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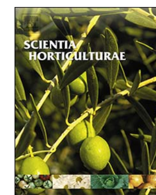

An investigation of ethylene sensitivity in three Australian native cut flower genera, *Calothamnus*, *Grevillea* and *Philotheca*

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ABSTRACT

Knowing a plant's sensitivity to ethylene is crucial in order to negate ethylene-mediated senescence with inhibitors such as silver thiosulphate (STS) or 1-methylcyclopropene (1-MCP). The ethylene sensitivity of three exported Australian native flowers, *Calothamnus quadrifidus*, *Grevillea* 'Superb' and *Philotheca myoporoides* was previously unknown. The first two of these three species produced steady amounts of ethylene during vase life. The vase life of all three species was not significantly shortened after exposing the flowers to ethylene (10 μL L^{-1} for 12 h). However, STS treatment (0.5 mM pulse for 12 h prior to ethylene exposure) significantly extended the vase life of *G.* 'Superb' from 7 d (control) to 10 d. In contrast, 1-MCP (10 nL L^{-1}), applied either at the same time as ethylene exposure, or 12 h prior to challenging plants with ethylene, did not result in significant differences in vase life over the control for any of the three species. Ethylene production was comparable between the STS and 1-MCP treatments for *G.* 'Superb' over the 11 d test period and was less than that of the control. Endogenous production of ethylene did not appear to relate to longevity or the effectiveness of the protectants. Our results indicate that the treatment of *C. quadrifidus* and *P. myoporoides* with ethylene inhibitors is unnecessary and is therefore not recommended. The positive effect of STS on *G.* 'Superb' was not linked to ethylene sensitivity, but may relate to another of the beneficial properties of STS, such as an ionic effect.

1. Introduction

The postharvest longevity of many cut flowers is limited by their sensitivity to ethylene. Not all flowers are ethylene-sensitive, but some that are, e.g. cultivars of rose and carnation, are among the most popular cut flowers in the world. If a plant is sensitive to ethylene, its vase life is markedly reduced, so much research has been directed towards inhibiting the effects of ethylene on cut flowers. Some flowers produce their own ethylene (endogenous), particularly as they senesce following pollination (Woltering and van Doorn 1988). In contrast, exogenous sources of ethylene are ubiquitous and include car exhaust fumes, cigarette smoke and gas heaters, so it is common practice to protect against ethylene with the use of protectants such as silver thiosulphate (STS) or 1-methylcyclopropene (1-MCP) after harvest and prior to export. STS is used as a liquid pulse, usually for up to 12 h, at concentrations between 0.2 and 0.5 mM. STS works because, when the Ag^{+} replaces the Cu^{+}, conformation of the primary receptor molecule is modified, blocking enzymatic action and preventing the ethylene biosynthesis pathway from proceeding (Beyer 1976; Veen 1983). 1-MCP is a gas and can be used as a pulse in an enclosed 'tent', usually for up to 24 h (Macnish et al., 2000a; Blankenship and Dole 2003). The plants are then protected against ethylene because 1-MCP has already bound to and blocked the receptor site (Serek et al., 1994).

There are several arguments regarding the relative merits of 1-MCP versus STS, including: (a) pollution: 1-MCP does not contain heavy metals as STS does; (b) transience: 1-MCP does not protect plants for as long as STS (Macnish et al., 2000a; Chamani et al., 2005; In et al., 2013); and (c) treatment time: 6–12 h is a common 1-MCP pulsing time for cut flowers (Blankenship and Dole 2003), but how long is really required? Clearly, the comparative benefits of STS and 1-MCP for particular cut flowers need clarification.

However, the STS/1-MCP decision is premature if the plants are not sensitive to ethylene. Therefore, ascertaining ethylene sensitivity is required for genera and species for which we have no information. Little is known about the ethylene sensitivity and response to ethylene inhibitors of many Australian native cut flowers, even those that are currently exported (Faragher et al., 2010).

Knowledge of ethylene sensitivity is important because vase life could be adversely affected if a non-sensitive flower is treated against ethylene. Furthermore, for those flowers that are found to be sensitive,

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commercial vase life could be extended if the shortest possible effective treatment, whether 1-MCP or STS, is used.

The aims of this research were therefore to: test the ethylene sensitivity of three Australian native cut flower species, *Calothamnus quadrifidus*, *Grevillea* 'Superb' and *Philotheca myoporoides*, that are currently exported as major (*Calothamnus*, *Grevillea*) or minor/filler (*Philotheca*) crops; ascertain whether they produce ethylene; and determine the effects of both 1-MCP (applied before or at the same time as ethylene) and STS on vase life.

2. Materials and methods

2.1. Plant material and vase life determination

Flowering stems 30 cm long of *Calothamnus quadrifidus* R.Br., *Grevillea* 'Superb' and *Philotheca myoporoides* (DC.) Bayly were harvested during spring in the early morning from the gardens of the Burnley Campus, The University of Melbourne. The stems were placed in plastic buckets containing distilled water and immediately brought to the laboratory. Stems were recut under distilled water, removing 10 cm from the base to reduce the likelihood of emboli in xylem conduits, and the lower leaves were removed so that none was below the water level, which could have stimulated microbial proliferation. Within one hour of harvest, stems were placed into individual glass vials containing distilled water and sealed with Parafilm. There were 10 replicates per treatment, arranged in a completely randomised blocks design. Experiments were repeated over two successive flowering seasons.

The stems were kept in a ventilated room at 20 ± 1 °C, 60 ± 10% relative humidity and 12 h Cool White fluorescent light of 20 μmol $m^{-2} s^{-1}$ photosynthetically active radiation at flower level. Stems and vials were weighed daily and assessed for signs of senescence. The end of vase life was reached when more than 50% of flowers on the stem had senesced (either via wilting, flower/petal drop, or change of colour).

2.2. Treatments and chemicals

Stems in individual vials were placed into gas-tight Perspex tubs sealed with water and randomly allocated to the following treatments: control; ethylene (10 μL L^{-1}) for 12 h; 1-MCP (10 nL L^{-1}) for 12 h then ethylene as above; 1-MCP and ethylene at the same time; or STS (0.5 mM for 12 h) then ethylene. The 1-MCP concentration was in accordance with Macnish et al. (2000a, 2000b), Setyadjit et al. (1997) and Serek et al. (1995). The STS concentration and time were as described by Joyce and Haynes (1989), Macnish et al. (2000a) and Williamson and Joyce (2013).

The tubs also contained potassium hydroxide (20% w/v) to absorb any respiratory build-up of carbon dioxide (Kang et al., 1967), which is a competitive inhibitor of ethylene (Burg and Burg 1965). Tubs were vented outside after enclosure treatments had ceased in order to avoid any cross-contamination between treatments in the vase life room. Just prior to use, 1-MCP was prepared and released into the gas-tight tubs according to the protocols from the manufacturer, AgroFresh.

2.3. Ethylene determination

In the vase life experiment, gas samples were taken after 24 h from all tubs, including an empty tub, to test for ethylene production by the plants or tubs (Faragher et al., 2010). A 20 mL gas sample was injected into 12 mL evacuated glass vials through a gas septum, the overpressure enabling ease of sampling and sampling checks on the gas chromatograph. Samples were analysed by gas chromatography (Shimadzu, Model GC-14B; temperature of Flame Ionisation Detector 200 °C; injector temperature 180 °C; internal oven temperature 50 °C; column Porapak P).

In an additional experiment, native ethylene production was determined on days 1, 3, 5 and 7 of vase life for all genera on a replicate set of plants (10 stems per treatment). For this, the lids were placed on the tubs every alternate 24 h; gas samples were then taken after enclosure for 24 h and analysed as described above. This experiment was required to avoid confounding the vase life experimental results because different humidity levels would have occurred every alternate 24 h when the plants were enclosed for ethylene determination.

In a third ethylene experiment, ethylene production of *G.* 'Superb' was determined every alternate 24 h (i.e. on days 1, 3, 5, 7, 9 and 11) following treatment with or without the ethylene protectants. This experiment was done because *G.* 'Superb' was the only plant that showed a response to STS (see Section 3.2 below). A set of replicate plants (10 stems per treatment) was used, where the effect of ethylene production after five treatments (including control, as described in 2.2 above) was assessed. Gas sampling occurred as described above.

2.4. Water status measurements

The relative fresh weight (RFW) (as a % of initial fresh weight, FW) was calculated daily as (FW/initial FW) × 100; the water content (WC) (g g^{-1} dry weight, DW) was also calculated daily as (FW − stem dry weight)/stem dry weight.

2.5. Statistical analysis

Analysis of variance was performed on the vase life data. Residuals were checked for normality and homogeneity of the variances and data were transformed as necessary. If *F*-values were significant, means were compared using Tukey's post-hoc test at the $p \ll 0.05$ level. The RFW and WC data were analysed for the effects of treatment and time using a linear mixed model (Patterson and Thompson 1971), which accounted for (i) the effects of repeated measures, i.e. data collected from the same stem over time were not independent; and (ii) design imbalance owing to missing values that accumulated progressively over time (i.e. plants that died were no longer measured). All statistical analyses were performed using Genstat 18.0 (VSN International, Hemel Hempstead).

3. Results

3.1. *C. quadrifidus*

Control plants of *C. quadrifidus* had a vase life of 7 d, which was not

Table 1
Vase life and senescence symptoms of *Calothamnus quadrifidus*.

Treatment	Vase life (d) ± SE	Range (d)	Senescence symptoms
Control	7^{a} ± 0.92	3–10	Petal wilting, colour change, abscission
Ethylene for 12 h	6.9^{a} ± 0.70	3–9	Petal wilting, colour change, abscission
1-MCP & then ethylene for 12 h	8^{a} ± 0.86	3–10	Petal wilting, colour change, abscission
Ethylene & 1-MCP at same time for 12 h	6.5^{a} ± 0.84	3–10	Petal wilting, colour change, abscission
STS & then ethylene for 12 h	6^{a} ± 0.56	3–8	Petal wilting, colour change, abscission

Vase life is the mean of 10 replicates; SE = standard error.
Numbers followed by the same letter are not significantly different from each other ($p < 0.05$).

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