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Coolstorage enhances ethylene production and reduces vase life of carnation flowers

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Abstract Cut flowers are typically coolstored for varying periods following harvest and subsequently may be exposed to large temperature fluctuations, particularly during transit. This investigation determined the influence of low temperature coolstorage and silver thiosulphate (STS) on subsequent ethylene production and vase life duration of carnation (*Dianthus caryophyllus* L. cv. Scania) flowers. Carnations were coolstored for 1, 2, or 5 days at 2°C and then transferred to 20°C. Coolstorage for 5 days accelerated the onset of ethylene production by 1.9 days and the onset of peak ethylene production, which occurred 1.5 days earlier than control flowers. The total number of days of ethylene production was c. 4 days for all treatments. Peak ethylene production from flowers coolstored for 5 days was 150 nlitre/flower per h greater than control flowers. A linear relationship was established between total ethylene production and the duration of coolstorage. The increased ethylene production induced in flowers coolstored for 5 days reduced the vase life by 14%. Silver thiosulphate was applied before and after 5 days of coolstorage at 2°C. The application of STS prevented overshoot ethylene production and resulted in an approximate three-fold increase in vase life for flowers treated with STS before coolstorage and an approximate two-fold increase for flowers treated after coolstorage. This study has established that coolstorage and subsequent warming of carnations results in a surge of ethylene which is earlier and greater than normally associated with senescence. This overshoot ethylene reduces the vase life of cut carnation; flowers but can be overcome by the application of STS.

Keywords ethylene; coolstorage; vase life; STS; silver thiosulphate; flower; post harvest physiology; senescence; carnation; *Dianthus caryophyllus* L; low temperature

INTRODUCTION

A recent cut flower transportation study has revealed that conditions during transit often include a cooling and warming cycle (Downs 1985). He outlined that flowers exported from New Zealand are subjected to many large temperature fluctuations during transit to overseas markets. Flowers are harvested and graded in temperatures often exceeding 20°C. They may then be coolstored for a few hours, or up to several days at temperatures of 1–5°C. Flowers are then packed and, by the time they reach the point of export, temperatures may reach 17.5°C. Temperatures experienced in the cargo holds during flight are often high, with peaks of 30°C in some instances. Substantial periods of time exist in flight when temperatures exceed 15°C. Frequently, exported carnation flowers have suffered from premature inrolling of the petals, a sign of ethylene injury (Downs & Lovell 1986).

Field (1981) found that variations in temperature influenced the ethylene production of bean (*Phaseolus vulgaris* L.) leaf tissue. Transfer of leaf tissue from 5°C, where the rate of ethylene production was extremely low, to 25°C resulted in a very rapid increase in ethylene production that exceeded the normal wound response at 25°C. Leaf discs maintained at 5°C continued to produce ethylene at very low rates (< 0.3 nlitre/g per h). Other workers

other plant tissues. Sfakiotakis & Dilley (1974) showed that cold stress induced metabolic changes leading to increased ethylene production in "Bosc" pears (*Pyrus communis* L.) upon their removal from coolstorage. Chilling at 2.5°C for 2 days accelerated the synthesis of 1-aminocyclopropane-1-carboxylic acid (ACC) and ethylene production in cucumber (*Cucumis sativus* L.) fruit (Wang & Adams 1982). Eaks (1980) found that ethylene production and the ethylene content of the internal atmosphere of lemon (*Citrus limon* L.) fruit increased at 20°C following exposure to chilling temperatures (0°C and 5°C), compared to fruit placed directly at 20°C. The increases were greater the longer the chilling exposure, and greater following exposure to 0°C than following exposure to 5°C. Faragher & Mayak (1984) showed that cut rose (*Rosa hybrida* L.) flowers previously held at 2°C for 10 or 17 days senesced earlier at 22°C than freshly cut flowers. The maximum rate of ethylene production at 22°C was nine times higher in the petals of flowers previously held at 2°C.

An investigation was carried out into a possible connection between the problem of the inrolling of the petals of export carnations and the phenomenon noted by Field (1981, 1984) and other workers, that a cooling and warming cycle could induce a surge of ethylene greater than that normally associated with senescence. The influence of temperature on ethylene production has been reviewed by Field (1985) and, more recently, in relation to specific mechanisms operating in chilling sensitive species (Field 1990).

It is common practice for flower growers to delay senescence by treating flowers in solutions containing silver ions. Silver salts have long been used as a germicide in floral preservatives (Ryan 1957). It was not until Beyer (1976) discovered that silver ions inhibited the effects of ethylene in a number of plant responses and delayed senescence in flowers, that its potential as a preservative in cut flower production was fully realised. Veen & Van de Geijn (1978) measured the rate of uptake of radioactive solutions of different silver salts and found that silver must be applied in the anionic thiosulphate complex (STS) to be of practical use. In addition to inhibiting ethylene's effects, silver ions in the STS complex were also found to inhibit ethylene production in carnation (Veen 1979; Cook et al. 1985).

The primary objective of this study was to determine whether coolstorage and subsequent warming of cut standard carnation would result in a surge of ethylene greater than normally associated with senescence. If the surge did occur, the effects of this surge on flower vase life were to be observed. Second, would the application of STS via the stem prevent this surge of ethylene and therefore extend the vase life of the flowers, and would the timing of this application alter its effectiveness in prolonging vase life?

MATERIALS AND METHODS

Flower preparation

Standard carnation (*Dianthus caryophyllus* L. cv. Scania) flowers were cut at the normal commercial harvesting point, at the start of flower opening (Downs & Lovell 1986). All flowers were cut and graded into four sizes and stages of development and allocated to treatments as evenly as possible. All leaves were removed to eliminate variations in water use (Carpenter & Rasmussen 1974). Stems were cut to 300 mm and the cut end immersed in a reservoir of distilled water which was sealed with aluminium foil to prevent contamination. The total time between cutting flowers and placing in distilled water was less than 3 h.

Environmental conditions

Flowers to be chilled were placed in a completely randomised design in an incubator at 2 ± 0.5°C in the dark. Control flowers and those transferred from periods of cool storage were placed in a completely randomised design in a controlled environment growth cabinet under the following conditions:

Temperature	20 ± 1°C day and night
Light	14 mole/m^2 per s (photosynthetically active photon flux density)
Day length	12 h (7.30 am – 7.30 pm)
Relative humidity	60%
Cabinet volume	4.8 m^3

These environmental conditions were based on recommendations made for the evaluation of the vase life of cut flowers by Reid & Kofranek (1980).

In a preliminary experiment, thermocouples attached to the petals or inserted in the flower receptacle established that flowers re-equilibrated after temperature transfer within 30 min.

Ethylene measurement

Ethylene measurements were made by enclosing single flowers in glass jars (586 ml). A rubber septum in the lid allowed the internal gas volume to be

sampled. The system was completely gas-tight and enabled ethylene production from single flowers to be determined with minimum enclosure periods (30 min). Ethylene in 1 ml gas samples was determined by gas chromatography using an alumina F1 column and flame ionisation detector (Field 1981).

Leakage tests on the sampling system and determination of ethylene production rates were carried out using known ethylene standards.

STS treatments

Flowers were treated singly with 1 μmole of silver contained in 1 ml (Reid & Kofranek 1980). A single cut stem was placed in the STS solution and loss of liquid resulting from transpiration monitored. When the STS had been almost completely transpired, an additional volume of 2 ml of distilled water was added to ensure that any residual STS solution was allowed to enter the stem, before the flower stem was re-immersed in the main distilled water reservoir. This method ensured that all flower/stem units received the same mass of STS.

Vase life duration and flower senescence

Visual assessment of all flowers was carried out daily to determine the stage of senescence and the point of termination of the useful vase life. The visual assessments were supported by measurements of petal angle, flower diameter, and flower height. Vase life duration was determined as completed when petal inrolling, loss of flower colour, and sepal tip necrosis commenced (Downs & Lovell 1986). The addition of STS changed the pattern of flower senescence, and termination of vase life duration was recognised by necrosis to petals and sepals rather than major changes to petal orientation.

Experimental design

In the first experiment, flowers were placed either directly at 20°C or initially at 2°C. The latter flowers were then transferred to 20°C after 1, 2, or 5 days at low temperature. There were five replicates for each treatment. Ethylene measurements were carried out every 12 h from the start of the initial incubation period at either 2°C or 20°C.

In the second experiment, flowers for the control treatment were incubated directly at 20°C, whereas in three other treatments flowers were coolstored at 2°C for 5 days before transfer to 20°C. In two of the latter treatments, flowers were treated with STS, either before incubation at 2°C or immediately before transfer to 20°C. There were six replicates of each treatment. Ethylene production was determined every 24 h in all treatments. On the final day of this experiment (Day 28), the STS-treated flowers were enclosed for 2 h before a gas sample was taken for ethylene measurement.

Statistical analysis

Both experiments were completely randomised designs with five or six replicates per treatment. Repetitions of treatments from the experiments were carried with only minor variations in treatment response. Curves for mean ethylene production were calculated using count data. To calculate total ethylene production, the area under each curve of each replicate was integrated and an analysis of variance was calculated on the result. A regression of the mean total ethylene production per treatment was made against the days of coolstorage for each treatment. The mean time for peak ethylene production was calculated separately and an analysis of variance carried out, using all replicates.

An analysis of variance was also completed on the following count data: days at 20°C until the first ethylene production; days at 20°C until the peak ethylene production; total days of ethylene production; and vase life duration of the flowers at 20°C.

A covariate analysis was made using flower dry weight at the end of the experiment as a covariate to determine whether flower size had any effect on the amount of ethylene produced.

RESULTS

Coolstorage accelerated the onset of ethylene production (Table 1). Control flowers produced ethylene after 7.7 days at 20°C, whereas flowers that had previously been coolstored at 2°C for 5 days produced ethylene after 5.8 days at 20°C. Coolstorage also accelerated the onset of peak ethylene production. The total number of days over which ethylene was produced was not altered by coolstorage. All flowers produced ethylene for approximately 4 days, regardless of treatment. Peak ethylene production was increased from 167 nlitre/flower per h in control flowers to 317 nlitre/flower per h in flowers that were coolstored for 5 days. A covariate analysis of peak ethylene production using flower dry weight as a covariate was not significant, indicating that peak ethylene production was not influenced by the dry weight of the flowers. The total amount of ethylene produced per flower was a linear function of the duration of coolstorage (Table 1).

The vase life of flowers at 20°C that had previously been coolstored was reduced compared to control flowers, although the relationship appears exponential (Table 1). Significant reduction in vase life was apparent only after 2–5 days coolstorage. Similarly, total ethylene production or peak ethylene production versus vase life showed indications of exponential relationships (Table 1).

In the second experiment the control flowers and the flowers that had been coolstored for 5 days without STS showed a similar pattern of ethylene production to the equivalent treatments in the first experiment (Table 2). Coolstored flowers had earlier ethylene production and earlier peak ethylene production, but the duration of ethylene production was the same for both treatments.

Peak ethylene production for control flowers was 144 nlitre/flower per h after 7.7 days at 20°C, compared to 184 nlitre/flower per h after 6.8 days at 20°C for flowers that had previously been coolstored for 5 days without STS (Table 2). These differences were not significant. The total amount of ethylene produced per flower was increased by coolstorage (Table 2).

No ethylene was produced by flowers that had been treated with STS, except for on the 28th day of the experiment when the enclosure period was increased to 2 h. On this occasion, two flowers produced ethylene, 4 and 6 nlitre/flower per h respectively. The application of STS before coolstorage extended flower vase life duration to 20.3 days, which was 24.5% longer than for flowers treated with STS after coolstorage (Table 2).

DISCUSSION

The covariate analysis established that flower dry weight had little effect on the amount of ethylene produced. This agrees with Nichols (1977) who found that 40–50% of the ethylene produced from senescing, unpollinated carnations was produced by the styles and most of the remainder from the petals. Since styles produce nearly half the total ethylene, yet contribute less than 4% of the fresh weight of the flower, they are the most active centres of ethylene production. This means experiments that have measured ethylene production in flowers in the absence of the styles, i.e., from excised petal discs, cannot be compared to experiments measuring ethylene from intact flowers.

Coolstorage reduced the subsequent vase life of flowers at 20°C (Table 1). This was primarily associated with a surge in ethylene production by the flowers following transfer to 20°C (Table 1). This surge was greater than the normal peak in ethylene production associated with senescence, and was similar to that found in dwarf bean (Field 1981), pear (Sfakiotakis & Dilley 1974), cucumber (Wang & Adams 1982), citrus (Eaks 1980), and rose (Faragher & Mayak 1984) after coolstorage of the tissues. The defined overshoot in ethylene appeared earlier than control ethylene and its peak production was greater, although its duration remained unchanged (Table 1).

In contrast, Halevy et al. (1983) found that a spray carnation (cv. Pink Royalette) coolstored at 2°C and a relative humidity of 95%, with the stems in water, did not produce overshoot ethylene. There is

Table 1 Parameters of ethylene production at 20°C by carnation following coolstorage at 2°C.

	Coolstorage (days)				
	0	1	2	5	LSD
Days at 20°C until first ethylene production	7.7	6.8	6.9	5.8	0.88
Days at 20°C until peak ethylene production	8.5	7.7	7.9	7.0	1.03
Days of ethylene production	4.0	3.9	3.8	4.1	0.56
Peak ethylene production irrespective of day (nlitre/flower per h)	167	176	236	317	68.60
Total ethylene production (µlitre/flower)	7.6	9.3	10.0	16.0	2.40
(the equation for the linear regression is: $y = 7.54 + 1.72\ d$, where d = days of coolstorage; the percentage variation accounted for is 97.0%)					
Vase life duration (days) at 20°C	9.0	8.8	8.2	7.8	0.74

no adequate explanation for this conflicting result, but it may suggest that overshoot ethylene production is not always a consequence of coolstorage and that cultivars that are more suitable for transport to distant export markets may exist. However, such a simple interpretation needs to be investigated carefully as, even within the present and subsequent experiments, there were substantial differences in ethylene production levels associated with warming previously coolstored flowers (Tables 1 and 2). Some of the differences in the magnitude of ethylene production between the two experiments were attributed to the differences in sampling frequency.

The timing of the overshoot ethylene surge was different in this experiment compared to that found by others. In this study, the surge of ethylene was not immediately apparent, but appeared after a delay of several days (Table 1). Other work suggests that overshoot ethylene usually occurs within hours or minutes (Field 1981; Wang & Adams 1982). The delay found in this experiment was probably because ethylene production was measured from an intact flower, not, as is the case with most other work, from excised plant tissue. Excised plant tissue, whether it be petals or leaves, probably represents a more responsive system where wound-induced ethylene is of great significance. The model system using excised discs of dwarf bean leaves (Field 1981, 1984) involved the effect of temperature regimes on plant tissue where the physiological and biochemical processes required for ethylene production have already been induced by wounding. The lag time reduction induced by low temperature was not determined for dwarf bean where, in both temperature manipulated and control tissues, it is short (≤ 20 min). The lag in the induction of ACC synthesis is easier to determine and may be less than 10 min when the warming temperature is 35°C (Field & Barrowclough 1989).

In contrast to the excised tissue systems, in a young, intact carnation flower, induction of ethylene requires the onset of senescence (Downs & Lovell 1986). It seems certain that low temperature exposure per se, in a non chilling-sensitive species such as carnation, does not induce rapid ethylene production, even when tissue is returned to a favourable temperature environment (Table 1). However, tissues that receive low temperature treatments may store the potential for enhanced ethylene production when ethylene biosynthesis is triggered naturally by the onset of senescence events or when ethylene biosynthesis is induced artificially by wounding. In the present experimental system, the low temperature exposure did influence ethylene forming processes by reducing the lag time for induction (Table 1).

Irrespective of the duration of the lag time to ethylene production, there is some suggestion that the mechanism for storing the potential for overshoot ethylene production is the same in all plant systems (Field & Barrowclough 1989; Field 1990). The critical issue is the mechanism that allows for the induction of ethylene on warming and how this is quantitatively related to the duration of coolstorage. There is currently no evidence to support the low temperature accumulation of 1-(malonylamino) cyclopropane-1-carboxylic acid (MACC) or any

Table 2 Parameters of ethylene production at 20°C and vase life duration by carnation following coolstorage at 2°C and STS treatment before or after coolstorage.

	Coolstorage (days)				
	0 No STS	5 No STS	5 STS before	5 STS after	LSD
Days at 20°C until first ethylene production	6.5	5.7	–	–	1.05
Days at 20°C until peak ethylene production	7.7	6.8	–	–	1.00
Days of ethylene production	4.3	4.8	–	–	1.15
Peak ethylene production irrespective of day (nlitre/flower per h)	144	184	0	0	75.60
Total ethylene production (µlitre/flower)	7.6	10.1	0	0	2.52
Vase life duration (days)	7.3	6.5	20.3	16.3	1.05 (–STS) 2.15 (+STS)

other conjugate that then releases ACC, and subsequently ethylene, on tissue warming. A current view involves the expression of gene activity controlling ACC synthase and the subsequent formation of ACC. It is likely that incomplete synthesis of ACC synthase occurs at low temperature and that translation of the protein is completed only on warming (Wang & Adams 1982; Wang 1988; Field 1990). The formation of ACC synthase would be quantitatively related to the amount of a pre-translation product, presumably m-RNA, produced at low temperature and ACC synthase would only be formed following tissue warming, provided the tissue was in an ethylene inducible state. A more detailed understanding of the system in carnation that stores the potential for enhanced ethylene production would be invaluable in establishing the use of chemical inhibitors that may delay ethylene production and prolong flower vase life. It is likely that carnation flowers must reach a critical point in maturation or senescence before they are able to produce the overshoot in ethylene production.

One possible criticism of the ethylene measurement procedure employed in these experiments is that it did not detect adequately the low levels of ethylene produced before the major peak of production. It is not clear what relationships exist between the early phase low level production, the onset of flower senescence, the subsequent rapid rise in ethylene production and the subsequent duration of vase life. The experiment employing STS application before and after coolstorage may emphasise the significance of low level ethylene production during coolstorage in predisposing tissue to higher level production at a later stage. Application of STS before tissue coolstorage prolonged vase life by 4 days, an increase of 24.5% (Table 2), and this may be linked to the delay of normal ethylene-induced senescence.

The mechanism whereby STS interferes with ethylene-mediated responses such as flower senescence was not investigated specifically in this work. However, it is clear that no major ethylene production occurred in STS-treated flowers (Table 2), although low levels (6 nlitre/flower per h) were detected by prolonging the enclosure period to 2 h on the final day of assessment.

These data support the criterion by Veen (1979) that silver ions substantially prevent the surge of ethylene production that precedes the wilting of petals in carnations. There will almost certainly be a reduced sensitivity of STS-treated flowers to both endogenous and exogenous ethylene (Beyer 1976). In this regard, there is a practical advantage in administering STS to flowers immediately after cutting and before coolstorage.

The vase life duration of non-STS treated flowers showed an apparent exponential relationship with the duration of coolstorage, whereas the increase in total ethylene had a highly significant linear relationship against days of coolstorage. The linearity of the latter relationship is maintained only for c. 5 days as similar ethylene production levels from flowers warmed at 20°C, following 4 and 10 days at 2°C, have been noted (Gibbs & Field unpubl. data). Prolonged coolstorage may lead to irreversible deterioration in biochemical integrity of the system that converts ACC to ethylene (Wang & Adams 1982).

There is a critical relationship between duration of vase life and ethylene production, whether determined as total or peak production (Table 1). From the form of the relationship, it appears that low temperature-induced ethylene production must reach a minimum value before it has a major influence on enhancing flower senescence and reducing vase life.

The present results suggest that maximum effective vase life duration can be attained by keeping coolstorage periods to a minimum, preferably no more than 1 day (Table 1). It is recognised that coolstorage periods of up to 5 days plus the period of vase life duration result in an overall postharvest life in excess of flowers not receiving a coolstorage treatment. Although this acknowledges that coolstorage can slow down postharvest deterioration the major practical concern is to ensure maximum post-retail vase life duration. Therefore, it is necessary to establish the optimum coolstorage conditions that will give both an extended period for transportation and the maximum vase life duration. However, this is not always practical and when it is necessary to coolstore for longer periods flowers should be treated with STS. The treatment of flowers with STS has a profound effect on prolonging vase life and this may be further enhanced by ensuring that application is made immediately after cutting and before coolstorage (Table 1).

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