Effects of Some Preservative Solutions on Vase Life in *Gerbera jamesonii*

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Abstract

Gerbera (*Gerbera jamesonii* H. Bolus ex. Hooker) is an important ornamental flower in global flower market. Consumers are attracted by its bright colour and beautiful shape; but as with other cut flowers, quality loss after harvest is a major concern. Moreover, in certain cultivars, its vase life is dramatically shortened by a stem bending problem. 'Navy', an attractive new variety, caused customer complaints resulting from its short vase life, as a result of a high incidence of stem bending.

In this thesis, 2 – 6% sucrose was shown to be an effective preservative for preventing 'Navy' from stem bending. Furthermore, deeper research showed sucrose improved stem rigidity and did so by improving lignification of sclerenchyma fibres in the phloem caps and interfascicular region.

Once stem bending has been prevented by sucrose, it is also possible to delay underlying flower senescence. Certain antibacterial materials were tested and a preservative solution containing 4% sucrose and colloidal silver (3 or 5 ppm) was shown to be the best. This may be mainly due to effective control of bacteria and resulting reduction in water stress; but also it may delay flower senescence by inhibiting ethylene action (although most gerbera varieties that have been tested are ethylene insensitive). Just sucrose and colloidal silver is sufficient to keep 'Navy' flowers alive for three or four weeks; which should be enough for consumer demand.

There is quite limited knowledge on the mechanism of gerbera flower senescence. The sequence of 'Navy' senescence was shown to involve first a change in head angle which always occurred on day 11 after harvest. Water uptake mostly started to be affected from day 13 to 15. Most of the senescence-associated colour changes, including the values of 'L', 'a', 'b', began to change during day 15 to 20. Flower weight generally did not change too much, and accompanying with water uptake reduced (apart from the first experiment). Therefore, the results suggest visible initiation of 'Navy' senescence might start

at around 11 – 13 days after harvest, so investigations into underlying genetic regulation would need to start before this time.

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Abbreviations

8-HQC：	8-hydroxyquinoline citrate
8-HQS:	8-hydroxyquinoline sulfate
ABA:	abscisic acid
ACC:	1-aminocyclopropane-1-carboxylic acid
ACO:	ACC oxidase
ACS:	ACC synthase
$AgNO_3$:	silver nitrate
AOA:	amino-oxyacetic acid
AOPP:	α-aminooxi-β-phenylpropionic acid
AP:	apoptotic-like mechanism
AU:	autophagous-like mechanism
BA:	benzyl adenine
$CaCl_2$:	calcium chloride
CHS:	chalcone synthase
CMS:	cytoplasmic male sterility
DFR:	dihydroflavonol-4-reductase
Dfs:	disc florets
ER:	endoplasmic reticulum
GA:	gibberellic acids
GDP:	geranyl diphosphate
JA:	jasmonic acid
MAP:	modified atmosphere packaging
MAPK:	MAP kinase
MTs:	cortical microtubules
PAL:	phenylalanine ammonia-lyase
PAs:	polyamines
PGRs:	plant growth regulators

PCD:	programmed cell death
PE:	polyethylene
PP:	polypropylene
PVC:	polyvinylchloride
Rf:	ray floret
ROS:	reactive oxygen species
UV:	ultraviolet
SA:	salicylic acid
SAGs:	senescence associated genes
SAM:	S-adenosyl methionine
SI:	self-incompatibility
TFs:	signal transduction and transcription factor

Chapter 1 Introduction

1.1 Literature Review

1.1.1 Background of Gerbera

1.1.1.1 Brief for Gerbera and Its Industry

Gerbera (*Gerbera jamesonii* H. Bolus ex. Hooker), a member of Asteraceae (Compositae) family, popularly known as Transvaal daisy or Barberton daisy, was named in honour of the German botanist and medical doctor Traugott Gerber (Ambrosius, 2003; Cardoso & Teixeira da Silva, 2013; Salunkhe, Bhat, & Desai, 1990). Actually the first person who discovered it in 1880s was a Scotsman, called Robert Jameson, who is recognized by its specific name - *jamesonii*. Gerbera was firstly found in the Transvaal area of South Africa (between lat. 20 °S and 30 °S, and east of long. 25 °E) and was established as a commercial crop in 1930s (Kumar, Lavania, Bhatulkar, & Chonkar, 2010; Rogers & Tjia, 1990; Tourjee, Harding, & Byrne, 1994). Nowadays, gerbera has become one of the ten most popular commercial cut flowers in the world (Emongor, 2004; Jafarpour, Golparvar, Askarikhorasgani, & Amini, 2015).

One of the most significant reasons why gerbera has become such a popular floricultural crop is attributed to its wonderful shape and bright colours attracting enormous numbers of consumers (Nair, Singh, & Sharma, 2003; Solgi, Kafi, Taghavi, & Naderi, 2009). In current flower markets, for satisfying different demand of flower users, gerbera has a relatively wide range of size, the flower diameter of smallest ones being about 5 cm, while the biggest ones can reach 15 cm. Approximately 70% of the gerbera market is occupied by Mini-*Gerbera*, whose flower diameter is about 7-9 cm (Acharyya, Mukherjee, Chakraborty, & Chakraborty, 2012; Esendam, 2014; Stravers & Van Os, 2008). Gerbera flowers have three kinds of florets: ray florets, trans florets and disc florets (Figure 1.1) (Gerbera Lab, 2016; Helariutta, Elomaa, Kotilainen, Seppänen, & Teeri, 1993).

According to gerbera's floret form, gerbera could be classified into five groups (Table 1.1), which include single, double or duplex, crested doubles, full crested double, and quilled full crested doubles (Tjia, Black, & Park-Brown, 1991).

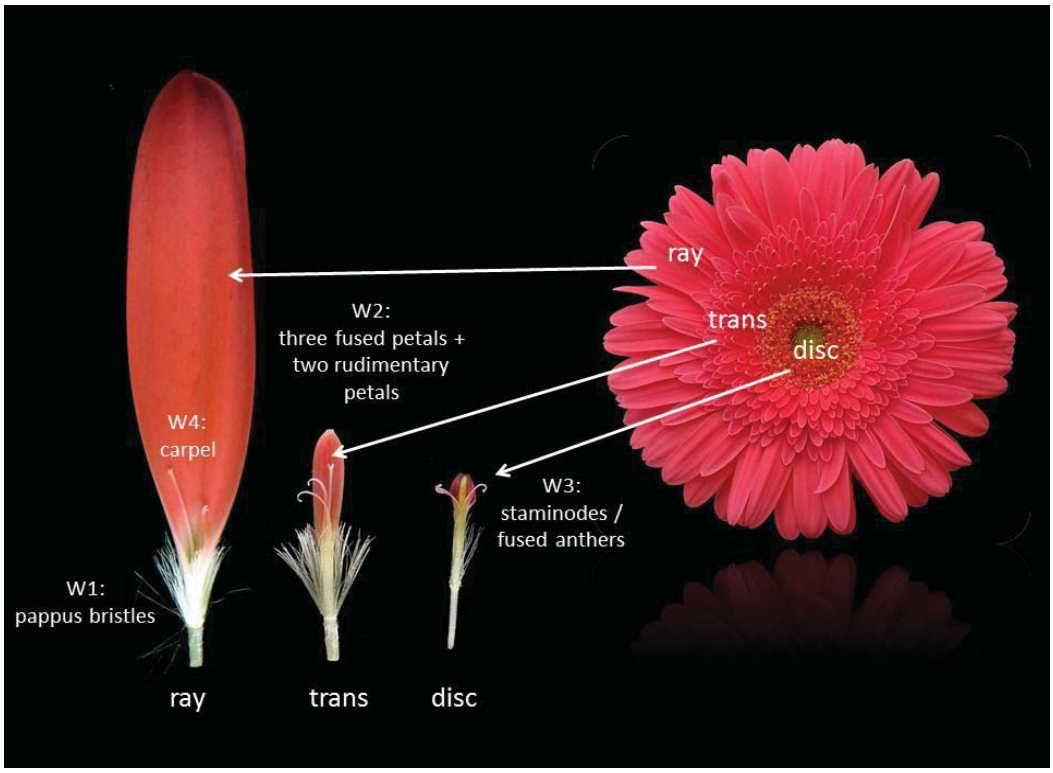

Figure 1.1: Gerbera flower and its petal category (Gerbera Lab, 2016)

Table 1.1: Five gerbera categories based on flower forms (Tjia et al., 1991)

Gerbera category	Ray florets (Rfs) form	Disc florets (Dfs) form
Single	A row of non-overlapping Rfs	A green center (disc florets, Dfs)
Double/ Duplex	A double row of overlapping Rfs	Green, black, or dark red Dfs
Crested doubles	Two rows of overlapping Rfs with one or more inner rows of shorter Rfs	Green, black, or dark red Dfs
Full crested doubles	Solid overlapping rows of Rfs with an inner row diminishing in size	No obvious Dfs
Quilled full crested doubles	Solid overlapping rows of split Rfs, having a fine textured appearance	No Dfs

Apart from its big range of size, gerbera has a wide range of colours, including white, yellow, orange, pink, crimson, red, and purple (Kumar et al., 2010; Salunkhe et al., 1990). Besides its attractive external appearance, gerbera's longer shelf life is another factor making it popular in cities (Kumar et al., 2010). Depending on the variety, and methods and conditions of postharvest handling, the longevity of gerbera can vary from 1-4 weeks after harvest (Acharyya et al., 2012).

Since gerbera has highly desirable attributes, it consistently occupies one of the leading places in floral industry. After rose, carnation, chrysanthemum, and tulip, gerbera is the fifth most-used cut flower worldwide (National Garden Bureau, 2013). Meanwhile, on the basis of auction sales, in the global trade, gerbera is also on the fifth position (FlowerZone Turners Limited, 2016). In 2013, gerbera occupied 5.3 % of cut flower turnover at the Dutch Auctions, the largest auction in the world (Figure 1.2) (CBI Market Intelligence, 2015; FloraHolland, 2013).

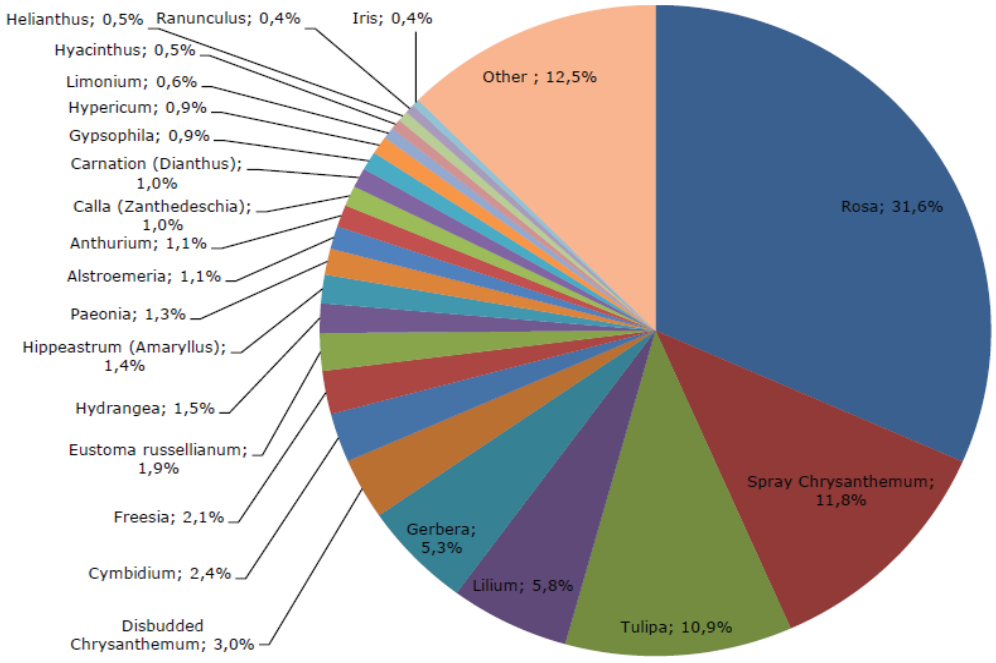

Figure 1.2: Cut flowers at the Dutch Auctions, in % of turnover in 2013 (CBI Market Intelligence, 2015; FloraHolland, 2013)

Gerbera is one of the most popular cut flowers for trading around the world, and is grown and exported by the majority of cut-flower production countries, such as Netherlands, USA and China (Hanks, 2015). In New Zealand, the production supplies only the domestic market (Neave, 2015). As well as local production, gerbera are imported from several other countries, which include Australia, Colombia, India, Mexico, Netherlands, Singapore, Malaysia, Thailand and USA (Ministry for Primary Industries, 2015).

PlentyFlora (38°14'58.1" S, 176°10'21.9" E) in Rotorua is one of the larger gerbera growers in New Zealand with over 10 years' experience in growing gerbera. PlentyFlora has a 2,688 m² glasshouse, in which more than 600,000 cut-flower gerberas are produced annually for florists all over the North Island (Esendam, 2014; Rotorua District Council, 2012). The grower produces both the standard diameter size gerberas and the mini-size gerberas, growing a total of seventy different varieties (Esendam, 2014). In this project, all gerberas were supplied by this company.

1.1.1.2 The Gerbera Industry

The postharvest quality loss of cut flowers, including gerbera, is a consistent issue for floricultural industry, which seriously affects their marketability, commercial value and consumers' benefit (Ferrante, Alberici, Antonacci, & Serra, 2007). Physiological disorders and senescence are the major problems causing cut flower quality loss (Ferrante et al., 2007). In this project, the longevity of gerbera is studied.

The determining factors of vase life include pre-harvest conditions and postharvest situations (Davarynejad, Tehranifar, Ghayoor, & Davarynejad, 2008). In this chapter, the pre-harvest essentials on producing gerbera will be discussed.

1.1.1.2.1 Growing

Firstly, high quality flowers must have proper pre-harvest growing conditions. For example, flower weight of gerbera at harvest is an important index for identifying flower quality and influencing its vase life, which maybe

mainly caused by the different growing seasons. Floral characteristics of gerbera cultivars were diverse in different months as a result of variable production conditions, as stated by Kumar and Kumar (2000). Furthermore, Acharya, Baral, Gautam, and Pun (2010) reported gerberas had highest stalk length and floral diameter when they grew in autumn, compared with growing in spring and winter. Meanwhile diameter of the stalk was the highest in spring (Acharya et al., 2010). Therefore, growing season should be a very important factor to influence flower quality. Apart from growing season, other growth factors, such as temperature, humidity, light, plant density, pest and disease control and nutrition supplement, also can affect gerbera vase life (Davarynejad et al., 2008; Rogers & Tjia, 1990). But as the impact of pre-harvest growing conditions on gerbera vase life during post-harvest is limited, the introduction of this part will be brief.

Lots of experience showed around 24 °C on daytime, and around 15 °C at night is an ideal temperature for gerbera, including its growth and its later vase life during the postharvest period (Reddy, 2016; Agriculture Forestry and Fisheries, 2011; Davarynejad et al., 2008; Esendam, 2014). Humidity controlled below 70% during the day and 85% at night is good for gerbera health (Agriculture Forestry and Fisheries, 2011; Reddy, 2016). It was found gerbera as a day-neutral plant, was not importantly affected by day-length; it can flower in day-lengths from 8 to 16 hours (Rogers & Tjia, 1990; Stinson, 1957; Tjia et al., 1991). Generally, 25 cm – 30 cm deep ground beds are suitable for two to four rows of gerberas. But 30 × 40 cm spacing produced flowers with longer stalk length (52.50 cm) and larger diameter (11.60 cm), and those longer flowers have potential to have a longer vase life (Buisman, 1985; Gurav et al., 2005; Keditsu, 2013; Singh & Sangama, 2002). Pest and disease control is consistently a significant issue for growers which directly and seriously influences the quantity and quality of cut flowers, and is an important factor affecting their vase life. For gerbera, there still are various factors that can cause injuries. The main pests include whitefly, aphid, leaf miner, thrips, and mites; major diseases are

powdery mildew, collar rot, root rot, stem rot, leaf spot; and particular nematodes such as root-knot nematode and spiral nematode (Reddy, 2016; Rogers & Tjia, 1990). Gerbera requires high fertility levels depending on the growing medium. For dolomitic limestone soil in Florida, a complete fertilizer, like 8-8-8 (the ratio of nitrogen (N), phosphate (P) and potash (K)), should be implemented at a rate of 1.5 kg/10 m^2 of bed area every month during the growing season (Hotta, Tanaka, Takaoka, Takeuchi, & Konnai, 1997; Tjia et al., 1991).

1.1.1.2.2 Harvesting

Since the 1970s, peer-reviewed scientific articles on gerbera have demonstrated the optimum maturity for harvesting. It is suitable for gerbera flowers to be harvested after the capitulum has 1 – 3 rows of bisexual disc florets opened, because the flowers have reached their final size, although the stalks at this period are still not fully mature structurally (Steinitz, 1983; van Meeteren, 1978a; Wernett et al., 1996). Commercial maturity standard for the majority of gerbera varieties is defined as when the two outer whorls of flowers in the floral head show mature stamens. This harvest maturity results in the longest vase life of gerbera flowers (Hannweg, 2008; Perik, Razé, Harkema, Zhong, & van Doorn, 2012; Rogers & Tjia, 1990; Shoub, 2013).

It is preferable to pull gerbera stems rather than cut them. Cutting makes plant vulnerable to attack by disease organisms, since it leaves behind senescent stubs. The better way to harvest flowers is to gently remove the entire stem at its uppermost internodes (Perik et al., 2012; Rogers & Tjia, 1990; Salunkhe et al., 1990; Shoub, 2013).

1.1.1.2.3 Postharvest Handling

Gerbera cut flowers generally are packed in boxes (Berlingieri Durigan & Mattiuz, 2009; Prashanth & Chandrasekar, 2010; Salunkhe et al., 1990). Specifically, in India, 100 x 40 x 15 cm telescopic corrugated fibre board boxes are used to pack gerbera, with two 1.5 cm diameter holes in the box. Tissue

paper or newspaper is utilized as bedding (Prashanth & Chandrasekar, 2010). By contrast, in the Netherlands gerberas are always packed upright in plastic-coated metal grids (50 × 70 cm) with a mesh (2 × 2 cm), and grid partitions that support each flower head. The grids are hung above a plastic tray 48 × 78 ×30 cm (Salunkhe et al., 1990). Later on, gerbera growers prefer to select a kind of sophisticated container, which is excellent for avoiding desiccation and reducing stem bending for gerbera, called Procona System container (Çelikel & Reid, 2002). Cardboard cups or plastic sleeves are used for protecting flower heads in most countries (Prashanth & Chandrasekar, 2010; Salunkhe et al., 1990).

Gerbera cannot withstand long-term storage and transport, and its quality is affected by the situations of its surroundings during those periods (Hannweg, 2008; Salunkhe et al., 1990). Dry storage is convenient and is the most common method for gerbera in many countries (Berlingieri Durigan & Mattiuz, 2009; van Doorn, Veken, & Bakker, 1994). However, if wet transport is feasible it is a better method for shelf life (Goszczyńska, Rudnicki, & Nowak, 1986; van Doorn et al., 1994). According to van Doorn, Veken, & Bakker (1994), dry storage at 1 °C for 4 days didn't affect most gerbera varieties (nine varieties were tested in this experiment, including Cora, Donatella, Liesbeth, Mickey, Nikita, Regina, Rosamunde, Simonetta and Terrafame). Cultivars with a lower dry weight showed more stem bending, especially in summer. In winter, stem bending was higher for all cultivars after dry storage (van Doorn et al., 1994). An evaluation testing was developed for use during retail display which worked well for gerbera. The results showed the incidence of stem bending increased dramatically during display time, but displaying flower in low temperature condition using floral coolers can improve the situation, and prolong the display time. As for the comparison of wet and dry displaying condition, the result showed for ‘Foske’ gerbera, holding dry is better. They suggested this situation occurred as a result of bacteria growing easier in wet. Although the commercial hydration solution they selected has anti-bacterial compounds, it still did not

completely protect flowers from bacterial injury (Nell, Leonard, & Alexander, 2009).

The environmental conditions after harvest affect the vase life of gerbera. Temperature is always a key variable, because it directly influences the respiratory activity and vase life of flowers (Berlingieri Durigan & Mattiuz, 2009; Rogers & Tjia, 1990; Wills, McGlasson, Graham, & Joyce, 1998). Most articles demonstrated the optimum temperature for gerbera during post-harvest handling was just above the freezing point (0 °C) (Çelikel & Reid, 2002; Reid, 2001). For instance, Salunkhe et al. (1990) recommended 1.65 °C; Rogers & Tjia (1990) suggested 4.44 °C; while, Berlingieri Durigan & Mattiuz (2009) found 2°C was the best temperature for Gerbera 'Suzanne'. Once flowers are rehydrated, Nell et al. (2009) found that displaying gerbera in 2, 6 or 10 °C prolonged its shelf life up to 44% compared to room temperature (21°C), particularly for flowers which had been held dry for 2 - 3 days (Nell et al., 2009). Light is mainly required for leafy cut flowers as it affects photosynthesis and leaf senescence, and has less influence on the leafless scapes of gerbera. Nevertheless, in recent years, UV (λ = 254 nm) irradiation was found beneficial to maintain 'Ice cream' and 'Ecco' gerbera quality after harvest by diminishing floret specking caused by *Botrytis cinerea*, and reducing stem bending (Darras, Demopoulos, & Tiniakou, 2012). Modified atmospheres during postharvest handling are another tool to moderate disorders and can prolong shelf life of cut flowers (Akbudak & Murat, 2013; de Pascale, Maturi, & Nicolais, 2005; Rogers & Tjia, 1990), but there is little research on gerbera in this field. De Pascale, et al. (2005) used modified atmosphere packaging (MAP) (the practice of adjusting the constitution of the internal atmosphere of a package with the aim of improving the shelf life) system to preserve cut flowers, and figured out that packaging in air is a good constituent for gerbera 'Igloo' to have a longer vase life, compared with non-packed control, which may primarily be caused by packaging reducing the water consumption of gerbera stems (de Pascale et al., 2005). Moreover, Akbudak & Murat (2013) found storing in 1-Methylcyclopropene (1-MCP) (a cyclopropene

derivative used as a synthetic plant growth regulator) (625 ppb at 4 ± 1 °C for 4 hours) and MAP (in low-density polyethylene (PE), with an O_2 permeability of 5371 ml m^{-2} day^{-1} atm^{-1} at 23 °C and a water vapor permeability of 7.50 g m^{-2} day^{-1} atm^{-1} at 37.8 ± 1.1 °C and 90 ± 2% RH; polypropylene(PP), with an O_2 permeability of 1128 ml m^{-2} day^{-1} atm^{-1} at 23 °C and a water vapor permeability of 3.10 g m^{-2} day^{-1} atm^{-1} at 37.8 ± 1.1 °C and 90 ± 2% RH; and polyvinylchloride (PVC), with an O_2 permeability of 62.70 ml m^{-2} day^{-1} atm^{-1} at 23 °C and a water vapor permeability of 4.80 g m^{-2} day^{-1} atm^{-1} at 37.8 ± 1.1 °C and 90 ± 2% RH) condition reduced disorders and the loss of quality of gerbera 'Rosalin'.

There are different perspectives for the gerbera's reaction from ethylene. Gebera is considered as ethylene insensitive flower based on the classification system from Woltering and van Doorn (1988), because exogenous ethylene and ethylene inhibitors (STS) have been found to have no effect on petal senescence. But there is still controversy about this statement (Constanta, Vintila, Lamureanu, & Madalina, 2012; Serek, Sisler, & Mibus, 2015; Tripathi & Tuteja, 2007; van Doorn & Stead, 1994; Woltering & van Doorn, 1988). For example, Akbudak & Murat (2013) showed ethylene was produced at the end period of flower senescence and the ethylene inhibitor 1-MCP delayed senescence for some cultivars of gerbera. But in this article, there was an ambiguous definition of end of vase life: it may imply, stem bending (which usually occurs relatively soon after harvest), or petal senescence (which is the final step in vase life). However, there is an interesting result showing that increasing ethylene application may reduce gerbera stem bending by increasing PAL (phenylalanine ammonia-lyase) activity. PAL is a significant regulatory enzyme of secondary metabolism which catalyzes the deamination of phenylalanine to trans-cinnamic acid, used to synthesize phenolic and lignin-like compounds (which are the compounds that strengthen vascular tissue in the stems) (de Witte, Harkema, & van Doorn, 2014; Ferrante et al., 2007; Gerasopoulos & Chebli, 1998; Perik et al., 2012). Based on this information, different varieties may have different

sensitivities to ethylene. But in general ethylene does not appear to regulate flower senescence in gerberas, although it may promote stem strength.

Pulse treatment should not be ignored for cut flowers during postharvest handling. Its benefit has been investigated in a large number of cut flowers, such as rose, carnation, eustoma, and it showed a positive result to extend vase life for all of them (Lü et al., 2010; Serrano, Amorós, Pretel, Martínez-Madrid, & Romojaro, 2001; Shimizu-Yumoto & Ichimura, 2010). Gerbera has the same situation that there was large number of peer-reviewed studies showing that a pulse treatment is beneficial for prolonging vase life (Danaee, Mostofi, & Moradi, 2011; Liu et al., 2009; Perik, Razé, Ferrante, & van Doorn, 2014). The majority of preservatives used in pulse treatments supply energy, control or kill bacteria, and overcome the effect of air bubbles in the stem. These are the essential factors influencing gerbera health, vase life, and commercial value. Pulse treatment can be applied at various times, but primarily are recommended to be applied just after harvesting, re-cutting the stem before putting into the pulse treatment. It can therefore be applied by growers, or retailers (Abdel-Kader & Rogers, 1986; Nell et al., 2009; Prashanth & Chandrasekar, 2010). Many solutions have been investigated, and several chemicals have been shown to be effective for gerbera and are commonly used. Sucrose with high concentration and an anti-bacterial agent are needed in practically every recipe of preservatives. Rogers and Tija (1990) suggested gerbera should be hydrated in distilled or deionized water with 9% sucrose and a non-toxic, anti-bacterial agent (e.g. 5% sodium hypochlorite at 7 mL per liter, 200 mg/L 8-HQC or 8-HQS) for 6 to 24 hours before settling into vase. Amiri, Rabiei, & Zanjani (2009) demonstrated that sucrose with 8-HQS, calcium chloride ($CaCl_2$) and silver nitrate ($AgNO_3$) enhanced gerbera vase life, reduced stem bending, and reduced wilting (Amiri et al., 2009). Furthermore, Perik et al. (2014) indicated sugar with an efficient antimicrobial compound delayed the time to gerbera bending, and they demonstrated 25 g L^{-1} sucrose, 50 mM calcium chloride, buffered at pH 3.5 by citric acid / K_2HPO_4, was suitable as a 24 h pulse treatment at 20 °C (the

buffer could be replaced by chlorine bleach) (Perik et al., 2014). As stem bending is a key disorder for gerbera, some unique additives working on cell wall rigidity may be useful for keeping gerbera quality. In line with Perik et al. (2014), Ca^{2+}, H^{+} concentration and vanadate may help prevent stem bending because of their effects on cell membranes and cell walls. Ca^{2+} can cross-link pectin molecules, and is a key compound increasing cell wall stiffness and maintaining its rigidity (Brummell, 2006; Cosgrove, 2005; Perik et al., 2014). Cell wall hydrolases during elongation growth require an increase in H^{+} concentration in the cell wall, and they break the bonds in several polymers in the cell walls which allows these molecules to slide apart (Koizumi, Hara, Yazaki, Sakano, & Ishizawa, 2011; Marre, 1979). Vanadate, as an inhibitor of active proton transport from the cyto-plasm to the cell wall, is an effective compound to reduce the acid environment that cell wall hydrolases need (Koizumi et al., 2011; Marre, 1979; Perik et al., 2014).

Apart from pulse treatment, long term treatments supplied throughout the vase life of flowers may be useful for consumers to use in their home to improve flower vase life. The main composition and the aim of those additives are generally similar to pulse treatments, but the concentration of additives is always lower than pulse treatment. For example, van Meeteren (1980), used 20% sucrose as a pulse treatment, but 4% sucrose as a vase solution for gerbera.

1.1.2 Vase Life for Cut Gerbera Flowers

The longevity of flowers is remarkably different for diverse species, and it varies from less than one hour (*Desmotrichum appendiculatum*) to almost 80 days (*Odontoglossum rossii*) (Stead & van Doorn, 1994). For gerbera, its flowers show perianth wilting after approximately 16 - 24 days (Stead & van Doorn, 1994). Generally, for ornamental perishables, the major problems that determine the end of vase life are flower or leaf senescence, characterized by symptoms of flower or leaf wilting, flower abscission, and leaf yellowing (Ferrante et al., 2007; Mishra & Dwivedi S. K., 2015; Mutui, Emongor, & Hutchinson, 2001; van Doorn & Woltering, 2008). Among these symptoms, petal

senescence is the most common and important sign for the end of vase life. According to van Doorn & Woltering (2008), petal senescence may result from a change in water relations. Petals also frequently show a colour change during senescence. Monitoring water relations (uptake, growth and transpiration) and petal colour change are therefore useful objective means of describing flower senescence. Moreover, as cut gerbera flowers are just composed of a capitulum (inflorescence), and scape (stem), without leaves, leaf senescence is not important for cut gerberas. Apart from flower senescence, there is another significant disorder, which may occur before wilting of the ray petals which is generally named stem bending (Ferrante et al., 2007; Hema, Bhaskar, Bhanusree, & Suneetha, 2015). It can result in significant shortening of the vase life of cut gerbera. For instance, 'Miria' showed stem bending on 5th day of its vase life (Ferrante et al., 2007).

1.1.2.1 Stem Bending

Stem bending is a phenomenon where the cut flower stem collapses, leaving the flower hanging downwards. It is referred to in different ways in different articles, such as stem break, stem collapse, or stem folding (Balestra, Agostini, Bellincontro, Mencarelli, & Varvaro, 2005; Perik et al., 2012; Steinitz, 1982, 1983; Wernett et al., 1996). What is more, Steinitz (1983) listed stem malformations of 'Clementine', and determined that apart from what he called 'stem folding', there are two other distinct stem problems: confusingly called 'stem bending' and 'neck folding'; and these problems in different positions at different times with different phenologies. In his terminology, 'stem bending' occurs as an inclined bend between the lower part of the stem and the younger upper part; 'stem folding' (called in this thesis 'stem bending' as well) occurs at the midregion of the stem with a sharp break; and 'neck folding' appears very few centimeters below the flower head with a sharp break. Stem bending and folding occurred at a similar early period; but 'neck folding' appeared much later than the previous two (two weeks later) when sucrose was provided (Steinitz,

1983). Most authors have not made these precise distinctions. Most authors use ‘stem bending’ to refer to abrupt stem curvature of over 90° that occurs before normal flower senescence (Javad, Mahmood, Roya, & Hamideh, 2012; Perik et al., 2012; van Doorn et al., 1994). It always occurs approx. 10 cm below the inflorescence, although the exact location is variable between cultivars. It was found at the position below flower head from ca. 2 cm for ‘Schilino’ to ca. 16 cm for ‘Maorussia’ when the bending appears (Ferrante et al., 2007; Wilberg, 1973). Moreover, depending on different varieties, the incidence of stem bending is diverse. Several cultivars rarely show the disorder, while some others have this problem very seriously, and for some, the probability of occurrence reaches 100%, like ‘Sensation’, ‘Venice’ and ‘Olina’ (Ferrante et al., 2007; Javad, Ahmad, Mostafa, & Roya, 2011).

Several possible reasons for gerbera stem bending have been presented; the problem is considered not to result from a single factor (Ferrante et al., 2007; Javad et al., 2011; Nazarideljou & Azizi, 2015; Perik et al., 2014; Perik et al., 2012). The majority of authors agreed stem vascular blockage, which mainly appears in the bottom 5 cm of the cut stem, affects gerbera water uptake and is an important cause of stem bending (de Witte et al., 2014; He et al., 2009; Hema et al., 2015; Perik et al., 2014; van Meeteren, 1978a; Wang, Zheng, & Xu, 2014). According to van Meeteren (1978b), two factors can be responsible for vascular blockage: bacterial growth and physiological stem plugging (physical stem plugging was the author’s word, but physiological stem plugging may be more apt according to his meaning). But this author showed microbial activity was the main reason for gerbera stem bending, not physiological stem plugging, because those two problems happened in different period, and have different symptoms when they occur. During the first two days, microbial blockage occurs, water relations are adversely affected and water potential of flower petal drops quickly, leading to stem breakage; if physiological plugging happens (it occurs after four days), no visible symptoms are seen and the water potential of the flower petals decreases more slowly. Therefore, he suggested, gerbera bending

closely relates to water relations, which is due to the bacteria in vase solution. While it is still have some other view of it, de Witte et al. (2014) found bacteria was not the most cause for reducing water uptake and occluding the xylem leading to stem bending. They raised the reason may due to the material released by dead cells, which resulted from antimicrobial compounds made a toxic effect on the superficial stem cells. But those authors both agree that once there is a water deficit, the stem has a loss of turgor and lacks the ability to maintain the weight of capitulum (10 - 13 g when harvested) leading to stem bending (Ferrante et al., 2007; Perik et al., 2012).

Another major focus of research is on stem mechanical support. Since the floral head is heavy, sometimes the stem cannot hold it, and the stem breaks naturally, even without prior water stress. Perik et al. (2012) found removal of the floral head prevented stem bending. But they suggested the gravitational pull on the floral head is not the only factor contributing to stem bending, because varieties with the same size of capitulum and same stem diameter, did not all show the same proportion of stem bending. Inadequate stem wall thickening is therefore another probable cause of stem bending. Some evidence showed improving stem rigidity and texture with sucrose, which promoted stem cell wall structure by thickening and lignification process of phloem-associated-cells, contributed to reducing stem bending incidence (Steinitz, 1982, 1983). Gerbera stem mainly consists of vascular bundles (phloem and xylem), and surrounding parenchyma (Steinitz, 1982). During development, between vascular bundles, there is a collateral arrangement with an interfascicular cambium which makes stem's sclerenchyma eventually form a complete cylinder (Marousky, 1986; Perik et al., 2012). The strength of gerbera stem is closely related to lignification of xylem cells and interfascicular sclerenchyma cells, which are fiber cells with thick cell wall (Marousky, 1986; Perik et al., 2012). In terms of the upper part of gerbera stem, there is less lignified xylem, and a lack of sclerenchyma (Marousky, 1986; Perik et al., 2014; Perik et al., 2012). These combine to make the mechanical stem strength lower in the upper part of the

stem and may be an essential variable in gerbera stem bending, if there is a lack of lignification at the upper part of stem. As well as the stem tissue anatomy, stem elongation and the cavity present in stem center may also contribute to a loss of mechanical stem strength, and therefore be associated with stem bending (Perik et al., 2012). Gerbera stems are still elongating after harvest when placed in water, and the elongation zones are weak with less development of xylem and sclerenchyma (Botondi, Esposito, Massantini, & Mencarelli, 1998; Perik et al., 2012). Some evidence shows bending mainly occurred in this zone (Botondi et al., 1998; de Jong, 1978; Wilberg, 1973). Besides, because flowers are generally placed in containers at an angle, when stems elongate, the gravitational pull promotes stem bending, and the internal cavity may also be thought of as a zone of weakness (Perik et al., 2012). However, Perik et al. (2012) demonstrated those two factors are not greatly important for stem bending. Instead, the extent of sclerenchyma in the upper part of stem wall has the biggest influence on the mechanical strength of stem (Perik et al., 2014; Perik et al., 2012).

In conclusion, two main reasons were shown closely related to stem bending for gerbera, which were bacterial growth or the material released by dead cells leading to stem plugging and lack of sclerenchyma at the top of stem resulting in low stem mechanical strength, to the point where it is unable to hold flower head.

1.1.2.2 Flower Senescence

After the initial post-harvest stem bending period, gerbera enter a period when water stress (due to bacterial growth or physiological stem plugging) or natural aging can lead to petal senescence and death (Jones & Hill, 1993; van Meeteren, 1978b).

Senescence has many individual definitions, which relate to the different concerns of the authors, some focus on macro view, and some on micro. In macro view, authors highlight the importance of this being a development phase

or program process affecting whole plant or several tissues' lifespan and the symptoms when it happens. From micro aspect, the change of cell viability and the expression of specific genes is the focus of senescence which is usually referred to as programmed cell death (PCD) (Dar, Tahir, & Ahmad, 2014; Rogers, 2006; Thomas, 2013; Tripathi & Tuteja, 2007; van Doorn & Woltering, 2008; Yamada, Ichimura, Kanekatsu, & van Doorn, 2009). Overall, the sophisticated definition of senescence should involve those two parts: it is the final event of the life time of a plant or its tissues; and comprises a series of structural, biochemical and molecular changes which generally bear the hallmark of PCD (Makrides & Goldthwaite, 1981; Shahri & Tahir, 2011; Tripathi & Tuteja, 2007). However, in some papers and for some specific tissues, like petal, authors use senescence and PCD almost interchangeably (Rogers, 2006, 2013; van Doorn & Woltering, 2008), because its deterioration inevitably results in PCD and the time of PCD generally occurs at the beginning of petal senescence (Rogers, 2006, 2013). But for whole flower, its senescence implies a degree of remobilization of nutrients, which served for developing the ovary or to that of new flowers, and cell death is the terminal event (Rogers, 2013; Thomas, Ougham, Wagstaff, & Stead, 2003). Specifically, for the period of senescence in macro view, it is an episode following the completion of growth, and it can be defined as the last stage of some tissue's lifespan, such as flowers (Rabiza-Świder, Rochala, Jędrzejuk, Skutnik, & Łukaszewska, 2016; Thomas, 2013).

Flower senescence is more complicated than for example leaf senescence because each flower contains many different units (including petals, sepals, anthers, and stigma), and those individual units may senesce at different rates and interact with each other (Shahri & Tahir, 2011). For example, when pollination triggers flower senescence, almost all the units in flowers change, including petal/sepal, megaspore, pollen-tube and so on (Figure 1.3), but for those units the change may not be initiated at the same time (Rogers, 2006). In carnations, whose senescence is regulated by ethylene, stigma is the unit producing ethylene, and ethylene can be translocated via the style and ovary, to

up-regulate the ethylene biosynthetic genes and produce ethylene in the petals (ten Have & Woltering, 1997). For ornamental flowers, petals (or sepals for some flowers, e.g. *Consolida*, *Delphinium*) are generally the most valuable units, and limit the lifespan of the flower (Shahri & Tahir, 2011).

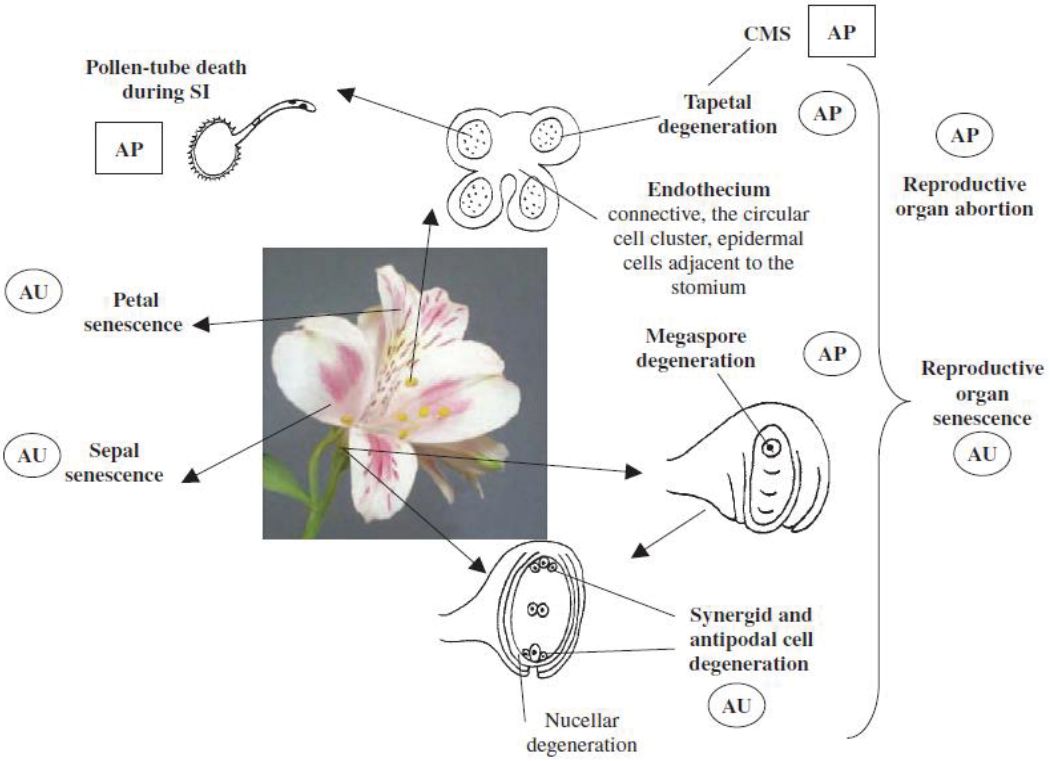

Figure 1.3: Positions of programmed cell death in floral organs (Rogers, 2006) (SI, self-incompatibility; CMS, cytoplasmic male sterility; AU, autophagous-like mechanism; AP, apoptotic-like mechanism; NS: square points to strong evidence, circle points to weaker evidence)

Petal senescence is broadly separated into wilting or abscission although specifically for some species (e.g. *Solanaceae*, *Alstroemeria*, and carnation), flower abscission may occur accompanied by wilting or withering (Breeze E. et al., 2004; Shahri & Tahir, 2011; van Doorn, 2002; van Doorn & Woltering, 2008). According to the symptom of petal senescence and the diverse reactions for ethylene, flowers can be grouped into five patterns (Table 1.2) (Shahri & Tahir, 2011). In addition, according to Woltering and van Doorn (1998), gerbera

generally fit into category 2, which is insensitive to ethylene and its senescence symptom being petal wilting. More discussion will give below.

Table 1.2: Categories of flowers responding to ethylene (Shahri & Tahir, 2011)

Group	Senescence symptoms	Effectiveness to ethylene	Examples
1	Petal wilting	Strongly affected by endogenous ethylene, and ethylene inhibitors delay wilting	Carnation, *Petunia*
2	Petal wilting	Very little or not affected by endogenous ethylene, and ethylene inhibitors don't delay wilting	*Chrysanthemum, Narcissus, Ranunculus*
3	Petal abscission	Generally stimulated by exogenous ethylene and ethylene inhibitors delay abscission	*Delphinium, Digitalis, Purpurea*
4	Petal abscission	Not stimulated by exogenous ethylene and ethylene inhibitors don't delay abscission	*Tulipa, Plectranthus*
5	Petal wilting with/without abscission	Without pollination, little affected by endogenous ethylene, and ethylene inhibitors limited delay senescence; after pollination, the application of exogenous ethylene accelerates senescence	Daffodil

However, no matter whether the species is sensitive to ethylene or not, their underlying senescence programme is similar, and involves the initiation and the procession of senescence (Bleecker & Patterson, 1997; Tripathi & Tuteja, 2007). Flower senescence can be triggered by developmental signals or accelerated by environmental factors; and then is regulated through a signalling network by endogenous and exogenous signals; moreover, the cells show characteristic biochemical and structural changes during the process (Figure 1.4) (Dar et al., 2014; ten Have & Woltering, 1997; Tripathi & Tuteja, 2007).

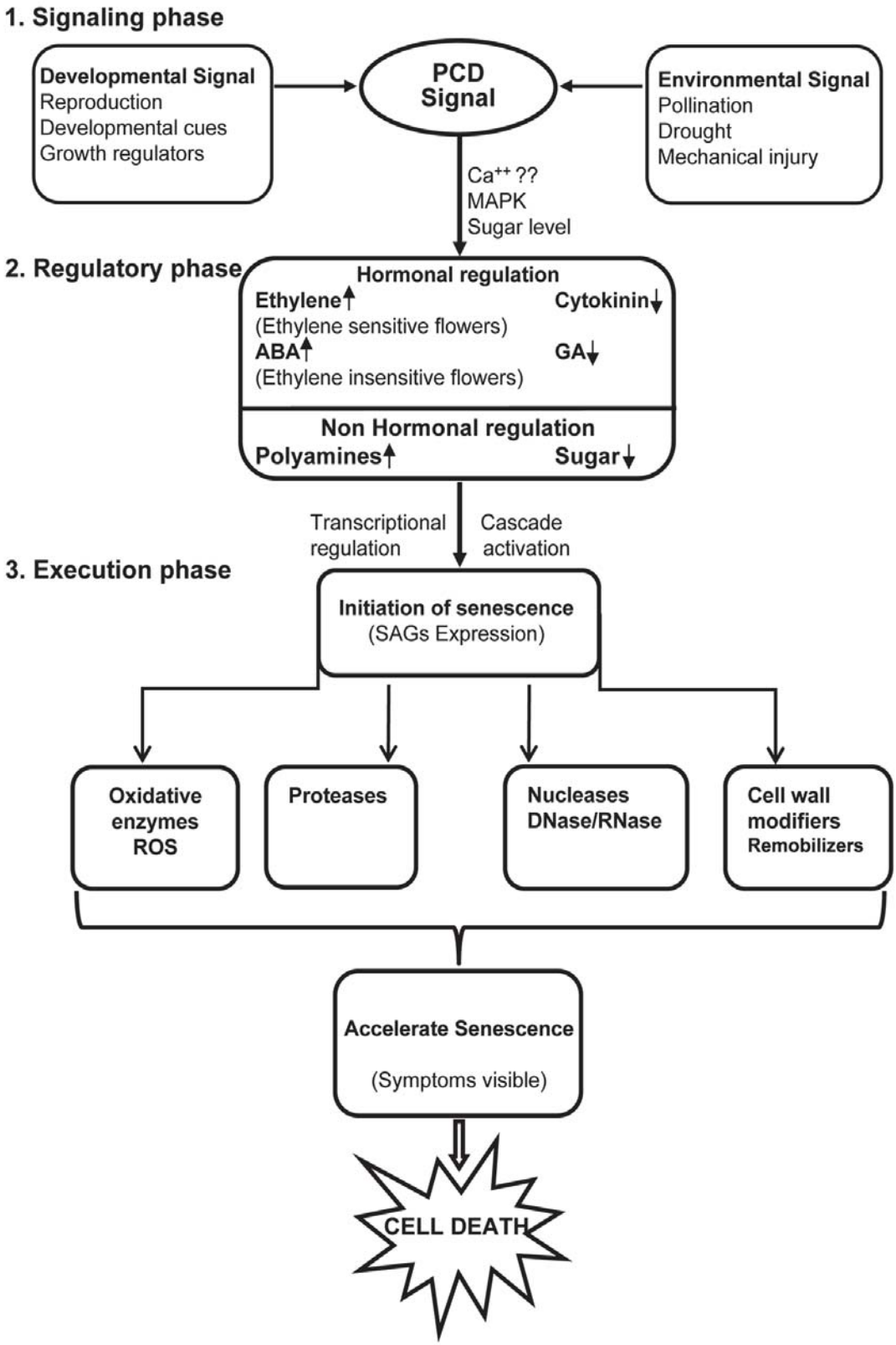

Figure 1.4: A model for regulatory pathways in flower senescence (Tripathi & Tuteja, 2007)

It has been known that flower senescence is coordinated by several plant growth regulators (PGRs) which include ethylene, cytokinin, auxin, abscisic acid (ABA), gibberellic acids (GA), salicylic acid (SA) and jasmonic acid (JA) (Dar et al., 2014; Shibuya & Ichimura, 2016; Tripathi & Tuteja, 2007). But for some PGRs, such as GA, their role in flower senescence has not been well demonstrated, though they can be effective for regulating flower senescence in some species. For example, GA is able to delay the onset of senescence in carnation with acting as an antagonist to ethylene (Dar et al., 2014). Figure 1.4 shows how ethylene can drive PCD in species where it is an important regulator of senescence. Firstly, pollination and/or age related process (it can lead to a drop of cytokinins and increase of ABA) activate up-regulation of ethylene biosynthetic genes and promote ethylene sensitivity. Then ethylene-responsive signal transduction and transcription factors (the process of senescence-associated genes (SAGs) expression in Figure 1.5) are triggered directly or through a MAPK (MAP kinase) signaling cascade (Rogers, 2013; Tripathi & Tuteja, 2007). Besides, auxin and some other signals, such as ROS can activate several signal transduction and transcription factors (TFs). Subsequently, C-terminal KDEL sequence proteases act on the endoplasmic reticulum (ER) and then ricinosomes. Vacuolar processing enzymes (VPEs) in the cell vacuole may be required for processing of proteases, such as metacaspases, and then lead to cell death. Apart from VPEs, the upregulation of autophagy genes may lead to the formation of auotophagosomes that fuse with the vacuole and contribute to death (Rogers, 2013).

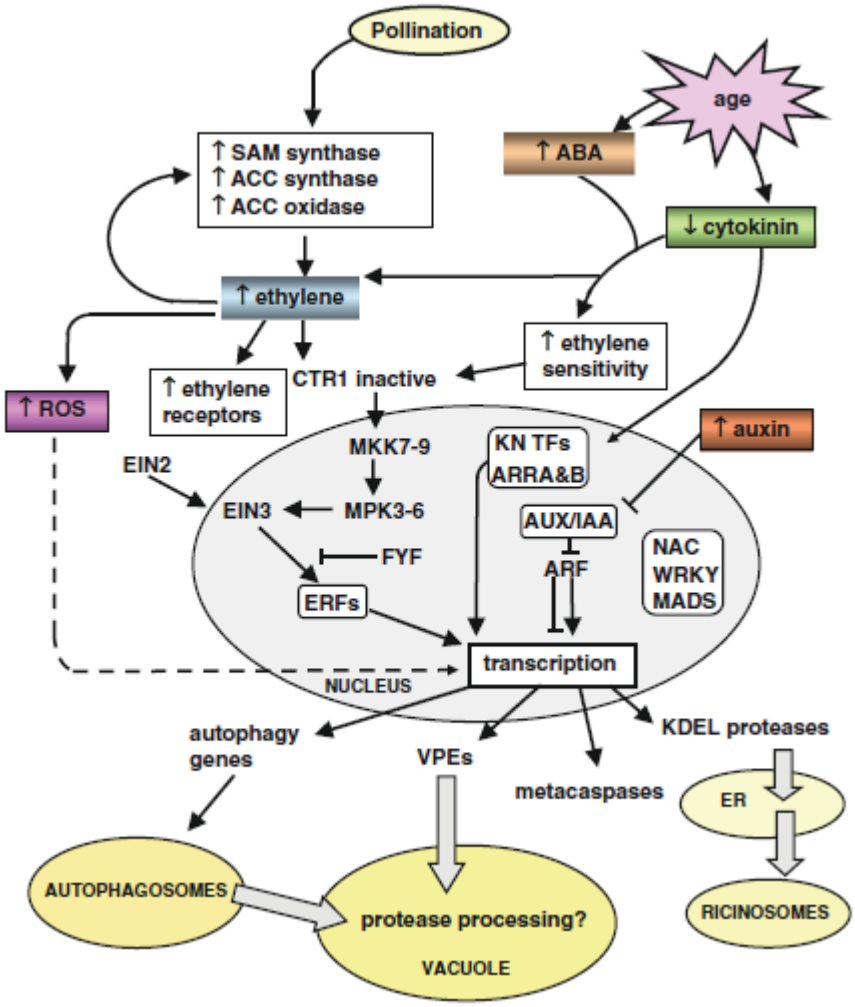

Figure 1.5: Components of the ethylene downstream signaling pathway (Rogers, 2013)

For ethylene insensitive flowers, the mechanisms regulating its senescence are much less known, their PGR regulation is likely to be quite complicated (Rogers, 2013; Shibuya & Ichimura, 2016). ABA seems be an important PGR intermediate signal for regulating senescence in some species of ethylene insensitive flowers, such as daffoduil, daylily and *Lilium* (Arrom & Munné-Bosch, 2012a, 2012b; Hunter, Ferrante, Vernieri, & Reid, 2004; Hunter, Yi, Xu, & Reid, 2004; Panavas, Walker, & Rubinstein, 1998; Rogers, 2013; Shahri & Tahir, 2011; Tripathi & Tuteja, 2007). Specifically for daylily, the primary hormonal regulator is considered to be ABA, because ABA gives rise to several senescence changes, such as ion leakage, changes in lipid peroxidation, protease activity and expression of novel DNases and RNases (Panavas et al., 1998; Shahri & Tahir,

2011). But for daffodil, ABA is not an early regulator, due to its increase after the increase of mRNA abundance of senescence associated genes, and ABA biosynthesis inhibitor is not able to delay senescence (Hunter, Ferrante, et al., 2004). Apart from ABA, some other PGRs also influence flower senescence in several specific species, such as, GA_3 for daffodil, cytokinin and jasmonates for *Iris* (Hunter, Ferrante, et al., 2004; van der Kop et al., 2003; van Doorn, Çelikel, Pak, & Harkema, 2013; van Doorn & Woltering, 2008). As there are different PGRs for each species, it may be much more promising to study the regulation of the signaling and TF intermediaries (Rogers, 2013).

Apart from PGRs, factors such as cytosolic Ca^{2+}, sugar and polyamines, affect flower senescence, through several distinct signaling pathways (Cai et al., 2015; Dar et al., 2014; Tripathi & Tuteja, 2007; van Doorn et al., 2013). Some evidence showed that excessive exogenous calcium is able to accelerate cell death (Tripathi & Tuteja, 2007; van Doorn & Woltering, 2008), possibly because the increase of exogenous calcium increases the cytosolic Ca^{2+} concentration (overwhelming the normal homeostatic process that keeps cytosolic Ca^{2+} at a low concentration)leading to Ca^{2+}-activated calmodulin action and activation of protein kinases, and altered hormone signal transduction (Kudla, Batistič, & Hashimoto, 2012; van Doorn et al., 2013). Sugar has been found to delay senescence in a great number of species, including ethylene sensitive and insensitive flowers. Sugar supplementation can delay protein degradation and reduce expression of several senescence-associated genes (SAGs) expression. For instance, in carnation, soluble sugars play a role of a repressor in the process of ethylene signal transduction (Eason, de Vré, Somerfield, & Heyes, 1997; Hoeberichts, van Doorn, Vorst, Hall, & van Wordragen, 2007; Tripathi & Tuteja, 2007). Polyamines (PAs), which include spermine, spermidine and putrescine, also are anti-senescence agents (Dar et al., 2014; Tripathi & Tuteja, 2007; van Doorn et al., 2013). It has been reported that in carnation, PAs retard membrane deterioration, lower lipoxygenase and phospholipase D activities (Nambeesan, Handa, & Mattoo, 2008). Moreover, van Doorn et al. (2013) found 5 or 10 mM

spermine is effective to delay the oneset of tepal senescence in *Iris*.

The normal flower senescence for gerbera is identified as petal wilting, and de Jong (1978) considered the stem bending also may lead to petal senescence and mentioned a specific identification for gerbera senescence which is that wilting occurs when the ligulae of an inflorescence on an upright stem have visibly lost their turgidity (de Jong, 1978; Wernett et al., 1996). But so far, little is known about the mechanism of gerbera senescence, and there just is a limited amount of data relating to the metabolic changes which occur in the inflorescences of gerbera after harvest (Constanta et al., 2012).

Gerbera senescence seems very complex, and may differ among varieties. As mentioned before, ethylene is a controversial topic for gerbera, but most researchers consider it belongs to the group of ethylene insensitive flowers, and a large number of tested varieties were not sensitive to ethylene (Woltering & van Doorn, 1988). Moreover, ethylene may even help to promote vase life in several gerbera cultivars, such as 'Testarossa', 'Olina' and 'Dalma', due to reducing the incidence of stem bending (Ferrante et al., 2007; Gerasopoulos & Chebli, 1998). Other PGRs, including cytokinin, GA, and SA, may be important to gerbera senescence, as they are able to extend vase life when supplied exogenously (Asgari & Moghadam, 2015; Danaee et al., 2011; Emongor, 2004). But no published paper exactly shows the mechanism of how those PGRs work. The only paper working on the expression patterns of hormone regulated transcription factors in gerbera is from Li et al. (2015), but they focused on petal growth responses to ABA and GA. However, they found some information that may be useful for understanding hormone regulation of senescence. They found GA and ABA could target the same gene, and their biosynthetic and signaling pathways are relevant for each other, due to the effect on their components of the other's pathways. Moreover, some other hormone pathways, such as cytokinin, JA, and SA signaling, are also influenced by GA and/or ABA. It shows gerbera hormone regulation is a complicated and precise system, and it can be influenced by each other among PGRs. However, we still have limited knowledge

of the exact process of regulation which links PGRs to altered protease activity and gene expression leading to PCD. Non-hormone regulation is also very important to gerbera senescence. Sugar has been shown to be an important regulator for extending gerbera senescence through delaying the increase of ion leakage and increasing pressure potential of petals, although the principle and mechanism is not fully understood (Constanta et al., 2012; van Meeteren, 1980).

The knowledge of metabolic changes for gerbera is limited, but relatively more data has been presented on physiological modifications. Several physiological changes have been found during gerbera flower senescence, including changes in membrane permeability (ion leakage), lower average values of respiratory intensity and peroxide activity, higher average content of total dry substance and total glucose, and reduced growth of ligules' anthocyanic pigment content (Javad et al., 2012; Shahri & Tahir, 2010).

It should be mentioned that water balance may be important for gerbera petal senescence (in the absence of stem bending); (Kumar, Ahmed, Sharma, Mahendiran, & Lal, 2013) found the cultivars with a higher water balance had longer vase life and maintained higher water potential in the vascular tissues and flower petals. It can be explained that higher water uptake maintains turgidity, freshness of flowers leading to extended vase life. Moreover, water loss is related to drop in uptake of water and transpiration and can result in water stress (Halevy & Mayak, 1981; Kumar et al., 2013). As gerbera has no stomata on the petals, the ability of stems to absorb and petals to hold water may be the most important factors for water balance of gerbera (van Doorn, 1997). And preventing stem physical plugging and bacterial problems can benefit water balance (van Meeteren, 1978b).

1.1.2.3 Preservative Solutions Choices for Gerbera

There are numerous chemicals that have been tested for gerbera, but the results are not always similar. For example, sugar is always an essential additive for flower holding solutions, due to its benefits for delaying altered

metabolism during flower senescence, including being a food source, promoting petal water balance and delaying the degradation of proteins (Acharyya et al., 2012; Ahmadi & Hassani, 2015; Nair et al., 2003; Whitehead, O'Reilly, Weerts, Zaayman, & Gaum, 2003). Some authors have shown sugar can extend gerbera vase life by reducing stem bending and promoting lignin synthesis (Amthor, 2003; Ferrante & Serra, 2009; Steinitz, 1982, 1983). However, some evidence showed sugar was not effective, or even harmful, for extending gerbera vase life (Banaee, Hadavi, & Moradi, 2013; Nahrabadi, Rood, Danyaei, Babarabie, & Shadbash, 2015). It may be due to the use of different cultivars (Nahrabadi et al., 2015).

This situation also showed in other chemicals sometimes; but the chemicals mentioned here, are generally beneficial for most gerbera flowers.

Apart from sugar, germicides appear indispensable for flower preservative (de Witte et al., 2014; Vaidya & Collis, 2013). No matter for stem bending or final petal senescence problem, bacterial or micro-organism accumulation that leads to the blockage of xylem vessels and water stress of flowers is one of the most important possibilities to cause those problems. A large number of biocides have been tested on specific gerbera varieties. But the recommended biocides were diverse, which may be due to the different requirements of varieties, concentration selection, and even experimental season. The most commonly used effective biocides include chlorine (e.g. sodium hypochlorite (NaClO), chlorine dioxide (ClO_2)), 8-HQ (e.g. 8-HQS, 8-HQC), and silver ions (e.g. $AgNO_3$, nano-silver) (Banaee et al., 2013; de Witte et al., 2014; Hema et al., 2015; Jafarpour et al., 2015; Macnish, Leonard, & Nell, 2008; Rabiza-Świder et al., 2016; Safa, Hashemabadi, & Kaviani, 2012; Vaidya & Collis, 2013). Several different kinds of essential oil, including *Eucalyptus*, *Rosa damascena*, thyme, and zataria, have been shown in some varieties to increase water uptake leading to extended vase life due to their antimicrobial and antibacterial character (Nahrabadi et al., 2015; Solgi et al., 2009).

For improving stem bending of gerbera, several PGRs have been tested. As mentioned before, ethylene is effective to reducing stem bending by

strengthening stem vascular lignification and inhibiting stem elongation (Botondi et al., 1998; Ferrante et al., 2007). And injecting ACC promoting ethylene production was found as a method to reduce the incidence of stem bending of 'Testarossa' cut gerbera (Gerasopoulos & Chebli, 1998). There are a great number of hormones or inhibitors that have been found to delay gerbera senescence successfully, such as SA, GA, (Asgari & Moghadam, 2015; Danaee et al., 2011; Emongor, 2004). Moreover, some other secondary metabolites are found beneficial to prolong vase life of gerbera, such as geranyl diphosphate or benzyl adenine (BA) (Ardebili, Abdossi, Zargarani, & Ardebili, 2013; Danaee et al., 2011).

Acid is another familiar additive for gerbera holding solutions. It helps to hydrate flowers and prevent bacterial growth (Hannweg, 2008; Taj, Sangeetha, & Kumar, 2013; van Meeteren, 1978b). Citric acid is commonly used. Some research used it at particular concentrations, but others decided the optimal quantity depended on final solution pH (Javad et al., 2011; Perik et al., 2014; van Meeteren, 1978b).

In addition, some ions, such as calcium, have been tested by researchers. As mentioned before, calcium supplementation was beneficial to extend the vase life of gerbera, mainly reducing stem bending incidence (Gerasopoulos & Chebli, 1999; Hatamzadeh & Shafyii-Masouleh, 2013; Perik et al., 2014).

1.2 Aims and Hypotheses

The information of gerbera disorders or senescence on postharvest, including stem bending and petal senescence, is still limited, and the varying demands of different cultivars make the research more complicated. One new variety, 'Navy', has attractive flowers but appears to have a short vase life as it has a serious bending problem. In this research, we use 'Navy', because of customer complaints that it suffers from this typical early postharvest problem. In addition, this project aimed to determine the underlying reason for the end of vase life, and establish some effective and cheap preservatives for longer term treatment to delay senescence. Consumer recommendations were developed to keep gerbera flowers longer at home.

Specifically, based on the literature, 'Navy' gerbera has a shorter vase life because of stem bending, which may be caused by stem plugging or low stem mechanical strength. The first aim is finding out which reason caused 'Navy' stem bending, and trying to find technical evidences for the underlying mechanism. Secondly, since we have limited knowledge on gerbera senescence mechanism, especially for gene changes, it will be very helpful if we can give more information on the sequence of events that happen during gerbera senescence. Lastly, a specific preservative for gerbera 'Navy' will be developed to help consumers to extend their flower life at home.

Based on this literature review, two hypotheses need to be tested:

- *That vase life of 'Navy' gerbera can be extended by the use of a sucrose solution (in combination with a bactericide)*
- *That successful vase life extension will be accompanied by increased lignification of the vascular bundles, reducing the risk of stem bending.*

Chapter 2 Comparing the Effectiveness of Different Vase Life Solutions in 'Navy'

2.1 Introduction and Aim

Based on literature review, the main issues which shorten the vase life of gerbera were stem bending, which may be caused by blocking vascular system or a shortage of stem mechanical support; and water stress leading to premature flower senescence, resulting from physical stem plugging: caused by bacterial or physiological stem plugging. Aiming to solve stem bending, nutritional supplementation and antibacterial chemicals may be needed. As for water stress issue, it is necessary to control the number of bacteria and the pH of solution. The following chemicals were selected for investigation: sucrose (nutritional supplement); Sodium hypochlorite (NaClO), 8-HQC, and colloidal silver/ nano-silver (antibacterial); and citric acid (hydration) (Table 2.1). At first, it is important to try to find effective solution to reduce stem bending and determine which reason, low mechanical strength or bacterial stem plugging, is the main reason leading to 'Navy' stem bending. Secondly, it is necessary to find out whether vase life can be extended, by testing a range of chemicals and concentrations that may reduce bacteria growth, support water uptake and delay senescence. Several important indexes, including flower weight, water uptake, colour and angle, were measured for objectively identifying the physiological changes affecting flower quality in vase.

Overall, the aim of experiments in this part is to figure out the effectiveness of chemicals that are not too expensive to prolong the vase life of 'Navy' or improve 'display life' after harvest. Moreover, physiological understanding for the steps in 'Navy' senescence should be determined.

Table 2.1: Chemicals and their concentration used in experiments

Experiment	Treatment	Sucrose			Sodium hypochlorite		8-HQC				Colloidal silver		Citric acid	
		2%	4%	6%	50 ppm	80 ppm	100 ppm	200 ppm	300 ppm	400 ppm	3 ppm	5 ppm	200 ppm	300 ppm
1	1													
	2		√											
	3				√									
	4		√		√									
2	1		√					√						
	2		√				√							
	3		√							√				
	4	√						√						
	5			√				√						
3	1		√						√					
	2		√									√		
	3		√			√								
4	1		√										√	
	2		√									√	√	
	3		√									√		√
	4		√								√		√	
	5		√								√			√

2.2 Materials and Methods

2.2.1 Plant Materials

Flowers, mini gerberas variety cv. Navy, were grown in a geothermally-heated glasshouse by PlentyFlora in Rotorua, New Zealand. They were harvested at commercial maturity, when between 1 and 2 whorls of stamens had opened, by using twisting technique that separated each flower from the crown at its abscission zone in the afternoon before transportation. Flowers were held overnight in water containing 10% solution 'White Brite' (which includes 5% Bleach) at 8 +/- 2 °C. Gerberas were packed horizontally in cardboard box in the morning, and transported to the laboratory by overnight Fastway courier.

Upon arrival, they were recut under water with a 'Flower Power' stem cutter, provided by PlentyFlora, to a length of 40 ± 1 cm; and then completely randomized into different solutions (Table 2.1). Solutions were made the day before flower arrival; and in each experiment, distilled water was added to return the water volume to that of the first day every two days. Moreover, distilled water was added to replace liquid removed for bacterial sampling. Except for expt. 3, flowers in other three experiments were placed in 100 ml glass test tubes (25 cm height and 1 cm diameter) with a single tube for each flower containing 80 ml test solution. In experiment 3, 2000 ml Erlenmeyer flasks were used; and 5 flowers were stored in each flask in 1600 ml test solution. All experiments were done with 10 flowers per treatment.

In all treatments, flowers were kept in a temperature controlled room (Figure 2.1) at 20 ± 2 °C, 70 ± 5% RH and a photosynthetically active photon flux of 20 ± 5 µmol*m^{-2}*s^{-1} (from Philips TLD 58W/865 cool daylight (China)) from 8 a.m. to 8 p.m. for 12 h day^{-1}.

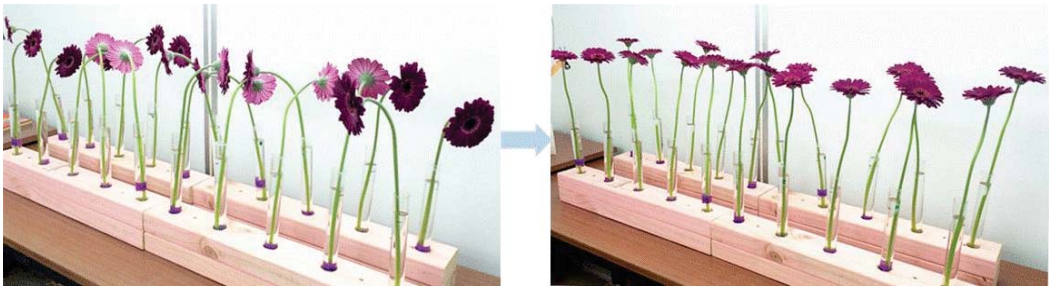

Figure 2.1: 'Navy' stored in controlled temperature room (NB flower appearance shows negatively geotropic neck bending after transport (left) which disappears by second day (right) after arrival)

Table 2.2: The number of flowers being tested in different day after flower arrival

Day	Experiment 1				Experiment 2					Experiment 4				
	T1	T2	T3	T4	T1	T2	T3	T4	T5	T1	T2	T3	T4	T5
1	10	10	10	10	10	10	10	10	10	10	10	10	10	10
2	10	10	10	10	10	10	10	10	10	10	10	10	10	10
3	10	10	10	10	10	10	10	10	10	10	10	10	10	10
4	10	10	10	10	10	10	10	10	10	10	10	10	10	10
5	10	10	10	10	10	10	10	10	10	10	10	10	10	10
6	10	10	10	10	10	10	10	10	10	10	10	10	10	10
7	10	10	10	10	10	10	10	10	10	10	10	10	10	10
8	10	10	10	10	10	10	10	10	10	10	10	10	10	10
9	10	10	10	10	10	10	10	10	10	10	10	10	10	10
10	9	10	9	10	10	10	10	10	10	10	10	10	10	10
11	7	10	8	10	10	10	10	10	10	10	10	10	10	10
12	6	10	7	10	10	10	10	10	10	10	10	10	10	10
13	5	10	6	10	10	10	10	10	10	10	10	10	10	10
14	4	10	5	10	10	10	10	10	10	10	10	10	10	10
15	3	9	4	10	10	10	10	10	10	10	10	10	10	10
16	3	9	4	10	10	10	10	10	10	10	10	10	10	10
17	2	9	3	10	10	10	10	10	10	10	10	10	10	10
18	1	9	2	10	10	10	10	10	10	10	10	10	10	10
19	0	9	0	10	10	10	10	10	10	10	10	10	10	10
20	0	9	0	10	10	10	10	10	10	10	10	10	10	10
21	0	9	0	10	10	10	10	10	10	10	10	10	10	10
22	0	9	0	10	10	10	10	10	10	10	10	10	10	10
23	0	8	0	10	10	10	10	10	10	10	10	10	10	10
24	0	5	0	10	10	10	10	10	10	8	10	10	10	10
25	0	5	0	10	10	10	8	10	10	7	9	10	10	9

26	0	5	0	9	10	9	7	10	10	7	9	10	10	9
27	0	3	0	5	10	9	5	7	9	4	8	10	10	9
28	0	0	0	0	10	8	5	6	9	4	8	10	10	9
29	0	0	0	0	5	4	1	1	4	1	4	6	7	8
30	0	0	0	0	4	4	0	0	3	1	4	5	5	5
31	0	0	0	0	0	0	0	0	0	0	3	4	4	4
32	0	0	0	0	0	0	0	0	0	0	2	4	4	1
33	0	0	0	0	0	0	0	0	0	0	2	1	2	1
34	0	0	0	0	0	0	0	0	0	0	0	0	0	0

2.2.2 Measurements

Flower weight, water uptake, flower colour, stem angle, bacterial load, and vase life were measured in experiment 1, 2, 4. In experiment 3, bacterial load and vase life were measured. Flowers were removed as they died, so flower numbers reduced over time (Table 2.2). At the end of vase life data analysis was stopped more than 3 flowers had died in a treatment.

2.2.2.1 Flower and Solution Weight

Flower weight and bottle weight (container weight + solution weight) were measured at the same time each two days in grams from first day arrival with one decimal point using Mettler Toledo digital balance (Lower Hutt, New Zealand) (Jafarpour et al., 2015; Pompodakis, Joyce, Terry, & Lydakis, 2004). Empty bottles were weighted before the experiment began. Solutions were topped up to the original depth with distilled water, and the weights of solutions were recorded before and after topping up. Three empty tubes containing distilled water were run in each experiment to measure the evaporation of solutions.

2.2.2.2 Flower Colour

Flower colour was measured by Minolta CR-200 chroma meter (Japan). The measurement is the Commission Internationale de l'Eclairage (CIELAB) colour score. The lightness coefficient, 'L' 'a' 'b' colour involve lightness coefficient, 'L' (ranges from black = 0 to white = 100), corresponding to the vertical axis, and two chromatic components: 'a' (from green (negative) to red (positive)) and 'b' (from blue (negative) to yellow (positive)). The chroma parameter 'C' value is calculated with an equation: $C = (a^2 + b^2)^{1/2}$, which indicates the vividness of color, and hue angle ($h° = \arctan(b/a)$) represents the basic colour (Voss, 1992; Xue et al., 2016).

2.2.2.3 Stem Angle

Stem angle was estimated each two days to the nearest 10° using a 15

cm 360° Celco protractor (China). As shown in figure 2.2, the angle is estimated between a line drawn through the main stem and a line drawn through the stem just below the capitulum; i.e. 0° represents a fully upright flower, 90° is a flower bent fully at right angles to the stem (Asgari & Moghadam, 2015; Çelikel & Reid, 2002).

Figure 2.2: Measurement of flower angle (NB angle becomes bigger from left to right)

2.2.2.4 Vase Life

The definition of the end of vase life (EOVL) of 'Navy' was flowers showing at least one of the following symptoms: stem bending (≥ 90°) (Figure 2.3 A and B), petal curling (Figure 2.3 D) or wilting (Figure 2.3 E) (Solgi et al., 2009), neck bending (Figure 2.3 C) or petal abscission (Figure 2.3 F) (in these experiments, the time when almost 100% flower petals had changed (curling or wilting), or when the first petals had started abscission, was defined as the end of vase life). Moreover, the date of flowers death was recorded, and any measurements from this date, except angle, were deleted.

Figure 2.3: The symptoms of the end of vase life (NB picture A, B for stem bending; C for neck bending; D for petals curling; E for petals wilting; F for petals abscission)

2.2.2.5 Bacteria Load

The number of microorganisms was counted by Julia Good, using pour plates counting method in Cook (1991) to generate the number of Colony Forming Units ml^{-1} (CFU ml^{-1}). Serial dilutions are used for estimating bacterial numbers but the initial load has to be estimated based on experience. The bacteria test results of several samples were inadequate to allow accurate estimation (7 in experiment 1; 3 in experiment 4). Three samples were randomly selected in each treatment of experiment 1 (day 13 after flower arrival), experiment 2 (day 8 after flower arrival), experiment 4 (day 8, 15, 22 after flower arrival). In experiment 3 (day 11, 21, 31 after flower arrival), only two samples were measured for each treatment. Each sample was 3 ml, removed

with a precision pipette (Hamilton, Switzerland).

2.2.3 Statistics

Results were analysed using SAS 9.4. All data were analysed using an analysis of variance (ANOVA), followed by a Tukey's studentised range test, which included flower weight, water uptake, plant growth, transpiration, colour ('L', 'a', 'b'), vase life and bacteria number. On each experiment, ANOVA is just allowed to use for comparing amongst treatments, instead of each measurement; since repeated measurements in same samples each time leading to the data not be independent, which is a precondition for using ANOVA.

2.3 Results

2.3.1 Flower Weight and Water Relations

As it was mentioned, flower weight is an essential index for identifying flower quality. As the time is different when those experiments were undertaken, it is necessary to know whether flowers in different experiments had same quality at the beginning of each experiment. In experiment 1, original flower weights (g) were significantly heavier than in expt. 2 and 4 (Table 2.3) ($p < 0.05$), while within each experiment there were no significant differences amongst treatments ($P > 0.05$).

Table 2.3: Original flower weight (g) of flower on the first day after flower arrival (NB MSD requires the amount of data being balance, while the number of flowers is 40 in experiment 1, and other two experiments is 50; so the MSD of three experiments cannot be obtained)

Experiment	Treatment	Mean of flower weight (g)
1	1	15.42
	2	16.37
	3	15.35
	4	16.3
	MSD	2.5419
	Mean	15.86[a]
2	1	13.02
	2	12.67
	3	12.31
	4	12.59
	5	12.92
	MSD	1.941
	Mean	12.70[b]
4	1	11.91
	2	12.58
	3	13.44
	4	12.81
	5	13.61
	MSD	2.0752
	Mean	12.87[b]

As the flower weight (g) at first arrival day were different in different experiments, the flower quality may not be identical among experiments. But there was a similar pattern of change in all experiments; flowers were slightly

dehydrated on arrival so weights initially increased for several days; then retained a stable weight (although this period is not obvious in experiment 1), and then decreased in weight (Figure 2.4).

According to Tukey's studentised range test, there is no significant difference ($P > 0.05$) in flower weight amongst treatments within each experiment. Finding breakpoint of each line was tried, but the results were not very convincing to use this method. Breakpoint needs to set the exact day when data is showed; but the measurement date has interval, and when each measurement date was tried and each curve was divided into to line in this day, the fitness of two lines (R square) at each measurement all was low and the two lines were disconnected at the setting date. Basically a few consecutive days where (a) the weight was relatively constant and then (b) the later data where weight is clearly decreasing by eye this change happens around day 11 to 15 (exclude first experiment), but the 'breakpoint' date is always not in this area. Therefore, this approach was not used, and trend changes of line were observed visibly. The trends of flower weight changes in different treatments seem similar in each treatment. In first experiment, flower weights of four treatments gradually declined after day 3, and flowers in treatment 1 and treatment 3 (solutions without sucrose), began to die from day 9 (Table 2.2), but from data of flower weight, there was no significant decrease even by day 9. In experiment 2, except for treatment 2 (4% sucrose with 100 ppm 8-HQC), which decreased after day 9, other four treatments had a relatively obvious drop in fresh weight that began around day 15 to 17. In experiment 4 (Figure 2.4 C), the steeper decline in weight began about day 11, but in treatment 4, flower weight seemed to decrease a little earlier.

If flower fresh weight begins to decrease from a certain date it is important to establish whether this is because of accelerated weight loss (wilting induced by accelerated transpiration) or reduced water uptake. Water uptake (g/h), is defined as the difference in solution weight in a vase across a two-day period, with the flower lifted out of the solution each time. A small

correction is included to allow for evaporation (mean of three empty vases in the same period).

$$\frac{SW_{d+2} - SW_d - SE}{h}$$

=Water uptake (g/h)

Equation 2.1: Water uptake of gerbera each hour in each two days (SW_d is solution weight on day d; h is the exact number of hours between measurements; SE is solution evaporation during those two days)

Water uptake (g/h) is very variable on the first two days as flowers have been transported dry and are rehydrating in the vases. This period is basically complete by day 3 and the data settle to very consistent rates in each experiment for about two weeks thereafter (Figure 2.5).

Water uptake is positive throughout the experiments, and the initial period from day 3 to day 13 shows a remarkably consistent rate of water uptake, ca. 0.11 in each experiment (0.11 g/h in expt. 1; 0.10 g/h in expt. 2; 0.11 g/h in expt. 4). Subsequently, from some point, the rate of water uptake starts to decline in all experiments. Visually this appears to begin on day 13 in expt. 1 (As the stem bending occurred in treatment 1 and 3, flowers were dead before day 13) and on day 15 in expt. 2 and 4 (Figure 2.5, A, B and C).

Some of the data are more 'noisy' on particular days, and this is likely to be a response to inconsistent temperature control on those days. The room temperature is graphed in figure 2.6 and shows that there was a period when the room heating must have been accidentally turned off from day 15 to 18, and the temperature fell to between 4 and 18 °C. Method of missing data was used by SAS, and the new expected data got and was showed on figure 2.7.

Specifically, some treatment differences should be mentioned. Experiment 2 showed the largest variability between treatments, and using repeated measures ANOVA it is true that treatment 5 (6% sucrose) significantly reduces ($P < 0.05$) uptake compared to treatment 4 (2% sucrose), with the other data lying

in between, from days 7 – 11. The other treatment difference was an early difference ($P < 0.05$) in water uptake between treatments 2 and 4 in experiment 1. According to Tukey's Studentized Range (MSD), there is a significant difference between treatments on day 3 and 5 ($P < 0.05$), and sodium hypochlorite is the better treatment. Moreover, as mentioned before, reduced water uptake can be an important contributor for stem bending. Therefore, it is necessary to observe the original data to analyse the situation of water uptake when stem bending occurred to get more information about stem bending. Figure 2.8 shows that before stem bending happened in treatment 1 and 3, water uptake was still high and only began to decrease around day 10 – 12. This makes it clear that reduce water uptake was not the trigger for early stem bending.

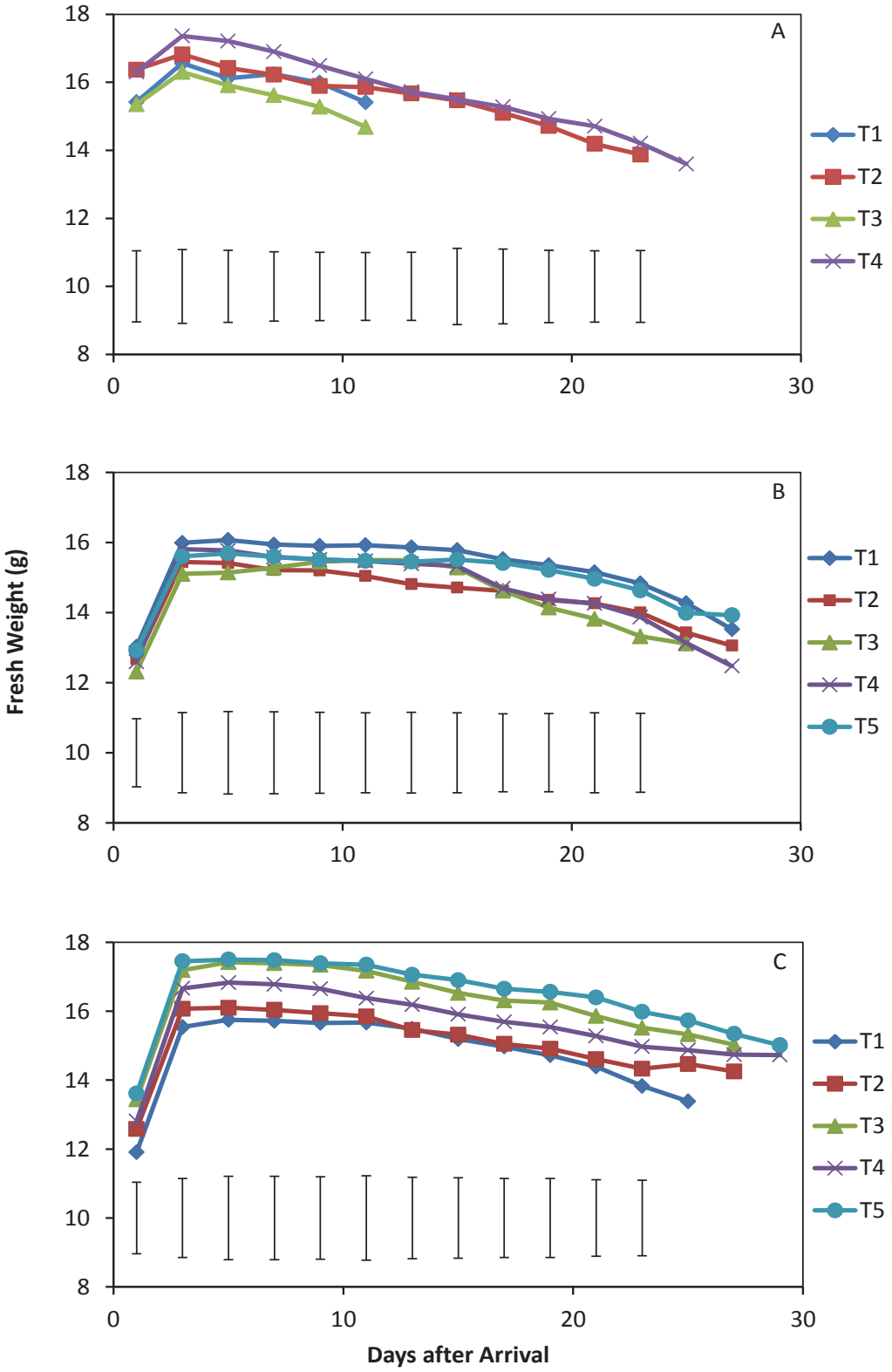

Figure 2.4: Fresh weight (g) of 'Navy' every two days in experiment 1(A), 2 (B), 4 (C) on the day after flower arrival (NB Tukey's MSD of each treatment showed at the bottom of figures; MSD in experiment 1 was only for T2 and T4; solutions of each treatment are shown in table 2.1)

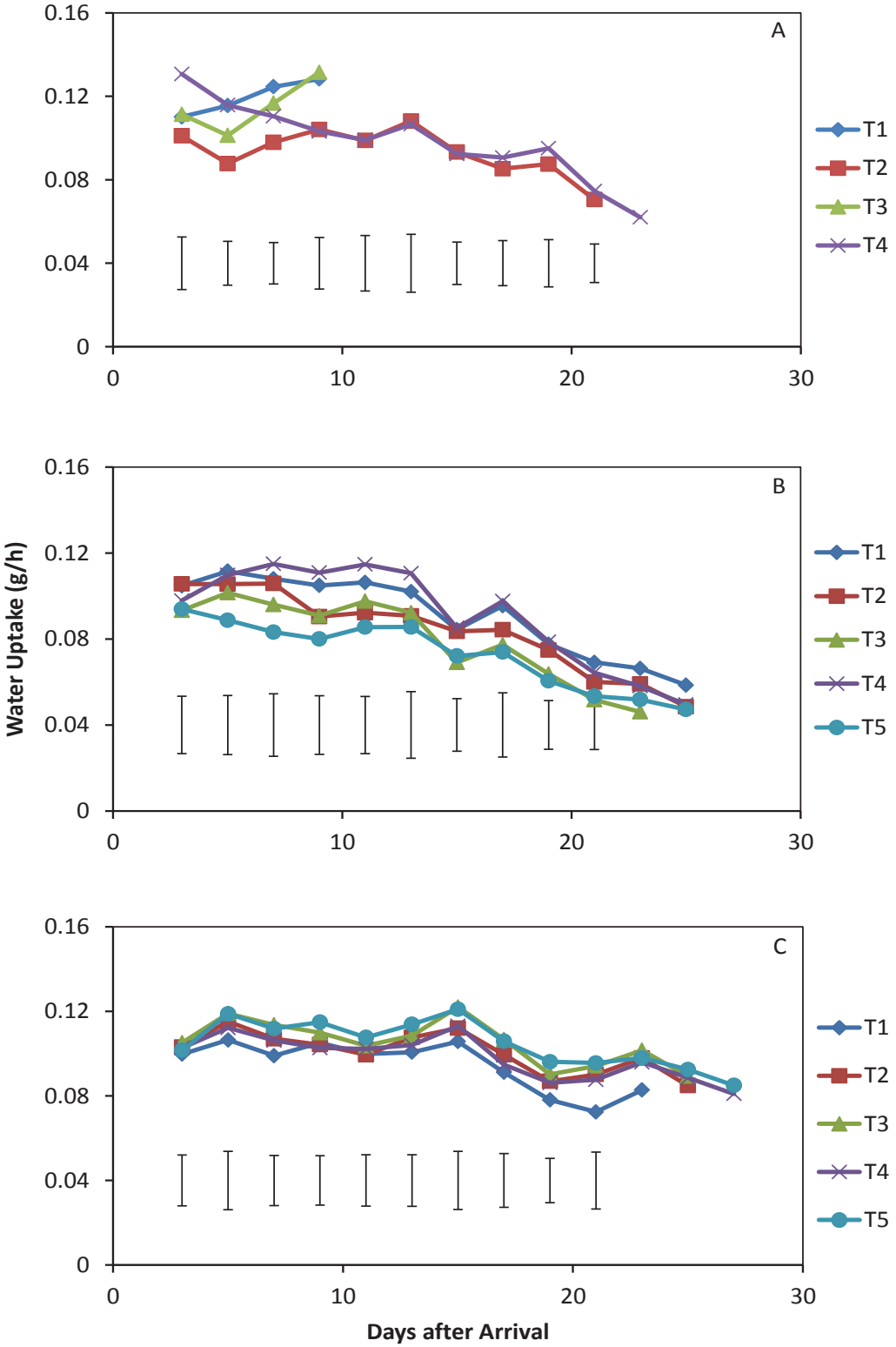

Figure 2.5: Water uptake (g/h) of 'Navy' every two days in experiment 1(A), 2 (B), 4 (C) on the day after flower arrival (NB Tukey's MSD of each treatment showed at the bottom of figures; MSD in experiment 1 was only for T2 and T4; solutions of each treatment are shown in table 2.1)

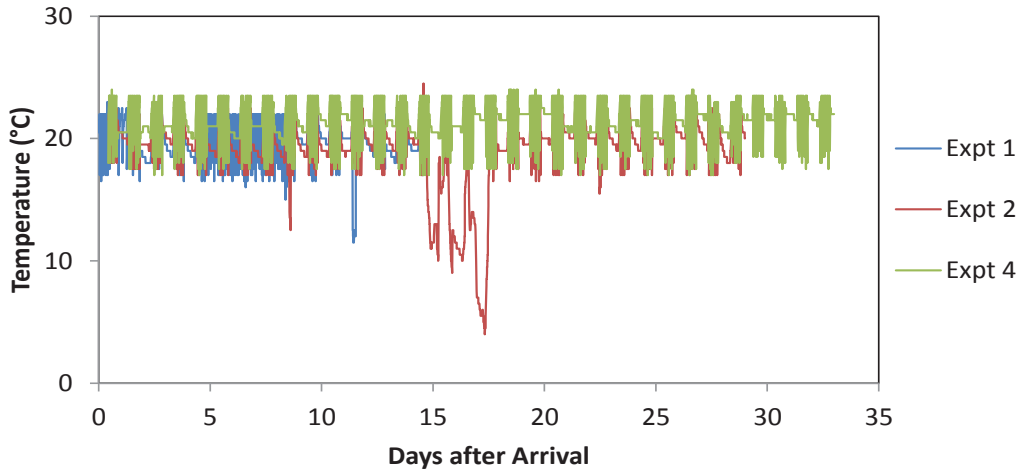

Figure 2.6: Temperature of experiment 1, 2 and 4 in each day from the day after arrival to the day ending the experiment (NB temperature data of experiment 1 stopped on day 15 after arrival for logger failure)

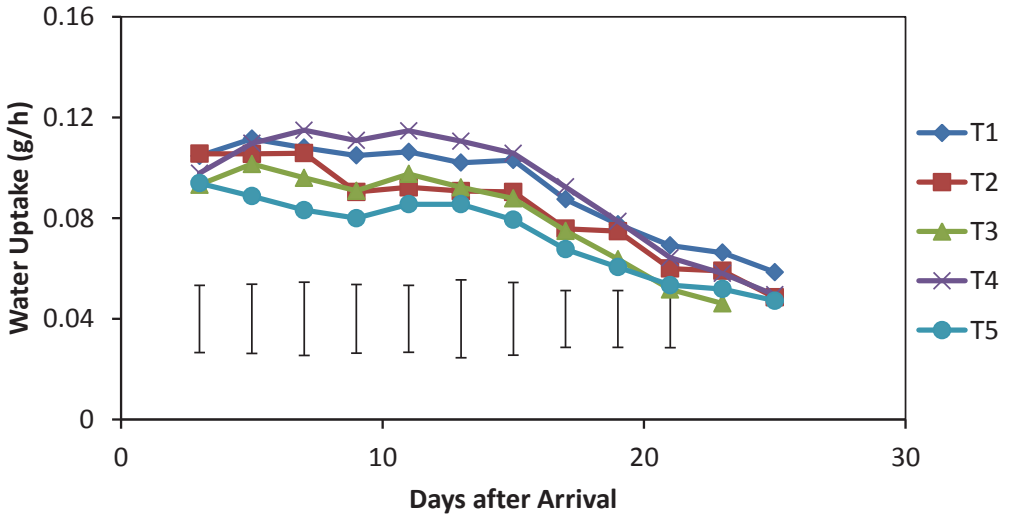

Figure 2.7: Water uptake (g/h) of 'Navy' every two days on the day after flower arrival after modifying data in experiment 2 (NB Tukey's MSD of each treatment showed at the bottom of figures)

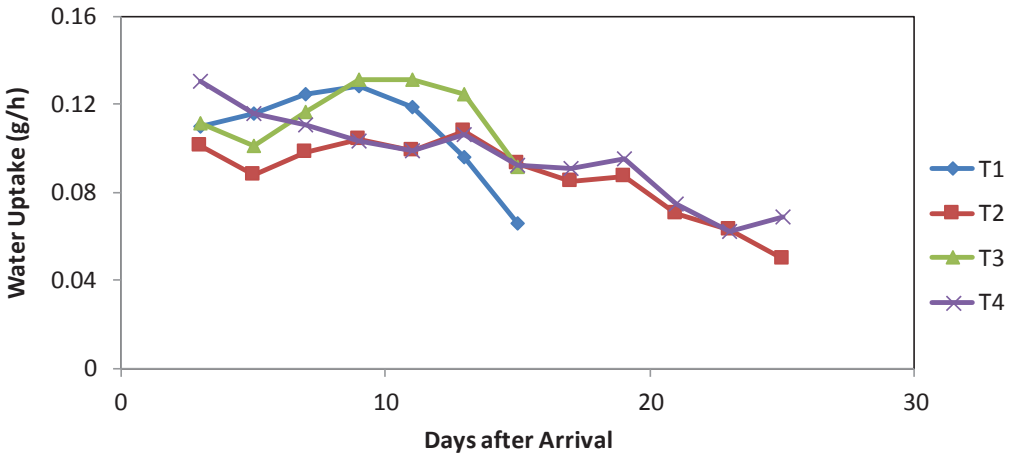

Figure 2.8: The original data of water uptake on the day after flower arrival in experiment 1

Transpiration ($\frac{WU_d - FW_d}{h}$) (g/h) is the difference between water uptake and plant weight change (plant growth). In these experiments, the results of transpiration are almost the same as water uptake, because plant growth (g/h) is very small (-0.02 to 0.01 g/h, Figure 1 in appendix). Therefore, transpiration almost has the same trend and result as water uptake (Figure 2 in appendix); there is certainly no acceleration in transpiration that might have contributed to flower wilting or stem bending.

2.3.2 Flower Colour Changes

'L', 'a', 'b' of flower colour for CIELAB have been shown on figure 2.9, 2.10, and 2.11, and 'C' and 'h°' were also calculated, but 'b' value is so tiny that 'C' is almost the same as 'a' value and 'h°' is closely related to 'b' value; therefore, their figures are not shown here, but can be found in appendix (Figure 3 and 4 in appendix).

'L' value is for the lightness coefficient. From the results, flowers became whiter and then go back to dark (Figure 2.9). Except for day 21 and 24 in experiment 2, 'L' value is no different amongst treatments ($P > 0.05$) in each experiment. Visibly, in experiment 1, 'L' value starts to drop at a very late date, almost day 20 for treatment 2 and day 22 in treatment 4. In experiment 2, the

big drop is not so clear, with a gradual decline after day 10, but the final drop should be from day 17. Experiment 4 has an obvious trend, and 'L' value drops from day 16.

Flower colour 'a' presents the green to red colour axis. From all experiments, the 'a' value increased to a peak, and then declined (Figure 2.10). There was no consistent difference amongst different treatments. In experiment 2, on day 1, 14 and 24, there were significant differences ($P < 0.05$) among treatments; and in experiment 4, on day 23, treatment 1 is significantly lower than others. But those differences just occurred on specific days, and disappeared in later measurements. Therefore, they may represent random variability and could be ignored. In experiment 1, the biggest drop in 'a' value occurred on day 19; and experiment 2 on day 17; and experiment 4 on day 15.

Colour 'b' represents the colour axis from yellow to blue. The flowers in three experiments basically increased in 'b' value and then decreased again. For 'Navy', 'b' value can be an important indicator associated with flower wilting, for its colour visibly became more bluish when it happened (Figure 2.11). The day when the big drop began was different amongst different treatments within an experiment. In experiment 1, treatment 2 and 4 had an obvious decrease on day 19 to 20. Moreover, based on the analyses of Tukey test, the previous measurement (day 16) and the later measurement (day 22) of those two days were significantly different ($P < 0.05$). It seems treatment 4 falls faster, but difference may be not significant. In addition, it should be mentioned that treatments 1 and 3 in experiment 1 had a decline in colour 'b' when they showed stem bending. Usually data are not presented once more than 3 flowers have died in a treatment; but when all the original data are retained the decrease can be seen from Day 9 (Figure 2.12). Tukey's Studentized Range (MSD) Test showed that for almost each measurement date (apart from the first day, when just treatment 3 is different from 2 and 4), treatments 1 and 3 were significantly lower ($P < 0.05$) than other two treatments for 'b' value. However, in experiment 2, only treatment 4 was significantly different ($P < 0.05$) from

other four treatments on day 7, 10, 14, 17 and 24. And it seems other four treatments did not show a rapid decline until day 15, while treatment 4 started to decline from day 10. All treatments in experiment 4 obviously have a big decrease on day 16.

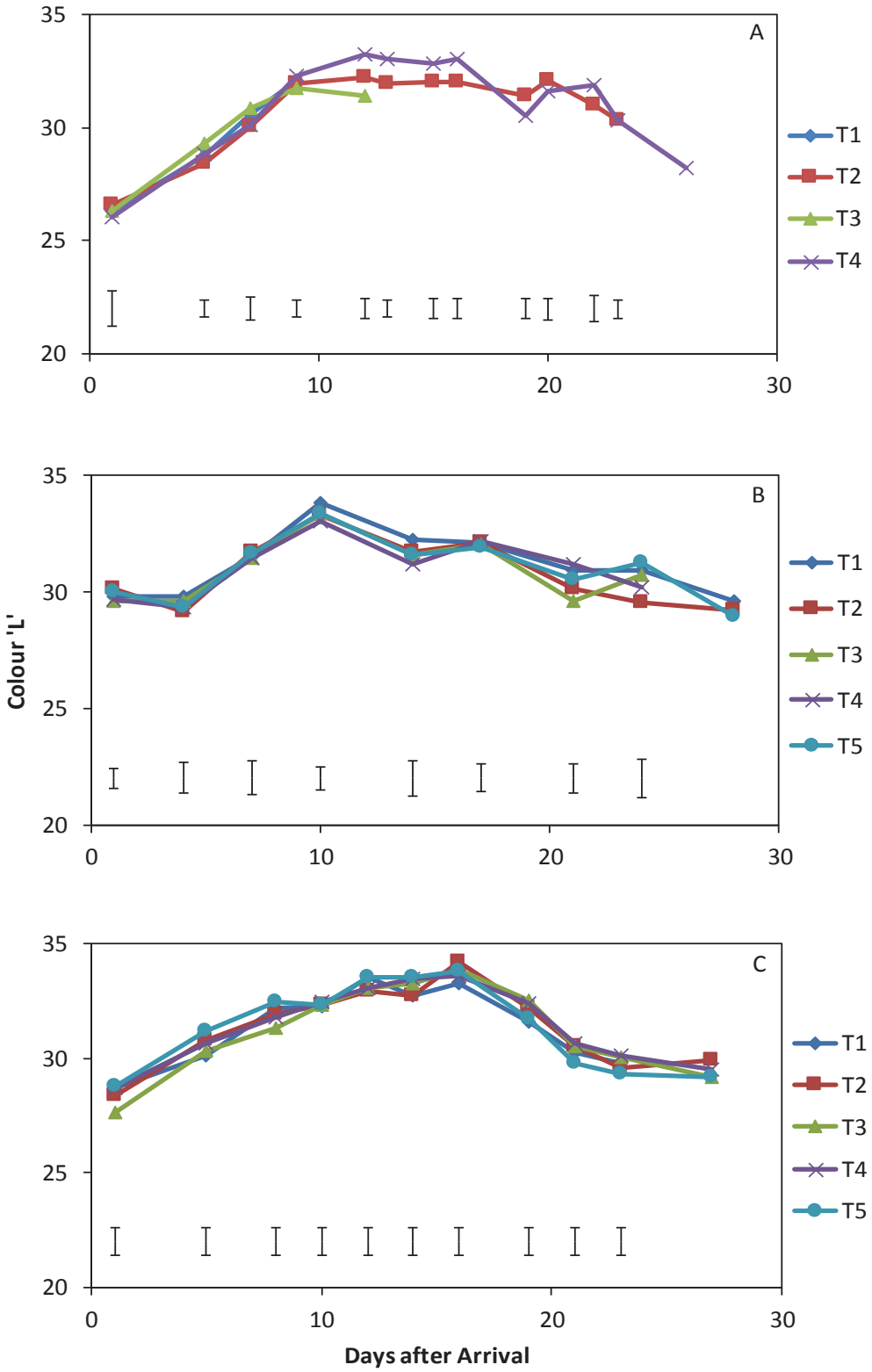

Figure 2.9: Colour 'L' of 'Navy' every two days in experiment 1(A), 2 (B), 4 (C) on the day after flower arrival (NB Tukey's MSD of each treatment showed at the bottom of figures; MSD in experiment 1 was only for T2 and T4; solutions of each treatment are shown in table 2.1)

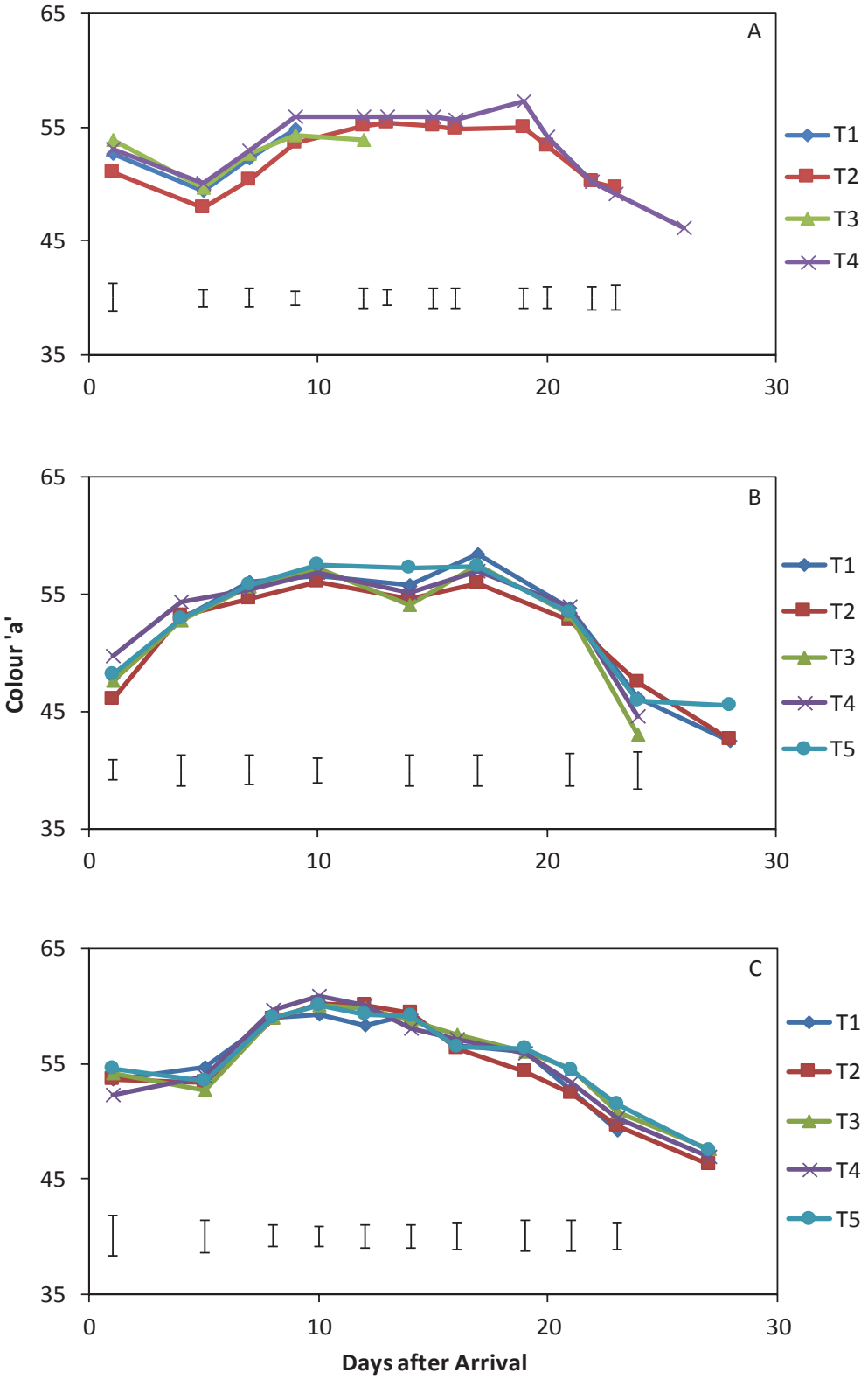

Figure 2.10: Colour 'a' of 'Navy' every two days in experiment 1(A), 2 (B), 4 (C) on the day after flower arrival (NB Tukey's MSD of each treatment showed at the bottom of figures; MSD in experiment 1 was only for T2 and T4; solutions of each treatment are shown in table 2.1)

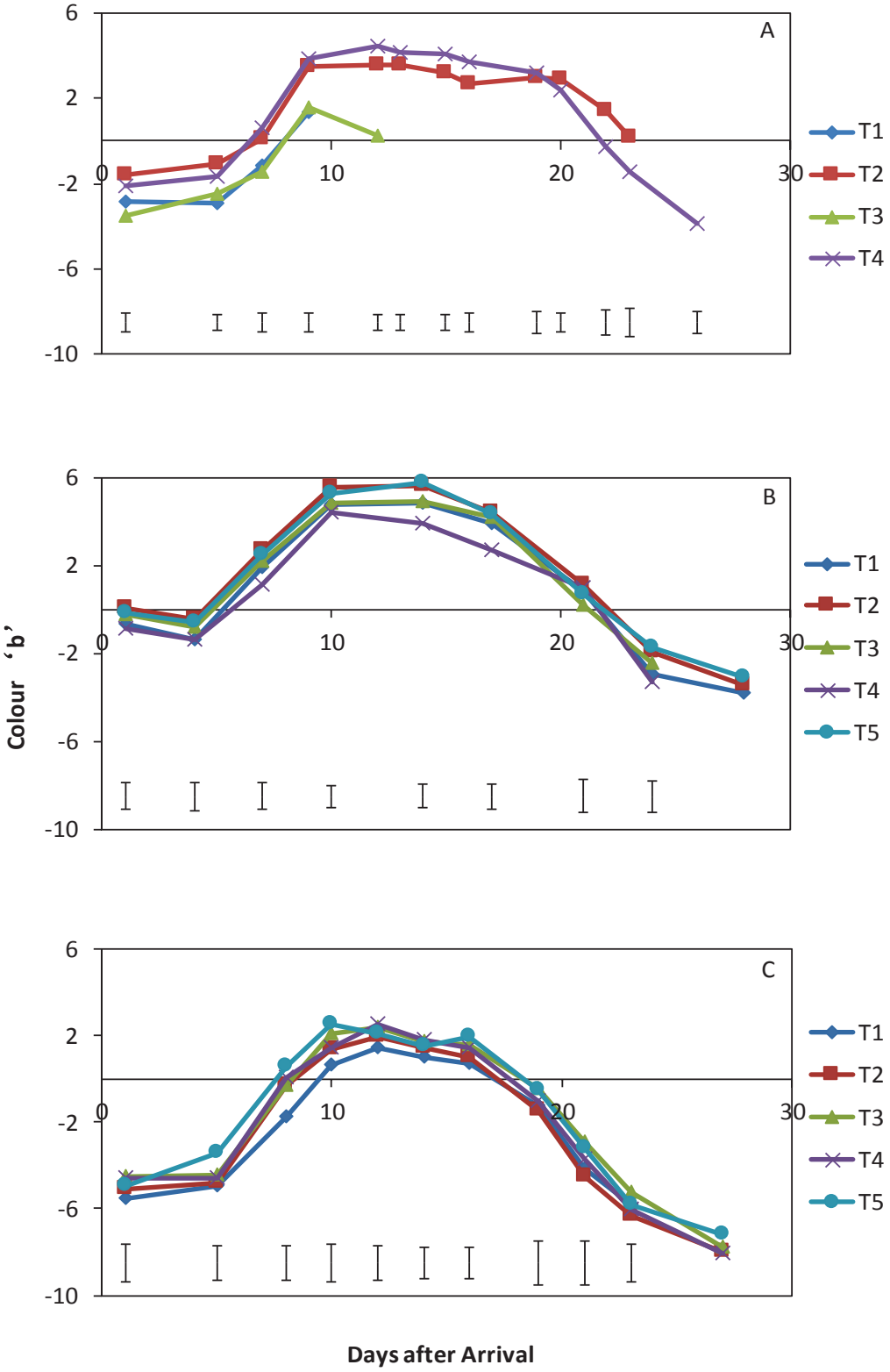

Figure 2.11: Colour 'b' of 'Navy' every two days in experiment 1(A), 2 (B), 4 (C) on the day after flower arrival (NB Tukey's MSD of each treatment showed at the bottom of figures; MSD in experiment 1 was only for T2 and T4; solutions of each treatment are shown in table 2.1)

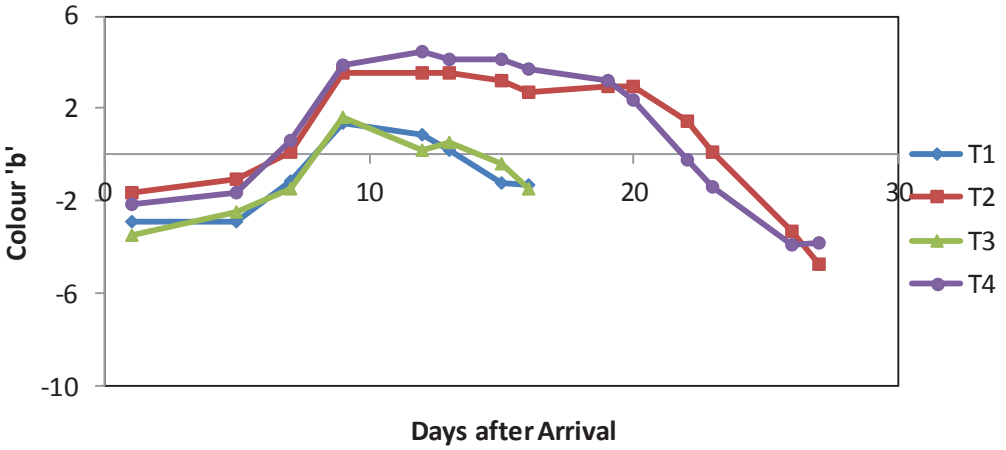

Figure 2.12: The original data of colour 'b' on the day after flower arrival in experiment 1

2.3.3 Angle Changes

According to figure 2.13, we know flowers look bent on the first day of arrival after their dry transportation, and then recover over first two days. According to Tukey's Studentized Range (MSD), there was no significant difference ($P > 0.05$) among treatments in any experiment (Figure 2.13).

When stem bending occurred, the stem bent approximately 10 cm below flower head (Figure 2.3 A and B), and flower angle sharply increased on day 9 (treatment 1 and 3 in figure 2.13 A). Otherwise, flower angle began to increase gradually (treatment 2 and 4 in experiment 1 and all treatment in experiment 2 and 4) at a certain point: this change occurred on day 11 in almost all treatments, except for treatment 2 (a little later) and treatment 3 (a little earlier) in experiment 2; and the stem may also change shape as shown on the picture C of figure 2.3, which is caused by stem wilting approximate 1 cm below the flower head.

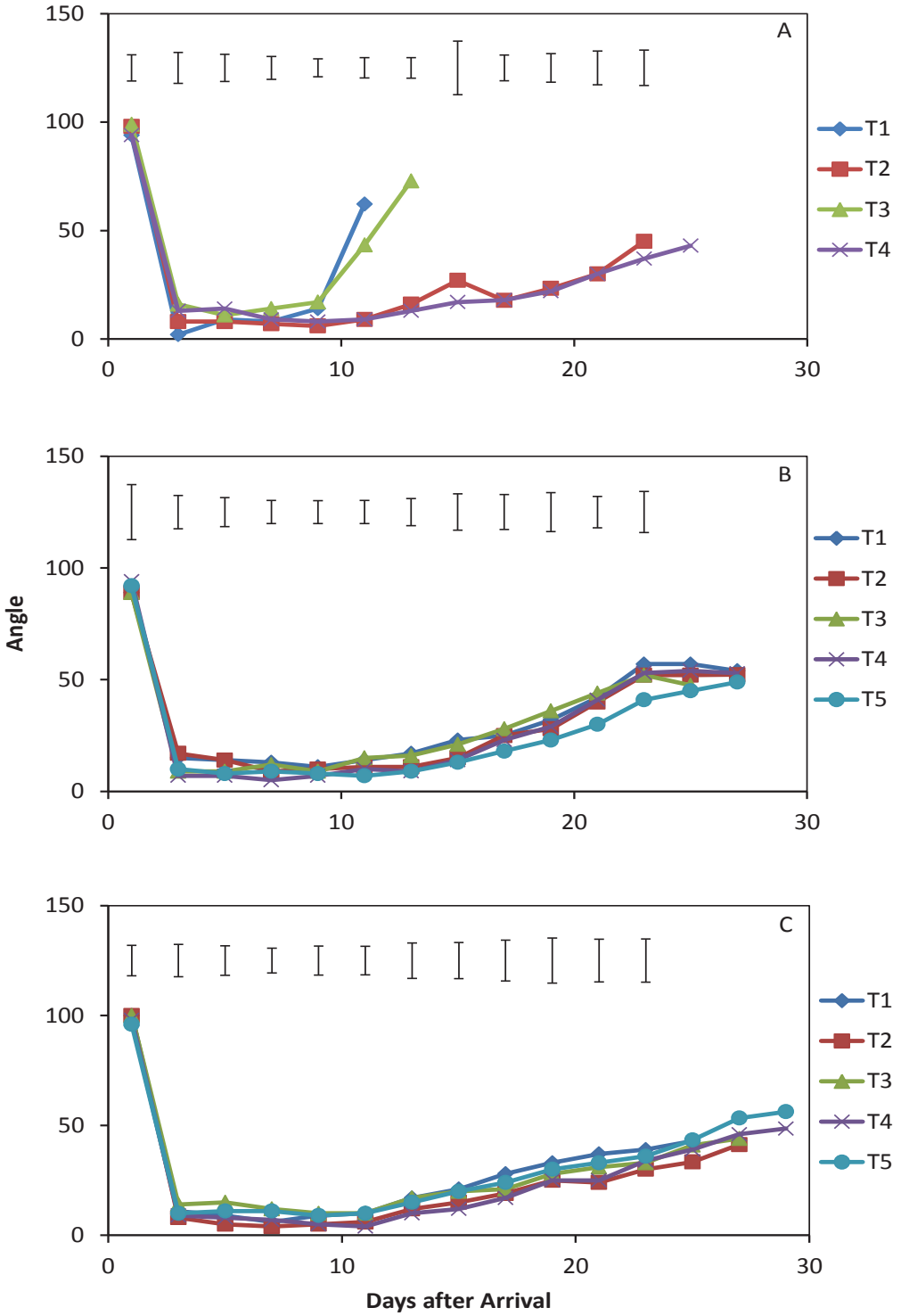

Figure 2.13: Angle of 'Navy' every two days in experiment 1(A), 2 (B), 4 (C) on the day after flower arrival (NB Tukey's MSD of each treatment showed at the top of figures; MSD in experiment 1 was only for T2 and T4; solutions of each treatment are shown in table 2.1)

2.3.4 Vase Life Difference

Vase life based on subjective assessment is shown in figure 2.14. It is clear that in expt. 1, treatment 1 (water) and 3 (50 ppm sodium hypochlorite) have a significantly shorter ($P < 0.05$) vase life than treatment 2 and 4. As for experiment 2 (4% sucrose and 100 ppm 8-HQC), treatment 3 is significantly shorter ($P < 0.05$) than the other four treatments. In experiment 4, the only treatment without colloidal silver (4% sucrose with 200 ppm citric acid) was the worst treatment ($P < 0.05$) for vase life.

Moreover, figure 2.15 shows in experiment 3, 4% sucrose with 5 ppm colloidal silver is best solution ($P < 0.05$), and the 4% sucrose with 80 ppm sodium hypochlorite is the worst one ($P < 0.05$) for vase life of gerbera.

An attempt was made to define an objective measurement for end of vase life. Because of the very consistent pattern of change of head angle (Figure 2.13), an arbitrary figure of 35 degrees was chosen that generally coincided with the subjectively-defined day for end of vase life. However, since data were collected only every two days, and rounded to 10 degrees precision, using this figure to calculate end of vase life for each stem did not help to separate treatments within an experiment (data not shown).

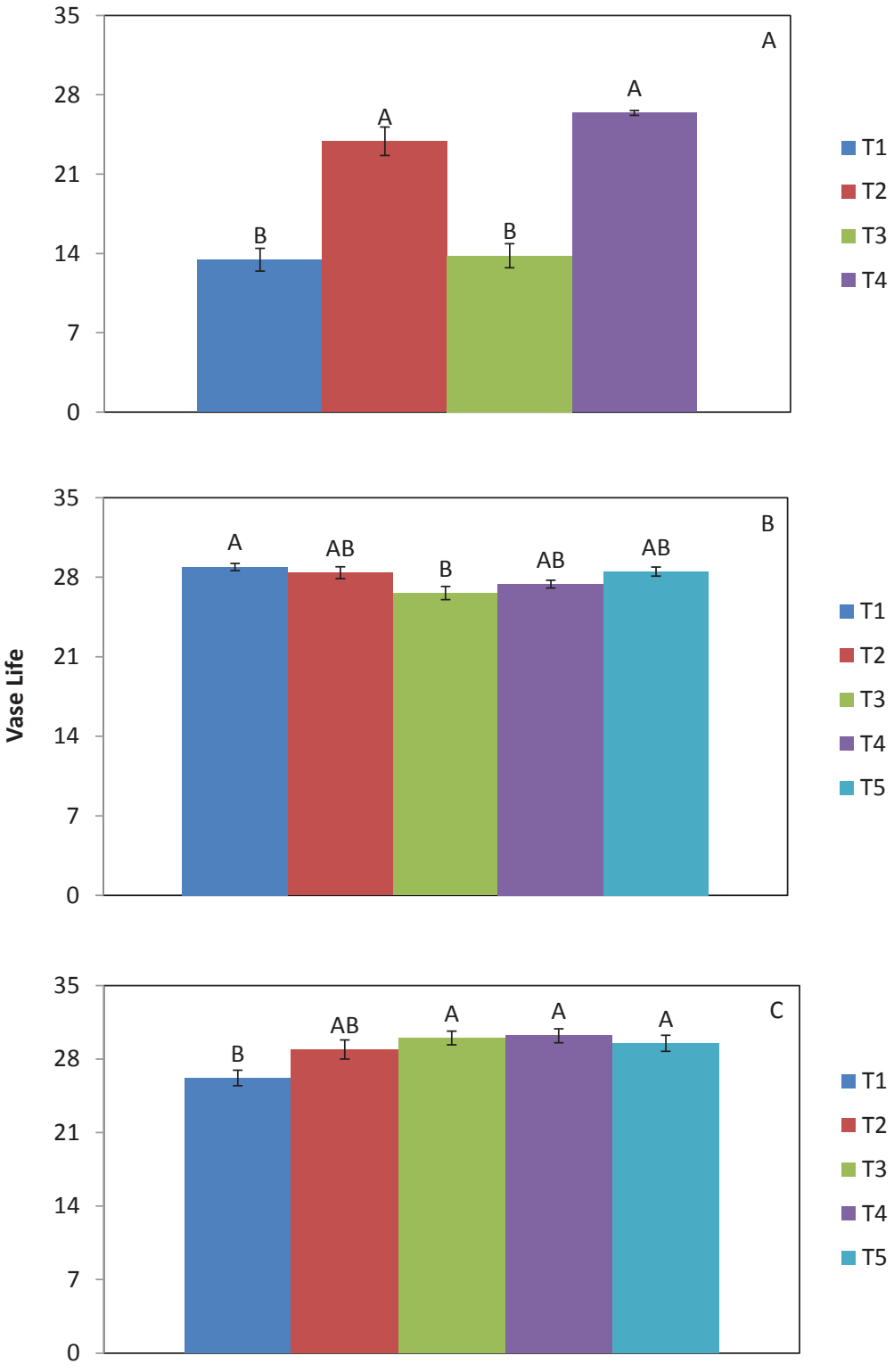

Figure 2.14: Vase life of 'Navy' in experiment 1(A), 2 (B), 4 (C)

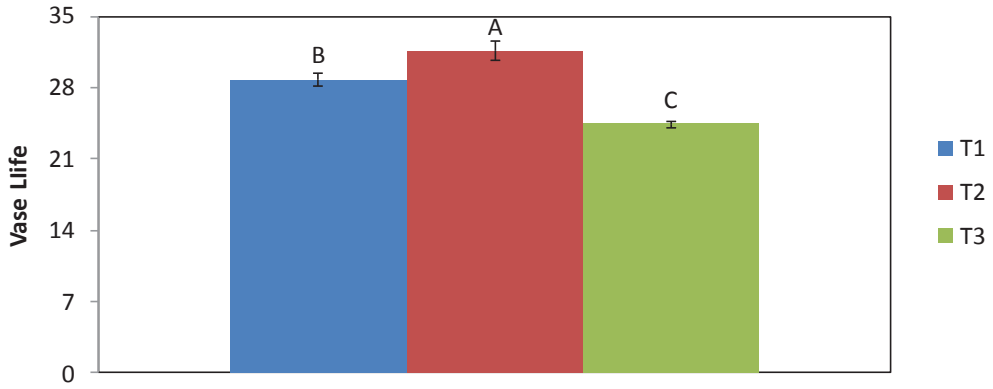

Figure 2.15: Vase life of 'Navy' in experiment 3

2.3.5 Bacteria Number in Different Solution

Estimates of bacteria number in vases are cumbersome to make and may not accurately represent the number of bacteria in biofilms on stems. In experiment 1, the first estimate of bacterial number was made on day 13, but the samples were all too concentrated for the chosen dilution series so the results are not useful (Table 1 in appendix). With greater dilution, there were no significant differences amongst treatments in experiment 2 (Table 2.4), although the higher 8-HQC concentration (treatment 3 with 4% sucrose and 400 ppm 8-HQC), gave a bacterial concentration at the lower end of the range (10^4 CFU ml^{-1}, compared to almost 10^6 CFU ml^{-1} for all other treatments on day 8 after arrival (Table 2.4). In experiment 3, the difference among treatments is also not significant on any of the three measurement days (which may be due to the low number of samples making MSD large), but treatment 2 which contained colloidal silver was always at the lower end of the range (Table 2.5). Experiment 4 gave some different insights. Bacteria number in treatment 1 (4% sucrose with 200 ppm citric acid) was significantly higher than other treatments (which all contained bactericides) on day 8. But by day 15 and 22, there were no significant differences amongst treatments (Table 2.6).

Table 2.4: The log count of bacteria number in experiment 2 on day 8 after flower arrival

Treatment	Mean of log count on bacteria number
1	6.106[a]
2	6.599[a]
3	3.870[a]
4	6.242[a]
5	5.799[a]
MSD	3.154

Table 2.5: The log count of bacteria number in experiment 3 on day 11, 21 and 31 after flower arrival (NB n.d. = not determined; no living flowers remaining)

The date of determination after arrival	Treatment	Mean of log count on bacteria number
11	1	5.427[a]
	2	1[a]
	3	4.988[a]
	MSD	4.683
21	1	6.762[a]
	2	5.476[a]
	3	5.491[a]
	MSD	7.127
31	1	n.d.
	2	5.946
	3	n.d.
	MSD	—

Table 2.6: The log count of bacteria number on in experiment 4 on day 8, 15 and 22 after flower arrival (NB because the samples were too concentrated for the certain chosen dilution series which were one sample in treatment 4 and two samples in treatment 5 on day 15, those results were not useful leading to MSD cannot be got for those uncompleted data)

The date of determination after arrival	Treatment	Mean of log count on bacteria number
8	1	6.985[a]
	2	0.900[b]
	3	1.181[b]
	4	1.181[b]
	5	1.282[b]
	MSD	1.241
15	1	7.449[a]
	2	6.985[a]
	3	7.161[a]
	4	7.273[a]
	5	6.447[a]
	MSD	—
22	1	7.614[a]
	2	7.250[a]
	3	7.698[a]
	4	6.650[a]
	5	6.830[a]
	MSD	1.940

2.4 Discussion

The quality of flowers tested within each experiment was similar, as shown by their original flower weights. But for different experiments, the original weight was different; which was probably caused by the different growing and harvesting seasons, based on literature. For this project, the flowers were harvested in expt. 1, 2 and 4 on 3 June, 3 August and 21 October, so these flowers were grown in autumn, winter and spring, respectively. Therefore, the difference among experiments in original weight means fair comparisons should be made within each experiment.

Furthermore, it should be mentioned that experiments were designed iteratively based on results of previous one. In the first experiment, it was found that 'Navy' stem bending problem can be solved by supplying sucrose alone. After the absence of this issue, next step was figuring out whether 'Navy' can be affected by water stress leading to premature senescence and shorter vase life; which according to previous studies may be mainly caused by bacteria or physiological plugging. But according to the data in experiment 1, it is hard to confirm, because there was no obvious evidence for bleach being beneficial to keep flower quality and extend vase life. Unfortunately, in this experiment, the result of bacteria test was not precise as it had too high bacteria content and the dilution multiple was not enough. It is reasonable to consider that sodium hypochlorite may be not good enough to control bacteria, and it was shown in previous research that the effectiveness of bleach is not lasting, especially when it is presented with flowers which may produce organic matter and reduce the solution pH which can significantly reduce its effectiveness (the effective compound of antibacterial) (Xie, Joyce, Irving, & Eyre, 2008). Therefore, it was necessary to try other bactericides. Some former studies showed 8-HQC was excellent to control bacteria and positive to improve the vase life of gerbera (Vaidya & Collis, 2013; Wang et al., 2014); and it was used to examine on 'Navy' to see whether it worked as well, testing a range of concentrations. The bacteria results showed 200 ppm 8-HQC was not effective to control bacteria; by day 8,

there were over 10^6 CFU/ml bacteria, which was high enough to influence gerbera (Balestra et al., 2005). There did seem to be some benefit in reducing bacteria at 400 ppm 8-HQC but these flowers showed a phenomenon of stem damage (stem under the water solution became brown after just 1 or 2 days); and the toxicity of 8-HQC for other gerbera variety was discovered in previous study as well (de Witte et al., 2014). So it shows 8-HQC may be effective, but only at a concentration that is too high to be supported by 'Navy'. More generally, the results of bacterial assessment suggest that numbers rise steadily in the vases and although the data were not statistically significant it seems clear that the few very low measurements were found only up to day 11. It would seem that the beneficial effects of tested bactericides, as assessed by numbers of free bacteria in the vase, were lost after that time; yet there were differences in vase life amongst treatments. At the least this suggests that while bacterial contamination may shorten the final vase life (e.g. in experiment 3 and 4, Figure 2.14 and 2.15), it does not correlate with any earlier, measurable changes in flower weight, water uptake or flower colour amongst treatments. Interestingly, it was found some antimicrobial compounds lead to the selection of tolerant bacterial species (de Witte et al., 2014; van Doorn, 1997); so increasing bactericidal concentrations is not a good strategy on environmental grounds. It found that 2% sucrose can be enough to eliminate stem bending, but 4% sucrose was better for keeping flower quality in some indexes, including flower colour 'b' value change, which is a very important index to determine petal senescence (the drop in b value during senescence indicates when increases in membrane permeability raise the vacuolar pH, which is a metabolic change central to petal senescence during post-harvest life of cut flowers (Borohov, Tirosh, & Halevy, 1976; Rani & Singh, 2014)). Experiment 3 is a simple one just for quickly to testing the effectiveness of different antibacterial materials on controlling bacteria and affect the longevity of 'Navy' vase life. And the positive results were gained which showed colloidal silver was a good one. The following experiment used the colloidal silver as a bactericide. Additionally,

citric acid was supplied as well to try to reduce physiological plugging (van Meeteren, 1978b).

Stem bending will be discussed separately first. As mentioned before, Steinitz (1983) grouped stem malformations into stem bending, stem folding and neck folding. 'Navy' have similar situation with 'Clementine'. But in 'Navy', stem bending and folding seem be the same situation, they occurred in similar zone area during the same period, and both can be solved by sucrose. Based on the indexes that we got, some details should be mentioned. According to the data, we found a drop for water uptake and b value of colour started at the same time as stem bending, i.e. over 9 days after flower in vase. As mentioned according to previous study, stem bending is very close to water balance of gerbera, and before stem bending occurred, water uptake reduces were found by lots of researchers (Danaee et al., 2011; de Witte et al., 2014; Javad et al., 2012; van Meeteren, 1978a, 1978b). Authors found lots of varieties had this situation mainly caused by bacteria problem (Balestra et al., 2005; Perik et al., 2012; van Doorn & de Witte, 1994; van Meeteren, 1978a, 1978b); or the material released by dead cells, which was from stem surface resulted from antibacterial toxic effect(de Witte et al., 2014). As it mentioned, there was a precise description and explain from van Meeteren (1978b), he found there was the decline of water uptake and flower weight caused by microbial blockage that occurred in first two days. And this article presented that if gerbera went through the period of microbial blockage leading to the declining of water uptake, stem bending would not happen later, even the physiological stem plugging occurring later. But our 'Navy' results showed there was no obvious decrease in flower weight with other two treatments without stem bending; and although water uptake dropped faster after bending, there was no difference between treatments in first several days, before stem bending happened. And more important is that the surviving two treatments were not in the solution with sodium hypochlorite (anti-bacteria chemical), but in sucrose solution. And water uptake decrease was not caused by some toxic effect of hypochlorite

either, because flowers in distilled water bent as well. Other than those possibilities, there is another possible reason that it may be related to early senescence which can also result in water uptake decline. There was an evidence shown on colour change. We found 'b' value also had a relative big decrease after day 9 which showed petal senescence may have been initiated, while sucrose had an effect on delaying flower senescence. However, it seems more likely that stem bending caused the reduced water uptake which in turn caused petal blueing. According to ANOVA analysis, water uptake was not significantly different with or without sucrose; and although 'b' value of colour was different, this was an artefact that started on the first day of measurement (i.e. it must have been established by accident during randomisation). Apart from the effectiveness of sucrose in delaying senescence, as mentioned before, it has another potential important impact on improving stem mechanical strength (Perik et al., 2012; Steinitz, 1982). Therefore, it is necessary to carry out more experiments to see whether the hypothesis is right.

Since stem bending is absent once 2 – 6% sugar is included in the vase life medium, the effectiveness of antibacterial compounds needs to be compared in more detail. At the first experiment, from the results of all determinations, the benefit of 50 ppm sodium hypochlorite is not obvious, except for having a positive effect on water uptake for first few days, and it may have delayed a little the change of colour 'L'. The results of experiment 2 were more complicated, and solutions may have different impact on different aspects of senescence. In treatment 5, 6% sucrose and 200 ppm 8-HC significantly reduced water uptake compared to treatment 4 (2% sucrose with 200 ppm 8-HQC). This is likely to be an effect of the osmotic strength of the sucrose: higher concentrations for 'Navy' will make it more difficult for the stem to take up the water. However, interestingly, flower colour in treatment 4 (2% sucrose with 200 ppm 8-HQC) changed to blue earlier than other treatments, which indicated lower concentration of sucrose may make petal senescence start earlier. The conflicting results on sucrose concentrations (6% sucrose had lower water

uptake, while 2% sucrose started senescence earlier) may be because 6% sucrose is too high for 'Navy' absorbing solution by an effect of the osmotic strength of the sucrose, but sucrose has a positive effect on delaying senescence by supplying of substrates for respiration (Ghale-shahi, Babarabie, Zarei, Danyaei, & Gorgan, 2015; Halevy, 1976; Shahri, Tahir, Islam, & Ahmad, 2010). It has been known the most suitable concentration for water uptake depends on different varieties (Han, 2016), and for gerbera of 'Navy', 4% sucrose may be the best for its quality, but if only for pretending stem bending, 2% sucrose should be enough. Visibly, treatment 3 (4% sucrose & 400 ppm 8-HQC) had the shortest life, which suggests 8-HQC may be toxic at higher concentrations as mentioned. In experiment 3, visible senescence and death were used to define the end of vase life and sucrose with colloidal silver emerged as the best choice compared with sucrose with sodium hypochlorite and sucrose with 8-HQC, which may be caused by its the higher bactericidal ability. Specifically, although there is no significant difference among those treatment as the MSD is big for the sample number is too small, but after 10 days, the bacteria number of other two treatments almost reached 10^5 CFU ml^{-1}, while silver treatment just was 10 CFU ml^{-1}. The effectiveness of silver delaying gerbera senescence had been presented by lot of cultivars, such as 'Goodtimming', 'Dune', 'Carambole' (Ansari, Hadavi, Salehi, & Moradi, 2011; Geshnizjany, Ramezanian, & Khosh-Khui, 2014; Solgi et al., 2009). There was a large part of former study focused on its antibacterial effect, but still had some views on its ability to reducing ethylene production on gerbera, such as Geshnizjany et al. (2014). Moreover, some bacteria can produce ethylene (Primrose & Dilworth, 1976), it is not sure whether it is also a reason for less effective bactericides, including sodium hypochlorite and 8-HQC, having no obvious positive result for affecting 'Navy' vase life. It is necessary to have more experiments to test whether the senescence of 'Navy' is affected by ethylene. Experiment 4 suggested again silver is a good antibacterial, keeping good colour (treatments with colloidal silver have obvious higher 'b' value before 10 days) and vase life and bacteria

number in treatment 1 are significant different from other treatments. And the trend of all indexes is quite similar in all treatments. There is no obvious evidence show the positive effectiveness of citric acid.

In conclusion, sucrose is a very effective chemical to prevent ‘Navy’ stem bending problem, even with a low concentration. This could be because sucrose is improving stem mechanical strength, but this needs to be verified. Moreover, sucrose did maintain flower quality; but too high a concentration reduced solution absorption, and so 4% sucrose is recommended a suitable concentration for ‘Navy’. Among the antibacterial compounds tested, colloidal silver was best for controlling bacteria number, and prolonging vase life of ‘Navy’. Moreover, 3 ppm colloidal silver was enough for keeping flower quality.

Chapter 3 Sucrose Affects the Lignification of 'Navy' Scape

3.1 Introduction and Aim

From previous results of experiment 1, it was clear that the problem of stem bending for 'Navy' was solved by adding 4% sucrose into distilled water, with or without a biocide (sodium hypochlorite). In a second experiment, 2% sucrose was shown to be sufficient to prevent bending (in this case, with 200 ppm 8-HQC as biocide). Sucrose is clearly sufficient to control stem bending of 'Navy', even in low concentration. Furthermore, there was no significantly decline on water uptake and flower weight for stem bending treatments. Therefore, these data indicated that lack of mechanical support in the stem is likely to be the reason for premature stem bending of 'Navy', rather than problems with water uptake caused by stem plugging, which was previously found to be a main cause of stem bending in different varieties of gerbera (Balestra et al., 2005; de Witte et al., 2014; van Meeteren, 1978a; Wang et al., 2014).

Based on this hypothesis, additional experiments concentrated on giving evidence for sucrose feeding accelerating stem lignification leading to the change of stem mechanical strength. Two separate approaches were tested: testing the effect of sucrose addition on 'Navy' stem strength; and investigating underlying anatomical features that may give more subtle insights into how sucrose strengthens gerbera stems.

3.2 Materials and Methods

3.2.1 Plant Materials

Flowers were obtained from Plentyflora with the same methods as in chapter 2, and were established in the controlled temperature room under identical conditions. In the two experiments, the flowers were placed in vases containing 5 or 10 stems instead of in single-bloom tubes. In the preliminary test, 6 flowers were measured per treatment, while for main experiments, over 10 flowers were used per treatment. For the main experiment of stem mechanical strength, the replication of flower stems was not balanced for each treatment. The experiment was established with 40 flowers in the sucrose treatment, and 50 flowers in water treatment. This was to ensure there would be enough stems bending in the predetermined 'assessment window'. In addition, two stems from the sucrose treatment and four stems from the water treatment bent before the assessment window, perhaps through disease before harvesting; rots were observed on their stem before they bent.

3.2.2 Determination of Stem Mechanical Strength

3.2.2.1 Preliminary Test: Testing the Feasibility of Three-point Bend on Gerbera Stem

Flowers at the stage of end of vase life (ca. 30 days after harvest) from a previous experiment were used to test whether three-point bend method, also called Flexure bending test, was efficient and feasible to measure the diverse strengths of different positions below the flower head on flower stems (Ampofo, Ofori, & Ibrahim, 2013; Heyes, Burton, & De Vré, 1998). The strength of stem sections centred at 2 cm, 10 cm, 20 cm, and 30 cm in the stem below the capitulum (which were marked when they first arrived) was tested. Stem sections were cut by sharp razor blades into 10 cm sections (5 cm either side of the named midpoints) and were measured by TA XT plus Texture Analyser (Hamilton, Massachusetts, USA) from Stable Micro Systems. Stem diameter was

measured with Mitutoyo digital calipers (Japan); and then stem sections were laid horizontally on a testing rig with a distance of 6 cm between two rollers, and flexed by a metal arm travelling downwards at a rate of 5 mm/sec for a maximum 15 mm travel distance. 'Trigger force' at which the rig determined it was contacting the stem was set at 0.001 N.

Strictly speaking, force and displacement data should be recalculated into stress and strain in order to determine Young's Modulus. This would require calculation of detailed stem morphology, because the gerbera stem is a hollow cylinder (Ampofo et al., 2013; Speck, 1994). However, according to Perik et al. (2012), the size of the central cavity of gerbera stem does not affect stem bending; and stem diameters were reasonably consistent; so it was decided to compare strength and displacement data directly.

3.2.2.2 Strength of Gerbera Stem in Sucrose or Water

Flowers were marked at 5 cm and 10 cm on the first day of arrival, and placed in distilled water or 2% sucrose solution within jars. Seven days later, the stem strength of 10 cm sections centred at those points was measured using the same method as in the preliminary test.

3.2.3 Determination of Anatomical Traits

3.2.3.1 Preliminary Test: Distinguishing the Degrees of Lignification

Flowers in distilled water were used for distinguishing the stages of lignification. Once flowers arrived, they were marked at 1 cm, 5 cm, 10 cm, 15 cm, 20 cm, 30 cm, and 40 cm below the capitulum. Once stems began to bend, those positions marked on the stem were cut into thin transverse sections by hand, using a sharp razor blade (Perik et al., 2012). Sections were mounted in a drop of water on slides and coved with a cover slip, and were observed at λexc = 530 – 550 nm (green light) using the U - FGW filter block on an Olympus BX53 (Tokyo, Japan) fluorescence microscope equipped with a XC50 camera (Tokyo, Japan). Auto-fluorescence of lignin was visible as a yellowish - green colour

(Vavrčík, Gryc, & Rybníček, 2007).

The degree of lignification was distinguished by lignin or vascular bundle colour and width of interfascicular region. Those two aspects were separately classified into different stages. In addition, the number of vascular bundles and interfascicular region observed in different stages at different positions on the scape were counted.

3.2.3.2 Lignification Degree of Gerbera Stem in Sucrose or Water

Flowers in distilled water and in 2% sucrose solution were marked at 5 cm or 10 cm below the capitulum on the first day of arrival, and were utilised to measure the degree of lignification. Whenever a flower in distilled water became bent, a single flower in sucrose solution was randomly selected and measured at the same time. Stem transverse sections at original marked 5 cm and 10 cm below the flower head were cut by hand, and were observed by fluorescence microscope. The degree of lignification of a number of vascular bundles and interfascicular regions observed in different stages at original 5 cm and 10 cm of scape were counted.

3.2.4 Statistics

Results were analysed using SAS 9.4. ANOVA ($P < 0.05$) was used to determine the difference between control and 2% sucrose on stem diameter, stem strength and deflection under load. Chi-squared ($P < 0.05$) was used to analyse the different distribution of vascular bundle 'colour categories' and interfascicular region width between treatments.

3.3 Results

3.3.1 Determination of Stem Mechanical Strength

3.3.1.1 Preliminary Test: Testing the Feasibility of Three-point Bend on Gerbera Stem

At different distances from flower head, the stem diameter, strength, and deflection under load were different. At the top of stem, diameter and strength were significantly lower than lower down the stem (Figure 3.1, 3.2). Deflection under load, by contrast, was significantly higher in the young tissues at the top of the stem (Figure 3.3). The small increase in stem diameter measured in segments from 2 to 10 cm may have made a small contribution to the larger increase in stem strength; but the main conclusion was that the method was suitable for use in more controlled experiments.

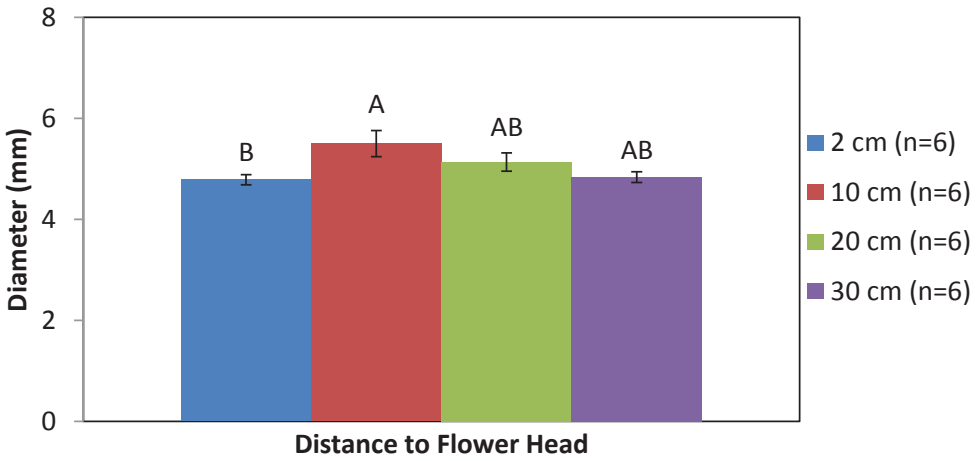

Figure 3.1: Diameter (mm) of gerbera stem at different distances (cm) to flower head (NB the distance is the original distance which was marked on the first day after arrival; data are means ± SE; the number of replicate stems used is shown on legend)

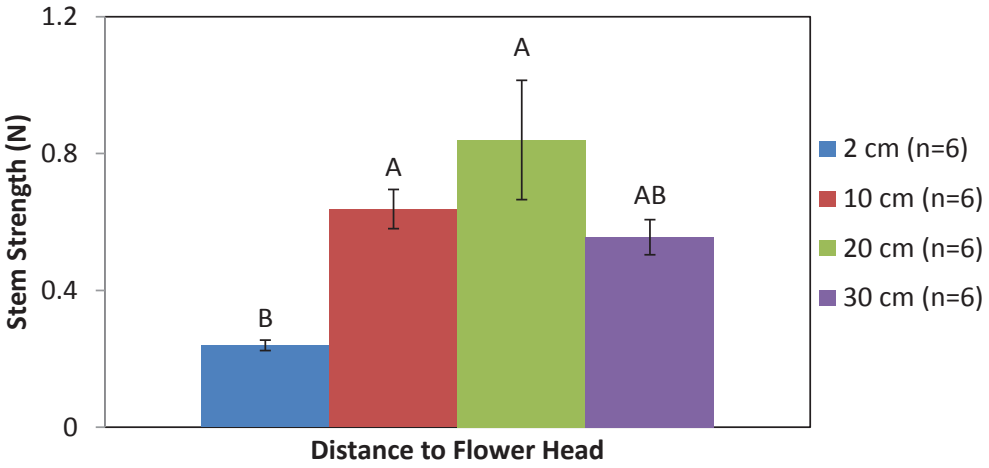

Figure 3.2: Stem strength (N) of gerbera stem at different distances (cm) to flower head (NB the distance is the original distance which was marked on the first day after arrival; data are means ± SE; the number of replicate stems used is shown on legend)

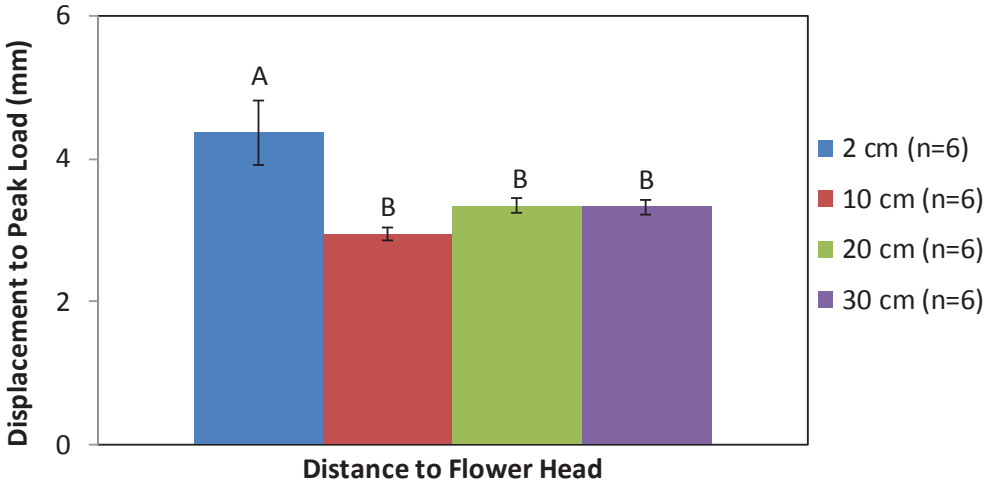

Figure 3.3: Displacement to peak load (mm) of gerbera stem at different distances (cm) to flower head (NB The distance is the original distance which was marked on the first day after arrival; data are means ± SE; the number of replicate stems used is shown on legend)

3.3.1.2 Strength of Gerbera Stem in Sucrose or Water

Diameter, strength, and deflection under load of stem at the original 5 cm in water or 2% sucrose are shown in figure 3.4, 3.5 and 3.6. It should be noted that the length of original 5 cm below flower head changed to be 7.69 cm

(mean) in sucrose treatment, and 7.47 cm (mean) in water treatment after seven days. Based on the MSD being 0.39 cm, there is no significant difference ($P > 0.05$) between those two treatments. Sucrose led to a significant reduction in deflection under load ($p < 0.05$), whereas no significant difference was found for stem diameter or strength. The results indicate the stem in distilled water is more flexible at 5 cm.

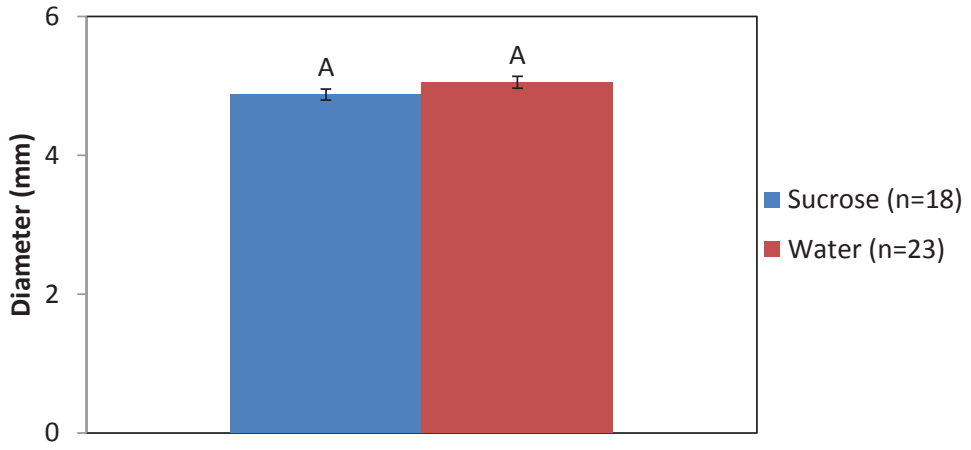

Figure 3.4: Diameter (mm) of gerbera stem at original 5 cm point below flower head (NB the number of replicate stems used is shown on legend; data are means ± SE)

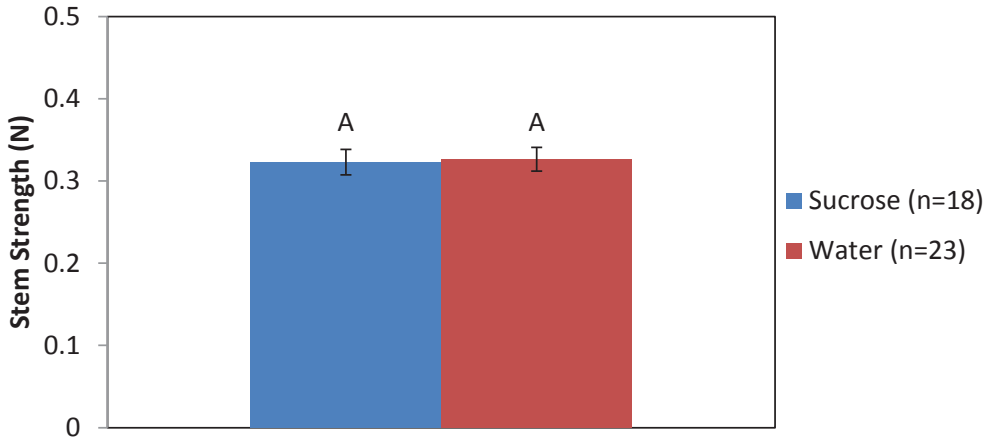

Figure 3.5: Stem strength (N) of gerbera stem at original 5 cm point below flower head (NB the number of replicate stems used is shown on legend; data are means ± SE)

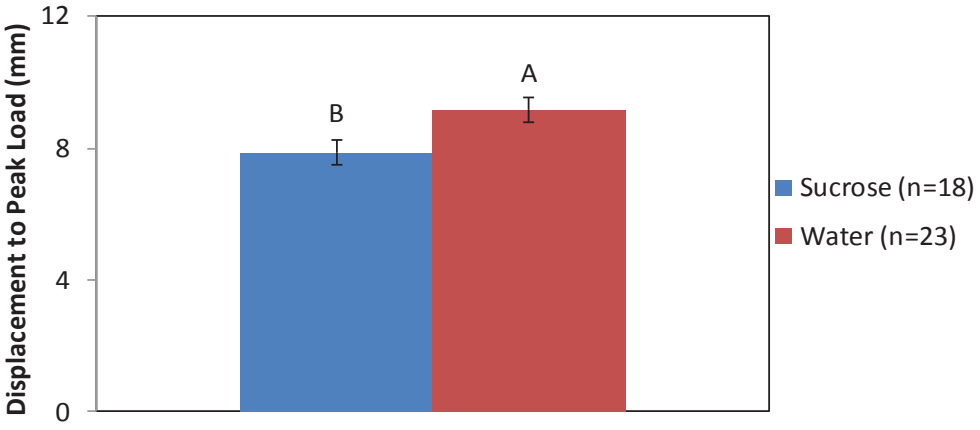

Figure 3.6: Displacement to peak load (mm) of gerbera stem at original 5 cm point below flower head (NB the number of replicate stems used is shown on legend; data are means ± SE)

But at the original 10 cm, there is no big difference ($P > 0.05$) in those parameters between sucrose treatment and the water control (Figure 3.7, 3.8 and 3.9). Moreover, there is also no treatment effect ($P > 0.05$) on flower growth, the original 10 cm point became 12.84 cm (MSD: 0.48 cm) below flower head in both treatments after seven days' growth. Comparing this result with extension growth at 5 cm, it is clear that almost all of the total 2.84 cm extension growth occurred in the region between 0 and 5 cm below the capitulum.

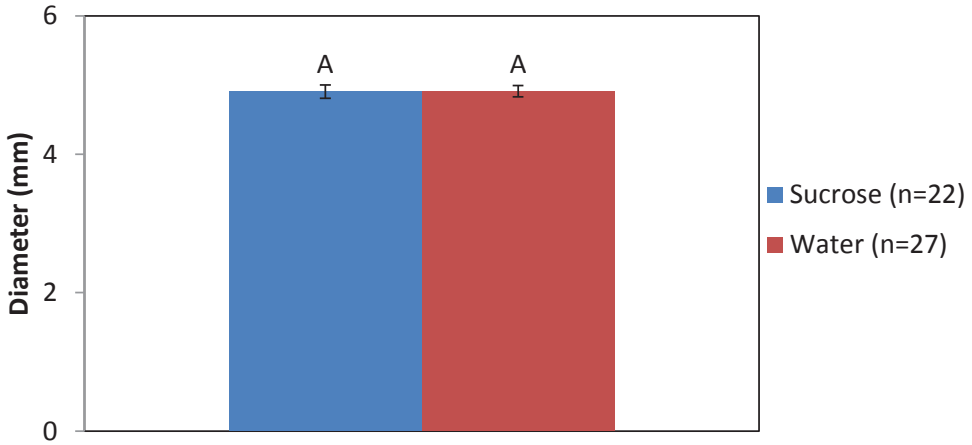

Figure 3.7: Diameter (mm) of gerbera stem at original 10 cm point below flower head (NB the number of replicate stems used is shown on legend; data are means ± SE)

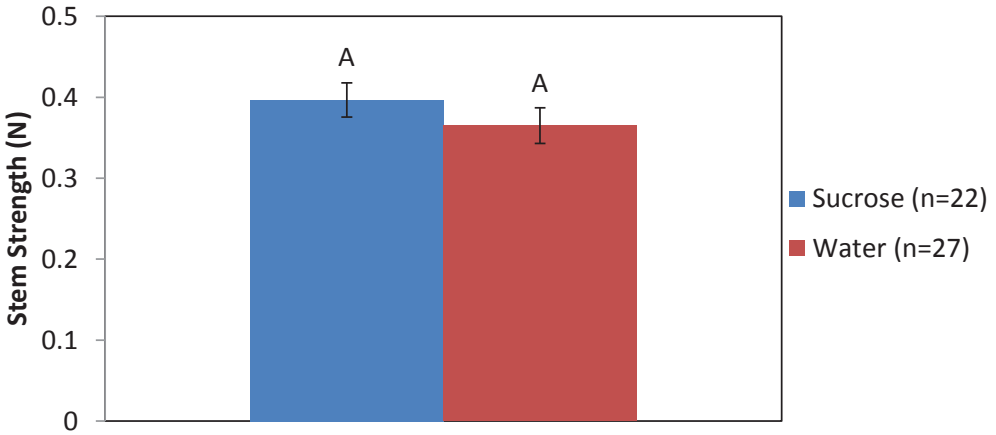

Figure 3.8: Stem strength (N) of gerbera stem at original 10 cm point below flower head (NB the number of replicate stems used is shown on legend; data are means ± SE)

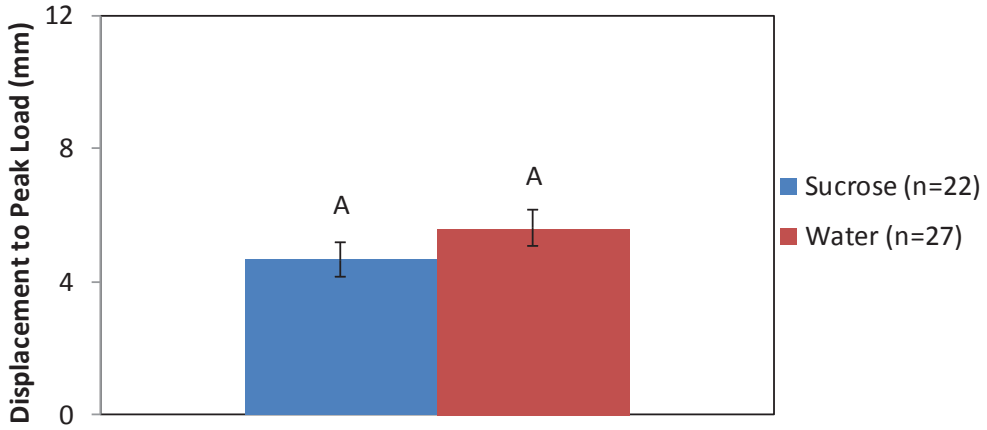

Figure 3.9: Displacement to peak load (mm) of gerbera stem at original 10 cm point below flower head (NB the number of replicate stems used is shown on legend; data are means ± SE)

The difference in flexibility between distilled water and sucrose is interesting but it was somewhat surprising that there was no significant difference in strength. This suggests treatment effects are subtler than can be measured by this flexural testing. It is necessary to design further experiments to see whether there is difference in stems on micro view.

3.3.2 Determination of Anatomical Traits

3.3.2.1 Preliminary Test: Distinguishing the Degrees of Lignification

Lignin as an auto-fluorescent material, is clearly detectable with a fluorescence microscope (Vavrčík et al., 2007). According to the observation of microscopic transverse sections, the structure of stem wall, which mainly contains vascular bundles and interfascicular regions, was seen matching previous description of gerbera stem anatomical features (Marousky, 1986; Perik et al., 2012; Steinitz, 1982). Furthermore, apparently, it seems that upper stem (younger tissues) had less bright yellow colour on vascular bundles and narrower interfascicular regions than the base stem (older tissues). In order to quantify the observed increase in lignification, based on the diversity of interfascicular regions and vascular bundles colour in cambium, they were classified into several degrees.

The colour of vascular bundles, specifically in phloem area, can be categorized into five different stages: full black (A); black inside, orange outside (B); fully orange (C); orange inside, yellow or green outside (D_1/D_2); fully yellow or green (E_1/E_2) (Figure 3.10). Yellow and green are hard to distinguish and may relate to section thickness or background room illumination. Thin section edges had more of a green colour, thicker sections were more yellow. For analysis, both colours were grouped into one stage.

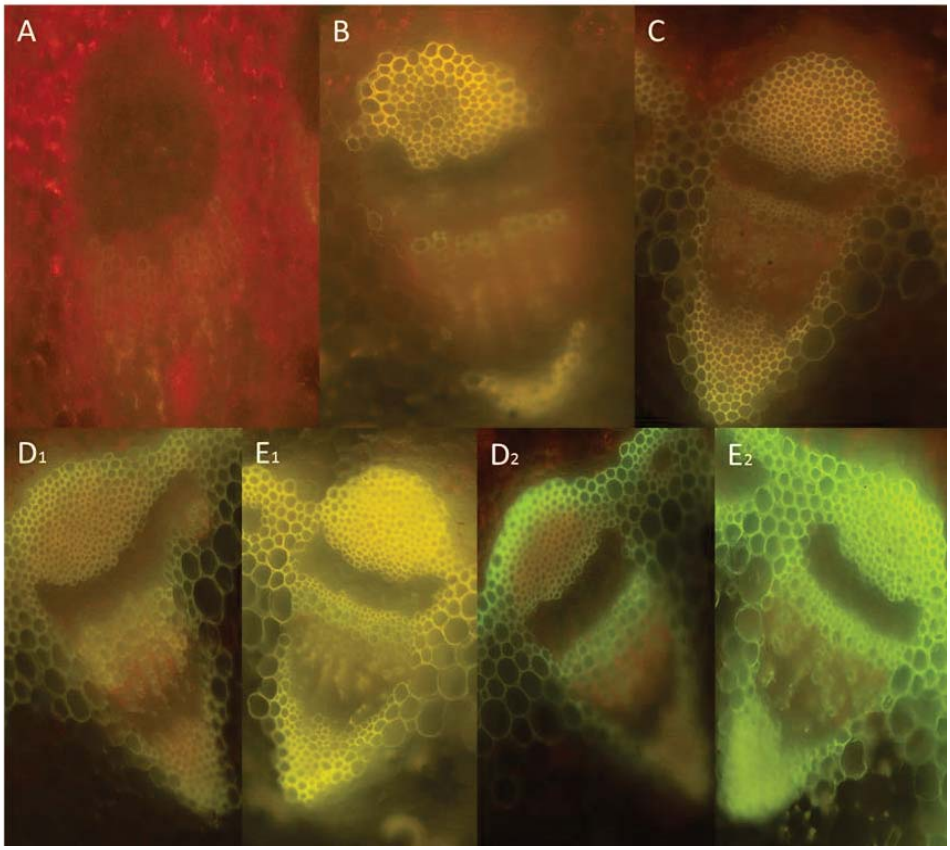

Figure 3.10: Different colour degree for vascular bundles in gerbera stem (NB the five different stages: full black (A); black inside, orange outside (B); fully orange (C); orange inside, yellow or green outside (D_1/D_2); fully yellow or green (E_1/E_2))

Four different stages are classified for Interfascicular region by its width, which are 0% (A), 0% to 10% (B), 10% to 50% (C), and 50% (D) of the length of the two vascular bundles on each side (Figure 3.11).

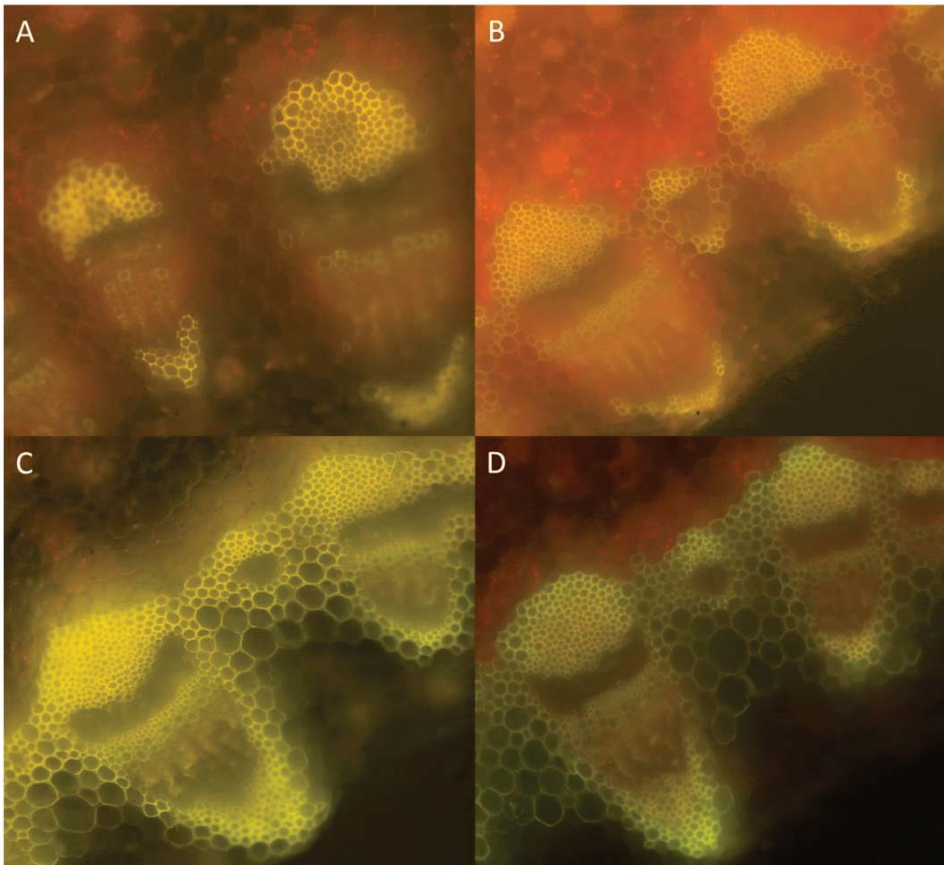

Figure 3.11: Different Interfascicular region width degree in gerbera stem (NB the four different stages: 0% (A), 0% to 10% (B), 10% to 50% (C), and 50% (D))

There were large differences in phloem fibre lignification and interfascicular lignification at different distances below flower head (Figure 3.12 and 3.13). At the original 1 cm below disc, there is hardly any lignification of phloem fibres (i.e. the cap looks black), and no interfascicular lignification. While at the original 5 cm below flower head, the major colour of phloem caps is orange with dark inside; moreover, the majority of interfascicular region had narrow thickening (mainly 0 – 10%). By 10 cm, the vascular bundles are mainly mature, with fully orange, yellow and green, and big interfascicular regions (10 – 50% and over 50%) were always found. Furthermore, our results showed that from 15 cm, even 10 cm below inflorescence, colour of lignified tissues and proportion of interfascicular region were quite similar (Figure 3.12 and 3.13).

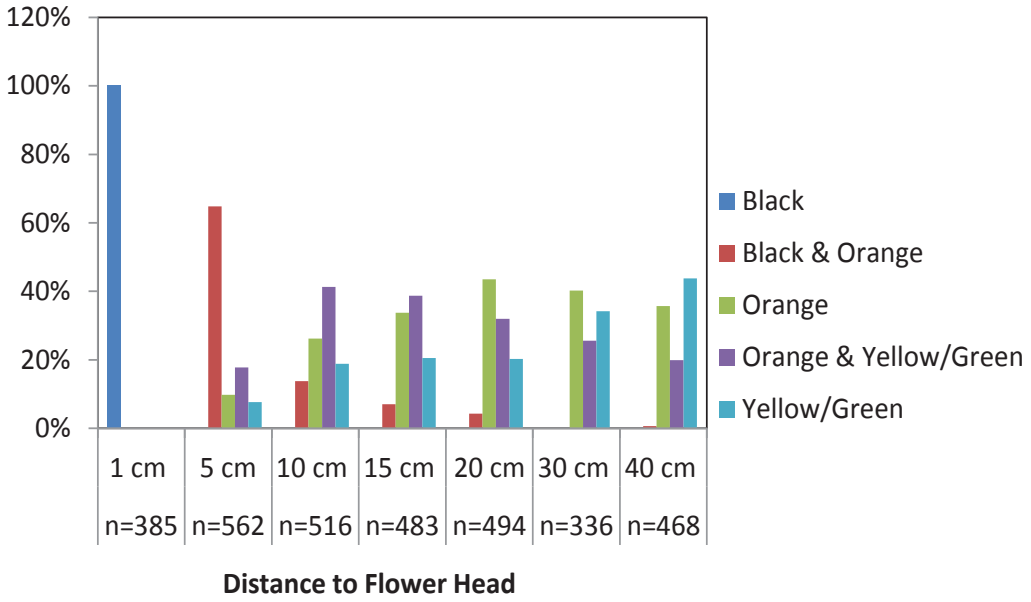

Figure 3.12: Auto-fluorescence colour of lignified tissues at different distance to flower head (NB The distance is the original distance which was marked on the first day arrival; the number of vascular bundles counted is shown on axis)

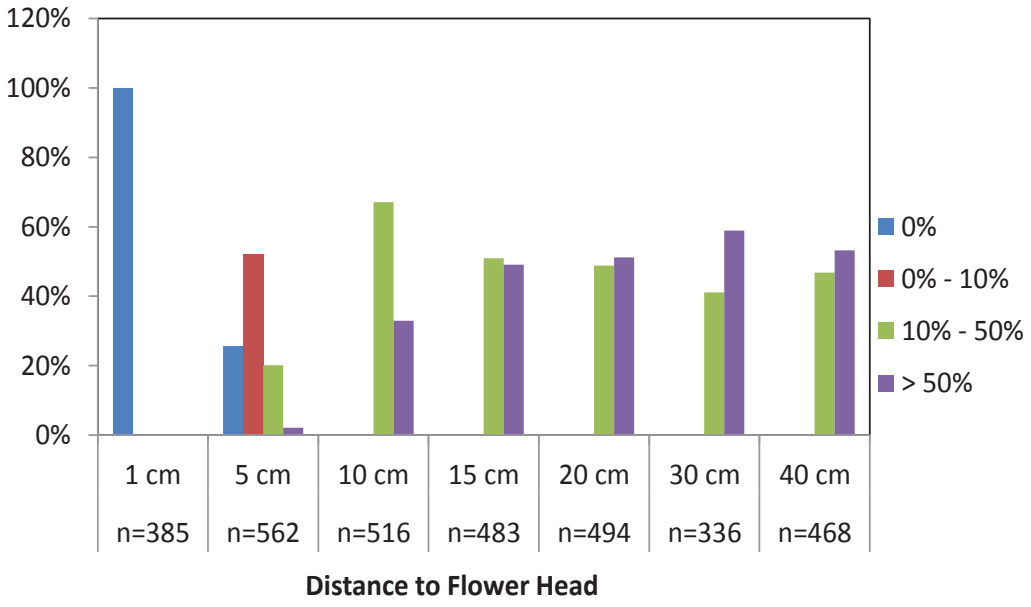

Figure 3.13: Proportion of thickness of interfascicular region (compared to adjacent vascular bundle thickness) at different distances to flower head (NB The distance is the original distance which was marked on the first day arrival; the number of vascular bundles counted is shown on axis)

3.3.2.2 Lignification Degree of Gerbera Stem in Sucrose or Water

The percentage distribution of vascular bundle 'colour categories' on

control and 2% sucrose at the original 5 cm are shown in figure 3.14 which shows that whether in sucrose or water, the colour of vascular bundles is just black & orange, orange and orange & yellow/green; and black & orange is the most common colour of vascular bundle at original 5 cm. Sucrose treatment leads to a significant increase in lignification, showing as greater orange and orange & yellow/green fluorescence. In table 3.1 of chi-squared analysis, we can see clearly that in some different 'colour categories', black & orange and orange & yellow/green, there are significant difference between treatments ($P < 0.05$). In addition, the flower growth length in sucrose (2.75 cm) was significantly longer ($P < 0.05$) than growth length in water (1.85 cm) (MSD is 0.56) after growing average on twelve days.

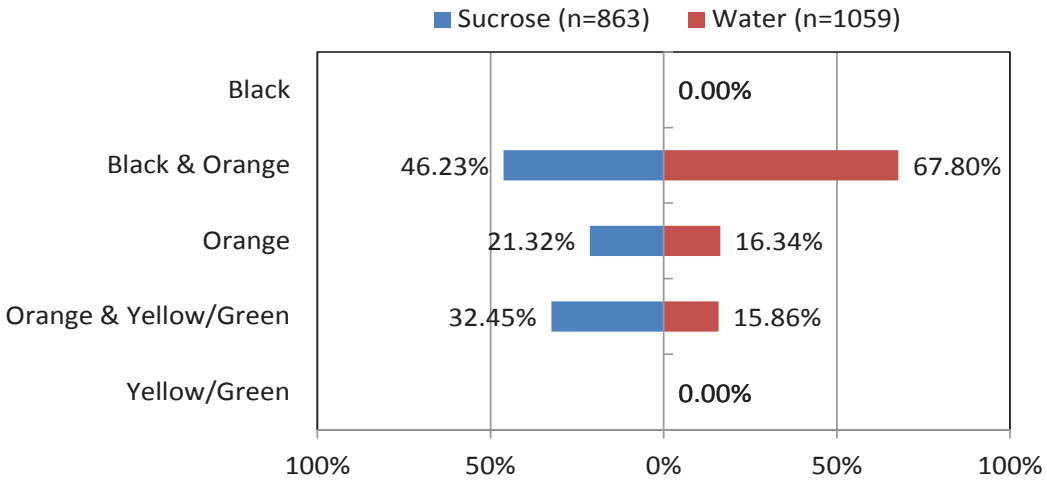

Figure 3.14: Percentage distribution of vascular bundle 'colour categories' by treatment at original 5 cm point below flower head

Table 3.1: Chi-squared analysis of distribution of vascular bundle 'colour categories' by treatment at original 5 cm point below flower head (NB % values add to 100 within colour categories; data with different letters are statistically different ($P < 0.05$))

		Black	Black & Orange	Orange	Orange & Yellow/Green	Yellow/Green	Total Number
Sucrose	Number	0	399[b]	184[a]	280[a]	0	863
	Percentage	—	35.72%	51.54%	62.50%	—	100%
Water	Number	0	718[a]	173[a]	168[b]	0	1059
	Percentage	—	64.28%	48.46%	37.50%	—	100%
	Total Number	0	1117	357	448	0	1922

At original 10 cm, the results show orange & yellow/green has become the most common colour of vascular bundle in sucrose or water, but in sucrose, the percentage of vascular bundles with orange & yellow/green colour is higher than in water, whereas black & orange and orange are lower (Figure 3.15). Using chi-squared analysis, it is clear that black & orange and orange & yellow/green have significant difference between treatments ($P < 0.05$) (Table 3.2). Sucrose clearly promotes lignification at both the original 5 cm and 10 cm zones.

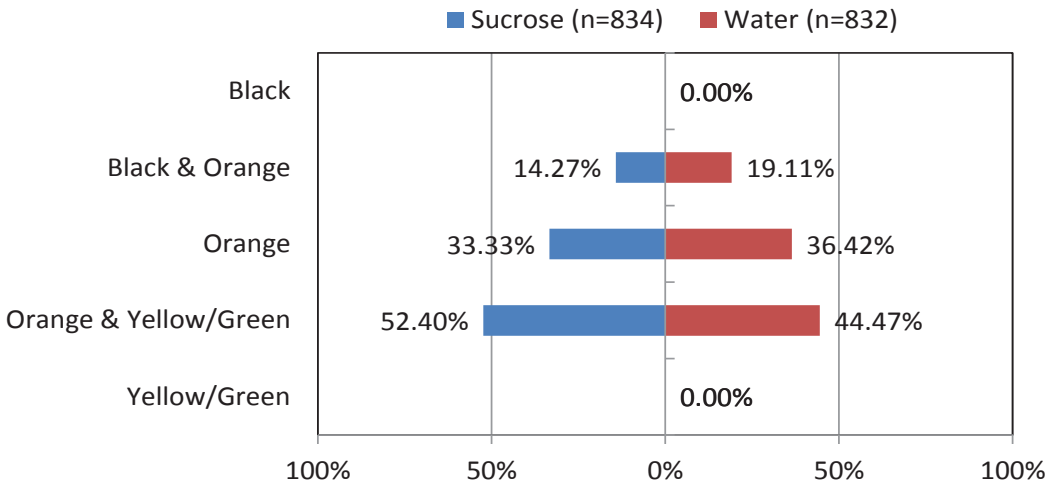

Figure 3.15: Percentage distribution of vascular bundle 'colour categories' by treatment at original 10 cm point below flower head

Table 3.2: Chi-squared analysis of distribution of vascular bundle 'colour categories' by treatment at original 10 cm point below flower head (NB % values add to 100 within colour categories; data with different letters are statistically different ($P < 0.05$))

		Black	Black & Orange	Orange	Orange & Yellow/Green	Yellow/Green	Total Number
Sucrose	Number	0	119[b]	278[a]	437[a]	0	834
	Percentage	—	42.81%	47.85%	54.15%	—	100%
Water	Number	0	159[a]	303[a]	370[b]	0	832
	Percentage	—	57.19%	52.15%	45.85%	—	100%
	Total Number	0	278	581	807	0	1666

Figure 3.16 shows flower in water, the width of interfascicular region mainly occupies only 0% - 10% of size of vascular bundle; while in sucrose, many

of the measured bundles had interfascicular cambium that had reached 10% to 50% of the adjacent vascular bundle length. When these data were analysed by chi squared, there are significant differences between sucrose and water in all three size categories (0%, over 0% to 10 %, and over 10% to 50%; ($P < 0.05$); Table 3.3).

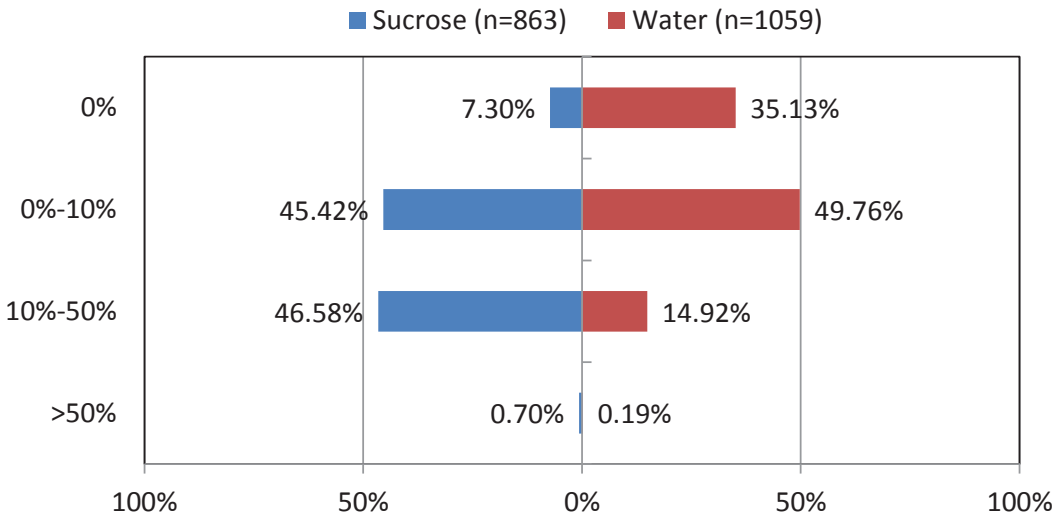

Figure 3.16: Percentage distribution of interfascicular region width by treatment at original 5 cm point below flower head

Table 3.3: Chi-squared analysis of distribution of interfascicular lignification extent by treatment at original 5 cm point below flower head (NB % values add to 100 within categories; data with different letters are statistically different ($P < 0.05$))

		0%	0%-10%	10%-50%	>50%	Total Number
Sucrose	Number	63^{b}	392^{b}	402^{a}	6^{a}	863
	Percentage	14.48%	42.66%	71.79%	75.00%	100%
Water	Number	372^{a}	527^{a}	158^{b}	2^{a}	1059
	Percentage	85.52%	57.34%	28.21%	25%	100%
	Total Number	435	919	560	8	1922

In the more mature region at the original 10 cm mark, which reached 12.65 cm in sucrose and 11.95 cm in water (there was big difference (MSD: 0.57 cm; $P < 0.05$) between them) after growing average on twelve days, the most common percentage distribution of interfascicular region width is now always

over 10% (Figure 3.17). Furthermore, in sucrose, a higher proportion of bundles had over 50% interfascicular thickening ($P < 0.05$) (Figure 3.17; Table 3.4).

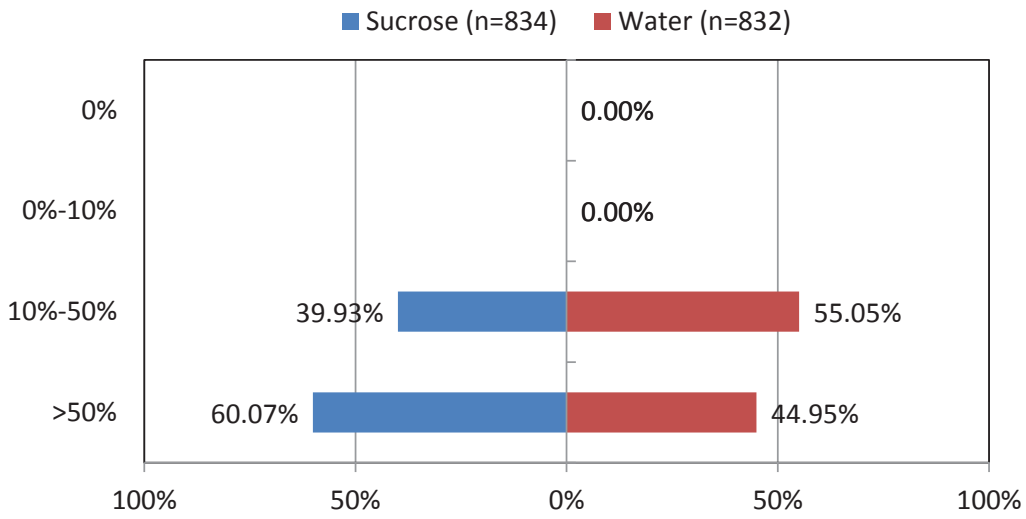

Figure 3.17: Percentage distribution of interfascicular region width by treatment at original 10 cm point below flower head

Table 3.4: Chi-squared analysis of distribution of interfascicular lignification extent by treatment at original 10 cm point below flower head (NB % values add to 100 within colour categories; data with different letters are statistically different ($P < 0.05$))

		0%	0%-10%	10%-50%	>50%	Total Number
Sucrose	Number	0	0	333[b]	501[a]	834
	Percentage	—	—	42.10%	57.26%	100%
Water	Number	0	0	458[a]	374[b]	832
	Percentage	—	—	57.90%	42.74%	100%
	Total Number	0	0	791	875	1666

3.4 Discussion

From the preliminary test of stem bending, stem strength close to the flower head is obviously weaker than at the bottom of the stem, which shows the top of the stem is less mature and more easily bent, even 30 days after harvest. The top of the stem has younger tissues that are still elongating and do not have fully developed cell walls, as noted by Steinitz (1982). Comparing control and sucrose, there was no big difference in the stem strength at original 5 cm and 10 cm below inflorescence; while at 5 cm, the distance from touching stem to reaching to highest strength in sucrose treatment was significantly less than in control, which demonstrated the flexibility of the stem in sucrose was lower. In this experiment, it was shown that the top of stem was still growing and much weaker than the bottom, but during the first 7 days, before stem bending had occurred, the extension growth in sucrose and water was similar, but stem in sucrose had higher rigidity. The results may indicate the extension leads to new tissues, but this may be not the most important reason for stem bending occurrence, in agreement with Perik et al. (2012); moreover, sucrose is able to improve stem bending as noted by Steinitz (1982), which may be due to it being the necessity for stem sclerenchyma formation (especially for lignin production) which toughens those tissues when they elongate. It is because sucrose is beneficial to lignin synthesis, since three alcohol monomers (p-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol) which are the building blocks of lignin, are all transferred by sucrose (Amthor, 2003; Ferrante & Serra, 2009). To investigate these changes in more detail, anatomical study was necessary.

Under fluorescence microscope, the transverse section of gerbera stem is a single almost actinomorphic (like its petal) ring of vascular tissue. In ‘Navy’, this ring was quite uniform, unlike the asymmetrical lignification noted by Perik et al. (2012). Vascular bundles (phloem and xylem), parenchyma and interfascicular sclerenchyma make up this ring, although in young tissues of gerbera stems, there was no interfascicular sclerenchyma between vascular

bundles. Additionally, in general, vascular bundles are arranged with large bundles alternating with smaller ones. In previous research, there is rarely information about gerbera stem lignification based on anatomical observation; and even in some parts of the articles mentioned, the results are ambiguous. In this experiment, as lignin is auto-fluorescent and lignin deposition can be clearly shown in cell wall, we can easily figure out the lignin distribution and formation. It indicates that the lignification should be measured depending on the form of vascular bundles (phloem cap) and the expansion of the sclerenchyma zone linking those bundles. The lignification scoring system developed in the thesis effectively identifies and describes progressive lignification.

Colour of vascular bundles is good to identify accumulation and maturation of lignin. Under λexc = 530 – 550 nm fluorescence, chloroplasts in parenchyma generally fluoresce red, and the yellow fluorescence of lignin shows clearly against this red background. As lignification develops, the colour changes from orange to a bright yellow-green. Sometimes, green lignin appears in the edge of specimen with mature vascular bundles; it is not clear whether this relates to yellow lignin with less red background overexposure. Because black backgrounds can unintentionally signal light meters to cause overexposure; and exposure times can be long, during exposure, the specimen's fluorescence may fade (Abramowitz & Davidson, 2000). This probably explains the distinction between yellow and green signals, which could change over time. The proportion of black or orange in the phloem caps was very consistent and not affected by fading. Moreover, the degree of interfascicular sclerenchyma formation is obviously different between new tissues and old tissues. In very new tissues, vascular bundles are totally isolated; while in old ones, there are a large number of lignified cells between them. Therefore, it also can be a useful scoring index. In addition, between two big vascular bundles, the small one were not counted in this scoring degree. The colour of vascular bundles and the connecting lignin degree are related. When phloem caps are generally black, there is always no lignin between them; while when vascular bundles fluoresce

yellow or green, the proportion of interfascicular thickening is high.

Based on the scoring system, we can statistically know that the top of gerbera stem has less lignification in phloem and interfascicular sclerenchyma region is less developed than the older parts. These results significantly extend earlier findings (Marousky, 1986). Specifically, they found that scapes at 15 and 30 cm below crown had no difference in number and size of vascular bundles, but our results now extend this observation and show there was little difference between the extent of lignification (as seen by colour in auto-fluorescence) or in the extent of interfascicular thickening in these regions. According to the results of sucrose with water experiment, sucrose has now been shown to truly advance lignification of vascular bundles, both in the sclerenchyma caps of the bundles and in the interfascicular sclerenchyma regions, at both the original 5 cm and 10 cm below inflorescence. This is precise confirmation that sucrose feeding is beneficial for lignin production. Besides, in this experiment there is evidence to show sucrose increased stem extension. As the flowers in sucrose were randomly selected for measurement once flowers in water bent, the flowers in both treatments had same age. But the flower growth in sucrose was significantly longer than in water, which indicates sucrose increased stem extension. Those evidences indicate that sucrose feeding accelerates sclerenchyma formation and leads to increased stem rigidity.

In conclusion, these results support the hypothesis that sucrose increased the mechanical support of ‘Navy’ stem resulting in improving its rigidity and reducing bending symptom. It is not possible to rule out an additional effect on stem strength, if the method used to assess stem bending is not yet sensitive enough to precisely describe the stem strength of gerbera. Because the shape of gerbera stem is oval rather than circular, and hollow, it would be excellent to develop, other methods in future work to test the stem strength or rigidity in other ways, such as cutting test, tensile test, compression test and elasticity test (de Jong, 1978; Heyes, Blaikie, Downs, & Sealey, 1994; Heyes et al., 1998; Hoseinzadeh & Shirneshan, 2012; Iwaasa, Beauchemin,

Buchanan-Smith, & Acharya, 1996; Steinitz, 1982).

Additionally, sucrose significantly promoted stem extension after 12 days' growth on average. But for the stem strength experiment, there was no difference in stem length between the two treatments after 7 days' growth. This suggests that sucrose may keep stem consistently growing. As for the exact length of growth in the two experiments, the greater growth seen in 7 days than in 12 days may be due to the different seasons in which the experiments were done: the way the flowers grow before harvest, and the postharvest handling conditions, could influence extension growth.

Chapter 4 Conclusion and Recommendations for Future Work

'Navy' gerbera has a commercial problem in that it suffers from a high incidence of stem bending which occurs a little over a week after harvest. The work in this thesis has demonstrated that it is possible to prevent this problem by including 2% – 6% sucrose in the vase solution. Once neck bending is prevented, the variety has a long vaselife (over three weeks).

Careful analysis of the sequence of physiological events accompanying senescence in 'Navy' gerbera has clarified a number of points that are debated in the gerbera senescence literature. Firstly, unlike many other varieties, such as 'Wageningen Rood', 'Tamara' and 'Cora' (de Witte et al., 2014; van Meeteren, 1978b), there was no strong evidence for reduced water uptake or flower weight preceding stem bending. This suggests that bacterial growth or stem plugging are not prerequisites for stem bending in 'Navy'. Secondly, once sucrose is included in the medium (preferably at 4%), there is evidence for a number of senescence-associated changes in physiology occurring from around 11 days in vase, although the precise sequence was variable amongst experiments and treatments. Flower angle always started to change around day 11, caused by neck bending; water uptake began to fall from around day 13 to 15, with accompanying reduction in flower weight; and colour change became evident in the range of day 15 to 20 (particularly darkening (reduced L value) and 'blueing' (reduced 'b' value)). It is logical to infer that these changes mark the visible progression of senescence and future researchers wanting to study the underlying regulation of 'Navy' senescence would need to look for changes in expression of e.g. transcription factors starting before day 11 (Rogers, 2013).

Prior to the end of vase life these changes accelerated: there was a marked reduction in the rate of water uptake, the rate of change of the flower angle accelerated and the 'b' value fell below its original starting value. Together

these changes just preceded the end of vase life, which was found to be marked most often by petal wilting and discolouration, sometimes accompanied by petal abscission. The possibility of bacterial plugging of the stem contributing to this final phase is quite strong: the best vase life of over 30 days was found in a treatment containing 4% sucrose and colloidal silver as a bactericide.

One of the major contributions of this thesis has been the demonstration of anatomical changes accompanying stem bending and the likely mechanism by which sucrose prevents stem bending in 'Navy'. Although other researchers have suggested sucrose may promote lignification, for the first time there are hard data showing the normal process of progressive lignification of sclerenchyma fibres in the phloem caps of the vascular bundles and progressive lignification of interfascicular tissue linking vascular bundles. Sucrose feeding was clearly shown to accelerate both lignification of the phloem sclerenchyma fibres and to promote lignification of the interfascicular tissue. The net effect of these changes was found to be an increase in stiffness of the stem (not an increase in strength) which is apparently all that is required to prevent stem bending in 'Navy'.

Finally, this thesis was focused on recommending an effective long term persavative solution to extend the vaselife of 'Navy' gerbera. It appears that 4% sucrose is adequate to prevent stem bending and support normal water relations for around three weeks. Further extension of vaselife could be achieved by adding an effective, non-toxic bactericide; 8-HQS was effective at high concentration but was toxic to 'Navy' stems, and therefore colloidal silver appears most suitable for use in achieving over 4 weeks of vase life. Further delays in senescence may be achievable with solutions not tested here, such as hormonal mixes, but the economic benefit of further vase life extension is unlikely to be significant as consumers are likely to be satisfied with a 3 – 4 week vaselife, with 2- 3 weeks perfect flower visible quality (Grower Direct, 2006).

Further research is desirable to confirm that the lignification changes seen here are truly causal in preventing stem bending by increasing stem rigidity.

Further refinement of the textural testing should be possible. It would also be interesting to know if the sclerenchyma scoring systems developed here can be applied to other gerbera varieties or even other flower species.

Secondly, colloidal silver was shown to be an effective bactericide in this work. However other researchers state that it may also prevent ethylene production which could delay flower senescence (Geshnizjany et al., 2014; Sharma & Bhardwaj, 2015), if 'Navy' is indeed sensitive to ethylene. Many gerbera varieties are not sensitive to ethylene, but it would be useful to test the rate of ethylene production from silver-treated 'Navy' stems and to determine whether exogenous 1-methylcyclopropene or ethylene application extend or shorten vaselife in this variety.

From a physiological point of view it would be useful to investigate the early changes in flower angle that started around day 11. Stem extension growth may have continued for more than 12 days, so neck bending may be caused by unilateral neck growth, tipping the head over. This could be tested with careful investigation; it may mark a deliberate 'strategy' to tip up the flower head, rather than simple wilting of the neck, since water uptake did not usually begin to decline until day 13.

In conclusion, this study has successfully solved the stem bending problem for 'Navy', with the use of an effective and cheap preservative solution that would allow consumers to keep 'Navy' flowers for 3 to 4 weeks. The sequence of physiological changes accompanying senescence in 'Navy' flowers has been defined which is a prerequisite for subsequent genetic research into the mechanism of gerbera senescence. Finally, a novel , fast and efficient scoring system for lignification progression based on autofluorescence measurements of unfixed hand-cut sections has been designed and used to describe the role of sucrose in promoting lignification. This method may be able to be applied more widely.

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Appendix

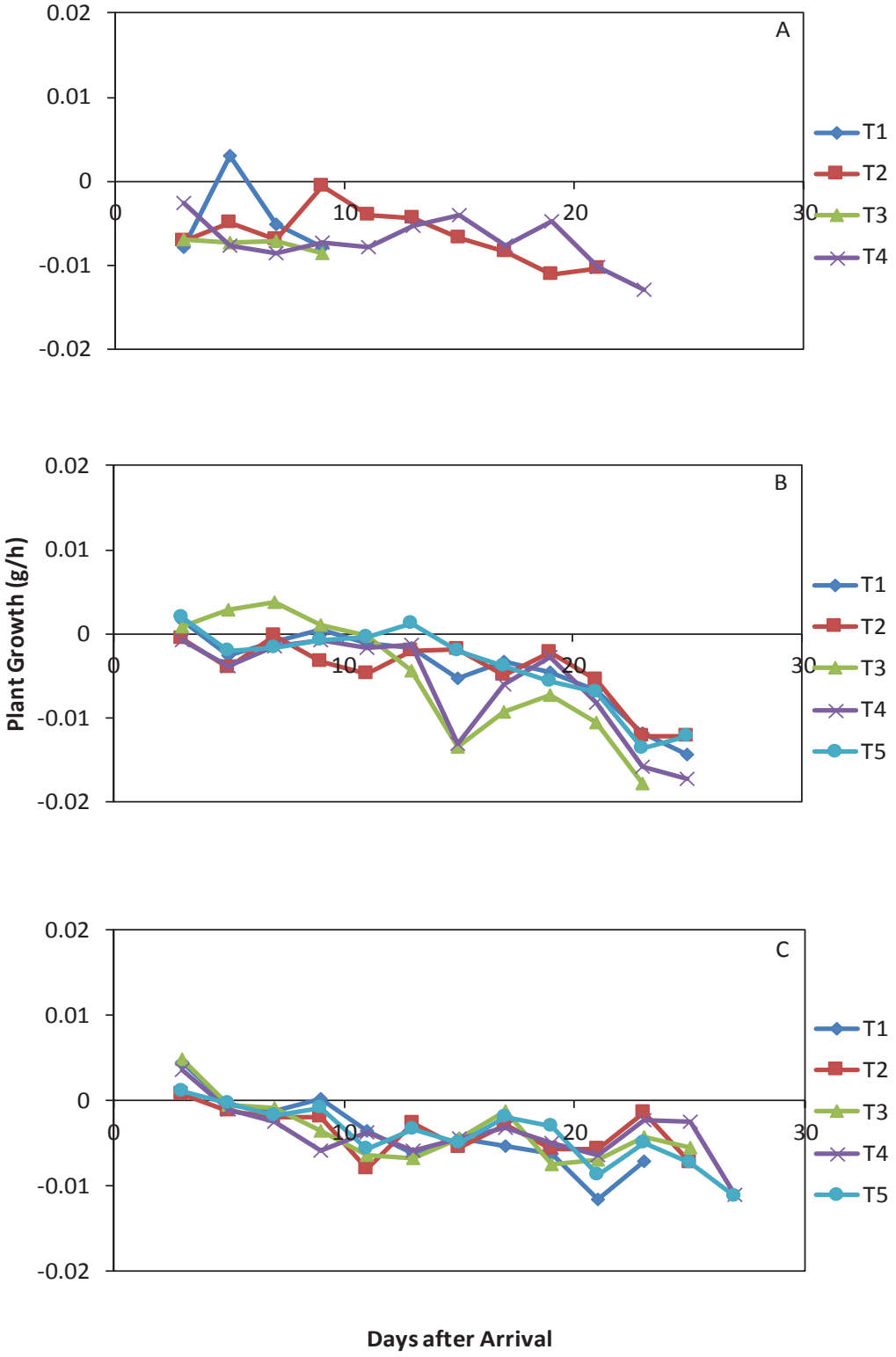

Figure 1: Plant growth (g/h) of 'Navy' every two days in experiment 1(A), 2 (B), 4 (C) on the day after flower arrival (NB solutions of each treatment are shown in table 2.1)

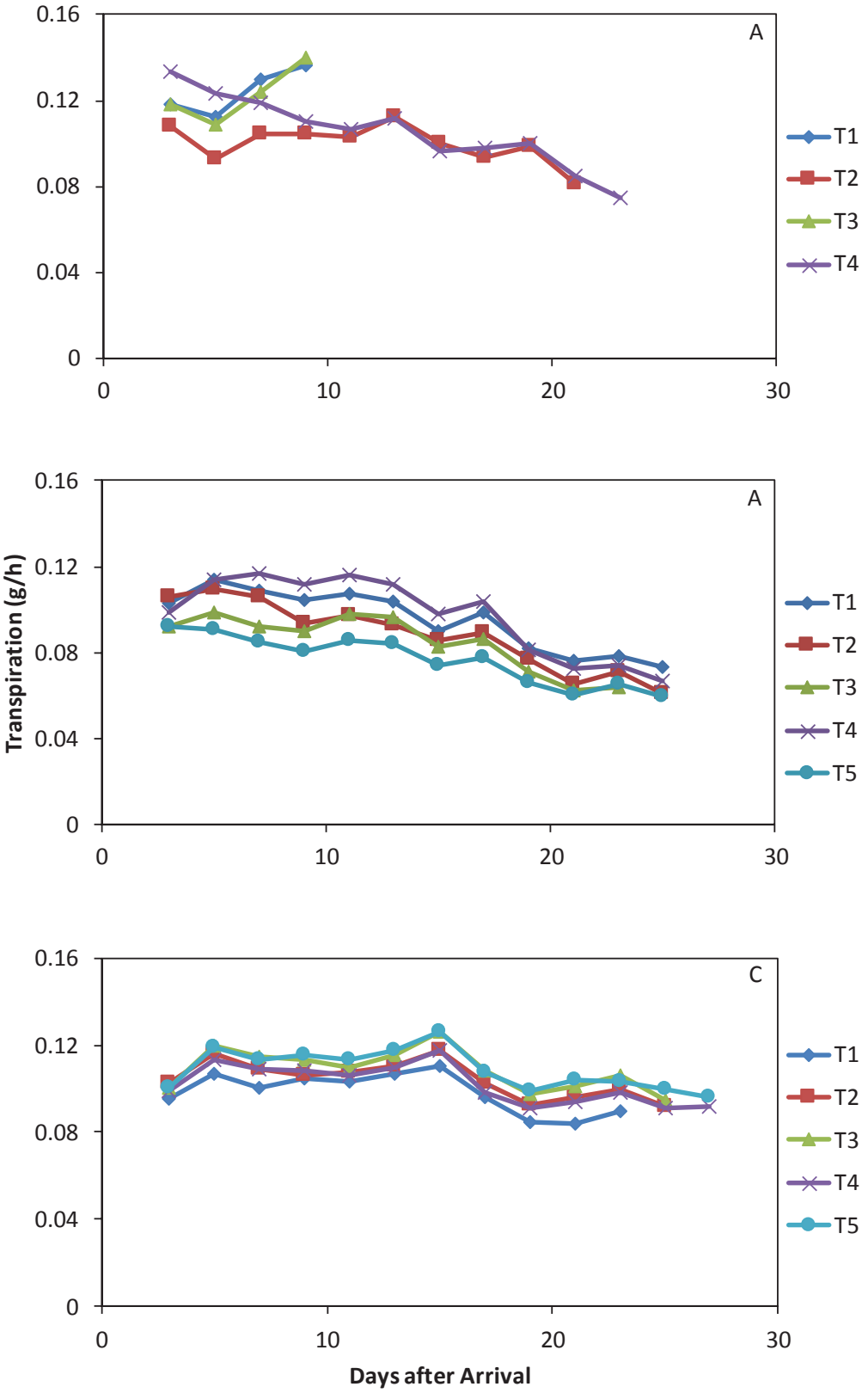

Figure 2: Transpiration (g/h) of 'Navy' every two days in experiment 1(A), 2 (B), 4 (C) on the day after flower arrival (NB solutions of each treatment are shown in table 2.1)

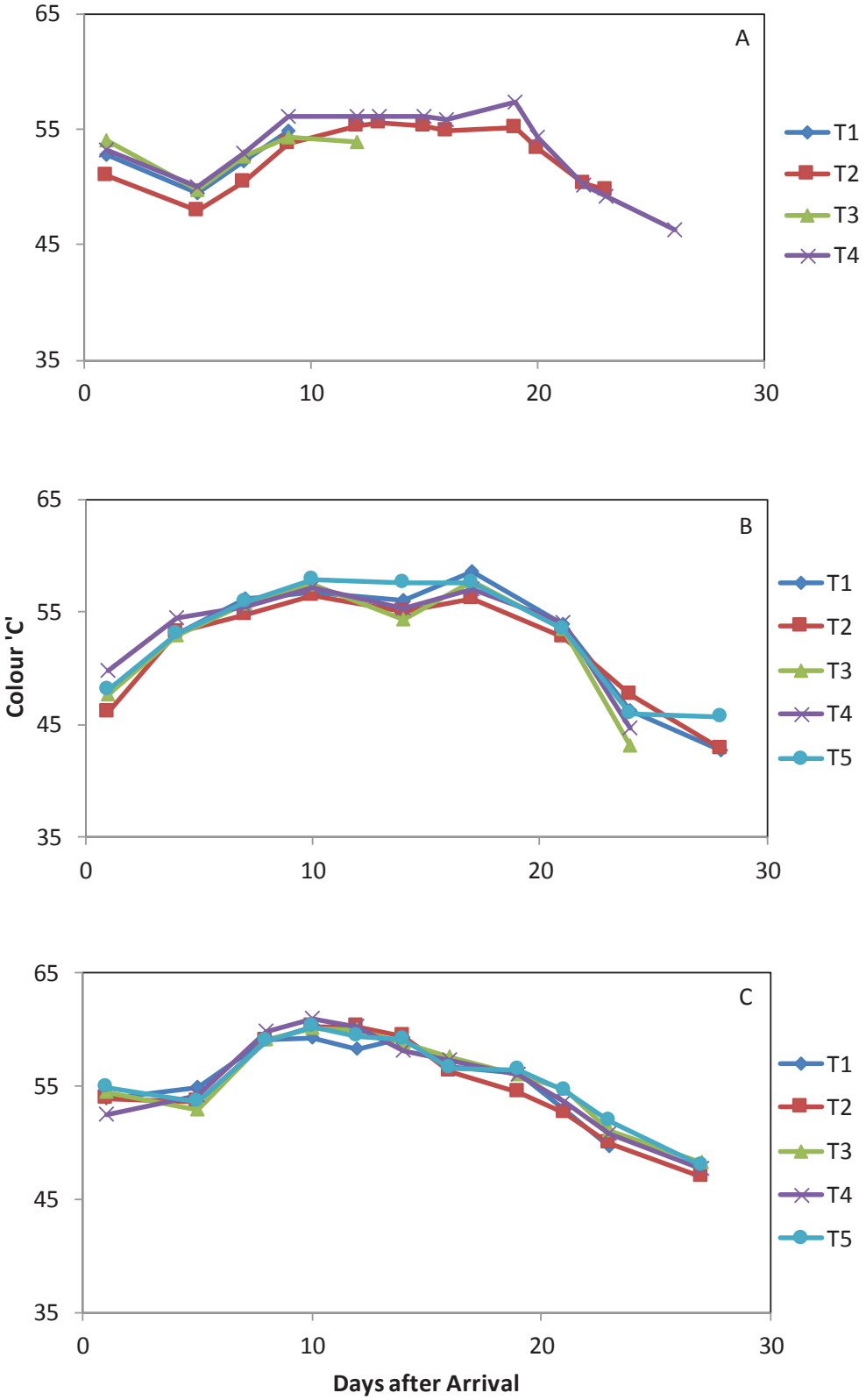

Figure 3: Colour 'C' of 'Navy' every two days in experiment 1(A), 2 (B), 4 (C) on the day after flower arrival (NB solutions of each treatment are shown in table 2.1)

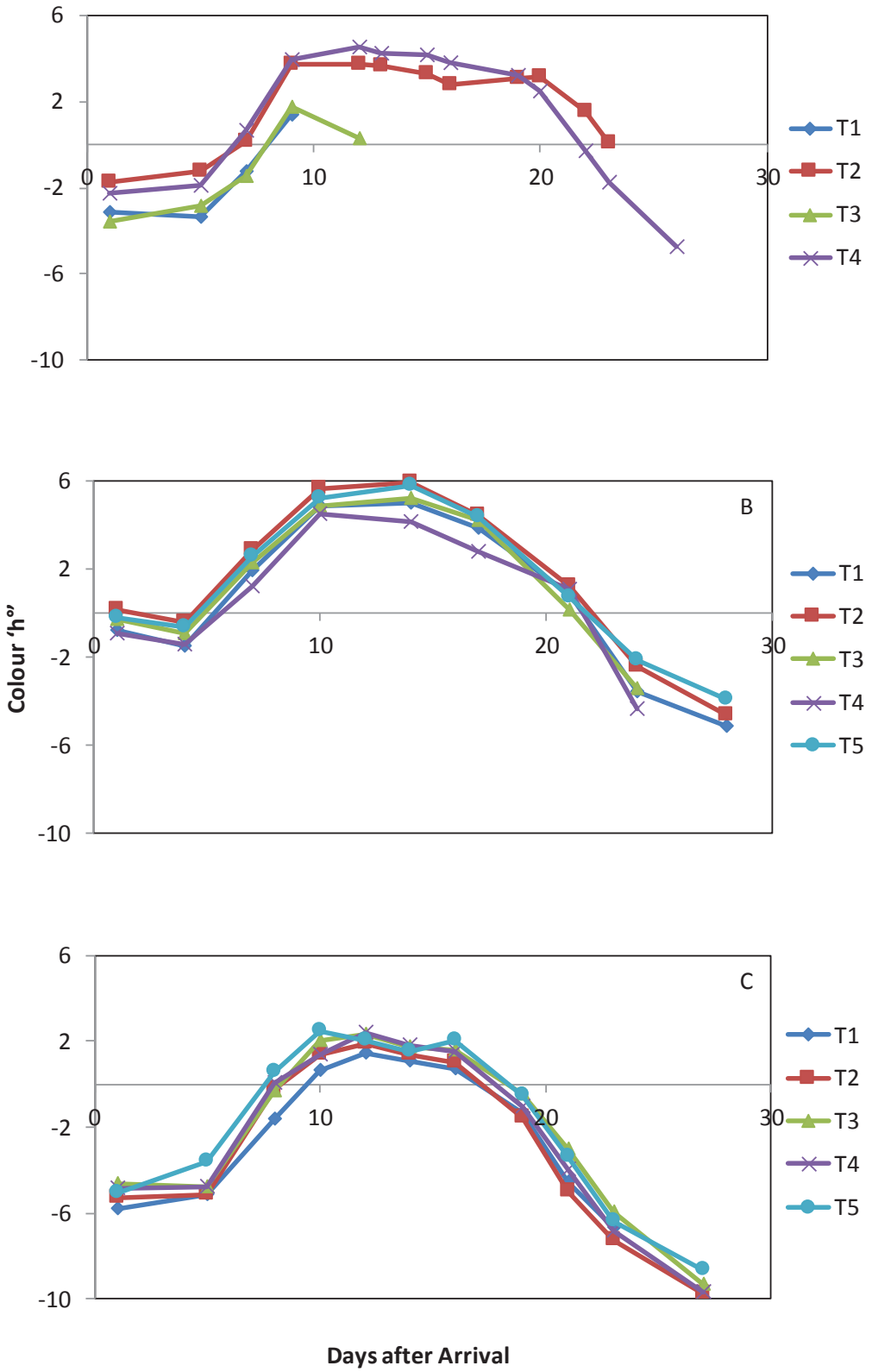

Figure 4: Colour 'h°' of 'Navy' every two days in experiment 1(A), 2 (B), 4 (C) on the day after flower arrival (NB solutions of each treatment are shown in table 2.1)

Table 1: The log count of bacteria number on experiment 1 on day 13 after flower arrival

Sample number	Sample name	Aerobic plate count (30 °C)
1	T1N1	$>10^3$ CFU/ml
2	T1N2	$6.2x10^5$ CFU/ml[e]
3	T1N3	$>10^3$ CFU/ml
4	T2N1	$>10^3$ CFU/ml
5	T2N2	$>10^3$ CFU/ml
6	T2N3	$3.6x10^4$ CFU/ml
7	T3N1	$>10^3$ CFU/ml
8	T3N2	$4.5x10^5$ CFU/ml[e]
9	T3N3	$>10^3$ CFU/ml
10	T4N1	$>10^3$ CFU/ml
11	T4N2	$5.6x10^5$ CFU/ml[e]
12	T4N3	$4.2x10^5$ CFU/ml[e]