate in the GA_3/light-treated leaves was relatively high (Fig. 2B) as compared to GA_3/dark- or control/light-treated leaves, whereas the photosynthesis rate of the control/dark-treated cut flowers was even lower. The decreases in photosynthesis rate became more prominent with time and after 9 days only the GA_3/light-treated cut flowers exhibited a low but significant photosynthetic activity (Fig. 2D).

A decline in photosynthetic activities observed at 950 µmol m^{-2} s^{-1} PAR was prevented to a certain extent by treatment with light and/or GA_3 (Fig. 3). The photosynthetic activities determined at other light intensities showed a similar decline in time (data not shown). In ranking order the effects on photosynthesis rates reflect the effects on chlorophyll levels in the various treatments, but there are important differences in the time courses of both features. After 5 days of incubation, chlorophyll levels in the leaves from control/light-, GA_3/dark- and GA_3/light-treated cut flowers were indistinguishable from the initial chlorophyll content. In contrast, after such an incubation period the rates of photosynthesis in control/light- and GA_3/dark-treated cut flowers, and to a lesser extent also in the GA_3/light-treated cut flowers were lower than the initial photosynthetic rates. This shows that chlorophyll content and photosynthesis are not linked proportionally under these experimental conditions and can therefore *not* be used as a reliable parameter to study senescence (Grover 1993). Other authors have arrived at similar conclusions using non-yellowing mutants of *Festuca pratensis* L. (Thomas and Stoddart 1975) and *Phaseolus vulgaris* L. (Ronning et al. 1991). There may be other limiting factors, either stomatal or at the chloroplast level. The first possibility could be excluded on the basis of calculated internal CO_2 concentrations at different steady-state light intensities. With senescence, the internal CO_2 concentration increased (data not shown), which indicates that there was no diffusion limitation but a limitation at the chloroplast level.

Fluorescence

Figure 4 shows the time courses of the photosynthetic efficiency (Φ_{pc}) of open reaction centres of photosystem II. A comparison of Figs 1 and 4 shows that the decrease of Φ_{pc} follows the decrease in the chlorophyll content of the leaves. In principal, Φ_{pc} is a ratio of two fluorescence signals, which excludes the absolute chlorophyll content as a variable that affects Φ_{pc}. However, because the structure of the leaves changed drastically over the measuring period, there might be a spatial effect due to the breakdown of chlorophyll and changes in optical properties of the leaves that biased the results due to changes in scattering, self absorption or photosystem I fluorescence. Disregarding these effects, it seems that the breakdown of chlorophyll and a concomitant decrease of absorbance parallels the decrease of the functional status of the remaining photosynthetic reaction centres. To study whether additional light reactions, particularly in the electron transport chain, were affected, its activity was estimated under low light conditions. Under these conditions, the supply of energy to regenerate ribulose 1,5-bisphosphate in the Calvin cycle becomes rate limiting for the photosynthetic process. Under 2% oxygen, which prevents photorespiration, the electron transport rate is equivalent to the rate of the carbon reduction cycle. The electron transport rate was determined by steady-state fluorescence measurements and analyses were made ac-

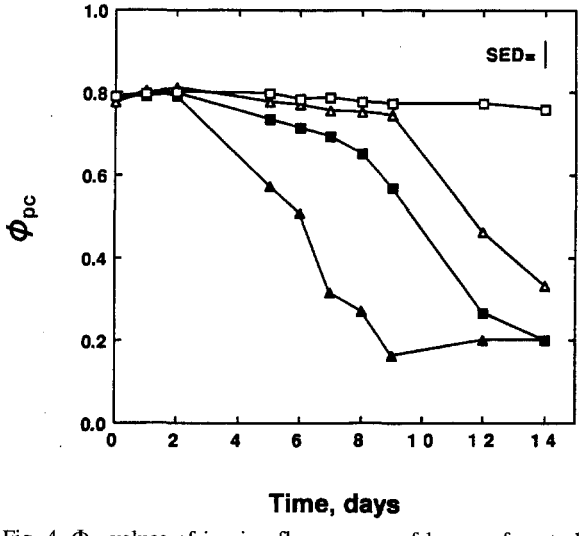

Fig. 4. Φ_{pc} values of in vivo fluorescence of leaves of control (triangles) and 10 µ*M* GA_3-treated (squares) cut flowers under light (open symbols) or dark (closed symbols) conditions. Each data point represents the mean of leaves from 4 cut flowers. The bar in the figure indicates the standard error of differences of means.

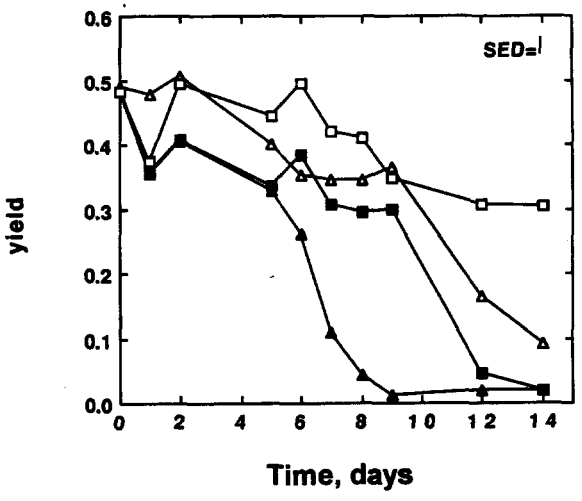

Fig. 5. Yield values of in vivo fluorescence of leaves of control (triangles) and 10 m*M* GA_3-treated (squares) cut flowers under light (open symbols) or dark (closed symbols) conditions. Each data point represents the mean of leaves from 4 cut flowers. The bar in the figure indicates the standard error of differences of means.

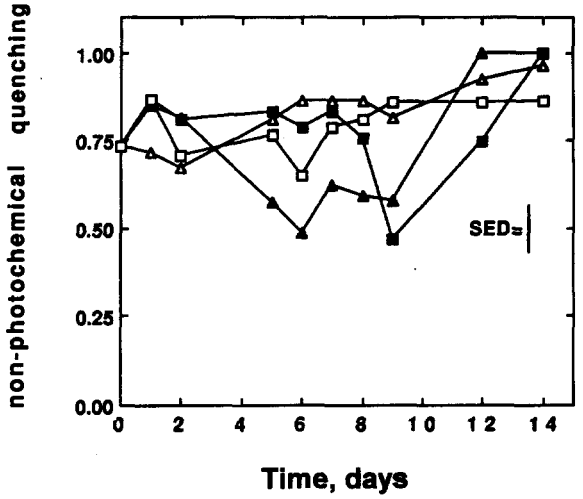

Fig. 6. Non-photochemical quenching values of in vivo fluorescence of leaves of control (triangles) and 10 μ*M* GA_3-treated (squares) cut flowers under light (open symbols) or dark (closed symbols) conditions. Each data point represents the mean of leaves from 4 cut flowers. The bar in the figure indicates the standard error of differences of means.

cording to Genty et al. (1989). The results are summarised in Fig. 5. The yield presents the number of electrons going through photosystem II per unit of irradiance absorbed by photosystem II. The data indicate that at low irradiance, 68 μmol m^{-2} s^{-1}, the patterns of electron transport rate closely resemble those for Φ_{pc} (Fig. 4), which is to be expected because under those light conditions most of the photosystem II centres will be open such that electron flow is mainly determined by the efficiency of open centres. In the case of the GA_3/light treatment, however, the efficiency of open photosystem II reaction centers remained stable over the period, but the electron transport rate decreased by 40%. This shows that optical signs of senescence lag behind functional senescence.

The low efficiency of photosystem II could be due to a feedback control of photosynthesis as a result of severe sink limitation. Previously, we demonstrated that the content of reducing sugars in the leaves increased from 80 μmol g^{-1} fresh weight at the start of the experiment to approximately 200 μmol g^{-1} fresh weight after 17 days in the light, both in the absence and presence of GA_3 (J. H. M. Overbeek, unpublished data). In addition, we demonstrated that developing buds are *not* important for chlorophyll loss and/or retention of chlorophyll in the presence of GA_3 (Jordi et al. 1993). Both observations are consistent with a limited sink activity of the buds. Down-regulation of the photosynthetic efficiency in case of sink-limitation is probably one of the most efficient mechanisms to prevent irreversible damage to the leaves. This is accompanied by an increase of energy dissipation as heat and a concomitant decrease of fluorescence (non-photochemical quenching). From our data it became apparent that nonphotochemical quenching was extremely high for all treatments and during the whole period, even under low light conditions (Fig. 6), indicating that the photosynthetic system is in a highly reduced state despite the low irradiance. This means that the reducing power is consumed at a very low rate in the Calvin cycle. Under such conditions photosynthesis will become down-regulated. The data suggest that the nonphotochemical quenching increased with time as might be expected from the observed decrease in yield. However, at nonphotochemical quenching values beyond 0.6, data analyses may be erroneous and conclusions are difficult to draw. Nevertheless it is quite clear that the plants were severely limited in using the energy provided by photosynthetic processes.

Conclusions

Photosynthetic rates of alstroemeria cut flowers are very low and the differences under the various experimental conditions are *not* caused by stomatal limitations and not solely due to different chlorophyll levels. The decrease in photosynthetic rate is, in order of occurrence, caused by a decrease of the Rubisco concentration or its activity (Thimann 1980, Grover 1993), a decrease of the absorbance and a concomitant decrease in the electron transport rate, and possibly by an increase of photorespiration. The chlorophyll remaining after 9 days incubation of leaves with GA_3 in the dark should be considered as a 'cosmetic' pigment without any function in the supply of assimilates to the flowers.

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