School of Science

Department of Environment and Agriculture

Factors Controlling Vase Life of Waxflowers (*Chamelaucium* Desf. Varieties and Hybrids)

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Declaration

"To the best of my knowledge and belief this thesis contains no material previously published by any other person except where due acknowledgment has been made. This thesis contains no material which has been accepted for the award of any other degree or diploma in any university."

Signature:  ……

Date: 1/10/2013

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Abstract

Factors affecting vase life of cultivars of *C. uncinatum* Schauer and hybrids between *C. uncinatum* and *C. megalopetalum* F. Muell. ex Benth., *C. sp. Gingin* Marchant, *C. floriferum* Marchant and Keighery, *Verticordia grandis* Desf. or *Verticordia plumosa* Desf. were studied. Variation in vase life of waxflowers in deionized (DI) water was affected by genetic make-up of the species and the particular cross. Vase life varied for 16 cultivars from 7.4 to 24.9 days for flowers and from 5.5 to 31.0 days for leaves. Vase life of flowers of *C. megalopetalum* hybrids averaged 16.6 days being longer than the vase life of hybrids of *C. uncinatum* with *C. floriferum* (15.5 days), *C. sp. Gingin* (12.4 days) or *Verticordia grandis* (11.4 days). Vase life of flowers of *C. uncinatum* cultivars alone was shortest with an average 9.4 days. While *Verticordia plumosa* hybrid 'Southern Stars' had longest vase life of 24.9 days, another *Verticordia plumosa* hybrid 'Jasper' had shorter vase life with 15.4 days. Vase life of flowers of cultivars within *C. uncinatum* or *C. megalopetalum* hybrid varied by 1.6 folds. Vase life of leaves of *C. megalopetalum* hybrids averaged 24.7 days being greater than vase life of hybrids of *C. uncinatum* with *C. sp. Gingin* (22.9 days), *Verticordia plumosa* (22.1 days), *C. floriferum* (18.5 days) or *Verticordia grandis* (17.0 days). Alternatively, vase life of leaves of *C. uncinatum* cultivars alone was shortest with 8.9 days. Vase life of leaves of cultivars within *C. uncinatum* alone or *C. megalopetalum* hybrid varied by 2.0 or 1.7 folds respectively.

Vase life response of cultivars was affected by different types of vase solutions within a genotype. For overall cultivars, the vase solutions containing sucrose was more effective in improving vase life than the vase solutions containing 8-hydroxyquniline sulphate (HQS) alone or HQS and silver thiosultphate (STS) (applied as a 20-min pulse at 4 mmol). Vase solution of sucrose, HQS and STS extended vase life of flowers of *C. uncinatum* cultivars by 1.9 folds, followed by cultivars with *C. megalopetalum* (1.6 folds), *C. sp. Gingin* (1.4 folds), *C. floriferum* (1.4 folds) and *Verticordia* (1.4 folds) as a parent. Vase life of leaves of *C. uncinatum* cultivars was extended by 1.6 folds, followed by cultivars with *C. floriferum* (1.4 folds) or *C. sp. Gingin* (1.2 folds) as a parent; whereas, vase life of leaves of cultivars with *C. megalopetalum* or *Verticordia* as a parent decreased in vase solution of sucrose, HQS and STS.

Addition of sucrose, maltose, fructose, glucose and galactose all at concentration of 58.5 mmol to vase solution containing 200 mg L^{-1} HQS extended flower vase life of overall cultivars, but not for leaf vase life compared to the DI controls. Sucrose at 58.5 mmol (2% w/v) had a similar effect in extending vase life of cultivars compared to fructose or glucose, but was more effective as compared to maltose or galactose. Vase life of cultivars extended with increased sucrose concentrations from 14.6 to 117 mmol supplemented with 200 mg L^{-1} HQS. However, the concentration of sugar where vase life was improved for both flowers and leaves was from 14.6 to 29.2 mmol in combination with low level of HQS (50 mg L^{-1}) as a quarter as concentration normally used. Long vase life of flowers of cultivars in vase solutions had longer day for stems fresh weight above initial stems fresh weight and vase life of flowers was terminated when stem fresh weight reached 75%.

Flowers of Geraldton wax strongly competed with leaves for water and carbohydrates. Cultivars with higher weight ratio of flowers to stems gave a higher flower to leaf vase life ratio. Vase life of flowers of cultivars increased to a maximum with stems flowering up to 50%.

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×	Hybrid/ multiply/interaction
µ	Micro
±	Plus/minus
√	Positive/no response/ same the previous one
$	US dollar
%	Percentage
°C	Degree Celsius
<	Less than
mmol	Millimolar
g	Gram
mg	Milligram
L	Litrer
nL	Nanolitrer
L^{-1}	Per litrer
h	Hour
cm	centimeter
m^{-2}	Per meter squared
s^{-2}	Per second squared
–	Hyphen
/	Divide
P	Probability
ABA	Abscisic acid
ACC	1–aminocyclopropane–1–carboxylate
Ag^{+}	Silver ion
$AgNO_3$	Silver nitrate
ANOVA	Analysis of variance
AOA	Aminooxyacetic acid
BA	Benzyladenine
ClO_2	Chlorine dioxide
DAFWA	Department of Agriculture and Food of Western Australia
DI	Deionized
E	Eastern

EC	Electrical conductivity
Fig.	Figure
GA_3	Gibberellic acid
8-HQ	8-hydroxyquinoline
8-HQC	8-hydroxyquinolinecitrate
8-HQS	8–hydroxyquniline sulphate
IAA	Indole−3−acetic acid
KCl	Potassium chloride
LSD	Least significant difference
1–MCP	1–methylcyclopropene
mRNA	messenger Ribonucleic acid
NAA	Naphthalene acetic acid
S	Southern
SA	Salicylic acid
SE	Standard error
STS	Silver thiosulphate
UK	United Kingdom
RH	Relative humidity

CHAPTER 1
Introduction

1.1. Introduction

Australian native flowers including fresh and dried cut flowers play an important role in floricultural export of this country, accounting for $41 million (Anon, 2002). Australian flowers are mainly exported to Japan (40%), America (26%) and The Netherlands (10%) (Sutton, 2004). Waxflower is a major contributor to Australian native cut flowers for the export market (Shan and Seaton, 2009; Shan et al., 2010), contributing annually up to $20 million farm gate to the Australian farming industry (Michael, 2011). Annual production of cut flowers in the world is approximately 600 million stems of waxflowers (Ratanasanobon and Seaton, 2009). In Western Australia, waxflower accounts for approximately 25% of cut flower exports, contributing about $4 million to the State annually. Exploiting native flowers as cut flower for export markets has received much attention from the Department of Agriculture and Food of Western Australia (DAFWA). The development of waxflowers (*Chamelaucium* species) as a cut flower has occurred over the past 30 years, but particularly in Australia in the last twenty years. Waxflower is Australia's leading commercial native flower and is in the top 20 species in terms of volume sold in Europe (Considine and Growns, 1998). About 100 cultivars are now grown for this purpose. The DAFWA waxflowers selection, breeding and development project began in 1991 with a focus on collecting the genotypic variance of waxflowers. Many superior genotypes have been selected for further trialing at sites around Australia, and in Israel and California (Growns et al., 2000). Breeding new varieties of waxflowers through selection and hybridizing in the past few years has gained a great impetus and more than 20 new cultivars were released to waxflowers growers by DAFWA (WADAF, 2004; Seaton and Poulish, 2010) with other cultivars by commercial breeders (Seaton and Poulish, 2010). However, vase life of waxflowers varied among cultivars with shorter vase life found in *C. uncinatum* cultivars of 'Alba' and 'Purple Pride' (Joyce and Jones, 1992; Seaton et al., 2010) and longer vase life found in *C. megalopetalum* hybrids of 'Albany Pearl' and 'Bridal Pearl' (Seaton et al., 2010). Improvement of vase life is critical to the future of waxflower cultivars and requires an understanding the factors controlling vase life.

Postharvest senescence and organ loss are major limitations to cut flower quality and their marketing of many species of cut flowers and considerable effort has been devoted in developing postharvest treatments to improve cut flower quality (Bowyer et al., 2003). At markets, the quality of transported cut flowers is often far from optimal (Hoogerwerf et al., 1994) .The estimated loss of the total flower production in the Netherlands ranged from 15 to 20% (Boon and Groot, 1980). In Bangladesh, postharvest losses of cut rose, tuberose, marigold and gladiolus was highest at the retail level (39.82%) followed by the wholesaler (27.52%), the producer (18.87%) and the local trader (13.78%) (Bagchi and Raha, 2011). Loss of vase life of cut flowers during all stages of postharvest handing ranged from 20 to 40% with the mean loss of vase life per day of 6–7% (Hoogerwerf et al., 1994). Reduction of vase life of cut flowers from bacterial contamination of water varied from 0.2 to 12% (Hoogerwerf et al., 1994). Long vase life of cut waxflowers is highly desirable and beneficial as the price of cut stems can be increased up to 20% more if flowers last more than five days (Sutton, 2004). Postharvest waxflowers floral organ abscission and senescence are; therefore, the most important and key factor for the success of the commercial waxflowers industry (Eyre et al., 2006).

Vase life of cultivars was strongly affected by genetic variation. Large differences in vase life of cut rose have been observed among cultivars which were grown and tested under identical condition (Mortensen and Gislerød, 1999; Marissen, 2001; Fanourakis et al., 2012). Vase-lives of cut *Verticordia* cultivars (Family *Myrtaceae*) in the same family as *Chamelaucium* varied from 5.2 to 19.1 days for flowers, and from 4.7 to 22.4 days for leaves (Seaton, 2006a). Research work has been reported on the effects of genotype on flower abscission (Macnish et al., 2004) but genotypic factors affecting variation in vase life of cut waxflowers has not been investigated on extensive level.

STS is very effective in reducing flowers drop in waxflowers (Joyce, 1993; Seaton, 2005; Seaton, 2006b), but it was ineffective in extending vase life of cut waxflowers (Joyce, 1988). Applying sucrose at concentration of 58.5 mmol (2% w/v) in combination with 200 mg L^{-1} HQS to vase water significantly increased vase life of cultivars of *C. uncinatum*. Increasing sucrose concentration up to 146.2 mmol (5% w/v) was harmful for flowers and leaves of cultivars of *C. uncinatum* (Joyce, 1988).

No research work has been reported on the effects of different types and concentrations of sugars, HQS and STS on newly developed different genotypes of waxflowers and yet warrant to be investigated.

Leaves are known source of carbohydrates for the developing flower buds during vase life (Marissen and La Brijn, 1995), but can lead to an increase in water loss of cut stem, resulting in a decrease in vase life of flowers of *Grevillea* 'Crimson Yu-lo' (He et al., 2006). The competition for metabolites between buds and flowers as occurs in *Alstroemeria* where lack of sugar also negatively affected vase life of flowers (Chanasut et al., 2003). The effects of changes in sources and sinks on vase life of different genotypes of waxflowers justify to be further investigated.

The understanding of influence of genetic variation on vase life of cut waxflowers and of the effect of several vase solutions containing various combinations between sugar, HQS and STS are key points for improving vase life of cut waxflowers. Through improvement of vase life and quality, profits of farmers are expected to increase from waxflowers-cultivated stems receiving a much higher price.

1.2. Research aims

The present study aimed to determine the factors controlling the variation in vase life of various intra- and interspecific and intergeneric waxflower genotypes, examining genotypic variation, response to vase solutions, the role of sugars and source and sink relationships in leaves and flowers.

The study comprised three hypotheses (Chapter 4–6, below), which aimed to identify the factors affecting variation in vase life of waxflower genotypes. The aim of each chapter was to:

(a) (i) compare the vase life response of a range of waxflower cultivars, (ii) determine how this response varies depending on vase solution?. The hypothesis tested was that the degree of waxflowers vase life response depended on a genotype's ability to maintain water balance in different vase solutions.

(b) investigate the effects of different types and concentrations of sugar supplemented with 200 mg L^{-1} 8-hydroxyquniline sulphate on vase life

of a range of waxflower cultivars. The hypothesis tested was that vase life response of cultivars to different sugar types and concentrations was different.

(c) determine the relationship between source and sink as well as the effect of changes in weight ratios of flowers and stems on the changes in vase life of flowers and leaves of cultivars. The hypothesis tested was that vase life of flowers of waxflowers was controlled by availability of water and sucrose from leaves.

CHAPTER 2

Literature Review

2.1. Waxflowers

Waxflower is the common name of *Chamelaucium uncinatum* Schauer and its hybrids being the most widely cultivated species with hybrids now accounting for approximately 80% of the volume of all *Chamelaucium* cultivars being exported from Australia (Seaton and Poulish, 2010). *Chamelaucium* , a member of Myrtaceae family being a short day plant (Shillo et al., 1984; Dawson and King, 1993), is a perennial evergreen shrub endemic to southwest of Australia (Akilan et al., 1995; Beasley and Joyce, 2002), from Perth (31°56'S) to Kalbarri (27°71'S) (Yan, 2001) (Fig. 2.1). It is well adapted to a Mediterranean climate (Elliot and Jones, 1984; Martínez et al., 2007) and survives in low nutrient conditions while it responds to added nutrients (Seaton, 2008; Ratanasanobon and Seaton, 2009). *Chamelaucium* is a garden and cut flower species (Klyne et al., 2003; Ratanasanobon and Seaton, 2009) and also cultivated for the cut-flower export trade (Seaton, 2008; Seaton and Poulish, 2010). The flowering season of waxflower species are from June to November under Mediterranean temperatures (Growns and Geraldton, 2007). Waxflower has long been grown as cut-flower in Australia, and was introduced into Israel in 1970 (Sexton, 1995), California in 1980s (Ichimura et al., 2000). It has been now grown around the world in places such as Peru, Thailand, India, South Africa and New Zealand (Witte and Van Doorn, 1991; Tomas et al., 1995). Waxflower inflorescence forms a corymb (Wu et al., 1991) bearing flowers in cluster of 2–5 small attractive flowers, 12 to 25 mm in diameter, with small erect green leaves. Flower colour varies from white to cream, pink, mauve, and purple and typically become darker with age (Sankat and Mujaffar, 1994; Dinh et al., 2011). Waxflower flowers contain 5 minute sepals, 5 broad waxy petals and 10 stamens (Wu et al., 1991). Waxflower flowers are protandrous with anthers opening before the stigma becomes a receptive, thereby reducing the likelihood of shelf–pollination (Wu et al., 1991). Cut flower stems are used alone in vases or as fillers in bouquets (Dinh et al., 2011) (Fig. 2.2).

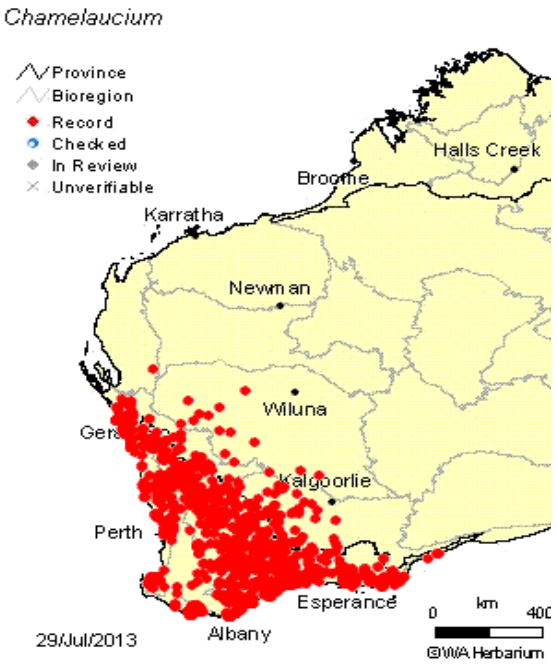

Figure 2.1. Distribution of waxflowers (Red points) in Western Australia (colour picture available online at http://florabase.dpaw.wa.gov.au).

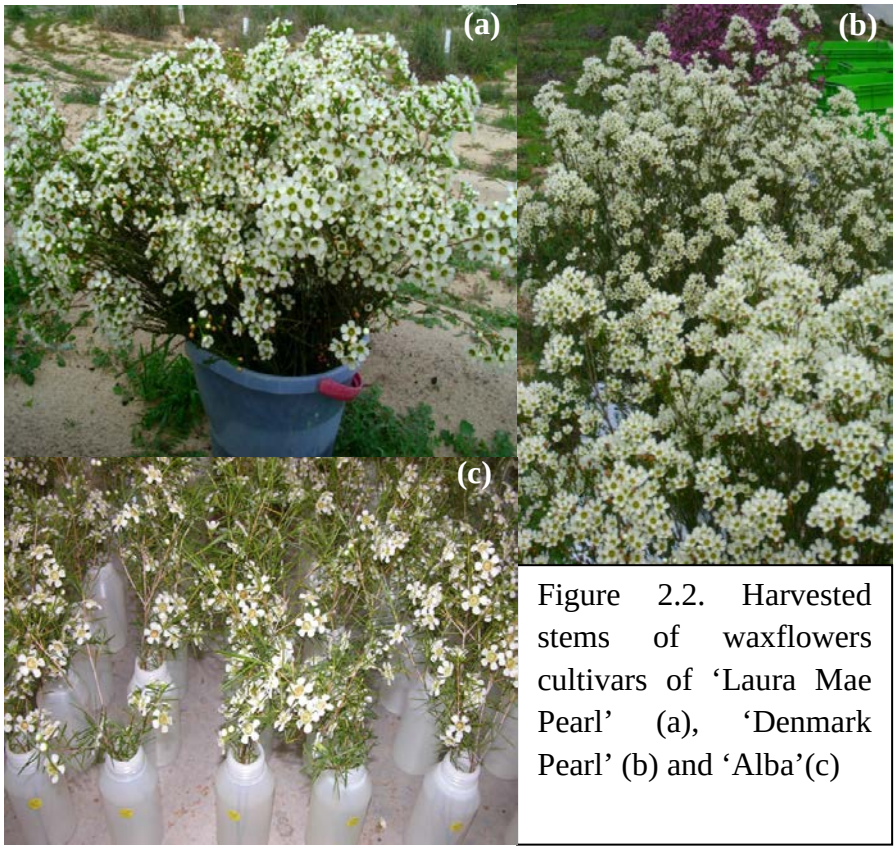

Figure 2.2. Harvested stems of waxflowers cultivars of 'Laura Mae Pearl' (a), 'Denmark Pearl' (b) and 'Alba'(c)

2.2. The variation in vase life of cut flowers based on genetic background

Genetic variability in postharvest longevity of flowers has been found in many types of cut flowers such as *Gerbera* sp. Bolus et Hook (van Meeteren, 1978), *Rosa hybrida* L. (Zeislin et al., 1978; Macnish et al., 2010), *Antirrhinum majus* L. (Weber et al., 2005) and *Verticordia* Desf. (Seaton, 2006a). The length of vase life of cut flowers is determined by genetic make-up of species as occurs for *Campanula* L. where vase life of cultivars of *C. rapunculoides* in pure water was longer than vase life of cultivars of *C. latifolia* and *C. trachelium* and doubled vase life of cultivars of *C. barbata* (Scariot et al., 2008). Also vase life is determined by genetic make-up of particular cross as occurs for cut *Rosa* L. hybrids where vase life of cultivars in vase water varied by 4.2 folds (Macnish et al., 2010). Different response of stomata to closing stimuli (e.g. light/dark transition) has been shown to account for cultivar differences in vase life (Mayak et al., 1974). Cultivars with high dehydration resistance or slow dehydration rate showed longer vase life (Spinarova and Hendriks, 2007). Water uptake ability of a cultivar also accounts for genetic variation in vase life. Cultivar with high ability to increase fresh weight upon rehydration had longer vase life (van Doorn and D'Hont, 1994). Soluble carbohydrates in petals were also a factor contributing to the difference in vase life among cultivars. Cultivar with higher content of soluble carbohydrates in petals has longer vase life (Ichimura et al., 2005). Vase life response of cut flower cultivars between and within species to vase solutions also varied. Response of cultivars of *Campanula* species to commercial vase solution of 9.38 g L^{-1} Chrysal Clear® (Pokon & Chrysal, Naarden, Holland) was different with vase life of cultivars of *C. rapunculoides* and *C. barbata* being extended by 1.2 folds, while vase life of cultivars of *C. latifolia* and *C. trachelium* did not response (Scariot et al., 2008). Studies on vase life response of different cultivars of *Proteaceae* Benth. & Hook. f., *Leucospermum* L. (Stephens et al., 2003), *Anigozanthos* sp. (Kangaroo paw) (Teagle et al., 1991) and *Eucalyptus* L'Hér. (Delaporte et al., 2005) to sugar by pulsing or continuously holding in vase solution indicated that effect of types of sugar on vase life of such flower cultivars differed with cultivar (Table 2.1).

Table 2.1. Variation in vase life of flowers of *Proteaceae, Leucospermum, Anigozanthos* and *Eucalyptus* cultivars in response to sugar

Cultivars	Reference	Sugar treatment	Flower response	
			Positive	No
Protea	Stephens et al., 2003	Glucose pulsing		
'Brenda'	√	√	√	
'Carnival'	√	√	√	
'Pink Ice'	√	√	√	
'Susara'	√	√	√	
'Sylvia'	√	√	√	
'Cardinal'	√	√		√
'King'	√	√		√
Leucospermum	Stephens et al., 2003	Glucose pulsing		
'Cordi'	√	√	√	
'Gold Dust'	√	√	√	
'High Gold'	√	√	√	
'Succession'	√	√	√	
'Scarlet Ribbon'	√	Glucose pulsing		√
'Tango'	√	√		√
Anigozanthos	Teagle et al., 1991	Sucrose pulsing		
'Gold Fever'	√	√	√	
'Regal Claw'	√	√		√
Eucalyptus lesouefii	Delaporte et al., 2005	Sucrose pulsing	√	
Eucalyptus yalatensis	√	√		√

Note: √ means positive or no response to sugar or similar to the previous one

Sensitivity to ethylene causing flower abscission and reducing the visual appearance and display life (van Doorn and Stead, 1997) and promoting flower senescence in ethylene sensitive flowers (Trobacher, 2009) varied among species and crosses or hybrids. Sensitivity to ethylene of cut waxflowers appeared to be an inherited trait (Macnish et al., 2004). Flowers of *C. uncinatum* × *C. micranthum* hybrid 'Sweet Georgia' and *C. uncinatum* 'Early Nir', 'Paddy's Late', 'Purple Pride',

'CWA Pink' and 'Early Hard' were highly sensitive to ethylene. These genotypes shed 10% of their flowers in response to a 12 h treatment with less than 0.01 μL L^{-1} ethylene at 20°C. Response to ethylene of *C. megalopetalum* × *C. uncinatum* varied among cultivars. Cultivars of 'Albany' and 'Crystal Pearl' were insensitive to ethylene and also flowers of *C. megalopetalum* 'Winter White' and 'Iceberg' were insensitive to ethylene even at 100 μL L^{-1} for 12 h at 20°C, while 'Painted Lady' shed 10% of its flowers with 1−9.9 μL L^{-1} (Macnish et al., 2004). Recent work indicated that the amount of endogenous ethylene production of flowers was not related to variation in vase life of cut roses (Borda et al., 2011). Vase life of *Verticordia* being related to waxflowers decreased with increasing floral drop. The regression relationships between vase life of flower and floral drop were described as: vase life of flowers (days) = 13.343 × $\exp^{(-0.0102\ (\%\ \text{floral organ drop}))}$ with r^2 = 0.36 and vase life of leaves (days) = 20.154 × $\exp^{((-0.0163\ (\%\ \text{floral organ drop}))}$ with r^2 = 0.63 (Seaton, 2006a). Consequently, genotypic variation in vase life was related to cultivar differences in ethylene response and tolerance to ethylene was critical for a long vase life in ethylene-rich environments (Fanourakis et al., 2012).

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2.3. Abscission and senescence

2.3.1. Abscission

Abscission is a process whereby organs such as leaves, fruit and floral structures are shed from plants, which is mainly due to the acceleration of ethylene production (Sexton, 1995). Abscission in waxflower cultivars is mediated by exposure to ethylene (Joyce, 1988; Macnish et al., 2000) which can be endogenously or exogenously derived. For example, infection of *Botrytis* (Joyce, 1993) stimulates endogenous ethylene production while exhaust fumes or climacteric fruit such as apples nearby in cool room produce exogenous ethylene (Shafiq et al., 2011; Singh et al., 2013). Ethylene production in plants is induced by two enzymes, 1–aminocyclopropane–1–carboxylate (ACC) synthase and ACC oxidase (Kende, 1993; Mathooko, 1996). Ethylene binds to membrane-located protein receptors in plant cells and activates a phosphorylation signal transduction pathway that initiates downstream ethylene responses (Bleecker and Kende, 2000). In waxflowers, the sites for ethylene-induced abscission of flowers and buds are at a narrow two– to four–cell–wide abscission zone at the distal end of the flower receptacle and the flower

petiole (Fig. 2.3). These abscission cells on the abscised surface of both the pedicels and floral tubes are typically spherical and loosely packed (Macnish et al., 2005). Flower buds enclosed in shiny bracteoles and aged flowers were generally less exposed and sensitive to exogenous ethylene than were flower buds with opening petals and flowers with a nectiferous hypanthium (Macnish et al., 2005).

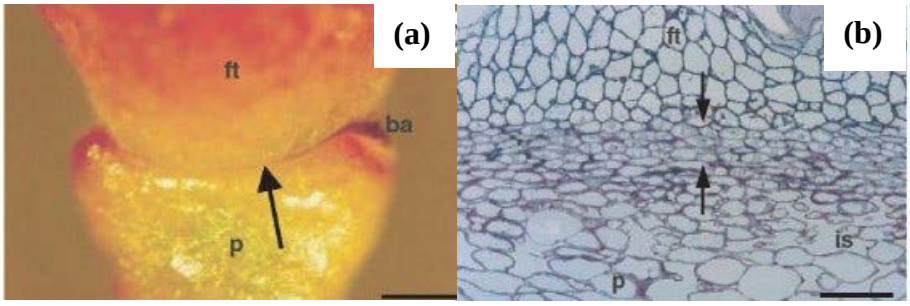


Figure 2.3. Morphology and anatomy of floral-organ abscission−zone tissues in flowers of *C. uncinatum* 'Purple Pride'. Side view of the abscission–zone junction of a flower (a) and point to the abscission zone at the junction between the floral tube and pedicel (b) (Macnish et al., 2005).

2.3.2. Senescence

In contrast to abscission, senescence of flower is a highly coordinated, developmentally regulated process, which requires active gene expression and protein synthesis (Borochov and Woodson, 1989). Senescence of cut flowers is basically induced by several factors, e.g., carbon availability (Ichimura et al., 2000), carbohydrate depletion and water stress (Sankat and Mujaffar, 1994), micro–organisms (Witte and Van Doorn, 1991) and ethylene effects (Wu et al., 1991). A study on three lily cultivars with a different petal life span by van der Meulen-Muisers et al. (2001) showed that the time to visible senescence of non–cut flowers coincided with the time until carbohydrate content had been reduced to about half. This indicates a correlation between the onset of senescence and lack of carbohydrates in lily petals. The competition for carbohydrates between flower buds and opened flowers also induces the senescence. The presence of flower buds and opening flowers apparently induced earlier senescence in the older flowers of cut lily (van der Meulen-Muisers et al., 1995). Stress promotes ethylene production in many plant species (Muller et al., 1999). Water stress or water deficit induced flower drop, leaf abscission and accelerated rate of flower wilting (Serek, 1993). Bacterial

infection caused vascular occlusion (Jones and Hill, 1993), resulting in a reduction in water uptake and consequently decrease vase life.

2.4. Postharvest

The Australian export cut flower industry mainly based on Myrtaceous species in which waxflower has been dominated faces increasing in competition from other oversea producers (Webb et al., 1997). Short vase life also limits the marketing of many Australian native cut flowers. Extending postharvest and vase life of cut waxflowers is a prerequisite to improve the sale and competition in cut flower market.

Flowers can be protected against flower drop and extended vase life by the use of chemical and non–chemical methods.

2.4.1. None chemical-used methods

None chemical-used methods have been used after the harvesting or during the shipment of cut waxflowers. Cold storage is commonly used with the purpose of reducing respiration rate, water loss, sensitivity to ethylene or suppression of ethylene production and microbial growth (Reid and Seaton, 2001). Flower abscission can be significantly reduced by ensuring that the cold chain is maintained through transport or handling (Joyce, 1997; Taylor et al., 1997). Some of *C. uncintum* and *Verticodia monodelpha* and *V. plumosa* cultivars can be stored in dry conditions at 1°C from 1 to 3 weeks (Reid and Seaton, 2001); however, vase life was reduced after the storage (Table 2.2).

Table 2.2. Storage period of conditions for Australian native *Chamelaucium* and *Verticordia* cutflowers

Species	Storage period (days)	Conditions	Initial vase life (days)	Final vase life (days)
C. uncinatum 'Alba'	14–21	1°C, dry	8.8	4,3
C. uncinatum 'Newmarracarra'	7–14	1°C, dry	15	14,8
C. uncinatum 'Purple Pride'	7	1°C, dry	12	8.5
V. grandiflora	21	1°C, dry	13	10
V. monodelpha, and V. plumosa	14	1°C, dry	13,13	0,0
V. ninteens	14	1°C, dry	7	3

Source: Reid and Seaton, 2001. Storage conditions for ornamental crops, Farmnote. Published by WADAF (data available online)

Cold store is an effective method to improve shelf life of cut flowers during the shipment, but decreased vase life of waxflowers at display places. Bark removal combined with stem-end splitting increased the vase life of cut *Rosa* hybrid 'High & Mighty' when applied after short term storage for 24 h at 4°C (Ahmad et al., 2011). Immersion of cut stems of *Chrysanthemum morifolium* 'Cassa' into cold water (0 – 5°C) for 2 h restored water balance resulting in an increase in vase life of flowers (van Meeteren, 1992). Dipping cut stem of Asiatic hybrid lily 'Elite' (*Lilium* L.) in hot water treatment of 50°C for 5 min and 52°C for 2.5 min delayed leaf yellowing and consequently increased vase life of flowers (Woolfa et al., 2012). However, hot water or vapour heat treatments were unsuitable for waxflowers (Seaton and Joyce, 1993).

2.4.2. Chemical-used methods

2.4.2.1. Effect of postharvest chemicals on vase life of cut flowers

Methanol, ethanol, benzyladenine (BA) and paclobutrazol were used in prolonging vase life of chrysanthemum (Petridou et al., 2001). All substances extended the vase life, limited fresh weight loss and increased chlorophyll content. Chlorine dioxide (ClO_2) was also effective in extending vase life of cut flowers (Macnish et al., 2008).

Salicylic acid (SA) improved vase life of cut rose 'Black Magic' by improving water balance of cut stems (Alaey et al., 2011).

Plant growth regulators were effective in extending vase life of cut flowers such as naphthalene acetic acid (NAA) which significantly reduced bract abscission in *Bougainvillea spectabilis* Willd (Gago and Monteiro, 2011) and improved postharvest life of cut *Alstroemeria* hybrid (Bagheri et al., 2013). Aminooxyacetic acid (AOA) in combination with sucrose extended vase life of cut *Dendrobium* (Rattanawisalanon et al., 2002). Indole−3−acetic acid (IAA) decreased ethylene production and petal wilting in cut carnations (van Staden, 1995). Application of gibberellic acid (GA_3) at concentration of 50 mL L^{-1} in combination with 50 g L^{-1} of sucrose improved fresh weight, delayed petal senescence and increased vase life of gladioli (Singn et al., 2008).

Mineral ions such as aluminium, cobalt, copper, nickel and zinc in form of various salts at appropriate concentration have been used to increase vase life of cut flowers (Halevy and Mayak, 1981). Silver nitrate ($AgNO_3$) was also used to inhibit ethylene production (Kofranek and Paul, 1975). However, STS was more mobile in cut flower stems than $AgNO_3$ (Veen, 1983).

The non–toxic gaseous ethylene action inhibitor, 1–methylcyclopropene (1–MCP), has been used to prevent ethylene causing flower abscission and to replace the use of STS damaging to the environment (Serek and Sisler, 2001; Macnish et al., 2004). Fumigation with 1–MCP (10 nL L^{-1}) for 12 h at 20°C afforded the flowers of waxflowers up to 4 days protection from ethylene (Macnish et al., 2000). Although 1–MCP is a non-toxic gas and has a good effect on waxflower shelf life, but its efficacy is limited by its short-term residual activity in stems (Macnish et al., 2004). 1–MCP is difficult to use because it is an unstable gas (Reid and Seaton, 2001; Macnish et al., 2010); however, easier means such as EthylBloc™ Sachets have been used (Smithers-Oasis, 2013). Fumigation for 6 h at 20°C with 1–hexylcyclopropene (1,000 nL L^{-1}) and 1–octylcyclopropene (200 nL L^{-1}) were more effective in delaying in−rolling and extending display life of *Kalanchoe¨ blossfeldiana* Poelln. 'Alexandra' compared to the treatment of 1–MCP (200 nL L^{-1}) or water as control (Kebenei et al., 2003).

Biocides are an important component of vase solutions, preventing the growth of bacteria and fungi and accordingly improve water uptake of cut flowers stems. 8-hydroxyquinoline (8-HQ), 8-hydroxyquinolinecitrate (8-HQC) and 8-hydroxyquniline sulphate (HQS) prevented growth of the microorganisms in xylem vessels, maintained water uptake and prolonged vase life of cut 'Shiraz' Narcissus (*Narcissus tazetta* L. 'Shiraz') (Jowker, 2005), *Asparagus densiflorus* L. 'Meyerii' (Skutnik et al., 2006) and snapdragon (*Antirrhinum*) (Asrar, 2012).

2.4.2.2. Effect of postharvest chemicals on vase life of cut waxflowers

To prolong the vase life of cut waxflowers, different types of chemical have been used. Application of of 10 mg L^{-1} abscisic acid (ABA) or the combination of ABA (10 mg L^{-1}) and 10 mmol KCl in vase water extended vase life of *C. uncinatum* 'Purple Bride' and 'Alba' (Joyce and Jones, 1992). These chemicals improved water uptake of cut waxflower, thereby prolonging its vase life. However, ABA is expensive and the solution is awkward to prepare (Joyce et al., 1996). To overcome the dowside effect of ABA, Triazole compound, triadimenol and triadimenol metluidide (10 mg L^{-1}) were used with the purpose of increasing endogenous ABA concentration, and subsequently extending vase life of *C. uncinatum* 'Alba' and 'Mullering Brook'. S–carvone has also been proved effective in extending vase life of flowers of *C. uncinatum* (Damunupola et al., 2010).

2.4.2.3. Effect of STS on flower drop and vase life of waxflowers

Silver (Ag^+) ion in silver thiosulphate (STS) was found to be extremely antagonistic towards ethylene (Beyer, 1976) and was particularly important in interrupting senescence (Abeles, 1973). The presence of Ag^+ ion in the receptacle tissues inhibited the action of ethylene in cut carnations (Beyer, 1977). Further, STS inhibited the accumulation of 1-aminocyclopropane–1–carboxylic acid (ACC) being the immediate precursor of ethylene (Adams and Yang, 1979) in the receptacle and ovary tissues and consequently increased vase life of cut carnation (Veen and Kwakkenbos, 1982) and *Thalictrum delavayi* (Hansen et al., 1996). Similarly, Buffer et al. (1980) reported that the onset of senescence was accompanied with the rise in the activity of the ACC oxidase enzyme converting ACC to ethylene and also in the ACC synthase involved in the synthesis of ACC. STS has been used to decrease the

reduction of endogenous ethylene (Joyce, 1993; Seaton, 2005) and consequently may extend vase life of waxflower. STS also reduced the formation of ABA that shortens vase life of flowers. In the petal tissue, the concentration of ABA rose during senescence, but an STS pre–treatment blocked this increase (Nowak and Veen, 1982). The Ag^+ may reduce microbial activity in vase solution (Mayak et al., 1997) and thereby increase water uptake, or Ag^+ could be improving water conductivity of the xylem vessels (Veen, 1983). Vase life of waxflowers was the same when pre–treated with STS either as a pulse of 0.5 mmol for approximately 24 h in a cold room (0°C), or for 20 or 30 min at room temperature (20°C), as a pulse of 4 mmol (Joyce, 1993). The application of STS as a pulse at higher concentration but shorter pulsing time has been commonly used. Applying STS (4 mmol) for 20 min as a pulse was effective in reducing floral drop (Seaton, 2005), but did not improve vase life of *C. uncinatum* cultivars held in air (Joyce, 1988).

2.4.2.4. Effect of sugars and HQS on vase life of cut flowers and waxflowers

2.4.2.4.1 Effect of sucrose and other types of sugar

Sugar provides carbohydrate source for flower respiration and reduces the induction of endogenous ethylene (Pun and Ichimura, 2003). The addition of sugars into vase solutions has therefore been a standard or recommended practice to improve the vase life of a number of cut flower species. Additional supply of substrates for respiration resulted in a higher respiration rate and in a longer vase life, thereby extending the vase life of carnation flowers (Nichols, 1973). Sugars accelerated flower buds opening by supplying carbohydrates as a source for petal expansion and carbon skeleton for floral structure as occur in gladiolus (Kofranek and Halevy, 1976), carnation (Borochov and Mayak, 1984) and gypsophila (Downs, 1988). Sugars concentration in the petal tissues increased during the flower opening period and then declined during senescence period as occur in *Ranunculus asiaticus* L. (Shahri and Tahir, 2011). Sugars also supplied materials for building structures for the plant organs and contributed to cell wall synthesis. Prolongation of the vase life of several cut flowers by sugars was attributed to the synthesis of cell walls (Ichimura, 1998).

The increase in the vase life of cut flowers with sugar application has also been attributed to the increase in water uptake of the flowers. Sucrose at lower

concentrations prolonged the vase life of gladiolus florets by increasing water uptake; whereas, higher concentrations seemed to impede water uptake (Bravdo et al., 1974). It is suggested that the increase in the water uptake by sucrose treatments could be due to the increase in the osmotic concentration of the florets and leaves (Pun and Ichimura, 2003). Sucrose decreased water loss of gladiolus leaves, suggesting that sucrose maintained a positive water balance in the spikes by inducing the closure of stomata (Bravdo et al., 1974).

Exogenous application of sugars considerably delays visible senescence in flowers with ethylene–sensitive petal senescence, but it has only a small effect on flowers with ethylene–insensitive petal senescence (van Doorn, 2004). Sucrose delayed the increase in mRNA abundance of almost all senescence-associated genes in carnation flowers (Hoeberichts et al., 2007) and may also temporarily impair the activity of ACC synthase and ACC oxidase enzymes (Pun and Ichimura, 2003), very similarly to the effect of STS. Benefits such as better flower opening, increased flower size and improved colour were also observed (Han, 1992; Meir et al., 1995; Ichimura, 1998).

Sucrose has been found commonly used sugar among the different types of sugars in prolonging vase life of cut flowers (Pun and Ichimura, 2003). Other sugars such as trehalose, mannitol and inositol in the vase water also delayed senescence of cut tulips (*Tulipa gesneriana* L.) (Iwaya-Inoue and Nonami, 2003) and *Gladiolus* spp. (Yamada et al., 2003). These sugars may delay senescence in a way similar to sucrose or glucose (van Doorn and Woltering, 2008). However, Verlinden and Garcia (2004) reported that sucrose (146.2 mmol) delayed ethylene production in *Dianthus caryophyllus* L. ‘White Sim’; whereas, fructose, glucose, maltose and sorbitol at the same concentration did not delay ethylene production. Contrarily, the study on cut flowers of *Protea* ‘Sylvia’ by Stephens et al. (2011) showed that glucose decreased leaf blackening and increased vase life by 12.0 days, while sucrose did not improve vase life compared to that of control. However, no detail research work has reported on the effects of different sugars on vase life of newly bred cultivars of waxflowers.

2.4.2.4.1. Effect of 8-hydroxyquniline sulphate

Cut flower stems xylem vessels was quickly blocked by the contamination of bacteria and fungi multiplying in stems tissues resulting from the use of pure water in vases. Microorganisms in vase water can cause physical blockages and released toxic metabolites (Alvarez, 2000). 8-hydroxyquniline sulphate (HQS) known as a germicide prevented growth of the microorganisms in xylem vessels and maintained water uptake consequently prolonging vase life of cut flowers (Asrar, 2012). HQS prevented the activity of ACC enzyme and consequently reduced ethylene generation and promoted longevity of carnation flowers (Pun et al., 2005). HQS coupled with sucrose increased flower quality, water uptake, fresh weight and flower freshness and reduced respiration rate and physiological weight loss of gladiolus (Beura et al., 2011) and *Dendrobium* spp. (Dineshbabu et al., 2002). The combination between sucrose, HQS and STS was more effective in extending vase life of flowers and diameter of rose than sucrose and HQS (Liao, 2000). Sucrose at concetrantion of 58.5 mmol in vase water can improve vase life of flowers, but at this concentration sucrose damaged to foliage of *C. uncinatum* 'Alba' (Joyce and Jones, 1992). Sucrose in combination with HQS significantly increased vase life of cut *C. uncinatum* (Joyce, 1988). Pre-treated with STS followed by holding in vase solution of sucrose and HQS was more effective than sucrose and HQS in terms of extending vase life of rose (Liao et al., 2000). However, the effect of HQS on vase life of newly bred waxflower cultivars is unknown.

2.4.2.5. The relationship between leaf and flower

The amount of carbohydrates present in flower buds is not sufficient to support respiration and maintain longevity during vase life (Kuiper et al., 1995) there must be import of carbohydrates in to flower buds, which is considered to be a sink (Marissen, 1991). The leaves are the most probable source to provide carbohydrates to flower buds (Marissen and La Brijn, 1995). Paulin (1981) stated that the leaves are the main place where sucrose is hydrolyzed, and the resulting glucose and fructose are transported to flower buds. Removal of leaves from cut rose led to a decrease in carbohydrate content in flower buds and carbohydrate content in leaves was higher when corolla was removed (Marissen and La Brijn, 1995). Similarly, removal of inflorescence of cut *Protea neriifolia* L. delayed the onset of the leaf blackening and increased starch and carbohydrate content in leaves (Dail and Paull, 1995). However,

the presence of leaves on stem resulted in a greater loss of water in inflorescence of *Grevillea* ‘Crimson Yu-lo’ and consequently decreased vase life of inflorescence. Vase life of flowers on cut stem with 4 or 6 leaves was significantly shorter when compared with the vase life of flowers on zero or 2 leaves–stem (He et al., 2006). Leaves of waxflowers have higher turgor and lower osmotic potential than flowers resulting in a greater decrease in water potential in leaves than in flowers. Increasing water potential in leaves led to an increase in leaf vase life (Joyce and Jones, 1992). However, the relationship between flowers and leaves of waxflowers has not been studied yet.

CHAPTER 3
Materials and Methods

3.1. Plants materials

Flowering stems of up to 21 cultivars derived from four species and hybrids of *Chamelaucium* and three hybrids of *Chamelaucium* and *Verticordia* (Table 3.1) were harvested in the field from mature bushes (approximately five years old) being grown under irrigation and fertigation (Seaton and Poulish, 2010) at Medina Research Station (32°13'18"S, 115°38'50"E) the Department of Agriculture and Food Western Australia (DAFWA).

Table 3.1. Parentage of different waxflowers cultivars used in experiments

Cultivars	Parents
'Alba'	*C. uncinatum* Schauer
'Monica's Blush'	*C. uncinatum* Schauer
'Purple Pride'	*C. uncinatum* Schauer
'Mullering Brook'	*C. uncinatum* Schauer
'Dancing Queen'	*C. uncinatum* Schauer
'Micro wax'	*C. uncinatum* Schauer
'WX69'	*C. uncinatum* Schauer
'WX17'	*C. uncinatum* Schauer
'Chrystal Pearl'	*C. megalopetalum* F. Muell. ex Benth. × *C. uncinatum* Schauer
'Denmark Pearl'	*C. megalopetalum* F. Muell. ex Benth. × *C. uncinatum* Schauer
'Laura Mae Pearl'	*C. megalopetalum* F. Muell. ex Benth. × *C. uncinatum* Schauer
'Bridal Pearl'	*C. megalopetalum* F. Muell. ex Benth. × *C. uncinatum* Schauer
'WX102'	*C. megalopetalum* F. Muell. ex Benth. × *C. uncinatum* Schauer

Table 3.1. Continue

'WX87'	*C. uncinatum* Schauer × *C. megalopetalum* F. Muell. ex Benth.
'Matilda'	(*C. uncinatum* Schauer × *C. uncinatum* Schauer) × *C. megalopetalum*
'Lady Stephanie'	*C. floriferum* Marchant and Keighery × *C. uncinatum* Schauer
'WX97'	*C. sp. Gingin* Marchant × (*C. uncinatum* Schauer × *C. uncinatum* Schauer)
'Southern Stars'	*C. uncinatum* Schauer × *Verticordia plumosa* Desf.
'Jasper'	*C. uncinatum* Schauer × *Verticordia plumosa* Desf.
'WX35'	*C. uncinatum* Schauer × *Verticordia plumosa* Desf.
'WX73'	(*C. uncinatum* Schauer × *Verticordia grandis* Desf.) × *Verticordia grandis* Desf.

3.2. Methods

3.2.1. Flowering stems harvest

Flowering stems were harvested from an average 5 bushes in the early morning, and the cuts ends of stems were immediately placed upright in buckets of clean water in the field, and then transported to the laboratory of DAFWA at South Perth, taking about 35 min, by air-conditioned vehicle at approximately 20°C. At the laboratory flowering stems cut from several field plants were spread across a bench and stems were then randomly chosen to make an 8 stem replicate needed for each treatment. For vase life treatments, flower stems were recut with secateurs in water to 30 cm in length (from the cuts ends to the most extreme opened-flowers) (Seaton and Joyce, 1996; Seaton et al., 2010). All source plants were cultivated using irrigation and fertigation best practice (Seaton and Poulish, 2010).

3.2.2. Removal of organs

Depending on the experiment flowers, buds and leaves were removed by hands or by scissors.

3.2.3. Silver thiosulphate (STS) treatment

3.2.3.1. STS preparation

The STS solution was freshly prepared using sodium thiosulphate and silver nitrate where 80 mL stock sodium thiosulphate solution at 0.1 M was made by dissolving 1.264 g into 80 mL of deionized water and 20 mL of silver nitrate of stock solution was made by dissolving 0.34 g in 20 mL of deionized water. Twenty milliliters of the 0.1 M silver nitrate solution was slowly poured, with stirring, into 80 mL of the 0.1 M sodium thiosulphate making a 100 mL of 20 mmol STS solution. This was then diluted further to prepare 4 mmol STS solution for trials.

3.2.3.2. STS pulsing

Harvested stems were recut under water to a length of 60 cm, and then held in a 4 mmol STS solution for 20 min at 20°C. Immediately after pulsing, stem ends were rinsed under a running tap water and then stood with cut end in buckets of clean water. Buckets containing pulsed-stems were placed in cold store at approximately 5°C for 24 h in order to redistribute Ag^{+} in flower stems and then recut under water to 30 cm in length for trials.

3.2.4. Sugars and HQS preparation

Fresh aqueous solutions were prepared containing sucrose (Table sugar, CSR white sugar), maltose, glucose, fructose, galactose or HQS for each experiment.

3.2.5. Measurements

3.2.5.1. Assessment of vase life and flower drop

Vase life of flowers was determined when more than 50% of opened flowers had more than 50% closed or the petals showed damage. For genotypes, where flowers dropped before closing, the end of vase life was determined when more than 50% of opened flowers had dropped. Vase life of leaves was determined when more than 50% of the leaves were fully desiccated or yellow for their full length.

3.2.5.2. Assessment of the relationship between flower vase life, stem fresh weight and days to stem fresh weight reaching 75% of initial weight

Stem fresh weight was recorded daily until the end of flower vase life and expressed as a percentage of initial fresh weight. Analyzing stem fresh weight at the end of flower vase life showed that flowers ended vase life when stem fresh weight reaching 75.5% of initial weight. Thus, days for stems fresh weight to reach 75.5% of initial weight was determined to analyze the relationship between flower vase life and days for stem fresh weight at 75% of initial weight.

3.2.5.3. Organs measurement

Eight flowering stems were recut under water to 30 cm in length (measured from the cut end to the furthest open flower) and were then dried the surface water on leaves and flowers with tissue paper. Flowers, buds and leaves of cut stems were weighted with an electronic scale in an air-conditioned room at 20 ± 2°C, 60 ± 10% RH.

3.2.5.4. Ratio measurement

3.2.5.4.1. Weight ratio of flowers to stems

Flower weight ratio was calculated as a ratio of fresh weight of flowers to fresh weight of stems.

3.2.5.4.2. Vase life ratio of flowers to leaves

Flower to leaf vase life ratio was calculated as a vase life of flowers to vase life of leaves.

3.3. Experimental design and statistical analysis of data

Experiments were arranged in a completely randomized block design with four to six treatments per cultivars, and each treatment was replicated five to eight times consisting of a single stem in individual vases.

Vase life of flowers and leaves from treatments effect were analyzed by 1-way and 2-way ANOVA using the statistical package Genstat XV (Lawes Agricultural Trust, Rothamsted Experimental station, UK). Treatment means were compared by LSD at $P < 0.05$ and standard errors of the mean (± SE) were shown as appropriate. Where possible, mean comparisons were made using Duncan's Multiple

Range Test. All the assumptions of ANOVA were checked to ensure the validity of the statistical analysis.

The relationship between flower ratio and vase life ratio was determined by regression analysis.

CHAPTER 4

Factors affecting variation in vase life response of waxflower cultivars (Myrtaceae: *Chamelaucium* and *Verticordia* spp.) tested under various vase solutions

Abstract

Sixteen cultivars of waxflower derived from various species of *Chamelaucium* and crosses between four *Chamelaucium* species and crosses between *C. uncinatum* and two *Verticordia* species were compared in terms of vase life response, and the relationship between flower vase life and stem ability to maintain water balance by examining stem fresh weight changes. Vase life of *C. uncinatum* cultivars was generally the shortest with less than 12.0 days and over 4 cultivars averaged 9.5 days for flowers and 8.9 days for leaves. *C. uncinatum* cultivars vase life was 7.0 days for flowers and 16.0 days for leaves less than the lives for *C. megalopetalum* hybrids. Flower vase-lives of cultivars with *Verticordia grandis* ('WX73'), *C. sp. Gingin* ('WX97') and *C. floriferum* ('Lady Stephanie') as a parent were 11.4 to 15.5 days, while those with *Verticordia plumosa* ('Southern Stars') as a parent had the longest flower vase life of 24.9 days, but another *Verticordia plumosa* hybrid 'Jasper' had 9.5 days shorter. Vase life for certain cultivars could be improved by addition of sucrose, 8-hydroxyquniline sulphate (HQS) and silver thiosulphate (STS) to vase solution with 1.9 folds increase for *C. uncinatum* being more than *C. megalopetalum* (1.7 folds) and compared to cultivars with *C. sp. Gingin* (1.4 folds), *C. floriferum* (1.4 folds) or *Verticordia* (1.4 folds). The flower vase life of waxflowers, averaged for all vase solutions, ended when stems fresh weight reached 75 ± 5%. Cultivars with longer vase life maintained positive water balance for longer and had a slower reduction in fresh weight loss. In conclusion, the variation in vase life of waxflowers derived from different genetic backgrounds which were mainly affected by ability of cut stems to maintain water balance for longer. This ability was improved by adding sucrose to vase solutions, with *C. uncinatum* cultivars showing the greatest response.

4.1. Introduction

The vase life of cut flowers is highly depended on the inheritance or genotype of the cultivars (Kende, 1993). Vase-lives of 17 *Anthurium andraeanum* Hort. cultivars varied from 14.0 days for 'Evergreen' to 49.0 days for 'Cuba', which was mainly

depended on the ability of cultivars to maintain positive water balance of cut stems (Elibox and Umaharan, 2010). Vase life of Gerbera cultivars was also variable (Tesi, 1978) where some flowers lasted only 8.9 days while other flowers of the same clone lasted 16.0 days (Jong and Garretsn, 1985). Natural variation in the postharvest quality and organ retention of ornamental plants can often be related to differences in their response to ethylene sensitivity (Macnish et al., 2010), which for carnations is heritable (Woltering et al., 1993; Onozaki et al., 2001). The benefits of sugar, HQS and STS in extending flower vase life and reducing flower drop had been reported for many types of cut flowers including Geraldton waxflowers both *C. uncinatum* (Joyce, 1988; Seaton, 2006b) and *C. megalopectalum* hybrids 'Bridal Pearl' and 'Albany Pearl' (Seaton, 2005), *Dendrobium* (Dineshbabu et al., 2002), gladiolus (Beura et al., 2011) and snapdragon (Asrar, 2012). Sugar provided carbohydrate as an energy source for flower respiration, reduced the induction of endogenous ethylene (Pun and Ichimura, 2003) and improved water balance (Halevy and Mayak, 1974; Kuiper et al., 1995). 8−hydroxyquniline sulphate (HQS) prevented growth of the microorganisms in xylem vessels allowing maintenance of water uptake and extended flower vase life of snapdragons (Asrar, 2012). Combining HQS and sucrose increased flower quality, and vase life of gladioli (Beura et al., 2011) and dendrobiums (Dineshbabu et al., 2002). Silver thiosulphate (STS) has been used to reduce of endogenous ethylene production, and consequently extends the vase life of waxflowers (Joyce, 1993; Seaton, 2005). STS also inhibited microbial population which caused vascular occlusions in stems of snapdragons (Asrar, 2012). Vase life response varies and is not always heritable where addition of sugar to vase solutions containing 'Golden Light', an intergenetic hybrid obtained between *Sandersonia aurantiaca* and *Littonia modesta*, did not improve vase life (Eason et al., 2001) while sugar was effective in increasing vase life of their parents (Morgan et al., 2000). Vase life response of Asiatic hybrid lilies (*Lilium* L.) to STS was dependent on genotype (van der Meulen-Muisers et al., 1999). The aim of this study was to compare vase life response for different waxflower genotypes derived from *Chamelaucium* and *Verticordia* genomes and determined how this response was affected by different preserving solutions, using a combination of sucrose, HQS and STS. Stem fresh weight was measured during vase life to determine how water balance of stems influenced waxflower vase life response.

4.2. Materials and methods

4.2.1. Plant materials

Flowering stems of sixteen cultivars deriving from four species and hybrids of *Chamelaucium* and three hybrids of *Chamelaucium* and *Verticordia* (Table 4.1) were harvested in the field from mature bushes at Medina Research Station (32°13'18"S, 115°38'50"E) the Department of Agriculture and Food Western Australia (DAFWA). Flowering stems of approximately 70 cm were picked with 50−70% of flowers open followed by harvesting and transferring procedures as described in Chapter 3, section 3.2.1.

Table 4.1. The sixteen cultivars used in genetic variation study, and their parents

Cultivars	Parents	Harvest times
'WX102'	*C. megalopetalum* × *C. uncinatum*	June
'Purple Pride'	*C. uncinatum*	July
'Matilda'	(*C. uncinatum* × *C. uncinatum*) × *C. megalopetalum*	July
'Bridal Pearl'	*C. megalopetalum* × *C. uncinatum*	July
'Denmark Pearl'	*C. megalopetalum* × *C. uncinatum*	August
'Laura Mae Pearl'	*C. megalopetalum* × *C. uncinatum*	August
'Chrystal Pearl'	*C. megalopetalum* × *C. uncinatum*	September
'WX97'	*C. sp. Gingin* × (*C. uncinatum* × *C. uncinatum*)	September
'Mullering Brook'	*C. uncinatum*	October
'WX87'	*C. uncinatum* × *C. megalopetalum*	October
'Lady Stephanie'	*C. floriferum* × *C. uncinatum*	October
'Jasper'	*C. uncinatum* × *Verticordia plumosa*	October
'Southern Stars'	*C. uncinatum* × *Verticordia plumosa*	October
'Monica's Blush'	*C. uncinatum*	November
'WX17'	*C. uncinatum*	November
'WX73'	(*C. uncinatum* × *Verticordia grandis*) × *Verticordia grandis*	November

4.2.2. Vase solutions

The following vase solution treatments were used to test vase life response of waxflower cultivars

1. Deionized water (Control)
2. 200 mg L^{-1} 8-hydroxyquniline sulphate
3. 200 mg L^{-1} 8-hydroxyquniline sulphate and silver thiosultphate (applied as a 20-min pulse at 4 mmol)
4. 58.5 mmol sucrose and 200 mg L^{-1} 8-hydroxyquniline sulphate
5. 58.5 mmol sucrose and 200 mg L^{-1} 8-hydroxyquniline sulphate (HQS) and silver thiosultphate (STS) (applied as a 20-min pulse at 4 mmol)

2.2.1. Sucrose and 8HQS preparation

Sucrose and 8HQS solution were freshly prepared for each experiment. A 20 g of table sugar (CSR white sugar) and 200 mg 8HQS were diluted in 1 L of deionized water to make a vase solution of 58.5 mmol sucrose and 200 mg L^{-1} 8HQS.

4.2.2.2. Silver thiosulphate preparation

The STS solution was freshly prepared as described in Chapter 3, section 3.2.3.1.

4.2.2.3. Silver thiosulphate pulsing

Silver thiosulphate pulsing procedure as described in Chapter 3, section 3.2.3.2.

4.2.3. Assessment of vase life

Vase life was determined as described in Chapter 3, section 3.2.5.1.

4.2.4. Assessment of the relationship between flower vase life, stem fresh weight and Day to stem fresh weight reaching 75% of initial weight

Stem fresh weight was recorded as described in Chapter 3, section 3.2.5.2.

All vase life assessment trials were held in an air-conditioned room at 20 ± 2°C, 60 ± 10% RH with a 12 h photoperiod. The light flux densities are 8 μm m^{-2} s^{-2}.

4.2.5. Statistical design and analysis

Experiments were arranged in a completely randomized design with five treatments per cultivars, and each treatment was replicated eight times, excluding 'WX73' where the replication was five times.

Vase life of flowers and leaves from treatment effects (genotype × vase solution) was analyzed by 2-way ANOVA using the statistical package Genstat XV (Lawes Agricultural Trust, Rothamsted Experimental station, UK). Replication was 8 folds consisting of a single stem in individual vases. Treatment means were compared by LSD at $P < 0.05$ and standard errors of the mean (± SE) were shown as appropriate. Where possible, mean comparisons were made using Duncan's Multiple Range Test.

4.3. Results

4.3.1. Effect of genotype in DI water

There was a significant ($P < 0.05$) difference in vase life of flowers and leaves among cultivars ranging from 7.4 to 24.9 days for flowers and 5.5 and 21.5 days for leaves. Vase life of cultivars of *C. uncinatum* was the shortest being less than 12.0 days for flowers (ranging from 7.4 days for 'WX17' to 11.8 days for 'Mullering Brook') and less than 11.0 days for leaves (ranging from 5.5 days for 'WX17' to 11.0 days for 'Purple Pride'). For *C. megalopetalum* hybrids, vase life was longer than 12 days for flowers (ranging from 12.6 days for 'WX102' to 20.1 days for 'Crystal Pearl') and longer than 18.0 days for leaves (ranging from 18.1 days for 'WX87' to 31.0 days for 'Chrystal Pearl'). Within the cultivars of *C. uncinatum* and *C. megalopetalum* vase life was significantly higher for 'Chrystal Pearl', 'Laura Mae Pearl' and 'Bridal Pearl' all having a vase life of 27.0 to 31.0 days, while the other *C. uncinatum* and *C. megalopetalum* of 'Denmark Pearl', 'Matilda', 'WX87' and 'WX102' had 5.0 to 9.0 days shorter vase life. Similarly, for vase life of leaves of the *C. megalopetalum* hybrids 'WX87' and 'WX102' had significantly ($P < 0.05$) shorter vase life of 12.0 to 13.0 days compared to 'Chrystal Pearl', 'Denmark Pearl', 'Laura Mae Pearl', 'Matilda' and 'Bridal Pearl' with 16.0 to 20.0 days vase life (Fig. 4.2). For three *Verticordia* hybrids, flower vase life showed a similar range to *C. megalopetalum* hybrids and ranged from 11.4 days for 'WX73', a *C. uncinatum* × *V.*

grandis hybrid to 24.9 days or 'Southern Stars', a *Chamelaucium* × *V. plumosa* hybrid and for vase life of leaves ranged from 17.0 days for 'WX73' to 21.5 days for 'Southern Stars'. The *Verticordia* hybrid of 'Jasper' with a similar parentage as 'Southern Stars' in contrast had a flower vase life that was 10 days shorter than 'Southern Stars' and while leaf vase life which was not significantly ($P < 0.05$) different to 'Southern Stars'. While for *C. floriferum* hybrid 'Lady Stephanie' vase life was longer than *C. uncinatum* cultivars with 15.5 days for flowers and 18.5 days for leaves and *C. sp. Gingin* hybrid 'WX97' flower vase life was 12.4 days, similar to *C. uncinatum* cultivars while leaf vase life was longer and similar to *C. megalopetalum* hybrids of 22.9 days.

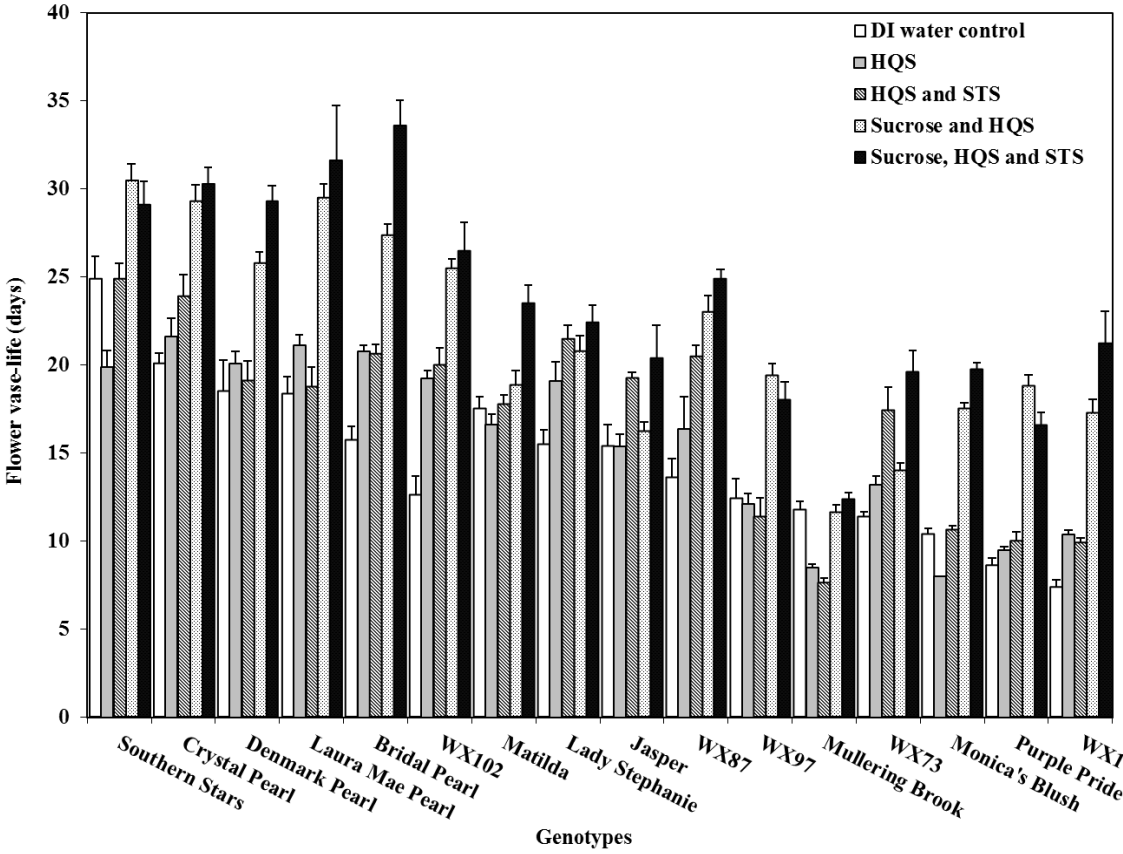


Figure 4.1. Vase life of flowers of different cultivars held in various vase solutions and DI water as control. LSD ($P < 0.05$) for genotype = 1.2 days, for vase solution = 0.7 day and for genotype × vase solution = 2.7 days, n =8. Vertical bars are standard errors of the mean.

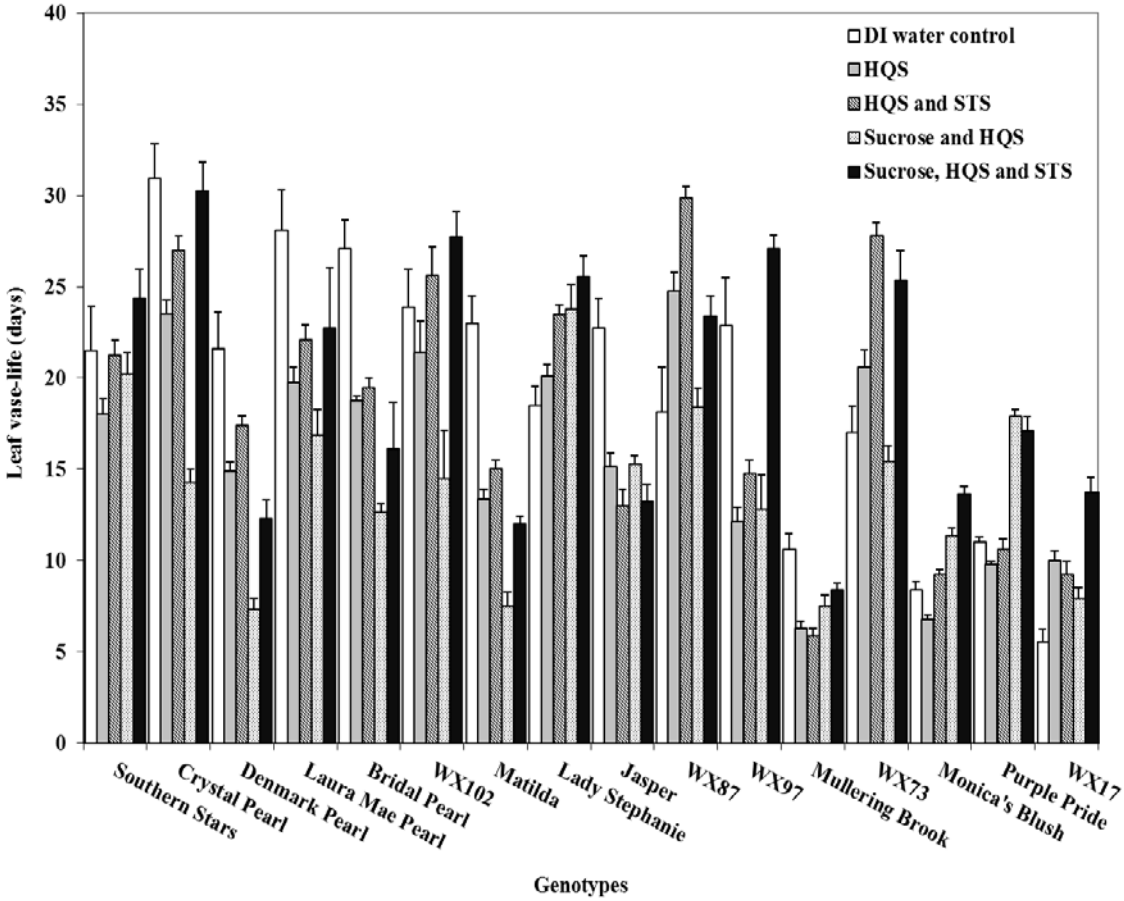


Figure 4.2. Vase life of leaves of different cultivars held in various vase solutions and DI water as control. LSD ($P < 0.05$) for genotype = 1.5 days, LSD for vase solution = 0.9 day and LSD for genotype × vase solution = 3.4 days, n = 8. Vertical bars are standard errors of the mean.

4.3.2. Effect of vase solutions

4.3.2.1 Flower vase life

Compared to DI water as a vase solution, sucrose, HQS and STS significantly ($P < 0.05$) extended flower vase life over all cultivars by 1.6 folds, followed by vase solution of sucrose and HQS by 1.5 folds; whereas, vase solution of HQS and STS increased flower vase life of cultivars by 1.2 folds and vase solution of HQS was less effective and in half of the cultivars caused vase life of flowers to decrease. A vase solution containing sucrose, HQS and STS was more effective in *C. uncinatum* increasing vase life by 1.8 folds compared to cultivars with *C. megalopetalum* as a parent (1.7 folds) and compared to cultivars with *C. sp. Gingin* (1.4 folds), *C. floriferum* (1.4 folds) or *Verticordia* (1.4 folds) as a parent. Cultivars with shorter

vase life tested in DI water had a larger response to vase solutions with flower vase life of 'WX17' increased by 2.9 folds in sucrose, HQS and STS (Fig. 4.1) and similarly 2.5 folds increase in leaf vase life (Fig. 4.2). Cultivar of 'Mullering Brook' was an exception and did not show a significant ($P < 0.05$) response to sucrose, HQS and STS. The effect of vase solutions did not increased vase life of the cultivar compared to the DI controls (Fig. 4.1). Flower vase life of *C. uncinatum* cultivars was significantly ($P < 0.05$) extended by 1.9 folds in vase solution of sucrose, HQS and STS and by 1.7 folds in vase solution of sucrose and HQS, but did not increase in vase solutions containing HQS or HQS and STS. Flower vase life of cultivars with *C. megalopetalum* as another parent was extended by 1.7 and 1.6 folds in vase solutions containing sucrose and HQS with and without STS respectively, but by 1.2 folds in HQS or HQS and STS vase solutions (Fig. 4.1). Flower vase life of cultivars with *Verticordia, C. sp. Gingin* 'WX97' and *C. floriferum* 'Lady Stephanie' as another parent was significantly ($P < 0.05$) extended by 1.4 folds in vase solution of sucrose, HQS and STS, while vase solution of sucrose and HQS significantly ($P < 0.05$) extended flower vase life of 'WX97' by 1.5 folds, followed cultivars of 'Lady Stephanie' and cultivars with *Verticordia* as another parent by 1.3 and 1.2 folds respectively. Vase solution of HQS and STS significantly ($P < 0.05$) prolonged flower vase life of 'Lady Stephanie' and cultivars with *Verticordia* as another parent by 1.4 and 1.3 folds respectively, but did not improved flower vase life of 'WX97' compared to the DI controls. Vase solution of HQS also significantly ($P < 0.05$) prolonged flower vase life of 'Lady Stephanie' by 1.2 folds, but did not prolonged vase life of 'WX97' or cultivars with *Verticordia* as another parent (Fig. 4.1). Flower vase life of 'WX102' and 'WX17' was doubled in vase solutions fortified with sucrose and HQS with or without STS, but increased by 1.5 folds in vase solutions containing HQS or HQS and STS compared to the DI controls. Similarly, flower vase life of 'Purple Pride' doubled in vase solutions containing sucrose and HQS or sucrose, HQS and STS, but vase life in HQS or HQS and STS vase solutions was not significantly ($P < 0.05$) different to those in the DI controls. Flower vase life of 'Jasper' and 'WX73' in vase solution containing sucrose, HQS and STS was not significantly ($P < 0.05$) different to those in vase solution of HQS and STS, but was significantly ($P < 0.05$) longer with 1.3 to 1.4 folds increase than those in vase solution of sucrose and HQS (Fig. 4.1).

4.3.2.2. Leaf vase life

Leaf vase life of all cultivars in vase solution of sucrose, HQS and STS did not significantly ($P < 0.05$) differ to that in vase solution of HQS and STS or the DI controls, but was significantly ($P < 0.05$) longer with 1.2 folds increase than that in HQS and 1.4 folds increase than that in sucrose and HQS (Fig. 4.2). Leaf vase life of almost cultivars except for 'Lady Stephanie', 'WX73' and 'WX17' decreased in vase solution of HQS compared to DI controls. Leaf vase life of *C. uncinatum* cultivars were significantly ($P < 0.05$) improved by 1.6 folds in vase solutions containing sucrose, HQS and STS, and by 1.2 folds in vase solution of sucrose and HQS, but did not increase in vase solutions containing HQS or HQS and STS compared to the DI controls. Leaf vase life of cultivars with *C. megalopetalum* as another parent in vase solution of HQS and STS was not significantly ($P < 0.05$) different to vase life in the DI water which was significantly ($P < 0.05$) greater with 1.3 folds increase than that in vase solutions containing HQS or sucrose, HQS and STS and with 2.0 folds increase than leaf vase life in vase solution of sucrose and HQS. Leaf vase life of cultivars with *Verticordia* as another parent in vase solutions containing HQS and STS or sucrose, HQS and STS was similar to leaf vase life in the DI controls which was significantly ($P < 0.05$) greater with 1.2 folds increase than that of in vase solutions containing HQS or sucrose and HQS. Similarly, leaf vase life of 'WX97' was significantly ($P < 0.05$) longer with 1.2 folds increase in vase solutions containing sucrose, HQS and STS, but decrease half in the remaining vase solutions compared to the DI controls. Alternatively, leaf vase life of 'Lady Stephanie' was significantly ($P < 0.05$) longer with 1.4 folds increase in vase solutions containing sucrose, HQS and STS and 1.3 folds in vase solutions containing sucrose and HQS or HQS and STS compared to the DI control which similar to vase solution of HQS in increasing vase life (Fig. 4.2). Leaf vase life of 'Monica's Blush' and 'Purple Pride' was significantly ($P < 0.05$) longer with 1.6 folds increase than vase life in the DI controls or vase solutions containing HQS or HQS and STS. Leaf vase life of 'WX17' was significantly ($P < 0.05$) longer with 2.5 folds increase than vase life in the DI controls or with 1.8 folds increase than leaf vase life in vase solution of sucrose and HQS. Compared to the DI control, vase solution of HQS and STS significantly ($P < 0.05$) increased leaf vase life of 'WX87' by 1.7 folds compared to 1.3 folds of vase solutions containing HQS or sucrose, HQS and STS and 1.0 fold of vase solution of sucrose and HQS (Fig. 4.2).

4.3.3. Relationship between vase life of flowers and leaves

Leaf vase life, for all cultivars in the DI water, except for three out of four cultivars of *C. uncinatum* and two out of three *Verticordia* hybrids exceeded flower vase life by an average 5.0 days. Also leaf vase life of *C. megalopetalum* × *C. uncinatum* hybrids exceeded vase life of flowers by an average of 44% or 8.0 days. While leaf vase life of three out of four *C. uncinatum* cultivars was 13% less than flower vase with only the cultivar 'Purple Pride' where leaf vase life was 2.0 days longer than flower vase life (Fig. 4.1 and 4.2). Leaf vase life of cultivars with *C. sp. Gingin* as a parent in DI water was longer with 1.8 folds increase than flower vase life, followed by cultivars with *C. megalopetalum* as a parent with 1.5 folds. Leaf vase life of cultivars with *Verticordia* as a parent in DI water was greater with 1.5 folds for 'Jasper' and 'WX73' and 13% less for 'Southern Stars' than flower vase life and with 1.2 folds for *C. floriferum* hybrid.

Leaf vase life of cultivars was similar to flower vase life in vase solutions containing HQS or HQS and STS, but was significantly ($P < 0.05$) 54% and 17% shorter in vase solutions containing sucrose and HQS or sucrose, HQS and STS (Fig. 4.1 and 4.2).

4.3.4. The relationship between flower vase life, stem fresh weight and day for when stem fresh weight reaching 75% of initial weight

The percentage of fresh weight at the end of vase life was on average 75.5 ± 5% over all cultivars and vase solutions (Table 4.2). In a vase solution of HQS there was a significant ($P < 0.05$) decrease on the average of 9% fresh weight at the end of vase life compared to in DI water. While for other vase solutions no difference occurred in average fresh weight at the end of vase life for all cultivars compared to DI water controls. On average for all cultivars only those in vase solution containing HQS had significantly ($P < 0.05$) 15% lower fresh weight than in DI water and other vase solutions. For 'Southern Stars', 'Denmark Pearl', 'Matilda', 'Bridal Pearl', 'Lady Stephanie', 'Jasper', 'WX102', 'WX97', 'Mullering Brook' and 'Purple Pride' in vase solution of HQS reached the end of vase life at 42.7% which was significantly ($P < 0.05$) lower than in DI water and other vase solutions with an average of 68.4% whilst, 'WX102' in vase solutions containing sucrose, HQS and STS reached the end of vase life at significantly ($P < 0.05$) higher percentage of fresh weight than in

the DI control, HQS or sucrose and HQS as did 'Laura Mae Pearl' in vase solution of sucrose and HQS (Table 4.2). On average for all cultivars only those in vase solution containing HQS had significantly ($P < 0.05$) 12.4% lower fresh weight than in DI water and other vase solutions. Across all vase solutions, cultivars of 'Crystal Pearl', 'Denmark Pearl', 'Matilda', 'Lady Stephanie', 'Mullering Brook' and 'Purple Pride' reached the end of vase life at an average 67.9% fresh weight which was significantly ($P < 0.05$) lower than that of 'Southern Stars', 'Bridal Pearl', 'WX73' and 'WX17' with an average of 80.3% and also was significantly ($P < 0.05$) lower than that of 'Laura Mae Pearl' and 'WX102' with an average fresh weight of 91.0% (Table 4.2).

4.3.5. Changes in fresh weight of cut stems

Relative fresh mass increased during the first 2.0 to 3.0 days of vase life then gradually decreased when cut stems of all cultivars were held in DI water, except for 'Laura Mae Pearl' where fresh weight decreased sharply in the first 2.0 days then gradually decreased until end of flowers vase life (Fig. 4.4). Flower vase life between cultivars was closely correlated with time to reach 75% fresh weight changes, where cultivars with short vase life took a short time to reach 75% fresh weight and cultivars or vase solution treatments with longer vase life took a long time to reach 75% fresh weight (Fig. 4.3). For instance in DI control, *C. uncinatum* cultivars with the shortest flower vase life reached stem fresh weight at 75% at day 10 compared to day 20 for cultivars with *C. megalopetalum* as a parent or day 25 for *Verticordia plumosa* 'Southern Stars' with the longest flower vase life. In vase solutions containing sucrose flower vase life took a longer time to reach 75% fresh weight. For instance in sucrose, HQS and STS, overall cultivars reached stem fresh weight at 75% at day 26 compared to day 17 in DI water (Fig 4.4). Also *C. uncinatum* 'Monica's Blush' in sucrose, HQS and STS reached stem fresh weight at 75% at day 22 compared to day 11 in DI water (Fig 4.4c) while *C. megalopetalum* hybrid 'Crystal Pearl' reached stem fresh weight at 75% at day 32 compared to day 19 in DI water (Fig 4.4p). The correlation between flower vase life (days) and day for stem fresh weight reaching 75% of initial weight could be described as: in DI control, flower vase life = 1.0763x + 1.1008, $r^2 = 0.8118$; in HQS, flower vase life = 1.0438x – 0.5658, $r^2 = 0.8511$; in HQS and STS, flower vase life = 1.0196x + 1.764, $r^2 = 0.8371$; in sucrose and HQS, flower vase life = 1.524x – 10.565, r^2 =

0.8608 and in sucrose, HQS and STS, flower vase life = 1.0214x + 1.9172, r^2 = 0.7535 (Fig. 4.3).

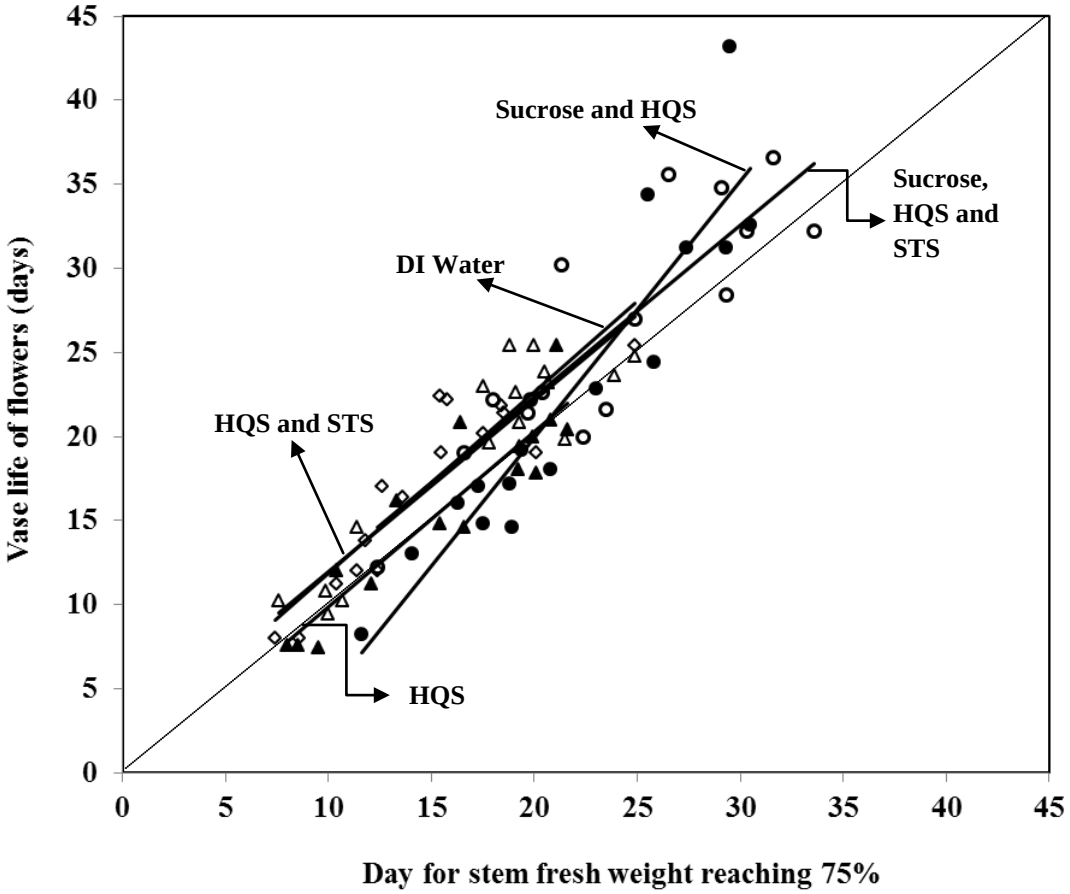
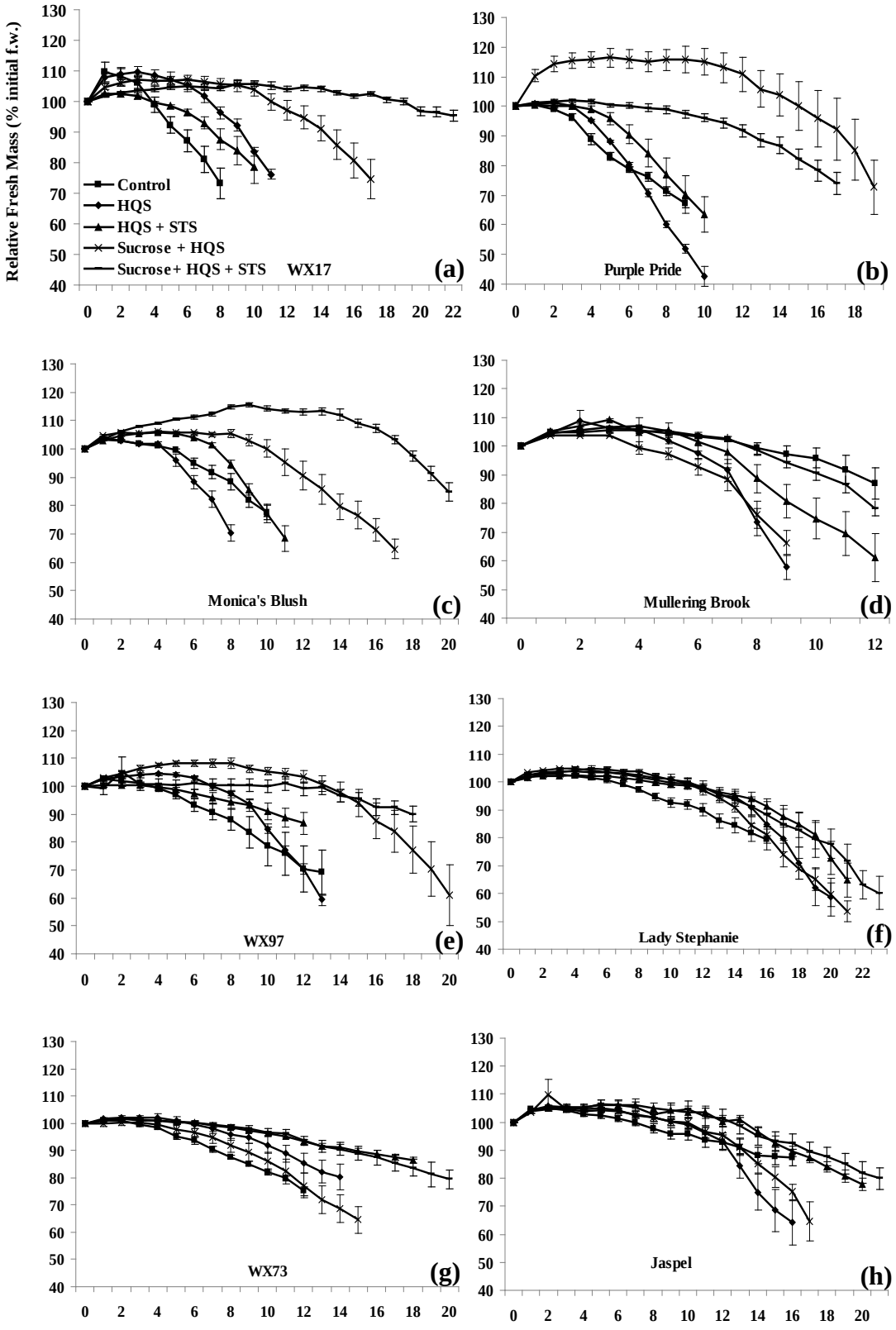


Figure 4.3. Regression relationships between flower vase life (days) and day for stems to reach a fresh weight of 75% of initial fresh weight for cultivars in the DI control (◊), HQS (▲), HQS and STS (Δ), sucrose and HQS (●) and sucrose, HQS and STS (o), n = 5. Equations for lines are shown in text.

Table 4.2. Percentage of stem fresh weight at the end of flower vase life in vase solutions

Cultivars	Control	HQS	HQS and STS	Sucrose and HQS	Sucrose, HQS and STS	Average fresh weight
'Southern Stars'	77.8 ± 3.5	64.4 ± 8.8	73.1 ± 6.9	83.9 ± 2.0	96.4 ± 6.8	79.1 ± 5.6
'Chrystal Pearl'	69.4 ± 2.8	65.7 ± 8.8	74.3 ± 4.4	70.2 ± 9.3	86.4 ± 4.7	73.2 ± 6.0
'Denmark Pearl'	79.3 ± 4.8	51.5 ± 7.7	90.4 ± 2.2	56.4 ± 11.8	66.1 ± 4.4	68.7 ± 6.2
'Laura Mae Pearl'	82.9 ± 3.6	86.3 ± 2.0	92.2 ± 3.2	105.1 ± 1.9	98.4 ± 9.6	93.0 ± 4.1
'Matilda'	79.5 ± 5.6	63.8 ± 4.3	78.1 ± 2.9	48.8 ± 4.8	70.7 ± 3.7	68.2 ± 4.3
'Bridal Pearl'	87.0 ± 3.1	74.5 ± 2.5	83.3 ± 0.6	89.3 ± 1.4	92.7 ± 5.5	85.4 ± 2.6
'Lady Stephanie'	79.4 ± 3.8	58.7 ± 6.7	64.8 ± 6.2	53.6 ± 3.8	60.2 ± 5.9	63.3 ± 5.3
'Jasper'	87.5 ± 3.0	64.2 ± 7.9	77.7 ± 2.2	64.7 ± 7.1	79.9 ± 3.9	74.8 ± 4.8
'WX87'	68.4 ± 5.4	69.8 ± 8.5	81.7 ± 2.1	65.1 ± 13.2	91.0 ± 7.0	75.2 ± 7.2
'WX102'	83.4 ± 4.1	72.3 ± 5.1	87.0 ± 1.9	101.3 ± 4.4	100.5 ± 4.2	88.9 ± 3.9
'WX97'	69.1 ± 8.2	59.4 ± 5.0	86.7 ± 3.9	60.9 ± 11.0	90.0 ± 3.0	73.2 ± 6.2
'Mullering Brook'	86.9 ± 5.4	57.7 ± 4.2	61.1 ± 8.4	66.4 ± 4.2	78.3 ± 2.6	70.1 ± 5.0
'WX73'	75.1 ± 1.6	80.3 ± 4.8	86.5 ± 1.2	64.6 ± 4.9	79.5 ± 3.4	77.2 ± 3.2
'Monica's Blush'	77.7 ± 2.7	70.5 ± 2.8	68.4 ± 2.8	64.7 ± 3.3	84.9 ± 3.3	73.2 ± 3.0
'Purple Pride'	67.4 ± 1.5	42.7 ± 3.0	63.5 ± 6.0	72.8 ± 9.1	74.0 ± 3.9	64.1 ± 4.7
'WX17'	73.3 ± 5.0	76.2 ± 1.7	78.6 ± 5.3	74.6 ± 6.4	95.4 ± 1.9	79.6 ± 4.1
Average fresh weight	77.8 ± 4.0	66.1 ± 5.0	78.0 ± 3.8	71.4 ± 6.2	84.0 ± 6.0	75.5 ± 5.0

LSD ($P < 0.05$) for cultivars = 6.8 days, for vase solutions = 3.8 days and for cultivar × vase solution =15.3 days, n= 5. ± = standard errors of the mean.


Relative Fresh Mass (% initial f.w.)
Control
HQS
HQS + STS
Sucrose + HQS
Sucrose+ HQS + STS
WX17
(a)
Purple Pride
(b)
Monica's Blush
(c)
Mullering Brook
(d)
WX97
(e)
Lady Stephanie
(f)
WX73
(g)
Jaspel
(h)

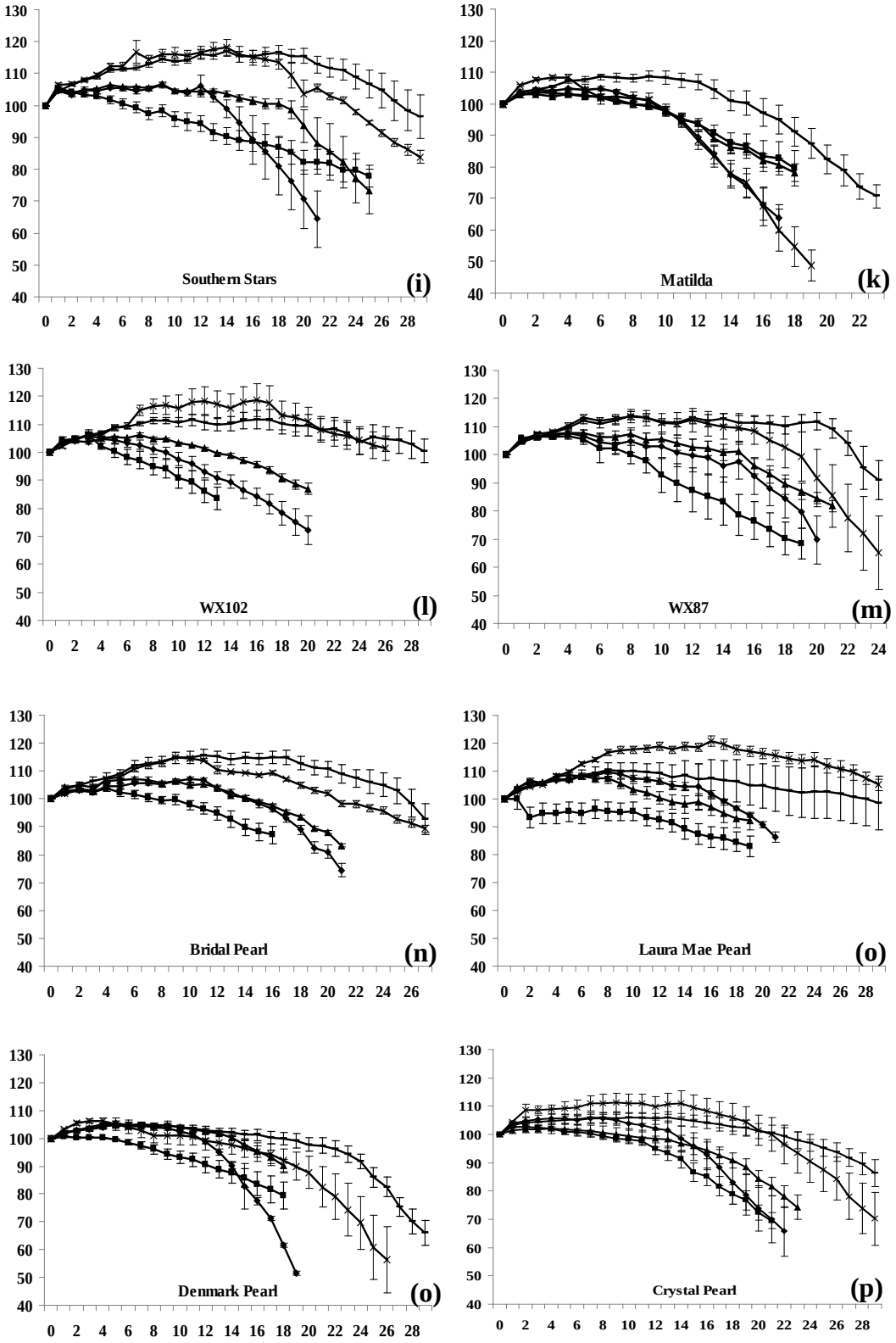


Figure 4.4. Changes in relative fresh mass during assessment of vase life of waxflower cultivars held in various vase solutions and DI water as control. Each point represents a mean ± SE, n = 5.

4.4. Discussion

4.4.1. Effect of genotype

Variation in vase life among cultivars was not always dependent on the particular genetic background of a cultivar although cultivars solely with *C. uncinatum* in their genotype tended to have shorter vase life than cultivars with *C. megalopetalum* or a different species such as *V. plumosa*. This appears to be dependent on the ability of genotypes to maintain water balance. Symptoms associated with the end of vase life in *Anthurium* cultivars are typical of water stress when the balance between water loss and water uptake exceed 1.5 (Mujaffar and Sankat, 2003). Similarly, in cut waxflowers the ability of able to maintain a positive water balance of cut stems determined vase life as found in *Anthurium andraeanum* Hort. where flowers with a longer vase life had greater ability to maintain a positive water balance, which appears to be an inherited trait (Elibox and Umaharan, 2010). The short vase life of *C. uncinatum* cultivars is consistent to findings reported by Joyce and Jones (1992) and Seaton et al. (2010) and was attributed a greater water loss in leaves than in flowers decreasing water potential in leaves (Joyce and Jones, 1992). The longer vase life of leaves compared to flowers of *Megalopetalum*, *sp. Gingin* are similar to result obtained for *Verticordia* flowers (Seaton, 2006a) and may indicate that leaves dry out more slowly than flowers and do not compete with flowers for water reducing flower vase life. Observations (data not included) showed that 'Lady Stephanie' and 'WX97' had shorter leaves than *C. uncinatum* cultivars, but longer than *C. megalopetalum* hybrids possibly prompting greater water loss. Accordingly 'Lady Stephanie' and 'WX97' had longer flower vase life than *C. uncinatum* cultivars but shorter flower vase life than cultivars with *C. megalopetalum* as a parent. The variation in vase life of waxflower genotypes was consistent with the findings on *Verticordia* (Seaton, 2006a), Asiatic hybrid *Lilium* (van der Meulen-Muisers et al., 1999), *Dianthus caryophyllus* (Downs, 1988; Olley, 1996) and *Antirrhinum majus* (Weber et al., 2005). Also flower vase life of 'Southern Stars' (long vase life) being 2.2 times the vase life of 'WX73' (short vase life) was consistent with differences in parents of these cultivars where *V. plumosa* flowers and leaves had 2 to 3 times the length of vase life than *V. grandis* (Seaton, 2006a).

4.4.2. Effect of vase solutions

The ability of sucrose to improve water balance (Halevy and Mayak, 1974; Kuiper et al., 1995) may have been a critical factor in improving vase life in waxflower cultivars found in this study where flowers with longer vase life dehydrated slower with sugar present in the vase solution. Different cultivar of waxflower response to sucrose may indicate a different sensitivity of cultivars to sucrose as lower concentrations prolonged the vase life of gladiolus florets by increasing water uptake; whereas, higher concentrations seemed to impede water uptake (Bravdo et al., 1974). As sucrose was more effective when HQS was added (Ichimura et al., 1999), suggesting that sucrose may encourage microbial blockage of stems limiting water uptake. Significant increase in vase life of *C. uncinatum* cultivars when treated with sucrose and HQS was similar to the result for 'Purple Pride' and 'Alba' (Joyce and Jones, 1992). HQS acts an antimicrobial agent (Ketsa et al., 1995), resulting in increased in water uptake of cut flowers (Reddy et al., 1996). HQS at 200 mg L^{-1} in vase water prolonged flower vase life of cut snapdragon (Asrar, 2012) and rose (Ichimura et al., 1999). However, in this study HQS was ineffective in extending vase life of waxflowers, supported by results of van Doorn et al. (1990) and Liao et al. (2000), and also shortened leaf vase life which could be caused by HQS being toxic. The reduction in leaf vase life of some cultivars treated with sucrose and HQS may indicate that concentrations of HQS were toxic to some cultivars especially their leaves. Sucrose at 58.5 mmol may also have been toxic to some cultivars as Joyce (1993) found that high sucrose concentration (i.e 146.2 mmol) may cause leaf tip injury, through osmotic stress of sucrose accumulation. Also in this study sucrose at 58.5 mmol caused leaf tip injury of 'Purple Pride' and desiccated leaves of the *C. megapetalum* hybrids. This indicates the leaves of 'Purple Pride' experienced more turgor loss than leaves of the *C. megapetalum* hybrids. Variation in leaf vase life of waxflowers caused by genotypic variance was consistent with the finding on *Protea neriifolia* where pulsing with 584.8 mmol sucrose (24 h, 25°C) significantly reduced leaf blackening in *Protea neriifolia* (McConchie and Lang, 1993), but not for *Protea* 'Sylvia' (Stephens et al., 2011). STS was more effective in prolonging vase life of leaves than flowers of waxflowers with leaf vase life significantly increased with the presence of STS in HQS vase solution, suggesting that STS can reduce the deleterious effect of HQS. STS prevents ethylene induced absicssion (Serek and Sisler, 2001) and was very effective in preventing flower abscission of *C.*

megalopectalum hybrids (Seaton, 2005) and *C. uncinatum* cultivars (Joyce, 1993), but was ineffective in increasing vase life of cut *C. uncinatum* (Joyce, 1988). STS combined with sucrose and HQS was more effective than sucrose and HQS in terms of increasing vase life of cut waxflowers, supported by study on cut rose (Liao et al., 2000). This may be due to role of STS as an ethylene blocker (Joyce, 1988) delaying senescence of ethylene-sensitive flowers (Veen, 1983; Knee, 1995) and inhibiting microbial population which causes vascular occlusion of stems (Asrar, 2012) .

4.5. Conclusions

The influence of genotype was instrumental in improving the vase life of different cultivars and this appeared to depend on its ability to maintain stem water balance, with parents of hybrids of *C. megalopetalum* and *V. plumosa* having the most influence. Vase solution especially sucrose and STS were effective in improving water balance and vase life. HQS appears necessary to prevent toxicity effects of sucrose at the levels used in this trial. Leaves appear to be more affected by sucrose in certain cultivars especially where there was *C. megalopetalum* present in the hybrids.

Next chapter will examine the effect of different types and concentrations of sugars on vase life of several selected cultivars of waxflower.

CHAPTER 5

Influence of type and concentration of sugars, supplemented with 8-hydroxyquniline sulphate (HQS), on vase life of waxflowers

Abstract

A study was conducted to test the effect of 58.5 mmol maltose, glucose, fructose or galactose compared to 58.5 mmol (or 2% w/v) sucrose, and different sucrose concentrations supplemented with 200 mg L^{-1} 8-hydroxyquniline sulphate (HQS) on vase life and stems fresh weight changes of waxflowers cultivars including *Chamelaucium uncinatum* selections and *Chamelaucium* hybrids of *C. megalopetalum, C. floriferum, C. sp. Gingin* and *Verticordia* spp., and the effect on vase life of *C. uncinatum* 'Alba' of the interaction between sucrose and HQS concentrations was also investigated. There was no significant improvement in vase life of flowers of cultivars between using fructose and glucose compared to sucrose, except for 'Laura Mae Pearl' where flower vase life in fructose was significantly higher (9.7%) than vase life in sucrose vase solution. Overall cultivars, sucrose, fructose and glucose were more effective in improving vase life of flowers than maltose and galactose. All types of sugar decreased vase life of leaves of cultivars compared to the DI controls, except for 'Lady Stephanie' where vase life of leaves in sucrose, maltose and fructose was significant higher with an average of 35% than vase life in the DI controls, while for *C. uncinatum* cultivars vase life in all types of sugar was similar to vase life in the DI controls. At sucrose concentration up to 117 mmol, in combination with 200 mg L^{-1} HQS, vase life of flowers of six out of eight cultivars significantly increased, except for 'Laura Mae Pearl' and 'Mullering Brook' where flower vase life maximized at concentrations of 29.2 and 58.5 mmol respectively, while vase life of leaves decreased. Sucrose concentrations from 14.6 to 29.2 mmol in combination with 50 mg L^{-1} HQS maximized vase life for both flowers and leaves of 'Alba'. Vase-lives of flowers of *C. megalopetalum* hybrids 'Laura Mae Pearl' and 'Denmark Pearl' and *C. sp. Gingin* hybrid 'WX97' were more increased in vase solution of sugars than that of cultivars of *C. uncinatum* 'Mullering Brook', *C. floriferum* hybrid 'Lady Stephanie' and *Verticordia grandis* hybrid 'WX73'. Cultivars with longer vase life of flowers had longer the number of days of stem fresh weight was above initial stems fresh weight rather than days below 100% fresh weight. This applied for cultivars in different types and concentrations of sugars.

5.1. Introduction

Improvement of vase life of cut flowers by supplying sugar in vase solution has been widely reported (Nichols, 1973; van Doorn, 2001; Arrom and Munné-Bosch, 2012). Cut flower demand on carbohydrates is mainly provided by application of exogenous sugar (Stephens et al., 2011) because cut flowers reduced ability to photosynthesis after detachment from plant (van Staden, 1995). Vase life of cut flowers has been correlated with sugar content of flowers where with higher sugar content promoting longer vase life (Holly, 1963). Adding sugar to vase solutions or pulsing in higher sugar concentration extended the longevity of cut tulip (Ranwala and Miller, 2009), rose (Gholami et al., 2011) and lily flowers (van Doorn and Hanb, 2011). Sugars supply materials for building structures for the plant organs and contribute to cell wall synthesis, resulting in increased vase life of flowers (Ichimura, 1998). Sugar also reduced ethylene production of the petals of cut flowers as found with cut carnations which when treated with sucrose significantly decreased the ethylene production of petals (Verlinden and Garcia, 2004). The osmotic (Kuiper et al., 1995) and delaying deterioration (Kikuchi et al., 1995) and preserving effects of sugar on flower membranes enhanced vase life of cut flowers. Both mono– and di–saccharides have been found in flower cells of many types of cut flowers such as S*andersonia aurantiaca* (Eason et al., 1997), rose (Ichimura et al., 1997), *Petunia* (O'Donoghue et al., 2008) and snapdragon (Asrar, 2012) (Table 5.1). Among different types of sugars, sucrose has been commonly used commercially to prolong longevity of cut flowers (Pun and Ichimura, 2003). Sucrose is a main component in commercial vase solutions such as Chrysal (Chrysal, Naarden, The Netherlands) providing carbon source for flowers and showed a positive effect in extending vase life as occu`rs in cut *Dendrobium* 'Kao Sanan', 'Lovely Pink' and 'Suree Peach' (Obsuwan et al., 2012). In higher plants, hydrolysis of sucrose provides mono–saccharides of glucose and fructose as was the case for cut roses (Yamada et al., 2007) which are then further metabolized by acid invertase (β–fructosidase, EC 3.2.1.26) which is present in the vacuole (soluble form) of the petals (ap Ree, 1974; Kaltaler and Steponkus, 1974; Roitsch and Gonzalez, 2004).

HQS combined with sucrose were found to be very effective in increasing vase life and reducing respiration rate and physiological weight loss of dendrobium

(Dineshbabu et al., 2002) and gladiolus (Beura et al., 2011). The use of biocides such as 8−hydroxyquniline sulphate (HQS) is a very important germicide in preservatives used by the floral industry (Nowak and Rudnicki, 1990) to keep the cut flowers fresh over extended periods. Biocides reduce entry of bacteria and fungi, particularly through blockage of xylem vessels by air and microorganisms that caused xylem occlusion (Hardenburg, 1968). Use of HQS preventing microbial growth results in an increase in water uptake of cut flowers (Reddy et al., 1996).

The effect of types and concentrations of sugar on vase life of cultivars also differed. While sucrose at concentration of 146.2 mmol delayed ethylene production in *Dianthus caryophyllus* 'White Sim', fructose, glucose and maltose at the same concentration did not delay ethylene production (Verlinden and Garcia, 2004). Similarly, the combinations of glucose and aminooxyacetic (AOA) showed a prolonged vase life of flowers of dendrobium longer than sucrose plus AOA at the same rate (Rattanawisalanon et al., 2003). Response of vase life of flowers to sucrose concentration also differed as occur in cut snapdragon where flower vase life increased with increasing sucrose concentration from 7.31 to 14.6 mmol and reached a maximum at concentration of 14.6 mmol and then decreased with increasing concentrations to 29.2 mmol in combination with 200 mg L^{-1} HQS (Ichimura and Hisamatsu, 1999).

Vase life of flowers of *C. uncinatum* 'Alba' was extended with the presence of sucrose at concetration of 58.5 mmol in vase water, but this concentration of sucrose damaged to leaves (Joyce and Jones, 1992). In combination, 58.5 mmol sucrose plus 200 mg L^{-1} HQS significantly increased vase life of *C. uncinatum* cultivars (Joyce, 1988). Increasing sucrose concentrations up to 146.2 mmol combined with 200 mg L^{-1} HQS decreased vase life of flowers and leaves of cultivars of *C. uncinatum* (Joyce, 1988). However, there has been no extensive research studying on the effects of different types and concentrations of sugars and the effect of interaction between sucrose and HQS concentrations on vase life of cultivars of different waxflowers species. The aim of this study was to evaluate the effect of different types and concentrations of sugars on vase life of a range of waxflowers cultivars. It also studied the effect of HQS in combination with sucrose as an effective vase solution.

Table 5.1. Literature references to the presence of mono– and di–saccharides types in flower cells of cut flowers

Cultivars	Reference	Type of sugars			
		Mono–saccharides			Di–saccharides
		Fructose	Glucose	Galactose	Sucrose
Rosa 'Carl Red'	Ichimura et al., 1997	√	√		√
Rosa 'Rote rose'	Ichimura et al., 1997	√	√		√
Rosa 'Sonia'	Ichimura et al., 1997	√	√		√
Sandersonia tubers	Eason et al., 1997	√	√		√
Petunia 'Mitchell'	O'Donoghue et al., 2008		√	√	
Antirrhinum majus 'Yellow Butterfly'	Asrar, 2012	√	√		

Note: √ means similar to the previous one

5.2. Materials and Methods

5.2.1. Plants materials

Flowering stems of eleven cultivars of *C. uncinatum* 'Purple Pride', 'Alba' and 'Mullering Brook', *C. megalopetalum* × *C. uncinatum* 'Bridal Pearl', 'Denmark Pearl', 'Laura Mae Pearl' and 'Chrystal Pearl', *C. uncinatum* × *C. megalopetalum* 'WX87', *C. sp. Gingin* × (*C. uncinatum* × *C. uncinatum*) 'WX97', *C. floriferum* × *C. uncinatum* 'Lady Stephanie' and (*C. uncinatum* × *Verticordia grandis*) × *Verticordia grandis* 'WX73' were harvested in the early morning from mature bushes cultivated using irrigation and fertigation (Seaton and Poulish, 2010) at Medina Research Station (32°13'18"S, 115°38'50"E) the Department of Agriculture and Food Western Australia (DAFWA). Flowering stems of approximately 70 cm were picked with 50−70% flower open followed by harvesting and transferring procedures as described in Chapter 3, section 3.2.1.

5.2.2. Experimental design

Experiment 1: Sugar types treatments

Two disaccharides ((sucrose (table sugar − a CSR white sugar) and maltose) and three mono–saccharides (glucose, fructose and galactose) all at concentrations of 58.5 mmol were prepared by dissolving for sucrose 20,000 mg, maltose 21,071 mg, glucose 10,538 mg, fructose 10,538 mg or galactose 10,535 mg into 1 L of deionized water supplemented with 200 mg L^{-1} HQS. Vase life of flowers and leaves was compared in different sugars for cultivars of 'Bridal Pearl', 'Denmark Pearl', 'Laura Mae Pearl', 'Crystal Pearl', 'WX87', 'WX97', 'Lady Stephanie', 'WX73', 'Mullering Brook' and 'Purple Pride' with deionized water (DI) was used as a control. Experiment was arranged in a completely randomized design, and each treatment was repeated eight times.

Experiment 2: Sucrose concentrations

A two factors experiment was conducted to test the effect of different sucrose concentrations on vase life for different cultivars. Factor of sucrose concentrations included 0, 14.6, 29.2, 58.5 and 117 mmol sucrose being prepared by dissolving 0, 5,000; 10,000; 20,000 and 40,000 mg table sugar (CSR white sugar) into 1 L of deionized water. Factor of cultivars included cultivars of 'Bridal Pearl', 'Denmark Pearl', 'Laura Mae Pearl', 'WX97', 'Lady Stephanie', 'WX73', 'Mullering Brook' and 'Purple Pride'. HQS at concentration of 200 mg L^{-1} was added to vase water containing sucrose. A completely randomized experiment was designed with each treatment was replicated eight times.

Experiment 3: Sucrose vs. HQS

A factorial experiment was conducted to test the effect of the combination between sucrose at concentrations of 14.6, 29.2, 58.5, and 117 mmol and HQS at concentrations of 50, 100, 200, and 400 mg L^{-1} on vase life of cultivars 'Alba'. Deionized water was used as a control. The experiment was arranged in a completely randomized design with eight replications for each treatment.

5.2.3 Experimental conditions

All experiments were conducted in a vase life evaluation room at 20 ± 2°C, 60 ± 10% RH with a 12 h photoperiod. The light flux densities are 8 μmol m^{-2} s^{-2}. In each experiment, all stems were individually placed in 250 mL capacity plastic vases containing each of the above vase solution.

5.2.4. Measurements

5.2.4.1. Vase life measurements

Vase life was recorded as described in Chapter 3, section 3.2.5.1.

5.2.4.2. Assessment of the relationship between flower vase life and day for stem fresh weight above or below initial stem fresh weight

Day for stem fresh weight above initial stems fresh weight (days) was recorded as a period of times from stems being placed in vase solutions to the day when stem fresh weight equal initial stem fresh weight. Day for stem fresh weight below initial stems fresh weight (days) was determined as vase life of flowers (days) minus day for stem fresh weight above initial stems fresh weight (days).

5.2.4.3. Assessment of the relationship between flower vase life and day to stem fresh weight reaching 75% of initial weight of cultivars in different sucrose concentrations

The relationship between flower vase life and day to stem fresh weight reaching 75% of initial weight were recorded as described in Chapter 3, section 3.2.5.2.

5.2.4. Statistical analysis

Vase life of flowers and leaves from treatment effects (sugar type × genotype, sucrose concentration × genotype and a factorial treatment of sucrose concentration × HQS concentration) were analyzed 2–way ANOVA using the statistical package Genstat XV (Lawes Agricultural Trust, Rothamsted Experimental station, UK). Treatment means were compared by LSD at $P < 0.05$ and means (± SE) were shown as appropriate. Where possible, mean comparisons were made using Duncan's Multiple Range Test.

5.3. Results

5.3.1. Effect of sugars types supplemented with 200 mg L^{-1} HQS on vase life of waxflowers

5.3.1.1. Flower vase life

Flower vase life of overall cultivars was significantly ($P < 0.05$) extended in vase solutions containing 58.5 mmol sugars coupled with 200 mg L^{-1} HQS compared to the DI control (Table 5.2). Sucrose had a similar effect on increasing flower vase life of cultivars when compared to fructose or glucose, but was more effective in improving vase life compared to maltose and galactose (Table 5.2). However, flower vase life response of individual cultivar to different types of sugar was different. Flower vase life of cultivars was significantly ($P < 0.05$) increased by addition of sugars except for, 'WX73' in sucrose, 'Crystal Pearl', 'WX73' and 'WX97' in maltose, 'Purple Pride' and 'WX73' in glucose, 'Lady Stephanie', 'Purple Pride', 'WX87', 'WX73' in galactose and 'Mullering Brook' in all types of sugars where flower vase life was similar to vase life in the DI controls (Table 5.2). There was no significant ($P < 0.05$) gain in flower vase life between using sucrose compared to glucose or fructose for all cultivars, except for 'Laura Mae Pearl' where vase life in fructose was significantly ($P < 0.05$) greater than in sucrose. For sucrose, fructose and glucose flower vase life increased by an average 1.4 folds and by 1.3 for maltose and 1.2 folds for galactose compared to the DI controls (Table 5.2). The addition of sucrose had a significantly ($P < 0.05$) greater effect of 1.5 folds on extending flower vase life of cultivars with *C. megalopetalum* or 1.7 folds for *C. sp. Gingin* hybrid 'WX97' as a parent compared to other cultivars including all cultivars with only *C. uncinatum* as a parent (1.4 folds) or *C. floriferum* hybrid 'Lady Stephanie' (1.3 folds) or *Verticordia* hybrid 'WX73' (1.2 folds) compared to the DI controls (Table 5.2).

Table 5.2. Effect of different types of sugars on vase life of flowers of cultivars

Cultivars	Vase life of flowers (days)					
	Control	Sucrose	Maltose	Glucose	Fructose	Galactose
'Crystal Pearl'	23.9	33.3	25.3	31.6	30.8	31.4
'Laura Mae Pearl'	21.5	34.9	34.1	36.3	38.3	30.9
'Bridal Pearl'	18.4	25.0	28.0	26.1	27.8	25.1
'Denmark Pearl'	15.5	28.4	25.8	27.3	27.9	22.4
'Lady Stephanie'	15.5	20.8	18.6	19.0	23.3	18.0
'WX87'	14.4	18.1	17.5	18.5	18.1	15.9
'WX73'	13.4	15.4	12.8	14.9	17.6	13.1
'Mullering Brook'	12.0	15.0	10.3	13.4	13.9	12.4
'WX97'	11.8	19.6	13.4	18.8	18.1	16.6
'Purple Pride'	9.3	13.6	14.6	12.0	15.9	10.3
Average means of vase life	15.6	22.4	20.0	21.8	23.2	19.6

LSD ($P < 0.05$) for sugar types = 0.8 day, for cultivars = 1.0 day and for sugar types × cultivars = 2.4 days by 2-way analysis of variation.

5.3.1.2. Leaf vase life

Vase life of overall cultivars was significantly ($P < 0.05$) decreased by addition of 58.5 mmol sugars in vase water containing 200 mg L^{-1} HQS compared to vase life in the DI controls (Table 5.3). For sucrose, fructose and glucose vase life decreased by an average 30% and by 50% for maltose and galactose compared to the DI controls (Table 5.3). Leaf vase life response of individual cultivars to different types of sugar was different. Leaf vase life of cultivars 'Lady Stephanie' significantly ($P < 0.05$) increased by an average 1.3 folds in all sugar types and leaf vase life of cultivars of 'Purple Pride', 'Mullering Brook' and 'WX87' in all types of sugars, 'Denmark Pearl' in sucrose and fructose and 'WX97' in glucose was similar to vase life in the DI controls (Table 5.3). Vase life of leaves of cultivars of *C. uncinatum* in vase solutions containing sugars was similar to those in the DI controls (Table 5.3). Alternatively, vase life of leaves of cultivars with *C. megalopetalum*, *C. sp. Gingin* or *Verticordia grandis* as a parent was significantly ($P < 0.05$) decreased half in vase solutions containing sugars compared to the DI controls (Table 5.3). The addition of

sucrose significantly ($P < 0.05$) extended leaf vase life of *C. floriferum* hybrid 'Lady Stephanie' by 1.3 folds and by 1.1 folds for *C. uncinatum* cultivars while decreased leaf vase life of cultivars with *C. megalopetalum*, *C. sp. Gingin* and *Verticordia grandis* as a parent by half (Table 5.3).

Table 5.3. Effect of different types of sugar on vase life of leaves of cultivars

Cultivars	Vase life (days)					
	Control	Sucrose	Maltose	Glucose	Fructose	Galactose
'Crystal Pearl'	38.5	19.62	9.5	22.4	19.5	20.3
'Laura Mae Pearl'	34.9	21.3	8.8	26.6	25.9	19.5
'Bridal Pearl'	29.3	16.4	8.3	11.4	10.3	10.0
'Denmark Pearl'	19.5	16.5	12.4	14.9	15.8	13.4
'Lady Stephanie'	18.5	24.6	23.3	20.9	27.1	22.1
'WX87'	23.8	20.1	23.0	21.8	20.6	18.3
'WX73'	19.1	13.3	7.0	12.9	16.1	12.4
'Mullering Brook'	9.9	11.5	7.5	10.5	10.3	10.5
'WX97'	17.4	11.9	7.8	14.4	10.6	11.8
'Purple Pride'	13.8	13.4	13.5	12.9	14.1	12.3
Average means of vase life	22.5	16.9	12.1	16.9	16.6	15.1

LSD ($P < 0.05$) for sugar types = 1.3 days, for cultivars = 1.7 days and for sugar types × cultivars = 4.1 days by 2-way analysis of variation.

Vase life of flowers of *C. sp. Gingin* hybrid 'WX97' reported in this chapter was more responded to sucrose and HQS than cultivars with *C. megalopetalum* as a parent or *C. uncinatum* cultivars, while in chapter 4 the result was reversed (Fig. 4.1 and Table 5.1). Vase life of leaves of *C. uncinatum* cultivars in this chapter was also less responded to vase solution of sucrose and HQS compared to that in chapter 4 (Fig. 4.2 and Table 5.2).

5.3.2. Effect of sucrose concentrations supplemented with 200 mg L^{-1} HQS on vase life of waxflower cultivars

5.3.2.1. Vase life of flowers

Overall, increasing sucrose concentrations increased flower vase life of cultivars (Fig. 5.1A). However, flower vase life response to sucrose concentrations depended on cultivars. Vase life of 'Laura Mae Pearl' was significantly ($P < 0.05$) extended by 1.7 folds at a sucrose concentration of 29.2 mmol compared to the DI water and also significantly ($P < 0.05$) higher than those in 14.6 or 117 mmol sucrose but similar to that in 58.5 mmol sucrose. At a concentration of 58.5 mmol 'Mullering Brook' showed the greatest response to sucrose where flower vase life reached a maximum which was 1.3 folds increase but did not significantly ($P < 0.05$) differ to those in 29.2 or 117 mmol; whereas, vase life of other cultivars continued to increase with higher concentrations with 'Bridal Pearl' and 'Purple Pride' increasing 1.8 folds and 1.6 folds respectively in 117 mmol compared to the DI controls and was significantly ($P < 0.05$) higher than those in any sucrose concentrations (Fig. 5.1A). Vase life of 'Lady Stephanie' or 'Denmark Pearl' in sucrose concentrations was similar to each other but was significantly ($P < 0.05$) higher than that in the DI controls. Cultivar of 'WX97' had maximal vase life in 117 mmol sucrose but was similar to that in 58.5 mmol and was significantly ($P < 0.05$) higher than that in 14.6, 29.2 mmol and the DI control. Another cultivar of 'WX73' did not respond to increasing sucrose concentration compared to the DI control (Fig. 5.1A).

5.3.2.2. Vase life of leaves

Leaf vase life of all cultivars tested except for 'Lady Stephanie', 'Mullering Brook' 'Purple Pride' and 'WX97' decreased with increasing sucrose concentrations. Leaf vase life of 'Lady Stephanie' was significantly ($P < 0.05$) increased by 1.3 folds to a maximum at concentrations of 14.6 mmol compared to the DI control. Leaf vase life of *C. uncinatum* cultivars was significantly ($P < 0.05$) increased by 1.2 folds at concentration of 58.5 mmol and was similar to those in the DI controls when being treated with 29.2 mmol sucrose. Lower concentration at 14.6 mmol or higher concentration at 117 mmol significantly ($P < 0.05$) decreased leaf vase life by 13.5% and 16.4% respectively. Alternatively, vase life of leaves of the remaining cultivars

was deceased by 37% to 54% in vase solutions containing sucrose compared to the DI controls (Fig. 5.1B).

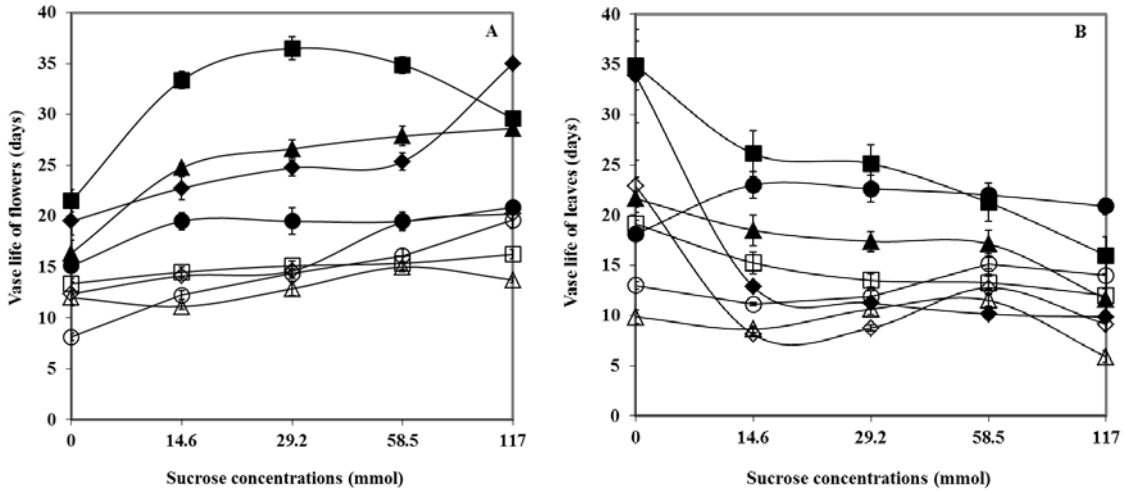


Figure 5.1. Vase life of flower (Panel A) and leaves (Panel B) of 'Laura Mae Pearl' (■), 'Bridal Pearl' (♦), 'Denmark Pearl' (▲), 'Lady Stephanie' (•), 'WX73' (□), 'WX97' (◊), 'Mullering Brook' (Δ), and 'Purple Pride' (o) in different sucrose concentrations supplemented with 200 mg L^{-1} HQS, and deionized water. LSD (P < 0.05) for flowers = 2.2 days and for leaves = 4.0 days by 2-way analysis of variation, n = 8. Vertical bars are standard errors of means.

5.3.4. Sucrose vs. HQS concentrations

Maximal vase life of cultivar 'Alba' was obtained with low concentration of sucrose in combination with low concentration of HQS (Fig. 5.2A and 5.2B). Vase life of flowers of 'Alba' was significantly (P < 0.05) extended by 1.6 folds to a maximum in vase solution of 50 mg L^{-1} HQS and 14.6 mmol sucrose. As HQS concentrations increased the maximum vase life of flowers of 'Alba' decreased and occurred at high sucrose concentrations with the maximum vase life decreased by 35% in 117 mmol sucrose and 400 mg L^{-1} HQS compare to a vase solution of 14.6 mmol sucrose and 50 mg L^{-1} HQS (Fig. 5.2A). Vase life of flowers in increasing sucrose concentrations could not be improved by increasing HQS concentrations with a 26% decrease in vase life occurring in 117 mmol sucrose and 400 mg L^{-1} HQS compared to 50 mg L^{-1} HQS (Fig. 5.2A).

Vase life of leaves was significantly (P < 0.05) extended by 1.6 folds to a maximum in vase solution of 50 mg L^{-1} HQS plus 14.6 mmol sucrose, and remained

at a similar level to that in 50 mg L^{-1} HQS plus 29.2 mmol sucrose (Fig. 5.2B). At higher levels of sucrose of 58.5 and 117 mmol sucrose, leaf vase life significantly ($P < 0.05$) decreased (Fig. 5.2B). For higher concentrations of HQS of 100 and 200 mg L^{-1}, leaf vase life reached a maximum at 29.2 mmol sucrose. At 400 mg L^{-1} HQS, leaf vase life decreased in 14.6 to 117 mmol sucrose (Fig. 5.2B).

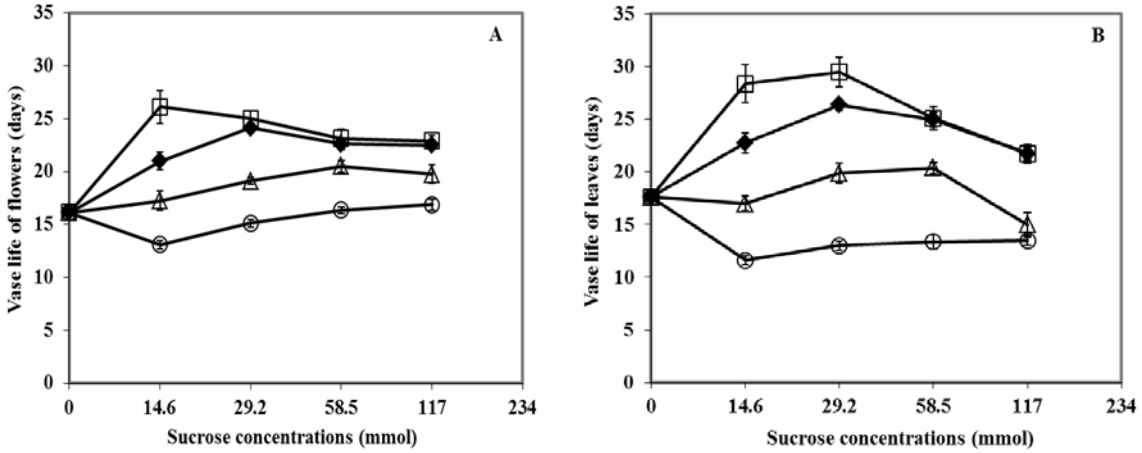


Figure 5.2. Effect on vase life of flowers (Panel A) and leaves (Panel B) of *C. uncinatum* 'Alba' treated with 14.6, 29.2, 58.5 or 117 mmol sucrose in combination with 50 (□), 100 (♦), 200 (Δ) or 400 (o) mg L^{-1} HQS. Nil concentration of sucrose on the graph indicates flower stems held in deionized water. Vertical bars are standard errors of means. LSD ($P < 0.05$) for flowers = 2.0 days and for leaves = 2.6 days, n = 8.

5.3.5. Relationship between vase life of flowers and day for stems fresh weight above or below initial stems weight of cultivars in different types of sugars

Long vase life of cultivars in sugars had higher days for stems fresh weight above initial stems fresh weight (DFWA) compared to days for stems fresh weight below initial stems fresh weight (DFWB) (Fig. 5.3). DFWA of cultivars in all types of sugar was higher with 2.0 folds increase than that in the DI controls. Overall cultivars, DFWA for sucrose, fructose or glucose was 108%, 85.2% or 51.3% higher than DFWB respectively while DFWA for galactose was 40.3% higher than DFWB. Alternatively, DFWA for maltose or DI controls was 13.4% or 35.3% lower than DFWB respectively. DFWA of cultivars with *C. megalopetalum* as a parent or *C. uncinatum* 'Mullering Brook' in vase solutions was 116.3% and 81.9% greater than DFWB while DFWA of *C. floriferum* hybrid 'Lady Stephanie', *C. sp. Gingin* hybrid

'WX97' and *Verticordia grandis* hybrid 'WX73' was 17.2%, 48.1% and 35.6% lower than DFWB respectively (Fig. 5.3).

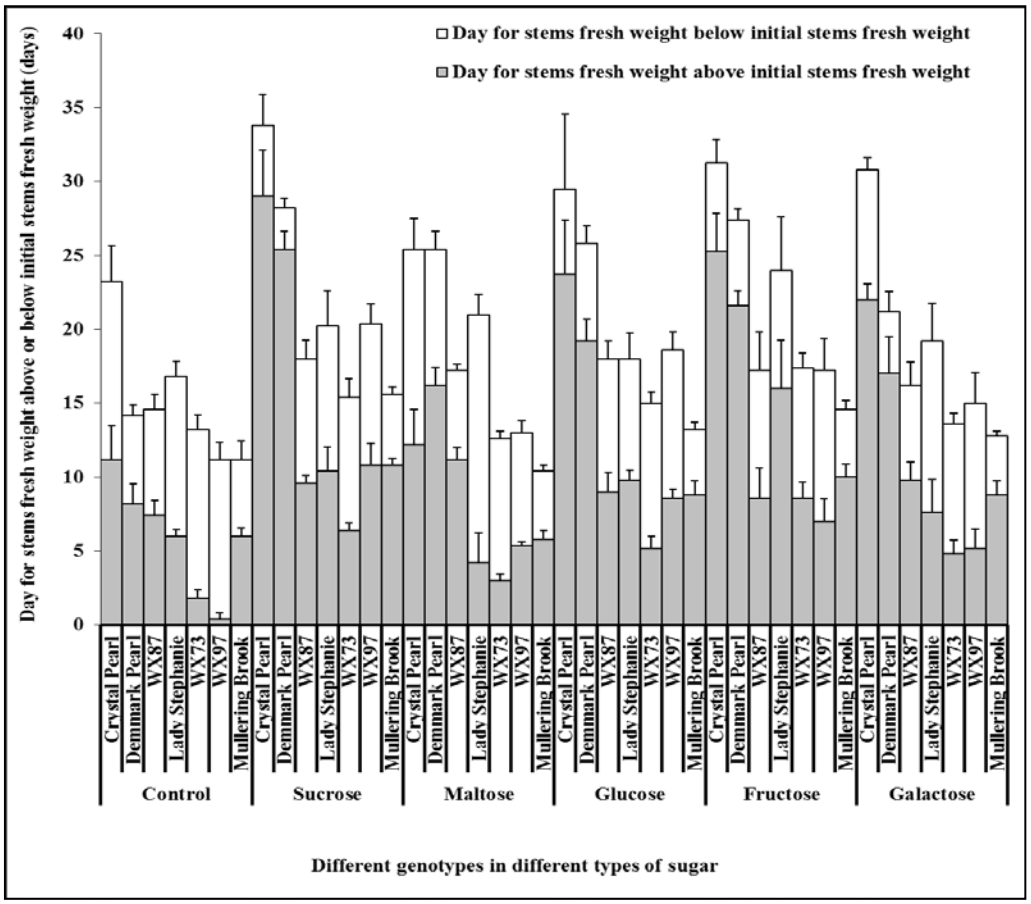


Figure 5.3. Relationship between vase life of flowers and day for stems fresh weight above or below initial stems fresh weight of different cultivars in 58.5 mmol of sucrose, maltose, glucose, fructose or galactose supplemented with 200 mg L^{-1} HQS. DI water was used as a control. Vertical bars are standard errors of means, n = 5.

5.3.6. Relationship between vase life of flowers and day for stems fresh weight above or below initial stems weight of cultivars in different concentrations of sucrose, fructose or glucose

Vase life of flowers of cultivars in different concentrations of sucrose (Fig. 5.4), fructose (Fig. 5.5) or glucose (Fig. 5.6) increased with increasing DFWA over DFWB. DFWA of cultivars in all sucrose, fructose or glucose concentrations was higher with 2.0, 1.9 or 2.0 folds increase respectively than those in the DI controls. For sucrose, DFWA of cultivars in 58.5 or 117 mmol was 90.6% or 92.9% greater than DFWB respectively while DFWA of cultivars in 29.2 or 14.6 mmol was 60.9%

or 18.7% higher than DFWB respectively. Alternatively, DFWA of cultivars in the DI control was 14.0% lower than DFWB (Fig. 5.4). Similarly, DFWA of cultivars in 117 mmol fructose or glucose was 71.9 % or 167.2% higher than DFWB respectively while DFWA of cultivars in 14.6 mmol fructose was 18% lower and DFWA of cultivars in 14.6 mmol glucose was only 1.3% higher than DFWB (Fig. 5.5 and 5.6).

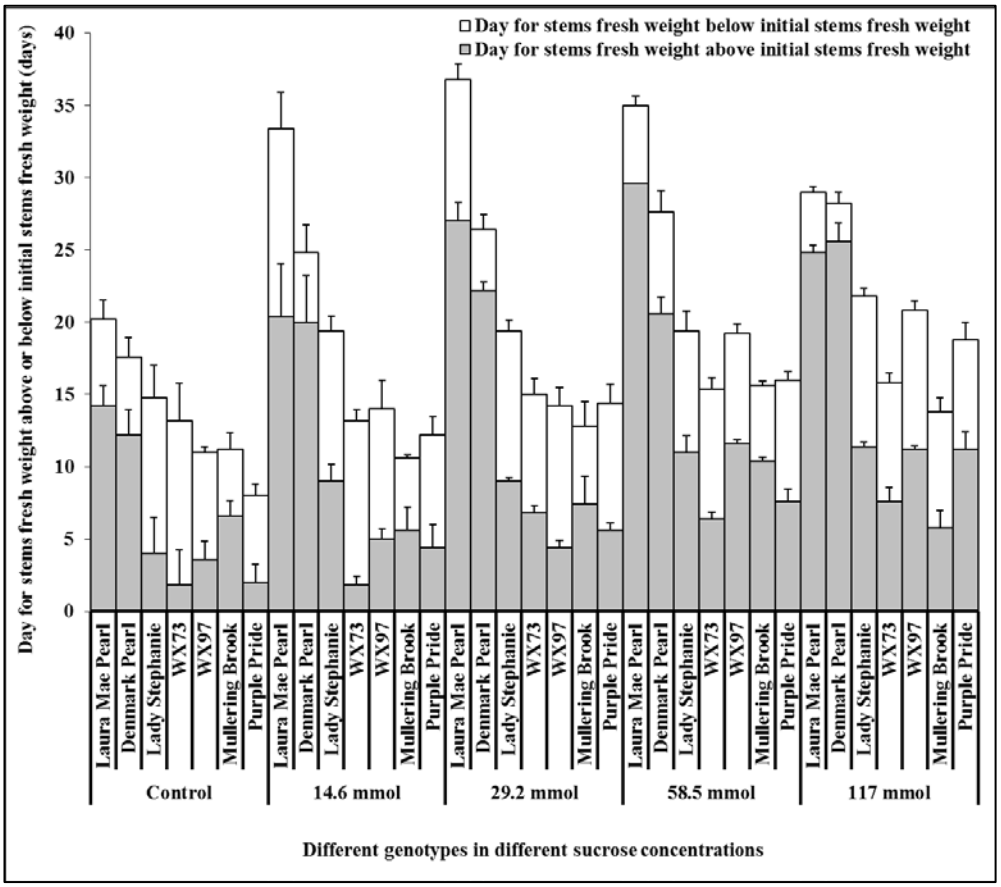


Figure 5.4. Relationship between vase life of flowers and day for stems fresh weight above or below initial stems fresh weight of different cultivars in different sucrose concentrations supplemented with 200 mg L^{-1} HQS. DI water was used as a control. Vertical bars are standard errors of means, n = 5.

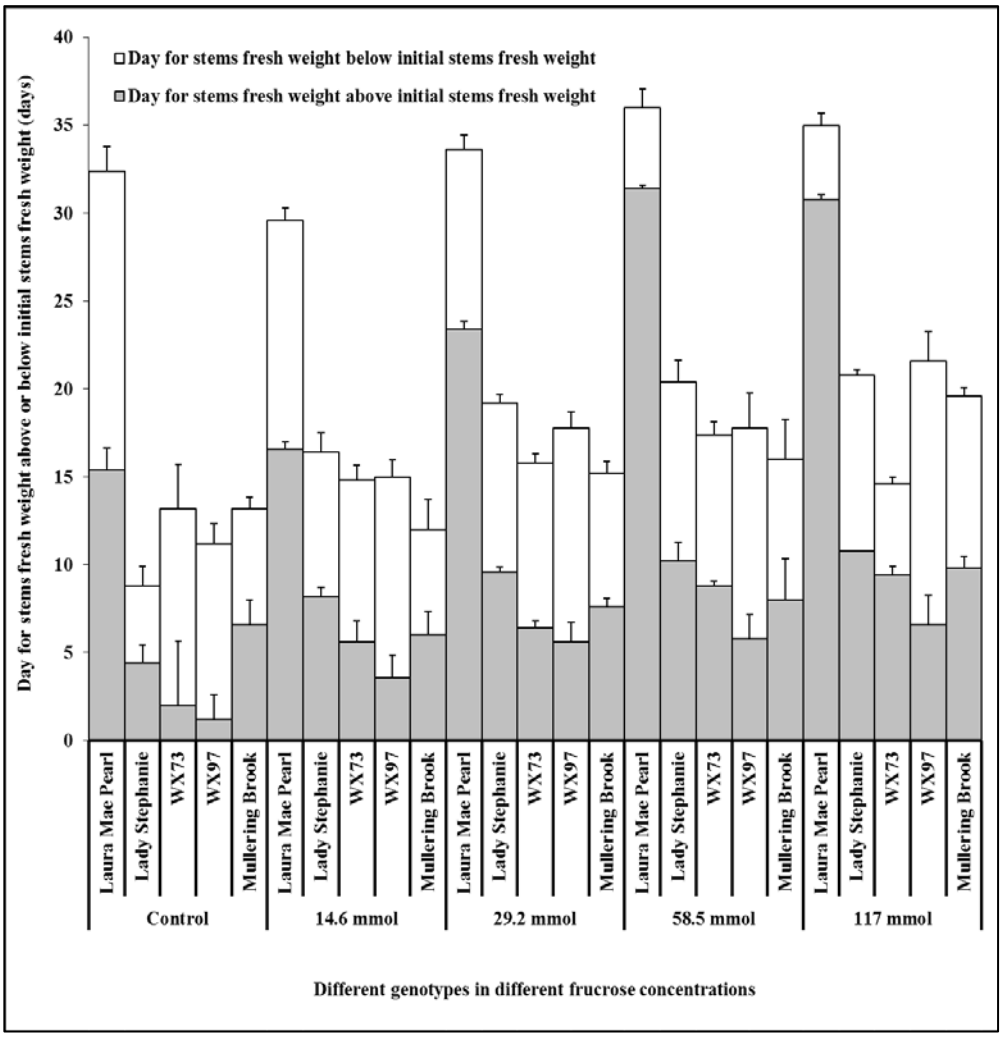


Figure 5.5. Relationship between vase life of flowers and day for stems fresh weight above or below initial stems fresh weight of different cultivars in different fructose concentrations supplemented with 200 mg L^{-1} HQS. DI water was used as a control. Vertical bars are standard errors of means, n = 5.

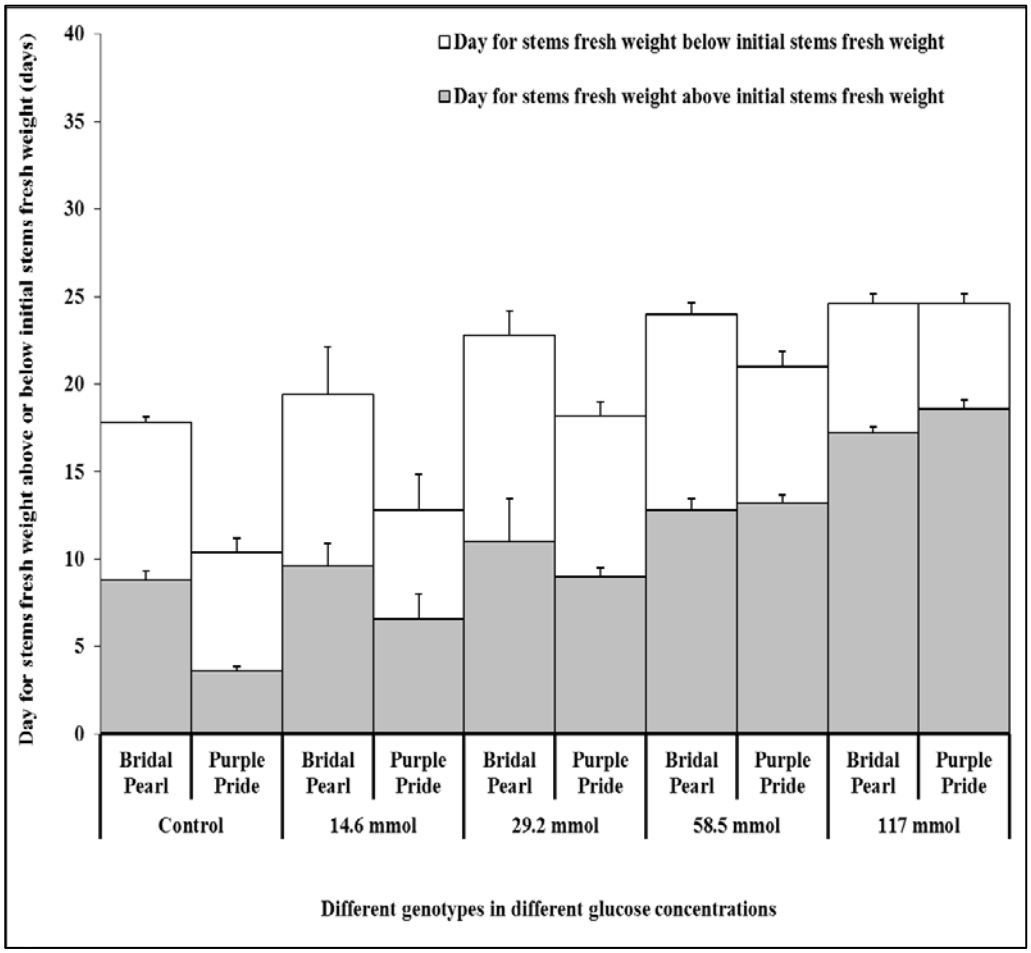


Figure 5.6. Relationship between vase life of flowers and day for stems fresh weight above or below initial stems fresh weight of different cultivars in different glucose concentrations supplemented with 200 mg L^{-1} HQS. DI water was used as a control. Vertical bars are standard errors of means, n = 5.

5.3.7. The relationship between flower vase life, stem fresh weight and day for stem fresh weight reaching 75% of initial weight

The percentage of fresh weight at the end of vase life was on average 74.6 ± 4% for over all cultivars and sucrose concentrations (Table 5.4). This result was consistent with resulted obtained for vase solutions in Chapter 4. In a vase solution of 14.6 mmol sucrose there was a significant ($P < 0.05$) decrease on the average of 9.9% fresh weight at the end of vase life compared to in the DI control. While for other concentrations no difference occurred in average fresh weight at the end of vase life for all cultivars compared to the DI controls.

Table 5.4. Percentage of stem fresh weight at the end of flower vase life in vase solution containing sucrose at different concentrations (mmol) in combination with 200 mg L^{-1} HQS

Cultivars	Control	14.6	29.2	58.5	117
'Laura Mae Pearl'	89.9 ± 2.4	82.6 ± 1.7	84.4 ± 2.9	94.1 ± 3.3	93.3 ± 2.7
'Bridal Pearl'	86.5 ± 1.5	70.8 ± 3.1	77.6 ± 2.4	88.5 ± 2.5	103.3 ± 2.4
'Denmark Pearl'	86.4 ± 3.8	85.3 ± 3.3	91.2 ± 7.3	85.1 ± 6.1	105.4 ± 4.8
'Lady Stephanie'	75.1 ± 3.6	63.6 ± 7.9	68.7 ± 4.3	61.0 ± 6.8	59.0 ± 3.3
'WX73'	64.5 ± 5.2	58.7 ± 4.0	55.6 ± 4.8	60.9 ± 3.9	65.2 ± 3.9
'WX97'	74.8 ± 5.7	58.7 ± 4.1	70.6 ± 2.7	68.4 ± 10.4	65.2 ± 3.9
'Mullering Brook'	76.9 ± 1.0	64.8 ± 1.1	66.2 ± 2.4	72.1 ± 3.7	63.5 ± 3.3
'Purple Pride'	72.2 ± 4.4	62.9 ± 4.2	55.9 ± 6.9	76.0 ± 4.2	74.9 ± 2.2
Average means of fresh weight	78.3 ± 3.4	68.4 ± 3.7	71.3 ± 4.2	75.8 ± 5.1	79.4 ± 3.6

± = standard errors of the means.

Figure 5.7. Vase life of leaves of 'Denmark Pearl' in DI water (a) and in 58.5 mmol sucrose and 200 mg L^{-1} HQS (b) at day 16. Arrow points show visible symptom of chemicals injury on leaves.

5.4. Discussion

The increase in vase life of flowers by supplying sucrose and HQS in chapter 4 and this chapter is consistent with results obtained for *C. uncinatum* cultivars (Joyce, 1988). It is also consistent with sugar providing carbohydrate source for flower respiration and reducing the induction of endogenous ethylene of cut sweet pea (Ichimura and Suto, 1999). Increased vase life of cut flowers by sugar application has been attributed to the increased water uptake of the flowers through increasing the osmotic concentration of the florets and leaves (Pun and Ichimura, 2003). Waxflowers responded to increasing sugars concentration (i.e. increasing amount of sugars) with increased vase life of flowers but not for leaves. These effects may have been due to the increasing osmotic concentration. In the case of flowers this may have allowed flowers to take up more water during vase life while for leaves they may have lost water. However these effects may become unbeneficial at high concentrations due to toxic effects. In Geraldton wax sugar was most effective when applied to vase solutions with a biocide such as HQS to prevent growth of the microorganisms in xylem vessels and maintain water uptake, prolonging longevity of cut flowers (Asrar, 2012) but can become toxic. The difference in vase life response to sugar of cultivars within specie in chapter 4 this chapter could have been affected by seasonal condition as occurred for Gerbera (Acharya et al., 2010).

The greater response of Geraldton wax to normally respired sugars in plants of glucose and fructose is consistent with cut roses where fructose, glucose and sucrose were the main soluble carbohydrates decreasing slowly during the vase life (Ichimura et al., 1999) and decreased sucrose conversion to a respirable carbohydrate occurs when there is loss of sucrose synthase and invertase activities (Yamada et al., 2007; Kumar et al., 2008,). The osmotic effects of fructose, glucose and sucrose would have been expected to be similar as the same number of moles was used. In the case of fructose or glucose where concentration was approximately half as amount of sucrose, but their effect on vase life of flowers were similar. The lower vase life response to maltose and galactose are rare types of sugar in flowers (Table 5.1) and therefore are less effective in increasing vase life of waxflowers. Vase life increase in vase solutions containing fructose or glucose may be due to the flower cells being supplied with increased respiratory substrate to maintain stems water

balance and maintain stems fresh weight above initial stems fresh weight longer when compared to maltose or galactose. The affecting way of different sugar types on vase life of flowers is unclear. However, a study on *Petunia* 'Mitchell' by O'Donoghue et al. (2008) showed that galactose content in petals doubled when flowers opened and then decreased sharply by 2.0 days after flowers opening; whereas, amounts of other sugars showed little change over this time. This suggested that the loss of galactose may also affect vase life of flowers. Variation in genotype postharvest response to sucrose was reported in *Protea* 'Sylvia' where vase life did not improve with a sucrose pulse at 584.8 mmol (Stephens et al., 2011) while a 584.8 mmol sucrose pulse (24 h, 25°C) reduced leaves blackening in *Protea neriifolia* R. Br. and increased vase life of flowers (McConchie and Lang, 1993).

Increasing concentrations of sucrose resulted in an increase in supplied carbohydrates source (Nichols, 1973) and osmosis concentration (Pun and Ichimura, 2003) and increased flower vase life of waxflowers, confirming the result obtained for *C. uncinatum* cultivars (Joyce, 1988). Competition for sugar between flower-buds, opening-flowers and older flowers shortened vase life of flowers of lily (van der Meulen-Muisers et al., 1995). Flowers of 'Purple Pride' and cultivars with *C. megalopetalum* as a parent are bigger than flowers of the remaining cultivars (Macnish et al., 2004), which may have resulted in a higher demand for carbohydrates. Increasing sucrose concentrations may have satisfied the requirement for carbohydrates of these cultivars and consequently increased vase life of flowers. The decrease in vase life of leaves in increasing sucrose concentrations could have been caused by osmotic stress in leaves due to high concentration use of sugar. Joyce (1988) reported that sucrose at concentration of 146.2 mmol in combination with 200 mg L^{-1} HQS desiccated leaves of *C. uncinatum* cultivars through osmotic stress. However, in this study a 40% lower sucrose at concentration of 58.5 mmol damaged to leaves of almost all cultivars (Fig. 5.7), except for 'Lady Stephanie' and cultivars of *C. uncinatum*. Leaves of these cultivars may have not been damaged by sugar, indicating leaves did not take up as much sugar as leaves of stressed cultivars. Vase life response of cultivars to glucose or fructose concentrations was also similar to vase life response of cultivars to sucrose concentrations (Fig. 1 and 2, Appendix I).

Increasing concentrations of sucrose in higher concentrations of HQS increased vase life of 'Alba'. Response to sucrose of cultivars 'Alba' may indicate a

different sensitivity as at lower concentrations prolonged the vase life of gladiolus florets by increasing water uptake; whereas, higher concentrations seemed to impede water uptake (Bravdo et al., 1974). Sucrose with biocides has become important preservatives for floral several cut flowers (Pun and Ichimura, 2003). Application of HQS, an antimicrobial agent (Ketsa et al., 1995) increased water uptake and fresh weight and consequently increased vase life of cut rose (Ichimura et al., 1999). Supplying HQS leads to prevention from activity of ACC enzyme; as a result, reduces ethylene generation and promotes longevity of flowers. HQS coupled with sucrose increased flower quality, water uptake, fresh weight, flower freshness and reduced respiration rate and physiological weight loss of dendrobium (Dineshbabu et al., 2002) and gladiolus (Beura et al., 2011). However, HQS at high concentrations can become toxic and decrease vase life of ‘Alba’. This was consistent with result obtained in chapter 4. The optimal concentrations of sucrose and HQS for increasing vase life of ‘Alba’ were far below those reported for waxflowers (Joyce, 1988).

5.5. Conclusions

Critical levels of sugars were found for a range of cultivars to be less (approximately half) that recommended and this depended on the types of sugar. Fructose, glucose and sucrose were the most effective sugars on increasing vase life of waxflowers. The concentration of sugar where vase life was improved for both flowers and leaves was from 14.6 to 29.2 mmol. The advantage of using sugar depended on the level of biocide present such as HQS at this can become toxic at concentration normally used and levels of one-quarter of this extended vase life. Vase life of flowers of cultivars increased with increasing day for stems fresh weight above initial stems fresh weight.

The next chapter will examine the relationship between sink and source of waxflowers.

CHAPTER 6

Effect of changes in weight of sources and sinks on vase life of waxflowers

Abstract

The relationship between vase life of flowers and leaves of waxflowers was studied by examining the effect of changes in proportion of flowers to leaves on vase life changes of flowers and leaves. Competition for water and carbohydrates between flowers and leaves influenced vase life. Removal of flowers had at least 4 times the effect on leaf vase life as removal of leaves on flower vase life. Supplying exogenous sucrose to satisfy the demand for carbohydrates negated this effect, indicating that flowers dependence on carbohydrates being supplied from leaves to maintain vase life. Cultivars having greater proportion of flowers (on a weight basis) improved vase life of flowers at the expense of leaves. Cultivars with large flowers or many small flowers or greater weight ratio of flowers to stem appeared to draw more carbohydrates and water from leaves giving them longer vase life and decreasing vase life of leaves. The vase life of flowers increased with stage of opening of flowers up to 50% opening and then decreased up to 100% opening.

6.1. Introduction

Improved vase life of cut waxflowers increased the price of cut stems (Sutton, 2004). Competition between flowers and leaves for carbohydrates and water may change vase life of flowers and leaves, as occurred for *Protea neriifolia* L. where flowers drew carbohydrates from leaves and consequently decreased vase life of leaves (Dail and Paull, 1995); whereas, leaves of *Grevillea* 'Crimson Yu-lo' competed with flowers for water, resulting in a decrease in vase life of the inflorescence (He et al., 2006).

Flowers are a significant sink for carbohydrates, deriving them from leaves where sucrose is hydrolyzed and the resulting glucose and fructose are transported to flower buds (Paulin, 1981; Marissen and La Brijn, 1995). Removal of leaves of cut rose led to a decrease in carbohydrates concentration in flower buds and the removal of the corolla resulted in an increase in carbohydrates level in leaves (Marissen and La Brijn, 1995). Similarly, removal of inflorescence of cut *Protea neriifolia* L. delayed the onset of the leaf blackening and increased levels of starch and carbohydrate in leaves (Dail and Paull, 1995). Removal of flowers of cut rose

'Forever Yours' and cut carnation 'White Sim' reduced water uptake by 20.4% and 27.1%; however, the removal of leaves from those cut flowers decreased water uptake by 78.5% and 37.3% respectively (Carpenter and Rasmussen, 1974).

The reduction of longevity of cut flowers in vases is often caused by water stress (van Doorn, 1997). The presence of leaves on stems resulted in a greater loss of water in inflorescence of *Grevillea* 'Crimson Yu-lo' and consequently decreased vase life of the inflorescence. Vase life of *Grevillea* 'Crimson Yu-lo' flowers on cut stem with 4 or 6 leaves was significantly shorter when compared to the vase life of flowers with zero or 2 leaves per stem (He et al., 2006). Leaves of *C. uncinatum* 'Purple Pride' and 'Alba' had higher turgor and lower osmotic potential than flowers, resulting in a greater decrease in water potential in leaves than in the flowers. Increased water balance led to an increase in vase life of leaves of cultivars of *C. uncinatum* (Joyce and Jones, 1992).

Increased vase life of *C. uncinatum* 'Alba' and 'Purple Pride' with exogenously applied sucrose, abscisic acid and/or KCl was mainly due to improved water uptake and water balance (Joyce and Jones, 1992). Vase life was further improved by also adding the antibacterial chemical (Joyce, 1988; Joyce and Jones, 1992). In conclusion, leaf is a source of carbohydrates for the cut flower contributing to extend vase life, whilst evapotranspiration from the leaf surface leads to decreased water balance causing water stress in the stems therefore diminishes vase life of cut flowers. The aim of this study was to determine the relationship between source and sink as well as the effect of changes in weight of flowers on the changes in vase life of flowers and leaves of cultivar.

6.2. Materials and methods

6.2.1. Plant materials

Cultivars of *C. uncinatum* 'Purple Pride', 'Micro wax', 'Dancing Queen' and 'WX69', *Verticordia* hybrids 'Southern Stars', 'WX73' and 'WX35' and *C. megalopetalum* hybrids 'Laura Mae Pearl' and 'WX87' were cultivated using irrigation and fertigation at Medina Research Station (32°13'18"S, 115°38'50"E) of the Department of Agriculture and Food Western Australia (DAFWA). Flowering

stems were harvested from July through November, 2012. Flowering stems of approximately 50% flower opens were picked followed by harvesting and transferring procedures as described in Chapter 3, section 3.2.1.

6.2.2. Experimental design and treatments

Experiment 1: Effect of removal of flowers or leaves on vase life of different cultivars

Cut stems of cultivars of 'Purple Pride', 'Micro wax', 'Dancing Queen', 'WX69', 'Southern Stars', 'WX73', 'WX35' and 'Laura Mae Pearl' were subject to following treatments (i) intact stems, (ii) all leaves removed from stems and (iii) all flowers and buds removed from stems. The stems were placed in vases of either deionized water or 29.2 mmol sucrose and 100 mg L^{-1} HQS for vase life comparison.

Experiment 2: Effect of changes in weight ratio of flowers to stems on the changes in vase life ratio of flowers to leaves

Thirty-cm flowering stems of cultivars 'Purple Pride', 'Micro wax', 'Dancing Queen', 'WX69', 'Southern Stars', 'WX73' and 'WX35' with a different flower weights and densities to give a range of weight ratios of flowers to stems were selected for the study. The weight ratio of flowers to stems was determined by weighting flowers and the whole stems and an average value was used to determine the relationship. Thirty-cm flowering stems of cultivars were either placed in DI water or 29.2 mmol sucrose and 100 mg L^{-1} HQS for vase life assessment and the vase life ratio of flowers to leaves was determined. There were eight replicates for vase life each treatment.

Experiment 3: Effect of changes in weight ratio of flowers to stems of 'WX87' through different flowering stages on the changes in vase life ratio of flowers to leaves

Cut stems of 'WX87' at 25, 50 and 100% flowers opening stages (Table 6.1) were harvested during September, 2012. An opened flower was determined when the entire receptacle and anthers was visible (Seaton, pers. com.) (Fig. 6.1). The weight of flowers and stems of thirty-cm flowering stems were weighted eight times to determine the weight ratio and averaged values were used. Vase life in DI water

alone or 29.2 mmol sucrose plus 100 mg L^{-1} HQS for eight thirty-cm stems at each flowering stage was recorded and vase life ratio of flowers to leaves was determined.

Table 6.1. Stages of opening of flowers of 'WX 87'

Stage	Degree of opening
1	25%
2	50%
3	100%

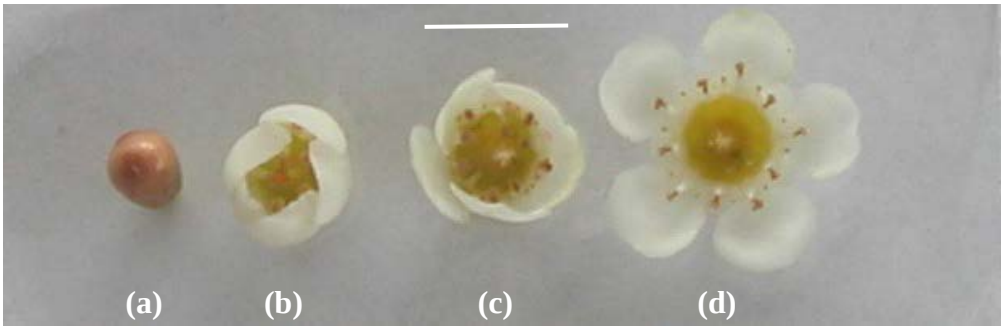


Figure 6.1 Determination of an opened–flower of 'WX87': bud (a), partially opened–flower (b), open–flower (c), and fully opened–flower (d). Scale bar represents 1cm.

All experimental vase life assessment was conducted in a vase room maintained at 20 ± 2°C, 60 ± 10% RH with a 12 h photoperiod. The light flux densities are 8 μmol m^{-2} s^{-2}. In each treatment, individual stems were placed in a 250ml–vase containing various vase solution treatments. Experiments were arranged in a completely randomized design and each treatment was repeated eight times.

6.2.3. Measurements

6.2.3.1 Vase life and flower drop

Vase life was recorded as described in Chapter 3, section 3.2.5.1.

6.2.3.2. Organs measurement

Each organ of cut stems was measurement as described in Chapter 3, section 3.2.5.3.

6.2.3.3. Ratio measurement

6.2.3.3.1. Weight ratio of flowers to stems

Weight ratio of flowers to stems was determined as weight of flowers over weight of stems.

6.2.3.3.2. Vase life ratio of flowers to leaves

Vase life ratio of flower to leaf was determined as flower vase life over leaf vase life.

6.2.4. Vase solution preparation

Vase solutions were prepared by dissolving 10 g of table sugar (CSR white sugar) and 100 mg HQS into 1 L of deionized water (DI) to making a vase solution of 29.2 mmol sucrose and 100 mg L^{-1} HQS.

6.2.5. Data analysis

Vase life of flowers and leaves from treatments effects were analyzed by 2-way ANOVA using the statistical package Genstat XV (Lawes Agricultural Trust, Rothamsted Experimental station, UK). Replication was eight folds consisting of a single stem in individual vases. Treatment means were compared by LSD at $P < 0.05$ and standard errors of the mean (± SE) are shown as appropriate. Correlation coefficient between flower to leaf vase life ratio and weight ratio of flowers to stems was analyzed with the statistical package Genstat XV by using simple linear model or polynomial regression.

6.3. Results

6.3.1. Effect of removal of leaves or flowers on vase life of waxflowers

Removal of leaves significantly ($P < 0.05$) increased vase life of flowers of all cultivars in DI water by an average of 4.8 days or 30% while removal of flowers significantly ($P < 0.05$) increased vase life of leaves of all cultivars by an average of 21.7 days or 117.5% compared to intact stems (Fig. 6.2a and 6.2b). The addition of sucrose reversed the gain in vase life from removal of leaves for most (six out of eight) cultivars (Fig. 6.2a) with a decrease in vase life of 2.2 days or 8.2%. Removal of flowers of stems held in a sucrose vase solution increased vase life of leaves of all cultivars by 7.2 days or 29.5% compared to those of intact stems (Fig. 6.2b). Exceptions were for cultivars of ‘WX73’, ‘WX35’ and ‘Dancing Queen’ where

removal of leaves in DI water did not significantly ($P < 0.05$) increase vase life compared to that of intact stems (Fig. 6.2a).

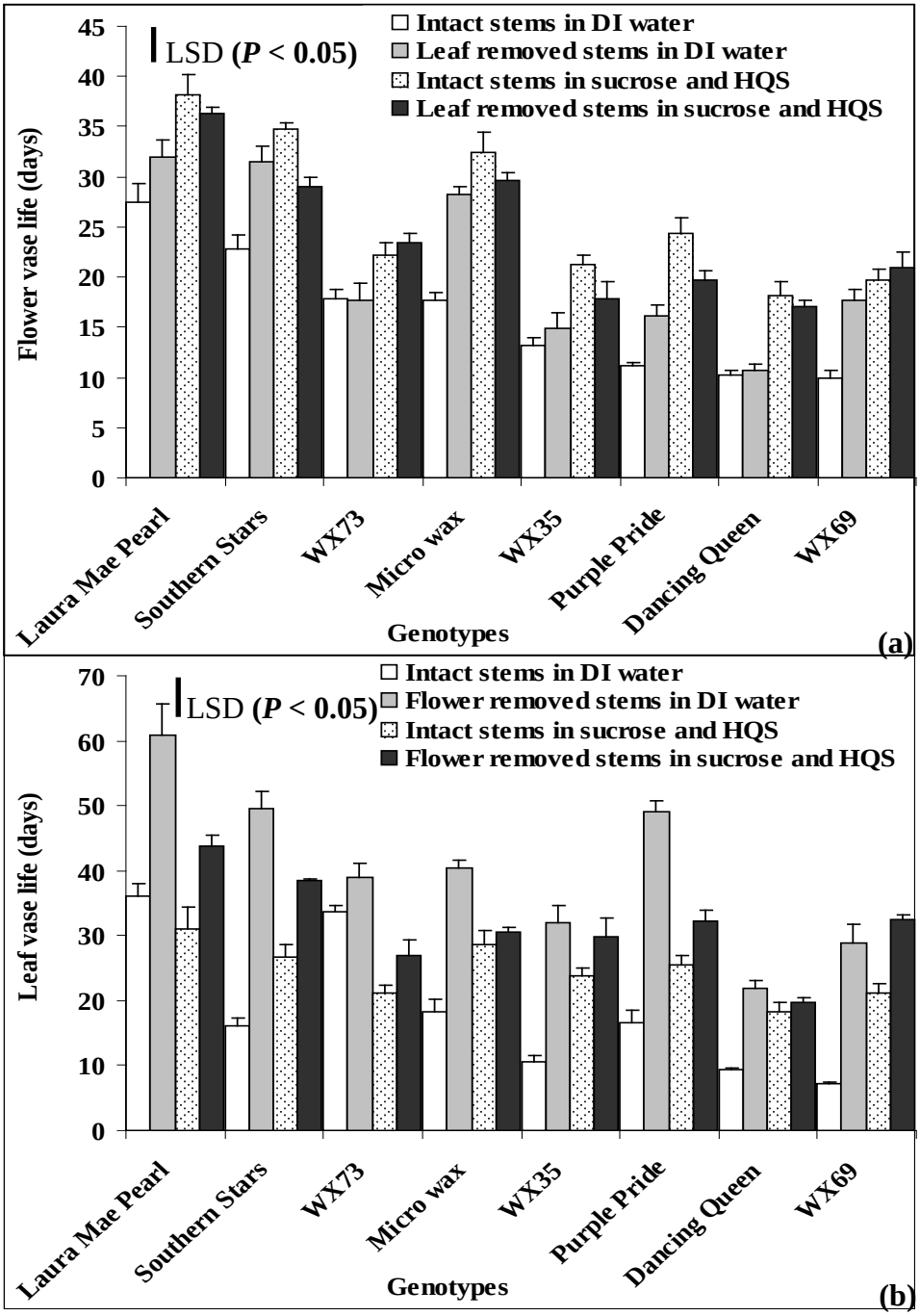


Figure 6.2. Vase life of flowers (a) and leaves (b) of leaf or flower removed stems respectively and of intact stems of cultivars in DI water and in 29.2 mmol sucrose and 100 mg L^{-1} HQS. Vertical bars are standard error of means was shown. LSD at $P < 0.05$ by 2-way analysis of variation.

6.3.2. Effect of changes in weight ratio of flower to leaves on vase life ratio of flowers to leaves of cultivars

Cultivar having a greater proportion of their weight in flowers compared to leaves gave a higher flower to leaf vase life ratio in DI water. Positive correlation between flower to leaf vase life ratio and weight ratio of flowers to stems was accorded to: y = 1.2532x + 0.6478, r^2 = 0.177 (P < 0.001, n= 56) (Fig. 6.3a). For flowers in sucrose and HQS, cultivar with higher flower weight ratio had lower flower to leaf vase life ratio. The correlation between flower to leaf vase life ratio and weight ratio of flowers to stems was accorded to: y = - 0.4242x + 1.2002, r^2 = 0.0743 (P < 0.04, n= 56) (Fig. 6.3b).

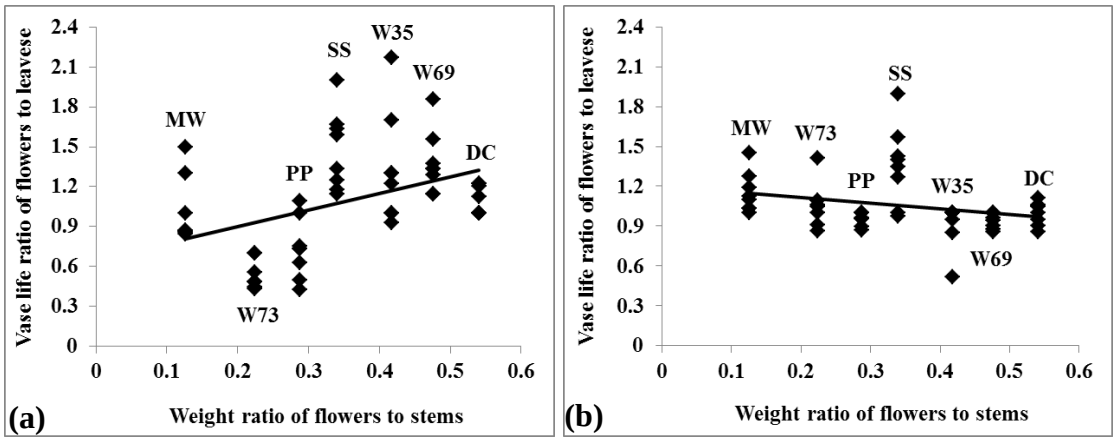


Figure 6.3 Regression relationships between flowers to leaves vase life ratio and flower weight ratio of cultivars of 'Micro wax' (MW), 'WX73' (W73), 'Purple Pride' (PP), 'Southern Stars' (SS), 'WX35' (W35), 'WX69' (W69) and 'Dancing Queen' (DQ) in DI water (a) and in 29.2 mmol sucrose and 100 mg L^{-1} HQS (b), n= 56.

6.3.3. Effect of changes in weight ratio of flowers to stems on vase life ratio of flowers to leaves through developmental stages of cultivar 'WX87'

Flower to leaf vase life ratio of 'WX87' in DI water increased as flowers opened up to 50% then decreased with increasing weight ratio of flowers to stems up to 100% open according to: y = - 0.10703x^2 + 5.5317x + 0.2725, r^2 = 0.2996 (P < 0.07, n= 24) (Fig. 6.4a) while flower to leaf vase life ratio in sucrose and HQS tended to decrease with increasing weight ratio of flowers and buds to stem according to: y = - 1.796x^2 – 0.0807 x + 1.12317, r^2 = 0.4309 (P < 0.001, n= 24) (Fig. 6.4b).

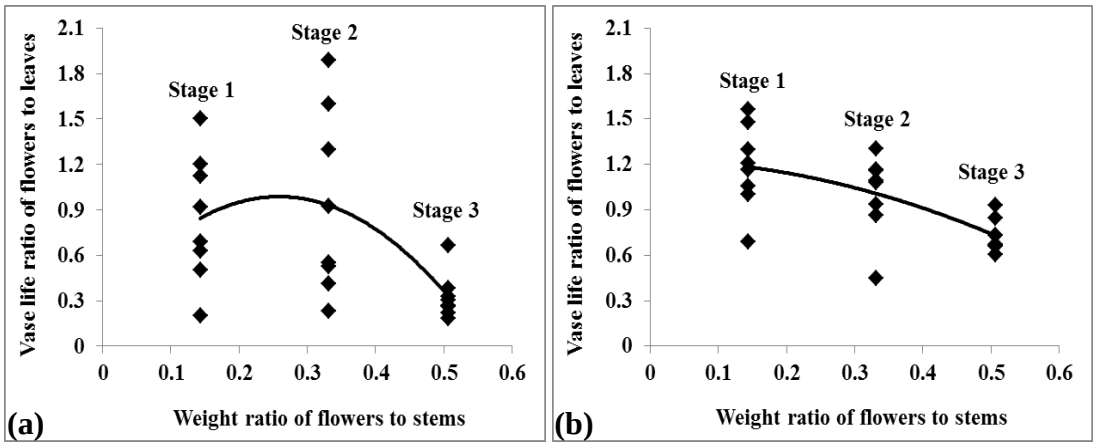


Figure 6.4 Regression relationships between flower weight ratio and flower to leaf vase life ratio of 'WX87' at different developmental stages in DI water (a) and in 29.2 mmol sucrose and 100 mg L^{-1} HQS (b), n = 24.

6.4. Discussion

Competing demand between flowers and stems appeared to be controlling the vase life of flowers and leaves in waxflowers. Under conditions of no added sugar vase life of flowers increased when leaves were removed, indicating that leaves competed with flowers for sugar and water. In the presence of sugar, the effect of removal leaves on vase life of flowers was less or non-existent. Response of vase life of leaves to loss of flowers was much greater than response vase life of flowers to loss of leaves while in sugar there was not much gain in vase life of leaves with removal flowers compared to that in DI water. This indicated that leaves of Geraldton wax were less competitive than flowers for water and particularly carbohydrates and flowers are a much stronger sink for carbohydrates than leaves as in *Protea neriifolia* where carbohydrates depletion in leaves, resulting in leaf blackening (Dail and Paull, 1995). These findings support the theory that leaves are considered a supporter providing carbohydrates for the development of flower buds (Marissen and La Brijn, 1995) but may compete for water as found for *Grevillea* 'Crimson Yu-lo' where removal of leaves increased vase life of flowers (He et al., 2006). The lack of response in vase life of flowers between leaf removed and intact stems of cultivars of 'WX73' may have been affected by physical injury producing endogenous ethylene and causing flower drop (Joyce, 1993) and shortening vase life of waxflowers (Seaton, pers. com.). Observation during vase life assessment (data not shown) showed that the flowers drop rate increased with leaf removed stems of 'WX73' than

that in other cultivars negating the gains in flower vase life of cultivars having leaves as a source of carbohydrates. Alternatively, the cultivars of 'Dancing Queen' and 'WX35' had higher flower weight than leaf weight. Therefore, leaf removal may not have had a significant effect on vase life of flowers; whereas, removal of flowers notably increased vase life of leaves.

Sucrose provided carbohydrates for flowers (Pun and Ichimura, 2003) and improved water uptake (Bravdo et al., 1974), and HQS reduced the growth of bacterial and xylem occlusion and increased water uptake, resulting in an increase in vase life of cut snapdragon (Asrar, 2012). Difference in vase life response of Geraldton wax cultivars having higher flowers weight ratios suggested that the large flower sink compared to leaves and thereby gained sugars and water so improving vase life.

During stages of flower opening (development) the sinks and sources balance changes allowing the open flowers to gain more sugars from leaves improving vase life. This agreed with the finding of leaf blackening in *Proteas* where depletion carbohydrates by the flowers increased with increasing flower open stages (Dail and Paull, 1995). However, as flower become fully open they aged and possible breakdown of cellular membranes may have reduced the size of the sink.

6.5. Conclusions

Flowers were strongly competitive with leaves for water and carbohydrates. Changes in vase life of flowers and leaves were caused by the changes in weight ratio of flowers to stems, depending on genotype. Cultivars had a higher flower weight ratio had more gain in vase life of flowers than cultivars with lower flower weight ratio. Vase life of flowers of cultivars maximized when flowers opened up to 50%.

CHAPTER 7

General discussion

A range of new waxflower cultivars have been recently bred for domestic and international markets and understanding their postharvest characteristics is important for management of these flowers and extending their postharvest life. Hence, an extensive study examining the effect of genotype, vase solutions and the relationship between flowers and leaves on vase life variation of waxflower cultivars derived from different species and hybrids was studied. This was done to provide for testing a wide range of genetic variation, developing a greater understanding of the mechanisms (or principles) determining plant vase life response and predicting vase life response for different cultivars. In this study it was found that:

(1) Genetic make-up of species or a particular cross controlled the length of vase life.

(2) The vase life of cultivars responded differently to vase solutions depending on genotypes.

(3) Different vase life expression of cultivars was also affected by types of sugars.

(4) The length of vase life of flowers was positively correlated with stems fresh weight changes.

(5) Competition between flowers and leaves for water and carbohydrates controlled vase life of waxflowers.

Although the genetic species composing a cross determines the length of vase life, the particular selection from a genetic cross is also important. Vase life of cultivars of genus *Campanula* was affected by genetic make-up of species where vase life of cultivars of *C. rapunculoides* was the longest with 4.6 days when compared to vase life of cultivars of *C. trachelium* (3.1 days), *C. barbata* (2.9 days) or *C. latifolia* (2.1 days) (Scariot et al., 2008). Similarly, a study on vase life of cultivars derived from various *Verticordia* species showed that vase life may be

affected by genetic make-up of species (Seaton, 2006a). Also sensitivity to ethylene causing flowers drop (Joyce, 1993) and possible shortening vase life of waxflower cultivars (Seaton, pers. com.) was affected by genetic make-up of species (Macnish et al., 2004). Cultivars of *C. megalopetalum* were less sensitive to ethylene than cultivars of *C. uncinatum* (Macnish et al., 2004). The vase life of cultivars with *C. megalopetalum* or *Verticordia plomosa* as a parent appeared to be longer than vase life of cultivars with only *C. uncinatum*. This may cause by the effect of *C. megalopetalum* or *Verticordia plumosa* where cultivars within those species tended to have longer vase life than cultivars of *C. uncinatum* (Seaton, 2006b; Seaton et al., 2010). Similarly, vase life of flowers of cultivars with *Verticordia grandis* as a parent being similar to those of cultivars with *C. sp. Gingin* as a parent and *C. uncinatum* cultivars but was shorter than vase life of flowers of cultivars with *Verticordia plomosa* as a parent because flower vase life of *Verticordia plomosa* cultivars tended to be greater than that of *Verticordia grandis* cultivars (Seaton, 2006a). However, the vase life of cut flowers was also affected by genotype of cross (Halevy and Mayak, 1979; Kende, 1993). Vase life of cultivars within *C. uncinatum* or *C. megalopetalum* or *Verticordia* hybrid was largely varied. This was consistent with the findings on Gerbera (Tesi, 1978), *Verticordia* (Seaton, 2006a) and *Anthurium* (Elibox and Umaharan, 2010) where vase life of cultivars with the same species was highly varied.

The vase life ratio of flowers to leaves depended on the genetic make-up of a hybrid. For waxflower the highest ratios were found for cultivars with *C. sp. Gingin*, followed by cultivars with *C. megalopetalum* as a parent compared to cultivars with *Verticordia* and *C. floriferum* as a parent. Lowest ratios were found for *C. uncinatum* cultivars. However, the vase life ratio of flowers to leaves also depended on cultivar. As the vase life of flowers of almost all *C. uncinatum* cultivars was longer than vase life of leaves but vase life of flowers of *C. uncinatum* 'Purple Pride' was shorter than vase life of leaves. Longer vase life of leaves than flowers of cultivars with *C. megalopetalum* as a parent was consistent with the findings of Seaton (2006b) and Seaton et al. (2010) and shorter vase life of leaves than flowers of cultivars with *C. uncinatum* as a parent was consistent with the findings of Joyce and Jones (1992). A similar variation between vase life of flowers and leaves was observed for different *Verticordia* species with leaves generally 1.4 folds longer (Seaton, 2006a).

For all genotypes, sugar improved vase life but the response was greater for *C. uncinatum* cultivars, followed by cultivars with *C. megalopetalum* compared to cultivars with *Verticordia, C. sp. Gingin* or *C. floriferum* as a parent; whereas, HQS alone was ineffective in improving vase life. Although HQS prevented growth of the microorganisms in xylem vessels and maintained water uptake of stems of cut snapdragons (Asrar, 2012), vase life of almost all waxflower cultivars studied tended to decrease with the presence of HQS at concentration of 200 mg L^{-1} in vase water. This suggests that HQS at this concentration was toxic for these cultivars. Contrarily, vase life of some cultivars was extended with HQS at this rate in vase water. This agreed with findings for cut rose where vase life of cultivar 'Sonia' increased after being treated with HQS (Ichimura et al., 1999) while cultivar 'Diana' did not increase vase life in treatment of HQS at the same rate (Liao et al., 2000). Silver thiosulphate (STS) has been used to reduce of endogenous ethylene production causing flower drop (Joyce, 1993; Seaton, 2005) and may increase vase life of waxflowers. STS also inhibited microbial population which caused vascular occlusions in stems of snapdragons (Asrar, 2012). In combination, vase solution of HQS and STS was more effective on improving vase life of waxflowers than HQS alone. Alternatively, vase life of cultivars responded the most to vase solutions containing sucrose, confirming results obtained on *C. uncinatum* cultivars (Joyce, 1988), dendrobiums (Dineshbabu et al., 2002) and gladioli (Beura et al., 2011). However, vase life of cultivars responded differently to vase solutions containing sucrose with more response found for cultivars of *C. uncinatum* or *C. megalopetalum* compared to cultivars with *C. sp. Gingin, C. floriferum* or *Verticordia* as a parent. The degree of increase in vase life of crosses within *Chamelaucium* species being treated with sucrose was also different. There also had an exception of *C. uncinatum* 'Mullering Brook' where vase life did not increase in vase solution of sucrose. Variation in vase life response of cultivars to sugar was found for *Anigozanthos* sp. (Kangaroo paw) (Teagle et al., 1991), *Proteaceae* and *Leucospermum* (Stephens et al., 2003) and *Eucalyptus* (Delaporte et al., 2005).

Vase life expression was differentially influenced by types and concentrations of sugars depending on genotype. Cultivars of *C. sp. Gingin* hybrid 'WX97' and *C. uncinatum* 'Purple Pride' and cultivars with *C. megalopetalum* as a parent responded

more than cultivars with *C. floriferum* or *Verticordia* as a parent. Cultivars within *Chamelaucium* species also responded differently to types of sugar. This agreed with the findings on *Anigozanthos* sp. (Kangaroo paw) (Teagle et al., 1991), *Proteaceae* and *Leucospermum* (Stephens et al., 2003) and *Eucalyptus* (Delaporte et al., 2005). The similar effect on vase life of waxflowers being treated with sucrose compared to fructose and glucose disagreed with finding for dendrobium (Rattanawisalanon et al., 2003). The mechanism effecting different vase life responses of cut flowers to different types of sugars requires further study. However, the changes of sugars amount during flowers opening period may cause a different effect on improving vase life as found on *Petunia* 'Mitchell' where galactose content in petals decreased sharply by 2.0 days after flowers opening; whereas, amounts of other sugars showed little change over this times (O'Donoghue et al., 2008). Similarly, sucrose content in flower heads of *Protea* 'Sylvia' decreased quicker than that of glucose, resulting in a shorter vase life of flowers being treated with sucrose than glucose (Stephens et al., 2011). In this study, vase life of flowers of cultivars terminated when stems fresh weight reached 75% of initial fresh weight and longer vase life of flowers was positively correlated with longer time of fresh weight of stems remained above 100% than between 100% and 75%. In comparison, Joyce and Jones (1992) reported that vase life of leaves of 'Purple Pride' ended when stems fresh weight reached 85%.

Vase life of flowers increased with increasing sugars concentrations which may be due to providing an increased substrate for respiration (Yamada et al., 2007; Kumar et al., 2008) or due to increased osmotic effects, drawing more water from leaves to supply flowers. Sucrose at lower concentrations prolonged the vase life by increasing water uptake; whereas, higher concentrations seemed to impede water uptake by osmotic shock from high sugar levels as occurred in gladiolus florets (Bravdo et al., 1974). This indicates that a balance is struck in the sucrose concentration where flowers benefits from high sugar compared to detrimental effects on leaves. A concentration of sucrose and HQS (i.e sucrose at 14.6 mmol and HQS at 50 mg L^{-1}) were the most effective vase solution for vase life of flowers and leaves of 'Alba'. These levels of sucrose and HQS were far below those reported by Joyce (1988).

Competing for carbohydrates and water between flowers and leaves affected vase life of flowers and leaves of waxflowers. Flowers of Geraldton wax strongly competed with leaves for water and carbohydrates to give them longer vase life and decreased vase life of leaves. It appears that leaves of waxflowers provided carbohydrates for the development of flower buds, as found for cut roses (Marissen and La Brijn, 1995), while leaves competed with flowers for water, as occurred in *Grevillea* 'Crimson Yu-lo' where removal of leaves increased vase life of flowers (He et al., 2006). The competition between flowers and leaves was also depended on genotype. Cultivars of *C. uncinatum* had greater weight ratio of flowers to stems than cultivars with *Verticordia* as a parent, resulting in a higher competition for sugar and water in *C. uncinatum* cultivars than in *Verticordia* hybrids. Sucrose in vase solutions provided carbohydrates (Ichimura, 1998) and promoted increased water uptake of stems (Pun and Ichimura, 2003) and consequently satisfied the requirement of flowers for carbohydrates and water. Accordingly, flower vase life of *C. uncinatum* cultivars was more responded to sucrose than that of cultivars with *Verticordia* as a parent.

*** Conclusions**

1. Variation in vase life of waxflowers was partly explained by genetic make-up of species and also the particular cross with *C. megalopetalum* × *C. uncinatum* cultivars and some *V. plumosa* × *C. uncinatum* crosses had long vase life.
2. Vase life of leaves of cultivars appears to depend on parentage with *C. megalopetalum*, *C. sp. Gingin*, *C. floriferum* and *Verticordia* as a parent with longer vase life than flowers. Alternatively, vase life of leaves of most *C. uncinatum* cultivars was shorter than vase life of flowers.
3. The vase life of cultivars responded to vase solutions containing sucrose compared to DI water or vase solutions containing HQS and STS or HQS alone with *C. uncinatum* cultivars or *C. megalopetalum* hybrids responding more than cultivars with *C. sp. Gingin*, *C. floriferum* and *Verticordia* as a parent.

4. Sucrose had a similar effect on increasing vase life of cultivars when compared to glucose and fructose being more effective than maltose and galactose and
5. For flowers osmotic effect tended to be beneficial while for leaves was not beneficial.
6. The effective concentration of sucrose (from 0.5% to 1%) and HQS (50 mg L^{-1}) for cut waxflowers was a quarter as concentrations normally used.
7. Vase life of flowers of cultivars ended on average when stems fresh weight reached 75%.
8. Vase life of flowers of cultivars was longer when day for stems fresh weight above initial stems fresh weight was longer.
9. Flowers strongly competed with leaves for water and carbohydrates.
10. Vase life of flowers and leaves of waxflowers cultivars was determined by changes in weight ratio of flowers to stems with cultivar. Those having higher weight ratio had an increased flower to leaf vase life ratio. Later flower development stage was also associated with a higher flower to leaf vase life ratio up to 50% open.

*** Future research**

1. Differences of parents in influencing the vase life of crosses could be used to breed longer vase life cultivars. This would also need to take account of effect of vase solutions.
2. The mechanism of the effect of different sugars on vase life of cut waxflowers needs further study.

Appendix I

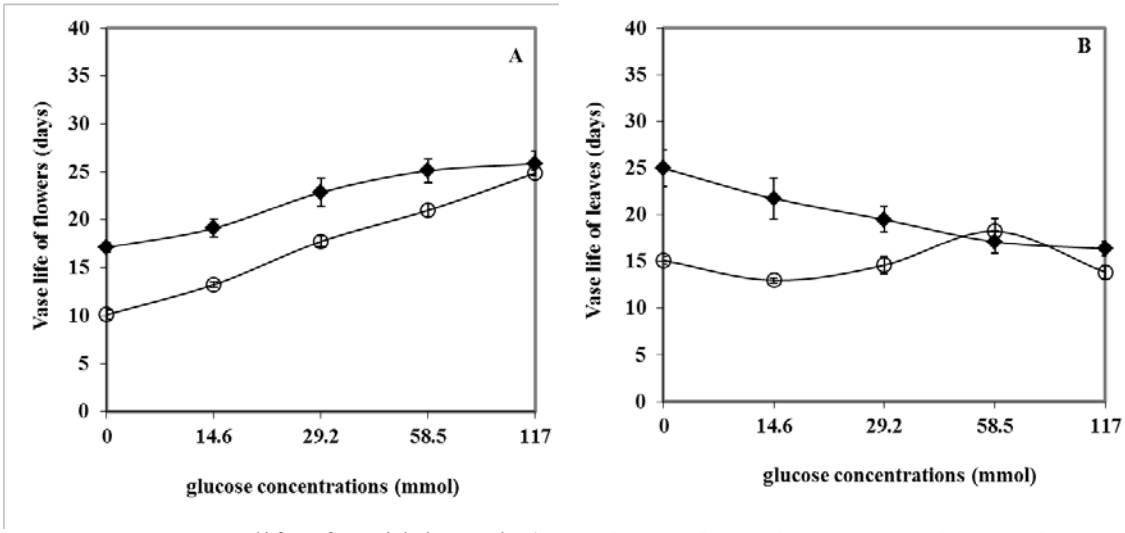


Figure 1. Vase life of 'Bridal Pearl' (♦) and 'Purple Pride' (o) (Panels A and B) in vase solutions containing 14.6, 29.2, 58.5, and 117 mmol glucose supplemented with 200 mg L^{-1} HQS, and deionized water. Standard of means are shown. LSD ($P < 0.05$) for flowers = 2.4 days and for leaves = 3.6 days, n = 8.

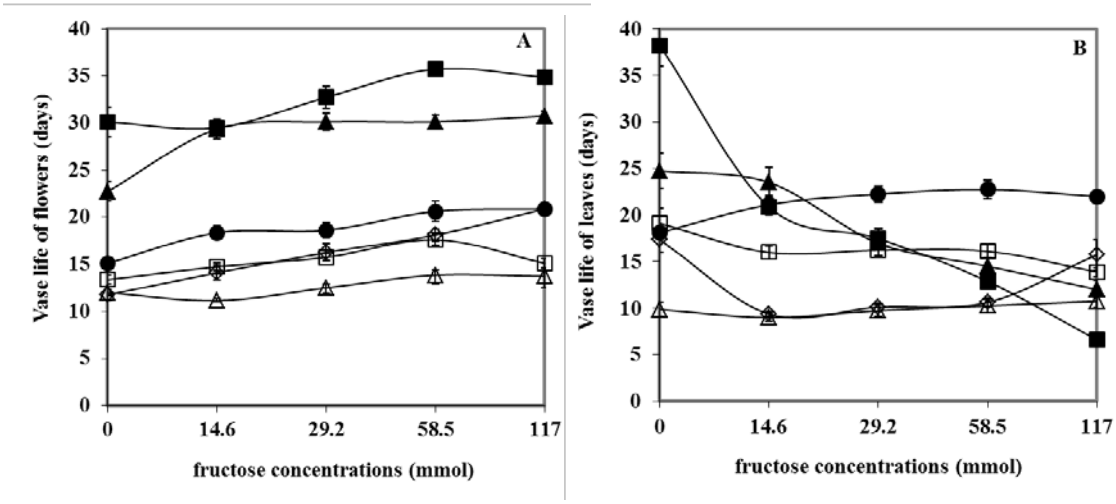


Figure 2. Vase life of 'Laura Mae Pearl' (■),'Denmark Pearl' (▲), 'Lady Stephanie' (•), 'WX73' (□), 'WX97'(◊) and 'Mullering Brook' (Δ) (Panels A and B) in vase solutions containing 14.6, 29.2, 58.5, and 117 mmol fructose supplemented with 200 mg L^{-1} HQS, and deionized water. Standard of means are shown. LSD ($P < 0.05$) for flowers = 2.1 days and for leaves = 2.8 days, n = 8.

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