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Australian Journal of Experimental Agriculture

Volume 39, 1999

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Involvement of ethylene in postharvest senescence of *Boronia heterophylla* flowers

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Summary. Treatment of cut flowering *Boronia heterophylla* (red boronia) stems with 10 L ethylene/L for 72 h at 20°C induced flower senescence and abscission, and thereby reduced stem fresh weight and vase life. Pre-treatment with 1-methylcyclopropene (1-MCP) reduced these ethylene effects. Treatment of *B. heterophylla* with 10 L ethylene/L for a shorter 12 h period at 20°C did not affect vase life. Rates of endogenous ethylene production by *B. heterophylla* flowers increased in association with wilting during flower senescence.

Introduction

Cut boronia flowers are a native Australian product traded internationally (Joyce *et al*. 1993). The vase life of *Boronia heterophylla* (red boronia) can be shortened when exposed to ethylene, whereupon loss in stem fresh weight (FW) and flower wilting are accelerated. Treatment with silver thiosulfate (STS) can prevent these ethylene-related effects (Joyce and Haynes 1989). Willamson (1999) showed that STS treatment extended the vase life of *B. heterophylla* stems not exposed to ethylene. A new ethylene binding inhibitor, 1-methylcyclopropene (1-MCP) gas, can also protect cut flowers against ethylene (Serek *et al*. 1995; Sisler *et al*. 1996). In this study, relationships between *B. heterophylla* flower senescence, exogenous and endogenous ethylene, and STS and 1-MCP treatments were examined.

Materials and methods

Flowering *B. heterophylla* stems were harvested from farms in Western Australia and air freighted dry to the University of Queensland postharvest laboratory within 48 h. Stem ends were recut under deionised water to 20–30 cm length by removal of about 2 cm of stem. They were stood for 3–6 h in deionised water to fully rehydrate. Stem ends were again recut and assigned to individual vases containing 10 mg/L available chlorine [dichloroisocyanurate (DICA)] in deionised water.

Flowering stems in their vases were enclosed in glass chambers (60 L volume) and pre-treated on day 0 with 0 or 10 nL 1-MCP/L for 12 h at 20°C. Half of the stems from each of these treatments were then exposed on day 1 to 10 L ethylene/L for 12 h at 20°C. In a second experiment, stems were pre-treated on day 0 with 0 or 10 nL 1-MCP/L or pulsed with STS (0.5 mmol Ag^+/L) for 12 h at 20°C. The ethylene treatment that followed was extended to 72 h (cf. experiment 1).

1-MCP was synthesised and quantified according to Sisler and Serek (1997), with the exception that lithium diisopropylamide was substituted for phenyllithium. Ethylene gas for treatment was diluted from a pressurised cylinder of pure ethylene. 1-MCP and ethylene were quantified on separate Shimadzu GC-8AIT gas chromatographs fitted with flame ionisation detectors. Detector temperatures were 50°C for 1-MCP and 120°C for ethylene. 1-MCP was separated at 40°C in a 1.22 m long by 3.2 mm internal diameter stainless steel column packed with Chromosorb P-AW mesh range 80/100. Ethylene was separated at 90°C in a 0.9 m long by 3.5 mm internal diameter glass column packed with activated alumina mesh range 80/100. STS was prepared according to Reid *et al*. (1980).

Flowers were weighed daily to allow calculation of relative fresh weight as a percentage of initial day 0 fresh weight. Flower wilting was assessed using the following scale: 1, none–slight; 2, moderate; 3, advanced. Flower discolouration, observed as fading from bright pink to white, was assessed daily using the following rating scale: 1, 0–25%; 2, 26–50%; 3, 51–75%; 4, 76–100% discolouration over the multi-flowered stem. Vase life of flowering stems was judged as the time in days to loss of visual appeal; i.e. moderate flower wilting and/or 50% of flowers with > 50% flower discolouration. Ethylene production by *B. heterophylla* flowering stems and by individual flowers sealed in jars or test tubes, respectively, was also measured daily.

Vases were arranged in a completely randomised design with 8–10 replicate stems for each treatment. Treatment means are presented with standard errors and were analysed as 2 x 2 (experiment 1) or 3 x 2 (experiment 2) factorial ANOVAs using MINITAB (Release 11.12). The least significant difference (l.s.d.) test at $P = 0.05$ was used to separate means. Replication was 5-fold for ethylene production measurements.

 10.1071/EA99046

Table 1. Vase life of flowering *B. heterophylla* stems treated with 1-MCP or STS (Ag^+) for 12 h at 20°C on day 0 and then exposed to ethylene for 72 h at 20°C on day 1

Vase life values followed by different letters are significantly different at $P = 0.05$

Ethylene (L/L)	1-MCP (nL/L)	Ag^+ (mmol/L)	Vase life (days)
0	0	0	10.8 (± 0.9) b
0	10	0	10.0 (± 0.7) b
0	0	0.5	9.1 (± 0.2) a
10	0	0	8.2 (± 0.4) a
10	10	0	10.4 (± 0.5) b
10	0	0.5	9.7 (± 0.5) a

Results and discussion

Exposure of *B. heterophylla* stems to 10 L ethylene/L for 12 h at 20°C did not significantly ($P>0.05$) reduce vase life. Thus, flower wilting and discolouration and stem fresh weight were not affected by this ethylene treatment protocol (data not presented). Also, 1-MCP had no effect on postharvest quality (data not presented). However, increasing the duration of ethylene treatment to 72 h reduced vase life (Table 1) by inducing flower and leaf abscission (>10% of total number of flowers and leaves) and wilting (Fig. 1*e*). These effects are reflected in accelerated loss of stem fresh weight (Fig. 1*f*). Joyce and Haynes (1989) found that treatment of *B. heterophylla* with 10 L ethylene/L for 72 h at 22°C induced rapid loss of stem fresh weight and flower wilting. However, flower abscission was not observed in that study.

The 1-MCP pre-treatment protocol was more effective than the STS pre-treatment protocol in preventing exogenous ethylene-mediated flower wilting, loss of stem fresh weight (Fig. 1*e*, *f*) and vase life reduction (Table 1). Nonetheless, stems pre-treated with STS lost less fresh weight (Fig. 1*f*) and had slightly longer vase lives (Table 1) than stems exposed only to ethylene. Silver toxicity may have limited vase life as evidenced by premature flower wilting for the STS pre-treatment (Fig. 1*b*, *e*). Based on solution volume uptake measured for STS pulsing, 0.25 ± 0.01 mol Ag^+/g stem fresh weight ($n = 20$) was accumulated by stems. A safe, effective range for Ag^+ uptake by waxflower (*Chamelaucium uncinatum*) is 0.1–0.6 mol Ag^+/g stem fresh weight (Joyce 1988). However, 0.35 mol Ag^+/g stem fresh weight was found to be toxic for *C. uncinatum* 'Lollypop' (A. J. Macnish, D. C. Joyce, P. J. Hofman and D. H. Simons unpublished data). The effective treatment range may vary with genotype and/or phenotype. A problem with STS pre-treatment is that the effective concentration is often close to the phytotoxic level (Cameron and Reid 1981). In contrast, 1-MCP is thought to be non-phytotoxic (Serek *et al.* 1994). Possibly as a result of silver toxicity, discolouration of flowers on STS pre-treated stems was delayed (Fig. 1*a*, *d*). 1-MCP pre-treatment did not affect flower discolouration (Fig. 1*a*, *d*).

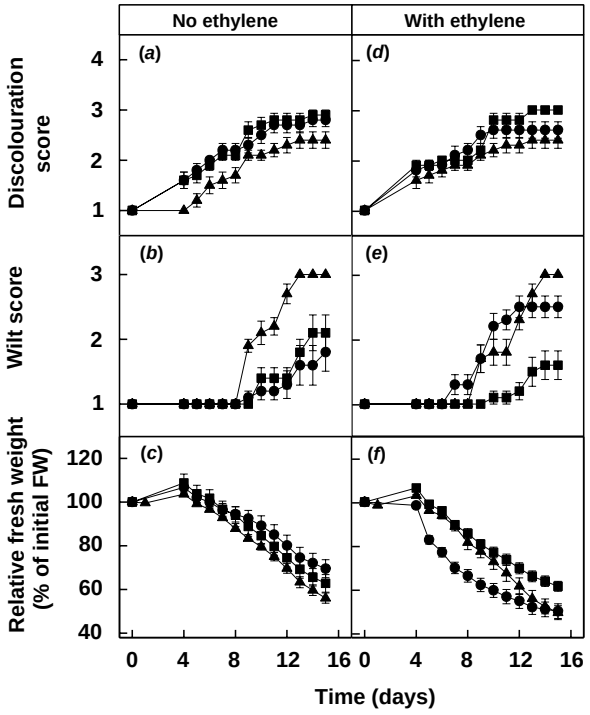

Figure 1. Flower discolouration (*a*, *d*), wilting (*b*, *e*) and stem relative fresh weight (*c*, *f*) for flowering *B. heterophylla* stems not treated (control) (●), treated with 10 nL 1-MCP/L (■), or pulsed with STS (0.5 mmol Ag^+/L) (▲) for 12 h at 20°C on day 0. Half of the stems from each treatment were then exposed to 10 L ethylene/L for 72 h at 20°C on day 1. Vertical bars show the standard errors of means ($n = 10$). The l.s.d. ($P = 0.05$) for flower discolouration is 1.1 days when analysed as the time to a score of 2. Factorial level significance for flower wilting is 1-MCP x ethylene, $P = 0.011$. The l.s.d. ($P = 0.05$) for relative fresh weight is 5.6% at day 5.

The observation that STS pre-treatment has been shown in another study (Williamson 1999) to extend the vase lives of *B. heterophylla* flowering stems not treated with ethylene provides correlative evidence that endogenous ethylene is involved in flower senescence. This proposition is supported in the present study as ethylene production measured for isolated *B. heterophylla* flowers was correlated with their

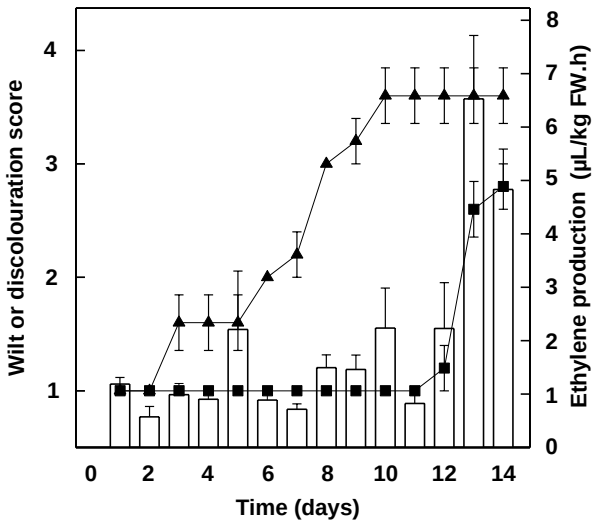

Figure 2. Flower wilting (■), discolouration (▲) and ethylene production (open bars) by individual *B. heterophylla* flowers at 20°C. Vertical lines indicate the standard error of mean (n = 5).

senescence. Ethylene production increased in association with wilting at advanced stages of discolouration (Fig. 2). Thus, ethylene production by flowers may increase in response to a water-deficit stress. Elevated ethylene production by *C. uncinatum* has been related to post-harvest water stress (Joyce 1993). Ethylene production by whole flowering *B. heterophylla* stems ranged from 1.4 to 3.1 L/kg FW.h during vase life. Kader (1992) classified rates of ethylene production by fresh produce between 1 and 10 L/kg FW.h as being 'moderate'.

Senescence and abscission of *B. heterophylla* flowers can be initiated and/or accelerated by exogenous ethylene, and rates of endogenous ethylene production increase in association with flower senescence. Pre-treatment with 1-MCP was effective in preventing senescence caused by exogenous ethylene.

Acknowledgments

Financial support from the Rural Industries Research and Development Corporation is gratefully acknowledged. The authors thank Jamie Creer and Philip Watkins for providing flowers, and Allan Lisle for advice on statistics.

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Received 31 March 1999, accepted 5 July 1999