Productschap Tuinbouw

Betrouwbaarheid en Uniformiteit Proteas

Gezamenlijk project van de IPA (International Protea Association), bestaande uit 8 deelprojecten in samenwerking met Oudendijk Import, OZ Import (Dutch Flower Group), de Mooy en een aantal lokale universiteiten.

Uitgevoerd door: Vereniging van Groothandelaren in Bloemkwekerijprodukten (VGB)

Nutrient and irrigation requirements of cultivated and wild-harvested Proteaceae: Interactive fertilizer scheduling

Executive institution: Department of Biological Science, University of Cape Town

Recommendations

On the basis of our research we recommend that the ratio of nutrients supplied should be considered, above the actual concentration of the nutrients delivered. This has been tested in pots but not in the field. Therefore field trials would be appropriate.

Web summary

Control of the ratios of fertilizers applied (e.g. N:P:K) is a standard agricultural practice, although these ratios are often barely considered in modern agricultural practice due to the now prevalent understanding that plants regulate their nutrient uptake. We assert that mass-flow of nutrients to roots results in nutrient toxicity or deficiency if incorrect nutrient ratios are supplied. The correct ratios of nutrients have not, however, been established for Proteaceae. From foliar analyses we have determined that the ratio of N:P:K:Ca:Mg for Proteaceae on farms is 13:1:6:5:3. Surprisingly there is little variation between tissue nutrient concentrations of Proteaceae genera (*Leucospermum*, *Leucodendron*, *Protea*). We have determined that the soils of the Cape generally have low binding capacity for P. Therefore we suggest that N:P:K fertilizer should be supplied as 6:1:3 to 8:1:5 (agricultural specification) for Proteaceae cultivation in this region.

Public Summary

The uptake of nutrients from soil that roots are in contact with is biochemically regulated. However, the content of the soil volume adjacent to the root is determined by the diffusion and mass-flow of nutrients into this soil volume, and the uptake of nutrients by the roots from that volume. This together with the fact that plants partially control the flow of water through the soil towards the root on the basis of their nutritional status (in particular nitrogen; see below) means that managing the chemistry of the soil volume adjacent to the root is extremely imortant. If the cocktail of nutrients available in the soil is not appropriate for plant demand, then the plant may experience either deficiencies or toxicities. Toxicities occur because plants either do not take up nutrients that flow towards the roots or take them up and accumuate them in the plant tissue. Since the flow of nutrients into the soil volume is dictated by the availability of N (the form of the nutrient and soil characterisitcs are also important), it is important to get the ratio of N to other nutrients right. Proteaceae have evolved in a nutrient poor context and have very distinct leaf nutrient compositions to most crop plants. Therefore, it is imortant to establish what the correct ratios of nutrients are that may be required by the plants. This is complicated by the fact that soils vary and bind different nutrients to variable extents. However, many soils of the Cape region of South Africa have low cation exchange capacity. On the basis of the foliar requirements and the nutrients removed during harvesting of stems, we have thus determined what the N:P:K ratio of nutrients supplied as fertilisers should be between 6:1:3 and 8:1:5.

We first set out to determine the foliar nutrient concentrations and ratios in a range of Proteaceae in cultivation. Foliar samples were collected from a number of farms throught the Cape region. Proteaceae growing wild were also sampled. We also collected soils to determine the forms and availabilities of nutrients in the soils from these sites. From leaf analyses we have determined that the ratio of N:P:K:Ca:Mg for Proteaceae on farms was 13:1:6:5:3. The equivalent ratios for wild Proteaceae were 19:1:10:28:9. The farm leaf N:P is consistent with the reported N:P ratios of harvested material (12.5).Thus the farm-grown Proteacea have a deficiency (relative to wild Proteaceae) of N supplied relative to P and are probably also deficient in Ca and Mg. There was little variation between tissue nutrient concentrations of Proteaceae genera (*Leucospermum*, *Leucodendron*, *Protea*). By progressively adding P to sampled soils and then assaying the P availbale in the soils we were able to measure the

binding capacity of the soils. Although this varied significantly between different farms, overall there was relatively little retention of N or P by the soils. This is not too surprising since the soils of the Cape are highly leached. As a consequence we were able to predict that the N:P:K ratios for fertilizer should 6:1:3 (agricultural specification; i.e. $N:P_2O_5:K_2O$) based on the measured foliar concentrations on farms. However, the equivalent foliar data from wild Proteaceae yielded a ratio of 8:1:5, which is probably more suitable. For a limited number of farms resampled between 2000 and 2010 we note a change in foliar nutrient ratios from N:P:K:Ca:Mg 19:1:10:10:3, close to the values of wild Proteaceae, to 13:1:5:4:5 in 2010, possibly as a consequence of a lack of fertilization combined with sustained harvesting.

The control of Protea Sylvia transpiration exercised by N was investigated by supplying different forms of N to the plants and examining the gas exchange characteristics. We supplied $NO_3^-$and NH_4^+ such that NO_3^- made up 0, 25, 50, 75 and 100% of the total N. The flux of water through Syliva decreased with increasing proportions of NO_3^- from 0.12 mol m^{-2} s^{-1} to 0.04 mol m^{-2} s^{-1}. This is consistent with our theory that NO_3^- is more effective at suppressing water flux than NH_4^+. While Proteaceae have generally been assumed to utilize NH_4^+ in preference to NO_3^-, we have shown previously that fertilization of Proteaceae with NO_3^- is equally, if not more effective, than fertilization with NH_4^+. The suppression of transpiration by NO_3^- is consistent with the regulation soil mass-flow by N availability and emphasizes the importance of supplying the correct ratios of fertilizers to the plants.

Screening Proteaceae material for resistance against *Phytophthora* root rot

Executive institution: Agricultural Research Council (ARC), Horticulture Division, Stellenbosch, South Africa

Recommendations

Further research should be done to design a hot water treatment of cuttings before screening tests are performed so that any latent infections can be killed. Latent infections interfere with the re-isolation process of the inoculated pathogen and with the recording of visual symptoms.

Web summary

The most important root disease of cut-flower Proteaceae is root rot caused by *Phytophthora cinnamomi.* The goal of this project was to design a protocol for screening new material from breeding programmes, for resistance against Phytophthora root rot, before large scale commercialisation. A selection of isolates was used to inoculate potted, rooted cuttings of *Leucospermum* 'High Gold' to confirm pathogenicity. Thereafter trials were conducted to test various inoculation techniques to determine the technique(s) with the most consistent results obtained in the shortest possible time. One of the most efficient techniques tested, was used to inoculate *Leucospermum*, *Leucadendron* and *Protea* cuttings with *Phytophthora* isolates.

Public Summary

The most important root disease of cut-flower Proteaceae is root rot caused by *Phytophthora cinnamomi*. This pathogen is widespread in the soil and river water of the Western Cape Province of South Africa, which makes it very difficult to control. One way of avoiding the effects of disease is by making use of resistant plant material. The long term aim of this project was to design a protocol for screening Proteaceae material under controlled conditions for resistance against *Phytophthora* root rot.

During a survey conducted in 2008/09, which was not part of the current study, *Phytophthora* cultures were collected from all fynbos production areas in South Africa, as well as from various Proteaceae hosts. During 2010/11 pathogenic isolates were selected from the collected isolates so that these could be used in glasshouse trials. The objectives for 2011/12 was to test various inoculation techniques, using the selected isolates, in order to develop an easy, repeatable technique for testing tolerance to Phytophthora root rot.

During 2011, a technique using rooted cuttings planted in vermiculite, resulted in only 4% of the *Leucospermum* 'Spider' cuttings being dead or dying after 8 weeks incubation and none of the 'Spider' control plants to be dead. This was expected since 'Spider' is seen as resistant by the South African fynbos industry. 74% of the *Leucospermum* 'High Gold' (susceptible cultivar) inoculated cuttings were dead after 8 weeks incubation. However 40% of the control plants also died, probably from transplant shock, since the inoculation protocol entailed the washing of roots before planting into the vermiculite. Another technique using infected orchard soil to water potted, rooted 'High Gold' cuttings, did not result in any symptomatic plants or deaths. In the 2012 trial, using sand-bran inoculum and rooted cuttings (from three different genera) planted in seedling mix, the individual isolates caused 52-86% of cuttings to die and the isolate mix caused 81% of cuttings to die. 10% of the control plants died, but no *Phytophthora* was isolated from these plants. Therefore the technique proved to be effective and repeatable.

The confirmed pathogenicity of the selected isolates will be very useful in future tests such as resistance screening or fungicide testing under controlled conditions. It has to be kept in mind however, that these isolates may lose some virulence in long-term storage. The deaths noted in the control plants during the inoculation technique trials, remain a stumbling block in the pathogenicity testing of Proteaceae pot plants, and may be attributed to apparently healthy looking cuttings that are naturally infected with pathogenic fungi. Research is required to develop a method for cleaning cutting material of pathogens before it is used in pot trials.

The sand-bran technique and the vermiculite - liquid inoculum technique proved to be promising techniques to be used in screening trials and also proved to be effective for Proteaceae species other than *Leucospermum*, such as *Leucadendron* 'Magenta Sunset' and *Protea* 'Sylvia'. Future projects could now use the above mentioned techniques together with the collected isolates to screen Proteaceae material for resistance or tolerance to Phytophthora root rot.

Investigation into the suitability of an environmentally friendly plant defense activator for desease control and yield enhancement in the protea industry.

Executive institution: Instituto de Productos Naturales y Agrobiología – Consejo Superior de Investigaciones Científicas – Tenerife – Spain

Recommendations

The research was discontinued after the first year

Web summary

Water soluble vitamin K3, or menadione derivative (MSB), has been proved to be a plant defence activator, that also enhances yields and crop quality of several plants. Research was undertaken to verify the control of this product on *Botryosphaeriaceae* fungal canker disease of *Leucospermum cordifolium* cultivar 'Succession II', as well as its effect on mineral nutrition. Fortnightly sprays of MSB and a control treatment began in November 2009, in two plantations of this Leucospermum cultivar, on La Palma island (Canarian Archipelago, Spain). The plants were tested periodically for the presence of canker, and foliar samples were taken during the vegetative stage to determine their nutrient content. Plant yield and flower qualities were also measured. MSB did not control 'Succession II' canker, and no significant differences in nutrient content were found between treated and non-treated plants. No effect of the treatments was observed on yield and quality of the flowers.

Public Summary

Menadione sodium bisulphite (MSB) is a water-soluble addition compound of vitamin K_3, or pro-vitamin K. MSB induce resistance against vascular wilt disease of bananas, stem canker of oilsedd rape plants and downy mildew in pearl millet. Other applications of MSB in agriculture have shown that it accelerates plant blooming and enhances quality and yields of crops.

Members of the Instituto de Productos Naturales y Agrobiología and of Proteas de La Palma Sociedad Cooperativa (Canarian Archipelago, Spain) made a research in two commercial farms where protea plants of the species *Leucospermum cordifolium* 'Succesion II' were grown. They were situated on La Palma island, and received fortnightly foliar applications of either 80 ppm of MSB or a control treatment (sprayed as treated plants but without MSB). The 'Succession II' plants were tested periodically for the presence of canker produced by *Botryosphaeriaceae*. Foliar samples throughout the vegetative stage were taken to determine if treatments affected the nutrient balance. During the flowering stage, we measured the growth of floral stems and their buds.

After treating throughout one year with MSB, we foud that this product had no effect on the studied protea plant, because it did not control 'Succession II' canker. No significant differences in nutrient content were found between treated and non-treated plants, and No effect of the treatments was observed on yield and quality of the flowers.
Since we did not find any interesting results, the experiment was cancelled.

Control of leaf blackening as a postharvest disorder in *Protea*

Executive institution: Stellenbosch University, South Africa

Recommendations

1. Harvesting times with associated high leaf blackening risks have been identified for 'Sylvia' over a three year period and can serve as a guideline for the implementation of glucose pulsing regimes or when sea freight for this product should be avoided.
2. Stems should be harvested later during the day rather than early-morning when non-structural carbohydrate levels are low and the water content is very high.
3. Ethanol vapour or pulsing alone is not currently a commercial alternative to pulsing with glucose in the control of leaf blackening in *Protea*.
4. The rate of transpiration, the osmotic potential of the pulsing solution and the water potential (tension) in the stem will determine the pulsing time required. All these factors should be carefully monitored and incorporated in a pulsing regime to ensure that stems accumulate sufficient levels of glucose, but also to avoid glucose toxicity.
5. Glucose pulse may play a role in protecting stomatal functionality and therefore assist in delaying and lower the incidence of leaf blackening. Other factors that may influence stomatal conductivity such as photomorphogenetic effects results from exposure to low light levels may thus also possibly alleviate leaf blackening and requires further investigation.

Web summary

The postharvest disorder of leaf blackening poses a high risk for *Protea* products, both at the producer, exporter and retail level. Under South African conditions this disorder varies significantly throughout the year, with peak incidence from June to November. Harvesting stems in the afternoon, rather than morning could significantly reduce leaf blackening in 'Sylvia', primarily due to higher sucrose levels at the time of harvest. Glucose pulsing remains the most effective and low risk control remedy available for this disorder, as opposed to ethanol fuming or pulsing. The rate of glucose uptake during pulsing can be more effectively control by the manipulations of the transpiration rate, as well as the osmotic potential of the pulsing solution together with the water potential of the stem. Mechanisms protecting stomatal functionality may assist in delaying or lower the incidence of leaf blackening.

Public Summary

Leaf blackening, a serious postharvest disorder in Protea cut flowers, that can develops within 3-7 days after harvest, remains a significant impediment for Protea to compete successfully on the international market with other commodity, high quality floricultural products. For South Africa and other production areas with remote markets, the incidence of leaf blackening is exacerbated by increasing airfreight costs and a need for the industry to minimize its carbon footprint. Leaf blackening in several cultivars appears to fluctuate seasonally and is especially prevalent for South African producers during the highly profitable marketing window of August to November. The underlying cause (s) of leaf blackening remains largely elusive. In addition, limited post-harvest strategies such as glucose pulsing and girdling in combination with low temperature storage are available to minimise Protea leaf blackening. The aim of the study is to gain more in depth understanding of pre-harvest factors that may contribute to the development of leaf blackening in Protea and to develop pre- and post-harvest technologies that may offer additional control of leaf blackening, especially under conditions of long term storage and/or transport.

Significant findings in our understanding of leaf blackening in *Protea* have been made during this study. Extensive variation in leaf blackening have been recorded throughout the year, with significantly more leaf blackening during the late winter and spring months. The incidence of leaf blackening throughout the season correlated well with sucrose and % water content, but was not significantly correlated with starch and phenolic content. Furthermore, significant variation in the expression in leaf blackening occurred when stems were harvested in the morning when compared to an afternoon harvest, where harvesting in the morning consistently produced stems with more leaf blackening compared to harvesting any other time of the day, irrespective of season or daily average temperature. As in the seasonal study, a link was made between sucrose and water content of the leaves subtending the flower and

the incidence of leaf blackening. Stems harvested in the morning was also much slower to respond to glucose pulsing than stems harvested after 13h00 in the day, due both to a lower water tension in the stem and the reduced role of transpiration, ascribed to a lower water pressure deficit than would be present later during the day. The uptake rate of various glucose concentrations that ranged from 0-20% varied significantly. To achieve a non-toxic concentration of pulsed glucose in stems, pulsing with 10% glucose solution over a period of 4 hours was recommended.

The mechanism by means which glucose to control leaf blackening is yet unsure, but a link was made between glucose pulsing and the retention of stomatal functionality. This aspect requires further investigation as a lack of stomatal control after long term cold storage may be the pivotal in the expression of leaf blackening.

Studies on ethanol pulsing concluded that glucose pulsing together with a 2% ethanol pulse (not fuming) was most effective to control, but not eradicate leaf blackening. Ethanol fuming proved to be too variable in the rate of release to be able to use effective and safe as a current commercial recommendation.

Enhancing Proteaceae Flower and Foliage Postharvest Life

Executive institution: University of Hawaii at Manoa

Recommendations

Protea leaf blackening postharvest can be reduced by firstly, harvesting full-grown flowers with the bracts still closed at the the top of the flower, keeping the cut flowers cool and in water, use 2.5% glucose at least overnight before packing and if possible add a stem vial also containing 2.5% glucose during shipping. When unpacked at a retail store hold in a well-lite area or under plant growth lamps. In the long term, incorporate into protea breeding and selection programs leaf blackening as a crucial criteria for clone evaluation with simulated handling and shipping could lead to significant improvement

Web summary

The blackening of leaves on cut Protea flowers stems remains a significant problem for shippers and retailers. The blackening is strongly associated with nectar production by the flower that starts as the flower bracts at the top begin to separate and open. Harvesting the flower when the flowers are full-sized and the bracts are still closed limits nectar production and its demand from sugars from the leaves. Glucose in the vase solution helps but needs to be present from soon after harvest all the way to the consumer. Anti-blackening chemicals and leaf coating show limited usefulness.

Public Summary

The postharvest life of Proteaceae flower and foliage is limited by leaf blackening, and foliage and flowers by dehydration. Foliage blackening is a problem for flowers with Protea spp., and foliage and flower dehydration occurs in both Protea spp., and Leucospermum spp.
Leaf blacken in cut Protea flowers starts to occur soon after harvest and often dramatically increases during shipping. The leaf blackening causes a significantly loss in the cut flowers appeal, as the leaves, also as cut vegetative shoot, have a very pleasant colour and texture. The blackening often leads to the grower/packer or the wholesaler/retail to remove the discoloured leaves at extra expense and unsightly leaf scars on the stem.

For the cut flower, leaf blackening is known to be associated with the initiation of nectar production by the flower head. The nectar's role is to attract birds and insects to carry out cross pollination. Nectar production dos not start until the flower starts to open as seen by an opening at the top of the flower inflorescence as the colourful bracts reflex outwards when the flower is mature. The nectar (~10 mL) is very sweet with a soluble solids content of greater than 15% which is more than 5% greater than that found in sodas. Unfortunately, the sugar for nectar production is drawn from the cut flower stem and especially from the leaves. This demand for sugar for nectar production and final flower development depletes leaves' stores of sugars which the leaves also need to stay alive. Apparently when the stored sugars in the leaves fall below some critical level leaf senescence rapidly occurs, one of whose symptoms is leaf blackening. In the field, the blackening is not commonly seen as the flower can draw sugar from many more leaves that are still photosyntheszing in full sunlight and producing sugars. Cut Protea foliage stem have a similar problem as the young developing leaves at the tip also require sugars for te continued growth and development.

The demand for sugars and leaf blackening provides a number of approaches to reducing or eliminating leaf blackening. An related event is drying out of the flower with time after harvest that is associated with water uptake from the vase. The water uptake from the vase is the same system that we can use to provide sugars and other chemicals to the flower to reducing blackening that lead to our projects's objectives: 1. Re-evaluate plant growth regulators and sugar sources on minimizing leaf blackening, and, 2. Evaluate new coating that limit water loss for foliage and inflorescence.

In this project, to address the sugar needs of the developing flower and to minimize nectar demand we confirmed that glucose was by far the best sugar source for the cut flowers. However, the glucose needs to be supplied from harvest or soon after through the whole marketing chain to the consumer. A grower/shipper has control only until the carton is received by the wholesaler/retailer and his options are limited. Our results showed that the grower/shipper can hold the cut flower stems in a 2.5% (w/v) glucose solution before

packing. Large flower vials can be used on the stem with the same glucose solution. The vials should be as large as possible (50 mL or greater) as the water uptake by these flowers is significant (30 to 50 mL/day). Honey products have simple sugars such as glucose and contain chemicals that are known to delay browning. We tested a number of different honey brands including those that are reported to significantly inhibit bowning and found the a glucose treatment was still more effective. As we have shown leaving as many leaves on the stem, at least until received by the wholesaler/retailer and holding in high light after unpacking helps to reduce blackening. Our results in this project confirmed in other cultivars that cut flowers should be harvested before the colourful flower bracts have started to open at the top of the flower. This maturity standard for harvesting means that nectar production is dramatically limited and leaf blackening minimized.

The second approach is then to reduce the reactions that lead to leaf blackening. In this project we tested a number of chemicals that are used in the food industry to reduce browing in cut fruits and vegetables (apple that brown after being cut) and known inhibitors of the enzymes involving in generating these browing reactions. Chemical tested included: Arbutin, cyclodextrin, thiosemicarbazide, 4-hexylresorcinal, tropolone, kojic acid, ferulic acid. L-minosine and Mallard product (1:5, glycine/sucrose, 90^{o}C, 7 hours). A number of these gave a slight delay in leaf blackening when pulsed for seven hours as soon as possible after harvest in a simulated handling protocol that included packing in a carton. From a commercial position, a one day delay to 50% leaf blackening at around 10 days from harvest that included two days in a carton does not warrant the treatment.

It has been reported that an ethanol vapour treatment delay blackening. It is known that ethanol response play a role in postponing ethylene responses. Another compound that shows the same response 1-MCP did not delay leaf blackening in our studies. Collidal silver has also been shown to delay senescence in some flowers but was not effective in our tests. Attempts to delay water loss with three common wax and flower gloss sprays did not limit dehydration of the flower.
We had previously reported that Leucosperum leaves that do not blacken after harvest contain an inhibitor of leaf blacken. This was shown by mixing extracts from both species (Protea and Leucospermum). We tried extracting this inhibitor and supplying the extract to Protea flowers via the vase solution without success.

The conclusion from this projects and that of other published research is the best postharvest handling protocol for the Protea is:

1. Harvest the flowers when full size and before they have started to open. (Crucial)
2. Hold cut flowers in water after harvest or supply 2.5% (w/v) glucose.
3. Storage temperatures less than 10oC are desirable.
4. Leave as many leaves on the stem until the cut flower reaches the retailer.
5. Add a large vial to the stem during shipping in the carton that contains 2.5% (w/v) glucose.
6. Hold the stems in water and exposed to strong lighting or indirect sunlight.

Post-harvest biology and technology of *Leucadendron* and *Leucospermum* under prolonged cold storage

Executive institution: Stellenbosch University, South Africa

Recommendations

1. Storage at low temperatures (-0.5°C and 2°C) posed a greater risk earlier in the season (March – April) when the *Leucadendron* involucral bracts are not yet fully mature.
2. Irrigated stems were more susceptible to chilling injury at the higher temperatures than the dry-land stems.
3. The quality loss in foliage of long-term cold stored *Leucospermum* potted plants and cutflower stems may be linked to loss of stomatal control during and after cold storage under dark conditions of consistent high humidity.
4. Shoots became less susceptible to chilling injury as stems matured with the progressing season, irrespective of the storage temperature. The higher susceptibility of younger stems to chilling injury are linked to a possible lower free sugar and storage carbohydrate availability to stems as respiration reserves during storage.

Web summary

Increasing costs of air freight and the requirement to reduce carbon footprints necessitates a major switch to sea freight for the transportation of *Proteaceae* cut flowers. However, the extended period of cold storage has lead to a major increases in post-harvest losses due to leaf desiccation resulting from the subsequent chilling injury and *Botrytis* infection. Susceptibility of stems of *Leucadendron* and *Leucospermum* varies with the maturity of the shoots at harvest. Chilling injury were mostly observed when storage duration exceeded 14 days at temperatures of 1-4°C. Production practices such irrigation regimes or temperature at harvest as well as the postharvest pulsing of stems with sugars prior to storage may alleviate chilling injury, although inconsistency with results were obtained. Methods such as heat shock treatment to control chilling injury as well as the use of growth regulator dips or systemic acquired resistance enhancers such as methyl-jasmonate requires further investigation.

Public Summary

Leucadendron and *Leucospermum* are both high value export commodities. It is aimed to export these products primarily by sea freight as it is the most cost effective and carbon conscious option. However, sea transport is linked with prolonged periods of cold storage. Some of the risks of prolonged cold storage are chilling injury and dessication for the foliage and infection by *Botrytis* in both the flower and foliage, all of which would negatively affect the quality and vaselife of the product.

Leucadendron 'Safari Sunset' (*Ld. Laureolum x Ld. salignum*) were harvested from two areas, Hopefield, being the warmer area and Piketberg, being the cooler area in order to study the effect of different harvesting times and temperatures (07:00, 11:00 and 15:00) on the development of chilling injury under different storage regimes (-0.5°C, 2°C and 6°C) and storage times. *L.* 'Safari Sunset' was also evaluated for the effect of a preventative heat shock treatment on the possible control of chilling injury.

Leucadendron 'Laurel Yellow' (Ld*. Laureolum x Ld. discolor)* from at Piketberg area was used to investigate the effect of irrigation regimes and post-harvest hydration. Material was harvested from an irrigated as well as a dry-land site. Post-harvest, prior to packing of the material, the stems received either a water hydration treatment or no hydration treatment. *Leucadendron* 'Laurel Yellow' for the same source was used in the study as pertaining to chemical and organic treatments to limit the development of chilling injury. After the postharvest treatments, all material was stored at -0.5°C, 2°C, 4°C and 6°C for a three week period. The time of harvesting during the day had no significant effect on the development of chilling injury during prolonged cold storage. Storage at low temperature (-0.5°C and 2°C) posed a greater risk earlier in the season (March-April) when the involucral bracts of the *Leucadendron* flowering head was not yet fully mature (hardened-off). Temperatures lower than 4°C are not recommended for prolonged cold storage in these early stages, however higher temperatures poses the risk of increased *Botrytis* infections. However, later in the season (May-July) when the stems were more mature, the impact of

lower temperature was much less severe. Chilling injury and desiccation symptoms are mostly observed when storage duration exceeds 14 days at temperatures of 1-4°C. A recommendation of -0.5°C as optimum storage temperature is made for *Leucadendron* with harvesting from May and onwards until July/August.

Irrigation regimes allow for the production of increased stem length and are therefore favoured by producers. However, these stems produced at a faster growth rate under irrigation may implicate a higher susceptibility to chilling injury. For both dry-land and irrigated produced stems, -0.5°C was the recommended optimum temperature. As pertaining to post-harvest pulsing before export, the combination of dry picking of dry-land produced stems and picking irrigated stems in water produced the best results during long-term storage.

The maturity of the shoots during harvest may have an influence on the development of chilling injury during cold storage where younger stems are considered be more susceptible to chilling injury and as the *Leucadendron* shoots mature, it is possible that the susceptibility to chilling injury would decline. Although characterization of the maturity of foliage products are not clearly defined in the literature, maturity of *Leucadendron* 'Chameleon' stems was assessed by means of sugars/starch ratio, the leaf water content as well as the dry mass content to leaf area ratio. During a maturity experiment where *Leucadendron* 'Chameleon' stems were assessed for optimum storage temperature from the first commercial stage of harvesting in February 2010 and thereafter on a biweekly basis until the end of the commercial harvest in June, it was confirmed that with progressing maturity the shoots became less susceptible to chilling injury as stem matured with the progressing season, irrespective of the storage temperature. The higher susceptibility of younger stems to chilling injury are linked to a possible lower free sugar and storage carbohydrate availability to stems as respiration reserves during storage. Stems were therefore pulsed with various carbohydrate solutions at various concentration to assess the efficacy of sugar pulses to alleviate the chilling injury during cold storage for three weeks.

The use of methods such as heat shock treatment to control chilling injury as well as the use of growth regulator dips or systemic acquired resistance enhancers such as methyljasmonate requires further investigation. The control of *Botrytis* under conditions that limit desiccation during long term cold storage of *Leucospermum* remains a serious challenge.

A preliminary investigation which studied chilling injury in potted *Leucospermum* plants showed that plants receiving low levels of light exposure under unsaturated levels of relative humidity during long-term cold storage were much less susceptible to chilling injury compared to plants stored at the same temperature at 100% relative humidity in the dark. Initial results suggest that stomatal functionality during storage may be of key importance to control desiccation that occurs when plants are removed from cold storage.

Post harvest processing and treatment of Leucospermum and Protea to transport to Duch market by sea shipping

Executive institution: Frutercoop – Cooperativa de Hortofrutifloricultores da Ilha Terceira

Web summary

The main goal of this research was to study the evolution of vase life time of some varieties of protea flowers, such as: *P. cynaroides* 'King', *P*. 'Susara', *P*. 'Grandicolor', *L.* 'Tango', *L.* 'Succession', *L.* 'High Gold' and *L*. 'Soleil', under some post-harvest treatment. During this project anti-Botrytis treatments were made in the field applications to evaluate the efficiency of control in Azores weather conditions during the seasons 2009/2010, 2010/2011 and 2011/2012.

With this experimental research we know the vase life time of flowers sent by Frutercoop to the Dutch market by sea freight exportation in which shows a positive result for almost all experimented varieties. About the anti-*Botritys* treatments, the best results were obtained for samples treated with cyprodinil+fludioxonil and thiophanate methyl.

Public Summary

Frutercoop is a cooperative with a protea flowers exportation department that is operating from Azores to the Dutch market. The main goal of this research was to study the evolution of vase life time of some varieties of protea flowers, such as: *P. cynaroides* 'King', *P*. 'Susara', *P*. 'Grandicolor', *L.* 'Tango', *L.* 'Succession', *L.* 'High Gold' and *L*. 'Soleil', under some post-harvest treatment. During this project anti-*Botrytis* treatments were made in the field applications to evaluate the efficiency of control in Azores weather conditions during the seasons 2009/2010, 2010/2011 and 2011/2012.

For the vase life time evaluation, some samples were harvested in the fields, submitted to different concentrations of ethanol and glucose, girdling or simple water processing. We used the normal chain of sea freight transportation from Azores until the Dutch market and vase life tests were made in Holland.

As conclusion, we observed a good vase life time for the varieties of *Protea* genus tested. With girdling treatment *P. cynaroides* 'King' gave a vase life time beyond 8 days and for flowers of *P*. 'Grandicolor' processed only in water we had a vase life time beyond 10 days. For *P*. 'Susara' results were not so conclusive because the quality of the leaves of the samples was not the best. Regarding *Leucospermum* genus, for *L*. 'Succession' flowers the best result was 18 days of vase life time and to *L.* 'Tango' the best result was 13 days of vase life time after the transport and conservation when flowers were processed only with water. For *L*. 'High Gold' best treatment was with the commercial product Chrysal that showed a vase life time of 12 days. The results obtained for *L*. 'Soleil' are not so conclusive, because they showed 8 days of vase life time when processed in water and less resistance to the sea freight exportation. Some rules of harvest, grading, packaging and temperature of transport and conservation are the key for successful sea freight transportation.

In reference to the anti-*Botrytis* treatments, spray applications were held in the field of some growers with *L*. 'Tango', that is one of the most susceptible variety to *Botrytis* in Azores. Seven active ingredients were tested: pyrimethanil, iprodione, cyprodinil+fludioxonil, thiabendazole, thiophanate methyl, fenhexamid and a biologic product. Flowers were harvested, packed and was a simulation of sea freight transportation. The *Botrytis* development observations during the vase life were made in Frutercoop facilities. Best results were obtained for samples treated with cyprodinil+fludioxonil and thiophanate methyl.

With this experimental research we know the vase life time of flowers sent by Frutercoop to the Dutch market by sea freight exportation. We had the opportunity to implement some rules of good practices on harvest, packaging and transportation. For anti-*Botrytis* treatments we got a good reference of efficiency for different active ingredients in the control of this disease and this could be an important tool to choose the best strategy to spray against *Botrytis*.

Leucadendron 'Safari Sunset': optimization of pre- and post-harvest management for quality improvement of the cut branches following prolonged sea transport

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Web summary

The main problem which limits the quality of 'Safari Sunset' cut branches is leaf blackening followed by foliage desiccation and pathogen development. We have confirmed our hypothesis that the seasonal sensitivity to leaf blackening during transport is associated with the flower development, which leads to changes in the sink-source relationships within the branch. The confirmation was gained by application of sugars to the cut branches before and after transport, as well as by performing girdling experiments in the field, with branches grown under two fertigation regimes. We have developed new postharvest treatments, based on carbohydrate supply, controlled atmosphere (CA), ethanol and fungicide treatments, which prevent leaf blackening and enable prolonged (1-4 weeks) sea shipment of Safari branches. These experiments enabled us to establish a protocol for the growers, including the best recommended treatments to obtain quality branches following prolonged sea transport from Israel to Europe or to USA.

Public Summary

Background: *Leucadendron* 'Safari Sunset' is one of the leading ornamental branches of the Israeli export. However, the profitable marketing of these branches depends on their successful sea transport from Israel, which requires a prolonged shipment (8-28 days to Europe or USA). The main problem which limits the quality of 'Safari Sunset' cut branches is leaf blackening followed by foliage desiccation (typical to other *Proteaceae* species), which enhances pathogen development later on. It was suggested that leaf blackening in proteas, which stems from increased oxidation of their reactive phenols, is enhanced by the carbohydrate depletion in leaves. Thus, the glycosilated phenols are cleaved to reactive phenols under periods of carbohydrate stress occurring during florogenesis and/or prolonged transport of cut branches. Our working hypothesis postulates that the seasonal sensitivity to leaf blackening during transport is associated with the flower development, which leads to changes in the sink-source relationships within the branch. Hence, the green leaves are depleted of soluble sugars, as they serve as source for the flower sink. Reduced fertigation before harvest, which occurs during flower differentiation, may also aggravate these symptoms, since florogenesis requires assimilates.

Objectives: The general goals of this research is to identify and characterize the physiological basis for leaf blackening in *Leucadendron* 'Safari Sunset', and to develop a protocol for plant cultivation, optimal harvest stage and postharvest handling to prevent this undesired syndrome.

During the first year of the project, we have established our initial hypothesis that the seasonal sensitivity to leaf blackening during transport is associated with the flower development, which leads to changes in the sink-source relationships within the branch. During the second year of the project, we have perfromed girdling experiments in the field, with branches grown under two fertigation regimes, to further establish the sink-source relationship between the flower meristem and the green leaves during growth. In the third year of the project we have focused on one main issue of the proposal, namely continue the development of new postharvest treatments for quality improvement and sea transport. We have continued to develope the new treatment, based on accumulation of endogenous ethanol in the tissue, to prevent leaf blackening, as an alternative to ethanol dipping, which caused damage.

Main results: We have elucidated the environmental and internal factors affecting florogenesis and flower maturation in *Leucadendron.* This enabled us to facilitate the extension of the flowering period and marketing season and improve the branch quality. Our results show that the flower meristem continues to develop and grow during prolonged storage at 2ºC and subsequent vase life. As a result, the soluble sugar content in the flower tissue is reduced, mainly during the November and December stages, and also starch content is reduced, mainly

during the December and January stages. The green leaves are depleted of soluble sugars, as they serve as source for the flower sink. Double fertigation keeps high sugar levels in the leaves and bracts mainly during the January stage when the meristem is fully developed. Soluble sugars, supplied either as a pulse treatment before storage (at the grower phase) or in the vase solution after storage (at the consumer phase), effectively reduced the incidence of leaf blackening in 'Safari Sunset' branches following prolonged sea transport, depending on the developmental stage of the flower meristem. Hence, these results further support our initial hypothesis that the seasonal sensitivity to leaf blackening during transport is associated with flower development, which leads to changes in the sink-source relationships within the branch.

Girdling of Safari branches in the field delayed slightly the rate of meristem development in branched grown in the two fertigation regimes. However, no significant effect of the girdling on the TSS levels in the leaves could be observed in the two fertigation regimes. In general, the changes in TSS levels during meristem development confirmed the pattern of changes in carbohydrate levels obtained in the first year, with higher TSS levels in the developing meristem compared to leaves.

In addition, we have developed new postharvest treatments, based on carbohydrate supply, controlled atmosphere (CA), ethanol and fungicide treatments, which prevent the leaf blackening problem and enable prolonged (1-4 weeks) sea shipment of Safari branches. Postharvest treatments with various fungicides reduced decay during the prolonged sea transport. We have developed a combined treatment of ethanol accumulation within the tissue followed by sucrose pulsing. The combined treatment was very efficient, and it reuslted in a reduction of leaf blackening to less than 10% of the branches, following sea transport simulation (14 days at 2ºC) and additional 10 days of vase life. Therefore, the combined treatment of ethanol and sucrose pulsing can be recommended as the most effective treatment to prevent leaf blackening of Safari branches during prolonged sea transport from Israel to Europe.