# Carbohydrates and leaf blackening of *Protea* cut flowers

**By**

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## DECLARATION

I, the undersigned, hereby declare that the work contained in this thesis is my own original work and has not previously in its entirety or in part been submitted at any university for a degree.

**Signature** **Date**

## SUMMARY

*Protea* cut flowers are exported worldwide but the vase life of some species and cultivars is considerably shortened by post-harvest leaf blackening. Research has established carbohydrate depletion to be positively correlated with this disorder. Consequently, a study had been made of the carbohydrate status of various species and cultivars, as well as the effect of supplemental glucose (pre and post-storage) on leaf blackening.

Glucose, fructose, sucrose and starch concentrations of various *Protea* species and cultivars held in water were measured at harvest, and again at leaf blackening initiation. All measured carbohydrates declined significantly in 'Carnival', 'Pink Ice' and 'Sheila'. In 'Cardinal' all carbohydrate concentrations decreased significantly, except the sucrose concentration in the inflorescence. 'Susara' and 'Ivy' had very high initial carbohydrate concentrations in the leaves which decreased significantly. The very high initial carbohydrate concentrations in the inflorescence of 'Ivy' declined significantly. 'Brenda' differed from the other cultivars and species in that glucose concentrations increased over time. Carbohydrate concentrations of most of the tested proteas declined significantly from harvest to the initiation of leaf blackening. This highlighted the dependence of the leaves and inflorescence on the carbohydrate reserves, further substantiating the carbohydrate depletion theory. The inflorescences were characterized by high fructose and glucose concentrations and low sucrose concentrations when compared to the leaves.

It was hypothesized that glucose pulsing and cold storage at 1°C for three weeks would significantly reduce leaf blackening. 'Brenda', 'Cardinal', 'Carnival', 'Pink Ice', 'Susara' and 'Sylvia' had significantly less leaf blackening with glucose treatments of 4 and 10%. Leaf blackening of 'Sheila', *P. cynaroides* and *P. grandiceps* was not significantly reduced by glucose pulsing. *P. magnifica* showed a small, but significant, reduction in leaf blackening in response to the 3, 6 and 9% treatments after 10 days only, but despite this, leaf blackening was unacceptably high. 'Pink Ice' harvested at the soft tip stage had less leaf blackening than those harvested open or closed. Toxicity symptoms on the leaves, and in some instances flowers, were observed at higher glucose concentrations (8 and 10%) on *P. grandiceps*, *P. cynaroides,* 'Cardinal' and 'Sheila'. All glucose treatments resulted in toxicity symptoms on *P. magnifica*. A decrease in non-structural carbohydrates post-harvest apparently occurs in all proteas but it appears that only members of the *Ligulatae* respond to glucose.

Glucose pulsing followed by cold storage at 1°C for three weeks in combination with post-storage glucose vase solutions, significantly reduced leaf blackening of some *Protea* cultivars. Glucose (1 and 2%), with hypochlorite, significantly delayed leaf blackening in 'Cardinal' and 'Sylvia' after seven days. Leaf blackening of 'Brenda', 'Carnival', 'Pink Ice' and 'Susara' was not significantly reduced by the glucose vase solutions. Other disinfectants, in combination with the sugar treatments, need to be evaluated since the hypochlorite treatment had a dehydrating effect on all the cultivars and resulted in increased leaf blackening.

Carbohydrate supplementation of protea flowers with glucose, pre and post-storage, will help meet the post-harvest carbohydrate requirements of certain *Protea* cultivars and species to an extent. Glucose treatments must be seen in conjunction with maintaining the cold chain and when combined with cold chain maintenance, can extend the storage and vase life.

## OPSOMMING

Protea snyblomme word wêreldwyd uitgevoer alhoewel die vaasleeftyd van sommige spesies en kultivars beduidend verkort word deur na-oes loof verbruining. Navorsing het koolhidraatverbruik positief gekorreleer met hierdie probleem. Gevolglik is 'n studie gemaak van die koolhidraatstatus van verskeie spesies en kultivars asook die effek van addisionele glukose (voor en na opberging) op loofverbruining.

Glukose, fruktose, sukrose en stysel konsentrasies van verskeie *Protea* spesies en kultivars wat in water gehou is, is bepaal met oes en weer met die eerste tekens van loofverbruining. Al die gemete koolhidraatkonsentrasies het beduidend afgeneem in 'Carnival', 'Pink Ice' en 'Sheila'. In 'Cardinal' het al die koolhidraatkonsentrasies beduidend afgeneem, behalwe vir die sukrosekonsentrasie in die blom. 'Susara' en 'Ivy' het baie hoë begin koolhidraatkonsentrasies in die blare wat beduidend afneem. Die baie hoë inisiële koolhidraatkonsentrasies in die blom van 'Ivy' neem beduidend af met tyd. 'Brenda' verskil van die ander kultivars en spesies deurdat die glukosekonsentrasies toeneem met tyd. Koolhidraatkonsentrasies van die meeste getoetste proteas neem beduidend af vanaf oes totdat die eerste tekens van loofverbruining verskyn. Dit het die afhanklikheid van die blare en blom op die koolhidraatreserwes beklemtoon en daardeur verder die koolhidraatteorie ondersteun. Die blomme is gekarakteriseer deur hoë fruktose- en glukosekonsentrasies en lae sukrosekonsentrasies wanneer dit met die blare vergelyk is.

Die hipotese is gestel dat die voorsiening van glukose, vir 'n aantal ure, gekombineerd met koue opberging by 1°C vir drie weke loofverbruining beduidend sal verminder. 'Brenda', 'Cardinal', 'Carnival', 'Pink Ice', 'Susara' en 'Sylvia' het beduidend minder loofverbruining met glukose behandelings tussen 4 en 10%. Loofverbruining van 'Sheila', *P. cynaroides* en *P. grandiceps* is nie beduidend verminder deur glukose behandelings nie. *P. magnifica* het 'n klein, maar beduidende verlaging in loofverbruining getoon met die 3, 6 en 9% behandelings na 10 dae, maar ten spyte hiervan was loofverbruining onaanvaarbaar hoog. 'Pink Ice' is geoes by die sagte punt stadium en het minder loofverbruining gehad as blomme wat oop of toe geoes is. Toksisiteitsimptome op die blare, en in sommige gevalle blomme, is waargeneem met hoër glukose konsentrasies (8 en 10%) op *P. grandiceps*, *P. cynaroides*, 'Cardinal' en 'Sheila'. Alle glukosebehandelings het toksisiteitsimptome tot gevolg gehad op *P. magnifica*. 'n Afname in nie-strukturele koolhidrate na oes kom waarskynlik voor in alle proteas maar dit wil voorkom of slegs lede van die *Ligulatae* positief reageer op glukose.

Glukosebehandeling gevolg deur koue opberging by 1°C vir drie weke in kombinasie met na-stoor glukose vaasoplossings het loofverbruining van sommige *Protea* kultivars beduidend verminder. Glukose (1 en 2%), saam met hipochloriet, het loofverbruining beduidend verminder in 'Cardinal' en 'Sylvia' na sewe dae. Loofverbruining van 'Brenda', 'Carnival', 'Pink Ice' en 'Susara' is nie beduidend verminder deur die glukose vaasoplossings nie. Ander ontsmettingsmiddels in kombinasie met die suikerbehandelings moet geëvalueer word aangesien die hipochlorietbehandeling 'n

dehidrerende effek op al die kultivars gehad het en 'n toename in loofverbruining tot gevolg gehad het.

Byvoeging van glukose by proteablomme, voor en na opberging, sal tot 'n mate help om in die na-oes koolhidraatbehoeftes van sekere *Protea* kultivars en spesies te voorsien. Glukosebehandelings moet saam met die beheer van die koueketting gesien word en wanneer gekombineerd met koueketting beheer kan dit opberg en vaasleeftyd verleng.

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# CONTENTS

## 1. Introduction

Protea cut flowers are exported to foreign markets in large quantities. However, vase life and transport options of certain *Protea* species and cultivars are limited by leaf blackening symptoms, which usually appear three to seven days after harvest. Air freight is very expensive in comparison to sea freight, but sea freight is not a viable option for proteas prone to leaf blackening because shipping times are about three weeks.

Paull and Dai (1990) stated that the onset of leaf blackening is influenced by genetic variation, season, time of day flowers are harvested and the developmental stage of the flower. This highlights the complex set of factors contributing to leaf blackening. Many theories have been proposed to explain this phenomenon including post-harvest water stress (Paull et al., 1980), leaf contact with condensation (Reid et al., 1989), depletion of leaf carbohydrates (Reid et al., 1989; Paull & Dai, 1990), ethylene and the role of red and far-red light (van Doorn, 2001).

The developing inflorescence is a very strong sink because of its high respiration rate and the production of nectar (Dai & Paull, 1995). Several experiments supported the idea that the transport of carbohydrates to this very strong sink initiates the leaf blackening process (McConchie et al, 1991; Bieleski et al., 1992). Since the important role of carbohydrates has been established various approaches have been taken to alleviate the problem. The change in carbohydrate concentration over time and under different treatment regimes has been investigated to determine the carbohydrate status of the *Protea*. Cold storage and supplementation with various sugars have been tried with varying degrees of success.

The focus of this study was to determine carbohydrate changes in various *Protea* species and cultivars and understand the role played by carbohydrate supplementation. Furthering this approach, glucose pulsing and storage at low temperatures were evaluated for their effectiveness in reducing leaf blackening and, in effect, making sea freight a viable alternative. Consequently, glucose post-storage vase solution combinations were assessed for additional benefits in reducing leaf blackening. A combination of these approaches could aid in developing successful post-harvest strategies that prevent or minimize protea leaf blackening.

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# 2. Post-harvest carbohydrate concentrations of *Protea* and interacting factors

## 2.1 Introduction

Vase life and transport options of certain *Protea* species are limited by post-harvest leaf blackening. Leaf blackening symptoms appear three to seven days after harvest with the onset influenced by genetic variation, season, time of day the flowers are harvested and developmental stage of the flower (Paull & Dai, 1990). Deep brown to black discoloured areas, starting at the tips or margins of leaves are characteristic of post-harvest leaf blackening (Jones et al., 1995). *P. eximia, P. neriifolia* and their hybrids are more susceptible to leaf blackening than *P. compacta* hybrids (Ferreira, 1986).

Different theories have been proposed to explain the triggers and mechanisms of post-harvest leaf blackening. Jones et al. (1995) highlighted three of the most important theories in their review, namely, post-harvest water stress (Paull et al., 1980), leaf contact with condensation (Reid et al., 1989) and depletion of leaf carbohydrates (Reid et al., 1989; Paull & Dai, 1990).

The post-harvest water stress theory was substantiated by the removal of the flower head that delayed leaf blackening (Paull et al., 1980). Later research related the positive effect of flower head removal to a decrease in carbohydrate depletion (Dai & Paull, 1995) and not to the reduction in water loss from the flower head (Paull et al., 1980). Reid et al. (1989) and Californian producers reported a positive link between condensation on leaves and occurrence of leaf blackening. However, condensation has not explained all the cases of leaf blackening and no work has been done to

corroborate this observation or explain the possible mechanism of free water initiating leaf blackening (van Doorn, 2001).

Carbohydrates are transported from the leaves to the developing inflorescence, which has a high respiration rate and produces nectar (Dai & Paull, 1995). This chain of events is thought to trigger the leaf blackening process (McConchie et al., 1991; Bieleski et al., 1992; McConchie & Lang, 1993b). Since girdling below the inflorescence significantly reduces the severity of leaf blackening, this strongly supports the hypothesis that blackening is a result of the high carbohydrate demand by the inflorescence (Newman et al., 1990). Girdling disrupts phloem transport, and hence the flow of carbohydrates from the leaves (source) to the flowers (sink), and in this way reduces the demand placed on reserves by the flower head. Artificial lighting of sufficient intensity for photosynthesis, used after harvest has been shown to reduce leaf blackening (Bieleski et al., 1992).

Van Doorn (2001) investigated two additional causes of leaf blackening in an updated review, namely ethylene and red/far red light. Ethylene does not contribute to leaf blackening (Reid et al., 1989; Bieleski et al., 1992). Low levels of incandescent light that keep phytochrome in Pr form are reported to prevent leaf blackening. Hence, van Doorn (2001) hypothesized that the conversion of the phytochrome from Pr to Pfr may be a pivotal step in the leaf blackening process, but this remains a hypothesis.

Carbohydrate metabolism and partitioning have not been examined during a typical post-harvest period despite the correlation between depleted carbohydrates and leaf blackening. Since the mechanism of leaf blackening is not clear we will look at

factors that impose stress on the product and possibly initiate leaf blackening. This chapter reviews the published literature on carbohydrate status and distribution in *Protea* and cut flowers, factors that influence it and subsequent changes after harvest. The effect of pulsing on carbohydrate status will also be investigated.

## 2.2 Carbohydrate content and post-harvest changes of *Protea*

*Protea* typically stores carbohydrates as 1,5-anhydro-D-glucitol (polygalatol), a simple derivative of sorbitol (D-glucitol) (Figure 1). The high polygalatol concentrations remain relatively constant after harvest over time, with sucrose, glucose, fructose and starch decreasing to relatively low concentrations after harvest (Bieleski et al, 1992; McConchie et al., 1991, 1994; McConchie & Lang, 1993a, b). Polyol formation and accumulation occurs mainly in the leaves of plants of selected species (Bieleski, 1982). Plants that contain large amounts of sugar alcohols and do not readily metabolise them are likely, under certain stressful conditions, to use these sugar alcohols to maintain a high osmotic pressure in the cell through high, inert, non-polar polyol concentrations (Lewis & Smith, 1967). Osmotic stress, especially an increase in salinity results in a corresponding increase in polyol levels in several plants. These concentrations decrease when the stress is relieved. It is possible that a polyol 'hydrated' protein is more tolerant of high intrinsic salt levels than a protein hydrated in the normal manner (Bieleski, 1982).

The plant parts of *Protea* will be discussed separately, because of different concentrations found in each. Various experiments are summarized in Tables 1-3, with changes in carbohydrate concentrations in the different plant parts documented.

Carbohydrate content at harvest and subsequent changes with regard to different post-harvest manipulations are considered.

### 2.2.1 Leaves

Freshly harvested *P. eximia* leaves contained sucrose (4-6 $mg.g^{-1}$ fresh weight), glucose (0.3 $mg.g^{-1}$), fructose (0.1 $mg.g^{-1}$), raffinose or similar trisaccharides (0.1 $mg.g^{-1}$), oligosaccharides (2 $mg.g^{-1}$) and polygalatol (25-30 $mg.g^{-1}$) (Bieleski, 1992). Polygalatol concentrations are the highest in apical leaves of *P. neriifolia* declining significantly in a basipetal pattern (McConchie et al., 1991). The phyllotactic division does not significantly influence leaf sucrose concentration. Phyllotactic division is defined as one complete spiral of leaves (ca. 5-7 leaves) numbered from the distal end (1) to basal end (7), of the floral stem. A source to sink transition occurs with an increased ability for synthesizing transport carbohydrates, which is a sign of increased photosynthetic competence. The similarity of sucrose profiles across floral stem phyllotactic divisions suggests that most leaves are source leaves after flower initiation (McConchie & Lang, 1993b). A significant reduction in percentage leaf blackening and flower senescence was observed with an increasing number of leaves left on the flowering stem (Dai & Paull, 1995).

An 82% reduction in starch reserves was observed 24 hours after harvest in *P. neriifolia* stems kept under dark conditions (McConchie & Lang, 1993b). After four days in the dark, sucrose decreased to an undetectable concentration in 'Pink Ice' stems, coinciding with the rapid onset of leaf blackening. Polygalatol concentrations did not show this decline over time and were four times higher than sucrose concentrations (McConchie et al., 1994). Leaf blackening occurrence is preceded by

starch declining to very low concentrations (McConchie et al., 1994). Development of the flower head and the consequent nectar production increased sink strength, leading to starch reserves being metabolised to transport sucrose (McConchie & Lang, 1993a).

Storage carbohydrates (starch and sucrose) showed a three-fold increase after three days under natural light conditions, but were depleted after three days in the dark at 20°C (Bieleski et al., 1992). These results are consistent with the hypothesis that carbohydrate depletion leads to visible leaf blackening symptoms, which is preceded by storage carbohydrates reaching a minimum.

The high concentrations of phenolic glycosides observed in *Protea* spp. (Perold et al., 1979), may be hydrolysed under low carbohydrate conditions, releasing glucose for metabolism and releasing free phenols which are susceptible to oxidation (Dey & Dixon, 1985). Significantly, greater phenolic concentrations were found in subtending flush leaves than in remaining flush leaves, both at harvest and in storage. The phenolic concentrations increased significantly during storage, although there were no differences between flowers stored between 0°C and 10°C (Stephens et al., 2000).

Increasing storage temperatures correspond with decreasing carbohydrate content. Flowering stems stored at 0°C showed no significant difference in starch and reducing sugar concentration in the leaves, compared with freshly harvested flowers, whereas a significant decrease in starch and sugar concentrations was observed after three days of storage at 0°C, 4.5°C, 7°C and 10°C. The decline in carbohydrate content with

increasing storage temperatures was attributed to the increased respiration rate at higher temperatures (Stephens et al., 2000).

### 2.2.2 Stem

The carbohydrate status of leaves and stems of over-wintering shoots of 'Lady Di' was found to be very low (Gerber et al., 2001). Starch accumulates mainly in leaves, and not stems, of 'Sylvia' shoots and the carbohydrate concentration in leaves is five times that of stems (Hettasch et al., 2001). Significantly higher total sugars and starch concentrations are found in both 'Sylvia' and 'Cardinal' leaves than stems of all growth flushes. Characteristically, carbohydrate storage in evergreen plants is in the leaves rather than the stem (Hettasch et al., 2001).

### 2.2.3 Inflorescence

The maturity of the developing inflorescence plays a significant role in the demand placed on reserves. Leaf blackening occurs more rapidly when flowers are harvested in the closed bud stage than when bracts are just unfolding (Paull & Dai, 1990). Generally, it is better to harvest flowers in the bud stage to reduce airfreight costs and damage occurring during transport (Reid & Evans, 1986), but in proteas this may result in increased leaf blackening, because of the high carbohydrate demand of the developing inflorescence.

Rapid floral development favours the partitioning of fixed carbon into transport carbohydrates. With a fully expanded or senescing flower the photosynthetic products are partitioned into starch and stored in the leaves. A reduction in demand for stored or newly fixed carbohydrates is correlated with a decrease in leaf

blackening. Leaf blackening is more related to post-harvest inflorescence sink demand than to total pre-harvest carbohydrate status (McConchie & Lang, 1993a). Newman et al. (1990) suggested that leaf blackening could be delayed by harvesting flowers in the afternoon when their carbohydrate status is at its highest.

### 2.2.4 Nectar

Nectar production in proteas is significant and is a very strong sink for carbohydrates. Dai and Paull (1995) reported a nectar production of 2.7 mL in *P. neriifolia* flowers harvested at stage four (open, cylindrical flower), which increased to 9.8 mL per flower as the flower opened (Table 4). Cowling & Mitchell (1981) measured 5 to 6 mL of glucose and fructose rich nectar in *P. neriifolia* when the flower head opened.

Nectar sugars have been analysed (Table 4) using HPLC (high performance liquid chromatography). Most *Proteaceae,* including *P. repens* and most of the bearded proteas are characterized by nectar composed of mainly glucose and fructose, with xylose accounting for only a small percentage in most proteas (Van Wyk & Nicolson, 1995). *P. coronata* and *P. grandiceps* are exceptions and produce sucrose-rich nectar (van Wyk & Nicolson, 1995). Dai and Paull (1995) found that when $^{14}$C-sucrose was applied to middle leaves of floral stems picked at five different growth stages, 58% was detected in the nectar 24 hours after harvest. This provides evidence that nectar is the primary sink for carbohydrates from the leaves.

## 2.3 Post-harvest carbohydrate supplementation

Exogenous sugars are used to supply an additional energy source for completion of blooming and to prolong vase life (delay senescence). The sugars promote *de novo*

protein and amide synthesis and maintain osmotic pressure (Paulin, 1986; Halevy, 1976). The effect of carbohydrate supplementation on cut flowers, and specifically on *Protea,* is examined in the following sections. Data from experiments on carbohydrate analysis and supplementation on *Protea* are presented in detail in Tables 1-3.

### 2.3.1 Cut flowers

A lower sugar concentration is required with a longer exposure to the chemical solution. High concentrations are used for pulsing and low concentrations for holding solutions. Green leaves are more sensitive to high sugar concentrations than petals, because their ability for osmotic adjustment is less than that of petals (Halevy, 1976). Consequently, sugar concentrations in pulsing solutions are not always determined by the optimal effect on the flower, but in some cases by the sensitivity of the leaves (Halevy, 1976). Halevy and Mayak (1979) explained this sensitivity of foliage in roses by showing that excess sugar accumulates primarily in leaves, since the uptake of these sugars follows the same translocation pattern as naturally formed carbohydrates, i.e., from leaves to petals. The respiratory substrate pool is actively maintained by several exogenously supplied metabolic sugars. This has a positive effect on respiration (Rogers, 1973).

Sucrose is needed as a carbohydrate source for dry weight gain with the bud developing into a mature flower (Rogers, 1973). Uptake and sucrose metabolism was investigated in cut roses (*Rosa hybrida* 'Red American Beauty') placed in a modified Cornell Solution (2% sucrose + 200 $mg.L^{-1}$ 8-hydroxyquinoline sulphate) at 23°C with a 10-hour photoperiod. Fructose and glucose concentrations increased and the endogenous sucrose concentration remained low and constant in the petals after

treatment, indicating that sucrose was hydrolysed before it reached the petals. This increase in reducing sugars after pulsing suggests that the sucrose invertase capacity in the stem was high (Kaltaler & Steponkus, 1974).

*Gladiolus* shoots (*Gladiolus grandiflora* 'Oscar') treated with sucrose solutions showed that with an increasing sucrose concentration the volume of solution absorbed decreased. The amount of sucrose absorbed affected the subsequent water uptake, i.e., sucrose treatment concentrations above 30% decreased the water uptake. *Gladiolus* absorbed 4.2 g per shoot within the first 24 hours of being placed in a 40% sucrose solution although there was a decline in the amount of solution absorbed. Sucrose is accumulated at a lower rate by leaves than florets, because of stomatal closure being induced (Bravdo et al., 1974). Brink and De Swardt (1986) found that with a sucrose pulse period of 18 hours the flower head of *P. neriifolia* was the preferred sink, while with a sucrose pulse of less than 12 hours the leaves were the preferred sinks.

The translocation of $^{14}$C-sucrose was studied in relation to carbohydrate content changes in rose corollas (*Rosa hybrida* 'Sonia'), of roses cut at different development stages. In water-fed flowers, starch breakdown supplied an increase in soluble sugars, which depleted rapidly after day 0. Soluble sugar accumulation in the petals was higher than could be accounted for by starch conversion. Diminishing radioactivity concentrations in the leaves supported the supposition that movement of soluble sugars from the leaves to the petals took place. Direct translocation of $^{14}$C from the feeding solution to the petals also occurred as shown by the radioactivity recovered from the petals during the first four hours (Ho & Nichols, 1977).

### 2.3.2 *Protea*

Carbohydrate supplementation of *Protea* flowers had a positive impact on leaf blackening, although high sugar concentrations accelerated the problem in cases. Leaf blackening of *P. neriifolia* was significantly delayed by 0.5 and 1% sucrose holding solutions (Brink & de Swardt, 1986). The most effective sucrose vase solution for 'Sylvia' was 3% with a vase life period of 10 days (Ligawa et al., 1997). *P. neriifolia* vegetative and floral stems, after a 24 hour 20% sucrose pulse, had significantly less leaf blackening than decapitated stems or stems kept in 0.5% sucrose holding solution (McConchie & Lang, 1993a). Although the addition of 0.5% sucrose to the vase solution of *P. neriifolia* resulted in reduced leaf blackening, this was not significant (McConchie et al., 1991). The addition of 0.5% sucrose to the vase solution of *P. eximia* significantly delayed leaf blackening, resulting in a vase life period of 16 days (Newman et al., 1990). Sucrose pulsing solutions of 10 and 20% did not significantly improve 'Sylvia' vase life. A glucose holding solution of 2.5% had a significant effect on leaf blackening and the vase life was terminated after 20 days due to flower head collapse (Stephens et al., 2001).

A sucrose pulse (20%) for 24 hours resulted in an increased starch concentration in *P. neriifolia* 48 hours after the initiation of the pulse, suggesting the partitioning of sucrose into starch reserves (McConchie & Lang, 1993a). Starch concentrations of *P. neriifolia* differed significantly between 0 and 0.5% sucrose vase solutions, with the 0.5% treatment resulting in higher starch concentrations from day 5 (McConchie et al., 1991). Brink and De Swardt (1986) found that with a sucrose pulse of 18 hours the *P. neriifolia* flower head was the preferred sink, while with a sucrose pulse of less

than 12 hours the leaves were the preferred sinks. A 5% sucrose solution seemed to stimulate nectar production in *P. neriifolia* (Dai & Paull, 1995).

There are, however, negative implications in using high sugar concentrations for pulsing or holding solutions. A 3% sucrose vase solution had a detrimental effect on leaf blackening of *P. neriifolia* (Brink & de Swardt, 1986). Sucrose holding solutions of 4 to 6% caused leaf blackening of 'Sylvia' (Ligawa et al., 1997). Leaf spotting in *P. eximia* is associated with high concentrations of exogenous sugars (3% sucrose) supplied in the vase solution resulting in an over supply. Newman et al. (1990) observed black spots developing on *P. eximia* leaves, with 1% and higher sucrose holding solutions. *P. neriifolia* stems showed an acceleration of leaf blackening, when pulsed with sucrose concentrations higher than 7.5% (Paull & Dai, 1990). Vase solutions of 2 and 5% sucrose promoted leaf blackening in 'Sylvia' proteas with 1% sucrose having no significant effect (Stephens et al., 2001). Leaf blackening is delayed due to the endogenous production of carbohydrates under high light conditions (Bieleski et al., 1992). Bieleski et al. (1992) suggested that the compartmentation of sugars depends whether carbohydrates were produced endogenous or supplied exogenously.

## 2.4 Effects of respiration on the carbohydrate status

Sugars are the major substrate for respiration of flowers, and the availability is influenced by the hydrolysis of starch and other polysaccharides, the rate of respiration, translocation and photosynthesis (Ho & Nichols, 1977). In the field, the high demand for carbohydrates is met by ongoing photosynthesis. However, in harvested flowers light levels are too low for photosynthesis to manufacture substrates

for respiration (Anon., 2002). Under these conditions depletion of the free sugars occurs and this triggers hydrolysis of starch, sucrose or proteins, to supply alternative substrates for respiration. This utilization of protein is demonstrated by the delay of excessive protein degradation by an exogenous sugar supply (Coorts, 1973).

The rate of respiration, the main metabolic activity in utilization sinks, controls the import rate into these sinks (Ho, 1988). Sink demand controls starch and sucrose partitioning of photosynthate. The synthesis and storage of starch are accelerated when the utilization of sucrose is lower than the rate of synthesis. At elevated temperatures plants can respire large amounts of carbohydrate (Rajapakse et al., 1994) and these high respiratory rates shorten the life of flowers. Lowering the storage temperatures of cut flowers is standard practice to delay senescence (Coorts, 1973).

The 'dry' storage method, whereby flowers are sealed in a container, simulates modified atmosphere storage since the carbon dioxide ($CO_2$) produced by the flower's respiration accumulates and the oxygen used is not replenished. To prevent accumulation of excessive $CO_2$ concentrations it is preferable to store flowers in a selectively permeable material (Halevy & Mayak, 1981). The effect of respiration on the carbohydrate status of cut flowers and *Protea*, and measures to control this process will be discussed in the following sections.

### 2.4.1 Cut flowers

Enzymes, which are temperature sensitive, regulate respiration. For each 10°C rise in temperature, up to 25°C to 30°C, enzyme activity increases two to four times. An exponential increase in respiration of *Gerbera* and sunflower (*Helianthus annuus L.*),

as with most horticultural commodities, is observed with increasing storage temperatures. A significant negative linear relationship is noted between vase life after storage and respiration rate during storage. This highlights the importance of maintenance of temperatures close to freezing point during the handling chain for optimum vase life (Çelikel & Reid, 2002).

Carbohydrates serve as respirable material and different carbohydrates are utilized at different rates and various enzymes are involved in this process. In *Freesia*, glucose and fructose concentrations decreased faster than sucrose concentrations, with different stem lengths strongly influencing the amount of available carbohydrates (van Meeteren et al., 1995). Amylase activity in rose buds increased in the starch fraction after harvest, making it likely that amylase attack is the first step in starch breakdown. Artificial suppression of amylase activity could therefore retard the opening of harvested rose buds (Hammond, 1982). The respirable substrate pool is actively maintained by several exogenously supplied metabolic sugars, which has a positive effect on respiration (Rogers, 1973).

Enrichment of the atmosphere with carbon dioxide can enhance carbohydrate and water status of plants during growth due to its influence on photosynthesis, dark respiration and stomata characteristics. In potted miniature roses both leaf and stem sucrose: starch ratios of plants grown in 700 or 1050 $uL.L^{-1}$ $CO_2$ were reduced by about 70% compared to those of plants grown under 350 $uL.L^{-1}$ $CO_2$. Leaf and stem starch concentrations increase with elevated $CO_2$ concentrations. The high concentrations have relatively little effect on sucrose and glucose concentrations.

High $CO_2$ concentrations probably altered the chemical form of storage carbohydrates (Rajapakse et al., 1994).

Cut roses placed in modified Cornell solution (2% sucrose + 200 $mg.L^{-1}$ 8-hydroxyquinoline sulphate) showed a very gradual rate of decline and loss of respiratory control (RC) did not occur. The RC associated with mitochondria of flowers held in distilled water declined at a rapid rate until day seven when complete loss of RC occurred. RC is a useful measure of mitochondria integrity since the degree of coupling of electron transport and oxidative phosphorylation gives an indication of the integrity of the isolated mitochondria. Hence it was hypothesised that exogenous sugars extend vase life by maintaining mitochondria structure and function rather than by providing substrates (Kaltaler & Steponkus, 1976).

### 2.4.2 *Protea*

The expanding flower heads of 'Sylvia' (Stephens et al., 2000) and *P. neriifolia* (Dai & Paull, 1995; Ferreira, 1986) have a high respiration rate after harvest. This high respiration rate is thought to be typical of *Protea* although it has not been measured in many of the flowers. The cooling of flowers as rapidly as possible after harvest is essential to slow the development of the flower head and reduce respiration (Newman et al., 1990). A lower respiration rate is observed in the more mature stages of harvested 'Sylvia' flowers when compared to the more immature stages (Joyce et al., 1995). Ferreira (1986) also reported higher respiration rates in immature florets than mature florets of *P. neriifolia,* which resulted in a higher demand on carbohydrate reserves. Paull et al. (1981) recommended that proteas be stored at temperatures of 2

to 8°C. However, Stephens et al. (2000) found that no chilling injury occurred at a storage temperature of 0°C and leaf blackening was significantly lower.

The leaf starch concentrations in 'Pink Ice' (*P. susannae x P.* compacta) decreased by 58% during the 24-hour dark shipping period after harvest (McConchie et al., 1994). Starch concentrations in the leaves of *P. neriifolia* declined significantly (70 to 82%) 24 hours after harvest (McConchie and Lang, 1993b). This decrease in starch is due to the combined result of the high respiratory demand of the flower head as well as nectar production. When sucrose was provided in the vase solution of 'Sylvia', a higher steady state respiration level was maintained on days one through six (Joyce et al., 1995).

When *P. neriifolia* flowers were removed from controlled atmosphere (1% $O_2$, 5% $CO_2$) to an ambient atmosphere, 80% of leaves blackened within 16 hours (Jones & Clayton-Greene, 1992). Ferreira (1983) showed complete browning of *P. neriifolia* leaves could be induced within two hours at 40°C and 100% RH.

## 2.5 Conclusions

The post harvest aspects of commercial cut flowers have been researched extensively and their carbohydrate status and the effect of various storage regimes and treatments are well documented. *Protea* has not been produced commercially for as long as other commercial cut flowers. The leaf blackening problem of *Protea* is not fully understood, although it has been established that carbohydrate depletion is an initiating factor. A comprehensive study of the carbohydrate status of *Protea,* and changes over time, needs to be undertaken to formulate possible treatment regimes.

Pulsing flowers before storage and vase solutions will help meet the post harvest carbohydrate requirements of certain *Protea* cultivars and species. When preparing flowers for the export market, it is necessary to cool them in order to lower respiration rates and metabolic activities. Relative humidity control and maintenance of the cold chain are very important in lowering transpiration and avoiding water stress. The importance of all these factors needs to be taken into account when looking at research opportunities in this field.

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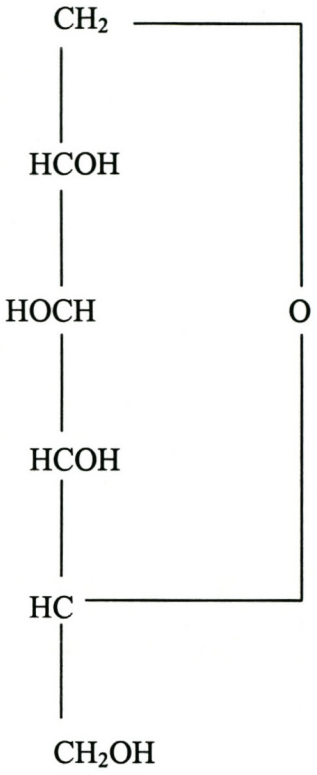


**Figure 1.** 1,5-anhydro-D-glucitol (polygalatol) (Lewis & Smith, 1976).

 

**Table 1.** Summary of carbohydrate data in the nectar of various *Protea* species.

***Protea***

5-6 mL nectar produced with fructose and glucose being dominant

(Cowling & Mitchell, 1981)

***P. neriifolia***

- ~ 3 mL/flower head at stage 4 (open with cylindrical shape)
- ~ 10 mL/flower head at stage 5 (reflexing outward)
- After seven days in 5% sucrose solution – nectar at all opening stages except tightly closed stage 1
- After seven days in 5% sucrose solution – highest nectar production when harvested at stages 3 (starting to separate) and 4
- After seven days in 5% sucrose solution/water - significant decline in nectar production

(Dai & Paull, 1995)

**Table 2.** Summary of carbohydrate data of the flower head and stem of various *Protea* cultivars.

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**Flower head**

**'Sylvia' (mg.g$^{-1}$ dry weight)**

- Consistent decrease in glucose concentration during all treatments
- No significant change in fructose concentration during experiment

**Harvest**

- Sucrose 0.5
- Glucose 3.2
- Fructose 7.9

**Three days at 0°C**

**Three days at 0°C + two days at 18°C**

- Significant increase in sucrose concentration

(Stephens et al., 2001)

**Three days at 0°C + six days at 18°C**

**Stem**

***'Sylvia' & 'Cardinal'***

Significantly higher total sugars and starch accumulation in leaves than in stems of all flushes.

(Hettasch et al., 2001).

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**Table 3.1** Summary of carbohydrate data in the leaves of *P. eximia* (Bieleski et al., 1992).

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***P. eximia*** **(mg.g$^{-1}$ fresh weight)**

- Sucrose 4-6
- Inositol ~ 1
- Glucose ~ 0.3
- Fructose ~ 0.1
- Raffinose/similar trisaccharide 0.1
- Oligosaccharide ~ 2
- Polygalatol 25-30

**Vases (containing 50 mg.L$^{-1}$ hypochlorite, replaced each day)**

- Polygalatol concentration remained relatively constant
- No appreciable change in glucose, fructose, inositol, raffinose and oligosaccharide concentrations

**Light (greenhouse under full natural light)**

- Starch content increased from 2.5 to ~ 14 mg.g$^{-1}$ fresh weight after three days
- Sucrose rose to 7 mg.g$^{-1}$ fresh weight
- Storage carbohydrates (starch + sucrose) increased three-fold over three days to 19 mg.g$^{-1}$ fresh weight

**Dark (20°C, 60% RH)/beheaded treatments**

**(darkness after removing flower head from stem)**

- Starch and sucrose fell rapidly over first one to two days reaching concentrations of ~ 0.5 and 0.9 mg.g$^{-1}$ fresh weight respectively

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**Table 3.2** Summary of carbohydrate data in the leaves of *P. neriifolia* (McConchie et al., 1991).

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***P. neriifolia* (0% & 0.5% sucrose with 50 mg.L$^{-1}$ hypochlorite)**

**Dark experiment (25 ± 1°C)**

- fructose, glucose & maltose < 1.9 mg.dm$^{-2}$
- 1,5-anhydro-D-glucitol – 3x the concentration of sucrose

**Light experiment (25 ± 1°C, 12 h light in 24 h period)**

- significant differences in starch concentrations between sugar treatments
- 0.5% sugar – significantly higher starch concentrations from day five onward
- 0% sugar – decrease of starch concentration over first seven days to concentration similar in dark experiment, slight increase in starch concentration after day seven
- general increase in leaf sucrose concentrations

**Uncut vegetative stems divided into 10 phyllotactic divisions with one division equalling one complete spiral of leaves.**

- basipetal increase in [starch], sharp rise below 5$^{th}$ phyllotactic division
- 1,5-anhydro-D-glucitol (polygalatol) highest in young leaves, declining linearly as leaves became older
- leaf sucrose concentrations remain generally constant regardless of position
- starch concentrations – higher below 3$^{rd}$ phyllotactic division than in leaves of both sugar treatments in light and dark experiments
- polygalatol – similar concentrations in light experiments and most basipetal phyllotactic divisions on uncut stems; similar concentrations in dark experiments and most acropetal phyllotactic divisions of uncut stems

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**Table 3.3** Summary of carbohydrate data in the leaves of *P. neriifolia* (McConchie & Lang, 1993a).

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**Floral and vegetative stems (*P. neriifolia*)**

**Pre-harvest**

- one phyllotactic division = one complete spiral of leaves (ca. five to seven leaves) from the distal end (1) to basal end (7)
- starch concentrations fluctuated across seven phyllotactic divisions ranging from 32.7 - 88.3 $mg.dm^{-2}$ glucose
- fructose, glucose and maltose, concentrations < 1.7 $mg.dm^{-2}$
- neither polygalatol nor sucrose differed significantly between floral and vegetative stems
- [polygalatol] higher than [sucrose] in both stems across all divisions
- polygalatol concentrations decreased basipetally
- sucrose concentrations increased basipetally

**Post-harvest**

- starch concentrations of leaves of vegetative stems declined more gradually than those of floral stems
- starch concentrations of leaves in 0.5% sucrose dropped to 4.2 $mg.dm^{-2}$ within 24 h and remained low
- 24 h 20% pulse: after initial decline during shipping, significant increase in starch concentration 48 h after initiation of pulse treatment
- dramatic decline in sucrose concentrations
- polygalatol concentrations significantly higher in vegetative stems

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**Table 3.4** Summary of carbohydrate data in the leaves of various *Protea* species and cultivars.

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***P. neriifolia* (PN), *P. eximia* (PE), *P. susannae* x *P. compacta* (PS)**

- fructose, glucose and maltose < 1.9 $mg.dm^{-2}$
- [starch]of PN significantly higher, for both floral and vegetative stems, than other species
- [starch] for vegetative stems of all three species were lower in first two phyllotactic divisions
- similar starch pattern in floral stems, except PS, which had highest starch concentration at first division
- significant difference in sucrose concentrations: PS highest, PE lowest
- floral stems: [sucrose] not significantly influenced by phyllotactic division
- vegetative stems: phyllotactic division significantly influence [sucrose]
- floral and vegetative [polygalatol] declined significantly in a basipetal pattern

(McConchie & Lang, 1993b)

**'Pink Ice' ($mg.g^{-1}$ fresh weight)**

- 58% decline in [starch] during 24 hours shipping period following harvest
- in dark, starch had declined by 88% by day four
- in light, similar decline, rate of decline significantly less than in dark
- [polygalatol] ~ 4x higher than [sucrose] throughout postharvest period
- in dark sucrose undetectable on day four; in light sucrose concentrations maintained until day eight

(McConchie et al., 1994)

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**Table 3.5** Summary table of carbohydrate data in the leaves of 'Sylvia'.

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**'Sylvia' (mg.g$^{-1}$ dry weight)**

- Consistent decrease in glucose concentration during all treatments
- Significant decrease in fructose concentration after removal from storage

**Harvest**

- Sucrose 0.86
- Glucose 3.5
- Fructose 11.8

**Three days at 0°C**

- significant decrease in glucose and sucrose concentration

**Three days at 0°C + two days at 18°C**

**Three days at 0°C + six days at 18°C**

(Stephens et al., 2001)

**'Sylvia' (dry mass basis)**

Storage temperatures of 0, 4.5, 7 or 10°C used and stems stored for three days

- significant decrease in starch and reducing sugar content with increasing temperatures
- no significant difference in starch and reducing sugar content of leaves stored at 0°C and at harvest

(Stephens et al., 2000)

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**Table 4.** Nectar sugar compositions in species of *Protea* (Van Wyk & Nicolson, 1995).

| **Sample** | **Percentage of total sugar** | | | |
|---|---|---|---|---|
| **Genus & species** | **Xylose** | **Fructose** | **Glucose** | **Sucrose** |
| ***P. cynaroides*** | 3 | 47 | 50 | - |
| ***P. grandiceps*** | | | | |
| Sample 1 | 3 | 2 | 2 | 93 |
| Sample 2 | Trace | 14 | 10 | 76 |
| ***P. eximia*** | 3 | 44 | 51 | 2 |
| ***P. neriifolia*** | 2 | 45 | 50 | 3 |
| ***P. burchellii*** | 2 | 21 | 22 | 55 |
| ***P. compacta*** | 2 | 47 | 51 | - |
| ***P. magnifica*** | - | 47 | 47 | 6 |
| ***P. susannae*** | | | | |
| Sample 1 | 1 | 36 | 36 | 27 |
| Sample 2 | 2 | 38 | 41 | 19 |
| Sample 3 | 1 | 36 | 40 | 23 |
| ***P. repens*** | 5 | 46 | 49 | - |

**PAPER I** - Carbohydrate Utilisation Patterns of Various *Protea* Cultivars and Species.

**Abstract**

The concentrations of glucose, fructose, sucrose and starch of various *Protea* species and cultivars were measured at harvest, and again at the initiation of leaf blackening, after being held in water for several days. Flowering stems were divided into the inflorescence and the upper and lower leaves. All measured carbohydrates declined significantly in 'Carnival', 'Pink Ice' and 'Sheila'. In 'Cardinal' all carbohydrate concentrations decreased significantly, except the sucrose concentration in the inflorescence. 'Susara' and 'Ivy' had very high initial carbohydrate concentrations in the leaves and these decreased significantly. The very high initial carbohydrate concentrations in the inflorescence of 'Ivy' declined significantly, but were higher than the initial carbohydrate concentrations of most of the other cultivars in this experiment. 'Brenda' differed from the other cultivars and species in that glucose concentrations increased over time. In most tested cultivars glucose concentrations declined significantly from harvest to the initiation of leaf blackening, highlighting the dependence of the leaves and inflorescence on the carbohydrate reserves. Starch concentrations tended to be high in the leaves and low in the inflorescences, and decreased to very low concentrations prior to leaf blackening. Higher fructose and glucose concentrations were reported in the inflorescence than in the leaves. Sucrose concentrations were generally higher in the leaves than in the inflorescences.

## Introduction

A positive correlation was found between the depletion of carbohydrates in the leaves and the onset of post-harvest leaf blackening (Reid et al., 1989; Paull & Dai, 1990). This was substantiated by the removal of the inflorescence leading to a reduction of the carbohydrate demand on the leaves and a concurrent decrease in leaf blackening (Paull & Dai, 1990). Girdling below the inflorescence, which interrupts phloem transport, also significantly reduced leaf blackening (Newman et al., 1990). The use of post-harvest lighting of sufficient intensity for photosynthesis also diminished the occurrence of leaf blackening (Bieleski et al., 1992). Jones and Clayton-Greene (1992) suggested that the inhibitory effect of net photosynthesis on leaf blackening could be either a direct effect on carbohydrate production, or an indirect effect of maintaining cellular integrity, thereby preventing phenol oxidation. Leaves have an excess of carbohydrates (mainly starch or sucrose) that serve as respiratory substrates (Coorts, 1973). Increasing temperatures were positively correlated with an increase in leaf blackening (Ferreira, 1986; Stephens et al., 2000). Carbohydrates are rapidly transported from the leaves to the developing inflorescence, therefore an increasing number of leaves on the stem correspond with an improvement of vase life and less leaf blackening (Dai & Paull, 1995).

*Protea* typically stores carbohydrates as 1,5-anhydro-D-glucitol (polygalatol), a simple derivative of sorbitol (D-glucitol). The high polygalatol concentrations remain relatively constant after harvest over time, with sucrose, glucose, fructose and starch decreasing to relatively low concentrations after harvest (Bieleski et al, 1992; McConchie et al., 1991, 1994; McConchie & Lang, 1993a, b). Jones et al. (1995) hypothesised that polygalatol synthesis could form a secondary competitive sink for

leaf carbohydrate pools. Membrane destabilisation can occur, because of a deficiency in available energy resources. A constant energy supply is required to keep membranes intact.

Various cut flowers have different sugar compositions and concentrations. Mature cut Freesia flowers ('Aladin' and 'Polaris') have 1.5-2 times the total carbohydrate concentration of immature flowers. The highest concentrations of glucose and fructose are found in the largest flower buds of both cultivars (Sytsema-Kalkman et al., 1995). Fructose is the primary soluble carbohydrate fraction in gladiolus florets ('New Rose') with substantially lower concentrations of glucose and sucrose characteristic of the soluble carbohydrate pool (Waithaka et al., 2001). Kaltaler and Steponkus (1974) noted that the sugar pool of mature rose petals (*Rosa hybrida* L. cv. Forever Yours) is mainly made up of the reducing sugars, glucose and fructose.

We hypothesised that by analysing carbohydrate utilisation patterns in different *Protea* cultivars and species it would be possible to better understand the role played by carbohydrate supplementation. A clear picture could be obtained of what happens in the carbohydrate composition of proteas over time with measurements taken from freshly harvested material and when first signs of leaf blackening are observed. This could aid in developing post-harvest strategies and, possibly, in predicting leaf blackening severity.

## Materials and Methods

### Plant material.

'Brenda' (*P. compacta* x *P. burchellii*), 'Cardinal' (*P. eximia* x *P. susannae*), 'Carnival' (*P. compacta* x *P. neriifolia*), *P. cynaroides*, *P. grandiceps*, 'Ivy', 'Pink Ice' (*P. compacta* x *P. susannae*), 'Sheila' (*P. magnifica* x *P. burchellii*) and 'Susara' (*P. magnifica* x *P. susannae*) flowers were harvested into SAPPEX S14 fibreboard boxes and brought to the laboratory within one hour. Two single flowering stems were used as one replicate and five replicates per treatment were used, except for 'Ivy' where ten flowers were used per replicate and five replicates per treatment.

Flowering stems were either processed at harvest (control), or placed in tap water (treatment) at standard conditions of 19°C ± 2°C and processed as soon as the leaves showed signs of leaf blackening. Leaves were stripped from the flowering stem leaving 16 subtending leaves. These were divided into eight leaves below the flower (upper leaves) and the remaining eight leaves on the stem (lower leaves) (Figure 1). A longitudinal section was taken from the inflorescence.

### Carbohydrate analysis.

Samples were freeze-dried and milled to a fine powder and 0.5 g of this powder was analysed for sucrose, fructose, glucose and starch concentrations. Samples were extracted overnight at 20±1°C in 1% acetic acid on an automatic shaker. The samples were centrifuged (4 000$g_n$, 15 min, 20±1°C); the supernatant filtered and made up to 100 mL with 1% acetic acid. The pellet was re-suspended in an acetate buffer (pH 4.8) and gelatinised in a boiling steam bath for two hours. The suspension was cooled to 55°C and the starch fraction hydrolysed with amyloglucosidase (208 469,

Boehringer, Mannheim, W-Germany). An incubator (55°C) was used for hydrolysis (18 hours). The supernatant was filtered and made up to 100 mL with distilled water. Glucose, fructose, sucrose and starch were analysed on a Sanplus Segmented Flow Analysis System from Skalar, using Method Numbers 551-965 w/r issue 070798/MH and 356-001 w/r issue 012998/MH97203066 (Skalar, De Breda, The Netherlands).

**Statistical analysis.**

Standard analysis of variance was performed on the data using the SAS program (Statistical Analysis Systems Institute, 1996). Means are separated by Dunnet's t test ($P \leq 0.05$).

## Results

In general the carbohydrates declined significantly over time with the exception of 'Brenda' where glucose concentrations increased after seven days, although not significantly. Glucose and fructose concentrations were higher in the inflorescence than the leaves of all tested cultivars/species. However, sucrose and starch concentrations were higher in the leaves than the inflorescence, this however was not consistent.

In 'Brenda' the starch, fructose and sucrose concentrations in the upper leaves decreased after seven days in water and all of these reductions were significant ($P \leq 0.05$) (Table 1). During this time the glucose concentration increased from 11.0 $mg.g^{-1}$ to 17.0 $mg.g^{-1}$, although this was not significant. The pattern was similar in the lower leaves. This increase in glucose was only found in 'Brenda'. In the inflorescence, fructose and sucrose concentrations decreased significantly over the

same time, but the changes in glucose and starch concentrations were not significant. Leaf blackening was observed after seven days.

'Cardinal' showed a significant decline in all the concentrations of all measured carbohydrates in both the upper and the lower leaves (Table 2). The sucrose concentration in the inflorescence did not change significantly over the four days that it took for leaf blackening to occur for the first time.

Low initial starch concentrations of 3.5 $mg.g^{-1}$ and 3.6 $mg.g^{-1}$ were observed in the upper and lower leaves of 'Carnival', respectively (Table 3) and these concentrations did not decline significantly after six days when leaf blackening first occurred. However, the starch concentration in the inflorescence decreased significantly in the inflorescence over the same time. Glucose, fructose and sucrose concentrations decreased significantly in the leaves and inflorescence after six days in water.

At harvest, high starch concentrations were found in the leaves of 'Ivy' and high concentrations of glucose, fructose and sucrose were found in the leaves and the inflorescence (Table 4). These concentrations declined significantly after six days when the first leaf blackening symptoms were observed. However, all the tested carbohydrate concentrations were still higher in the inflorescence of 'Ivy' after six days, compared to the other *Protea* cultivars and species in this experiment.

Glucose and fructose concentrations in the separated leaves and inflorescence of 'Pink Ice' declined significantly after six days in water when leaves showed the first

symptoms of leaf blackening (Table 5). A similar pattern was observed in the sucrose and starch concentrations of the upper and lower leaves, but not in the inflorescence.

After six days in water the measured carbohydrate concentrations in the leaves and the inflorescence of 'Sheila' declined significantly (Table 6). Leaf blackening was observed after six days.

In contrast to other tested species and cultivars, the initial glucose concentrations were very similar in the leaves and inflorescence of 'Susara' (Table 7). Glucose, fructose, sucrose and starch concentrations decreased in the upper and lower leaves and inflorescence after seven days in water when the first signs of leaf blackening occurred.

## Discussion and Conclusions

Bieleski et al. (1992) found that *P. eximia* leaves contained only trace amounts of glucose (0.3 mg) and fructose (0.1 mg) per gram fresh weight. Apart from polygalatol, sucrose and starch at 4 and 2.5 mg per gram fresh weight, respectively, were the major non-structural carbohydrates in *P. eximia* leaves. Compensating for water content of between 66 and 80 percent, these values translate to 0.9 to 1.2 mg glucose, 0.3 to 0.4 mg fructose, 12 to 16 mg sucrose and 7.5 to 10 mg starch per gram dry weight. McConchie and Lang (1993b) also found that glucose, fructose and maltose at <1.9 $mg.dm^{-2}$ were present in lower concentrations than sucrose and starch in leaves of *P. neriifolia*, *P. susannae* x *P. compacta* and *P. eximia*. Sucrose varied from 2 to 10 $mg.dm^{-2}$ and starch from 12 to 80 $mg.dm^{-2}$ leaf area. In contrast, Stephens et al. (2001) found that fructose (11.8 $mg.g^{-1}$ dry weight) was present in a

higher concentration than either glucose (3.5 $mg.g^{-1}$ dry weight) or sucrose (0.9 $mg.g^{-1}$ dry weight) in leaves of 'Sylvia'. In another study, Stephens (2003) found that reducing sugars in the leaves of pre-harvest stems of 'Sylvia' varied from 12 to 45 $mg.g^{-1}$ dry weight.

Our results reveal that both glucose and fructose contribute significantly to the soluble carbohydrates in both leaves and inflorescences of proteas. For all the proteas included in our study, glucose and fructose added together were present in higher concentrations than sucrose in both leaves and inflorescences.

There is general consensus in the published literature that with the exception of polygalatol, carbohydrate concentrations in *Protea* leaves decrease rapidly when harvested flower stems are kept at low light levels or in the dark. In *P. eximia* leaves, starch and sucrose declined by ca. 80% in two days in a vase and leaf blackening was evident by the third day (Bieleski, 1992). Even at low temperatures the decline was rapid in 'Sylvia'. Stephens (2000) found that after three days at 10°C starch decreased by 60% and reducing sugars by 37%, and during a three-week period at 1°C, glucose decreased by 80%.

The dependence of both flowers and leaves on carbohydrate reserves is highlighted by the sharp decline in the concentration of both sugars and starch from the time of harvest until the onset of leaf blackening for the seven *Protea* cultivars/species studied (Tables 1-7). With the exception of 'Carnival', the concentration of starch was higher in the leaves than in the inflorescence by a factor that varied from 1.5 for 'Sheila' to 12 for 'Susara'. Leaves have higher concentrations of starch and these

concentrations are depleted to a greater extent during the period from harvest to the onset of leaf blackening than in the inflorescences. Starch depletion of the inflorescences varied from 40-50% as compared to the 70-90% for leaves. At harvest, glucose, fructose and sucrose combined were consistently higher in the inflorescences than in the leaves. However, leaves were depleted of sugars to a greater extent than inflorescences during the period from harvest to the onset of leaf blackening.

The finding that both starch and sugars are depleted to a greater extent in the leaves may imply that the inflorescence acts as a strong sink and mobilises sugars from the leaves. This concurs with the conclusion drawn from earlier results. The respiration rate of *Protea* flowers is high (Ferreira, 1983) and they produce large quantities of nectar (Dai & Paull, 1995). In harvested flowers kept in the dark or at low light intensities leaf starch is hydrolysed and mobile sugars translocated to the inflorescences to meet the respiratory needs and for nectar production (McConchie & Lang, 1994). However, it is clear that sugars exported to the inflorescence from the leaves are not adequate to meet the demands of the inflorescence. From the time of harvest, sugars and starch present in the inflorescences are consumed with the resultant decrease in their concentrations. We analysed upper and lower leaves separately in an attempt to show that upper leaves are depleted to a greater extent. However, the data do not support this notion.

The argument above can also be applied to individual sugars in most of the proteas studied, but there are a number of discrepancies which are difficult to explain. The increase in the concentration of glucose seven days after harvest in the leaves of

'Brenda' serves as an example, although it is not significant and probably not of great importance.

Indirect evidence that the decline in starch and sugars triggers the blackening reaction in leaves has mounted over the years. These include reduced leaf blackening when flower stems are kept under high light intensities (McConchie & Lang, 1991) and ring barking the stem just below the inflorescence (Reid et al., 1989; Stephens et al., 2000). However, supplementation of the vase solution with sucrose was only partially effective in reducing leaf blackening in *P. eximia*, *P. neriifolia* and *P. compacta*. Glucose in the vase solution was far more effective in reducing leaf blackening of the cultivar 'Sylvia' than sucrose (Stephens et al., 2001). The extent to which this result applies to other *Protea* is part of this investigation and warrants investigation on a wide range of cultivars grown worldwide.

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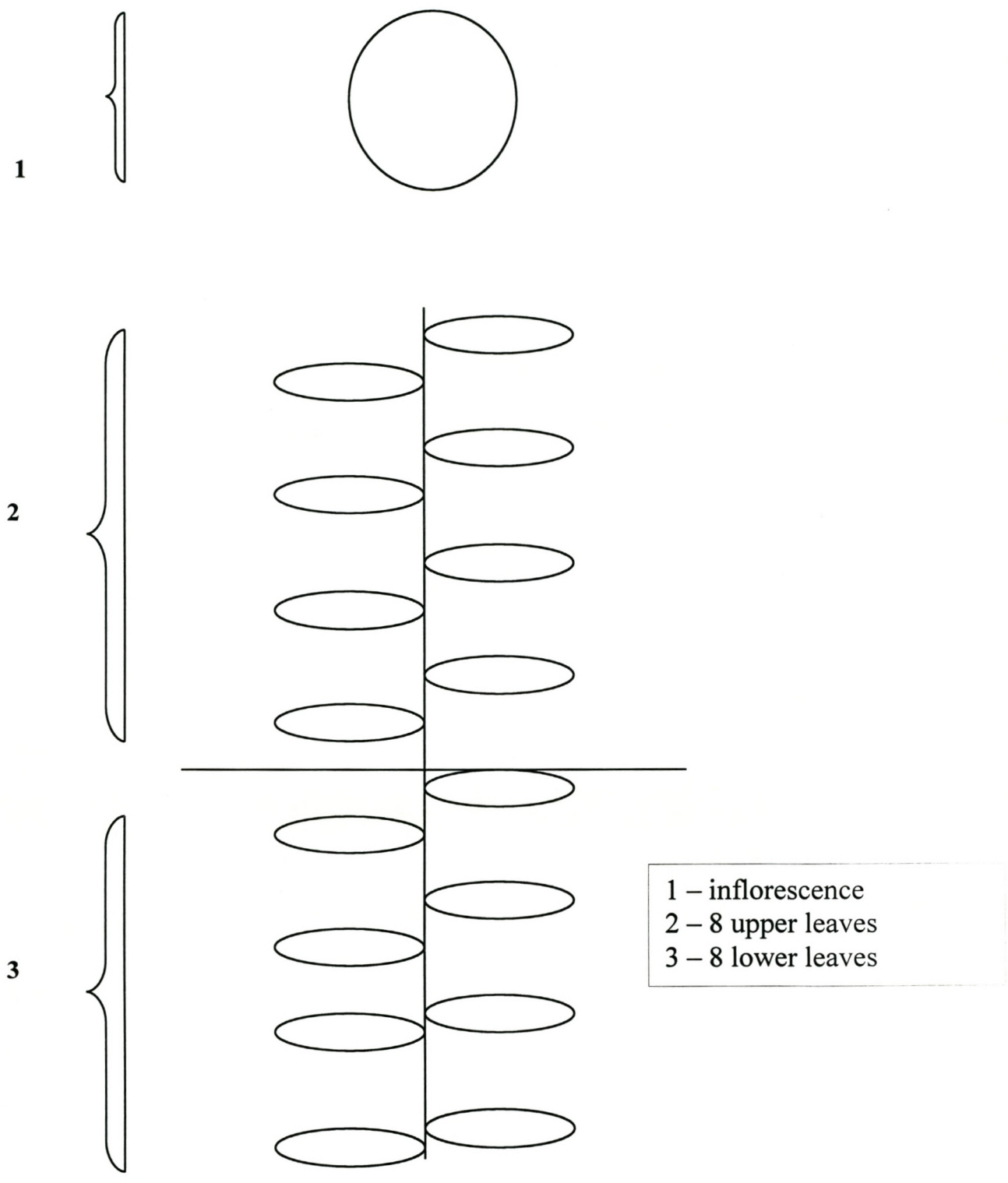


**Figure 1.** A schematic representation of the separation of plant material for carbohydrate analysis.

**Table 1.** Concentration of sucrose, glucose, fructose and starch ($mg.g^{-1}$ dry weight) in upper leaves, lower leaves and inflorescence of 'Brenda' (*P. compacta* x *P. burchellii*) flower stems at harvest and after 7 days at the onset of leaf blackening.

| | | Glucose | Fructose | Sucrose | Starch |
|---|---|---|---|---|---|
| **Inflorescence** | At harvest | $23.2^{a}$ | $38.2^{a}$ | $13.8^{a}$ | $6.4^{a}$ |
| | After 7 days | $17.2^{a}$ | $27.8^{b}$ | $3.3^{b}$ | $3.3^{a}$ |
| **Upper leaves** | At harvest | $11.0^{a}$ | $11.8^{a}$ | $9.3^{a}$ | $11.4^{a}$ |
| | After 7 days | $17.0^{a}$ | $3.1^{b}$ | $1.9^{b}$ | $2.4^{b}$ |
| **Lower leaves** | At harvest | $11.4^{a}$ | $11.9^{a}$ | $11.3^{a}$ | $20.9^{a}$ |
| | After 7 days | $17.3^{a}$ | $2.7^{b}$ | $3.6^{b}$ | $2.3^{b}$ |

Values are means of 5 replicates (2 flower stems per replicate).

Within carbohydrate fraction and plant part values not followed by the same superscript differ significantly at $P \leq 0.05$.

**Table 2.** Concentration of sucrose, glucose, fructose and starch ($mg.g^{-1}$ dry weight) in upper leaves, lower leaves and inflorescence of 'Cardinal' (*P. eximia* x *P. susannae*) flower stems at harvest and after 4 days at the onset of leaf blackening.

| | | Glucose | Fructose | Sucrose | Starch |
|---|---|---|---|---|---|
| **Inflorescence** | At harvest | $25.2^{a}$ | $35.1^{a}$ | $8.1^{a}$ | $5.7^{a}$ |
| | After 4 days | $10.6^{b}$ | $19.3^{b}$ | $5.4^{a}$ | $3.1^{b}$ |
| **Upper leaves** | At harvest | $12.0^{a}$ | $10.5^{a}$ | $13.9^{a}$ | $20.2^{a}$ |
| | After 4 days | $4.2^{b}$ | $1.5^{b}$ | $0.9^{b}$ | $1.7^{b}$ |
| **Lower leaves** | At harvest | $12.2^{a}$ | $9.6^{a}$ | $12.5^{a}$ | $24.9^{a}$ |
| | After 4 days | $4.0^{b}$ | $1.2^{b}$ | $0.9^{b}$ | $1.7^{b}$ |

Values are means of 5 replicates (2 flower stems per replicate).

Within carbohydrate fraction and plant part values not followed by the same superscript differ significantly at $P \leq 0.05$.

**Table 3.** Concentration of sucrose, glucose, fructose and starch ($mg.g^{-1}$ dry weight) in upper leaves, lower leaves and inflorescence of 'Carnival' (*P. compacta* x *P. neriifolia*) flower stems at harvest and after 6 days at the onset of leaf blackening.

| | | Glucose | Fructose | Sucrose | Starch |
|---|---|---|---|---|---|
| **Inflorescence** | At harvest | $21.5^{a}$ | $31.5^{a}$ | $8.7^{a}$ | $5.4^{a}$ |
| | After 6 days | $6.2^{b}$ | $13.7^{b}$ | $3.9^{b}$ | $1.6^{b}$ |
| **Upper leaves** | At harvest | $10.5^{a}$ | $10.9^{a}$ | $6.9^{a}$ | $3.5^{a}$ |
| | After 6 days | $3.2^{b}$ | $0.6^{b}$ | $1.3^{b}$ | $3.1^{a}$ |
| **Lower leaves** | At harvest | $12.7^{a}$ | $13.6^{a}$ | $11.6^{a}$ | $3.6^{a}$ |
| | After 6 days | $4.5^{b}$ | $1.1^{b}$ | $1.9^{b}$ | $2.4^{a}$ |

Values are means of 5 replicates (2 flower stems per replicate).

Within carbohydrate fraction and plant part values not followed by the same superscript differ significantly at $P \leq 0.05$.

**Table 4.** Concentration of sucrose, glucose, fructose and starch ($mg.g^{-1}$ dry weight) in upper leaves, lower leaves and inflorescence of 'Ivy' flower stems at harvest and after 6 days at the onset of leaf blackening.

| | | Glucose | Fructose | Sucrose | Starch |
|---|---|---|---|---|---|
| **Inflorescence** | At harvest | 34.2[a] | 48.6[a] | 27.6[a] | 7.6[a] |
| | After 6 days | 21.0[b] | 34.3[b] | 12.8[b] | 4.5[b] |
| **Upper leaves** | At harvest | 17.9[a] | 23.5[a] | 34.1[a] | 35.4[a] |
| | After 6 days | 6.4[b] | 5.0[b] | 4.4[b] | 3.0[b] |
| **Lower leaves** | At harvest | 19.2[a] | 24.6[a] | 34.2[a] | 40.8[a] |
| | After 6 days | 5.8[b] | 5.8[b] | 4.7[b] | 2.8[b] |

Values are means of 5 replicates (10 flower stems per replicate).

Within carbohydrate fraction and plant part values not followed by the same superscript differ significantly at $P \leq 0.05$.

 

**Table 5.** Concentration of sucrose, glucose, fructose and starch ($mg.g^{-1}$ dry weight) in upper leaves, lower leaves and inflorescence of 'Pink Ice' (*P. compacta* x *P. susannae*) flower stems at harvest and after 6 days at the onset of leaf blackening.

| | | Glucose | Fructose | Sucrose | Starch |
|---|---|---|---|---|---|
| **Inflorescence** | At harvest | $21.2^{a}$ | $32.5^{a}$ | $12.5^{a}$ | $4.2^{a}$ |
| | After 6 days | $18.3^{b}$ | $28.8^{b}$ | $10.9^{a}$ | $3.2^{a}$ |
| **Upper leaves** | At harvest | $12.8^{a}$ | $11.9^{a}$ | $18.9^{a}$ | $16.0^{a}$ |
| | After 6 days | $4.3^{b}$ | $2.0^{b}$ | $2.3^{b}$ | $3.3^{b}$ |
| **Lower leaves** | At harvest | $13.0^{a}$ | $11.9^{a}$ | $23.7^{a}$ | $15.9^{a}$ |
| | After 6 days | $4.6^{b}$ | $1.3^{b}$ | $2.3^{b}$ | $4.3^{b}$ |

Values are means of 5 replicates (2 flower stems per replicate).

Within carbohydrate fraction and plant part values not followed by the same superscript differ significantly at $P \leq 0.05$.

 

**Table 6.** Concentration of sucrose, glucose, fructose and starch ($mg.g^{-1}$ dry weight) in upper leaves, lower leaves and inflorescence of 'Sheila' (*P. magnifica* x *P. burchellii*) flower stems at harvest and after 6 days at the onset of leaf blackening.

| | | Glucose | Fructose | Sucrose | Starch |
|---|---|---|---|---|---|
| **Inflorescence** | At harvest | $19.1^{a}$ | $33.1^{a}$ | $14.0^{a}$ | $5.2^{a}$ |
| | After 6 days | $7.3^{b}$ | $14.5^{b}$ | $4.4^{b}$ | $2.1^{b}$ |
| **Upper leaves** | At harvest | $14.0^{a}$ | $14.4^{a}$ | $17.0^{a}$ | $8.5^{a}$ |
| | After 6 days | $6.7^{b}$ | $3.7^{b}$ | $4.1^{b}$ | $1.7^{b}$ |
| **Lower leaves** | At harvest | $13.5^{a}$ | $13.7^{a}$ | $18.3^{a}$ | $10.4^{a}$ |
| | After 6 days | $5.5^{b}$ | $2.6^{b}$ | $10.4^{b}$ | $1.8^{b}$ |

Values are means of 5 replicates (2 flower stems per replicate).

Within carbohydrate fraction and plant part values not followed by the same superscript differ significantly at $P \leq 0.05$.

**Table 7.** Concentration of sucrose, glucose, fructose and starch ($mg.g^{-1}$ dry weight) in upper leaves, lower leaves and inflorescence of 'Susara' (*P. magnifica* x *P. susannae*) flower stems at harvest and after 7 days at the onset of leaf blackening.

| | | Glucose | Fructose | Sucrose | Starch |
|---|---|---|---|---|---|
| **Inflorescence** | At harvest | $20.7^{a}$ | $32.1^{a}$ | $14.3^{a}$ | $3.3^{a}$ |
| | After 7 days | $8.4^{b}$ | $16.6^{b}$ | $6.5^{b}$ | $1.7^{b}$ |
| **Upper leaves** | At harvest | $19.6^{a}$ | $16.0^{a}$ | $22.5^{a}$ | $32.2^{a}$ |
| | After 7 days | $6.8^{b}$ | $4.5^{b}$ | $5.5^{b}$ | $1.7^{b}$ |
| **Lower leaves** | At harvest | $20.0^{a}$ | $18.9^{a}$ | $23.5^{a}$ | $40.4^{a}$ |
| | After 7 days | $7.7^{b}$ | $5.5^{b}$ | $6.7^{b}$ | $2.8^{b}$ |

Values are means of 5 replicates (2 flower stems per replicate).

Within carbohydrate fraction and plant part values not followed by the same superscript differ significantly at $P \leq 0.05$.

**PAPER II** - Glucose Pulse Treatments Prolong Storage Period of Certain *Protea* Cultivars and Species.

**Abstract**

Leaf blackening was significantly reduced in some *Protea* cultivars and species by glucose pulsing prior to three weeks of storage at 1°C (simulating sea transport). Evaluation was done one, seven and 10 days following storage. The most effective glucose pulsing concentrations for 'Brenda', 'Cardinal', 'Carnival', 'Pink Ice', 'Susara' and 'Sylvia' were between 4 and 10%. Leaf blackening of 'Sheila' and *P. cynaroides* was not significantly reduced by glucose pulsing, while *P. grandiceps* showed a significant reduction in leaf blackening with 2% glucose on the first evaluation date only. *P. magnifica* showed a small, but significant, reduction in leaf blackening in response to the 3, 6 and 9% treatments after 10 days only, but despite this, leaf blackening was unacceptably high. 'Pink Ice' harvested at the soft tip stage had less leaf blackening than those harvested open or closed. Toxicity symptoms on the leaves, and in some instances flowers, were observed at higher glucose concentrations (8 and 10%) on *P. grandiceps*, *P. cynaroides,* 'Cardinal' and 'Sheila'. All glucose treatments (3, 6 and 9%) resulted in toxicity symptoms on *P. magnifica*. The flower quality for the majority of tested cultivars and species was acceptable on the last evaluation date. A post harvest decrease in non-structural carbohydrates apparently occurs in all proteas but it appears that members of the *Ligulatae* are more likely to respond to glucose treatments.

## Introduction

Quality perception is subjective and the vase life of certain *Protea* cultivars is shortened by leaf blackening occurring three to seven days after harvest (Paull & Dai, 1990). Leaf blackening is characterized by unsightly black/dark brown discoloured areas on the leaves (Jones et al., 1995). It spreads very quickly to cover the whole leaf. Leaf blackening has been positively correlated with carbohydrate depletion (Paull & Dai, 1990; Reid et al., 1989). In addition to temperature and humidity control, this research supports the importance of a carbohydrate source given as a pre-storage pulse.

Delaying senescence of cut flowers is achieved by reducing respiration and metabolism through lower storage temperatures. Significant decreases in respiration rates, number of blackened leaves and appearance of leaf blackening of 'Sylvia' proteas were observed at lower storage temperatures (Stephens, 2001). An extended storage period at low temperatures offers greater flexibility in marketing and permits sea transport, which is more economically viable than airfreight. The control of relative humidity is also important in reducing transpiration and water loss from the product.

Immature buds require an external supply of carbohydrates as is evident with sucrose solutions improving vase life (Halevy & Mayak, 1979). Carbohydrate reserves in stems and leaves are used for nectar production if no additional source, such as a sugar pulse, is supplied (Dai & Paull, 1995). Flower head demand for carbohydrates is usually fulfilled by substrates from vegetative parts of the growing plant, but this is limited once the flower has been harvested (Ho & Nichols, 1977). The effects of

exogenous sugar on delaying senescence of cut flowers are complex since it serves as respiratory substrate and basic metabolite (Coorts, 1973), improves water relations by increasing osmotic solutes (van Doorn, 2001), maintains mitochondria (Kaltaler & Steponkus, 1976) and membrane integrity (Coorts, 1973), interacts with several growth hormones (Borohov et al., 1976), and delays gene expression related to cell death (van Doorn, 2001).

Sacalis and Durkin (1972) pulsed cut hybrid tea roses ('Forever Yours') and carnations ('Scania') with $^{14}$C-sucrose and followed it's subsequent distribution in the flower. The radioactive C moved selectively to the leaves and stems during the pulse but not into the flower heads. However, when the flowers were held in distilled water for different times following the pulse, the $^{14}$C moved from the leaves and stems to the flower heads. Diminished movement of the labelled C was observed in the senescing carnations while age had no effect on movement into rose flower heads. Most of the exogenous sugars are exported from the green leaves in the phloem as sucrose in hybrid tea roses and carnation flowers, but a small amount is directly transported in the xylem to the petals (Sacalis & Durkin, 1972). However, when *P. neriifolia* stems were pulsed for 18 hours with 1% $^{14}$C-sucrose, significantly more $^{14}$C accumulated in the flower head than in the leaves. A 12-hour pulse period allows for even distribution of sucrose throughout the flowering stem of *P. neriifolia* (Brink & de Swardt, 1986). The water absorption rate for the first one to two hours was higher in flowers that were kept dry (not placed in water) for a few hours before pre-treatment. To increase solution uptake, the transpiration rate needs to be high. This is achieved through high temperatures and low relative humidity (Harkema & van Doorn, 1986).

*P. neriifolia* (Brink & de Swardt, 1986) and *P. eximia* (Newman et al., 1990) responded positively to sucrose holding solutions and leaf blackening was significantly reduced. Sucrose pulsing, however, did not significantly improve 'Sylvia' vase life (Stephens et al., 2001). A reduction in glucose concentration of the leaves and flower head of 'Sylvia' was measured during storage and vase life, and as a result 2.5% glucose was added to the vase solution. This significantly reduced leaf blackening (Stephens et al., 2001). Stephens et al. (2001) reported sugar concentrations at harvest and after different storage regimes and correlated it to leaf blackening. In Paper I, sugar and starch concentrations of various *Protea* cultivars and species were determined at harvest and when first leaf blackening symptoms were observed. This research indicated that sugars can be used for supplementation by pulsing or in vase solutions (Paper I).

We hypothesized that glucose pulsing and storage at low temperatures would prolong the storage and vase life period of various *Protea* cultivars and species. Different pulsing concentrations were used to determine the optimum for alleviating leaf blackening.

## Materials and Methods

### Plant material.

In 2002, flowering shoots of 'Cardinal' (*P. eximia* x *P. susannae*), *P. cynaroides*, *P. grandiceps*, 'Ivy', *P. magnifica*, 'Pink Ice' (*P. compacta* x *P. susannae*), 'Sheila' (*P. magnifica* x *P. burchellii*), 'Susara' (*P. magnifica* x *P. susannae*) and 'Sylvia' (*P. eximia* x *P. susannae*) were harvested at the soft tip stage in the morning, placed in

SAPPEX S14 fibreboard cartons with lids and brought to the laboratory within one hour. Different numbers of replicates were used for experiments depending on availability of plant material (Tables 1-11). Flowering stems were re cut to 50 cm. The bottom leaves were removed leaving an average of 25±5 leaves per stem. In 2003, 'Brenda' (*P. compacta x P. burchellii*) and 'Carnival' (*P. compacta* x *P. neriifolia*) were harvested in a similar manner. 'Pink Ice' flowers were harvested at three different stages: open, soft tip and closed.

**Pulsing.**

The stems were tagged with different colours and numbered to distinguish between the various treatments. A stock solution of 20% glucose in water was diluted to give the required concentrations for pulsing. The following treatments were given: 0, 2, 4, 6, 8 and 10% glucose for all cultivars, as well as for *P. grandiceps*. *P. cynaroides* and *P. magnifica* were treated with 0, 3, 6 and 9% glucose. The 'Pink Ice' flowers harvested at different stages were pulsed with 0% and 7% glucose. Flowers were pulsed at 23±2°C under light levels of 300 $\mu mol.m^{-2}.s^{-1}$ PAR (photosynthetically active radiation). Buckets were filled with 1 L of the pulsing solution and flowers were held in the solution until an average of 10 mL per stem was taken up. This was measured by weighing the buckets with the solution initially, and then at hourly intervals, thereby determining the rate of uptake. Consequently, the pulsing time varied from 2 to 10 hours depending on the cultivar or species (Tables 1-11). After pulsing, the flowers were randomly packed into SAPPEX S14 fibreboard boxes with lids. The boxes were held overnight at 4.5°C before being wrapped in black polyethylene bags and placed in a 10 m integral container at 1°C and high relative humidity, simulating sea freight.

**Evaluation.**

After three weeks the boxes were removed from the container and the stems re-cut. The flowering stems were randomly placed in buckets of tap water and held at 19±2°C with natural light. The flowers were evaluated one, seven and 10 days after removal from storage. On each evaluation date leaves showing ≥ 5% of leaf area blackening or with toxicity symptoms were removed and counted. Toxicity symptoms were expressed as leaves with dried out or necrotic areas. On the last evaluation day (three weeks plus 10 days) the remaining leaves were counted. Leaf blackening was expressed as a percentage of the total number of leaves. Flower quality was subjectively assessed based on bract discoloration and wilting, and is only discussed when it was considered unacceptable.

**Statistical analysis.**

Each evaluation date was analysed as a separate experiment. With *P. magnifica* a randomised block design was used to take the seedling bush effect into account. Percentage data was transformed using the log transformation. Standard analysis of variance was performed on the data using the SAS program (Statistical Analysis Systems Institute, 1996). LSD was calculated at a 5% significance level.

## Results

A decrease in solution uptake rate (note: total uptake was the same e.g.10 mL/stem, but the rate varied) was observed with increasing solution concentrations of glucose in all the flowers evaluated (data not shown). For flowers in the lower concentrations of

glucose (2 to 6%) this took between four and seven hours, while in the higher concentration (8 to 10%) this took seven to 12 hours.

Glucose pulses of 4, to 10% resulted in less leaf blackening in 'Brenda' after 10 days, compared to the control (94% leaf blackening) (Table 1). Since toxicity symptoms were observed with the 8% and 10% treatments, the 6% glucose pulse is recommended for this cultivar. Flower quality was excellent for all treatments and showed very little decline over time.

There was a significant decrease in leaf blackening of 'Cardinal' pulsed with 4, 6, 8 and 10% glucose compared to the control from the second evaluation date onwards (Table 2). After 10 days, the lowest percentage blackened leaves (32%) was found with the 6% glucose pulse. Flower quality was still deemed acceptable on the third evaluation date. Toxicity was observed on a few leaves of flowers pulsed with 8 and 10% glucose.

On the first evaluation date of 'Carnival', leaf blackening was significantly worse in the 2% glucose treatment than the other treatments, and the control did not differ significantly from the higher glucose concentrations at this stage (Table 3). However, after seven and 10 days, the flowers treated with 4, 6, 8 and 10% glucose were significantly better than the control flowers. Flower quality was acceptable for up to seven days at room temperature.

No significant differences in leaf blackening were observed between treatments on *P. cynaroides* (Table 4). After 10 days, 100% of the leaves from the control flowers had

blackened. There was a large clonal variation in the plant material with regard to stem thickness, flower head size and leaf size. Signs of glucose toxicity, indicated by browning of the leaves and flowers, were present after seven days. Leaf blackening in this species started at the leaf petiole, consequently leaves with blackened petioles were removed as well. A sticky substance was observed on some of the leaves. Flower quality was very poor with flowers collapsing as soon as they opened. The tips of the flower bracts were blackened and/or dried out in some instances.

The percentage blackened leaves did not exceed 60% on *P. grandiceps* on the last evaluation date for all treatments (Table 5). From the second evaluation date onwards the percent black leaves removed did not differ significantly between the treatments. Leaves of flowers treated with higher glucose concentrations (6, 8, and 10%) showed typical toxicity symptoms. Some flower bracts turned black, especially at the bottom of the flower head and some of the flower bracts from high glucose concentrations were dried out. Since this was not observed on the control flowers, it is likely a symptom of glucose toxicity. Despite these symptoms, flower quality tended to be good for all the treatments, even on the last evaluation date. There was considerable genetic variation in this species with some flowers having a yellow blush and the top bracts turning white with time, while others remained yellow.

As soon as the flowers of 'Ivy' opened they collapsed and consequently no data on leaf blackening could be collected for this cultivar. The bottom flower bracts were black and this was attributed to nectar collecting in the flower head. Glucose treatments seemed to stimulate this nectar formation. According to local producers, 'Ivy' is not very prone to leaf blackening.

Significant differences were observed on the last evaluation date of *P. magnifica,* but the lowest percentage blackened leaves (76%) was unacceptably high (Table 6). After seven days of vase life, glucose toxicity was observed as dehydration of the leaves and flowers, with higher glucose concentrations resulting in more severe dehydration.

The results from 'Pink Ice' were affected by poor flower quality (Table 7), consequently this trial was repeated in 2003 (Table 8). In 2002, blackening of the lower flower bracts occurred and appeared to be more severe on the one side of the flower head. This was attributed to nectar accumulating on the lower side when the flowers were stored flat in the box for three weeks. After 10 days, the flowers treated with 6, 8 and 10% glucose had less leaf blackening than the flowers treated with lower concentrations (Table 7). Flower quality was not evaluated for these experiments, since it was consistently poor.

In 2003, the flower quality of 'Pink Ice' was better than observed in 2002, however the flower quality was unacceptable after seven days. The harvest stage did not have a noticeable effect on the quality. There were significant differences in leaf blackening between the 0% and 7% glucose treatments irrespective of the stage of harvest (Table 8). With increasing picking maturity of the flower head a decrease in leaf blackening was observed with the 7% glucose pulse, although the difference were not statistically significant.

Glucose did not have a beneficial effect on 'Sheila' and the incidence of leaf blackening was high in all treatments (Table 9). A leaf blackening percentage of 47% was reported for the 2% glucose treatment in comparison to 59% in the control after 10 days vase life. The 8% and 10% glucose treatments resulted in toxicity symptoms on the leaves.

Flower quality of 'Susara' was unacceptable on the third evaluation date. After 10 days leaf blackening was 100% and 99% for the control and 2% pulse, respectively, while flowers treated with 8% glucose had only 52% leaf blackening (Table 10).

On the first evaluation date only 2% leaf blackening was recorded on 'Sylvia' as a result of the 8% glucose pulse compared to 25% on control flowers (Table 11). This beneficial effect was also observed on the second and third evaluation dates with 28% and 53% blackened leaves, respectively. Flowers pulsed with 8% glucose were significantly better, with regard to leaf blackening, on all evaluation dates than those treated with 0 to 6% glucose. Flower quality was unacceptable on the third evaluation date.

## Discussion and Conclusions

Glucose pulses effectively reduced the incidence of post-harvest leaf blackening in the following proteas: 'Brenda' (*P. compacta* x *P. burchellii*), 'Cardinal' (*P. eximia* x *P. susannae*), 'Carnival' (*P. compacta* x *P. neriifolia*), 'Pink Ice' (*P. compacta* x *P. susannae*), 'Susara' (*P. magnifica* x *P. susannae*) and 'Sylvia' (*P. eximia* x *P. susannae*) (Tables 1-3, 7, 10, 11). On the other hand glucose had little or no effect in *P. magnifica*, *P. grandiceps* and *P. cynaroides* and 'Sheila' (*P. magnifica* x *P.*

*burchellii*) (Tables 4-6, 9). Proteas can therefore be classified as those cultivars and species 'responsive' and those 'not responsive' to glucose.

Within the responsive *Protea* cultivars at least one of the parents belongs to the section of *Ligulatae* of the genus *Protea* (Rousseau, 1970). 'Sheila' (*P. magnifica* x *P. burchellii*) appears not to follow this rule (Table 9). However, leaf blackening of 'Sheila' was suppressed when the vase solution contained 0.5 or 1.0% glucose (Jacobs, unpublished data). Apparently 'Sheila' has a lower concentration threshold for glucose as revealed by the toxicity response when pulsed with glucose at 4% or higher (Table 9). Other cultivars responsive to glucose include 'White Pride' (*P. longifolia* selection), 'Lady Di' (*P. magnifica* x *P. compacta*) and 'Candida' (*P. magnifica* x *P. obtusifolia*) (Jacobs, unpublished data). These three glucose responsive cultivars also have one parent belonging to the *Ligulatae*.

*P. magnifica* and *P. grandiceps* (section *Speciosae)* and *P. cynaroides* (section *Cynaroideae*) are not, or only marginally, responsive to glucose applied either as a pulse (Tables 4-6) or a vase solution (Jacobs, unpublished data). 'Ivy' (section *Exsertae*) (Meyer, unpublished data) and *P. repens* (section *Melliferae*) (Malan, unpublished data) are also not responsive to glucose. Sucrose, as a vase solution, reduced leaf blackening in *P. neriifolia* (section *Speciosae*) (Dai & Paull, 1995) and *P. eximia* (section *Ligulatae*) (Newman et al., 1990), but glucose has not been evaluated on these species.

In all the flowers evaluated, except 'Brenda', glucose, fructose, sucrose and starch decreased in both the leaves and inflorescences from harvest to the onset of leaf

blackening irrespective of whether the cultivar responded to glucose (Paper I). Despite this decrease in non-structural carbohydrates after harvest that occurs in all proteas, it appears that only members of the *Ligulatae* respond to glucose. At present, it is uncertain why these specific cultivars are responsive to glucose. Although glucose was proven to be effective in reducing leaf blackening in proteas belonging to the division *Ligulatae* it should be interesting to test other sugars in this regard.

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**Table 1.** Effect of a glucose pulse (%) applied after harvest on the development of leaf blackening (expressed as cumulative percentage; leaves removed if ≥ 5% leaf area blackened), of 'Brenda' (*P. compacta x P. burchellii*), after 3 weeks at 1°C and 1, 7 or 10 days at ambient conditions in tap water.

| | **Glucose concentration (%)** | | | | | |
|---|---|---|---|---|---|---|
| Days of vase life | **0** | **2** | **4** | **6** | **8** | **10** |
| **1** | $68^{a}$ | $70^{a}$ | $26^{bc}$ | $9^{d}$ | $17^{cd}$ | $35^{b}$ |
| **7** | $93^{a}$ | $96^{a}$ | $66^{b}$ | $31^{c}$ | $33^{c}$ | $42^{c}$ |
| **10** | $94^{a}$ | $99^{a}$ | $79^{b}$ | $60^{c}$ | $54^{c}$ | $48^{c}$ |

Means (n = 10) within rows with different superscripts differ significantly at LSD $_{(P \leq 0.05)}$ level.

**Table 2.** Effect of a glucose pulse (%) applied after harvest on the development of leaf blackening (expressed as cumulative percentage; leaves removed if ≥ 5% leaf area blackened), of 'Cardinal' (*P. eximia* x *P. susannae*), after 3 weeks at 1°C and either 1, 7 and 10 days at ambient conditions in tap water.

| | **Glucose concentration (%)** | | | | | |
|---|---|---|---|---|---|---|
| Days of vase life | **0** | **2** | **4** | **6** | **8** | **10** |
| **1** | $17^{a}$ | $16^{a}$ | $16^{a}$ | $2^{a}$ | $11^{a}$ | $6^{a}$ |
| **7** | $93^{a}$ | $53^{b}$ | $22^{bc}$ | $12^{c}$ | $14^{c}$ | $11^{c}$ |
| **10** | $98^{a}$ | $72^{ab}$ | $57^{bc}$ | $32^{c}$ | $57^{bc}$ | $53^{bc}$ |

Means (n = 5) within rows with different superscripts differ significantly at LSD $_{(P \leq 0.05)}$ level.

**Table 3.** Effect of a glucose pulse (%) applied after harvest on the development of leaf blackening (expressed as cumulative percentage; leaves removed if ≥ 5% leaf area blackened), of 'Carnival' (*P. compacta* x *P. neriifolia*), after 3 weeks at 1°C and either 1, 7 and 10 days at ambient conditions in tap water.

| | **Glucose concentration (%)** | | | | | |
|---|---|---|---|---|---|---|
| Days of vase life | **0** | **2** | **4** | **6** | **8** | **10** |
| **1** | $2^{b}$ | $18^{a}$ | $0^{b}$ | $4^{b}$ | $2^{b}$ | $1^{b}$ |
| **7** | $85^{a}$ | $67^{a}$ | $3^{b}$ | $12^{b}$ | $8^{b}$ | $11^{b}$ |
| **10** | $90^{a}$ | $96^{a}$ | $7^{b}$ | $19^{b}$ | $18^{b}$ | $29^{b}$ |

Means (n = 6) within rows with different superscripts differ significantly at LSD $_{(P \leq 0.05)}$ level.

**Table 4.** Effect of a glucose pulse (%) applied after harvest on the development of leaf blackening (expressed as cumulative percentage; leaves removed if ≥ 5% leaf area blackened), of *P. cynaroides*, after 3 weeks at 1°C and either 1, 7 and 10 days at ambient conditions in tap water.

| | **Glucose concentration (%)** | | | |
|---|---|---|---|---|
| Days of vase life | **0** | **3** | **6** | **9** |
| **1** | 11[a] | 6[a] | 12[a] | 2[a] |
| **7** | 86[a] | 63[a] | 55[a] | 55[a] |
| **10** | 100[a] | 90[a] | 78[a] | 76[a] |

Means (n = 8) within rows with different superscripts differ significantly at LSD $_{(P \leq 0.05)}$ level.

**Table 5.** Effect of a glucose pulse (%) applied after harvest on the development of leaf blackening (expressed as cumulative percentage; leaves removed if ≥ 5% leaf area blackened), of *P. grandiceps*, after 3 weeks at 1°C and either 1, 7 and 10 days at ambient conditions in tap water.

| | **Glucose concentration (%)** | | | | | |
|---|---|---|---|---|---|---|
| Days of vase life | **0** | **2** | **4** | **6** | **8** | **10** |
| **1** | $14^{bcd}$ | $0^{d}$ | $5^{cd}$ | $26^{a}$ | $14^{abc}$ | $17^{ab}$ |
| **7** | $43^{a}$ | $29^{a}$ | $26^{a}$ | $51^{a}$ | $36^{a}$ | $28^{a}$ |
| **10** | $53^{a}$ | $40^{a}$ | $32^{a}$ | $56^{a}$ | $39^{a}$ | $35^{a}$ |

Means (n = 10) within rows with different superscripts differ significantly at LSD $_{(P \leq 0.05)}$ level.

**Table 6.** Effect of a glucose pulse (%) applied after harvest on the development of leaf blackening (expressed as cumulative percentage; leaves removed if ≥ 5% leaf area blackened), of *P. magnifica*, after 3 weeks at 1°C and either 1, 7 and 10 days at ambient conditions in tap water.

| | **Glucose concentration (%)** | | | |
|---|---|---|---|---|
| Days of vase life | **0** | **3** | **6** | **9** |
| **1** | $14^{a}$ | $12^{a}$ | $15^{a}$ | $8^{a}$ |
| **7** | $54^{a}$ | $57^{a}$ | $58^{a}$ | $65^{a}$ |
| **10** | $96^{a}$ | $76^{b}$ | $90^{b}$ | $88^{b}$ |

Means (n = 8) within rows with different superscripts differ significantly at LSD $_{(P \leq 0.05)}$ level.

**Table 7.** Effect of a glucose pulse (%) applied after harvest on the development of leaf blackening (expressed as cumulative percentage; leaves removed if ≥ 5% leaf area blackened), of 'Pink Ice' (*P. compacta* x *P. susannae*), after 3 weeks at 1°C and either 1, 7 and 10 days at ambient conditions in tap water.

| | **Glucose concentration (%)** | | | | | |
|---|---|---|---|---|---|---|
| Days of vase life | **0** | **2** | **4** | **6** | **8** | **10** |
| **1** | $43^{a}$ | $14^{b}$ | $11^{b}$ | $0^{c}$ | $4^{c}$ | $2^{c}$ |
| **7** | $82^{a}$ | $41^{b}$ | $35^{bc}$ | $16^{cd}$ | $8^{d}$ | $9^{d}$ |
| **10** | $85^{a}$ | $71^{ab}$ | $60^{b}$ | $37^{c}$ | $11^{c}$ | $17^{c}$ |

Means (n = 10) within rows with different superscripts differ significantly at LSD $_{(P \leq 0.05)}$ level.

 

**Table 8.** Effect of a glucose pulse (0% or 7%) applied after harvest on the development of leaf blackening (expressed as cumulative percentage; leaves removed if ≥ 5% leaf area blackened), of 'Pink Ice' (*P. compacta* x *P. susannae*) harvested at different picking stages (open, soft tip or closed), after 3 weeks at 1°C and either 1, 7 and 10 days at ambient conditions in tap water.

| | **Stage of harvest** | | | | | |
|---|---|---|---|---|---|---|
| | **Open** | | **Soft tip** | | **Closed** | |
| Days of vase life | **0% glucose** | **7% glucose** | **0% glucose** | **7% glucose** | **0% glucose** | **7% glucose** |
| **1** | $7^{a}$ | $3^{a}$ | $5^{a}$ | $6^{a}$ | $9^{a}$ | $6^{a}$ |
| **7** | $84^{a}$ | $3^{b}$ | $85^{a}$ | $6^{b}$ | $80^{a}$ | $11^{b}$ |
| **10** | $89^{a}$ | $5^{b}$ | $89^{a}$ | $11^{b}$ | $94^{a}$ | $22^{b}$ |

Means (n = 10) within rows with different superscripts differ significantly at LSD $_{(P \leq 0.05)}$ level.

**Table 9.** Effect of a glucose pulse (%) applied after harvest on the development of leaf blackening (expressed as cumulative percentage; leaves removed if ≥ 5% leaf area blackened), of 'Sheila' (*P. magnifica* x *P. burchellii*), after 3 weeks at 1°C and either 1, 7 and 10 days at ambient conditions in tap water.

| | **Glucose concentration (%)** | | | | | |
|---|---|---|---|---|---|---|
| Days of vase life | **0** | **2** | **4** | **6** | **8** | **10** |
| **1** | $19^{bc}$ | $12^{c}$ | $20^{bc}$ | $28^{ab}$ | $44^{a}$ | $48^{a}$ |
| **7** | $48^{c}$ | $38^{c}$ | $56^{bc}$ | $66^{ab}$ | $66^{ab}$ | $76^{a}$ |
| **10** | $59^{c}$ | $47^{c}$ | $63^{bc}$ | $71^{ab}$ | $81^{ab}$ | $81^{a}$ |

Means (n = 10) within rows with different superscripts differ significantly at LSD $_{(P \leq 0.05)}$ level.

**Table 10.** Effect of a glucose pulse (%) applied after harvest on the development of leaf blackening (expressed as cumulative percentage; leaves removed if ≥ 5% leaf area blackened), of 'Susara' (*P. magnifica* x *P. susannae*), after 3 weeks at 1°C and either 1, 7 and 10 days at ambient conditions in tap water.

| | **Glucose concentration (%)** | | | | | |
|---|---|---|---|---|---|---|
| Days of vase life | **0** | **2** | **4** | **6** | **8** | **10** |
| **1** | $55^{ab}$ | $66^{a}$ | $25^{bc}$ | $12^{c}$ | $16^{c}$ | $14^{c}$ |
| **7** | $99^{a}$ | $94^{a}$ | $52^{b}$ | $41^{b}$ | $48^{b}$ | $52^{b}$ |
| **10** | $100^{a}$ | $99^{a}$ | $62^{b}$ | $58^{b}$ | $52^{b}$ | $62^{b}$ |

Means (n = 8) within rows with different superscripts differ significantly at LSD $_{(P \leq 0.05)}$ level.

**Table 11.** Effect of a glucose pulse (%) applied after harvest on the development of leaf blackening (expressed as cumulative percentage; leaves removed if ≥ 5% leaf area blackened), of 'Sylvia' (*P. eximia* x *P. susannae*), after 3 weeks at 1°C and either 1, 7 and 10 days at ambient conditions in tap water.

| | **Glucose concentration (%)** | | | | | |
|---|---|---|---|---|---|---|
| Days of vase life | **0** | **2** | **4** | **6** | **8** | **10** |
| **1** | $25^{ab}$ | $39^{a}$ | $39^{a}$ | $30^{ab}$ | $2^{c}$ | $13^{bc}$ |
| **7** | $62^{ab}$ | $54^{ab}$ | $74^{a}$ | $55^{ab}$ | $28^{c}$ | $41^{bc}$ |
| **10** | $83^{a}$ | $90^{a}$ | $88^{a}$ | $75^{ab}$ | $53^{c}$ | $59^{bc}$ |

Means (n = 10) within rows with different superscripts differ significantly at LSD $_{(P \leq 0.05)}$ level.

## PAPER III - Extending Post Storage Vase Life of *Proteas* with Glucose.


### Abstract

Glucose pulsing followed by cold storage at 1°C for three weeks in combination with post-storage glucose containing vase solutions, significantly reduced leaf blackening of the *Protea* cultivars tested. Evaluation was done one, seven and 10 days following storage. On the first evaluation date there were no significant differences for all cultivars. The 1 or 2% glucose plus hypochlorite (0.05 $g.L^{-1}$) combinations significantly delayed leaf blackening in 'Cardinal' and 'Sylvia' after seven and 10 days compared with water alone or water containing hypochlorite. Leaf blackening of 'Cardinal' and 'Sylvia' stems kept in water alone or water plus hypochlorite, did not differ between these treatments. Leaf blackening of 'Brenda' and 'Susara' was not significantly reduced by 1% glucose. Leaf blackening was significantly less with 1 or 2% glucose for 'Carnival' and 'Pink Ice' when compared to vase solutions containing hypochlorite but not when compared to water alone. The hypochlorite treatment, which was included as a disinfectant, resulted in increased leaf blackening in these cultivars. Hypochlorite should not be used in the holding solutions for proteas and other disinfectants need to be evaluated. Flower quality was unacceptable after 10 days vase life for most of the cultivars. Glucose in the vase solution reduces leaf blackening at ambient temperatures of protea cultivars pulsed with glucose and stored cold for 3 weeks. The importance of the poorly understood effect of pre-harvest conditions on leaf blackening was highlighted by this study.


### Introduction

Post-storage vase solutions are used to supply the cut flower's developmental needs of an additional carbohydrate source and to improve the water balance. Conditioning

solutions usually consist of a disinfectant, a weak acid and sugar. Conditioning with solutions without sugar is beneficial, but is usually not as effective as those with sugars. Disinfectants and acids improve water conductance by preventing bacterial growth and plugging of xylem vessels (Halevy & Mayak, 1973).

Previous work has shown that carbohydrate reserves in leaves and inflorescences decrease rapidly in the dark or under low light conditions at both ambient or low temperatures (Bieleski et al, 1992; McConchie et al. 1994) (Paper I). Stephens et al. (2001) reported on the efficacy of glucose in the vase solution to reduce leaf blackening of freshly harvested 'Sylvia'. The efficacy of glucose as a pulse treatment to suppress the development of leaf blackening during long-term cold storage for a number of protea cultivars was reported in Paper 2.

We hypothesised that the vase life of the different tested *Protea* cultivars could be further extended by using post-storage holding solutions. Pulsing with the optimum glucose concentration (as determined in Paper II) prior to three weeks of storage at 1°C was followed by treatment with various post-storage glucose containing holding solutions.

## Materials and Methods

### Plant material.

In 2003, flowering shoots of 'Brenda' (*P. compacta x P. burchellii*), 'Cardinal' (*P. eximia* x *P. susannae*), 'Carnival' (*P. compacta* x *P. neriifolia*), 'Pink Ice' (*P. compacta* x *P. susannae*), 'Susara' (*P. magnifica* x *P. susannae*) and 'Sylvia' (*P. eximia* x *P. susannae*) were harvested in the morning, placed in SAPPEX S14

fibreboard cartons with lids and brought to the laboratory within one hour. The number of replicates varied depending on the availability of plant material (Table 1-6). Flowering stems were re-cut to 50 cm and the bottom leaves were stripped leaving an average of 25±5 leaves per stem.

**Pulsing.**

All stems were pulsed with a 7% glucose solution, except 'Brenda', which was pulsed with a 6% glucose solution. Pulsing was done at 23±2°C under light levels of 300 $\mu mol.m^{-2}.s^{-1}$ PAR (photosynthetically active radiation). Pulsing time varied from four to 10 hours depending on the cultivar or species. Buckets were filled with 1 L of the pulsing solution and an average of 10 mL per stem was taken up. This was measured by weighing the buckets with the solution initially, and then at hourly intervals, thereby determining the rate of uptake. After pulsing the flowers were randomly packed into SAPPEX S14 fibreboard boxes with lids. Flowers were placed overnight in the cold room at a temperature of 4.5°C. The next morning the cardboard boxes were wrapped in black polyethylene bags and placed in a 10 m integral container kept at high relative humidity and 1°C, simulating sea freight.

**Vase solutions.**

After three weeks the boxes were removed from the container and the stems were re-cut. The flowering stems were randomly assigned to buckets with different holding solutions and numbered for evaluation purposes. The following vase solutions were used: water, water containing 0.05 $g.L^{-1}$ hypochlorite, 1% glucose and 0.05 $g.L^{-1}$ hypochlorite, and 2% glucose and 0.05 $g.L^{-1}$ hypochlorite. Sodium hypochlorite

(bleach) was used as an anti-microbial agent. Flowers were placed in a controlled temperature room at 19±2°C and natural light conditions.

**Evaluation.**

The flowers were evaluated one, seven and 10 days after removal from storage. On each evaluation date leaves showing ≥ 5% leaf area blackening or with toxicity symptoms were removed and counted. Toxicity symptoms were expressed as leaves with dried out or necrotic areas. On the last evaluation day (three weeks plus 10 days) the remaining leaves were counted. Leaf blackening at each evaluation date was expressed as a percentage of the total number of leaves. Flower quality was subjectively assessed based on bract discoloration and wilting, and is only discussed when it was considered unacceptable.

**Statistical analysis.**

Each evaluation date was analysed as a separate experiment. Percent data was analysed using the log transformation. Standard analysis of variance was performed on the data using the SAS program (Statistical Analysis Systems Institute, 1996). LSD was calculated at a 5% significance level.

## Results

Precipitation of glucose on the stems of all tested cultivars treated with the 2% glucose vase solution was observed on the second evaluation date. The bleach treatment had a deleterious effect on the flowering stems, and the flowers and leaves were dehydrated. On the first evaluation date there was no significant treatment effects for all cultivars. After seven days, leaf blackening was significantly reduced

in 'Cardinal' (Table 2) and 'Sylvia' (Table 6) with the glucose treatments. The other tested cultivars, however, had no significant difference in leaf blackening on the second evaluation date.

The 'Brenda' flowers were picked at the end of the season and half of the flowering stems were of poor quality. When the flowers were taken out of storage, some of the flowers were infected with *Botrytis*. The first two evaluation dates showed no significant treatment in percent between blackened leaves removed (Table 1). After 10 days flowers treated with 1% glucose and hypochlorite had significantly less leaf blackening (39%) than flowers held in water containing hypochlorite (67% leaf blackening).

Leaf blackening was significantly lower in 'Cardinal' flowers held in 1 and 2% glucose compared to control flowers held in water alone or water containing hypochlorite after seven and 10 days (Table 2). Flower quality was unacceptable on day 10 and the tips of the flower bracts were dessicated.

Only after 10 days vase life did the glucose treatments significantly reduce leaf blackening on 'Carnival' (Table 3) compared to flowers held in water containing hypochlorite, which had 51% blackened leaves. Flowers held in water alone had 33% leaf blackening and did not significantly differ from the other treatments. Flower quality was deemed unacceptable on the third evaluation date.

Significant differences between the treatments were only observed after 10 days vase life for 'Pink Ice' (Table 4). Flowers held in water containing hypochlorite had 66%

blackened leaves at this stage, while flowers held in water alone, 1% glucose and 2% glucose had 17%, 26% and 24% leaf blackening, respectively. On day 10 flower quality was unacceptable and flowers with thin stems were dehydrated. The hypochlorite solution turned pink after seven days. This change of solution colour was not observed for any of the other tested cultivars or solutions.

Percentage leaf blackening of 'Susara' ranged from 21% to 33% after seven days vase life (Table 5). Leaf blackening did not exceed 47%, with the water containing hypochlorite treatment, on the third evaluation date. This was significantly higher than the treatment containing 1% glucose plus hypochlorite.

A significant difference in leaf blackening percentage between the glucose treatments and treatments without glucose was observed at the second and third evaluation date of 'Sylvia' (Table 6). After seven days, 7% and 15% leaf blackening was observed with the respective glucose treatments of 1% and 2%. On the third evaluation date leaf blackening did not exceed 25% on the glucose treatments while flowers held in water containing hypochlorite had 82% leaf blackening and those held in water alone had 73% leaf blackening.

## Discussion and Conclusions

After three weeks cold storage 'Carnival', 'Cardinal' and 'Sylvia' pulsed with 7% glucose had less than 5% black leaves. This is in agreement with an earlier study (Paper II). However for 'Susara' results were better and for 'Pink Ice' and 'Brenda' it was poorer than an earlier study (Paper II). These differences highlight the importance of the poorly understood pre-harvest factors on leaf blackening.

Stephens (2003) has shown that after pulsing with 5% glucose the leaves of Sylvia flower stems contained 170 mg starch and 100 mg glucose as compared to 95 mg starch and 50 mg glucose in non-pulsed controls. After three weeks of cold storage at 1°C leaf starch decreased to 40 and 30 mg for pulsed and non-pulsed stems, respectively, and leaf glucose content decreased to 37 and 10 mg for pulsed and non-pulsed stems, respectively. After three weeks of cold storage it is clear that carbohydrate levels were low and additional benefits may be derived after cold storage by supplementing the holding solution with glucose. This conclusion is supported by the results in Tables 2 & 6. For both 'Sylvia' and 'Cardinal' most leaves were black after 10 days in a holding solution of water alone or water containing hypochlorite. When the water containing hypochlorite holding solution was supplemented with 1 or 2% glucose leaf blackening was greatly reduced.

Hypochlorite increased leaf blackening and should not be used in the holding solutions for 'Brenda', 'Susara', 'Pink Ice', 'Sylvia' and 'Carnival'. The beneficial effect of glucose in reducing leaf blackening is also evident when results obtained with holding solutions containing hypochlorite are compared when glucose is added or withheld.

To optimise the use of glucose in a holding solution to reduce leaf blackening it is imperative to find a preservative other than hypochlorite, which does not enhance leaf blackening, for as wide a range of *Protea* cultivars/species as possible.

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**Table 1.** Effect of post-storage holding solutions on the development of leaf blackening (expressed as cumulative percentage; leaves with more than 5% leaf area black) of 'Brenda' (*P. compacta x P. burchellii*) evaluated after 1, 7 or 10 days at ambient conditions, following a pre storage pulsed of 6% glucose and 3 weeks at 1°C.

| | **Holding solutions** | | | |
|---|---|---|---|---|
| Days of vase life | **Water** | **Water + Hypochlorite** | **1% glucose + hypochlorite** | **2% glucose + hypochlorite** |
| **1** | $19^{a}$ | $22^{a}$ | $21^{a}$ | $24^{a}$ |
| **7** | $42^{a}$ | $44^{a}$ | $33^{a}$ | $42^{a}$ |
| **10** | $57^{ab}$ | $67^{a}$ | $39^{b}$ | $51^{ab}$ |

Means (n = 8) within rows with different superscripts differ significantly at LSD $_{(P \leq 0.05)}$ level.

**Table 2.** Effect of post-storage holding solutions on the development of leaf blackening (expressed as cumulative percentage; leaves with more than 5% leaf area black) of 'Cardinal' (*P. eximia* x *P. susannae*), evaluated after 1, 7 or 10 days at ambient conditions, following a pre storage pulsed of 7% glucose and 3 weeks at 1°C.

| | **Holding solutions** | | | |
|---|---|---|---|---|
| Days of vase life | **Water** | **Water + Hypochlorite** | **1% glucose + hypochlorite** | **2% glucose + hypochlorite** |
| **1** | $4^{a}$ | $6^{a}$ | $2^{a}$ | $5^{a}$ |
| **7** | $65^{a}$ | $58^{a}$ | $11^{b}$ | $15^{b}$ |
| **10** | $94^{a}$ | $86^{a}$ | $13^{b}$ | $21^{b}$ |

Means (n = 10) within rows with different superscripts differ significantly at LSD ($P \leq 0.05$) level.

**Table 3.** Effect of post-storage holding solutions on the development of leaf blackening (expressed as cumulative percentage; leaves with more than 5% leaf area black) of 'Carnival' (*P. compacta* x *P. neriifolia*), evaluated after 1, 7 or 10 days at ambient conditions, following a pre storage pulsed of 7% glucose and 3 weeks at 1°C.

| | **Holding solutions** | | | |
|---|---|---|---|---|
| Days of vase life | **Water** | **Water + Hypochlorite** | **1% glucose + hypochlorite** | **2% glucose + hypochlorite** |
| **1** | $4^{a}$ | $3^{a}$ | $3^{a}$ | $9^{a}$ |
| **7** | $21^{a}$ | $17^{a}$ | $8^{a}$ | $12^{a}$ |
| **10** | $33^{ab}$ | $51^{a}$ | $9^{b}$ | $12^{b}$ |

Means (n = 10) within rows with different superscripts differ significantly at LSD $_{(P \leq 0.05)}$ level.

**Table 4.** Effect of post-storage holding solutions on the development of leaf blackening (expressed as cumulative percentage; leaves with more than 5% leaf area black) of 'Pink Ice' (*P. compacta* x *P. susannae*), evaluated after 1, 7 or 10 days at ambient conditions, following a pre storage pulsed of 7% glucose and 3 weeks at 1°C.

| | **Holding solutions** | | | |
|---|---|---|---|---|
| Days of vase life | **Water** | **Water + Hypochlorite** | **1% glucose + hypochlorite** | **2% glucose + hypochlorite** |
| **1** | $11^{a}$ | $24^{a}$ | $20^{a}$ | $17^{a}$ |
| **7** | $16^{a}$ | $38^{a}$ | $21^{a}$ | $23^{a}$ |
| **10** | $17^{b}$ | $66^{a}$ | $26^{b}$ | $24^{b}$ |

Means (n = 10) within rows with different superscripts differ significantly at LSD $_{(P \leq 0.05)}$ level.

**Table 5.** Effect of post-storage holding solutions on the development of leaf blackening (expressed as cumulative percentage; leaves with more than 5% leaf area black) of 'Susara' (*P. magnifica* x *P. susannae*), evaluated after 1, 7 or 10 days at ambient conditions, following a pre storage pulsed of 7% glucose and 3 weeks at 1°C.

| | **Holding solutions** | | | |
|---|---|---|---|---|
| Days of vase life | **Water** | **Water + Hypochlorite** | **1% glucose + hypochlorite** | **2% glucose + hypochlorite** |
| **1** | $0^{a}$ | $0^{a}$ | $1^{a}$ | $0^{a}$ |
| **7** | $21^{a}$ | $22^{a}$ | $23^{a}$ | $33^{a}$ |
| **10** | $26^{ab}$ | $47^{a}$ | $23^{b}$ | $34^{ab}$ |

Means (n = 10) within rows with different superscripts differ significantly at LSD $_{(P \leq 0.05)}$ level.

**Table 6.** Effect of post-storage holding solutions on the development of leaf blackening (expressed as cumulative percentage; leaves with more than 5% leaf area black) of 'Sylvia' (*P. eximia* x *P. susannae*) evaluated after 1, 7 or 10 days at ambient conditions, following a pre storage pulsed of 7% glucose and 3 weeks at 1°C.

| | **Holding solutions** | | | |
|---|---|---|---|---|
| Days of vase life | **Water** | **Water + Hypochlorite** | **1% glucose + hypochlorite** | **2% glucose + hypochlorite** |
| **1** | $5^{a}$ | $2^{a}$ | $2^{a}$ | $5^{a}$ |
| **7** | $46^{a}$ | $58^{a}$ | $7^{b}$ | $15^{b}$ |
| **10** | $73^{a}$ | $82^{a}$ | $25^{b}$ | $20^{b}$ |

Means (n = 10) within rows with different superscripts differ significantly at LSD $_{(P \leq 0.05)}$ level.

# CONCLUSION

Post harvest leaf blackening of *Protea* is not fully understood although research has established that carbohydrate depletion is an initiating factor. A comprehensive study of the carbohydrate status of *Protea,* changes over time as well as sugar supplementation was undertaken to formulate possible treatment regimes.

The dependence of both flowers and leaves on carbohydrate reserves was highlighted by the sharp decline in the concentration of both sugars and starch from the time of harvest until the onset of leaf blackening for the seven *Protea* cultivars/species studied (Paper I). With the exception of 'Carnival', the concentration of starch was higher in the leaves than in the inflorescence. At harvest, glucose, fructose and sucrose combined were consistently higher in the inflorescences than in the leaves. The finding that both starch and sugars are depleted to a greater extent in the leaves may imply that the inflorescence acts as a strong sink and mobilises sugars from the leaves. This concurs with the conclusion drawn from earlier results.

Glucose pulses effectively reduced the incidence of post-harvest leaf blackening in the following proteas: 'Brenda' (*P. compacta* x *P. burchellii*), 'Cardinal' (*P. eximia* x *P. susannae*), 'Carnival' (*P. compacta* x *P. neriifolia*), 'Pink Ice' (*P. compacta* x *P. susannae*), 'Susara' (*P. magnifica* x *P. susannae*) and 'Sylvia' (*P. eximia* x *P. susannae*) (Paper II). On the other hand, glucose had little or no effect in *P. magnifica*, *P. grandiceps* and *P. cynaroides.* Proteas can therefore be classified as those cultivars and

species 'responsive' and those 'not responsive' to glucose. Within the responsive *Protea* cultivars at least one of the parents belongs to the botanical division of *Ligulatae* of the genus *Protea*. Apparently, 'Sheila' has a lower concentration threshold for glucose as revealed by the toxicity response when pulsed with glucose at 4% or higher. Despite this decrease in non-structural carbohydrates after harvest that occurs in all proteas, it appears that only members of the *Ligulatae* respond to glucose. Although glucose has proved to be effective in reducing leaf blackening in proteas belonging to the division *Ligulatae* it should be rewarding to test other sugars in this regard.

Hypochlorite increased leaf blackening of some cultivars and should not be used in the holding solutions for 'Brenda', 'Susara', 'Pink Ice', 'Sylvia' and 'Carnival'. The beneficial effect of glucose in reducing leaf blackening is also evident when results obtained with holding solutions containing hypochlorite are compared when glucose is added or withheld. To optimize the use of glucose in a holding solution to reduce leaf blackening it is imperative to find a preservative other than hypochlorite, which does not enhance leaf blackening, for as wide a range of *Protea* cultivars/species as possible.

All tested sugars depleted significantly in tested cultivars and species although this was not always an indication of which sugars would be successful in sugar supplementation. When parentage of the tested cultivars was investigated a distinction could be made with cultivars with one of the parents belonging to the botanical division of *Ligulatae* being glucose responsive although exceptions were found with 'Sheila' having a low glucose concentration threshold. It could be interesting to assess any further similarities in the

*Ligulatae* division in regards to the carbohydrate profile and why specifically glucose is effective. Other sugars for species belonging to other botanical divisions can also be tested to see if similar results can be obtained. Supplementation with different commercial post-storage vase solutions, as well as various combinations of sugars and an alternative disinfectant need to be tested. Variability between different season's results suggested the effect of poorly understood pre-harvest factors on post harvest leaf blackening and probable interaction that needs to be investigated.