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# Evidence for the involvement of gibberellins in developmental phenomena associated with carnation flower senescence

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**Abstract**

The application of $10^{-4}$ M $GA_3$ to preclimacteric carnation flowers delayed senescence, climateric ethylene production reduced the rate of loss in fresh weight of intact flowers and the decrease in moisture content of the petals. The loss in flower fresh weight commenced prior to the ethylene climacteric. The increased membrane permeability which was observed when intact, control flowers were half opened, was delayed by $GA_3$ application. This effect was only significant when $GA_3$ was applied to young flowers. In addition to slowing down the loss in fresh mass, $GA_3$ inhibited ethylene production by the style and stigma. The increase in ovary dry weight and chlorophyll content and the associated decrease in petal dry weight was slowed down by $GA_3$ but not arrested, this despite reduced ethylene production by the ovary. It is proposed that a decline in endogenous gibberellin may be a correlative event associated with the onset of the senescence process in carnation flowers.

*Abbreviations:* $GA_3$, gibberellic acid; STS, silver thiosulphate

## 1. Introduction

Flower senescence is a multi-faceted process in which events follow a set pattern during most parts of the developmental processes [9]. Some of these physiological, biochemical and/or physical events which precede or follow the ethylene climacteric have been investigated [5, 7, 9]. These include flower water relations [1, 15, 25], membrane permeability [1, 7] and source-sink relationships associated with carbohydrate translocation [3, 16, 23].

It is well-established that hormones are involved in the regulation of carnation flower senescence [5]. Changes in hormonal levels have been suggested to act as important regulatory signals for the various physiological, biochemical and biophysical processes associated with the senescence syndrome [7, 9, 26]. An important hormonal change which results in petal inrolling (a feature of senescence) is the production of climacteric ehtylene, which has received considerable attention [5, 11, 12, 15, 22].

Endogenous gibberellin levels decline in flowers prior to full bloom [10]. This might suggest that changes in the flower which reduces endogenous gibberellin levels may enhance their senescence. It has been shown recently [18, 20] that gibberellic acid ($GA_3$), applied to young flowers, delayed carnation senescence [20]. This delayed and reduced level of ethylene evolution resulted in petal shrivelling rather than the typical inrolling (sleepiness) symptom usually associated with the ethylene climacteric.

We report here the effect of $GA_3$ applied to preclimacteric carnation flowers, on changes in water balance, membrane permeability and the development of the ovary and style.

## 2. Material and methods

Carnations *Dianthus caryophyllus* L. cv. *White Sim* grown in a commercial nursery were cut at Stages IV (open brush buds), VI (marketable stage, fully open flowers) and VII (petals reflexed) [20] and transported overnight at 4°C to the laboratory. Upon arrival the stems were cut to 10 cm and placed in distilled water for 4 h. Subsequently the stems were reduced to 3 cm, so as to reduce the effects of leaves and stems on flower senescence to a minimum, and then immersed either in distilled water (controls) or $10^{-4}$ M $GA_3$ for 48 h [20]. Each flower was subsequently maintained individually in a test tube of distilled water. All other experimental conditions were as outlined previously [20]. The day of flower arrival was designated 'day 0'. Flowers were inspected daily for *petal inrolling*, symptomatic of natural senescence, or *petal shrivelling*, which occurs in $GA_3$-treated flowers.

The fresh weight of intact flowers, as well as the fresh and dry weights of individual flower components (petals, ovaries and styles), were determined daily for flowers harvested at Stage VI (which show petal inrolling about 6 days later). Ten flowers were used per treatment for measurements of whole (intact) flowers. These were weighed daily for the duration of the experiment. The results are expressed as the average daily change as a percentage of the day 0 values $\pm$ SE. Where flower components were monitored, 5 flowers were destructively harvested each day and fresh and dry weights of each component determined. The ovaries and styles were weighed individually while, in the case of the petals, three petals from the outer whorl, were used for each flower. Dry weights were determined after 48 h drying at 80°C. The moisture content of the petals was expressed as a percentage on a wet weight basis.

Ethylene production by intact flowers and flower components was measured as described previously [19]. Membrane permeability in attached petals was determined by measuring changes in conductivity as described earlier [7]. To reduce the background readings, particularly with the low values obtained with flowers at Stage IV, the flowers were rinsed three times with distilled water before taking a reading. As whole flowers were used for these determinations the possibility of the styles contributing to the recorded values had to be considered. This was monitored in preliminary experiments where the styles were removed from the flowers. Changes in membrane permeability were measured (A) for flowers harvested at Stages IV, VI and VII and (B) for untreated, control flowers harvested at Stage IV or treated with $GA_3$. In the latter instance this was done (i) upon arrival, (ii) when they reached Stage VI or (iii) Stage VII of development. Measurements for the different Stages of development were made on the day of flower arrival and then daily until they senesced. All results are the mean of five measurements. All experiments were repeated at least twice.

Chlorophyll was extracted and quantified for individual ovaries immediately upon removal from the flowers using the technique outlined by Cook and Van Staden [3].

## 3. Results

The fresh weight of both the control and $GA_3$-treated flowers decreased over the 12 days of the experiment (Fig. 1A). The rate of loss was significantly faster in the control flowers. A difference in fresh weight loss was already evident on day 3 when the control flowers had reached Stage VII of development. It became more pronounced with time. By day 10 an 80% loss in fresh weight was recorded for the control while in the $GA_3$-treated flowers it was less than 50%. Fresh weight loss commenced prior to the ethylene climateric. The moisture content of the outer petals decreased very sharply and significantly between days 7 and 8 once the flowers had reached Stage VIII of development and began to inroll at the edges (Fig. 1B). In the $GA_3$ treated flowers this water loss was later and more gradual and they did not reach Stage IX until day 12.

Membrane permeability changes for the flowers at different stages of development mirrored the above changes seen in water loss. The increase was rapid and without an appreciable lag phase for flowers harvested at Stage VII of development, in contrast to the pattern seen for flowers harvested at Stages IV and VI (Fig. 2A). Applied $GA_3$ decreased membrane permeability of Stage IV and VI flowers but was not effective when applied at Stage VII (Fig. 2B). $GA_3$ reduced permeability by about 50%,

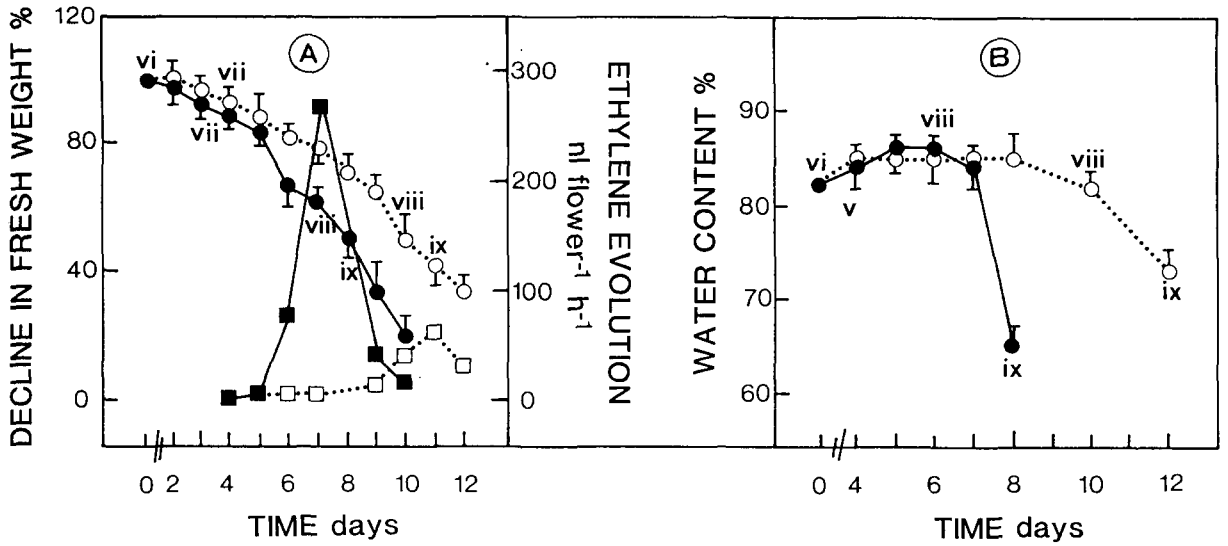


*Fig. 1.* The effect of a 48 h $GA_3$ pretreatment ($10^{-4}$ M) on carnation flower senescence. (A) Changes in fresh weight and ethylene evolution. Fresh weight ● = control, ○ = $GA_3$ – treated. Ethylene production ■ = control, □ = $GA_3$-treated. (B) Changes in the moisture content of petals from the outer whorl (values are expressed as a percentage of the fresh weight) ● = control, ○ = $GA_3$-treated. The stages of flower development reached during the course of the experiment are: Stage VI = outer petals 90° to the stem, petals white and turgid; Stage VI = outer petals 45° to the stem, petals white and turgid; Stage VIII for the controls = all petals inrolled at the edges (sleepiness), petal changes from white to cream with turgor loss. Stage VIII for $GA_3$-treated flowers = petals randomly change from white to cream, loss of turgidity, petal edges dried out. Stage IX for the control = advanced petal inrolling and collapse, tips turn brown, flower closed. Stage IX for $GA_3$ -treated flowers = petals shrivelled. Vertical bars represent SE. n = 5, 10 or 15 for ethylene, flower fresh weight and petal water content respectively.

as indicated by diffusate conductivity when applied to flowers at the two earlier developmental stages.

The styles of flowers harvested at Stage VI were underdeveloped but showed an increase in length and their fresh weight reached a maximum roughly consistent with the ethylene climacteric (Figs 3A and B). $GA_3$ application suppressed style development and markedly reduced ethylene production.

The dry weight of the ovaries of flowers harvested at or shortly after opening (Stage VI) increased slowly during the preclimacteric Stages (VI and VIII) (Fig. 4A). With the onset of climacteric ethylene production (Fig. 4B) the dry weight increased more rapidly. Treatment with $GA_3$ decreased ovary development and markedly delayed and reduced ethylene production. The ovaries from both control and $GA_3$-treated carnations had higher levels of chlorophyll (Fig. 4C). Chlorophyll accumulation was, however, slower in the ovaries of $GA_3$-treated flowers than in the controls. This was probably a reflection of the fact that they developed at a slower rate (Fig. 4A). The dry weights of petals, detached from the same flowers from which the ovaries were obtained, decreased in both the control and $GA_3$-treated flowers, but the decrease was slower with $GA_3$ application (Fig. 4D).

## 4. Discussion

Gibberellic acid has recently been shown to delay flower senescence and to alter the final manifestation of the process from petal inrolling to petal shrivelling [18, 19, 20]. This study shows that several others developmental processes which lead up to the final demise of the flower are affected by $GA_3$ pretreatment.

$GA_3$ treatment decreased the rate of loss in fresh weight which starts early during the preclimacteric of the cut flower. This indicates that, as with other compounds such as STS, which delays flower senescence, it is affecting some of the developmental processes which lead up to the climacteric [25]. An impaired capacity of the petals to ultilize available water accelerates senescence [4]. $GA_3$ delayed and reduced the water loss by the petals. This was ac-

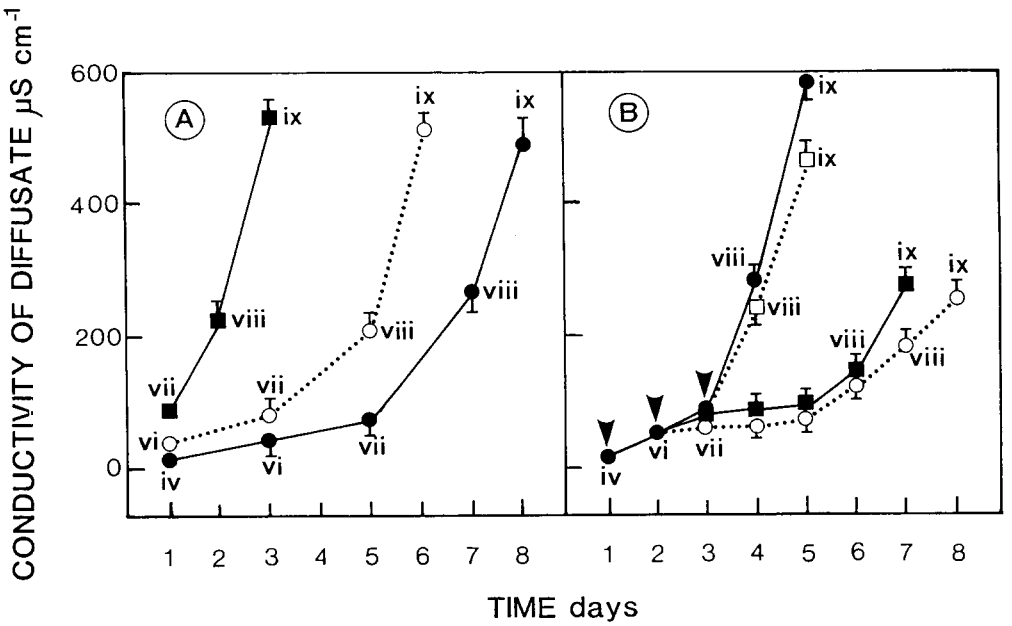


*Fig. 2.* Changes in the membrane permeability of attached petals of carnations pretreated with $GA_3$ ($10^{-4}$ M) for 48 h. (A) Flowers harvested at Stages IV, VI and VII of development (● = Stage IV; ○ = Stage VI; ■ = Stage VII). (B) The effect of $GA_3$ on flowers that were harvested at Stage IV and only treated with $GA_3$ when they had reached Stages IV, VI and VII of development. Times of $GA_3$ treatment are indicated by arrows. (● = control; ○ = $GA_3$ applied at Stage IV; ■ = $GA_3$ applied at Stage VI; □ = $GA_3$ applied at Stage VII). Vertical bars represent SE. Details of developmental stages are outlined in Figure 1.

companied by a reduction of membrane permeability of flowers from the early half open stage. This process, which commences in very young flowers [7, 21], together with ethylene production, are key regulatory factors in the sequence of events, leading to senescence. As petal sensescence is associated with a decline in protein synthesis [2], which influences changes in membrane perme-

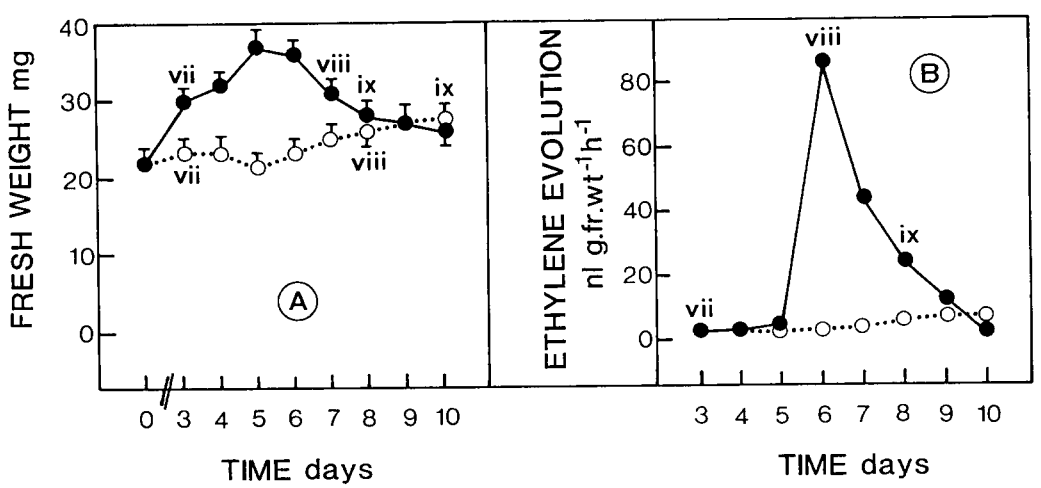


*Fig. 3.* The effect of a 48 h $GA_3$ pretreatment ($10^{-4}$ M) on carnation flowers harvested at Stage VI of development on (A) the fresh weight of the styles, and (B) ethylene evolution. Stages of flower development are as outlined in Figure 1. Vertical bars represent the SE. n = 5. ● = control; ○ = $GA_3$-treated.

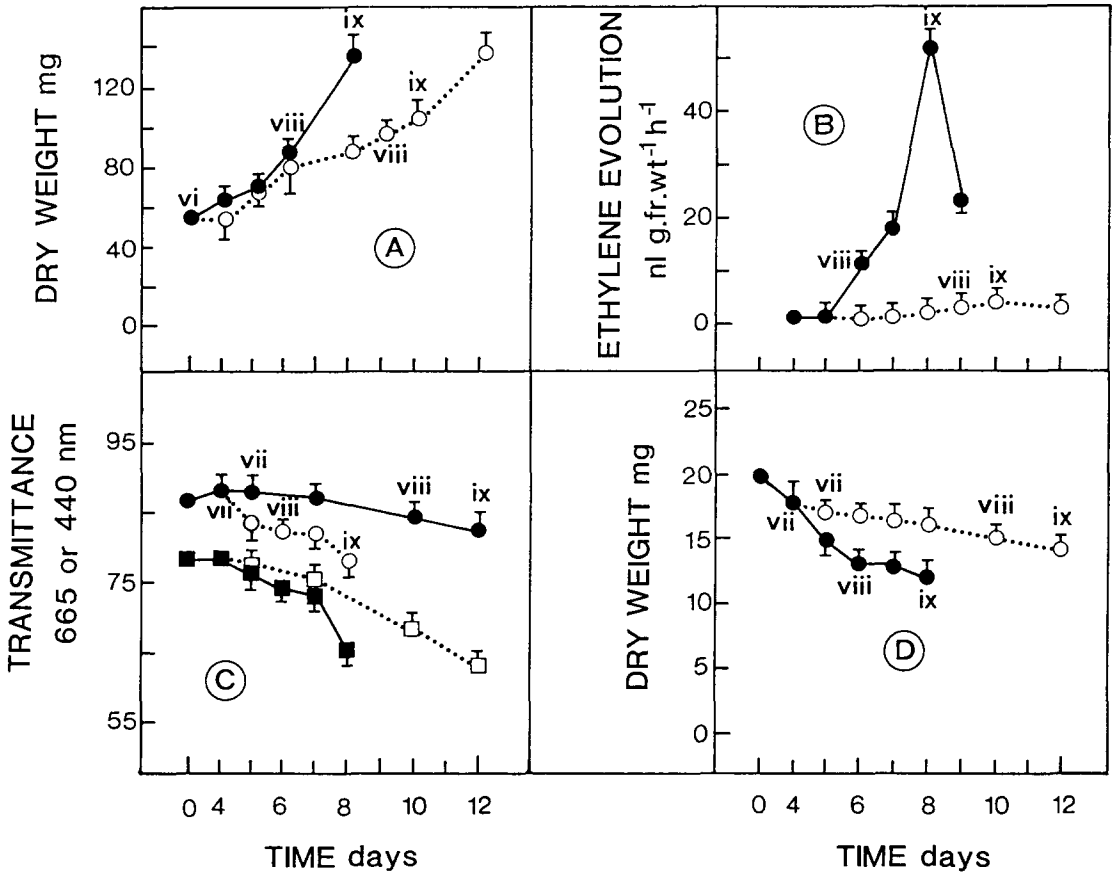


*Fig. 4.* The effect of a 48 h $GA_3$ pretreatment ($10^{-4}$ M) on carnation flowers harvested at Stage VI of development on the ovaries and petals during senescence. (A) Changes in ovary dry weight, (B) ethylene evolution by the ovary, (C) changes in the chlorophyll content of the ovaries measured at 665 (circles) and 440 nm (squares), and (D) changes in the dry weight of the petals. (● and ■ = control; ○ and □ = $GA_3$-treated). Stages of flower development are as outlined in Figure 1. Vertical bars indicate SE. n = 5 for Figures A, B and C and 15 for Figure D.

ability, the question arises as to whether $GA_3$, as is the case for cytokinins [8], may exert its effect via enhancement of protein synthesis.

The styles, and particularly the stigmas, have been shown to contribute considerably to the ethylene production of the cut carnation [11, 15]. This may act as an important senescence cue associated with pollination. $GA_3$ application inhibited both style and stigma growth, as well as their resultant ethylene evolution. The present results are not seen to be in conflict with the reports that GAs promote flower growth and opening [13] as, at the time of full bloom, gibberellin levels usually decline [10]. Thus, they would be low at the time of style and stigma growth. Applying $GA_3$ delayed growth while ethylene production was both reduced and delayed.

A decline in petal mass, correlated with an increase in both ovary dry weight and chlorophyll content, associated with climateric ethylene production, is well documented [5, 14, 16, 24]. The causative factor(s), however, remains unclear [9]. While $GA_3$ inhibited ethylene production by petals and ovaries it only decreased the rate of dry mass accumulation and chlorophyll content, as well as the rate of dry mass loss of the petals. The final values obtained were not different to those of the controls. This contrasts with the response seen with STS which also inhibits ethylene production, but seriously impaired ovary growth and the development of chloroplasts [3, 4]. This suggests an element

of selectivity of $GA_3$ action on certain aspects of the senescence processes [6, 17]. $GA_3$ is known to cause regreening of tissues [17] and this may explain why the ovaries of $GA_3$-treated flowers turned green despite the low level of ethylene production. The greater growth of the ovary following $GA_3$ treatment may indirectly have caused a stronger sink for carbohydrate export from the petals [23].

The present results indicate that an early decline in endogenous gibberellin levels in cut carnations may be one of the correlative events associated with the termination of maturation and the onset of the senescence process. By increasing the levels of $GA_3$ early in flower development the processes leading to maturation and senescence were severely retarded.

## Acknowledgements

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