RURAL INDUSTRIES RESEARCH
& DEVELOPMENT CORPORATION

Delaying postharvest senescence of cut flowers
using nitric oxide

A report for the Rural Industries Research and Development Corporation

By M.C. Bowyer and R.B.H. Wills

May 2003

RIRDC Publication No 03/51
RIRDC Project No UNC-16A

ISBN 0642 58618 7
ISSN 1440-6845

Delaying postharvest senescence of cut flowers using nitric oxide
Publication No. 03/051
Project No. UNC-16A

Researcher Contact Details

Name: Dr. M. C. Bowyer
Address: School of Applied Sciences,
University of Newcastle
PO Box 127
Ourimbah NSW 2258

Phone: 02 43484119
Fax: 02 43484145
Email: chmcb@cc.newcastle.edu.au

In submitting this report, the researcher has agreed to RIRDC publishing this material in its edited form.

RIRDC Contact Details

Rural Industries Research and Development Corporation
Level 1, AMA House
42 Macquarie Street
BARTON ACT 2600
PO Box 4776
KINGSTON ACT 2604

Phone: 02 6272 4539
Fax: 02 6272 5877
Email: rirdc@rirdc.gov.au
Website: http://www.rirdc.gov.au

Published in May 2003
Printed on environmentally friendly paper by Canprint

Foreword

The cut flower industry is expanding world-wide and Australia is actively seeking to generate niche markets for its unique range of native flora. Because Australia is geographically isolated from the high volume markets of Europe and North America, growers are heavily reliant on technologies that maintain the quality of postharvest produce during transportation. Australia must therefore take a leading role in the development and marketing of technologies that provide Australian producers a competitive advantage over their foreign counterparts.

This report details a brief project undertaken to evaluate the use of a soluble nitric oxide donor chemical (DETA/NO) to delay senescence in a range of Australian native cut flowers. The findings indicate that DETA/NO can delay the appearance of senescence features in some flower species. The report also details recommendations for future work to be undertaken to gain a fuller understanding of the results generated by this preliminary study.

This project was funded from RIRDC Core Funds which are provided by the Federal Government.

This report is an addition to RIRDC's diverse range of over 900 research publications, forms part of our Optimised productivity of crops and pastures R&D program, which aims to improve the quality of Australian native cut flowers.

Most of our publications are available for viewing, downloading or purchasing online through our website:

- downloads at www.rirdc.gov.au/reports/Index.htm
- purchases at www.rirdc.gov.au/eshop

Simon Hearn
Managing Director
Rural Industries Research and Development Corporation

Acknowledgments

The authors wish to thank the staff of *The Wildflower Place*, Erina Heights for their assistance in coordinating the supply and delivery of wildflowers for this project, Paul Hasking of *Crows Nest Wildflowers*, Queensland for his kind donations of grevillea, Dr Brian Freeman IHM, Gosford for his encouragement and support in developing the project, and the Flower Export Council of Australia (FECA) for its funding support making the project a reality.

Abbreviations

DETA/NO	2,2'-(hydroxynitrosohydrazino)-bisethanamine.
1-MCP	1-methylcyclopropene.
NO	nitric oxide.
PBN	N-*t*-butyl-α-phenylnitrone.
Sin-1	3-morpholinosyl-nonimine.
STS	silver thiosulfate.

Contents

Executive Summary

Nitric oxide (NO) is a highly reactive, toxic gas that occurs naturally in the environment as a by-product of lightening strikes and from anthropogenic sources such as combustion processes. Recently, NO has been found to be an important bioactive molecule in mammalian physiology, where it acts as a signalling agent in a diverse range of physiological processes including blood pressure regulation, blood clotting, the transmission of neural impulses and the generation of immunological responses. NO performs similar roles in other more phylogenetically distant animal species, including both vertebrates and invertebrates.

In plants, nitric oxide has recently been linked to a range of physiological processes including cell growth, seed germination, phytopathological stress and plant senescence. It is this latter characteristic that forms the keystone of the investigations in this report.

Previous investigations undertaken by our research group have demonstrated that fumigation with NO gas can delay senescence in selected fruits, vegetables and cut flowers. We have also showed that water-soluble chemicals releasing NO gas into solution can extend the postharvest life of a number of species of commercially important exotic cut flowers. Importantly, these studies found that nitric oxide was effective on both ethylene sensitive and ethylene insensitive flower species suggesting that the mode of action of NO differs from current commercial treatment protocols such as silver thiosulfate (STS) and 1-methylcyclopropene (1-MCP) that exclusively target the effects of endogenous and exogenous ethylene.

The aim of this study was to assess the impact of a single solid NO donor compound, 2,2'-(hydroxynitrosohydrazino)-bisethanamine, (DETA/NO) on the postharvest life of selected native cut flowers. Seven flower species (paper daisy, ptilotus, kangaroo paw, isopogon, grevillea, Geraldton wax and waratah) were investigated. Of these, four (ptilotus, kangaroo paw, grevillea and waratah) responded to pulse treatment with mid range concentrations (10–1000 ppm) of DETA/NO. In three of these cases (ptilotus, kangaroo paw and waratah), the extension in postharvest life was greater than that observed for the current industry standard treatment, STS.

Most significantly, the DETA/NO treatment produced changes in the senescence pattern of each flower species that was not observed in the corresponding STS treatment. This included reductions in the occurrence of stem wilt and flower head wilt (in the case of ptilotis and kangaroo paw), and decreased incidence of mould growth in the case of waratah.

The results from this brief study suggest that DETA/NO is effective in counteracting some of the undesirable postharvest characteristics that afflict certain species of native cut flowers. However, a more extensive long-term study is required to gain a comprehensive understanding of the actions of NO donor compounds on Australian native flowers.

1. Introduction

Postharvest senescence is a major limitation to the marketing of many species of cut flowers and considerable effort has been devoted to developing postharvest treatments to extend the marketing period. Silver ion, applied as STS, is in widespread use to delay senescence in ethylene-sensitive cut flowers. Silver reduces ethylene-binding capacity and suppresses endogenous ethylene production (Van Doorn *et al.*, 1991) thereby delaying the appearance of characteristics such as premature wilting, petal inrolling and abscission of flowers and buds (Nichols, 1966; Wu *et al.*, 1991). However, concerns have been raised over the use of silver as it is a heavy metal salt and environmental toxin and many countries are actively working towards its elimination from commercial use (Nell, 1992; Serek *et al.*, 1995). 1-MCP has been shown to prevent the action of ethylene through competitive inhibition. 1-MCP has been demonstrated to extend the storage life of a range of cut flowers and potted flowering plants (Sisler *et al.*, 1994; Serek *et al.*, 1994; 1995; Porat *et al.*, 1995). Since 1-MCP is considered non-toxic to humans, studies have extended to fruit and vegetables where it has also been successful in extending shelf life (Abdi *et al.*, 1998; Ku and Wills, 1999; Porat *et al.*, 1999; Fan *et al.*, 1999; Wills and Ku, 2002). 1-MCP has been approved for use with flowers in various countries and is seen as an environmentally acceptable alternative to STS.

Interest in the use of nitric oxide gas (NO) as a means of extending the postharvest life of horticultural commodities is of recent origin. NO was first characterised in plants in 1996 (Leshem *et al.*, 1996). Subsequent investigations have linked its occurrence to a range of physiological processes including modulation of endogenous ethylene and vegetative stress (Leshem *et al.*, 2000), water loss (Ku *et al.*, 2000), plant immunity (Hausladen *et al.*, 1998), anthocyanin biosynthesis and chlorophyll production (Giba et al, 1998; Laxalt *et al.*, 1998), root growth and fruit and flower formation (Lamattina, 2001). Postharvest application of NO has been shown to be effective in extending the shelf life of a range of flowers, fruits and vegetables when applied as a short term fumigation treatment at low concentrations (Wills and Leshem, 1998; Leshem *et al.*, 1998b; Wills *et al.*, 2000). While fumigation with NO and 1-MCP represent competitive alternatives to STS, the gaseous nature of both materials is a barrier to their use as a commercial treatment because of the need to develop and construct the infrastructure to undertake large scale fumigation. A measure of technical operational expertise is also required in the case of NO, which is a highly reactive biologically active toxin. Efficient usage is particularly problematical when the site of production is geographically isolated from key European and North American markets, as is the case when the farms are located in developing countries.

The role of NO as a key regulator in mammalian physiology has been the major force driving advances in nitric oxide research in recent years (Feldman *et al.*, 1993; Keefer, 1998). Of particular interest is the emergence of NO donor technology, that is, solid materials that store NO chemically but allow it to be regenerated in a controlled manner under appropriate physical conditions. Several subclasses of NO donor compounds based on the nature of the attachment of the NO releasing moiety have been developed, providing materials that possess a range of chemical and physical properties (Hou *et al.*, 1999). Of these, the diazeniumdiolates (a class in which the NO moiety is bound to the nitrogen atom of a secondary polyamine unit) are of particular interest because of favourable characteristics that include high water solubility, chemical stability and ease of synthesis (Hrabie *et al.*, 2002).

While diazeniumdiolates have been used extensively for investigations targeting the role of nitric oxide in mammalian physiology, they have been used in only one plant based study (Noritake *et al.*, 1996), where the role of nitric oxide in biotic stress was investigated. Application of 2,2'-(hydroxynitrosohydrazino)-bisethanamine (DETA/NO) led to an accumulation of the phytoalexin rishitin, an endogenous antibiotic. The result strongly suggests a key role for NO in the stimulation of the defence mechanism in plants.

Other NO donors such as N-*t*-butyl-α-phenylnitrone (PBN) and 3-morpholinosyl-nonimine (Sin-1) have been employed to investigate the mechanistic of nitric oxide in plant senescence. Leshem *et al.* (1998) showed that carnations (*Dianthus caryophyllus* L. cv. White-Sim) immersed in solutions containing low concentrations of these materials in the presence of the ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC) significantly reduced endogenous ethylene output, suggesting that NO inhibits in the ACC → ethylene step in the production cycle. However, the experimental design failed to conclusively identify the mechanism of action, that is, whether the experimental observations stemmed from exposure of the flower to gaseous NO escaping the surface of the liquid or were the result of *in vivo* NO release.

Studies undertaken at the University of Newcastle, Ourimbah show that fumigation with NO gas inhibits senescence in selected fruits and vegetables (Wills *et al.*, 2000). Preliminary studies on exotic flower species have been undertaken to assess the ability of NO donors to counteract the effects of exogenous ethylene. Results to date have proved encouraging, with ethylene sensitive species such as carnations displaying extensions in postharvest life similar to those obtained using STS.

1.1 Objectives

The geographic isolation of Australia from the flower markets of Europe and North America requires the use of efficient postharvest technology to ensure the maintenance of high quality in the flowers, on arrival at the market place. To maintain an international competitive advantage, Australia must remain an active research program to explore, develop and implement effective and environmentally sustainable postharvest technologies.

This project was a short six month study that aimed to conduct a quick evaluation to determine if the use of NO through donor technology had the potential to extend the postharvest life of native cut flowers, and thus offer an environmentally friendly alternative to silver based treatments.

The twin experimental objectives of this project were to:

- Assess the ability of the NO releasing solid DETA/NO to extend the postharvest life of seven types of Australian native cut flowers, and
- Benchmark the performance of DETA/NO against the current protocol for STS.

2. Methods and Results

2.1 Materials and Methods

Seven species of native flowers were evaluated - paper daisy (*Bracteantha bracteata*), ptilotus (*Ptilotus exattatus* cv Abell Star), grevillea (*Grevillea sp.* cv Majestica), isopogan (*Isopogon latifolius*), kangaroo paw (*Anigozanthos flavidus*), Geraldton wax (*Chamelaucium uncinatum*), waratah (*Telopea speciosissima*) and backhousia (*Backhousia citriodora*). Flowers were obtained from the Flemington Flower Markets and delivered by courier (under refrigeration) to Erina Heights (~90 km) where they were stored in water at room temperature for approximately 1 hr prior to being transported by car to the University laboratories (~10 km). Flower stems were immersed in tap water at 20°C until treated. All flower stems were re-cut to a uniform length of approximately 22 cm immediately prior to treatment. Flowers for each treatment unit were placed in an Erylenmeyer flask containing 250 ml of a treatment solution and stored in air at 20°C for 24 hr. Control flowers were placed in distilled water for the same period.

Treatment solutions consisted of:

- DETA/NO, which was manufactured in the laboratory according to the methods of Hrabie *et al.* (1993) Solutions were prepared to contain DETA/NO at 10, 100 and 1000 parts per million (ppm) w/v in distilled water. These concentrations were chosen as previous investigations with exotic species of cut flower showed concentrations ranging between 10 and 1000 ppm gave good extensions in postharvest life.

- STS, with a solution prepared in accordance with commercial practices. This involved dissolving Solution A, sodium thiosulphate, (0.25 g) in 5 ml distilled water, Solution B, silver nitrate, (0.0425 g) in 5 ml distilled water then adding 4 ml of each solution into 992 ml distilled water.

A randomised block design was employed for all experiments. Two to three replicates were generally carried out for each flower with each replicate comprising three treatment units of three flowers per unit. Following treatment, flowers were placed in individually numbered specimen containers filled with water (100 ml), then transferred to sealed 20 litre styrofoam containers connected to a positive pressure ventilation system circulating humidified air (75% R.H.) containing ethylene (0.1 ppm) at a flow rate of 20 L hr^{-1} to simulate a commercial storage environment.

Monitoring of ethylene concentration in the storage environment was undertaken using a flame ionisation gas chromatograph (Gow-Mac Model 580, Bound Brook N.J.) equipped with a stainless steel column (1.8 m x 3mm i.d.) packed with activated alumina (80-100 mesh). Detector, column, and injector temperatures were 110, 90 and 70°C respectively, and gas flow rates were 30, 30, and 300(ml min^{-1} for N_2, H_2 and air, respectively.

Treatments were periodically removed from the containers, randomised, subjectively ranked, scored, re-randomised then returned to the containers. Ranking was conducted using a 1 to 5 scale to indicate flower quality. Assessment criteria for the flowers examined were:

- Paper daisy, ptilotus, kangaroo paw and isopogon: **5,** no deterioration; **4,** sight wilting or discolouration or mould growth; **3,** moderate wilting / discolouration / mould growth; **2,** severe wilting / discolouration / mould growth; **1,** very severe wilting / discolouration / mould growth.

- Grevillea, Geraldton wax and backhousia: **5,** no deterioration; **4,** 10% flower drop or slight discolouration or wilting; **3,** 30% flower drop and/or moderate discolouration / wilting;

2, 50% flower drop and/or serious discolouration / wilting; **1,** more than 50% flower drop and/or serious discolouration / wilting.

- Waratah: **5,** no deterioration; **4,** 10% flower wilting / drop /colour fading blue, <50% flower opening / slight wilting / fading blue / bract browning; **3,** 30% flower wilting / drop/ fading blue, all flower opened, bracts slight mould, moderate wilting / fading blue / brown; **2,** 50% flower wilting / drop / fading blue, bracts moderate mould, severe wilting /fading blue / brown; **1,** >70% flower wilting / drop / fading blue, severe mould, bracts very severe wilting / fading blue / brown.

Regression analysis was performed on the data generated by individual flowers to calculate the time taken for flower quality to diminish to a level considered to be the minimum acceptability for retail sale (score = 3.0). Experiments were continued until all flowers in the replicate had deteriorated to an unacceptable level. Statistical procedures for storage life values were performed using SPSS version 10.0 (Chicago IL). Least significant differences (LSD) at P=0.05 were calculated to compare differences between means, and where appropriate, regression equations were fitted.

In preliminary experiments, the water uptake of individual flower species was determined. Measurements were conducted on a 24 hour cycle of the loss of weight of the solution and container, which equates to the uptake of solution by the flower. The transpiration rate of the flower was calculated by difference of the solution uptake and the weight of the flower. The experiments were conducted in air at 20°C and 60% RH.

2.2 Effect of Nitric Oxide on Water Uptake and Transpiration

The water uptake by flowers was determined to provide an estimate of DETA/NO uptake by individual flower species, thereby allowing a means of comparing across species and between individuals with respect to changes in flower quality.

The data in Table 1 shows the water uptake and transpiration data for each flower species when stems were placed in a solution of DETA/NO or STS for 24 hr. Water uptake varied significantly between species with species native to more temperate climates such as waratah having a higher solution uptake than those native to drier regions such as kangaroo paw, isopogon and ptilotus. There was, however, no significant change in water uptake by individual flower species due to DETA/NO or STS.

Transpiration rates showed a similar pattern and order, with significance differences between flower species with waratah being the highest and ptilotis the lowest, but no differences in any flower species due to DETA/NO or STS. In all cases, the rates of solution uptake and transpiration were similar, indicating that the flowers remained fully hydrated for the duration of the experiment.

Table 1. Rate of water uptake and transpiration of native cut flowers during exposure to DETA/NO and STS for 24 hr.

Flower	Rate of water uptake (g/hr/flower)					
	Control	DETA/NO			STS	
	(H_2O)	10 ppm	100 ppm	1000 ppm		Mean
Ptilotus	0.06	0.06	0.05	0.06	0.06	0.06f
Grevillea	0.34	0.27	0.27	0.22	0.25	0.27c
Geraldton wax*	0.46	0.44	0.43	0.32	0.31	0.39b
Isopogon	0.17	0.18	0.18	0.14	0.16	0.17e
Kangaroo paw	0.18	0.25	0.22	0.2	0.2	0.21d
Waratah	*0.67*	0.66	0.66	0.66	0.64	0.66a
Backhousia*	0.26	0.23	0.25	0.25	0.22	0.24c
Mean	0.31	0.30	0.29	0.26	0.26	
LSD 5%	*±0.08*	*±0.12*	*±0.10*	*±0.09*	*±0.10*	*±0.04*
	Transpiration rate (g/hr/flower)					
Ptilotus	0.06	0.06	0.05	0.06	0.06	0.06f
Grevillea	0.37	0.30	0.32	0.28	0.30	0.31c
Geraldton wax*	0.41	0.40	0.39	0.32	0.31	0.37b
Isopogon	0.17	0.18	0.19	0.15	0.18	0.17e
Kangaroo paw	0.16	0.22	0.2	0.19	0.18	0.19e
Waratah	0.63	0.65	0.63	0.66	0.62	0.64a
Backhousia*	0.26	0.23	0.24	0.25	0.22	0.24d
Mean	0.29	0.29	0.29	0.27	0.27	
LSD 5%	*±0.07*	*±0.12*	*±0.10*	*±0.09*	*±0.10*	*±0.03*

* Water uptake and transpiration of Geraldton wax and backhousia is g/hr/100flowers
Each value is the mean of two or three replicates.
Values in a column followed by different character are significantly different at P=0.05.

Table 2. Rate of water uptake and transpiration of flowers during post-treatment storage.

Flower species	Water uptake (g/hr/flower)					
	day 1	day 2	day 3	day 4	day 5	day 6
Ptilotus	0.05	0.04	0.04	0.03	0.02	0.02
Grevillea	0.27	0.19	0.14	0.08	0.06	0.05
Geraldton wax	0.21	0.43	0.18	0.19	0.10	0.09
Isopogon	0.14	0.21	0.12	0.13	0.11	
Kangaroo paw	0.20	0.25	0.31	0.27	0.34	0.29
Waratah	0.84	0.65	0.39	0.42	0.57	0.35
Backhousia	0.22	0.24	0.14	0.21	0.23	0.14
	Transpiration rate (g/hr/flower)					
Ptilotus	0.05	0.04	0.05	0.04	0.03	0.03
Grevillea	0.34	0.26	0.18	0.17	0.13	0.11
Geraldton wax	0.22	0.48	0.21	0.24	0.12	0.13
Isopogon	0.14	0.21	0.12	0.13	0.11	
Kangaroo paw	0.20	0.25	0.31	0.28	0.35	0.29
Waratah	0.66	0.68	0.42	0.49	0.67	0.41
Backhousia	0.22	0.24	0.15	0.22	0.24	0.14

The rates of water uptake and transpiration for each flower species was measured during the six days immediately following the 24 hr treatment phase. Both the rate of water uptake and transpiration rate were found to diminish with time but there was no significant difference between the rates for any treatment on a flower species. Since there was no effect of treatment, Table 2 presents the time course change in values for rates of water uptake and transpiration averaged across all flower species.

2.3 Effect of Nitric Oxide on Postharvest Life

The major investigation of the project was to examine the effect on postharvest life of seven native cut flowers of a 24 hr exposure of stems to aqueous solutions of 10, 100 and 1000 ppm DETA/NO to compare the effect with that obtained with STS used according to current commercial protocol. The post-treatment atmosphere contained 0.1 ppm ethylene to simulate a commercial storage environment where many flowers are held in close proximity. The visual symptoms of senescence for cut flowers varied considerably and the characteristics for each species are summarised in Table 3.

Table 3. Major deterioration symptoms of native cut flowers at 20°C

Flower	Stem wilting and discolouration
Paper daisy	Stem wilting and discolouration
Ptilotus	Wilting
Grevillea	Flower dropping
Isopogon	Discolouration
Kangaroo paw	Mouldy, wilting & discolouration
Geraldton wax	Flower dropping
Waratah	Mouldy and discolouration

Table 4 Postharvest life of native cut flowers pulsed with DETA/NO and STS for 24 hr.

Flower	Postharvest life (days)					
	Control	DETA/NO (ppm)			STS	LSD 5%
		10	100	1000		
Ptilotus	5.3d	7.5a	6.7b	4.9d	5.9c	:±0.5
Grevillea	3.6c	3.8bc	4.0bc	4.3b	6.7a	:±0.7
Geraldton wax	7.6b	6.1b	5.5b	5.1b	15.9a	:±4.9
Isopogon	2.7	2.7	2.8	2.8	3.1	
Kangaroo paw	17.1c	21.6a b	22.9a	21.2a b	16.0c	:±5.2
Waratah	4.1c	4.2c	5.0a	4.5ab	4.7ab	:±0.5
Paper daisy	10.6	10.8	10.3	9.6	9.7	

Each value is the mean of two or three replicates.
Values in a row followed by a different character are significantly different at P=0.05.

The postharvest life of the flowers is given in Table 4. This shows that for four of the seven native species examined - ptilotus, grevillea, kangaroo paw and waratah, DETA/NO treatments generated a significant increase in postharvest life compared to the water only control flowers. In three of these cases, ptilotus, kangaroo paw and waratah, DETA/NO treatments yielded increases in postharvest life that were significantly greater than the STS control flowers.

Ptilotus: This species is known to be susceptible to stem collapse soon after harvest. Treatment with 10 and 100 ppm DETA/NO proved effective in delaying this symptom and thereby extended postharvest life by about 40% and 25%, respectively, relative to the water control. DETA/NO was more effective than STS, which increased postharvest life by about 10%. The flower head itself

showed little evidence of sensitivity to ethylene. The appearance of ptilotus flowers after 4 days is shown in Figure 1.

Grevillea: This cultivar of grevillea showed considerable sensitivity towards ethylene, exhibiting high levels of flower drop soon after exposure to ethylene. STS was found to be a highly effective treatment for counteracting this symptom and produced about 90% extension in postharvest life relative to the water control. Treatment with DETA/NO did show some benefit with exposure at 1000 ppm proving the most effective concentration with an extension of about 30% in postharvest life.

Waratah: Waratah flowers treated with DETA/NO showed reductions in mould growth and exhibited good levels of colour retention. The most effective treatment was with 100 ppm DETA/NO where a 22% extension in postharvest life was achieved relative to the water control. Treatment with STS extended the postharvest life by 14%.

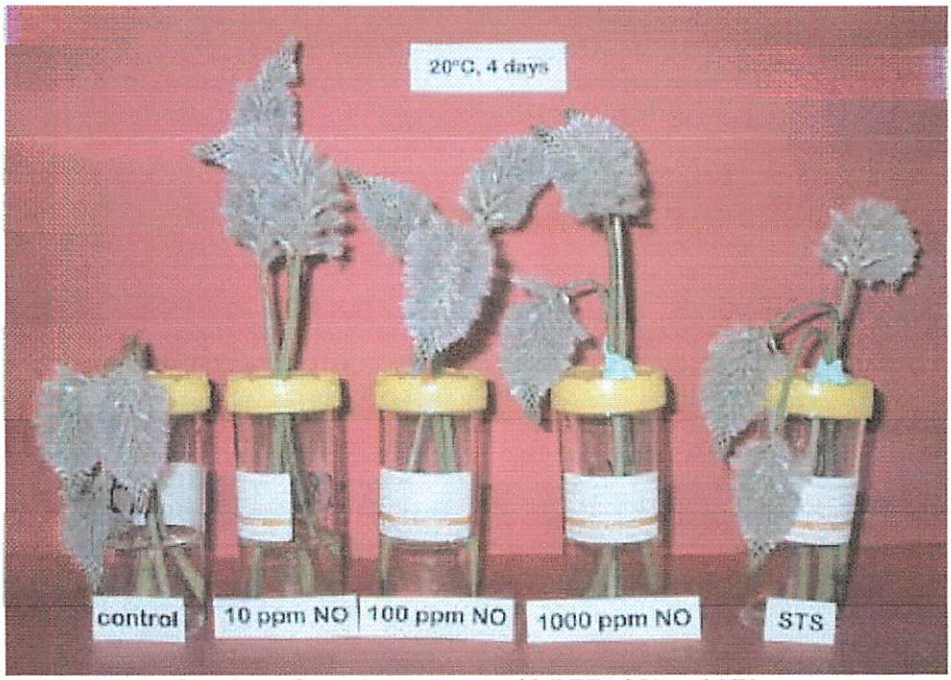

Figure 1. Ptilotus four days after pulse treatment with DETA/NO and STS.

Kangaroo Paw: Like ptilotis stems, the flower head of the kangaroo paw is known to be susceptible to collapse soon after harvest. Treatment with 10 and 100 ppm DETA/NO proved effective in delaying this symptom, leading to extensions in postharvest life of about 34% and 26% respectively, relative to the water control. DETA/NO was found to be more effective than STS, which increased postharvest life by only 10%.

Geraldton wax: The rapid dropping of flowers during storage was not affected by treatment with DETA/NO. Although the postharvest life of the flowers treated with STS, the recognized industry protocol, appeared to be much greater than the other treatments, the result was not statistically significant because of considerable variation in the flower quality between individual replicates. Further replicates could not be conducted due to a lack of supply during the short duration of the project.

Paper daisy and isopogon showed no significant effect on postharvest life when treated with DETA/NO or STS.

3. Conclusions & Recommendations

A consequence of the brief nature of this six month study is that it was not possible to fully evaluate DETA/NO as a treatment for extending the postharvest life of native cut flowers. Only those flowers in season during the six months could be evaluated and even for these only a limited number of varieties and sources of supply could be assessed. Notwithstanding these limitations, the following conclusions can be drawn from the study:

- The use of DETA/NO appears not to be a universal treatment applicable to all native cut flowers as not all the species examined showed a significant extension in postharvest life. This is in contrast to the greater effect seen with exotic flowers.

- Use of DETA/NO on ptilotus would appear to be quite promising as a 40% increase in postharvest life was achieved with the relatively low DETA/NO concentration of 10 ppm and the treatment was much more effective than STS.

- Use of DETA/NO on waratah would also seem to be worthy of further study as the flowers examined in this study achieved a 22% increase in postharvest life and was more effective than STS.

- Use of DETA/NO on kangaroo paw seems to be worthy of further study as the flowers examined in this study achieved a 34% increase in postharvest life and was more effective than STS.

- For Geraldton wax, STS appears to be the preferred treatment as it achieved a 100% increase in postharvest life compared to no significant effect by DETA/NO. However, the Geraldton wax used in the study was obtained from Western Australia and the flowers were not in of high quality on arrival at the laboratory.

- For grevillea, STS would appear to also be the preferred treatment as it achieved a 90% increase in postharvest life compared to 30% by DETA/NO. However, only one type of grevillea was evaluated and it is recognised that there is considerable variation in flower and plant types within the species.

- Paper daisy and isopogon appear to be unreceptive to both DETA/NO and STS.

An effective action of DETA/NO was to inhibit drooping of flower heads as seen with ptilotus and kangaroo paw. The absence of measurable differences in either transpiration or water uptake rates in these species suggests that the phenomenon is not directly related to total water retention in the flower. It may, however, relate to water distribution within the flower. It may be a stress-induced response that is countered through nitric oxide metabolism as found in other plant tissues (García Mata, 2003, Corpas, 2001). Other native flowers with a similar postharvest problem would appear worthy of study.

In addition, the reduction in mould growth shown by waratah is an effect that is not normally associated with ethylene action. McElhaney-Feser (1998) suggested that the diazeniumdiolates have antimicrobial properties although no formal study of the microbiological profile of plants treated with such solutions has been undertaken.

It is concluded that further research is required to substantiate the outcomes of this short study. It is recommended that comprehensive trials incorporating a wider range of species and types within species be conducted to fully discriminate the actions of NO donor technology. Initial studies could be on ptilotus, waratah and grevillea. The effect of DETA/NO on flowers susceptible to stem wilting

or mould growth would also appear to be worthy of investigation. The likely differential action of STS and NO raise the possibility that DETA/NO may be useful as a synergistic co-treatment with STS.

4. References

Abdi, N., McGlasson, W.B., Holford, P., William, M., 1998. Responses of climacteric and suppressed-climacteric plums to treatment with propylene and 1-methylcyclopropene. Postharvest Biol. Technol. 14, 29–39.

Beynon, R.J., Easterby, J.S., 1996. Buffer Solutions: The Basics, BIOS Scientific Publishers, Oxford.

Çelikel, F.G., Dodge, L.L., Reid, M.S., 2002. Efficacy of 1-MCP (1-methylcyclopropene) and Promalin for extending the post-harvest life of Oriental lilies (*Lilium* × 'Mona Lisa' and 'Stargazer').

Corpas, F.J., Barroso, J.B., del Río, L.A., 2001, Peroxisomes as a source of reactive oxygen species and nitric oxide signal molecules in plant cells. Trends Plant Sci., 6, 145-150.

Davies, K.M., Wink, D.A., Saavedra, J.E., Keefer, L.K., 2001. Chemistry of diazeniumdiolates 2. Kinetics and mechanism of dissociation to nitric oxide in aqueous solution. J. Am. Chem. Soc. 123, 5473-5481.

Fan X., Blankenship, S.M., Mattheis, J.P., 1999. 1-Methylcyclopropene inhibits apple ripening. J. Amer. Soc. Hortic. Sci. 124, 690–695.

Feldman, P.L., Griffith, O.W., Stuehr, D.J., 1993. The surprising life of nitric oxide, Chem. Eng. News, 71, 26-38.

García Mata, C., Lamattina, L., 2003. Abscisic acid, nitric oxide and stomatal closure – is nitrate reductase one of the missing links? Trends Plant Sci., 8, 20-26,

García Mata, C., Lamattina, L., 2001. Nitric oxide induces stomatal closure and enhances the adaptive plant responses against drought stress. Plant Physiol., 126, 1196–1204.

Giba, Z., Grubisic, D., Todorovic, S., Sajc, L., Stojakovic, D., Konjevic, R., 1998. Effect of nitric oxide – releasing compounds on phytochrome – controlled germination of Empress tree seeds. Plant Growth Regul., 26, 175–181.

Hausladen, A., Stamler, J.S., 1998. Nitric oxide in plant immunity. Proc. Natl. Acad. Sci. USA, 95, 10345-10347.

Hrabie, J.A., Klose, J.R., Wink, D.A., Keefer, L.K., 1993. New nitric oxide-releasing zwitterions derived from polyamines. J. Org. Chem., 58, 1472-1476.

Hrabie, J.A., Keefer, L.K., 2002. Chemistry of nitric oxide-releasing diazeniumdiolate ("nitrosohydroxylamine") functional group and its Oxygen substituted derivatives. Chem. Rev., 102, 1135-1154 and references therein.

Hou, Y.C., Janczuk, A., Wang, P.G., 1999. Current trends in the development of nitric oxide donors. Curr. Pharm. Des., 5, 417-441.

Keefer, L. K., 1998. Nitric oxide-releasing compounds: from basic research to promising drugs. Chemtech, 28, 30–35.

Ku, V.V.V., Wills, R.B.H., 1999. Effect of 1-Methylcyclopropene on the storage life of broccoli. Postharvest. Biol. Technol. 17, 127–132.

Ku, V.V.V., Wills, R.B.H., Leshem, Y.Y., 2000. Use of nitric oxide to reduce postharvest water loss from horticultural produce. J. Hort. Sci. Biotech., 75, 268-270.

Lamattina, L., Beligni, G.L., Garcia Mata, C., Laxalt, A.M., 2001, US Patent 6,242,384 B1.

Laxalt, A.M., Beligni, M.V. Lamattina, L., 1997. Nitric oxide preserves the level of chlorophyll in potato leaves infected by *Phytophthora infestans*. Eur. J. Plant Pathol., 103, 643–651.

Leshem, Y.Y., Haramaty, E., 1996. The characterisation and contrasting effects of the nitric oxide free radical in vegetable stress and senescence of *Pisum sativum* Linn. foliage. J. Plant Physiol., 148, 258-263.

Leshem, Y.Y., Wills, R.B.H., 1998. Harnessing senescence delaying gases nitric oxide and nitrous oxide: a novel approach to postharvest control of fresh horticultural produce. Biol. Plant. 41, 1-10.

Leshem, Y.Y., Wills, R.B.H., Ku, V.V.V., 1998. Evidence for the function of the free radical gas - nitric oxide (NO•) - as an endogenous maturation and senescence regulating factor in higher plants. Plant Physiol. Biochem. 36, 825-855.

Leshem, Y.Y., 2000. *Nitric oxide in plants; occurrence, function and use*. Kluwer Academic Publishers, Dordrecht, p13.

Leshem, Y.Y., Pinchasov, Y., 2000. Non-invasive photoacoustic spectroscopic determination of relative endogenous nitric oxide and ethylene content stoichiometry during the ripeneing of strawberries *Fragaria anannasa* (Dutch.) and avocados *Persea americana* (Mill.). J. Expt. Bot. 51, 1471-1473.

McElhaney-Feser, Gail E.; Raulli, Robert E.; Cihlar, Ronald L, 1998. Synergy of nitric oxide and azoles against Candida species *in vitro*. Antimicrobial Agents and Chemotherapy, 42, 2342-2346.

Nell, T.A. 1992. Taking silver safely out of the longevity picture. Growertalks, 6, 35-38.

Nichols, R. 1966. Ethylene Production During Senescence of Flowers. J. Hort. Sci., 41, 279-290.

Noritake, T., Kawakita, K. Doke, N., 1996. Nitric oxide induces phytoalexin accumulation in potato tuber tissues. Plant Cell Physiol. 37, 113-116.

Pfeiffer, S., Mayer, B., Hemmens, B., 1999. Nitric oxide: Chemical puzzles posed by a biological messenger. Angew. Chem. Int. Ed., 38, 1714-1731.

Porat, R., Shlomo, E., Serek, M., Sisler, E.C., Borochov, A., 1995. 1-Methylcyclopropene inhibits ethylene action in cut phlox flowers. Postharvest Biol. Technol. 6, 313-319.

Porat, R., Weiss, B., Cohen, L., Daus, A., Goren, R., Droby, S., 1999. Effects of ethylene and 1-methylcyclopropene on the postharvest qualities of 'Shamouti' oranges. Postharvest Biol. Technol. 15, 155–163.

Serek, M., Sisler, E. C., Tamarini, G., Borochov, A., 1995. Inhibition of ethylene-induced cellular senescence symptoms by 1-methycyclopropene. Acta. Horticulturae, 405, 264-268.

Serek, M., Sisler, E.C., Reid, M.S., 1994. Novel gaseous ethylene binding inhibitor prevents ethylene effects in potted flowering plants. J. Amer. Soc. Hortic. Sci. 119, 1230 – 1233.

Serek M., Sisler E.C., Reid M.S., 1995. 1-Methylcyclopropene, a novel gaseous inhibitor of ethylene action, improves the life of fruits, cut flowers and potted plants. Acta Hortic. 394, 337– 45.

Snyder, M.S. 1992. Nitric oxide: first in a new class of neurotransmitters. Science, 257, 494-496.

Van Doorn, W.G., Wothering, E.J., 1991. Developments in use of growth regulators for the maintenance of postharvest quality in cut flowers and potted plants. Acta. Horti., 298, 195-208.

Wills, R.B.H., Ku, V.V.V., 2002. Use of 1-MCP to extend the time to ripen of green tomatoes and postharvest life of ripe tomatoes. Postharvest Biol. Technol. 26, 85-90.

Wills, R.B.H., Leshem, Y.Y., 1998. Method for reducing the rate of deterioration of perishable horticultural produce. Australian Patent No. 738169.

Wills, R.B.H., Ku. V.V.V., Leshem, Y.Y., 2000. Fumigation with nitric oxide to extend the postharvest life of strawberries. Postharv. Biol. Technol. 18, 75-9.

Wu, M.J., Zacarias, L., Reid, M.S., 1991. Variations in the senescence of carnation (*Dianthus caryophyllus* L.) cultivars II. Comparisons of sensitivity to exogenous ethylene and of ethylene binding. Scientia Hort., 48, 109-116.