CHAPTER TEN

GENERAL DISCUSSION

10.1 GENERAL DISCUSSION

After at least 90 years of research towards increased cut flower longevity, it is somewhat disconcerting that the earliest problems and recommendations are still valid today. In 1906, Fourton and Ducomet observed that:

> "The flower, detached from its natural connection, ... [is] capable of maintaining its turgidity as long as on the plant, provided that it is furnished with water and that putrid fermentations are avoided, probably harmful by the obstructions which they produce in the canals and which oppose the passage of water."
>
> (translated from French)

Since that time, there has been much speculation about the cause of the 'obstructed canals' described by Fourton and Ducomet (1906). The results of this thesis have shown that bacteria, *per se*, (the "putrid fermentations" referred to by Fourton and Ducomet 1906) are not responsible for decreased longevity, at least in *Acacia* and *Boronia*. Blockage of xylem conduits by micro-organisms is an attractive and logical hypothesis, however, its general applicability should be questioned seriously. Bacterial plugging should not be accepted as the primary reason for premature blockage of xylem conduits.

In 1934, Dorner noted that flowers and leaves died because water uptake was less than water loss. He postulated that further work in this area may unravel the problem of keeping quality (Dorner 1934). Certainly, the longevity of some flowers, such as carnations, has been extended dramatically (by breeding and STS pulsing) from five to approximately 20 d, and the promise of genetically engineered 'long-life' flowers portends much for the future. However, the initial problems recognised by the early researchers still remain incompletely answered, specifically, what is the cause of impaired water uptake?

Impaired water uptake was associated with *Acacia* cut flower senescence. Uptake rates in distilled water were significantly less than in citric acid, and fell dramatically after the first 24 h in distilled water (Chapter 3). In citric acid, however, the rate of solution uptake only began to decrease after 60 h. These findings lead to the extension of preliminary *Acacia* cavitation detection experiments begun with distilled water during the author's B.Sc.(Hons) year.

It was found (Chapter 4) that cut stems cavitate when kept in solutions, thereby providing the first direct evidence of this often-surmised event (Dixon *et al.* 1988; de Stigter and Broekhuysen 1989; Dixon and Peterson 1989) in cut flowers. However, initial cavitation rates appeared to be too low to account for rapidly decreasing water potentials, unless the larger, more vulnerable conduits with, according to the Hagen-Poiseuille equation, flow rates proportional to the fourth power of the capillary radius, cavitated first. To provide evidence that cavitation is responsible for decreased hydraulic conduction in cut flowers, it is necessary to determine if the larger and more vulnerable xylem conduits cavitate first. This would indicate that the relatively few early clicks evident before the peak are disproportionately important. Indeed, Dixon *et al.* (1988), measuring rose stems dehydrating in air, revealed that the larger conduits cavitated first because the few early cavitation events were associated with disproportionate amounts of water loss and significant decreases in hydraulic conductance. Tyree and Dixon (1986) and Ritman (1988) also found that more water was lost per early AAE than at the end of stem segment dehydration. The results of the hydraulic conductivity experiment in Chapter 9 (Fig. 9.4) lend further support to these findings. Thus, the early AAE detected in cut flowers are likely to be of major significance in the disruption of water uptake and flow.

The results of this thesis have established that cavitation is a common occurrence in cut flowers in vase solutions. However, the first major event curtailing vase life remains problematic, unless the plant tissues respond to injury by initiating gas bubbles within xylem conduits and the larger, more vulnerable conduits cavitate first. If this is the case, then cavitation may indeed be the first and most important event precluding water uptake. Embolised conduits would then inititate the series of events that occur after water flow is disrupted such as, *inter alia*, tylose formation, cell wall breakdown and pectin deposition in xylem conduits.

Stems kept in citric acid (10 mol m^{-3}) produced few AAE and no discernible peak in AAE production compared with stems in other solutions (Chapter 4). However, the xylem water potential of stems in citric acid decreased markedly between 48 and 60 h. These results corresponded in time with the reduction in solution uptake in the cut flower longevity experiments (Chapter 3). However, it was uncertain why stems in citric acid would produce so few AAE, despite a decreasing xylem water potential. Citric acid is known to increase "stem porosity" (Durkin 1986b) but, according to Halevy and Mayak (1981), "the mode of action of the acidification in promoting hydration is not clear". Based on the low incidence of AAE production in citric acid, the following hypothesis was proposed for the action of citric acid. It was hypothesised that citric acid degraded pit membranes, which resulted in less potentially cavitatable compartments (Chapter 4) and improved hydraulic conduction rates (Chapter 9). This hypothesis was supported by the distance travelled by paint in xylem conduits of stems kept in either distilled water or citric acid for 2 d (Chapter 5). Paint particles, which should be stopped by pit membranes, were carried over 100% farther in stems kept in citric acid than in

distilled water. This finding provides a plausible and factual explanation for the mechanism by which citric acid hydrates stems. However, as warned by Durkin (1986a), citric acid "is not a preservative". Its action can perhaps best be described as "palliative" (Milburn and Williamson 1996). Nevertheless, the beneficial property of citric acid can be exploited by adding it along with other vase solution components, or by using it as a "conditioning" treatment. A weaker citric acid solution (<10 mol m^{-3}) may be more suitable for flowers which are sensitive to it, possibly violets and primulas (Fourton and Ducomet 1906). Additional visual evidence of the citric acid pit membrane digestion hypothesis could be provided by examination of pit membranes under the high resolution afforded by transmission electron microscopy (TEM). Pit membrane degradation by citric acid compared with distilled water and freshly cut stems would provide compelling evidence for its proposed action. It may also be possible to observe the effect on pit membrane structure of different citric acid concentrations using TEM. It is the author's intention to pursue the TEM study upon completion of this thesis.

A possible alternative to using citric acid to improve hydraulic conduction is to breed cut flowers which root readily in water. The adventitious roots would thereby maintain a water-xylem continuum, by-passing the blocked cut end of the stem. Such rooting characteristics already exist in several plants, e.g. the mint family (Lamiaceae), including common mint (*Mentha* × *piperita* L.) and basil (*Ocimum basilicum* L.); weeping willow (*Salix babylonica* L., Salicaceae) and African violets (*Saintpaulia* spp. Wendl., Gesneriaceae). Although essentially no longer cut flowers, the hydroponic cuttings would undoubtedly provide a long vase life.

High concentrations (>10^6 cfu mL^{-1}) of mixed and pure (*Ps. fluorescens*) bacterial populations did not decrease *A. amoena* longevity or RWC. Nor was AAE production expedited by inclusion of *Ps. fluorescens* (10^6 and 10^8 cfu mL^{-1}) in the vase solution (Chapters 4 and 7). Furthermore, none of the bacteria isolated produced pectolytic enzymes (Chapters 7 and 9), a finding also recorded by de Witte and van Doorn (1988). In addition, SEM observations (Chapter 8) did not reveal any obvious increases in bacterial colonisation and blockage of cut stems or anatomical changes, even after 5 d in water. These results provide evidence against the proposition that bacteria commonly cause xylem blockage, either directly or by the production of pectic enzymes. However, it is possible that different bacteria may exert variable effects on vase life (Zagory and Reid 1986b). Some (unidentified) bacteria which were isolated from carnation vase water significantly decreased the vase life of 'Gabrielle' roses (Jones and Hill 1993). Yet these appear to be rare instances. Others have found that different bacterial genera, species and strains had similar effects in decreasing water uptake and vase life (de Witte and van Doorn 1988; Put and Jansen 1989). Thus, in view of the results for two unrelated genera presented in this thesis, and the numerous studies which have found that bacteria did not decrease longevity (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), bacteria should no longer be implicitly assumed to play a major role in cut flower senescence.

The validity of many of the "amorphous" deposits observed by several researchers (Fujino *et al.* 1983; Put and Rombouts 1989; van Doorn *et al.* 1991a, b; van Doorn, de Stigter, de Witte and Boekestein 1991) could be ascertained using the method described by Exley *et al.* (1974). They recommended soaking wood samples in a sodium hypochlorite solution (section 8.4) to remove cytoplasmic debris prior to SEM preparation and examination. Use of this method would reveal whether the "amorphous" substances remained in the xylem of treated specimens, and therefore were either vestured pits or warts (Chapter 8). If the "amorphous" deposits disappeared after sodium hypochlorite treatment, they were likely to have been cytoplasmic debris. However, whilst this method could clarify the "amorphous deposit" issue, it is likely to be peripheral to the crucial issues involved in cut flower senescence. Certainly, cell wall breakdown and changes in membrane integrity are involved in the senescence process, but the relative coarseness of SEM examination is unlikely to reveal the very fine detail required. TEM or electrolyte (solution conductivity) measurements are, perhaps, more appropriate techniques with which to pursue this line of research.

The increase in pectinaceous deposits over time in *A. amoena* stems kept in distilled water was not caused by the secretion of pectolytic enzymes from isolated bacteria (Chapter 9). Pectin deposition began <u>after</u> both AAE production and decreased hydraulic conduction had occurred. These results support Durkin's (1979a) hypothesis that carbohydrate blockages were the result of the loss of xylem function and not the cause. Such blockages did not form after hydraulic conduction decreased in stems kept in citric acid (Chapter 9). The enzymes responsible for pectin deposition may be inhibited by the pH of citric acid. This area requires further investigation.

The vase solution used by Accati and Sulis (1980) to increase *Acacia dealbata* longevity produced the shortest vase life of all treatments in *A. floribunda* (Chapter 3). Nevertheless, the solution has been recommended to extend *Acacia* vase life (Sedgley 1989; Sedgley and Parletta 1993), and perhaps highlights the lack of postharvest research available on *Acacia*. The largest botanical genus in Australia is *Acacia*, with over 750 of the world's 1,200 species (Elliott and Jones 1982; Harden 1991). The European demand for *Acacia* as a cut flower is such that many years have been spent researching its cultural requirements in the northern hemisphere (de Ravel d'Esclapon 1962; Timmermans 1989). Potentially, Australians have a temporal advantage in being able to bypass such preliminary cultural research, and instead concentrate on the development of effective postharvest recommendations for *Acacia* spp. Such research needs to be performed if Australia is to realise a potential export advantage, particularly in terms of *Acacia* product variety.

Ethylene is not responsible for the premature senescence of the two *Acacia* spp. (*A. floribunda* and *A. subulata*) examined in this thesis (Chapter 3). However, caution should be exercised in proclaiming *Acacia* as an ethylene-insensitive genus. The vase life of *A. baileyana* was shortened in the presence of ethylene (J. Truett, pers. comm. 1993), and several other acacias have exhibited ethylene sensitivity (section 3.1). Differing responses to ethylene within the genus suggests that scope exists for breeding *Acacia* cultivars [as has been done in France for over 30 years (de Ravel d'Esclapon 1962)] without an ethylene based senescence.

In contrast to *Acacia*, the vase lives of *Boronia heterophylla* and the natural hybrid, *B. crassipes* × *B. heterophylla* 'Lipstick' were affected by ethylene. Longevity was significantly extended in these two *Boronia* spp. by use of an STS pulse (Chapter 6), which indicates an ethylene-related senescence. Woltering and van Doorn (1988) observed that the response of cut flowers to an STS pulse is an indication of whether the plant is affected by the presence of ethylene in its natural senescence process. However, two other *Boronia* spp. tested, *B. muelleri* 'Sunset Serenade' and *B. clavata*, did not demonstrate an ethylene-based senescence. Thus, an STS pulse should not be regarded as a general 'panacea' for prevention of premature senescence in *Boronia* spp.

Although vase life was significantly greater in *B. heterophylla* when treated with an STS pulse, this treatment also had the highest number of vase solution bacteria (Chapter 6). However, no statistical relationship was found between the number of bacteria in the vase solutions and longevity. Furthermore, high numbers of bacteria in stem segments did not reduce vase life. Therefore, it would appear that the main factor increasing longevity in ethylene-sensitive species is prevention of ethylene action by an STS pulse. Nevertheless, a treatment (citric acid and sucrose) that (i) did not protect against ethylene action; and (ii) contained high numbers of bacteria in the vase solution and stem segments, produced the second longest vase life. These results lend further weight to the *Acacia* results, in which high bacterial numbers were found in solutions that produced a long vase life. The reason why high concentrations of bacteria in vase solutions and stem segments did not limit, and might actually promote, vase life needs further investigation. Bacteria decrease vase solution oxygen content, thereby conceivably removing a potential source of cavitation nuclei. However, lower oxygen concentrations did not increase vase life (Chapter 5). It is hoped that this thesis has opened new areas of debate, as well as provided direct evidence of a novel mechanism explaining impaired water uptake in cut flowers.

10.2 RECOMMENDATIONS

This thesis has shown that it is imperative, when undertaking any initial longevity experiments with a new species or cultivar, to ascertain whether the plant is ethylene-sensitive or not. If the plant is sensitive to ethylene, pulsing with STS (or an environmentally acceptable, and effective

alternative) is a base treatment which will improve longevity. Traditional vase solution components such as sucrose, citric acid and germicides can then be added to vase solutions after the STS pulse. A germicide concentration which is sufficient to reduce microbial numbers, but which is not injurious to the plant will need to be determined for each species and cultivar. However, inclusion of a germicide is, in the author's opinion, largely for aesthetic reasons. In this thesis, it was demonstrated that longevity can actually be enhanced by high microbial numbers in both vase solutions and stem segments. If it is desired to use a germicide, 8-HQC should no longer be recommended. It is a mutagen/carcinogen, and is also ineffective against most gram negative bacteria (Albert *et al.* 1947), the most common bacterial type found in vase water. Finally, it is strongly recommended that cut flower researchers adopt sound plant physiological practices when measuring hydraulic conductivity, and use stem segments which are longer than the longest conduit length.

"The flower in the vase still smiles, but no longer laughs."

Malcolm de Chazal
(1902 - ?)

but

"A flower unplucked is but left to the falling,
And nothing is gained by not gathering roses."

Robert Frost
(1874 - 1963)