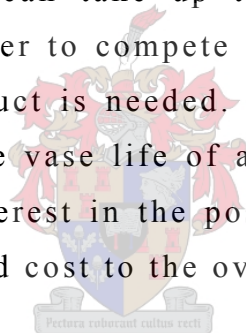


1. Introduction

Many *Protea* species hybrids are exported to foreign markets in substantial quantities annually. The occurrence of both pre- and postharvest leaf blackening is a problem that severely limits the marketability, vase life and transport options of certain *Protea* species and cultivars (pers comm. G. Jacobs).

Postharvest leaf blackening symptoms have been described many times (de Swart *et al.*, 1987; Paull *et al.*, 1980; Brink and De Swart, 1986; Ferreira, 1983; Haasbrock *et al.*, 1973). Postharvest leaf blackening usually appears within the first seven to ten days after harvest. Expensive airfreight is thus the primary means of transport as the shipping of fresh produce can take up to three weeks to reach the overseas markets and in order to compete in the export flower markets an exceptional quality product is needed. In recent years progress has been made in prolonging the vase life of a number of protea cultivars. This has led to renewed interest in the possibility of shipping *Proteas* in large quantities at reduced cost to the overseas markets.



The extent and rate of leaf blackening in *Protea* plants after harvest appears to vary widely between species (McConchie and Lang, 1993), clones within species as well as the maturity, the time of year and even the time of day at which they are harvested (Paull and Dai, 1990). Whitehead and de Swardt (1982) hypothesized that leaf blackening is induced after cellular membrane breakdown has been facilitated by some external stress, resulting in uncontrolled substrate-enzyme interactions which leads to the oxidation of polyphenol compounds by the enzymes polyphenol oxidase (PPO) and peroxidase (POD).

Another hypothesis directs attention to the abundant occurrence of phenolic glycosides in *Protea* leaves (Perold *et al.*, 1979), and is based on the possible enzymatic cleavage of these compounds by glucosidases

followed by the nonenzymatic oxidation of the highly reactive aglycone moiety (Jones, 1995).

Many experiments have supported the idea that the transport of carbohydrates to the very strong sink that is the developing inflorescence initiates the process of leaf blackening (McConchie *et al.*, 1991; Bielecki *et al.*, 1992). Once the important role of carbohydrates was discovered in leaf blackening many different approaches were used to try and solve this problem. All with the aim of slowing carbohydrate loss and eliminating or decreasing leaf blackening.

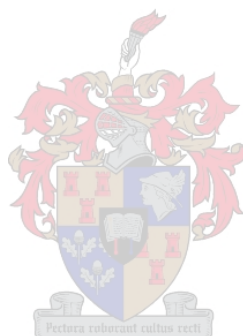
The aim of this study was two fold: (I) To examine the distribution of carbohydrates in inflorescence bearing stems of certain *Protea* cultivars from harvest, following pulsing with a 10g.L⁻¹ glucose solution until four weeks postharvest and (II) The suppression of *Protea* postharvest leaf blackening with specific focus on the cultivar 'Sylvia' (*P. eximia* x *P. susannae*).



Literature cited

- Bielecki, R.L., J. Ripperda, J.P. Newman and M.S. Reid. 1992. Carbohydrate changes and leaf blackening in cut flower stems of *Protea eximia*. J. Amer. Soc. Hort. Sci. 117:124-127.
- Brink, J.A. and G.H. de Swardt. 1986. The effect of sucrose in a vase solution on leaf browning of *Protea neriifolia* R. Br. Acta Hort. 185:111-119.
- De Swardt, G.H., J. Pretorius, and L. Burger. 1987. The browning of foliage leaves in Proteas-a review. *Protea News* 6, 4-9.
- Ferreira, D.I. 1983. Prevention of browning of leaves of *Protea neriifolia* R. Br. Acta Hort. 138:273-276.
- Haasbroek, F.J., G.G. Rousseau, and J.F. de Villiers. 1973. Effect of gamma-rays on cut blooms of *Protea compacta* R.Br., *P. longiflora* Lamarck and *Leucospermum cordifolium* Salisb. Ex Knight. *Agroplantae* 5:33-42.
- Jones, R. B., 1995. Sucrose prevents foliage desiccation in cut *Leucadendron* 'Silvan Red' during cold storage. *Postharvest Bio. Tech.* 6:293-301.

- McConchie, R., N.S. Lang, and K.C. Gross. 1991. Carbohydrate depletion and leaf blackening in *Protea neriifolia*. *J. Amer. Soc. Hort. Sci.* 116:1019-1024.
- McConchie, R. and N.S. Lang. 1993. Carbohydrate metabolism and possible mechanisms of leaf blackening in *Protea neriifolia* under dark postharvest conditions. *J. Amer. Soc. Hort. Sci.* 118:355-361.
- Paull, R.E., T. Goo, R.A. Criley and P.E. Parvin. 1980. Leaf blackening in cut *Protea eximia*: importance of water relations. *Acta Hort.* 113:159-166.
- Paull, R.E. and J.W. Dai. 1990. *Protea* postharvest leaf blackening a problem in search of a solution. *Acta Hort.* 264:93-101.
- Perold, G.W., M.E.K. Rosenberg, A.S. Howard and P.A. Huddle. 1979. Metabolites of Proteaceae. Part 9. eximin (6-O-benzoylarbutin) and the synthesis of aryl glycoside esters. *J. Chem. Soc. Perkin Trans I* 1979, 239-234.
- Whitehead, C.S. and G.H. de Swardt. 1982. Extraction and activity of polyphenoloxidase and peroxidase from senescing leaves of *Protea neriifolia*. *S. Afr. J. Bot.* 1:127-130.



2. Literature Review

2.1 Introduction

Many South African *Protea* species and hybrids have been identified as having commercial value as cut flower products and are exported to foreign markets in substantial quantities. The occurrence of both pre- and postharvest leaf blackening is a problem that severely limits the marketability, vase life and transport options of certain *Protea* species and cultivars. Postharvest leaf blackening symptoms have been described many times (De Swart *et al.*, 1987; Paull *et al.*, 1980; Brink and De Swart, 1986; Ferreira, 1983; Haasbrock *et al.*, 1973). According to De Swart *et al.* (1987) leaf blackening can be divided into four types based on the first appearance of the discoloration: (1) tip blackening; (2) marginal blackening; (3) spot blackening and (4) midrib blackening. The rapid subsequent blackening across the remainder of the leaf indicates that the same process is involved no matter what initial symptom is displayed. Postharvest leaf blackening usually appears within the first seven to ten days after harvest. Expensive airfreight is thus the primary means of transport as the shipping of fresh produce can take up to three weeks to reach the overseas markets and in order to compete in the export flower markets an exceptional quality product is needed. In recent years progress has been made in prolonging the vase life of a number of *Protea* species. This has led to renewed interest in the possibility of shipping *Proteas* in large quantities at reduced costs to the overseas markets.

Preharvest leaf blackening can be caused by a number of factors all involving some form of physical damage to the leaf. According to Jones *et al.* (1995) mechanical damage, insect or fungal attack, or excessive heat can all induce discoloration of *Protea* leaves. Water stress has also been found to play a role in preharvest leaf blackening (Starke, 1979; Forsberg, 1988 & 1993).

The extent and rate of leaf blackening in *Protea* plants after harvest appears to vary widely between species (McConchie and Lang, 1993b), clones within species as well as the maturity, the time of year and even the time of day at which they are harvested (Paull and Dai, 1990). Paull and Dai (1990) reported that postharvest leaf blackening of *P. neriifolia* leaves in Hawaii was more severe during late summer than at any other time of year. Unpublished data of van Doorn established the same pattern in *Protea* flowers exported from South Africa during late summer (Jones *et al.*, 1995). It has, however, been reported by numerous people that more severe postharvest leaf blackening occurs during the spring months in the Western Cape area of South Africa. Whitehead and de Swardt (1982) hypothesized that leaf blackening is induced after cellular membrane breakdown has been facilitated by some external stress/es, resulting in uncontrolled substrate-enzyme interactions which leads to the oxidation of polyphenol compounds by the enzymes polyphenol oxidase (PPO) and peroxidase (POD).

Hypothesized physiological causes of leaf blackening include; 1) water stress, 2) carbohydrate stress [the carbohydrate demand of the inflorescence for expansion, respiration and nectar production (Dai and Paull, 1995)], 3) water condensation on the leaves, 4) ethylene, 5) red and far red light (van Doorn, 2001) as well as 6) chilling injury and 7) anoxia (Halevy and Mayak, 1979; Sacher, 1973). Jones *et al.* (1995) concluded that there were no reports that confirmed *Protea* leaf blackening resulted from membrane degradation by physiological events, followed by the actions of PPO and POD. At present there is no clear role for oxidative enzymes in *Protea* leaf blackening (Stephens, 2003).

Another hypothesis directs attention to the abundant occurrence of phenolic glycosides in *Protea* leaves (Perold *et al.*, 1979), and is based on the possible enzymatic cleavage of these compounds by glucosidases followed by the nonenzymatic oxidation of the highly reactive aglycone moiety (Jones *et al.* 1995).

2.2. Physiological causes of leaf blackening

2.2.1 Water Stress

Many different theories have been put forward to explain the hypothesized physiological activation and mechanisms of postharvest leaf blackening in *Protea* species.

Water stress resulting from rapid water loss by the leaves and via transpiration from the flowerhead has been suggested to contribute to cell membrane damage and leaf blackening (De Swardt, 1979; Paull *et al.*, 1980; De Swardt and Pretorius, 1980; Ferreira, 1983; Paull and Dai, 1990).

Halevy and Mayak (1979) reported that the vase life of cut flowers is seriously reduced by their limited water uptake that results from vascular blockage caused by air embolism, microbial growth or cellular metabolites. Leaf blackening was reduced in *P. neriifolia* by recutting the base of the stems and replacing the water on a daily basis (Du Plessis, 1978). Paull and Dai (1990) came to the conclusion that blockages at the base of *P. neriifolia* stems limited water uptake resulting in osmotically induced cellular membrane disruption leading to leaf blackening. De Swardt *et al.* (1987) reported that cut *Protea* stems leached leucoanthocyanidins which were rapidly oxidized to form tannins in the vase water. This was thought to induce stem plugging and hence water stress when taken up by *Protea* shoots (De Swardt *et al.*, 1987). The use of various chemicals; phenylmercury acetate (Masie, 1979) and lead acetate (Du Plessis, 1978; De Swardt, 1979) resulted in the precipitation of these compounds in the vase solution and the reduction of leaf blackening. These studies, however, did not resolve the role played by tannins in leaf blackening, the exact effect that the application of the chemicals had on stem occlusions or if the chemicals themselves had an inherently negative effect on the occurrence of leaf blackening (Jones *et al.*, 1995).

Other compounds including proteins, carbohydrates and pectins are also released from cut *Protea* stems (de Swardt *et al.* 1987). The presence of these compounds in the vase solution could lead to an increase in the microbial population in the vase water, resulting in vascular blockage (Jones *et al.*, 1995) and hence water stress and leaf blackening. Newman *et al.* (1990) found that a wide range of antimicrobial compounds in a wide range of concentrations in the vase water did not suppress the occurrence of leaf blackening. As a result there is no evidence that vascular blockages caused by microorganisms play a role in leaf blackening in *Protea* species.

Paull *et al.* (1980) theorized that the rapid loss of water from the shoot via transpiration from the flowerhead led to water stress and damage to cellular membranes and leaf blackening. However, Jones *et al.* (1995) found that there is no evidence to support this. In contrast to this, Reid *et al.* (1989) found that encasing *P. eximia* inflorescences in polyethylene bags, thus limiting transpiration, or leaving the shoots overnight in a controlled environment (20°C, 60% RH) to induce water stress, had little effect on leaf blackening during vase life. It was, in fact, shown that the leaves in contact with free water inside the plastic bags blackend at an increased rate (Reid *et al.*, 1989). Stems that are girdled just below the inflorescence have the same rate of transpiration as untreated stems, yet the rate of leaf blackening decreases (van Doorn, 2001). There is very little evidence in literature to support the hypothesis that water stress is the primary cause of leaf blackening of *Protea* cut flowers. However, the fact that water stress may be a contributing factor to the process may not be ruled out entirely.

2.2.2 Water on the leaves

It has been observed that direct contact between *Protea* leaves and free water in the form of condensation leads to an increase in the occurrence and severity of leaf blackening (Reid *et al.*, 1989). Newman *et al.* (1990) showed that when leaves of cut *Protea eximia* still attached to a complete stem, were covered with polyethylene bags, the incidence and

severity of leaf blackening increased at the point of contact with free water in the interior of the bag compared to the leaves attached to uncovered stems. There have been many reports stating that condensation should be avoided during the postharvest treatment of *Protea* species as it results in increased leaf blackening (van Doorn, 2001). The physiological implications of the effect of condensation on the leaf surface in relation to the occurrence and severity of leaf blackening are unknown (Jones *et al.*, 1995). However, because of the fact that the occurrence of free water leads to an increase in the rate and severity of leaf blackening, extra weight is added to the argument that water stress does not play a leading role in the process.

2.2.3 Carbohydrate Stress

Carbohydrates are synthesized by all green plants and are used both as building blocks and as a source of energy to keep the plant functioning normally. When a flower is picked, its ability to synthesize carbohydrates is severely curtailed. Recent experiments have built up a considerable amount of evidence indicating that postharvest leaf blackening is mainly due to low leaf carbohydrate status (Jones *et al.*, 1995; van Doorn, 2001). Reid *et al.* (1989) showed by girdling *Protea* stems just below the inflorescence and by using darkness and additional light, that leaves act as a source of carbohydrates for the inflorescence. Photosynthetically active radiation (PAR) of around $15 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ reduced the extent of postharvest leaf blackening on *P. eximia* stems by 35% after four days of storage when compared to stems stored in darkness (Newman *et al.*, 1990). McConchie *et al.* (1991) and Bieleski *et al.* (1992) indicated that the delay and reduction of leaf blackening under light was deemed to result from net carbon assimilation under these conditions. McConchie *et al.* (1991) demonstrated that *P. neriifolia* leaves actively photosynthesized and contributed to the carbohydrate pool of the leaf during postharvest storage when PAR light was present. When flower-bearing shoots of *P. neriifolia* were exposed to light intensities of $\geq 25 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ postharvest leaf

blackening was significantly inhibited (Jones and Clayton-Greene, 1992). Through the use of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) by Jones and Clayton-Greene (1992), the photosynthetic electron transport chain of photosystem II was inhibited. This resulted in a significant increase in postharvest leaf blackening, indicating the importance of photosynthesis in the inhibition of leaf blackening.

Data on carbohydrate levels show that starch and sucrose are the main non-structural metabolic compounds in most *Protea* species (McConchie *et al.*, 1991; Bieleski *et al.*, 1992; McConchie and Lang, 1993a,b). However, Stephens *et al.* (2001) indicated that both fructose and glucose occur in higher concentrations in the inflorescence of 'Sylvia' (*P. eximia* x *P. susannae*) than does sucrose. The leaves of 'Sylvia' were shown to contain comparable amounts of these three sugars directly after harvest. McConchie *et al.* (1991) and Bieleski *et al.* (1992) demonstrated through carbohydrate analysis, that starch and sucrose concentrations decline rapidly in shoots held under dark postharvest conditions but increase when held under PAR conditions of over 300 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ in *P. neriifolia* and *P. eximia* stems. Many other simple sugars are components of the nectar of certain *Protea* species. These include glucose, fructose, sucrose and xylose (Cowling and Mitchell, 1981; van Wyk and Nicholson, 1995). A soluble polygalatol (1,5-anhydro-D-glucitol) carbohydrate has also been found to occur in substantial concentrations in many *Protea* species (Bieleski *et al.*, 1992; McConchie and Lang, 1993a,b). The concentrations of both sucrose and starch in *Protea* leaves tend to drop rapidly after harvest (McConchie *et al.*, 1991; Bieleski *et al.*, 1992; McConchie and Lang, 1993a,b) but the concentration of polygalatol is not significantly altered, even when levels of starch and sucrose are low and leaf blackening has commenced. This finding, according to Bieleski *et al.* (1992) shows that metabolically inert sugars could play a leading role in osmotic buffering in *Protea* species and that if this high concentration of polygalatol was fully accessible for remetabolism it

could potentially double the shelf life time of *Protea* species held under dark environmental postharvest conditions.

The developing inflorescence, with its high respiration rate and nectar production, acts as a strong sink (Dai and Paull, 1995) causing photosynthetic products to be transported from the *Protea* leaves to the developing inflorescence. Newman *et al.* (1990) initially hypothesized that this chain of events led to the initiation of leaf blackening. Strong evidence supporting this hypothesis has been obtained by many researchers who have proved that the removal of the inflorescence or the girdling of the shoot just below the inflorescence delayed or reduced the occurrence of postharvest leaf blackening significantly (Stephens, 2003). Dai and Paull (1995) concluded that nectar production plays a large role in the carbohydrate demand of the inflorescence preharvest but may then become relatively insignificant in comparison to the ongoing respiration of the inflorescence postharvest. The maturity of the inflorescence has a direct effect on the rate of its respiration, with less developed inflorescences having significantly higher rates of respiration than mature inflorescences (Ferreira, 1986). This partially helped to explain the findings by Paull and Dai (1990) that shoots with less developed inflorescence tended to have increased rates of leaf blackening when compared to harvested shoots with more mature inflorescence. High temperatures may also result in a reduced rate of carbon dioxide fixation as water stress causes stomatal closure, and over time, this may lead to reduced levels of stored carbohydrates in the leaves and leaf blackening in intact plants in the field (van Doorn, 2001).

2.2.4 Ethylene

Ethylene is a naturally occurring plant hormone that plays a major role in the ripening of fruit and the senescence of many commercially important agricultural crops. Ethylene can be extremely detrimental to sensitive crop types and can cause early ageing and reduced vase life in many cut flower species. There is little evidence to suggest that

ethylene is involved in the occurrence of leaf blackening in any of the *Protea* species that are susceptible (van Doorn, 2001), even though there have been suggestions that the presence of fruit such as apples during postharvest storage and transport of *Protea* species, leads to an increase in the occurrence of leaf blackening. McConchie and Lang (1993b) found that the ethylene released by the leaves of a number of species of *Protea* was not related to the occurrence of leaf blackening. The use of ethylene inhibitors such as silver thiosulfate have been of little success in reducing the rate and intensity of leaf blackening in all *Protea* species tested to date. Newman *et al.* (1990) and Bielecki *et al.* (1992) treated *P. eximia* shoots with 4 nM silver thiosulfate, but no inhibition of leaf blackening was noted. However, according to Van Doorn (2001), studies testing ethylene inhibitors have only used a limited concentration range and, therefore, must be viewed as inconclusive.

2.2.5 Red and far red light

A number of separate papers has examined the role of photosynthesis and light in postharvest leaf blackening, and has shown that leaf blackening can be delayed in shoots that are exposed to sufficient light to allow for carbon assimilation to occur (Newman *et al.*, 1990; Paull and Dai, 1990; McConchie *et al.*, 1991; Bielecki *et al.*, 1992). The delay in leaf blackening that occurs when stems are held under lighted conditions indicates that carbon assimilation takes place under these conditions, and leads to this delay (McConchie *et al.*, 1991, Bielecki *et al.*, 1992). Van Doorn (2001) reported that leaf blackening was reduced when stems were stored under incandescent lighting in pack houses and cold rooms. In contrast, leaves on stems that were stored under the same lighting conditions, but that were placed in enclosed cardboard containers, became black at an increased rate. It is known that incandescent lamps emit large enough amounts of red light to saturate the phytochrome occurring in *Protea* leaves and keep it in the Pr form.

Van Doorn (2001) hypothesized that the conversion of phytochrome from the Pr form to the Pfr form may be an important step in the pathway that results in leaf blackening or it may lead to an increased rate of leaf blackening. However, this remains a hypothesis and there is a lack of scientific work relating to this subject concerning *Protea* leaf blackening.

2.3 Leaf blackening biochemistry

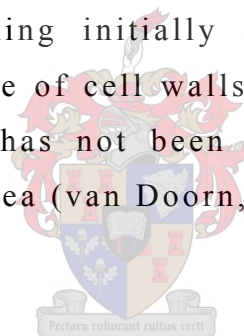
It has become clear that carbohydrate stress is the main cause in the pathway that leads to leaf blackening. The link between carbohydrate starvation and the resulting blackening is, however, still not clear (van Doorn, 2001). One fact is relatively clear, leaf cells continue to consume oxygen during blackening, whether this is due to the cells respiring during leaf blackening or is the result of oxidative reactions that are involved in the process of leaf blackening is, however, unclear (van Doorn, 2001). The leaves of *Protea* contain many colorless phenolic and flavonoid compounds (van Rheede van Oudtshoorn, 1963; Perold *et al.*, 1973a,b; Perold *et al.*, 1979; Perold, 1993). These compounds rapidly turn black when oxidation and polymerization take place (de Swardt, 1979). Perold *et al.* (1973a,b), Perold *et al.* (1979) and Perold (1993) identified the presence of unstable *O*-glycoside esters in the leaves of *Protea* species susceptible to leaf blackening. These unstable esters are formed from β -D-sugars and aglycones. More stable *C*-glycoside esters were found to occur in the leaves of those *Protea* species that are not prone to leaf blackening (Perold, 1993). Phenolic glucoside esters, when hydrolyzed by glucosidase enzymes result in a free sugar and a reactive phenolic moiety (Dey and Dixon, 1985). Perold (1993), supported by findings from McConchie and Lang (1993b), hypothesized that the cleavage of these esters under low cellular leaf carbohydrate status, brought about by the developing inflorescence need for energy, would release glucose to supply the sink, and highly reactive phenols. These phenols could then undergo non-

enzymatic oxidation with free O₂ within the cell resulting in leaf blackening. Marty *et al* (1980) found that glycosidase enzymes like β -D-glucosidase occur in the plant vacuole along with phenolic glycosides (Lagrimi, 1992). This supports McConchie *et al.* (1994) and McConchie and Lang (1993b) who found that membrane degradation is not a precondition that must occur before enzyme-substrate interaction can take place and visible leaf blackening occurs. Jones and Cass (1996) found that the activity of β -D-glucosidase increased significantly in the attached leaves of certain *Protea* species prior to the appearance of leaf blackening.

The use of antioxidant compounds to inhibit oxidation in the leaf and the development of leaf blackening has met with only partial success (Jones *et al.*, 1995). The inhibition of the activity of β -D-glucosidase *in vitro* has been achieved through the use of solutions containing copper and zinc ions, but the same compounds supplied through the stems did not prove to be effective at retarding the appearance of leaf blackening (Jones and Cass, 1996). Van Doorn (2001) states that the positively charged copper and zinc ions bind to the negatively charged cell walls and thus remain at the base of the stem and therefore no inhibition of β -D-glucosidase occurs in the leaves. It is recommended that negatively charged complexes containing copper and zinc be used for further investigation into the role played by β -D-glucosidase in leaf blackening (van Doorn, 2001), as this would allow the complex to move up the stem along the negatively charged cell walls. Crick and McConchie (1999) showed that ethanol vapor significantly reduced leaf blackening of 'Pink Ice' inside plastic bags stored for 19 days at a constant temperature of 20°C. No data for leaf blackening post-bag removal were presented.

It is still unclear as to what role, if any, enzymes and their activity play in leaf blackening (van Doorn, 2001). Whitehead and de Swart (1982) showed that oxidation by air played a bigger role in leaf blackening than enzyme activity. Many blackening reactions in plants are due to the activity of either peroxidase or polyphenol oxidase (van

Doorn, 2001). However, work focusing on PPO and POD enzyme activity and its role in leaf blackening in *Protea* has only produced inconclusive results (Newman *et al.*, 1990, Jones and Clayton-Greene, 1992). According to this hypothesis on leaf blackening, the substrate and enzymes mix in an uncontrolled manner. For this to occur there has to be some form of cell membrane degradation as the substrate and enzymes are separated in different organelles within the cell (van Doorn, 2001). McConchie *et al.* (1994) discovered that cellular membrane degradation occurring prior to leaf blackening is very unlikely as no evidence of degradation products were found in the cell and the anti-oxidant system remained intact (van Doorn, 2001). Therefore, McConchie *et al.* (1994) concluded that the activity of PPO is unrelated to the appearance of leaf blackening symptoms. However, the presence of PPO and POD in cell walls is a known fact and the possibility of leaf blackening initially occurring in the cell walls cannot be excluded. The role of cell walls in the complex cascade that results in leaf blackening has not been fully investigated and more work must be done in this area (van Doorn, 2001).



2.4 Treatments to suppress and prevent postharvest leaf blackening

One of the aims of this study was to investigate possible pre- and postharvest treatments to improve the shipping potential of various *Protea* species and cultivars. In order to determine the best possible treatment regime a number of different procedures were examined. The carbohydrate composition and distribution in various *Protea* cultivars was determined after pulsing with a 2% glucose solution. The effectiveness of various holding solutions on the rate of leaf blackening of different *Protea* species and cultivars that are susceptible to leaf blackening were determined. The effect of various preharvest treatment during winter on the occurrence of postharvest leaf blackening during

the “spring flush” was evaluated and some postharvest treatments were performed on ‘Sylvia’ (*P. eximia* x *P. susannae*) to try and suppress leaf blackening during spring when this cultivar is particularly susceptible to leaf blackening.

2.4.1 Carbohydrate content and exogenous sugar pulsing

Postharvest leaf blackening in *Protea* species and cultivars does not appear to be the product of one factor but rather the result of a complex chain of events (Jones *et al.*, 1995) that eventually culminate in the oxidation of phenolic compounds. The developing inflorescence’s high respiration rate and nectar production (Dai and Paull, 1995) combined with a relatively low availability of translocatable carbohydrates in the leaves (Jones *et al.*, 1995) results in a dramatic decline of translocatable carbohydrates in the leaves followed by the initiation of leaf blackening. This lack of an immediately available energy source may play a role in the destabilization of membranes in the plant cell, as most membranes have a very high energy demand (Jones *et al.*, 1995) and a constant source of metabolites is needed to keep the membranes intact. Soluble sugars are known to protect membranes from desiccation by preventing membrane fusion, phase transition and phase separation (Crowe and Crowe, 1986; Caffrey *et al.*, 1988; Crowe *et al.*, 1988; Crowe and Crowe, 1992). The application of an endogenous supply of nutrients, such as sucrose, can extend cut flower shelf life by aiding flower opening (van Doorn *et al.*, 1991) and maintaining the membrane integrity of the delicate petal tissue (Halevy and Mayak, 1979). Goszezynska *et al.* (1990) determined that the application of exogenous sucrose to roses reduced the age induced increase in membrane lipid microviscosity. Jones *et al.* (1995) found similar results in the leaf tissue of a few species of *Leucadendron* after they were treated with sucrose.

Dai and Paull (1995) reported that carbohydrates were transported from the leaves to the developing inflorescence of *P. neriifolia* (a strong

sink). This translocation of carbohydrates is thought to be the catalyst for the process of leaf blackening (McConchie *et al.*, 1991; Bieleski *et al.*, 1992; McConchie and Lang 1993b). Newman *et al.* (1990) discovered that girdling the stem just below the flowerhead resulted in a significant reduction in the rate and severity of leaf blackening, this indicating that the carbohydrate demand of the flowerhead is a leading cause of leaf blackening. Many *Protea* species store large amounts of carbohydrates in the form of 1,5-anhydro-D-glucitol (polygalatol), a derivative of sorbitol. After harvest the concentrations of sucrose, glucose, fructose and starch (all reducing sugars) decrease to relatively low concentrations while the concentration of polygalatol (non-reducing sugar alcohol) remains fairly constant over time (McConchie *et al.*, 1991; Bieleski *et al.*, 1992; McConchie and Lang, 1993a,b). The concentration of polygalatol was found to be highest in the apical leaves of *P. neriifolia* and declined significantly in the leaves near the base of the stem (McConchie *et al.*, 1991). The concentration of sucrose was fairly constant in all of the leaves on the stem. This seems to indicate that most leaves are source leaves after flower initiation (McConchie and Lang, 1993a). A significant discovery was made by Dai and Paull (1995), when they determined that the greater the number of mature leaves on the stem the slower the rate of leaf blackening and flower senescence. This seems to indicate that the greater the carbohydrate storage potential in the plant the slower the rate of leaf blackening. *Protea* plants follow the characteristic carbohydrate storage pattern of evergreen plant species with significantly higher concentrations of sugars and starch occurring in the leaves of the plant than in the stem (Hettasch *et al.*, 2001). While the leaves and stem of a *Protea* plant act as a source, the inflorescence acts exclusively as a sink. The stage of the inflorescence development at harvest has a significant impact on the demands placed on the reserves in the cut shoot. Paull and Dai (1990) found that the stage of flower development plays a critical role in the rate of leaf blackening with blackening occurring at a far more pronounced rate on stems that were harvested with mature inflorescences at a closed bud stage in comparison to stems

with more open mature inflorescences attached. The developing inflorescence, with its high respiration rate and nectar production, acts as a strong sink (Dai and Paull, 1995) causing photosynthetic products to be transported to the developing inflorescence where they act as an energy source. Ferreira (1986) found that the maturity of the inflorescence has a direct effect on the rate of its respiration, with less developed inflorescences having significantly higher rates of respiration than mature inflorescences. Increased respiration rates were shown by Stephens *et al.* (2001) to correlate with an increase in leaf blackening of 'Sylvia' *Protea* leaves. It may, therefore, be beneficial to harvest *Protea* shoots at a stage when the inflorescence is more open to avoid the high carbohydrate demands of the developing inflorescence. This should result in a decrease in the occurrence of postharvest leaf blackening as a decline in the demand for fixed carbohydrates correlates well to a drop in the rate of leaf blackening (Meyer, 2003). Dai and Paull (1995) supplied ^{14}C -sucrose to leaves subtending freshly harvested *P. neriifolia* inflorescences. Twenty-four hours later 58% of the radiolabeled carbon could be accounted for in the nectar. This indicates that nectar production is the most likely reason for the high carbohydrate demand of the developing inflorescence (Dai and Paull, 1995).

The application of endogenous sugars, to supply an alternate form of energy to prolong flower vase life has become mandatory in order to compete in the international flower markets and is a recognized form of postharvest storage and vase life extension in many cut flower crops (Halevy and Mayak, 1979, 1981). The application of exogenous sugars as carbohydrate supplementation to *Protea* cut flowers has only been partially successful (Stephens, 2003) with leaf blackening partially reduced (Jones *et al.*, 1995) and in some cases, high sugar concentrations actually causing an increase in the rate and intensity of leaf blackening (Meyer, 2003). The type of sugar and the concentration used for the pulsing and holding solutions appears to be specific to each *Protea* species or cultivar as the severity of leaf blackening ranges

widely between species (McConchie and Lang, 1993a,b) clones within a species (Paull and Dai, 1990) and the time of year (de Swardt *et al.*, 1987). Brink and de Swardt (1986) determined that a sucrose holding solution of 0.5 and 1% significantly reduced and delayed leaf blackening of *P. neriifolia*, while Ligawa *et al.* (1997) found that a sucrose vase solution concentration of 3% extended the vase life of 'Sylvia' by 10 days. McConchie *et al.* (1991) discovered that sucrose did not inhibit leaf blackening significantly in *P. neriifolia*. The use of a 0.5% sucrose vase solution delayed the occurrence of leaf blackening in *P. eximia* and extended the vase life to 16 days (Newman *et al.*, 1990). Holding solutions containing sucrose at $\leq 2 \text{ g.L}^{-1}$ effectively reduced leaf blackening of *P. compacta*, *P. cynaroides* and *P. magnifica* (Ireland *et al.*, 1967). Brink (1987) and Jones (1991) found that sucrose holding solutions at higher concentrations enhanced the rate and intensity of leaf blackening in *P. neriifolia*. The vase life of 'Sylvia' held in a 2.5% glucose solution was extended by 10 days as a result of a significant reduction in leaf blackening (Stephens, 2003). These findings indicate the importance of evaluating postharvest carbohydrate treatments at cultivar level and further research is needed on carbohydrate source, concentration, conditions of uptake and storage temperatures in order to improve the shipping potential of various *Protea* species and cultivars.

2.4.2 Spring flush leaf blackening

Producers are able to manipulate the flowering time of certain species and cultivars of *Protea* through pruning in order for the flowering time to occur within the optimum marketing period in Europe of September-January (Gerber *et al.*, 2001). Pruning is also used to control and direct shoot growth. Shoot growth, flower initiation and development cycles of *Protea* are not as predictable as in many other commercial cut flower types (Malan and le Roux, 1995). Shoots initiate growth from upper axillary buds on stems that have been cut back. The time of pruning plays a major role in the later sprouting and rate of development of the

shoots (Malan and le Roux, 1995). The time taken from pruning to inflorescence initiation is cultivar specific (Malan and le Roux, 1995). Growth flushes following pruning occur seasonally on the terminal growth points. There are four periods of distinct vegetative growth flushes: (1) a March to August winter growth period that produces a short flush; (2) an extensive spring flush that occurs from September to November; (3) medium to long summer flushes that are produced from December to January; and a (4) February to March autumn growth period that produce medium to long stem flushes (Malan and le Roux, 1995). Different *Protea* species and cultivars experience vegetative growth flushes at different times of the year. *Protea* inflorescence bearing shoot development occurs three to 11 months after pruning. This demonstrates the enormous difference in shoot growth between species and cultivars and again puts emphasis on treating each cultivar separately. Hettasch *et al.* (2001) determined that the concentration of carbohydrates in the leaves and stems of the whole shoot increased from one flush to the next up to and incorporating the next summer flush. In some cultivars no stem growth occurs during the winter months (Malan and le Roux, 1995). During winter the carbohydrate concentration of the leaves on a flower-bearing shoot declined (Hettasch *et al.*, 2001). This reduced carbohydrate state is followed immediately by the 'spring flush' (growth period between September and October). In 'Sylvia' plants this is the most vigorous growth flush and extensive new growth occurs in a relatively short space of time (Gerber *et al.*, 2001). This cultivar is prone to suffer from extensive pre- and postharvest leaf blackening during this time period. The increase in postharvest leaf blackening corresponds to the vigorous growth period following the decreased day length and low light levels of the South African winter (Stephens., 2003). McConchie *et al.* (1991), Bieleski *et al.* (1992) and McConchie and Lang, (1993a,b) all correlated the occurrence of leaf blackening with the decrease in the carbohydrate content of numerous *Protea* species. It is thought that a similar process may be involved with the development of the greater susceptibility, both pre- and postharvest, of 'Sylvia' shoots to leaf blackening during

this time of the year. This is a serious problem for producers as this period of susceptibility correlates with the period when there is an increasing demand for fresh flowers in the European market place. Most current postharvest treatments have little to no effect on the rate of leaf blackening during this time period of late September to mid October. Therefore more research into alternative treatments needs to be carried out as a matter of urgency.

2.4.3 Plant growth regulating chemicals

‘Sylvia’ flower-bearing shoots are most susceptible to pre- and postharvest leaf blackening during or just post the development of the spring flush, the most extensive and vigorous of the four growth stages for this cultivar. It has been hypothesized (pers. com. G. Jacobs) that in order for the ‘Sylvia’ plant to meet the carbohydrate demands of the developing shoots during this time period, translocatable carbohydrates move from source leaves to the rapidly developing young shoots (strong sinks). This reduces the size of the readily available energy source that occurs in the mature leaves, thereby making them more susceptible to leaf blackening if subjected to some form of stress, either pre- or postharvest.

It is hypothesized that the application of bioregulators/plant growth regulators (PGR’s) can suppress the spring growth flush, thereby decreasing the demand for mobile carbohydrates to be exported from the mature leaves to the new growth. This would occur as a result of the decreased demand by the slower developing spring flush for carbohydrates both as an energy source and as building blocks.

The majority of PGR’s used today are plant growth retardants. Plant growth retardants reduce the shoot length of plants without altering developmental patterns or being phytotoxic (Dicks, 1980; Davis and Curry, 1991; Rademacher, 1995, 2000). This is normally achieved

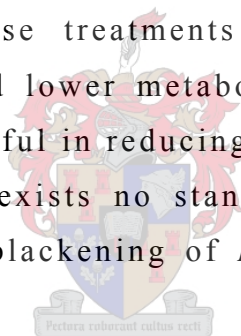
primarily through reducing cell elongation but also through reducing the rate of cellular division (Rademacher, 1991, 1995, 2000). To achieve this, most growth retardants block some step in the synthesis of gibberellins (GA's) or auxins, the two plant hormones that are primarily responsible for shoot elongation. The existing growth retardants can be classified into three main groups; (a) ethylene-releasing compounds, (b) inhibitors of GA translocation, and (c) inhibitors of GA biosynthesis (Rademacher, 1991). At this time four groups of GA biosynthesis inhibitors are recognized; (a) "onium" compounds, (b) compounds with N-containing heterocycles, (c) acylcyclohexanediones and related compounds and (d) 16,17-dihydro GA's. Each separate group inhibits GA metabolism at different stages of GA biosynthesis (Rademacher, 2000).

Horticultural uses of plant growth retardants include the reduction of vegetative growth in fruit bearing trees, grape vines and other woody species, the control of turf growth and regulation of height in ornamentals (Rademacher, 1995). Numerous PGR's may have application in suppressing the vegetative shoot growth of *Proteas* during the growth flush in spring. Two of these are Prohexadione-calcium and Paclobutrazol. Prohexadione-calcium (P-Ca) (calcium 3-oxido-4-propionyl-5-oxo-3-cyclohexenecarboxylate Evans *et al.* 1999) is a foliar applied acylcyclohexanedione PGR, the uptake of which is normally complete four hours following application (Rademacher, 2001). P-Ca has been shown to inhibit the later steps in GA biosynthesis directly, thereby reducing the extension of shoots (Rademacher, 1991). P-Ca is primarily transported acropetally to growing points of individual shoots (Evans *et al.*, 1997, 1999). Normally only the development of treated shoots is affected. (Evans *et al.*, 1999). Paclobutrazol, (2RS, 3RS)-1-(4-chlorophenyl)-4, 4-dimethyl-2-(1, 2, 4-triazol-1-yl) pentan-3-ol Grossman, 1990), trade name Cultar, is a compound with an N-containing heterocycle (Rademacher, 1995). This PGR is applied as a soil drench (as it is more easily transported in the xylem, Rademacher, 1995), to soil that is free of

organic material directly above the root system of the plant that is to be treated. The persistence of Paclobutrazol in the soil is relatively high and it remains active for an extended period of time (Rademacher, 1995).

2.5 Conclusions

The postharvest leaf blackening that occurs on many *Protea* plants is not fully understood. It has however been established that the depletion of carbohydrates is an initiating factor in the complex cascade of events that result in the phenomenon. Pulsing shoots after harvest and vase holding solutions containing exogenous carbohydrates have been shown to partially meet the carbohydrate requirements of certain *Protea* cultivars and species. These treatments combined with cooling to reduce respiration rates and lower metabolic activities have, in some cases, been partially successful in reducing postharvest leaf blackening. However, at present there exists no standard commercial practice to eliminate or suppress leaf blackening of *Protea* cut flowers both pre- and postharvest.



2.6 Literature Cited

- Bieleski, R.L., J. Ripperda, J.P. Newman and M.S. Reid. 1992. Carbohydrate changes and leaf blackening in cut flower stems of *Protea eximia*. *J. Amer. Soc. Hort. Sci.* 117:124-127.
- Brink, J.A., 1987. The influence of the flower-head on leaf browning of cut *Protea neriifolia*. *Protea News* 5: 11-12.
- Brink, J.A. and G.H. de Swardt. 1986. The effect of sucrose in a vase solution on leaf browning of *Protea neriifolia* R. Br. *Acta Hort.* 185:111-119.
- Caffrey, M., V. Fonseca, and A.C. Leopold. 1988. Lipid-Sugar Interactions. *Plant Physiol.* 86:754-758.
- Cowling, R.M. and D.T. Mitchell. 1981. Sugar composition, total nitrogen and accumulation of C-14 assimilates in the floral nectaries of *Protea* species. *J. S. Afr. Bot.* 47:743-750.
- Crick, S.G. and R. McConchie. 1999. Ethanol vapour reduces leaf blackening in cut flower *Protea* 'Pink Ice' stems. *Postharvest Biol. Technol.* 17, 227-231.
- Crowe, J.H. and L.M. Crowe. 1986. Stabilization of membranes in anhydrobiotic organisms. pp. 188-209. In: A.C. Leopold (ed.). *Membranes, metabolism, and dry organisms*. Comstock Publication Assoc., Ithaca, NY.
- Crowe, J.H. and L.M. Crowe. 1992. Membrane integrity in anhydrobiotic organisms: toward a mechanism for stabilizing dry cells. pp. 87-103. In: G.N. Somero, C.B. Osmond and C.L. Bolis (Editors). *Water and Life*. Springer-Verlag, Berlin.
- Crowe, J.H., L.M. Crowe, J.F. Carpenter, A.S. Rudolph, C. Aurell Wistrom, B.J. Spargo and T.J. Anchooguy. 1988. Interactions of sugars with membranes. *Biochem Biophys Acta.* 947:367-384.
- Dai, J-W. and R.E. Paull. 1995. Source-sink relationship and *Protea* postharvest leaf blackening. *J. Amer. Soc. Hort. Sci.* 120:475-480.

- Davis, T.D. and E.A. Curry. 1991. Chemical regulation of vegetative growth. *Critical Reviews in Plant Sciences* 10(2):151-188.
- De Swardt, G.H., 1979. Blackening of Protea leaves. *SAPPEX Res. Paper* 13.
- De Swardt, G.H. and J. Pretorius. 1980. Browning of foliage leaves in Proteas. *Farming in South Africa: Flowers and Ornamentals Publ.* B.13.
- De Swardt, G.H., J. Pretorius, and L. Burger. 1987. The browning of foliage leaves in Proteas-a review. *Protea News* 6, 4-9.
- Dey, P.M. and R.A. Dixon. 1985. Glycosides. pp.131-148. In: P.M. Dey and R.A. Dixon (eds.). *Biochemistry of storage carbohydrates in green plants*. Academic Press. New York.
- Dicks, J.W. 1980. Modes of action of growth retardants. pp. 1-14. In: D.R. Clifford and J.R. Lenton (eds.). *Recent developments in the use of growth retardants*. British Plant Growth Regulator Group, Wantage, England.
- Du Plessis, D.G.C. 1978. Leuko-antosianienbinders as beheermaatreël vir die voorkoming van bruinwording in Protealoofblare. M.Sc. Thesis, Randse Afrikaans Univ., Johannesburg, South Africa.
- Evans, R.R., J.R. Evans, and W. Rademacher. 1997. Prohexadione calcium for suppression of vegetative growth in Eastern apples. *Acta Hort.* 451:663-666.
- Evans, J.R., R.R. Evans, C.L. Regusci, and W. Rademacher. 1999. Mode of action, metabolism, and uptake of BAS125W, Prohexadione-calcium. *HortScience* 34(7):1200-1201.
- Ferreira, D.I. 1983. Prevention of browning of leaves of *Protea neriifolia* R. Br. *Acta Hort.* 138:273-276.
- Ferreira, D.I. 1986. The influence of temperature on the respiration rate and browning of *Protea neriifolia* R. Br. Inflorescences. *Acta Hort.* 185:121-125.
- Forsberg, L.I. 1988. Protea diseases and their control. *Queensland Agr.J.* (Nov.-Dec.):347-359.
- Forsberg, L.I., 1993. Protea diseases and their control. Queensland Dept Primary Industries, Brisbane, Queensland.

- Gerber, A.I., K.I. Theron and G. Jacobs. 2001. The role of leaves and carbohydrates in flowering of *Protea* cv. Lady Di. *HortScience* 36(5):905-908.
- Goszezynska, D., H. Itzhaki, A. Borochoy, and A.H. Halevy, 1990. Effects of sugar on physical and compositional properties of rose petal membranes. *Scientia Hort.*, 43:313-320.
- Grossmann, K. 1990. Plant growth retardants as tools in physiological research. *Physiol. Plant.* 78:640-648.
- Haasbroek, F.J., G.G. Rousseau, and J.F. de Villiers. 1973. Effect of gamma-rays on cut blooms of *Protea compacta* R.Br., *P. longiflora* Lamarck and *Leucospermum cordifolium* Salisb. Ex Knight. *Agroplanta* 5:33-42.
- Halevy, A.H. and Mayak. 1979. Senescence and postharvest physiology of cut flowers-Part 1. *Hort. Rev.* 1:205-236.
- Halevy, A.H. and Mayak. 1981. Senescence and postharvest physiology of cut flowers-Part 2. *Hort. Rev.* 3:59-143.
- Hettasch, H.B., K.I. Theron, and G. Jacobs. 2001. Dry mass allocation and carbohydrate allocation in successive growth flushes of *Protea* cultivar 'Sylvia' and *Protea* cultivar 'Cardinal' shoots. *Acta Hort.* 545:215-225.
- Ireland, J.P., J.T. Meynhardt, and J.M. Strauss. 1967. When proteas become sailors: treatment before shipping. *Farming in South Africa* 43, 33-35.
- Jones, R.B., 1991. Understanding and controlling leaf blackening in *Protea* leaves: the use of high concentrations of sucrose. *Proc. Int. Protea Assoc. 6th Biennial Conf.*, Perth, Western Australia. Pp 313-322.
- Jones, R.B. and K.A. Clayton-Greene. 1992. The role of photosynthesis and oxidative reactions in leaf blackening of *Protea neriifolia* R. Br. leaves. *Scientia Hort.* 50:137-145.
- Jones, R.B., R. McConchie, W.G. van Doorn and M.S. Reid. 1995. Leaf blackening in cut *Protea* flowers. *Hort. Rev.* 17:173-201.

- Jones, R.B. and A. Cass. 1996. The possible involvement of a β -D-glucosidase in postharvest leaf blackening in cut *Protea* flowers. J. Int. Protea Assoc. 31, 36-44.
- Lagrimi, L.M., 1992. Wound-induced deposition of polyphenols in transgenic plants over expressing peroxidase. Plant Physiol. 96:577-583.
- Ligawa, J.K., D.C. Joyce and S.E. Hetherington. 1997. Exogenously supplied sucrose improves the postharvest quality of *Grevillea* 'Sylvia' inflorescences. Aus. J. Exp. Agr. 37:809-816.
- Malan, D.G., and R.D. le Roux. 1995. Preliminary investigation into the effect of time of pruning on shoot growth and flowering time of *Protea*. Acta Horticulturae 387:91-97.
- Marty, F., D. Branton, and R.A. Leigh. 1980. Plant vacuoles. Pp 625-658. In: N.E. Tolbert (ed). The biochemistry of plants. Vol. 1. Academic Press, New York.
- Masie, W.E. 1979. Fenielermerkuri-asetaat as voorkomingsmiddel vir loofblaarverbruining by *Protea neriifolia* R.Br. M.Sc. Thesis, Randse Afrikaans Univ., Johannesburg, South Africa.
- McConchie, R and N.S. Lang. 1993a. Postharvest leaf blackening and pre-harvest carbohydrate status in three *Protea* species. HortScience 28:313-316.
- McConchie, R and N.S. Lang. 1993b. Carbohydrate metabolism and possible mechanisms of leaf blackening in *Protea neriifolia* under dark postharvest conditions. J. Amer. Soc. Hort. Sci. 118:355-361.
- McConchie, R., N.S. Lang, and K.C. Gross. 1991. Carbohydrate depletion and leaf blackening in *Protea neriifolia*. J. Amer. Soc. Hort. Sci. 116:1019-1024.
- Meyer, C. 2003. Carbohydrates and leaf blackening of protea cut flowers. M.Sc. Thesis, University of Stellenbosch., Stellenbosch, South Africa.
- Newman J.P., W.G. van Doorn and M.S. Reid. 1990. Carbohydrate stress causes leaf blackening in *Proteas*. Acta Hort. 264:103-108.

- Paull, R.E. and J.W. Dai. 1990. Protea postharvest leaf blackening a problem in search of a solution. *Acta Hort.* 264:93-101.
- Paull, R.E., T. Goo, R.A. Criley and P.E. Parvin. 1980. Leaf blackening in cut Protea eximia: importance of water relations. *Acta Hort.* 113:159-166.
- Perold, G.W., 1993. Consistency and variation in metabolite patterns of South African Proteaceae: a chemical perspective. *S. Afr. J. Sci.* 89:90-93.
- Perold, G.W, P. Beylis, and A.S. Howard. 1973a. Metabolites of Proteaceae. Part VII. Lacticolorin, a phenolic glucoside ester and other metabolites of *P. lacticolor* Salisb. *J. Chem. Soc. Perkin Trans I* 1973, 638-642.
- Perold, G.W, P. Beylis, and A.S. Howard. 1973b. Metabolites of Proteaceae. Part VII. The occurrence of (+)-D-allose in nature: rubropilosin and pilorubrosin from *Protea rubropilosa* Beard. *J. Chem. Soc. Perkin Trans I* 1973, 643-649.
- Perold, G.W., M.E.K. Rosenberg, A.S. Howard and P.A.Huddle. 1979. Metabolites of Proteaceae. Part 9. Eximin (6-O-benzoylarbutin) and the synthesis of aryl glycoside esters. *J. Chem. Soc. Perkin Trans I* 239-243.
- Rademacher, W. 1991. Biochemical effects of plant growth retardants. Pp. 169-200. In: H.W. Gausman (ed.). *Plant biochemical regulators*. Marcel Dekker, Inc., New York.
- Rademacher, W. 1995. Growth retardants: Biochemical features and applications in horticulture. *Acta Hort.* 394:57-73.
- Rademacher, W. 2000. Growth retardants: Effects on gibberellin biosynthesis and other metabolic pathways. *Annu. Rev. Plant Physiol Plant Mol. Biol.* 51:501-531.
- Rademacher, W. 2001. Technical Data Sheet, BASF Agricultural Center, p. 7.
- Reid, M.S., W. van Doorn and J.P. Newman. 1989. Leaf blackening in Proteas. *Acta Hort.* 261:81-84.
- Sacher, J.A., 1973. Senescence and postharvest physiology. *Annu. Rev. Plant Physiol.* 24:197-224.

- Starke, L.C., 1979. Common Protea pests. Farming in South Africa: Flowers and Ornamental Shrubs Publ. B.11.
- Stephens, I.A. 2003. Leaf blackening of proteas. Ph. D. Dissertation, University of Stellenbosch., Stellenbosch South Africa.
- Stephens, I.A., D.M. Holcroft, and G. Jacobs. 2001. Low temperatures and girdling extend vase life of 'Sylvia' Proteas. Acta Hort. 545:205-214.
- Van Doorn, W.G. 2001. Leaf blackening: Recent developments. Acta Hort. 545:197-204.
- Van Doorn, W. G., G. Groenewegen, P.A. van der Pol, and C.E.M. Berkholst. 1991. Effects of carbohydrate and water status on flower opening of cut Madelon roses. Postharvest Biol. Technol., 1:47-57.
- Van Rheede van Oudtshoorn, M.C.B., 1963. Distribution of phenolic compounds in some South African Proteaceae: a contribution to the chemotaxonomy of the family. Planta Med. 4:399-406.
- Van Wyk, B.E. and S.W. Nicholson. 1995. Xylose is a major nectar sugar in Protea and Faurea. Suid-Afrikaanse Tydskrif vir Wetenskap. 91:151-153.
- Whitehead, C.S. and G.H. de Swardt. 1982. Extraction and activity of polyphenoloxidase and peroxidase from senescing leaves of Protea neriifolia. S. Afr. J. Bot. 1:127-130.

3. Paper I Post-harvest Carbohydrate Metabolism and Partitioning in *Protea* cut flowers

Abstract.

Glucose, sucrose, fructose and starch concentrations were determined in the leaves, inflorescence and stems of two *Protea* cultivars, namely 'Sylvia' and 'Lady Di' shortly after harvest. At harvest polygalatol is a major component of the soluble carbohydrate fraction in the inflorescence, leaves and stems, occurring in the highest concentration in the leaves. Sucrose was found to occur in lower concentrations in the inflorescence than both glucose and fructose. The leaves contained higher concentrations of sucrose than glucose and fructose. Stems do contain significant amounts of reserve carbohydrates in the form of glucose, fructose and sucrose but very little starch. This, however, seems to be cultivar specific. The metabolically active carbohydrate pool consists of sugars that occur in the inflorescences, leaves and stems as well as starch from the leaves. Individual 'Sylvia' and 'Lady Di' inflorescence bearing shoots were then pulsed with a 10g.L⁻¹ glucose solution until 10mL of the solution had been taken up by each stem. Water served as the control. After the pulsing process and storage at room temperature as well as 4.5°C for different lengths of time the shoots were separated into stem, leaf and inflorescence components and the concentrations of glucose, sucrose, fructose and starch determined. Directly after pulsing most of the glucose is located in the stems. An increase in starch concentration occurred in the leaves of 'Sylvia' directly after the pulsing treatment. The conversion of glucose to starch did not take place in 'Lady Di'. It appears that glucose and starch are the main reserve carbohydrate present in glucose pulsed 'Sylvia' shoots. 'Sylvia' shoots stored at room temperature lose between 25-30% of reserve carbohydrates within the first day after pulsing and a further 10-15% during the next 24h storage. In contrast it takes 10 days for reserve carbohydrates to decrease by 50% at a storage temperature of 4.5°C.

Introduction

Recent experiments have built up a considerable amount of evidence indicating that there is a strong correlation between postharvest leaf blackening and low leaf carbohydrate status (Jones *et al.*, 1995; van Doorn, 2001). McConchie and Lang (1993a) suggested that postharvest floral sink demand plays a bigger role in leaf blackening than the preharvest carbohydrate status of *Protea neriifolia*. The concentrations of nonstructural metabolic carbohydrates in *Protea* leaves tend to drop rapidly after harvest (McConchie *et al.*, 1991; Bieleski *et al.*, 1992; McConchie and Lang, 1993a, 1993b), and elevated temperatures were shown to further exacerbate the situation (Stephens *et al.*, 2001a). Reid *et al.* (1989), by girdling *Protea* stems just below the inflorescence and by using darkness and additional light, indicated that the leaves act as a source of carbohydrates for the inflorescence. This, together with the results achieved by pulsing with C^{14} (Dai, 1993) supported the hypothesis that the depletion of carbohydrates in the leaves of *Proteas*, caused by the demand of the developing inflorescence and nectar production, initiates leaf blackening (Ferreira, 1986; Paull and Dai, 1989). It has been shown by many authors that when postharvest leaf blackening occurs there is a correlating drop in the carbohydrate content of the leaves. Data on carbohydrate levels show that starch and sucrose are the main non-structural metabolic simple carbohydrate compounds found in *Protea* species (McConchie *et al.*, 1991; Bieleski *et al.*, 1992; McConchie and Lang, 1993a, 1993b). However, Stephens *et al.* (2001a) indicated that both fructose and glucose occur in higher concentrations in the inflorescence of 'Sylvia' (*P. eximia* x *P. susannae*) than does sucrose. The leaves of 'Sylvia' were shown to contain comparable amounts of these three sugars directly after harvest. Many other simple sugars are components of the nectar of certain *Protea* species. These include glucose, fructose, sucrose and xylose (Cowling and Mitchell, 1981; van Wyk and Nicholson, 1995). A soluble polygalatol (1, 5-anhydro-D-glucitol) carbohydrate, a simple derivative of sorbitol, has also been found to occur in substantial concentrations in many *Protea* species (Bieleski *et al.*, 1992; McConchie and Lang,

1993a, 1993b). As the concentrations of other carbohydrates (in particular sucrose and starch) decrease rapidly in the leaves of many *Protea* species after harvest (McConchie *et al.*, 1991; Bielecki *et al.*, 1992; McConchie and Lang, 1993a, 1993b) the concentration of polygalatol does not significantly alter, even when levels of starch and sucrose are extremely low and leaf blackening has commenced. This finding, according to Bielecki *et al.* (1992), shows that metabolically inert sugars could play a leading role in osmotic buffering in *Protea* species and that if this high concentration of polygalatol was fully accessible for remetabolism it could potentially double the survival time of *Protea* species held under dark environmental postharvest conditions.

The application of exogenous sugars as an additional energy source to delay senescence during storage and vase life is a recognized practice in both climacteric and non-climacteric cut flower crops (Goszczyńska *et al.*, 1990; Paulin, 1986; Whitehead *et al.*, 2003). The extension of vase life seems to be related to a consistent fresh weight and a regular gain in dry matter (Paulin, 1986). According to Halevy and Mayak (1979) the most advantageous sugar and optimum concentration of this sugar will vary with the species of flower crop treated, the length of this treatment and the stage of floral development. The use of exogenous sugars as a postharvest treatment to replace depleted photosynthates in *Protea* cut flowers has only been moderately successful. Postharvest leaf blackening has been reduced in species such as *P. neriifolia*, *P. eximia*, *P. compacta*, and *P. longiflora* with low concentration sucrose holding solutions ($\approx 2\text{g.L}^{-1}$). Leaf blackening was, however, never totally inhibited (Haasbroek *et al.*, 1973; Mulder, 1977; Akamine *et al.*, 1979; Brink and de Swardt, 1986; Brink, 1987; Paull and Dai, 1989; McConchie *et al.*, 1991; Bielecki *et al.*, 1992). Sucrose holding solutions at higher concentrations were found to actually magnify the occurrence and severity of leaf blackening in *P. neriifolia* (Jones, 1991). However, Akamine *et al.* (1979) showed that sucrose holding solutions of 30g.L^{-1} could significantly suppress leaf

blackening of *P. eximia*. Likewise, Jones (1991) successfully repressed leaf blackening of *P. cynaroides* during long-term dry storage (>14 days) at 1°C using a 200 g.L⁻¹ sucrose pulse delivered over a 24 hour period. McConchie and Lang (1993b) were able to reduce leaf blackening in *P. neriifolia*; during dark, wet storage at 25°C for seven days, by 70% through an initial 24 hour pulsing period using a 200 g.L⁻¹ sucrose solution. In contrast Stephens *et al.* (2001b) indicated that sucrose holding solutions of 1 g.L⁻¹ did not significantly improve the vase life of ‘Sylvia’ (*P. eximia* x *P. susannae*) and, in fact, higher concentrations of the solution actually promoted leaf blackening of this cultivar. Alternatively, it was shown that a 25 g.L⁻¹ glucose holding solution significantly suppressed leaf blackening in this cultivar.

Carbohydrate metabolism and partitioning have not been examined in great detail during a postharvest period even though there is a strong correlation between depleted carbohydrates and leaf blackening. Some information does, however, exist. Carbohydrate concentration profiles, taken after pulsing *P. neriifolia* with a 200 g.L⁻¹ sucrose solution showed peaks in leaf sucrose and starch concentrations 48 and 72 hours after pulsing respectively, indicating that exogenous sucrose moves through the stem into the leaves (McConchie and Lang, 1993a). Stephens *et al.* (2005) reported on the change in concentrations of glucose, fructose, sucrose and starch from harvest to the onset of leaf blackening in the leaves and inflorescence of the *Protea* cultivars ‘Cardinal’ (*P. eximia* x *P. susannae*), ‘Carnival’ (*P. compacta* x *P. neriifolia*), ‘Ivy’ (*P. laticolor* selection), ‘Pink Ice’ (*P. compacta* x *P. susannae*), ‘Sheila’ (*P. magnifica* x *P. burchellii*) and ‘Susara’ (*P. magnifica* x *P. susannae*). At harvest all cultivars had higher concentrations of glucose, sucrose, fructose and starch than when leaf blackening began to occur (Stephens *et al.*, 2005). It was also shown that the concentrations of glucose and/or fructose in the inflorescence at harvest were higher than the corresponding concentration of sucrose. This held true for all of the cultivars tested. The concentrations of the three sugars in the leaves were comparable. The concentrations of

glucose and fructose were consistently higher in the inflorescence than in the leaves for most of the cultivars, but the reverse held true for the sucrose and starch concentrations (Stephens *et al.*, 2005). At leaf blackening it was concluded that the concentrations of both sugars and starch, for all cultivars, had dropped (in most cases significantly) from the levels recorded at harvest. The levels of the sugars and starch had decreased proportionately more in the leaves than in the inflorescence. This evidence supports the hypothesis that the translocation of carbohydrates from the leaves to the strong inflorescence sink, may play a role in the initiation of a series of events that lead to the occurrence of leaf blackening (McConchie and Lang, 1991).

A 50 g.L⁻¹ glucose pulsing solution was used by Stephens *et al.* (2005) to determine the change in carbohydrate concentrations and distribution in different parts of 'Sylvia' (*P. eximia* x *P. susannae*) either directly following the pulse or after 3 weeks of cold storage at 1°C. Immediately after pulsing the glucose concentration of pulsed shoots, for all shoot parts, was significantly higher than the control shoots. After the storage period the concentration of glucose in the leaves of the pulsed shoots remained significantly greater than the control. There was, however, no significant difference in the glucose concentration in the inflorescence and stems of the pulsed and control shoots after the storage period. The levels of starch increased significantly in the leaves of the pulsed shoots compared with those of the control (Stephens *et al.*, 2005). There was no significant difference in the concentration of starch in the inflorescence and stems of the two treatments. During the storage period there was a significant decrease in the concentration of starch for all plant parts in both treatments. However there was no significant difference between the two treatments.

Here we report on carbohydrate metabolism and partitioning during a typical postharvest period for 'Sylvia' (*P. eximia* x *P. susannae*) and 'Lady Di' (*P. magnifica* x *P. compacta*) shoots that are used as cut

flowers. The changes in shoot glucose, sucrose, fructose, polygalatol, inositol and starch concentrations from harvest to two days after a pulsing treatment (100 g.L^{-1} glucose solution) and from harvest until four weeks after the pulsing treatment held under cold storage at 4.5°C are presented and discussed.

Materials and Methods

Plant material. *Protea* cultivars ‘Sylvia’ (*P. eximia* x *P. susannae*) and ‘Lady Di’ (*P. magnifica* x *P. compacta*) were harvested from mature plants in a commercial plantation located in the Stellenbosch region (lat. $33^\circ15'S$; long. $19^\circ07'E$) of the Western Cape in South Africa. The climate of this area is classed as Mediterranean. The summers are hot and dry with an average annual rainfall of between 600-700 mm falling predominantly in the winter months.

Sample preparation. Shoots were harvested in June and July 2003 when the inflorescences were ready for commercial harvest (11/06/03 for ‘Sylvia’ and 7/07/03 for ‘Lady Di’).

The following procedure was followed for the *Protea* cv ‘Sylvia’. Harvesting took place early in the day and was complete by 10:00 am. 44 Shoots were harvested and placed into a cardboard transport carton before being moved to a controlled environment with a fixed temperature of $18\pm1^\circ\text{C}$ within half an hour of harvest. The shoots were cut to a uniform length of 40 cm and most of the leaves were removed below the subtending flush. A total of twenty leaves per stem were left. Four of the shoots were immediately separated into stem, leaf and inflorescence portions, sealed in clearly marked brown paper bags and stored in a -20°C refrigerator. The remaining shoots were placed into flat-bottomed test tubes that contained a 10% glucose solution. A specially designed holding apparatus was used to position the shoots securely under high-pressure sodium (HPS) lamps (400W, SON-T; Osram, Munich, Germany) that produced photoperiodic light at an

intensity of approximately $110 \mu\text{mol.m}^{-2}.\text{s}^{-1}$ while the stems stood suspended in the 10 % glucose solution. Once the stems were fixed into position the level of liquid was marked on the test tubes using a felt tipped permanent marker, 10 ml of the glucose solution was then added to each test tube using a suitable pipette. The lamps were immediately switched on to help speed up the process of transpiration and thus liquid uptake. The temperature of the room was also increased to $24 \pm 1^\circ\text{C}$. The process of liquid uptake or 'pulsing' was considered complete when the level of liquid had dropped to the previously marked level on the test tubes, this normally took between six and eight hours to occur under the conditions described above. Once the level of liquid had dropped to the marked line it could be concluded with a fair degree of certainty that 10 ml of the 10 % glucose solution had been taken up by the stems. This effectively meant that one gram of glucose had moved into the stem during the 'pulsing' process. As pulsing was completed for each shoot, it was removed from the solution and placed directly into cardboard transport cartons. Two cartons were used, eight shoots being placed in the one and 28 shoots into the other. Four of the shoots were immediately separated into leaf, stem and inflorescence portions following the completion of the pulsing process. The various portions were placed into clearly marked brown paper bags and stored in the -20°C refrigerator. The HPS lamps were immediately switched off after 'pulsing' was complete and the room temperature set again to $\pm 18^\circ\text{C}$. One of the transport cartons was allowed to remain in this room for a further two days with four shoots been removed one day after pulsing had been completed and again two days after pulsing. These shoots were separated into portions and stored in the same manner as previously described. The other carton was moved directly into a cold room where it was stored at a temperature of $\pm 4.5^\circ\text{C}$ for a period of four weeks. Four shoots were removed from this carton at the following time intervals after pulsing had being completed; 1d, 2.5d, 7d, 10.5d, 14d, 21d and 28d. The shoots were separated into portions as previously described and stored in the -20°C refrigerator. Once all the portions of the plants had been allowed to freeze they were extracted and prepared

for freeze drying in the Vacuum Freeze Drying Unit (Model 30 P.2.T.S serial number 126) from Edwards High Vacuum L.T.D (Crawley, England). Upon the completion of the freeze-drying process the dry weights for the various plant parts were recorded before being milled to a fine powder.

The same procedure was carried out for the *Protea* cv 'Lady Di', but only 20 shoots of the cultivar were harvested. Therefore the break up of the treatments for this cultivar differed from that of 'Sylvia'. The initial four treatments remained the same but the fifth treatment comprised of four shoots that had been pulsed with water and held at a room temperature of $18\pm 1^{\circ}\text{C}$ for 1d before being partitioned. No further treatments were done for this cultivar. The partitioned flower parts underwent the same procedures as outlined above for 'Sylvia'.

Carbohydrate analysis.

Reducing sugars. A 0.2 g sample of the dried tissue was used for carbohydrate analysis. The weighed samples were placed in test tubes and extracted overnight in a 5 ml solution of methanol, chloroform and water (MCW) in the proportions of 12:5:3. After approximately 16 hours of extraction the samples were centrifuged at $4000g_n$ for 10 minutes at $20\pm 1^{\circ}\text{C}$, and the ensuing clear supernatant was pipetted out into a second set of clearly marked tubes. One ml of the MCW was added to the residue, which was vortexed and centrifuged again. The resulting supernatant was pipetted into the marked tubes that contained the first extract. After these two extractions with MCW were completed it was assumed that most of the reducing sugars had been removed from the residue (this residue was retained for starch analysis). One ml of chloroform was added to the clear pooled MCW extract which was shaken vigorously before one ml of deionized water was added to the solution, whereupon the solution was again shaken vigorously. The samples were centrifuged at $4000g_n$ to allow a separation of layers to occur. The top aqueous layer that contained the more polar substances was pipetted quantitatively into marked tubes; these were placed into a

SC 210 A Speed Vac[®] Plus (manufactured by Thermo Savant) for evaporation. The evaporation procedure was separated into two parts, the first, which lasted for 80 minutes, took place with the heater on. The second, which lasted from between 30 – 90 minutes depending on the intensity of vacuum in the Savant, took place with the heater off. Once the samples were dry five ml of deionized water was added to the tubes containing the residues, the tubes were then placed on a shaker set at 300 revolutions per minute for 10 minutes. While this was taking place the C18 cartridges [containing preparative C18 bulk packing material (Wat 020594) from Waters], were drained of methanol in which the C18 had been conditioned overnight and rinsed with four portions of three ml deionized water. This was done with the aid of a VacMaster[®] Sample Processing Station manufactured by International Sorbent Technology. Marked 10 ml volumetric flasks were placed beneath the C18 cartridges and one ml of the relevant dissolved sample was added onto the C18 column that had the corresponding volumetric flask beneath it. The vacuum was applied and the samples were allowed to move through the column into the volumetric flasks. The cartridges were then washed with four portions of two ml deionized water. (Extreme care was always taken during cartridge conditioning and sample clean-up never to allow them to run dry as this severely impacts on the ability of the C18 bulk packing material to perform its function of removing apolar substances from the sample draining through it). Upon completion, the volumetric flasks were removed from the vacuum manifold and brought to volume with deionized water and shaken. The cleaned and diluted extract was poured into 10 ml disposable syringes and filtered through a 0.45 μm filter directly into marked High-Performance Liquid Chromatography (HPLC) vials. These vials were placed into the HPLC for further analysis. Sugars were separated and quantified using an Agilent 1100 HPLC system equipped with a Refractive Index Detector. A Transgenomic[™] IC Sep ICE-ION-300 column was used at 30°C and the sugars were eluted with a filtered 4.5 mM H₂SO₄ solution, a flow rate of 0.4 ml minute⁻¹ was maintained throughout the analysis. The sugars were quantified against external

standards of the individual sugars that were being analysed (glucose, sucrose, fructose, polygalatol and inositol).

Starch. The 'starch residue' in the original test tubes was 'washed' with two ml of 20% methanol, vortexed and centrifuged for 10 minutes at 4000g_n. The resulting supernatant was discarded. The residue was washed a further three times using five ml of deionized water. Each time the residue was vortexed and centrifuged in the same manner as previously mentioned before the supernatant was discarded. After washing away all the possible remaining free sugar from the residue a further 4.5 ml of deionized water was added to the residue as well as 0.5 ml of a 5 g/l sorbitol solution that acted as an internal standard. The tubes containing the residues were placed into a heating block that had been set at 100°C, where they remained for two hours. This was done to rewet the tissue and to allow the starch fraction to gelatinize. After the samples had been removed and allowed to cool, 100 µl of a 2.5 mM buffer solution (potassium hydrogen succinate, buffered at pH 4.6) was added. In order to absorb any phenolic compounds in the solution 0.05 g of polyvinylpyrrolidone (PVPP), (P-6755) from Sigma, was then added to each individual tube. The tubes were placed on a shaker for 15 minutes (300 revolutions per minute). After this time period 200 µl of a buffered Amyloglucosidase (Fluka from *Aspergillus niger*, 20 U/ml in 0.6 mM potassium succinate) enzyme solution was added. The tubes were lightly shaken to allow for gentle mixing and placed into a heating block that had been set at 56°C where they were allowed to remain overnight in order for the enzymatic hydrolysis of the starch to take place. Approximately 17 hours later the tubes were removed from the heating block and placed into boiling water for five minutes to denaturise the enzyme. The tubes were cooled and centrifuged at 3000 rpm for 10 minutes. The supernatant was pipetted into marked 10 ml volumetric flasks. A further 2 ml of deionized water was added to the individual residues, this was vortexed and centrifuged for 10 minutes at 4000g_n. The resulting supernatant was added to the first in the various volumetric flasks. This process was repeated twice to ensure that all the glucose was removed from the residues. The 10 ml

volumetric flasks were brought to volume using deionized water and shaken. The suspensions were poured into individual 10 ml disposable syringes and filtered through 0.45 μm filters into marked HPLC vials. The glucose concentration was determined by the HPLC using the same instruments and settings as before. The concentrations determined represented the total amount of starch that occurred in the original 0.2 g sample of dried tissue.

Statistical analysis. The SAS program (Statistical Analysis Systems Institute, 1996) was used for a one-way or two-way classification of variance for the data, and LSD values were calculated for a 5% level of significance.

Results

Experiment 1: Carbohydrate distribution in ‘Sylvia’ (*P. eximia* x *P. susanna*) and ‘Lady Di’ (*P. compacta* x *P. magnifica*) at room temperature.

The glucose concentration of ‘Sylvia’ inflorescences determined upon completion of the pulsing treatment did not increase significantly (Table 1a). There was, however, a significant decrease one and two days after the treatment. Pulsing caused a significant increase in the glucose concentration of leaves. Concentrations in the leaves again followed the trend previously described and decreased significantly at 1d and 2d. The levels of glucose in the leaves at day two were comparable to the concentration before pulsing. There was a significant decrease in the glucose content in the stems of the pulsed shoots at day one and day two. However, at this stage the glucose concentration of the stems was still far higher than levels that occurred in control shoots.

Fructose levels in leaves and stems but not inflorescences were significantly higher at completion of glucose pulsing (Table 1b). Thereafter fructose levels in inflorescences and leaves declined. Stem fructose concentrations were even higher 1d and 2d after the pulse treatment. The concentration of sucrose (Table 1c) in inflorescences

and stems remained unchanged. Only at 2d after the glucose pulse did levels increase in inflorescences and decrease in stems. The sucrose concentration was initially higher in the glucose pulse treatment, but thereafter it decreased to levels lower than in the control.

Inositol comprised a very small proportion of the carbohydrates measured in *Protea* 'Sylvia'. The concentration varied from a low of 0.23 to a high of 2.6 mg/kg (Table 1d). In contrast, the concentration of polygalatol (Table 1e) was the highest of the carbohydrates measured. Levels remained unchanged in all shoot parts. The starch concentration (Table 1f) was higher after pulsing in all shoot parts, significantly so in the inflorescences and leaves. Leaves contained the highest concentration. The starch concentration decreased in all shoot parts following the completion of the pulsing treatment.

The carbohydrate contents (Table 2) of the various plant parts followed much the same pattern as the concentrations (Table 1) and after initial significant increases directly after pulsing there was a decline over the remainder of the storage period.

The total carbohydrate content for 'Sylvia' (Table 3) shows a dramatic increase in all plant parts directly after the pulsing treatment. Thereafter significant decreases in the carbohydrate content of the leaves, inflorescences and stems occurred.

The glucose concentration in inflorescences (Table 4a) of 'Lady Di' decreased during the course of the experiment. In leaves and stems glucose levels were higher following pulsing but dropped thereafter. Fructose concentrations decreased in the inflorescences of 'Lady Di' but remained unchanged in leaves and stems (Table 4b). Sucrose levels (Table 4c) were very low in inflorescences and tended to decrease in other plant parts. The levels of inositol in all shoot parts of 'Lady Di' were low and remained relatively constant throughout (Table 4d). As with 'Sylvia', polygalatol (Table 4e) was the dominant carbohydrate in 'Lady Di' and remained relatively constant in all shoot parts. Starch levels (Table 4f) were low in stems and inflorescences and remained

relatively constant. Starch occurred in higher concentrations in the leaves and decreased only towards the end of the experiment.

The carbohydrate contents for 'Lady Di' (Table 5) of the various plant parts followed much the same pattern as the figures presented for 'Sylvia' (Table 2) with an initial increase and then a rapid decline in the levels of the various carbohydrates. The totals for 'Lady Di' of glucose, sucrose, fructose and starch (Table 6) followed the same trends outlined above.

Experiment 2: Carbohydrate distribution and use of supplemental glucose in 'Sylvia' (*P. eximia* x *P. susannae*) during cold storage.

After pulsing with 10% glucose the concentration of glucose in the inflorescences decreased rapidly, with 80% of the glucose lost within the first 10 days of cold storage (Fig. 1a). Most of the glucose had been metabolised by d28 of cold storage. The sucrose concentration decreased at an increasing rate after completion of the glucose pulse treatment (Fig. 1b). Fructose behaved in a manner comparable to that of glucose but higher concentrations of fructose were present after 28 days of cold storage (Fig. 1c). The concentration of starch in the 'Sylvia' inflorescences decreased rapidly to low levels during the first 10 days during cold storage (Fig. 1d). The concentration of polygalatol (Fig. 1d) in the inflorescences of 'Sylvia' remained constant throughout the experiment. The concentration of inositol was low (< 0.03 mg/g) in the inflorescences of 'Sylvia' (Fig. 1e).

Both the glucose and the sucrose concentrations in the leaves of 'Sylvia' decreased rapidly after the completion of the glucose pulse treatment (Fig. 2a & 2b). After 10 days of cold storage the glucose and sucrose concentrations decreased by 60 and 80 percent, respectively. Extending the cold storage period from 21 days to 28 days resulted in a further rapid decrease in the concentration of these two sugars. No significant trend in the fructose concentration during the 28 day storage period was found in the leaves of 'Sylvia' (Fig. 2c). Starch levels in

the leaves of 'Sylvia' (Fig. 2d) decrease slowly at first but rapidly after 7 days in cold storage with the result that most starch had been depleted by day 21 in cold storage. The concentration of both polygalatol (Fig. 2e) and inositol (Fig. 2f) increased in the leaves of 'Sylvia' during the 28 day cold storage period.

The concentration of glucose (Fig. 3a), sucrose (Fig. 3b) and starch (Fig. 3d) decreased in the stems of 'Sylvia' after the completion of the glucose pulse treatment. The decrease in the concentrations of sucrose and starch in the stems was gradual over time whereas for glucose the decrease was rapid until day 10 in cold storage and then the concentration remained at low levels. The concentration of fructose in the stems rose initially before decreasing to low levels (Fig. 3c). The levels of polygalatol remained unchanged in the stems (Fig. 3e) whilst inositol (Fig. 3f) was present in very low levels (less than 1.0 mg /g).

The carbohydrate content of the various shoot parts declined rapidly after the shoots were placed into cold storage at $\pm 4.5^{\circ}\text{C}$. As is evident from Figure 4 and Table 3, carbohydrate levels decreased at a slower rate in 'Sylvia' shoots held under cold storage conditions when compared to 'Sylvia' shoots held at room temperature.

Discussion and Conclusion

High levels of Polygalatol occurred in all shoot parts of both 'Sylvia' (Table 1 & 2) and 'Lady Di' (Table 4 & 5). Concentrations remained relatively constant in all shoot parts over time. Polygalatol forms a major component of the soluble carbohydrate fraction in *P. neriifolia* (McConchie and Lang, 1993a). It was determined by McConchie and Lang (1993a), that the highest concentrations of polygalatol occur in the most acropetal phyllotactic divisions of the plant and decreased basipetally. Bieleski *et al.* (1992) were able to show that polygalatol is one of the major non-structural carbohydrates in the leaves of *P.*

eximia. McConchie and Lang (1993a & 1993b) concluded that polygalatol occurs in high concentrations in the soluble carbohydrate fraction in the leaf of *P. neriifolia* but that starch occurs in higher concentrations throughout the whole plant. Our results differ slightly. We found that polygalatol is a major component of the soluble carbohydrate fraction in the inflorescences, leaves and stems. It occurs in the highest concentrations in the leaves of 'Sylvia' and 'Lady Di' (Tables 1-6) where it makes up the highest concentration in the soluble carbohydrate fraction. It has been determined that polygalatol does not contribute to the carbohydrate metabolic pool and is unavailable for metabolism as the concentration of this soluble carbohydrate does not decrease during postharvest storage periods even when a cut shoot experiences extreme carbohydrate stress (Bieleski *et al.*, 1992, McConchie and Lang, 1993a). We concur with this statement, as polygalatol remains at high levels even after extended periods of cold storage when reducing sugars and starch levels decrease. This indicates that polygalatol is not important to the metabolically active carbohydrate pool. Bieleski *et al.* (1992) estimated that if polygalatol was fully available for metabolism, the survival time of *P. eximia* stored in the dark could be doubled. It was concluded by Bieleski (1982) that inert polygalatol may have a role in osmotic buffering and that its presence may form part of the adaptation process of many *Protea* species to dry climates.

Inositol occurs in extremely low concentrations in the various plant parts (never more than 3.5 g.mg⁻¹). The levels of inositol present in 'Sylvia' and 'Lady Di' are so low it can be regarded as insignificant in terms of acting as a reserve carbohydrate.

Stephens *et al.* (2001a, 2005) showed that glucose and fructose were present in significantly higher concentrations than sucrose in the inflorescences of 'Sylvia' (*P. eximia* x *P. susannae*). Cultivars 'Cardinal' (*P. eximia* x *P. susannae*), 'Carnival' (*P. compacta* x *P. neriifolia*), 'Ivy' (*P. laticolor* selection), 'Pink Ice' (*P. compacta* x *P.*

susannae), 'Sheila' (*P. magnifica* x *P. burchellii*) and 'Susara' (*P. magnifica* x *P. susannae*) contained more glucose and fructose in the inflorescences than sucrose at harvest (Stephens *et al.*, 2005). McConchie and Lang (1993a, 1993b), using an HPLC procedure to determine soluble carbohydrates per unit leaf area, observed that in *P. neriifolia*, *P. eximia* and 'Pink Ice' (*P. susannae* x *P. compacta*), starch was the most abundantly occurring non-structural carbohydrate at harvest, and that sucrose occurred in higher concentrations in the leaves than both glucose and fructose (both of which were reported to occur in trace amounts). The concentration of starch was shown to be higher than that of polygalatol. Stephens *et al.* (2005) using an Auto analyser procedure, concluded that the concentrations of glucose, fructose and sucrose were comparable in the leaves of the cultivars that were studied by him.

Sucrose was determined, through the use of a HPLC procedure modified from Bieleski *et al.* (1992), to occur in lower concentrations in the inflorescences of 'Sylvia' and 'Lady Di' at harvest than glucose and fructose (Table 1 & 4). The leaves and stems contain higher concentrations of sucrose than glucose and fructose (Table 1 & 4). Very little published data exist on the carbohydrate profile of *Protea* stems. It appears that stems do contain significant amounts of reserve carbohydrates in the form of glucose, fructose and sucrose but very little starch, and can function as a storage area for carbohydrate reserves. This, however, seems to be cultivar specific (Table 1-6). The data compiled by Stephens *et al.* (2005) compare favourably to the data presented (Tables 1 & 4) for the glucose content in the inflorescences of 'Sylvia' and 'Lady Di' as well as the overall distribution of carbohydrates in 'Lady Di'. Compelling evidence thus exists suggesting that sucrose and starch may not be the main forms of non-structural reserve metabolic carbohydrates in many *Protea* species and cultivars as had previously been suggested by Bieleski *et al.* (1992), McConchie and Lang (1993a, 1993b) and McConchie *et al.* (1991). However, the carbohydrate profile of the 'Sylvia' leaves correlate well with the

results reported on by Bieleski *et al.* (1992), McConchie and Lang (1993a, 1993b) and McConchie *et al.* (1991). Individual species and cultivars appear to differ in respect to the type and amount of carbohydrates that are present both just prior to harvest and post pulsing treatment. The inflorescences, stems and leaves have all been shown (Tables 1-6) to contain carbohydrates that can function as reserve metabolites. The metabolically active carbohydrate pool therefore consists of sugars that occur in the inflorescences, leaves and stems as well as starch from the leaves.

Although, before pulsing, the glucose content in the stems of both cultivars is low when compared to the levels in the leaves and inflorescences, directly after pulsing, most of the glucose was located in the stems (Table 2 & 5). This implies that the stems act as an important reservoir of glucose when shoots are pulsed. The decrease in the glucose concentration in the stems after pulsing indicates, as suggested by Stephens *et al.* (2005), that slow transport of the glucose to other shoot parts occurs as transpiration takes place during storage. An increase in the starch concentration occurred in the leaves of 'Sylvia' directly after the glucose pulsing treatment (Table 1 & 2). It appears, therefore, that glucose is rapidly converted into starch in the leaves but not in the stems of 'Sylvia'. This is in agreement with the findings of Stephens *et al.* (2005). In the case of 'Lady Di' however, the conversion of glucose to starch in the leaves did not appear to take place (Table 4 & 5).

It can be deduced from the fact that the concentration of glucose in the leaves and stems, and starch in the leaves, decrease more rapidly than other reducing sugars in the various plant parts, that glucose and starch are the main reserve carbohydrates present in glucose pulsed 'Sylvia' shoots (Table 1, 2 & 3). These two carbohydrates are utilized more rapidly, possibly for either maintaining cellular function or acting as a source of energy for the inflorescences. Dai (1993) established that 24h after the application of C¹⁴ sucrose to *P. neriifolia* stems, more than

half the total amount of radioactivity was found in the nectar. Stephens *et al.* (2005) found that the levels of glucose, fructose and sucrose concentrations in the inflorescences of a number of *Protea* species did not decline as significantly as the concentrations of these sugars in the leaves. This lends weight to the hypothesis that carbohydrate depletion in the leaves, is partly caused by the energy requirements of the developing inflorescences and nectar production (Paull and Dai, 1990).

‘Sylvia’ shoots stored at room temperature lose between 25-30% of their reserve carbohydrates within the first day after pulsing and a further 10-15% during the next 24h storage period (Table 3). In contrast, it took 10 days for the reserve carbohydrates to decrease by 50% at a storage temperature of 4.5°C (Figure 4d). Higher respiration rates in ‘Sylvia’ have been correlated with increases in the occurrence and severity of leaf blackening. Increased respiration rates have been recorded for both ‘Sylvia’ (Stephens *et al.*, 2001a) and *P. neriifolia* (Dai and Paull, 1995, Ferreira, 1986) after harvest. The significance of low temperatures in reducing postharvest respiration to preserve reserve carbohydrates has been stressed by other reports (Ferreira and De Swardt, 1980, Stephens *et al.*, 2001a, Newman *et al.*, 1990). The rapid decrease in the carbohydrate concentration after 21 days in cold storage is possibly due to the rapid senescence of the product and, possibly, also the result of increased micro-organism activity.

The relationship between reserve carbohydrates and in particular glucose and postharvest leaf blackening is now well established. However, it remains unexplained as to why glucose is effective in reducing postharvest leaf blackening in only some cultivars/species. Furthermore, the apparent inability of ‘Lady Di’ to rapidly convert glucose taken up through pulsing to starch, as was the case with ‘Sylvia’, is worth further research to determine whether this phenomenon also applies to other *Protea* cultivars and species.

Literature Cited

- Akamine, E.K., T. Goo, and R. Suehisa. 1979. Relationship between leaf darkening and chemical composition of leaves of *Protea*. *Flor. Rev.* 163:62-63, 107-108.
- Bieleski, R.L. 1982. Sugar alcohols. In: Loewus, F.A and W. Tanner. (ed.), *Plant carbohydrates I: Intracellular carbohydrates*. Pp. 158-192. *Encyclopedia of Plant Physiology*, NS vol. 13A. Springer-Verlag, Berlin.
- Bieleski, R.L., J. Ripperda, J.P. Newman, and M.S. Reid. 1992. Carbohydrate changes and leaf blackening in cut flower stems of *Protea eximia*. *J. Am. Soc. Hort. Sci.* 117:124-127.
- Brink, J.A. 1987. The influence of the flower-head on leaf browning on cut *Protea neriifolia*. *Protea News* 5:11-12.
- Brink, J.A. and G.H. de Swardt. 1986. The effect of sucrose in vase solution on leaf browning of *Protea neriifolia* R. Br.. *Acta Hort.* 185:111-119.
- Cowling, R.M. and D.T. Mitchell. 1981. Sugar composition, total nitrogen accumulation of C₁₄ assimilates in floral nectaries of *Protea* species. *J. S. Afr. Bot.* 47:743-750.
- Dai, J.W. 1993. Postharvest leaf blackening in *Protea neriifolia* R. Br.. Ph.D. Diss, Univ. of Hawaii, Honolulu.
- Dai, J.W., Paull, R.E., 1995. Source-sink relationship and *Protea* postharvest leaf blackening. *J. Am. Soc. Hort. Sci.* 120:475-480
- Ferreira, D.I. 1986. The influence of temperature on the respiration rate and browning of *Protea neriifolia* R. BR.. *Inflorescences. Acta Hort.* 185:121-129.
- Ferreira, D.I. and G.H. De Swardt. 1980. Veranderinge in die respirasietempo, styselkonsentrasie, totale vry reduserende suikerkonsentrasie en totale vry aminosuur-konsentrasie in verouderende rose (cv. Sonia). *Agroplantae*, 12:23-28

- Goszczynska, D., H. Itzhaki, A. Borochoy, and A.H. Halevy, 1990. Effects of sugar on physical and compositional properties of rose petal membranes. *Scientia Hort.*, 43:313-320.
- Haasbroek, F.J., G.G. Rousseau, and J.P. de Villiers. 1973. Effect of gamma-rays on cut blooms of *Protea compacta* R. Br., *P. longiflora* Lamarck and *Leucospermum cordifolium* Salisb. ex Knight. *Agroplanta* 5:33-42.
- Halevy, A.H. and S. Mayak. 1979. Senescence and postharvest physiology of cut flowers, Part 1. *Hort Rev.* 1:204-236.
- Jones, R.B. 1991. Understanding and controlling leaf blackening in *Protea* leaves: the use of high concentrations of sucrose. Proc. Int. Protea Assn. 6th Biennial Conf., Perth, Western Australia. Pp 313-322.
- Jones, R.B., R. McConchie, W.G. van Doorn, and M.S. Reid. 1995. Leaf blackening in cut *Protea* flowers. *Hort. Rev.* 17:173-201.
- McConchie, R. and N.S. Lang. 1993a. Postharvest leaf blackening and preharvest carbohydrate status in three *Protea* species. *HortScience* 28:313-316.
- McConchie, R. and N.S. Lang. 1993b. Carbohydrate metabolism and possible mechanisms of leaf blackening in *Protea neriifolia* under dark postharvest conditions. *J. Am. Soc. Hort. Sci.* 118:355-361.
- McConchie, R., N.S. Lang, and G.A. Gross. 1991. Carbohydrate depletion and leaf blackening in *Protea neriifolia*. *J. Am. Soc. Hort. Sci.* 116:1019-1024.
- Mulder, P.W.A. 1977. Primêre meganismes betrokke by die bruinwording van loofblare in *Protea neriifolia*. M.Sc. Thesis. Randse Afrikaans Univ., Johannesburg, South Africa.
- Newman, J.P., W.G. van Doorn and M.S. Reid. 1990. Carbohydrate stress causes leaf blackening in Proteas. *Acta Hort.* 264:103-108.
- Paulin, A. 1986. Influence of exogenous sugars on the evolution of some senescence parameters of petals. *Acta Hort.* 181:183-193.
- Paull, R.E. and J.W. Dai. 1989. *Protea* postharvest black leaf a problem in search of a solution. *J. Int. Protea Assoc.* 17:26-31.

- Reid, M.S., W.G. van Doorn and J.P. Newman. 1989. Leaf blackening in proteas. *Acta Hort.* 261:81-84.
- Stephens, I.A., D.M. Holcroft, and G. Jacobs. 2001a. Low temperatures and girdling extend vase life of 'Sylvia' Proteas. *Acta Hort.* 545:205-214.
- Stephens, I.A., D.M. Holcroft, and G. Jacobs. 2001b. Glucose prevents leaf blackening in 'Sylvia' proteas. *Postharv. Biol. Technol.* 23:237-240.
- Stephens, I.A., C. Meyer, D.M. Holcroft, and G. Jacobs. 2005. Carbohydrates and Postharvest Leaf Blackening of Proteas. *HortScience* 40(1):181-184
- van Doorn, W.G. 2001. Leaf Blackening in Protea Flowers: Recent Developments. *Acta Hort.* 545:197-203.
- van Wyk, B. E. and S.W. Nicholson. 1995. Xylose is a major nectar sugar in *Protea* and *Faurea*. *J. S. Afr. Sci.* 91:151-152.
- Whitehead, C.S., L. O'Reilly, J. Weerts, M.M. Zaayman, and W. Gaum. 2003. The effect of Sucrose Pulsing on Senescing Climacteric Cut Flowers. *Acta Hort.* 599:549-557.

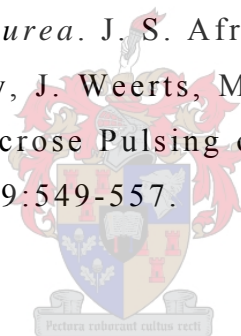


Table 1. Concentration (mg.g^{-1} dry weight) of glucose, fructose, sucrose, inositol, polygalatol and starch in shoot parts of *Protea* cv 'Sylvia' (*P. eximia* x *P. susannae*) before and at different times after pulsing with a 10% glucose solution (10 mL per stem taken up).

a								
Shoot Part	Glucose concentration per shoot part in mg.g^{-1} dry weight							
	Before Pulsing		After Pulsing		Pulsed+1d		Pulsed+2d	
Inflorescence	22.4	a	23.6	a	17.7	b	16.5	b
Leaves	3.8	c	11.6	a	6.2	b	4.6	bc
Stems	1.4	d	25.4	a	13	b	10.2	c
b								
Shoot Part	Fructose concentration per shoot part in mg.g^{-1} dry weight							
	Before Pulsing		After Pulsing		Pulsed+1d		Pulsed+2d	
Inflorescence	28.5	a	26.9	ab	23.6	bc	21.3	c
Leaves	7.2	b	11.3	a	9.8	a	7.2	b
Stems	4.1	c	6.6	b	8.6	a	8.4	a
c								
Shoot Part	Sucrose concentration per shoot part in mg.g^{-1} dry weight							
	Before Pulsing		After Pulsing		Pulsed+1d		Pulsed+2d	
Inflorescence	1.9	b	1.6	b	1.7	b	3	a
Leaves	18.3	b	23.7	a	14	bc	12.9	c
Stems	12.6	ab	13.4	a	11.3	b	9.4	c
d								
Shoot Part	Inositol concentration per shoot part in mg.g^{-1} dry weight							
	Before Pulsing		After Pulsing		Pulsed+1d		Pulsed+2d	
Inflorescence	0.43	b	0.58	a	0.25	c	0.28	c
Leaves	2.1	b	2.5	ab	2.3	ab	2.6	a
Stems	0.38	c	1	a	0.57	b	0.54	b
e								
Shoot Part	Polygalatol concentration per shoot part in mg.g^{-1} dry weight							
	Before Pulsing		After Pulsing		Pulsed+1d		Pulsed+2d	
Inflorescence	44.8	a	39.6	a	42	a	39.7	a
Leaves	51.5	a	49.3	ab	48.2	b	49.9	ab
Stems	32	a	31	a	30	a	33	a
f								
Shoot Part	Starch concentration per shoot part in mg.g^{-1} dry weight							
	Before Pulsing		After Pulsing		Pulsed+1d		Pulsed+2d	
Inflorescence	2.2	b	2.8	a	1.04	c	0.53	d
Leaves	10.9	b	21.4	a	19.5	a	4	b
Stems	0.71	ab	0.91	a	0.68	ab	0.47	b

Mean separation within plant part and the various carbohydrates at the 5% level, LSD test.

Table 2. Content of different carbohydrate types (glucose, fructose, sucrose, inositol, polygalatol and starch) in shoot parts of *Protea* cv 'Sylvia' (*P. eximia* x *P. susannae*) before and at different times after pulsing with a 10% glucose solution (10 mL per stem taken up).

a								
Shoot Part	Dry Weight (g)	Glucose content per shoot part in mg						
		Before Pulsing	After Pulsing	Pulsed+1d		Pulsed+2d		
Inflorescence	24.42	348.9	a 496.5	a	336.7	a	321	a
Leaves	19.31	97	b 282.3	a	143.2	b	115.4	b
Stems	15.15	29.4	c 509.5	a	244.5	b	239.1	b
b								
Shoot Part	Dry Weight (g)	Fructose content per shoot part in mg						
		Before Pulsing	After Pulsing	Pulsed+1d		Pulsed+2d		
Inflorescence	24.42	442.2	a 606.8	a	447.9	a	419.1	a
Leaves	19.31	179.1	b 190.2	b	225.4	a	178.7	a
Stems	15.15	82.9	a 85	a	165.2	a	193.6	a
c								
Shoot Part	Dry Weight (g)	Sucrose content per shoot part in mg						
		Before Pulsing	After Pulsing	Pulsed+1d		Pulsed+2d		
Inflorescence	24.42	30.8	a 36.6	a	33.5	a	57.2	a
Leaves	19.31	464.5	a 618.5	a	328.3	a	326.9	a
Stems	15.15	246.2	a 265.8	a	214.4	a	209.9	a
d								
Shoot Part	Dry Weight (g)	Inositol content per shoot part in mg						
		Before Pulsing	After Pulsing	Pulsed+1d		Pulsed+2d		
Inflorescence	24.42	6.6	a 12.4	a	5.1	a	5.4	a
Leaves	19.31	54.6	a 64.2	a	56.3	a	65.3	a
Stems	15.15	7.5	b 20.3	a	11.3	b	12.4	b
e								
Shoot Part	Dry Weight (g)	Polygalatol content per shoot part in mg						
		Before Pulsing	After Pulsing	Pulsed+1d		Pulsed+2d		
Inflorescence	24.42	683.8	a 846.8	a	798.5	a	788.7	a
Leaves	19.31	1270.2	a 1273.1	a	1154.2	a	1290.3	a
Stems	15.15	616.8	b 600.5	b	583.3	b	735.5	a

Table 2. Continue

f

Shoot Part	Dry Weight (g)	Starch content per shoot part in mg							
		Before Pulsing		After Pulsing		Pulsed+1d		Pulsed+2d	
Inflorescence	24.42	33.9	ab	55.9	a	20.4	b	10.4	b
Leaves	19.31	269.7	ab	535.2	a	448.6	ab	98.9	b
Stems	15.15	13.8	ab	17.3	a	13.3	ab	11.3	b

Mean separation within plant part and the various carbohydrates at the 5% level, LSD test.

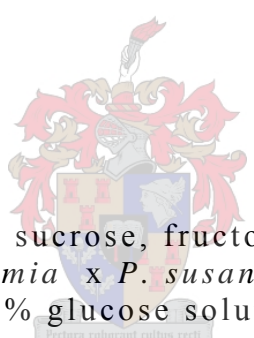


Table 3. Content of glucose, sucrose, fructose and starch in shoot parts of *Protea* cv 'Sylvia' (*P. eximia* x *P. susannae*) before and at different times after pulsing with a 10% glucose solution (10 mL per stem taken up).

Shoot Part	Dry Weight (g)	Actual Carbohydrate content per shoot part in mg							
		Before Pulsing		After Pulsing		Pulsed+1d		Pulsed+2d	
Inflorescence	24.42	855	a	1195	a	838	a	807	a
Leaves	19.31	1010	a	1626	a	1145	a	720	a
Stems	15.15	372	a	877	a	637	a	654	a
Total CHO	58.88	2238	b	3699	a	2621	b	2181	b

Mean separation within plant part and the various carbohydrates at the 5% level, LSD test.

Table 4. Concentration (mg.g^{-1} dry weight) of glucose, fructose, sucrose, inositol, polygalatol and starch in shoot parts of *Protea* cv 'Lady Di' (*P. magnifica* x *P. compacta*) before and at different times after pulsing with a 10% glucose solution (10 mL per stem taken up).

a								
Shoot Part	Glucose concentration per shoot part in mg.g^{-1} dry weight							
	Before Pulsing		After Pulsing		Pulsed+1d		Pulsed+2d	
Inflorescence	27	a	23	b	21	b	18	c
Leaves	11	b	18	a	12	b	9	b
Stems	6	c	28	a	20	b	21	b
b								
Shoot Part	Fructose concentration per shoot part in mg.g^{-1} dry weight							
	Before Pulsing		After Pulsing		Pulsed+1d		Pulsed+2d	
Inflorescence	35	a	30	b	29	b	25	c
Leaves	17	a	16	a	15	a	13	a
Stems	12	a	10	a	12	a	11	a
c								
Shoot Part	Sucrose concentration per shoot part in mg.g^{-1} dry weight							
	Before Pulsing		After Pulsing		Pulsed+1d		Pulsed+2d	
Inflorescence	2	a	1	b	1	b	2	a
Leaves	11	a	9	ab	5	b	8	ab
Stems	13	a	11	b	6	c	7	c
d								
Shoot Part	Inositol concentration per shoot part in mg.g^{-1} dry weight							
	Before Pulsing		After Pulsing		Pulsed+1d		Pulsed+2d	
Inflorescence	1.5	a	1	ab	0.6	b	0.5	b
Leaves	2	a	2	a	2	a	2	a
Stems	0.6	b	1	a	1	a	1	a
e								
Shoot Part	Polygalatol concentration per shoot part in mg.g^{-1} dry weight							
	Before Pulsing		After Pulsing		Pulsed+1d		Pulsed+2d	
Inflorescence	41	a	39	a	42	a	43	a
Leaves	62	a	52	a	53	a	53	a
Stems	31	a	27	a	27	a	28	a
f								
Shoot Part	Starch concentration per shoot part in mg.g^{-1} dry weight							
	Before Pulsing		After Pulsing		Pulsed+1d		Pulsed+2d	
Inflorescence	3	a	2	b	1.9	b	0.8	c
Leaves	9	a	10	a	7	a	4	a
Stems	0.53	ab	0.6	a	0.55	ab	0.46	b

Mean separation within plant part and the various carbohydrates at the 5% level, LSD test.

Table 5. Content of different carbohydrate types (glucose, fructose, sucrose, inositol, polygalatol starch in shoot parts of *Protea* cv ‘Lady Di’ (*P. magnifica* x *P. compacta*) before and at different times after pulsing with a 10% glucose solution (10 mL per stem taken up).

a									
Shoot Part	Dry Weight (g)	Glucose content per shoot part in mg							
		Before Pulsing		After Pulsing		Pulsed+1d		Pulsed+2d	
Inflorescence	23.61	643	a	569	ab	415	bc	320	c
Leaves	29.09	331	ab	438	a	331	ab	191	b
Stems	23.96	155	c	609	a	380	b	352	b
b									
Shoot Part	Dry Weight (g)	Fructose content per shoot part in mg							
		Before Pulsing		After Pulsing		Pulsed+1d		Pulsed+2d	
Inflorescence	23.61	818	a	736	ab	557	bc	452	c
Leaves	29.09	481	a	369	ab	421	ab	241	b
Stems	23.96	294	a	234	b	222	b	191	b
c									
Shoot Part	Dry Weight (g)	Sucrose content per shoot part in mg							
		Before Pulsing		After Pulsing		Pulsed+1d		Pulsed+2d	
Inflorescence	23.61	53	a	38	ab	28	b	40	a
Leaves	29.09	317	a	209	ab	140	b	157	a
Stems	23.96	302	a	237	b	117	c	116	c
d									
Shoot Part	Dry Weight (g)	Inositol content per shoot part in mg							
		Before Pulsing		After Pulsing		Pulsed+1d		Pulsed+2d	
Inflorescence	23.61	36	a	20	ab	13	b	9	b
Leaves	29.09	55	a	46	ab	55	ab	41	b
Stems	23.96	14	b	25	a	18	b	16	b
e									
Shoot Part	Dry Weight (g)	Polygalatol content per shoot part in mg							
		Before Pulsing		After Pulsing		Pulsed+1d		Pulsed+2d	
Inflorescence	23.61	965	a	965	a	815	a	750	a
Leaves	29.09	1808	a	1264	ab	1363	ab	1041	b
Stems	23.96	740	a	608	ab	520	bc	446	c

Table 5. Continue

f

Shoot Part	Dry Weight (g)	Starch content per shoot part in mg							
		Before Pulsing		After Pulsing		Pulsed+1d		Pulsed+2d	
Inflorescence	23.61	79	a	49	b	43	b	15	c
Leaves	29.09	268	a	279	a	197	a	90	a
Stems	23.96	13	a	12	ab	10	b	7	c

Mean separation within plant part and the various carbohydrates at the 5% level, LSD test.

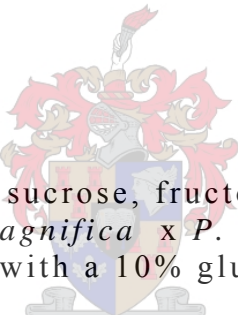


Table 6. Content of glucose, sucrose, fructose and starch in shoot parts of *Protea* cv 'Lady Di' (*P. magnifica* x *P. compacta*) before and at different times after pulsing with a 10% glucose solution (10 mL per stem taken up).

Shoot Part	Dry Weight (g)	Actual Carbohydrate content per shoot part in mg							
		Before Pulsing		After Pulsing		Pulsed+1d		Pulsed+2d	
Inflorescence	23.61	1593	a	1392	ab	1043	bc	827	c
Leaves	29.09	1397	a	1295	ab	1089	ab	679	b
Stems	23.96	764	ab	1092	a	729	ab	666	b
Total CHO	76.66	3754	a	3779	a	2861	b	2172	c

Mean separation within plant part and the various carbohydrates at the 5% level, LSD test.

Fig 1. Effect of postharvest pulsing with a 10% glucose solution (10 mL per shoot taken up) on *Sylvia* (*P. eximia* x *P. susannae*) leaf mg.g^{-1} weight of glucose (a), sucrose (b), fructose (c), starch (d), polygalatol (e), and inositol (f), measured from pulsing, and the time periods indicated after pulsing; 1d at 4.5°C, 2.5d at 4.5°C, 7d at 4.5°C, 10.5d at 4.5°C, 14d at 4.5°C, 21d at 4.5°C and 28d at 4.5°C.

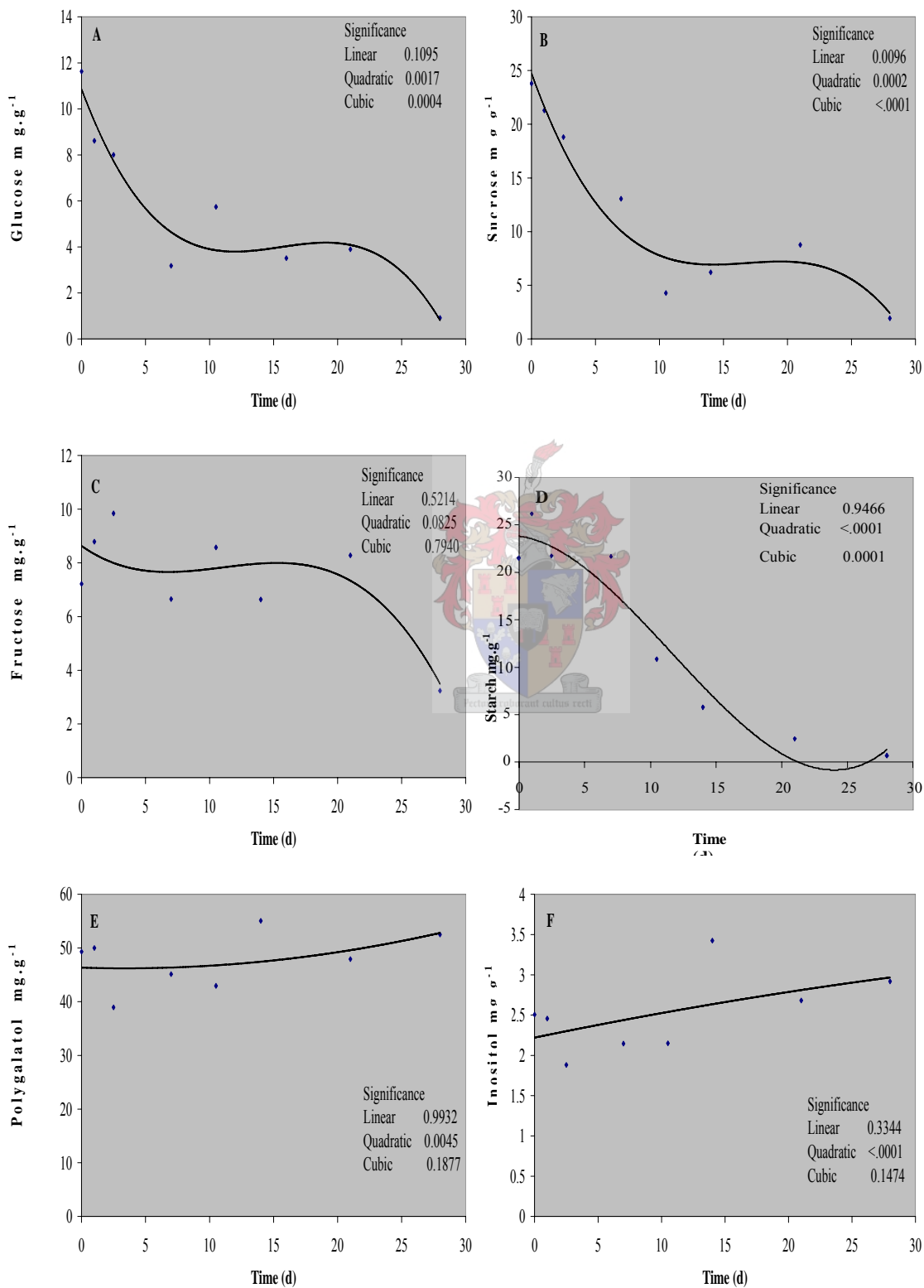


Fig 2. Effect of postharvest pulsing with a 10% glucose solution (10 mL per shoot taken up) on *Sylvia* (*P. eximia* x *P. susannae*) stem mg.g⁻¹ weight of glucose (a), sucrose (b), fructose (c), starch (d), polygalatol (e) and inositol (f), measured from pulsing, and the time periods indicated after pulsing; 1d at 4.5°C, 2.5d at 4.5°C, 7d at 4.5°C, 10.5d at 4.5°C, 14d at 4.5°C, 21d at 4.5°C and 28d at 4.5°C.

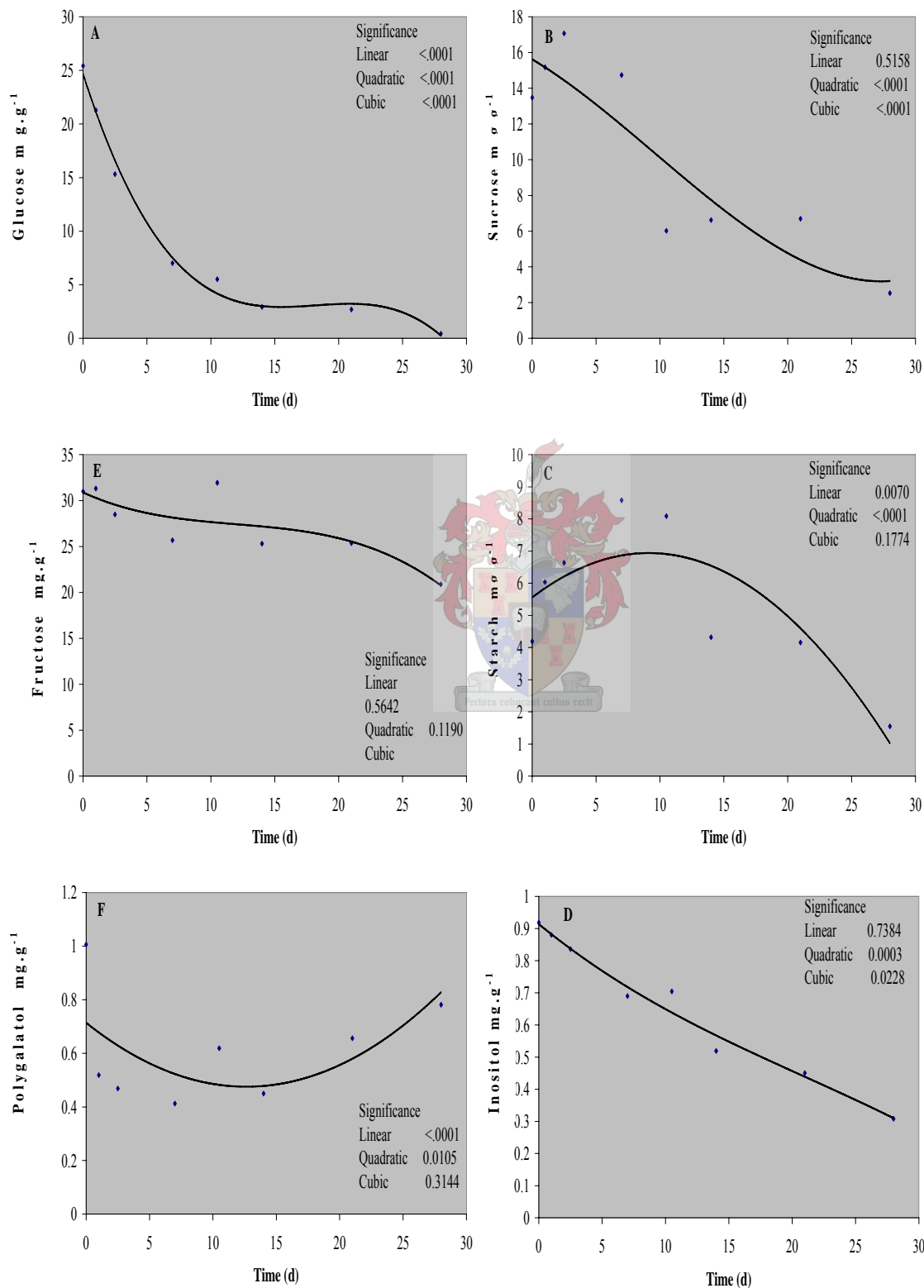


Fig 3. Effect of postharvest pulsing with a 10% glucose solution (10 mL per shoot taken up) on *Sylvia* (*P. eximia* x *P. susannae*) inflorescence mg.g⁻¹ weight of glucose (a), sucrose (b), fructose (c), starch (d), polygalatol (e) and inositol (f), measured from pulsing, and the time periods indicated after pulsing; 1d at 4.5°C, 2.5d at 4.5°C, 7d at 4.5°C, 10.5d at 4.5°C, 14d at 4.5°C, 21d at 4.5°C and 28d at 4.5°C.

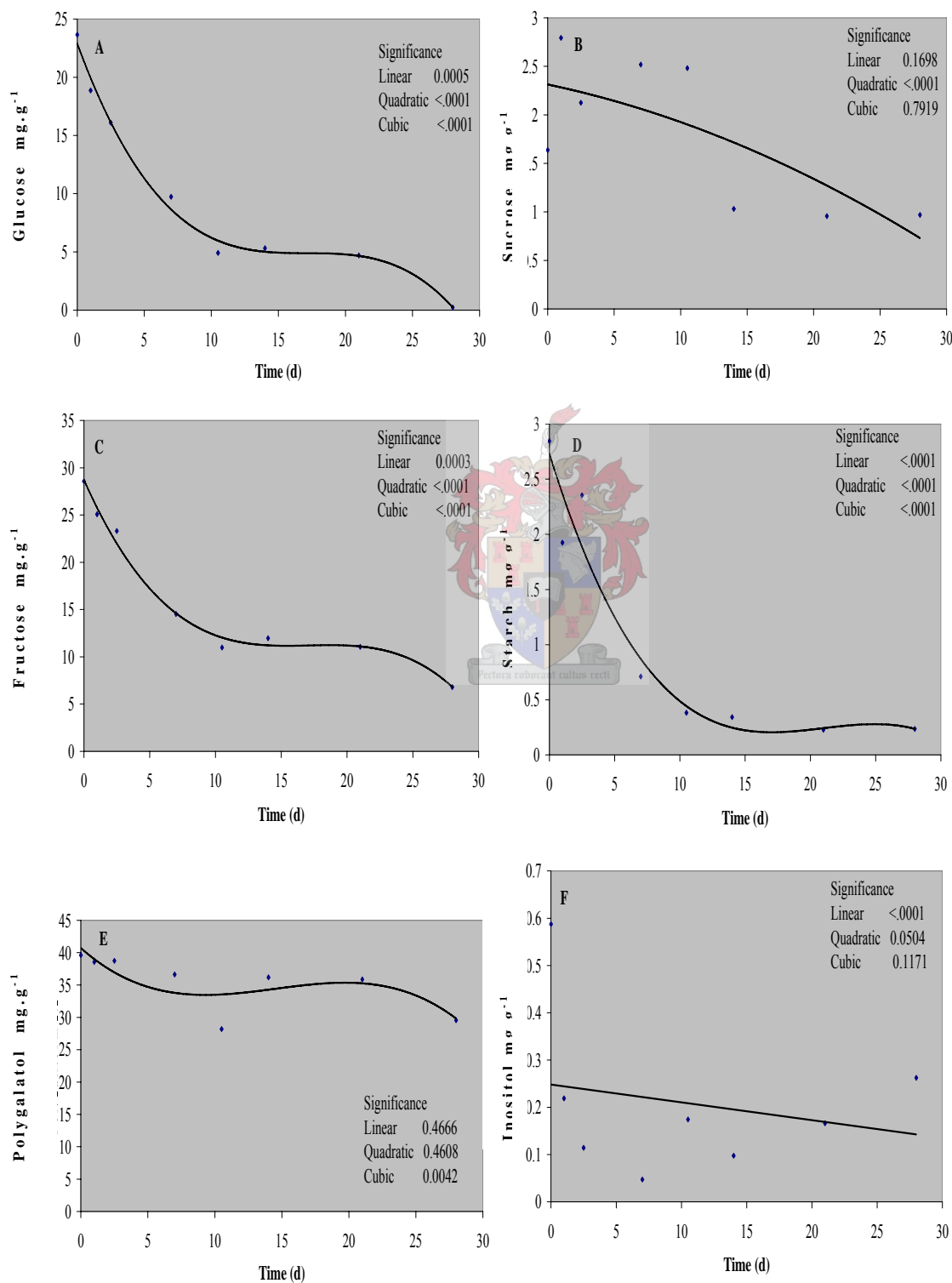
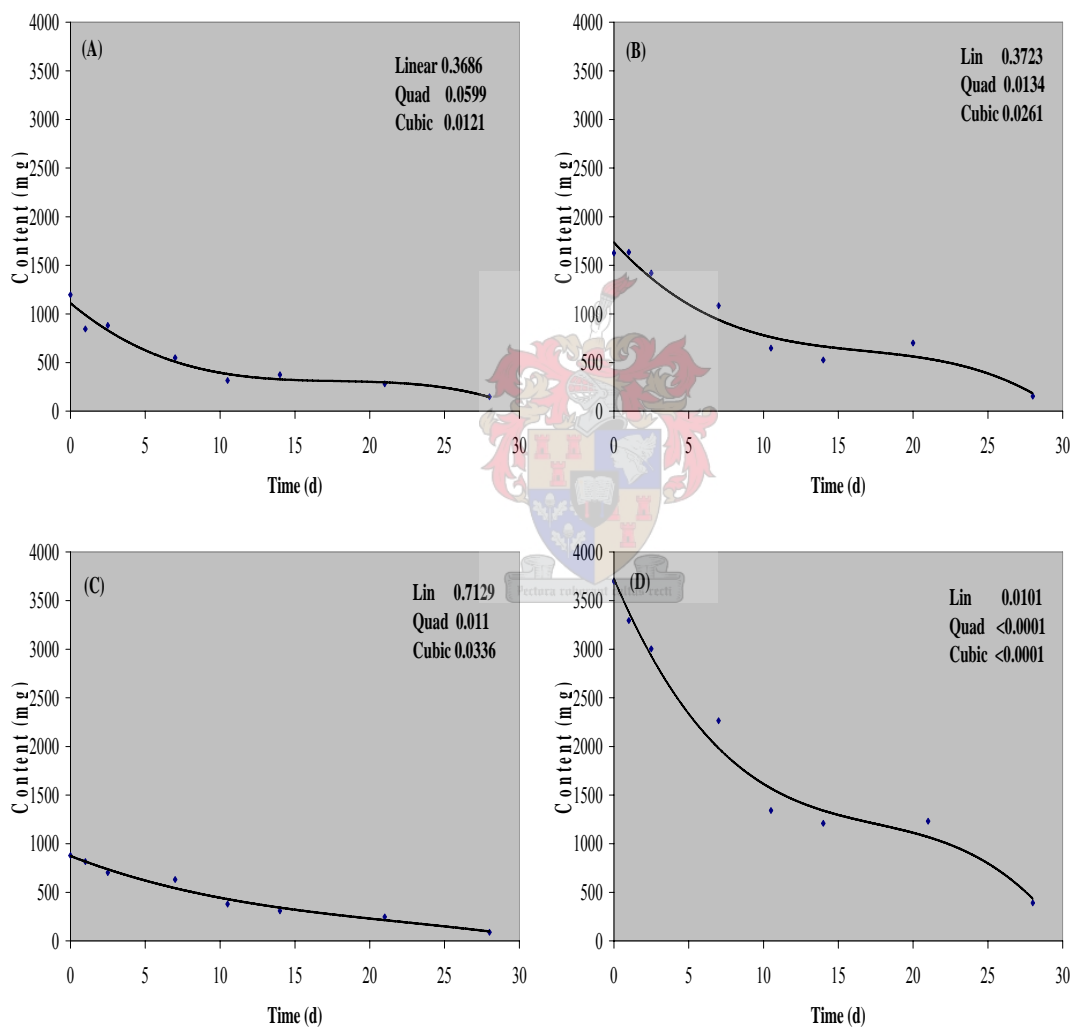


Fig 4. Effect of postharvest pulsing with a 10% glucose solution (10 mL per stem taken up) on actual dry mass carbohydrate content (glucose, sucrose, fructose and starch) of ‘Sylvia’ (*P. eximia* x *P. susannae*) inflorescence (a), leaves (b) and stems (c) measured from pulsing, and the time periods indicated after pulsing; 1d at 4.5°C, 2.5d at 4.5°C, 7d at 4.5°C, 10.5d at 4.5°C, 14d at 4.5°C, 21d at 4.5°C and 28d at 4.5°C. Total carbohydrates are presented in Fig d.



4 Paper II Pre- and post-harvest factors affecting leaf blackening in *Proteas*

Abstract.

The effect of the plant growth regulator Prohexadione-calcium (P-Ca) on the occurrence of postharvest leaf blackening as well as the suppression of reproductive shoot growth on 'Sylvia' (*P. eximia* x *P. susannae*) *Protea* shoots was determined. P-Ca had no effect on preventing or delaying postharvest leaf blackening when applied to 'Sylvia'. The lengths of reproductive shoots, as well as cumulative postharvest leaf blackening were also examined on 'Sylvia' plants treated with Paclobutrazol (cultar) prior to harvest. There was a significant reduction in the length of treated reproductive shoots when compared to the control. The levels of cumulative postharvest leaf blackening were also significantly lower on shoots that were harvested from treated plants when compared to control shoots. However, the removal of the spring growth flush had no significant influence on the level of postharvest leaf blackening when compared to the control. This indicates that Cultar does not inhibit postharvest leaf blackening through the suppression of stem elongation and that blackening is enhanced by high levels of gibberellins in the plants.

The rate and severity of postharvest leaf blackening on 'Sylvia' inflorescence bearing shoots increases dramatically during the spring and early summer months in the Western Cape Province of South Africa. Preharvest leaf discolouration in October 2003, appeared first, and was more severe on those shoots that subtended inflorescences that were nearing commercial maturity. Shoots that subtended less mature inflorescences were not as negatively affected. An excessively warm

period of weather occurred a few days prior to the appearance of the preharvest leaf blackening symptoms.

Introduction

The extent and rate of leaf blackening in *Protea* plants appears to vary widely between species (McConchie and Lang, 1993b) clones within species as well as the maturity, time of year and even the time of day at which they are harvested (Paull and Dai, 1990). Preharvest leaf blackening can be caused by a number of factors all involving some form of physical damage to the leaf. According to Jones *et al.* (1995) mechanical damage, insect or fungal attack, or excessive heat can all induce discoloration of *Protea* leaves. Water stress has also been found to play a major role in preharvest leaf blackening (Starke, 1979, Forsberg, 1988 & 1993). Postharvest leaf blackening in *Protea* species and cultivars does not appear to be the product of one factor, but rather the result of a complex chain of events (Jones *et al.*, 1995) that eventually culminate in the oxidation of phenolic compounds.

The developing inflorescences high respiration rate and nectar production (Dai and Paull, 1995) combined with the relatively low availability of translocatable carbohydrates in the leaves (Jones *et al.*, 1995) results in a dramatic decline of translocatable carbohydrates followed by the initiation of leaf blackening. This lack of an immediately available energy source may play a role in the destabilization of membranes in plant cells. Most membranes have a very high-energy demand (Jones *et al.*, 1995) and a constant source of metabolites is needed to keep the membranes intact. Soluble sugars are known to protect membranes from desiccation by preventing membrane fusion, phase transition and phase separation (Crowe and Crowe, 1986, Caffrey *et al.*, 1988, Crowe and Crowe, 1992). Application of an endogenous supply of nutrients, such as sucrose, can extend cut flower shelf life by aiding flower opening (van Doorn *et al.*, 2001) and

maintaining the membrane integrity of the delicate petal tissue (Halevy and Mayak, 1979). Goszezynska *et al.* (1990) determined that the application of exogenous sucrose to roses reduced the age induced increase in membrane lipid microviscosity. Jones (1995) found similar results in leaf tissue of a few species of *Leucadendron* post sucrose treatment. Dai and Paull (1995) reported that carbohydrates were transported from the leaves to the developing inflorescence of *P. neriifolia* (a strong sink). This translocation of carbohydrates is thought to be the catalyst for the process of leaf blackening (McConchie *et al.*, 1991, Bielecki *et al.*, 1992, McConchie and Lang 1993b). Newman *et al.* (1990) discovered that girdling the stem just below the inflorescence resulted in a significant reduction in the rate and severity of leaf blackening, this indicates that the carbohydrate demand of the inflorescence is a leading cause of leaf blackening.

Many *Protea* species store large amounts of carbohydrates in the form of 1,5-anhydro-D-glucitol (polygalatol), a derivative of sorbitol. After harvest the leaf concentrations of sucrose, glucose, fructose and starch decrease to relatively low concentrations while the concentration of polygalatol (non-reducible sugar alcohol) remains fairly constant over time (McConchie *et al.*, 1991, Bielecki *et al.*, 1992, McConchie and Lang, 1993a,b). This seems to indicate that most leaves are source leaves after flower initiation (McConchie and Lang, 1993a). A significant discovery was made by Dai and Paull (1995) when they determined that the greater the number of mature leaves on the stem the slower the rate of leaf blackening and flower senescence. This seems to indicate that the greater the carbohydrate storage potential in the plant the slower the rate of leaf blackening. *Protea* plants follow the characteristic carbohydrate storage pattern of evergreen plant species with significantly higher concentrations of sugars and starch occurring in the leaves of the plant than in the stem (Hettasch *et al.*, 2001). While the leaves and stem of a *Protea* plant act as a source, the inflorescence acts exclusively as a sink. The stage of the inflorescence development at harvest has a significant impact on the demands placed on the

reserves in the cut shoot. Paull and Dai (1990) found that the stage of flower development plays a critical role in the rate of leaf blackening, with blackening occurring at a far more pronounced rate on stems harvested with mature inflorescences at a closed bud stage in comparison to stems with more open mature inflorescences attached.

The developing inflorescence, with its high respiration rate and nectar production, acts as a strong sink (Dai and Paull, 1995) causing photosynthetic products to be transported to the developing inflorescence where they act as an energy source. Ferreira (1986) found that the maturity of the inflorescence has a direct effect on the rate of its respiration, with less developed inflorescence having significantly higher rates of respiration than mature open inflorescence. Increased respiration rates were shown by Stephens *et al.* (2001b) to correlate with an increase in leaf blackening of 'Sylvia' *Protea* leaves. It may, therefore, be beneficial to harvest *Protea* shoots at a stage when the inflorescence is more open to avoid the high carbohydrate demands of the developing inflorescence. This should result in a decrease in the occurrence of postharvest leaf blackening as a decline in the demand for fixed carbohydrates correlates well to a drop in the rate of leaf blackening (Meyer, 2003). Dai and Paull (1995) supplied ^{14}C -sucrose to leaves subtending freshly harvested *P. neriifolia* inflorescences, twenty-four hours later 58% of the radiolabeled carbon could be accounted for in the nectar. This indicates that nectar production is the most likely reason for the high carbohydrate demand of the developing inflorescence (Dai and Paull, 1995).

The application of exogenous sugars as an additional energy source to delay senescence during storage and vase life is a recognized practice in both climacteric and non-climacteric cut flower crops (Goszezynska *et al.*, 1990, Paulin, 1986, Whitehead *et al.*, 2003). The extended vase life seems to be related to a consistent fresh weight and a regular gain in dry matter (Paulin, 1986). According to Halevy and Mayak (1979) the most advantageous sugar and optimum concentration of this sugar,

will vary with the species of flower crop treated, the length of this treatment and the stage of floral development. The use of exogenous sugars as a postharvest treatment to replace depleted photosynthates in *Protea* cut flowers has only been moderately successful. Postharvest leaf blackening has been reduced in species such as *P. neriifolia*, *P. eximia*, *P. compacta*, and *P. longiflora* with low concentration sucrose holding solutions ($\approx 2 \text{ g.L}^{-1}$). Leaf blackening was however never totally inhibited (Haasbroek *et al.*, 1973, Mulder, 1977, Akamine *et al.*, 1979, Brink and de Swardt, 1986, Brink, 1987, Paull and Dai, 1989, McConchie *et al.*, 1991, Bieleski *et al.*, 1992). Sucrose holding solutions at higher concentrations were found to actually magnify the occurrence and severity of leaf blackening in *P. neriifolia* (Jones, 1991). Akamine *et al.* (1979) showed that sucrose holding solutions of 30 g.L^{-1} could significantly suppress leaf blackening of *P. eximia*. Likewise, Jones (1991) successfully repressed leaf blackening of *P. cynaroides* during long-term dry storage (>14 days) at 1°C using a 200 g.L^{-1} sucrose pulse delivered over a 24 hour period. Brink and de Swardt (1986) determined that a sucrose holding solution of 5 g.L^{-1} and 10 g.L^{-1} significantly reduced and delayed leaf blackening of *P. neriifolia*. Ligawa *et al.* (1997) found that a sucrose vase solution concentration of 30 g.L^{-1} extended the vase life of 'Sylvia' by 10 days. McConchie *et al.* (1991) discovered that sucrose did not inhibit leaf blackening significantly in *P. neriifolia*. The use of a 5 g.L^{-1} sucrose vase solution delayed the occurrence of leaf blackening in *P. eximia* and extended the vase life to 16 days (Newman *et al.*, 1990). Holding solutions containing sucrose at $\leq 2 \text{ g.L}^{-1}$ effectively reduced leaf blackening of *P. compacta*, *P. cynaroides* and *P. magnifica* (Ireland *et al.*, 1967). Brink (1987) and Jones (1991) found that sucrose-holding solutions at higher concentrations enhanced the rate and intensity of leaf blackening in *P. neriifolia*. McConchie and Lang (1993b) were able to reduce leaf blackening in *P. neriifolia* shoots held in dark wet storage at 25°C for seven days, by 70% through an initial 24 hour pulsing period using a 200 g.L^{-1} sucrose solution. In contrast Stephens *et al.* (2001b) indicated that sucrose holding solutions of 1 g.L^{-1} did

not significantly improve the vase life of 'Sylvia' (*P. eximia* x *P. susannae*) and in fact higher concentrations of the solution actually promoted leaf blackening of this cultivar. Pulsing and holding solutions appear to be specific to each *Protea* species or cultivar as the severity of leaf blackening ranges widely between species (McConchie and Lang, 1993a,b) clones within a species (Paull and Dai, 1990) and the time of year (De Swardt *et al.*, 1987). These findings indicate the importance of evaluating postharvest carbohydrate treatments at cultivar level. Further research is needed on carbohydrate source, concentration, conditions of uptake and storage temperatures in order to improve the shipping potential of various *Protea* species and cultivars.

After winter the carbohydrate concentration of the leaves on *Protea* inflorescence bearing shoots tends to be significantly reduced (Hettasch *et al.*, 2001). This reduced carbohydrate state is followed immediately by the 'spring flush' (growth period between September and October). In 'Sylvia' plants this is the most vigorous growth flush and extensive new growth occurs in a relatively short space of time (Gerber *et al.*, 2001). This cultivar is prone to suffer from extensive pre- and postharvest leaf blackening during this time period. The increase in postharvest leaf blackening corresponds to the vigorous growth period following the decreased day length and low light levels of winter in the Western Cape Province of South African (Stephens *et al.*, 2003). McConchie *et al.*, (1991), Bieleski *et al.*, (1992) and McConchie and Lang (1993a,b) all correlated the occurrence of postharvest leaf blackening with a decrease in the carbohydrate content of numerous *Protea* species. It is thought that a similar process may be involved with the development of greater susceptibility, both pre- and postharvest, of 'Sylvia' inflorescence bearing shoots to leaf blackening during this time of the year. This is a serious problem for producers, as this period of susceptibility correlates with the increasing demand for fresh flowerse in the European market place. Most current postharvest treatments have little to no effect on the rate of leaf blackening during this time period.

It is hypothesized that the spring growth flush could be suppressed through the application of plant growth regulators (PGR's), thereby, decreasing the demand for mobile carbohydrates to be exported from the mature leaves to the new growth. This would occur as a result of the decreased demand by the slower developing spring flush for mobile carbohydrates, both as an energy source and as building blocks. The majority of PGR's used today are plant growth retardants. These reduce the shoot length of plants without altering developmental patterns or being phytotoxic (Dicks, 1980, Davis and Curry, 1991, Rademacher, 2000, 1995). This is normally achieved primarily through reducing cell elongation but also through reducing the rate of cellular division (Rademacher, 2000, 1995, 1991a). To achieve this, most growth retardants block some step in the synthesis of gibberellins (GA's) or auxins. These two plant hormones are primarily responsible for shoot elongation. The existing growth retardants can be classified into three main groups; (a) ethylene-releasing compounds, (b) inhibitors of GA translocation and (c) inhibitors of GA biosynthesis (Rademacher, 1991a). At this time four groups of GA biosynthesis inhibitors are recognized; (a) "onium" compounds, (b) compounds with N-containing heterocycles, (c) acylcyclohexanediones and related compounds and (d) 16,17-dihydro GA's. Each separate group inhibits GA metabolism at different stages of GA biosynthesis (Rademacher, 2000).

Prohexadione-Calcium, calcium 3-oxido-4-propionyl-5-oxo-3-cyclohexenecarboxylate (Evans *et al.*, 1999) is a foliar applied PGR, the uptake of which is normally complete four hours following application (Rademacher, 2001). P-Ca has been shown to inhibit the later steps in GA biosynthesis directly, thereby, reducing shoot extension (Rademacher, 1991b). P-Ca is primarily transported acropetally to growing points of individual shoots (Evans *et al.*, 1999, 1997). Normally only the development of treated shoots are affected. (Evans *et al.*, 1999).

Paclobutrazol, (2RS, 3RS)-1-(4-chlorophenyl)-4, 4-dimethyl-2-(1, 2, 4-triazol-1-yl) pentan-3-ol (Grossman, 1990), trade name Cultar, is a compound with an N-containing heterocycle (Rademacher, 1995). This PGR is applied as a soil drench to soil that is free of organic material directly above the root system of the plant that is to be treated.

Here we report on the effectiveness of the various pre – and postharvest treatments at suppressing or reducing the amount of postharvest leaf blackening that occurs on relevant species and cultivars.

Materials and Methods

Plant Material. The plant material for all experiments, unless otherwise stated, was either collected or treated at two commercial *Protea* farms. One is located near the Stellenbosch region (lat. 33°15'S; long. 19°07'E) (site one) of the Western Cape in South Africa and the other near Grabouw (lat. 33°15S; long. 19°03'E) (site two) in the same general area. The climate of both areas is classed as Mediterranean. The summers are hot and dry with an average annual rainfall of between 600-700 mm falling predominantly in the winter months.

Experiment 1. Effect of Prohexadione-calcium (P-Ca) on leaf blackening of Protea 'Sylvia'.

Twenty mature 'Sylvia' (*P. eximia* x *P. susannae*) *Protea* bushes were selected at each site. The selection process comprised of three criteria; bushes had to be uniform and healthy, they had to show potential for vigorous spring growth and each bush had to have a range of inflorescences that would allow for harvest roughly every two weeks for a period of between six-eight weeks after the treatment was applied. This was achieved by measuring the diameter of the inflorescences and, by working from a graph (Gerber *et al.*, 2001), estimating the probable time when the inflorescence would reach commercial maturity. All selected shoots were marked accordingly. Five blocks containing four plants were demarcated. A randomized complete block design was used

to set out the experiment statistically. Blocking was based on the toss of a coin, heads signifying the position of a treatment and tails that of a control.

A motorized backpack mist-blower (SR 420) from Stihl was used to apply P-Ca to run-off, at 250 mg.L^{-1} on wind still day's, corresponding to 21/08/2003 (site one) and 28/08/2003 (site two). Agral90 'Dash' (BAS90470S) distributed by WP Landbou Beperk was used as a surfactant, 6 ml was added to the made-up 10 L solution of P-Ca. The solution that was applied was at the recommended concentration used for apple trees. Four shoots per block were harvested on the 5/09/2003, 19/09/2003 and the 3/10/2003 (site one) and on the 12/09/2003, 26/09/2003 and the 11/10/2003 (site two). Each block represented a single replicate of both the treatment and the control. Shoots were clearly marked and placed into enclosed SAPPEX S14 fiberboard mini-cartons before been transported back to our labs within half-an-hour (site one) and one and a half-hours (site two) of harvest. Shoots were re-cut in the laboratory to a uniform length of 40 cm and the stem below the subtending flush stripped clean of leaves, approximately 20 leaves remained/shoot. Four shoots (two representing the treatment and two the control) were placed together in 5 L plastic buckets containing water and Chrysal clear cut flower food (Pokon Chrysal International) at 10 g.L^{-1} . The number of leaves with 20% leaf area or more blackened were determined daily. This took place in a temperature controlled room ($18 \pm 2^\circ\text{C}$) under natural light conditions. To determine the effect of Prohexadione-calcium on shoot growth three shoots per plant (9-11mm in diameter) were marked. The diameter and the length of each shoot were recorded on the 24/10/2003.

Experiment 2. *The effect of Paclobutrazol (Cultar) on leaf blackening of Protea 'Sylvia'.*

'Cultar', produced by Zeneca Agrochemicals SA (Pty) Ltd, active ingredient 250 g.L^{-1} , 20 'Sylvia' (*P. eximia* x *P. susannae*) *Protea* were used. Twenty plants were treated and 20 untreated plants served as

controls. The same criteria for selection of plants as well as experimental lay out used, were described in Exp. 1. The surface areas under the shrubs that were to receive the 'Cultar' treatment were cleared of all organic material before application. Two ml Cultar in 500ml water was applied per plant on the 11/08/2004 as a soil drench. Four shoots per block were harvested on the 4/09/2004, 25/09/2004 and the 13/10/2004. Each block consisted of 4 treated and 4 control plants. Shoots were clearly marked and placed into enclosed SAPPEX S14 fiberboard mini-cartons before being transported back to our labs within half-an-hour. Upon arrival at the laboratory, shoots were re-cut to a uniform length of 40 cm and the leaves below the subtending flush removed, approximately 20 leaves remained/shoot. Two shoots of both treatment and control were placed together in 5 L plastic buckets containing water and Chrysal clear cut flower food (Pokon Chrysal International) at 10 g.L⁻¹. The number of leaves with a minimum of 20% blackened leaf surface area were determined daily, this took place in a temperature controlled room (18±2°C) under natural light conditions.

To determine the effect of Cultar on shoot growth three shoots per plant (9-11mm in diameter) were marked. The diameter and the length of each shoot were recorded on the 9/10/2004.

Experiment 3. Effect of removal of spring growth flush on postharvest leaf blackening of Protea 'Sylvia'.

A randomized complete block design consisting of eight blocks, each containing four plants was established on site one. All the plants were of a similar size and vigor and all had inflorescences that would become commercially mature during mid October 2004. Spring growth was removed from treatment plants when it had reached a length of approximately 100 mm. No removal took place on the controls. Flower bearing shoots were harvested on the 13/10/2004. Shoots were clearly marked and placed into enclosed SAPPEX S14 fiberboard mini-cartons before being transported back to our labs within half-an-hour. Upon

arrival, shoots were re-cut to a uniform length of 40 cm and the stem below the subtending flush stripped clean of leaves, approximately 20 leaves/shoot remained. Two shoots of the treatment and control were placed together in 5 L plastic buckets containing water and Chrysal clear cut flower food (Pokon Chrysal International) at 10 g.L⁻¹. The number of leaves with a minimum of 20% blackened leaf surface area were determined daily for a period of 14 days postharvest. This took place in a temperature controlled room (18±2°C) under natural light conditions.

Experiment 4. Effect of harvest date on postharvest leaf blackening of Protea 'Sylvia'.

From February to November 2003 shoots were harvested from site one on the 14/03, 14/04, 14/08, 5/09, 19/09, 3/10 and the 3/11. Twenty uniform shoots were harvested randomly at each date when the inflorescences had reached the soft point stage. Shoots were clearly marked and placed into enclosed SAPPEX S14 fiberboard mini-cartons before being transported back to our laboratory within half-an-hour. Shoots were then re-cut to a uniform length of 40 cm and the leaves below the subtending flush removed. Approximately 20 leaves remained per shoot. Five pairs of shoots were placed into separate 5 L plastic buckets containing water and Chrysal clear cut flower food (Pokon Chrysal International) at 10 g.L⁻¹. The number of leaves with a minimum of 20% blackened leaf surface area were determined daily for a period not exceeding 14 days in a temperature controlled room (18±2°C) under natural light conditions.

Experiment 5. Effect of developmental stage of the inflorescence on preharvest leaf blackening following a heat wave in spring.

Three stages of inflorescence development were randomly harvested from site one on the 16/10/2003. Stages of inflorescence maturity were based on flower diameter and tip hardness. An immature stage (30-59mm diameter), medium maturation stage (60-79mm diameter, hard tip) and a commercially mature stage (>80mm diameter, soft tip) were

collected. Ten uniform shoots per stage, each representing a single replication, were harvested. The amount of preharvest leaf blackening was recorded for each inflorescence stage.

Experiment 6. Effect of holding solutions containing different sugars on leaf blackening of different species and cultivars of Protea.

The flower-bearing shoots of a number of *Protea* cultivars and species, namely; ‘Sylvia’ (*P. eximia* x *P. susannae*), ‘Cardinal’ (*P. eximia* x *P. susannae*), ‘Susara’ (*P. magnifica* x *P. susannae*), ‘Carnival’ (*P. compacta* x *P. neriifolia*), ‘Ivy’ (*P. laticolor*), *P. eximia*, *P. neriifolia*, *P. coronata*, *P. repens*, *P. mundii*, and *P. compacta* were brought to the laboratory. All the stems were re-cut to a uniform length of 50 cm and the leaves below the subtending flush stripped away, leaving approximately 20 leaves on each stem.

All the mature flower-bearing shoots were harvested from commercial plantations located near the Stellenbosch region (lat. 33°15’S; long. 19°07’E) of the Western Cape in South Africa. Two shoot of each *Protea* species and cultivar were placed together in 5 L plastic buckets containing 2% (20 g.L⁻¹) holding solutions of glucose, galactose, fructose or sucrose. Water acted as a control and ten replicates of two shoots per treatment were used. The number of leaves with a minimum of 20% blackened leaf surface area were determined daily, this took place in a temperature controlled room (18±2°C) under natural light conditions for 14 days postharvest.

Statistical analysis. The Statistica program and the SAS program (Statistical Analysis Systems Institute, 1996) were used for one-way classifications of variance for the data, LSD values were calculated for a 5% level of significance.

Results and Discussion

Inhibitors of Gibberellin synthesis and shoot removal.

Neither post-harvest leaf blackening nor shoot length of the new spring flush were affected by spray application with P-Ca to 'Sylvia' plants (data not presented, Experiment 1). In contrast when Cultar was administered as a soil drench (data not presented Experiment 2) post-harvest leaf blackening, as well as the length of the new spring flush of shoot growth were reduced by the Cultar treatment (Table 1) (Table 2). The failure of P-Ca to reduce shoot growth may be an indication that absorption of P-Ca was restricted by the waxy leaf surface. The efficacy of Cultar to reduce post-harvest leaf blackening in 'Sylvia' appears to be related to the inhibition of shoot growth by the chemical. Since carbohydrate stress is one of the physiological causes of leaf blackening (Dai and Paull, 1995; Jones *et al.*, 1995; Stephens *et al.*, 2005) it can be argued that the suppression of new growth (strong sink) during the spring flush may result in higher levels of mobile CHO's being converted to storage carbohydrates that could meet the various sinks demands for energy during post-harvest and, thus, delay post-harvest leaf blackening.

Removal of the spring growth flush (Experiment 3) had no significant effect on the incidence of postharvest leaf blackening when compared to the control (Table 3). This contradicts those results obtained from Tables 1 and 2, as the removal of the spring flush should create the same end effect as the application of PGR's, i.e. removal of competing sinks. This indicates that Cultar does not inhibit postharvest leaf blackening through the suppression of shoot growth, but rather, that leaf blackening is enhanced by high levels of gibberellins in the plants. Alternatively removal of the new spring flush of shoot growth was done to late with the result that carbohydrate allocation to new growth prevented a build up in the leaves on the flowering shoot.

Climatic conditions

The incidence of post-harvest leaf blackening in 'Sylvia' (Experiment 4) was significantly higher during the period September to November when compared to the period March to August (Table 4). Since 'Sylvia'

flowers that reach anthesis during spring developed during conditions of low winter light intensities it is conceivable that leaves will be low in carbohydrates compared to leaves that were exposed to high light conditions for flowers that reached anthesis during autumn. This, however, does not explain the difference in the post-harvest leaf blackening between flowers that reach anthesis in mid August and early September, respectively. Spring bud break in *Sylvia* occurred during the last week of August. It appears, therefore, that the extreme susceptibility of 'Sylvia' to blacken post harvest is due to low status in carbohydrates caused by low light conditions of winter, a condition that is compounded by new shoot growth that occurs during spring. It is also possible that the hormone status of the plant eg. high gibberellin levels makes leaves more susceptible to leaf blackening.

Field observations (Experiment 5) revealed that leaf blackening in 'Sylvia' can occur in the field (Table 5) following a period of very high temperatures (Table 6). Shoots without an inflorescence showed no leaf blackening in the field and the incidence of blackening increased the more advanced the inflorescence was at the time of the heat wave. These results again point to the importance of carbohydrate and leaf blackening. It is feasible to assume that in more mature inflorescences more carbohydrates were transported from the leaves to the inflorescence. This resulted in a lower carbohydrate status of the leaves which made them more susceptible to leaf blackening as compared to leaves on shoots without flowers.

Genotypes

Results of Experiment 6 (Table 7) reveal that for all the cultivars tested the experiment was terminated prematurely and no meaningful results were recorded. For *P. repens* and *P. compacta* glucose was better than the control, whereas all other sugars did not differ from the control. None of the sugars was better than the control in the case of *P. mundii*. All the sugars tested did better than the control in the case of *P. compacta*. For *P. coronata* all the sugars except glucose were better

than the control. Only fructose was better than the control in the case of *P. eximia*. The incidence of leaf blackening in *P. neriifolia* was higher with glucose while the other sugars were without effect. It was not possible to come to general conclusions regarding the interaction between different sugars and protea genotypes with regard to suppression of leaf blackening. More experiments along these lines are required.

Literature Cited

- Akamine, E.K., T. Goo, and R. Suehisa. 1979. Relationship between leaf darkening and chemical composition of leaves of Protea. *Flor. Rev.* 163:62-63, 107-108.
- Bieleski, R.L., J. Ripperda, J.P. Newman and M.S. Reid. 1992. Carbohydrate changes and leaf blackening in cut flower stems of *Protea eximia*. *J. Amer. Soc. Hort. Sci.* 117:124-127.
- Brink, J.A., 1987. The influence of the flower-head on leaf browning of cut *Protea neriifolia*. *Protea News* 5:11-12.
- Brink, J.A. and G.H. de Swardt. 1986. The effect of sucrose in a vase solution on leaf browning of *Protea neriifolia* R. Br. *Acta Hort.* 185:111-119.
- Caffrey, M., V. Fonseca, and A.C. Leopold. 1988. Lipid-Sugar Interactions. *Plant Physiol.* 86:754-758.
- Crowe, J.H. and L.M. Crowe. 1992. Membrane integrity in anhydrobiotic organisms: toward a mechanism for stabilizing dry cells. In: G.N. Somero, C.B. Osmond and C.L. Bolis (Editors). *Water and Life*. Springer-Verlag, Berlin, pp. 87-103.
- Crowe, J.H. and L.M. Crowe. 1986. Stabilization of membranes in anhydrobiotic organisms. P. 188-209. In: A.C. Leopold (ed.). *Membranes, metabolism, and dry organisms*. Comstock Publication Assoc., Ithaca, NY.
- Dai, J-W. and R.E. Paull. 1995. Source-sink relationship and *Protea* postharvest leaf blackening. *J. Amer. Soc. Hort. Sci.* 120:475-480.

- Davis, T.D. and E.A. Curry. 1991. Chemical regulation of vegetative growth. *Critical Reviews in Plant Sciences* 10(2):151-188.
- De Swardt, G.H., J. Pretorius, and L. Burger. 1987. The browning of foliage leaves in Proteas-a review. *Protea News* 6, 4-9.
- Dicks, J.W. 1980. Modes of action of growth retardants. In: D.R. Clifford and J.R. Lenton (eds.). *Recent developments in the use of growth retardants*. pp. 1-14. British Plant Growth Regulator Group, Wantage, England.
- Evans, J.R., R.R. Evans, C.L. Regusci, and W. Rademacher. 1999. Mode of action, metabolism, and uptake of BAS125W, Prohexadione-calcium. *HortScience* 34(7):1200-1201.
- Evans, R.R., J.R. Evans, and W. Rademacher. 1997. Prohexadione calcium for suppression of vegetative growth in Eastern apples. *Acta Hort.* 451:663-666.
- Ferreira, D.I. 1986. The influence of temperature on the respiration rate and browning of *Protea neriifolia* R. Br. Inflorescences. *Acta Hort.* 185:121-125.
- Forsberg, L.I., 1993. *Protea diseases and their control*. Queensland Dept Primary Industries, Brisbane, Queensland.
- Forsberg, L.I. 1988. *Protea diseases and their control*. Queensland Agr.J. (Nov.-Dec.):347-359.
- Gerber, A.I., K.I. Theron and G. Jacobs. 2001. The role of leaves and carbohydrates in flowering of *Protea* cv. Lady Di. *HortScience* 36(5):905-908.
- Goszezynska, D., H. Itzhaki, A. Borochoy, and A.H. Halevy, 1990. Effects of sugar on physical and compositional properties of rose petal membranes. *Scientia Hort.*, 43:313-320.
- Grossmann, K. 1990. Plant growth retardants as tools in physiological research. *Physiol. Plant.* 78:640-648.
- Haasbroek, F.J., G.G. Rousseau, and J.F. de Villiers. 1973. Effect of gamma-rays on cut blooms of *Protea compacta* R.Br., *P. longiflora* Lamarck and *Leucospermum cordifolium* Salisb. *Ex Knight. Agroplantae* 5:33-42.

- Halevy, A.H. and Mayak. 1981. Senescence and postharvest physiology of cut flowers-Part 2. Hort. Rev. 3:59-143.
- Halevy, A.H. and Mayak. 1979. Senescence and postharvest physiology of cut flowers-Part1. Hort. Rev. 1:205-236.
- Hettasch, H.B., K.I. Theron, and G. Jacobs. 2001. Dry mass allocation and carbohydrate allocation in successive growth flushes of Protea cultivars 'Sylvia' and Protea cultivar 'Cardinal' shoots. Acta Hort. 545:215-225.
- Ireland, J.P., J.T. Meynhardt, and J.M. Strauss. 1967. When proteas become sailors: treatment before shipping. Farming in South Africa 43, 33-35.
- Jones, R. B., 1995. Sucrose prevents foliage desiccation in cut Leucadendron 'Silvan Red' during cold storage. Postharvest Bio Tech. 6:293-301.
- Jones, R.B., 1991. Understanding and controlling leaf blackening in Protea leaves: the use of high concentrations of sucrose. Proc. Int. Protea Assoc. 6th Biennial Conf., Perth, Western Australia. Pp 313-322.
- Jones, R.B., R. McConchie, W.G. van Doorn and M.S. Reid. 1995. Leaf blackening in cut Protea flowers. Hort. Rev. 17:173-201.
- Ligawa, J.K., D.C. Joyce and S.E. Hetherington. 1997. Exogenously supplied sucrose improves the postharvest quality of Grevillea 'Sylvia' inflorescences. Aus. J. Exp. Agr. 37:809-816.
- McConchie, R., N.S. Lang. 1993a. Postharvest leaf blackening and pre-harvest carbohydrate status in three Protea species. HortScience 28:313-316.
- McConchie, R., N.S. Lang. 1993b. Carbohydrate metabolism and possible mechanisms of leaf blackening in Protea neriifolia under dark postharvest conditions. J. Amer. Soc. Hort. Sci. 118:355-361.
- McConchie, R., N.S. Lang, and K.C. Gross. 1991. Carbohydrate depletion and leaf blackening in Protea neriifolia. J. Amer. Soc. Hort. Sci. 116:1019-1024.

- Meyer, C. 2003. Carbohydrates and leaf blackening of protea cut flowers. MSc. (Agric.) thesis, University of Stellenbosch, South Africa.
- Mulder, P.W.A. 1977. Primere meganismes betrokke by die bruinwording van loofblare in *Protea neriifolia*. MSc thesis. Randse Afrikaans Univ. Johannesburg. South Africa.
- Newman J.P., W.G. van Doorn and M.S. Reid. 1990. Carbohydrate stress causes leaf blackening in *Proteas*. *Acta Hort.* 264:103-108.
- Paulin, A., 1986. Influence of exogeneous sugars on the evolution of some senescence parameters of petals. *Acta Hort.* 181:183-193.
- Paull, R.E. and J.W. Dai. 1990. *Protea* postharvest leaf blackening a problem in search of a solution. *Acta Hort.* 264:93-101.
- Rademacher, W. 2001. Technical Data Sheet, BASF Agricultural Center, p. 7.
- Rademacher, W. 2000. Growth retardants: Effects on gibberellin biosynthesis and other metabolic pathways. *Annu. Rev. Plant Physiol: Plant Mol. Biol.* 51:501-531.
- Rademacher, W. 1995. Growth retardants: Biochemical features and applications in horticulture. *Acta Hort.* 394:57-73.
- Rademacher, W. 1991a. Biochemical effects of plant growth retardants. In: H.W. Gausman (ed.). *Plant biochemical regulators*. pp. 169-200. Marcel Dekker, Inc., New York.
- Rademacher, W. 1991b. Inhibitors of gibberellin biosynthesis: Applications in agriculture and horticulture, In: *Gibberellins*, Takahashi, N., Phinney, B.O., MacMillan, J. (Editors.), Springer Verlag, New York: 296-310.
- Starke, L.C., 1979. Common *Protea* pests. *Farming in South Africa: Flowers and Ornamental Shrubs Publ.* B.11.
- Stephens, I.A., C. Meyer, D.M. Holcroft, and G. Jacobs. 2005. Carbohydrates and Postharvest Leaf Blackening of *Proteas*. *HortScience* 40(1):181-184.
- Stephens, I.A. 2003. Leaf blackening of *proteas*. Ph. D. (Agric.) dissertation, University of Stellenbosch, South Africa.

- Stephens, I.A., D.M. Holcroft, and G. Jacobs. 2001a. Low temperatures and girdling extend vase life of 'Sylvia' Proteas. *Acta Hort.* 545:205-214.
- Stephens, I.A., D.M. Holcroft, and G. Jacobs. 2001b. Glucose prevents leaf blackening in 'Sylvia' proteas. *Postharv. Biol. Technol.* 23:237-240.
- Van Doorn, W.G. 2001. Leaf blackening in Protea Flowers: Recent developments. *Acta Hort.* 545:197-204.
- Whitehead, C.S., O'Reilly, J. Weerts, M.M. Zaayman, and W. Gaum. 2003. The effect of Sucrose Pulsing on senescing Climacteric Cut Flowers. *Acta Hort.* 599:549-557.

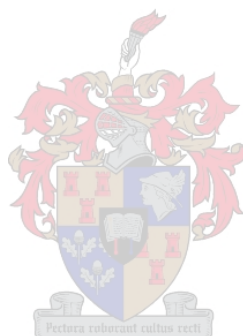


Table 1 . Effect of a soil drench with Cultar (2ml in 500ml water per plant) on post-harvest leaf blackening of ‘Sylvia’ (*P. eximia* x *P. susannae*) flowering shoots expressed as percentage leaves with more than 20 per cent of leaf area black after 14 days in a holding solution at 18C.

% Leaf Blackening	
Control	Cultar
60.9 a	37.2 b

Values with different letters differ significantly at the 5% level, LSD test.

Table 2. Comparison of shoot length (mm) on ‘Sylvia’ (*P. eximia* x *P. susannae*) *Protea* plants treated with ‘Cultar’(2ml in 500ml water per plant)

% Leaf Blackening	
Control	Cultar
245.5 a	194.7 b

Values with different letters differ significantly at the 5% level, LSD test.

Table 3. Effect of removing spring flush shoots on October 2004 from ‘Sylvia’ plants (*P. eximia* x *P. susannae*) on post-harvest leaf blackening of flowering shoots expressed as percentage leaves with more than 20 per cent of leaf area black after 14 days in a holding solution at 18C

%Leaf blackening	
Control	Spring flush removed
42.18 a	37.78 a

Values with different letters differ significantly at the 5% level, LSD test.

Table 4. Effect of harvest date of flowering shoots of Sylvania' (*P. eximia* x *P. susannae*) on post-harvest leaf blackening expressed as percentage leaves with more than 20 per cent of leaf area black after 14 days in a holding solution at 18C.

Date of harvest	% Leaf Blackening	
	14/03	7.64
14/04	5.09	b
14/08	16.80	b
05/09	47.75	a
19/09	48.68	a
03/10	59.18	a
03/11	46.93	a

Values with different letters differ significantly at the 5% level, LSD test.

Table 5. Percentage of preharvest leaf blackening following a heat wave during 11 and 12 October 2003 (see Table 6) on shoots of 'Sylvia' (*P. eximia* x *P. susannae*) with inflorescences at different developmental stage. Recorded 16/10/2003.

Inflorescence Maturity	Inflorescence Diameter (mm)	% Preharvest Black Leaves	
Commercially Mature	≥80	4.6	a
Medium Maturation	60-79	1.7	b
Immature	30-59	0.1	c
Vegetative	0	0	c

Values with different letters differ significantly at the 5% level, LSD test.

Table 6. Temperature in degrees Celsius over a five day period in October 2003.

Time of day	09-Oct	10-Oct	11-Oct	12-Oct	13-Oct
12:00	22	20.64	29.81	34.7	20.45
13:00	22.44	21.15	30.57	35.67	21.37
14:00	22.35	20.89	30.01	33.05	21.17
15:00	24.22	20.83	28.64	28.59	20.8
16:00	24.37	20.5	26.35	24.81	20.16

Table 7. Effect of holding solutions containing different sugars at a concentration of 2% on post-harvest leaf blackening of ‘Sylvia’ (*P. eximia* x *P. susannae*), ‘Cardinal’ (*P. eximia* x *P. susannae*), ‘Susara’ (*P. magnifica* x *P. susannae*), ‘Carnival’ (*P. compacta* x *P. neriifolia*), ‘Ivy’ (*P. lacticolor*), *P. eximia*, *P. neriifolia*, *P. coronata*, *P. repens*, *P. mundii*, and *P. compacta* Sylvia’ (*P. eximia* x *P. susannae*) expressed as a percentage leaves with more than 20 per cent of leaf area black after 14 days 18C.

Protea cultivar/ species	%Leaf blackening									
	Control		Sucrose 2%		Fructose 2%		Galactose 2%		Glucose 2%	
Sylvia	8.34	ab	15.00	a	2.62	ab	3.05	ab	0.50	b
Ivy	0	a	0	a	0	a	0	a	0	a
Cardinal	4.09	ab	14.81	a	6.11	ab	7.44	ab	0	b
Carnival	4.76	ab	2.95	ab	3.96	ab	5.21	a	0	b
<i>P. repens</i>	27.83	a	32.08	ab	15.21	b	26.29	ab	11.66	b
<i>P. mundii</i>	16.67	a	29.93	a	12.33	a	4.86	a	23.76	a
<i>P. compacta</i>	54.50	a	61.63	a	48.61	a	21.25	ab	9.69	b
<i>P. coronata</i>	41.49	a	13.05	b	11.39	b	10.86	b	17.01	ab
<i>P. eximia</i>	36.99	ab	52.85	a	16.56	b	40.33	ab	35.07	b
Susara	10.72	a	5.40	ab	5.95	ab	9.26	a	3.45	b
<i>P. neriifolia</i>	15.03	b	26.14	b	36.02	b	18.98	b	61.8	a

Values with different letters between treatments differ significantly at the 5% level, LSD test.



5. General Conclusions

Leaf blackening is a serious disorder that threatens the profitability of the South African *Protea* industry.

Postharvest decreases in the level of carbohydrates have been hypothesized to initiate the process of leaf blackening. It is evident that the levels of polygalatol in all stem parts of 'Sylvia' and 'Lady Di' remain relatively constant over time. It therefore appears that polygalatol does not contribute to the carbohydrate metabolic pool and is unavailable for metabolism. Sucrose occurs in lower concentrations in the inflorescences of 'Sylvia' and 'Lady Di' at harvest than glucose and fructose. The leaves and stems contain higher concentrations of sucrose than glucose and fructose. It appears that stems do contain significant amounts of reserve carbohydrates in the form of glucose, fructose and sucrose but very little starch and function as storage areas for carbohydrate reserves. This however, seems to be cultivar specific. Compelling evidence exists suggesting that sucrose and starch may not be the main forms of nonstructural reserve metabolic carbohydrates in many *Protea* species and cultivars. Individual species and cultivars appear to differ in respect to the type and amount of carbohydrate that are present both just prior to harvest and post pulsing treatment.

Directly after the pulsing treatment most glucose is located in the stems. This implies that the stems act as an important reservoir of glucose when shoots are pulsed. The decrease in the glucose concentration in the stems after pulsing indicates that slow transport of the glucose to other shoot parts occurs as transpiration takes place during storage. Glucose seems to be rapidly converted into starch in the leaves but not in the stems of 'Sylvia'. In the case of 'Lady Di' however, the conversion of glucose to starch in the leaves did not take place. It can be deduced from the fact that the concentrations of

glucose in the leaves and stems and starch in the leaves, decrease more rapidly than other sugars in the various plant parts, that glucose and starch are the main reserve carbohydrates present in 'Sylvia' shoots. 'Sylvia' shoots stored at room temperature loose between 25-30% of the reserve carbohydrates within the first day after pulsing and a further 10-15% during the next 24h storage. In contrast, it took 10 days for the reserve carbohydrates to decrease by 50% at a storage temperature of 4.5°C. The rapid decrease in the carbohydrate concentration after 21 days in cold storage is possibly due to rapid senescence of the product and possibly also the result of increased micro-organism activity.

The relationship between reserve carbohydrates, in particular glucose, and postharvest leaf blackening is now well established. However, it remains unexplained as to why glucose is effective in reducing postharvest leaf blackening in only some species/cultivars.

Prohexadione-calcium (P-Ca) had no effect in delaying or preventing postharvest leaf blackening when applied to 'Sylvia' *Protea* plants. The surface structure of 'Sylvia' *Protea* leaves may have prevented absorption of P-Ca by the plant and further investigation is needed. P-Ca had no effect on reducing the length of reproductive shoot growth.

Paclobutrazol significantly inhibited the elongation of reproductive shoots on treated plants. The levels of cumulative postharvest leaf blackening were also significantly lower on shoots that were harvested from treated plants when compared to control shoots. More research is needed in the direction of hormonal control of postharvest leaf blackening prevention/control. The removal of the spring growth flush had no significant influence on the level of postharvest leaf blackening when compared to the control. This indicates that Paclobutrazol does not inhibit postharvest leaf blackening through the suppression of stem elongation, but rather, that blackening is enhanced by high levels of gibberellins in the plant or the new growth was removed too late to have an effect.

The rate and severity of postharvest leaf blackening on 'Sylvia' inflorescence bearing shoots increases dramatically during the spring and early summer months in the Western Cape Province of South Africa. This cultivar is prone to suffer from extensive pre- and postharvest leaf blackening during this time period. It is thought that a decrease in the carbohydrate content of the leaves, as a result of the low light intensities of the South African winter, results in the development of greater susceptibility, both pre- and postharvest, of 'Sylvia' shoots to leaf blackening during this time period in the Western Cape. It is possible that in order for the 'Sylvia' plants to meet the carbohydrate demands of the developing shoots during the growth flush from September to October that mobile carbohydrates move from source leaves to the rapidly developing young shoots (strong sinks), thereby making them more susceptible to leaf blackening.

Preharvest leaf blackening that occurred in October 2003 began to appear first and was more severe, on those shoots that subtended inflorescences that were nearing commercial maturity. Shoots that subtended less mature inflorescences were not as negatively affected. It was noted that an excessively warm period of weather occurred few days prior to the appearance of the preharvest leaf blackening symptoms. These results, together with previous work, suggest that adverse preharvest conditions such as high temperatures do have a negative effect on the susceptibility of 'Sylvia' *Protea* plants to leaf blackening. More research is needed in this area.

Four simple carbohydrates were used to investigate the possible relationship between *Protea* species and cultivars and the source of exogenous carbohydrate supplementation in terms of effective postharvest leaf blackening prevention/control. No consistent trend with regard to the interaction between type of sugar and genotype in respect of leaf blackening could be established.