



## Research note

## Effect of S-carvone on vase life parameters of selected cut flower and foliage species

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## ABSTRACT

Insufficient water uptake by cut flowers and foliage species is often due to stem end occlusion and poor stem hydraulic conductance that involve bacterial growth and/or wound healing. S-carvone has putative antibacterial and anti-wound healing activity. S-carvone (0, 0.318 and 0.636 mM) was evaluated as a vase solution for *Acacia holosericea* (Mimosaceae), *Baeckea frutescens* (Myrtaceae), *Chamelaucium uncinatum* cv. 'Mullering Brook' (Myrtaceae) and *Chrysanthemum* sp. cv. 'Dark Splendid Reagan' (Asteraceae). S-carvone was also tested *in vitro* for activity against a vase solution bacterium. S-carvone at 0.318 and 0.636 mM showed significant ( $P < 0.05$ ) positive effects on relative fresh weight, solution uptake and/or vase life for *B. frutescens* foliage and *C. uncinatum* flowering stems, but did not have positive effects for *A. holosericea* and *Chrysanthemum* sp. S-carvone did not suppress vase solution bacterial populations. Moreover, there was no *in vitro* activity at vase solution concentrations against the specific vase water bacterium, *Bacillus cereus*. For the two Myrtaceous genotypes, *B. frutescens* and *C. uncinatum*, S-carvone apparently extended vase life by inhibiting wound healing.

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## 1. Introduction

Cut flowers and foliage develop water deficit stress even when standing in water (van Doorn, 1997). Reductions in stem hydraulic conductivity ( $K_h$ ) are typically caused by occlusion of cut ends and xylem conduits by microbes and their products (Loubaud and van Doorn, 2004), by physiological plugging processes (van Doorn and Cruz, 2000) and by disruption of water columns in xylem vessels from cavitation and air emboli (Nijse et al., 2000).

S-carvone is a monoterpene found in caraway (*Carum carvi*) and dill (*Anethum graveoleus*) seeds (De Carvalho et al., 2006). When applied exogenously to plant tissues, it can prevent or reduce the rate of synthesis of wound healing compounds, such as suberin, and/or can have antibacterial and antifungal activity (Oosterhaven et al., 1995a,b). Provision of S-carvone in the vase water extended the vase life of cut stems of *Hakea francisiana* (Proteaceae) (Williamson et al., 2002) and cut *Grevillea* 'Crimson Yul-lo' (Proteaceae) (He et al., 2006) inflorescences. He et al. (2006) found that S-carvone at 0.318 and 0.636 mM delayed decreases in vase

solution uptake rate and relative fresh weight of cut flowering *Grevillea* stems. The authors argued that S-carvone acted as an inhibitor of the wound response.

This research examined S-carvone effects on several non-proteaceous cut flower and foliage species: *Acacia holosericea* (Velvet Leaf Wattle; Mimosaceae), *Baeckea frutescens* (Maiden's Blush; Myrtaceae), *Chamelaucium uncinatum* (Geraldton waxflower; Myrtaceae) and *Chrysanthemum* sp. (syn. *Dendranthema* × *grandiflorum*; 'florist's chrysanthemum'; Asteraceae). It was hypothesized that low concentrations of S-carvone as vase solutions would improve vase life parameters for the non-proteaceous species tested. It was also hypothesised that S-carvone at 0.318 and 0.636 mM could have antibacterial activity.

## 2. Materials and methods

Cut stems of *A. holosericea*, *B. frutescens*, *C. uncinatum* cv. 'Mullering Brook' and *Chrysanthemum* sp. cv. 'Dark Splendid Reagan' were harvested from plants in the greater Brisbane region, Australia. Stems with mature leaves for *B. frutescens*, at least four fully expanded mature phyllodes for *A. holosericea*, fully open flowers for *Chrysanthemum* sp. and ~75–90% flowers fully opened on sprigs for *C. uncinatum* were used in three experiments.

In experiment 1, deionised (DI) water (control), 0.318 and 0.636 mM S-carvone were used with ten single stem replicates. In

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experiment 2, the same treatments were factorially combined with stem end cutting by removal of 1 cm from the stem base every 2 d. In experiment 3, the S-carvone concentrations were prepared with two different water types; DI water and standard tap water (STW: 0.7 mM CaCl<sub>2</sub>, 1.5 mM NaHCO<sub>3</sub>, and 0.05 mM CuSO<sub>4</sub> diluted with DI water) (van Meeteren et al., 2000, 2006). There were five single stem replicates in the two factorial experiments. Stem end re-cutting and water type treatments were included as comparative treatments against S-carvone treatments.

Vase life was evaluated at 20 ± 1 °C, 60 ± 10% RH and 14 μmol m<sup>-2</sup> s<sup>-1</sup> light intensity from white fluorescent tubes at foliage level with a daily light period of 12 h. Relative fresh weights (RFW) of cut stems and vase solution uptake rate were calculated as per He et al. (2006). Cut stems were assessed daily for visual appeal during vase life evaluation periods.

An agar well diffusion assay was used to assess antibacterial activity of S-carvone (Parente et al., 1995). *Bacillus cereus* test bacterium was isolated from vases containing *A. holosericea*. *B. cereus* was identified at the Australian Collection of Microorganisms (University of Queensland) and is a Gram positive spore forming rod. Inoculum suspension was prepared in phosphate buffer solution (PBS, pH 7.2). Turbidity (OD<sub>600nm</sub>) was adjusted by dilution with PBS to 0.5 units using a UV/Visible spectrophotometer (Pharmacia LKB, Ultraspec III). Then, 1% (v/v) was incorporated into 15 mL of 1.5% plate count agar (van Doorn et al., 1989) and poured into Petri plates. After setting, 6 mm diameter wells were bored into the centre of each agar plate and 20 μL of each treatment solution was added into the wells. In addition to pure S-carvone (6.39 M), four dilutions (0.318, 0.636, 1 and 10 mM) were prepared in DI water. Ethanol (80%) was used as a primary solvent to dissolve pure S-carvone. The final concentration of ethanol in treatment solutions was 0.05% (v/v). DI water and benzalkonium chloride (15 g/L) were used as negative and positive controls, respectively. Inoculated Petri plates were incubated at 29 ± 1 °C for 48 h and diameters of bacterial inhibition zones were measured using a digital caliper.

Numbers of bacteria from vase solutions sampled in the vase life evaluation room were evaluated for *A. holosericea* in experiment 3.

S-carvone solutions (0, 0.318 and 0.636 mM) prepared with DI water were used in this evaluation. Numbers of bacteria [colony forming units (cfu) per milliliter of vase water] were determined (van Doorn et al., 1989) for 1 mL aliquots from each vase on days 3, 5, 8 and 11.

Completely randomized experiment designs were used. Data were subject to analysis of variance using the General Linear Model program of the Statistical Analysis System® (SAS Institute Inc., Cary, NC, USA) release 8.02.

### 3. Results and discussion

*B. frutescens* showed a significant ( $P < 0.05$ ) positive RFW response to S-carvone in experiment 1 (Table 1). Significant positive effects of S-carvone on solution uptake rate were recorded for *C. uncinatum* in experiments 1 and 3 and *B. frutescens* in experiment 3 (Table 1). S-carvone treatment significantly ( $P < 0.05$ ) enhanced vase life of *B. frutescens* foliage in experiments 1 and 2 (Table 2). *C. uncinatum* cut foliage and flower vase lives were significantly improved by S-carvone in experiment 3. However, longevity of *A. holosericea* and *Chrysanthemum* sp. were not increased with S-carvone.

Recutting stems significantly ( $P < 0.05$ ) reduced the rate of decline in RFW and solution uptake in all three species in experiment 2 (Table 1). Vase lives of the cut flower and foliage species tested were also significantly prolonged by recutting (Table 2). Maintenance of RFW and solution uptake by regular recutting of *A. holosericea*, *B. frutescens* and *C. uncinatum* stems suggests that occlusions at the cut stem ends were removed, thereby facilitating continued water uptake. Similarly, regular recutting of 1 cm from stem bases significantly improved vase lives of *Acacia baileyana*, *Leptospermum polygalifolium* and *L. obovatum* (Williamson et al., 2002).

Average RFW and solution uptake for cut *A. holosericea* foliage were significantly ( $P < 0.05$ ) improved by STW in experiment 3 (Table 1). In contrast, these parameters were significantly lower when stems of the other three species were held in STW.

**Table 1**

Mean values of relative fresh weight (RFW; % of initial fresh weight) and vase solution uptake rate (Vrate; g g<sup>-1</sup> initial fresh weight) over the total vase period for cut flower and foliage species tested in three experiments.

Main factors	Species and characteristics of relative fresh weight and water uptake							
	<i>A. holosericea</i>		<i>B. frutescens</i>		<i>C. uncinatum</i>		<i>Chrysanthemum</i> sp.	
	RFW	Vrate	RFW	Vrate	RFW	Vrate	RFW	Vrate
Experiment 1								
0 mM	74.4	0.344	71.2b	0.393	88.4	0.040b	–	–
0.318 mM	78.4	0.368	84.3a	0.416	91.3	0.050a	–	–
0.636 mM	76.4	0.382	85.0a	0.518	88.1	0.053a	–	–
LSD <sub>0.05</sub> (n = 10)	ns	ns	7.22	ns	ns	0.009	–	–
Experiment 2								
0 mM	84.4	0.298	81.7ab	0.773	91.6	0.052	–	–
0.318 mM	82.0	0.282	87.0a	0.755	93.1	0.059	–	–
0.636 mM	81.7	0.289	75.5b	0.699	94.4	0.049	–	–
LSD <sub>0.05</sub> (n = 10)	ns	ns	9.16	ns	ns	ns	–	–
Intact	79.2b	0.268b	74.5b	0.609b	89.5b	0.045b	–	–
Recut	86.3a	0.312a	88.3a	0.876a	96.6a	0.062a	–	–
LSD <sub>0.05</sub> (n = 15)	7.02	0.038	7.48	0.126	4.54	0.007	–	–
Experiment 3								
0 mM	94.7	0.173	82.3	0.393b	85.2	0.037b	–	–
0.318 mM	93.0	0.171	84.4	0.464ab	86.9	0.050a	–	–
0.636 mM	94.0	0.178	85.9	0.490a	90.2	0.055a	–	–
LSD <sub>0.05</sub> (n = 10)	ns	ns	ns	0.073	ns	0.008	–	–
DI water	91.3b	0.147b	86.1a	0.486a	90.9a	0.050a	–	–
STW	96.5a	0.200a	82.3b	0.412b	84.0b	0.043b	–	–
LSD <sub>0.05</sub> (n = 15)	1.56	0.024	3.40	0.060	4.13	0.007	–	–

(–) *Chrysanthemum* sp. was not included in experiments 1 and 2. (–) Data not captured. ns: F-test was not significant ( $P > 0.05$ ). Within any experiment and column, means followed by different letters are significantly different ( $P < 0.05$ ).

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