

Bacterial Growth and Stem Water Relations in Cut Flowers

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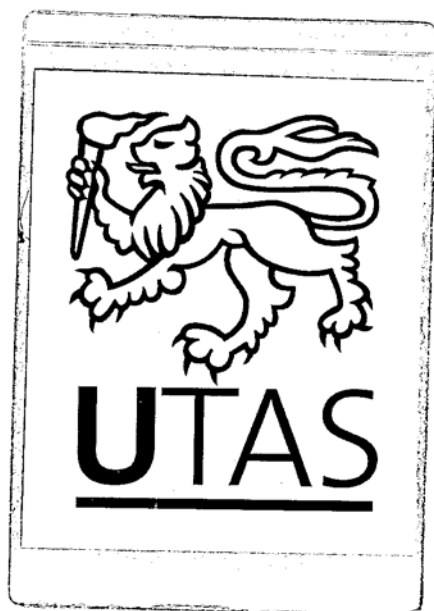
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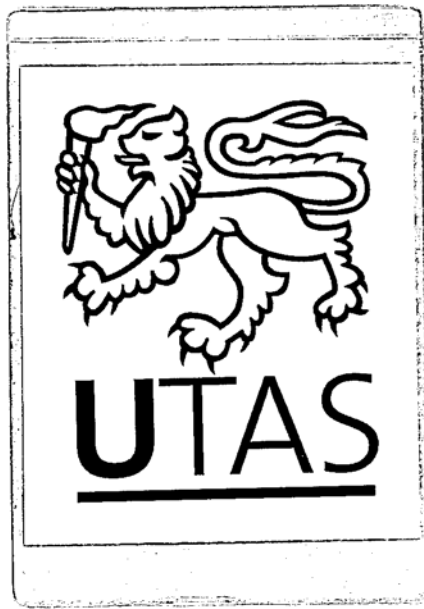
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I. ABSTRACT

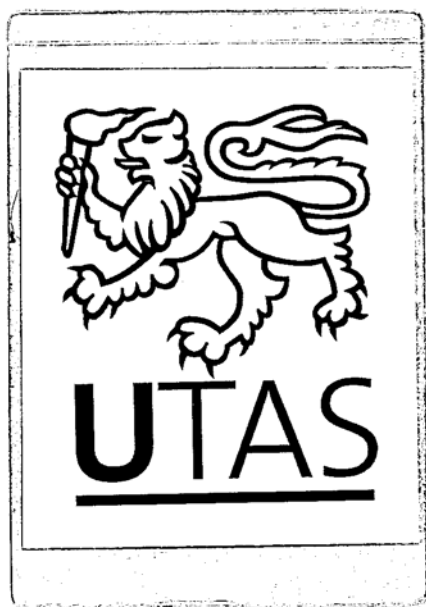
I. Abstract

Longevity of vase life is a significant quality parameter in the cut flower industry. A number of factors have been postulated to influence longevity of vase life, and many of these involve disruption to water relations within the stem, particularly increased hydraulic resistance as a result of air embolisms, physical occlusions in the xylem vessels or bacterial interference. The purpose of this study was to investigate the role of bacteria in disrupting hydraulic resistance in the stems of three important cut flower species.

Xylem vessel length distributions of carnation, iris and chrysanthemum were determined, the maximum vessel length of each flower species being less than 4, 1 and 6 cm respectively. Stem sections longer than these vessel lengths were then used to determine the relationship between bacterial number and hydraulic resistance in various parts of the stem. Bacterial populations increased in all parts of the flower stem during the vase life period. The largest populations were found in the basal section of stems, particularly within 2 mm from the cut surface. The basal section was also the site of highest stem hydraulic resistance. However, only a very weak correlation existed between bacterial numbers in various parts of the stem and hydraulic resistance. Furthermore, bacterial cells were found to travel higher up the stem than the length of the longest vessels, indicating that the bacterial cells were unlikely to be the major cause of occlusions at intervessel pits. Xylem occlusion was more likely to be located at or close to the stem cut surface as removal of a 5 mm segment from the stem base resulted in significantly decreased hydraulic resistance.

Positive correlations were obtained between stem hydraulic resistance and concentrations of a bacterial exudate, bacterial exopolysaccharide (EPS), which was found to be present primarily at the base of the stem. Bacterial

exopolysaccharides were also found in xylem vessels further up the flower stem but distribution was generally restricted to a small number of vessels. In contrast, the bacterial EPS at the base of cut flower stems covered all cut vessels within 2-4 days of vase life. Bacterial EPS extracted from cultured bacterial cells was found to significantly increase hydraulic resistance when attached to filter paper disks covering one end of a section of open tubing. The degree to which EPS caused increased hydraulic resistance was found to be influenced by the substrate on which the bacteria were established suggesting that EPS composition influenced its capacity to induce hydraulic resistance. Non-EPS producing bacteria were found to have minimal effect on hydraulic resistance in the filtered tubing section, even when applied in greater bacterial cell concentrations than EPS producing bacteria. It was concluded that bacterial exopolysaccharides play an important role in the development of hydraulic resistance around the stem cut surface, thus restricting water uptake and shortening cut flower vase life. The results of this study provide a basis for further research which may lead to substantial improvements in post-harvest techniques for the cut-flower industry.



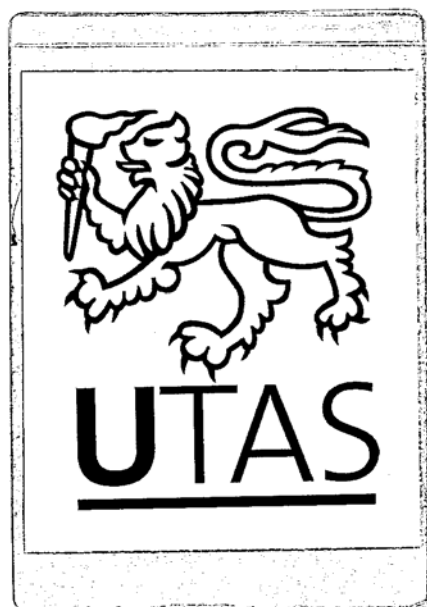
II. GENERAL INTRODUCTION

II. General Introduction

High quality and maximum vase life of cut flowers are the most desirable product characteristics in the cut flower industry. A large number of studies have examined the effect of both preharvest and postharvest conditions on flower longevity (Jones, 1991; Halevy and Mayak, 1979; Halevy and Mayak, 1981; Parups and Chan, 1973, Burge et al, 1996; Shvarts et al, 1997) and it has been widely accepted that bacteria are the most common cause of loss of vase life in cut flowers. Bacterial cells are thought to cause vascular occlusion, leading to a decrease in stem hydraulic conductance and consequentially earlier end of vase life (Put and Jansen, 1989; Burdett, 1970; de Witte and van Doorn, 1988; van Doorn and de Witte, 1991; Put and van der Meyden, 1988; van Doorn et al, 1991). However, evidence supporting this hypothesis is not complete and a number of alternative hypotheses have been proposed (Zagory and Reid, 1986; Williamson and Milburn, 1995; Kaus et al, 1996; Lineberger and Steponkus, 1976). Thus, despite years of intensive research examining cut flower physiology, the mechanism of bacterial reduction of vase life is still debated.

The aim of this study was to examine the role which bacteria play in disrupting water uptake in cut flower stems.

III. LITERATURE REVIEW



III. Literature Review

1 Introduction

Maximising cut flower vase life has been a major objective of floriculture research for many years. Numerous publications have reported on studies demonstrating the effects of preharvest conditions, stage of development and time of harvest, environmental conditions, relative humidity during crop growth, vase solution additives and other postharvest treatments on flower longevity. Much of this information has been reviewed in texts (eg. Jones, 1991) and review articles (Lewis and Borst, 1993; Kyalo et al, 1996; Shvarts et al, 1997; Jones and Hill, 1993; Burge et al, 1996; Parups and Chan, 1973; Mortensent et al, 1997, 1998; van Doorn, 1997a). While the optimum postharvest treatments for most important cut flower species are well documented, relatively little has been published on the physiological basis of cut flower postharvest handling. The published information on cut flower physiology has been comprehensively reviewed by Halevy and Mayak (1979; 1981) but there has been no detailed review since then. Significant advances have been made in recent years in understanding the importance of gene expression (Eason and de Vre, 1995; van Doorn and Stead, 1994; Woodson, 1994) and protein synthesis (Jones et al, 1994) during senescence of cut flowers. Attention has centred on the senescence reactions in petals as the commercial longevity of flowers is generally linked to these floral organs. While the genetic, biochemical and physiological processes occurring during petal senescence have received much attention over the past five years, other aspects of cut flower physiology such as water relations and the role of bacteria in reducing cut flower vase life still merit further attention given their importance to cut flower vase life. This literature review will summarise

major aspects of cut flower postharvest management and postharvest physiology, with emphasis on water relations and provide a more comprehensive review of the role of bacteria in reduction of cut flower vase life.

2 Cut Flower Postharvest Management

The floral organs of higher plants are generally short lived structures which persist only for the duration of the pollination and fertilization processes. Cut flowers typically exhibit high rates of respiration which leaves are the major transpiring surface (Jones, 1991). The photosynthetic capacity of flowers is generally low and they are reliant on assimilates translocated from other parts of the plant to maintain the high rates of metabolic activity. Harvesting of cut flowers separates the structures from the normal source of carbohydrates and water supply. The majority of postharvest treatments for cut flowers are thus aimed at extending vase life by providing a supply of water and carbohydrates and/or reducing water loss and metabolic activity within the flower structure.

2.1 Vase solution

Cut flowers are very sensitive to desiccation, the vase water in which cut flowers are placed is thus an important determinant of cut flower vase life. A large number of studies have been conducted on the effects of water quality and a vast array of vase water additives on cut flower vase life. These treatments can be grouped into those which influence the microbial activity in the vase water (van Doorn et al, 1989; 1990), those which influence the behaviour of water during uptake by the flower (Durkin, 1979a), and those which supply nutrients to the cut flower (Halevy and Mayak, 1979; Olley et al, 1996). While many treatments have been described in the literature, the most

important commercial treatments are the use of bacteriocides (Jones, 1991), acidification of the vase water (Jones, 1991), and the addition of sucrose to the vase water (Doi and Reid, 1995). For the flower species that are sensitive to ethylene, addition of the ethylene synthesis inhibitor silver thiosulphate is also required (Barrowclough et al, 1991).

Inhibition of microbial growth

In general, vase life of cut flowers is negatively correlated with the increase in bacterial number in vase water (Put and Jansen, 1989). Bacteriocides have been shown to decrease vascular blockage in flower stems, increase water uptake, increase stomatal closure (Burdett, 1970; Marousky, 1969; Parups and Chan, 1973; Burge et al, 1996), improve flower longevity (Marousky, 1971; Burge et al, 1996), increase flower diameter and inhibit growth of microorganisms (Larsen and Scholes, 1965; van Doorn, 1998; Larsen and Cromarty, 1967). Bacteriocides, therefore, are included in all preservative formulations added to vase water. 8-hydroxyquinoline sulphate (HQS) and 8-hydroxyquinoline citrate (HQC) are commonly used bacteriocides. Other antimicrobial compounds which have been found to be effective in inhibiting growth of bacteria and preventing decrease of hydraulic conductance in stems are NaOCl and other compounds generating OCl⁻, benzalkonium chloride (a quaternary ammonium compound), aluminium sulphate and sodium isoascorbic acid (Na-isoAA) (van Doorn et al, 1989, 1990; Parups and Chan, 1973).

Despite the many reports in the literature of beneficial effects of various bacteriocides, there are many conflicting accounts of the most effective compounds and optimum concentrations, and it is therefore likely that the effects of bacteriocides vary among flower species or even between flower cultivars. Jones and Hill (1993) showed that HQC prolonged vase life and improved solution uptake in *Rosa* and *Gypsophila* but did not extend vase life

of a number of cultivars of *Lilium*, *Gerbera*, *Narcissus* and *Freesia*. Jones and Hill (1993) also showed that longevity of *Gerbera* 'Mercy' was improved by sodium dichloroisocyanuric acid (DICA) and 1-bromo-3-chloro-5, 5-dimethylhydantoin (BCDMH), while longevity of *Gerbera* 'Double Delight' was not improved. Parups and Chan (1973) showed that, at equimolar concentrations, Na-isoAA was considerably more effective than 8-HQS in promoting the water uptake or retention in cut rose stems.

Supply of nutrients

The supply of nutrients and water to the flower is interrupted when the flower is excised from the mother plant. The most significant change is the reduction in phloem delivery of sucrose to the floral organs, but reduced supply of mineral ions, proteins and plant hormones also occurs and may impact on cut flower vase life.

Sucrose is an essential component of most cut flower vase solutions and is used both in preservative or holding solutions, and in pulsing solutions, where the flowers are exposed to a pulse of the solution for a short period of time. Sucrose is included in most preservative formulations and is generally accepted to have a benefit in extending cut flower vase life (Doi and Reid, 1995; Geertsen, 1990; Larsen and Scholes, 1965; Parups and Chan, 1973). Sucrose in the vase solution has a dual role, providing osmotic potential and to providing respirable substrate (Halevy and Mayak, 1979; Olley et al, 1996). The recommended concentration of sucrose in vase solution was normally in the range of 1 to 5% depending on the type of flower, temperature and treatment time (Jones, 1991; Larsen and Scholes, 1965). Ligawa et al (1997) showed that 6% (w/v) sucrose concentration caused flower discolouration in *Grevillea* 'Sylvia'. Sucrose has also been demonstrated to promote bud opening in hybrid *Limonium* and *Gladiolus*. Applying sucrose is essential for continued bud opening as there is competition among florets for the available

carbohydrate (Doi and Reid, 1995; Bravdo et al, 1974). Bravdo et al (1974) found an improvement in water uptake in *Gladiolus* spikes held in up to 18% sucrose solutions and suggested that this effect was due to increased osmotic potential of the florets and leaves. Adding sucrose into vase water was found to increase flower size (Larsen and Scholes, 1965; Geertsen, 1990) and induce stomatal closure resulting in decreased transpiration and weight loss in cut rose flowers (Marousky, 1969; 1971) and in *Grevillea* 'Sylvia' (Ligawa et al, 1997).

Adding sucrose alone into vase water has been shown to reduce flow rate and retard hydration of cut flowers (Durkin, 1979a; 1979b). However, this could be overcome by combination with acid which has been found to increase vase life of *Gladiolus*, *Thryptomene*, *Dianthus*, *Gypsophila* and *Rosa* (Jones, 1991; Parups and Chan, 1973). The addition of sucrose alone to vase water is thought to promote microbial growth and hence reduce cut flower vase life. Preservative solutions containing sucrose therefore always contain a bactericide.

Nutrient status of cut flowers may be improved through the applications of pulsing solutions. Pulsing is a short-term treatment which is usually performed by growers or shippers, especially with ethylene-sensitive flowers. The main ingredient of the pulsing solutions is sucrose, where optimum concentrations vary to the type of flowers but are often higher than those used in preservative formulations. The other important factors in pulsing are duration of pulsing, temperature and lighting conditions. Normally, the period of pulsing is between 12 to 24 hours, with light of 1000 lux and temperature of 20°C to 27°C (Halevy and Mayak, 1981). However, Wirthensohn et al (1996) showed that cut stems of *Eucalyptus* (L. Her.) foliage given a pulsing treatment at low temperature (3°C) for 24 hours had longer vase life than stems pulsed at high temperature (24°C) for 2 hours. It is not clear whether this response was due to the lower temperature or the longer period of pulsing or a combination of the two factors.

Properties of water

Acidification of the vase solution can significantly extend cut flower vase life (Conrado et al, 1980; Halevy and Mayak, 1981; Marousky, 1971). The beneficial effect of low pH on vase life has generally been attributed to reduction of microbial population. It has been shown that number of bacteria in rose stems was reduced from 10^4 colony forming units (cfu)/g fresh weight to 10^2 cfu/g fresh weight, when pH of the vase solution decreased from pH 6 to pH 3 (Van Doorn and Perik, 1990).

Apart from suppression of bacterial growth, acidification of the vase solution also has an instant effect on conductivity. Application of iso-ascorbic acid was observed to reduce gum formation, thus promoting water uptake in cut rose flowers (Parups and Chan, 1973). Durkin (1979a) found an increase in flow rate of water through rose stem segments when the pH of the vase solution was decreased from 6 to 3. Conrado et al (1980) also concluded that solution uptake was increased at pH below 3, while uptake was inhibited at pH 6 or higher. Marousky (1971) also found that freshly harvested roses held in acid solution (pH 3) had longer life than flowers held in higher pH solution. Most preservative solutions contain an acidifier to reduce vase water pH. The type of acid added appears unimportant as various kinds of acidifier including citric acid, acetic acid, tartaric acid, iso-ascorbic acid and glycolic acid (Jones, 1991, Parups and Chan, 1973 and Halevy and Mayak, 1981) have been shown to increase cut flower vase life.

Jones (1991) concluded that solutions with pH of around 3.5 were effective in overcoming air embolisms in flower stems. Acidified vase solution was shown to move more rapidly through the stems of cut flowers than neutral or alkaline solutions. Durkin (1979b; 1980) suggested that solution acidification enhanced the rate of hydration of cut rose flowers. In addition, acidification of vase solution may have a synergistic effect with germicide treatments. Marousky

(1971) found that 8-HQC was more effective in reducing vascular blockage in a solution at pH 4 than at pH 6. However, Zagory and Reid (1986) demonstrated that lowering the vase solution pH was not effective in increasing carnation vase life if certain microorganisms were present. The presence of yeast in the vase solution reduced vase life regardless of vase solution pH. This finding further emphasises the role of microbial population in reducing cut flower vase life.

2.2 Temperature management

Temperature is one of the most important factors that affects the postharvest life of cut flowers. Plant cell respiration rate is proportional to temperature within the physiological range, and cut flower vase life may be extended by reducing respiration rate (Strafford, 1965; Jones, 1991). Cold storage, which reduces the respiration rate of floral tissue and also minimises water loss, is therefore widely used to slow senescence and prolong overall display life of most cut flowers. However, tropical varieties (eg. *Anthurium*, *Heliconia*, and tropical orchids) are damaged by cool temperatures and have to be kept above 10°C at all time (Jones, 1991). Recommendations for temperature management of cut flowers are widely reported in the literature. The ideal storage temperature of temperate flowers such as *Rosa*, *Dianthus*, *Chrysanthemum* (Halevy and Mayak, 1981), *Tulipa* (Jones, 1991) is just above 0°C. However, most commercial cool rooms are set around 0°C and are effective in maintaining quality of cut flowers. Subtropical flowers (eg. *Sterlitzia*) are recommended to be stored at moderate temperatures of 2°C to 8°C (Jones, 1991; Halevy and Mayak, 1981).

Preharvest temperature may also affect the postharvest life of cut flowers. Growing plants at high temperature was reported to reduce tissue carbohydrate levels and flower longevity (Rogers, 1962; Stanby et al, 1976).

Production of crops under low temperatures has also been shown to reduce the postharvest life of cut flowers. Moe (1975) found that decreasing the temperature from 24°C to 12°C in the three weeks before harvesting, or increasing the temperature above 27°C, reduced the vase life of cut *Rosa* flowers. However, it has been reported that growing at high temperatures enhanced the postharvest longevity of petunia flowers (Shvarts et al, 1997) and carnations (Verlinden and Woodson, 1998). Kyalo et al (1996) also reported that the postproduction longevity of potted miniature *Rosa* was enhanced when plants were grown under high temperature long day conditions (30°C day/21°C night for 14 hours per day) compared to the low temperature short day conditions (21°C day/16°C night for 10 hours per day). The authors did not study the postharvest life of *Rosa* cut from the treated plants and it is therefore possible that the high temperature treatment while stimulating increased postproduction life of flowers left on the plants would not have resulted in increased vase life of cut flowers.

Apart from reducing respiration rate, cool temperatures have also been reported to improve water balance in cut flowers. Van Meeteren (1992) showed that water balance of dehydrated *Chrysanthemum* could be improved by placing the stems for 2 hours in cold water (5°C). The mechanism involved was concluded to be an increase in the solubility of gases in water at low temperature (van Meeteren, 1992; Colt, 1984).

2.3 Ethylene management

Ethylene promotes senescence in many cut flower species. Flowers which are considered to be ethylene-sensitive have been reported in the following families: Campanulaceae, Ranunculaceae, Caryophyllaceae, Geraniaceae, Libiatae, Malvaceae, Orchidaceae, Primulaceae and Rosaceae (Woltering and van Doorn 1988; Jones, 1991). However, in some flower species the onset of

senescence symptoms were not hastened by exogenous ethylene. These ethylene insensitive species mostly belong to the families Compositae, Iridaceae, Amaryllidaceae and Liliaceae (Woltering and van Doorn, 1988).

Ethylene synthesis can be induced by trauma caused by chemical exposure, insect damage, temperature extremes, drought, gamma irradiation, disease, mechanical wounding (Abeles, 1973) or pollination (van Doorn, 1997b). Ethylene can also be produced by vehicle exhausts, damaged flowers, ripening fruit and cigarette smoke (Jones, 1991).

The effects of ethylene vary between flower types but exposure to ethylene commonly results in colour changes in petals (blueing or reddening), abscission of flower parts (eg, snapdragon, geranium and sweet pea), in-rolling of petals (eg, carnation and morning glory), translocation of nutrients out of the flower organs and accelerated water loss (eg, petunia and some orchids) (Halevy, 1985). The sensitivity to exogenous ethylene and the petal senescence symptoms (wilting or abscission) are apparently related. Apart from a few families (Campanulaceae, Caryophyllaceae, Malvaceae and most Orchidaceae), most flowers that showed wilting as the primary senescence symptom were not sensitive to exogenous ethylene. Most flowers that showed initial abscission (Geraniaceae, Labiatae, Ranunculaceae, Rosaceae, Scrophulariaceae) were sensitive to exogenous ethylene (Woltering and van Doorn, 1988). The sensitivity of cut flowers to ethylene varies not only between species but also with stage of development of the flower. *Cyclamen*, for example, does not respond to exogenous ethylene before pollination but an increase in the rate of petal abscission was induced by exposure to exogenous ethylene after pollination (Halevy et al, 1984).

The concentration of ethylene required to cause a response in flower parts is typically less than 1 ppm. Ethylene at concentrations as low as 0.002 ppm caused the sepals to separate from one another in orchid flowers (Abeles, 1973; Salisbury and Ross, 1985). Akamine (1976) also reported that exposure of

Vanda flowers to emanations of ripening fruits at 0.05 ppm of ethylene caused fading.

Cut flowers are commonly treated with silver thiosulphate (STS) or silver nitrate which STS is an effective anti-ethylene agent while silver nitrate is thought to act primarily as a bactericide. Halevy and Kofranek (1977) examined the effects of silver treatments on longevity of *Dianthus* and found that direct coating of flowers with silver nitrate (50-100 ppm) extended flower longevity and counteracted the senescence enhancing effect of ethylene exposure. However, at least two hours was required between the silver nitrate treatment and exposure to ethylene to get the maximum benefit. In addition, STS markedly improved the opening of *Gladiolus* spikes but did not, however, prolong vase life (Serek et al, 1994). Serek et al (1994) also concluded that the wilting of *Gladiolus* was not hastened by application of exogenous ethylene.

Silver thiosulphate does not always have positive effect on cut flowers. For flowers that are not sensitive to ethylene, STS may be detrious to cut flower vase life (Woltering and van Doorn, 1988).

An ethylene-free environment is recommended in cut flower storage facilities. Merodio and de la Plaza (1989) found that removal of ethylene from the storage atmosphere improved the opening of *Gladiolus* spikes, decreased water loss and prevented changes in colour and stem rigidity. Maintenance of low ethylene concentration in the storage environment is generally achieved by :

- (1) providing adequate air exchange in storage, (2) avoiding storage with fruits, vegetables and flowers which produce significant quantities of ethylene, (3) avoiding internal combustion vehicles in transport, (4) removal of all diseased and decayed tissue, and (5) use of ethylene scrubbers (brominated charcoal or permanganate) (Halevy and Mayak, 1981).

Carbon dioxide (CO₂), a competitive inhibitor of ethylene (Burg, 1962), is used extensively in controlled atmospheric storage to improve keeping quality of

agricultural commodities and can be used to delay or prevent the onset of ethylene related cut flower senescence reactions. General effects of controlled atmospheric storage (reduced oxygen and increased carbon dioxide) are decreased respiration and destructive oxidation processes and reduced ethylene effects (Halevy and Mayak, 1981). Leshem et al (1986) concluded that ethylene production can be delayed by exposing flowers to high carbon dioxide or low oxygen atmospheres. A storage atmosphere containing 3% carbon dioxide was found to prevent fading in cut flowers (Akamine, 1976). The use of higher carbon dioxide concentration is, however, often accompanied by carbon dioxide injury. Seaton and Joyce (1993) showed that 15% carbon dioxide in cool room caused petal blueing in *Anigozanthus* sp. (kangaroo paw) and *Chamelaucium uncinatum* (geraldton wax). Modified atmosphere storage of cut flowers by sealing in containers designed to regulate gas exchange with reduced oxygen and increased carbon dioxide levels created by the flower respiration, is an alternative method for cut flower storage. An appropriate sealing material is, therefore, essential to prevent accumulation of excessive carbon dioxide levels (Halevy and Mayak, 1981).

Apart from being a competitive inhibitor of ethylene, carbon dioxide also found to maintain the stability of anthocyanin (Gil et al, 1997) therefore maintaining petal colour. An additional advantage of an enriched carbon dioxide treatment was controlling decay (Holcroft et al, 1998) due to lack of oxygen for microbial metabolism.

2.4 Other treatments

While vase solution, temperature and ethylene managements have been widely reported as the most important factors influencing cut flower vase life, a number of other postharvest treatments have also been reported in the literature. These treatments may relate directly or indirectly to improvement of water uptake or metabolic processes in cut flowers. Amongst those

treatments, recutting flower stems, and application of cycloheximide and lysophosphatidylethanolamine (LPE) deserve more attention since they have been shown to have a significant effect in prolonging vase life of cut flowers.

Recutting flower stems

Impairment of water uptake in flower stems was thought to be the result of microbial or physical blockage or air embolisms in xylem vessels (Burdett, 1970; Durkin, 1980 ; Lineberger and Steponkus, 1976). Van Doorn (1990) and van Doorn et al (1993) showed that when cut *rosa* stems were exposed to air for longer than 24 hours, the rate of water uptake was significantly reduced. In several types of cut flowers (eg. *Rosa*, *Chrysanthemum*, *Gladiolus* and *Antirrhinum*), air embolism could be overcome by recutting stems under water (Jones, 1991). Moreover, van Meeteren (1992) concluded that recutting *Chrysanthemum* stems under water could restore the water balance of the flowers. However, Fujino and Reid (1983) suggested that recutting stems under water did not increase vase life of cut maidenhair fronds.

Cycloheximide

Cycloheximide, a protein synthesis inhibitor, has been found to prolong vase life of several cut flowers. The improved flower longevity following cycloheximide treatment was not due to increased water uptake or reduced bacterial growth, but an inhibition of *de novo* protein synthesis (Jones et al, 1994). Besides inhibition of protein synthesis, treatment of flowers with cycloheximide also resulted in delayed wilting, reduction of membrane permeability, inhibition of ethylene production and increased electrolyte content (Drory et al, 1995). Including cycloheximide in the vase solution delayed wilting in *Iris* (van Doorn et al, 1995; Jones et al, 1994), *Dianthus*

(Drory et al, 1995), *Gladiolus* and *Narcissus* (Jones et al, 1994). However, vase life of cut *Tulipa* flowers was shortened when treated with cycloheximide as the treatment resulted in almost complete inhibition of petal expansion (Jones et al, 1994). It is evident, therefore, that stage of development of cut flowers during treatments seems to be an important factor in obtaining satisfactory results. Van Doorn et al (1995) found that cycloheximide delayed tepal wilting in *Iris* flowers when applied to fully opened flowers, but completely inhibited petal expansion when applied prior to flower bud opening. Bud development of *Hemerocallis* hybrid was also inhibited by treatment with cycloheximide (Bieleski, 1993). The effectiveness of cycloheximide treatments is influenced by the concentration used. Eason and de Vre (1995) showed that exposing *Sandersonia* flowers to vase solution containing 100 μM cycloheximide inhibited tepal fading but the flower wilted prematurely. Treatment with 1 μM , however, only delayed the fading without premature wilting.

Lysophosphatidylethanolamine

Lysophosphatidylethanolamine (LPE) is a naturally occurring phospholipid that has been used to prolong vase life of *Antirrhinum* (Kaur and Palta, 1997). Effects of LPE treatment may include a promotion of flower opening, delayed fresh mass loss, lowered ethylene production and reduced ion leakage (Kaur and Palta, 1997). The mode of action of LPE is still unclear. Results led the authors to conclude that LPE may improve vase life of *Antrrhinum* flowers by retarding loss of water and/or maintaining water uptake by the spikes or suppressing ethylene production. However, evidence that LPE promoted flower opening suggested that LPE might also move to the petal resulting in decreased water potential and thus water influx to the petal tissue and promoted cell enlargement. This is similar to the mechanism proposed for the effect of sugar treatment on flower opening (Ho and Nichols, 1977).

3 Physiological Changes

Cut flower postharvest treatments are generally aimed at maintaining a favourable water balance and reducing metabolic activity in the floral organs. Therefore the processes involved in regulation of water status and metabolic activity in cut flowers are critical to understanding of cut flower physiology. The termination of vase life in many cut flowers is characterized by water stress symptoms and decreasing stem hydraulic conductivity is widely accepted as a key physiological change during senescence of cut flowers (Halevy and Mayak, 1981). Changes in plant hormone concentrations (van Altvorst and Bovy, 1995; Borochoy et al, 1976; Aharoni et al, 1979; Wu et al, 1991a and 1991b), carbohydrate metabolism (Nichols, 1973; Olley et al, 1996; Bieleski, 1993), protein turnover and gene expression (Eason and de Vre, 1995, Celikel and van Doorn, 1995; Jones et al, 1994) have also been well documented during cut flower senescence. These changes may be causal or consequential to changes in water relations, or may result from entirely separate and unrelated mechanisms. The major physiological changes occurring during cut flower vase life are discussed in this chapter.

3.1 Water relations

Changes in water conductivity

After flower stems have been cut and placed in water, water potential, stem hydraulic conductivity and fresh weight of the cut flowers always declines (van Doorn and Vojinovic, 1996) but when flowers senesce on the plant, the rate of stem hydraulic conductivity remains constant (Mayak et al, 1974). Flower turgidity is the result of the balance between rate of water uptake and water loss, both of which may fluctuate according to external conditions, principally temperature and relative humidity (Mayak et al, 1974). However,

it has been shown that loss of flower turgidity and fresh weight in cut flowers was preceded by a decrease in rate of water uptake (Burdett, 1970) which indicated that reduced water uptake, rather than excess water loss, was the major cause of water balance impairment. Reduced water uptake has been documented in many cut flower species but did not occur in some such as *Tulipa* and *Narcissus* (Halevy and Mayak, 1981). Thus mechanisms involved in reduced water uptake are amongst the most important in cut flower post harvest physiological processes.

Causes of reduction in water conductivity

Several mechanisms have been proposed to explain the reduction in stem conductivity in cut flower stems. The most widely accepted theory is that the decrease in stem hydraulic conductivity results from a blockage of xylem vessels by bacterial cells (de Witte and van Doorn, 1988; Put and van der Meyden, 1988; van Doorn and de Witte, 1991; van Doorn et al, 1991). Support for this theory came from the many reports that vase solution containing bactericide reduced number of bacteria and delayed the characteristic decrease in stem conductance of cut flowers (Burdett, 1970; Jones and Hill, 1993; Morousky, 1969; van Doorn, 1998). A correlation between a decrease in stem hydraulic conductance and high number of bacteria (higher than 10^6 cfu/g fresh weight) has also been documented (van Doorn et al, 1989; van Doorn and Perik, 1990). Bacterial cells may move into the flower stem with the xylem stream up to the maximum vessel length as it has been shown that bacterial cells were unable to pass the inter-vessel pit membranes of the xylem system of cut *Rosa* (Put and Meyden, 1988). However, despite the transpirational flow of water through the stem and the demonstrated capacity of cells to move within xylem vessels, most bacterial cells adhered around the base of the flower stem (Lineberger and Steponkus, 1976; Put and Clerkx, 1988; Put and van der Meyden, 1988; van Doorn et al, 1991). The mechanism of

attachment of bacterial cells to the base of the flower stem has not been reported in the literature.

Decrease in hydraulic conductivity may not be solely dependent on the presence of microorganisms, since *Rosa* stems held in sterile water also displayed decreased rates of water uptake (Marousky, 1969). Unfortunately assessment of bacterial numbers in the stems was not undertaken so the presence of microorganisms can not be ruled out. Rasmussen and Carpenter (1974) found that exudate from the phloem and breakdown of secondary tissue caused xylem blockage. Other authors showed that material blocking xylem vessels may contain carbohydrates, pectin-, lipid- and protein-like compounds and some enzymes (Parups and Molnar, 1972; Dixon and Peterson, 1989). Durkin (1979a) concluded that the decrease in solution uptake in cut flowers was a response to loss of xylem element function and that the appearance of carbohydrate type occlusions was a consequence of lost function and not a primary cause. This conclusion was based on the assumption that the development of vascular occlusions in flower stems is similar to the mechanism of vascular gum development in heartwood of woody plants. The loss of xylem element function was attributed to water column breakage by factors thus far unidentified in anatomical and cytological studies on blockage.

Air embolisms due to cavitation have been proposed to have a role in reducing stem conductance. The role of cavitation may in turn be linked to xylem water potential and therefore influenced by any other factors which reduce stem conductance (Dixon et al, 1988; Ranasinghe and Milburn, 1995). Air entering the xylem vessels at the moment of stem cutting has also been proposed to disrupt water uptake in the stem (Durkin, 1979a; 1979b; van Meeteren, 1992; van Doorn and Otma, 1995). However, this kind of embolism, alone, is unlikely to be sufficient to impair the xylem water movement. Van Doorn (1990) showed that cut *Rosa* stems could be left dry for up to 3 hours without inhibition of water uptake. It has also been shown that the aspiration of air into the cut open vessels needed to result in a high number of cavitated xylem

elements to reduce the rate of water uptake in cut *Rosa* flowers (van Doorn, 1990; van Doorn and Suiro, 1996). In commercial cut flower postharvest management, dry storage at low temperature is recommended for long distance transport (Halevy and Mayak, 1981), indicating that air embolism is not a major concern for reduction in cut flower vase life. In addition, Fujino and Reid (1983) suggested that poor vase life of maiden hair fern does not result from the presence of air embolisms in the xylem.

A number of other theories to explain the loss in stem conductance have been proposed but have been the focus of few studies and little experimental evidence has been presented to support them. These theories included the formation of tyloses or other plant derived materials blocking the xylem and bacterial metabolites either directly blocking vessels or triggering a plant defence response which led to a decrease in stem conductance. Tylosis and gum are regarded as sealing materials produced by plants to seal off the injured or infected xylem (Kaus et al, 1996; Zimmermann, 1983). Gum has been found in some studies of cut *Rosa* flowers, although, tylosis as well as tannin, lignin, callose or hydrophobic material, were not observed (Parups and Molnar, 1972; Gilman and Steponkus, 1972; van Doorn and Reid, 1995, Lineberger and Steponkus, 1976; van Doorn, 1994). Other authors have questioned the relevance of vascular occlusions to reduced water flow, with Rassmussen and Carpenter (1974) observing only 4% of small vessels were blocked when flowers wilted. However the majority of studies have indicated a link between stem plugging and loss of conductivity so it is likely that vascular occlusion is a significant cause of impaired water balance in cut flower stems.

3.2 Plant hormones

Cytokinins, abscisic acid, auxins, gibberellins and ethylenes have all been shown to influence flower senescence. However, from a floriculture postharvest view point, ethylene is the most important hormone influencing this process. Changes in ethylene production follow a similar pattern in many flower types. Initially there is a steady phase of low ethylene production followed by a marked increase to the climacteric peak and then a rapid declines in synthesis. However, this peak did not occur in nonclimacteric flowers, for example *Chrysanthemum* and *Gerbera* (Leshem et al, 1986).

In climacteric flowers such as *Dianthus* and *Rosa*, the visual senescence symptoms could be distinguished at the end of the second phase of ethylene production (Leshem et al, 1986). This second phase of ethylene production could be enhanced by several factors. The ethylene production is autocatalytic, which means that exposure to ethylene stimulates ethylene biosynthesis (Leshem et al, 1986; van Altvorst and Bovy, 1995). This may explain the effect of ethylene produced from ripening or damaged plant tissue on acceleration of senescence in cut flowers when they are stored in the same room.

The involvement of other plant hormones in regulation of flower senescence is not clear. Cytokinin is thought to be a senescence retarding hormone. The endogenous level of cytokinins in *Rosa* petals increased during flower opening and decreased after the full bloom (Mayak and Halevy, 1970; Mayak et al, 1972). However, the changes in cytokinin level vary in the different part of the flowers. In cut *Dianthus*, the cytokinin-like activity in the ovaries increased when the petals were irreversibly wilting and declined during later stages of senescence (van Staden and Dimalla, 1980).

Abscisic acid appears to act as a promoter of petal senescence. Endogenous level of abscisic acid may increase before or during senescence in a very wide range of tissue and often decrease during late senescence (Nooden and Leopold, 1988). Increased levels of abscisic acid in plant tissue have been

shown to be induced by water stress (Borochoy et al, 1976). The increase in abscisic acid level could possibly be a secondary reaction linked to ethylene concentrations as ethylene production has been demonstrated to be stimulated by water stress (Halevy and Mayak, 1981).

Generally, the level of endogenous auxin and gibberellin activity decrease during or before senescence. Gilbert and Sink (1971) found that level of auxin in two poinsettia cultivars decreased with age, but declined faster in the short-lived one. Application of exogenous gibberellin to *Lilium* leaves resulted in delayed foliar chlorosis (Franco and Han, 1997) and also halted further development of leaf yellowing (Han, 1997). In addition, gibberellin also inhibited the decomposition of protein (Sabehat and Zieslin, 1994) and may be able to extend vase life.

Application of silver ions in the form of silver thiosulphate (STS), which is mobile in plant tissue and the xylem stream (Veen and van de Geijn, 1978 and van Altvorst and Bovy, 1995), resulted in extended vase life of various types of cut flowers, especially in the ethylene-sensitive species (Halevy and Kofranek, 1977; Barrowclough et al, 1991; Woltering and van Doorn, 1988). Roles of silver ions in extending vase life have been studied by several workers. Application of silver ions might reduce ethylene binding in cut flowers (Altvorst and Bovy; 1995). Halevy and Kofranek (1977) compared effects of silver treatments on longevity of *Dianthus* and found that direct coating of flowers of *Dianthus* with silver nitrate (50-100 ppm) extended flower longevity and encountered the enhancing effect of ethephon on senescence. Moreover, Alvorst and Bovy (1995) reported that, in cut *Dianthus*, after treatment with STS, there was no climacteric rise in ethylene production nor ACC accumulation. However, at least 2 hours was required between the silver treatment and the exposure to ethylene to get the maximum benefit. The conclusion drawn from these studies was that silver ions had an anti-ethylene effect through competitive inhibition at the ethylene action sites or reaction

with another substance to form the anti-ethylene agent (Halevy and Kofranek, 1977).

Silver ions in forms other than STS do not readily move in flower stems. Kofranek and Paul (1972) showed that a basal treatment with AgNO_3 , the silver ions coated only the base of the stem and were poorly translocated into the stem. An additive effect was found when basal and floral treatment were combined, indicating that there were separate sites of ethylene action within the cut flower. Hence, besides the anti-ethylene effect, silver ions might also have a bacteriocidal effect, from the basal treatment, on extending vase life of *Dianthus* (Halevy and Kofranek, 1977).

Silver ions did not appear to have a bacteriocidal effect in studies of maidenhair fern (Fujino et al, 1983b and Fujino and Reid, 1983). Fujino and Reid (1983) found that some biocides (8-hydroxyquinoline citrate, a quaternary ammonium compound, or 8-hydroxyquinoline citrate + NaOCl) had little effect on vase life of maidenhair fern, while inhibitors of ethylene production increased vase life. Moreover, adding AgNO_3 to the vase solution maintained water potential, water balance and turgidity of the fronds for up to 2 weeks. The authors proposed that ethylene production by wounded cells may stimulate the formation of vascular blockage and AgNO_3 might inhibit this effect of ethylene rather than acting as an anti-bacterial agent (Fujino et al, 1983b and Fujino and Reid, 1983).

3.3 Protein and carbohydrate content

Protein content

Changes in protein levels in petals is associated with the onset of senescence symptoms in the flower. Senescence of flowers follows a programmed

succession of changes. Early changes occurring in the petals include loss of membrane integrity, ion leakage and increased respiration rate (Eason and de Vre, 1995). These changes were accompanied by both quantitative and qualitative changes in both protein and nucleic acids and may involve expression of senescence related genes (van Doorn and Stead, 1994). While the genetic regulation of cut flower senescence is now receiving much research attention, our understanding of the subject is far from complete.

Celikel and van Doorn (1995) found a rapid drop in protein levels of the tepal edges in dutch *Iris* flowers and this decrease was concomitant with the onset of visible in-rolling of the tepals. Lay-Yee et al (1992) also found a sharp decrease in protein levels preceded the visible symptoms of senescence in *Hemerocallis* petals. The decrease in protein levels was found both in the insoluble (membrane and/or cell wall bound protein) and soluble protein fractions (Nooden and Leopold, 1988; Olley et al, 1996). However, the trends of total protein levels (sum of soluble and insoluble protein concentrations) mainly reflect the patterns of the insoluble protein component (Olley et al, 1996). The decrease in protein level may be due to reduced protein synthesis (Celikel and van Doorn, 1995) and/or increased degradation (Nooden and Leopold, 1988). Trippi and Tran Thanh Van (1971) found an increase in peroxidases activity in *Phalaenopsis* during aging of the corolla. Moreover, a sharp increase was found in the activity of the hydrolytic enzymes: Rnase, Dnase, and hydrolases of cell wall polysaccharides in morning glory at the onset of flower senescence (Matile and Winkenbach, 1971; Weinken-Gehring et al, 1974).

Treatment of flowers with the protein synthesis inhibitors cycloheximide (CHI) has been shown to result in increased longevity of several cut flower species (van Doorn et al, 1995; Jones et al, 1994; Drory et al, 1995). The treatment was effective in delaying the decrease in protein level (Courtney et al, 1994) and reduction of membrane permeability (Drory et al, 1995). This indicated that protein breakdown might induce certain senescence processes through an increase in cell leakage. Further evidence of the role of membrane

permeability has been provided by Celikel and van Doorn (1995) who noted an increase in ion and anthocyanin leakage in *Iris* tepals prior to the visible senescence symptoms. Colour changes in the petals of cut flowers may also be attributable to other reactions mediated by changes in protein levels. During protein degradation free ammonia was released, increasing vacuolar pH and resulting in colour changes in petals (Halevy and Mayak, 1979).

Changes in protein levels have been linked to other flower senescence changes such as ethylene production, membrane modifications and water and solute loss (Drory et al, 1995; Mayak et al, 1985). While these senescence processes occur in all flowers, they may be accelerated in cut flowers. In particular accelerated rates of ethylene production and loss of membrane integrity were involved in the response of cut flowers to water stress (Drory et al, 1995). Accelerated loss of vase life in cut flowers may therefore be induced by stress (eg. water stress) and the development of senescence symptoms regulated by mechanisms characterised by changes in proteins and nucleic acids.

Carbohydrate content

The normal development of flower petals is accompanied by substantial increases in dry matter until the petals are fully expanded. Fifty percent of the petal dry weight was soluble carbohydrate (Biesleski, 1993; Ho and Nichols, 1977). After full development, a decline in dry weight and the content of carbohydrates of petals were observed (Biesleski, 1993; Ho and Nichols, 1977; Leshem et al, 1986). The sugar pool of mature and aging petals was composed mainly of reducing sugars rather than sucrose (Lesham et al, 1986). The changes in sugar concentrations were accompanied by starch hydrolysis (Ho and Nichols, 1977).

Respiration rate patterns in many flowers have two peaks. The first one is observed when flowers start to open, followed by a gradual decline. The

respiration rate then increases over a short period and finally declines again. The second peak was considered to indicate the final senescence stage (Halevy and Mayak, 1979; Leshem et al, 1986). The decline in respiration in aging flowers may be due to reduced availability of readily respirable substrates. The size of the respirable substrate pool was affected by the rate of hydrolysis of starch and other polysaccharides, the rate of respiration, and translocation of carbohydrates between the flower and other plant parts (Halevy and Mayak, 1979; Ho and Nichols, 1977; Leshem et al, 1986; Nichols, 1976). In senescing flowers, translocation of metabolites within the flower from petal to ovary has been observed (Nichols, 1976). This translocation was stimulated by pollination and ethylene. Supplying cut flowers with exogenous sugar maintained the pool of available respirable substrates, especially in petals, thus extending longevity (Leshem et al, 1986). However, substantial amounts of sugars have been found in *Rosa* and *Dianthus* flowers, after wilting, which indicates that carbohydrate substrate limitation is not the sole cause of the onset of senescence (Halevy and Mayak, 1979).

The possible roles of applied sugar in extending longevity via mechanisms other than as a supplied respirable substrate have been studied. It has been suggested that the gradual decline in respiration was due to a progressive deterioration of mitochondrial systems (Kaltaler and Steponkus, 1976; Leshem et al, 1986). In mitochondria isolated from cut flowers pretreated with sucrose, respiratory control values were maintained over an extended period of time. This indicated that an effect of exogenous sugar in extending longevity may be to maintain mitochondrial structure and functions (Kaltaler and Steponkus, 1976).

Applied sugar also interacts with plant hormones. Mayak and Dilly (1976) concluded that sucrose enhanced the effect of cytokinins in delaying senescence of flowers and reduced the effect of ethylene. Moreover, sucrose was found to antagonise the effect of abscisic acid in promoting the senescence of carnations (Mayak and Dille, 1976).

It is also known that applied sugars improve the water balance in cut flowers (Leshem et al, 1986). This has been attributed to the effect of sugars on inducing stomata closure and reduction of water loss but not on increased water uptake (Marousky, 1969; 1971). Indeed, sucrose-treated *Rosa* flowers were observed to initially absorb less solution than control flowers which were held in water (Marousky, 1971). This result was explained by the lower water potential of sugar solution causing a lower rate of water uptake due to a reduced water potential gradient. Later, after the supplied sugar reached the flower, probably increasing the osmotic concentration, an improvement in water balance was observed (Bravdo et al, 1974; Halevy and Mayak, 1979).

Sugar was also found to affect the physical and compositional properties of flower petal membranes. It has been shown that the membrane lipid composition changes with senescence. The change was mainly the result of a decrease in membrane phospholipid content and type (Borochoy and Woodson, 1989). Adding 2% sucrose into vase water resulted in maintenance of the content of membrane phospholipid per cell in cut *Rosa* flowers (Goszczyńska et al, 1990) and prevented the deterioration in *Chrysanthemum* induced by gamma irradiation treatment (Hayashi and Todoriki, 1996).

Parups and Chan (1973) found that addition of sucrose prevented the rise of ammonia content and changes in pH of the petals and thus delayed senescence related colour changes in petals. Olley et al (1996) also suggested that supply of exogenous sucrose might delay or reduce protein degradation, since protein is an alternative substrate for respiratory process.

4 Effects of Bacteria

A strong relationship between bacterial numbers in vase water and stem conductivity has been demonstrated (van Doorn, 1998), and it is generally accepted that the presence of bacteria in the vase water hastens the onset of senescence in a number of species of cut flowers. The effectiveness of bacteriocides in extending cut flower vase life are widely reported although few studies have actually documented bacterial numbers in flower stems receiving different bacteriocide treatments. Studies which have been done have shown that vase life of cut flowers was negatively correlated with increasing bacterial numbers in the vase water (Put and Jansen, 1989). However much of the evidence implicating microorganisms in reduced vase life of cut flowers is largely circumstantial, and many of the studies undertaken so far have been flawed by inadequate control of microbial numbers, external contamination or uncertainty as to microbial populations present (Zagory and Ried, 1986). Thus the link between bacterial numbers and vase life is far from proven despite the wide acceptance in the literature.

While the negative effect of microorganisms on cut flower vase life is widely accepted, the mechanism by which microorganisms reduce vase life is still a subject debated in the literature. The most widely accepted hypothesis was that bacterial cells blocked the xylem vessels, leading to a decrease in stem hydraulic conductivity and development of water stress symptoms in the flower (de Witte and van Doorn, 1988; Put and van de Meyden, 1988; van Doorn and de Witte, 1991; van Doorn et al, 1991). Again the evidence supporting this hypothesis is not complete and a number of alternative explanations of published results have been proposed. Vascular plugging due to injury, enzymatic action, or ethylene (Zagory and Reid, 1986) have also been suggested, along with cavitation (Williamson and Milburn, 1995), tylosis or other plant derived occluding material (Kaus et al, 1996; Lineberger and Steponkus, 1976). The literature concerning the effects of bacteria on cut flower vase life therefore requires greater attention given the volume of published information on the topic and the questions still surrounding the

mechanism by which bacteria reduce vase life and even the involvement of bacteria in reduced vase life.

4.1 Bacterial populations in cut flowers

Changes in bacterial populations in cut flower stems have been examined in only a small number of flower species. The paucity of information on bacterial populations is surprising given their apparent importance in cut flower vase life. The bacterial genera most commonly found in vase water or from flower stems are *Pseudomonas* spp., *Enterobacter* spp., and *Bacillus* spp. (de Witte and van Doorn, 1988; Put, 1990; van Doorn et al, 1991). *Pseudomonas* spp have been found in all cases where bacterial identification has been reported from cut flower stems. Bacterial strains belonging to the genus *Pseudomonas* have been isolated from cut *Rosa* stems (Put, 1990; Put and Jansen, 1989; van Doorn et al, 1991; van Doorn and de Witte, 1997; van Doorn and de Witte, 1991; van Doorn et al, 1990), *Dianthus* stems (Zagory and Reid, 1986; Accati et al, 1980), *Gerbera* stems (van Doorn and de Witte, 1994) and in vase water (Put and Jansen, 1989; Put, 1990; Put and van der Meyden, 1988; de Witte and van Doorn, 1988). Where further identification has been undertaken, the following species have been reported; *P. aeruginosa*, *P. cepacia*, *P. maltophilia*, *P. putida*, *P. stutzeri*, *P. vesiculosis* (de Witte and van Doorn, 1988), *P. tupida* (Put and van der Meyden, 1988), *P. aeruginosa*, *P. cepacia*, *P. fluorescens*, *P. putida*, *P. maltophilia*, *P. putrefaciens*, *P. stutzeri*, *P. vesicularis* (Put, 1990), *P. fluorescens*, *P. putida* (Put and Jansen, 1989), *P. aeruginosa*, *P. cepacia*, *P. fluorescens*, *P. maltophilia*, *P. mendocina*, *P. pikettii*, *P. putida*, *P. stutzeri*, *P. vesicularis* (van Doorn et al, 1991), *P. fluorescens* (van Doorn et al, 1990) and *P. aeruginosa* (van Doorn and de Witte, 1994). Apart from *Pseudomonas*, the other bacterial genera commonly found associated with cut flower stems and/or vase water are *Bacillus* and *Enterobacter* (Put and Clercx, 1988; Put, 1990; Put and Jansen, 1989; van Doorn et al, 1991). Many other microorganisms including yeast and fungi have been

isolated from cut flower stems, with Put (1990) reporting over 60 species of microorganisms isolated from cut *Chrysanthemum*, *Gerbera* and *Rosa* stems. The majority of these microorganisms make up only a small percentage of the total population on cut flower stems held in vase water and have only been observed occasionally.

The majority of microbial species isolated from cut flower stems were normal inhabitants of the upper layer of agricultural soil (Put, 1990). Contamination of flower stems from the soil during crop growth was likely to be a source of inoculum for cut stems, while other sources of exogenous contamination such as secateurs, flower grading machinery, and tap water in holding and vase solutions may contribute to initial microbial levels at the start of vase life. Tap water was identified as the major source of contamination in cut *Rosa* stems (van Doorn and de Witte, 1997), while the plant surface has been implicated in *Gerbera* and *Chrysanthemum* which are lower growing plants and do not have smooth stems (Put, 1990).

Regardless of the source of inoculum, the microbial population on cut flower stems has been shown to increase rapidly after stems are placed in vase water. Van Doorn and de Witte (1994) observed an increase in bacterial population in cut *Gerbera* stems from 0 at the start of vase life to greater than 10^5 cfu/g fresh weight after one day and greater than 10^8 cfu/g after eight days. A similar increase in population was noted in the vase solution. Bacterial concentrations exceeding 10^8 cfu/g have been reported in cut *Rosa* stems after seven days of vase life (van Doorn et al, 1990). Bacterial concentrations greater than 10^5 cfu/mL have also been reported in vase water of cut *Rosa* after three days (de Witte and van Doorn, 1988). The rapid increase in bacterial number can be prevented by the addition of bacteriocides to the vase solution (van Doorn et al, 1990; de Witte and van Doorn, 1988). The population composition in the vase water and cut stems changes over the vase life with *Pseudomonas* sp. reported as the dominant bacteria in cut *Chrysanthemum*, *Gerbera* and *Rosa* stems after three days vase life (Put, 1990).

and accounting for 70% or more of the total population within 1 day of vase life in *Rosa* (van Doorn et al, 1991). *Enterobacter* sp. replaced *Pseudomonas* sp. as the dominant species on cut stem after more than six days of vase life (Put, 1990). The major location of bacteria in cut flower stems has been shown to be the cut surface and basal section of the stem (van Doorn et al, 1991; Put and Clerkx, 1988). The number of bacteria found in the basal 0.1 cm of cut *Rosa* stems was shown to account for approximately 90% of the population extracted from the basal 5 cm of stems (van Doorn and de Witte, 1991). Location of bacteria in the basal section and cut surface of vessels in flower stems has been attributed to attachment mechanisms involving bacterial exopolysaccharides and to a filtering effect due to water movement into the xylem vessels (van Doorn et al, 1991). SEM studies by Put and Clerkx (1988) and van Doorn et al (1991) have shown bacterial cells in xylem vessels, particularly associated with pits in the vessels, and both concluded that bacterial cells were not able to pass through inter vessel pit membranes and thus were only able to access cut vessels. Following infiltration of bacterial suspensions into *Rosa* stems, bacterial numbers in excess of 10^2 cells per cm stem section were found in stem sections 15-16 cm from the cut surface after seven days of vase life (Put and van der Meyden, 1988). These results were reported as being consistent with movement within cut vessels since vessel length was reported as 2-250 mm.

4.2 Mechanisms of bacterial reduction of vase life

The rapid increase in bacterial population at the base of flower stems coincides with the decrease in conductivity within stems which generally occurs within two to three days of the onset of vase life (Durkin, 1979a). Physical blockage of vessels by bacteria has thus been suggested as the most likely mechanisms of bacterial reduction of cut flower vase life. A number of studies (Put and van der Meyden, 1988; de Witte and van Doorn, 1988; van

Doorn and de Witte, 1994; van Doorn and de Witte, 1991; van Doorn and D'hont, 1994; Put and Jansen, 1989; Zagory and Reid, 1986) have demonstrated that infiltration of bacterial suspensions into flower stems could decrease rates of water uptake, stem conductance and vase life. While these studies generally support the hypothesis that bacterial cells may cause vascular occlusions, a number of findings appeared contradictory to the hypothesis. Zagory and Reid (1986) demonstrated that only 3 out of 25 pure cultures of microorganisms caused a significant reduction in cut *Dianthus* vase life. The three microorganisms were identified as a yeast, a fluorescent *Pseudomonad* and a nonfluorescent *Pseudomonad*. The authors concluded that vascular blockage by bacterial cells was not an important mechanism in reduced vase life as 2 species of *Pseudomonas* which were similar morphologically had very different effect on vase life when infiltrated into stems at the same concentration. The effects of different microbes on vase life were not consistent across different flower types, indicating that a diversity of microbial effects may be involved and that no single hypothesis for microbial action will explain the effects of all microorganisms.

In contrast to the findings of Zagory and Reid (1986), Put and Jansen (1989) found that infiltration of *Rosa* stems with pure cultures of *Bacillus subtilis*, *Enterobacter agglomeratus*, *Pseudomonas fluorescens* or *P. putida* had a similar effect on vase life. Heat-inactivated suspensions of the bacteria also reduced vase life. Suspensions of six *Pseudomonas* species and *Alcaligenes faecalis* have been shown to have a similar effect on vase life when infiltrated into stems at concentrations of 10^5 and 10^7 cfu/mL (de Witte and van Doorn, 1988), and inactivated suspensions of *P. aeruginosa* had the same effect on water uptake in cut *Rosa* flowers (van Doorn and de Witte, 1991). The concentration of bacteria required to cause a significant reduction in water uptake has been reported as 10^6 cfu/mL (Zagory and Reid, 1986; Put and Jansen, 1989) and 10^7 cfu/mL (de Witte and van Doorn, 1988).

This concentration of bacteria was generally not observed in cut flower stems until 3 days of vase life or greater but reduced water flow has been observed prior to this time leading to the conclusion that physical blockage of vessels by bacteria could not fully explain the reduction in stem conductance (van Doorn and de Witte, 1991).

A suspension of lysed bacterial cells has been shown to be more effective in reducing hydraulic conductance than a suspension of non-lysed bacteria (van Doorn and de Witte, 1991) suggesting an effect of particles of a smaller size than bacteria. Several authors have suggested that toxins or enzymes produced by bacteria may cause damaging effects. Accati et al (1980) and Mayak et al (1977) concluded that some species of bacteria released toxic metabolites into holding water and these toxins induced vascular blockage and/or senescence reactions in cut flowers. Put and Rombouts (1989) demonstrated that the addition of purified microbial pectic enzymes (pectate lyase isolated from *Pseudomonas fluorescens* and polygalacturonase from *Kluyvermyces fragilis*) to the vase water caused degradation of xylem vessel wall structure, reduced water uptake and decreased vase life in cut *Rosa*. However, the speed at which infiltrated bacterial suspensions (living and inactivated cells) induced a significant decrease in stem hydraulic conductance (within 30 minutes) is indicative of a physical process rather than a physiological one (van Doorn and de Witte, 1991). Further evidence against the hypothesis that bacterial pectic enzymes are responsible for inducing vascular blockage come from the study of de Witte and van Doorn (1988) who demonstrated that 2 bacterial isolates which induced vascular blockage did not show pectolytic activity.

Microscopic and chemical examination of vascular occlusions in cut flower stems have also failed to produce a definitive answer to the mechanism of bacterial blockage of cut flower stems. Bacterial cells and an amorphous substance presumed to be of bacterial origin have been observed in xylem vessels of cut flower stems (Burdett, 1970; Parups and Molnar, 1972; van

Doorn et al, 1991; Lineberger and Steponkus, 1970), but the number of occluded vessels was usually only a small percentage of the total vessel number. Van Doorn et al (1989) demonstrated that more than two-thirds of the total transverse area of the xylem of cut *Rosa* must be blocked before a reduction in water uptake was observed. Microscopic observations of numbers of occluded vessels in cut flower stems have not been able to account for this proportion of blocked vessels and therefore vascular occlusion with bacterial cells and/or substances of bacterial origin has been discounted as a mechanism of action of bacteria in reducing cut flower vase life.

Several authors have, however, noted a layer of amorphous material at the cut surface of flower stems and the role of this material remains largely unexplained (Put and van der Meyden, 1988; van Doorn et al, 1991). The material has been reported to stain with ruthenium red indicating the presence of pectinaceous compounds, and may consist of bacterial extracellular polysaccharides (van Doorn et al, 1991). While the layer of amorphous material has been observed covering the cut surface of flower stems (van Doorn et al, 1991), it has been suggested that gaps through the layer may allow function of vessels as has been observed when suspensions of particulate matter accumulate at the base of flower stems (de Stigter and Broekhyzen, 1986). The rate of water uptake in *Rosa* stems was not related to the amount of material at the base of cut *Rosa* stems as the stem with the thickest layer of material had the highest rate of water uptake (van Doorn et al, 1991). This conclusion was, however, based purely on observation and the authors suggested that further investigation into the role of the material was warranted. Likewise, Put and van der Meyden (1988) concluded that further research into gummosis, or formation of the amorphous material was required.

4.3 Bacterial extracellular polysaccharides

Bacterial extracellular polysaccharides (EPS) occur in two forms : loose slime which is nonadherent to the cell and microcapsules and capsules which adhere to the cell wall (Denny, 1995; Wilkinson, 1958). EPS may be comprised of either a single sugar (homopolysaccharide) or complex mixtures of sugars (heteropolysaccharide). The commonest sugar components of EPS are the hexoses D-glucose, D-galactose and D-mannose. Other neutral sugars found include the 6-deoxy-hexoses L-rhamnose (6-deoxy-L-mannose) and L-fucose (6-deoxy-L-galactose) (Powell, 1979).

Formation of EPS, in media, by bacteria has been widely studied. The synthesis was affected by several factors : bacterial species and strain (Put and Klop, 1990), nutrient, oxygen, temperature and pH (Wilkinson, 1958). A carbon source was necessary for polysaccharide synthesis (Wilkinson, 1958), and microorganisms appeared to be able to produce EPS from various kinds of carbohydrate substrate (Garma et al, 1997). However, particular bacterial types needed specific types of carbohydrate to maximize polysaccharide production. *D. pneumoniae* were found to produce the highest amount of EPS in presence of glucose (Bernheimer, 1953), while for *Lactobacillus casei* CRL 87, the highest amount of polymer was found in presence of galactose (ATPgal) (Mozzi et al, 1995). Other nutrients may not be necessary for EPS formation but stimulate or inhibit synthesis. Calcium ions strongly stimulated EPS synthesis in *Lactobacillus casei* CRL87 (Mozzi et al, 1995) and *K. aerogenes* (Wilkinson and Stark, 1956). Phosphate was found to be inhibitory for *K. aerogenes* but was stimulatory for *D. pneumoniae* (Duguid and Wilkinson, 1953; Wilkinson and Stark, 1956).

The effect of oxygen on polysaccharide synthesis was generally related to the metabolic character of the bacteria and the relative amounts of energy

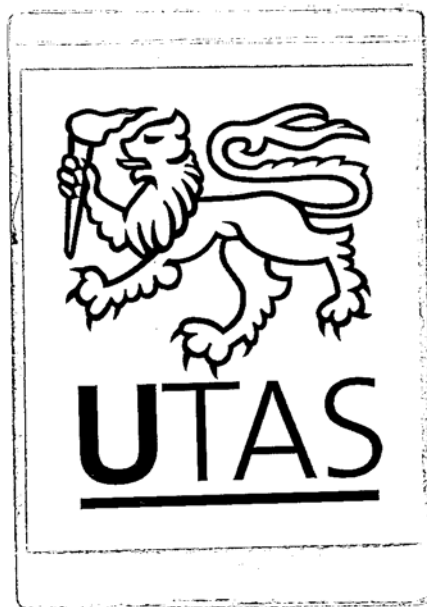
produced under aerobic and anaerobic conditions. In *D. pneumoniae* and *K. aerogenes*, the polysaccharide production in anaerobic condition was from 25 to 40% of that in aerobic condition (Wilkinson, 1958). Temperature was often critically important in determining the extent of extracellular polysaccharide formation (Wilkinson, 1958). The optimum temperature for polysaccharide production was between 15 and 25 °C for some strains of *E. coli* (Wilkinson et al, 1955), *Aeromonas salmonicida* (Bonet et al, 1993) and *Lactobacillus rhamnosus* C83 (Garma et al, 1997). However, extracellular polysaccharide synthesis in *K. aerogenes* was not affected by temperature (Duguid and Wilkinson, 1953). Medium pH maximized the formation of the polysaccharide from most bacterial species (Wilkinson, 1958), and considerable amounts of polysaccharide have been demonstrated to be formed between pH 5 and 8 (Bernheimer, 1953; Bonet et al, 1993). It should be noted here that the recommended pH of vase solution was also below this range, it is therefore possible that low pH (3) might reduce/inhibit the formation of EPS in flower stem or in vase water and therefore improve vase life by reducing stem occlusion.

The role of EPS on cut flower vase life is not clearly defined. EPS itself may disturb water relations in cut flower stems by blocking xylem vessels or pit pores. Fujino et al (1983a) found the vascular plugs in maidenhair fronds strongly stained with ruthenium red, which indicated the presence of pectic material. Moreover, Sutton and Williams (1969) found that EPS was a major component of xylem plugs and blocked water movement in infected xylem vessels thus inducing water stress in cabbage leaves.

EPS might also provide a selective advantage for bacteria based on its hydrophilic and anionic properties (Denny, 1995). Van Doorn (1998) suggested that EPS contributed to the growth rate of bacteria, while Denny (1995) reported that in phytopathogenic bacteria EPS protected bacteria from desiccation, concentrated minerals and nutrients, reduced contact with hydrophobic or charged macromolecules, enhanced attachment to surfaces,

prolonged water-soaking of host tissues, reduced contact with toxic molecules, minimized interaction with plant cells and promoted colonization.

As EPS provides numerous advantages for bacteria, they might partly influence cut flower vase life. While the formation of EPS in media has been widely studied, their production under a natural environment (in plant stems) has not been reported. Further studies detailing EPS formation *in situ* are required.



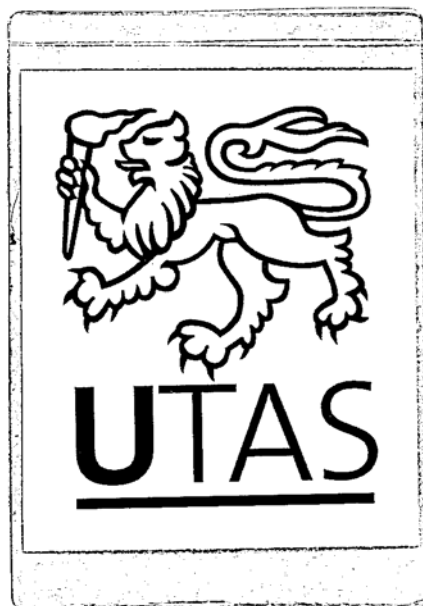
5 Summary

Postharvest physiology of cut flowers has been, and remains, an important area of horticultural research. Much work has been done in the development of postharvest management treatments and in understanding the physiological changes occurring in cut flowers during vase life. While more recent research has concentrated on the biochemical and molecular events of flower senescence, much of the earlier research identified the key factors influencing vase life. It is generally accepted that the presence of bacteria in vase water reduce cut flower vase life. Effects of postharvest treatments have been studied widely and most of those treatments have been focused on control of bacterial growth, leading to an improvement in water uptake. A number of studies have shown that infiltration of bacteria into flower stems resulted in early senescence, while numerous studies have demonstrated that use of bactericide could extend vase life. Despite the volume of literature indicating a role for bacteria in reduced cut flower vase life, the mechanisms by which bacteria affect vase life are still debated. The most accepted theory was that bacterial cells entering the cut vessels caused vascular blockage at the pit pores and thus restrict water uptake and hence reduce vase life. This theory has been supported by the numerous finding that impairment of water balance generally coincided with increases in bacterial numbers. The evidence in favour of the theory has, however, not been conclusive and several arguments against bacterial cell blockage of vessel have been raised. Other mechanisms such as air embolisms, tylosis, and vessel wall degradation have been proposed to explain the reduction in stem hydraulic conductance observed during cut flower vase life. Therefore, despite the volume of evidence suggesting a role of bacteria in reducing cut flower vase life, the

mechanism of action has still not been fully established and even the importance of bacteria in reduced vase life has been questioned.

The dominant bacterial genera identified on cut flower stems and in vase water has been *Pseudomonas*. The ubiquitous presence of this genera may provide a clue to the role of bacteria in vase life. One of the characteristics of this genera is the capacity to produce extracellular polysaccharide. Electron microscope studies have shown the presence of bacterial cells and amorphous substance presumed to be of bacterial origin in stem xylem vessels. This material may contribute the vascular blockage in flower stems but only a small body of evidence support a role for bacterial extracellular polysaccharide in vascular occlusion exists. Further investigation into the role of bacterial extracellular polysaccharide on cut flower water relation is required.

IV. MATERIALS AND METHODS



IV. General Materials and Methods

The relationship between bacterial populations and stem water relations was investigated in *Dianthus*, *Iris* and *Chrysanthemum* cut flowers. The sequence of experimentation in the project began with characterisation of the plant material used, followed by examination of changes in bacterial cell numbers and stem hydraulic resistance during vase life, identification of the location of stem blockages, and identification of the nature of the blockage. Experimental procedures used routinely in the project were dilution plating for determination of bacterial cell number, pressurised water column procedures to measure stem hydraulic resistance, and scholander type pressure bomb apparatus for measurement of water potential. These methods and details of the plant material and general experimental conditions and procedures are described in this chapter. Details of the specific methodology followed in each experiment are provided in Chapter V.

1 Plant material

Dianthus (cultivar Candy), *Iris* (cultivar Prof. Blaauw) and *Chrysanthemum* (cultivar White Reagan) flowers were obtained from a commercial retail florist and transported dry to the laboratory within 30 minutes of purchase. Flowers were ordered in advance to ensure that recently harvested material was used in all experiments. All flowers were placed in distilled water and held at 4 °C until required for experiments. Flowers were held for a maximum of two days under these conditions during this study. Prior to commencement of experiments, flower stems were recut to a final stem length of approximately 45 cm for *Dianthus*, 40 cm for *Iris*, and 60 cm for *Chrysanthemum*. In one experiment, single flower stems from spray *Chrysanthemum* were used, and in

this case stems were recut to the final length of approximately 15 cm. Leaves were removed from all flower stems with the uppermost three and four leaves left on carnation and iris stems respectively. Spray *Chrysanthemum* were used in the majority of experiments, and all leaves were removed from the main stem leaving only the leaves on lateral branches. In one experiment, single flower stems from spray *Chrysanthemum* were used, and in this case all leaves from the stem were removed. Flowers were then placed in flasks containing sterile distilled water (prepared by autoclaving the flasks and distilled water for 15 minutes). Flowers were kept either in a controlled environmental cabinet (20 °C, 60% RH and a photon flux density of 150 micromol. m⁻²s⁻¹, 12 hours of light per day) or on a shelf in the horticultural laboratory.

2 Bacterial cell numbers

Bacterial cell numbers were measured using dilution plating following extraction from flower stem sections. Stem sections were cut from the base of the flower stem and from positions further up the stem. Before extraction of bacterial cells from stems, the epidermal layer of the flower stem was removed using sterile equipment. The internal stem sections were placed individually in stomacher bags and weighed. Ten times this weight of sterile 0.85% NaCl solution was added. The bag was placed in a stomacher (Colworth stomacher 400) apparatus for 5 minutes, and the resultant bacterial suspension was taken through a series dilution and plated on trypticase soy agar medium (Appendix 2A) for determination of bacterial cell number. After 10, 100, 1000, 10000 or 100000 times dilution of the extracted bacterial suspension, 0.1 mL of bacterial suspensions from each dilution were spread on agar plates and incubated at 25 °C for 48 hours. Plates containing 30-400 bacterial colonies were selected from the dilution series for counting bacteria. Results were expressed as colony forming units (cfu)/g fresh weight. Each determination included five replications.

3 Stem hydraulic resistance measurement

Stem hydraulic resistance was determined by measuring water flow through a flower stem segment attached to a pressurised water column. While this method does not truly reflect water flow in an intact stem where xylem water is moved under tension rather than positive pressure, the method may be used to approximate the magnitude of stem vascular blockage. Stem hydraulic resistance in flower stems has been recorded previously using the pressurised water column method (Put and Rombouts, 1989; Durkin, 1979a; Gilman and Steponkus, 1972; Put and van der Meyden, 1988). Two methods were used to measure stem hydraulic resistance in this project. The first method, using a 2.6 m head of water as the pressurised water column, was used to determine hydraulic resistance when vascular blockage was low, while the second method utilised a modified pressure chamber apparatus to determine changes in hydraulic resistance when significant blockage was present. Each measurement included five replications.

The basic apparatus for measuring stem resistance consisted of a burette, containing water, connected to a flower stem section by latex tubing. Stem sections were inserted horizontally into the tubing in such a way that water passed through the stem sections in the same direction as in intact plants. The other end of the segment was placed in a vial containing cotton wool for collecting water which passed through the segments. In order to obtain accurate results, water was allowed to flow through the stem segments for at least 5 minutes to allow the system to equilibrate before collection commenced. The water flow through the segments was assessed by determining the weight of water accumulating in the vial over a 5 minute period following equilibration. Water level in the burette was maintained at the initial height by addition of water during the period of the measurement.

The average water flow rate in millilitres per hour was then calculated. Results were expressed in term of KPa/mL/hr. The stem hydraulic resistance was calculated using the following equation:

$$\text{stem hydraulic resistance} = \frac{\text{Height from meniscus to stem (cm)}}{\text{Flow rate in mL/hr}} \quad (\text{KPa/mL/hr})$$

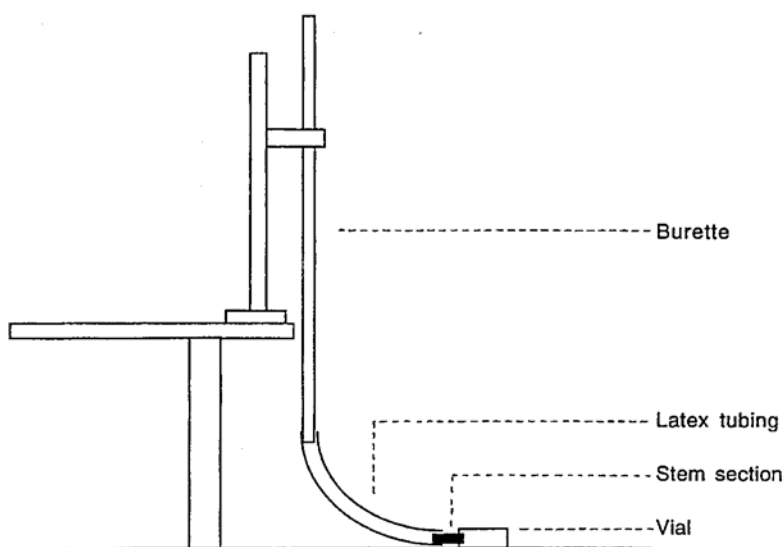


Figure 1: Burette apparatus for measuring flower stem hydraulic resistance.

The first method for measuring stem hydraulic resistance was limited by the head of pressure which could be applied (26 KPa at 2.6 m water column

height) and could thus only provide reliable flow rates when stem hydraulic resistance was low. In order to determine more accurately the changes in stem hydraulic resistance when vascular blockage increased later in flower vase life, a modified pressure chamber apparatus was used to apply greater water pressure to the stem sections. The apparatus consisted of a modified pressure cooker (Namco pressure cooker 10 L) attached to a water flow regulator. The pressure chamber was filled with water and the pressure was controlled by a pressure controller (Calorex Pty. Ltd., Huntingdale). Flower stems were inserted into holes at the lids of the pressure chamber. Before inserting the stems, water level in the chamber was adjusted to the top of the hole thus eliminating air spaces in the chamber. The bottom ends of the stems were inserted with the chamber so that water passed through the stems in the same direction as in intact plants. Water flow rate were recorded at four different pressures with each section. In order to obtain accurate results, water was allowed to flow through the stem segments for at least 5 minutes to allow the system to equilibrate before collection commenced. The water flow through the segments was assessed by determining the weight of water accumulating in the vial over a 5 minute period following equilibration and average stem resistance was calculated from the data of all 4 pressure levels.

$$\text{stem hydraulic resistance} \quad = \quad \frac{\text{Pressure (KPa)}}{\text{Flow rate in mL./hr}}$$

(KPa/mL/hr)

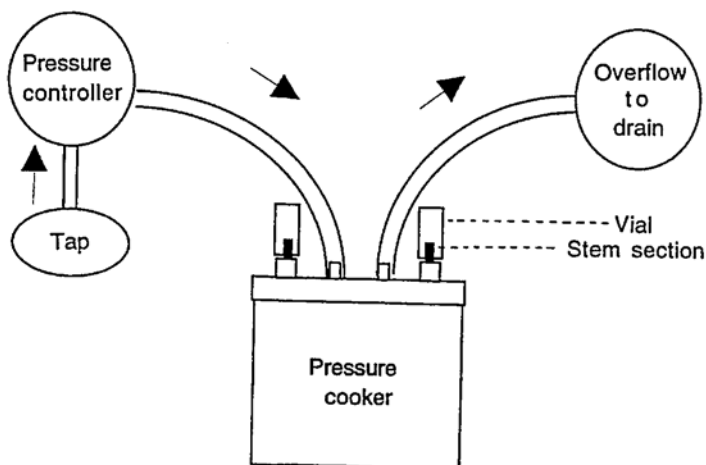


Figure 2: Pressure chamber apparatus for measuring flower stem hydraulic resistance.

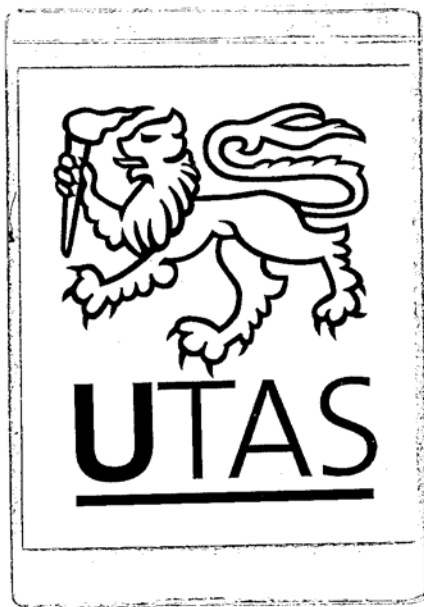
4 Water potential measurement

Water potential measurement was carried out in order to monitor water stress (if present) over the period of the experiment. Three centimetre *Chrysanthemum* and *Dianthus* stem sections removed from the flower stem approximately 2 cm below the flower were taken to measure water potential using a scholander type pressure bomb. As *Iris* stems were too big to be inserted into the pressure bomb, leaf sections approximately 3 cm in length were taken to measure water potential instead of stem sections. The flower stem or leaf section was inserted into the pressure bomb chamber with the cut end of the stem protruding from the lid. A dissecting microscope was placed over the lid in order to view the cut end of the stem. Pressure was added to the chamber until water was observed to bubble at the cut surface of the stem/leaf. The air pressure in the chamber at this point was recorded and

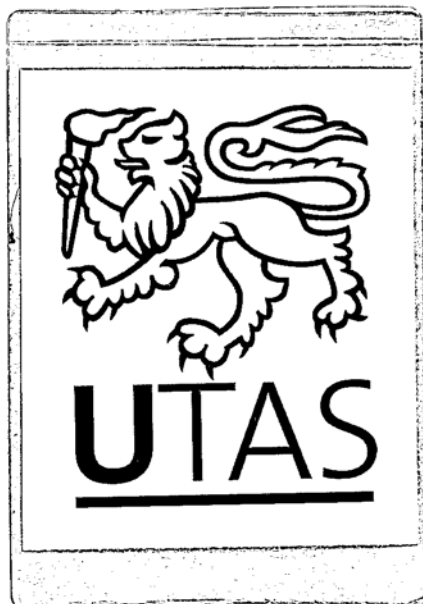
converted to a negative stem water potential reading. Triplicate stem and leaf sections were used for each determination of stem water potential.

5 Statistics

Data were analysed using Systat v.5.2 for MacIntosh. Analysis of variance (ANOVA) was undertaken for each data set generated in the project. Where the p value was significant, a least significant difference (LSD) was calculated at the 5% probability level and used to determine treatment differences.



V. RESULTS AND DISCUSSION



1 Xylem vessel length and diameter

1.1 Introduction

One xylem vessel consists of many xylem elements with a perforation plate separating each element. Water moves within a xylem vessel through the perforation plates and moves between vessels through pit membranes on their cell walls. The vessel length represents the maximum distance that water can travel without passing through a pit membrane. A knowledge of vessel length is important for measuring stem hydraulic resistance. If the stem section used is shorter than vessel length, hydraulic conductance will be overestimated as the resistance of the pit membranes to water flow would not be recorded. Xylem vessel length and diameter, including pit and perforation plate structure, have been studied extensively in woody plants (Ewer and Fisher, 1989; Zimmermann, 1983; Langan et al, 1997; Tyree and Dixon, 1986; Hacke and Sauter, 1996) but relative few studies has been undertaken on cut flower stems (van Doorn et al, 1989; Put and van der Meyden, 1988). The aim of these experiments was to measure the maximum vessel length of *Dianthus*, *Iris* and *Chrysanthemum* stems, thus providing information about the minimum length of stem sections which could be used to determine stem hydraulic resistance. The vessel diameter distribution in the three species of cut flowers was also investigated.

1.2 Materials and Methods

A. Vessel length distribution

Chrysanthemum, *Iris* and *Dianthus* flowers were obtained from a retail florist and then transferred dry to the laboratory. Vessel length distribution was determined using a modification of the methods described by Ewers and

Fisher (1989). The flower stems were recut under water to remove the bottom 20 cm and prevent formation of air embolisms, and the next 15 cm section was then cut and inserted, under water, into a tubing containing dilute acrylic paint solution.

The acrylic paint solution was prepared by a 1:100 (v/v) dilution of paint in distilled water followed by filtering (Whatman no.1) to remove paint particles greater than 5 μm in diameter. Thus the resulting paint particles were small enough to pass through pores in perforation plates. The paint solution was gravity fed into the stem section from a 2.5 m column. The solution was allowed to pass through the stem until flow completely stopped which indicated that the paint particles had blocked all the pit membranes (Williamson and Milburn, 1995).

The stem was then cut into 1 cm long sections. A freehand transverse section of each segment was then prepared, mounted on a glass microscope slide, examined at 100x magnification under a light microscope (Ernst Leitz Wetzlar Ortholux, Germany) and the number of paint-containing vessels was counted. For iris, before taking the transverse sections, the stems were longitudinally cut into quarters. Only one quarter from each stem was used for the further vessel counting, and results multiplied by four to give an approximate vessel number per stem. Longitudinal sections were also examined to confirm that the paint particles were blocking the pit membranes and not the perforation plates. The experiment included three replicates.

Calculation of vessel length distribution was carried out following the method of Ewers and Fisher (1989). The raw vessel count represented the number of vessels continuous from the cut surface, x_0 . The first difference represented the number of vessel ends between the distances where the raw counts were made. The second difference represented the rate of linear increase for vessels of this length class. The second difference multiplied by the number of increments (step to zero) gave the number of vessels at that length class.

B. Vessel diameter distribution

Freehand transverse sections of *Dianthus*, *Iris* and *Chrysanthemum* stems were taken to examined with 100x magnification under light microscope (Ernst Leitz Wetzlar Ortholux, Germany). The inner vessel lumen diameters were measured using an eyepiece graticule calibrated against a slide micrometer. When a vessel lumen was not circular, the widest and narrowest length of the lumen were measured and the vessel diameter was recorded as the average of the two data. Vessels were grouped into size classes of less than 20 μm , 20-30 μm , 30-40 μm and greater than 40 μm .

Before cutting hand sections, each *Dianthus* stem was cut longitudinally into quarters and one quarter from each stem was used for determination of vessel diameter distributions. For *Chrysanthemum* stems, the total number of vascular bundles was counted and 5 bundles randomly selected for vessel diameter measurements. Vessel diameter distributions were recorded for the main stem of spray *Chrysanthemum* as well as for peduncles of individual flowers. For *Iris*, the stems were cut longitudinally into eight segments prior to transverse sectioning. One segment was taken and ten vascular bundles were randomly selected for vessel diameter measurements. Total number of vascular bundles in the stem was approximated by recording the number of vascular bundles in two segments and multiplying by four.

Total xylem area was calculated using the vessel diameter distribution data and the equation below. Where diameter was <20 μm the vessel class was calculated as having a mean diameter of 15 μm ; diameter was 20-30 μm the vessel class was calculated as having a mean diameter of 25 μm ; diameter was 30-40 μm was calculated as having a mean diameter of 35 μm ; and diameter was >40 μm was calculated as having a mean diameter of 45 μm .

$$\text{Total vascular area (mm}^2\text{)} = \Pi \sum_{i=1}^4 n_i r_i^2$$

Where n_1 = number of vessels in size class <20 μm

n_2 = number of vessels in size class 20-30 μm

n_3 = number of vessels in size class 30-40 μm

n_4 = number of vessels in size class >40 μm

r_1 = radius of size class <20 μm

r_2 = radius of size class 20-30 μm

r_3 = radius of size class 30-40 μm

r_4 = radius of size class >40 μm

1.3 Results

A. Vessel length distribution

The flow through the stem section completely stopped within three days and numbers of paint-containing vessels in stem sections every 1 cm from cut surface were counted. Stem sections did not fit into one field of view under the microscope. The counting therefore started at a recognizable position on the stem section and moved along the edge of the stem.

In the present study, for *Dianthus*, one out of three replicates contained vessels in the 3-4 cm length class while the other two replicates contained only vessels shorter than 3 cm. However, there was not much variation in total vessel number and percent in length class between stems. Mean value of vessels in each length class of three replicates is shown in Table 1 and the results suggested that maximum vessel length of carnation was less than 4 cm. For *Chrysanthemum*, no paint-containing vessels were observed in stem sections higher than 6 cm (Table 2) which indicated that maximum vessel length of *Chrysanthemum* was less than 6 cm. One out of the three stem samples

contained vessels in the 4-5 cm length class. There was a large variation in total vessel number between stems but percent in length class of all three replicates were quite similar. For *Iris*, paint-filled vessels were found only in the first 1 cm section from the cut surface which indicated that maximum vessel length of *Iris* stem was less than 1 cm. The experiment, with *Iris*, was repeated to confirm the result.

Distance in Length (cm) class	Raw vessel count	1' dif	2' dif	Steps	No. of to zero	Length class vessels	% (cm.)
4	0	0	0	5	0	4-5	0.0
3	0.3	0.3	0.3	4	1.2	3-4	0.2
2	6	5.7	5.4	3	16.2	2-3	2.8
1	46.3	40.3	34.6	2	69.2	1-2	11.8
0(x_0)	586	539.7	499.4	1	499.4	0-1	85.2

Table 1 : Vessel length distribution of *Dianthus* stems. Data are means of 3 replicates. 1' dif = first difference ; 2' dif = second difference.

Distance Length (cm) class	Raw vessel count	1' dif	2' dif	Steps	No. of to zero	Length class vessels	% in (cm.)
6	0	0	0	7	0	6-7	0.0
5	0.7	0.7	0.7	6	4	5-6	0.8
4	6	5.3	4.7	5	23.3	4-5	4.5
3	12	6	1	4	4	3-4	0.8
2	37	25	19	3	57	2-3	11.0
1	117	80	55	2	110	1-2	21.2
0(x_0)	520	403	323	1	323	0-1	62.1

Table 2 : Vessel length distribution of *Chrysanthemum* stems. Data are means of 3 replicates. 1' dif = first difference ; 2' dif = second difference.

B. Vessel diameter distribution

In *Dianthus* flower, most xylem vessel diameters (85.7%) were smaller than 20 μm . No vessel, which was larger than 40 μm in diameter, was found. In *Iris* flower, 67.3% of xylem vessels were smaller than 20 μm , and as with *Dianthus*, no vessel larger than 40 μm was found. In *Chrysanthemum*, average total number of xylem vessels in the main stem was approximately 5 fold more than in lateral stems. The diameter class of main stem *Chrysanthemum* varied from the smallest (less than 20 μm) to the largest (>40 μm) group with the major vessel group (39.9%) between 20-30 μm in diameter. The lateral stem *Chrysanthemum* apparently contained only two groups of diameter class : <20 μm and 20-30 μm . Most xylem vessels (84.2%) were smaller than 20 μm in diameter (Figure 3). Average vessel numbers of *Dianthus*, *Iris*, main stem

Chrysanthemum and lateral stem *Chrysanthemum* were 1535, 1323, 1760, and 356 per stem respectively. Results were the average of data obtained from stems examined at different times of the year and replicates represented different flower samples obtained from the florist rather than three replicates from the same sample.

Average vascular areas for *Dianthus*, *Iris*, main stem *Chrysanthemum* and lateral stem *Chrysanthemum* were 0.35, 0.40, 0.90 and 0.08 mm² per stem respectively.

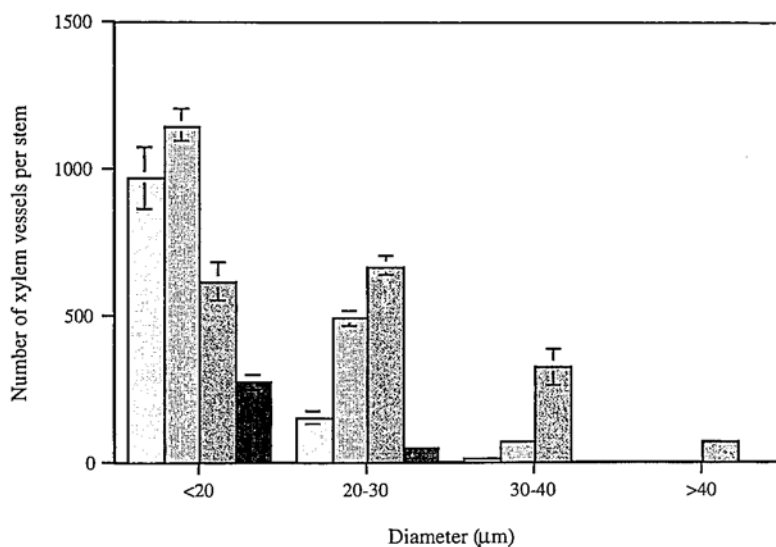


Figure 3 : Vessel diameter distribution in stem of *Dianthus* (□); *Iris* (▤); main stem *Chrysanthemum* (▨); and lateral stem *Chrysanthemum* (■). Data are means of 5 replicates.

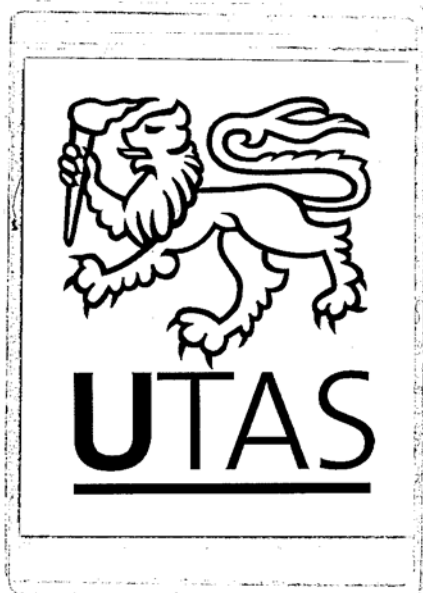
1.4 Discussion

Knowledge of flower stem xylem vessel length and diameter distributions and total xylem cross sectional area is valuable in interpreting data on cut flower

water relations. Attention has been focused on the effects of bacteria on stem hydraulic conductance, and it is surprising that very few studies have reported on xylem characteristics in the species being examined. In this study, the maximum vessel lengths of *Dianthus*, *Iris* and *Chrysanthemum* stems were less than 4, 1 and 6 cm respectively. These were relatively short in comparison to the maximum vessel length reported for cut Sonia rose stem which was approximately 25-30 cm (van Doorn et al, 1989). However minimum vessel length in cut Sonia rose stem was approximately 0.2 cm (Put and van der Meyden, 1988) which suggested that flower stems contained a large range of vessel lengths. The actual vessel length distribution was not recorded for *Rosa*, so it is impossible to determine the proportion of vessels which fit into the longest size category. In *Dianthus*, more than 90% of the vessels were shorter than 1 cm, while in *Chrysanthemum* approximately 80% were shorter than 1 cm and 100% of vessels in *Iris* stems were less than 1 cm in length. Thus, while maximum vessel length varied between the three species, the majority of vessels in each case were less than 1 cm in length. Based on the maximum vessel length results, the minimum lengths of stem sections to measure stem hydraulic resistance for *Dianthus*, *Iris* and *Chrysanthemum* must be 4, 1 and 6 cm respectively.

Dianthus and *Iris* were quite similar in both average vessel numbers per stem and vessel diameter distribution (most smaller than 20 μm and no vessel which was bigger than 40 μm was found) and therefore have a comparable vascular xylem area. The main stem of spray *Chrysanthemum* contained a greater proportion of larger diameter vessels and a higher average vessel number per stem which resulted in a greater vascular area. The lateral stem of *Chrysanthemum*, on the other hand, had the smallest vascular area which was approximately 10 times less than of the main *Chrysanthemum* stem. The results showed that *Dianthus* vessels were generally small to medium with various length up to 4 cm. *Iris* stems also contained small to medium vessel size but with very short vessels (all shorter than 1 cm). *Chrysanthemum* main stems contained various diameter size and length (up to 6 cm) while vessels of lateral

stem *Chrysanthemum* were generally small. The number of vessels per stem of *Dianthus*, *Iris* and *Chrysanthemum* (except for small stem) were comparable to that of Sonia roses which contained approximately 1,500 vessels (Put and van der Meyden, 1988).



2 Relationship between bacterial population, stem hydraulic resistance and water potential

2.1 Introduction

Bacteria have been proposed to be one of the main causes of vascular blockage which leads to the impairment of water flow in the flower stem hence reducing cut flower vase life (van Doorn and de Witte, 1994; van Doorn and D'hont, 1994; Put and Jansen, 1989). Infiltration of bacterial cells into flower stems has been demonstrated to cause a decrease in stem hydraulic conductance (van Doorn and de Witte, 1991). With the exception of studies by (Put and Jansen, 1989; van Doorn and de Witte, 1994; Durkin, 1979a), changes in stem hydraulic resistance in cut flower stems have not been undertaken in conjunction with determination of bacterial numbers. The studies which have been conducted have not examined hydraulic resistance in different parts of the stem (van Doorn and de Witte, 1991). The aim of this experiment was to examine the relationship between the number of bacteria and stem hydraulic resistance in stem samples taken from different parts of the flower stem. Water potential was measured to monitor the effects of changes in stem hydraulic resistance. Stem hydraulic resistance measurements were taken using stem sections longer than the maximum vessel length and shorter than the maximum vessel length as a preliminary investigation of the location and nature of stem blockage.

2.2 Materials and Methods

A. Stem section longer than the maximum vessel length

Flowers were prepared as described in the general materials and methods section. Flowers were placed in a controlled environment cabinet for the duration of the trial. Stem samples were taken at regular intervals to examine number of bacteria and stem resistance over the vase life of the flowers. The length of stem samples varied according to flower species with sections 4 cm long, 3 cm long and 6 cm long for *Dianthus*, *Iris* and *Chrysanthemum* respectively. Each section was longer than the maximum vessel length recorded for the flower species. Hydraulic resistance of each section was measured using water pressure in a gravity fed burette apparatus (Chapter IV, page 40). Stem samples were collected for analysis at the following times;

<i>Dianthus</i>	: 0, 3, 8 days of vase life
<i>Iris</i>	: 0, 2, 4, 6, 8 days of vase life
<i>Chrysanthemum</i>	: 0, 4, 8 days of vase life

Fifteen stems were taken at each sample date. Five stems were used for stem hydraulic resistance measurements, five stems were used for bacterial number determinations and five stems were used for water potential measurements. Stem sections for hydraulic resistance and bacterial number determinations were dissected from the following positions on the stem (0 cm is the base or cut surface of the flower stem);

<i>Dianthus</i>	: 0-4 cm, 4-8 cm
<i>Iris</i>	: 0-3 cm, 3-6 cm, 6-9 cm, 9-12 cm
<i>Chrysanthemum</i>	: 0-6 cm, 6-12 cm

For each of the flower stems, the lowermost section taken for determination of bacterial number was further divided into two sections, the bottom 0.2 cm part of the stem containing the original cut surface of the stem, and the remainder of the section. This division was undertaken in order to gain more information about bacterial distribution within the stem. Bacterial numbers were estimated using a serial dilution technique as describe in Chapter IV, page 40.

Another 10 stems of each flower species were used for vase life determination. End of vase life was determined by visually observing the condition of flowers. *Dianthus* flowers were considered to have senesced when more than one-third of their petals had lost turgor. *Chrysanthemum* flowers (flower peduncles were used for vase life observation) were considered to have senesced when more than one-third of petals were necrotic or wilted, all disc florets were necrotic or loss of turgor in the flower stem. Other negative features were also observed eg. first sign of necrosis or petal wilting or first sign of leaf chlorosis. *Iris* flowers were considered to have senesced when more than one-third of their petals had lost turgor.

B. Stem section shorter than the maximum vessel length

Flowers were prepared and placed in the same conditions as described in section A. *Iris* stems were not used in this trial as stem segments shorter than 1 cm could not be used for stem hydraulic resistance measurements. *Chrysanthemum* stems used in this experiment were flower peduncles. The length of stem section for *Dianthus* and *Chrysanthemum* was 3 cm and 2 cm respectively. Stem sections were taken from the following positions for *Dianthus* and *Chrysanthemum* respectively: 0-3 cm, 3-6 cm, 6-9 cm, 9-12 cm and 0-2 cm, 2-4 cm, 4-6 cm. Bacterial number and stem hydraulic were measured at regular intervals over the duration of flower vase life. Stem samples were collected for analysis at the following times;

<i>Dianthus</i>	: 0, 2, 5, 7 days of vase life
<i>Chrysanthemum</i>	: 0, 2, 4, 6, 8, 10, 13 days of vase life

Ten stems were taken at each sample date. Five stems were used for stem hydraulic resistance measurements and five stems were used for bacterial number determinations. For each of the flower stems, the lowermost section taken for determined of bacterial number was further divided into two sections; the bottom 0.2 cm part of the stem containing the original cut surface of the stem, and the remainder of the section. Bacterial numbers were estimated using a serial dilution technique as described in Chapter IV, page 40.

2.3 Results

A. Stem section longer than the maximum vessel length

Dianthus started to senesce (petals had lost turgor but no abscission was observed) on day 7 of vase life and 80% reached the end of vase life on day 10. For *Iris*, the first symptoms of senescence were observed at the distal edges of the wide petals, which became water-logged and showed inrolling after four days of vase life and all the *Iris* flowers senesced by day 6. For *Chrysanthemum*, only 10% of the flowers showed the signs of loss quality of the cut flowers (necrosis on the disc and ray florets) by day 13 of vase life.

The changes in bacterial population were similar in all three species of cut flowers. On the first day of the experiment, there was no significant difference between bacterial number of the bottom stem section and of the upper stem section. Number of bacteria increased rapidly within the first few days of vase life in both stem segments. Most bacteria were found at the bottom section especially at the 0.2 cm section which including cut surface. The number of bacteria in the bottom stem section and the upper stem section were

significantly different after the first day of the experiment (Table 3, Table 4, Table 5). Bacteria were found in the top section from each flower species despite the fact that each section was longer than the maximum xylem vessel length.

Days vase life	Number of bacteria (cfu/g.fresh weight)				LSD
	cut surface	0.2-4 cm.	0-4 cm.	4-8 cm.	
0	1.35×10^5	1.13×10^5	1.24×10^5	1.10×10^4	NS
3	1.54×10^7	1.39×10^5	7.77×10^6	1.36×10^3	5.58×10^6
8	7.30×10^7	2.08×10^5	3.66×10^7	9.80×10^3	4.19×10^7

Table 3 : Mean number of bacteria per gram fresh weight of *Dianthus* stems. NS= not significant. All data are means of 5 replicates.

Days vase life	Number of bacteria (cfu/g. fresh weight)						LSD
	cut surface	0.2-3 cm.	0-3 cm.	3-6 cm.	6-9 cm.	9-12 cm.	
0	1.73×10^5	6.49×10^4	1.19×10^5	3.80×10^3	1.58×10^2	3.20×10^2	NS
2	5.57×10^7	2.17×10^6	2.90×10^7	3.90×10^4	3.63×10^5	2.09×10^5	1.64×10^7
4	7.09×10^7	4.50×10^6	3.77×10^7	3.38×10^5	4.20×10^5	2.90×10^4	3.47×10^7
6	1.15×10^8	3.01×10^6	5.90×10^7	2.54×10^4	7.68×10^4	4.04×10^4	5.33×10^7
8	1.27×10^8	4.55×10^6	6.60×10^7	1.88×10^6	3.66×10^5	8.58×10^5	9.07×10^7

Table 4 : Mean number of bacteria per gram fresh weight of *Iris* stems. NS = not significant. All data are means of 5 replicates.

Days vase life	Number of bacteria (cfu/g.fresh weight)				LSD
	cut surface	0.2-6 cm.	0-6 cm.	6-12 cm.	
0	8.00X10 ²	0	4.00X10 ²	0	NS
4	7.88X10 ⁶	1.16X10 ⁶	4.52X10 ⁶	1.13X10 ⁵	2.95X10 ⁶
8	8.92X10 ⁷	8.44X10 ⁶	4.88X10 ⁷	6.50X10 ⁵	4.78X10 ⁷

Table 5 : Mean number of bacteria per gram fresh weight of *Chrysanthemum* stems. NS = not significant. All data are means of 5 replicates.

Bacterial population in the vase water of *Chrysanthemum* was also monitored during the trial. Mean number of bacteria found in vase water increased with time from 76 cfu/mL at the commencement of the experiment to 7.3X10⁴ cfu/mL after four days and 4.9X10⁵ cells/mL after eight days. The results agree with a previous studies that reported an increase in bacterial population in vase water of cut *Rosa* over the duration of vase life (van Doorn and de Witte, 1994; de Witte and van Doorn, 1988).

Stem hydraulic resistance in the three species of cut flowers increased in the similar pattern to bacterial number. The resistance of both basal section and upper section was very low at the commencement of the experiment, but the resistance to water flow of the bottom section increased within four days of vase life and was significantly different from the resistance of the upper stem segments at this time. The hydraulic resistance of the basal section increased and reached the maximum measurable level (1,500 KPa/mL/hr) while hydraulic resistance of the upper stem sections in each flower species remained low through the period of vase life (Figure 4a and 4b). The stem resistance of the bottom section in *Chrysanthemum* was significantly lower than that of *Iris* and *Dianthus*, but was still significantly higher than the resistance of the upper stem section (Figure 4c).

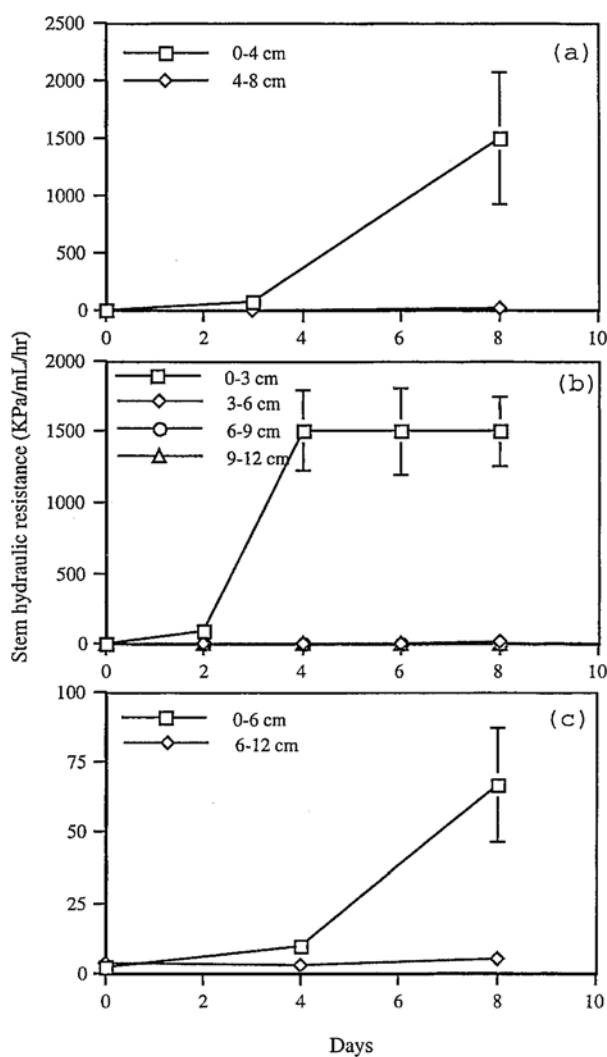


Figure 4 : The mean stem hydraulic resistance of *Dianthus* (a), *Iris* (b), and *Chrysanthemum* (c) in the different parts of the stems. The maximum stem hydraulic resistance which could be measured using the gravity fed technique was 1,500 KPa/mL/hr. All data are means of 5 replicates.

The largest increases in stem hydraulic resistance occurred in the bottom stem section between days 3 and 8 for *Dianthus*, 2 and 4 for *Iris* and 4 and 8 for *Chrysanthemum*. In each case the increase in bacterial population over the same time period was one order of magnitude or less, and it appears unlikely that bacterial number could account for the dramatic changes in stem hydraulic resistance. For example, hydraulic resistance in basal section of *Iris* stems increased from 90 to 1,500 KPa/mL/hr between days 2 and 4 while bacterial numbers increased from 2.9×10^7 to 3.8×10^7 cfu/g. fresh weight. Bacterial numbers in excess of 6.0×10^5 cfu/g. fresh weight were recorded in upper sections of *Iris* and *Chrysanthemum* stems but hydraulic resistance in these sections was always less than 20 KPa/mL/hr. Thus, while bacterial numbers and stem hydraulic resistance followed the same trend during vase life in all flowers, the timing and magnitude of changes did not always correspond. However, in all cases where large hydraulic resistance measurement were recorded, the bacterial numbers in the stem sections were in excess of 3.0×10^7 cfu/g fresh weight.

Water potential of *Dianthus* and *Iris* flowers decreased over period of the experiment while water potential of *Chrysanthemum* fluctuated with a decreasing trend (Table 6). This indicated a development of water stress, however, the changes in water potential of *Iris* and *Chrysanthemum* were not significant. The lack of water stress in the flowers was surprising and that it may have been due to limited water loss as the flowers were held in low light, moderate temperature, high humidity conditions.

Day	carnation	iris	chrysanthemum
0	-0.06	-0.28	-0.29
2	-0.11	-0.30	-0.51
4	-	-0.34	-0.36
5	-0.11	-	-
6	-	-0.48	-0.77
7	-0.20	-	-
8	-	-0.55	-0.48
10	-0.44	-	-0.73
13	-	-	-0.32
LSD	0.06	NS	NS

Table 6 : Average stem water potential(MPa) of *Dianthus* and *Chrysanthemum* and leaf water potential of *Iris*. NS= not significant. All data are means of 5 replicates.

B. Stem section shorter than the maximum vessel length

Bacterial numbers in both *Dianthus* and *Chrysanthemum* stems increased rapidly during the first 5 or 6 days of vase life, with the highest number of bacterial isolated from the basal stem segments (Table 7 and Table 8). The number of bacteria at the base of the flower stems began to decline towards the end of the experiment for both flower species.

Days vase life	Number of bacteria (cfu/g. fresh weight)						LSD
	cut surface	0.2-3 cm.	0-3 cm.	3-6 cm.	6-9 cm.	9-12 cm.	
0	0	0	0	0	0	0	-
2	5.33X10 ⁵	9.62X10 ⁴	3.15X10 ⁵	1.62X10 ⁴	1.84X10 ³	2.00X10 ²	1.61X10 ⁵
5	1.01X10 ⁶	1.26X10 ⁵	5.67X10 ⁵	8.62X10 ⁴	1.08X10 ³	0.20X10 ²	3.44X10 ⁵
7	9.00X10 ⁵	8.78X10 ⁴	4.94X10 ⁵	4.96X10 ⁴	8.16X10 ³	2.20X10 ²	1.91X10 ⁵
10	5.36X10 ⁵	7.62X10 ⁴	3.06X10 ⁵	2.60X10 ³	3.48X10 ³	2.60X10 ²	2.26X10 ⁵

Table 7 : Mean number of bacteria per gram fresh weight of *Dianthus* stems.
All data are means of 5 replicates.

Days vase life	Number of bacteria (cfu/g. fresh weight)					LSD
	cut surface	0.2-2 cm.	0-2 cm.	2-4 cm.	4-6 cm.	
0	0	0	0	0	0	-
2	6.20X10 ⁴	1.30X10 ⁴	3.75X10 ⁴	1.20X10 ⁴	0	3.56X10 ⁴
4	5.70X10 ⁶	1.57X10 ⁶	3.65X10 ⁶	1.80X10 ⁴	6.00X10 ²	NS
6	1.87X10 ⁷	2.70X10 ⁶	1.07X10 ⁷	1.15X10 ⁶	1.60X10 ³	9.92X10 ⁶
8	6.59X10 ⁷	7.17X10 ⁶	3.66X10 ⁷	8.47X10 ⁵	4.20X10 ⁴	1.59X10 ⁷
10	2.06X10 ⁸	6.63X10 ⁶	1.06X10 ⁸	1.13X10 ⁶	3.20X10 ³	NS
13	2.62X10 ⁷	9.48X10 ⁶	1.79X10 ⁷	2.18X10 ⁶	1.38X10 ⁵	1.59X10 ⁷

Table 8 : Mean number of bacteria per gram fresh weight of *Chrysanthemum* stems. NS = not significant. All data are means of 5 replicates.

Stem hydraulic resistance of both the basal section and the top sections of *Dianthus* and *Chrysanthemum* stems was very low at the first day of the experiment. In *Dianthus*, the hydraulic resistance of the bottom section increased significantly between day 0 and day 2 of vase life and continued to increase slowly after that time. Hydraulic resistance of the top sections remained low through the period of the experiment (Figure 5a). The hydraulic resistance of the bottom and top sections was significantly different at the 5% probability level from day 2 of vase life. In *Chrysanthemum*, hydraulic resistance of the bottom and top sections remained low for two days, but hydraulic resistance of the basal section started to increase on day 4 of vase life and by day 6 the resistance of the bottom and top sections was significantly different (Figure 5b).

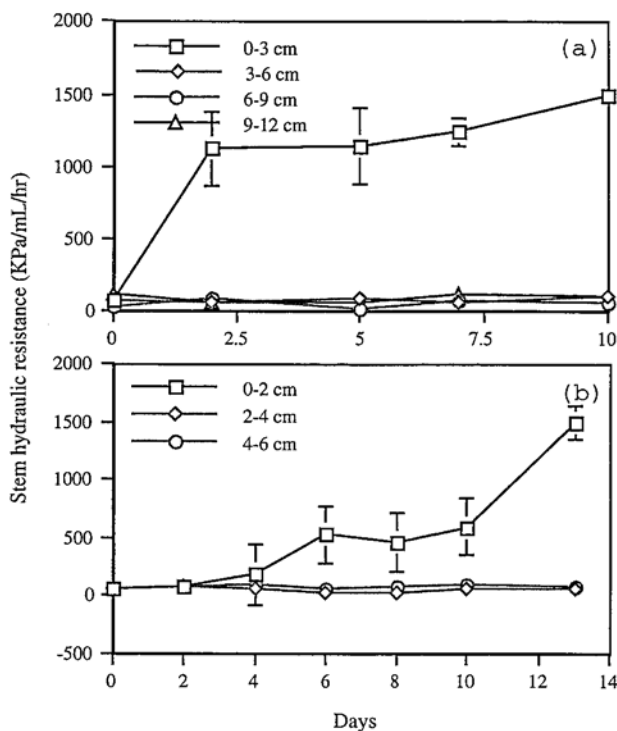


Figure 5 : The mean stem resistance of *Dianthus* (a) and *Chrysanthemum* (b) in the different parts of the stems. The maximum stem hydraulic resistance which could be measured using the gravity fed technique was 1,500 KPa/mL/hr. All data are means of 5 replicates.

Stem hydraulic resistance values in *Dianthus* stem sections shorter than the longest vessel length increased faster than the values recorded in the first trial using longer stem segments, while bacterial numbers increased more slowly. For *Chrysanthemum*, both stem hydraulic resistance and bacterial numbers in stem sections shorter than the longest vessel length increased slower than in the trial using longer stem sections. It is evident that increases in number of bacteria and stem hydraulic resistance did not correspond. The critical

bacterial numbers noted in the first experiment for all high hydraulic resistance measurements were not repeated in this experiment as hydraulic resistance in excess of 1000 KPa/mL/hr were recorded in *Dianthus* stem segments when bacterial numbers less than 5×10^5 cfu/g fresh weight were present. Hydraulic resistance in both *Chrysanthemum* and *Dianthus* stem segments taken from the base of the stem continued to increase even though bacterial numbers declined towards the end of the experiment.

2.4 Discussion

In all three flower species, significant increase in stem hydraulic resistance occurred prior to flower senescence indicating that restriction of water supply may lead to loss of cut flower quality. Decreases in water potential over vase life were also observed which suggested the onset of water stress. In *Dianthus*, decrease in water potential started to be significantly different on day 7 of vase life which coincided with the occurrence of first signs of the flower senescence. The difference, however, was not significant in *Iris* and *Chrysanthemum*.

Bacterial numbers in *Dianthus*, *Iris* and *Chrysanthemum* stems were shown to increase rapidly during vase life, with the highest concentration found in the bottom sections of the stems and in particular at the cut surface. This finding is in agreement with previous studies which showed that the major location of bacteria was at the cut surface and the basal section of the stem (van Doorn et al, 1991; Put and Clerkx, 1988; van Doorn and de Witte, 1991). Bacteria were also found in the stems up to 12 cm from the cut surface, suggesting that bacteria could move through intervessel pits from one xylem vessel to an adjacent vessel. However, the conflict between bacterial distribution in flower stem and the vessel length data may suggested that the method used to estimate vessel length distribution may not have been accurate enough to pick up a small number of long vessels which could have been present. In each of the three flower species, bacteria were found in segments higher in the stem

than the length of the longest vessels measured in the species (Chapter 1, page 47). Bacterial populations in excess of 10^5 cfu/g. fresh weight of stem were recorded in many of these stem sections. These populations suggest that significant movement of bacterial cells occurred between xylem vessels or that rapid multiplication of bacteria can occur in xylem vessels. These results appear to contradict the conclusion of van Doorn et al (1991) who stated that bacterial cells were unable to pass through the inter-vessel pits in cut *Rosa* stems. However, in their study, determination of bacterial number in the stem higher than the maximum vessel length was not undertaken, and the conclusion was reached based on SEM images of bacterial cells located around intervessel pits.

The method of movement of bacterial cells from one vessel to an adjacent vessel is unclear. While sizes and shapes of pit apertures in cut *Rosa* flower stems (Sonia) have been shown using a scanning electron microscope (Put and Meyden, 1988; Put and Rombouts, 1989; Put and Klop, 1990), pore sizes in pit membranes in other cut flower stems have not yet been reported. However, determinations of pore sizes in pit membrane in woody plants have been undertaken. It has been reported that the approximate range of pit opening diameters in softwoods probably extends from 0.02 to 4 μm , while in hardwoods was from 0.005 to 0.17 μm (Siau, 1984). Sperry and Tyree (1988) stated that diameter of pores in pit membranes of sugar maple (*Acer saccharum* Marsh) measured with scanning electron microscope were less than 0.4 μm . Van Alfen et al (1983) reported that the pore sizes of pit membranes in stems of alfalfa (*Medicago sativa* L. var DuPuits) were approximately 0.2 to 0.8 μm . This suggested that pore sizes in pit membranes vary between plant species and could be bigger or smaller than bacterial cells, based on *Pseudomonas* spp. with dimensions of 0.5 to 1.0 μm by 1.5 to 4.0 μm (Holt, 1977). If the pores in pit membranes of cut flower stems were bigger than bacterial cells, bacteria may move from one vessel to the adjacent vessel via the transpiration stream. However, if the pores in pit membranes were smaller than bacterial cells, it is possible that bacteria may produce pectic enzymes (Put and Rombouts, 1989)

to digest pit membrane or produce some metabolically active compound which could affect the flexibility of pit membrane to allow passage of the cells between vessels. Moreover, Siau (1984) also stated that tori and pit membranes were extensively degraded by bacterial attack.

Despite the presence of high numbers of bacterial cells in the upper stem sections, stem hydraulic resistance in these sections was always very low, and did not vary significantly during vase life. In contrast, stem hydraulic resistance in the basal sections of all flower stems increased rapidly during vase life. While bacterial numbers in the basal sections also increased significantly during vase life, the timing and magnitude of changes in hydraulic resistance and bacterial numbers did not correspond. The highest hydraulic resistance measurements generally occurred when bacterial numbers in excess of 10^7 cfu/g fresh weight of stem were present, but this relationship was not always observed suggesting that the critical bacterial concentration proposed by van Doorn et al (1989) was not universally applicable. Links between bacterial numbers in flower stems (van Doorn et al, 1990) or in vase water (Jones and Hill, 1993) and vase life have been suggested, but this approach appears inaccurate given the lack of correlation between bacterial numbers and stem water relations in this experiment and the speed at which bacterial populations increased. The large difference between bacterial numbers in vase water and flower stems recorded in this trial and by other researchers (Put, 1990; van Doorn and Perik, 1990) also suggests that determination of vase water bacterial numbers cannot be used to accurately predict level of stem blockage.

The presence of single vessels (no pit membranes separating the cut end of the vessel at one end of the segment from the cut and at the other) running the length of the stem sections used to determine stem hydraulic resistance did not result in decreased values. Stem hydraulic resistance in *Chrysanthemum* was much higher when shorter segments were used compared to the longer stem sections. The results were, however, difficult to interpret as different stem

types were used in the two experiments. In the experiment with longer sections, the main-stems which have a greater vascular area were used while in the experiment with shorter stem section, the flower peduncles which have less vascular cross-sectional area were used. While the effect of stem section length was not clearly demonstrated, there was still no correlation between bacterial number and stem resistance for either flower type. Hydraulic resistance in basal stem segments was higher than in upper stem segments for each flower type and segment length. The high hydraulic resistances recorded in stem segments cut shorter than the length of the longest vessels suggests that occlusion at the pit membranes may not be the sole cause of increasing stem hydraulic resistance during vase life. However as the shorter stem sections (3 cm for *Dianthus* and 2 cm for *Chrysanthemum*) contained more than 80% intact vessels (based on vessel length distributions calculated in Chapter 1, page 47), blockage at the pit membrane cannot be ruled out. Further investigation of the location of vascular occlusions is required to identify the major cause of increased stem hydraulic resistance during vase life.

3 Location of the blockage

3.1 Introduction

The major vascular blockage in cut flower stems was located in the bottom 3-6 cm of the stems (Chapter 2, page 56). This finding has been supported by a number of other studies where the general conclusion has been that vascular occlusion in basal sections of the stem but with little evidence of the precise location of the blockage presented (Dixon and Peterson, 1989; Lineberger and Steponkus, 1976; van Doorn and de Witte, 1991). Stem hydraulic resistance of the basal stem sections examined in this study increased over vase life and were significantly higher than that of sections from the top of the stem. The highest bacterial populations were found within the basal 2 mm section of the stem which suggested that the main location of vascular blockage may be at or very close to the cut surface. This experiment was carried out to further investigate the location of vascular occlusion in the flower stems.

3.2 Materials and Methods

Hydraulic resistance measurements were undertaken on stem sections taken from the base of flower stems. Sections of equal length with 10 mm, 5 mm or none of the base of the stem removed were dissected from replicate stems for measurements. These measurements were used to calculate stem hydraulic resistance for 5 mm long stem sections at the base and 5 mm from the cut surface of the stems.

Dianthus, *Iris* and *Chrysanthemum* flowers were obtained from a florist and the stems were recut and put in vases (5 stems per vase) containing sterile distilled

water. Flowers were placed in a controlled environment cabinet at 20°C, 60% RH, a photon flux density of 150 $\mu\text{mol.m}^{-2}.\text{s}^{-1}$ and 12 hour day length. Stem sections were taken from the bottom part of the stems to determine stem hydraulic resistance on day 0, 4 and 8 of vase life. Fifteen stems of each flower species were dissected at each sample date. Prior to measurement of hydraulic resistance, five stems were recut to remove the basal 10 mm, five were recut to remove the basal 5 mm and the remaining stems were left uncut. Following the trimming treatment, each sample section was 4 cm long for *Dianthus*, 3 cm long for *Iris*, and 6 cm long for *Chrysanthemum*. Hydraulic resistance in the sections was determined using the burette apparatus (Chapter IV, page 40).

Stem hydraulic resistance in the basal 5 mm stem section was calculated by subtracting the mean hydraulic resistance value for the stems which had the bottom 5 mm removed from the mean value recorded for stems which were not trimmed. Hydraulic resistance of the 5 mm section located 5 mm from the cut surface was calculated in the same way using mean values obtained for the two recut treatments.

3.3 Results

The changes of stem resistance over time followed a similar trend in all three types of cut flowers. Stem hydraulic resistance of each segment was low on the first day of the experiment. Afterwards, resistance to water flow of the bottom 5 mm section increased rapidly from day 0 to day 4 and reached 1,500 KPa/mL/hr/cm by day 8 of vase life. Stem hydraulic resistance of the section located 5 mm and the section located 10 mm from the cut surface remained low over the duration of the trial (Figure 6a, 6b, 6c). The results showed that the major resistance to water flow was located within the bottom 5 mm of the flower stem.

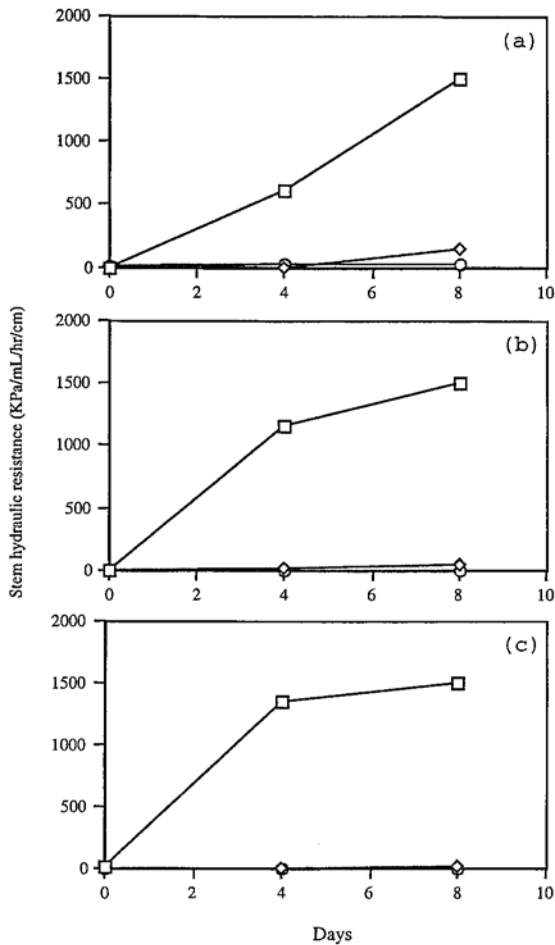


Figure 6 : Mean stem resistance of *Dianthus* (a), *Iris* (b), *Chrysanthemum* (c) in bottom 5 mm section including stem cut surface (—□—), a 5 mm section cut at 0.5 cm further up the stem (.....◇.....) and sections cut at 1 cm further up the stem (.....○.....). The maximum hydraulic resistance which could be measured using the gravity fed technique was 1,500 KPa/mL/hr.

Stem hydraulic resistance of *Dianthus* increased slower than of *Iris* and *Chrysanthemum*. The resistance to flow of *Iris* and *Chrysanthemum* was over 1,000 KPa/mL/hr/cm by day 4 of vase life while of *Dianthus* was less than 1,000 KPa/mL/hr/cm on the same day.

3.4 Discussion

In this experiment, the analysis of variance was calculated using the original data, prior to calculation of hydraulic resistance in 5 mm sections. This was because data for each treatment (stems not recut, 5 mm removed or 10 mm removed) was obtained from different stems. Therefore, only mean data of each treatment was taken to calculate hydraulic resistance in 5 mm sections and hence it was impossible to calculate the analysis of variance from the transformed data. The analysis results showed that the difference in resistance between section with and without removal of basal sections was statistically significant but the difference between sections with 5 and 10 mm removed was not significant. This indicated that the major vascular occlusion was located within 5 mm from the stem cut surface as removal of the 10 mm section did not further improve water flow in the stem.

In all three flower species studied, the highest stem hydraulic resistance was found in the lowermost 5 mm segment while resistance of the section higher than 5 mm from cut surface almost did not change over the period of vase life. The similarity in the changes between different flower species indicating a uniform mechanism of vascular blockage in various types of cut flowers. The results showed that the major vascular occlusion was located no further than 5 mm from stem cut surface. These results agree with the histochemical study of Dixon and Peterson (1989) who reported that plugging materials always found within 200 mm of the cut surface and the largest percentage of plugged vessels was near the cut end. Nevertheless, the results were consistent with blockage at the cut surface but could not preclude the blockage at pit pores of cut vessels. This area was then investigated further in the next experiment.

4 Polysaccharide assays

4.1 Introduction

Reduction in stem hydraulic conductance during vase life was shown in the previous experiments to be poorly related to bacterial numbers but linked to vascular occlusions within 5 mm of the cut surface of the stem. High numbers of bacteria were found in the top stem section but stem resistance to water flow was always low in these sections. Moreover, the highest concentration of bacteria was found in the basal 5 mm of stems, which was not consistent with xylem vessel perforation plates forming a block to bacterial cell movement as few perforation plates were likely to be present at the location of bacterial cell concentration. Attachment of bacterial cells to the base of the stem was occurring, suggesting that the attachment mechanism may be linked to the vascular occlusion noted at the base of the stem. Electron microscope examination revealed an amorphous substance covering the vascular bundles at the stem cut surface of *Chrysanthemum* flowers (Brown et al, 1998). Similar observations of what has generally been concluded to be bacterial exopolysaccharide (EPS) have been made previously (van Doorn et al, 1991). *Pseudomonas* sp. have been documented as the dominant bacteria found on cut flower stems (de Witte and van Doorn, 1988; Put, 1990; van Doorn et al, 1991) and this genera produce EPS (de Witte and van Doorn, 1988). It is therefore possible that bacterial EPS facilitates attachment of bacterial cells to the plant tissue and contributes to vascular blockage at the base of the flower stem. Changes in concentration of bacterial exopolysaccharide would thus be expected to correlate to the changes in stem hydraulic resistance. Qualitative analysis of carbohydrate level in flower stems has been attempted using staining and microscopy (Gilman and Steponkus, 1972; Lineberger and Steponkus, 1976) but the inability to separate carbohydrate of bacteria and plant origin has restricted interpretation of results. Examination of the role of

bacterial EPS on cut flower water relations requires the development of an sensitive assay for carbohydrate. Carbohydrate assay procedures based on colourimetry, viscometer and chemical reaction with anthrone were examined to determine the most appropriate assay for flower stem extracts.

4.2 Colourimetry method

This semiquantitative assay was adapted from the method of Vandevivere and Kirchman (1993) who studied the effect of solid surfaces on exopolysaccharide production by attached bacterial cells. The method was based on staining of bacterial EPS with alcian blue 8GX, a stain for mucosubstances. Bacterial cells and EPS were extracted from flower stems by put a stem section in a McCartney bottle containing distilled water (1 stem/mL) and shaken with vortex mixture (Chiltern Scientific, MT17V) at maximum speed for 3 minutes. The resulting suspensions were then stained with alcian blue 8GX for five minutes and the absorbance measured at 606 nm. Absorbance of alcian blue solution without extract was also measured. The solutions were then centrifuged at 6,000 rpm to remove bacterial cells and EPS. This step aimed to remove the alcian blue which had bound to the EPS, leaving free alcian blue in solution. After centrifuge, absorbance of the supernatant was measured. The difference in absorbance before and after centrifuge should thus be proportional to the concentration of bacterial exopolysaccharides present.

Preliminary experimentation with this procedure using seven day old flower stems revealed no difference in solution absorbance before and after centrifugation. This result was surprising as significant hydraulic resistance was revealed in stems at this stage of vase life. The results suggested that the method was not able to detect carbohydrate levels at the low concentrations expected in the flower stem extract, or that centrifugation did not separate the stained bacterial EPS from the solution. No pellet was observed following centrifugation, so aliquots of solution were sampled from the top third and the

bottom third of tubes following centrifugation to assay bacterial cell numbers. This revealed that even when centrifuged at 20,000 rpm for 30 minutes, more than twenty five percent of the bacteria originally present in the solution were located in the supernatant. It was concluded that the bacterial EPS remained in solution or suspension during centrifugation at the speeds used in this study, thus restricting separation of bound and free alcian blue and preventing the use of this procedure to assay bacterial EPS.

4.3 Viscometer method

The method was based on measurement of changes in viscosity of extract from flower stems with changes in bacterial exopolysaccharide concentration. A microviscometer (Brookfield Synchro Lectric Viscometer) was used in this experiment. Bacterial cells and EPS were extracted in distilled water (3 stems in 3 mL) using the vortex extraction method. One millilitre of extract was applied to the rotating disc surface of the microviscometer and the viscosity values obtained were calibrated against reading for distilled water. No repeatable differences in viscosity between extract and water were obtained. Eighty percent ethanol was then used for extraction instead of distilled water in case that 80% ethanol would be more efficient in extracting EPS from flower stems. However, the viscometer still showed poor repeatability for low viscosity bacterial suspension extracts for flower stems, and could not be used as a reliable measurement of EPS concentration.

4.4 Anthrone method

Total carbohydrate levels in extracts from the base and upper sections of the stems were determined. Stem extracts were obtained using the vortex extraction method. Plant extracts were filtering through a membrane filter paper. Results from a preliminary experiment demonstrated that sucrose

could pass through a membrane filter paper (data not shown). Therefore carbohydrate which was trapped on the filter was assumed to be polysaccharide.

The total carbohydrate assay was adapted from the anthrone method of Yemm and Willis (1954). Carbohydrates heated in a strong sulphuric acid solution form furfural derivatives which then react with anthrone. The rate of formation of the furfural derivatives corresponds with the development of a green colour of the anthrone-furfural derivative complex. The stability of this complex during heating then determines the final colour yield, which can be determined spectrophotometrically.

The anthrone reagent was prepared by dissolving 0.2 g anthrone in 100 mL cold diluted sulphuric acid made by adding 500 mL of concentrated acid to 200 mL of water. The reagent was stirred until thoroughly clear and kept on ice. The reagent was freshly prepared each day and used within 6 hours. All reagents used were analytical grade.

The reagent (2.5 mL) was dispensed into a clean tube and chilled on ice. The sample (200 μ L or 100 μ L plus 100 μ L distilled water) was layered on top of the reagent, allowed to cool for at least 5 minutes and then thoroughly mixed. The top of each tube was covered with aluminium foil and placed in a boiling water bath for 15 minutes and subsequently cooled in ice water for 5 minutes.

The absorbance of sample solutions and standard solutions (containing 5-40 g/L fructose) was measured at 630 nm at room temperature with a UV/VIS Spectrometer (Lambda 20, Perkin Elmer) with water as a reference. Fructose was chosen as standard because carbohydrate types of the sample extractions were unknown and preliminary experiments showed acceptable R^2 value.

4.5 Modification of anthrone method

When determining carbohydrate concentrations using the anthrone method maximum colour development is achieved at a point in time where the rates of formation of the chromogen and its destruction in hot acid just balance. This point is dependent on the type of sugar present in the sample (Jermyn, 1975). As extractions from flower stems contain more than one type of carbohydrate, standardisation of this method is required. It has been shown that by adding of hydrochloric and formic acids, with an appropriate heating time, approximately identical colour yields can be obtained from several carbohydrate types (Jermyn, 1975). Therefore, hydrochloric acid and formic acid were added to increase the robustness of the assay.

Samples (0.2 mL) were chilled on ice and a mixed reagent (1.82 mL) was added. The reagent was prepared by adding 10 mL 90% formic acid to concentrated hydrochloric acid (100 mL). Eight hundred millilitres of 80% sulphuric acid was then slowly added to the other acids. Anthrone (20 mg/100mL sulphuric acid) was then added to the reagent no longer than 6 hours before used. After thorough mixing of all the contents, the tube was heated for 12 minutes in a boiling-water bath and immediately put into cold water for 5 minutes. Absorbance was read at 630 nm after thorough mixing and allowing to stand in ice for one hour to disperse bubbles.

The absorbance of sample solutions and standard solutions (containing 5-40 g/L fructose) was measured at 630 nm at room temperature with a UV/VIS Spectrometer (Lambda 20, Perkin Elmer) with water as a reference.

5 Relationship between bacterial population, stem hydraulic resistance and polysaccharide accumulation

5.1 Introduction

Accumulation of polysaccharides of either plant or bacterial origin at the base of cut flower stems has been noted previously (Parups and Molnar, 1972; Dixon and Peterson, 1989; Lineberger and Steponkus, 1976; Fujino et al, 1983a). However, quantitative assessment of changes in polysaccharide concentration during vase life has not been reported. Changes in stem hydraulic resistance in flower stems would be expected to mirror changes in polysaccharide concentration if bacterial EPS was the major contributor to stem vascular occlusion. The aim of this experiment was to examine the relationship between stem hydraulic resistance, polysaccharide accumulation and bacterial population in flower stems.

5.2 Materials and Methods

A. Stem hydraulic resistance, bacterial numbers and polysaccharide concentration

Dianthus, *Iris* and *Chrysanthemum* flowers were prepared as described in the general materials and methods. Stem samples were taken from the base of the stem and 6 cm from the cut surface to measure stem hydraulic resistance, carbohydrate concentration, and bacterial population on day 0, 2, 4, 6 and 8 of the flower vase life. Stem hydraulic resistance and bacterial number determinations were performed using five replicate stems of each flower species were sampled at each date. Carbohydrate analysis was conducted on

five stem extracts at each sample date for each flower species, with two stems used for each stem extracts. Bacterial numbers were determined as described in the general materials and methods (Chapter IV, page 40).

Stem hydraulic resistance was measured using the pressure chamber technique (Chapter IV, page 40). The lengths of stem sections used were 4, 3 and 6 cm for *Dianthus*, *Iris* and *Chrysanthemum* respectively. For the bottom section, resistance to water flow before and after removal of 2 mm of cut surface was measured; for the top section, the hydraulic resistance was measured without recutting. The pith area tissue at the top end of each *Chrysanthemum* section was removed to a depth of approximately 5 mm and replaced with melted paraplast plus wax to prevent water flow through the pith. A thin layer of the stem section at the top end was then removed to eliminate any xylem vessels blockage by the wax. The basal end of the section was in water through the process.

Carbohydrate was extracted from flower stems as described in chapter 4, page 76. One and a half millilitres of the extract was passed through a membrane filter paper (pore size 0.45 μm , mixed cellulose ester type) to separate bacterial EPS and other polysaccharides from soluble carbohydrates such as sucrose, glucose and fructose which were likely to originate from the cut plant cells. Two hundred microlitres of the extract was collected before and after filtration for measurement of carbohydrate concentration using the anthrone method (Chapter 4, page 76). Polysaccharide concentration was calculated as the difference in carbohydrate concentration before and after filtration.

Carbohydrate contamination from the filter paper was accounted for by passing 1.5 mL of distilled water through a filter paper and determining the carbohydrate concentration in the solution. This value was subtracted from all carbohydrate values from filtered extract.

B. Effect of pressure on stem hydraulic resistance

Measurement of stem hydraulic resistance using the pressure chamber apparatus provided significant lower results than using the burette apparatus. The major difference between these two techniques was the water pressures applied to the stem sections. As pressure is taken into account when calculating stem hydraulic resistance, the results suggested either a change in the hydraulic properties of the stem sections with water pressure, or an error in the pressure measurements from the pressure chamber apparatus. This experiment was therefore carried out in order to examine the effect of different pressures on stem hydraulic resistance.

Dianthus, *Iris* and *Chrysanthemum* flowers were placed in flasks (5 stems per flask) and held under controlled environment conditions for four days. Stem samples were cut from the base of the stem including the cut surface. Length of the stem segments were 4 cm for *Dianthus*, 3 cm for *Iris*, and 6 cm for *Chrysanthemum*. Stem hydraulic resistance was measured using the gravity fed burette apparatus, with water column heights of 100, 200, 300 and 400 cm used to exert 10, 20, 30 and 40 KPa pressure respectively.

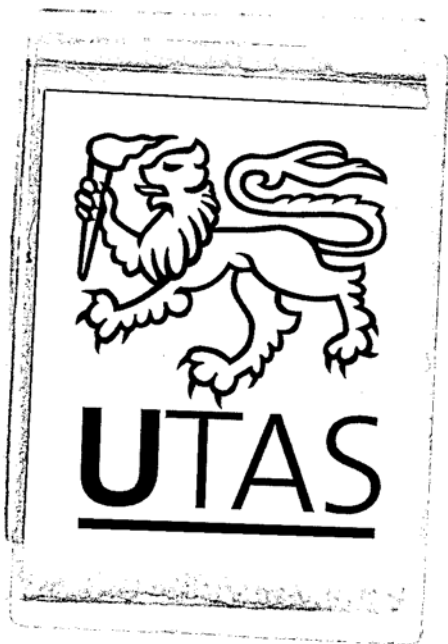
5.3 Results

A. Stem hydraulic resistance, bacterial numbers and polysaccharide concentration

Stem hydraulic resistance of the bottom sections including the stem cut surface increased rapidly within two days of the flower vase life while the hydraulic resistance of the top sections and of the bottom sections which the original cut surface had been removed remained low throughout this trial (Figure 7a, 7b, 7c). The difference in stem hydraulic resistance between the bottom and top

sections was statistically significant at the 5% probability level. Hydraulic resistance recorded in this experiment was lower than the measurements obtained in the previous experiment using the gravity fed water pressure technique. The highest hydraulic resistance recorded in the bottom sections of *Dianthus*, *Iris* and *Chrysanthemum* were 717, 87, and 57 KPa/mL/hr respectively. Stem hydraulic resistance of *Dianthus* was higher than *Iris* and *Chrysanthemum* and the resistance in excess of 500 KPa/mL/hr was observed by day 4 of vase life while resistance to water flow of *Iris* and *Chrysanthemum* was lower than 100 KPa/mL/hr throughout the duration of the experiment.

During vase life there was a drop in stem hydraulic resistance of the bottom section in each of the three flower species. Hydraulic resistance of *Dianthus*, *Iris* and *Chrysanthemum* dropped on day 6, 4 and 8 respectively.



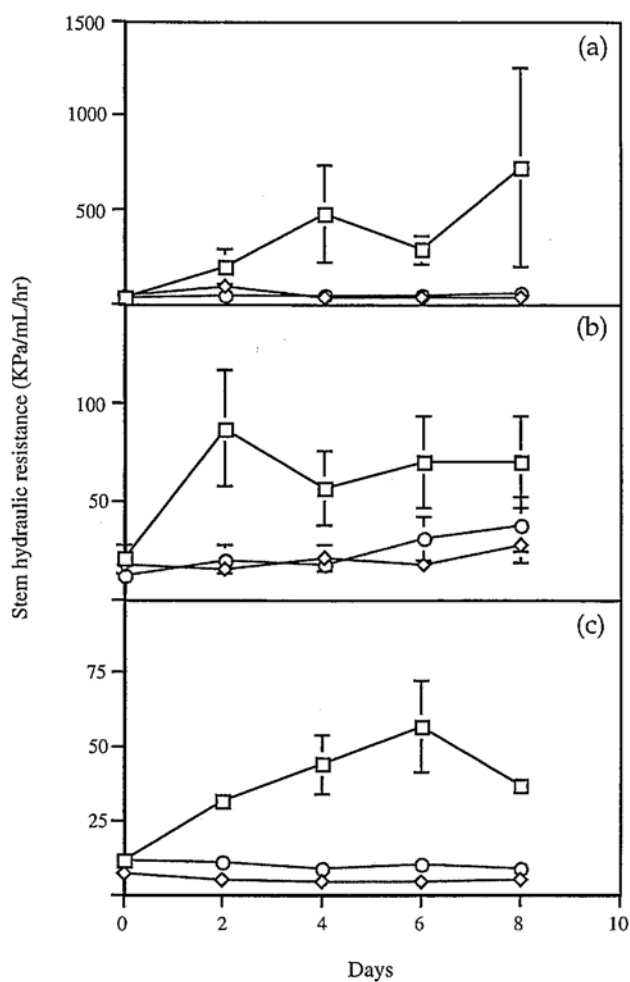


Figure 7 : Mean stem hydraulic resistance of *Dianthus* (a), *Iris* (b), and *Chrysanthemum* (c) in bottom section including cut surface (—□—), top section (—◇—), and bottom section which 2 mm of the cut surface was removed (—○—). The values represent the mean stem hydraulic resistance of 5 stems measured consecutively at 4 different pressures.

The carbohydrate concentration of extracts prior to filtration was significantly higher from top stem sections than basal sections, suggesting that a significant

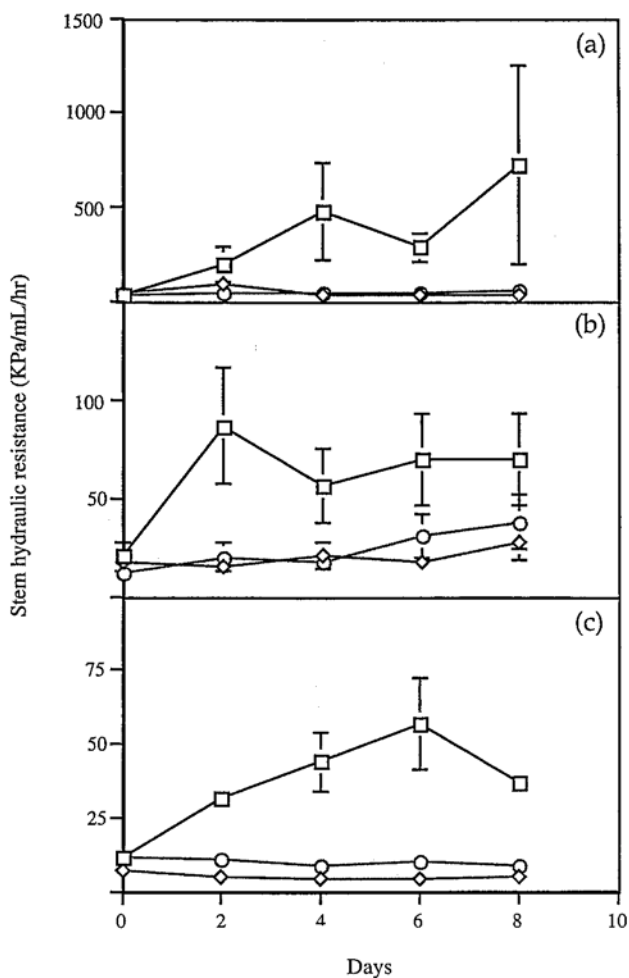


Figure 7 : Mean stem hydraulic resistance of *Dianthus* (a), *Iris* (b), and *Chrysanthemum* (c) in bottom section including cut surface (—□—), top section (—◇—), and bottom section which 2 mm of the cut surface was removed (—○—). The values represent the mean stem hydraulic resistance of 5 stems measured consecutively at 4 different pressures.

The carbohydrate concentration of extracts prior to filtration was significantly higher from top stem sections than basal sections, suggesting that a significant

proportion of carbohydrate extracted from stems was soluble sugars from the stem cells, or phloem exudate released following excision of the sections. Polysaccharides from *Dianthus* stem sections increased over the duration of vase life while concentrations in *Iris* and *Chrysanthemum* sections fluctuated over vase life (Figure 8). It was noted over the duration of the experiment that the carbohydrate assay was very sensitive to length of incubation period prior to measurement of solution absorbance. This may have contributed to variability in the results, which should thus be interpreted with caution.

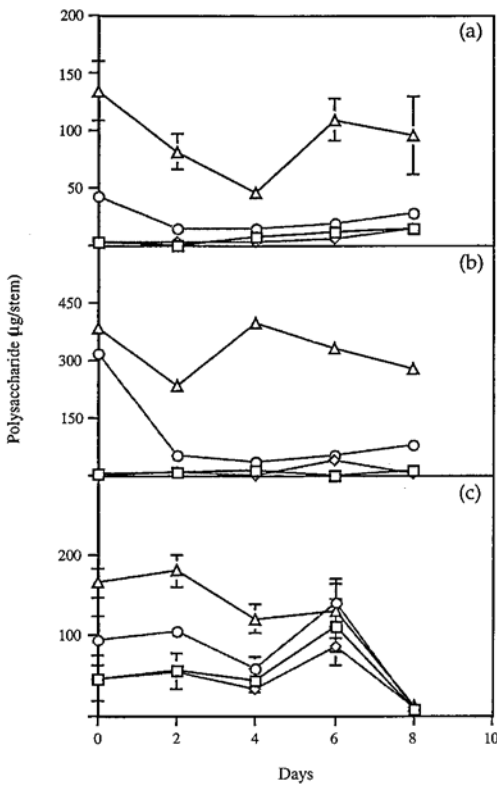


Figure 8 : Mean carbohydrate before filtrate and polysaccharide extracted from stems of *Dianthus* (a), *Iris* (b), and *Chrysanthemum* (c). Polysaccharide in bottom section (—□—), top section (-----◇-----). Carbohydrate in bottom section (.....○.....), top section (—△—). Data are means of 5 replicates.

In all three flower species, bacterial numbers in both basal and upper stem sections increased rapidly within two days of vase life (Table 9, 10, 11). In basal section, the increase in bacterial numbers was significantly different within two days in *Dianthus* and within four days in *Chrysanthemum* while in *Iris* was not significant throughout duration of the trial. After two days of vase life, only a small increase was observed in the basal sections. In upper sections, bacterial numbers increased more steady over vase life of the cut flowers and the difference was significant by day 8 of vase life except for *Iris*.

The highest bacterial numbers were found in the bottom sections however in excess of 10^6 cells/g. fresh weight in *Iris* and 10^5 cells/g. fresh weight in *Dianthus* and *Chrysanthemum* were found in upper stem sections. Generally, the difference in numbers of bacteria in basal and upper section was significant at the 5% probability level.

Days	Number of bacteria (cfu/g.fresh weight)		LSD
	bottom sections (0-4 cm)	top sections (4-8 cm)	
0	4.96×10^4	2.82×10^3	2.47×10^4
2	3.96×10^6	1.45×10^4	3.65×10^6
4	3.36×10^6	3.70×10^4	2.50×10^6
6	3.94×10^6	6.68×10^4	7.60×10^5
8	4.20×10^6	2.70×10^5	2.11×10^6
LSD	2.39×10^6	1.66×10^5	

Table 9 : Mean number of bacteria per gram fresh weight of *Dianthus* stems. Data are means of 5 replicates.

Days	Number of bacteria (cfu/g.fresh weight)		LSD
	bottom sections (0-3 cm)	top sections (3-6 cm)	
0	3.89×10^4	3.22×10^3	NS
2	6.70×10^6	2.04×10^6	4.53×10^6
4	1.47×10^7	2.27×10^6	NS
6	1.06×10^7	1.80×10^6	3.67×10^6
8	6.83×10^7	1.01×10^6	NS
LSD	NS	NS	

Table 10 : Mean number of bacteria per gram fresh weight of *Iris* stems. Data are means of 5 replicates. NS = not significant.

Days	Number of bacteria (cfu/g.fresh weight)		LSD
	bottom sections (0-6 cm)	top sections (6-12 cm)	
0	4.26×10^3	0	NS
2	1.68×10^6	1.66×10^4	1.19×10^6
4	7.50×10^6	1.43×10^5	4.52×10^6
6	4.56×10^6	1.78×10^5	2.22×10^6
8	1.56×10^7	4.25×10^5	8.68×10^6
LSD	5.19×10^6	1.67×10^5	

Table 11 : Mean number of bacteria per gram fresh weight of *Chrysanthemum* stems. Data are means of 5 replicates. NS = not significant

B. Effect of pressure on stem hydraulic resistance

The results obtained using the burette system. Stem hydraulic resistance of the three flower species decreased significantly as water pressure increased. The resistance dropped dramatically when the pressure increased from 10 KPa to 20 KPa and decreased slightly when pressures higher than 20 KPa were applied (Figure 9). This might be explained that with substantial pressure, air emboli, occluded by a plug of bacteria and slime in the vessels might be compressed, thereby opening pit membranes or other passages for the movement of water. Stem hydraulic resistances at 40 KPa were low and of a similar magnitude to the resistance of stem sections of the same age measured using the pressure chamber apparatus. These results clearly indicate that the hydraulic properties of the vascular occlusions present in four day old flower stems change with the water pressure applied to measure stem hydraulic resistance.

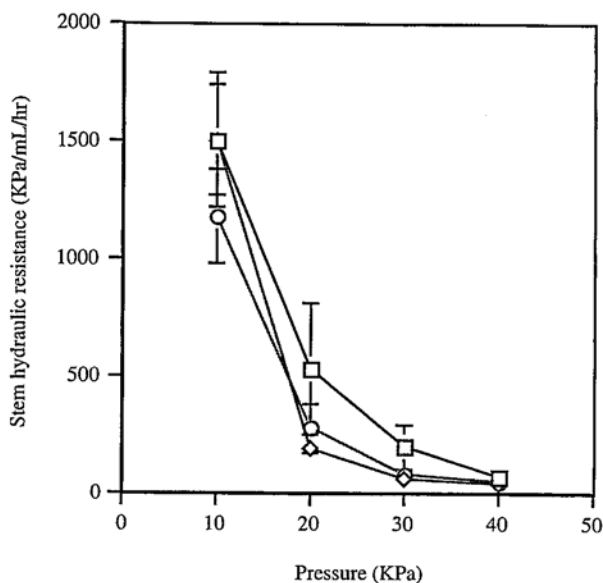


Figure 9 : The mean stem resistance of *Dianthus* (—□—); *Iris* (.....◇.....); and *Chrysanthemum* (.....○.....) stem sections. The maximum stem hydraulic resistance which could be measured using the gravity fed technique was 1,500 KPa/mL/hr. Data are means of 5 replicates.

5.4 Discussion

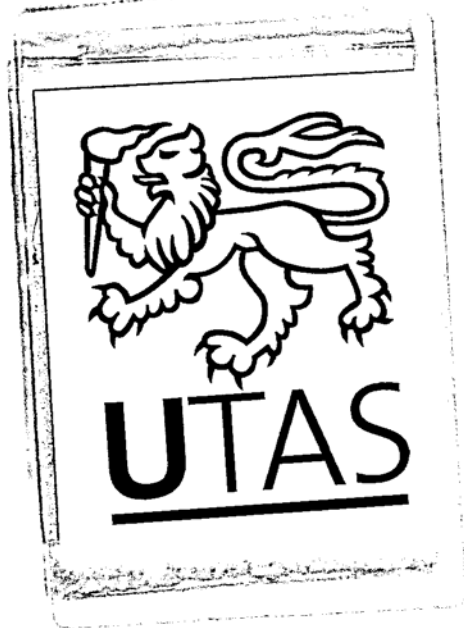
The hypothesis being tested in this set of experiments was not supported by the experimental results. The pattern of change in stem hydraulic resistance was similar to that observed in the earlier experiments. Stem hydraulic resistance of basal stem section increased over vase life while of the top section remained low. However, the stem resistance determined in this experiment, using the pressure chamber apparatus to force water through stem sections, was much lower than in the previous experiments using the gravity fed apparatus. This discrepancy can be explained by the effect of water pressure

on the hydraulic properties of the stem sections and in particular the vascular occlusions in older flower stems, with higher water pressures causing a decrease in stem hydraulic resistance. As the effect of higher water pressure was reversible, with higher resistance measurements obtained when pressure was decreased (data not shown), the effect was due to altered characteristics of the occluding material rather than forcing of material through the stems to reduce hydraulic resistance.

Changes in stem hydraulic resistance and bacterial population were similar to the results of the previous experiments, providing further evidence that the mechanism of vascular blockage was reproducible, always following the same general trend. The pressure chamber apparatus allowed measurement of stem hydraulic resistance during late vase life when the gravity fed burette apparatus was restricted in range. The results revealed a decrease in stem hydraulic resistance following an initial sharp increase within the first two days of vase life. While bacterial numbers followed a similar overall trend to stem hydraulic resistance, the timing of the decline in hydraulic resistance in each flower species did not correspond to decreasing bacterial populations. The stem hydraulic resistance in the upper stem sections appeared unrelated to bacterial numbers, with low resistance values recorded at each sample date and increasing bacterial populations over the duration of vase life.

Changes in polysaccharide concentration extracted from stem sections also did not correlate well with stem hydraulic resistance. Although higher stem hydraulic resistance and polysaccharide concentration were found in basal sections, the timing of the increase between these two values did not correspond. Variable results due to small differences in incubation time when using the assay may have occurred, making interpretation of the results difficult. Also, the anthrone assay for carbohydrate concentration gives variable results if carbohydrate types within the sample vary (Jermyn, 1975), introducing a further potential source of variability in the results. Stem extracts were likely to have contained various types of carbohydrate of both

plant and bacterial origin. Use of the modified anthrone carbohydrate assay needs to be conducted to clearly establish the relationship between bacterial exopolysaccharides and flower stem hydraulic resistance.



6 Microscopic examination

6.1 Introduction

Increasing stem hydraulic resistance over the duration of cut flower vase life was always found in the earlier experiments. While limited evidence of the involvement of bacterial cells and bacterial exopolysaccharides in vascular blockage has been presented, further evidence has been limited due to the difficulties in quantitative assessment of bacterial EPS. Qualitative measurement using microscopic and staining techniques could be an alternative method to examine the accumulation of bacterial EPS during the cut flower vase life. Alcian Blue, a stain for mucopolysaccharide (Lev and Spicer, 1964) and exopolysaccharide (Vandevivere and Kirchman, 1993), was used in this study. The purpose of this experiment was to detect changes in location and amount of bacterial EPS in basal and upper sections of flower stems over vase life.

6.2 Materials and Methods

Five stems each of *Dianthus*, *Iris* and *Chrysanthemum* were obtained from a retail florist and the stems were recut and put in vases containing sterile distilled water. Stem samples approximately 5 mm long were taken from the base of the stem and 6 cm from the cut surface for sectioning and staining on day 0, 2, 4, 6 and 8 of the flower vase life.

The sections were then cut longitudinally into 4 or 8 parts depending on the size of the stem. The stem segments were then fixed, dehydrated and embedded (Appendix 1A). After embedding in wax, 10 μm thick longitudinal

sections from each sample were prepared using a base sledge microtome (Ernst Leitz Optical Works G.m.b.H., Wetzlar, Germany No. 1300). Ribbons of the thin sections were floated in a water bath at approximately 45 °C and then attached to a microscope slide using Mayer's adhesive (20 mL white egg, 20 mL glycerine, 1 gm sodium salicylate). The sections were rehydrated through a xylene and ethanol series (Appendix 1B) and stained with alcian blue solution (Appendix 1C). The sections were examined under a light microscope (Leica Leitz DM RBE).

6.3 Results

The changes in location and area of stained tissue, assumed to be bacterial polysaccharide, were very similar in all three flower species examined. On day 0 of the flower vase life, samples of both the basal and upper sections did not show any evidence of bacterial EPS in the xylem tissue. By day 2 of vase life, a thin layer of blue stained bacterial EPS covered the edge of the basal cut end of the section and was also observed in some xylem vessels in the basal sections. Bacterial EPS was found in xylem vessels in the upper stem sections at day 2, but was only present in less than 5% of vessels in each section. From day 2 to day 8 of the flower vase life, only minor changes in area of bacterial EPS were observed. The basal cut surface of the stem was always covered with bacterial EPS (Figure 10), but the area of stained tissue did not increase after day 4. Up to 10% of vessels in the upper stem sections were completely occluded with stained material by day 8 of vase life.

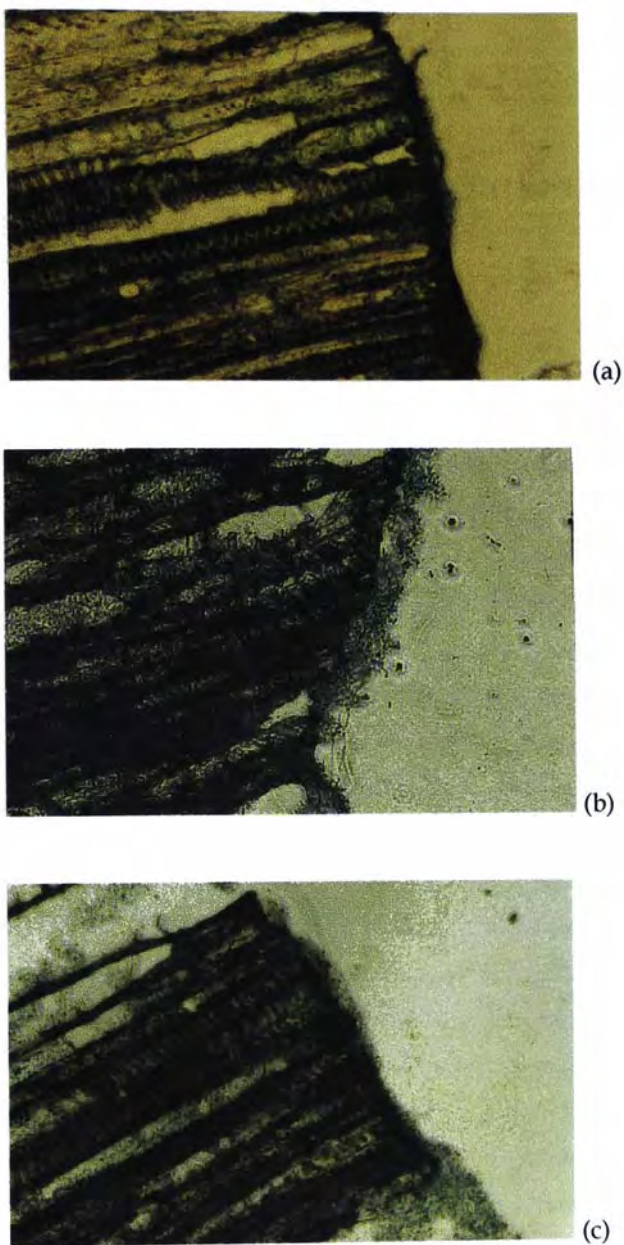
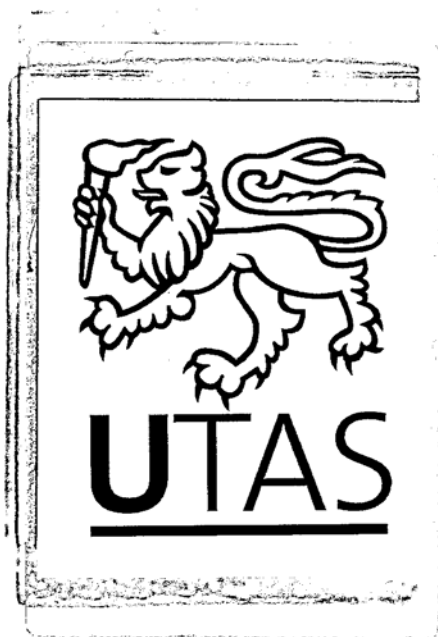


Figure 10 : Longitudinal section of chrysanthemum stem; (a) 2 days old, (b) 4 days old, (c) 8 days old. Samples were cut from basal part of the flower stems. Bacterial EPS stained blue.

6.4 Discussion

Alcian blue is a specific stain for mucosubstance (Lev and Spicer, 1964) and has been used to stain bacterial exopolysaccharide (Vandevivere and Kirchman, 1993). In this study, the results showed that xylem vessel walls were not stained with alcian blue therefore the staining substance was assumed to be bacterial EPS. The presence of what was presumed to be bacterial EPS covering the base of the flower stem within two days of vase life confirms to observations made in previous studies. An amorphous substance presumed to be of bacterial origin has been observed in xylem vessel of cut *Rosa* stems (van Doorn et al, 1991). Accumulation of bacterial EPS has been shown to be very rapid. On day 0 of vase life, there was no evidence of bacterial EPS in either basal or upper stem sections. However, by day 2 of vase life, bacterial EPS was observed in both sections. In basal sections, most bacterial EPS was found to be distributed over the base of flower stems, while in upper section, bacterial EPS was found within a small number of xylem vessels. This rapid increase in bacterial EPS occurred over a similar timeframe to the increase in stem hydraulic resistance in basal stem sections observed in previous experiments. A relationship between bacterial EPS and stem hydraulic resistance may be present, but would require significant impedance to water flow to be caused by a thin (approximately 1 mm) layer of EPS covering the majority of vessels at the cut surface of the stem. This layer was unlikely to completely block the water uptake as gaps in the slime layer were usually present (de Stigter and Broekhysen, 1986). Thickness of the slime layer did not seem to be an indicator of increasing hydraulic resistance rate as van Doorn et al (1991) showed that the cut *Rosa* stem with the thickest layer of material had the highest rate of water uptake. An artificial blockage of approximately two third of the xylem vessels in cut *Rosa* stems did not interrupt water uptake (van Doorn et al, 1989). However, the results of van Doorn need to be interpreted

cautiously as significant xylem occlusion under relatively mild environmental conditions may not result in water stress as the area of non-occluded xylem may be sufficient to supply water at a similar rate to transpirational loss. Nevertheless, their results suggested that more than 66% of xylem vessels had to be blocked to induce an increase in stem hydraulic resistance. Thus the accumulation of EPS in less than 10% of vessel in upper stem sections would be expected to have little effect on stem hydraulic resistance. In contrast, a small quantity of EPS covering the majority of vessels at the stem cut surface may restrict water uptake if the EPS has properties which slow water movement.



7 Hydraulic properties of bacterial EPS

7.1 Introduction

The role of bacterial EPS in flower stem water relations has proved difficult to investigate as effective methods to detect and qualify the material in flower stems have not been developed. The presence of bacterial EPS in flower stems has been proposed based on microscopic examination in this study and in a number of other studies (van Doorn et al, 1991; Lineberger and Steponkus, 1970; Parups and Molnar, 1972). However, the origin of the material may be questioned as plant derived polysaccharides are likely to stain in the same way as bacterial EPS. The presence of bacteria of the EPS producing genera *Pseudomonas* in flower stems (de Witte and van Doorn, 1988; Put and van der Meyden, 1988; Put, 1990; van Doorn and de Witte, 1994; Put and Jansen, 1989; van Doorn et al, 1991) provides further circumstantial evidence of the presence of bacterial EPS. Quantification of bacterial EPS concentrations is also difficult as contamination with plant polysaccharides may occur and concentrations present are likely to be low. In addition, bacterial EPS may consist of a number of different polysaccharides (Powell, 1979) and the composition may change according to the substrate on which the bacteria are growing.

Observations of location of polysaccharide material on cut flower stems suggested that coverage of all xylem vessels at the cut surface may contribute to increasing stem hydraulic resistance during vase life. However, the layer of polysaccharide covering the cut surface was generally less than 1 mm thick, and the permeability of this layer of material to water has not been determined. Therefore the aim of this experiment was to determine if low concentrations of bacterial EPS could significantly increase hydraulic resistance through filter paper.

7.2 Materials and Methods

A. Bacterial EPS and hydraulic resistance

Bacteria were isolated from the stems of *Chrysanthemum*, *Dianthus* and *Iris* stems following the procedures described in general materials and methods (Chapter IV, page 40). One hundred microlitres of extract from stem sections was cultured onto either trypticase soy agar medium (TSA), Basal mineral salt medium (BMS) or plant extract medium (PEM) and incubated at 25°C for two days. The combinations of plant species, time of vase life, growing media and position of stem section used in the experiment are shown in Table 12.

Flower species	Days of vase life	Growing media	Part of the stem
chrysanthemum	1	TSA	basal
chrysanthemum	1	BMS	basal
chrysanthemum	3	BMS	basal
carnation	4	BMS	basal
iris	2	TSA	basal
chrysanthemum	8	BMS	basal
carnation	7	PEM	basal
iris	5	PEM	basal
iris	7	BMS	top

Table 12: Sources of bacteria and types of cultured media; TSA = trypticase soy agar medium (Appendix 2A), BMS = Basal mineral salt sucrose medium (Appendix 2B), PEM = plant extract medium (Appendix 2C).

Bacterial suspensions were obtained by washing off the 2 day old mixed cultures on the agar with 10 mL. distilled water and the resulting suspensions

were used to produce solutions at 100%, 75%, 50% and 25% of the original concentration. One millilitre of each concentration was passed through a membrane filter paper (pore size 0.45 μm , mixed cellulose ester type) and the hydraulic resistance of the filter was measured. The filter was attached to a piece of aluminium foil containing holes totally approximately 2 mm^2 in area, which is larger than total vascular cross areas in the three flower species, for water to move through.

Holes on the aluminium foil were prepared by penetrating the foil with a needle. Area of the hole was measured using the same equipment and technique as measurement of vessel diameter (Chapter 1, page 47). The area of one hole was approximately 0.23 mm^2 therefore nine holes were made and distributed on the aluminium foil.

Following hydraulic resistance determination, the filter was shaken for 3 minutes, at maximum speed, in 1 mL distilled water using a vortex mixer to extract carbohydrate. Amount of carbohydrate in the resulting extract was measured using the validated anthrone carbohydrate assay. Three replicates of each bacterial source were conducted in the experiment.

Carbohydrate contamination from the filter paper was accounted for by shaking a filter paper in 1 mL distilled water for 3 minutes at maximum speed using a vortex mixer and determining the carbohydrate in the solution. This value was subtracted from all carbohydrate values from filter extract. Carbohydrate contaminations from culture media could not be taken to account because surface properties of the culture media may change once bacteria were grown therefore carbohydrate determination of the washing off solution from a new culture media may not be a reliable reference.

B. Effect of bacterial cells and EPS plus cells

The suspensions used in the first part of the experiment contained bacterial cells and EPS, and either may have contributed to changes in hydraulic resistance. In order to differentiate between the effect of bacterial cells and EPS, suspensions of the non-EPS producing species, *Bacillus subtilis*, were compared to mixed colony suspensions from flower stem extracts. Extract from a thirteen day old *Chrysanthemum* stem was used to produce a mixed colony suspension follow two days in culture. The extract was cultured on TSA and BMS media while *B. subtilis* was cultured only on TSA medium. The plates were then incubated at 25 °C for 48 hours. Suspensions were prepared as described in the first part of the experiment and both hydraulic resistance and carbohydrate concentrations were detected as described previously.

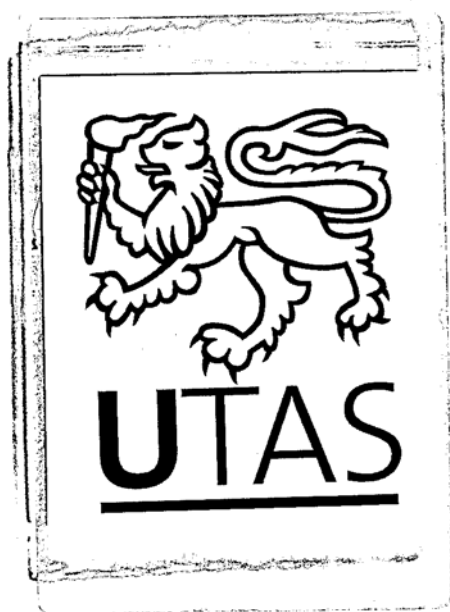
Regression analysis of the results of hydraulic resistance and carbohydrate assays from each bacterial source was undertaken.

7.3 Results

A. Bacterial EPS and hydraulic resistance

A strong positive correlation was found between concentration of carbohydrate produced by bacteria and hydraulic resistance (Figure 11). The correlation did not appear to vary between flower species, duration of vase life prior to isolation of bacteria and position on the flower stems. Bacteria extracted from an older stem did not produce more carbohydrate than bacteria extracted from a younger stem which were grown on the same medium. Regression equation of each bacterial source also revealed a close relationship between hydraulic resistance and carbohydrate produced by bacteria from different flower species, vase life prior to extraction and position on the flower

stems (data not shown). However, bacterial suspensions obtained from TSA and PEM media contained more carbohydrate and caused higher hydraulic resistance than the suspensions which obtained from BMS medium. Based on regression equations, relationship between carbohydrate and hydraulic resistance of bacterial suspensions obtained from TSA and PEM was close but different from the suspension obtained from BMS (Figure 12). This indicated that media used for growing bacteria affected the production of bacterial polysaccharides and the increase in hydraulic resistance.



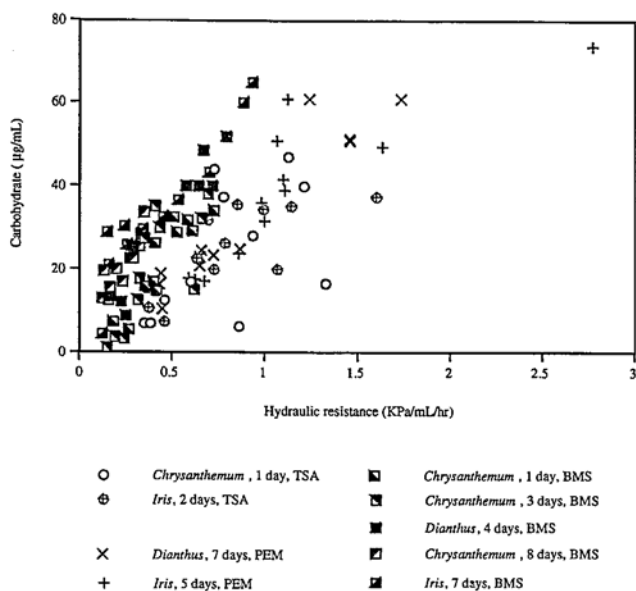
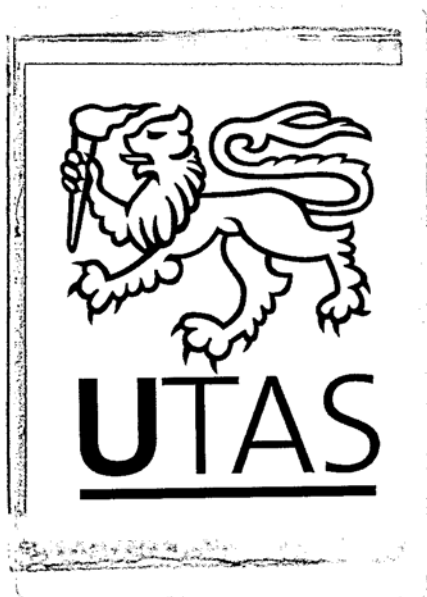


Figure 11: Hydraulic resistance versus carbohydrate produced by bacteria which were extracted from different flower species, ages, parts of the stems and cultured onto different media.



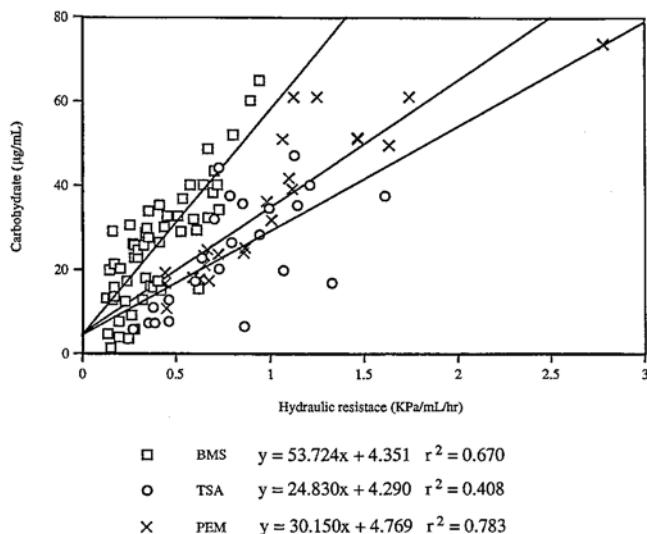


Figure 12: Regression analysis of hydraulic resistance versus carbohydrate produced by bacteria which were cultured onto different media.

B. Effect of bacterial cells and EPS plus cells

Positive correlations between carbohydrate concentration and hydraulic resistance were also found in this trial (Figure 13). At the original extraction, bacterial suspension of *B. subtilis* cultured on TSA contained 14.7 µg/mL carbohydrate and caused 0.30 KPa/mL/hr hydraulic resistance while mixed bacterial colonies isolated from flower stems and cultured on TSA medium produced 62.6 µg/mL carbohydrate and 1.79 KPa/mL/hr hydraulic resistance. The suspension from mixed colonies cultured on BMS produced a hydraulic resistance of 1.02 KPa/mL/hr and 40.2 µg/mL carbohydrate was isolated from the filter paper. Regression equations also showed three different relationships between hydraulic resistance and carbohydrate which indicated that the correlation was dependent on type of bacteria (bacterial cells or EPS plus cells) and substrate where the bacteria established.

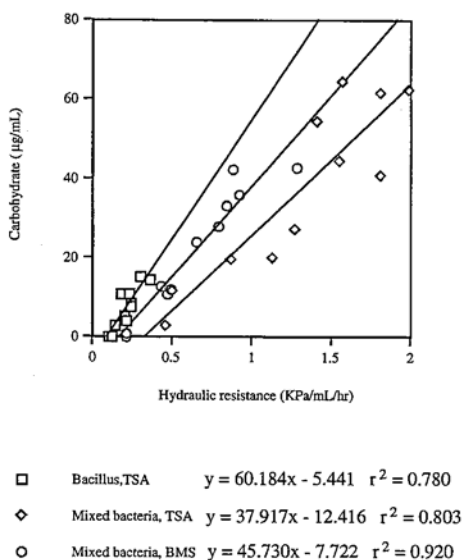


Figure 13: Hydraulic resistance versus carbohydrate of *B. subtilis* cultured on TSA(\square), bulk bacteria cultured on TSA (\diamond), bulk bacteria cultured on BMS (\circ).

There was no apparent relationship between number of bacteria and hydraulic resistance. While the bacterial suspensions of *B. subtilis* caused the lowest hydraulic resistance, they contained the highest numbers of bacteria (Table 13). For example, *B. subtilis* suspension from colonies cultured on BMS contained 7.1×10^7 cfu/mL and lead to hydraulic resistance at 0.30 KPa/mL/hr while bacterial number in the suspension of mixed colonies cultured on TSA was 5.8×10^7 cfu/mL and resulted in hydraulic resistance of 1.79 KPa/mL/hr which was approximately six times higher than the resistance caused by *B. subtilis*. This finding confirmed that bacterial cells alone did not lead to an increase in hydraulic resistance.

Dilution of the original suspension	Bacterial number (cfu/mL.)			LSD
	<i>B. subtilis</i>	Mixed culture (TSA)	Mixed culture (BMS)	
1	7.1×10^7	5.8×10^7	5.3×10^7	0.95×10^7
3/4	6.2×10^7	5.7×10^7	4.1×10^7	1.26×10^7
1/2	6.0×10^7	5.0×10^7	1.6×10^7	1.81×10^7
1/4	5.5×10^7	4.1×10^7	0.2×10^7	0.69×10^7

Table 13: Mean number of bacteria (cfu/mL), at different concentrations, of *B. subtilis* cultured on TSA; mixed bacteria cultured on TSA and BMS for 48 hours. Data are means of 3 replicates.

7.4 Discussion

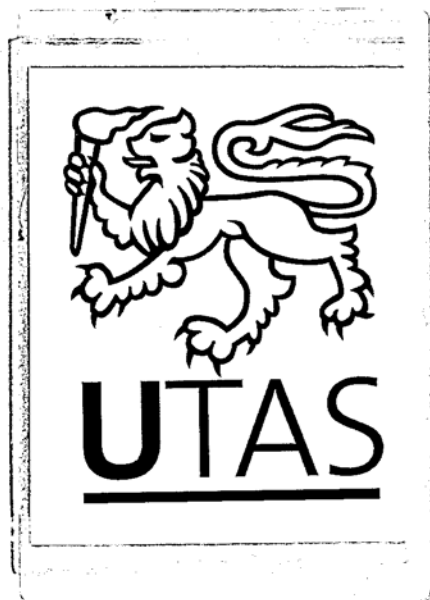
In this experiment, bacterial suspensions were obtained from bacterial culture and were therefore free from contamination with carbohydrate derived from plant tissues. There was a strong positive correlation between carbohydrate concentration and hydraulic resistance regardless of the source of bacteria, vase life of the stems from which bacteria were cultured and parts of the flower stems from which bacteria were cultured. Bacteria extracted from an older stem did not produce more carbohydrate than bacteria extracted from a younger stem when they were cultured onto the same medium, even if older stems usually contained higher bacterial number. This may be due to a limited food source, bacteria extracted from younger stems contained less bacterial cells therefore they had more food supplied per cell thus producing more carbohydrate per cell. Rates of carbohydrate production were affected

by the culture media, with bacteria cultured on TSA and PEM media producing more EPS than bacteria cultured on BMS medium. It has been documented that rate of bacterial polysaccharide production is dependent on carbohydrate types and nutrients in the medium (Bernheimer, 1953; Mozzi et al, 1995; Wilkinson and Stark, 1956; Garma et al, 1997). It is therefore probable that bacteria cultured on TSA and PEM media produced more polysaccharide than bacteria cultured on BMS medium due to the higher glucose content in the former media.

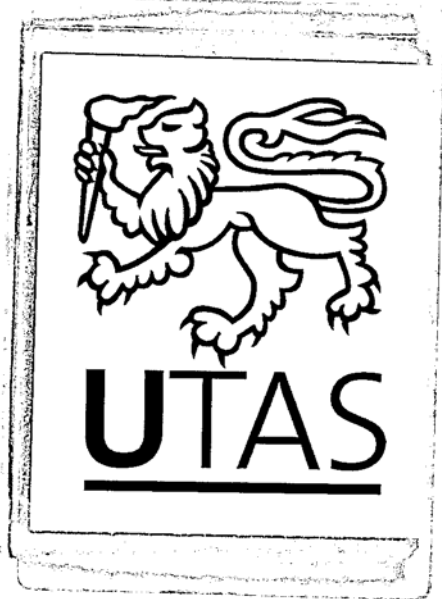
Clear evidence was presented to demonstrate that the increase in hydraulic resistance was due to bacterial EPS rather than bacterial cells. Numbers of *B. subtilis* in excess of 5.0×10^7 cfu/mL caused only a small increase in hydraulic resistance, whereas a corresponding cell number in the suspension produced by mixed bacterial cultures isolated from flower stems induced a six fold increase in hydraulic resistance. While this difference may have been due to differences in cell size, if we assume that the majority of bacteria isolated from stems were *Pseudomonas* spp., then this explanation can be discounted. The size of *B. subtilis* cell has been recorded as 0.7-0.8 μm . by 2.0-3.0 μm . (Holt, 1977) while the size of *Pseudomonas* spp. cell was in the range 0.5-1.0 μm . by 1.5-4.0 μm . (Holt, 1977; Palleroni, 1989). This indicated that the increase in hydraulic resistance was more likely to be due to bacterial polysaccharides rather than bacterial cells.

The apparatus used in this experiment had a cross sectional area through which water could flow which was at least two times larger than the total cross sectional area of vascular tissue in the flower species studied. Although the pore size in the filter used was smaller than the smallest diameter of xylem vessels in *Dianthus*, *Iris* and *Chrysanthemum* stems, low hydraulic resistance was still detected when the non-EPS producing bacteria or low concentration of EPS producing bacteria were used.

Determination of polysaccharide concentration in flower stem extracts using the validated anthrone assay was undertaken on extracts of two day old *Chrysanthemum* basal sections and upper sections, with 73.95 and 29.74 $\mu\text{g/mL}$ recorded respectively. While these values may be slightly initiated due to the presence of soluble sugars released from plant cells, the carbohydrate concentrations were similar to those obtained from two days old mixed bacterial cultures. Thus the effect of carbohydrate derived from cultured cells may be used to indicate a possible effect of bacterial EPS in cut flower stems. As the effect was shown to be linked to bacterial EPS concentration and not bacterial cell number, the results indicated that bacterial EPS covering the xylem vessels at the cut surface of flower stems could explain the increase in stem hydraulic resistance recorded within two days of vase life.



VI. GENERAL DISCUSSION



VI. General Discussion

Maximizing vase life of cut flowers has been the focus of floricultural research for many years. Impairment of water balance in cut flowers is regarded as one of the major causes of reduced vase life. While many theories to explain the reduction in water uptake during vase life have been proposed, the development of vascular occlusions in flower stem due to the presence of bacteria has received the most support (Lineberger and Steponkus, 1976; Put and Jansen, 1989; van Doorn and Perik, 1990; Put and Klop, 1990; van Doorn et al, 1991). The efficiency of bacteriocides in prolonging vase life of cut flowers also adds weight to this theory (Larsen and Scholes, 1965; Burge et al, 1996; Marousky, 1969; 1971; Larsen and Cromarty, 1967; Parups and Chan, 1973; Wirthensohn et al, 1996; Jones and Hill, 1993). It has been proposed that bacterial cells from the vase water infiltrate cut xylem vessels, leading to vessel occlusion at the pit membranes (Put and van Meyden, 1988; van Doorn et al, 1991), thus inhibiting water uptake and accelerating flower senescence. The principal argument against this theory has been that the majority of studies supporting the theory were flawed by inadequate control of microbial numbers, external contamination or uncertainty as to microbial populations present (Zagory and Reid, 1986). The main location of bacteria in cut flower stems has been shown to be near the cut surface, which is not consistent with occlusion at vessel pit membranes unless vessel length in flower stems is very short. Thus, while circumstantial evidence may support the theory of vascular occlusion caused by bacterial cells, some doubt remains as to the mechanism by which bacteria reduce vase life. The results presented in this study support the hypothesis that exopolysaccharide producing bacteria colonizing the cut surface of flower stems and cause an increase in stem hydraulic resistance principally through the exopolysaccharides covering the cut vessels.

In all measurements of bacterial cell numbers in this study the majority of bacteria were found in the basal stem section, and in particular within 0.2 cm from the cut surface. This finding is consistent with previous studies (van Doorn et al, 1991; Put and Clerkx, 1988). Highest stem hydraulic resistance was also found in this section, indicating that bacteria may cause the vascular blockage around the stem cut surface. Further investigation in the precise location of blockage showed that the major vascular occlusion was located no further than 5 mm from stem cut surface. Moreover, results in the trial using stem sections shorter than the longest vessel length did not show a decreased value in hydraulic resistance, indicating that vascular blockage caused by bacteria may not have occurred at the pit pores but was more likely to be at the stem cut surface. However, the timing and magnitude of change in bacterial numbers and hydraulic resistance in basal stem section was not consistent with a strong relationship between cell number and stem hydraulic resistance. Moreover, high numbers of bacteria (greater than 6×10^5 cfu/g fresh weight) were recorded in upper stem sections while hydraulic resistance in these sections remained low throughout the duration of vase life.

High hydraulic resistance measurements in basal sections and low values in upper stem sections were recorded for all three flower species in this study, indicating a common mechanism that was not specific to stem anatomical and physiological characteristics. The lack of correlation between bacterial numbers and stem hydraulic resistance suggested that the critical bacterial concentrations proposed by Zagory and Reid (1986) and Put and Jansen (1989) cannot be universally applied to explain the effect of bacteria on stem water relations. However the highest hydraulic resistance measurements in basal stem sections generally but not always occurred when bacterial numbers in excess of 10^7 cfu/g fresh weight of stem were present. These bacterial numbers were higher than those reported in *Rosa* stems (de Witte and van Doorn, 1988), and other cut flowers (Zagory and Reid, 1986) but similar to the critical bacterial concentration proposed by de Witte and van Doorn (1988). Further evidence against the use of critical concentrations to predict disruption in stem

water relations comes from the timing of changes in stem hydraulic resistance and bacterial numbers. Hydraulic resistance increased rapidly in cut stems and was significantly higher in basal stem sections than upper sections within two to three days of vase life, while bacterial numbers at this time were lower than the proposed critical concentrations. Similar observations have been made previously (van Doorn and de Witte, 1991). Put and van der Meyden (1988) also noted that bacterial numbers were too low to explain observed decreases in hydraulic conductance. Thus the relationship between bacterial cell numbers and stem hydraulic resistance appears weak and other factors are required to explain the reduction in stem conductance.

High bacterial cell numbers were isolated from sections of stems at a distance from the cut surface greater than the length of the longest vessels. This finding indicated that bacteria could move through pit membranes thus implying that the vascular occlusion may not be located at the pit pores. This finding may not be consistent with the conclusion of van Doorn et al (1991) who stated that bacterial cells could not pass through the pit membranes in cut *Rosa* flowers. However, in their study the conclusion was drawn based on SEM images and the number of bacteria in the stem higher than the maximum vessel length was not recorded. Sizes of pores in the pit membrane of cut flower stems have not yet been reported, making it difficult to draw a conclusion on transport mechanism of bacterial cells in xylem vessels. Pit membrane pore sizes of 0.02 to 4 μm in woody species (Siau, 1984) and 0.2 to 0.8 μm in alfalfa (van Alfen et al, 1983) have been reported, suggesting that *Pseudomonas* sp. cells of 1.5 to 4 μm length may move through some inter-vessel pits. It has also been proposed that bacteria may produced pectic enzymes to digest or increase the flexibility of pit membrane to allow the passage of the cells between vessels (Put and Rombouts, 1989). Further research will be required to identify the mechanism of bacterial movement between vessels in cut flower stems.

While vascular blockage at or close to the stem cut surface was recorded in each of the three flower species examined in this study, the response of each species to the restriction of water uptake was different. Water potential of

Dianthus and *Iris* decreased over the duration of vase life, while water potential of *Chrysanthemum* fluctuated over a wide range. Vase life of *Chrysanthemum* was also always found to be much longer than that of *Dianthus* and *Iris*. Mayak et al (1974) also found that a drop in water potential was associated with petal wilting in cut *Rosa* flowers and they concluded that early senescence of cut flowers was promoted by the development of water stress in the tissue which is reflected by the decline in water potential.

In this study, an amorphous substance presumed to be bacterial exopolysaccharides were observed at the base of flower stems within two days of vase life. The bacterial EPS was found to cover the cut surface of the flower stems and to infiltrate a few xylem vessels. Similar observations have been reported for xylem vessels in cut *Rosa* flowers (van Doorn et al, 1991). *Pseudomonas* sp., which is considered to be an EPS-producing bacterial genera, have been reported to be the most common bacterial genera found on flower stems (de Witte and van Doorn, 1988; Put, 1990; van Doorn et al, 1991). In addition, vascular plugging materials found in flower stems have been reported to contained polysaccharides or pectinaceous compound (Dixon and Peterson, 1989; Parups and Molnar, 1972; van Doorn et al, 1991). Thus, while bacterial cells alone could not fully explain the significant increase in stem hydraulic resistance observed early in vase life, bacterial EPS may be an important contributing factor to vascular blockage at the base of the flower stem. A strong positive correlation between hydraulic resistance and bacterial exopolysaccharide (EPS) concentrations demonstrated that concentrations of bacterial EPS similar to those extracted from flower stems could cause a significant increase in hydraulic resistance. Further evidence presented confirmed that bacterial EPS, rather than bacterial cells, caused a high resistance to water flow. Non-slime producing bacteria had minimal effect on hydraulic resistance, compared to slime producing bacteria, even when applied in greater number.

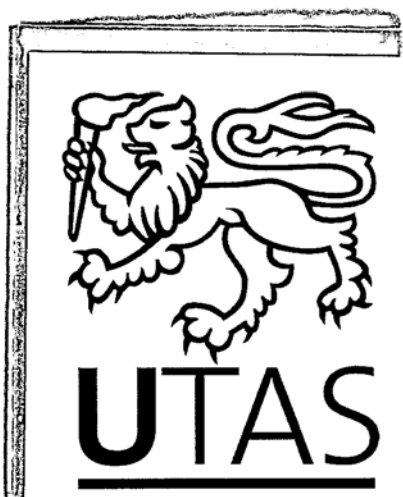
Postharvest management practices such as temperature and pH control, and addition of bacteriocides to vase water have been shown to prolong cut flower vase life (Jones, 1991; Halevy and Mayak, 1981; Condora et al, 1980; Marousky, 1971). Generally, the success of these treatments has been assumed to be due in part to controlling bacterial growth, thus decreasing vascular blockage and improving water uptake (van Doorn and Perik, 1990; van Doorn, 1998; Burge et al, 1996). However, these treatments may also inhibit production of bacterial EPS. Temperature in most commercial cool rooms is approximately 4 °C while the optimum temperature for polysaccharide production is between 15 and 25 °C (Wilkinson et al, 1955). Recommended pH of vase solution is also below the optimum pH range for EPS production (Bernheimer, 1953; Bonet et al, 1993). Moreover, degassed water has been shown to improve solution uptake and to extend *Acacia* vase life (Williamson and Milburn, 1995). This effect was explained by reduced rate of cavitation in the stems, but reduction of EPS due to restricted oxygen supply is also possible. It has been documented that polysaccharide production in anaerobic conditions was 25 to 40% of that in aerobic condition (Wilkinson, 1958). It is therefore possible that the postharvest treatments used in floriculture industry had effects on controlling of bacterial EPS production as well as on controlling of bacterial growth.

Cavitation has been suggested to be a factor causing increased hydraulic resistance (Williamson and Milburn, 1995). However, cavitation is unlikely to be the major event causing vascular blockage as approximately 66% of vascular area in *Rosa* had to be blocked before water uptake would be restricted (van Doorn et al, 1989). As stem hydraulic resistance increased rapidly within two days of vase life, a very rapid increase in rate of cavitation would be required to occlude over 66% of the xylem vessels in this period of time. Dixon and Peterson (1989) concluded that initial water deficits were caused by physical blockage at the stem cut end and cavitation was the second event induced by water stress. The identification of the major location of vascular occlusion in the basal 5 mm of stems also casts doubt on the

cavitation theory. Toxins or enzymes produced by bacteria have been thought to damage plant tissues, thus inducing vascular blockage (Accati et al, 1980; Mayak et al, 1977). In this study, suspensions of mixed bacteria cultured on agar media caused high resistance on filter papers indicating that physical blockage due to bacterial EPS could explain the changes in hydraulic resistance observed in flower stems. Moreover, van Doorn and de Witte (1991) showed that both living and inactivated bacteria induced a significant decrease in stem hydraulic conductance within 30 minutes of application suggesting a physical process rather than a physiological one.

The evidence presented in this study suggested that bacterial cells alone did not cause a significant resistance to water flow in flower stems. The increase in stem hydraulic resistance during vase life was more likely to have resulted from bacterial EPS accumulation at or around stem cut surface, thus restricting water uptake and stimulating flower senescence. This theory is consistent with much of the published literature, and deserves further attention to assess its validity. The implications of this conclusion to the floriculture industry are significant as the focus of research on postharvest treatments should change from control of bacterial numbers to control of bacterial extracellular polysaccharide production.

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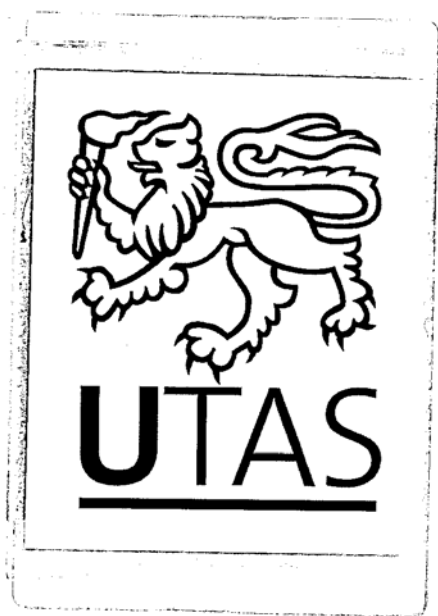
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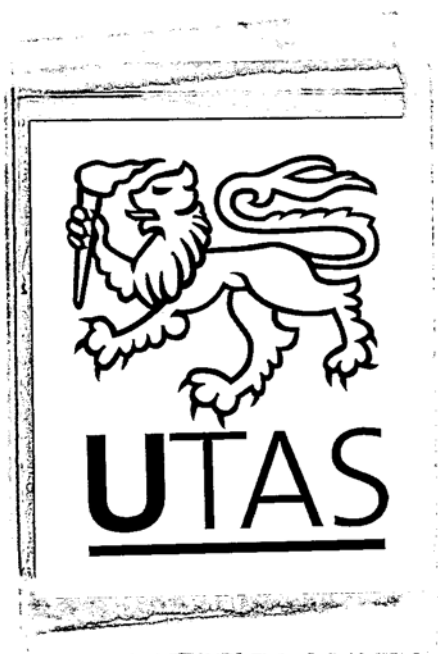
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VIII. APPENDICES



VIII. Appendices

Appendix 1. Histochemical technique

A. Fixation, dehydration and embedding

4% Gluteraldehyde	2 days
water wash	2 times
water	2 hours
25% Ethanol	2 hours
35% Ethanol	2 hours
50% Ethanol	2 hours
70% Ethanol	15 hours
80% Ethanol	2 hours
95% Ethanol	2 hours
Absolute Ethanol	2 hours
Ethanol/Xylene (50:50)	2 hours
Xylene I	2 hours
Xylene II	2 hours
Xylene III	2 hours
Xylene/Parafin (vacuum)	1 day
Parafin (vacuum)	2 hours
Parafin (vacuum)	2 days

B. Rehydration

1. Place slides containing sections into slide racks.
2. Place slide rack containing slides into Xylene for 15 minutes.
3. Transfer to ETOH:xylene (50:50 v/v) for 5 minutes.
4. Transfer to 100% ETOH for 5 minutes.
5. Transfer to 95% ETOH for 5 minutes.
6. Transfer to 70% ETOH for 5 minutes.
7. Transfer to 50% ETOH for 5 minutes.

C. Alcian blue staining

(a) Staining procedure

1. Deparaffinize and hydrate to distilled water.
2. Mordant in 3% acetic acid solution for 3 minutes.
3. Alcian blue solution for 30 minutes.
4. Wash in running water for 10 minutes.
5. Rinse in distilled water.
6. Blot dry with tissue paper.

(b) 3% acetic acid solution

Glacial acetic acid	3 mL.
Distilled water	97 mL.

(c) 1% alcian blue solution

Alcian blue, 8GX	1 gm.
Glacial acetic acid, 3%	100 mL.
Adjust the pH to 2.5, filter and add a few crystals of thymol.	

Appendix 2. Culture media

A. Trypicase soy agar medium (TSA)

Yeast extract	1	g/L
Trypicase soy broth	3	g/L
Tech agar	15	g/L

B. Basal mineral salt sucrose medium (BMS)

Sucrose	5	g/L
K_2HPO_4	1	g/L
KH_2PO_4	0.2	g/L
$MgSO_4$	0.5	g/L
$FeCl_3$	0.1	g/L
NH_4NO_3	1	g/L
Tech agar	15	g/L

C. Plant extracted medium (PEM)

Ground dried stems	25	g.
Distilled water	1	L.
Boiling for 30 minutes, then decant		
K_2HPO_4	1	g/L (pH 6.5 to 7)
Yeast extract	0.5	g/L
Agar	15	g/L