CHAPTER FIVE

DETERMINATION OF THE MECHANISM/S OF CITRIC ACID ACTION

5.1 INTRODUCTION

Organic acids are recommended frequently as components of both vase solutions and 'conditioning' treatments. Conditioning treatments are used commercially to replace water that has been lost during postharvest handling. These treatments usually contain a germicide and an acidifier. Citric, tartaric, iso-ascorbic and benzoic acids have been recommended as acidifiers, but citric acid is most commonly used (Halevy and Mayak 1981). Citric acid is believed to reduce vascular blockage (Nowak and Rudnicki 1990) and to improve the hydration of cut flowers (Durkin 1980; 1986a). However, the mechanism by which it does these things remains unclear (Halevy and Mayak 1981).

Low pH solutions are thought to increase hydraulic flow by inhibiting microbial growth (Aarts 1957 in Halevy and Mayak 1981; Sperry *et al.* 1988), however, yeasts are frequently found in citric acid solutions (W. van Doorn, pers. comm. 1991). Also, bacterial numbers were higher in citric acid than in distilled water or DICA solutions (Table 4.2). Thus, the 'microbial inhibition' hypothesis of citric acid action is debatable.

Durkin (1979a) found that upon lowering of pH from 5.7 to 3.2, hydraulic conduction increased and blockage rates decreased. He hypothesised that low solution pH increased hydraulic conduction because of an anatomical change to stems. Later (1979b), he speculated that low solution pH might enable air to be expelled more rapidly from the xylem conduits. This was thought to occur because of enhanced lateral movement of water through vessel walls.

Ascorbic acid (7.1 mol m^{-3}) increased infusion rates (compared with distilled water) into seedlings of *Cercis* and *Liriodendron* trees (Duncan and Himelick 1990). Although citric acid was not tested by these authors, it is likely that it would also have increased solution uptake. The longevity of *Acacia* stems in citric and ascorbic acids was not significantly different in a preliminary vase life experiment (Williamson 1989). Duncan and Himelick (1990) speculated that ascorbic acid, like oxalic acid, may increase flow rates by disrupting calcium in the middle lamella of pit membranes, thereby making the membranes more open and elastic (Sperry and Tyree 1988).

Buys (1969) thought that oxygen in the vase water played a role in xylem conduit blockage by oxidising a "hindering substance", a polyphenol, found in the leaves of acacias, roses and lilacs 'forced' to flower out of season. He recommended removal of lower leaves, and use of cooled, boiled water to remove oxygen. Removal of air from water has been known for some time to improve conduction and, therefore, flower life (Dickson and Blackman 1938; Hamner *et al.* 1945; Stocking 1948; Durkin 1979a b). Conduits will remain functional if they do not contain entrapped air, resulting in the maintenance of hydraulic conduction and, therefore, increased cut flower longevity.

The difference between solution uptake and transpiration reflects whether a water deficit has occurred in cut flowers. Stomatal closure, therefore, is a way of inhibiting water loss from the plant by reducing the transpiration rate (Jones and Sutherland 1991). Treatment with abscisic acid, which induces stomatal closure, extended the vase life of cut roses (Halevy *et al.* 1974). The efficiency of stomatal closure was thought to explain the difference in longevity of short- and long-lived rose cultivars (Mayak *et al.* 1974). It is not known whether citric acid improves the water balance of cut flowers by inducing stomatal closure.

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

- test an hypothesis that xylem plugging (evidenced by decreased hydraulic conduction and shorter vase lives) was a response to elevated dissolved oxygen levels in cut stems;

- determine whether citric acid improves the water balance in cut stems by promoting stomatal closure; and

- examine the effect of citric acid on the conduction of paint particles (which are normally stopped by pit membranes) in excised *Acacia* stems.

5.2 MATERIALS AND METHODS

5.2.1 Measurement of dissolved oxygen content

The amount of dissolved oxygen present in degassed distilled water, distilled water and in citric acid (10 mol m^{-3}) was monitored daily with an oxygen electrode (Model 97-08, Orion Research Inc., Massachusetts) during the vase life experiment described below (section 5.2.3).

5.2.2 Degassing of distilled water

Degassed water was prepared by boiling distilled water for 20 min and then immediately and carefully pouring it down the side of a conical flask. A 10 mm thick layer of pharmaceutical

quality olive oil was then poured over the surface to prevent gaseous exchange between atmospheric oxygen and the degassed water (Kordan 1975). Kordan (1972) previously showed that a 10 mm layer of olive oil was the most effective barrier against diffusion of atmospheric gases into the water below. Cooled water was siphoned from underneath the oil layer when required. The stem to be tested was placed into the water and a 10 mm layer of olive oil was quickly added.

5.2.3 Solution uptake, transpiration and vase life of *Acacia amoena* stems kept in citric acid (10 mol m^{-3}), distilled water and degassed distilled water

Solution uptake, transpiration and vase life were monitored as described previously (sections 2.13 and 3.2.7).

5.2.4 Cavitation detection and xylem water potential in *A. amoena* stems kept in degassed distilled water

The methods for cavitation detection and xylem water potential of *A. amoena* stems kept in degassed distilled water was the same as described earlier (sections 4.2.2, 4.2.3 and 2.5).

5.2.5 Measurement of stomatal diffusive resistance

Stomatal apertures of *Acacia amoena* stems were measured using a Li-Cor Diffusive Resistance Meter (Model LI-60, Lambda Instruments Co. Inc., Nebraska). Stems were kept in citric acid and in distilled water in either constant light (Cool White fluorescent, 20 µmol m^{-2} s^{-1}) or complete darkness. It was necessary to calibrate the meter daily for both the light and dark conditions (see Appendix F for the calibration curves). A horizontal sensor was used, and the aperture area of the vapour cup was reduced by 3/4 to ensure that the *Acacia* phyllodes were larger than the aperture area. The area component of the calibration calculation equation was therefore reduced from 2 cm^2 to 0.5 cm^2. Thus, the calibration equation was as follows:

$$r\ (\text{sec cm}^{-1}) = L/\alpha = 4A(L_o+\frac{\pi d}{8})/\alpha n\pi d^2 \qquad (5.1)$$

where L is the effective diffusion path length;
α is the diffusivity of water vapour in air at a given temperature (values provided in the Diffusive Resistance Meter Manual);
L_o is the actual length of each hole (and also the plate thickness);
A is the aperture area of the vapour cup;
n is the number of holes;

d is the diameter of the holes; and

$\frac{\pi d}{8}$ is a one end effect correction to the actual diffusion path length when the resistance plate is placed on a water saturated surface.

(The incorrect equation first appeared in Kanemasu *et al.* 1969; the corrected equation as shown above, was taken from the Li-Cor Diffusive Resistance Meter Manual.)

5.2.6 Measurement of transpiration and xylem water potential

The transpiration rate was measured during the diffusive resistance experiment described above (section 5.2.5). Transpiration was determined as described previously (section 2.13).

At the end of the diffusive resistance experiment, the xylem water potential of the stems was determined as described previously (section 2.5).

5.2.7 Assessment, preparation and infiltration of dyes and paints for uptake analysis

Initial trials were made with paints dilute (1:100) latex paint (Sunproof Gloss, Mid Bristol Green, Taubmans Pty. Ltd., Villawood, Australia), and an oil-based paint (Dulux Galaxy Blue, Dulux Australia, Clayton, Victoria). However, both proved unsuitable. The latex paint was not carried more than 2 cm in the cut plant stem when it was mixed with citric acid (10 mol m^{-3}), and the oil-based paint did not mix with citric acid when tested on a glass slide.

A paint tint (Tradex Concentrated Universal Colormix, Permanent Blue, Taubmans Pty. Ltd., Villawood, Australia) was found to be miscible when mixed with citric acid (10 mol m^{-3}) on a glass slide, and was thus used for uptake analysis. The paint tint was diluted 1:1 with distilled water, centrifuged for 4 min at 15,000 × *g* and the supernatant was used for infiltration into cut stems. Prior to infiltration, stems were kept in either citric acid (10 mol m^{-3}) or distilled water for 2 d under acoustic detection lighting conditions (100 W incandescent light, 30 cm away). The stems were then transferred to Parafilm-covered vials containing the paint supernatant. Stems were returned to the acoustic detection lighting conditions for another 2 d, during which time the paint was drawn up by transpirational pull. Transverse sections of the stems were cut by hand every 1 cm and the number of paint filled conduits was counted.

5.2.8 Analysis of data

Means from the uptake, transpiration and vase life experiment were checked for normality, and homogeneity of the variances, and transformed if necessary (section 2.14). Significant means

were separated using Scheffé's test (StatView 4.0) at $P < 0.05$. Dissolved oxygen concentration data were analysed by time series analysis ($P < 0.05$). Stomatal diffusive resistance data within each light condition were analysed using Fisher's Protected Least Significant Difference (PLSD) test at $P < 0.05$. The significance of the distance travelled by paint in distilled water and in citric acid was determined using an unpaired t-test ($P < 0.001$).

5.3 RESULTS

5.3.1 Uptake, transpiration and vase life of *Acacia amoena* stems kept in citric acid, distilled water and degassed distilled water

The vase life of stems in citric acid (10 mol m^{-3}) and in degassed distilled water was significantly greater (5.7 d) than that of stems in distilled water (3.5 d) (Table 5.1).

Table 5.1
Vase life of *A. amoena* in citric acid, distilled water and degassed distilled water

Treatment	Vase life (d) ± SE◊	Range (d)
T1: Citric acid (10 mol m^{-3})	5.7^{a}* ± 0.367	4 - 7
T2: Distilled water	3.5^{b} ± 0.269	3 - 5
T3: Degassed distilled water	5.7^{a} ± 0.448	4 - 8

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

After day 0 to 1, solution uptake was always greater in stems kept in citric acid than in distilled water (Fig. 5.1). Significant differences in uptake occurred at days 1 to 2 and 2 to 3. (Solution uptake was not measured for stems in degassed distilled water because daily removal and replacement of stems through the oil layer would have coated the cut end with oil and prematurely blocked solution uptake. It is likely that uptake would also have been greater in degassed water at days 1 to 2 and 2 to 3 because transpiration closely parallels uptake.)

Transpiration was highest in stems kept in citric acid after day 0 to 1, although at day 4 to 5 it was slightly higher in degassed distilled water than in citric acid (Fig. 5.2). At days 1 to 2, and 2 to 3, transpiration was significantly greater in citric acid and in degassed distilled water than in distilled water. After day 0 to 1, stems kept in distilled water had the lowest rate of transpiration.

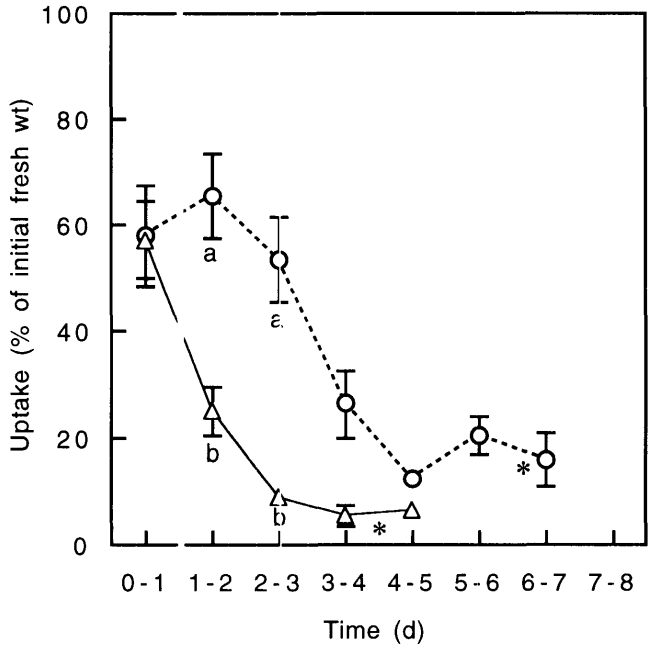

Fig. 5.1. Solution uptake (as a % of the initial fresh wt) of *A. amoena* stems during vase life. O = citric acid (10 mol m^{-3}); Δ = distilled water; * = end of vase life. Within each day, symbols followed by the same letter are not significantly different from each other ($P < 0.05$). Error bars represent SE. (Where no error bar appears, the SE was smaller than the size of the symbol.)

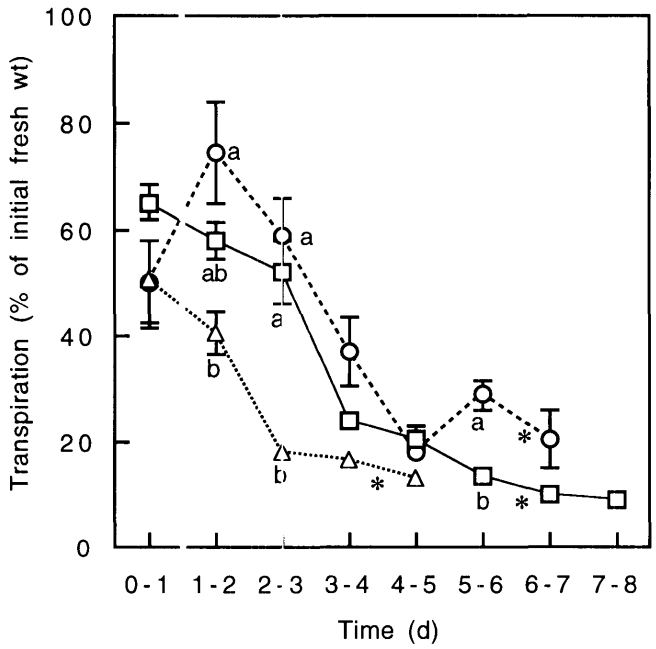

Fig. 5.2. Transpiration (as a % of the initial fresh wt) of *A. amoena* stems during vase life. O = citric acid (10 mol m^{-3}); □ = degassed distilled water; Δ = distilled water; * = end of vase life. Within each day, symbols followed by the same letter are not significantly different from each other ($P < 0.05$). Error bars represent SE. (Where no error bar appears, the SE was smaller than the size of the symbol.)

5.3.2 Changes in the dissolved oxygen content of citric acid, distilled water and degassed distilled water during vase life

Degassed water had the lowest oxygen concentration throughout the vase life, except at day 4, when citric acid was slightly higher (Fig. 5.3). After day 1, distilled water had the highest

oxygen concentration, although no significant differences were found between distilled water and citric acid until day 3 (Fig. 5.3; Appendix C). (Incomplete data sets after day 3 precluded further ANOVA tests between treatments.) Linear regression analysis between oxygen concentration and longevity revealed a weak (adjusted R^2 = 0.22) but significant negative relationship (t-value = -2.99) between the two variables for all treatments (Appendix C).

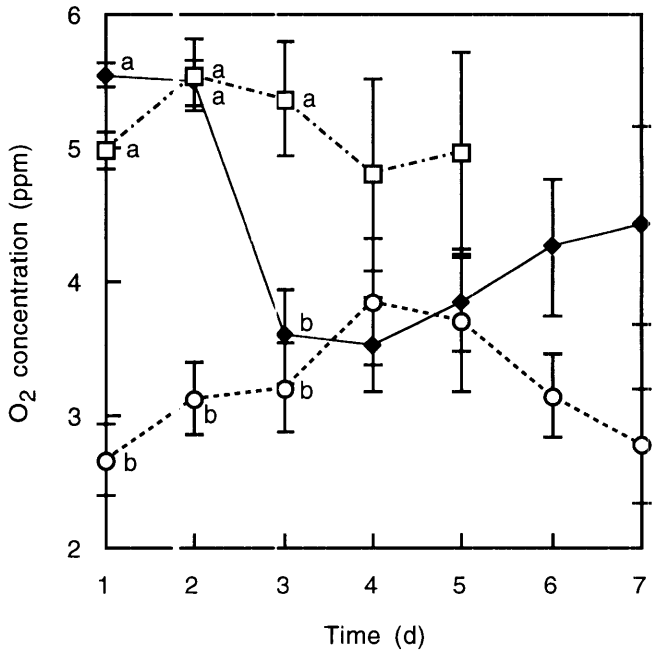

Fig. 5.3. The change in vase solution dissolved oxygen concentration (ppm) during the vase life of *A. amoena* stems kept in distilled water □, citric acid (10 mol m^{-3}) ◆ and degassed distilled water O. Within each day, symbols followed by the same letter are not significantly different from each other ($P < 0.05$). Error bars represent SE.

5.3.3 Cavitation events and xylem water potential of *A. amoena* stems kept in degassed distilled water

While stems were kept in degassed distilled water, a large peak in the rate of AAE production occurred at 108 h (Fig. 5.4). Xylem water potential decreased from -0.2 to -1.4 MPa between 60 and 72 h and reached -3.9 MPa at 108 h. Stems began to exhibit the first signs of desiccation between 72 and 96 h.

5.3.4 Stomatal diffusive resistances of vegetative *A. amoena* stems kept in: (a) Distilled water and citric acid under vase life lighting conditions; and (b) Distilled water and citric acid in complete darkness

(a) For the initial 4 h, the diffusive resistances for stems kept in citric acid and in distilled water were similar. However, from 28 h onwards, the diffusive resistances were significantly greater ($P < 0.05$) in distilled water (Fig. 5.5). Thus, from 4 h, cut stems in distilled water had

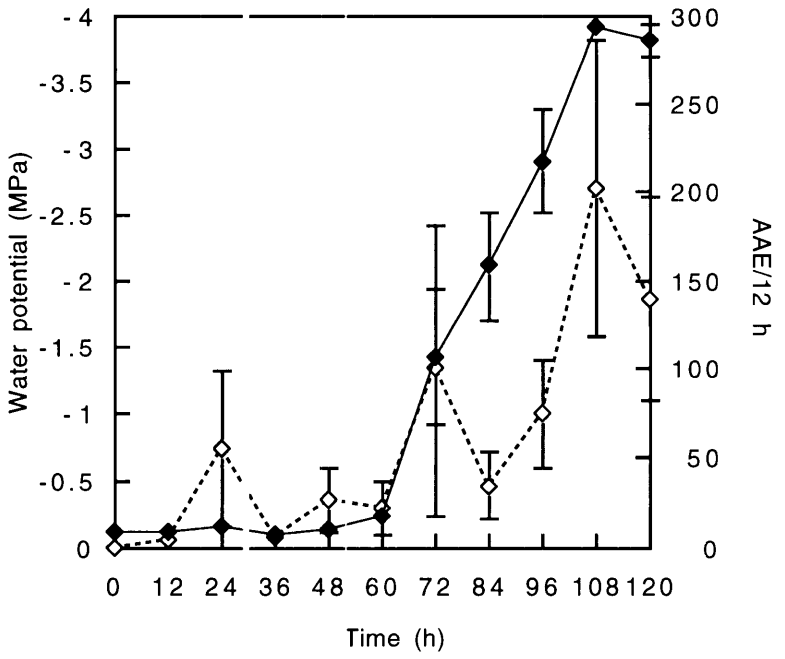

Fig. 5.4. The relationship between stem xylem water potential (MPa) and audible acoustic emission (AAE) production over time for *A. amoena* stems kept in degassed distilled water. ◇ = AAE; ◆ = xylem water potential. Water potential and AAE readings are both the means of 10 replicates ± standard error for each data point. (Each replicate = 1 stem.) (Where no error bar appears, the SE was smaller than the size of the symbol.)

a significantly greater stomatal diffusive resistance, indicating that stomata were not as open as in stems kept in citric acid. This result revealed that water stress developed rapidly when stems were kept in distilled water, particularly between 4 h and 30 h, when a steep rise in diffusive resistance occurred. In citric acid, however, diffusive resistances remained virtually unchanged from 4 to 74 h, indicating that water stress did not increase over the experimental period.

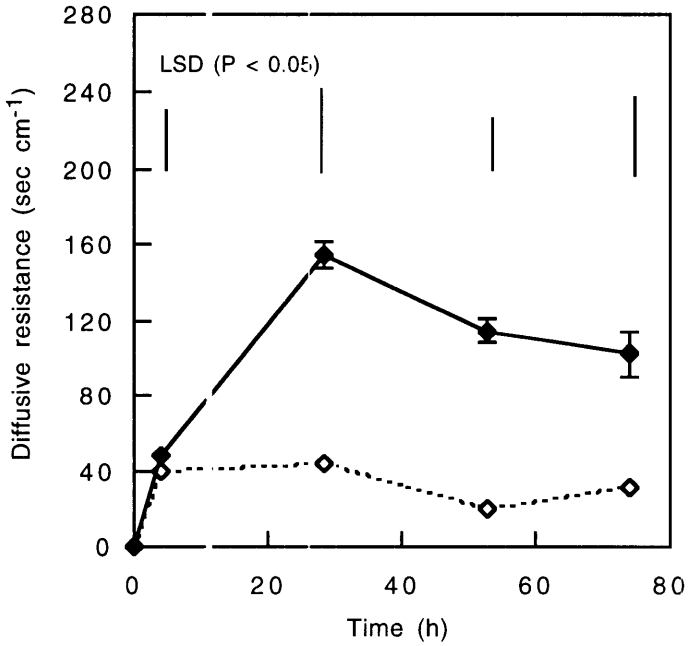

Fig. 5.5. The stomatal diffusive resistance (sec cm^{-1}) of *A. amoena* stems kept in either distilled water (◆) or citric acid (10 mol m^{-3}) (◇) under standard vase life lighting conditions. Each data point is the mean of five replicates ± standard error. (Where no error bar appears, the SE was smaller than the size of the symbol.) Means separated by more than the LSD bar are significantly different (P < 0.05).

(b) There were no significant differences in stomatal diffusive resistance of stems in citric acid or distilled water when kept in complete darkness (Fig. 5.6). A marked increase in stomatal diffusive resistance occurred at 27.5 h for both treatments, which indicated that the stomates were more tightly closed at this time than any other. However, after 27.5 h the diffusive resistance returned to similar figures to the 5 h reading for the duration of the experiment.

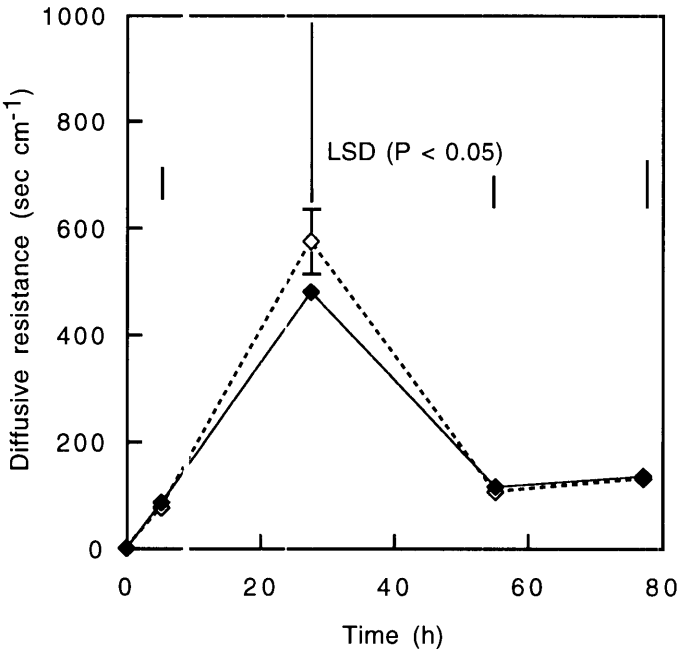

Fig. 5.6. The stomatal diffusive resistance (sec cm^{-1}) of *A. amoena* stems kept in either distilled water (◆) or citric acid (10 mol m^{-3}) (◇) in complete darkness. Each data point is the mean of five replicates ± standard error. (Where no error bar appears, the SE was smaller than the size of the symbol.) Means separated by more than the LSD bar are significantly different (P< 0.05).

5.3.5 Transpiration and xylem water potential of vegetative *A. amoena* stems during stomatal diffusive resistance experiments

The rate of transpiration was higher in distilled water than in citric acid for the first 5 h, but from 23 to 25 h onwards, transpiration was higher in stems kept in citric acid (Fig. 5.7). This indicates that solution uptake would also have been higher in citric acid as, although solution uptake was not measured, it closely parallels the transpiration rate.

The transpiration rate in complete darkness was also higher in distilled water than in citric acid for the first 23 to 25 h, but from then on it was slightly higher in stems kept in citric acid (Fig. 5.8).

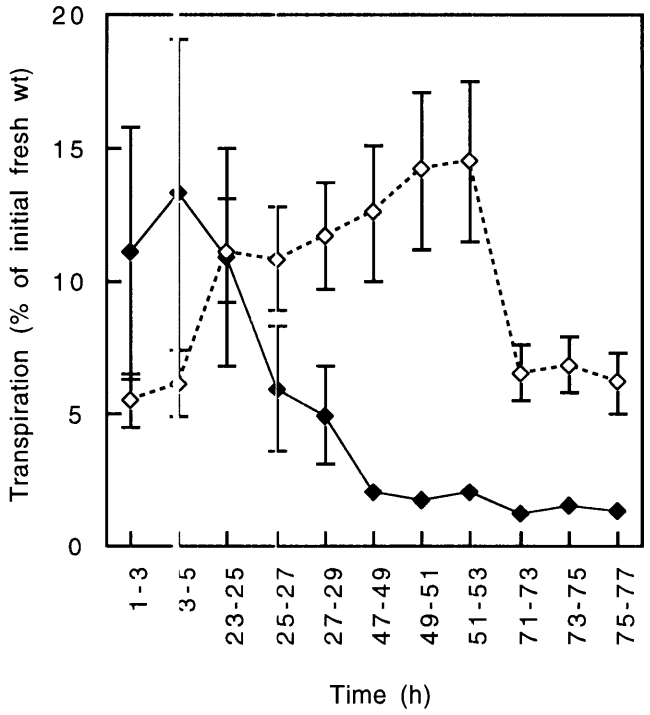

Fig. 5.7. Transpiration (as a % of the init al fresh wt) of *A. amoena* stems kept in either distilled water (◆) or citric acid (10 mol m^{-3}) (◇) during the diffi sive resistance experiment under standard vase life lighting conditions. Each data point is the mean of five replica es ± standard error. (Where no error bar appears, the SE was smaller than the size of the symbol.)

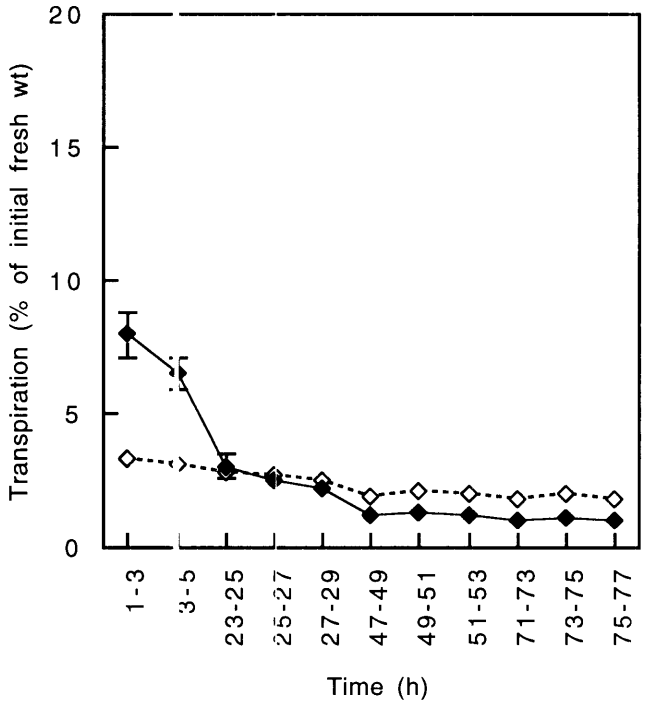

Fig. 5.8. Transpiration (as a % of the init al fresh wt) of *A. amoena* stems kept in either distilled water (◆) or citric acid (10 mol m^{-3}) (◇) during the diffusive resistance experiment in complete darkness. Each data point is the mean of five replicates ± standard error (Where no error bar appears, the SE was smaller than the size of the symbol.)

The xylem water potential of stems was determined at the end of the diffusive resistance experiments. There were significant differences between citric acid and distilled water for both the light and dark treatments (Table 5.2).

Table 5.2

Xylem water potential of *A. amoena* stems at end of diffusive resistance experiments (78 h)

Treatment	Mean xylem water potential (MPa)	
	Light	Dark
Distilled water	$-3.13^{b} \pm 0.53$	$-3.54^{b} \pm 0.21$
Citric acid (10 mol m^{-3})	$-1.03^{a} \pm 0.23$	$-0.97^{a} \pm 0.29$

Means within each column followed by different letters are significantly different ($P < 0.05$). Data are the means of five replicates per treatment ± standard error.

5.3.6 A comparison of the distance travelled by paint in stems kept in citric acid and in distilled water

Paint particles were carried over 100% farther and in more conduits in stems treated for 2 d in citric acid (10 mol m^{-3}) than in distilled water. The paint particles, which theoretically should be stopped by the pit membranes, travelled 9.8 cm in citric acid, which was significantly greater ($P < 0.001$) than the 4 cm travelled in distilled water (Fig. 5.9).

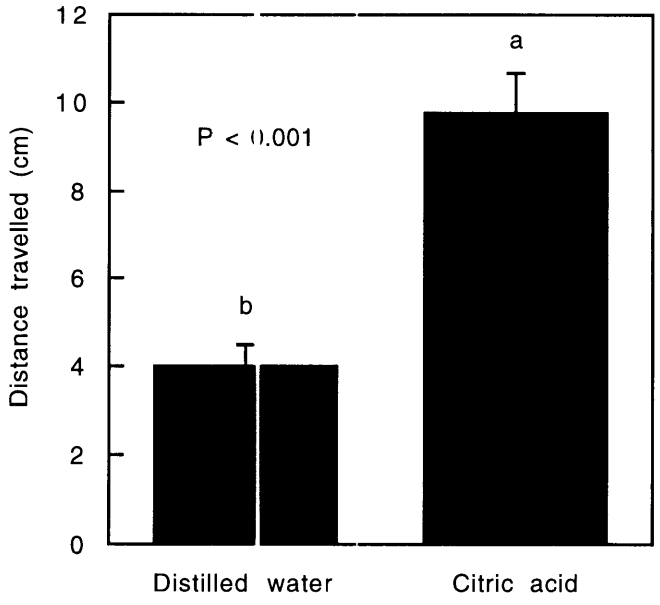

Fig. 5.9. The distance travelled by paint tint (Taubmans) in *A. amoena* stems after they were kept in either distilled water or citric acid (10 mol m^{-3}) for 2 d under acoustic detection lighting conditions. The stems were then transferred to the paint suspension and returned to the acoustic detection lighting conditions for another 2 d, whilst the paint was drawn up by transpirational pull. Transverse sections were cut by hand every 1 cm and the number of paint filled conduits was counted. Data are the means of five replicates ± standard error.

If these data are analysed according to Milburn and Covey-Crump (1971), the conduit lengths fall into the classes depicted below (Fig. 5.10). [See Appendix M for details of the Milburn and Covey-Crump (1971) method of conduit length allocation.] Conduit lengths fell into two classes in the distilled water treatment: the majority (88.9%) were in the 2 cm long class; and the remainder (11.1%) were in the 6 cm long class. In the citric acid treatment, there were four conduit length classes: 2 cm long (61.8%); 5 cm long (26.5%); 8 cm long (8.3%) and 14 cm long (3.4%).

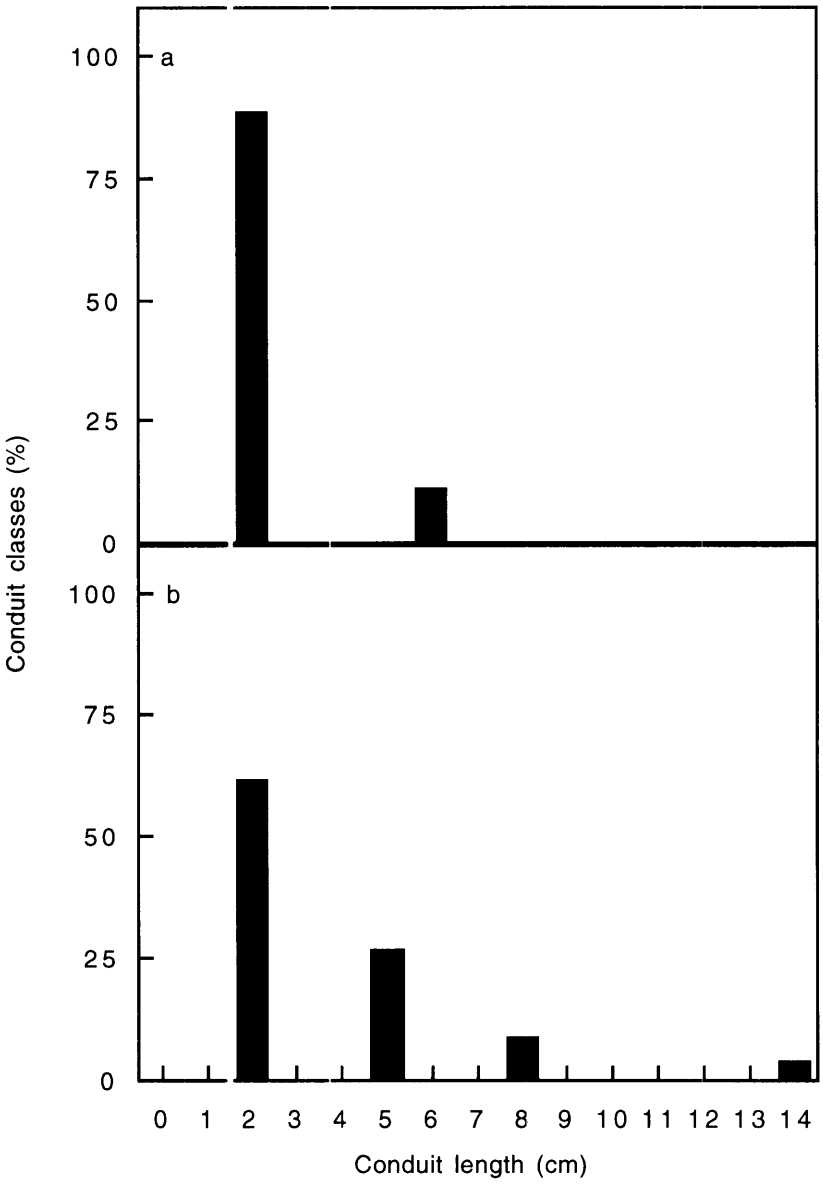

Fig. 5.10. Conduit length profile of *A. amoena* stems. The number of filled conduits per centimetre is expressed as a percentage of the number of initially filled conduits. Conduit length classes were allocated by linear regression analysis, according to Milburn and Covey-Crump (1971) (see Appendix M). (**a**) Injection of paint after 2 d in distilled water; (**b**) injection of paint after 2 d in citric acid (10 mol m^{-3}). Data are the means of five replicates.

5.4 DISCUSSION

The vase life of stems in citric acid and degassed water was the same (5.7 d), and significantly greater than that of stems in distilled water (3.5 d) (Table 5.1). Solution uptake was greater in citric acid than in distilled water (Fig. 5.1) (uptake could not be measured in degassed water). Transpiration was nearly always greater in citric acid than in distilled water and degassed water (Fig. 5.2), although frequently there were no significant differences between citric acid and degassed water. This latter result is surprising because Durkin (1979b) found that flower hydration was more rapid in millipore filtered water (which removes gas) than in citric acid (30 min cf. > 1 h). However, solution uptake was correlated with peduncle water potential (r = 0.96) and there was no significant difference between treatments in water potential (Durkin 1979b). The present results indicate that citric acid was more beneficial than degassed water because transpiration (and therefore probably uptake) was higher. However, both citric acid and degassed water improved water uptake, which resulted in significantly longer vase lives than in distilled water.

Overall, the average oxygen concentration during vase life was lowest in degassed water and next lowest in citric acid (Fig. 5.3). Longevity was not significantly different in these two treatments (Table 5.1), therefore, it is tempting to propose a relationship between lower vase oxygen concentration and longevity. However, linear regression analysis revealed only a weak (adjusted R^2 = 0.22) but significant negative relationship between longevity and oxygen concentration. Thus, 78% of the variation in longevity cannot be explained by oxygen concentration. When the average daily transpiration or uptake for each treatment replicate was regressed against average oxygen concentration per treatment replicate, only transpiration in degassed water showed a significant (t-value = 2.01), albeit weak (adjusted R^2 = 0.25), positive relationship (Appendix C). This was contrary to expectation, as it revealed that the replicates with the highest oxygen concentration during vase life had the highest rate of transpiration (≈ solution uptake). Therefore, little statistically convincing evidence was found to suggest a loss of hydraulic conductance (i.e. decreased solution uptake and transpiration) was promoted by high levels of dissolved oxygen. Conrado *et al.* (1980) found that oxygen concentration had no significant effect on solution uptake after 72 h. However, their results should be interpreted with caution because deoxygenated solutions were prepared by replacing oxygen with nitrogen, which is equally capable of initiating cavitation.

More AAE were recorded in degassed water than in citric acid (Fig. 5.4 cf. Fig. 4.4). The main AAE peak for stems kept in degassed water occurred at 108 h, which corresponded with a xylem water potential of -3.9 MPa. In citric acid, the number of AAE remained low throughout the experiment, although the xylem water potential decreased from -0.42 to -2.62 MPa between 48 and 60 h, and by 108 h it was <-4.0 MPa (Fig. 4.4). However, the time to phyllode desiccation

occurred much later in citric acid (08 h, section 4.3.3) than in degassed water (72 h, section 5.3.3). These results are surprising because it would be expected that as less air was available in degassed water for cavitation induction, the number of AAE would be lower. Yet citric acid had the lowest number of AAE of any cavitation experiment. The AAE results, together with the finding that oxygen concentration was only weakly related to longevity, therefore indicate that longevity and the beneficial effect of citric acid are not related to low solution oxygen concentrations. Nevertheless, water stress was delayed when *Acacia* stems were kept in citric acid or degassed water, thus extending vase life (Table 5.1) compared with distilled water. Perhaps vase life is a coarser measure of water stress than cavitation production, water potential and stomatal diffusive resistance. It has been found that stomatal resistance is a more sensitive measure of plant water status than RWC (Al-Ani and Bierhuizen 1971).

The significantly greater stomatal diffusive resistance (in light) after 4 h for stems in distilled water compared with citric acid (Fig. 5.5) indicated that the stomates were not as open. Thus, there was more water stress in stems kept in distilled water. Although these stems were kept under less intense lighting conditions (vase life lighting conditions) than for AAE detection (Fig. 4.2), and so are not strictly comparable, the results nevertheless show that increased water stress occurs when stems are kept in distilled water, irrespective of the lighting used. These diffusive resistance results revealed that water stress develops within 28 h of placing stems in distilled water, which was also indicated by the AAE production and water potential results (Fig. 4.2). Zelitch (1969) noted that stomatal opening depended primarily on water uptake by guard cells, therefore severe water stress would be indicated by decreased stomatal opening and hence increased stomatal diffusive resistance. Stomatal closure is thought to be a strategy to avoid catastrophic xylem failure ("runaway embolisation") if water potential falls to a particular cavitation threshold (Jones and Sutherland 1991).

The stomatal diffusive resistance of stems in complete darkness was not significantly different for the distilled water and citric acid treatments (Fig. 5.6). The sudden increase in diffusive resistance that occurred at 27.5 h was not likely to be the result of a water deficit as both treatments showed the same pattern of stomatal behaviour, yet stems in distilled water had a significantly lower xylem water potential than stems in citric acid (Table 5.2). Therefore, this unusual pattern of stomatal behaviour in darkness (Fig. 5.6) cannot be explained by Stålfelt's (1961) findings that stomatal opening can occur in the dark if a slight water deficit occurs. Perhaps biological variation, or equipment malfunction are more likely explanations.

The xylem water potential of stems in distilled water was significantly less than in citric acid, regardless of whether the stems were kept in light or complete darkness (Table 5.2). This indicated that stems in distilled water developed more water stress than those in citric acid. In darkness, although no significant differences were recorded in diffusive resistance, there were

significant differences in xylem water potential at the end of the experiment (78 h). Thus, it would appear that, in darkness, diffusive resistance alone is not a good indicator of water stress, because of the overriding influence of stomatal closure, regardless of treatment.

In darkness, transpiration was initially higher in distilled water than in citric acid, but after 25 h, the transpiration rate was slightly higher in citric acid (Fig. 5.8). Carpenter and Rasmussen (1973) found that water uptake rates (≈ transpiration) over 120 h were significantly greater in light than in darkness, and attributed this to stomatal closure in the dark. However, in the present results, after 49 h the transpiration rate of stems in distilled water and light was similar to that of stems kept in darkness. It was only stems in citric acid and light that had a higher transpiration rate than other treatments, which indicates that solution uptake was less impeded in citric acid than in distilled water. Light is necessary to instigate the transpiration stream, but in distilled water, it appears that a blockage occurring after 25 h precludes upward movement of water into the stem to replace water lost to the atmosphere. The stomates, by closing (Fig. 5.5), attempt to mitigate water loss, but this action alone cannot prevent a declining water balance. Closed stomates result in reduced transpiration, which translates to lower water uptake (de Stigter and Broekhuysen 1986).

Citric acid is employed in tissue culture because its anti-oxidant properties retard the browning of freshly-excised tissues (Erner *et al* 1975; Murashige 1977). The anti-oxidant properties of ascorbic acid were thought to be responsible for increased infusion rates (compared with distilled water) into tree seedlings (Duncan and Himelick 1990). However, Duncan and Himelick (1990) did not discount the possibility that ascorbic acid, like oxalic acid, may disrupt calcium in the middle lamella of pit membranes and make the membranes more open and elastic (Sperry and Tyree 1988). Ikeda *et al.* (1989) found that *Pinus* cuttings treated with benzoic acid (300 ppm) had openings on the torus of some bordered pits. Water movement was therefore not disturbed and remained high. Cavitation was thought to be prevented because the bordered pits did not aspirate. Oxalic acid is also known to cause structural modifications and degradation of the pit membrane layers (Wisniewski *et al.* 1991). Perhaps the effect with citric acid is similar to that of benzoic and oxalic acids. This hypothesis awaits testing under TEM.

The finding that paint particles travelled over 100% farther and in more conduits in citric acid than in distilled water (Figs. 5.9 and 5.10), together with the low number of AAE in citric acid (Fig. 4.4), also indicates that citric acid degrades pit membranes. This would explain the increased hydraulic conduction that always occurs in citric acid (Figs. 5.1, 3.6 to 3.10, 9.4). It is therefore likely that citric acid, by degrading pit membranes, acts in a similar way to oxalic and benzoic acids. Accordingly, the beneficial effect of citric acid is not likely to be attributed to inhibition of microbial growth (Table 4.2), but to an anatomical change in xylem conduits brought about by acidification. Durlin (1979a, b) believed that citric acid caused an anatomical

change to occur, but thought that lateral water movement was enhanced. The results of the paint uptake experiment (Figs. 5.9 and 5.10) revealed that an anatomical change did occur, but that it was longitudinal rather than lateral water movement that was enhanced.

Citric acid had a lower oxygen content and promoted stomatal closure compared with distilled water, but the most interesting effect is that of improving hydraulic conduction via pit membrane degradation. This finding explains the frequently observed increases in hydraulic conduction that occur with citric acid.

The following chapter examines the relationship between vase life and bacterial numbers in an ethylene-sensitive genus, *Boronia*.