Use of a Nitric Oxide Donor Compound to Extend the Vase Life of Cut Flowers

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Abstract. Snapdragon (*Antirrhinum majus* L. 'Chitchat'), delphinium (*Delphinium ajacis* L. 'Bellissimo'), chrysanthemum (*Dendranthema grandiflora* RAM. 'Regan'), tulip (*Tulipa hybrid* 'Golden Brush'), gerbera (*Gerbera jamesonii* H. Bolus 'Manovale'), oriental lily (*Lilium asiaticum* L. 'Specisim Simplon'), rose (*Rosa hybrid* L. 'Carnavale') and iris (*Iris hollandica* Tub. 'Blue Magic') cut flower stems were placed at 20 °C in water containing the NO donor compound 2,2'-(hydroxynitrosohydrazino)-bisethanamine (DETA/NO) at 10 and 100 mg·L⁻¹ and after 24 h, transferred to humidified air containing 0.1 µL·L⁻¹ ethylene. Compared with flowers kept in water, the vase life of all eight flowers was extended by DETA/NO with an average extension of about 60% with the range being about 200% for gerbera to 10% for chrysanthemum. DETA/NO appears to have widespread applicability to cut flowers and offers a simple technology to extend vase life.

Postharvest senescence is a limiting factor in the marketing of many species of cut flowers with ethylene being an important endogenous and exogenous factor that enhances this process. Flowers that are sensitive to ethylene are often pulse-treated soon after harvest with silver ion, applied as silver thiosulphate (STS) to delay senescence. Silver reduces ethylene-binding capacity and suppresses endogenous ethylene production thereby delaying the appearance of senescence characteristics (Van Doorn and Woltering, 1991). However, silver is a heavy metal and environmental concerns has many countries actively moving towards its elimination from commercial use. 1-Methylcyclopropene (1-MCP) has been found to inhibit the action of ethylene and thereby extend the storage life of a range of cut flowers and potted flowering plants (Serek et al., 1994, 1995). 1-MCP is considered nontoxic to humans and has been approved in an increasing number of countries for use as a short term fumigation treatment soon after harvest.

Nitric oxide (NO) was first characterised in plants in 1996 (Leshem and Haramaty, 1996) and has since been linked to a range of physiological processes including modulation of endogenous ethylene (Leshem and Pinchasov, 2000). Postharvest application of NO by fumigation with NO gas has been shown effective in extending the postharvest life of a range of flowers, fruits and vegetables when applied as a short term fumigation treatment (Wills and Leshem, 1998; Leshem et al., 1998). While NO and 1-MCP are alternative treatments to STS, the gaseous nature of both compounds increases commercial operational knowledge, requires infrastructure and is less suitable for developing countries.

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The importance of NO as a key regulator in mammalian physiology has led to substantial developments in NO donor technology, that is, solid compounds that store NO chemically but release it under appropriate physical conditions (Keefer, 1998). Leshem et al. (1998) found postharvest senescence was inhibited when Pink Sim and White-Sim carnation flowers (*Dianthus caryophyllus* L.) were continually exposed to the NO-releasing compounds N-*t*-butyl- α -phenylnitron (PBN) and 3-morpholiniosyl-nonimine (Sin-1). The experimental design, however, made it impossible to distinguish between a gaseous or in vivo release of NO. While NO donor compounds now exist based on different atoms attached to the NO releasing moiety, the diazeniumdiolates are of interest because of favourable characteristics that include high water solubility, chemical stability and ease of synthesis (Hrabie and Keefer, 2002). Bowyer et al. (2003) applied DETA/NO to carnation flowers by standing flower stems in an aqueous solution containing DETA/NO at 0.1 to 1000 mg·L⁻¹. An extension in vase life of 50% to 60% occurred for DETA/NO at 10 and 100 mg·L⁻¹ dissolved in distilled water. While the use of a buffer solution at pH 6.5 increases the extension in vase life to about 80%, the findings raised the potential for the simplified use of DETA/NO in unbuffered water during vase storage of flowers in domestic or commercial situations. This study explored this potential by examining the effect of DETA/NO on the vase life of eight flower types. The flowers were selected to span a range of sensitivity to ethylene.

Materials and methods

Iris (*Iris hollandica* Tub. 'Blue Magic'), tulip (*Tulipa hybrid* 'Golden Brush'), rose (*Rosa hybrid* L. 'Carnavale'), snapdragon (*An-*

tirrhinum majus L. 'Chitchat') and delphinium (*Delphinium ajacis* L. 'Bellissimo') flowers were obtained from farms in the Sydney basin and arrived at the laboratory within 24 h of harvest. Gerbera (*Gerbera jamesonii* H. Bolus 'Manovale'), oriental lily (*Lilium asiaticum* L. 'Specisim Simplon') and chrysanthemum (*Dendranthema grandiflora* RAM. 'Regan') were obtained from farms on the Central Coast of New South Wales and arrived at the laboratory within 1 h of harvest. Flowers were immediately placed at 20 °C and the stems immersed in distilled water until treated. Within each flower type, stems of similar maturity and visual quality were selected and cut under water to a stem length of about 12 cm. Stems were assigned to the various treatments in a randomised block design with 10 stems per treatment. Replication was by purchase of flowers on two occasions.

DETA/NO was synthesised by the method of Hrabie et al. (1993) and stored under nitrogen at -18 °C until required. Solutions of DETA/NO at 10 and 100 mg·L⁻¹ were freshly prepared for each replicate using distilled water (pH $\approx$ 5.5). Distilled water was used for the control treatment. Single flower stems were immersed in the treatment solution housed separately in plastic tubes (100 mL) by inserting the stem through hole in their lid. The gap around the stem was then sealed with a pliable putty, that is known from previous studies to be impermeable to gases, in order to prevent contact between any NO gas escaping from the solution and the flower head. Flowers were held in the treatment solution in ambient air at 20 °C and about 60% relative humidity (RH) for 24 h to permit compound uptake to occur in a low ethylene environment. The tubes containing the treatment solutions and flowers were then transferred to a sealed 20 L polystyrene container that was ventilated with humidified air (75% RH) containing ethylene at 0.1 µL·L⁻¹ at 20 L·h⁻¹. This ethylene concentration was used to simulate a likely commercial exposure (Wills et al., 2000). The ethylene concentration was generated by mixing gas from a cylinder containing 100 µL·L⁻¹ ethylene in air with humidified air. The ethylene concentration of the gas mixture was checked at regular intervals by gas chromatography (Wills et al., 2000). The flowers were examined every 12 h and the time for each stem to deteriorate to a quality that was considered as just acceptable on the basis of moderate discoloration, mold growth or wilting was recorded as the vase life. A mean value of vase life for all the stems in a treatment unit was calculated and the data for vase life were statistically analysed using SPSS version 11 (Chicago, Ill.). Least significant differences (LSD) at *P* = 0.05 were calculated to compare differences between means.

Results and discussion

Flower stems kept in water containing DETA/NO at 10 and 100 mg·L⁻¹ had significantly increased vase life relative to the water control for all eight flower types (Table 1). The use of 10 mg·L⁻¹ DETA/NO resulted in a greater extension in vase life than 100 mg·L⁻¹ for four

Table 1. Vase life at 20 °C of flowers continuously immersed in the water containing DETA/NO and stored in humidified air containing 0.1 µL·L⁻¹ ethylene.

Flower	Ethylene sensitivity	Vase life (h) in DETA/NO solution (mg·L ⁻¹)			Cause of senescence
		0	10	100	
Snapdragon	High	77 a ^c	111 b	108 b	Flower abscission
Delphinium	High	158 a	228 c	196 b	Flower abscission
Rose	High	120 a	148 c	139 b	Bud opening and dropping, petal abscission and browning
Chrysanthemum	Low	296 a	322 c	313 b	Stem mold, petal wilting and yellowing
Tulip	Low	75 a	116 b	119 b	Wilting and bud opening
Gerbera	Low	103 a	314 c	257 b	Petal discoloration, wilting and mold
Iris	Low	69 a	103 b	98 b	Wilting and petal inrolling
Oriental lily	Low	181 a	251 b	250 b	Bud opening, leaf yellowing and wilting

^cMean separation in rows by LSD at *P* = 0.05. Values are the mean of two replications each with 10 flowers per treatment.

flower types with the other four flowers having a similar effectiveness for 10 and 100 mg·L⁻¹. For flowers exposed to 10 mg·L⁻¹ DETA/NO, the greatest proportional extension in vase life was obtained for gerbera at about 200%. Tulip and iris showed about a 50% increase, snapdragon, delphinium and oriental lily about 40%, while the lowest extension was for rose at about 20% and chrysanthemum at about 10%. To these should be added our previously published value for carnation of about 50% (Bowyer et al., 2003). Thus, DETA/NO has generated a 40% to 50% extension in vase life for most flowers but with substantial variation from this level for a few types. It is of considerable interest that DETA/NO was highly effective on the four flowers that have been categorised as having a low sensitivity to ethylene (Woltering and Van Doorn, 1988) with gerbera the most responsive and rose which has a high sensitivity to ethylene giving a relatively low response. The apparent widespread effect of DETA/NO across many types of flowers suggests that it may have commercial application. Its effectiveness on flowers with low ethylene sensitivity would indicate a wider usage than STS and 1-MCP as they are claimed to only inhibit ethylene action. The multiple modes of action that have been found for NO in mammalian systems (Leshem 2000) would indicate a wider

modality for NO in plants than just on ethylene action but no such studies have thus far been conducted. As a solid that dissolves in water and with effectiveness from a single initial application at a relatively low concentration, DETA/NO could find usage in the florist trade and domestic situations to extend vase life. It is concluded that the relative effectiveness of DETA/NO and other NO-releasing compounds should be examined in more comprehensive studies including comparisons made with current commercial florist preparations as well as STS and 1-MCP. Stability of DETA/NO is an issue that will also need some investigation. Preliminary examination indicated a half-life for DETA/NO of about 2 months at 20 °C but in the absence of water vapour and carbon dioxide.

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