CHAPTER FOUR

CAVITATION EVENTS, XYLEM WATER POTENTIALS, RELATIVE WATER CONTENT AND BACTERIAL NUMBERS OF *ACACIA AMOENA* STEMS IN SOLUTIONS

4.1 INTRODUCTION

Elucidation of the factors involved in postharvest senescence has been the subject of cut flower research for nearly 100 years. Senescence symptoms are diverse and often species-specific (section 1.2.6). However, whilst the factors are varied, and there is at present no consensus on the first event to occur, most researchers agree on the importance of an unimpeded water supply for promoting cut flower longevity. External senescence indicators such as floral and vegetative wilting are frequently associated with impaired water uptake. Therefore much research has been directed towards the presence of vessel-occluding substances such as micro-organisms (Rasmussen and Carpenter 1974; Zagory and Reid 1986a, b; Put and Clerkx 1988; Put and van der Meyden 1988; Clerkx *et al.* 1989; Put and Jansen 1989; Put and Rombouts 1989; van Doorn *et al.* 1989; Put 1990; van Doorn, Clerkx and Boekestein 1991a; van Doorn, Zagory and Reid 1991) and the origin of vessel-plugging materials (Durkin and Kuc 1966; Burdett 1970; Parups and Molnar 1972; Lineberger and Steponkus 1976). However, the limited extent of these occlusions (Rasmussen and Carpenter 1974), and the time required for their formation, is not consistent with observed declines in hydraulic conductance, which are measurable from the time the stem is cut from the plant (Durkin and Kuc 1966; Carpenter and Rasmussen 1973; 1974; Mayak *et al.* 1974; Zimmermann 1978; Rappel 1985; Sperry *et al.* 1988; Williamson 1989).

Durkin and Kuc (1966) observed that losses in petal turgidity and fresh weight were preceded by decreased rates of water uptake. This indicated that reduced water uptake, rather than excessive water loss, was the cause. Rappel (1985) observed that the wilting of cut *Acacia* flowers was caused by an internal restriction to water uptake; however, the nature of the restriction was unknown. Stem blockage has been ascribed by several authors to unknown "physiological factors" (Mayak *et al.* 1974; Halevy and Mayak 1981; Put and van der Meyden 1988).

Stocking (1948) observed that floral vase life could be increased dramatically through removal of air by suction from cut xylem conduits, thereby enhancing continuity between xylem sap and the vase water. Durkin (1979a, b; 1980) showed that degassed water and citric acid increased hydraulic conduction, but did not determine the basis of this effect. He suggested that xylem embolisms inhibited water uptake by cut stems, but commented that embolised conduits would

be difficult to identify. Yet, long ago, Haines (1935) frequently observed air bubbles (presumably induced by embolisation) in the xylem of several plant species; and Milburn and Johnson detected cavitation acoustically in 1966.

Despite these indications of xylem sap cavitation in cut flowers, no direct studies have been made on cut stems kept in water over a sufficient period of time for monitored cavitation to occur. Milburn (1973) noted that cavitation was not detected to any extent when excised *Ricinus* leaves were kept in water. However, the introduction of indian ink suspensions caused cavitation to begin within 2 min. Dixon *et al.* (1988) measured the extent of cavitation when fully hydrated rose stems lost water in air; and Dixon and Peterson (1989) estimated cavitation indirectly in roses, by using a fluorescent tracer to reveal ratios of conducting to non-conducting conduits. However, the latter method does not take into account the number of conduits which may have already cavitated *in situ*, as has often been detected by the author in *Acacia*. De Stigter and Broekhuysen (1989) hypothesised that secondary' embolisms developed later in vase life, either from air liberated from dissolution in the vase water, or from cavitation within xylem conduits.

The aims of the experiments described in this chapter were to:

- determine whether acoustic emissions, indicating cavitation, could be detected directly whilst excised *Acacia* stems remained in water; thus providing evidence to explain losses in hydraulic conductance and decreased water uptake, despite an adequate water supply;

- determine the effect on the induction of cavitation of various treatments, including chemicals commonly added to vase solutions;

- measure the water status of cut *Acacia* stems in various treatment solutions, using indicators such as xylem water potential and relative water content; and

- enumerate the bacteria in vase solutions during cavitation monitoring to investigate the relationship between cavitation events (audible acoustic emissions, or AAE) and bacterial numbers in the vase solution.

4.2 MATERIALS AND METHODS

4.2.1 Plant material

Terminal shoots of *A. amoena* were used for cavitation detection studies. This species was chosen so that initial cavitation experiments begun on the species (Williamson 1989) could be

extended. The wide phyllodes (4.5 cm × 1.0 cm) of *A. amoena* were suitable because they provided a large surface area for transpiration (Photograph 4.1). Furthermore, none of the phyllodes was in contact with another, thereby removing a potential source of extraneous noise which could have arisen from 'contact' friction between phyllodes.

Photograph 4.1. Terminal shoot of *A. amoena*. Note the wide, discrete phyllodes.

4.2.2 Preparation of plant material for acoustic detection

The cavitation detection studies were performed on vegetative *A. amoena* shoots to avoid the possible distortion of AAE recordings by insect-produced noise, as first instar scale larvae (Super Family Coccoidea) were frequently found amongst the globular inflorescences of *Acacia* (Williamson 1989). Before the stems were recut under water, they were sprayed with a jet of distilled water to remove insects, then examined under a dissecting microscope to detect the

presence of larvae or insects. Any .arvae or insects were removed with dissecting needles or forceps.

Stems of similar weight and dimeiision were cut and brought to full turgor when required (sections 2.1 and 2.2). Hydrated plant material was used for all cavitation detection experiments. The recut, fully hydrated stem to be monitored was transferred to a beaker containing approximately 4 cm dep.h of solution. A total of 10 stems (i.e. 10 replicates) was used for all the cavitation detection e<periments, except where stated otherwise.

4.2.3 Acoustic monitoring

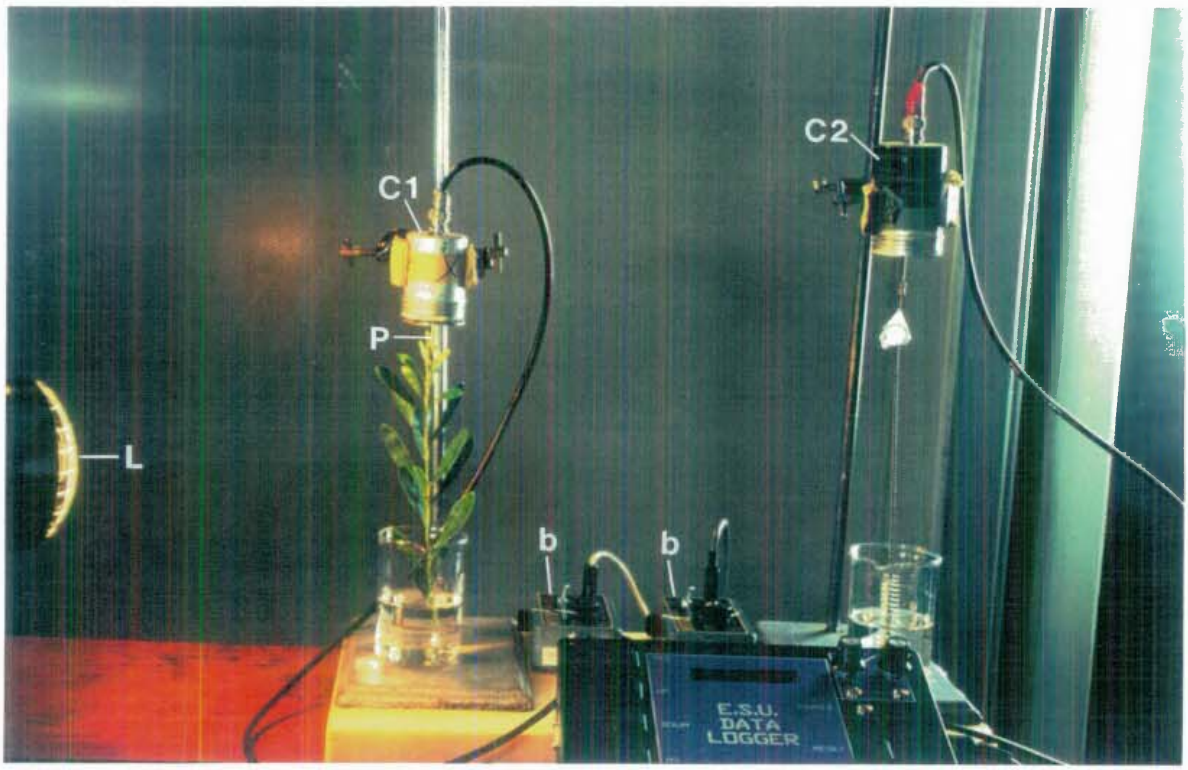
Xylem sap cavitation was detected ısing a method similar to that described by Crombie *et al.* (1985b), except that recently improved magnetic probes, amplifiers and loggers were utilised (J. Milburn, T. Brown and R. Kenny, p.rs. comm. 1993). (Circuit diagrams of the data logger and preamplifiers are shown in Appendi> E.)

The probe wire was inserted into the xylem region at right angles near the lengthwise mid-point of the stem to be monitored. This magnetic probe converted audio signals propagated by the tissue into electronic signals. The.e analog signals were then amplified and converted into digital signals. The threshold of the amplifier was set so that cavitation events were recorded singly, thus reducing multiple triggering to a minimum. The digital signals from the cavitation events (AAE) were recorded automa ically and continuously on a data logger over a 5-day period and then tabulated by computer.

A matched duplicate probe was set up with a dummy load replacing a plant stem. The two probes functioned as a coincidence counter, so that extraneous electrical or acoustic interference could be determined. Coincident signals appearing on both channels were then subtracted from the stem probe counts. The data logger had a headphone attachment, which facilitated the calibration process and also enabled checks of AAE counts to be made during the experiment. The apparatus stood on a 4-cm-thick foam pad within an acoustically isolated chamber (Model No. 105543, Industrial Acoustics Company, Inc., Bronx, New York). A light source (100 W incandescent bulb, 24 µmol m^{-2} s^{-1}) was placed 30 cm away from the stem to enhance the rate of transpiration. The cavitation detection set up is shown below (Photograph 4.2).

4.2.4 Calibration of acoustic detection equipment

The acoustic detection equipment was calibrated to ensure that each 'click' (i.e. cavitation event, or AAE) registered as only one count on the data logger. Two calibration methods were employed and each was cross-checked against the others to ensure accuracy. A vascular bundle



Photograph 4.2. Cavitation detection apparatus. The photo was taken through the window of a sound proof chamber. A light source (L) is 30 cm away from the plant stem, the base of which remains in a solution throughout the experiment. A wire probe (P) is inserted into the xylem of the stem, and the 'clicks' (or AAE) made by the stem as it cavitates are propagated along the wire. There are two sound detection channels: the channel on the left (C1) detects AAE made by the stem; the channel on the right (C2) functions as a coincidence counter, detecting any extraneous electrical or acoustic noise. Any counts appearing on both channels are then subtracted from the counts of the channel with the stem. Audio signals (acoustic energy) from the plant are converted into electronic signals (electrical energy) by an electromagnetic transducer (located in the suspended metal jars, C1 and C2), amplified and converted into digital signals (in the small black boxes, B), which are recorded on the data logger.

of *Plantago major* L., which had previously been isolated from the leaf and stored in $HgCl_2$ (0.1% w/v), was wound around the wire of the probe. A 100 W incandescent light bulb (24 $\mu mol\ m^{-2}\ s^{-1}$) was placed 30 cm away from the vascular bundle to enhance the rate of water loss. A set of headphones was plugged into the data logger and the clicks monitored by ear per time period (1 min) as the bundle dried out were recorded manually and checked against those recorded by the data logger. The other method involved running a pen along the twisted wire handle of a 'bottlebrush' cleaner and checking that the number of clicks registered on the data logger corresponded with the number of twists along the wire handle. An oscilloscope (Model DSS 6521, Kikusui Electronics Corp., Japan) was used to check that each click/twist registered as a pulse on the wave form monitor. The oscilloscope was also used to check that the wave form of each click was similar on both channels.

4.2.5 Limitations of the equipment

The data logger had a blanking time of 30 ms incorporated into the software program. Thus, following acquisition of a pulse (= click), a second click occurring within that time would not

register. In practice, such close cli:ks would occur only occasionally. However, a blanking time was necessary to ensure discre:e single pulses, without repeated pulses from the 'ringing' of the first event.

4.2.6 Measurement of leaf temperature during acoustic monitoring

During cavitation experiments in light, leaf (phyllode) temperatures at both base and top of the stem were monitored every 12 h with an infrared temperature sensor (Model 130AHCS, Everest Interscience Inc., California). The average leaf temperature was 23±2°C, which was within the normal physiological range of plant function during cavitation experiments in the light.

4.2.7 Determination of the relative water content of stems used in acoustic detection experiments

The Relative Water Content (RWC) of cut stems was calculated according to Weatherley's (1950) equation[1]:

$$RWC\ (\%) = 100 \times \frac{F - D}{T - D} \qquad (4.1)$$

where F is the fresh weight;
D is the dry weight (see section 2.6); and
T is the fully turgid weight (see section 2.2).

RWC was not determined on comparable stems via the standard 'evaporative desiccation method' of bench drying and regular weighings (Jones and Higgs 1979) because preliminary experiments revealed that this method gave results which were vastly inconsistent with the RWC of stems kept in solution (Fig. 4.1). Stems dried in air reached a RWC of 34% after 12 h, whereas stems kept in water reached 34% after 98 h. Excised stems exposed to air lose water via evaporation from the cut end as well as through the leaves (Ritchie and Hinckley 1975). Because these two sources of water loss do not represent what occurs in a vase, the evaporative desiccation method was not used.

If the latter method (see Fig. 4.1 description) had been used, inaccuracies would have occurred in the cavitation results because of intermittent exposure to air during weighing, when the stems were removed from their solutions. Therefore, the RWC of stems was not determined throughout the experiment, but was ascertained at the completion of the cavitation detection experiments.

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[1] This equation was originally defined by Weatherley (1950) as Relative Turgidity. He later changed it to RWC on the suggestion of Barrs (1968), because the measured value does not necessarily express turgor (Catsky in Slavík 1974).

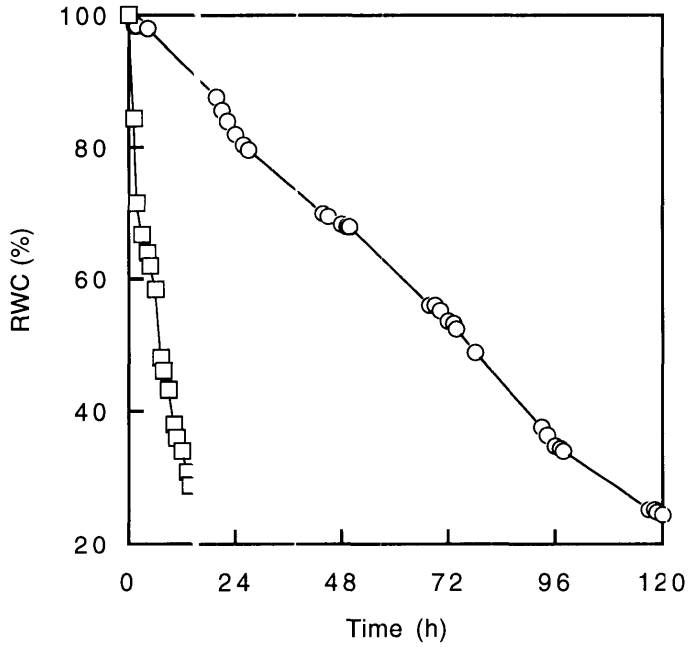


Fig. 4.1. A comparison between two methods of determining the RWC of *A. amoena* stems. □ = stem kept in air at room temperature and weighed regularly (the evaporative desiccation method, described by Jones and Higgs 1979); O = stem base kept in water at room temperature; removed from water, quickly dried and weighed, then returned to water; this same weighing procedure was repeated at regular intervals.

The construction of pressure-volume curves (Scholander *et al.* 1964; Hammel 1967; Tyree and Hammel 1972) to relate balancing pressures of *A. amoena* to particular RWCs, was not undertaken because previous experiments (Williamson 1989) had revealed that the overpressurisation method took an inordinate amount of time (cf. Tyree and Hammel 1972, who found that stabilisation occurred after 60 min) and required constant monitoring. Crombie (1983) also abandoned the method for similar reasons and found that evaporative water loss from the collection phials was a serious source of error. Jones and Higgs (1979) also reported finding discrepancies between the overpressurisation and evaporative desiccation methods.

4.2.8 Monitoring the change in solution bacterial numbers during acoustic detection experiments

Bacterial numbers in distilled water, citric acid and DICA solutions used in acoustic detection experiments were monitored at days 1, 3 and 5. One mL aliquots were taken with a sterile pipette from the solutions. The number of bacteria (colony forming units per mL, cfu mL^{-1}) was then determined by preparing a dilution series and plating out the diluent onto Plate Count Agar (Oxoid Ltd., Hampshire). (See sections 6.2.4 and 6.2.5 for details of the methods.)

4.2.9 Analysis of data

Means of RWC data and solution bacterial numbers were checked for normality, and heterogeneity of the variances using Cochran's test (Winer 1971; Underwood 1981).

Nonhomogeneous data were trans ormed using, as appropriate, square roots, cubic roots, quadratic roots or log transformtions (Appendix C). Means were then separated using Scheffé's test (StatView 4.0) at $P < 0.05$.

4.3 RESULTS

4.3.1 Cavitation and xylem water potential of *Acacia amoena* stems in distilled water and light

The relationship between xylem wat r potential and AAE production over time for stems kept in distilled water is shown in Fig. 4.2 After 24 h the stems were only slightly desiccated; and xylem water potential had fallen to 1.5 MPa. Maximum AAE production occurred after 48 h, by which time the majority of stems (eight of 10) had become very dry and brittle, and the mean xylem water potential was -3.7 MPa Tangential longitudinal sections of the stems, cut after 72 h, revealed a large proportion of emb olised xylem conduits (see Photograph 4.4).

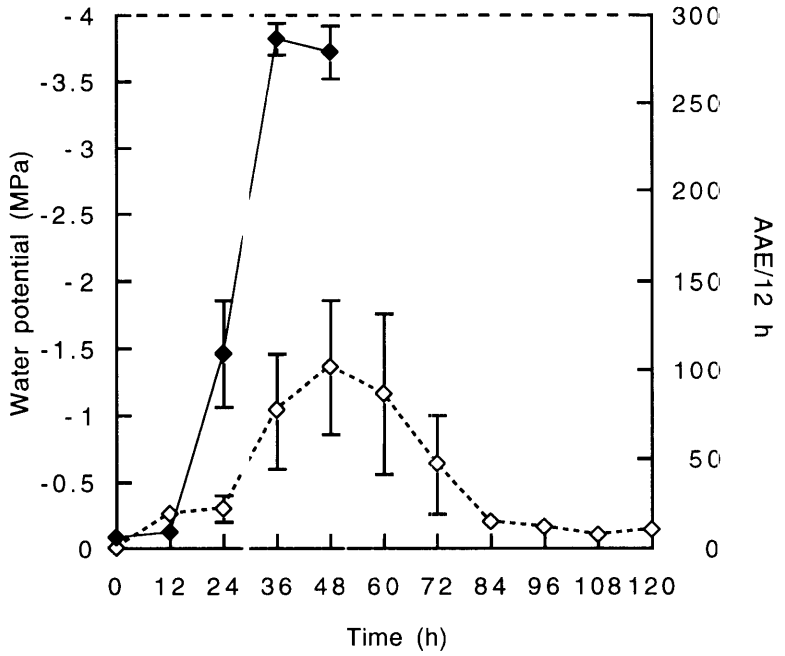


Fig. 4.2. The relationship between stem xylem water potential (MPa) and audible acoustic emission (AAE) production over time for *A. amoena* stems kept in distilled water. Water potential and AAE readings are both the means of 10 replicates ± standard error f r each data point. (Each replicate = 1 stem.) (Where no error bar appears, the SE was smaller than the size c f the symbol.) The dotted line across the top of the graph at -4.0 MPa indicates that the limit of the pressure regul tor in use was reached. Subsequent pressure readings could not exceed this figure and, to avoid the appearance of a stabilisation of pressure, no further readings were plotted. ◇ = AAE; ◆ = xylem water potential.

4.3.2 Cavitation of *A. amoena* stems in distilled water and darkness

The peak in AAE production for ste ns kept in distilled water without any light source occurred at 96 h (Fig. 4.3). This peak coinc ded wi h visible signs of water stress. Slight desiccation

was apparent after 72 h, and by 96 h the stems were very dry. After 72 h, white microbial growth was noted around the submerged basal 2 cm of stem. This growth resembled that observed on stems kept in citric acid solutions (see section 4.3.3 and Photograph 4.3). The cavitation profile of the distilled water and darkness treatment was similar to the degassed water and light treatment (Fig. 5.4).

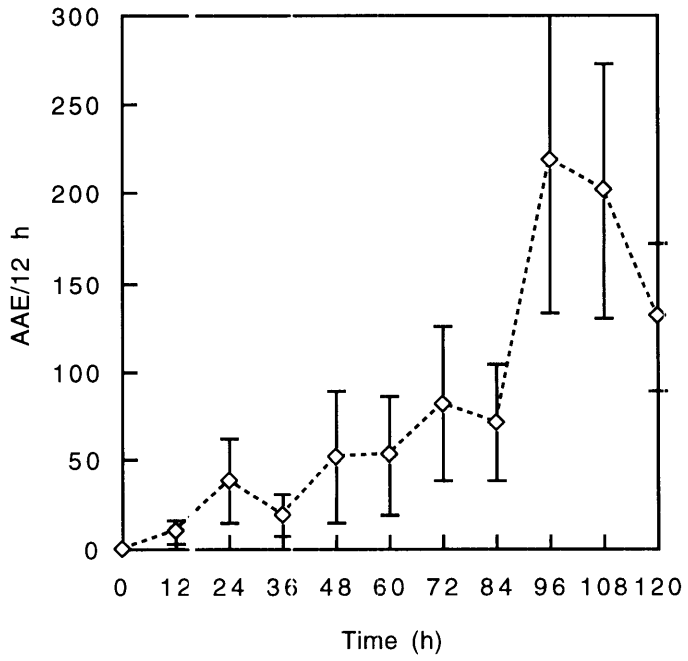


Fig. 4.3. The rate of AAE production for *A. amoena* stems kept in distilled water without a light source. Each data point is the mean of 10 replicates ± standard error. (Where no error bar appears, the SE was smaller than the size of the symbol.)

4.3.3 Cavitation and xylem water potential of *A. amoena* stems in citric acid (10 mol m^{-3})

While stems were kept in citric acid (10 mol m^{-3}) for 120 h, there was no discernible peak in AAE production. However, a marked decrease in xylem water potential occurred between 48 and 60 h: from -0.42 MPa to -2.62 MPa (Fig. 4.4). After 96 h, a white microbial growth was visible around the submerged basal 2 cm of stems in citric acid, but the stems nevertheless remained very fresh and turgid, indicating that the water supply had not been curtailed. Under microscopic examination, the microbial growth appeared to be a yeast with pseudohyphae and larger, thick-walled chlamydospores (Photograph 4.3). Although the yeast was not identified, such chlamydospores are typical of *Candida albicans*. Stems in citric acid began to dry out after 108 h.

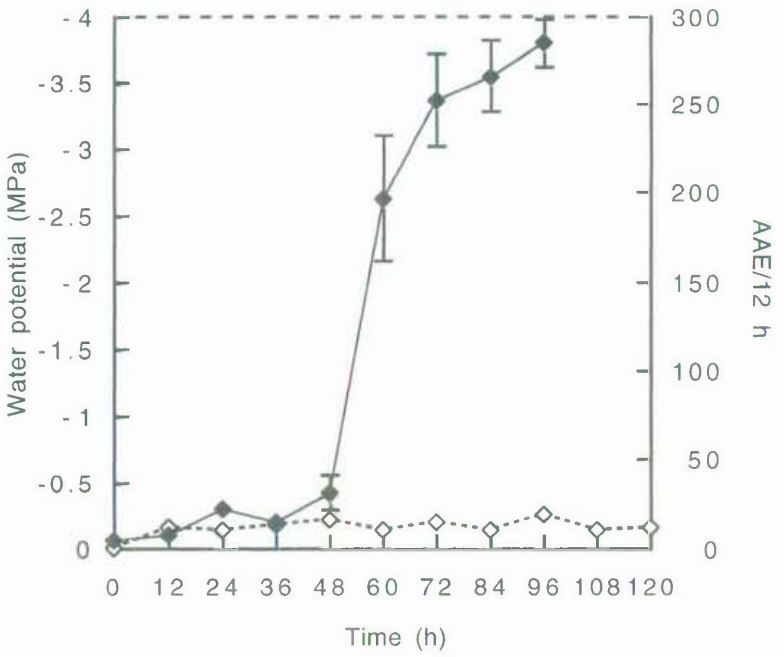
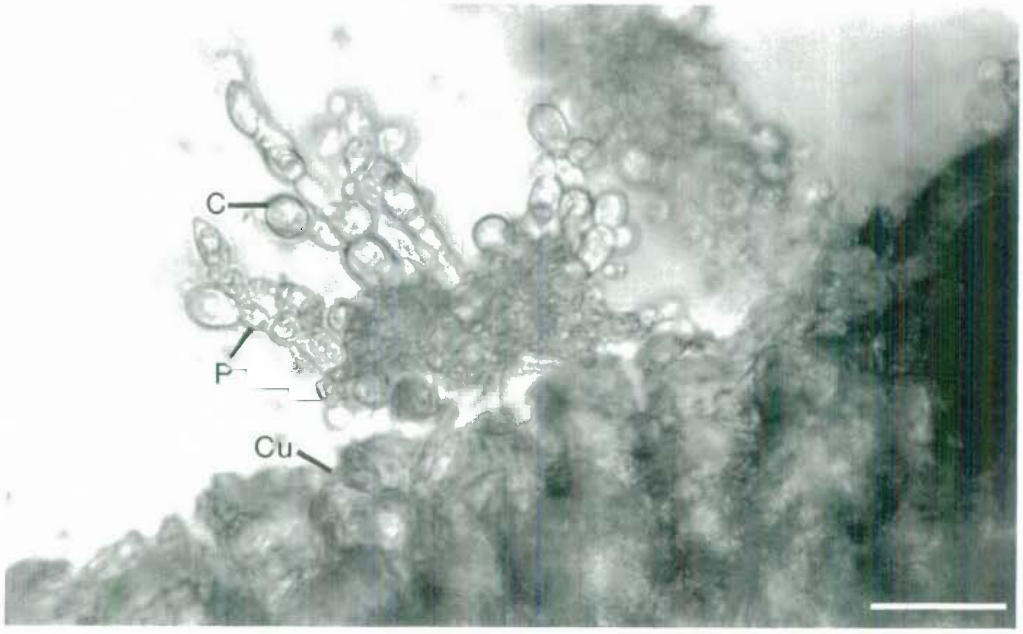


Fig. 4.4. The relationship between stem xylem water potential (MPa) and AAE production over time for *A. amoena* stems kept in citric acid (10 mol m^{-3}). Each data point is the mean of 10 replicates ± standard error. (Where no error bar appears, the SE was smaller than the size of the symbol.) The dotted line across the top of the graph at -4.0 MPa indicates that the limit of the pressure regulator in use was reached. Subsequent pressure readings could not exceed this figure and, to avoid the appearance of a stabilisation of pressure, no further readings were plotted. ◇ = AAE; ◆ = xylem water potential.



Photograph 4.3. A yeast with pseudohyphae (P) and larger, thick-walled chlamydospores (C) growing on the surface of an *A. amoena* stem kept in citric acid (10 mol m^{-3}) for 96 h. The tangential longitudinal section was cut by hand 1 cm from the stem base. Note the undulating nature of the cuticular surface (Cu). Numerous fungal spores and hyphae are frequently seen in these epidermal depressions under scanning electron microscopy (see Photograph 8.1 a). (bar = 20 μm)

4.3.4 Cavitation of non-turgid *A. amoena* stems in distilled water

When stems which had not been raised to full turgor were kept in distilled water, the rate of cavitation peaked after 96 h (Fig. 4.5), which was 48 h later than for turgid stems kept in distilled water. Stems began to exhibit the first signs of dryness (i.e. prominent phyllode venation; see Photograph 3.2 for a comparable level of phyllode desiccation) after 48 h, and by 72 h the stems were very dry (very prominent phyllode venation, phyllodes curled and light green; see Photograph 3.1a for a comparable level of phyllode desiccation).

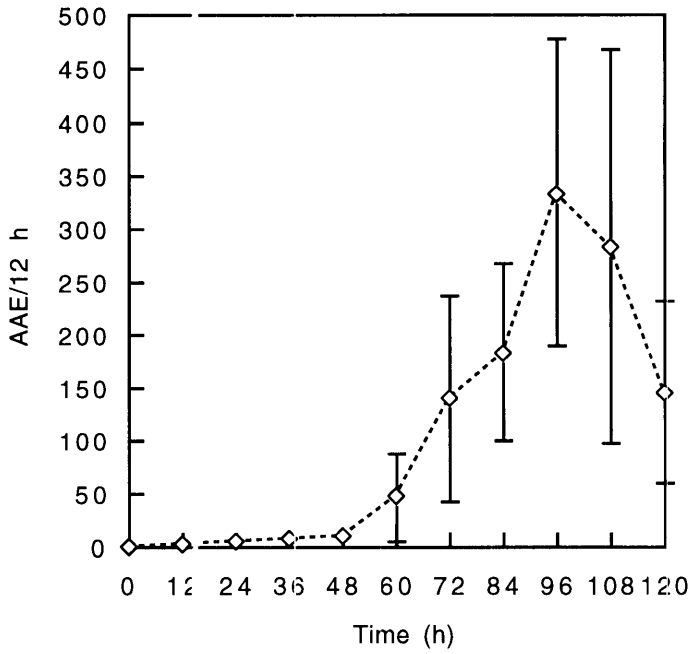


Fig. 4.5. The rate of AAE production for non-turgid *A. amoena* stems kept in distilled water. Each data point is the mean of five replicates ± standard error. (Where no error bar appears, the SE was smaller than the size of the symbol.)

4.3.5 Cavitation of *A. amoena* stems in stabilised chlorine (50 mg L^{-1} DICA)

The rate of AAE production while stems were kept in DICA (Fig. 4.6) was higher than that of all other treatments except the non turgid treatment (Fig. 4.5). Also, the peak in AAE production occurred at 120 h, which was later than in any other treatment. Although 120 h was the end of the experimental period, it is likely that the peak still would have occurred at 120 h even if further readings had been taken, as the rate of AAE production slowed down between 108 and 120 h. Furthermore, except for the citric acid treatment, all AAE production peaks exhibited a familiar parabolic pattern, and a decrease in AAE production after 120 h would fit that pattern. Stems in DICA began to dry out after 72 h, and were very dry (i.e. phyllodes light green and venation prominent; see, for example, Photographs 3.1a and 3.2) after 96 h.

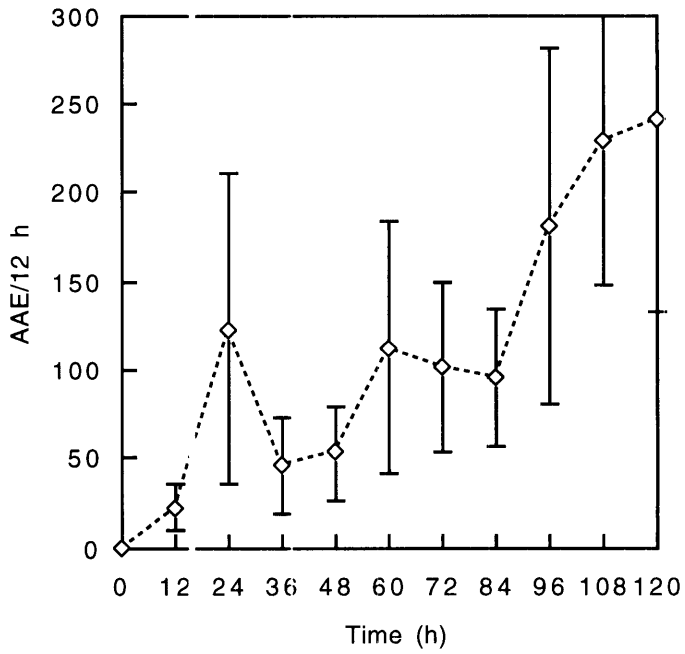


Fig. 4.6. The rate of AAE production for *A. amoena* stems kept in DICA (50 mg L^{-1}). Each data point is the mean of 10 replicates ± standard error. (Where no error bar appears, the SE was smaller than the size of the symbol.)

4.3.6 The relative water content of stems at the end of acoustic detection experiments

The RWC of stems at the conclusion of the 120 h acoustic detection experiments was significantly greater for stems kept in citric acid than for any other treatment (distilled water, DICA, and degassed distilled water; Table 4.1). The RWC of stems in citric acid was more than double that of stems in DICA, the next closest result. Stems in distilled water had the lowest RWC, and the result for degassed distilled water was intermediate between DICA and distilled water. The means of these last three treatments were not significantly different from each other ($P < 0.05$).

Table 4.1

Relative water content (RWC) of *A. amoena* stems at the end of cavitation experiments

Treatment	RWC (%) after 120 h
Distilled water (Fig. 4.2)	19.4±2.3[b]
Citric acid (10 mol m^{-3}) (Fig. 4.4)	71.4±4.7[a]
DICA (Chlorine) (Fig. 4.6)	34.6±4.5[b]
Degassed distilled water (Fig. 5.4)*	27.7±3.9[b]

Means followed by different letters are significantly different ($P < 0.05$).
Standard error is shown as ± value after mean.
* See section 5.3.3 for the cavitation graph of degassed distilled water.

4.3.7 Xylem water potentials under cavitation (incandescent) lighting conditions in flowering and vegetative *A. amoena* stems kept in distilled water and in citric acid (10 mol m^{-3})

The decreases in xylem water potentials of flowering *A. amoena* stems in distilled water and in citric acid had similar patterns, except that stems in distilled water exhibited greater water stress than those in citric acid from 12 h onwards (Fig. 4.7). For flowering stems, the limit of the pressure regulator (-4.0 MPa) was reached after 84 h in distilled water, whereas stems in citric acid did not reach this extreme during the 120 h experimental period. Within each treatment, flowering stems developed lower water potentials more rapidly than vegetative stems.

The xylem water potentials of vegetative *A. amoena* stems in distilled water and in citric acid were similar until 12 h (Fig. 4.7). After 12 h, the xylem water potential rose rapidly in stems kept in distilled water, whereas this did not occur until after 48 h in citric acid. Stems in distilled water reached the -4.0 MPa limit after 48 h, whereas in citric acid the stems did not reach -4.0 MPa until after 96 h.

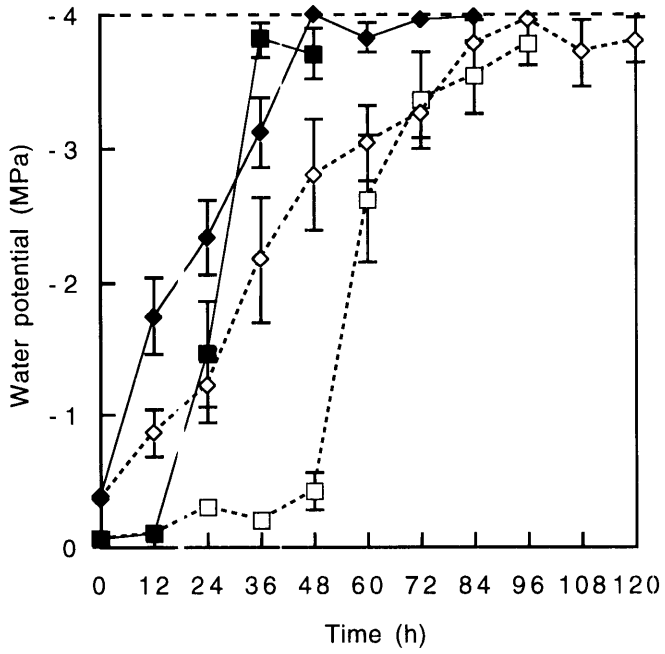


Fig. 4.7. Xylem water potentials of flowering and vegetative *A. amoena* stems kept under incandescent light (24 µmol m^{-2} s^{-1}). ◆ = flowering stems in distilled water; ◇ = flowering stems in citric acid (10 mol m^{-3}); ■ = vegetative stems in distilled water; □ = vegetative stems in citric acid (10 mol m^{-3}). Data are the means of 10 replicates ± standard error. (Where no error bar appears, the SE was smaller than the size of the symbol.) The dotted line across the top of the graph at -4.0 MPa indicates that the limit of the pressure regulator in use was reached. Subsequent pressure readings could not exceed this figure and, to avoid the appearance of a stabilisation of pressure, no further readings were plotted.

4.3.8 Xylem water pote ıtials under vase life (fluorescent) lighting conditions in vegetat ve *A. amoena* stems kept in different solutions

The xylem water potentials of vege ative *A. amoena* stems kept in distilled water and in citric acid under fluorescent light (Fig. 4.8) showed similar overall trends to when kept under incandescent light (Fig. 4.7). That is, regardless of lighting conditions, or whether stems were vegetative or flowering, stems in distilled water exhibited water stress more rapidly than those in citric acid. The results in Figs. 4.7 a ıd 4.8 are similar for vegetative stems in distilled water and reveal sudden decreases in xylem w ater potential after 24 and 12 h, respectively. Vegetative stems in citric acid developed less water stress under fluorescent than incandescent lighting, reaching -2.4 MPa after 120 h ur der fluorescent light, and <-4.0 MPa after 96 h under incandescent light.

The xylem water potentials of veget ıtive *A. amoena* stems pulsed with STS for 16.5 h and then kept in distilled water; and those in 'SCC' (pulsed with STS for 16.5 h, then kept in citric acid and DICA) (Fig. 4.8), again revealed that stems kept in distilled water exhibited water stress more quickly than stems in a solutic n containing citric acid. The STS pulse did not extend the time until severe water stress occurred (<-4.0 MPa). Irrespective of whether stems were pulsed with STS before being kept in disti led water or not, after 60 h the xylem water potential fell below <-4.0 MPa. The SCC solution, containing citric acid and DICA after an STS pulse, had the highest water potential of all trea ments, -0.8 MPa after 120 h.

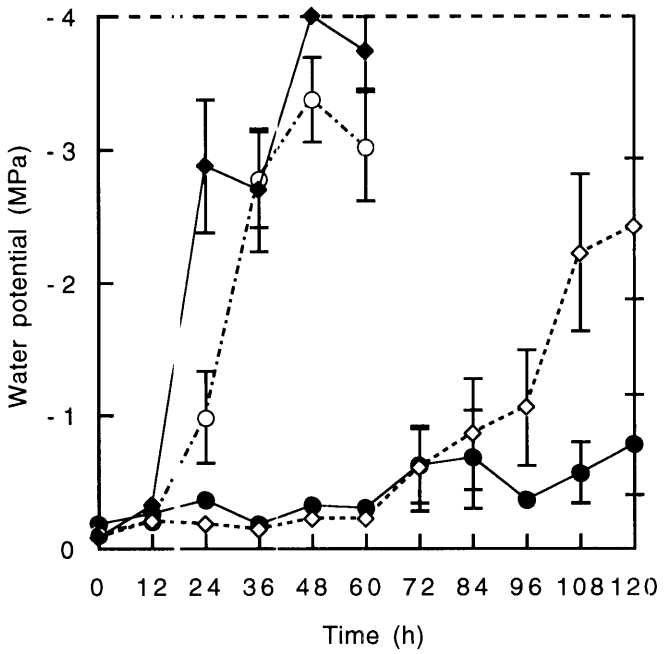
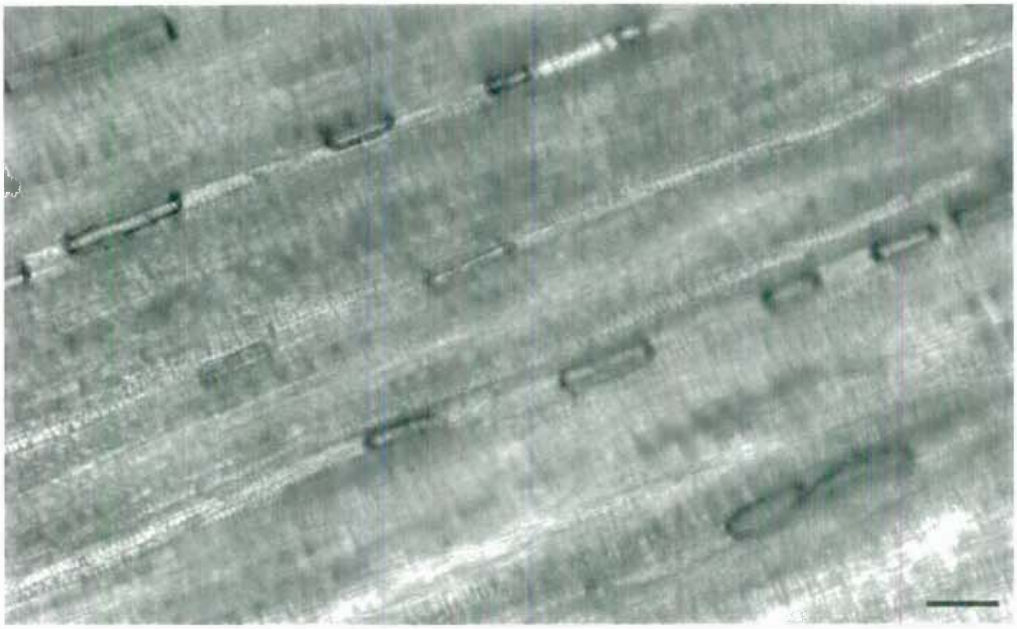


Fig. 4.8. Xylem water potentials of veget ıtive *A. amoena* stems in four solutions when kept under fluorescent light (20±2 µmol m^{-2} s^{-1}). ◆ = distilled w ater; ◇ = citric acid (10 mol m^{-3}); O = STS pulse for 16.5 h, then kept in distilled water; ● = 'SCC': STS pul se for 16 5 h, then kept in citric acid (10 mol m^{-3}) and DICA (50 mg L^{-1}). Data are the means of 10 replicates ± standard error. (Where no error bar appears, the SE was smaller than the size of the symbol.) The dotted line across the top of the graph at -4.0 MPa indicates that the limit of the pressure regulator in use was reached. Sub sequent pressure readings could not exceed this figure and, to avoid the appearance of a stabilisation of pressure, no further readings were plotted.

4.3.9 Anatomical evidence of cavitated conduits in *A. amoena*

Cavitated conduits were frequently observed in the xylem of stems which had been kept in distilled water (Photograph 4.4), particularly after 48 h. Stems kept in citric acid and in SCC, however, exhibited fewer cavitated conduits (data not shown), and they occurred much later than in stems kept in distilled water.



Photograph 4.4. Cavitated xylem conduits, indicated by the presence of air bubbles within conduits, in an *A. amoena* stem which had been kept in distilled water for 72 h. The tangential longitudinal section was cut by hand 5 cm from the stem base and mounted immediately in distilled water. (bar = 100 µm)

4.3.10 Changes in solution bacterial numbers during acoustic detection experiments

Bacterial numbers were significantly less in the DICA solution over the 5 day cavitation detection period compared with the distilled water and citric acid treatments (Table 4.2). On day 1, distilled water contained significantly greater numbers of bacteria. By day 3, the citric treatment contained more bacteria than distilled water, although the difference was not significant ($P < 0.05$). After 5 d, the citric acid treatment contained significantly greater numbers of bacteria than both the distilled water and DICA treatments.

Table 4.2

The number of bacteria (cfu mL^{-1}) in solutions during cavitation experiments

Treatment	Bacterial numbers (cfu mL^{-1})		
	Time (d)		
	1	3	5
Distilled water	8.2×10^{3a}	1.3×10^{5a}	3.4×10^{5b}
Citric acid	2.3×10^{3b}	3.2×10^{5a}	1.6×10^{6a}
DICA (Chlorine)	3.6×10^{2c}	2.2×10^{3b}	3.6×10^{3c}

Means within each column followed by different letters are significantly different ($P < 0.05$).

4.3.11 The relationship between AAE and solution bacterial numbers

The relationship between AAE and the number of bacteria in the vase solutions during cavitation experiments was examined using simple linear regression analysis. Only four significant correlations were found (Table 4.3, significance denoted by asterisks). On day 1, there was a significant but negative relationship for both citric acid and DICA and the number of bacteria (i.e. as the number of bacteria increased, the number of AAE decreased). However, by day 3 the relationship between bacterial numbers and AAE was positive for both citric acid and DICA.

Table 4.3

The relationship between AAE and the number of bacteria (cfu mL^{-1}) in solutions during cavitation experiments using simple linear regression

Treatment	Time (d)	Dependent variable	Intercept	Slope coefficient of cfu mL^{-1}	R^2
Distilled water	1	AAE	715.140 (2.662)	-0.027 (-0.922)	0.096
	3	AAE	2740.498 (1.655)	0.007 (0.907)	0.093
	5	AAE	1273.982 (1.748)	-0.005 (-0.879)	0.436
Citric acid	1	AAE	524.950 (4.473)	-0.115 (-2.482*)	0.468
	3	AAE	400.750 (3.952)	0.001 (4.260*)	0.722
	5	AAE	500.188 (4.029)	0.000 (0.957)	0.132
DICA (Chlorine)	1	AAE	743.065 (3.667)	-1.661 (-3.090*)	0.544
	3	AAE	-47.960 (-0.567)	0.166 (5.728*)	0.804
	5	AAE	955.138 (3.110)	-0.059 (-0.787)	0.072

* denotes significant at $P < 0.05$; t-statistics are in brackets.

4.4 DISCUSSION

While cut vegetative stems of *A. amoena* stood in distilled water, their rate of AAE production exhibited the familiar parabolic pattern (Fig. 4.2) previously noted in leaves and stems dehydrating in air (e.g. Milburn and Johnson 1966; Crombie *et al.* 1985b; Ritman and Milburn 1991). In the present experiments, the peak still occurred, but it was delayed, indicating that the xylem sap became progressively subjected to increasing stress, even though it was kept in an abundant supply of water. A stem xylem water potential of -3.7 MPa at the cavitation peak at 48 h and the embolised xylem conduits (Photograph 4.4), confirmed the severity of the water stress that developed within the xylem conduits.

The peak rate of AAE production was probably the result of positive feedback, whereby cavitating conduits place increasing strain on the remaining functional xylem, thereby inducing further cavitation. Hargrave *et al.* (1994) observed groups of embolised or non-embolised conduits and suggested that when one conduit embolised, it increased the chances of embolisation in adjacent conduits. Such a phenomenon supports the air seeding hypothesis of embolism induction, whereby air is drawn through from the embolised conduit into an adjacent wet pit membrane pore (Zimmermann 1983). However, the cause(s) behind the initial AAE event is(are) still uncertain. A build-up of a microbial population in the vase solution could theoretically cause such water stress. Put and van der Meyden (1988) found that bacteria infiltrated the xylem conduits of cut roses, but thought that 24 h was too short for colonisation of infiltrated bacterial cells. They noted that bacterial numbers were too low to explain observed decreases in hydraulic conductance and hypothesised that other unknown factors were involved. Sperry and Tyree (1988) suggested that the initial blockage occurring after infection by vascular pathogens resulted in decreased hydraulic conductance. This was not due to vessel occlusion by the pathogen but, rather, via the induction of embolisation resistances.

Cavitation has been found to begin at a variety of water potentials, e.g. -0.5 MPa in *Plantago major* L. (Milburn and McLaughlin 1974); -1.2 MPa in *Malus sylvestris* Mill. (West and Gaff 1976) and -1.8 MPa in *Eucalyptus globulus* Labill. (Crombie *et al.* 1985b). In those plants, the pressure at which maximum AAE production occurred was -1.0, -3.3 and -2.6 MPa respectively. In the present experiments, AAE production began at -0.1 MPa and the peak rate occurred at -3.7 MPa. Acacias are well-known for their ability to withstand xeric habitats. Pressure potentials have been reported down to -6.0 MPa, corresponding to a mere 15% water loss (Connor and Tunstall 1968). Tyree and Dixon (1986) observed that little was known about the water potentials at which cavitation begins to have a major effect on water conduction. This is especially the case with cut flowers, which lack nightly root pressure to reduce water stress, as may occur *in vivo*.

When air-dried specimens of *A. anoena* were tested for cavitation events and relative water content (Williamson 1989), the results corroborated with those obtained for roses (*Rosa hybrida* 'Samantha') by Dixon *et al.* (1988). In *Acacia*, peak AAE production occurred at 51% RWC and xylem water potential of -3.95 MPa, compared with 50% RWC and -4.0 MPa xylem water potential in roses. Such similar results are surprising if the anatomical and ecological differences of these two plants are considered. Acacias are more adapted to a xeric habitat than roses, as evidenced by anatomical features such as glaucous reduced isobilateral leaves (phyllodes), protected stomata, and a thick cuticle (see Photograph 8.3). Thus, it would be anticipated that acacias and roses would have quite different cavitation and water release profiles.

Two questions therefore arise: (1) is the occurrence of cavitation a measure of a plant's ability to withstand water stress; and (2) is there a pressure at which all excised plants can no longer withstand the tensions placed upon them, both mechanical cell wall tension and physical water tension? Crombie *et al.* (1985b) found that acoustic emissions were produced when the xylem water potential fell beyond a cavitation threshold. They observed that the sap tensions at which cavitation began were similar within each species but quite different between species, and thought that xerophytes would cavitate at lower sap tensions than mesic species. This was partly the case in the present experiments: the pressure at cavitation commencement was unexpectedly similar in the woody xerophyte *Acacia* (-0.1 MPa) and the mesophytic herb, *Plantago major* (-0.5 MPa, Milburn and McLaughlin 1974). However, the pressure at which maximum AAE production occurred, -3.7 MPa and -1.0 MPa respectively, was very different. This supports the hypothesis of Crombie *et al.* (1985b), that xerophytes would cavitate at lower sap tensions than mesophytes.

Crombie *et al.* (1985a) and Sperry and Tyree (1988) have suggested that vulnerability to xylem embolisation may be due to larger pit membrane pores. They proposed that pit membrane pore size was an adaptive feature reflecting a species' habitat. The pore sizes of 'Samantha' roses are unknown, but would be expected to be larger and more vulnerable than those in *Acacia* if their respective habitats are considered. SEM micrographs of *Acacia* revealed that the pit pores and apertures are very small (see Photograph 8.1c), especially compared with those of roses (Put and van der Meyden 1988; van Doorn, de Stigter, de Witte and Boekestein 1991; Put *et al.* 1992). Hargrave *et al.* (1994) hypothesised that wider xylem conduits may have more and larger pit membrane pores than narrower conduits, which may increase their vulnerability to embolisation. The air seeding hypothesis (Zimmermann 1983), which is governed by the capillarity equation (section 1.2.6.2), supports this idea. Put simply, the wider the pit membrane pore diameter, the lower the pressure differential required to draw air through a wet pit membrane pore from one conduit to another, and hence the greater ease of embolisation. Experimental evidence from several workers has revealed that wider xylem conduits were more likely to cavitate than narrower conduits (Tyree and Dixon 1986; Salleo and Lo Gullo 1986; 1989; Cochard and Tyree 1990).

When the time of peak AAE production is compared for stems kept in light (Fig. 4.2) and darkness (Fig. 4.3), it can be seen that cavitation events peaked 48 h later in darkness than in light. These data indicate that cavitation is precipitated, but not caused, by water lost under incandescent light. Leaf temperatures remained well within the normal physiological range of plant function throughout experiments, so an early onset of cavitation in illuminated stems could not be attributed to excessive stem temperatures.

Stems kept in citric acid did not exhibit a discernible peak in AAE production, despite a sudden decrease in xylem water potential between 48 and 60 h (Fig. 4.4). Water potentials of <-4.0 MPa were reached between 96 and 108 h and corresponded with the observed onset of phyllode desiccation in citric acid. Sperry *et al.* (1991) have questioned whether embolisation is in fact mainly a function of xylem tension, suggesting that it may be precipitated by changes in pit membrane permeability.

The increased hydraulic conductance that results from citric acid treatment (Durkin 1979a; b; 1980; see also Fig. 9.4) might be explained by degradation of the pit membranes. This hypothesis could account for the lower numbers of detectable AAE in citric acid treated stems, because the potentially cavitatable compartments would effectively fuse together. Wisniewski *et al.* (1991) observed partial pit membrane degradation in oxalic acid treated stems (5 to 50 mol m^{-3}), and both oxalic and citric acids (at 10 mol m^{-3}) have been found to prevent long-term reduction in hydraulic conductivity (Sperry *et al.* 1988). This hypothesis is explored further in experiments comparing the distance travelled by paint particles in distilled water and citric acid treated stems (sections 5.3.6 and 5.4).

Citric acid extended the vase life of *Acacia* stems (Tables 3.3 and 3.4), increased hydraulic conductances (Fig. 9.4) and improved water potentials (Figs. 4.7 and 4.8). Also, an extremely low number of AAE were produced. Two possible explanations exist: (1) when Durkin (1979a, b) found that water acidified with citric acid increased flow rates in rose stems, he hypothesised (Durkin 1980) that acidified water increased vessel wall porosity, possibly by breaking calcium pectate bonds. This may be regarded as the 'pit membrane digestion' hypothesis. (2) Microbial growth may be inhibited by low pH. Sperry *et al.* (1988) observed that solutions with a pH below 3 (including 10 mol m^{-3} citric acid) prevented long-term declines in hydraulic conductivity by limiting microbial growth. However, yeasts are frequently found in citric acid (W. van Doorn, pers. comm. 1991). Although yeasts were growing around the basal portion of the stem after 96 h (Photograph 4.3), this was long after the hydraulic crisis, i.e. the increase in xylem water potential between 48 and 60 h (Fig. 4.4), had occurred. Furthermore, after 3 and 5 d, citric acid contained higher numbers of bacteria than the distilled water and DICA treatments (Table 4.2), yet the number of AAE was lower. The occurrence of positive and negative relationships, together with the relatively few (four) significant correlations between the number of bacteria and AAE (Table 4.3), indicates that there is no clear relationship between the number of AAE and bacteria in the vase solution. Van Doorn's (1989) SEM studies failed to detect yeasts inside the xylem conduits or at the cut surface of rose stems after several days, despite their presence, albeit in low concentrations, in the vase water. Even after 11 d, no yeasts were found at the cut surface or inside rose stems, leading to the conclusion that yeasts played no part in rose vascular blockage (van Doorn, de Stigter, de Witte and Boekestein 1991). Furthermore, the suggestion of Sperry *et al.* (1988) that low pH reduces microbial growth was not borne out

by the number of bacteria in 11-day old *Boronia* vase solutions (section 6.3.3). Distilled water (pH 5.3) had significantly lower numbers of bacteria than citric acid (pH 2.6) and citric acid with 2% sucrose (pH 2.5) (Fig. 6.10). Thus, in view of the reduced numbers of AAE, which indicate that conduits have become amalgamated through loss of the integrity of intervening pit membranes, the data support the former (pit membrane digestion) hypothesis.

West and Gaff (1976), in their studies of air-dehydrated apple leaves, found that there was a time lag of approximately 10 min in peak AAE production for turgid compared with non-turgid leaves. The results of the present experiments contrast with those of West and Gaff (1976) as the peak in AAE production occurred 48 h earlier in turgid (Fig. 4.2) than in non-turgid (Fig. 4.5) stems. Additionally, the peak rate of AAE production was much higher in non-turgid stems (332 AAE 12 h^{-1} at 96 h) compared with turgid stems (101 AAE 12 h^{-1} at 48 h). The initial xylem water potentials of the two treatments were quite different, -0.07 and -2.2 MPa for turgid and non-turgid stems respectively (data not shown). The xylem water potentials in West and Gaff's (1976) experiments were -0.23 and -0.62 MPa for turgid and non-turgid leaves respectively. Thus, turgid and non-turgid readings were quite similar for the mesic species, apple (*Malus sylvestris* Mill.), compared with those of *A. amoena*, a xeric shrub. The results of the present AAE experiments are surprising when the initial water potentials are considered. It would be expected that a more negative xylem water potential (as occurred with non-turgid stems) would precipitate the peak in AAE production, however, this was not the case. Perhaps time since harvest is more crucial to the timing of the AAE peak than starting with a higher RWC and xylem water potential. Wound responses may develop more fully with time (sections 9.3.4 and 9.4).

The high AAE production rate while stems were kept in DICA (Fig. 4.6) is surprising if the number of bacteria in the solution is considered. Bacterial numbers were significantly lower in DICA than in the distilled water and citric acid treatments during the 120 h (5 d) experimental period. As mentioned above, the results of Table 4.3 indicated that there was no clear relationship between the number of bacteria in the 'vase' solutions and AAE production. Furthermore, the RWC results (Table 4.1) revealed that stems in citric acid had a significantly greater RWC after 120 h than stems in DICA, distilled water or degassed distilled water. Yet stems in citric acid had significantly greater numbers of bacteria after 120 h. Stems in DICA had the highest RWC of the remaining treatments, yet AAE production was highest in these stems. Distilled water had the lowest RWC of all treatments, a not unexpected result considering the early cavitation peak and rapid decrease in xylem water potential. The low RWC result for distilled water was also consistent with the vase life, solution uptake and transpiration results described in Chapters 3 and 5. The effect on cavitation production of the bacterium, *Pseudomonas*, which had been isolated from vase water is explored further in Chapter 7.

Two questions remain: (1) is AAE production an indicator of water stress in cut stems? (2) Is there a relationship between bacterial numbers in the vase solution and water stress? A vast amount of experimental evidence (both acoustic and ultrasonic) has accumulated (Table 1.5) since the first acoustic experiment detected cavitation production (Milburn and Johnson 1966), lending support to the first question. However, there are numerous conflicting reports concerning the second question. For example, neither DICA nor high numbers of mixed bacterial populations (10^6 cfu mL^{-1}) in the vase water affected carnation vase life (van Doorn, Zagory, de Witte and Harkema 1991; Jones and Hill 1993). However, the number of bacteria in the vase solution does not necessarily indicate the number of bacteria infiltrating xylem. Before bacteria could be detected in the vase water of roses, they reached 10^6 cfu g^{-1} fw in the basal stem segments (van Doorn and Perik 1990).

Despite the numerous conflicting reports on the relationship between longevity and bacterial numbers in the vase solution and stem (longevity unaffected: Camprubi and Fontarnau 1977; van Doorn, de Witte and Waltmann 1986; van Doorn, Harkema and Otma 1991; van Doorn, Zagory, de Witte and Harkema 1991; Jones and Hill 1993; van Doorn, Pak and Buddendorf 1993; longevity affected: Put and Conway 1986; Put and Clerkx 1988; Clerkx *et al.* 1989; Put and Jansen 1989; van Doorn *et al.* 1989; van Doorn and Perik 1990; van Doorn *et al.* 1991a; van Doorn, Zagory and Reid 1991), inclusion of slow release antimicrobial compounds such as DICA in the buckets of cut flowers during shipping may at least be of aesthetic benefit to the consumer. If germicides are left out, the water becomes putrid and the stems slimy, which does not appeal to consumers, and may even be a human health hazard. For example, *Pseudomonas aeruginosa*, which is pathogenic to humans, is the bacterium most frequently isolated from vase water (Taplin and Mertz 1973; McCleary and Layne 1977; de Witte and van Doorn 1988). It is responsible for the majority of nosocomial infections in burn patients, and flowers are often banned from such hospital wards (Taplin and Mertz 1973). It is possible that routine use of antimicrobial compounds may eliminate an opportunistic plant or human pathogen from the vase water, so their usage must be recommended.

The AAE detection studies were performed on vegetative *A. amoena* stems for the reasons outlined in section 4.2.2. It is likely that if flowering stems had been used, the AAE peaks would have occurred earlier because of the extra demand for water imposed by flowers. The xylem water potential results of flowering and vegetative *A. amoena* stems (Fig. 4.7) support this hypothesis, as flowering stems developed water stress earlier than their vegetative counterparts.

Xylem water potentials of *A. amoena* stems under fluorescent light (Fig. 4.8) were ascertained to relate the AAE production results (under incandescent light) to those of vase life experiments (under fluorescent light). Stems in distilled water developed severe water stress after 12 to 24 h,

irrespective of lighting. However, water stress was delayed when stems in citric acid were kept under fluorescent lighting. This indicates that in distilled water, a blockage/senescence process occurs readily, whereas citric acid has an ameliorative effect on blockage/senescence. The effect of an STS pulse (Fig. 4.8) was minimal if stems were then placed in distilled water, as was found in the vase life result with *A. floribunda* in Chapter 3 (Table 3.3). However, when the STS pulse was replaced with citric acid and SDIC (SCC), the xylem water potential remained high throughout the 120 h experimental period (Fig. 4.8). This result was also supported by the vase life of *A. floribunda* in SCC (Table 3.3), which had a longer vase life than either citric acid or SDIC individually. Perhaps the beneficial effect of citric acid combines with the antimicrobial properties of chlorine (SDIC) to provide a stable solution for water uptake. Unfortunately, restrictions in time and equipment prevented conclusive testing of AAE production in SCC.

The experiments described in this chapter have shown that cut stems cavitate when kept in solutions, thus providing the first direct evidence of this often-surmised event in cut flowers (de Witte and van Doorn 1988; de Stigter and Broekhuysen 1989; Urban and Lemattre 1991; Jones *et al.* 1993). Cavitation occurred earlier in distilled water than in treatments which improved longevity. However, the first major event curtailing vase life in cut flowers remains problematic. The initial rates of cavitation in cut stems seem too low to explain the familiar decreases in hydraulic conductance, unless the larger, more vulnerable xylem conduits, with flow rates proportional to the fourth power of the capillary radius (Zimmermann 1983), cavitate first. Several studies have shown this to be the case (Salleo and Lo Gullo 1986; Tyree and Dixon 1986; Cochard and Tyree 1990). Furthermore, the results of Dixon *et al.* (1988) indicated that larger conduits do cavitate first, because the few early cavitation events were associated with large amounts of water loss. However, if cavitation is a result of water stress, there must be an earlier cause. Yet if plant tissues respond to injury by initiating gas bubbles within conduits, then such cavitation could indeed be the first and most significant event.

The following chapter (Chapter 5) investigates several hypotheses of the action of citric acid in increasing hydraulic conduction and longevity, while also decreasing the number of AAE produced.