Postharvest Leaf Blackening and Preharvest Carbohydrate Status in Three *Protea* Species

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Abstract. *Protea neriifolia* R. Br., *P. susannae* E.P. Phillips $\times$ *compacta* R. Br., and *P. eximia* (Salis. ex Knight) Fourcade cut flower stems were examined to determine the relationship between postharvest leaf blackening rate and preharvest carbohydrate status. Postharvest leaf blackening was highest (83% by day 4) in *P. eximia* floral stems, which had the lowest preharvest sucrose concentrations. In contrast, *P. susannae* $\times$ *compacta* had <5% leaf blackening by day 4 and the highest preharvest leaf sucrose concentrations. Starch concentrations were highest in *P. neriifolia*; however, leaf blackening was intermediate between *P. susannae* $\times$ *compacta* and *P. eximia* and reached 52% at day 4. Preharvest carbon-exchange rate and stomatal conductance in all three species were extremely low, despite high photosynthetically active radiation and apparent lack of water stress. Comparing preharvest carbohydrate profiles in vegetative and floral stems suggests that vegetative stems may have a sink-to-source transition zone between the second and third divisions, while most leaves on floral stems may have transferred carbohydrates to source leaves at harvest. While preharvest floral stem sucrose concentrations can be linked to leaf blackening rate, the high starch reserves in *P. neriifolia* reduced leaf blackening little in this species. We conclude that leaf blackening may be related more to inflorescence sink demand after harvest and oxidative substrate availability than preharvest reserve carbohydrate concentrations in each species.

Premature leaf blackening on many cut-flower *Protea* species during postharvest holding seriously reduces vase life and market value. Blackening symptoms usually appear 3 to 7 days after harvest when stems are kept in darkness. Leaf blackening in *P. neriifolia* (PN) (McConchie et al., 1991) and *P. eximia* (PE) (Bielecki et al., 1992) has been attributed to carbohydrate depletion in leaves. During the postharvest period, nonstructural carbohydrates are translocated from leaves to the floral sink to continue floral development. McConchie and Lang (1993) have demonstrated that, under standard shipping conditions, up to 82% of starch reserves is depleted from PN leaves within 24 h of harvest. However, stems with delayed leaf blackening maintain higher carbohydrate concentrations in the initial post-harvest period. In contrast, early leaf blackening in floral stems is preceded by rapid leaf carbohydrate depletion (McConchie and Lang, 1993).

Developing postharvest treatments, such as stem illumination and sugar-based vase solutions, to reduce blackening symptoms have been the focus of much research (Jones, 1991; McConchie and Lang, 1993; McConchie et al., 1991; Newman et al., 1989; Reid et al., 1991). An alternative approach may be investigating species or cultivars less prone to leaf blackening. Susceptibility to leaf blackening may be species- or cultivar-specific due to heritable characteristics that influence carbohydrate metabolism. For example, cultivars with high carbohydrate concentrations before harvest may have sufficient substrate to support postharvest floral development; therefore, leaf carbohydrates are not depleted and blackening symptoms do not appear. Alternatively, differences in leaf sink-to-source transition along stems could influence the total amount of stored carbohydrate available to support postharvest inflorescence.

The purpose of this study was to evaluate the relationship between preharvest carbohydrate concentrations and leaf blackening rate in three *Protea* species considered by the industry to be differentially prone to leaf blackening. In addition, the sink-to-source status of leaves on floral and vegetative stems was determined for each species.

Six floral stems (at soft-tip maturity) were harvested from PN, *P. susannae* $\times$ *compacta* (PS), and PE plants growing at a commercial plantation in Goleta, Calif., in Sept. 1991 and air-freighted overnight to Baton Rouge, La. Stems were recut on arrival and placed in 1

liter deionized distilled water containing 50 ppm hypochlorite. Stems were kept in darkness for 24 h at 25C ($\pm$ 1C) in a growth chamber (Environmental Growth Chambers, Chagrin Falls, Ohio). The number of leaves per stem with $\geq$ 10% blackened surface area was recorded daily for 11 days. Blackening was expressed as a percentage of the total number of leaves per stem.

Physiological studies

Field-grown plants. Based on uniformity, one vegetative and one floral stem (at soft-tip maturity) were selected on three plants of each species noted growing at the same commercial plantation described above. Seven phyllotactic divisions (one division equals one complete leaf spiral) were tagged on each stem beginning at the apex. Carbon-exchange rate (CER) and stomatal conductance were measured for each division, as described by McConchie et al. (1991), under ambient photosynthetically active radiation (PAR) (1500 to 1975 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) between 1030 and 1330 HR. Four leaf disks (0.5 cm in diameter) were removed from two leaves in each phyllotactic division, frozen at -80C, and lyophilized for carbohydrate analysis (McConchie et al., 1991). Soluble carbohydrates were extracted and quantified using high-performance liquid chromatography as described by McConchie and Lang (1993). Starch concentrations were determined using the method of Robbins and Pharr (1988).

Glasshouse-grown plants. Based on uniformity, three vegetative stems were selected on each of six well-watered glasshouse-grown PS plants, and the third and sixth phyllotactic divisions were tagged and measured for photosynthetic rate and stomatal conductance during a 16-h interval that began before dawn and ended after sunset. Three plants were placed in a growth chamber (Environmental Growth Chambers, Chagrin Falls, Ohio) and kept at 25C ($\pm$ 1C) under 12 h of light each 24 h from 0730 to 1930 HR. The remaining three plants were placed outside under ambient temperature and PAR. Measurements were taken every 2 h from 0530 to 2130 HR; each data point represents an average of measurements from the third and sixth phyllotactic divisions. PAR and temperature were monitored and recorded during CER measurement.

Statistical analysis. The postharvest leaf blackening data set was analyzed as a repeated-measures design with a one-way treatment structure (three plant species, 11 days, $n = 6$) (Milliken and Johnson, 1984). The preharvest data sets were analyzed as completely randomized designs with two-way treatment structures (three plant species, seven phyllotactic divisions, $n = 3$). Means were separated using Duncan's new multiple range test. All analyses were performed using the SAS statistical package (SAS, 1987).

Due to the extremely low CER and degree of stomatal closure in PS and PE, these characteristics could not be measured on three replicate stems in the allotted time. Therefore, CER and stomatal conductance data presented

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for these two species represent an average of the seven phyllotactic divisions of one floral and one vegetative stem.

Leaf blackening. Leaf blackening development was significantly different ($P = 0.003$) in the three species examined (Fig. 1). PS leaf blackening was significantly less ($P < 0.05$) than that of PN or PE. PS had <5% leaf blackening by day 4. In contrast, PE leaf blackening developed rapidly, rising from 17% to 83% between days 3 and 4 and reaching 100% by day 5. Leaf blackening was intermediate in PN, reaching 52% on day 4. All three species developed $\approx 100\%$ leaf blackening by day 9.

CER and stomatal conductance. In field-grown plants of all three species, CER and stomatal conductance were low and did not vary significantly between floral and vegetative stems or across the seven phyllotactic divisions. PN CER averaged 1.13 and 0.93 mg $\text{CH}_2\text{O}/\text{dm}^2$ per hand stomatal conductance averaged 0.27 and 0.30 $\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ for floral and vegetative stems, respectively. PS CER in floral and vegetative stems averaged 0.09 and 0.11 mg $\text{CH}_2\text{O}/\text{dm}^2$ per h and stomatal conductance averaged 0.07 and 0.06 $\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, respectively. PE CER averaged 0.07 and 0.02 mg $\text{CH}_2\text{O}/\text{dm}^2$ per h, and stomatal conductance averaged 0.02 and 0.05 $\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ for floral and vegetative stems, respectively. The low stomatal conductance measured in PS and PE probably was due to the virtual cessation of carbon fixation in these two species.

The low CER in PN was similar to that measured previously for cut stems of this species in a growth chamber under low PAR (120 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) (McConchie et al., 1991). It is particularly striking that CER was even lower ($\approx 10\times$) in PS and PE. Water stress probably did not cause low stomatal conductance and CER in this experiment, since soil tensiometer readings (38 kPa at 30 cm deep) indicated that there was an adequate water supply. Further, plants did not have any visual symptoms of stress. The evolution of these *Protea* species under xerophytic environmental conditions may have resulted in consistently low carbon-fixation rates or alternative carbon-fixing mechanisms. CER in other xerophytic woody plant species such as *Nerium oleander*

Fig. 1. Percentage of leaves with $\geq 10\%$ area blackened on floral stems at 25°C ($\pm 1^\circ\text{C}$) in the dark. Species were (■) *Protea nerifolia*, (●) *P. susannae x compacta*, and (▲) *P. eximia*. Bars indicate the maximum SE associated with least square means ($n = 6$).

L. and Eucalyptus spp. ranges from ≈ 10 to 25 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (25°C) (Ferrar et al., 1989). Additionally, many evergreen shrubs adapted to desert environments have relatively low ($\approx 11 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) photosynthetic capacities (Smith and Nobel, 1986). CER measured in the three *Protea* species in this study was below these levels—0.22 to 10.52 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$.

The low CER and stomatal conductance measured in field-grown PS plants were confirmed later by measuring the same photosynthetic characteristics in vegetative glasshouse-grown PS plants (Fig. 2). Under ambient and growth-chamber PAR (≈ 800 and 100 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, respectively) (Fig. 2A), photosynthesis rates were $< 3 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (Fig. 2C). Stomatal conductance was also low and comparable to that measured in the field-grown plants (Fig. 2D). Stomatal conductance began to increase at dusk (1930 HR) in the ambient-held plants and at the end of the photoperiod (1930 HR) for the growth-chamber-held plants. Unlike C_3 plants, arid environment-adapted

plants using the Crassulacean acid metabolism (CAM) carbon-fixation mode typically open stomates at night to fix carbon into malic acid, which is decarboxylated during the day while stomates are closed to conserve water (Osmond, 1976). The data therefore suggest that PS plants may use the CAM or facultative CAM carbon-fixation mode. This characteristic would account for the low CER observed. Further inquiry into *Protea* carbon-fixing mechanisms is warranted.

Carbohydrate status. The major nonstructural carbohydrates found in floral and vegetative stems of all three species were starch, sucrose, and the sugar alcohol 1,5-anhydro-D-glucitol (polygalactol). Fructose, glucose, and maltose were detected in the soluble carbohydrate fraction at $< 1.9 \text{ mg}\cdot\text{dm}^{-2}$ and are not reported.

Leaf starch concentrations were similar for floral (Fig. 3A) and vegetative stems (Fig. 3B) for each species. However, starch concentrations in PN leaves were significantly higher (P

Fig. 2. Changes in average leaf photosynthetically active radiation (PAR), temperature, rate of photosynthesis, and stomatal conductance measured at the third and sixth phyllotactic divisions on vegetative glasshouse-grown *Protea susannae x compacta* plants placed in a growth chamber ($25 \pm 1^\circ\text{C}$, $\approx 100 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) under 12 h (0730–1930 HR) light each 24 h (—), or outside under ambient PAR (dawn = 0600 HR, dusk = 1930 HR) and temperature (---).

< 0.05) for both stem types than in leaves of the other species. Starch concentrations in PS and PE floral and vegetative stems did not differ significantly across all seven phyllotactic divisions (Fig. 3A and B). Starch concentrations for vegetative stems in all three species were lower in the first two than in higher phyllotactic divisions (Fig. 3B). A similar starch pattern existed in floral stems, except for PS, which had the highest starch concentration at the first division (Fig. 3A).

Floral stem leaf sucrose concentrations were significantly different ($P = 0.0001$) among the three species; PS had the highest and PE the lowest concentration (Fig. 4A). On floral stems, phyllotactic division did not influence sucrose concentration significantly; this result suggests that leaves subtending the inflorescence may be source leaves. In contrast, phyllotactic division significantly influenced sucrose concentration ($P = 0.0001$) in vegetative stems of the three species (Fig. 4B). Sucrose concentrations were lowest in the first two divisions and increased basipetally. Older PE leaves (2 fourth

division) on vegetative stems had significantly lower sucrose concentrations overall ($P < 0.05$) than the other two species.

Changes in leaf starch and sucrose concentrations across phyllotactic division may indicate a sink-to-source transition. In dicotyledonous plants, leaf transition from heterotrophy to autotrophy usually occurs when leaves are 30% to 60% fully expanded (Turgeon, 1989). In addition to leaf morphological changes, sink-to-source transition usually is characterized by an increased capacity to synthesize transport carbohydrates as a result of greater photosynthetic competence. Further, export capacity or source-leaf status has been correlated with photoassimilate partitioning into sucrose (Giaquinta, 1980). Based on the increase in sucrose concentration across phyllotactic division in all three species (Fig. 4B), a transition zone may exist between the second and third divisions in vegetative stems. The lower starch concentration in the first two phyllotactic divisions of vegetative stems (Fig. 3B) further supports the hypothesis of a tran-

sition zone. Developing leaves can be classified as utilization sinks, where imported carbohydrates are used for respiration and growth, rather than storage (Ho, 1988). Thus, low starch concentrations in leaves at the first division may indicate heterotrophic status. Starch metabolism in source leaves, however, is linked closely to photosynthetic capacity and sink requirements, which may vary diurnally (Beck and Ziegler, 1989). A phyllotactic starch pattern similar to that of stems in this study was found on intact vegetative stems of glasshouse-grown PS plants (McConchie et al., 1991); however, starch concentration rose sharply at the fifth division. The differences in transition zone between field-grown and glasshouse-grown plants may vary based on environmental influences to which field-grown plants are subjected and the resulting growth cycle. Consequently, a more active growth cycle may result in a more basipetal transition zone.

Of particular interest is the difference in carbohydrate patterns between floral and vegetative stems. Unlike vegetative stems, the similarity of sucrose profiles across floral stem phyllotactic divisions (Fig. 4A) suggests that, after flower initiation and during inflorescence, most leaves may be source leaves by the time floral stems are harvested. Additionally, floral stem sucrose concentrations at the first and second divisions are two to three times higher than comparable divisions in vegetative stems (Fig. 4A and B). The high starch concentration at the first division in PS (Fig. 3A) further supports the hypothesis that floral stem leaves may be source leaves. However, low starch concentrations at the first division in PN and PE suggests that the most acroptal leaves still may be undergoing transition from sink to source status. The time between flower initiation and anthesis for some members of the Proteaceae, such as *Banksia* spp., can be several months (Fuss et al., 1992). Since similar periods have been observed for floral development in the three *Protea* spp. in this study, transition of most subtending leaves to source-leaf status would not be unexpected.

Floral stem leaf polygalatol concentrations did not differ significantly ($P = 0.06$) between species (Fig. 5A); however, vegetative stem concentrations were highest ($P < 0.05$) in PS (Fig. 5B). Floral and vegetative stem leaf polygalatol concentrations declined significantly ($P = 0.009$ and 0.0001 , respectively) in a basipetal pattern. Thus, leaves closest to the apex consistently had the highest concentration of polygalatol, irrespective of species. Some sugar alcohols, such as sorbitol, are primarily transport carbohydrates and accumulate in source tissue where they are synthesized (Merlo and Passera, 1991). However, osmoregulation also has been hypothesized as an alternate role for sugar alcohols (Bielecki, 1982). Cheeseman (1988) suggested that such compounds used by the plant for osmotic adjustment in dry or saline habitats usually are not available for growth. Recent studies have shown that leaf polygalatol concentrations in PN and PE do not decrease under carbohydrate stress during postharvest holding

Fig. 3. Changes in preharvest leaf starch concentrations across phyllotactic division of (A) floral and (B) vegetative stems in (■) *Protea neriifolia*, (●) *P. susannae* × *compacta*, and (▲) *P. eximia*. Bars indicate the maximum SE associated with least square means ($n = 3$).

Fig. 4. Changes in preharvest leaf sucrose concentrations across phyllotactic division of (A) floral and (B) vegetative stems in (■) *Protea neriifolia*, (●) *P. susannae* × *compacta*, and (▲) *P. eximia*. Bars indicate the maximum SE associated with least square means ($n = 3$).

Fig. 5. Changes in preharvest leaf polygalatol concentrations across phyllotactic division of (A) floral and (B) vegetative stems in (■) *Protea neriifolia*, (●) *P. susannae* x *compacta*, and (▲) *P. eximia*. Bars indicate the maximum SE associated with least square means (n = 3).

(Bialeski et al., 1992; McConchie and Lang, 1993). Thus, a higher polygalatol concentration in the most acropetal leaves, coupled with its apparent unavailability for transport postharvest, suggests that polygalatol plays a role in osmotic adjustment in *Protea* spp.

Comparing preharvest carbohydrate profiles of the three species examined did not demonstrate a clear relationship between leaf blackening rate and preharvest reserve carbohydrate concentrations. Despite substantially higher starch levels in PN leaves (Fig. 3A), leaf blackening developed faster than in PS, which had lower starch reserves. Sucrose concentrations, however, are related more closely to differences in leaf blackening rate (Figs. 1 and 4A). Highest leaf sucrose concentrations were found in PS, which had a slower leaf blackening rate than the other two species. Further, PE had the lowest sucrose concentrations and developed leaf blackening symptoms most rapidly. Under high sink demand, starch reserves are metabolized to transport sucrose. Floral PN stems had about twice the leaf starch concentration of PS stems (Fig. 3A). This concentration, when combined with sucrose concentrations (Fig. 4A), represents a higher overall carbohydrate status. Collectively, these data suggest that leaf blackening rate may be related more to postharvest inflorescence sink demand than to total preharvest carbohydrate reserves. Sink strength of PN inflorescence seems to be substantial, since up to 82% of starch is depleted from leaves during the first 24 h after harvest (McConchie and Lang, 1993). Therefore, leaf blackening may be induced by the substantial carbohydrate depletion that occurs during continued darkness associated with shipping, irrespective of carbohydrate concentration in the leaves at harvest. Selecting *Protea* species and cultivars less prone to leaf blackening and using postharvest treatments that supply sufficient carbohydrate substrate to the cut flower stem remain the most effective means of preventing leaf blackening.

Whitehead and de Swardt (1982) hypothesized that leaf blackening symptoms are caused by the oxidation of polyphenols and leuco-anthocyanins by the enzymes polyphenol oxidase and peroxidase under carbohydrate depletion. Susceptibility to leaf blackening, in conjunction with depleting carbohydrate reserves, may be related more to enzyme activity and their substrate concentrations in the three species examined than preharvest carbohydrate concentrations. *Protea* species contain high concentrations of phenolic glycosides (Perold et al., 1979), which, under low carbohydrate status, may be hydrolyzed to release free phenols for oxidation and glucose for metabolism (Dey and Dixon, 1985). Thus, the reduction in leafblackening achieved when cut stems are placed under lighted postharvest conditions (Bialeski et al., 1992; McConchie et al., 1991) or treated with a 24-h 20% sucrose pulse (Jones, 1991; McConchie and Lang, 1993) may increase carbohydrate substrates and allow floral development to continue and decrease the requirement for phenolic glycoside cleavage. Additionally, further investigation into carbon-fixation mechanisms in *Protea* spp. may contribute to understanding the physiological mechanisms resulting in leaf blackening.

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