Longer vase life for Proteas

Leaf blackening is a post-harvest disorder that blots the protea's export copybook. New research suggests that the right sugars at the right time can help these iconic South African flowers to turn over a new leaf as far as quality and vase life are concerned.

> "The value of a cut flower crop is determined by the post-harvest quality and longevity of the flowers."

FOR THE SOUTH African Cape flora industry to be internationally competitive, it has to consistently deliver high quality stems with good storage and vase life characteristics. As air freight costs increase and a growing demand results in larger export volumes, Cape flora is increasingly being shipped to Europe. However, this leaves the industry with a quality management challenge, given that the flowers now spend 21 days at sea; air freight used to deliver them to market within three to five days. While some *Protea* cultivars can be successfully shipped, there are many that suffer from leaf blackening, resulting that these flowers be shipped with their leaves removed.

Currently, the only way to delay leaf blackening in some cultivars is to pulse the cut flowers with a glucose solution for a short period, usually less than four hours, as soon as possible after harvest.

1

Glucose pulsing prevents the flowers from using their own carbohydrate reserves once they have been removed from the mother plant. It also maintains osmotic pressure so that the flower can take up water after shipping, and helps to maintain quality and extend vase life.

Glucose pulsing is effective and reliable when administered under controlled laboratory conditions. However, it has proven to be somewhat problematic when applied on commercial scale on the farm, especially when different species, each with their own glucose concentration and exposure time preferences, are harvested at the same time.

Uptake rates are unpredictable, resulting in either ineffective control or glucose toxicity. The latter occurs when stems are exposed to the pulse solution too long, or when they absorb it too quickly.

The stem's water content, which is influenced by pre- and post-harvest conditions, affects its ability to absorb glucose. Ideally, the uptake of each stem should be measured, but it is impractical and a logistical nightmare.

"The reasons for these inconsistencies in glucose uptake and response are not known," says Dr Lynn Hoffman, lecturer in the Department of Horticultural Science at Stellenbosch University. "Further investigation is needed to find alternate solutions, such as pulsing with sugars other than glucose. We also need to improve our understanding of how the flowers metabolise carbohydrates in order to offer innovative strategies to control leaf blackening."

Filling the knowledge gap

With all these unanswered questions in mind, Lynn designed and conducted a research project financed by the Post-Harvest Innovation Programme, Cape Flora SA and Hortgro

2


Science. Working with her on this project is Werner Truter, a final year BSc Agric student, and Dr Waafeka Vardien, a post-PhD student.

Project objectives

1. Determine the ability of various *Protea* species to convert glucose to starch or sucrose, following glucose pulsing, and relate this phenomenon to the ability of glucose pulsing to alleviate leaf blackening.
2. Determine whether glycine betaine and trehalose, a sugar, are possible pulse alternatives to glucose.
3. Determine the potential of sodium nitroprusside (SNP) as a nitric oxide (NO) donor to extend the vase life of Cape flora cut flower products, and develop a method to generate the right amount of NO molecules required for fumigation.
4. Understand nectar biosynthesis inhibition as an alternative mechanism to maintain the carbohydrate status of selected proteas, thereby delaying the onset of leaf blackening.
5. Determine the natural carbohydrate store present in lignotuberous species of *Protea* and the availability of the carbohydrate pool to the storage ability and vase life associated with lignotubers.

Conducting vase life studies

Pulsing solutions trial – Objectives 1 and 2

The research was done at the laboratories of Stellenbosch University. The flowering cut stems were harvested at the 'soft-tip' stage when there is little nectar present in the flower head. The stems of selected, commercially important *Protea* species and cultivars, prone to leaf blackening, including *Protea magnifica*, 'Sylvia', 'Pink Ice', 'Brenda', 'Limelight', 'Susara', 'Sharonet' from Helshoogte, Riviersonderend, Napier and Porterville, were brought to the laboratory within hours of being harvested. The stems were stored for 24 hours at 4°C and then cut to a standard length of 45cm. The leaves were reduced to either 10 or 20, depending on the number that remained after commercial grading.

After 24 hours, the flowering stems were subjected to pulsing with one of the pulsing solutions (glucose, sucrose, lactulose, trehalose, glycine betaine, ascorbic acid

WHAT IS LEAF BLACKENING?

Leaf blackening is a brown-black discolouration on leaves closest to the inflorescence that occurs within three to seven days after harvest. Leaf blackening manifests differently in different species and cultivars.

The exact cause is not clear, but it is likely that leaf blackening occurs because of

- Harsh pre-harvest conditions, such as heat waves;
- The flowers draining carbohydrates stored in the green leaves closest to them to open, causing the leaves to turn black; and/or
- Unfavourable storage and transport conditions.

PROJECT TITLE
Developing innovative technologies to obtain a better understanding of the physiological processes critical to maintaining post-harvest quality in *Proteaceae* cut flower stems

PRINCIPAL INVESTIGATOR
Dr Lynn Hoffman

CONTACT DETAILS
+27 (0)21 808 2383
ewh@sun.ac.za

DURATION
One year and nine months

PHI CONTRIBUTION
R194 816

LEAD INSTITUTIONS
Cape Flora SA and Hortgro Science

BENEFICIARY
The protea and Cape flora industry

FOCUS AREA
Post-harvest physiology

HUMAN CAPITAL DEVELOPMENT
One post-PhD student and one final year BSc Agric student

PUBLICATIONS
Two

PRESENTATIONS
Two

1 The Cape flora PHI projects' research team consists of (from left) Dr Waafeka Vardien, Stenford Matsikidze, Dr Lynn Hoffman, Anton Huysamer and Dr Shelley Johnson
2 Vase life evaluation of 'Sylvia'.

Figure 1: Images of *Protea* cv. Brenda to depict flower quality and leaf blackening over the vase life period

> Understanding *Protea* plants' response to stress and the processing of carbohydrates in and by the lignotuber may provide meaningful insight into finding the most efficient pulsing solution to prolong the quality and vase life of these beautiful cut flowers.

and γ-aminobutyric acid (GABA) for three hours, or until 10ml of the pulse solution accumulated in the stem. Additional stems were placed in either tap water or a 6% glucose solution (industry standard) as controls for the pulsing period.

Thereafter, all stems were transferred to a vase with tap water and evaluated for flower quality and leaf blackening over a period of 10 to 14 days. Additional stems were stored for 21 days at 1°C, before evaluating them for an additional 10 days. Flowers were scored on a five-point scale, while leaf blackening was determined by the number of leaves that had more than 10% blackening.

Each treatment was replicated six to 10

PULSING SOLUTIONS ON TRIAL

As not all flower types respond in the same way to sugars, putting these solutions to the test (objective 1, 2 and 3) is the only way to see which may be effective to prolong vase life of Cape flora.

Sugars

Glucose ($C_6H_{12}O_6$) – a sugar made during photosynthesis from water and carbon dioxide, using energy from sunlight, and stored as starch for times when the plant needs it. Glucose and other sugars such as sucrose and fructose are soluble. Glucose needs to be linked together in long chains to make starch. Sucrose (table sugar), glucose and fructose (fruit sugar) are often used in pulsing solutions.

Lactulose ($C_{12}H_{22}O_{11}$) – a synthetic disaccharide sugar consisting of glucose and galactose.

Trehalose ($C_{12}H_{22}O_{11}$) – a sugar used by the flower industry to delay symptoms of senescence and associated programmed cell-death, to suppress water loss and provide enhanced vase life.

Glycine betaine ($C_5H_{11}NO_2$) (trading as Greenstim®) – this sugar, found in beetroot, is an organic osmotic compound taken up by cells for protection against osmotic stress, drought, high salinity, extreme temperatures, UV radiation and heavy metals. It plays a role in maintaining cell volume, enzyme and membrane integrity, and fluid balance, thus protecting cells from the effects of dehydration.

Senescence inhibitors

Nitric oxide (NO) fumigation – NO is a critical signalling molecule to down-regulate ethylene synthesis; acting as an antioxidant to delay decay of plant tissue and cellular membranes. Fumigation with NO gas reduces respiration rate, stem and flower head wilt (water loss), decreases the incidence of mould growth, and improves the vase life of cut flowers.

Sodium nitroprusside (SNP) – a NO donor which acts as a protective signal molecule in plants and is in particular responsible for the regulation of key stress/defence-related antioxidant enzymes to extend the shelf and vase life of fruit, vegetables and cut flowers.

times, depending on the amount of material received and the number of treatments per trial. A total of 17 pulsing experiments were conducted.

During vase life studies, the average vase life days, the water content and percentage leaf blackening were scored over the evaluation period.

Results

Leaf blackening develops rapidly in some species and cultivars, for example, at day one or two of vase life, whilst in others the onset of this post-harvest disorder is slower, developing only after day five. In 'Brenda', leaf blackening develops from day two onward. The quality of untreated (control) flowers declined rapidly, whereas those treated with glucose and lactulose maintained quality and had reduced levels of leaf blackening (Fig. 1 and 2).

In 'Sylvia', leaf blackening occurs between day one and four and flower quality can be maintained until day eight, after which it drops and the product is considered non-marketable. Glucose and trehalose significantly reduce leaf blackening in 'Sylvia' (Fig. 3 and 4).

For 'Pink Ice', leaf blackening was only visible from day five, and progressed slowly compared to 'Sylvia' and 'Brenda'. Glucose greatly reduced leaf blackening and good flower quality was maintained throughout the vase life period. For *P. magnifica*, the onset of leaf blackening was also slower, occurring between days eight and 10. For this cultivar, sucrose had the greatest effect in alleviating leaf blackening. Flower quality was retained throughout the vase life period.

Glycine betaine (trading as Greenstim) in combination with 6% glucose (industry standard) reduced leaf blackening. In cultivars such as 'Sylvia', the effect was better than when using glucose or Greenstim alone. The effect is similar for 'Susara'.

The positive effect of glucose, trehalose, sucrose and lactulose on leaf blackening confirms that the disorder may be largely linked to carbohydrate demand. These sugars not

Figure 2: Leaf blackening in *Protea* cv. Brenda

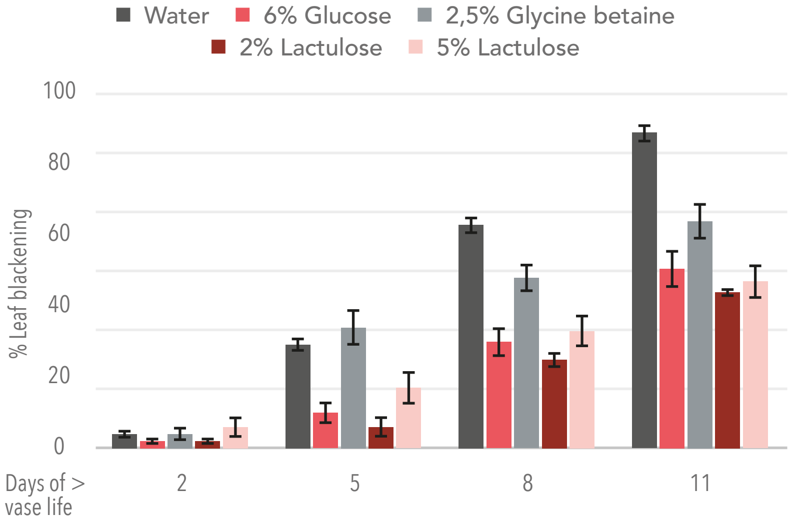

Figure 3: Leaf blackening in *Protea* cv. Sylvia

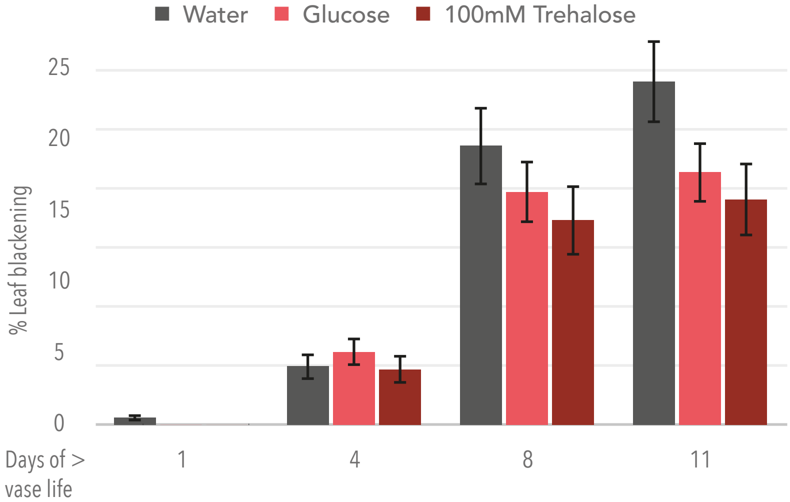

Figure 4: Images of *Protea* cv. Sylvia to depict flower quality and leaf blackening over the vase life period

Figure 5: Flower quality and leaf blackening in *Protea* cv. Sharonet during the SNP (Nitric oxide) trial

Day 2

Day 7

Water | 6% Glucose | 25μM SNP | 50μM SNP

> Understanding how Cape flora cut stems metabolise carbohydrates after harvest will enable us to identify which of the new alternatives currently being tested in other major commodity cut flowers, such as roses, will be most effective.
>
> Dr Lynn Hoffman

only delay senescence, but may also help to reduce water loss, which ultimately results in better flower quality and longer vase life.

GABA had a positive effect on flower quality in 'Sylvia' stems. Stems treated with 5, 10 and 20mM GABA had increased flower quality scores (averages of 2,25, 2,37 and 2,75, respectively) by day five5 of the vase life evaluation, which were better than the scores of flowers treated with water and glucose. The percentage leaf blackening also decreased with increasing concentrations of GABA and was lower than for water-treated stems. However, glucose-treated stems still exhibited the least amount of blackening.

1

Natural carbohydrate storage in lignotubers – Objective 5

Some species in the *Proteaceae* family have lignotubers, ie. they have the ability to re-sprout after their above-ground parts have been removed or killed by fire. The lignotubers, or underground stems, act as reservoirs of carbohydrates (sugars) and mineral nutrient reserves, which are assumed to play an important role in resprouting after fire. After a fire, shoots grow from the lignotuber and eventually develop into new trunks.

To determine the natural carbohydrate storage ability of lignotuberous species of the *Proteaceae* family, Lynn and her team milled lignotuber material from *P. cynaroides*, also known as 'King Pink', for carbohydrate (sugar) analyses for a preliminary assessment. Baseline samples for carbohydrate analysis (soluble sugars, water soluble polysaccharides and starch) were analysed for both lignotuberous and non-lignotuberous species.

Stems were stored for three weeks at 1°C, whereafter leaf, stem and inflorescence material was again sampled for post-storage carbohydrate analysis. In addition, the respiration rate of each representative was measured by placing five replications of two to three hydrated cut flower products (30cm stem with leaves and flower head) in a sealed airtight container for one hour, where after CO_2, possible C_2H_4 evolution and O_2 consumption were determined by means of gas chromatography. The species' vase life was determined over a 10-day period following long-term storage. Each treatment was replicated 10 times. Carbohydrate analyses for soluble sugars and starch were done.

To determine the effect that girdling might have on the storage ability of lignotubers, potted *Protea* plants, some girdled above the lignotuber, were obtained from Arnelia farm (Hopefield) in May 2016. The plants were monitored weekly for flower development and the quality of the leaves beneath the developing flower. The plants were harvested and separated into leaves, lignotubers and stems, and carbohydrate analyses were conducted on separated plant organs.

Results

The study showed that the lignotuber of 'King Pink' *Protea* contains more starch than the stem organs.

Nitric oxide donors – Objective 3

The application of SNP as a NO donor on freshly harvested 'Sylvia', 'Susara', 'Sharonet' (Fig. 5) and 'Brenda' cut stems did not show any positive effect on the reduction or control of leaf blackening. The only trial where SNP showed an efficacy was in the April-harvested 'Susara' trial. Both concentrations (25µM and 50µM) of SNP that were used in this trial had a positive effect on flower quality. It seems that the better the quality of the freshly harvested flowers, the lesser the incidence of leaf blackening. To maintain the quality of the cut stems, they can be stored in a controlled atmosphere with low oxygen and high carbon dioxide levels to reduce the metabolic activity and cellular processes of the cut stems. Applying SNP as a NO donor might be more effective under these conditions.

Slowing down nectar production – Objective 4

The production of nectar after harvest is a major carbohydrate drain, leaving the flower with less energy to look its best for the longest possible time. A better understanding of how nectar production can be held back or

2

slowed down may be a way to maintain the carbohydrate status of selected *Protea* cultivars, thereby delaying the onset of leaf blackening and extending vase life.

For this study, stems of 'Sylvia', 'Pink Ice' and 'Susara' were harvested just before the 'soft-tip' stage when only a little nectar is present in the flower head. Solutions of invertase inhibitors, ascorbic acid (Vit. C), Greenstim®, chloramphenicol and cycloheximide were prepared in a range of concentrations. The cut stems were treated with these pulsing solutions and kept at room temperature to allow further flower head development.

Once the flower heads opened, the volume and sugar composition of nectar in the flower head were determined and compared with controls (no inhibitor treatment). During this period, leaves from the stems were periodically sampled to analyse the starch and soluble sugar contents.

A subset of treated and untreated stems were held in water at room temperature to determine the rate of development of leaf blackening.

Results

Neither invertase inhibitor, cycloheximide or chloramphenicol, at different concentrations, delayed the onset of blackening or reduce it. In fact, their application resulted in a higher incidence of blackening. Ascorbic acid did not delay leaf blackening.

1 Leaf blackening on a *Protea magnifica* flower from the Porterville area. Hot and dry mid-summer conditions probably subjected the leaves to additional stress.
2 Leaf blackening, a post-harvest disorder characterised by a dark brown to black discolouration, is found in most commercially important *Protea* cut flower species and cultivars.

> "Currently glucose pulsing is seen as effective but cumbersome. Improving this technology, or finding an effective, easy-to-apply alternative, will ensure our beautiful Cape flora stand out in the highly competitive floricultural world markets."
>
> Dr Lynn Hoffman