CHAPTER THREE

ETHYLENE SENSITIVITY AND VASE LIFE OF *ACACIA* spp.

3.1 INTRODUCTION

Phan (1963) measured ethylene production by cut flowers and observed that different cultivars and forms of the same species produced very different amounts of ethylene. He also found that there was no relationship between "systematic groups" (a formal taxonomic level was not stated) of flowers and ethylene production. However, he cautioned that the results needed verification with gas chromatography, as he had used a mercuric perchlorate technique for ethylene detection (Phan 1963). More recently, Woltering (1987a) noted that differences in the response of ornamental pot plants to exogenous ethylene occurred at the species and species cultivar level. However, caution must be exercised in making direct comparisons between cut and intact flowers as cut flowers produce more ethylene than their intact counterparts (Kaltaler and Boodley 1970) and may exhibit greater sensitivity to its effects. Conversely, Woltering and van Doorn (1988), in exhaustive testing of 22 families and 93 species, found that the reaction to exogenous ethylene of either petal wilting or true abscission (i.e. abscission before wilting) occurred at the family level. In general, they observed that flowers which wilted showed little response to ethylene, while those in which petals abscissed were ethylene-sensitive. Nevertheless, some confusion exists within the Rosaceae, where different *Rosa hybrida* cultivars exhibit varying responses to ethylene exposure (Mor *et al.* 1989; Reid *et al.* 1989). Furthermore, the broad classification of flower sensitivity to ethylene based on whether petals wilt or abscise (Woltering and van Doorn 1988) does not appear to be substantiated by recent research. Both sweet peas (*Lathyrus odoratus* L.) and *Phalaenopsis* orchids are ethylene-sensitive, but *Phalaenopsis* orchids wilt rather than abscise, and sweet peas wilt and then abscise (Porat *et al.* 1995; Sexton *et al.* 1995).

It is unclear whether *Acacia*, a short-lived, but popular cut flower, especially in Europe, produces or is sensitive to ethylene. Cottrell (1968) stated that *Acacia* was "more resistant" to ethylene than susceptible crops, such as carnations and larkspurs [*Consolida ambigua* and *C. orientalis*, both formerly *Delphinium ajacis* (Armitage 1993)]. Nowak and Rudnicki (1990), in their book which mentioned, *inter alia*, guidelines for particular plant species, including *Acacia* spp., did not list *Acacia* as having a "high sensitivity, moderate sensitivity, sensitivity, low sensitivity", or as being "not sensitive" to ethylene. However, some *Acacia* spp. produce or are sensitive to ethylene, for example, ethylene gas was produced by leaf homogenates of *Acacia*

georginae (Peters and Shorthouse 1967). Doubt (1917) observed that leaf fall occurred in *Acacia horrida* Willd. exposed to 1000 ppm of illuminating gas (containing 4% ethylene and 25% carbon monoxide). *Mimosa pudica* L. (the sensitive plant, family Fabaceae), was also found by Doubt (1917) to be sensitive to illuminating gas (50 ppm) and ethylene (5 and 8 ppm). Leaf folding and drooping occurred, accompanied by pulvinar movement similar to the thigmonastic response. The plants lost their sensitivity to touch during exposure, but regained it after ethylene removal. After 1 d of exposure, the older leaflets and petioles turned yellow and fell (Doubt 1917). Funke *et al.* (1938) noted that after exposure to apple volatiles (containing ethylene), defoliation occurred in *Mimosa pudica* after 2 d and within two weeks in *Acacia linifolia*. McMillan and Cope (1969) found that *Acacia farnesiana* exhibited defoliation after exposure to ethylene (< 0.1%) in air. However, even though defoliation occurred in these *Acacia* species upon ethylene exposure, it is unknown whether there was a reaction in the flowers, and thus whether ethylene would decrease the vase life of these species.

Pokon & Chrysal make a floral preservative, Mimosa-Chrysal™ for shrub and tree cut stems of mimosa (*Acacia*), lilac (*Syringa vulgaris* L.), *Forsythia* spp., *Paeonia* spp., *Prunus* spp. and snowball tree (*Viburnum* spp.). Of these, Nowak and Rudnicki (1990) mentioned guidelines for postharvest care of *Acacia*, lilac, *Forsythia* spp., *Paeonia* spp. and *Prunus* spp. Only *Prunus* spp. was listed as being sensitive to ethylene, but it is possible that *Forsythia* spp. may also be ethylene sensitive as $AgNO_3$ (a protector against ethylene action, but also an effective germicide) was suggested as a component of a bud-opening solution. Phan (1963) found that *Prunus cerasus* L. produced ethylene, however, no detectable amount of ethylene was produced by *Syringa vulgaris*. Whilst Heuser and Evensen (1986) did not test the ethylene sensitivity of *Paeonia* spp. cultivars, they did note that the vase life of one cultivar was terminated abruptly when the unwilted petals abscissed. In light of Woltering and van Doorn's (1988) research (see above), which indicated that ethylene sensitivity occurs in those flowers that show abscission before wilting, it appears that this cultivar, at least, of *Paeonia* spp. is sensitive to ethylene. In addition, Davies *et al.* (1981) found that the vase life of flowering *Prunus persica* L. Batsch could be extended with ethanol, an inhibitor of ethylene production (Heins 1980) and action (Wu *et al.* 1992). Thus, there is a possibility that some form of ethylene inhibitor is one of the components of Mimosa-Chrysal, in which case the vase life of *Acacia* may be improved with the use of this compound because of its action against ethylene[1].

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[1] In February 1994, after the experimental part of this chapter had been completed, a leaflet was obtained which listed the cut flower foods manufactured by Pokon & Chrysal, one of which was "Mimosa-Chrysal". The existence of this *Acacia* preservative was not known prior to this time. The Australian distributor of Chrysal (John Dawson of Hortraco Pty. Ltd, Sydney) was contacted, but he said that Mimosa-Chrysal was not available in Australia because of its extremely low demand. The minimum order was four pallets, which was not feasible to import because of poor sales and the short shelf life of Mimosa-Chrysal. However, the components of the pretreatment product were given: pretreatment with HVB™ at 3 g L^{-1} (containing 15% citric acid at 975 mg kg^{-1} and 77% 8-HQS) for 24 h at 15-20°C, and then into the holding solution, Mimosa-Chrysal. Because it was not necessary to register the holding solution in Australia, the components were unknown to Hortraco. However, Mr Dawson said that *Acacia* was not ethylene-sensitive as STS was not one of the components of either the holding or pretreatment solution.

Very little work has been published on the vase life of flowering *Acacia*; a study by Accati and Sulis (1980) being an exception. They tested the vase life of two cultivars of *Acacia dealbata* Link. with a range of sucrose concentrations (from 1 to 20%) in solutions containing $AgNO_3$, 8-HQC and $Al_2(SO_4)_3$. The optimal sucrose concentration was 5%, although there was no significant difference in vase life when sucrose was omitted from the solution. None of the other vase components was tested on its own, so the influence of $AgNO_3$ is unclear. It is uncertain whether the beneficial effect of $AgNO_3$ on vase life was due to microbial inhibition (Aarts 1957 in Paull and Goo 1982) or protection against the action of ethylene. (Accati and Sulis' experiments with $AgNO_3$ were performed in 1978 and 1979, prior to the widespread acceptance of STS as the superior form of silver application.)

The aims of the experiments presented in this chapter were to determine:

- the effect of ethylene and an ethylene inhibitor (STS) on the vase life of *Acacia* spp.;
- whether ethylene is produced by two previously untested *Acacia* spp.; and
- the effect of various vase solutions on the vase life, solution uptake, transpiration, fresh weight, water content and net water loss of *Acacia* spp.

3.2 MATERIALS AND METHODS

3.2.1 Plant material

Acacia spp. plant material was obtained from the Botany Department garden, UNE, Armidale. Standard white carnations (*Dianthus caryophyllus* L.), cultivar unknown, were obtained from a local supermarket (Coles). It was unknown whether the carnations were pre-treated with STS, but their short vase life, terminated by petal inrolling, was indicative of an ethylene-sensitive senescence (Nichols 1968).

3.2.2 Apparatus used in vase life-ethylene experiments

The following experimental apparatus and design were based on Jones and Truett (1992). Ten 2.5 L glass jars were made into gas-tight sampling jars by drilling holes in the plastic lids and fitting a 2 cm-long hard plastic tube through the holes. The upper and lower junctions of the tube and lid were sealed with Epoxy-Plus Clag® (Inter-National Adhesives and Resins Pty. Ltd., Victoria, Australia). Rubber gas-sampling stoppers (Size 17, Suba-seal, Gallenkamp, England) were fitted into the tubes. In order to absorb respiratory carbon dioxide build up (Kang *et al.* 1967), since carbon dioxide is a competitive inhibitor of ethylene action (Burg and

Burg 1965), the jars contained potassium hydroxide (20 mL, 20% w/v) in beakers lined with filter paper (Keys *et al.* 1975). The flowering stem to be tested was then placed carefully in a vase, and the lip of the vase was sealed with Parafilm M (American Can Company, Connecticut). The vase was lowered into the jar, taking care to avoid contact with the flowering stem. This precaution was employed despite Nichols' (1966) finding that moderate handling did not increase ethylene production in carnations. Endogenous 'wound' ethylene production in excised stems can increase dramatically 30 min to several hours after cutting and handling (Jackson and Osborne 1970; Irvine and Osborne 1973; Jackson and Campbell 1976). The lid of the jar was then screwed on tightly The rubber stoppers, jars and lids were exposed to the atmosphere for at least 24 h before use. In addition, an empty, sealed jar was also tested for ethylene emanation (see Appendix D), to ensure that no ethylene was produced by the plastic materials of the experimental apparatus (Ward *et al.* 1978).

After the stems had been in the sealed jars for 1 h, ethylene gas was injected into the jars with a gas-tight syringe (Hamilton Co., Reno, Nevada) to give the required concentration. The ethylene concentration (peak area) was measured immediately after injection (time 0 h, which was approximately 1 h after flowers were placed in the jars), and after 24 h, by withdrawing 100 µL of headspace gas from the jars and injecting it into the gas chromatograph column. The jars were kept under low light (<1 µmol m^{-2} s^{-1}) at a temperature range of 20 to 22.5°C during the 24 h that flowering stems were enclosed within them.

3.2.3 Ethylene analysis

The amount of ethylene in the gas-tight jars was determined with a gas chromatograph (Model 3300, Varian Instrument Group, California) equipped with a flame ionisation detector (FID) and an integrator (Model HP 3396A, Hewlett-Packard Company, Pennsylvania). A 12 m long capillary column (S.G.E. GO 6), with a film coating of 0.5 µm and an internal diameter of 0.33 mm was used. The column temperature was 70°C, the injector temperature was 220°C, and the temperature of the detector was 300°C. The carrier gas was nitrogen, and the FID detector burnt a mixture of hydrogen and air. Under these conditions, the ethylene retention time was approximately 38 s (see Appendix D .

3.2.4 Vase solutions used for testing ethylene production and sensitivity in *Acacia subulata* and *Acacia floribunda*

The experiment described above (sections 3.2.2 and 3.2.3) was designed to test the effect of exposure to exogenous ethylene on the vase life of two acacia species, *Acacia subulata* Bonpl. and *Acacia floribunda* (Vent.) Willd., and also to determine whether these acacias produced

ethylene. An experimental design with the following treatments was used for *A. subulata* (note: T = Treatment):

T1: distilled water, in an ethylene atmosphere of 50 μL L^{-1};
T2: distilled water, without added ethylene; and
T3: STS pulse (0.5 mol m^{-3}) for 12 h, then distilled water, in an ethylene atmosphere of 50 μL L^{-1}.

The experimental design for *A. floribunda* was the same as for *A. subulata*, except that a higher level of ethylene was used (500 μL L^{-1}). Because a limited number of experimental jars were available for gas analysis, each experiment was run over three consecutive weeks, there being three replicates of each treatment per week. After 24 h, the flowering stems were removed from the jars and placed in the vase life room for vase life assessment (section 2.8). Measurements were made daily of solution uptake, transpiration, net water loss, fresh weight and water content during vase life (section 2.13).

To determine whether ethylene production could be detected in cut flowers after 0 and 24 h of enclosure, cut flowers known to produce ethylene were used. Carnations at different stages of senescence (Stages VI and VII, Cash *et al.* 1980) were placed in water in two gas sampling jars and the headspace gas was sampled after 0 and 24 h (see Appendix D).

3.2.5 Vase solutions used in vase life experiment with *A. floribunda*

The following solutions were used during a vase life experiment with *A. floribunda*:

T1 distilled water (control);
T2 citric acid (10 mol m^{-3})*;
T3 STS pulse (0.5 mol m^{-3} for 16.5 h), then into distilled water;
T4 SDIC (= Sodium dichloroisocyanurate: 50 mg L^{-1} available Cl^-; chemical name: sodium dichloro-5-triazine-trione);
T5 SCC (= "Silver, Citric and Chlorine": STS pulse @ 0.5 mol m^{-3} for 16.5 h; then into citric acid [10 mol m^{-3}] and SDIC [50 mg L^{-1}]);
T6 ethanol (8% v/v);

* The concentration of 10 mol m^{-3} citric acid (pH 2.6) was used in the *Acacia* vase life experiments because earlier experiments (Williamson 1989) revealed that this concentration of citric acid resulted in a longer vase life (3.8 d) of *Acacia* spp. than 1 mol m^{-3} citric acid (pH 3.2, 3.0 d). The citric acid concentration of 300 ppm (= 1.43 mol m^{-3}, pH 3.0) is the commonly used concentration in vase life experiments at the Institute for Horticultural Development, Knoxfield, Victoria. This latter concentration has been compared with a 10 mol m^{-3} concentration in *Boronia heterophylla* vase life experiments described in Chapter 6.

T7 A&S, 2% (= Accati and Sulis' solution: 200 mg L^{-1} 8-HQC, 50 mg L^{-1} $AgNO_3$, 50 mg L^{-1} $Al_2(SO_4)_3$ with 2% sucrose); and

T8 A&S, 5% (= Accati and Sulis' solution: 200 mg L^{-1} 8-HQC, 50 mg L^{-1} $AgNO_3$, 50 mg L^{-1} $Al_2(SO_4)_3$ with 5% sucrose).

Vase life parameters were measured as described in section 2.13.

3.2.6 Vase life experiment using *Acacia amoena*

Acacia amoena (Wendl.) was the species used in cavitation detection studies (Chapter 4) because of its wide, discrete phyllodes (see section 4.2.1). Cavitation detection results had revealed dramatic differences in the response of *A. amoena* stems kept in either distilled water or citric acid (10 mol m^{-3}) (see Figs. 4.2 and 4.4). Thus, a vase life experiment was designed to determine the effect of these two solutions on *A. amoena* solution uptake, transpiration, fresh weight, water content and net water loss.

3.2.7 Floral development and vase life evaluation of *A. subulata*, *A. floribunda* and *A. amoena*

The method of *Acacia* vase life evaluation followed that described by Williamson (1989). Flowering racemes were chosen which had at least one third of the flower heads on the raceme just exhibiting anthesis (Stage 2). At this stage of floral development, the perianth tips have just opened. The following stages of floral development (Williamson 1989) were observed:

Stage 1: pre-opening - all flower heads on raceme are closed;
Stage 2: anthesis - partial opening of sepals and petals;
Stage 3: the style is visible as it exserts through the open perianth tips;
Stage 4: 50% of flowers on head fully open - the stamens are turgid and bright yellow;
Stage 5: 100% of flowers on head fully open - the stamens are turgid and bright yellow;
Stage 6: <50% of flowers on head exhibiting visible symptoms of senescence - the stamens are infolded and appear disorderly, desiccated and discoloured;
Stage 7: >50% of flowers on head have disorderly, desiccated or discoloured stamens. The stamens are a golden brown colour (due to browning of the filaments).

The flowers were regarded as having reached the end of their vase life when they reached Stage 7. Flowering branches of *Acacia* spp. exhibit a range of floral developmental stages. Therefore, the raceme to be studied was noted by either recording which number raceme it was from the base, or by drawing a plan of the flowering stem, indicating its location.

3.2.8 Analysis of data

Data from experiments examining the effect of ethylene on vase life (sections 3.3.1 and 3.3.2) were not normally distributed. Therefore, these data were analysed using chi-square (χ^2) tests of independence, comparing each treatment separately with the control (I. Davies, pers. comm. 1995). (See Appendix C for all 2×2 χ^2 contingency tables.)

The *A. floribunda* vase life data (section 3.3.4, Table 3.3) were unable to be analysed statistically because the data were not normally distributed and the variances were unequal and unable to be transformed. (The smallest value for Cochran's test for homogeneity of the variances, achieved using a reciprocal of x transformation, was 1.5, but a value of < 0.29 was required.) Therefore, it was inappropriate to perform parametric or nonparametric statistical tests. Parametric tests assume that the data are normally distributed and have equal variances, and nonparametric tests require equal variances, the assumption being that the distributions differ only in location (Day and Quinn 1989). According to Day and Quinn (1989), "the effects of combinations of variance heterogeneity and non-normal distributions have not been studied in detail". After several discussions with two statisticians at UNE, (I. Davies and D. Smith, pers. comm. 1995), it was concluded that it was inappropriate to perform any statistical tests with the *A. floribunda* vase life data. However, means of solution uptake, transpiration, fresh weight, water content and net water loss per day from the *A. floribunda* vase life experiment were able to be separated using Scheffé's test ($P < 0.05$) (StatView 4.0), or a t-test if only two treatments remained. Data were transformed as necessary to fulfil the requirement of homogeneity of the variances using Cochran's test (Winer 1971). (See Appendix C for ANOVA tables and transformations used.)

A. amoena vase life experimental data (Table 3.4) were not normally distributed. Therefore, these data were analysed using chi-square (χ^2) tests of independence, comparing each treatment separately with the control (I. Davies, pers. comm. 1995). In the *A. amoena* experiment, solution uptake, transpiration, fresh weight, water content and net water loss per day of distilled water and citric acid stems were compared using t-tests to separate the treatment means for each measured variable.

3.3 RESULTS

3.3.1 Sensitivity of cut flowering *A. subulata* stems to 50 $\mu L\ L^{-1}$ exogenous ethylene

There were no significant differences between treatments in the vase life of flowering *A. subulata* stems (Table 3.1). Thus, neither exposure to exogenous ethylene (T1), nor protection against

exogenous ethylene with STS (T3) affected vase life of *A. subulata*, as compared with the distilled water control (T2). No flower drop was recorded in any treatment. Visible signs of senescence, such as stamen wilting and phyllode desiccation, did not differ between treatments.

Table 3.1

Vase life of *A. subulata* exposed to 50 μL L^{-1} exogenous ethylene

Treatment	Vase life* (d) ± SE◊	Range (d)
T1: Distilled water; added ethylene	3.8 ± 0.222	3 - 5
T2: Distilled water; no ethylene added (control)	3.8 ± 0.222	3 - 5
T3: STS pulse, then distilled water; added ethylene	3.4 ± 0.176	3 - 4

* There were no significant differences ($P < 0.05$) between the treatment means.
◊ Vase life is the mean of 9 replicates per treatment; SE = standard error.

3.3.2 Sensitivity of cut flowering *A. floribunda* stems to 500 μL L^{-1} exogenous ethylene

Vase lives of flowering *A. floribunda* stems exposed to high levels of exogenous ethylene (500 μL L^{-1}) and kept in distilled water (T1), or protected against exogenous ethylene with STS (T3), were not significantly different from one another or from the vase life of stems kept in the distilled water control (T2) (Table 3.2). Some flower drop occurred in all treatments, but only of older, senescing flowers which were not part of the raceme studied. There were no differences between treatments in visible signs of senescence.

Table 3.2

Vase life of *A. floribunda* exposed to 500 μL L^{-1} exogenous ethylene

Treatment	Vase life* (d) ± SE◊	Range (d)
T1: Distilled water; added ethylene	3.3 ± 0.167	3 - 4
T2: Distilled water; no ethylene added (control)	3.2 ± 0.147	3 - 4
T3: STS pulse, then distilled water; added ethylene	3.0 ± 0.0	3 - 3

* There were no significant differences ($P < 0.05$) between the treatment means.
◊ Vase life is the mean of 9 replicates per treatment; SE = standard error.

3.3.3 Production of ethylene by cut flowering *A. subulata* and *A. floribunda* stems

No detectable ethylene was produced by either *A. subulata* or *A. floribunda* during the 24 h period that the flowering stems were enclosed in sealed jars. In addition, the level of ethylene

did not increase in the jars which contained added ethylene (see Appendix D) over the 24 h period of stem enclosure.

The trial with carnation flowers was used to determine whether the experimental system was capable of detecting ethylene production in cut flowers known to produce ethylene. Ethylene was detected at time 0 h (approximately 1 h after enclosure) and, at an increased level, 24 h after enclosure (see Appendix D). No ethylene was added to the jars during that time.

3.3.4 Vase life, solution uptake, transpiration, fresh weight, water content and net water loss in *A. floribunda* using various vase solutions

The vase life of *A. floribunda* in the eight treatment solutions is shown in Table 3.3. The longest vase life (4.1 d) occurred in T5 (SCC), although individual replicates lasted the longest in T4 (SDIC), in which two replicates lasted 6 d. SDIC (T4) and citric acid (T2) were the next most beneficial treatments, lasting 3.5 and 3 d respectively.

The following five treatments had the poorest vase lives. T3 (STS pulse), resulted in a vase life of 1.4 d; and continuous treatment with ethanol (T6), also an inhibitor of ethylene action, produced a vase life of 1.7 d. Both these treatments resulted in shorter vase lives than in distilled water (T1), which was 1.8 d. The shortest vase life (1.3 d) occurred in Accati and Sulis' solution with 2% sucrose (T7) and, although their solution with 5% sucrose (T8) resulted in a longer vase life of 2.1 d, it was still one of the poorest treatments.

Table 3.3
Vase life of *A. floribunda* in various treatment solutions

Treatment	Vase life (d)* ± SE◊	Range (d)
T1: Distilled water	1.8 ± 0.133	1 - 2
T2: Citric acid (10 mol m^{-3})	3.0 ± 0.149	2 - 4
T3: STS pulse, then distilled water	1.4 ± 0.163	1 - 2
T4: SDIC (50 mg L^{-1})	3.5 ± 0.543	1 - 6
T5: SCC	4.1 ± 0.379	2 - 5
T6: Ethanol (8% v/v)	1.7 ± 0.153	1 - 2
T7: A&S, with 2% sucrose	1.3 ± 0.213	1 - 3
T8: A&S, with 5% sucrose	2.1 ± 0.233	1 - 3

◊ Vase life is the mean of 10 replicates per treatment; SE = standard error.
* Means were unable to be separated because the data were not normally distributed and the variances were heterogeneous and unable to be transformed.

The vase life parameters of solution uptake, transpiration, fresh weight, stem water content and net water loss (Figs. 3.1 - 3.5) all reflected the trends evident in the vase life data (Table 3.3). Although the vase life data could not be analysed statistically, the separation of means in the following graphs invariably reflected the order of the vase life results. The treatments which had the longest vase lives, SCC (T5), SDIC (T4) and citric acid (10 mol m^{-3}) (T2), had significantly greater values for all of the above parameters. Those treatments with the poorest vase lives, namely, Accati and Sulis' solution with 2% sucrose (T7), the STS pulse (T3), ethanol (T6), distilled water (T1) and Accati and Sulis' solution with 5% sucrose (T8) were usually in the lower statistical groupings of measured parameters. The exception to this occurred in the measurement of fresh weight (Fig. 3.3), in which Accati and Sulis' solution with 5% sucrose (T8) was in the statistically highest grouping. This result is not surprising, because sucrose increases the fresh weight of cut flowers, while also decreasing the amount of solution uptake and transpiration (Marousky 1969; 971). The increase in fresh weight of cut flowers kept in sucrose solutions is associated with stomatal closure and resultant decreased water loss (Marousky 1971). Stems in solutions containing sucrose (T7 and T8) lost less water than in most other treatments (Fig. 3.5), but this did not result in an increased vase life (Table 3.3).

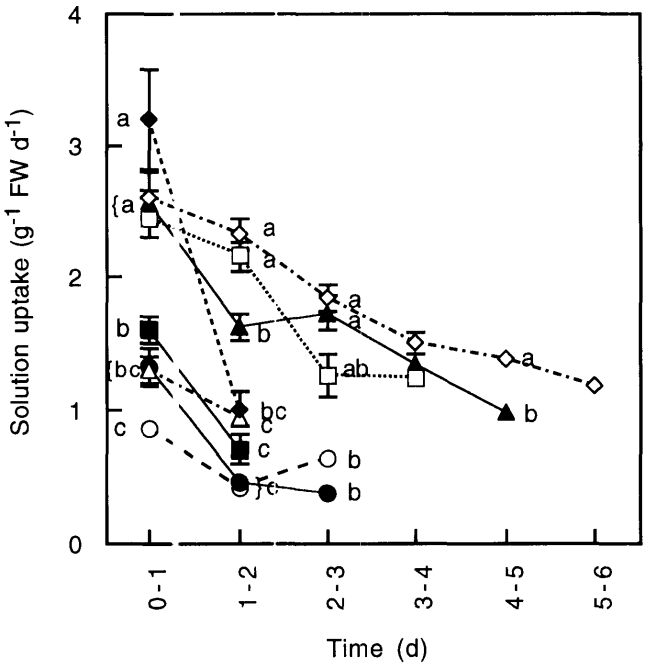


Fig. 3.1. The rate of solution uptake (expressed per gram of fresh weight per day) during the vase life of *A. floribunda* stems kept in eight treatment solutions (note: T = Treatment). **T1** (■) distilled water; **T2** (□) citric acid (10 mol m^{-3}); **T3** (◆) STS pulse, then distilled water; **T4** (◇) chlorine (SDIC 50 mg L^{-1}); **T5** (▲) SCC [STS pulse, then distilled water, citric acid (10 mol m^{-3}) and chlorine (SDIC 50 mg L^{-1})]; **T6** (△) ethanol (8% v/v); **T7** (●) Accati and Sulis' solution with 2% sucrose; **T8** (○) Accati and Sulis' solution with 5% sucrose. Within each day, symbols followed by different letters indicate significant differences between treatments ($P < 0.05$). Where no letters appear, the treatments were not significantly different ($P < 0.05$). (Where no error bar appears, the SE was smaller than the size of the symbol.)

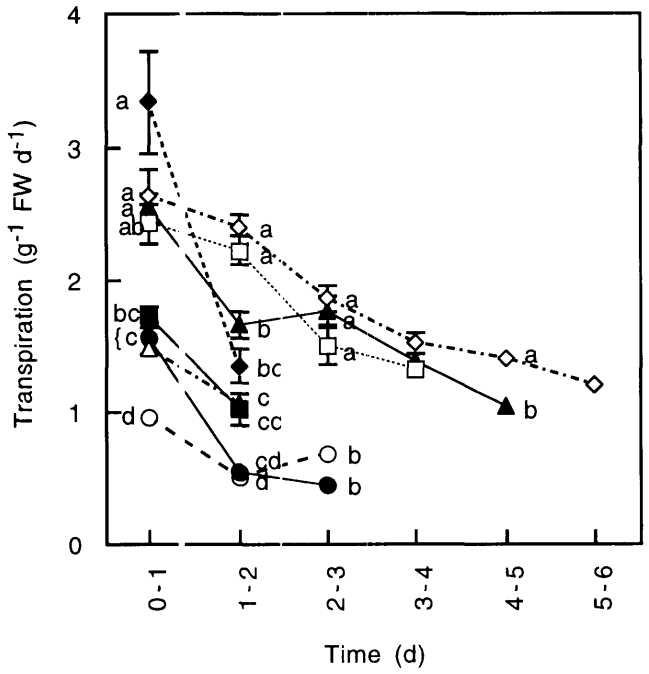


Fig. 3.2. The rate of transpiration (expressed per gram of fresh weight per day) during the vase life of *A. floribunda* stems kept in eight treatment solutions (note: **T** = Treatment). **T1** (■) distilled water; **T2** (□) citric acid (10 mol m^{-3}); **T3** (◆) STS pulse, then distilled water; **T4** (◇) chlorine (SDIC 50 mg L^{-1}); **T5** (▲) SCC [STS pulse, then distilled water, citric acid (10 mol m^{-3}) and chlorine (SDIC 50 mg L^{-1})]; **T6** (Δ) ethanol (8% v/v); **T7** (●) Accati and Sulis' solution with 2% sucrose; **T8** (O) Accati and Sulis' solution with 5% sucrose. Within each day, symbols followed by different letters indicate significant differences between treatments ($P < 0.05$). Where no letters appear, the treatments were not significantly different ($P < 0.05$). (Where no error bar appears, the SE was smaller than the size of the symbol.)

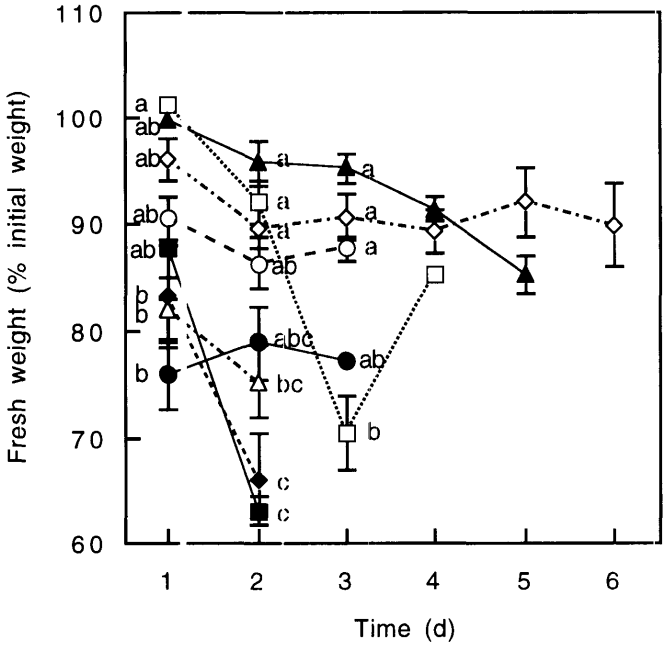


Fig. 3.3. The change in fresh weight (expressed as a percentage of the initial weight) during the vase life of *A. floribunda* stems kept in eight treatment solutions (note: **T** = Treatment). **T1** (■) distilled water; **T2** (□) citric acid (10 mol m^{-3}); **T3** (◆) STS pulse, then distilled water; **T4** (◇) chlorine (SDIC 50 mg L^{-1}); **T5** (▲) SCC [STS pulse, then distilled water, citric acid (10 mol m^{-3}) and chlorine (SDIC 50 mg L^{-1})]; **T6** (Δ) ethanol (8% v/v); **T7** (●) Accati and Sulis' solution with 2% sucrose; **T8** (O) Accati and Sulis' solution with 5% sucrose. Within each day, symbols followed by different letters indicate significant differences between treatments ($P < 0.05$). Where no letters appear, the treatments were not significantly different ($P < 0.05$). (Where no error bar appears, the SE was smaller than the size of the symbol.)

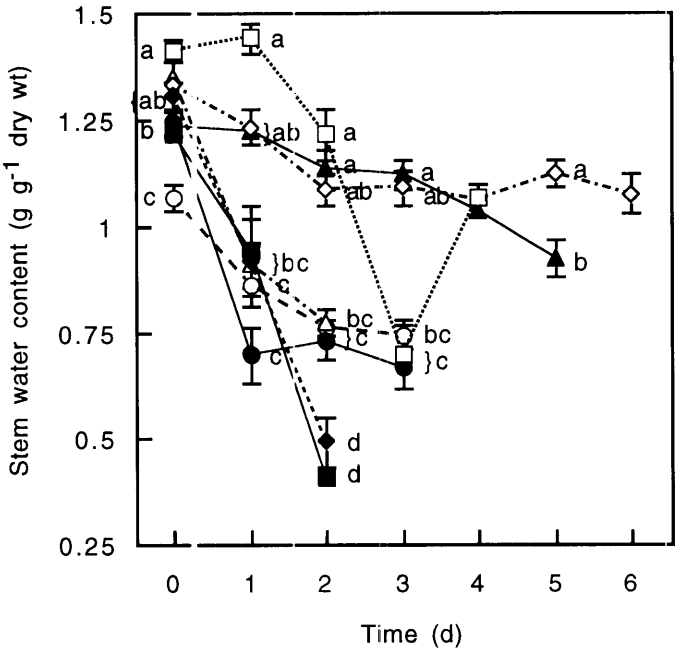


Fig. 3.4. The change in stem water content (expressed in grams of water per gram of dry weight) during the vase life of *A. floribunda* stems kept in eight treatment solutions (note T=Treatment). **T1** (■) distilled water; **T2** (□) citric acid (10 mol m^{-3}); **T3** (◆) STS pulse, then distilled water; **T4** (◇) chlorine (SDIC 50 mg L^{-1}); **T5** (▲) SCC [STS pulse, then distilled water, citric acid (10 mol m^{-3}) and chlorine (SDIC 50 mg L^{-1})]; **T6** (△) ethanol (8% v/v); **T7** (●) Accati and Sulis' solution with 2% sucrose; **T8** (○) Accati and Sulis' solution with 5% sucrose. Within each day, symbols followed by different letters indicate significant differences between treatments ($P < 0.05$). Where no letters appear, the treatments were not significantly different ($P < 0.05$). (Where no error bar appears, the SE was smaller than the size of the symbol.)

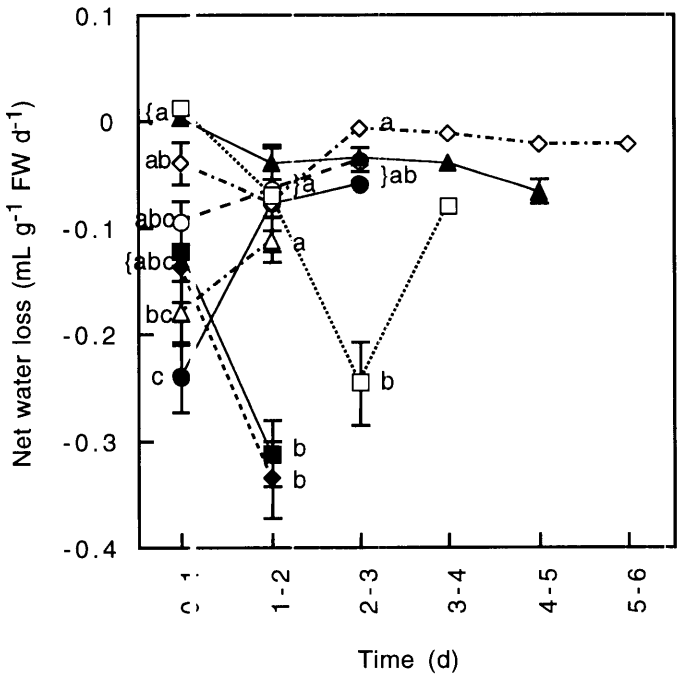
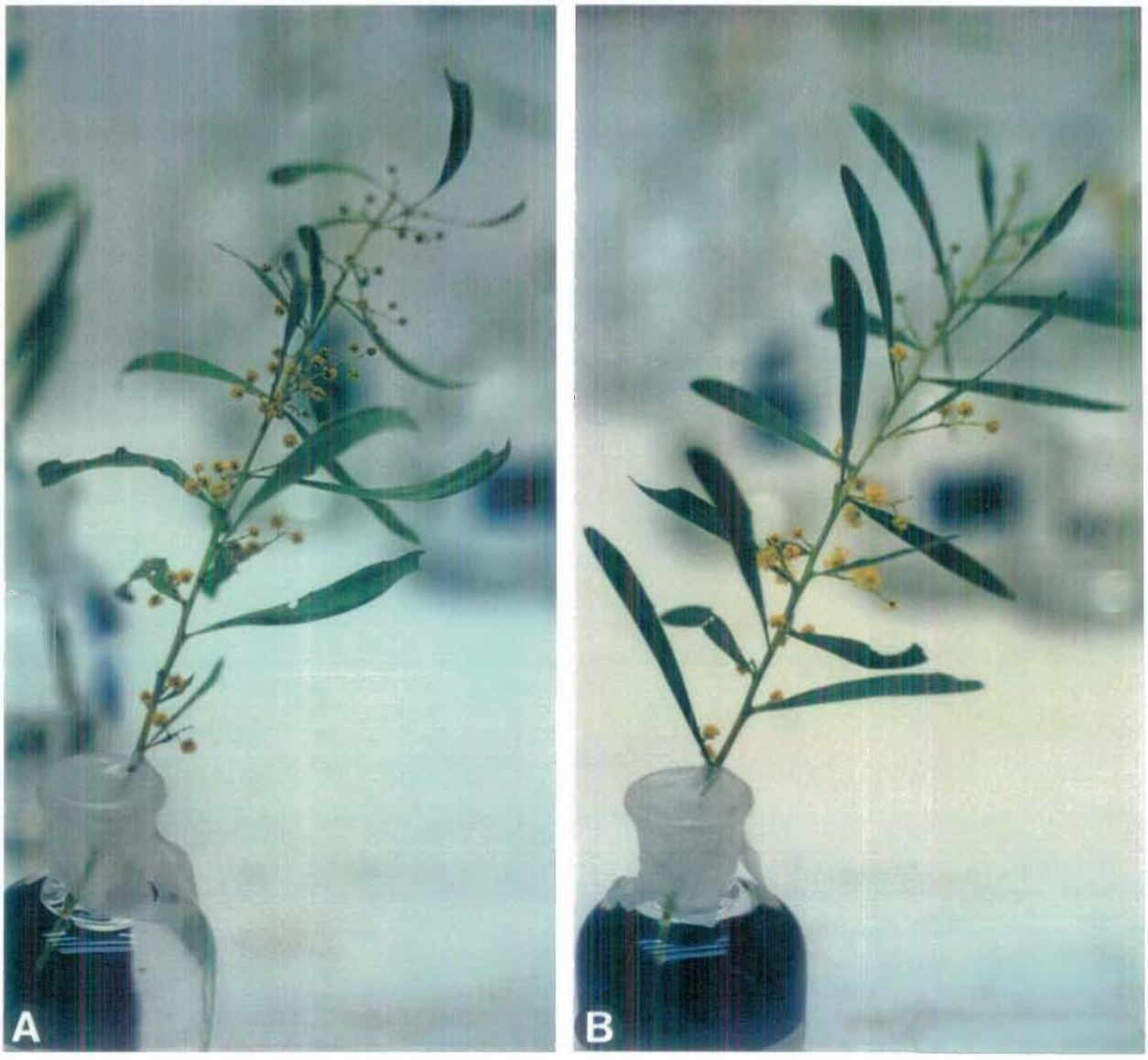


Fig. 3.5. The rate of net water loss (expressed in mL per gram of fresh weight per day) during the vase life of *A. floribunda* stems kept in eight treatment solutions (note: T=Treatment). **T1** (■) distilled water; **T2** (□) citric acid (10 mol m^{-3}); **T3** (◆) STS pulse, then distilled water; **T4** (◇) chlorine (SDIC 50 mg L^{-1}); **T5** (▲) SCC [STS pulse, then distilled water, citric acid (10 mol m^{-3}) and chlorine (SDIC 50 mg L^{-1})]; **T6** (△) ethanol (8% v/v); **T7** (●) Accati and Sulis' solution with 2% sucrose; **T8** (○) Accati and Sulis' solution with 5% sucrose. Within each day, symbols followed by different letters indicate significant differences between treatments ($P < 0.05$). Where no letters appear, the treatments were not significantly different ($P < 0.05$). (Where no error bar appears, the SE was smaller than the size of the symbol.)

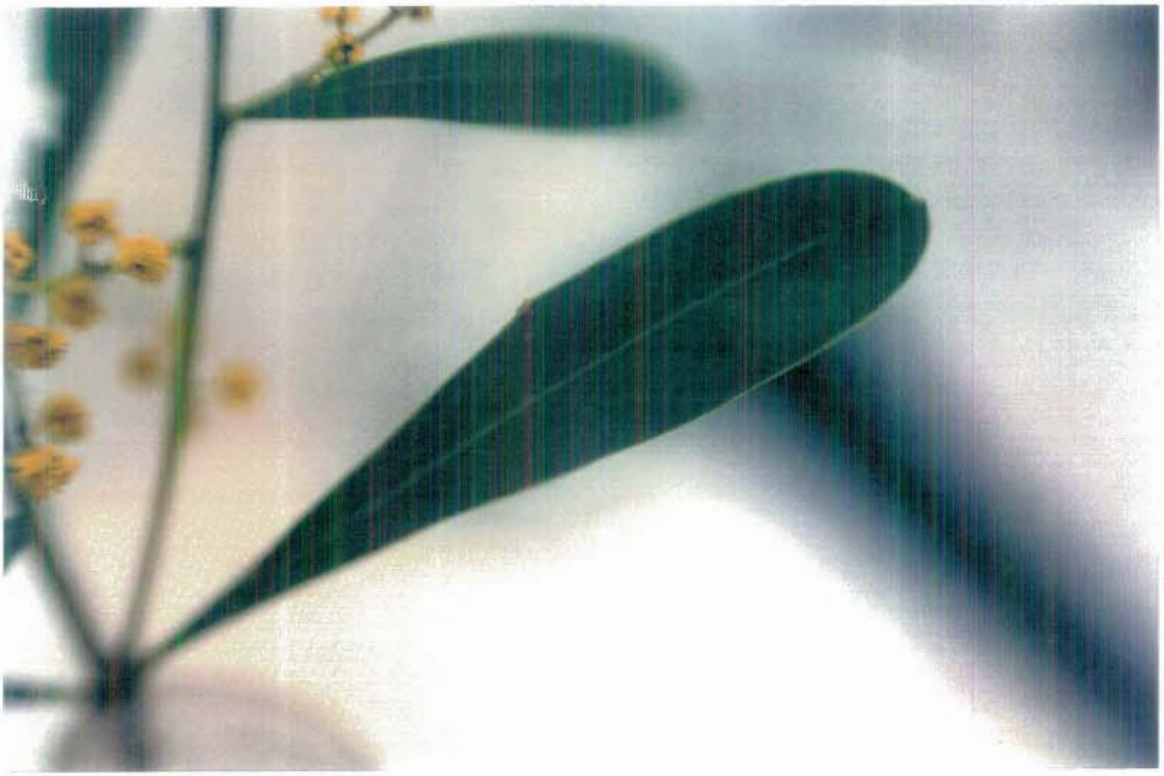
3.3.5 A comparison of vase life, solution uptake, transpiration, fresh weight, water content and net water loss in *A. amoena* using citric acid (10 mol m^{-3}) and distilled water

When phyllode desiccation was used to determine the end of vase life, cut flowering stems of *A. amoena* lasted more than three times longer in citric acid compared with distilled water (Table 3.4, Photographs 3.1 to 3.3). When flower senescence was used as the criterion of senescence, there was still a significant difference in the vase life of the two treatments (Table 3.4). In the stems kept in distilled water, phyllode desiccation and flower senescence occurred at the same time, whereas phyllode desiccation occurred over 4 d later than flower senescence in citric acid. There was no difference in the visible signs of flower senescence for either of the treatments: the flowers opened fully in both treatments, followed by a rapid (i.e. within 1 d) wilting of stamens.



Photograph 3.1. *A. amoena* flowering cut stems after 2 d of vase life. (**A**) Stem kept in distilled water. Note the dried, curled phyllodes and the shrunken appearance of the globular flower heads. (**B**) Stem kept in citric acid (10 mol m^{-3}). The phyllodes are fresh and the globular flower heads have a fluffy appearance.

Photograph 3.2. A close-up of an *A. amoena* phyllode after 2 d in distilled water. Note the prominent venation and light colour of the phyllode, which is indicative of desiccation, compared with fresh phyllodes (Photograph 4.1) and those kept in citric acid (10 mol m^{-3}) (Photograph 3.3).



Photograph 3.3. A close-up of an *A. amoena* phyllode after 2 d in citric acid (10 mol m^{-3}). The phyllodes are a dark green colour and no venation is prominent.

Table 3.4

Vase life of *A. an oena* in distilled water and in citric acid

Treatment	Vase life of phyllodes (d) ± SE◊	Range (d)	Vase life of flowers (d) ± SE◊	Range (d)
T1: Distilled water	2.4[b]* ± 0.155	2 - 3	2.4[b]* ± 0.155	2 - 3
T2: Citric acid (10 mol m^{-3})	7.6[a] ± 0.514	5 - 11	3.2[a] ± 0.126	3 - 4

◊ Vase life is the mean of 10 replicates per treatment; SE = standard error.

* Within each vase life criterion, numbers followed by different letters are significantly different from each other ($P < 0.05$).

The following graphs of the vase life parameters of solution uptake, transpiration, fresh weight, stem water content and net water loss (Figs. 3.6 - 3.10) all reflect the beneficial effect obtained with citric acid (10 mol m^{-3}) compared with distilled water.

After 1 d, solution uptake (Fig. 3.6) was significantly greater in citric acid (10 mol m^{-3}) than in distilled water. Solution uptake decreased markedly in stems kept in distilled water, whereas those in citric acid maintained their initial uptake rate for 3 d. A sudden decrease in solution uptake occurred between days 3 and 5 in citric acid, however, these stems took 11 d to reach the uptake rate attained by distilled water stems in 3 d.

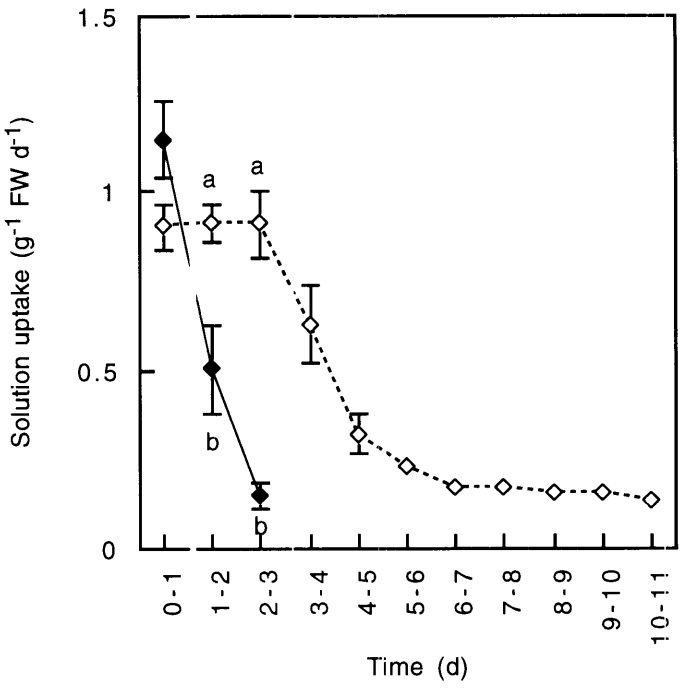


Fig. 3.6. The rate of solution uptake (expressed per gram of fresh weight per day) during the vase life of *A. amoena* stems kept in either distilled water (◆) or citric acid (10 mol m^{-3}) (◇). Within each day, symbols followed by letters indicate significant differences between treatments ($P < 0.05$). (Where no error bar appears, the SE was smaller than the size of the symbol)

The transpiration rate of stems in distilled water (Fig. 3.7) decreased rapidly from day 0-1, whereas in citric acid, the rate rose for the first 3 d. Transpiration of stems kept in citric acid declined after day 2-3, and reached the level of the distilled water stems 2 d later, i.e. after 4-5 d.

Stems in distilled water exhibited a marked and sudden decline in fresh weight (Fig 3.8) during vase life, particularly between days 1 and 2, compared with stems in citric acid. There was a significantly slower decline in fresh weight for stems in citric acid, and these stems took 10.5 d to reach the level of fresh weight attained by distilled water stems in 3 d.

There was a significant decrease in the stem water content of stems kept in distilled water compared with those in citric acid (Fig. 3.9). The greatest decrease in water content occurred between days 1 and 2 for stems in distilled water, whereas stems in citric acid exhibited an initial rise which, for the first 2 d, was greater than the initial water content. After day 2, there was a slow and steady decline in water content for the stems in citric acid. These stems took 11 d to reach the same level that stems in distilled water had reached in 3 d.

The net water loss of stems kept in either citric acid or distilled water (Fig. 3.10) was significantly different for the three comparable days (stems in distilled water were all dead by day 3, so no further readings were taken). Stems kept in distilled water lost more water and at a significantly faster rate than their citric acid counterparts. The amount of water lost from stems in citric acid remained close to the day 1-2 amount for most of the 11 d.

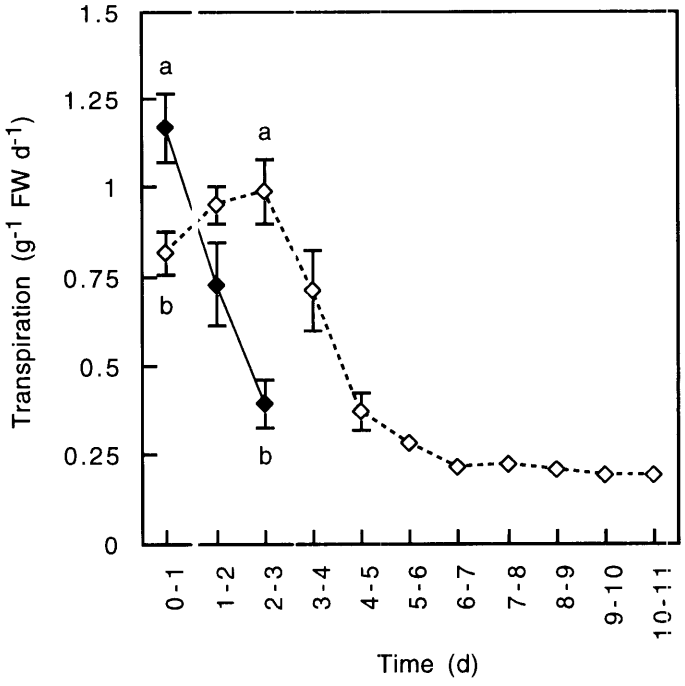


Fig. 3.7. The rate of transpiration (expressed per gram of fresh weight per day) during the vase life of *A. amoena* stems kept in either distilled water (◆) or citric acid (10 mol m^{-3}) (◇). Within each day, symbols followed by letters indicate significant differences between treatments ($P < 0.05$). (Where no error bar appears, the SE was smaller than the size of the symbol.)

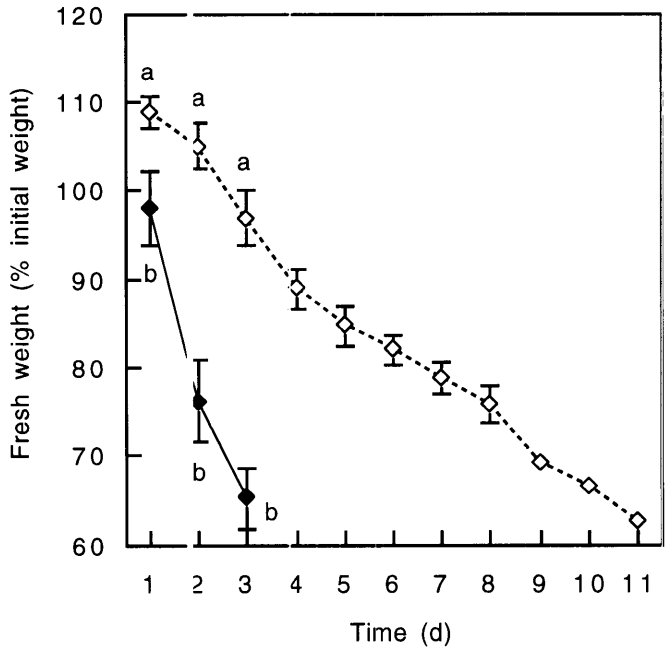


Fig. 3.8. The change in fresh weight (expressed as a percentage of the initial weight) during the vase life of *A. amoena* phyllodes kept in either distilled water (◆) or citric acid (10 mol m^{-3}) (◇). Within each day, symbols followed by letters indicate significant differences between treatments ($P < 0.05$). (Where no error bar appears, the SE was smaller than the size of the symbol)

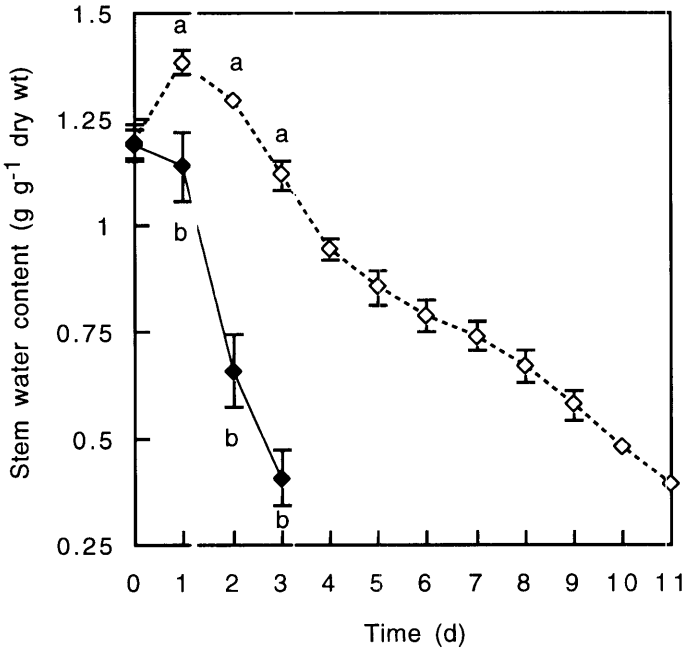


Fig. 3.9. The change in stem water content (expressed in grams of water per gram of dry weight) during the vase life of *A. amoena* stems kept in either distilled water (◆) or citric acid (10 mol m^{-3}) (◇). Within each day, symbols followed by letters indicate significant differences between treatments ($P < 0.05$). (Where no error bar appears, the SE was smaller than the size of the symbol.)

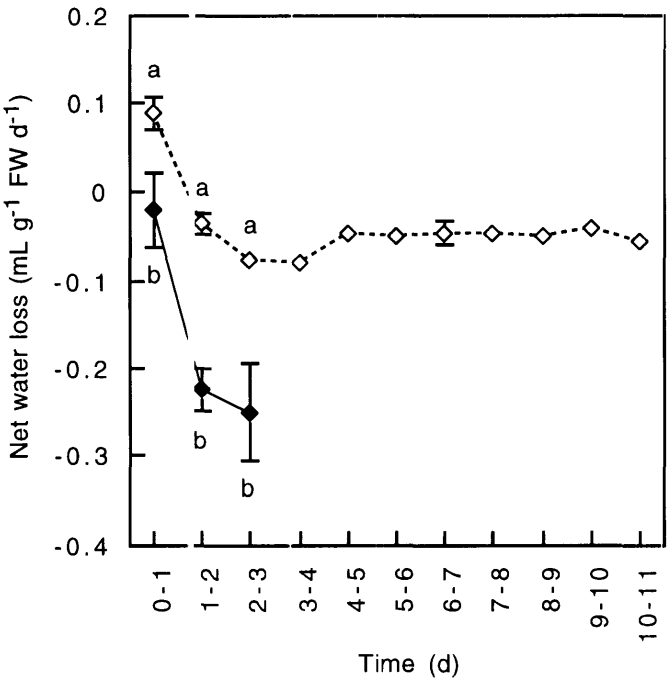


Fig. 3.10. The rate of net water loss (expressed in mL per gram of fresh weight per day) during the vase life of *A. amoena* stems kept in either distilled water (◆) or citric acid (10 mol m^{-3}) (◇). Within each day, symbols followed by letters indicate significant differences between treatments ($P < 0.05$). (Where no error bar appears, the SE was smaller than the size of the symbol)

3.4 DISCUSSION

Cut flowering stems of *Acacia subulata* and *A. floribunda* exposed to 50 and 500 μL L^{-1} exogenous ethylene respectively, did not have significantly different vase lives from those of control stems kept in distilled water without ethylene (Tables 3.1 and 3.2). Application of STS, an inhibitor of ethylene action, did not increase longevity in the presence of ethylene. Furthermore, there was no difference in the visual onset of floral senescence in any of the treatments or ethylene concentrations. Woltering and van Doorn (1988) stated that "the effects of STS are considered to be evidence for the presence or absence of a role of ethylene in the natural senescence of flowers". Therefore, from the results of the two ethylene vase life experiments, and the lack of vase life improvement when two inhibitors of ethylene action (STS and ethanol) were used (Table 3.3), floral senescence in the two species of *Acacia* tested is not mediated by ethylene.

In contrast, however, Jones and True tt (J. Tuett, pers. comm. 1993) found that the vase life of *Acacia baileyana* F. Muell. was shortened in the presence of ethylene (50 μL L^{-1}). No flower drop occurred, but the flowers had a shorter vase life after ethylene exposure. Thus, different *Acacia* species may possess different ethylene sensitivities. Such variations are not uncommon in the plant kingdom (Phan 1963; Mor *et al.* 1989; Reid *et al.* 1989; Wu, Zacarias and Reid 1991). It is now evident that sensitivity to ethylene varies within the genus *Acacia*, so the response to ethylene should be tested when conducting any vase life experiment with species other than those

listed here. The response of ethylene-sensitive *Boronia heterophylla* to an STS pulse revealed that the prevention of ethylene action was the most important factor in improving vase life in ethylene-sensitive species (Chapter 6). Therefore, it is imperative that any preliminary vase life experiment should determine the ethylene-sensitivity of the test species if it is not known.

In view of the differing *Acacia* spp. and *Mimosa pudica* reactions (section 3.1), as well as the varying effects observed with rose cultivars (Mor *et al.* 1989; Reid *et al.* 1989), it appears unlikely that classification of the reaction to ethylene may be as simple as occurring at the family level (Woltering and van Doorn 1988). Nevertheless, these different effects give some scope for breeding against, or genetic manipulation of, ethylene sensitivity.

No detectable ethylene was produced by *A. subulata* and *A. floribunda* during the 24 h enclosure period. It could be argued that *Acacia* flowers at Stage 2 of floral development may be too young to produce critical levels of ethylene. However, older flowers on the stem, although not directly part of the study, might have reached a higher stage of ethylene production, and yet no ethylene was detected. In ethylene-producing flowers, production is known to increase as the flowers age (Whitehead *et al.* 1984), as does sensitivity to exogenous ethylene (Camprubi and Nichols 1978). Camprubi and Nichols (1978), working with ethylene-sensitive carnations, found that doses of exogenous ethylene (10 ppm) induced ethylene production and hastened wilting. However, no ethylene was detected in either of the *Acacia* experiments (Appendix D) in which 10-fold differences in ethylene concentration were used.

Although ethylene production was found in senescing carnations after 0 and 24 h of enclosure (Appendix D), it is possible that the gas chromatograph was not sensitive enough to detect any extremely low levels of ethylene that may have been produced in *Acacia*. In initial experiments with daylily (*Hemerocallis* hybrid 'Cradle Song'), Lukaszewski and Reid (1989) were unable to detect the production of any ethylene, but when Lay-Yee *et al.* (1992) maximised apparatus sensitivity, they found that ethylene was produced, albeit in extremely low quantities (<1 nL g^{-1} h^{-1}). However, they questioned the role of such small amounts of ethylene, particularly as exogenously applied ethylene and inhibitors of ethylene biosynthesis or action had no effect on flower senescence (Lukaszewski and Reid 1989).

In the *A. floribunda* vase life experiment (Table 3.3), the two inhibitors of ethylene action, STS (T3) and ethanol (T6), did not improve longevity above that of distilled water. Those two treatments resulted in some of the poorest vase lives of the experiment, whereas ethylene-unrelated treatments such as citric acid (T2) and stabilised chlorine, SDIC (T4) improved longevity. Thus, it is unlikely that ethylene, or the inhibition of ethylene action, is a factor in the senescence of *A. floribunda*. It should be stressed that the object of the *A. floribunda* vase life experiment was not to find a 'magic' solution which would result in an increased vase life but,

rather, to determine the effect of the various vase solutions in an attempt to elucidate the cause/s of premature senescence in *Acacia*.

Accati and Sulis' solution with two different sucrose concentrations (2%: T7 and 5%: T8) was used to find out whether the beneficial results they obtained with *Acacia dealbata* 'Rustica' and 'Gaulois' would occur with *A. floribunda*. The solution has been recommended to increase *Acacia* vase life (Sedgley 1989; Sedgley and Parletta 1993), as Accati and Sulis (1980) found that their solution, with sucrose concentrations of both 1% and 5%, gave the longest vase life (6.0 d), whilst water (the quality was not mentioned) resulted in the shortest vase life of 2.0 d. The beneficial effects of Accati and Sulis' solution with *A. dealbata* were not repeated in the present experiment with *A. floribunda*. Both T7 (containing 2% sucrose) and T8 (containing 5% sucrose), with vase lives of 1.3 d and 2.1 d respectively, were no better than the ethanol (T6), STS (T3), or distilled water (T1) treatments, all of which produced the poorest vase lives. Lack of availability of material precluded examination of the two *A. dealbata* cultivars used by Accati and Sulis (1980) under standardised vase life conditions.

Accati and Sulis (1980) conducted two experiments. They determined optimal conditions for opening at two stages of floral development (equivalent to Stages 1 and 4; section 3.2.7); and they recorded the vase life response to several sucrose concentrations. Successful opening was achieved at 25°C and 70 to 90% RH with all sucrose concentrations except 20%[2]. Accati and Sulis' vase life experiment was conducted at 18 to 20°C, but the relative humidity was not stated. The recommended levels for determination of vase life are 20°C and 60% RH (Sytsema 1975; Reid and Kofranek 1980). Differences in humidity levels could explain the poorer results obtained in the present *A. floribunda* experiment. If a higher humidity was used by Accati and Sulis (1980), it would have slowed down water loss from the plant, a factor which, indirectly, is likely to be one of the major reasons for the premature senescence of *Acacia*. As dry air has a greater capacity for water vapour, evidenced by water potential differences (at 20°C) of -14.2 MPa at 90% RH and -93.5 MPa at 50% RH (Salisbury and Ross 1985), a much steeper water potential gradient would exist at 60% RH compared with 90% RH. The consequences of impaired water uptake appear only too readily in *Acacia* (Table 3.4, Figs. 3.6 - 3.10), and the results of Chapter 4 reveal that *Acacia* is particularly vulnerable to cavitation in distilled water. Therefore, if water loss was minimised via a reduction in transpiration, which would occur at high relative humidities, vase life could well have been prolonged. Jones *et al.* (1993), studying *Thryptomene*, a woody member of the Myrtaceae, found that high RH (95%) resulted in a significantly greater vase life than 60% RH. Water content was also significantly greater in stems kept at 95% RH than in other treatments after 3 d.

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[2] A similar forcing procedure has been practised in France for over 30 years. The stems are kept in cold water for 48 h in a dark, warm (22 to 25°C) and humid (85 to 90% RH) room (de Ravel d'Esclapon 1962).

The reason for the inclusion of $AgNO_3$ in Accati and Sulis' solution is uncertain, as already in the solution were two well-known bactericides, 8-HQC and $Al_2(SO_4)_3$. Silver, in the form of $AgNO_3$, is a very effective bactericide. The anti-microbial activity of the silver ion has largely been overshadowed by its effect as an inhibitor of ethylene action (Goszczynska and Rudnicki 1988), but this is in the form of STS, which has greater stem mobility than $AgNO_3$ (see section 1.2.6.1). Nevertheless, the action of $AgNO_3$ in Accati and Sulis' solution was not one of prolonging vase life in the present *A. floribunda* experiment. In view of the very poor vase life results obtained, as well as the toxicity of silver and 8-HQC, caution should be exercised in recommending this solution as an all-purpose *Acacia* floral preservative.

The reasons for the beneficial effect of citric acid in improving *Acacia* vase life (Tables 3.3 and 3.4), solution uptake (Figs. 3.1 and 3.6), transpiration (Figs. 3.2 and 3.7), fresh weight (Figs. 3.3 and 3.8), stem water content (Figs. 3.4 and 3.9), and reducing water loss (Figs. 3.5 and 3.10) will be explored more fully in Chapters 4 and 5. Suffice it to say here that citric acid is a common component of vase solutions, used to decrease the pH and thus improve water uptake (Durkin 1980). The low pH is also thought to inhibit the growth of bacteria (Reid 1987) and remove air embolisms (Durkin 1986a; b). It is thus not surprising that citric acid had a beneficial effect on longevity, although it should be noted that in some flowers, violets and primulas, it produces an unfavourable response (Fourton and Ducomet 1906). Nevertheless, it extends the vase life of lupins (Mohan Ram and Ramanuja Rao 1977), other *Acacia* species (Williamson 1989), maidenhair fern (*Adiantum raddianum*) (van Doorn, Zagory and Reid 1991) and improves water uptake in roses (Durkin 1979a; b).

Stabilised chlorine, in the form of sodium dichloroisocyanurate (SDIC), used alone (T4), or in combination with STS and citric acid (T5), resulted in longer vase lives than any of the other treatments. This bactericide has been found to increase vase life in roses (van Doorn, de Witte and Perik 1990), chrysanthemums and snapdragons (Halevy and Mayak 1981). Because of the detrimental effect on longevity when STS was used alone (T3), it is unlikely that this component of T5 played any beneficial role. Instead, the increased longevity from this solution is attributed to the inclusion of SDIC and citric acid, since both these solutions resulted in significantly longer vase lives.

In the *A. amoena* vase life experiment, citric acid always significantly improved the measured vase life parameters compared with distilled water. This was associated with a significantly longer phyllode and flower vase life (Table 3.4) for citric acid-kept stems. The temporal differences between vase life phyllode and flower death (Table 3.4), perhaps reflect the fragility and ephemerality of *Acacia* flowers. The flowers are, most conspicuously, a collection of showy stamens. In contrast, the four or five petals are small and inconspicuous, as are the small, basally connate sepals (Williamson 1989).

Percival (1965) stated that the function of an androecium is to produce and present the pollinator attractants of colour and scent. The life span of stamens is shorter than any other floral organ (unless premature petal abscission occurs in response to ethylene), possibly because it may not be energetically advantageous for the plant to maintain these organs once their attractive role has been fulfilled. The termination of *Acacia* vase life is determined by the appearance of the stamens which, at Stage 6 of floral development (section 3.2.7), show the first signs of browning, desiccation and disorderliness. Stamen desiccation is indicative of water stress, the latter being the most common reason for the termination of vase life (Halevy 1976). It is for this reason that the phenomenon of water stress in *Acacia* will be investigated in several ensuing chapters.