Effect of temperature on the growth, physiology and flowering of *Protea* cv. Pink ice

By

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Thesis presented in partial fulfilment of the requirements for the degree of Masters of Science at Stellenbosch University

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March 2010

DECLARATION

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the authorship owner thereof (unless to the extent explicitly otherwise stated) and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

Date: 20 February 2010

SUMMARY

As production areas expand to new, warmer areas, little information is available on the effect of high temperatures on the growth, physiology and flowering of *Protea*. In addition, high temperature effects on crops are becoming increasingly important as global temperatures rise.

In this study, the influence of high temperature on *Protea* ôPinkô Ice was investigated. The expectation was that optimal and supra-optimal temperature regimes could be identified with respect to parameters of importance to commercial protea production. Firstly, a greenhouse-based experiment with potted plants subjected to five levels of warming with a temperature gradient ranging from ambient to ambient+3.1°C, was established with infra-red lamps. A field verification experiment with two treatments, ambient and ambient+2.9°C, was established in a commercial orchard.

Leaf net CO_2 assimilation rate (A_{max}) and dark respiration rate (R_d) acclimated to the temperature gradient on a leaf area-basis. However, on a leaf mass-basis A_{max} and R_d decreased at higher temperatures. Stomatal conductance (g_s) remained approximately constant over the temperature gradient, but increased at higher temperatures on a mild spring day and decreased on hot days. On a seasonal basis, maximum A_{max} and g_s values were reached during spring, whilst maximum R_d rates were achieved during mid-summer. T_{opt} tracked seasonal temperatures closely.

At higher temperatures spring budbreak advanced by 1-2 weeks in both the greenhouse and field verification experiments. Flowering took place on the spring flush at ambient temperature, but this bearing habit shifted to summer flushes at the high temperature treatments. In the field verification experiment inflorescences on warmed plants were harvested earlier compared to those at ambient temperatures, as warming was possibly more optimal in the field than in the greenhouse (greenhouse ñambientò being warmer than field ñambientò).

In the second experiment conducted on a commercial farm, shoots bearing inflorescences which initiated on the autumn flush (April-May) were compared with shoots bearing inflorescences on the spring flush (August-September). Inflorescences initiated on the autumn flush, three months prior to those on the spring flush, were harvested one month earlier with a

significantly higher final dry mass. When comparing the gas exchange capacity of the two systems, seasonal climatic changes was found to have a stronger controlling influence than the phenological stage of the shoot.

For the third part of the study, vegetative and reproductive growth, physiology, gas exchange and carbohydrate trends were observed for one year in a commercial *Protea* ôPink Iceô orchard.

A threshold concentration of starch in mature leaves of the terminal flush, together with an estimated minimum stem diameter of 7.6 mm of a four- or five-flush shoot was suggested as a partial requirement for inflorescence initiation in *Protea* ôPink Iceô.

The results of this study show that *Protea* ôPink Iceô is well able to photosynthesise, grow and reproduce at temperatures 1-2°C higher than ambient. However, shifts towards weaker reproductive growth in favour of stronger vegetative growth under strong warming could have negative implications for commercial producers.

OPSOMMING

Die effek van temperatuur op die groei, fisiologie en blominisiasie van *Protea* cv. Pink Ice

Protea verbouing brei tans uit na nuwe, warmer produksie areas en daar is baie min inligting oor die effek van hoë temperatuur op die fenologie, groei, fisiologie en blominisiasie van *Protea*. Boonop word die impak van hoë temperature op gewasse toenemend belangrik as gevolg van aardverhitting.

Tydens hierdie studie is die invloed van hoë temperature op *Protea* ôPink Iceô ondersoek. Die verwagting was dat optimale en supra-optimale temperatuur grense geïdentifiseer kon word in terme van parameters wat belangrik is vir kommersiële proteaproduksie. Eerstens is twee jaar oue potplante in ón kweekhuis onderwerp aan ón temperatuur gradient van vyf verwarmings vlakke, van heersende temperatuur tot heersend+3.1°C, met behulp van infrarooi lampe. ón Boordbevestigings-eksperiment met twee temperatuur behandelings, heersende temperatuur en heersend+2.9°C, is opgerig op ón kommersiële protea plaas.

Die tempo van netto CO_2 vaslegging (A_{max}) en donker-respirasie tempo (R_d) het aangepas by die temperatuur gradiënt op ón blaar area-basis. Egter, indien uitgedruk op ón blaar massa-basis het A_{max} en R_d afgeneem by hoë temperature. Huidmondjie geleiding (g_s) het ongeveer konstant gebly oor die gradiënt, maar het toegeneem by hoë temperature op gematigde lente dae en afgeneem op baie warm dae. Seisonale maksimum A_{max} en g_s waardes is bereik tydens die lente maande, terwyl maksimum R_d waardes in die somer maande aangeteken is. T_{opt} het die seisonale temperatuur neigings nageboots.

By die hoë temperatuur behandelings van beide die kweekhuis en boordbevestigings-eksperimente het lente bot van die terminale groeistuwing 1-2 weke vroeër plaasgevind as by plante teen heersende temperature.

Blominisiasie het hoofsaaklik plaasgevind op die lente groeistuwing by plante by heersende temperatuur, maar by hoë temperatuur behandelings het blominisiasie later, op somer groeistuwings plaasgevind. In die boordbevestigings-eksperiment is blomme vanaf die verhitte plante twee weke vroeër geoes in vergelyking met blomme afkomstig van plante sonder verhitting. Dit dui daarop dat die verwarming in die boord heel moontlik meer

optimaal was as in die kweekhuis (kweekhuis ñheersende temperatuurò was warmer as boord ñheersende temperatuurò).

ôn Tweede eksperiment is uitgevoer op ón kommersiële plaas, waar lote met blomme wat op die herfs-groeistuwing (April-Mei) inisieër het vergelyk is met lote waarvan die blomme op die lente-groeistuwing (Augustus-September) inisieër het. Blomme op die herfs-groeistuwing wat drie maande voor blomme op die lente groeistuwing inisieër het, is een maand vroeër geoes met ón verhoogde finale droëmassa. Wanneer gaswisseling van die twee sisteme vergelyk is, was dit bevind dat seisonale temperature ón groter beherende invloed op die gaswisseling patroon het as die fenologiese stadium van die loot.

Vir die derde deel van die studie is vegetatiewe en reproduktiewe groei, fisiologie, gaswisseling en koolhidraat tendense waargeneem vir een jaar in ón kommersiële *Protea* ôPink Iceô boord.

ôn Drempelwaarde in die stysel konsentrasie van die volwasse blare van ôn vegetatiewe terminale groeistuwing, tesame met ôn minimum lootdikte van 7.6 mm by ôn vier- of vyf groeistuwingsloot word voorgestel as ôn gedeeltelike voorveiste vir blominisiasie by *Protea* ôPink Iceô.

Die resultate van hierdie studie wys dat *Protea* ôPink Iceô voldoende kan fotosinteer, groei en reproduseer by temperature 1-2°C hoër as heersend. Egter, ón verskuiwing na verlaagde reproduktiewe produksie kan voorkom, aangesien sterker vegetatiewe groei in lote bevoordeel word onder toestande van intense verwarming en dit mag negatiewe implikasies hê vir kommerisële produksie.

Dedicated to my friend, Samantha Reinecke.

Her passion for life, my example.

ACKNOWLEDGEMENTS

I am grateful to the following people and institutions:

My supervisor, Dr. Stephanie Midgley, for your commitment to my studies, good advice and for keeping me on my toes.

My co-supervisor, Dr. Lynn Hoffman, for your helpful insights, bright ideas and much needed emotional support.

Prof. Karen Theron for your motivation and statistical help.

The National Research Fund (NRF), Protea Producers of South Africa (PPSA) and Stellenbosch University for funding my project.

Hans Hettasch and John Sharpe (Arnelia Farms), Marius van der Merwe (Floraland-Etshwaleni), Lourens De Wet (Welgevallen Experimental farm), as well as Irene van Gent and Mardé Booyse at the ARC.

Technical and administrative staff in the Department of Horticultural Science, always willing to help, especially Shantl, Dene and Tikkie.

Hein Gerber and Micheal Schmeisser for motivation and a laugh and Jacques Fouché and Jacques Crous for making everything simple.

My friends on the outside of the Lombardi building, my diversion.

My parents, Pieter and Anne-Hilde for your love, support, cooked meals, patience and prayers, as well as my brothers Kristian and Ingmar.

All my ótanniesô!

Edu, for your love and support, always one step ahead.

My Father in heaven for His guidance and grace.

Table of contents

The referencing style in this thesis was written according to the requirements of the *South African Journal of Plant and Soil*. Chapter 2 provide the experimental design and layout of the research presented in Chapters 3 and 4, whilst Chapters 5 and 6 are discussed as separate entities. Some repetition between chapters may occur.

List of abbreviations

°C	degrees centigrade
ABA	abscisic acid
A/c_i	the response of A_{max} to intercellular CO_2 concentration, c_i
A_{max}	maximum rate of light-saturated net CO_2 assimilation, µmol m^{-2} s^{-1}
ANOVA	analysis of variance
ARC	agricultural research council
A_{sat}	maximum rate of light- and CO_2-saturated net CO_2 assimilation, µmol m^{-2} s^{-1}
CFR	cape floristic region
c_i	intercellular CO_2 concentration, µmol mol^{-1}
cv.	cultivar
ET	evapotranspiration rate
FATI	free air temperature increase
g_s	stomatal conductance, mol m^{-2} s^{-1}
LAR	leaf area ratio, cm^2 g^{-1}
LMA	leaf mass per unit area, g m^{-2}
OTC	open top chamber
PAR	photosynthetic active radiation
PFD	photon flux density, µmol m^{-2} s^{-1}
PNUE	photosynthetic nitrogen use efficiency, µmol mol^{-1} s^{-1}
PPFD	photosynthetic photon flux density, µmol m^{-2} s^{-1}
ppm	parts per million
R_d	dark respiration rate, µmol m^{-2} s^{-1}
R_{day}	predicted photorespiration rate, µmol m^{-2} s^{-1}
RH	relative humidity, %
ROS	reactive oxygen species
Rubisco	ribulose-1,5-bisphosphate carboxylase / oxygenase
SE	standard error
T_b	base temperature, °C
T_m	mean temperature, °C
T_{opt}	optimum temperature for net CO_2 assimilation rate, °C
VPD	vapour pressure deficit

Introduction

The South African Fynbos cut flower industry, focussing mainly on the genera *Protea, Leucospermum* and *Leucadendron,* is currently worth $40 million (Dorrington, 2008). The industry has grown from a steady market value of $23 million since 2004 and is still expanding (SAPPEX, 2006). Production is predominantly aimed at export, with only 3% of products sold on the domestic market (G. Malan, pers. comm., 2009). Of the total exports, 70% is sold to a discerning European market (Dorrington, 2008).

During the 2003/2004 season 600 000 stems of *Protea* cv. Pink Ice (*Protea compacta* R. Br. x *P. susannae* Phill.) was exported from South Africa, placing it third after *Protea* cv. Sylvia (*P. eximia* x *P. susannae*) and *Protea magnifica* (SAPPEX, 2006).

Protea cultivation is seen as a lucrative low-cost option for diversify existing agricultural activities. This perception leads to a continuous expansion of areas under cultivation with Proteaceae within South Africa to new regions outside the traditional Mediterranean-type climate *Protea* cultivation zones. These new production areas include the Gauteng province, where frost is a real threat, as well as the more temperate southern Cape region of South Africa (G. Nieuwoudt, pers. comm., 2009). However, as insufficient research on the effect of climatic factors on *Protea* growth and development is available, it is difficult to predict and make recommendations on the suitability and adaptability of existing cultivars to these new areas of cultivation with their different climatic regimes and production conditions.

The factors which influence flower bud induction, initiation and development in commercial *Protea* cultivars in South Africa are not clearly understood (Gerber, 2000). Seasonal temperature changes impact on the growth, phenology and physiology of plants, however the only information available on these interactions for *Protea* is sparse and based on fynbos species in their natural ecosystem. Mean temperatures during a year, which differ inter-annually, also impact on the time of harvest.

Furthermore, temperatures are increasing globally due to human fossil-fuel-based activities (Hughes, 2000). Assessments that have been made on the vulnerability of naturally occurring fynbos and renosterveld species to increased temperatures, showed that on average an alarming 58% of the fynbos bioclimatic zone may be lost by ~ 2050 (Midgley *et al*., 2002). Using spatial analysing tools Bomhard *et al*. (2005) predicted Proteaceae in low-lying coastal areas to have the highest extinction risk due to climate change and the continuous conversion

of uncultivated land for agricultural use. Does this indicate cultivated *Protea* to also be at risk under a changing climate?

This study focused on the impact of increasing temperatures on the growth, physiology and flowering of *Protea* 'Pink Ice'. An objective was to collect and interpret data on gas exchange and carbon allocation patterns over an increasing temperature range in potted plants as well as field verification experiments of 'Pink Ice'. Gas exchange was expected to acclimate to seasonal temperatures as well as to elevated temperatures. Spring budbreak was expected to advance at higher temperatures. At high temperatures later flowering dates was expected. Furthermore, it was aimed to assist producers in making accurate predictions (inter-annually and regionally) of the flowering period and harvest peaks both for specific production regions and on an annual basis for more effective marketing, and to allow for a timely adaptation of production methods and cultivar selection in response to a warmer climate and to new production areas.

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1. Literature review: Geographical and ecophysiological characteristics of the Proteaceae, trends in climate change and the effects of high temperature on plants, with a reference to *Protea*

1.1. The Cape Floristic Kingdom

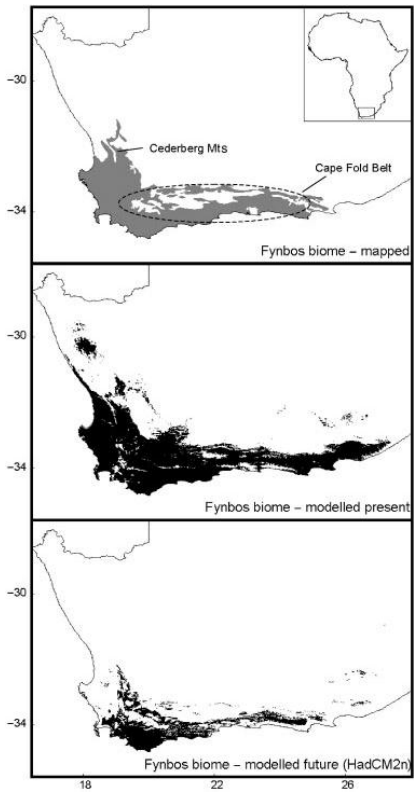
Today Proteaceae are mainly found in the southern hemisphere, with eastern and western Australia and the western Cape region of South Africa considered the richest areas. Other regions such as tropical Africa, tropical South America, Chile and central America also have some species (Paterson-Jones, 2007).

The first flowering plants date back 150 million years (Manning, 2007). After Gondwanaland began to break up 140 million years ago (Paterson-Jones, 2007) the Proteaceae followed different evolutionary paths on the separate continents. Around 60 million years ago southern Africa was covered by a subtropical forest, with fossil pollen records of fynbos families such as restios, proteas and ericas dating back 64 to 71 million years. However, 30 million years ago the climate generally became drier and 10 million years ago the central southern African plateau was elevated, whereafter the Benguela current was established. Modern fynbos evolved under increasing aridity, 3 to 5 million years ago (Manning, 2007). However, the quaternary period during which the Proteaceae has diversified was generally a cooler period than now (Midgley *et al*., 2005a).

The Cape floristic region (CFR) consisting of approximately 9000 endemic species, covers 90 000 km^2 of the western Cape of South Africa, from Van Rhynsdorp and Nieuwoudtville in the north to Port Elizabeth in the east (Fig. 1). The species of the CFR, often referred to as fynbos, can mainly be divided in the proteiod, ericoid and restioid families (Cowling, 1992). The Cape fynbos covers almost half the area of the CFR, but contribute 70 to 80% of the species richness. The CFR is renowned as the smallest of the worldôs six floral kingdoms, but, 94 species per 1000km^2 makes it more rich in species diversity than California or southwestern Australia with 14 and 12 species per 1000km^2 respectively (Manning, 2007). The CFR is therefore considered as a biodiversity hotspot (Bomhard *et al*., 2005).

From an ecological perspective, the ñSouth African Country Study on Climate Changeò predicted that future warming and aridity trends are sufficient to cause large reductions in species richness in the Mediterranean fynbos and succulent Karoo biomes (Midgley *et al*., 2002). Bomhard *et al*. (2005) estimated that the Proteaceae will be affected

the most severely by climate change, although land use change will critically affect other taxa. Of the 330 species endemic to the fynbos biome 29 species are predicted to become extinct as they are currently located in a range that is predicted to be lost by 2050 (Fig. 1).



Fynbos biome - mapped

Fynbos biome ï modelled present

Fynbos biome ï modelled future

Fig. 1. The predicted decrease in range of the fynbos biome by approximately 2050 (Midgley *et al*., 2002) with degrees of longitude and latitude on the x- and y-axis respectively.

Fynbos vegetation is mostly associated with Table Mountain Sandstone soils, which are sandy, nutrient-poor soils (Rebelo, 2001). There are a few exceptions where species favour calcareous and limestone soils, such as *Mimetes saxatilis, Leucadendron meridianum* and *Protea obtusifolia*. Cape fynbos experiences cool, wet winters and warm, dry summers. Some species experience snow high up on the mountain ranges for short periods, but frost is unusual. Cape fynbos rarely occurs where rainfall is less than 400 mm per annum or where droughts are common (Manning, 2007).

The Proteaceae, may be divided into more than 60 genera (Rebelo, 2001) of which *Protea* L., *Leucadendron* L. and *Leucospermum* R. Brown are the most widely cultivated as cut flowers (Coetzee & LittleJohn, 2001). The only food crop in the Proteaceae is *Macadamia integrifolia*, a nut crop native to Australia (Trochoulias & Lahav, 1983). Approximately ten to twelve species or species selections in the genus *Protea* are commercially cultivated (Crous *et*

al., 2004, Coetzee & Littlejohn, 2001) and marketed, including *P. cynaroides, P. magnifica and P. repens* (Gerber, 2000). These species and selections are exported together with popular hybrid cultivars such as ôPink Iceô (*P. compacta* x *P. susannae*), ôSylviaô (*P. eximia* x *P. susannae*) and ôSusaraô (*P. magnifica* x *P. susannae*). Widely use parents in breeding programmes include, *P. burchelli, P. compacta, P. eximia, P. longifolia P. magnifica, P. neriifolia, P. repens* and *P. susannae* (Gerber, 2000).

Protea cv. Pink Ice is an Australian bred hybrid cultivar. It was bred by P. Mathews and released in 1984, but only promoted internationally from 1987 (International Protea Register, 2007). ôPink Iceô was specifically selected for its vigorous growth habit and disease tolerance.

In South Africa, ôPink Iceô is harvested from late December to May and reaches the European market in the last third of the optimum marketing period and afterwards (Gerber, 2000). Currently, approximately 7 to 8 ha ôPink Iceô is in full production with another 2 to 3 ha being newly planted (G. Malan, pers. comm., 2009).

1.2 Ecophysiology of *Protea*

Leaf morphology: Proteaceae are evergreen, woody perennials with sclerophyllous leaves (Coetzee & Littlejohn, 2001). Young leaves are particularly pubescent, with the lower surface of mature leaves often protected by hairs (Manning, 2007). In *Protea* both sides of a leaf is covered with hairs. Four *Protea* species studied by Mooney *et al.* (1983) had stomata on both sides and in Fig. 2 a transverse section of *Protea cynaroides* is shown (Vogts, 1971). Fig. 3 shows a scanning electron micrography (SEM) image of *Grevillea floribunda*, where trichomes are abundant (Burrows, 2001).

In woody plants with thick, stiff leaves the capacity for stomatal opening is particularly low and the stomata are able to open only slightly (Larcher, 2003). This may limit the CO_2 supply to the leaves, although a mean internal CO_2 concentration of 249 ppm was recorded by Mooney *et al*. (1983).

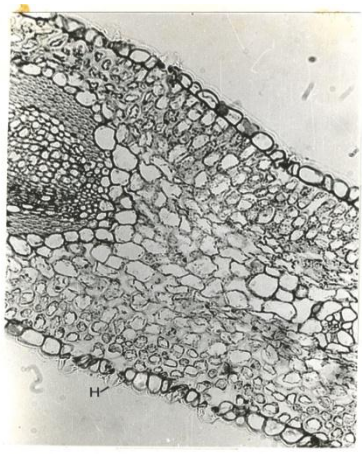


Fig. 2. Transverse section of a leaf of *Protea cynaroides*. The vascular bundle is visible with cuticular papillae (H) alongside stomata (Vogts, 1971).

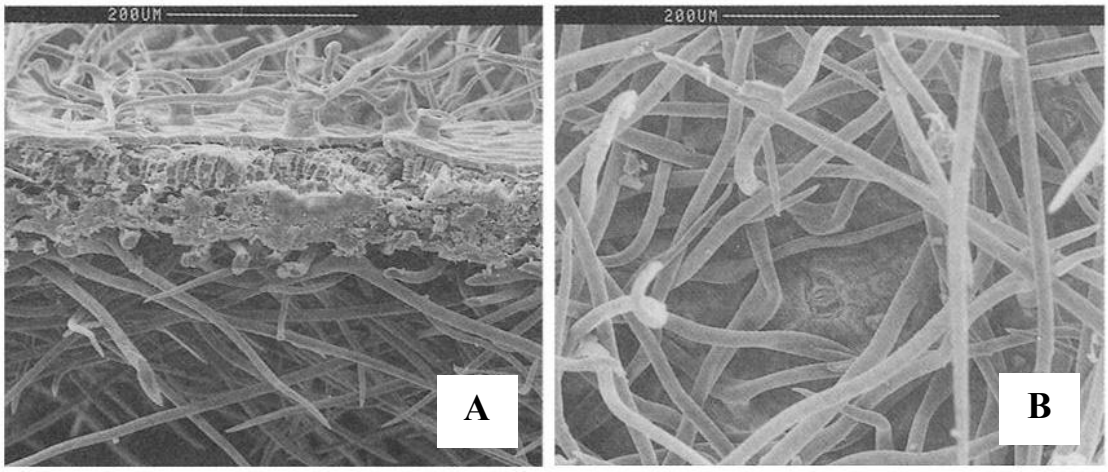


Fig. 3. Scanning electron micrography (SEM) images of the leaf of *Grevillea floribunda*, an Australian Proteaceae species, showing the abundance of trichomes. Scale = 50 times. A, SEM view of a leaf cut to show the trichomes on both surfaces. B, SEM view of the abaxial surface (Burrows, 2001).

Photosynthesis: Leaves of Proteaceae are considered to have a relatively low photosynthetic capacity, <14 ε mol m^{-2} s^{-1} (Mooney *et al*., 1983). More recently Smart (2005) confirmed the photosynthetic rate values of flowering *Protea* cv. Carnival (*P. compacta* x *P. neriifolia*) shoots to be in the same range. In deciduous fruits crops the rate of light saturated CO_2 assimilation (A_{max}) can vary between 16 and 22 ε mol m^{-2} s^{-1}, but values as high as 40 ε mol m^{-2} s^{-1} have been recorded under optimal conditions (Larcher, 2003).

Mooney *et al*. (1983) found *Protea* leaves to have low concentrations of nitrogen, between 1 and 1.4 % as well as a low phosphorous content on a leaf dry mass basis, together with relatively high specific leaf weights (~200 $g.m^{-2}$). Hawkins *et al*. (2007) determined that even fertilised Proteaceae such as *Leucadendron* ôSafari Sunsetô and *Leucospermum* ôSuccessionô contained similarly low levels of 0.98 ± 0.03 %N and 1.08 ± 0.04 %N of plant dry mass respectively. The main limitation to the photosynthetic capacity is therefore believed to be nitrogen related (Stock *et al*., 1992). These low leaf nutrient levels reflect the nutrient-poor soils to which these species are adapted. As native soil N and P are low in supply and therefore may restrict protein synthesis, excess organic carbon is produced from photosynthesis under conditions where light energy is mostly sufficient and hardly limiting. Additional carbon skeletons that are not used in protein synthesis are channelled towards the production of carbon-rich woody fibres such as lignin and tannins (Rebelo, 2001).

Light saturation of photosynthesis was determined for six fynbos shrub species, including *P. repens, P. neriifolia, P. nitida* and *P. acaulos*, in the Jonkershoek nature reserve

(Stellenbosch region) and was found to be reached at light levels of below 1000 ε mol m^{-2} s^{-1} (Mooney *et al*., 1983). This finding was contradicted during a study in the Cedarberg area by Von Willert *et al*. (1988) who found light saturation of photosynthesis of similar *Protea* species studied by Mooney *et al*. (1983), but also including *P. laurifolia* to take effect at much higher light levels of 2000 ε mol m^{-2} s^{-1}. The temperature optimum for net photosynthesis was found to be close to 25°C (Mooney *et al*., 1983) when measured during the spring months of September and October.

Van der Heyden & Lewis (1989) reported a decrease in the photosynthetic rate of five fynbos species, including *P. laurifolia, Leucadendron salignum* and *Erica plukenetii*, with increasing water stress. These species also showed summer depressions in the net photosynthetic rate. However, the deep-rooted *P. laurifolia* showed a smaller summer depression than the shallow-rooted *E. plukenetii*. Soil water availability is therefore important, especially during dry summer months to sustain carbohydrate assimilation rates.

Storage of reserves: *Protea* have a low allocation of carbohydrates to reserves (Greenfield, 1994). Starch concentrations in the bark and wood of ôCarnivalô varied from 0.16 to 0.36 % and 0.02 to 0.07% respectively (Greenfield *et al*., 1995). In spring, the buds on pruned bearers sprouted with minimal support in terms of stored carbohydrates for growth from bearers. In *Protea*, the leaves are the most important source of sugars which are derived from current photosynthesis (Gerber, 2000). In addition, total sugar and starch concentrations were found to be significantly higher in the leaves than in the stems of the uppermost flush (Hettasch *et al*., 2001). Distally on a mature shoot, there is a marked shift from stem to leaf in terms of dry mass allocation (Hettasch *et al*., 2001). Therefore, the more basal flushes have a greater allocation of carbohydrates to the stem, than the leaves. The allocation pattern changes successively with shoot growth.

1.3 Vegetative growth and flowering

Vegetative growth: *Protea* spp. grow vegetatively in flushes (Fig. 4), as described for ôCarnivalô, ôCardinalô (*P. eximia* x *P. susannae)* and ôSylviaô. These flushes are referred to from a basal position as a spring, first summer, second summer and then an autumn flush (Greenfield *et al*., 1994; Hettasch *et al*., 2001; Gerber *et al*., 2001) in ôCarnivalô. The number of flushes varies between cultivars, with ôSylviaô producing fewer flushes within a growing season, whereas ôPink Iceô could produce up to six flushes in one season (personal

observation), depending on environmental conditions. The spring flush is the most synchronized and robust flush and is also the first flush produced after the winter dormant period, where after the other flushes follow subsequently during late spring and into summer. The autumn flush, if present, may finish extension as late as May. Autumn flushes are mostly produced in warmer areas and on stronger shoots.

Studies on 'Sylvia', 'Carnival' and 'Lady Di' showed when growth ceases between flushes, the terminal bud contains almost all the appendages for the next flush (Gerber *et al*., 2001). A lag period of one to two weeks before the next flush begins ensures that the bud is entirely preformed before the next vegetative flush begins. Gerber *et al*. (2001) confirmed that a full complement of appendages for the new vegetative growth is present at budbreak. At the end of the autumn flush 70% of the appendages in the terminal bud that will constitute the spring flush are formed. During winter full bud development is then completed.

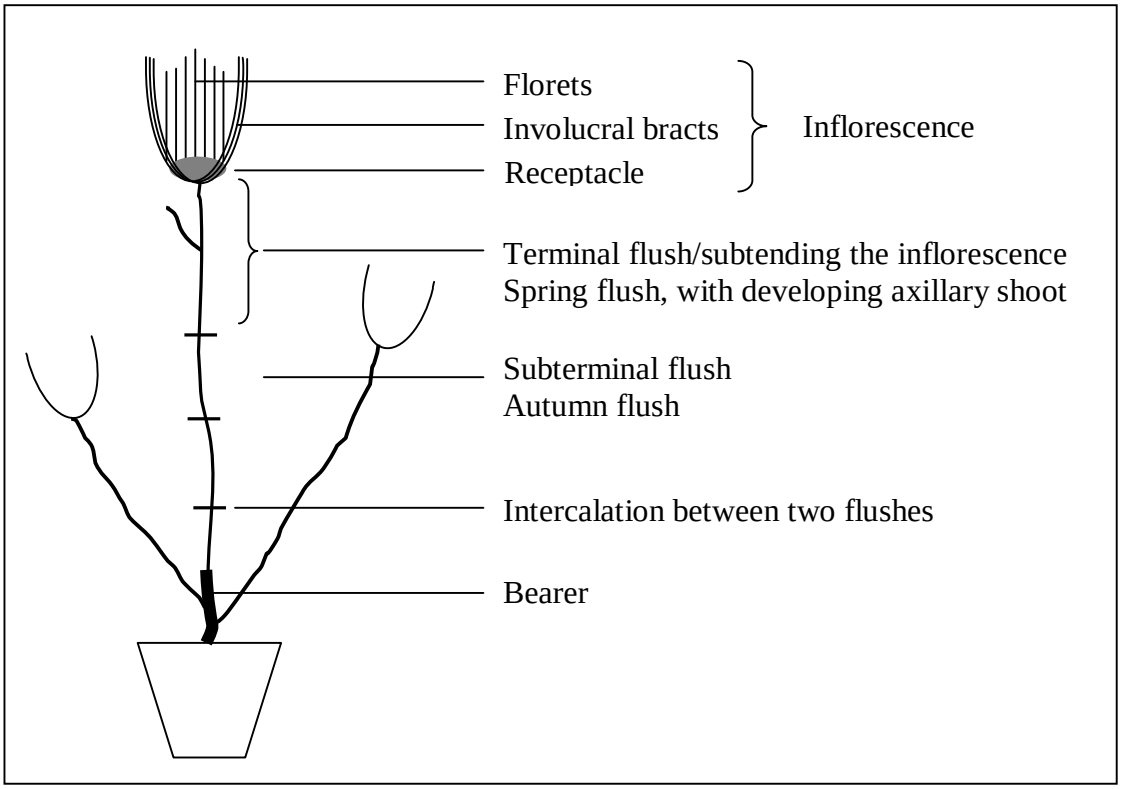


Fig. 4. Visual representation and terminology used to describe vegetative and reproductive growth in *Protea*.

Flowering: Most *Protea* species flower naturally from autumn to spring and the inflorescences are carried in the terminal position (Coetzee & Littlejohn, 2001). Flowering time of the species selections and hybrid cultivars varies considerably (Gerber, 2000; Gerber

et al., 2001). The inflorescence consists of a receptacle and numerous florets, which is enclosed by the involucral bracts (Fig. 4) (Coetzee & Littlejohn, 2001).

Rieger & Sedgley (1996) found vegetative growth to be a prerequisite for floral initiation in *Banksia*, a genus within the Proteaceae. *Leucospermum* (Malan & Jacobs, 1990) and *Leucadendron* are known to be short day plants (Hettasch & Jacobs, 2006). The requirement for short days in *Leucospermum* as a floral signal is probably facultative as flowering at the equator is possible, despite the absence of any variation in photoperiod.

Research by Agenbag (2006) on fynbos species, including seven *Protea* spp., in their native environment showed that on a north-facing slope, plants growing on the lower part of the slope have a longer growth period. This extended growth period was correlated with lower precipitation and higher temperature compared to plants growing at a higher elevation of north facing slopes, where the opposite pattern of higher precipitation and lower temperatures would prevail. Growth and flowering was generally found to be highly influenced by temperature and/or moisture and by the shift from the one to the other as would be prevalent with elevation (Agenbag, 2006).

For many plant species a vernalization period or exposure to low temperatures varying in length, is required (Salisbury & Ross, 1992). Many *Protea* species selections and hybrids flower preferably on the spring flush, after a period of lower temperatures during winter. Should proteas have a vernalization requirement, it would explain why most cultivated proteas flower after the winter period. High temperatures following on a period of vernalization can be detrimental to floral induction and cause devernalization (Kinet, 1993). However, some cultivars such as ‘Sylvia’ have the ability to flower all year round and therefore respond to a different floral stimulus to flower or is autonomously regulated.

In *Protea* a minimum of two flushes is considered necessary to support the inflorescence (Coetzee & Littlejohn, 2001). Furthermore, it is believed that a minimum stem diameter, which increases with each successive flush, is required before inflorescence initiation can occur (Coetzee & Littlejohn, 2001). A minimum stem diameter of 7mm has been determined for ‘Carnival’ by Hoffman (2006). Gerber *et al*. (2002) determined that floral induction in ‘Carnival’ takes place 6 to 7 weeks before spring budbreak. Shoots in the induced state remain committed to flowering.

Dupee & Goodwin (1990) indicated that visible flower initiation in *Protea* occurs when the extension growth of the spring flush subtending the inflorescence has ceased. However, Gerber *et al*. (2001) and Hoffman (2006) found that inflorescence initiation already occurred at the onset of elongation of the flush subtending the inflorescence. This would then

be the critical time period when changes in endogenous and/or environmental conditions would affect floral initiation most severely.

The spring flush bearing the inflorescence contains more bud scales, transitional leaves and leaves than any of the subtending flushes (Gerber *et al*., 2001). When the spring flush extension is completed, floret differentiation takes place, followed by enlargement of the inflorescence (Gerber *et al*., 2001).

Total defoliation 40 days before spring budbreak or earlier prevented flowering in ócarnivalô (Gerber *et al*., 2002). This demonstrated both the requirement for mature leaves to be present and for a minimum assimilate requirement, supplied largely by the flush subtending the inflorescence, is necessary in order for floral initiation to occur and inflorescence development to proceed. In *Banksia*, assimilate supply is critical for floral initiation (Rieger & Sedgley, 1996). Vegetative growth is necessary to sustain the developing inflorescence and this vegetative growth translates to stored reserves and a minimum stem diameter.

1.4 Climate change

Increasing temperatures and future predictions: Climate change is not a new phenomenon, as mean global temperatures have increased and decreased in cycles throughout time. However, the current rate of change is startling (Lessmann *et al*., 2001; IPCC, 2007). Long-term monitoring studies have indicated that current climatic and atmospheric trends are inconsistent with past climatic and atmospheric data and that it is already affecting the phenology, physiology and distribution of fynbos species (Musil *et al*., 2005).

Over the past 100 years the earthôs climate has warmed approximately 0.74°C (IPCC, 2007). Researchers have found two main periods of warming, between 1910 and 1945 and from 1976 onwards (Walther *et al*., 2002). By 2099 the global temperatures are predicted to be 1 to 6°C higher than 1999 (IPCC, 2007), while the predicted rise in temperature for the Western Cape, South Africa, will be between 1 to 3°C by 2045 (Midgley *et al*., 2007).

Interestingly, the diurnal temperature range (DTR) is decreasing, because minimum temperatures are increasing at almost double the rate of maximum temperatures (Turnbull *et al*., 2002; Hughes, 2000). Night temperatures are largely affected, and are predicted to increase more rapidly than daytime temperatures (Easterling *et al*., 1997). This has significant implications in terms of dark respiration and carbohydrate status of plants. The DTR rate of decrease also varies from one region to another (IPCC, 2007).

The primary cause for the rise in temperature is ascribed mainly to the rising concentrations of atmospheric greenhouse gases, largely from the use of fossil fuels and conversion of land to agriculture (IPCC, 2007). The principal greenhouse gasses include carbon dioxide (CO_2) (Midgley *et al*., 2005b). CO_2 levels have increased from the pre-industrial era level of 280 to 360 ppm and are still increasing at a rate of 1-2 ppm per annum (Lee *et al*., 2001) with CO_2 levels in 2005 estimated at 379 ppm (IPCC, 2007).

Water availability with climate change: Average rainfall is predicted to increase on a global scale, although some regions such as the Western Cape, South Africa, (Midgley *et al*., 2005b) may become drier (Hughes, 2000). Drying, especially during winter, is predicted to occur more severely in the south-west region of the Western Cape, together with an increased evaporation rate (Midgley *et al*., 2007). In addition to changes in rainfall patterns, the frequency and intensity of extreme climatic events is also expected to increase (IPCC, 2007). Therefore, water supply, especially for agricultural purposes will come under ever increasing pressure. As a result of significantly increased population growth in the Cape Town Metropolis water usage more than tripled during the last four decades, with the demand for water showing a phenomenal increase of 4% per annum in the last decade (Louw & Callaway, 2008). The current water reserve status is already restricted, with very little prospect to increase storage capacity (Midgley *et al*., 2005b), as the suitable dam sites available for development is limited (Louw & Callaway, 2008).

Effect of climate change on plant processes and distribution: The effects of anthropogenic climate change on plant life can be divided into four main categories, of which the first would be the effects on respiration and photosynthesis and how these processes are affected by temperature, increasing CO_2 levels and changes in precipitation (Hughes, 2000).

Secondly, climate change may have an effect on species distribution. The rates at which plants can shift to more suitable climatic areas differ greatly among and within species (Walther *et al*., 2002). It is expected that species migration will occur to higher altitudes and latitudes; therefore, a drift of high temperature sensitive plant species higher up slopes and more toward the poles are expected (Hughes, 2000; Walther *et al*., 2002). Additionally, climatic conditions favouring higher temperatures and a drying climate, will be conducive to veld fires, and therefore may increase the frequency of these events in the fynbos biome (Western Cape Strategy and Action Plan, 2007). Although fynbos is fire-dependant for soil

nutrient recycling and germination of serotinous seeds, too frequent fire events will be detrimental to the re-establishment of species and cause local extinction (Manning, 2007)

Thirdly, the general phenology of plants will also be affected, as it is expected that plants will advance to the next ontogenetic phase faster. Spring budbreak may be promoted together with earlier flowering in middle to higher latitudes (Badeck *et al*., 2004). There is evidence for a later onset of autumn phenological events, although this may not be as pronounced as the changes in spring events (Walther *et al*., 2002).

Lastly, the adaptation of species to a new climate, especially species with short generation times and rapid population growth is possible *in situ* (Hughes, 2000) where geographical barriers are present and species migration would not be possible. Indirectly, warm temperatures may disfavour genotypes with a delayed onset of growth in the spring and with premature dormancy in autumn (Saxe *et al*., 2001). Higher temperatures will exerts selection pressures and species with high temperature tolerant genotypes may survive and acclimate.

The effect of climate change on agriculture: Global warming has important economic and production implications for the agricultural sector. Crop productivity is predicted to increase slightly in temperate regions with a moderate temperature increase of 1 to 3°C (IPCC, 2007), as optimum growth will coincide with optimum temperatures. These optimum growth temperatures are crop specific (IPCC, 2007). However, a decrease in productivity is expected with further temperature increase. In response to climate change, producers will have to carefully align crops and cultivars used in agriculture production with the micro-climate of area in which crops are to be cultivated. For instance, a change in pollination behaviour may lead to different production peaks in fruit crops that may coincide with a period of low demand from importing countries, especially for chill-dependant deciduous fruits (Midgley *et al*., 2007).

At lower latitudes a decrease in productivity is expected, together with the increase of droughts. Health risks, such as malaria and other vector borne diseases may influence production outputs especially when the specific industry is heavily reliant on manual labour in packhouses and orchards. Pest risk management may need drastic adaptation as an increase in insect outbreaks is expected (IPCC, 2007).

Agriculturally, pressure on water sources will further affect crop production severely, especially in the Western Cape where current crop production is heavily reliant on irrigation.

Producers will have to improve and optimize present irrigation scheduling or use a different irrigation system altogether (Warrington, 2008).

1.5 Plant response to temperature

Plant response to increased temperature: Larcher (2003) states temperature to be the most important environmental factor influencing respiratory gas exchange. Temperature affects plant metabolic processes firstly by affecting the rate of chemical reactions and secondly, by controlling the activities of the multitude of enzymes involved in various reactions (Larcher, 2003). Photosynthetic models show that as temperature increases, photosynthesis and growth rise to an optimum, thereafter if temperature still increases, photosynthesis declines (Martínez-Carrasco *et al*., 2005). Therefore, a distinction must be made between slightly elevated temperatures leading to increased growth and productivity under conditions where water and nutrients are not limiting and supra-optimal temperatures that will result in decreasing gas exchange and growth.

Heat stress can be an acute event with immediate responses which include the formation of heat shock proteins, but it can also be a chronic state where higher temperatures become the norm and the plant then has an extended opportunity to adjust to its new environment. Therefore, heat stress is both time and intensity dependant (Larcher, 2003).

Plants in a Mediterranean climate are considered more plastic in terms of adjusting to different seasons and weather patterns than tropical plants, which occupy a narrow physical niche (Walther *et* al., 2002). Throughout a single year, plants in a Mediterranean climate will experience both dry, warm summers as well as cool, wet winters. The temperature in this type of climate rarely drops below 0°C, with a mean above 10°C for three months of the year and for the warmest months the average is in the upper 20-30°C range (Ritter, 2006). Maximum temperatures in summer often exceed 32°C and reach as high as 38°C.

If moderate higher temperature is considered to promote positive growth, a longer summer and a shorter winter will extend the period available for plant growth (Lee *et al*., 2001). In experiments by Lee *et al*. (2001) plant phenology such as earlier seedling emergence and flowering advanced proportionally to seasonal warming. The most consistent plant response in tundra vascular plants to increased temperature regimes was found in an increase in plant size (Hollister & Webber, 2000).

When temperatures increase supra-optimally it cause chronic heat stress. The movement of molecules are highly accelerated at these high temperatures, which weaken

bonds with macromolecules, making lipid bilayers more fluid. Metabolic activity and growth are therefore impaired (Larcher, 2003). The reproductive phase is most susceptible to heat damage (Klueva *et al*., 2001). Heat stress also directly inhibits vegetative growth and indirectly causes dehydration of plants due to of a high evaporative demand (Hall, 2001). Dehydration is also indicated as a secondary effect of heat stress. An additional secondary process is photo-inhibition linked to high light intensity and temperature. Excessive free photo-energy, not trapped by the light harvesting centra, is transferred to oxygen, producing reactive oxygen species (ROS). ROS damage the chloroplast and mitochondrial membranes (Klueva *et al*., 2001). Antioxidants, which are able to quench ROS, increase during photo inhibition and can alleviate damage to some degree. Plants compromise between yield and survival and therefore productivity cannot be maximised under such stress conditions. Therefore high temperature regimes are associated with decreases in yield along with a shift in time of flowering, and inhibition of flower bud development (Larkindale *et al*., 2005).

Night temperatures slightly higher than what plants are adapted to may cause an increase in respiration and the accumulative loss of carbohydrates (Lambers *et al*., 1998). As temperature increases further, dark respiration rate increases exponentially until biochemical reactions take place so rapidly that the substrate availability and metabolites cannot sustain the high rate of respiration. The respiration rate then decrease, even though temperatures remain high. Extremely high temperatures (50-60°C) cause irreversible heat damage to enzymes (Klueva *et al*., 2001) and membrane structures essential to respiration, leading finally to the termination of respiration.

Immediate responses of plants when introduced to higher temperatures that are accompanied by a high vapour pressure deficit (VPD) will include stomatal closure. This stomatal behaviour leads to a decreased photosynthesis due to a lower internal CO_2 concentration. If temperatures remain high, the CO_2/O_2 ratio decreases and is subsequently followed by a decrease in the carboxylation efficiency of ribulose bisphosphate carboxylase oxygenase (Rubisco) (Larcher, 2003). Under these conditions, Rubisco could be inactivated and photosynthetic membranes can become leaky at such high temperatures, further reducing photosynthesis. Extreme high temperatures will cause permanent damage to these membranes, the critical site of heat injury (Larcher, 2003; Klueva *et al*., 2001).

Acclimation of photosynthesis and respiration to increased temperature: Photosynthesis and respiration are able to adapt to changing environmental conditions by means of acclimation. An understanding of how plants acclimate is necessary to predict plant behaviour at higher

prevailing temperatures. The capacity of different genotypes to grow over a range of temperatures is apparently correlated with the seasonal and diurnal temperature range of their native habitats (Teeri, 1980).

Acclimation of photosynthesis to temperature can take variable forms. Firstly, full acclimation can occur with a pronounced shift in the optimum temperature of net CO_2 assimilation (T_{opt}). This means that during warming the T_{opt} and heat limit of photosynthesis can shift to a higher temperature (Larcher, 2003). Secondly, a compensatory increase in photosynthesis is possible across a wide temperature range. Thirdly, no adaptation takes place or lastly, negative adjustments are made to the new temperature regime where photosynthetic rates actually decrease (Björkman *et al*., 1980). Furthermore, a combination of the above acclimation options is possible (Gunderson *et al*., 2000). In addition, the strategy with which plants acclimate may differ across species. According to Björkman *et al*. (1980), high temperature acclimation is primarily the result of an increased capability of one or more chloroplast components at the higher temperature. Other mechanisms of acclimation include changes in substrate concentrations, replacement of enzymes by iso-enzymes, which has the same action, but operates at different temperature optima, and chemical and structural alterations in the membranes (Larcher, 2003).

Respiration rate is affected by the prevailing temperature during acclimation and will classically increase at elevated temperatures up to 40°C, whereafter the respiration rate decreases due to enzyme denaturation. The factor by which the respiration rate increases in the short term with a 10°C rise in temperature is referred to as the Q_{10} value. The Q_{10} factor for respiration rates between 5 to 25°C is between 2 to 2.5 for most plants. At higher temperatures respiration still increase, but the Q_{10} is lower (Salisbury & Ross, 1992). If respiration could acclimate perfectly the same intensity of respiration rates would be maintained across a relatively wide range of temperatures. According to Larcher (2003) respiratory activity is increased during cold adaptation and diminished by heat. Although this hypothesis is contrary to what would be expected, biochemical control of respiration ensures a controlled rate of reaction. For Mediterranean sclerophylls, such as *Protea*, the respiratory activity is proposed to increase in the cooler season to keep metabolic activity constant throughout the year (Larcher, 2003). Different plant speciesô respiratory activity is adapted to the average temperature of the distribution area.

The effects of temperature on flowering: Hollister & Webber (2000) stated that the time of flowering of tundra vascular plants is closely controlled by and associated with environmental

temperature. If temperatures are below a certain minimum, vegetative growth and floral initiation proceed slowly or not at all (Rieger & Sedgley, 1996). Alternatively, temperatures positively promoting growth will speed up anthesis (Kinet, 1993). At the other extreme, heat stress can be detrimental to reproductive development, with no flowers developing or flowers developing without fruit or seed formation (Hall, 2001). Supra-optimal temperatures are believed to cause floral reversion within a limited time during the early stages of floral differentiation (Hoffman, 2006).

Dupee & Goodwin (1990) found that with *Telopea speciosissi*, an Australian Proteaceae, a warm winter advanced and enhanced flowering, especially in cooler areas. The abortion of flower heads was caused by higher than normal temperatures and limited soil moisture (Dupee & Goodwin, 1990). Similarly in *Banksia*, very high temperatures during the floral initiation window were found to inhibit floral initiation, resulting in lower yields (Fuss & Sedgley, 1991). Stephenson & Gallagher (1986) investigated flower initiation of *Macadamia integrifolia* and found that warm nights (20°C) during flower initiation, followed by lower night temperatures (10.5°C) promoted floral bud production.

1.6 Interaction between air temperature and other environmental factors

Carbon dioxide, water availability and nitrogen: Exposure to elevated CO_2 concentration has been shown to increase photosynthesis in spring wheat (*Triticum aestivum* L.) adapted to ambient CO_2. However, wheat grown and adapted to elevated CO_2 concentration showed decreased photosynthesis and stomatal conductance (Martínez-Carrasco *et al*., 2005). Down regulation of stomatal conductance is greater at a lower VPD and higher temperatures. Such plants are frequently incapable of maintaining their initial photosynthesis rates as stimulated under enriched CO_2 conditions. When in combination with elevated temperature, high CO_2 levels also decreases Rubisco activity (Pérez *et al*., 2005) and therefore depress carbohydrate assimilation.

Within the global warming context more erratic rainfall is predicted in future. Therefore, temperature stress will most likely be accompanied by water stress. Abscisic acid (ABA) is produced in the roots when water stress is experienced, causing stomatal closure (Chaves *et al*., 2002). Water loss is then limited, but stomatal closure will decrease the rate of photosynthesis and therefore reduce the total carbon gain.

Heinsohn & Pammenter (1988) found that *Protea* adapted to a summer rainfall area (Eastern Transvaal, South Africa) exhibited shoot growth which was advanced by several

months compared to plants in their natural habitat, the Western Cape, a winter rainfall area. Subsequently, flowering was also advanced and took place in late summer to early winter compared to autumn or early winter as would be the case in its native habitat, the Western Cape region. This shift may be caused by the temporal change in water availability.

At higher temperatures an abundant supply of nitrogen decreases the negative effect (down regulation) of elevated CO_2 on photosynthesis. This is only evident where the higher temperature promotes growth and the plant is not already functioning in the optimal temperature regime (Martínez-Carrasco *et al*., 2005).

Temperature and plant phenology: Phenology can be defined as the timing of seasonal activities of animals and plants (Walther *et al*., 2002) or as the study of the timing of recurrent biological events (Badeck *et al*., 2004). The phenology of plants is one the most basic processes and can be tracked and used as a measurement of the changing environment. Temperature strongly influences and drives developmental processes and life cycles (Hughes, 2000). Specifically higher temperatures have been shown to increase the rate of plant development, therefore enabling plants to switch to the next ontogenetic stage earlier than expected (Badeck *et al*., 2004). The timing and progression of phenological stages can thus be expressed as a function of temperature.

In Europe, Menzel & Fabian (1999) have found that distinct spring events, such as leaf unfolding, have advanced by 6 days in the past 30 years. Earlier budbreak and flowering of plants studied in Europe and North America as well as in a woody evergreen tree, *Andromeda polifolia*, was also recorded by Walther *et al*. (2002) and Aerts *et al*. (2004) respectively. Autumn events (leaf colouring) have been delayed by 4.8 days and these shifts can mainly be attributed to changes in air temperature, which is one of the main effects of global warming (Menzel & Fabian, 1999).

Lee *et al*. (2001) studied annual weed species of various temperate habitats and found that plant phenology advanced in proportion to seasonal warming, while elevated CO_2 had little effect. Seedling emergence and flowering times were significantly earlier in all warmed plots.

Hollister & Webber (2000) determined that tundra vascular plants species flowered earlier and showed enhanced vegetative growth in response to elevated temperatures. This supports research on trees in Europe where increased temperatures also resulted in accelerated tree growth (Menzel & Fabian, 1999). However, the increased allocation of carbohydrates and dry mass to vegetative and reproductive growth requires elevated nutrient investments.

Therefore, in the long-term, these positive growth responses will most likely be constrained by nutrient limitations in most natural habitats (Aerts *et al*., 2004). As fynbos plants are mainly confined to acidic, low nutrient soils, an increase growth-driven requirement for nutrients will have serious ecological and economic implications for nutrient acquisition strategies and fertilisation regimes respectively.

1.7 Sensitivity of fynbos species to environmental stress

Protea leaves can be retained between one to six years on the plant and will survive several seasons of wet, cold winters and warm, dry summers (Coetzee & Littlejohn, 2001). Adaptive characteristics to drought tolerance include sclerophyllous leaf characteristics which include narrow leaf margins and a steep leaf inclination together with low leaf area indices (Richardson & Kruger, 1990). Drought avoidance adaptations include the tuberous taproots of *Protea cynaroides* to utilise deeper (Ó 1 m) below ground water resources (Chaves *et al*., 2002), whilst the leaves of some species have a dense trichome layer to increase reflectance, similar to *Olea europaea* (Larcher, 2003), another Mediterranean crop. Hawkins *et al*. (2009) reported deep-rooted *Protea* ‘Sylvia’ to redistribute ground water, 1.2m deep, to upper soil layers where shallow-rooted companion plants act as water parasites and this sustain their growth during dry summer periods. In addition to these deep roots, Proteaceae also have shallow lateral roots which emerge during the wet winter and spring months, namely proteoid roots, which absorbs mainly phosphorus from the soil (Lamont, 2003). In addition to morphological adaptations, Chaves *et al*. (2002) found antioxidant systems to offer increased protection against photo-inhibition during summer in Mediterranean woody species.

Van der Heyden & Lewis (1990) recorded the optimum temperature for photosynthesis for several fynbos species to be between 20-30°C during November and February. However, the absolute value of A_{max} decreased from ~16 µmol m^{-2} s^{-1} in November to ~11 µmol m^{-2} s^{-1} in February. On both recording dates A_{max} markedly decreased from 30 to 40°C, whereas temperatures above 40°C was shown to be detrimental to photosynthesis.

In experiments done with macadamia (*Macadamia integrifolia* cv. Keauhou), plants were placed in controlled growth chambers (Trochoulias & Lahav, 1983). Temperatures treatments ranged from 10-35°C in order to investigate the effect of temperature on the growth and dry matter accumulation of macadamia. Net photosynthesis was found to be at a maximum between 16-25°C. Dry matter accumulation was also reported to be the highest in the leaves at 20-25°C. However, the rate of photosynthesis decreased above 26°C and was

terminated at around 41-43°C. Plants grown at a constant temperature of 10°C showed no growth, whilst plants grown at 35°C had multiple, unsynchronised budbreak, followed by callus growth and later, desiccation (Trochoulias & Lahav, 1983).

Midgley *et al*. (2002) warned that the threatening rate of climate change can surpass the rate of migration of certain fynbos species. Spatial distribution of fynbos seeds naturally dispersed by ants or wind is limited, as these processes are slow when compared to water, birds or larger animals as dispersal agents. Certain species have serotinous seeds with post fire requirements for dispersal and germination (Manning, 2007). A possible time lapse of 15 to 25 years between fires implies that shifting of these species to a more suitable climatic area takes place very slowly and the chances of loss of diversity is increased (Midgley *et al*., 2002). A further threat to the survival of proteas is the inhibition of seedling germination after fires caused by very high fire temperatures (Musil *et al*., 2007).

Midgley *et al*. (2002) predicted that in the northern region (Van Rhynsdorp) of the fynbos species range, heat and drought stress will cause severe mortality of these species, whilst the phenology of the fynbos along the western coast is expected to change because of overall increasing minimum temperatures.

1.8 Experimental approaches to plant studies in climate change

Different facilities have been developed for studies of elevated temperature on plants, such as open-top chambers (OTC), growth chambers with fixed temperature, free air temperature increase (FATI), temperature gradient chambers (TGC) or tunnels, O_2 gradient chambers with screen-aided CO_2 control (SACC), which includes a combination of elevated CO_2 levels and increased temperature (Lee *et al*., 2001).

Hollister & Webber (2000) found that a comparison of growth response variables of a warmer summerôs control and a cooler summerôs OTC plants showed no statistical difference in 80% of parameters measured. This provides evidence toward the reliability of the OTC approach. The main benefits of the OTC system is that it does not require high technological maintenance and it allows for free air exchange when not sealed to the ground. The light levels, precipitation, CO_2 and O_2 levels and humidity are very similar to that of the control environment. Furthermore, pollinators and herbivores are not excluded. Inside an OTC air temperature can be as high as 5.5°C above ambient, due to passive warming of the natural vegetation (Musil *et al*., 2005). However, enclosures do modify the microclimate surrounding

the plants (Nijs *et al*., 1996a) and would be expensive to construct for large woody plants such as proteas.

When using temperature gradient tunnels, Pérez *et al*. (2005) could control the temperature and CO_2 levels inside the tunnel by using probes at both the in- and outlet. The outlet was connected to heaters and fans, with precise control over the system. However, a negative side-effect of a high performance CO_2-temperature gradient chamber is that a humidity gradient accompanies the temperature gradient, making it difficult to interpret the interactive effects between CO_2 and temperature (Lee *et al*., 2001).

With FATI, plants in the field or in pots can be artificially heated using infra-red lamps. Unlike sunlit chambers, the air around the plants is not warmed directly, therefore preventing the indirect transfer of heat from the air to the plants. In the FATI system, the infra-red lamps directly heat the plants. The lamps are relatively inexpensive, with little maintenance required. A cut-off filter can be used to eliminate any photosynthetic active radiation (PAR), but the PAR is very low and filters are generally not essential. No chambers or enclosures are necessary and effects such as reduced light intensity, differences in light quality, wind speed and a lack of uniformity between chambers are removed (Nijs *et al*., 1996b). The only serious disadvantage of this system is the inability to control the relative humidity. When Nijs *et al*. (1996b) used infra-red lamps on *Lolium perenne* L., a grass species; it was suggested that the design could be modified for use on plants with larger canopies.

1.9 Conclusion

The South African Fynbos industry is faced with various challenges in the immediate future. These include competition from new emerging southern hemisphere producing countries such as Ecuador and Colombia, changes in market trends, economic pressures and certainly, environmental changes. A limited understanding of the factors which influence growth and vegetative requirements, as well as requirements for floral induction, initiation and development will further complicate the development of strategies to optimize *Protea* production. Except for own historical production and climatic data, producers have few guidelines to assist in the prediction of harvests and to align production with market demands in order to obtain premium prizes for their product. Only through with research, innovative thinking, planning and timely adaptation by producers, will *Protea* production be maintained and expanded as a thriving and lucrative industry in South Africa.

1.10 Overall objectives

The objectives of this study were:

- To gain a better understanding of the response of *Protea* cv. Pink Ice under elevated temperatures in terms of gas exchange, physiology and flowering.
- To evaluate plant acclimation in terms of the optimum temperature for the rate of net CO_2 assimilation (T_{opt}) during the course of a season as well as under elevated temperatures. Furthermore, to establish whether shoot growth rates increase or decrease under supra-optimal temperatures and how changes in gas exchange, vegetative growth and carbohydrate levels caused by high temperature influence inflorescence production and, therefore, plant phenology.
- To compare inflorescences growth and development which initiated on either the autumn or spring flush and therefore developed during different climatic periods in the year as well as shoot growth prior to floral initiation.
- To establish baseline seasonal data as a reference to future studies and to be used during modelling of *Protea* adaptation to warming.

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2. General materials and methods of warming experiments

2.1 Greenhouse-based warming experiment

The greenhouse-based warming experiment was conducted from March 2008 to March 2009 on Welgevallen Experimental Farm, Stellenbosch University, Stellenbosch, South Africa (33°56ôS; 18°51ôE). The greenhouse structure (Figs. 1, 2, 3) consisted of a glass roof which excluded rain, whilst the glass panels of the walls were removed to allow free air-ventilation. One side of the greenhouse consisted of a brick wall. The wall, on the southern side of the experimental plot, did not cause a shade effect during the any season.

Plant material: Twenty-eight two-year-old, drip irrigated, potted *Protea* cv. Pink Ice (*P. compacta* R. Br. x *P. susannae* Phill.) plants were used in the study. Two 2ă hour^{-1} drippers were placed in each pot, one in each half of the pot. At the start of the experiment the irrigation schedule was set to pulses of two minutes each, two times a day at 08:00 and 18:00 to deliver a total of 260 ml per day per pot. From 3 November 2008 to the end of the experiment two additional times, 06:00 and 20:00 were added, giving a total of 520 ml per day per pot in summer.

The potting medium consisted entirely of Tsitsikamma forest bark to which dolomitic or calcitic lime was added to regulate the pH. Controlled release fertilizer was added to the potting medium according to commercial practice. All plants were transplanted once from 16 to 20 cm pots during a period of root dormancy (31 July 2008) to allow sufficient rooting volume. Proteiod roots were noticed during repotting. The pots, and subsequently the soil and roots, were covered with a straw mulch to reduce temperature fluctuations within the pots.

Four plants were allocated per treatment and were spaced within a north-south row orientation (Fig. 5). Replicate plants were randomised biweekly within the treatment row to minimise any localized micro-climatic effects. Two vegetative two- or three-flush shoots per plant were randomly tagged and baseline measurements were recorded (Chapter 4.2.1).

Experimental heating design: Infra-red warming lamps (Royal Philips, The Netherlands, PAR 38 IR 175R) were used to create a temperature gradient ranging from ambient to ambient+3.1°C (Table 1). This is a type of free air temperature increase (FATI) experiment, where plants are artificially heated. Unlike sunlit chambers, where plants are indirectly

warmed by the warmer air, in the FATI system, infra-red lamps directly heat the plants. No enclosures are needed, which reduce unwanted effects such as reduced light intensity, differences in wind speed and a lack of uniformity (Nijs *et al*., 1996). A cut-off filter can be used to eliminate any photosynthetic active radiation (PAR) below 800nm, but filters were not considered essential as the red-coloured version lamp was used to reduce visual light emission and glare and therefore filters were not included in the design. The only serious disadvantage of this system is that the relative humidity and thus vapour pressure deficit cannot be controlled.

Lamps were positioned 1.5 m above the plants. The number of lamps per treatment started at zero for the control at ambient temperature, and then increased from one to six lamps to establish a projected seven treatment temperature gradient (Fig. 5). The lamps were activated on 19 May 2008; whereafter lamps were run on a timer-controlled daily cycle from 05:00 to 23:00, allowing for a six-hour period without warming nightly. This also reduces any possible visible light effects.

Temperature calibration: A full temperature range calibration was done prior to the start of the experiment using a handheld thermometer (SAM 990DW, Mannix Instruments, Hewlett, New York, USA) on 28 May 2008 and repeated on 30 June 2008 (Fig. 8). Air temperature was recorded at plant height. An infra-red thermometer (Raynger® MX4Ê , Raytek Corporation, Santa Cruz, California, USA) measuring surface leaf temperature of the terminal flush was also used on 16 and 18 July 2008 to verify leaf temperature calibrations obtained through the handheld thermometer (Fig. 8). In addition, a calibration was done on 16 July 2008 with the infra-red thermometer recording leaf temperature on leaves of the basal, intermediary and terminal flushes (n = 13) and subsequently averaged for each treatment temperature (Fig. 9). On 23 September 2008 a comprehensive calibration was performed by means of the infra-red thermometer recording leaf temperature of the terminal flush of the four plants per treatment and subsequently averaged per treatment (n = 20) (Fig. 10).

Temperature monitoring: One TinyTag mini-datalogger (TGP-06, Gemini Dataloggers Ltd., Chichester, UK) was suspended at plant height, in the middle of each treatment position (Fig. 5). The radiation shields around each logger were abandoned due to unsatisfactory readings. Each logger recorded air temperature (°C) at fifteen-minute intervals. Tinytags were calibrated against each other as well as against the two professionally calibrated thermistors

used in the greenhouse weather station prior to the start of the experiment. Mean daily temperatures for the seven temperature positions were calculated for the duration of the experiment (Fig. 11). The mean daily temperatures of the respective temperature treatments in mid-summer (February 2009) are shown in Fig. 12. The temperature gradient established by the infra-red warming lamps changed slightly over time and the monthly trends are presented in Table 1. In relation to the first treatment position (ambient), the mean temperature changes at the six other positions along the gradient for the duration of the experiment were ambient, ambient-0.1°C, ambient+0.7°C, ambient+0.8°C, ambient+2.5°C, ambient+1.8°C and ambient+3.1°C. The actual temperature gradient was not suitably spaced as was initially aimed for; therefore two treatment positions, ambient-0.1°C and ambient+0.7°C were eliminated for data analysis and discussions.

Soil temperature: Two TGP-4510 Tinytag mini-dataloggers (Gemini Dataloggers Ltd., Chichester, UK) with external temperature probes were used to log soil temperature from 28 November 2008 to 23 March 2009 at position 1 (ambient) and position 7 (ambient+3.1°C) at fifteen-minute intervals. The probes were placed 10 cm deep in one pot and were not removed or shifted between pots throughout recordings.

Greenhouse and Stellenbosch climate: Microclimatic measurements representative of the greenhouse environment were recorded on the edge of the greenhouse on the ambient side of the gradient (Fig. 5), using a CR10X datalogger (Campbell Scientific, Inc., Logan, Utah, USA). Data was logged hourly from May to November 2008 and included air temperature (°C) and relative humidity (%RH) measured with a shielded thermistor, photosynthetic photon flux density (PPFD) (µmol m^{-2} s^{-1}) measured with a quantum sensor (LI190SB, LI-COR, Lincoln Nebraska, USA) and total radiation measured with a pyranometer (LI200X, LI-COR, Lincoln Nebraska, USA). Due to a faulty thermistor caused by tampering and an unsuccessful re-initiation following repair, no data from November 2008 onwards were collected. All sensors used for the weather station was professionally calibrated (M. Savage, University of KwaZulu-Natal) prior to the start of the experiment.

Hourly temperatures, daily minimum and maximum temperatures as well as mean hourly RH were obtained from the Nietvoorbij automatic weather station of the Agricultural Research Council (ARC) in Stellenbosch (33°55ôS; 18°54ôE), South Africa.

Seasonal climatic trends: Seasonal temperatures shown give an indication of the type of season experienced during the experiment. The greenhouse climate is compared to that of the surrounding area and any differences could be identified. Soil temperatures recorded with external probes is shown in Fig. 13.

The monthly means of mean daily air temperature recorded at the ambient position in the greenhouse (TinyTag) was compared to the air temperature from the shielded thermistor (CR10X) and from Nietvoorbij (Fig. 14). The greenhouse was found to be slightly warmer than true ambient temperatures for the Stellenbosch area, the difference increasing through spring and early summer. As TinyTags were not shielded, direct warming of TinyTags by the infra-red lamps is probably responsible for the difference between TinyTag and CR10X mean temperatures. Analysis showed that this was entirely due to higher readings during the warmest part of the day. Nonetheless, plants were warmed in an acceptable and calibrated gradient, whilst Tinytag recordings were used as a relative indication of warming.

The monthly means of mean daily photosynthetic photon flux density (PPFD) as well as maximum values recorded by the CR10X weather station is presented (Fig. 15), whilst relative humidity (CR10X) in the greenhouse and from Nietvoorbij is shown in Fig. 16.

Mean daily air temperature and PPFD as logged by the CR10X weather station in the greenhouse are presented in Fig. 17a and b. Mean daily relative humidity, logged by the CR10X weather station from May to November 2008 and the Nietvoorbij weather station from May 2008 to March 2009 is presented in Fig. 18 and shown similar absolute values and fluctuations, if not daily agreement.

A snapshot of hourly trends from 14 to 20 October 2008 in air temperature, PPFD and RH are presented in Fig. 19.

2.2 Field verification warming experiment

In the field verification warming experiment two temperature treatments, ambient and warmed, were established in July 2008, on a commercial protea farm, Floraland-Etshwaleni, outside Stellenbosch (33°54ôS; 18°48ôE), South Africa. The temperature treatments were established on eight-year-old *Protea* ôPink Iceô (*P. compacta* R. Br x *P. susannae* Phill.) plants managed in an annual cropping system. Plants were in a five row system, in a north-south row direction, spaced 1.5 x 1.5 m within rows with a 3 m service way. The soil was classified as a sandy soil. Plants were not irrigated from August to October 2008, as the farm

dam was situated directly above the orchard and overflow water saturates the soil during winter and spring. Therefore, plants within well drained rows were selected. Irrigation during February 2009 was 20.7 ă water per plant per week, and in all other months 6.9 ă per plant per week was given. The farm manager applied Kelpak® (Kelp Products (Pty) Ltd, www.kelpak.com) as a foliar spray in December 2008, January and February 2009, whilst K-max® (Gouws and Scheepers (Pty) Ltd, www.plaaskem.co.za/label/KMAX.pdf) was applied in March 2009, according to commercial recommendations. In June and July 2008, a supplement of 5 to 10g K_2SO_4 per plant was applied. Two shoots on six eight-year-old plants were completely randomly selected per treatment across three rows. Four- or five-flush vegetative shoots were tagged.

Experimental warming: The temperature treatments were established with the use of infra-red warming lamps as described in 2.1, positioned 80 cm above each plant. Lamps were activated on 15 July 2008. Lamps were timer-controlled on a daily warming cycle from 05:00 to 23:00, allowing for six hours of no heating every night.

TinyTag mini-dataloggers (TGP-06, Gemini Dataloggers Ltd., Chichester, UK) suspended at plant height were used to obtain the temperature difference between treatments, namely ambient and ambient+2.9°C. In addition, hourly temperatures (°C), daily minimum and maximum temperatures, as well as relative humidity (%) were obtained from the Nietvoorbij automatic weather station at the Agricultural Research Council (ARC) in Stellenbosch, South Africa.

2.3 References

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Table 1. The monthly means of daily mean temperature and the mean air temperature gradient for the experimental period as created by infra-red lamps and recorded by TinyTag mini-dataloggers in the greenhouse. The gradient varies between months, depending on solar movements, cloud cover and microclimate changes in the greenhouse. Monthly means of mean daily air temperature (°C) at each position (2 to 7) is shown relative to position 1 (ambient).

Month (2008-2009)	1	2	3	4	5	6	7
July	0.0	+0.2	+0.8	+0.9	+2.4	+1.6	+2.8
August	0.0	+0.3	+0.7	+1.0	+2.3	+2.3	+3.2
September	0.0	+0.5	+1.3	+1.2	+3.1	+2.4	+2.7
October	0.0	-0.7	+0.2	-0.1	+2.7	+1.2	+2.3
November	0.0	+0.0	+0.9	+0.9	+2.9	+2.2	+3.3
December	0.0	+0.0	+0.6	+1.1	+2.1	+1.8	+3.3
January	0.0	-0.1	+0.5	+0.9	+2.2	+1.9	+3.3
February	0.0	-0.3	+0.5	+0.7	+2.4	+1.7	+3.3
March	0.0	-0.5	+0.6	+0.7	+2.4	+1.5	+3.7
Mean for experimental period	**0.0**	**-0.1**	**+0.7**	**+0.8**	**+2.5**	**+1.8**	**+3.1**

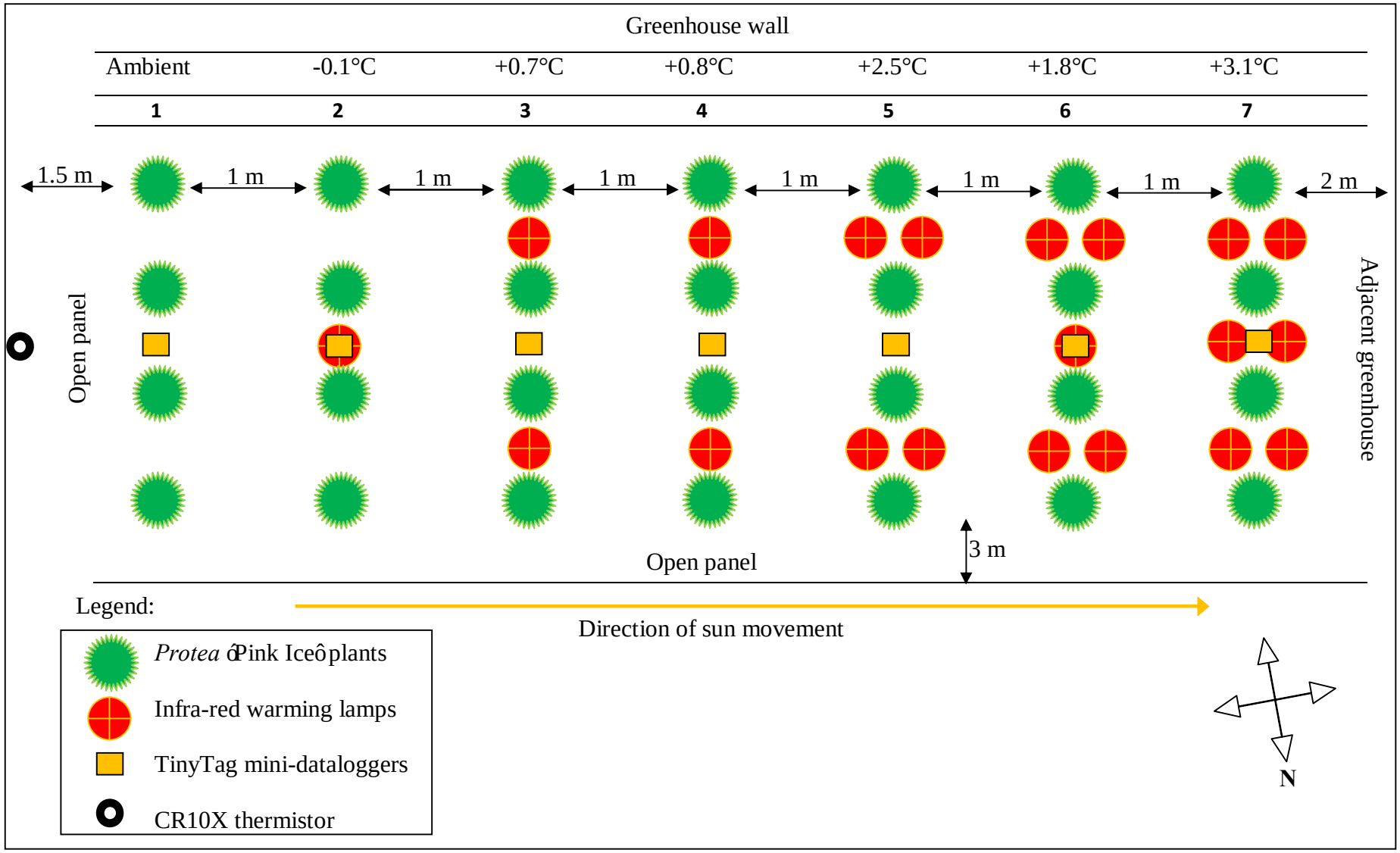

Fig. 5. Diagram of the experimental site layout from above. The open sides of the greenhouse and direction of sun movement are indicated.

Fig. 6. Arrangement of two-year-old *Protea* ôPink Iceô plants in the greenhouse. Treatments 0.0°C and -0.1°C are not visible on the left. View from the open panel.

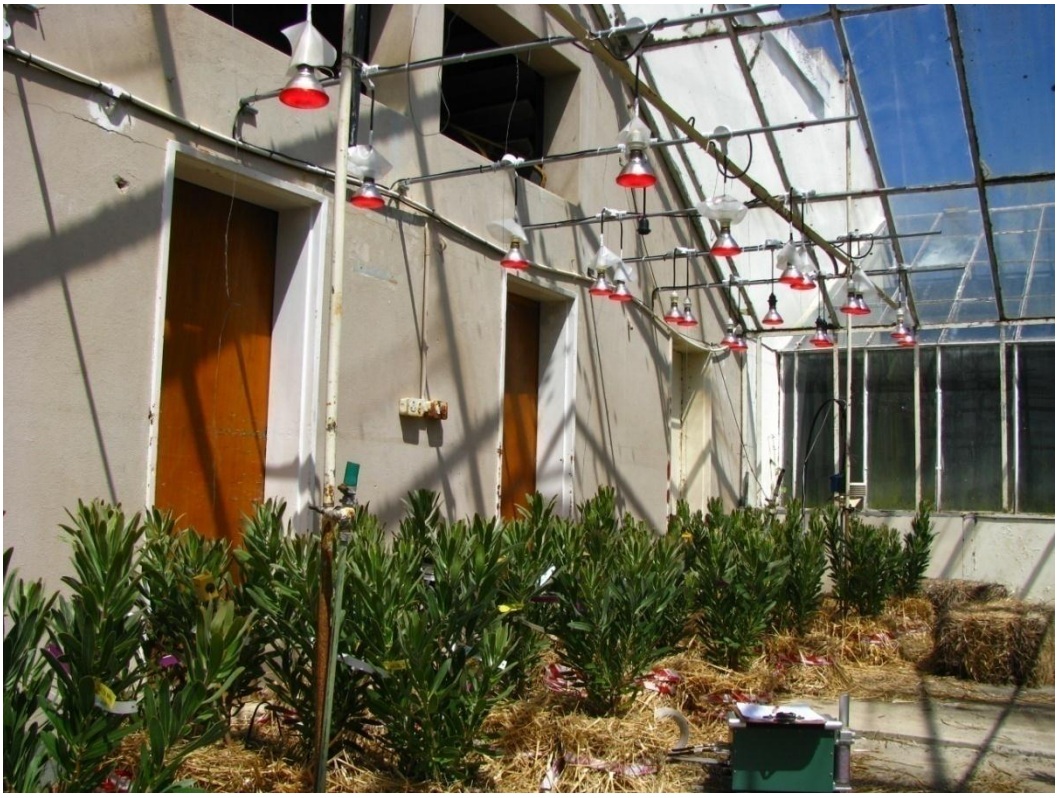

Fig. 7. A view of the glass-roofed greenhouse with 28 two-year-old *Protea* ôPink Iceô plants spaced under infra-red lamps suspended from a metal frame. The adjacent greenhouse and greenhouse wall is visible on the right.

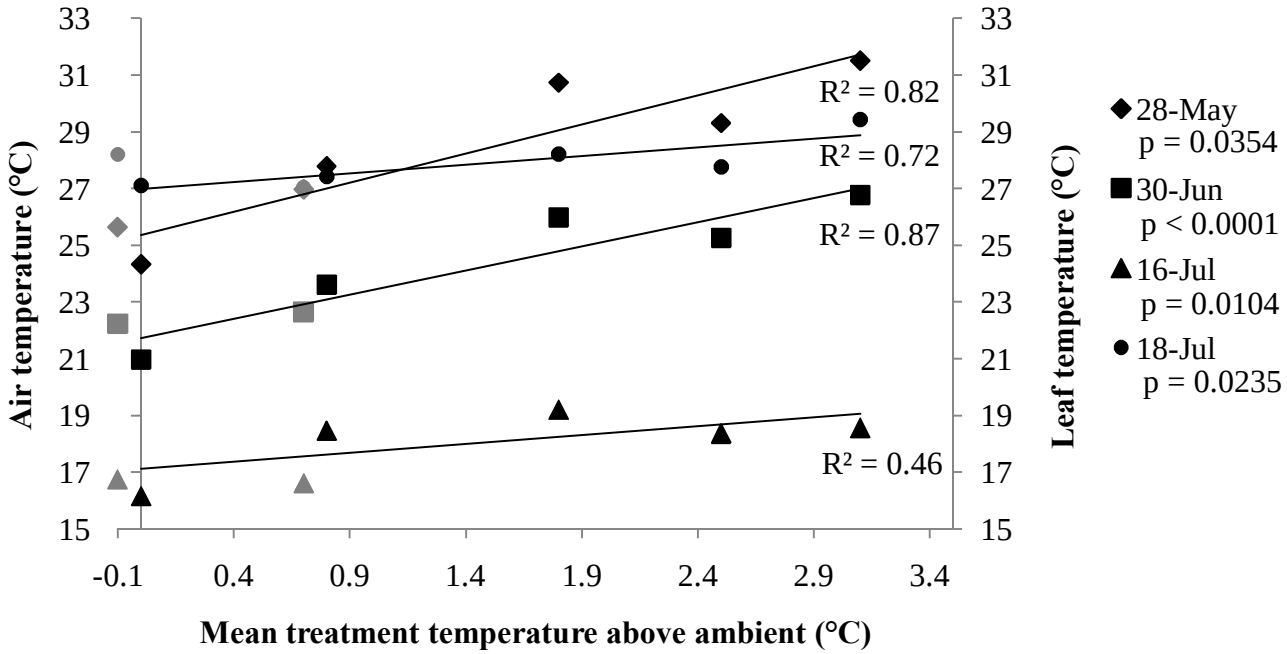

Fig. 8. Initial temperature calibrations performed on 28 May and 30 June 2008 with the handheld thermometer, recording air temperature, whilst calibrations on 16 and 18 July 2008 was taken with the infra-red thermometer, recording leaf temperature of the terminal flush of *Protea* óPink Iceô in a seven temperature gradient. Treatments presented in grey data points were eliminated during analysis and discussion.

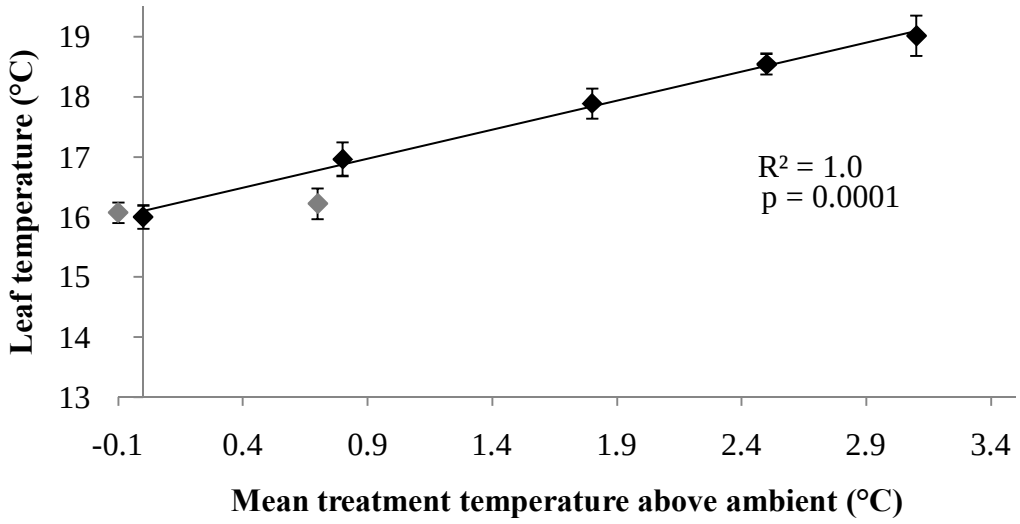

Fig. 9. A complete temperature calibration was performed on 16 July 2008 using the infra-red thermometer. Leaf temperature ± SE (n = 13) of the basal, intermediary and the terminal flushes was recorded and averaged of *Protea* óPink Iceô. Treatments presented in grey data points were eliminated during data analysis and discussions.

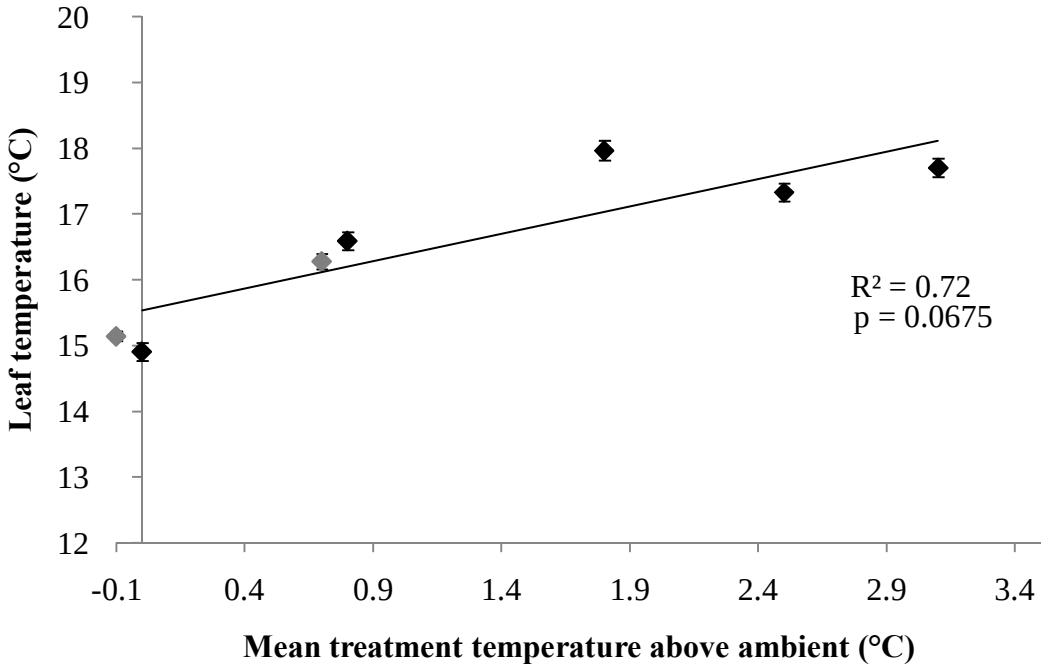

Fig. 10. A comprehensive temperature calibration was performed on 23 September 2008 recording leaf temperature ± SE (n = 20) with the use of the infra-red thermometer using leaves of the terminal flush of *Protea* ôPink Iceô. Treatments presented in grey data points were eliminated during analysis and discussion.

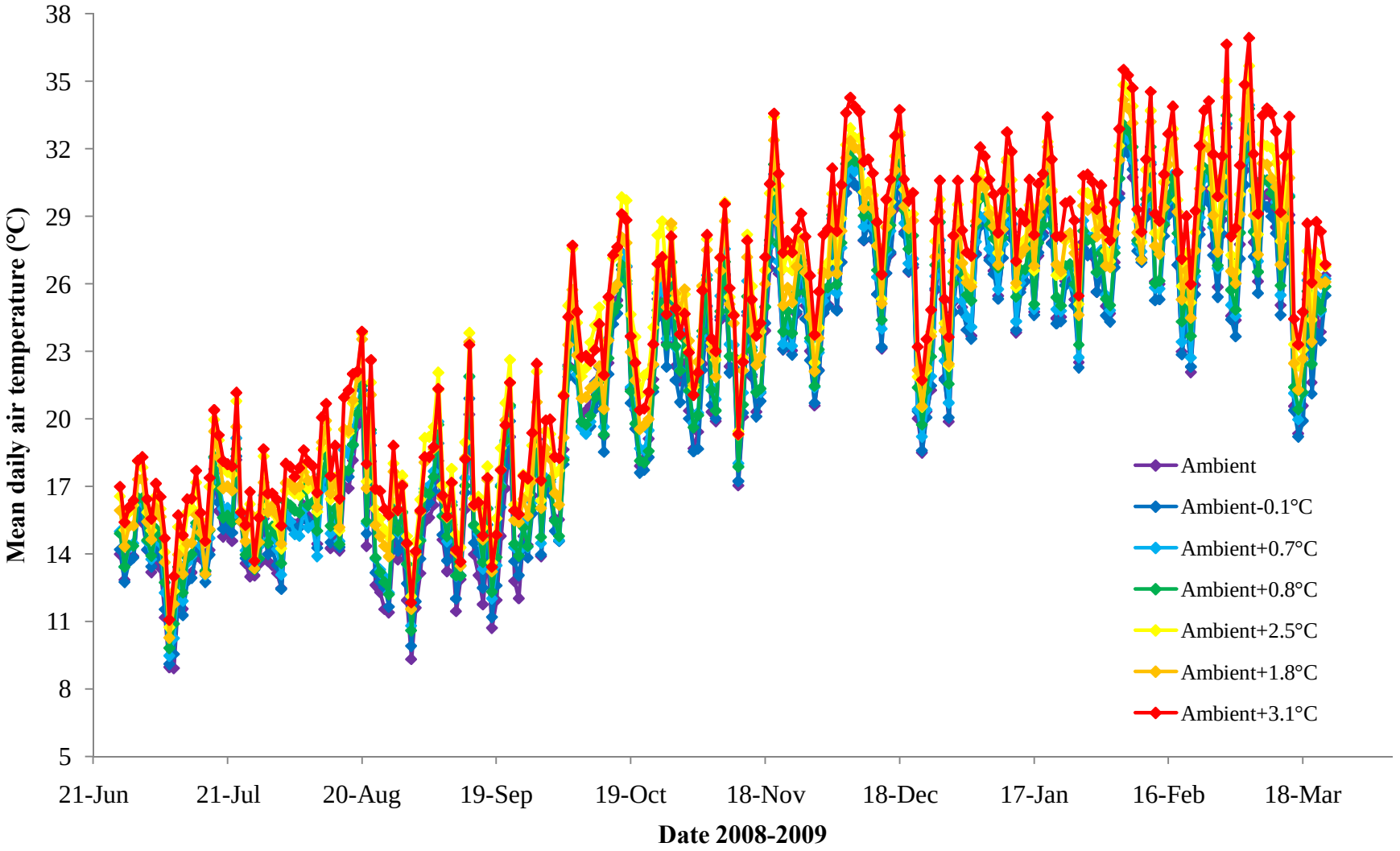

Fig. 11. Mean daily air temperatures logged by TinyTag mini-dataloggers at each of the seven infra-red heating positions in the greenhouse from 27 June 2008 to 23 March 2009.

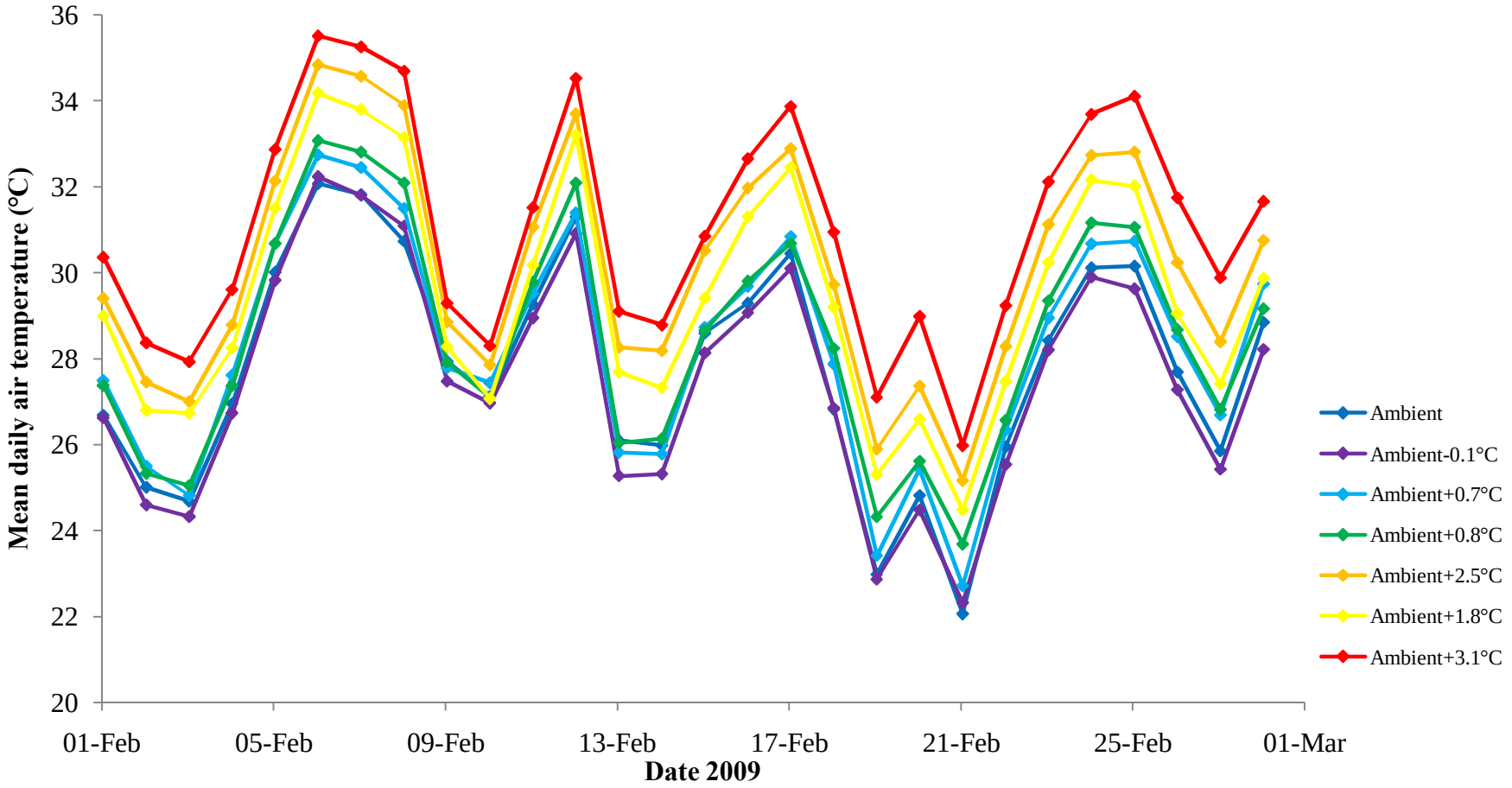

Fig. 12. Mean daily air temperatures logged by TinyTag mini-dataloggers at the seven infra-red heating positions in the greenhouse during February 2009.

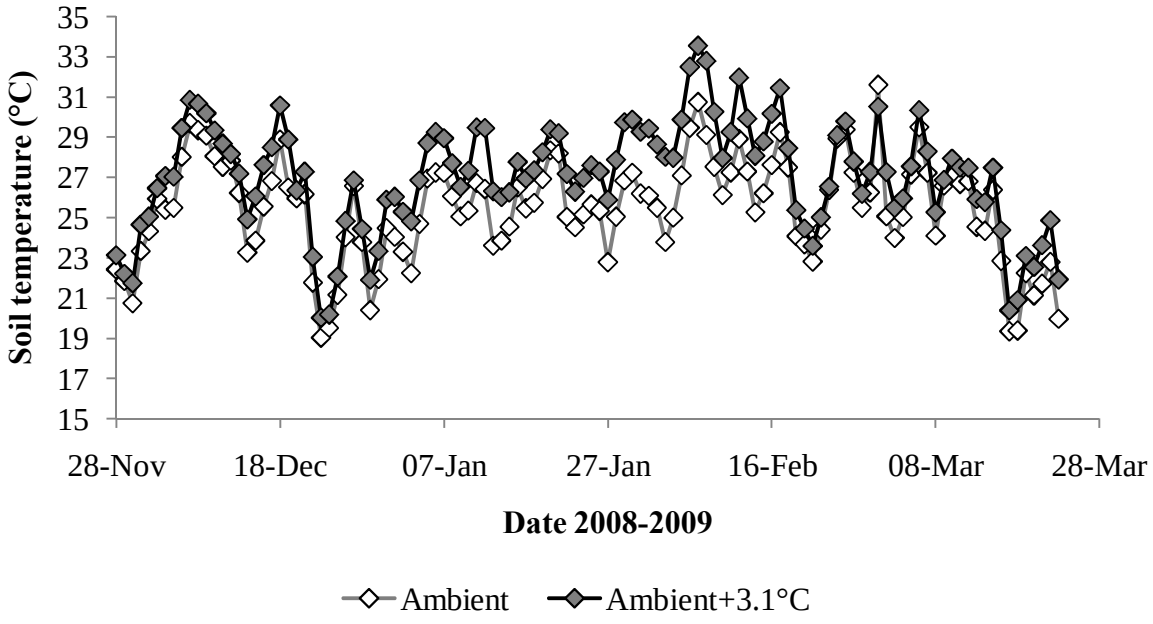

Fig. 13. Soil temperature at 10 cm depth recorded from 28 November 2008 to 23 March 2009 at position 1 (ambient) and position 7 (ambient+3.1°C) using TinyTag mini-dataloggers with an external temperature probes.

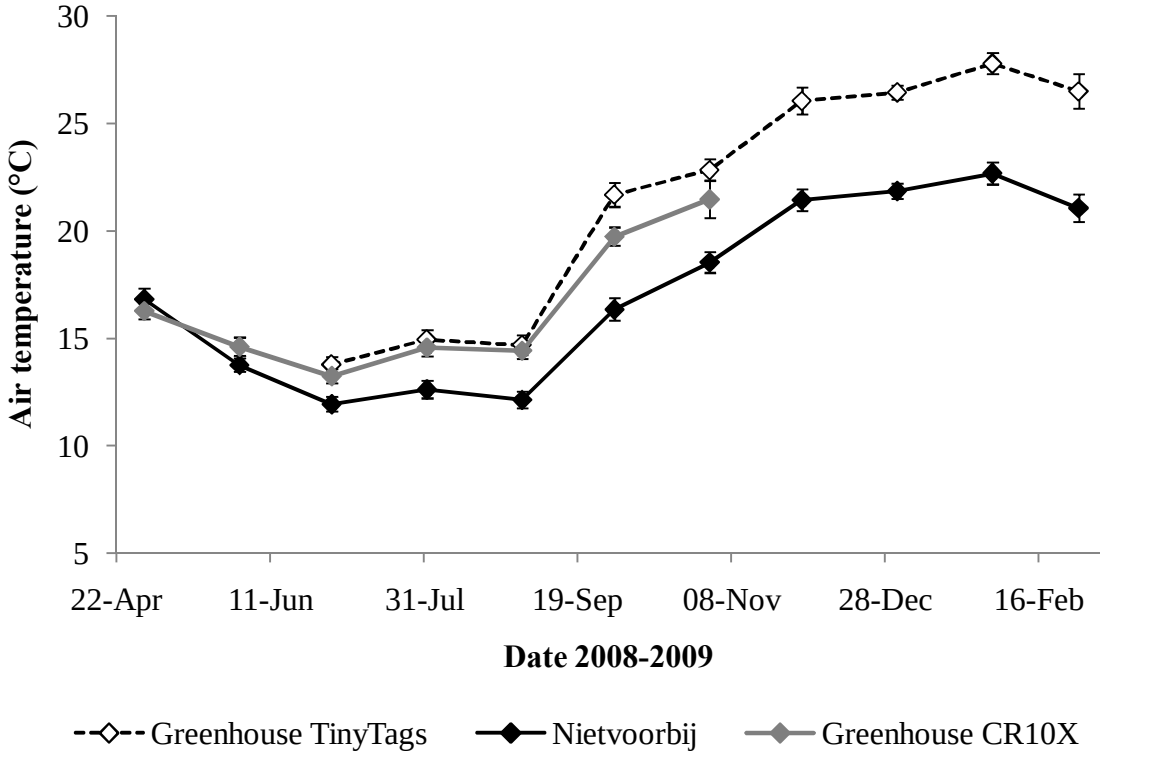

Fig. 14. Monthly means of daily mean temperature recorded from May 2008 to March 2009 using a TinyTag mini-datalogger at the ambient temperature treatment position, a shielded thermistor attached to an automated datalogger (CR10X, Campbell Scientific), as well as from an automatic weather station located at Nietvoorbij, in the greater Stellenbosch area.

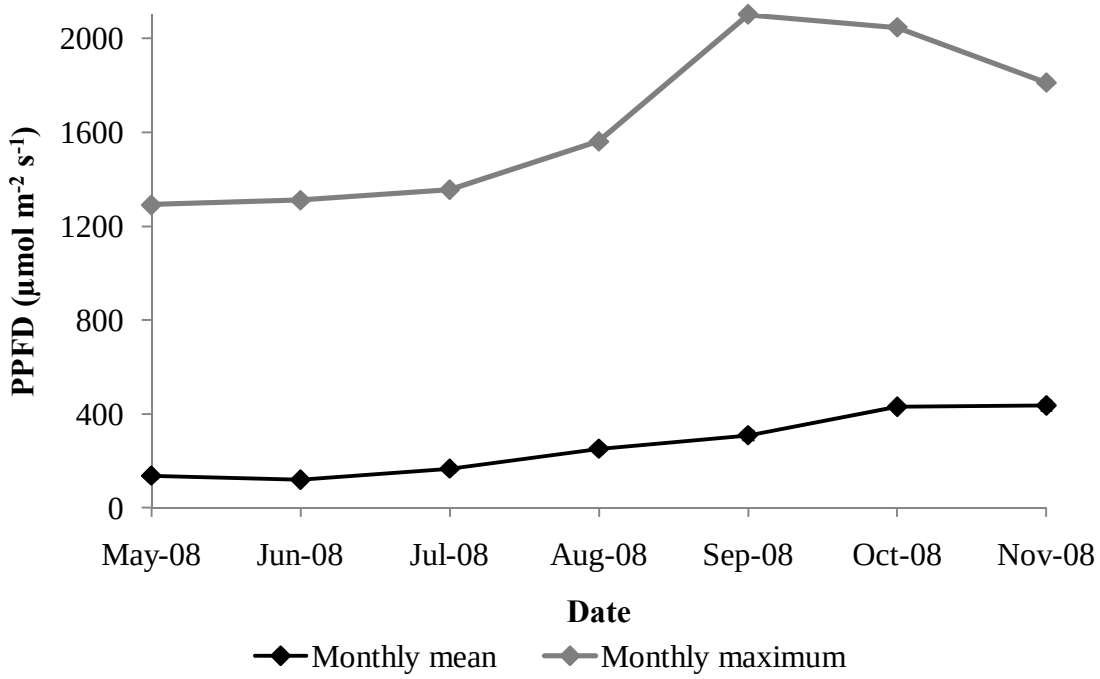

Fig. 15. Monthly means of daily mean and maximum monthly photosynthetic photon flux density (PPFD) logged by the CR10X weather station at the ambient treatment position in the greenhouse from May to November 2008.

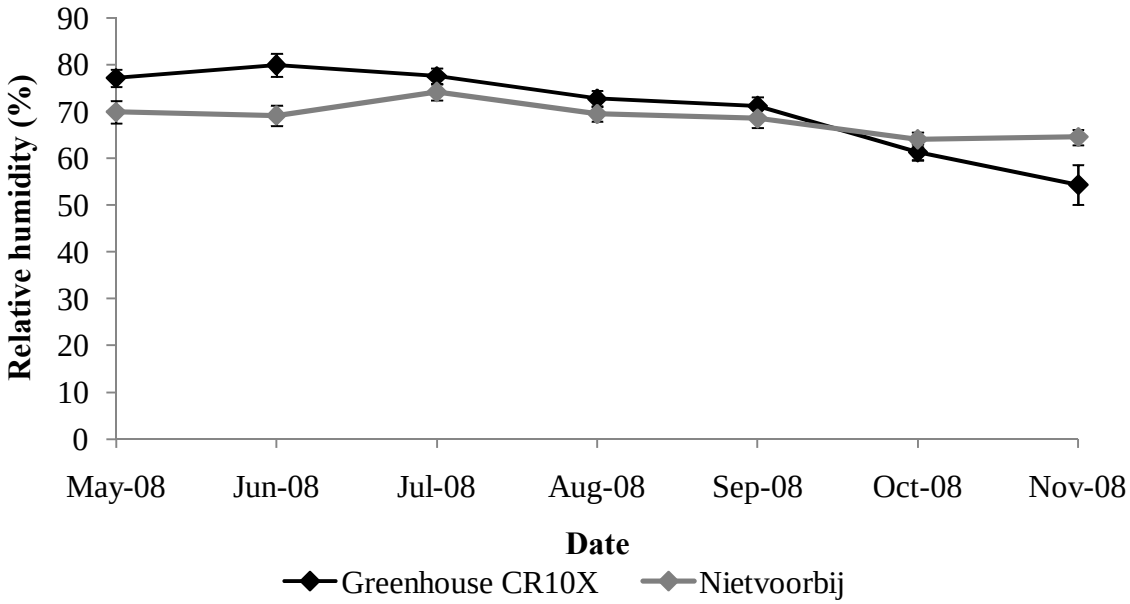

Fig. 16. Monthly means of daily mean relative humidity logged by the CR10X weather station from May to November 2008 in the greenhouse-based warming experiment at the ambient treatment position and at Nietvoorbij automatic weather station at the Agricultural Research Council (ARC).

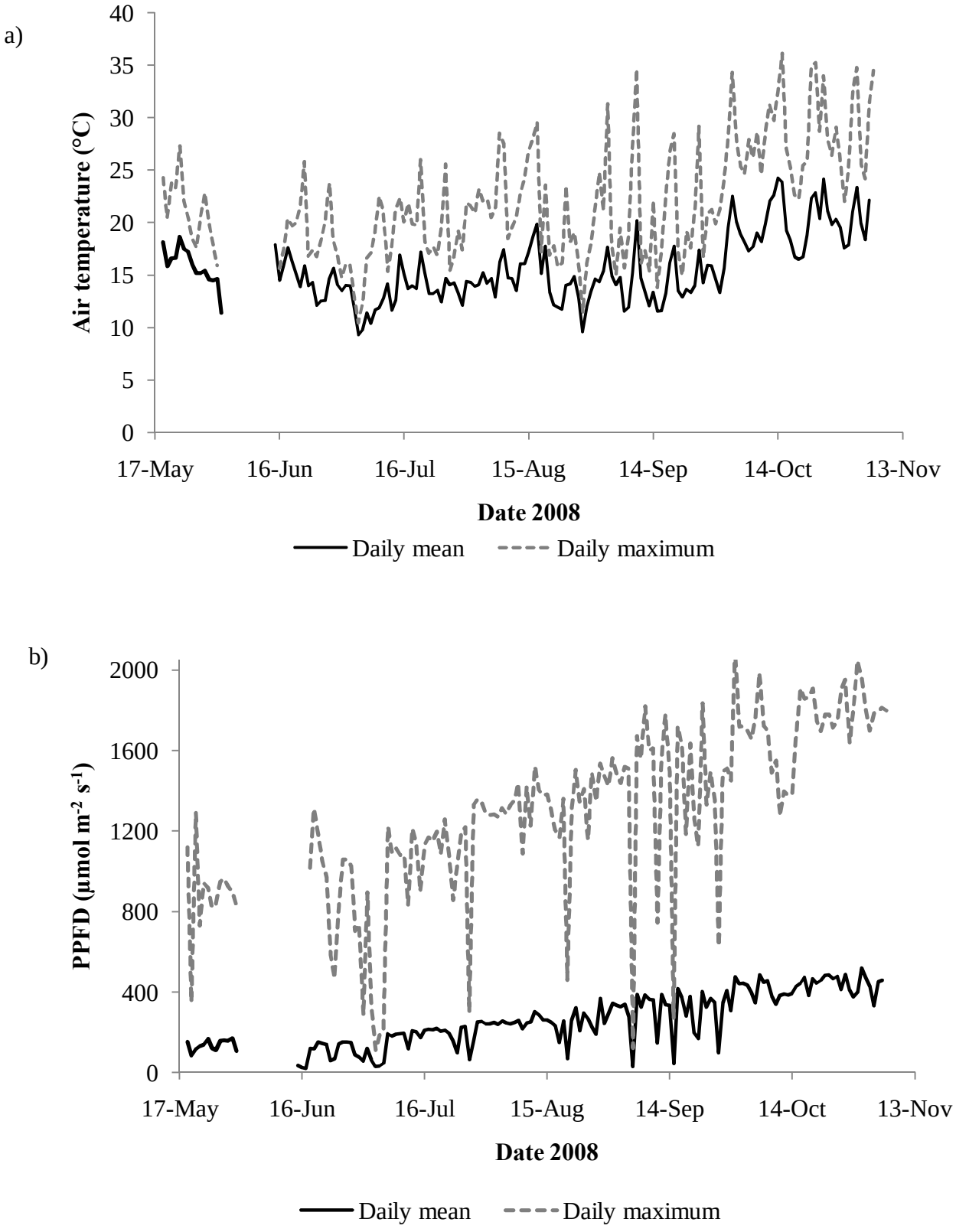

Fig. 17. Mean and maximum daily a) air temperature and b) photosynthetic photon flux density logged from 19 May to 6 November 2008 by the CR10X weather station in the greenhouse-based warming experiment.

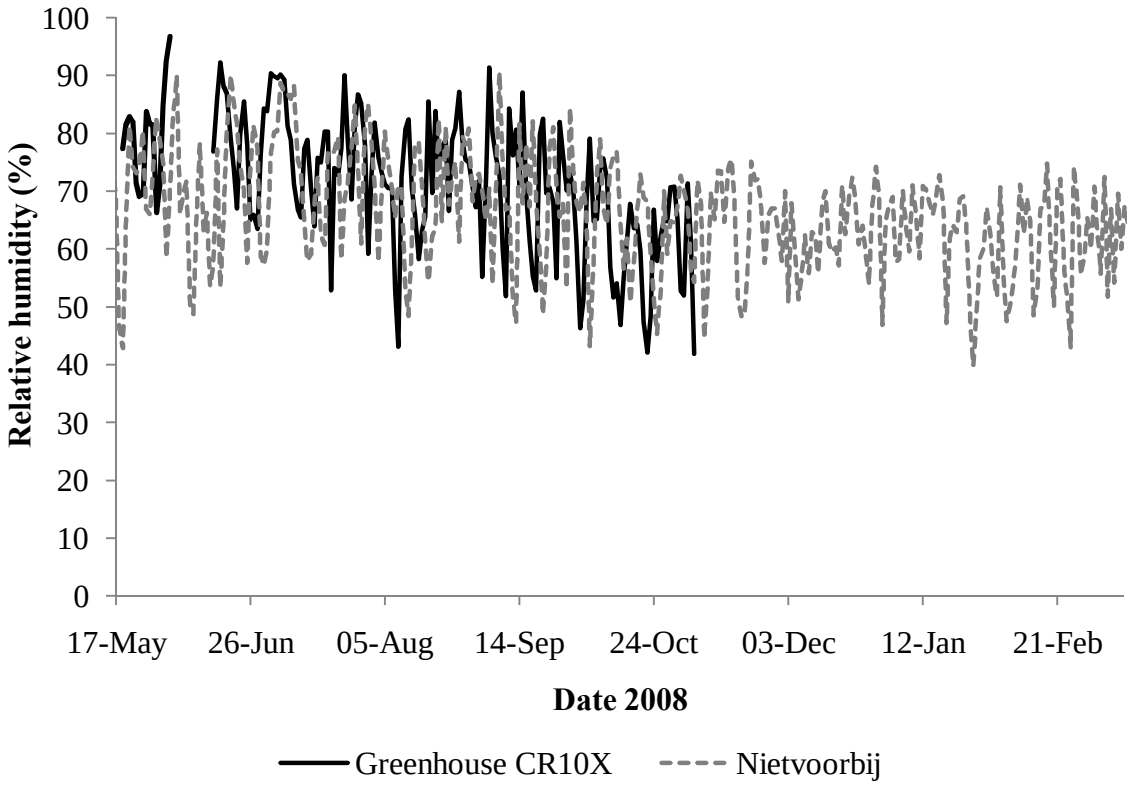

Fig. 18. Mean daily relative humidity logged from 19 May to 6 November 2008 by the CR10X weather station in the greenhouse-based warming experiment and from May 2008 to March 2009 by the automatic weather station at Nietvoorbij in the greater Stellenbosch area.

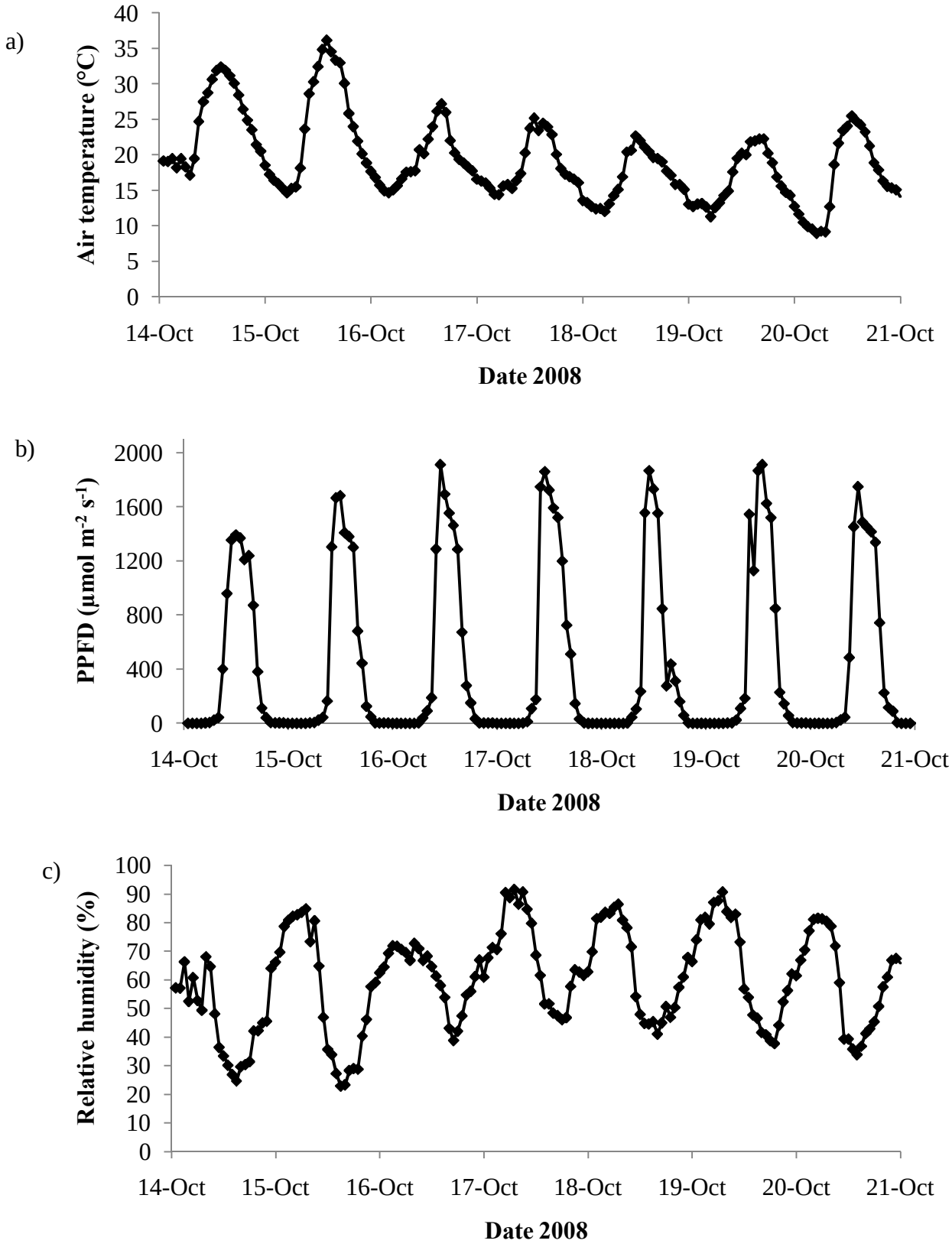

Fig. 19. Hourly trends of a) air temperature, b) photosynthetic photon flux density (PPFD) and c) relative humidity logged from 14 to 20 October 2008 by the CR10X weather station in the greenhouse-based warming experiment.

3. Gas exchange acclimation of *Protea* cv. Pink Ice cultivated under elevated temperatures

Abstract

The production of *Protea* selections and hybrids are expanding to new, warmer areas, as well as climates different from the Mediterranean-type climate traditionally associated with *Protea*. In addition, global temperature is increasing as a result of increased CO_2 emissions and there is a need to predict the effect of high temperature on the physiology, especially gas exchange, of *Protea. Protea* ôPink Iceô potted plants were warmed experimentally using infra-red lamps in a five-treatment temperature gradient that ranged from ambient to ambient+3.1°C in a greenhouse in Stellenbosch, South Africa, from May 2008 to March 2009. A field verification experiment consisting of two temperature treatments, ambient and ambient+2.9°C, was also established in a commercial orchard outside Stellenbosch from June 2008 to March 2009. Except for a limited number of days, the maximum rate of light-saturated net CO_2 assimilation (A_{max}), the maximum rate of light- and CO_2-saturated CO_2 assimilation (A_{sat}), the dark respiration rate (R_d) and stomatal conductance (g_s) showed good acclimation to the temperature gradient with no significant differences between treatments on a leaf area-basis. However, A_{max}, A_{sat} and R_d decreased significantly at higher temperatures on a leaf mass-basis from November 2008 to March 2009. Acclimation of the optimum temperature for the net CO_2 assimilation rate (T_{opt}) to the temperature gradient showed no consistent trend. A_{max} and R_d decreased during warm summer months, whilst R_d and A_{sat} increased during the same period. A_{max} and g_s increased again during autumn, when R_d and A_{sat} decreased slightly. *Protea* ôPink Iceô showed upward acclimation of T_{opt} to higher seasonal temperatures and tracked seasonal temperature changes closely. The apparent carboxylation efficiency, derived from A/c_i curves decreased at high temperatures. In general, *Protea* ôPink Iceô appeared to acclimate well to increased temperatures, although, prolonged midday exposure to supra-optimal temperatures was detrimental to gas exchange. Should temperature increases be limited to a few degrees plants will not suffer major reductions in gas exchange capacity.

3.1 Introduction

Photosynthetic models show that as temperature increases, photosynthesis and growth increases to an optimum, thereafter if temperature increases supra-optimal, photosynthesis decreases (Martínez-Carrasco *et al*., 2005). Therefore, a distinction must be made between slightly higher, more optimal, temperatures leading to increased growth and productivity under conditions where water and nutrients are not limiting and supra-optimal temperatures that will result in decreased gas exchange and growth.

Few reports in the literature could be sourced on gas exchange patterns of Cape flora, especially cultivated Proteaceae. Earlier studies on gas exchange mostly focussed on selected fynbos species in their natural habitat. Mooney *et al*. (1983) recorded basic photosynthetic characteristics of four *Protea* species. All species had low photosynthetic capacities (3-14 µmol m^{-2} s^{-1}) with high leaf specific weights of ~200 g m^{-2}. In deciduous fruit crops the rate of light saturated CO_2 assimilation (A_{max}) can vary between 16 and 22 ε mol m^{-2} s^{-1}, but values as high as 40 ε mol m^{-2} s^{-1} have been recorded under optimal conditions (Larcher, 2003). Von Willert *et al.* (1989) examined leaf water relations and daily variation in photosynthetic capacity of fynbos species, including *Protea laurifolia*, in the Cedarberg region, South Africa. Photosynthetic light response curves reached saturation at 2000 µmol m^{-2} s^{-1} photosynthetic photon flux density or even at higher levels. *P. laurifolia* displayed a modest midday depression, with the highest leaf water potential measured at dawn and dusk. Similarly, Richardson & Kruger (1990) studied a range of indigenous mountain flora including two *Protea* species (*P. nitida* and *P. repens*). Photosynthetic light response curves confirmed results by Von Willert *et al*. (1989) where daily variation in net photosynthesis showed low photosynthetic capacity together with minor midday depressions. Van der Heyden & Lewis (1989) recorded seasonal variation of photosynthetic capacity and water use of five species including *P. laurifolia* together with *Erica plukenetii* and *Thamnocortus lucens*. Photosynthetic response to irrigation, temperature and irradiance was also investigated using the same species (van der Heyden & Lewis, 1990). Irrigation had no effect on *P. laurifolia*, probably because the deep taproots had access to water in lower soil horizons. Highest net CO_2 assimilation rates was measured at 20°C, with very little activity at 40°C in combination with low light intensity (100 µmol m^{-2} s^{-1}). More recently, Smart (2005) studied inflorescence induction of a commercially cultivated *Protea* hybrid, ‘Carnivalô (*P. compacta* x *P. neriifolia*) and measured total shoot photosynthesis of flowering shoots in varying developmental stages.

The youngest, most terminal flush contributed a greater percentage of photosynthates to the developing inflorescence than the older, more basal flushes. This indicated the importance of assimilate supply from the terminal flush to sustain inflorescence development. However, the effects of temperature on gas exchange of *Protea*, as well as elevated temperature have not yet been studied previously.

Experimentally, it is difficult, if not impossible, to isolate temperature from other environmental interactions. These types of experiments are becoming increasingly important as global temperature increase. The rise in temperature linked to increased carbon dioxide and other greenhouse gas emissions, is threatening the productivity and survival of South Africaôs natural fynbos, as well as commercial plantings of *Protea* cultivars and selections. This threat is imminent as temperatures are predicted to rise in the Western Cape, South Africa, by 1-3°C by 2045 (Midgley *et al*., 2007).

The main objective of this study was to evaluate the response in gas exchange of *Protea* óPink Iceô (*P. compacta* R. Br x *P. susannae* Phill.) to both the natural variation in seasonal temperature and induced elevated temperatures.

A better understanding of the effects of high temperatures on *Protea* gas exchange will enable researchers and producers to better predict the performance of *Protea* in new, warmer cultivation areas, whilst results can be extended to possible future warming scenarios, as well as the suitability of a vigorous cultivar to warmer or cooler areas.

3.2 Materials and Methods

The experimental design, construction and micro-climatic measurements for this experiment were described in Chapter 2.

Gas exchange was performed on potted *Protea* óPink Iceô plants heated within a temperature gradient from ambient to ambient+3.1°C using infra-red lamps. A field verification experiment with two temperature treatments, ambient and ambient+2.9°C, was also established (Chapter 2.2). Gas exchange measurements were taken with a LI-6400 portable photosynthesis system (Li-Cor, Lincoln, Nebraska, USA). The photosynthetic photon flux density (PPFD) was provided with an internal red/blue LED (light emitting diode) light source (LI-6400-02B Li-Cor, Lincoln, Nebraska, USA). The carbon dioxide (CO_2) concentration inside the cuvette was controlled by the LI-6400 CO_2 injection system and compressed CO_2-cylinders. The air flow rate through the cuvette was set at 200 ε mol s^{-1}.

3.2.1 Spot gas exchange measurements

Spot gas exchange measurements are an immediate measurement of the gas exchange activity of the plant and were taken at ambient temperatures for both the greenhouse and field verification experiments. One set of spot readings per tagged shoot was done on a single, mature leaf located on the terminal flush. If the terminal flush was still immature or not yet unfolded, a leaf from the subtending flush was used. A_{max} (the maximum rate of light-saturated net CO_2 assimilation) was measured under light (PPFD) and CO_2 levels of 2000 ε mol m^{-2} s^{-1} and 380 µmol mol^{-1}, respectively. Stomatal conductance (g_s) was measured concurrently. The maximum rate of light- and CO_2-saturated net CO_2 assimilation (A_{sat}) was measured at 2000 µmol m^{-2} s^{-1} PPFD and 1200 µmol CO_2 mol^{-1}. Dark respiration rate (R_d) was measured when the CO_2 was set at 380 µmol mol^{-1}, with the LED light turned off. The photosynthetic photon flux density used was 2000 µmol m^{-2} s^{-1} throughout to eliminate variation caused by changes in time of day and year as well as cloud cover, and measurements then indicate photosynthetic potential under non-limiting light conditions.

Greenhouse-based warming experiment: Spot gas exchange measurements were taken on a monthly basis in 2008 on 14 July, 19 August, 25 September, 27 October, 17 November, 15 December and in 2009 on 14 January, 10 February and 12 March. One mature leaf on each of the two tagged shoots per plant was selected for observations (n = 4). The vapour pressure deficit (VPD) differed between measurement days and ranged from below 1 kPa winter to 6 kPa in mid-summer.

Field verification warming experiment: Spot gas exchange measurements were taken on 12 tagged shoots per treatment (n = 6) on 11 September, 24 October, 18 November and 10 December 2008, as well as 13 January and 5 February 2009. A mature leaf from the terminal flush was used.

Data analysis: Data was analysed by linear regression using the PROC REG procedure (SAS Institute Inc., 2003) to test significance (Pr Ò 0.05). All measurements of net CO_2 assimilation rate expressed on a leaf area-basis were re-calculated on a leaf dry mass-basis using leaf dry mass per area (LMA) for the conversion (see Chapter 4.2). In graphs presenting seasonal trends second degree polynomial trend lines were fitted with Microsoft® Office Excel® and R^2 values are shown.

3.2.2 Photosynthetic temperature response

Photosynthetic temperature response curves were performed to establish the optimum temperature for net CO_2 assimilation rate (T_{opt}) at a given time. Temperature response curves were generated by cooling the leaf 6-8°C below ambient temperature in the cuvette. The leaf temperature was then increased in increments of 2-3°C to 6-8°C above ambient temperature, whilst increases in vapour pressure deficit (VPD) were limited by manipulating the flow rate through the desiccant scrub. Irradiance (PPF) was set at 2000 ε mol m^{-2} s^{-1} and cuvette CO_2 concentration was kept constant at 380 µmol mol^{-1}. The range of temperatures at which T_{opt} was measured varied from 15°C to 45°C, depending on the time of year and the ambient temperature on the day of measurement.

Greenhouse-based warming experiment: Data was collected in 2008 on 22 July, 27 August, 10 October, 26 November and 19 December and in 2009 on 15 January, 9 February and 10 March. One mature leaf of the terminal flush per temperature treatment was used (n = 1). If the terminal flush was still immature or not yet unfolded, a leaf from the subtending flush was used.

Field verification warming experiment: Measurements were taken on 4 November and 11 December 2008, 13 January and 5 February 2009. Two to three leaves per treatment, located on the terminal flush of tagged shoots were used (n = 2; 3).

Data analysis: Curves were plotted in Microsoft® Office Excel® 2007. A polynomial trendline was fitted and the turning point was used to calculate the T_{opt} value.

3.2.3 A/c_i response curves

A/c_i curves (the response of A_{max} to intercellular CO_2 concentration, c_i) are a measure of the biochemical activity of photosynthetic enzymes (Larcher, 2003).

Greenhouse-based warming experiment: Two curves per temperature treatment were recorded on 18 February 2009. The first curve was performed at a leaf temperature 2-3°C below ambient temperature. Leaf temperature was subsequently increased by approximately 10°C for the second curve. Irradiance (PPFD) was set at 2000 ε mol $m^{-2}s^{-1}$ for both curves. The cuvette CO_2 concentration was initially set at 1200 µmol mol^{-1}, whereafter it was sequentially lowered (1000, 750, 500, 380, 250, 170, 100, 75, 50 µmol mol^{-1}). No curves at ambient+1.8°C were performed due to temporary equipment malfunction.

Data analysis: A/c_i response curves were fitted individually using an advanced non-linear estimation (Statistica 8.0, Statsoft, Inc., Tulsa, Oklahoma, USA) and the

monomolecular function $y = a(1-e^{(b-cx)})$ given by Causston & Dale (1990). The fitted curve coefficient ‘a’ represented the rate of light- and CO_2-saturated net CO_2 assimilation (A_{sat}), ‘b/c’ represented the CO_2 compensation point, ‘ace^b’ represented the apparent carboxylation efficiency (the slope of the A/c_i response curve at x = 0), and the predicted photorespiration rate (R_{day}) was calculated using ‘a(1-e^b)’ (Causston & Dale, 1990). There was no replication per temperature treatment, with n = 1 for each measurement temperature.

3.3 Results

3.3.1 Spot gas exchange measurements

Greenhouse-based warming experiment: In Fig. 20 (A_{max}) and (R_d), five of the nine measurement dates are presented (expressed on a leaf area-basis) and data expressed per mass-basis are shown for the last three of these dates when leaf structure (LMA, see Chapter 4.3) was expected to have changed. In Fig. 2 (A_{sat}) data collected on four dates are expressed on a leaf area-basis. Data expressed on a mass-basis are presented for two dates. No A_{sat} measurements were performed in February and March due to a faulty CO_2 injection system. Data not shown does not in any way change the pattern or interpretation, but is omitted for the sake of simplification. The graphs (dates) were selected to be representative of the whole season. For g_s (Fig. 23) all measurement dates are shown since the results show great sensitivity to daily climatic conditions (Table 2, 2).

A_{max} expressed on a leaf area-basis (Fig. 20a-e) did not differ significantly across the temperature gradient. However, when expressed on a leaf mass-basis (Fig. 20f-h), A_{max} decreased significantly with increasing temperature treatment on 17 November 2008 (Fig. 20f) and 12 March 2009 (Fig. 20h). The 12th of March was the warmest measurement day (Table 2) with mean and maximum temperatures inside the greenhouse of 30°C and 43.5°C respectively.

A_{sat} expressed on a leaf area-basis (Fig. 21a-d) increased significantly with the temperature gradient on 14 July 2008 (Fig. 21a). However, when expressed on a leaf mass-basis (Fig. 21e-f), A_{sat} decreased significantly with increasing temperature treatment on 17 November 2008 (Fig. 21e) and 14 January 2009 (Fig. 21f).

R_d expressed on a leaf area-basis (Fig. 3a-e) showed no differences across the temperature treatment gradient. When calculated on a leaf mass-basis (Fig. 3f-h), R_d

decreased significantly with increasing temperature treatment on 17 November 2008 (Fig. 3f) and 14 January 2009 (Fig. 3g).

Stomatal conductance (g_s) (Fig. 23) remained more or less constant over the temperature treatment gradient on all measuring dates, except on 19 August 2008 (Fig. 23b), 27 October 2008 (Fig. 23d) and 14 January 2009 (Fig. 4g). On 19 August and 14 January there was a significant increase in g_s across the temperature gradient. On 27 October, g_s significantly decreased from the ambient temperature to the elevated temperature treatments. On the August and October dates, temperatures were remarkably high for that time of year, with maximum temperatures reaching 34.5°C and 38.4°C, respectively (Table 2).

Seasonal trends in gas exchange recorded from July 2008 to March 2009 as presented in Fig. 24, show A_{max} values during August and September 2008 at the start of the growing season (Fig. 24a). Thereafter, A_{max} decreased during summer, but increased subsequently in early autumn under more favourable environmental conditions (Table 2, 2). A_{sat} increased from mid-winter to mid-summer, with lower values measured on isolated hot days in spring and summer (Fig. 24b). R_d increased from mid-winter to mid-summer, whereafter it stabilised at all temperature treatments, except at ambient, which decreased in early autumn (Fig. 24c). For g_s, the lowest values were recorded in mid-winter, late spring and summer, but g_s increased rapidly to a maximum in late winter (August 2008) and again in early autumn (Fig. 24d).

Field verification warming experiment: There was no significance difference in gas exchange parameters measured between ambient and ambient+2.9°C treatments on any of the measurement dates except on 24 October 2008, when A_{sat}, g_s and R_d were higher under the ambient than under the elevated temperature treatment (Fig. 6). On 13 January 2009, R_d was exceptionally low (Fig. 6c), but this might be due to a cool measurement day in summer with mean and maximum temperatures 21°C and 23.4°C, respectively (Table 3).

3.3.2 Photosynthetic temperature curves

Greenhouse-based warming experiment: The optimum temperature for net CO_2 assimilation rate (T_{opt}) increased from mid-winter to early autumn for the ambient, ambient+0.8°C and ambient+1.8°C treatments (Fig. 26a). T_{opt} similarly increased from mid-winter to early summer for the treatments ambient+2.5°C and ambient+3.1°C, but thereafter levelled off at approximately 29°C and 30°C, respectively. Acclimation to the temperature

gradient revealed no observable pattern as values differed noticeably between measurement days.

Field verification warming experiment: From late spring to mid-summer T_{opt} was found to be lower under the ambient treatment than under ambient+2.9°C (Fig. 26b). Apart from a decline under the ambient treatment on 11 December 2008, T_{opt} for both treatments remained fairly constant over the entire period.

3.3.3 A/c_i curves

Fig. 27a and 8b represent the A/c_i curves measured at a lower and a higher (approximately 10°C difference) leaf temperature, respectively. The curve measured at the lower leaf temperature of ambient+3.1°C was not used as it was considered unreliable.

The curves measured at lower leaf temperature approached CO_2 saturation at 800 µmol mol^{-1} c_i (Fig. 27a), whereas the curves measured at higher leaf temperature showed A_{max} still increasing at 800 µmol mol^{-1} c_i (Fig. 27b), especially in the ambient and ambient+0.8°C treatments. As a result, A_{sat} was considerably higher for the high leaf temperature curves, in particular for the ambient and ambient+0.8°C temperature treatments (Fig. 28a). The difference was less pronounced at ambient+2.5°C and ambient+3.1°C treatments, where A_{sat} decreased at the higher leaf temperature.

Similarly, there was a decrease in R_{day} from the ambient temperature treatment to ambient+3.1°C (Fig. 28b). Differences between lower and higher measurement temperature could not be tested statistically, as curves were measured without replication.

The initial linear gradient of the A/c_i curves at the lower leaf temperatures (Fig. 27a) were steeper compared to those measured at a higher leaf temperature (Fig. 27b). Therefore, the apparent carboxylation efficiency derived from the slope of the A/c_i curves was higher in the low leaf temperature than in the high leaf temperature curves for all temperature treatments (Fig. 28c).

3.4 Discussion

Plants have the ability to acclimate to environmental changes, including changes in light quality or intensity, water availability and temperature. Acclimation is a morphological and/or physical adjustment made by plants to compensate for the reduction in growth and/or photosynthetic capacity following exposure to a changed environment (Lambers *et al.*, 1998).

Plants respond in various ways and time-frames depending on the type and intensity of pressure brought about by the new environment.

In this study heating lamps were switched on in May 2008 (late autumn) when an average monthly temperature of 16.8°C prevailed. Even at ambient+3.1°C no immediate photosynthetic response was elicited (Fig. 20a-c). During July 2008 (mid-winter) A_{sat} increased at the higher temperature treatments (Fig. 21a). This enhanced photosynthetic activity may most likely have supported the earlier spring budbreak and growth as observed in both the greenhouse-based and field verification experiments (Chapter 4). During August 2008 increased stomatal conductance at the higher temperatures further facilitated and promoted growth at higher temperatures during the cooler part of the season (Fig. 23b).

However, after prolonged exposure to higher temperatures, physiological acclimation is possible. Acclimation could also be referred to as a homeostatic response. Ideally, metabolic activity should adjust so perfectly that the same level of photosynthetic or respiratory activity is maintained over a fairly broad range of temperatures (Larcher, 2003). In this manner *Protea* ôPink Iceô appeared to acclimate well on a leaf area-basis over the temperature gradient (Fig. 20a-e, 2a-d, 3a-e). Therefore, the absolute amount of carbon captured remained more or less similar across the temperature gradient from ambient to ambient+3.1°C. However, at higher treatment temperatures, sink demand may increase, mainly due to higher root activity and more vigorous flush growth, possibly reducing the availability of assimilates and storage products that may be critical for inflorescence induction and initiation and to sustain inflorescence growth (Chapter 4).

Generally, in the short term, respiration (R_d) increases as a function of temperature, depending on the temperature coefficient (Lambers *et al*., 1998). However, over time and expressed on a leaf area-basis, respiration acclimated apparently well across the temperature gradient, with no significant differences between treatments (Fig. 3a-e).

However, when expressed on a leaf mass-basis, a decrease in assimilation rates (Fig. 20f-h), and R_d (Fig. 3f-h), indicated reduced relative carbon gain. Battaglia *et al*. (1996) examined photosynthetic temperature responses of *Eucalyptus* and found that net photosynthesis declined above temperatures of 25°C together with reduced stomatal conductance. This finding was supported by Wright *et al*. (2005), who compared more than 2500 vascular species from 175 sites globally and reported net photosynthesis measured on a leaf mass-basis to decrease with increasing mean annual temperature. Similarly, a reduction in carbon gain at higher temperatures was also observed in black spruce seedlings grown in a

high temperature greenhouse compared to a lower temperature greenhouse (Way & Sage, 2008). R_d expressed on a leaf mass basis may be altered due to cellular changes in mitochondrial density and the total amount of protein invested (Atkin *et al*., 2005). In addition, Larcher (2003) states that during acclimation to prevailing temperatures respiratory activity is lowered (during adaptation to heat).

During the season, R_d reached maximum values during periods of increased sink activity (spring to summer), shoot elongation and inflorescence growth and development. This period also coincided with warmer temperatures.

The lower A_{max} (Fig. 20f-h) and R_d (Fig. 3f-h) when expressed on a leaf mass-basis at the higher temperatures may be attributed to greater allocation of biomass to structural components, such as cell walls and sclerenchyma tissue (Chapter 4). The change in biomass allocation could be caused by physiological water stress.

Physiological drought is a condition where soil water is ample, but due to several possible factors, including a high rate of evapotranspiration (ET) or a high salt concentration in the soil, the rate of water uptake by roots is too low to keep the plant sufficiently hydrated. In bananas it was found that inactive root hairs decreased the absorption capacity of the root system during winter, which caused internal plant stress, despite a well-water soil (Robinson & Bower, 1988). High evaporative demands cause a plant water deficit (Rachmilevitch *et al*., 2006) and plants, particularly leaves, wilt during the day and recover overnight. Changes in the leaf relative mass or density may occur under these conditions, to maintain turgor potential during the day, but this lowers net CO_2 assimilation on a leaf mass-basis.

Berry & Björkman (1980) described the term ôacclimation potentialô as the genetic predisposition of plants to acclimate. Evergreen desert shrubs have a greater acclimation potential as these plants are subjected to large seasonal temperature variation (Berry & Björkman, 1980). Similarly, *Protea* adapted to a Mediterranean climate with wide-ranging temperature fluctuations between seasons, are considered to be more plastic (Lambers *et al*., 1998), implying a higher acclimation potential. The shift of T_{opt} to higher temperatures at monthly intervals from winter to summer to coincide with seasonal temperature changes is an ordinary occurrence (Lambers *et al*., 1998). However, the degree of the T_{opt} shift observed for *Protea* in this study when subjected to a range of temperatures, illustrates the high acclimation potential of these plants (Fig. 26). Interestingly, although T_{opt} increased during the season, the net CO_2 assimilation rate declined (Fig. 24a). The decline in CO_2 assimilation rate can probably be ascribed to high evaporative losses and subsequent stomatal closure, not

only as the usual midday depression, but throughout a larger part of the day when supra-optimal temperatures prevailed (personal observation). High temperature also affects the oxygenating reaction of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) and photorespiration increases (Lambers *et al*., 1998). Therefore, changes in T_{opt} cannot be interpreted only as an indication of acclimation, but more importantly, the increase in T_{opt} during warm summer months raises the threshold temperature at which heat damage to photosynthetic components is likely to occur (Berry & Björkman, 1980). This further emphasizes the ability of ôPink Iceô to acclimate to higher temperature regimes.

Acclimation to higher temperatures is mostly a result of increased heat stability of the photosynthetic machinery. This may involve altered membrane fluidity due to changes in membrane lipid saturation, especially the thylakoid membranes associated with photosystem II (PS II) and electron transport (Berry & Björkman, 1980). Iso-enzymes that are better aligned to a higher temperature range are synthesized and substrate availability and concentration, for example sucrose, can also change (Larcher, 2003). In this study, acclimation may already have been attained at ambient+2.5°C and ambient+3.1°C early in the summer, explaining why a further increase in T_{opt} could not be sustained from mid-January to early March (Fig. 26a). In the field verification experiment the measured T_{opt} (Fig. 26b) closely followed monthly temperatures, although the optimum temperature of ambient+2.9°C shifted to a higher temperature compared to the ambient treatment as would be expected.

From the A/c_i curves it can be seen that the first (short-term) response of ôPink Iceô to higher temperatures in winter was likely an increased A_{sat} (Fig. 28a). Derived A_{sat} values almost doubled from more or less 30 µmol m^{-2} s^{-1} to just below 60 µmol m^{-2} s^{-1} (Fig. 27). This increase was evident in the A_{sat} spot gas exchange measurements of 14 July (Fig. 21a). However, at the higher growth temperatures, over time, A_{sat} measured at high leaf temperature, decreased to values similar to those obtained at ambient growth temperatures at the lower leaf temperature. The short-term boost in A_{sat} at high temperatures could indicate an increase in proteins associated with electron transport at elevated temperatures at the start of the experiment. However, the photosynthetic system was probably not able to sustain the high level of activity stimulated by high temperatures and therefore responded by down-regulation. The proposed down-regulation of photosynthesis is supported by the observed reduction in the apparent carboxylation efficiency. This occurred firstly from a lower to higher measurement temperature in the ambient and ambient+0.8°C treatments (short-term) and secondly, by progressively decreasing in a quadratic trend progressively over the temperature

gradient (long-term) (Fig. 28c). At temperatures above the optimal range for a specific species, the oxygenase component of Rubisco is favoured (Larcher, 2003) which would lead to lower carboxylation efficiency. Alternatively, less Rubisco was simply synthesized or activated under supra-optimal temperature conditions. At higher temperatures CO_2 is also less soluble in water, compared to O_2 (Ericsson *et al*., 1996).

Gas exchange patterns varied considerably during the course of the season (Fig. 24). Van der Heyden & Lewis (1989) conducted a study of seasonal variation in photosynthetic capacity of five fynbos species, including *Protea laurifolia*, in the Bainôs Kloof Forestry Reserve. Under natural field conditions A_{max} and g_s values were closely correlated, with the highest values obtained during the winter from June to August. Similarly, in the greenhouse and field verification experiments, A_{max} and g_s were also closely linked, but in the greenhouse experiment the optimum period for photosynthesis shifted by two months, occurring from August to October.

When measuring A_{max} at different leaf temperatures in November and February, Van der Heyden & Lewis (1990) found maximum photosynthesis values at 20°C. Photosynthesis then decreased from 20°C to 30°C and declined even further at 40°C. However, for ôPink Iceô in this study, the maximum rate of net CO_2 assimilation was measured at leaf temperatures between 26°C and 28°C. But similar to our results, Van der Heyden & Lewis (1990) obtained higher specific photosynthetic values in November compared to February.

Protea ôPink Iceô appeared to acclimate well to seasonal temperature changes with respect to gas exchange. This may largely be attributed to the adaptability of *Protea* to temperature extremes in their native Mediterranean habitat. Should global temperature increase be contained to a few degrees, commercial *Protea* plants will not suffer major reductions in gas exchange capacity.

3.5 References

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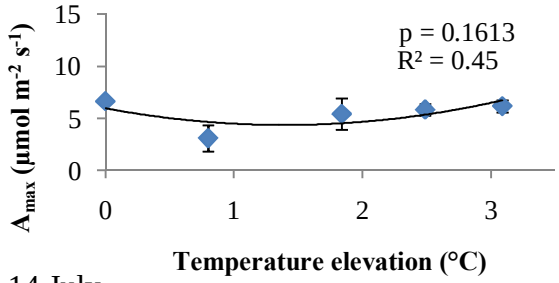
Table 2. Mean temperatures on the day of spot gas exchange measurements in a greenhouse-based warming experiment. The greenhouse temperatures were recorded with the TinyTag mini-datalogger at the ambient treatment, whereas Stellenbosch temperatures were obtained from the Agricultural Research Council (ARC) automatic weather station at Nietvoorbij.

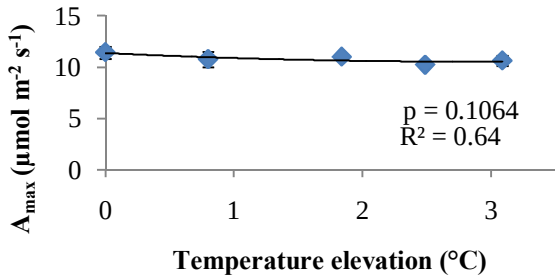
Date (2008-2009)	Mean greenhouse temperature (°C)	Mean Stellenbosch temperature (°C)
14 July	13.9	12.2
19 August	19.7	17.7
25 September	14.8	11.2
27 October	23.4	17.8
17 November	21.2	16.4
15 December	26.5	22.3
14 January	25.8	21.0
10 February	27.1	22.4
12 March	28.5	24.3

Table 3. Mean and maximum temperatures recorded during spot gas exchange measurements in a field verification experiment. Stellenbosch temperatures were obtained from the Agricultural Research Council (ARC) automatic weather station at Nietvoorbij.

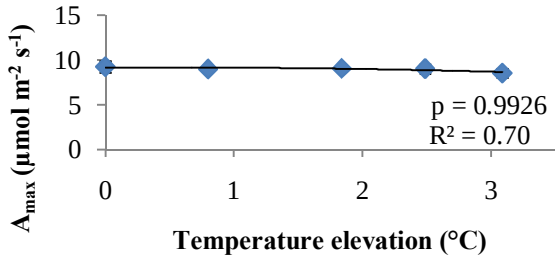
Date (2008-2009)	Mean Stellenbosch temperature (°C)	Maximum Stellenbosch temperature (°C)
11 September	9.3	14.7
24 October	15.7	20.1
18 November	19.1	24.8
10 December	22.4	28.9
13 January	21.0	23.4
5 February	26.1	33.4



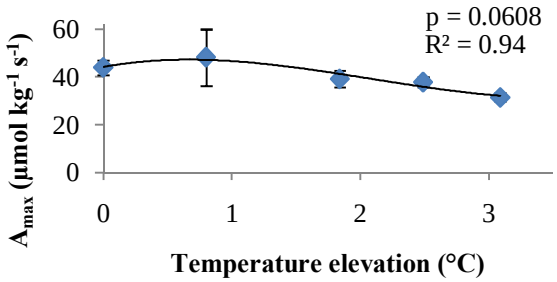
a) 14 July



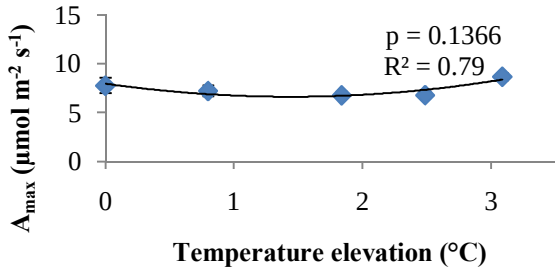
b) 25 September



c) 17 November



f) 17 November



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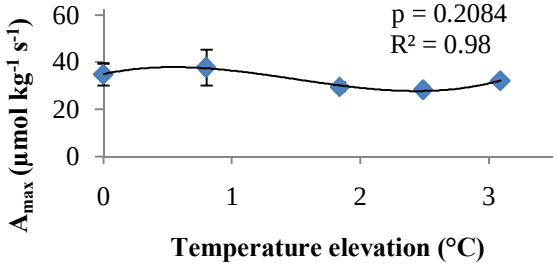
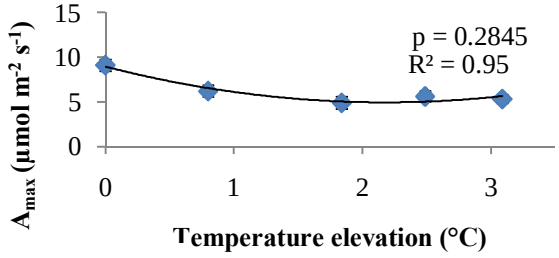
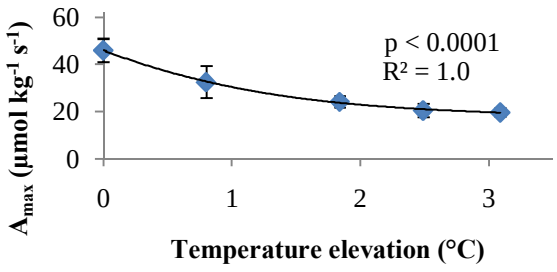


g) 14 January

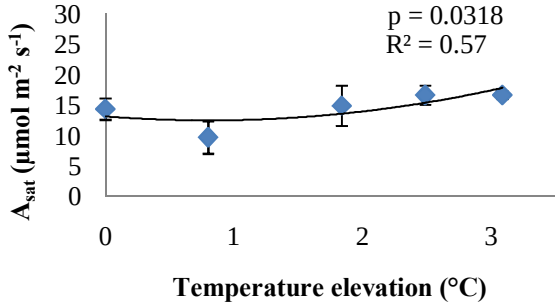


e) 12 March

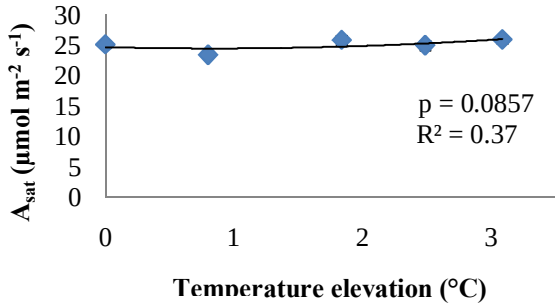


h) 12 March

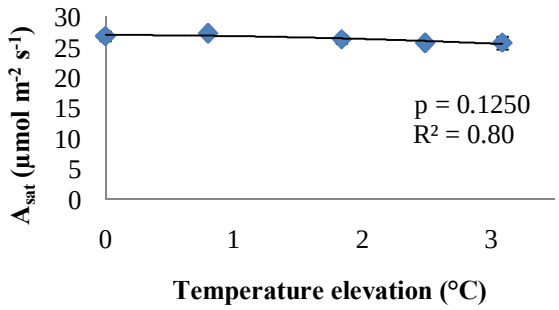
Fig. 20. Maximum light-saturated rate of net CO_2 assimilation (A_{max}) ± SE (n = 4) expressed on a leaf area basis (a - e) compared to a leaf mass basis (f - h) of *Protea* ôPink Iceô as measured on five dates (July 2008 to March 2009) for five temperature elevation treatments in a greenhouse-based warming experiment.



a) 14 July



b) 25 September



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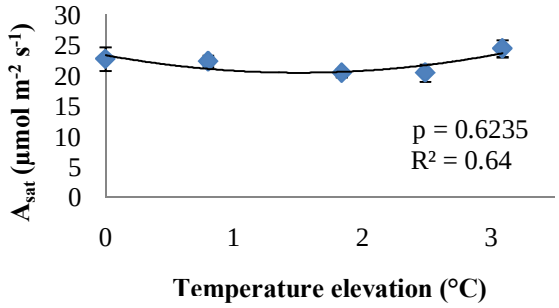

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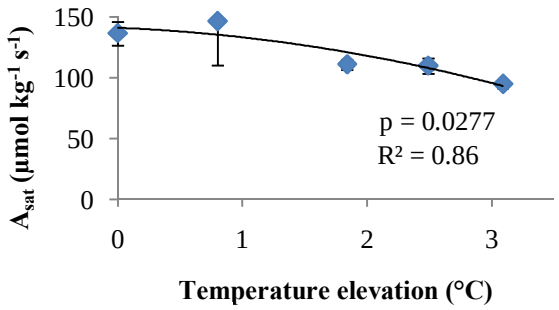
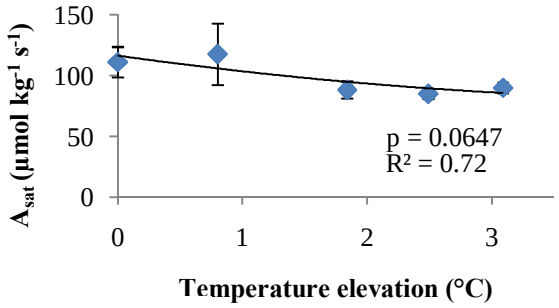
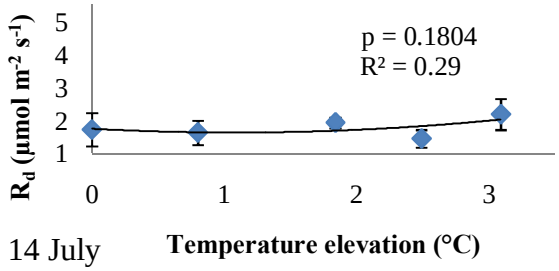


e) 17 November

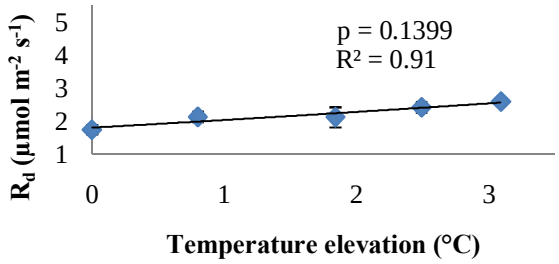


f) 14 January

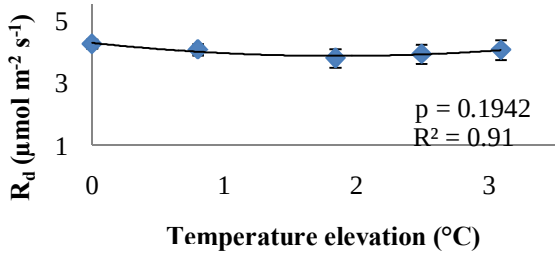
Fig. 21. Maximum light-and CO_2-saturated rate of net CO_2 assimilation (A_{sat}) ± SE (n = 4) expressed on a leaf area basis (a - d) compared to a mass basis (e - f) of *Protea* õPink Iceô as measured on four dates (July 2008 to January 2009) for five temperature elevation treatments in a greenhouse-based warming experiment.



a) 14 July



b) 25 September



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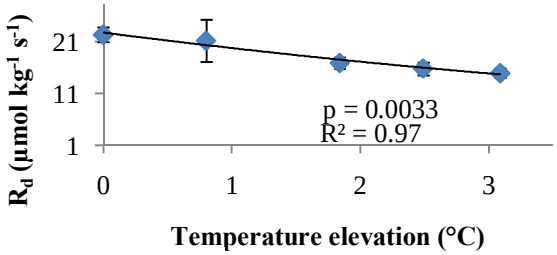
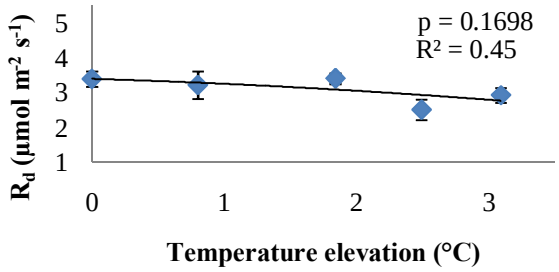


f) 17 November



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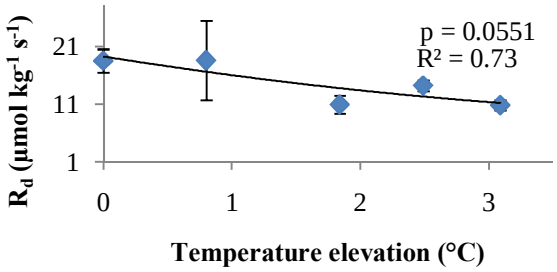

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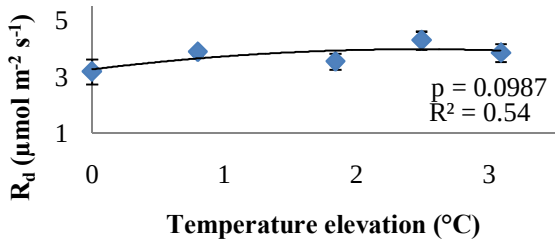
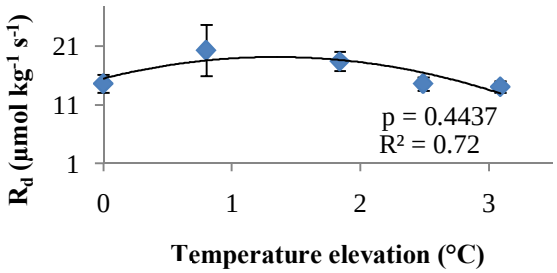
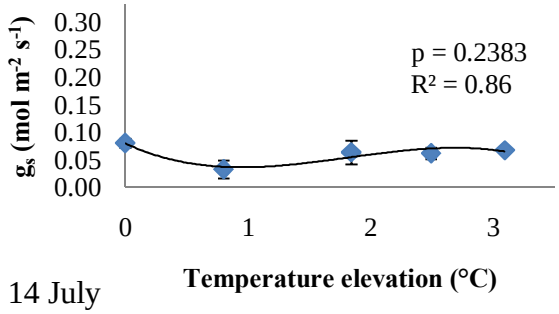


e) 12 March

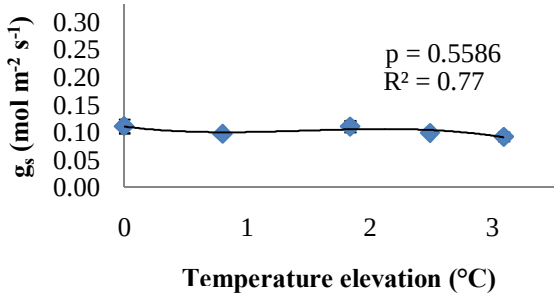


h) 12 March

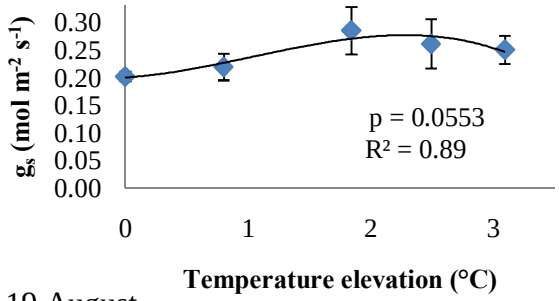
Fig. 22. Rate of dark respiration (R_d) ± SE (n = 4) expressed on a leaf area basis (a - e) compared to a mass basis (f - h) of *Protea* ôPink Iceô as measured on five dates (July 2008 to March 2009) for five temperature elevation treatments in a greenhouse-based warming experiment.



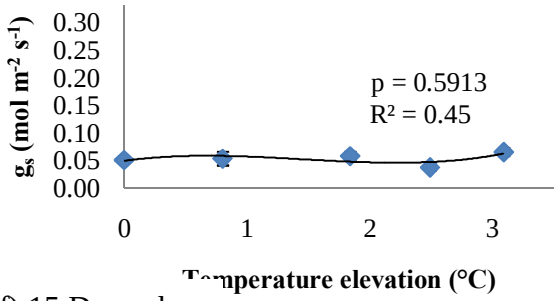
a) 14 July



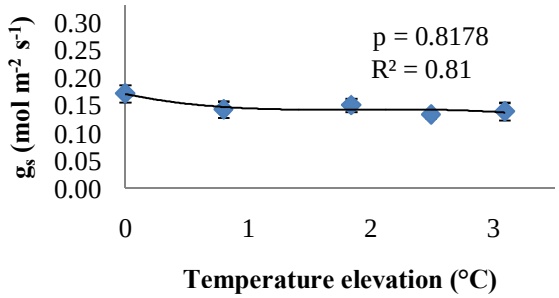
e) 17 November



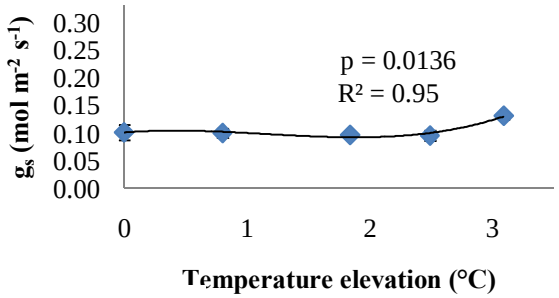
b) 19 August



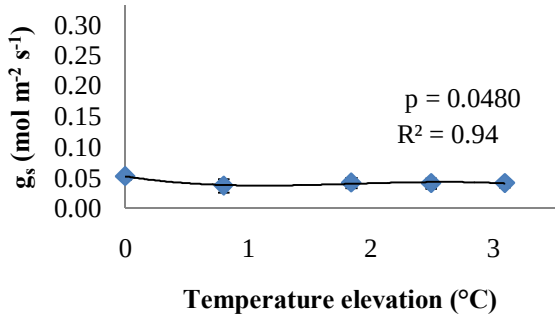
f) 15 December



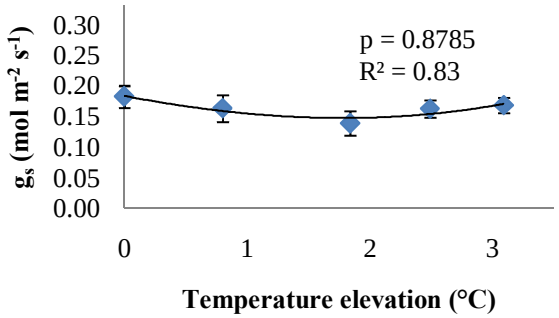
c) 25 September



g) 14 January



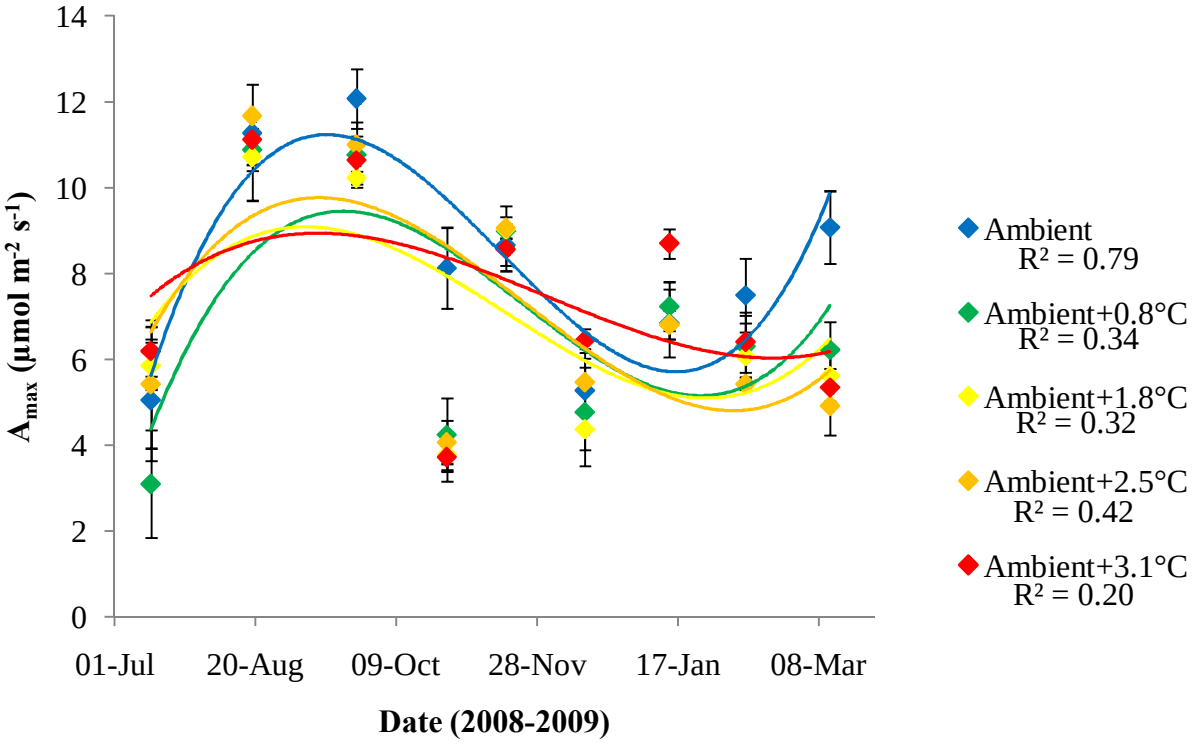
d) 27 October



i) 12 March

Fig. 23. Stomatal conductance (g_s) ± SE (n = 4) for *Protea* 'Pink Ice' expressed on a leaf area basis for five temperature elevation treatments recorded from July 2008 to March 2009 in the greenhouse-based warming experiment.

a)



b)

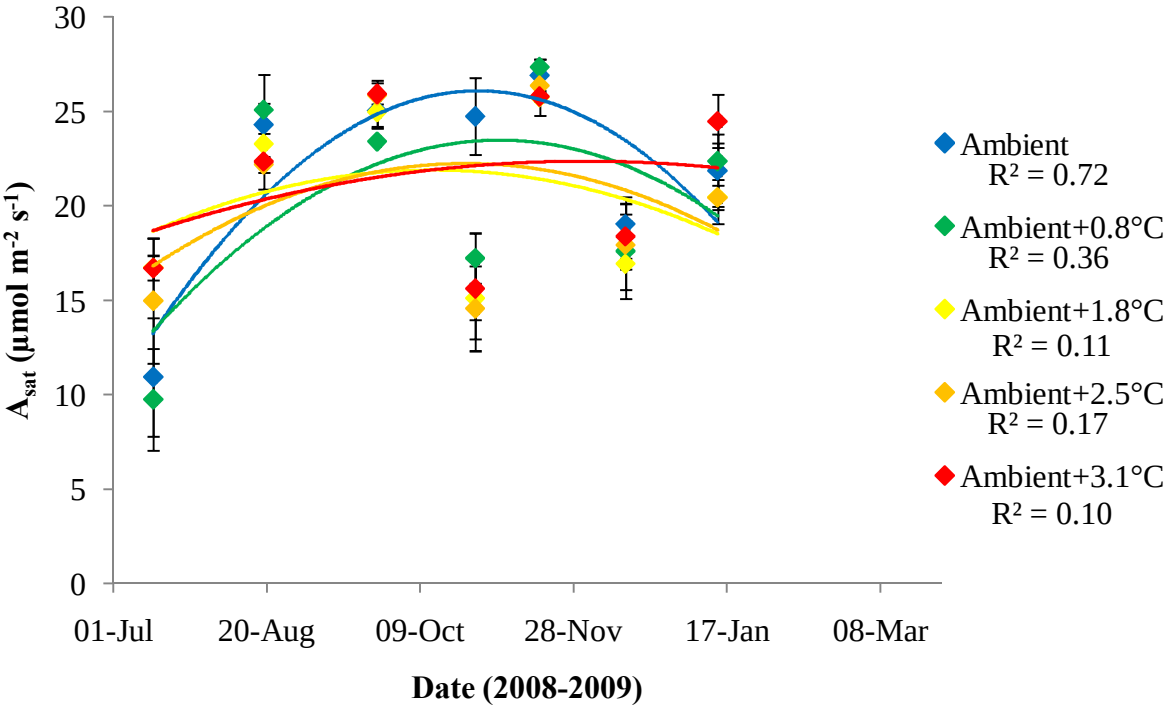
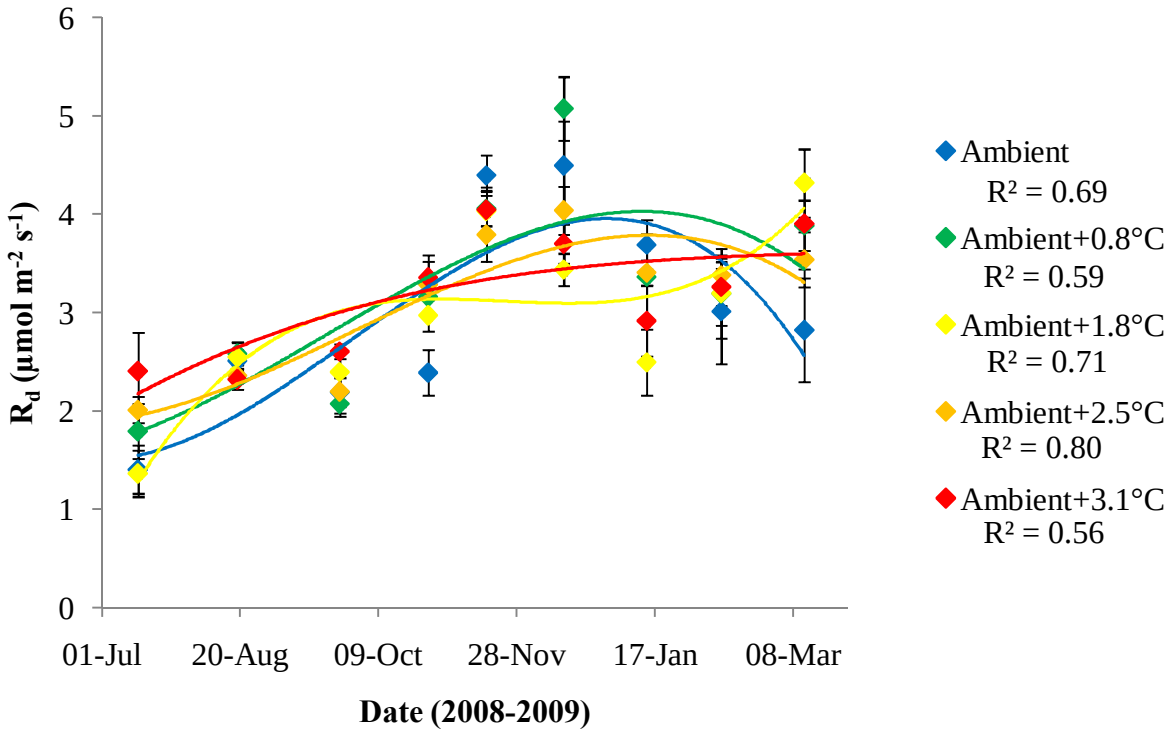


Fig. 24. Seasonal trends of (a) A_{max} and (b) A_{sat} ± SE (n = 4) from July 2008 to March 2009 for *Protea* õPink Iceô in a greenhouse-based warming experiment at five temperature treatments (ambient to ambient+3.1°C).

c)



d)

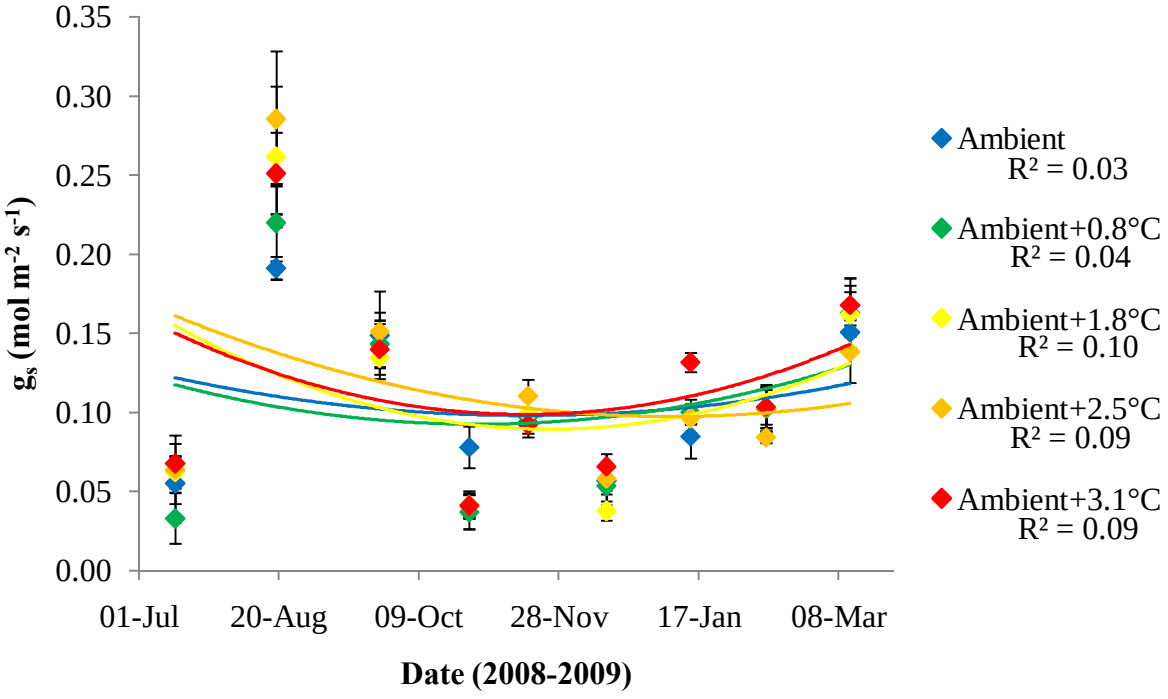


Fig. 5. Seasonal trends of (c) R_d and (d) g_s ± SE (n = 4) from July 2008 to March 2009 for *Protea* ôPink Iceô in a greenhouse-based warming experiment at five temperature treatments (ambient to ambient+3.1°C).

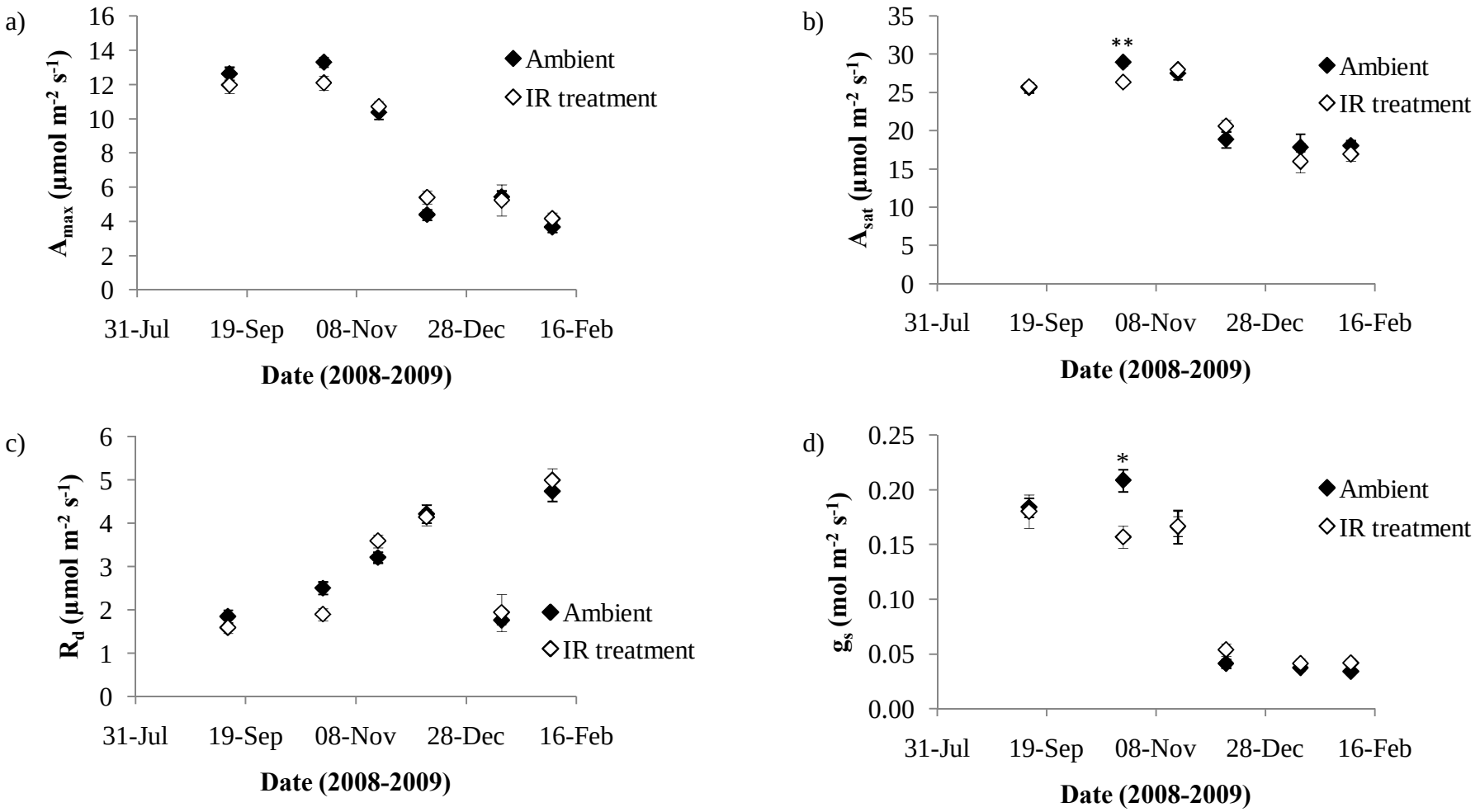
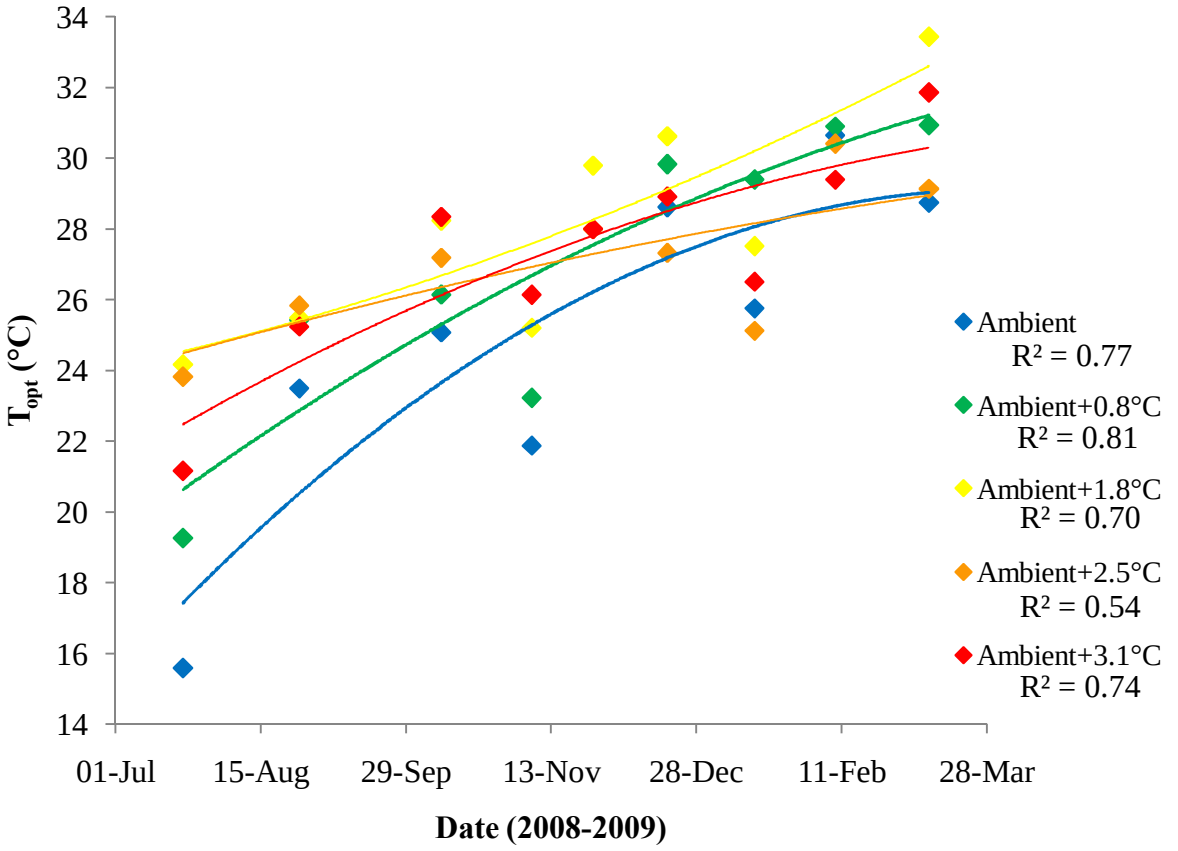


Fig. 25. Spot gas exchange measurements of a) A_{max}, b) A_{sat}, c) R_d and d) g_s respectively of *Protea* 'Pink Ice' ± SE (n = 6), expressed on a leaf area basis as measured from September 2008 to February 2009 for two heating treatments, namely ambient and ambient+2.9°C in a field-verification warming experiment.

* Significantly different at P<0.05; ** Significantly different at P<0.01; *** Significantly different at P<0.001.

a)



b)

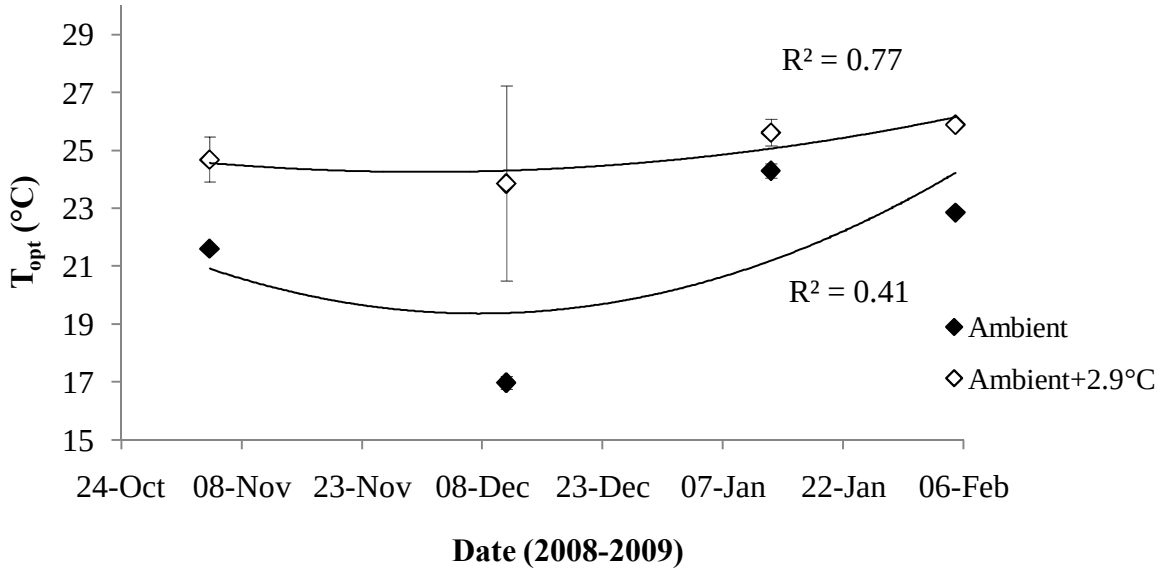


Fig. 26. The optimum temperature for the maximum rate of light-saturated net CO_2 assimilation (T_{opt}) of *Protea* 'Pink Ice' for a) a greenhouse-based warming experiment from July 2008 to March 2009 at five temperature treatments (ambient to ambient+3.1°C) (n = 1) and for b) T_{opt} ± SE (n = 2) a field verification warming experiment with two heating temperature regimes (ambient and ambient+2.9°C) from October 2008 to February 2009.

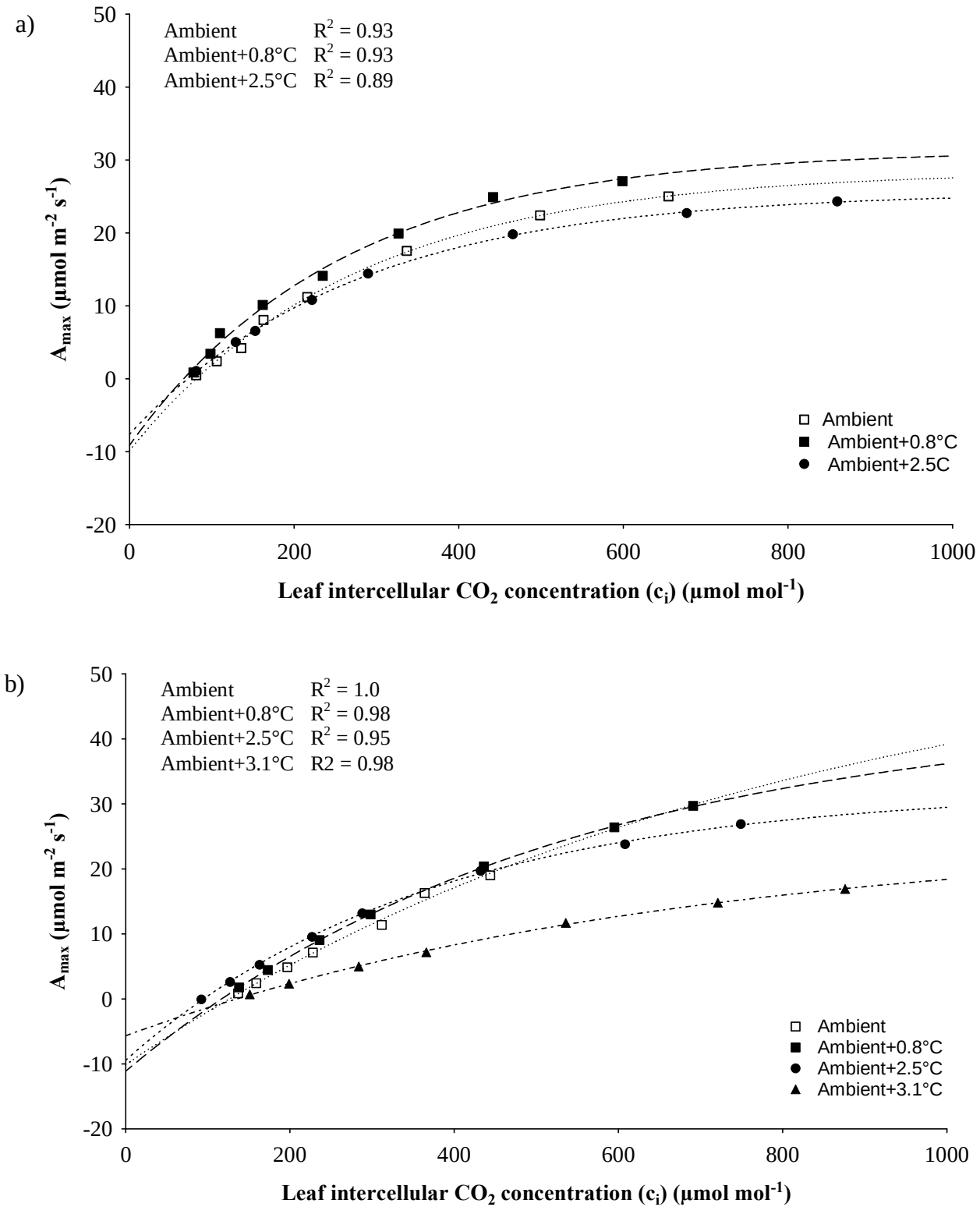


Fig. 27. A/c_i curves for *Protea* 'Pink Ice' at a) a lower leaf temperature (22 – 30°C) and b) a higher leaf temperature (28 – 40°C) for plants grown under four temperature treatments (ambient to ambient+3.1°C) in a greenhouse-based warming experiment. The monomolecular function $y=a*(1-e^{(b-(c*x))})$ was fitted and R^2-values are shown.

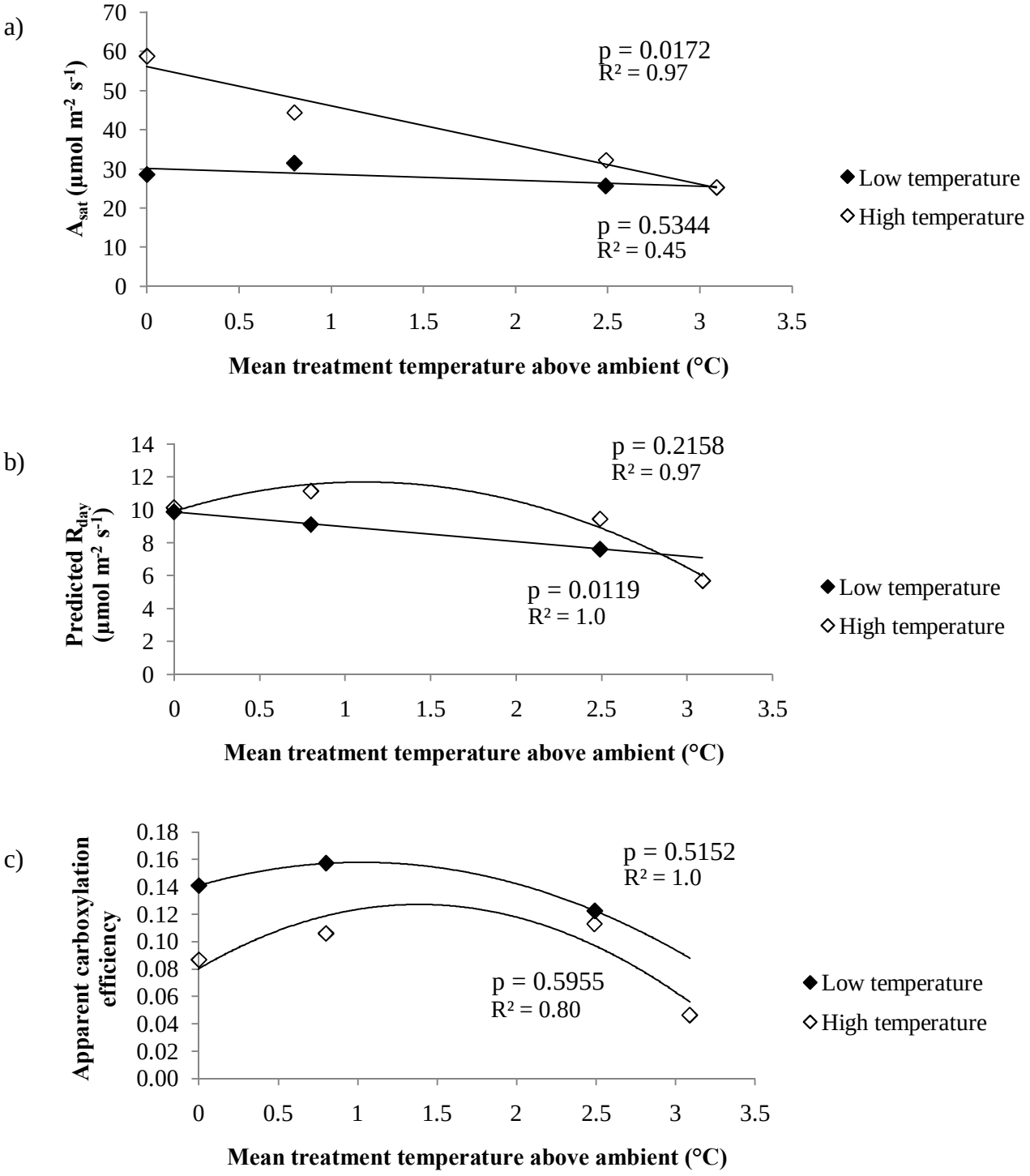


Fig. 28. Photosynthetic parameters calculated from A/c_i curve data (Fig. 8) of *Protea* ôPink Iceô showing a) the maximum rate of light- and CO_2-saturated net CO_2 assimilation (A_{sat}), b) the predicted rate of photorespiration (R_{day}) and c) the apparent carboxylation efficiency as measured at a lower or higher temperature, respectively in a green-house based warming experiment with five temperature regimes.

4. Changes in vegetative and reproductive growth and development of *Protea* cv. Pink Ice grown under elevated temperatures

Abstract

Exact requirements for floral induction in *Protea*, as well as the role of temperature in synchronizing and regulating vegetative and reproductive growth phases, are largely unclear. *Protea* cv. Pink Ice plants were warmed using infra-red lamps in a five-treatment temperature gradient from ambient to ambient+3.1°C in a greenhouse in Stellenbosch, South Africa, from May 2008 to March 2009. A field verification experiment in a commercial orchard outside Stellenbosch was established with two temperature treatments, ambient and ambient+2.9°C, from June 2008 to March 2009. Earlier budbreak was recorded in plants grown under elevated temperatures in both the greenhouse and field verification experiments. Higher temperatures favoured vegetative growth, as more non-flowering shoots with additional summer flushes was observed at elevated temperature treatments. Inflorescences were mostly carried on the spring flush for plants grown at ambient temperature, but this bearing habit shifted to the summer flushes for plants at elevated temperature treatments. In addition, fewer inflorescences initiated at elevated temperatures. In the greenhouse, inflorescences developing under higher temperatures were generally smaller or developing at a slower rate, whereas in the field verification experiment, harvest was approximately two weeks earlier at ambient+2.9°C compared to ambient temperatures. Root growth increased in a quadratic trend along the temperature gradient, whereas the leaf nitrogen concentration of the terminal flush decreased quadratically along the gradient. The leaf mass per area increased linearly with increasing temperature, indicating increased leaf density, whilst the leaf area ratio decreased under elevated temperature treatments. Plants grown at higher temperatures had a less dense above-ground structure and exhibited longer, spindly shoots. Leaf sugar and starch concentrations were higher in reproductive shoots when compared to vegetative shoots. Starch concentration followed a decreasing trend under elevated temperatures.

4.1 Introduction

Growth and development of plants is dependent on carbon assimilation and distribution to different organs. This, together with environmental stimuli, such as light (daylength and irradiance) and temperature determines to a large extent if and when plants flower.

Selected taxa in the Proteaceae, such as *Leucospermum* (Malan & Jacobs, 1990), as well as *Serruria florida* (Malan & Brits, 1990) have been found to initiate inflorescences in response to a short daylength signal. In *Banksia*, Rieger & Sedgley (1996) determined that daylength controlled floral initiation in *B. coccinea*, whereas floral initiation of *B. hookeriana* is linked to temperature. The stimulus required for induction and initiation of *Protea* species and cultivars is largely unclear. In South Africa, most protea selections and hybrids flower in spring, with the cool, winter dormant period an apparent requirement for flowering. Therefore temperature has been implicated in inflorescence induction (Gerber *et al*., 2002). In *Protea* cv. Carnival (*P. compacta* x *P. neriifolia*) floral initiation is believed to coincide with the start of spring flush elongation, which follows immediately after budbreak (Gerber *et al*., 2002; Hoffman *et al*., 2009), with floral induction completed 6 to 7 weeks prior to budbreak in spring (Gerber *et al*., 2002). However, some *Protea* cultivars such as ‘Sylvia’ (*P. eximia* x *P. susannae*) are capable of initiating inflorescences throughout the year, even though the spring period is favoured in terms of synchronization and volume (Gerber *et al*., 2001a).

An increasing and grave concern for agriculture is that average temperatures are predicted to increase 1-2°C in the next 30-40 years in the Western Cape, South Africa due to global warming, with further increases thereafter (Midgley *et al*., 2007). Global warming is strongly linked to increased emissions of greenhouse gases which include carbon dioxide (CO_2), nitrous oxide (N_2O), methane (CH_4) and fluorocarbons, as a result of anthropogenic activities. Climate change has important economic and production implications for the agricultural sector. Should temperatures increase supra-optimally a decrease in crop productivity is expected (IPCC, 2007). Changes in volume and time of harvest will force producers to carefully reconsider and align crops and cultivars with the micro-climate of their specific area.

Protea production is expanding to new, warmer areas as well as climatic areas which differ from that of the Mediterranean Western Cape from which *Protea* originate. Existing fruit producers use proteas to diversify farming activities, as the perception exists that proteas require less fertilizer, utilize less water and are adapted to warmer areas. Whether this is an

accurate assumption is still an unanswered question. For example, we have no scientific data on the temperature sensitivity of growth and flowering in *Protea*. Generally in plants, temperatures positively promoting growth will speed up anthesis (Kinet, 1993). At the other extreme, heat stress can be detrimental to reproductive development, with no flowers developing or flowers developing without fruit or seed formation (Hall, 2001). Supra-optimal temperatures can cause floral reversion within a limited time during the early stages of floral differentiation (Hoffman, 2006).

The objective of this study was to monitor the occurrence and progression of the various phenological stages of budbreak, flush elongation and inflorescence development of *Protea* 'Pink Ice' when grown under elevated temperatures using infra-red lamps. This study may provide a better understanding of the response of *Protea* 'Pink Ice' to elevated temperatures caused by climate change. It will also enable producers from production areas with different climates to better estimate the flowering time, align marketing strategies and choose production areas better suited to *Protea* 'Pink Ice'.

4.2 Materials and Methods

In a greenhouse-based experiment two-year-old *Protea* 'Pink Ice' plants were warmed using infra-red lamps in a five-treatment temperature gradient from ambient to ambient+3.1°C in Stellenbosch, South Africa (Chapter 2.1, p.29). A field verification experiment with two treatments, ambient and ambient+2.9°C, was also established in an eight-year-old 'Pink Ice' orchard on a commercial *Protea* farm outside Stellenbosch (Chapter 2.2, p.33). Refer to Chapter 2 for a detailed description of the experimental construction, design and micro-climatic measurements.

4.2.1 Baseline vegetative measurements

Greenhouse-based warming experiment: Non-destructive baseline vegetative measurements were recorded on 9 May 2008 on tagged shoots (n = 4) to establish the vegetative status and quality of plants and of individual shoots prior to any heating treatment. Parameters recorded included shoot length, shoot diameter measured at the upper intercalation between the terminal and subterminal flush, number of flushes from the bearer and numbers of leaves per flush. In addition, four representative non-experimental plants were harvested to obtain above-ground vegetative biomass baseline data prior to the start of the experiment.

Field verification warming experiment: Non-destructive baseline vegetative measurements as described above were recorded in the field on 16 July 2008 on 2 marked shoots per plant. Six plants were used per treatment (n = 6).

4.2.2 Vegetative and reproductive phenological observations

Greenhouse-based warming experiment: The timing of budbreak during spring 2008 was recorded from 11 August 2008 at two- to three-day intervals for a four week period. Thereafter, the progression of shoot growth (length and width) by means of flush extensions, the visible appearance of the inflorescence and the progression (length and width) of inflorescence development were measured. Lateral buds and shoots that could interfere with or delay inflorescence development were removed by hand on sprouting, as is done in commercial practice.

Field verification warming experiment: The timing of spring budbreak was recorded from 7 August to 11 September 2008 at four- to five-day intervals.

4.2.3 Harvest

Greenhouse-based warming experiment: Above-ground plant parts of the four pots per treatment were harvested on 22 March 2009 at soil level and separated into the individual shoots and the residual bearer stem. Shoots were further separated into flushes, whilst flushes were separated into the stem, leaves and inflorescence (if present on the terminal flush). Vegetative measurements recorded on every shoot included final shoot length, stem diameter measured at the upper intercalation between the terminal and subterminal flush, number of flushes and flush length, number of leaves per flush, total leaf area per flush (measured using a Portable Area Meter, Li-3000A, Li-Cor, Lincoln, Nebraska, USA), and fresh mass of individual flush stems and leaves. Each individual inflorescence was analysed by measuring the final length and width, whilst the fresh and dry mass of the inflorescence, separated into bracts, florets and receptacle, was also determined.

Harvested leaves, stems and inflorescence components were subsequently dried at 60°C (Forced circulation incubator, FSIE 16, Labcon (Pty) Ltd, Roodepoort, South Africa) until the dry mass remained stable.

The root component could not be accurately separated from the bark potting medium. Therefore, the approximate number of roots in a subsample on each of the four sides of the pot was estimated (Fig. 29a). The pot was divided into four quadrants according to the

placement of the drippers. The roots were found to be denser in the periphery of the two drippers, therefore these quadrants are referred to as ‘high density’ zones, whilst roots were less dense on the opposite two sides of the pot, thus referred to as ‘low density’ zones. Roots were counted vertically alongside a ruler as a guide from the top, middle and bottom 3 cm on the four sides of each pot respectively (Fig. 29b). This is based on a similar technique by Bennie *et al*. (1987).

Field verification warming experiment: All tagged shoots (n = 6) were harvested at the soft-tip commercial harvest stage. Harvest data was collected as described for the greenhouse experiment above. Leaves from the terminal flush of all tagged shoots were dried, milled and stored at -20°C for sugar and starch analysis.

4.2.4 Mass allocation, leaf structure and nitrogen

From harvest data (4.2.3), total fresh and dry mass allocations were calculated for the greenhouse-based experiment using the shoot growth increment produced after the heating treatments commenced. Fresh and dry mass allocations were also calculated for the terminal flushes subtending an inflorescence. Fresh mass allocation was included in the results to illustrate comparative treatment trends where dry mass data was not available (ambient+3.1°C) due to loss of material in a drying oven fire.

The leaf area ratio (LAR, adapted for above-ground biomass) was calculated as the ratio of leaf area to aboveground plant mass. The leaf dry mass per unit area (LMA) was calculated as the ratio of total shoot leaf area to total leaf dry mass.

Leaf nitrogen (N) concentration was determined by a commercial laboratory (Bemlab (Pty) Ltd, Somerset West) using a dried subsample from the milled terminal flush of each of the eight tagged shoots per temperature treatment. Leaf N content was used, together with the spot measurements of light-saturated net CO_2 assimilation rate (A_{max}) as recorded in January and February 2009 (Chapter 3.3.1), to calculate photosynthetic nitrogen use efficiency (PNUE).

4.2.5 Heat units

Heat units were calculated as growing degree hours (GDH) where,

GHD = (Measured mean hourly temperature (T_m°C)) ï (Base temperature (T_b°C)),

with GHD = 0 when $T_b > T_m$ as negative values do not implicate plant growth.

An upper temperature limit of 35°C was selected, following the assumption that growth ceases at these temperatures and above. The heat unit requirements for the two phenological stages, budbreak to the cessation of shoot growth, and inflorescence development from visible detection to anthesis, were calculated. The optimum base temperature (T_b) for each phenological stage was identified as the temperature where the GDH sum displayed the minimum coefficient of variation (Rattigan & Hill, 1986). Temperature data collected from the experimental sites in Stellenbosch (Floraland-Etshwaleni farm) and Hopefield (Arnelia farm) (Refer to Chapter 5) were used together with air temperatures recorded at the Agricultural Research Council (ARC) weather stations at Nietvoorbij (Stellenbosch) and Koperfontein (Hopefield) (33°06ôS; 18°24ôE).

4.2.5 Sugar and starch analysis

On 14 January 2009, in the greenhouse experiment, three leaves each were sampled from vegetative and reproductive shoots for every treatment. The leaves were dried at 60°C, milled as a pooled sample and stored at -20°C until sugar and starch analysis.

These leaves, as well as the leaves obtained at harvest in the field verification experiment, were analysed for sugar and starch concentration. Total soluble sugars were extracted using 80% ethanol extraction, a method described by Allen *et al*. (1974) and Hamid *et al*. (1985). Starch was hydrolysed to glucose using an acetic acid buffer method and amyloglucosidase enzyme (Hamid *et al*., 1985). Final sugar and starch concentrations were obtained by means of an adapted spectrophotometric method (Dische, 1962), also described by Reed *et al*. (2004) where anthrone was used as a colorimetric agent. Sample absorbance was read with a Cary 50, Bio UV-visible spectrophotometer (Varian, Varian Australia Pty Ltd., Victoria, Australia) at 620 nm against a blank consisting of deionised water and anthrone. A glucose standard was used for quantification, therefore sugar and starch concentrations are presented as glucose equivalents.

4.2.6 Data analysis and statistical procedures

Shoot growth was analysed using PROC NLIN (non-linear) (SAS Institute Inc., 2003) (Fig. 30). Seven parameters were obtained from growth curve analysis using the PROC NLIN procedure of SAS. These parameters include: the Y-axis intercepts a1, a2, a3, the gradient coefficients b1, b2, b3, and the line intercepts T1 and T2. The curve parameters were then

subjected to mean separation by one-way ANOVA using the PROC GLM (SAS Institute Inc., 2003).

Stem diameter growth, as well as inflorescence width development was analysed by repeated measures ANOVA using Statistica 8.0 (Statsoft, Inc., Tulsa, Oklahoma, USA).

Data obtained at harvest was subjected to analyses of means by linear and quadratic contrasts using the General Linear Method procedure generated by SAS Institute Inc. (2003). Logit transformations were performed on the data describing flowering percentages. Mean separation was accomplished by Tukey (p Ò 0.05), where applicable. Linear and quadratic contrasts were fitted using SAS Institute Inc. (2003).

LAR and LMA values were submitted to a linear regression analysis (SAS Institute Inc., 2003) at a significance level of pr<0.05.

4.3. Results

4.3.1 Vegetative and reproductive development

Baseline shoot characteristics and aboveground dry mass: There were no significant differences in the shoot lengths or stem diameters of tagged shoots (pÒ0.05) between plants of the various treatment plots, prior to the warming experiments, for both the greenhouse and field experiments (Table 4). There was also only small variability in shoot dry mass (16.9 ± 1.1 g) and leaf dry mass (38.2 ± 3.1 g) between the four non-experimental plants harvested for baseline data. Therefore, between-plant variability at the start of the experiments can be considered minimal, with no bias between treatments.

Vegetative development: Budbreak progressed more rapidly during late winter (mid-August) at the warmer temperature treatments with almost 30% of the ambient+3.1°C treatment shoots having obtained budbreak on 11 August 2008 compared to less than 12% for the other treatments in the greenhouse-based experiment (Fig. 31a). Three days later, about 40% of shoots exposed to the ambient+3.1°C treatment were at budbreak, compared to about 23-24% of shoots grown at ambient+1.8°C and ambient+2.5°C, with less than 15% of shoots having reached budbreak under ambient and ambient+0.8°C temperatures. Thereafter, differences in the rate of budbreak between treatments were reduced and had just about disappeared by 27 August 2008.

In the field verification experiment, 50% of shoots under the ambient+2.9°C treatment were at budbreak on 7 August 2008, whilst less than 10% of shoots under the ambient

treatment were at a similar stage (Fig. 31b). On 12 August 2008, the advancement of the percentage of shoots reaching budbreak under warming was even more pronounced, but thereafter the difference between treatments was reduced (20 August 2008) and finally disappeared when all shoots were at budbreak on 11 September 2008.

Of all the shoot growth parameters calculated by the NLIN procedure only growth rate (b2) exhibited a linear response with increasing temperature ($p = 0.0474$), whilst the final shoot length (a3) showed a quadratic trend ($p = 0.0681$) where the ambient+3.1°C treatment had the longest shoots, followed by shoots grown at ambient temperatures (Table 2). Other parameters were not significant (data not shown).

The stem diameter increase showed significant interaction between time and treatment ($p = 0.0099$) during the growth season (Fig. 32) as the stem diameter between temperature treatments did not increase similarly over time. The lowest diameter increase was observed in the shoots grown at ambient+1.8°C, whilst shoots grown at ambient temperatures were thickest, with no difference between the other three treatments (Fig. 32).

The increment of flushes produced after the winter dormant period on tagged shoots ranged from zero to two before inflorescences initiated (Fig. 33). At the ambient temperature treatment, all shoots ($n = 8$) completed a spring flush before inflorescence development commenced. Under warming treatments, shoots either flowered on the spring flush, but could also initiate inflorescences on the first or second summer flush. At both the ambient+0.8°C and ambient+2.5°C treatments a single, three-flush shoot initiated an inflorescence in autumn. Therefore no spring flush growth occurred (Fig. 33), as inflorescence initiation on the autumn flush took place before the experiment commenced.

Root development was the most pronounced in the periphery of the drippers in all the pots across the temperature range (Fig. 34). There was a significant quadratic trend across the temperature gradient for both the high density and low density roots, where the highest numbers of roots were counted at the ambient+1.8°C and ambient+2.5°C treatments, followed by a decrease at the ambient+3.1°C treatment.

Reproductive development: Following a natural heat wave in early February 2009, when maximum temperatures exceeded 36°C, several inflorescences across treatments were sun scorched and desiccated, whereafter development of these inflorescences aborted. Thus inflorescence width growth is presented only until early February 2009 (Fig. 35a). The first inflorescences to commence development were observed on shoots growing at ambient temperature and were closely followed by inflorescence developing under the ambient+0.8°C

treatment. Shoots growing under ambient+1.8°C and ambient+2.5°C treatments had smaller and/or later developing inflorescences, whilst inflorescences growing under ambient+3.1°C developed the slowest and were the last to mature, with the lowest basal diameter. Inflorescence width growth rates did not differ significantly (Fig. 35a).

In the field verification experiment, inflorescence growth rates, based on the basal width increase, were similar between the two temperature treatments (Fig. 35b).

Inflorescence initiation on tagged shoots were most abundant under the ambient+0.8°C treatment, thereafter the flowering percentage decreased progressively with increasing warming treatments, although the trend was not significant ($p = 0.1856$) (Fig. 36). Inflorescences were borne mainly on the spring flush when grown at ambient temperature, as only two shoots flowered on the first summer flush. However, at higher temperature treatments there were a clear shift towards flowering on both the spring and the first summer flush, and even on the second summer flush, as was observed at ambient+3.1°C.

4.3.2 Harvest

As all plants were harvested on the same day in the greenhouse experiment, shoots were necessarily at different developmental stages. Some vegetative shoots were growing actively, whilst others were dormant. Across treatments, inflorescences varied considerably in size and maturity. Some reached harvest maturity several weeks prior to the actual experimental harvest, whilst other inflorescences were very immature, measuring less than 2cm in basal diameter. If harvest took place at commercial soft tip stage harvest in the greenhouse, it would probably have commenced in December 2008 and have extended to late May 2009.

In general, reproductive shoots were thicker and had fewer flushes (Table 6) than vegetative shoots (Table 7), as was expected. Flowering shoots were generally shorter than vegetative shoots for all treatments, except for plants grown at ambient temperatures. The stem diameter and total leaf area of whole flowering shoots displayed a quadratic trend with increasing temperature with minimum values at ambient+1.8°C (Table 6). Similarly, flush length, number of leaves, stem- and leaf dry mass of the terminal flush subtending the inflorescence also showed quadratic responses over the temperature range, whilst individual leaf area of the terminal flush of flowering shoots exhibited a decreasing linear response from ambient to ambient+3.1°C (Table 6).

Non-flowering shoots had a significantly higher number of flushes at the warmer temperatures ($p = 0.0336$) (Table 7). Total shoot length revealed a quadratic trend with

increasing temperatures at p = 0.0752. The individual leaf area, stem- and leaf dry mass of the terminal flush of non-flowering shoots showed increasing linear trends over the temperature gradient, whereas the flush length exhibited a quadratic response with increasing temperatures, with a maximum at ambient+1.8°C (Table 7).

Whole shoot leaf area, together with stem fresh and dry mass of tagged flowering shoots showed quadratic trends with increasing temperature, where minimum values were recorded at the ambient+2.5°C treatment (Table 8). Flush length, number of flushes, leaf area, stem and leaf fresh mass also exhibited quadratic responses as temperature increased, with minimum values generally at ambient+1.8°C. Notably, the quadratic pattern observed at harvest in flowering shoots (Table 6) had the opposite trend to that exhibited in non-flowering shoots (Table 7).

In the field verification experiment harvest was on average two weeks earlier for the ambient+2.9°C treatment, on 1 March 2009 ± 2 days, compared to the ambient treatment that was harvested on 14 March 2009 ± 5 days (Fig. 35b). Except for the difference in harvest date, all other parameters were comparable, including flower size (Table 9).

4.3.3 Heat units

A base temperature of 9°C was calculated for the period from inflorescence initiation to anthesis (Table 10). The ambient treatment in the field verification experiment at Floraland-Etshwaleni accumulated 35903 growing degree hours (GDH) for this period, whilst the ambient+2.9°C treatment accumulated more units at 39780 GDH. On average, from inflorescence initiation to anthesis, 38887 GDH was accumulated for the five different temperature regimes within two field production areas, namely Stellenbosch and Hopefield. A base temperature for the period of budbreak to cessation of shoot elongation could not be calculated as no turning point could be obtained.

4.3.4 Fresh and dry mass allocation, leaf/plant structure and nitrogen

A trend of increasing allocation of fresh (Fig. 9a) and dry mass (Fig. 9b) to the stems was observed with increasing temperature treatments when only the new growth was taken into account. Similarly, an increasing proportion of fresh and dry mass was allocated to leaves with increasing temperature, whereas a decreased allocation of fresh and dry mass to inflorescences was found with increasing temperature (Fig. 37a).

The fresh (Fig. 10a) and dry mass (Fig. 10b) allocation of the terminal flush subtending the inflorescence showed a minor increase in biomass allocated to stem and leaf components with increasing temperature, and a slight decreasing trend for inflorescences, with a small anomaly in the trend for ambient+2.5°C.

The aboveground leaf area ratio (LAR) decreased ($p = 0.0505$) from ambient to the ambient+2.5°C treatments from 35 to 25 cm^2 g^{-1} (Fig. 39).

The leaf dry mass per area (LMA) increased significantly ($p = 0.0187$) across the entire temperature gradient from 200 g m^{-2} when grown at ambient temperatures to 270 g m^{-2} at the ambient+3.1°C treatment (Fig. 40).

Leaf nitrogen concentration was slightly lower in plants grown under ambient+1.8°C compared to the other temperature treatments (Fig. 41), but was non-significant. The photosynthetic nitrogen use efficiency (PNUE) decreased across the entire temperature gradient, but only significantly so in the February 2009 sampling date (Fig. 42).

4.3.5 Leaf sugar and starch concentration

Samples taken on 14 January 2009 from shoots in the greenhouse experiment revealed lower levels of leaf sugar and starch in vegetative shoots compared to reproductive shoots (Fig. 43). Leaf total soluble sugar concentrations were similar (80 to 90 mg glucose (g dry $mass^{-1}$)) across all the temperature treatments in reproductive shoots, but varied from 50 to 80 mg glucose (g dry $mass)^{-1}$ across the gradient in vegetative shoots (Fig. 43a). Leaf starch concentrations in reproductive shoots decreased from ambient to ambient+1.8°C, remained similar to ambient+1.8°C at ambient+2.5°C, and then increased greatly at ambient+3.1°C (Fig. 43b). Vegetative shoots showed a similar pattern, though less pronounced.

In the field verification experiment leaf sugar and starch concentrations did not differ statistically between the ambient and ambient+2.9°C treatments (Fig. 44).

Maximum potential whole plant carbon assimilation rate did not differ significantly between temperature treatments in January 2009, but decreased significantly in the gradient from ambient to ambient+3.1°C in February 2009 and even more so in March 2009 (Fig. 45).

4.3.6 Visual changes in morphology

Changes in leaf morphology were observed from broad leaves at ambient temperatures to longer and narrower leaves at elevated temperature treatments (Fig. 46). Leaves on the terminal flushes of plants grown under elevated temperatures had a lower presence of

trichomes (personal observation). Fig. 47a and b provides visual evidence of growth differences between the ambient and ambient+1.8°C treatments respectively, where the visual differences between treatments were most evident. Plants grown at higher treatment temperatures were less uniform within the treatment, with long vegetative shoots compared to a denser and more compact plant structure of the plants grown at ambient temperatures. One plant representing each treatment in Fig. 47a, clearly show the more robust and vegetative nature of the plants at the higher temperatures as they typically displayed longer, more spindly shoots compared to plants at lower temperatures.

4.4 Discussion

Temperature, as the driving force behind plant growth and development rates, also influenced *Protea* ôPink Iceô during vegetative and reproductive phases. In addition, temperature also indirectly affects plant performance by its effect on relative humidity and stomatal activity. It is difficult to evaluate the effect of temperature as a single factor, as several other factors, including water availability and vapour pressure deficits (VPD) cannot be overlooked.

Walther *et al.* (2002) observed that horticultural spring markers such as budbreak and flowering have steadily advanced during the past 30 to 63 years, which reflect plant responses to global warming. Similar to other crops studied, earlier budbreak in *Protea* was recorded in the greenhouse and field verification warming experiments grown under elevated temperatures (Fig. 31). Higher, more optimal temperatures in early spring may therefore advance flowering for ôPink Iceô, whilst supra-optimal temperatures may extend the growing season for ôPink Iceô.

In the Stellenbosch region ambient temperatures appeared to sub-optimal and up to an increase of approximately 3°C, the harvest period will advance as temperatures become more optimal. However, as the greenhouse ñambientò was slightly warmer than Stellenbosch ñambientò, temperature increasing with 4-5°C will be detrimental to protea production. These ranges will differ slightly between production areas. A longer season could result in a greater number of flushes produced for this vigorous cultivar before flowering commences, depending on the nature of the flowering signal. The timing of additional flushes caused by increased temperatures appears to be important. Should autumn temperatures increase and more flushes be produced before the winter period, flowering may occur as normal in spring,

however, should spring temperatures increase supra-optimally, increased number of flushes produced after the winter period may result in fewer inflorescences.

Such an increased number of flushes before floral initiation were found in the shoots at higher temperature treatments in the greenhouse-based experiment (Fig. 33). In addition, an accelerated growth rate was observed for shoots at higher treatment temperatures during spring (Table 2).

Of critical commercial importance is the finding that fewer inflorescences initiated and developed at the higher temperature treatments in the greenhouse experiment (Fig. 36). In addition, inflorescences were initiated later than normal on summer flushes, therefore delaying the harvest period.

Protea óPink Iceô is harvested in South Africa from late December to May (Gerber, 2000), whilst the most favourable period for export to Europe only extends up to early February, the week prior to Valentineôs Day. If average temperatures are to increase as predicted, the majority of *Protea* óPink Iceô produced in the Western Cape might be harvested later than February, reducing producer income due to competition from spring flowers produced in Europe. However, there may also be positive consequences in that the harvest window could possibly extend up to the first or second week in May, to include Motherôs Day, the most important flower day on the calendar, as a prime marketing opportunity.

In comparison to the greenhouse experiment, in the field verification experiment, increased temperatures did not appear to be supra-optimal, but rather closer to optimal. Budbreak advanced similarly (Fig. 31b), but was followed by a possibly slightly higher growth rate (Table 2) and subsequent earlier flower initiation and development was noted. On average, inflorescences were harvest up to two weeks earlier compared to inflorescences at ambient temperatures (Fig. 35b).

The base temperature of 9°C that was calculated for the period of inflorescence development to anthesis (Table 9) differed from the base temperature of 6°C obtained for the development of the secondary *Leucospermum* cv. Golden Star inflorescence (Jacobs & Honeyborne, 1979). This variation may be due to species differences and/or the alternate seasons during which the respective inflorescences developed. *Protea* óPink Iceô inflorescences generally develop during early summer to autumn, whilst *Leucospermum* óGolden Starô develops during late winter to spring. The base temperature of 9°C is also significantly higher than the 1°C calculated for *Protea* óCarnivalô (Hoffman, 2006). The difference could be ascribed to the fact that the óCarnivalô was treated with a cytokinin-like

hormone to advance the flowering time and inflorescence development took place during winter.

An upper temperature limit of 35°C was selected as it was reported that *Macadamia integrifolia* cv. Keauhou grown in controlled growth chambers from 10 to 35°C showed zero growth at 10°C, whilst plants grown at 35°C had multiple, unsynchronised budbreak, which developed calluses followed by desiccation (Trochoulias & Lahav, 1983). Ericsson *et al*. (1996) stated that physiological processes and growth in several tree species increased with temperature to reach a maximum between 20 and 35°C. Future considerations in selecting suitable production areas for *Protea* should include an analysis of average and maximum temperatures. It may become just as critical to ensure sustainable production in indigenous Fynbos crops as it is currently for temperate crops such as pome- or stonefruit having specific chilling requirements for reproductive success.

Allocation of biomass to the new growth was higher in leaves and stems, probably due to the increased number of flushes produced at the higher temperature treatments, together with fewer and smaller flowers at the time of harvest (Fig. 37).

Leaf dry mass per unit area (LMA) increased at the higher temperature treatments (Fig. 40). At high temperatures and low photosynthetic capacities a decrease in LMA would be expected (Poorter *et al.*, 2009) as carbon assimilation is limited. Alternatively, higher LMA values may result in increased net CO_2 assimilation rates, should higher protein and chloroplasts levels in the mesophyll be responsible for the increased LMA. However, in this study increased LMA is likely linked to an increased leaf density through the accumulation of carbon rich compounds and thickening or lignification of cell walls. This could explain the decreased assimilation rates on a leaf mass-basis (Chapter 3, Fig. 1). Wright *et al*. (2005) studied data compiled from 2548 species/site combinations and found that LMA increased with increasing mean annual temperature.

In the native habitat of *Protea*, soil N is in low supply and therefore may restrict protein synthesis, leading to excess production of organic carbon from photosynthesis as light energy is hardly limiting. Additional carbon skeletons not used in protein synthesis are channelled towards the production of woody fibres such as lignin and tannins (Rebelo, 2001). Whilst this is similar to increased LMA in this study (Fig. 40), N was probably not available due to a reduced transpiration stream as a result of stomatal closure. The increase in LMA in this study at the elevated temperatures was possibly an attempt to resist daytime wilting as a result of physiological drought.

Physiological drought is a condition where water supply to the roots remains sufficient, but due to a high rate of evapotranspiration at elevated temperatures and thus high VPD, where evaporation exceeds the rate of water uptake by the roots, plants experience wilting during the day, but recover at night time. In banana, high temperatures in combination with excessive VPD caused physiological drought stress as a result of evaporative demand exceeding water absorption potential by the roots (Robinson & Bower, 1988). The roots, however, do not experience drought stress and therefore, an abscisic acid (ABA) hormone signal from the roots to the leaves is probably not driving stomatal closure. Low stomatal conductance measured (Chapter 3, Fig. 4) at the high temperature treatments, was most likely caused by low leaf water status throughout the plant during warm summer months. Stomatal conductance has been known to decrease via a feedback response in reaction to decreasing turgor pressure (Mitchell *et al.*, 2008). Still, stomatal closure is often not enough to prevent tissue dehydration.

Leaf starch concentration in reproductive shoots decreased in the greenhouse experiment as was measured in January 2009 from ambient to ambient+2.5°C (Fig. 43b). This trend could have a dual cause. Firstly, the above mentioned shoots carried inflorescences. These flowers are strong sinks as they require large amounts of carbohydrates for growth and nectar production which is supplied mainly from the flush subtending the inflorescence. Smart (2005), calculated that as much as 35% of daily photosynthates were allocated to the inflorescence. Secondly, a combination of high temperature and physiological drought stress could have increased the demand for carbon and reduce carbohydrate reserves during the day. Under continuous physiological drought, excess carbon could be allocated to form osmotic agents, such as sucrose, mostly from stored starch, in order to maintain turgor pressure (Geiger & Servaites, 1991).

Maximum whole plant carbon assimilation rate decreased in the gradient from ambient to ambient+3.1°C treatments as temperatures increased inside the greenhouse (Fig. 45). The decrease at higher temperatures, as well as from January to March 2009 reflects the greenhouse environmental conditions, higher temperatures and VPD during summer.

Acclimation to a new environment or seasonal temperature changes can also include morphological changes (Lambers *et al.*, 1998). The spring flush of ‘Carnival’, comparable to ‘Pink Ice’ is always the most predominant flush in terms of flush length, number of leaves and leaf area (Gerber *et al.*, 2001b). In ‘Carnival’ this phenomenon may be ascribed to a period of carbohydrate accumulation from July to October (Gerber *et al.*, 2001c) during a

phase of high net CO_2 assimilation rates. Similarly, this period had high net CO_2 assimilation rates also for ôPink Iceô (Chapter 3, Fig.5a), but no seasonal carbohydrate data was collected in the greenhouse or field verification experiments. Summer and autumn flushes are as a rule, reported to be shorter, have fewer leaves and the leaves are narrower compared to the spring flush. A similar observation was made in plants grown in the greenhouse-based experiment (Fig. 46, 19). Flushes that developed during summer and autumn were shorter and had narrower leaves. However, these leaves were longer compared to summer and autumn flushes in the field grown plants and displayed a distinct vertical orientation (personal observation) (Fig. 46). Changing leaf morphology to narrower leaves during warm summer months may lower the peak surface leaf temperature and decrease the boundary layer compared to larger, broader leaves of the spring flush (Vogel, 2009).

Total plant leaf area to plant mass (LAR) decreased over the temperature range (Fig. 39). LAR is considered a measure of plant leafiness (Dent, 2000). A decrease in plant leafiness at higher temperatures are visible in Fig. 47a, b, where plants that were grown at ambient temperatures were denser and smaller compared to the plants exposed to elevated temperatures.

The interaction between vegetative and reproductive growth have been studied extensively in different crops, including pomefruit (Webster, 2005). The extensive research reflected on the use of vigorous cultivars, the importance of rootstocks and pruning regimes to ensure fruit size and high yield. A reduction in vegetative growth could increase fruit size or number as there is constant competition between vegetative and reproductive growth (Webster, 2005). The same interaction observed in fruit crops is noticeable in this study for *Protea* ôPink Iceô when analysing harvest shoot characteristics (Table 6-5). In general, the ambient+1.8°C and ambient+2.5°C treatments had the lowest number of inflorescences, but the highest vegetative shoot vigour and growth (Fig. 36), together with the strongest root development (Fig. 34). Sachs & Hackett (1983) describes source-sink relationships with respect to flowering, and reported that root initiation and growth inhibits floral initiation in the long day (LD) plant, *Anagallis arvensis*, especially when rapid root elongation coincides with long day induction conditions. Similarly in tobacco, root induction delayed flowering (Sachs & Hackett, 1983). In this study, floral induction could possibly have been inhibited by increased biomass allocation to root growth.

Vegetative growth of two flushes or more is a prerequisite for inflorescence development in *Protea* (Coetzee & Littlejohn, 2001). A minimum stem diameter has been

found to be significant for óCarnivalô (Hoffman, 2006). This vegetative growth prerequisite for floral initiation could also include a minimum number leaves or accumulated dry mass (Gerber *et al*., 2001c). For other tropical fruit crops exhibiting a flushing growth habit, such as mango, a latent or dormant period between flushes where the current flush is allowed to mature, is considered a prerequisite for inflorescence initiation (Wilkie *et al*., 2008). Flush maturation translates into accumulation and storage of reserves for new vegetative or reproductive growth. Davenport (2003) in a comparison of the flowering habit of avocado, lychee and mango concluded that reproductive flushes mostly occur after an extended period of stem rest or mild water stress where shoot growth is inhibited. Alternatively, reproductive growth may follow a period of cool winter months when no or little shoot or root growth occurs.

High temperature seems to favour vegetative growth as the reproductive phase is sensitive to high temperatures (Leclerc, 2003). High temperature conditions increase the sink demand, promoting continual flushing. For ôPink Iceô absolute amounts of carbon assimilated remained constant across all temperature treatments on a leaf area-basis (Chapter 3, Fig. 1a-e), but on a leaf mass-basis, less carbon was assimilated at elevated temperature treatments (Chapter 3, Fig. 1f-h). Source strength or assimilation capacity, was most likely limited as a result of reduced stomatal opening under high temperature during summer (Chapter 3, Fig. 4, 5b) caused by daytime physiological drought.

Furthermore, in mango (Wilkie *et al*., 2008) similar to *Protea* óCarnivalô (Hoffman, 2006), the apical meristem appears to be receptive for the floral stimulus only for a short period during which inductive temperatures need to coincide with bud release. Over-wintering shoots of *Protea* ôPink Iceô, as in óCarnivalô and ôLady Diô (Gerber *et al*., 2001a) may experience inductive conditions during the winter dormant period, which possibly includes lower temperatures and could explain why *Protea* ôPink Iceô preferentially flowers on the spring flush. However, low temperature may act indirectly by reducing plant activity, which in turn ensures a period of carbon assimilation, which leads to flower induction. Higher temperatures may interfere with the floral signal in two ways. Firstly, high temperature may reduce the low temperature signal during winter. However, as flowering of *Protea* occurs in the tropics (Hawaii), low temperature may not be critical. Therefore, secondly and more importantly, high temperature that promote continuous flushing may interfere with flush maturation and the required accumulation of a threshold of carbohydrates in order for floral induction to both occur and proceed.

Protea, similar to many other crops, appeared to tolerate high temperatures efficiently for short periods, but prolonged periods of high temperature exposure may be detrimental to plant reproductive success as the vegetative phase is favoured.

4.5 References

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Table 4. Baseline shoot length and stem diameter of two- or three-flush tagged shoots of potted two-year-old *Protea* 'Pink Ice' plants measured on 9 May 2008 in a greenhouse-based experiment (n = 4), and on four- or five-flush shoots of commercially grown eight-year-old 'Pink Ice' measured on 16 July 2008 in the field verification experiment (n = 6). Values are means ± SE.

	Vegetative characteristics	
Treatment	Shoot length (cm)	Stem diameter (mm)
Greenhouse-based warming experiment		
Ambient	32.9 ± 0.6 ns[z]	6.23 ± 0.23 ns
Ambient+0.8°C	32.6 ± 2.0	6.24 ± 0.27
Ambient+1.8°C	30.9 ± 2.9	5.95 ± 0.10
Ambient+2.5°C	29.8 ± 1.4	5.70 ± 0.24
Ambient+3.1°C	33.3 ± 2.3	6.32 ± 0.15
Pr>F	0.7040	0.1866
Field verification experiment		
Ambient	65.8 ± 2.6 ns	6.66 ± 0.20 ns
Ambient+2.9°C	63.9 ± 4.2	6.74 ± 0.12
Pr>F	0.7064	0.7276

[z] Nonsignificant

Table 5. Descriptive mathematical parameters obtained from the Proc NLIN (SAS) procedure describing the growth curve of two or three flush tagged shoots (n = 4) of three-year-old *Protea* ôPink Iceô grown under five temperature treatments in a greenhouse-based warming experiment.

	Parameter	
Treatment	b2	a3
Ambient	0.25	53.6
Ambient+0.8°C	0.28	52.7
Ambient+1.8°C	0.29	51.2
Ambient+2.5°C	0.28	51.4
Ambient+3.1°C	0.34	59.3
Contrasts	Pr>F	
Treatment	*0.2191*	*0.2174*
Linear	*0.0474*	*0.3237*
Quadratic	*0.7156*	*0.0681*

Table 6. Vegetative characteristics of all the flowering shoots at harvest of three-year-old *Protea* ôPink Iceô plants subjected to a greenhouse-based warming experiment with five temperature treatments (ambient to ambient+3.1°C).

	Total shoot				Terminal flush subtending the inflorescence				
Treatment	Number of flushes	Stem diameter (mm)	Shoot length (cm)	Total leaf area (cm^2)	Individual leaf area (cm^2)	Flush length (cm)	Number of leaves	Stem dry mass (g)	Leaf dry mass (g)
Ambient	3.5	9.5	51.9	326	21.9	19.6	27.5	4.3	13.6
Ambient+0.8°C	4.0	8.7	50.6	280	19.1	12.5	22.1	2.8	10.3
Ambient+1.8°C	3.9	8.3	48.3	247	16.8	10.9	21.3	2.2	9.2
Ambient+2.5°C	3.6	9.0	51.1	292	18.5	14.4	22.3	3.2	11.1
Ambient+3.1°C	3.8	8.9	53.8	298	18.1	13.9	24.5	4.5	14.2
Contrasts					Pr>F				
Treatment	*0.3129*	*0.0126*	*0.7256*	*0.0747*	*0.0047*	*0.0003*	*0.0510*	*0.0044*	*0.0326*
Linear	*0.8431*	*0.1129*	*0.7199*	*0.3154*	*0.0028*	*0.0063*	*0.1587*	*0.8878*	*0.7857*
Quadratic	*0.2444*	*0.0038*	*0.2170*	*0.0133*	*0.0099*	*0.0002*	*0.0073*	*0.0002*	*0.0022*

Table 7. Vegetative characteristics of all the non-flowering shoots at harvest of three-year-old Protea ôPink Iceô plants subjected to a greenhouse-based warming experiment with five temperature treatments (ambient to ambient+3.1°C).

	Total shoot					Terminal flush			
Treatment	Number of flushes	Stem diameter (mm)	Shoot length (cm)	Total leaf area (cm^2)	Individual leaf area (cm^2)	Flush length (cm)	Number of leaves	Stem dry mass (g)	Leaf dry mass (g)
Ambient	4.4	6.5	43.3	173	10.1	5.8	16.8	0.48	2.6
Ambient+0.8°C	4.5	7.7	64.0	193	10.0	10.0	22.5	1.31	3.0
Ambient+1.8°C	5.6	7.3	57.0	259	11.6	10.7	22.3	1.15	4.6
Ambient+2.5°C	5.8	7.7	68.9	246	12.9	8.2	19.0	1.16	5.3
Ambient+3.1°C	5.3	7.4	56.5	221	12.4	7.1	18.0	*	*
Contrasts					Pr>F				
Treatment	*0.0336*	*0.2989*	*0.0617*	*0.3308*	*0.1361*	*0.0332*	*0.4794*	*0.0141*	*0.0378*
Linear	*0.0161*	*0.2179*	*0.0811*	*0.1697*	*0.0256*	*0.6771*	*0.9703*	*0.0086*	*0.0049*
Quadratic	*0.2440*	*0.2660*	*0.0752*	*0.2736*	*0.9827*	*0.0052*	*0.1042*	*0.0608*	*0.7613*

* Missing data due to sample loss.

Table 8. Vegetative characteristics of three-year-old *Protea* 'Pink Ice' plants at harvest. Only growth produced after commencement of the experiment is shown for tagged shoots (n = 4). Plants were subjected to a greenhouse-based warming experiment with five temperature treatments (ambient to ambient+3.1°C).

	Whole shoot						Per flush						
Treatment	Leaf area (cm^2)	Stem fresh mass (g)	Stem dry mass (g)	Leaf fresh mass (g)	Leaf dry mass (g)	Number of flushes	Flush length (cm)	Number of leaves	Leaf area (cm^2)	Stem fresh mass (g)	Stem dry mass (g)	Leaf fresh mass (g)	Leaf dry mass (g)
Ambient	660	12.2	4.7	37	15.1	1.00	19.8	30.4	660	12.2	4.7	37.8	15.1
Ambient+0.8°C	563	10.4	4.4	30	14.6	1.43	13.8	21.1	398	7.6	3.2	21.2	10.2
Ambient+1.8°C	487	9.8	4.1	25	11.8	1.50	15.1	24.1	342	7.0	2.9	17.9	8.3
Ambient+2.5°C	420	8.7	3.8	25	12.1	1.13	21.0	21.0	370	7.5	3.2	21.4	10.3
Ambient+3.1°C	804	15.3	*	44	*	1.63	26.6	26.6	508	9.7	*	27.3	*
Contrasts							Pr>F						
Treatment	*0.0247*	*0.1116*	*0.7868*	*0.0260*	*0.6919*	*0.1696*	*0.1784*	*0.1580*	*0.0348*	*0.0482*	*0.3805*	*0.0131*	*0.3580*
Linear	*0.7680*	*0.5526*	*0.3529*	*0.8406*	*0.3214*	*0.1480*	*0.1938*	*0.3530*	*0.1083*	*0.1542*	*0.1597*	*0.0603*	*0.1653*
Quadratic	*0.0063*	*0.0282*	*0.9341*	*0.0031*	*0.9130*	*0.6172*	*0.0658*	*0.0501*	*0.0052*	*0.0069*	*0.1941*	*0.0022*	*0.1784*

* Missing data due to sample loss.

Table 9. Vegetative and reproductive characteristics of eight-year-old *Protea* 'Pink Ice' plants at harvest (n = 6) in a field verification experiment with two temperature treatments, ambient and ambient+2.9°C.

	Whole shoot					Inflorescence		
Treatment	Length (cm)	Diameter (mm)	Leaf area (cm^2)	Leaf dry mass (g)	Stem dry mass (g)	Width (mm)	Length (mm)	Dry mass (g)
Ambient	85 ns[z]	10.1 ns	1749 ns	38 ns	40 ns	54.4 ns	99.7 ns	28.6 ns
Ambient+2.9°C	91	9.6	1609	36	37	54.3	100.4	29.4
				Pr>F				
	0.4315	0.4426	0.3898	0.6702	0.5231	0.8812	0.6117	0.7768

[z] Nonsignificant

Table 10. The accumulated growing degree hours (GDH) for inflorescence development (initiation to anthesis) at various base temperatures (°C) are indicated. The number of days from inflorescence initiation to harvest ± SE is shown for *Protea* 'Pink Ice' plants in two field production areas, grown under five different temperature regimes. An upper temperature limit of 35°C was set. Values in **bold** specify the base temperature with the lowest %CV. Mean, standard deviation (SD) and coefficient of variation (%CV) are reported. For the Hopefield experiment, refer to Chapter 5.

Production Area	Cultivation conditions	Period	Days from inflorescence initiation to anthesis	Base temperature (T_b)						
				6°C	7°C	8°C	**9°C**	10°C	11°C	12°C
Stellenbosch	Floraland commercial	14-Nov-08 ï 24-Mar-09	130 ± 5	50439	47582	44725	**41868**	39012	36163	33322
Stellenbosch	Floraland warming - Ambient	05-Nov-08 ï 14-Mar-09	124 ± 3	44654	41737	38820	**35903**	32987	30076	27185
Stellenbosch	Floraland warming - Ambient+2.9°C	28-Oct-08 ï 01-Mar-09	131 ± 5	48009	45266	42523	**39780**	37037	34294	31553
Hopefield	Arnelia commercial autumn	01-Jun-08 ï 31-Jan-09	245 ± 14	56832	51350	46074	**41010**	36162	31538	27200
Hopefield	Arnelia commercial spring	01-Nov-08 ï 08-Mar-09	130 ± 4	44608	41695	38782	**35872**	32971	30086	27228
			Mean	48908	45526	42185	**38887**	35634	32431	29298
			SD	5063	4101	3339	**2837**	2634	2704	2934
			%CV	10.35	9.01	7.92	**7.29**	7.39	8.34	10.01

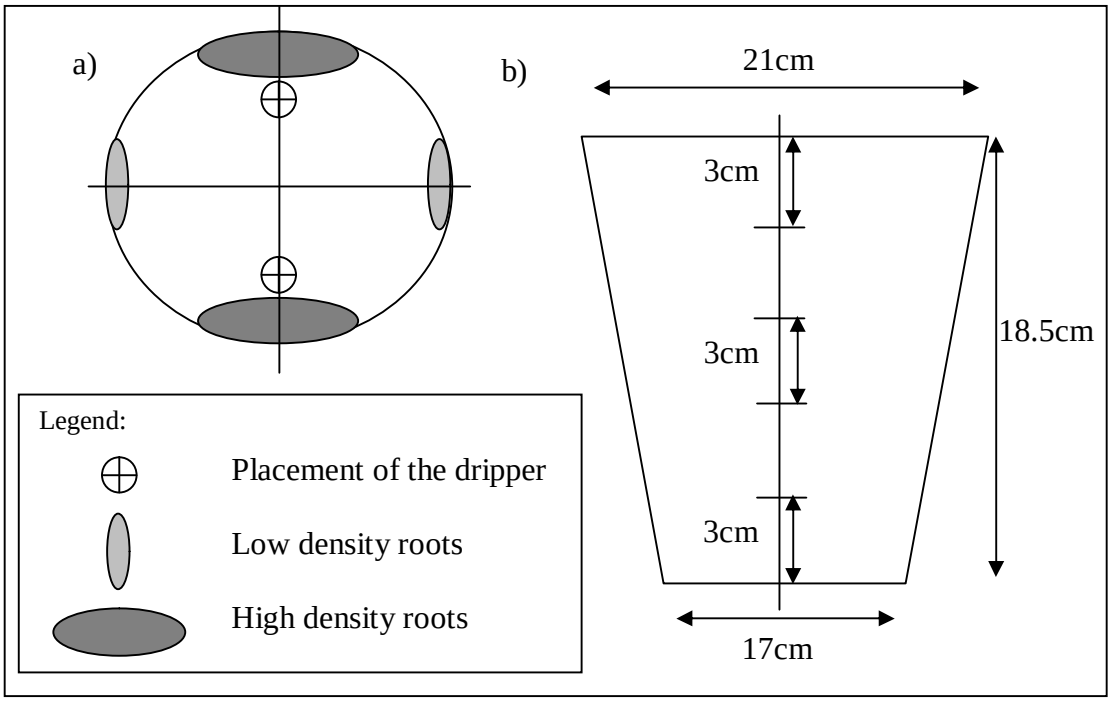


Fig. 29. a) The placement of the irrigation drippers in the pots of the three-year-old *Protea* ôPink Iceô plants are shown together with the distribution of high and low density roots within the pots. b) An illustration of root sampling positions within the experimental pot as was used to quantify root development and distribution.

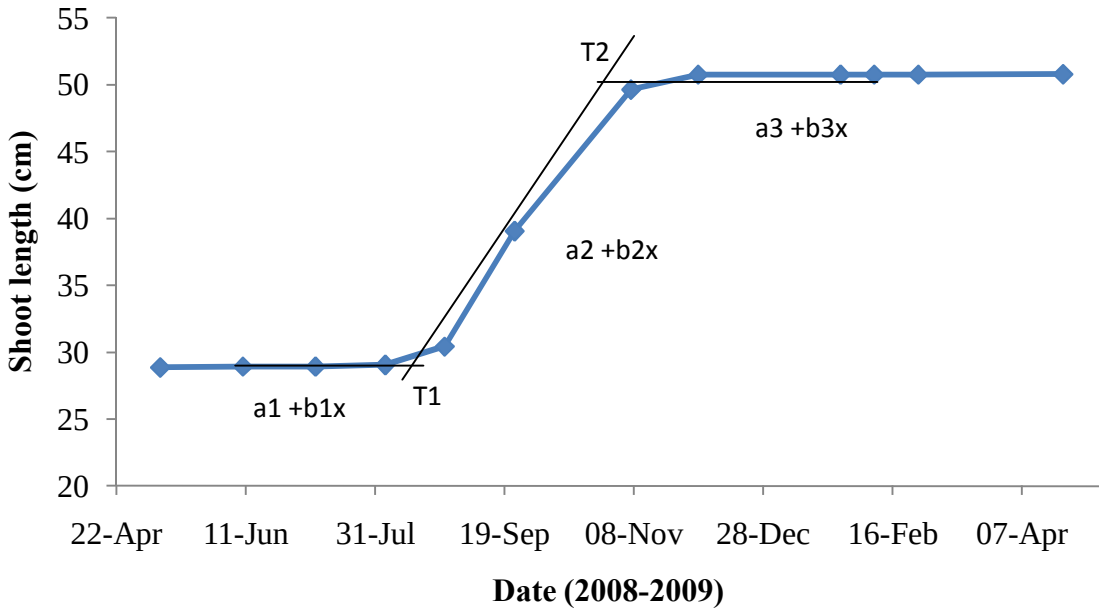
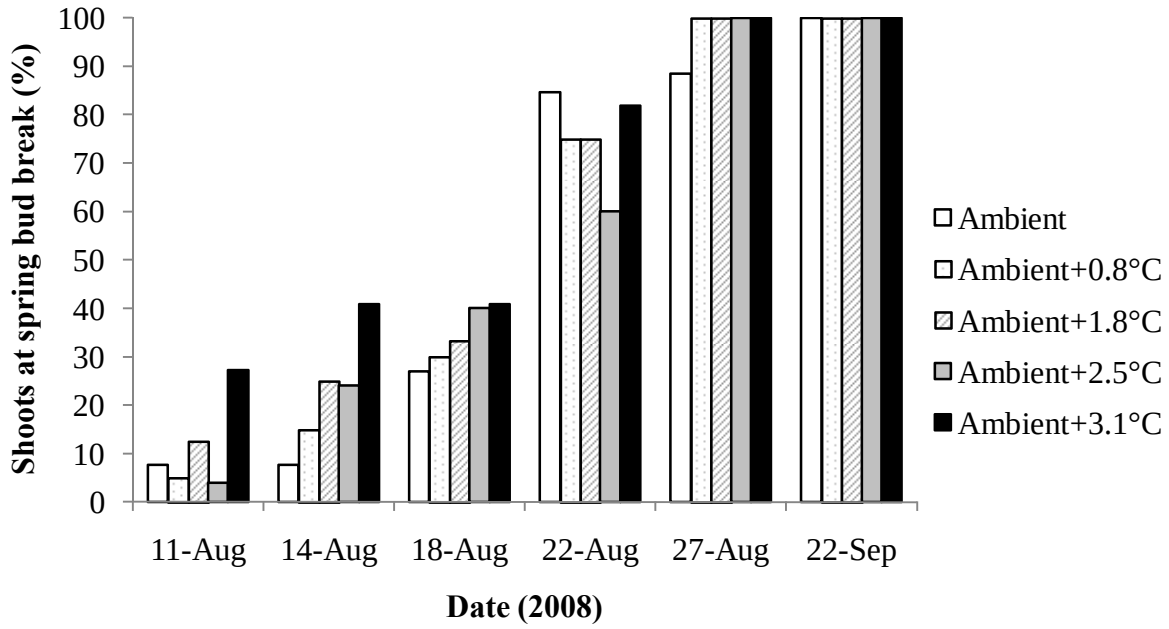


Fig. 30. Representation of the growth curve analysis of the spring flush of *Protea* ôPink Iceô using Proc NLIN (SAS v. 9.1.3) to obtain seven parameters namely the Y-axis intercepts a1, a2, a3, the gradient coefficients b1, b2, b3, and the line intercepts T1 and T2. No vegetative growth occurred during the winter dormant period (first linear regression), as well as after inflorescence initiation (third linear regression).

a)



b)

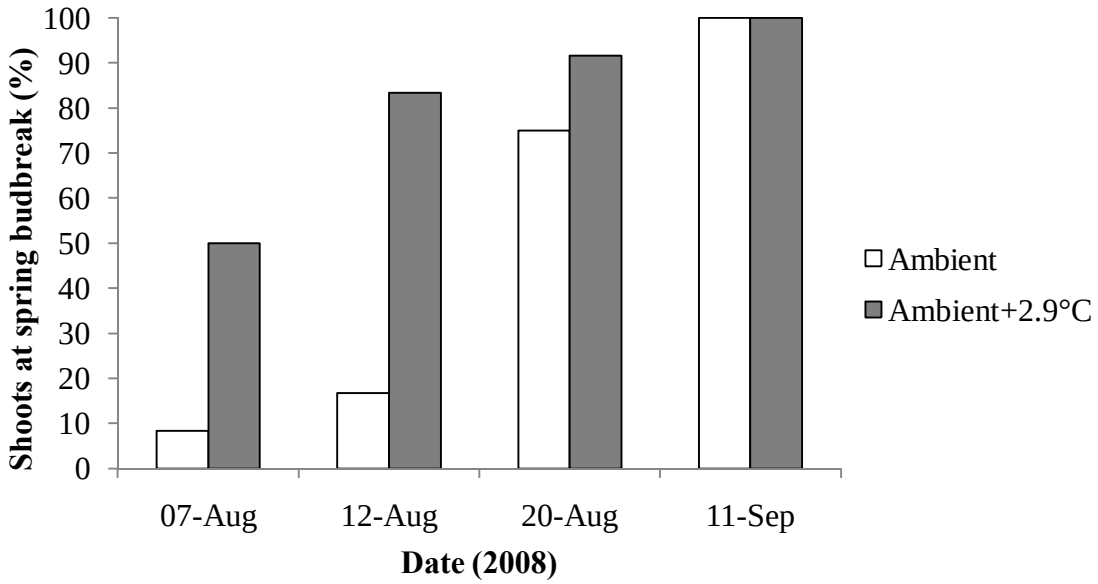


Fig. 31. (a) The percentage spring budbreak in vegetative two- or three-flush shoots (n = 4) of two-year-old potted *Protea* 'Pink Ice' plants in the greenhouse-based warming experiment under five temperature treatments (ambient to ambient+3.1°C) and (b) in the field verification experiment on four- or five-flush shoots (n = 6) of eight-year-old *Protea* 'Pink Ice' plants grown under two temperature treatments.

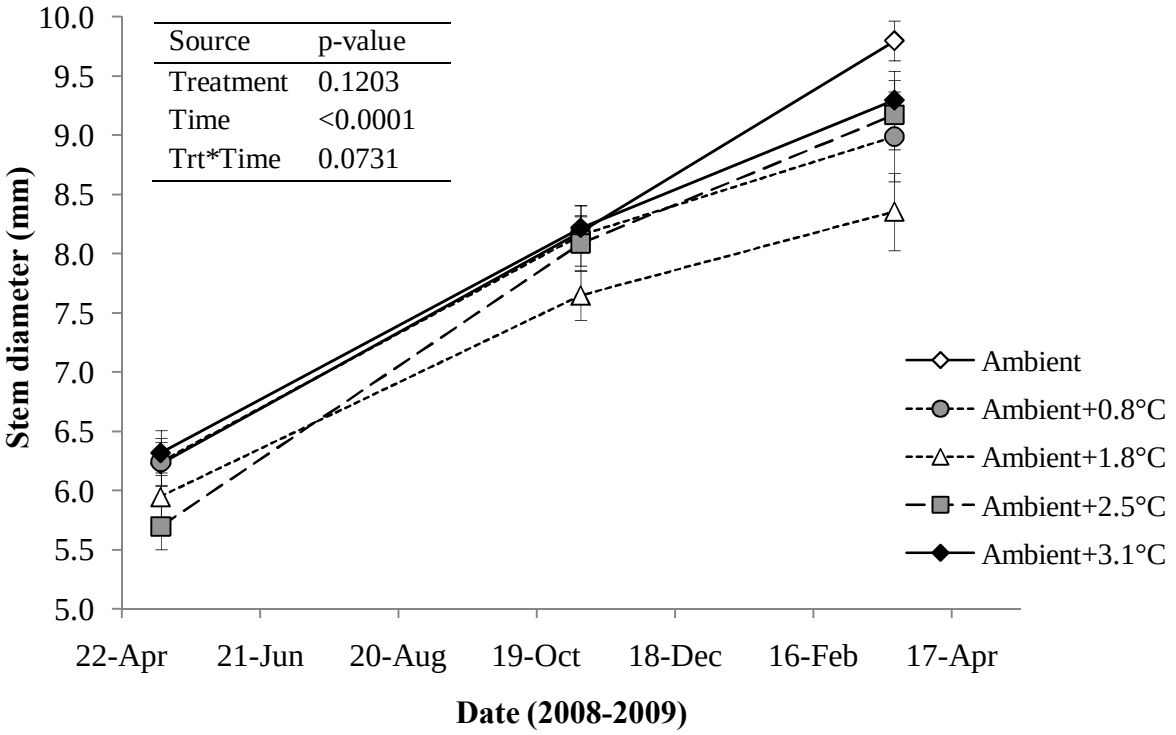


Fig. 32. The stem diameter increase (mm) ± SE (n = 4) from May 2008 to March 2009 for five temperature treatments (ambient to ambient+3.1°C) of *Protea* ôPink Iceô in a greenhouse-based warming experiment.

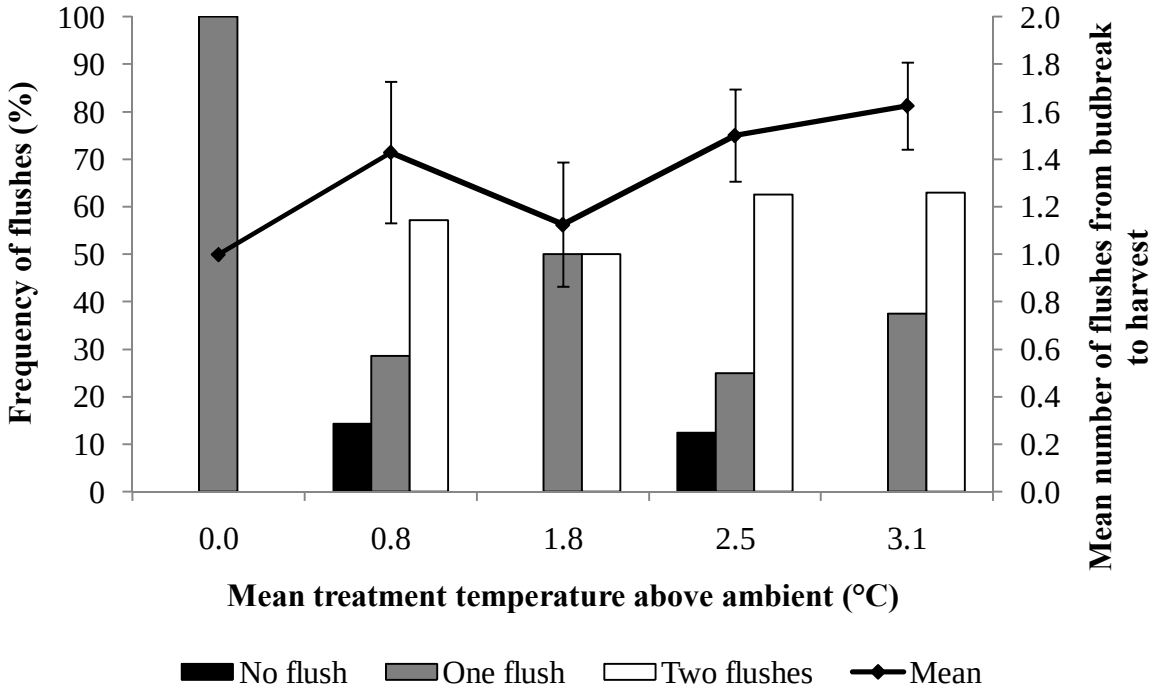


Fig. 33. The distribution and average number of vegetative growth flushes from budbreak to harvest on tagged flowering stems (n = 4) of three-year-old *Protea* 'Pink Ice' plants for five temperature treatments (ambient to ambient+3.1°C) in a greenhouse-based warming experiment.

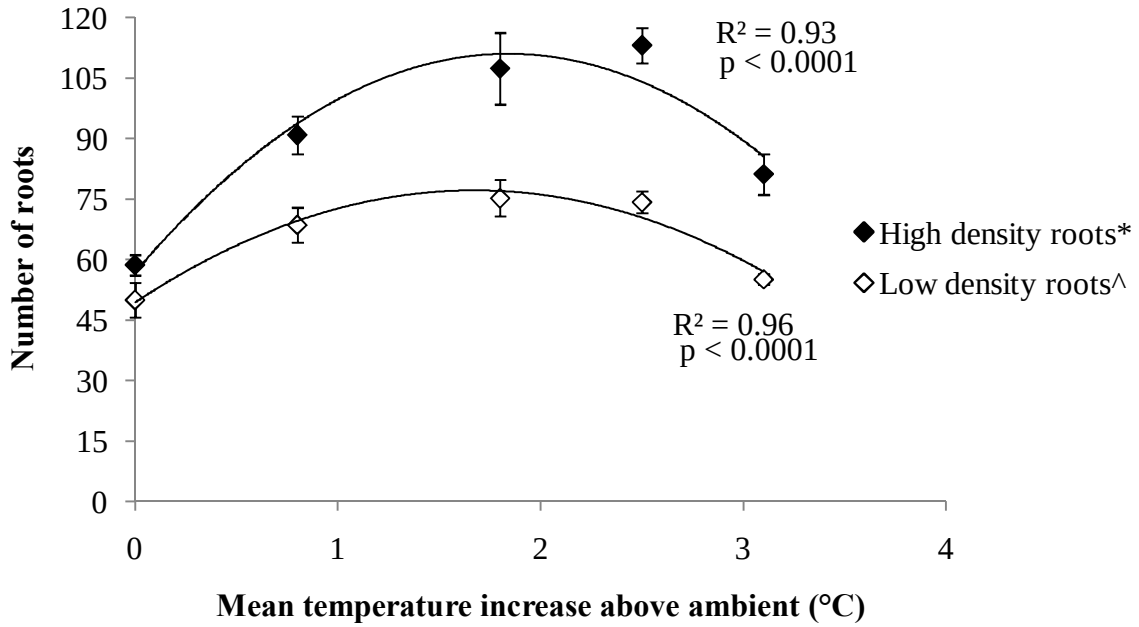


Fig. 34. The number of lateral and main roots in a high density (*in the wet periphery) and low density (^in the dry periphery) zone of potted *Protea* ôPink Iceô plants (n = 4) for five temperature treatments (ambient to ambient+3.1°C) in a greenhouse-based warming experiment.

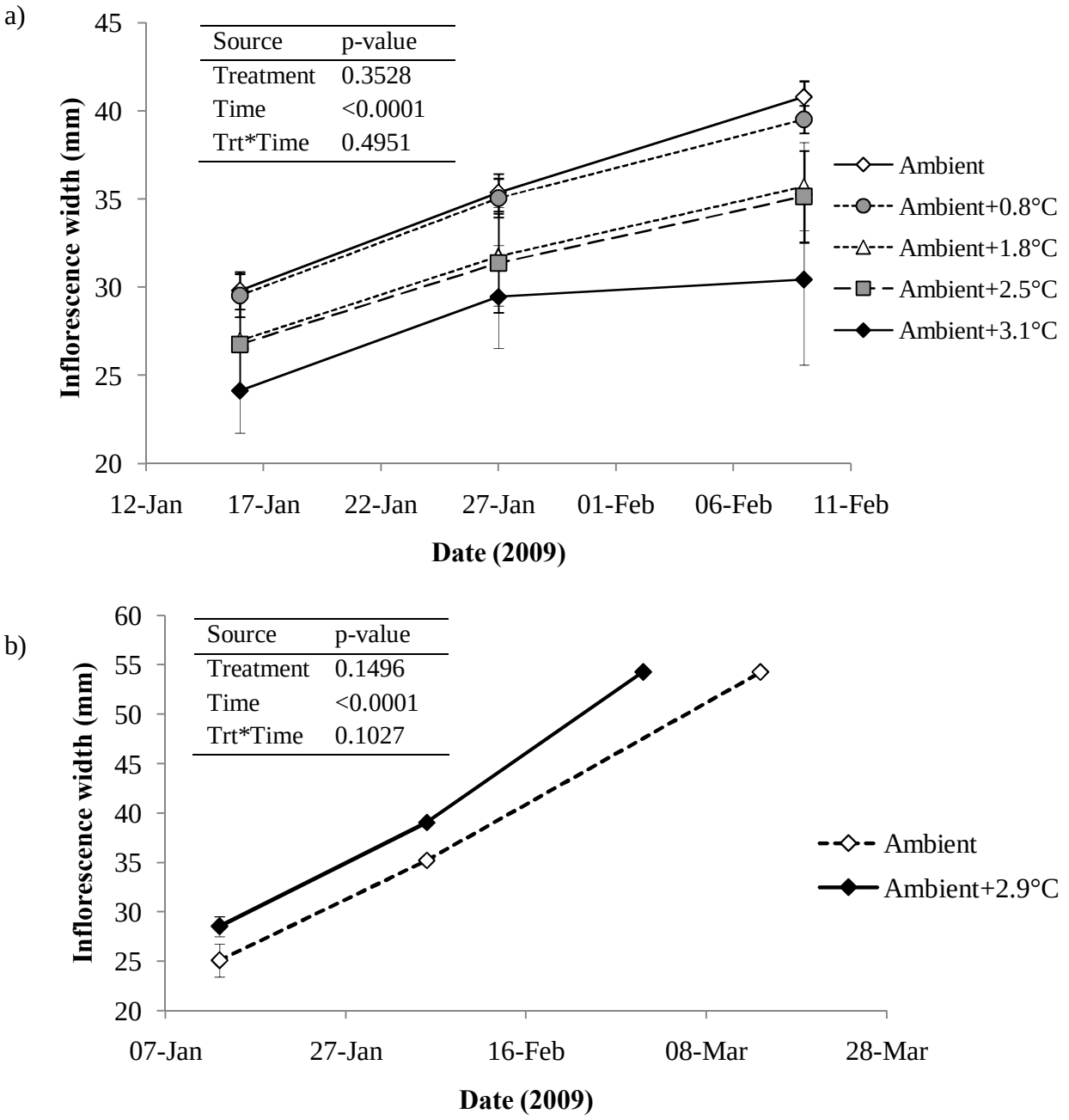


Fig. 35. (a) *Protea* ôPink Iceô inflorescence width development ± SE (n = 4) in a greenhouse-based warming experiment as recorded on 16 January, 27 January and 9 February 2009 and (b) in a field verification experiment (n = 6) as recorded on 13 January, 5 February and at harvest 2009.

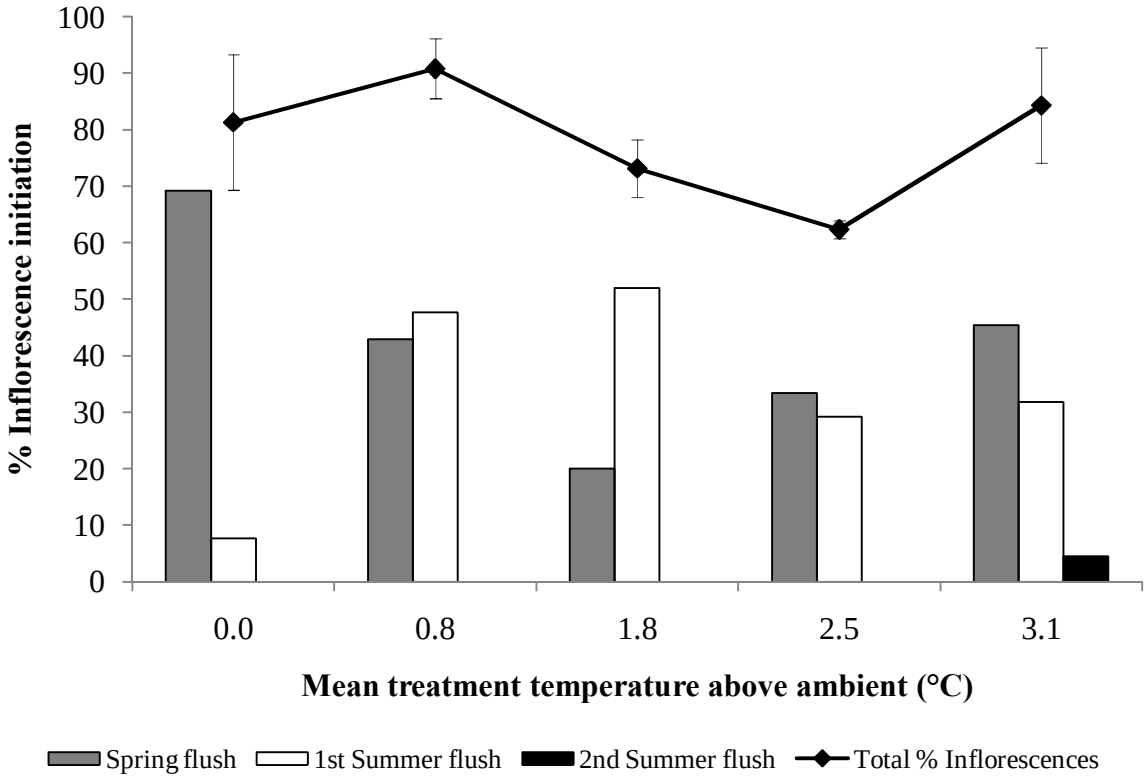
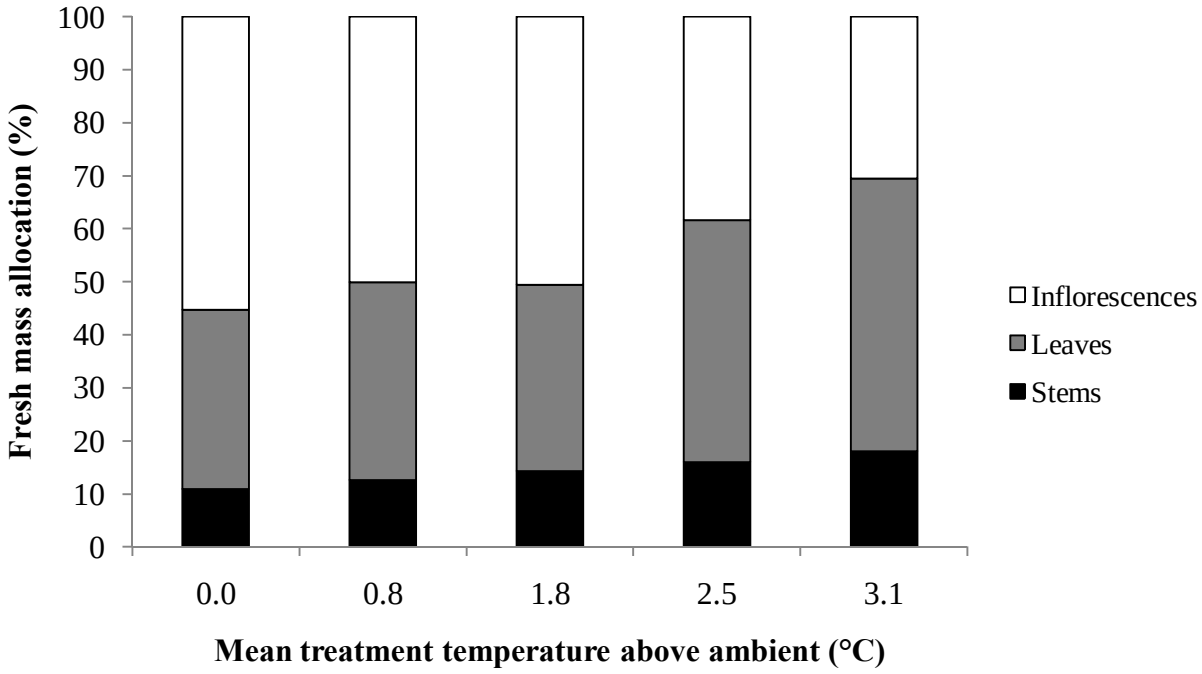


Fig. 36. The distribution of percentage inflorescence initiation on three-year-old *Protea* 'Pink Ice' plants for five temperature treatments (ambient to ambient+3.1°C) in a greenhouse-based warming experiment (n = 4). The figure specifies the flush on which the inflorescence was carried, namely the spring flush, first summer flush or the second summer flush.

a)



b)

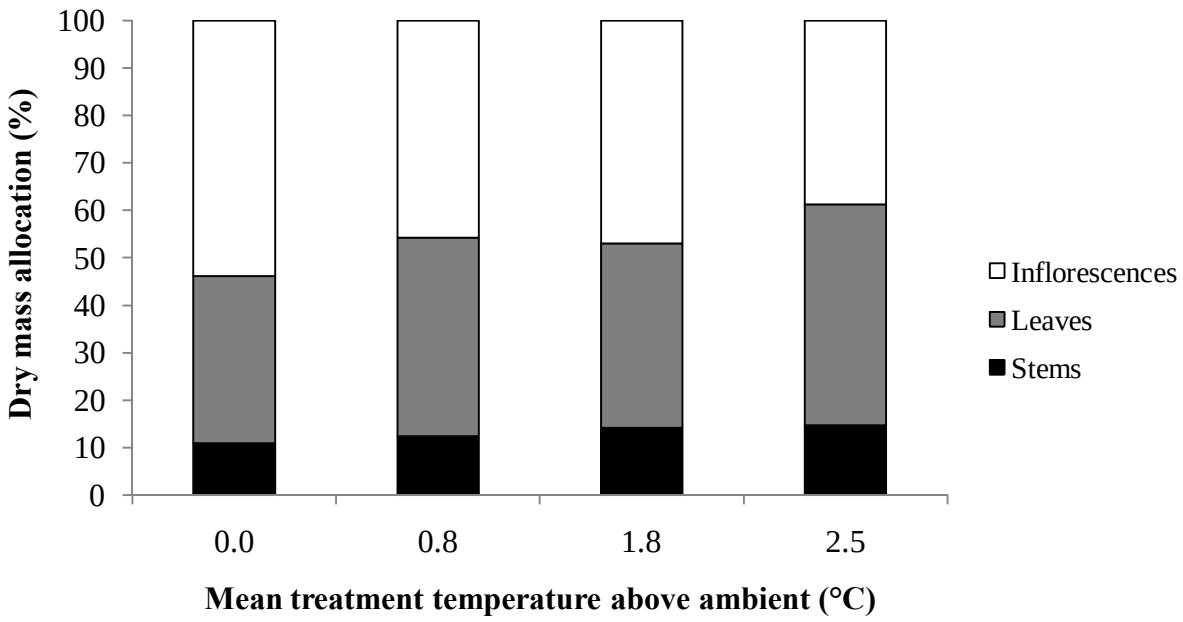


Fig. 37. The distribution percentages at harvest of shoot (a) fresh- and (b) dry mass allocation to new growth after commencement of an experiment of five temperature treatments (ambient to ambient+3.1°C) in a greenhouse-based warming experiment of three-year-old *Protea* ‘Pink Ice’. Shoots were separated into stems, leaves and inflorescences.

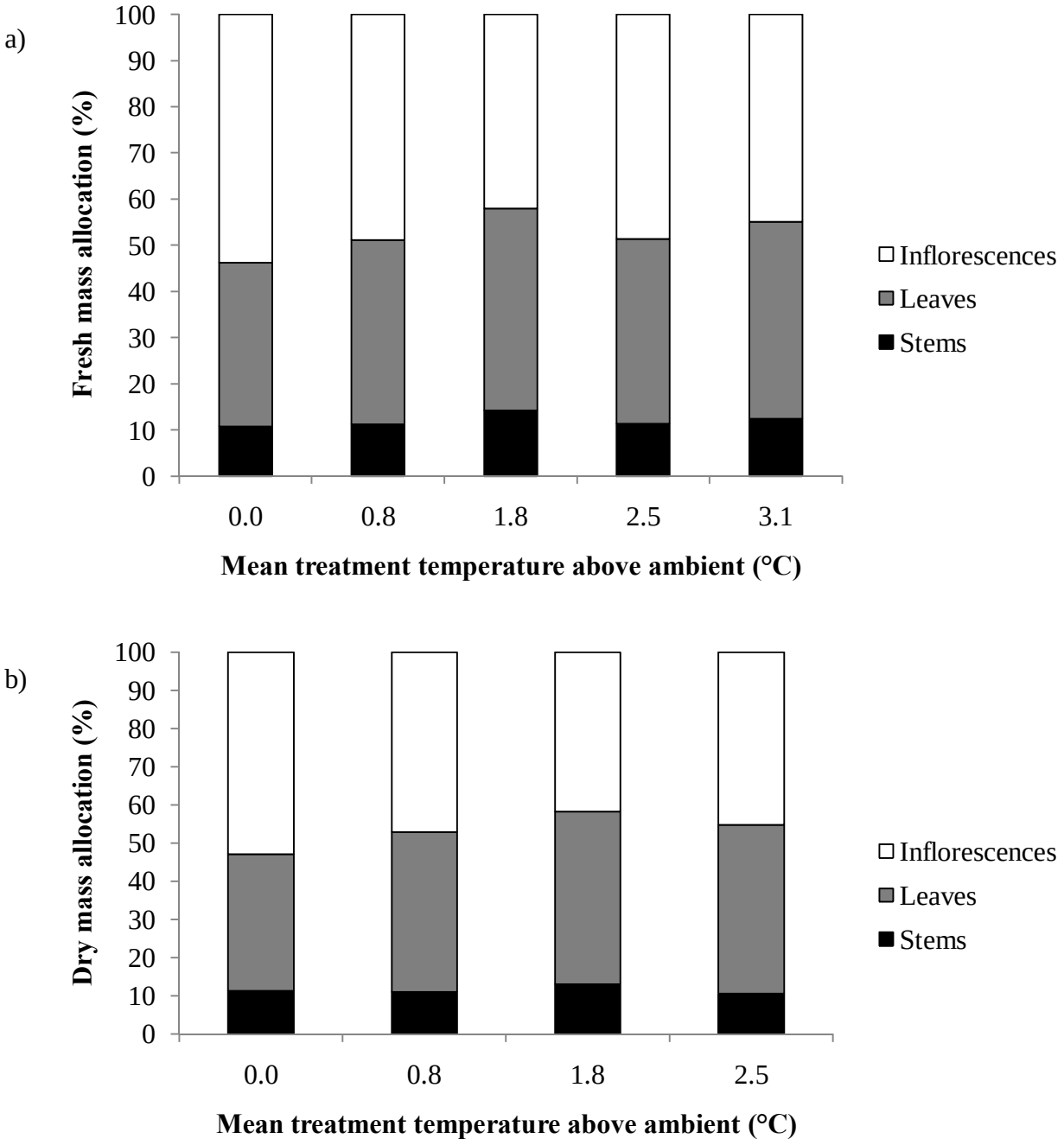


Fig. 38. The distribution percentages at harvest of shoot (a) fresh- and (b) dry mass allocation of the terminal flush subtending the inflorescence of *Protea* 'Pink Ice', separated into stems, leaves and inflorescences for the five temperature treatments (ambient to ambient+3.1°C) in a greenhouse-based warming experiment.

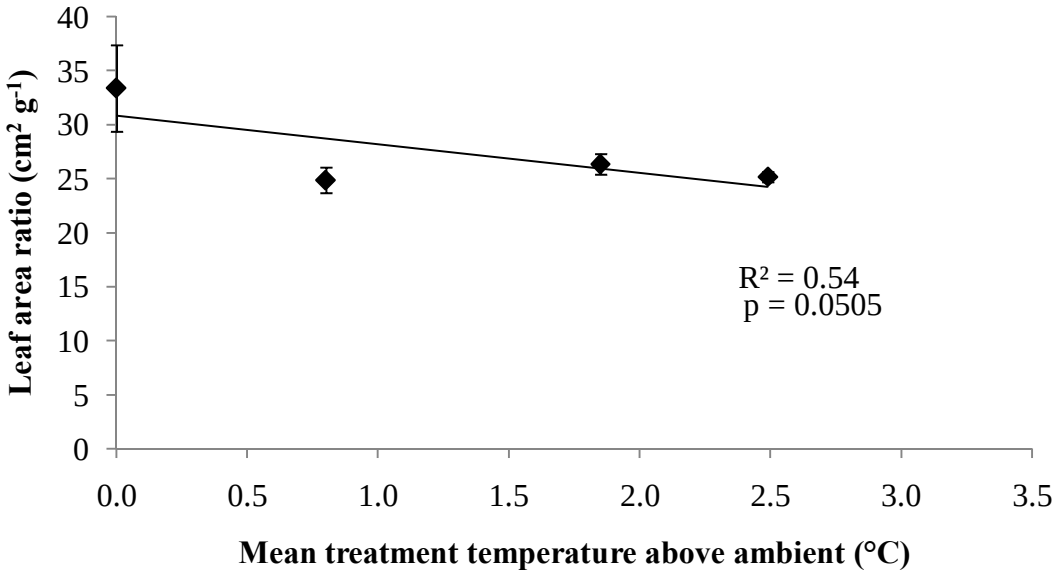


Fig. 39. Total plant leaf area to plant mass ratio (LAR) ± SE (n = 4) in four temperature treatments (ambient to ambient+2.5°C) for three-year-old potted *Protea* ôPink Iceô plants in a greenhouse-based warming experiment.

* No data was available for ambient+3.1°C.

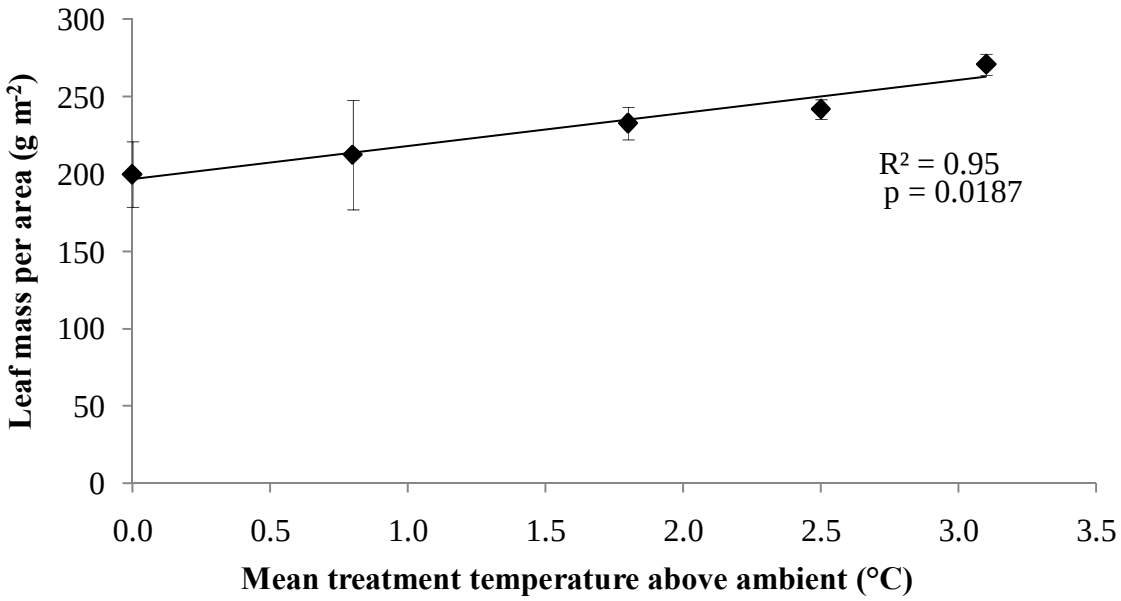


Fig. 40. The total shoot leaf dry mass per leaf area (LMA) ± SE (n = 4) calculated of three-year-old *Protea* ôPink Iceô plants for five temperature treatments (ambient to ambient+3.1°C) in a greenhouse-based warming experiment at harvest.

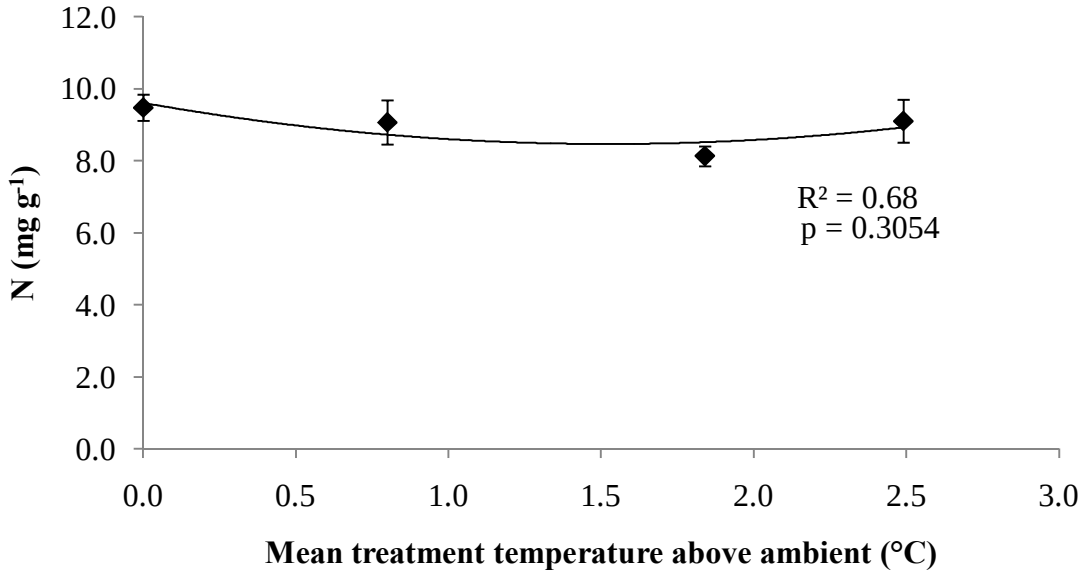


Fig. 41. Total leaf nitrogen (N) concentration ± SE (n = 4) of the terminal flush of three-year-old *Protea* 'Pink Ice' shoots at five temperature treatments (ambient to ambient+3.1°C) in a greenhouse-based warming experiment.

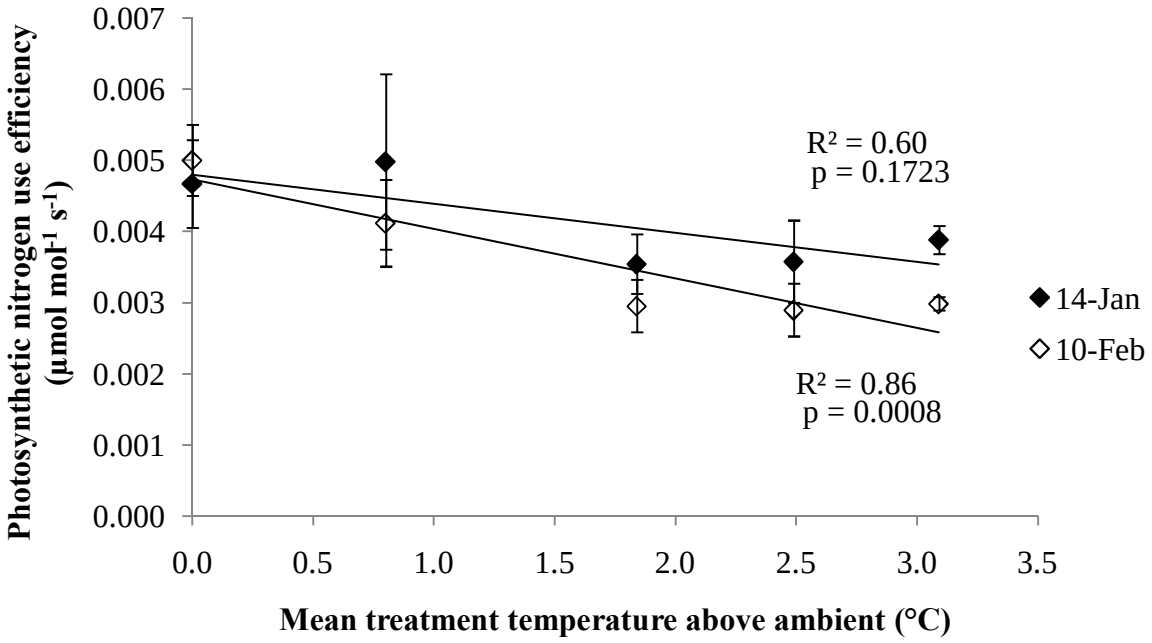
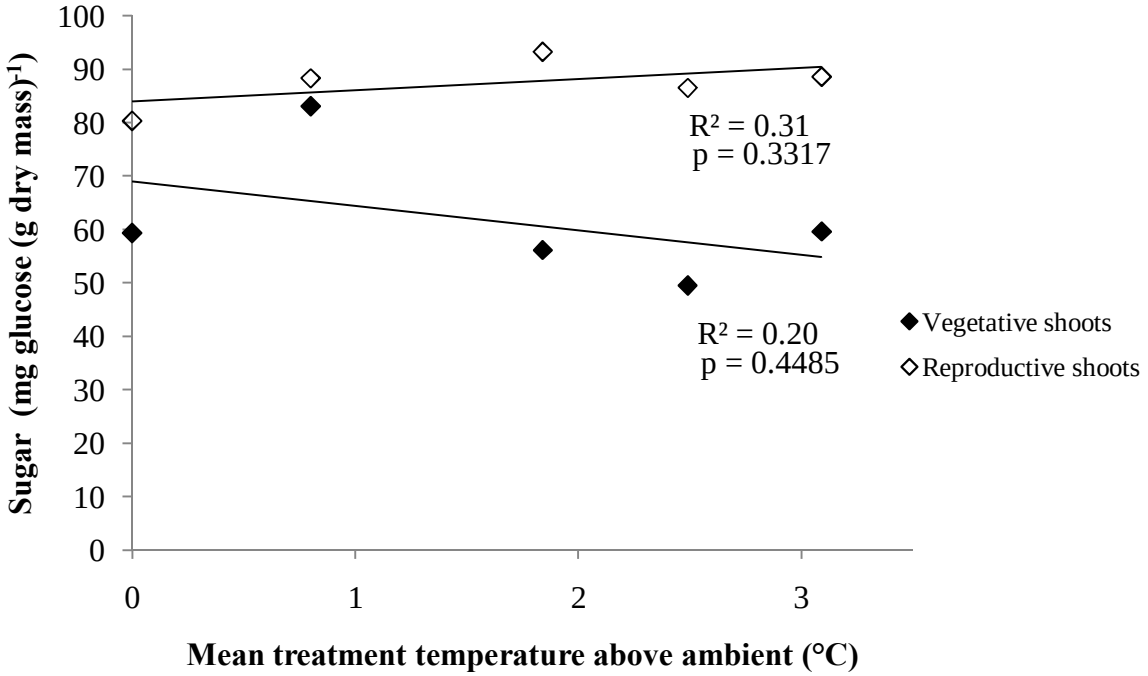


Fig. 42. Photosynthetic nitrogen use efficiency ± SE (n = 4) calculated for January and February 2009 for three-year-old *Protea* 'Pink Ice' plants at five temperature treatments (ambient to ambient +3.1°C) in a greenhouse-based warming experiment.

a)



b)

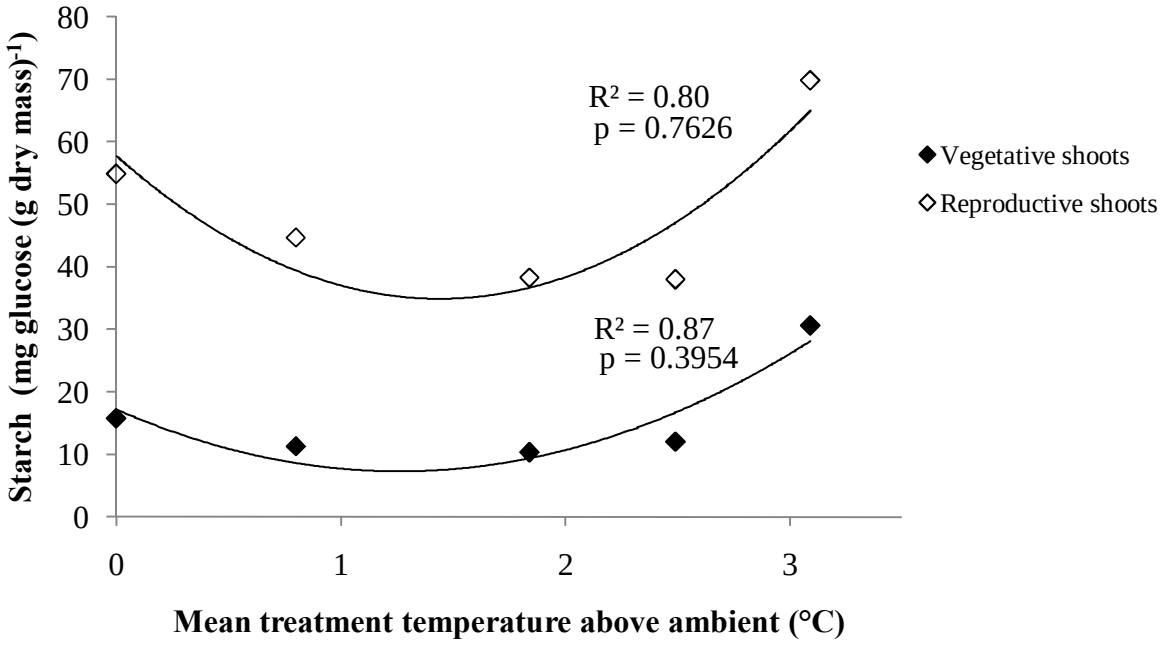


Fig. 43. Total soluble sugar (a) and starch (b) concentrations expressed as glucose equivalents in the leaves sampled on 14 January 2009 from the terminal flush of vegetative or reproductive shoots of three-year-old *Protea* ôPink Iceô plants for five temperature treatments (ambient to ambient+3.1°C) in a greenhouse-based warming experiment. (n = 1)

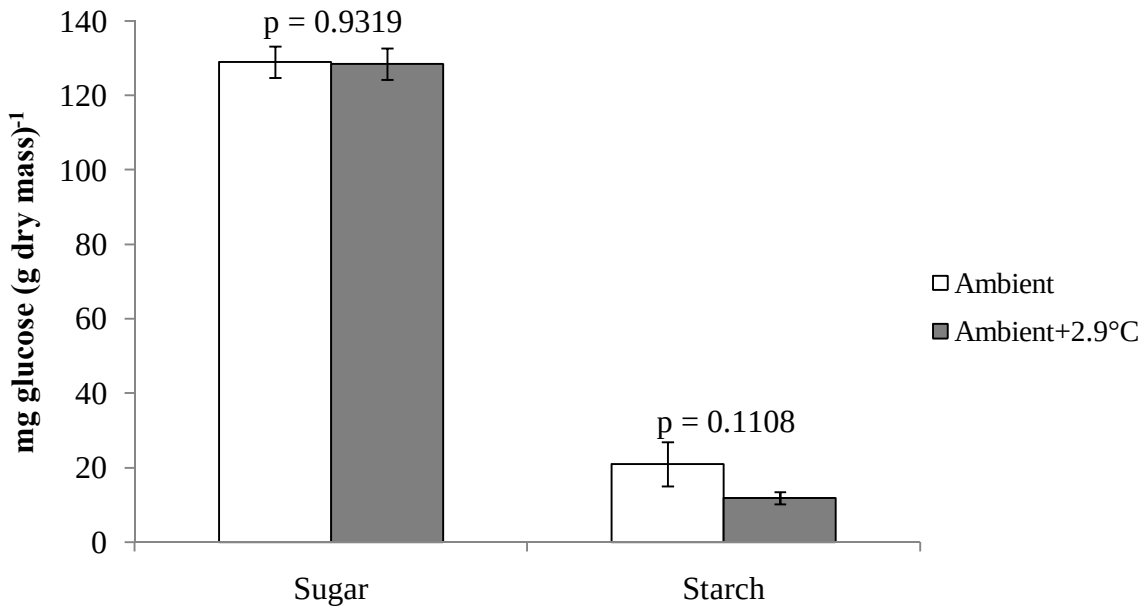


Fig. 44. Leaf soluble sugar and starch concentrations expressed as glucose equivalents in the terminal flush of flowering shoots of eight-year-old *Protea* 'Pink Ice' plants grown at two temperature treatments (ambient and ambient+2.9°C) in a field-based warming verification experiment.

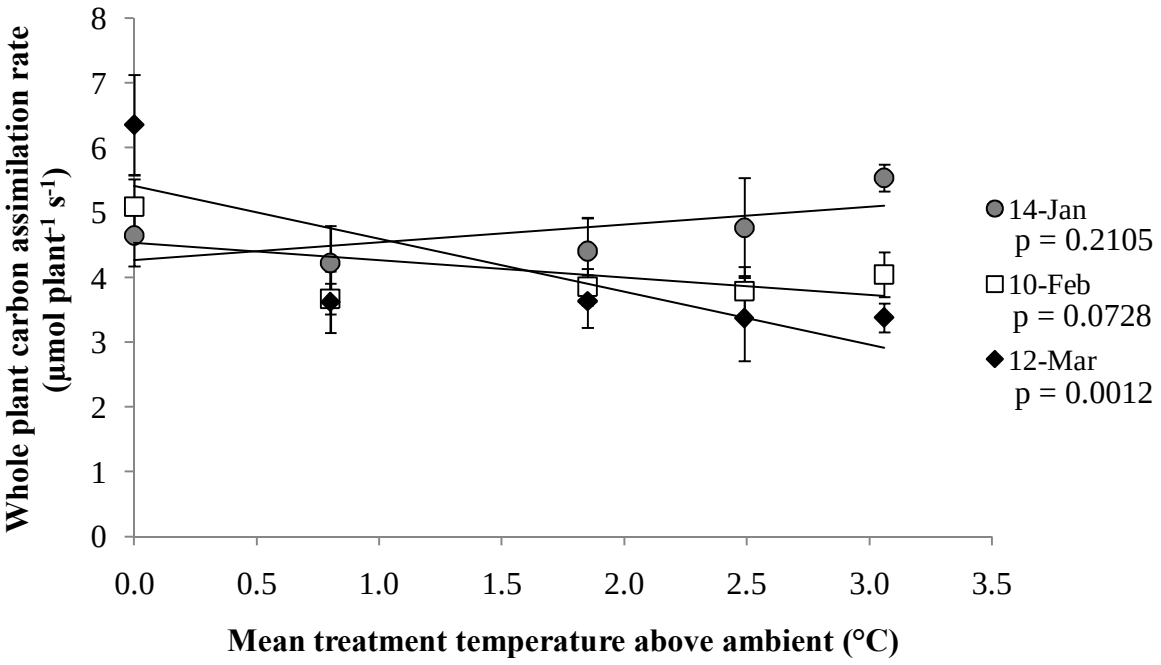


Fig. 45. Maximum potential whole plant carbon assimilation rate ± SE estimated from spot gas exchange data (A_{max}) recorded on 14 January, 10 February and 12 March 2009, and the total leaf area for three-year-old *Protea* 'Pink Ice' plants (n = 4) grown over five temperature treatments (ambient to ambient+3.1°C) in a greenhouse-based warming experiment.

Fig. 46. Shoot growth of three-year-old *Protea* ôPink Iceô in the greenhouse-based warming experiment. Longer vegetative and shorter reproductive shoots showing different leaf morphologies are compared.

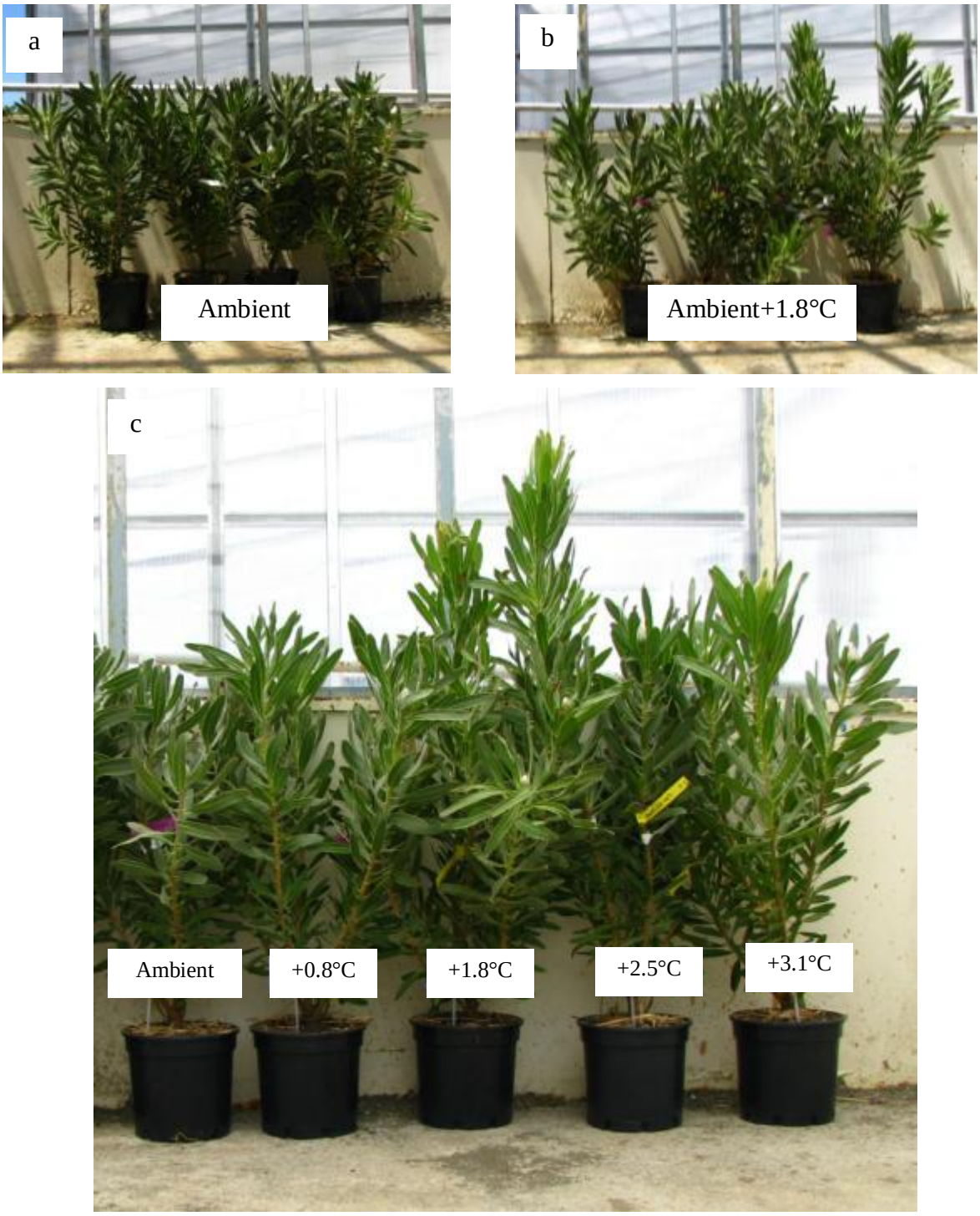


Fig. 47. Four three-year-old *Protea* ôPink Iceô plants grown under (a) ambient and (b) ambient+1.8°C treatment temperatures in a greenhouse-based warming experiment. A representative plant of each temperature treatment in a five temperature gradient (c) provide photographic evidence of a more vegetative nature (longer stems) of plants when grown at elevated compared to ambient temperatures.

5. Impact of post-initiation climate on autumn and spring initiated inflorescence systems of *Protea* cv. Pink Ice within a biennial pruning system

Abstract

In most of the commercially produced *Protea* selections and hybrids the majority of shoots initiate inflorescences on the spring flush. A limited number of shoots have the ability to initiate inflorescences earlier, on the autumn flush. These autumn initiated inflorescences display advanced flowering times which fall within a period of higher demand from European markets. Scientifically, this system where inflorescences can be initiated either in autumn or spring, during different climatic periods of the year, provides the opportunity to study and compare gas exchange, carbohydrate availability and vegetative as well as reproductive growth during cooler versus warmer periods. This information could assist in the modelling of impacts of increasing temperatures that, as a result of global climate change, might affect cultivated *Protea* inflorescence development, size and harvesting periods. In a commercial *Protea* orchard in Hopefield, South Africa, autumn flush initiated inflorescence development was compared to spring flush initiated inflorescence development. Gas exchange patterns were similar during the course of the season, with the highest net CO_2 assimilation rates measured during spring and early summer. The shoots which initiated inflorescences on the autumn flush generally had higher gas exchange capacities and on some measurement dates significantly so. It appeared as if a minimum stem diameter of 7.6 mm is required for floral initiation in *Protea* ôPink Iceô. Inflorescences which initiated on the spring flush, approximately three months after the inflorescences which initiated on the autumn flush, had a faster development rate, but had a significantly smaller final size (width) together with a lower dry mass. Spring flush initiated inflorescences were harvested on average a month later than autumn flush initiated inflorescences. The ambient temperature during inflorescence development almost certainly played a significant role in inflorescence growth rate and the time from inflorescence initiation to harvest. At the calculated optimum base temperatures of 9°C, the autumn flush initiated inflorescences required 41010 growing degree hours (GDH), whilst inflorescences which initiated on the spring flush required 35872 GDH. This study also revealed *Protea* ôPink Iceô shoots to require a minimum of four or five flushes to initiate an inflorescence.

5.1 Introduction

The European autumn and winter, September to February, is considered to be the optimum period for *Protea* export, as this is the period of highest demand (Hettasch *et al*., 1997; Gerber *et al*., 2001). The time of harvest, together with a minimum stem length and an unblemished bloom, determines the quality and price of these cut flowers, destined for a niche market (Hettasch *et al*., 1997). In the southern hemisphere, *Protea* ôPink Iceô (*P. compacta* R. Br x *P. susannae* Phill.) initiate inflorescences mainly on the spring flush. Anthesis is reached from January to May (Gerber, 2000). Commercially, flowering time in *Protea* can be manipulated by pruning (Greenfield *et al*., 1994; Nieuwoudt, 2006). Winter (June/July) pruning of *Protea* ôSylviaô advances the harvest time of inflorescences borne on the spring flush from January-February to October-December, when the majority of the inflorescences initiated on the autumn flush (Gerber *et al*., 2001). However, as the combined vegetative and reproductive cycles extend over 14 ï 16 months, a biennial bearing cycle with two blocks in alternating phases was recommended for commercial production (Gerber *et al*., 1995; Hettasch *et al*., 1997). The biennial management system ensures that inflorescences can be harvested annually, albeit from different blocks, and provides for a sustained income.

In a biennial bearing cycle *Protea* ôPink Iceô is pruned back to a basal bearer in June/July and grows vegetatively during the spring and summer of the first year, only to flower during the summer of the second year (Fig. 48). An advantage of the biennial cycle is that stems are longer, thus increasing the price (Gerber *et al*., 2001). It was observed that in a biennial cycle a limited number of shoots of ôPink Iceô initiate inflorescences terminally on the autumn flush, approximately nine to ten months after pruning (personal observation). The remaining shoots will only initiate inflorescences on the spring flush, five months later following the dormant winter period. These long-stemmed autumn-initiated inflorescences reach the European market from December to February, amidst the period of high demand, optimizing the price per stem.

As no information is available on how temperatures affect commercial *Protea* cultivation, especially floral induction and initiation, the different inflorescence initiation systems provides the opportunity to study the effect of post-initiation climate (including temperature as a primary variable) on the growth and development of inflorescence during different climatic periods.

The aim of this study was to monitor and compare the two distinctly different inflorescence initiation systems within a biennial bearing regime, namely the more common

spring-initiated system with the preferred ‘out of season’ autumn inflorescence initiated system. As such inflorescences initiate and develop during different times of the year, it provides an opportunity to monitor inflorescence development under two different temperature regimes. This comparative study could assist researchers in modelling how inflorescence development of *Protea* is influenced by cooler and warmer periods. Assessments were made with respect to gas exchange and carbohydrate availability, as well as the progression of vegetative and reproductive growth within the two flowering systems.

5.2 Materials and methods

Experimental site and pruning system: Arnelia farm, Hopefield, South Africa (33°02’S; 18°20’E) was selected as an experimental site. Six-year-old *Protea* ‘Pink Ice’, planted in a double row system, spaced 2.2 x 1 m within the rows with a 3 m service way in a north-east to south-west row orientation, was grown on a Strandveld sandy soil and were drip irrigated. In-line fertigation with nitrogen, potassium, phosphorous and iron was supplied weekly during winter and was increased progressively to once a day during summer, to coincide with the period of inflorescence development, according to commercial growers’ practice.

Plants were managed according to a biennial bearing pruning system, originally developed for *Protea* ‘Carnival’ (Fig. 48) (Gerber *et al*., 1995; Hettasch *et al*., 1997). Plants in the vegetative phase (‘off block’) were selected in 2008 for this study (Table 11). All plants were pruned back to twenty bearers per plant in July 2007. Newly sprouting lateral buds were thinned to two remaining shoots per bearer in November 2007, allowing 40 harvestable shoots to develop per plant. On 10 April 2008, 30 four- or five-flush shoots and 30 two- or three-flush shoots were tagged on randomly selected plants in a single row within the block. This selection was made to include both vigorous shoots that may have the capacity to initiate inflorescences on the autumn flush, as well as shoots of a lower quality that will require the later occurring and more vigorous spring flush to initiate an inflorescence.

Vegetative measurements: Baseline measurements recorded on 10 April 2008 on the 30 tagged shoots of each shoot type. Shoot length (cm), stem diameter (mm) measured at the upper intercalation between the terminal and subterminal flush, total number of flushes on the shoot and number of leaves per flush were recorded. Thereafter, shoot growth as well as timing of inflorescence initiation and the progression of inflorescence development were recorded on a biweekly basis throughout summer, autumn and spring of 2008 and up to the

end of summer 2009. Data was recorded only every four weeks during the winter of 2008 as no visibly active growth occurred. Lateral shoots sprouting in axillary positions after inflorescence initiation were removed as early as possible, as in commercial practice.

Gas exchange: Measurements of gas exchange were taken with a LI-6400 Portable Photosynthesis System (LI-COR, Lincoln Nebraska, USA) as described previously in Chapter 3.2.1. Spot gas exchange measurements at ambient temperature included the maximum rate of light-saturated net CO_2 assimilation (A_{max}), light- and CO_2-saturated rate of net CO_2 assimilation (A_{sat}), dark respiration rate (R_d) and stomatal conductance (g_s). These recordings were made every two to four weeks starting on 17 April 2008 on a single leaf on the terminal flush of 15 tagged shoots per shoot type. From 30 July 2008 onwards data collection proceeded only on the 30 four- or five-flush vigorous shoots. As the fate of the terminal bud was unknown in April 2008 when shoots were selected, only seven of the vigorous shoots initiated inflorescences on the autumn flush, whereas the other 23 shoots remained vegetative, whereafter it proceeded to flush in spring 2008 and then flowered.

On 30 October 2008, A/c_i curves were generated and photosynthetic parameters, which included the modelled A_{sat}, CO_2 compensation point, apparent carboxylation efficiency and the predicted R_{day}, were calculated as described in Chapter 3.2.3. A single leaf on the terminal flush was selected from four shoots per inflorescence initiation shoot type.

Total non-structural carbohydrate determination: Concurrent with gas exchange measurements 25 leaves were harvested from shoots comparable to the tagged shoots on which gas exchange measurements were performed. Leaf fresh mass, length, breadth, area (Portable Area Meter, Li-3000A, LI-COR, Lincoln, Nebraska, USA) and dry mass (Forced circulation incubator, FSIE 16, Labcon (Pty) Ltd, Roodepoort, South Africa) were determined. The oven-dried leaves were subsequently pooled in multiples of five, milled and stored at -20°C until sugar and starch analysis (described in Chapter 4.2.5). Total soluble sugar and starch were analysed spectrophotometrically (Cary 50, Bio UV-visible spectrophotometer, Varian, Varian Australia Pty Ltd., Mulgrave, Victoria, Australia) using anthrone as a colour reagent, and glucose as a standard.

Harvest: All flowering shoots were harvested at the commercial ósoft tipô harvestable stage. Harvest commenced in December 2008 and ceased in March 2009. Shoots were cut at the bearer, to include the entire basal flush. Shoot length, stem diameter at the upper intercalation

between the terminal and subterminal flush, and the final number of flushes were recorded. The leaves and stem of each flush were separated. Subsequently, the length of each flush, number of leaves per flush, total leaf area per flush (Portable Area Meter, Li-3000A, Li-Cor, Lincoln, Nebraska, USA), and the fresh and dry mass of flush stem and leaves were measured. Separated leaves and stems were then dried at 60°C in a draught oven (Forced circulation incubator, FSIE 16, Labcon (Pty) Ltd, Roodepoort, South Africa) until the dry weight remained stable.

Inflorescence length and width were recorded, with the width measured approximately one third from the base of the inflorescence. Thereafter the inflorescence was dissected into bracts, florets and receptacle. Fresh and dry mass of the respective floral parts were recorded.

Climatic data: Hourly air temperatures were obtained from the Koperfontein Agricultural Research Council (ARC) weather station (33°06ôS; 18°24ôE), within 10km of the experimental site. Mean, maximum and minimum temperatures as recorded at Koperfontein from March 2008 to March 2009 are presented in Fig. 49. Climatic conditions on days on which gas exchange was measured is presented in Fig. 50. Notably, the light intensity during winter is relatively high in Hopefield.

Heat units: Heat units were calculated as growing degree hours (GDH) where,

GDH = (Measured mean hourly temperature (T_m°C)) ï (Base temperature (T_b°C)),

with GDH = 0 when $T_b > T_m$ as negative values do not implicate plant growth.

An upper temperature limit of 35°C was selected as it may be proposed that growth ceases at these temperatures and above. The heat unit requirements for two phenological stages, budbreak to the cessation of shoot growth, and inflorescence development from visible detection to anthesis were calculated. The optimum base temperature (T_b) for each phenological stage was identified as the temperature where the GDH sum displayed the minimum coefficient of variation (Rattigan & Hill, 1986). ôPink Iceô data from the experimental sites in Stellenbosch (Floraland-Etshwaleni farm) and Hopefield (Arnelia farm) were used together with climatic temperature recorded at the Agricultural Research Council (ARC) weather stations at Nietvoorbij (Stellenbosch) and Koperfontein (Hopefield).

Statistical analysis: Data obtained from spot gas exchange measurements, the A/c_i curves and harvest parameters were analysed by ANOVA using the PROC GLM (SAS Institute Inc., 2003) and mean separation of LSD were performed at $p<0.05$.

5.3 Results

Gas exchange: A_{max} reached optimum values (>12 µmol m^{-2} s^{-1}) from August to mid-November 2008 (Fig. 51a). A_{max} values were slightly higher in the shoots which initiated inflorescences on the autumn flush compared to those initiating in spring and significantly so on 17 and 24 April, 9 October and 6 November 2008. The seasonal A_{max} pattern reflected the seasonal temperatures accurately, but inversely, in that A_{max} reached maximum values at lower temperatures and vice versa (Fig. 50). A_{sat} values were highest in October and November for both shoot types and significantly higher during April 2008 and on 6 November 2008 in shoots bearing autumn initiated inflorescences compared to shoots that initiated inflorescences in spring (Fig. 51b). R_d values, irrespective of shoots type, were the lowest during May and June 2008 (Fig. 51c). Highest R_d values were obtained from 23 October 2008 to 12 February 2009, a period which coincided with the highest mean temperatures of ~25°C since the previous autumn in April 2008 (Fig. 50). Shoots with autumn initiated inflorescences had higher R_d values from April to October 2008 and significantly so on 30 July and 23 October 2008, after which shoots with spring initiated inflorescences had higher R_d values, particularly during November 2008. For both shoot types stomatal conductance (g_s) peaked in July and August 2008 and was significantly higher in shoots with autumn initiated inflorescences during May, November and December 2008 compared to shoots that initiated inflorescences during spring (Fig. 51d). An exception to this trend was during late winter when g_s was similar for both shoot types (Fig. 51d). The highest g_s values were found to reflect the lowest maximum temperatures during the year (Fig. 50).

Parameters calculated from the A/c_i curve as recorded on 30 October 2008 showed that the modelled maximum rate of light- and CO_2-saturated net CO_2 assimilation (A_{sat}) was significantly higher in the autumn inflorescence initiation shoots than in shoots that initiated inflorescences in spring (Table 12), similar to the trend recorded for spot gas exchange A_{sat} measurements of 6 November 2008 (Fig. 51b). There were no significant differences in the CO_2 compensation point, apparent carboxylation efficiency or predicted rate of day respiration (R_{day}) (Table 12).

Vegetative growth: In April 2008, shoots which would initiate inflorescences on the autumn flush had significantly thicker stems than shoots which initiated inflorescences on the spring flush (Table 13). There were no differences in shoot length (Table 13). Shoot growth patterns of the two different inflorescence initiation shoot types are shown in Fig. 52. For the shoots which initiated inflorescences in autumn, stem elongation ceased in June 2008. The remaining shoots that did not initiate inflorescences in autumn produced a spring growth flush, after which inflorescence initiation naturally led to the cessation of shoot elongation during November 2008.

Reproductive growth: Inflorescences borne on the spring flush followed a linear growth trend for width (Fig. 53a), whereas inflorescence length displayed an exponential growth curve, with a lag phase or slower growth rate followed by a more rapid linear growth pattern (Fig. 53b). Inflorescences borne on the autumn flush had a slower growth rate during winter and early spring, exhibiting an exponential growth curve for both the width and length (Fig. 53). The growth rate increased rapidly in mid-spring and early summer. All fitted trendlines produced high R^2 values.

Harvest: Stem diameter, stem length, leaf dry mass and leaf area were significantly higher at harvest in shoots that initiated inflorescences in spring compared to shoots that initiated inflorescences in autumn (Table 14a). Mean days to harvest differed significantly as autumn flush initiated inflorescences were harvested 37 days earlier on average than the spring initiated inflorescences (Table 14a). Autumn initiated inflorescences were significantly wider, but not longer than spring initiated inflorescences (Table 14b). Total inflorescence dry mass as well as bract, floret and receptacle dry mass were significantly higher in the autumn initiated inflorescences compared to spring initiated inflorescences (Table 14b).

Heat units: A base temperature for the first phenological phase, bud break to end of shoot elongation, could not be calculated. For the reproductive phenological phase, inflorescence initiation to anthesis, the lowest coefficient of variance was calculated at 9°C. A total of 41010 GDH was required to complete inflorescence development of autumn initiated inflorescences, whereas 35872 GDH were required for spring initiated inflorescences to reach anthesis (Table 15).

Sugar and starch analysis: Leaf total soluble sugar content (Fig. 54a) in the terminal flush decreased from approximately 130 to 95 mg glucose (g dry weight)$^{-1}$ during winter (April 2008 to August 2008) for both shoot types. An increased was noted from September 2008 to harvest, with significantly higher values during summer (6 November 2008 to 29 January 2009) in autumn initiation shoots, except on 9 and 23 October 2008 when spring initiation shoots had higher leaf sugar concentrations. Leaf starch concentration of between 80-100 mg glucose (g dry mass)$^{-1}$ was calculated for autumn 2008. The starch content decreased during winter, whereafter an increase from July to October 2008 to a maximum of 120 mg glucose (g dry mass)$^{-1}$ was recorded (Fig. 54b). From October 2008 to harvest 2009 no clear pattern could be observed in the starch concentration, although shoots that initiated inflorescences in autumn had a significantly higher leaf starch concentration from October to November 2008, after which shoots with spring initiated inflorescences had a significantly higher starch content (Fig. 54b).

5.4 Discussion

It is apparent that natural variation in seasonal temperature determined gas exchange patterns as trends in seasonal gas exchange were similar in both the inflorescence initiation systems (Fig. 51). In general, shoots on which inflorescences initiated in autumn had slightly superior or similar gas exchange characteristics compared to shoots with spring initiated inflorescences. Secondary control of gas exchange was probably at the level of the phenological stage. It is known that fruiting trees have higher specific rates of photosynthesis (expressed on a leaf area basis) compared to trees with zero crop load (Webster, 2005) owing to the sink effect. The developing inflorescence borne on the autumn flush may have contributed to the increased photosynthetic capacity of the terminal flush, whilst the spring flush was still vegetative. This was validated during October 2008 by comparative data from A/c_i curves which indicated greater photosynthetic capacity of shoots that initiated inflorescences in autumn (Table 12). During this time, autumn initiated inflorescences were larger in size compared to the spring initiated inflorescences, therefore requiring more assimilates. Alternatively, autumn flush inflorescence shoots simply may have had a greater photosynthetic capacity to begin with, as they had significantly thicker stems in April 2008 as well as more leaves with a larger total leaf area (Table 13).

From September to October 2008 A_{max} reached maximum values, >10µmol m^{-2} s^{-1}, in both shoot types (Fig. 51a). This period coincided with favourable environmental conditions

promoting growth during spring (Fig. 49). During August and September 2008 A_{sat} values were similar in both shoot types, probably due to an increased photosynthetic capacity as upregulated by the growth demand of the new developing sink, the spring flush (Fig. 51b). After October 2008 dark respiration rate in the spring flush inflorescence shoots exceeded that of the autumn flush inflorescence shoots (Fig. 51c), possibly as a result of increased sink activity during the summer when first the maturation of the spring flush and then the inflorescence development was taking place at a rapid rate (Fig. 52, 6).

Studies on *Protea* óCarnivalô (Greenfield *et al*., 1994) and *Banksia* (Rieger & Sedgley, 1996) established that vegetative growth is a prerequisite for floral initiation. At least two flushes are considered essential for flowering in *Protea* (Coetzee & Littlejohn, 2001) The minimum number of flushes is probably species or cultivar specific. *Protea* óCarnivalô (*P. neriifolia* x *P. compacta*) initiates inflorescences on two- or three-flush shoots (Greenfield *et al*., 1994; Hoffman, 2006). However, in a greenhouse-based warming experiment (Chapter 4) with potted plants it was observed that only two ôPink Iceô shoots flowered on a three-flush shoot, whilst in various field experiments (Chapters 4, 6) only one shoot flowered on a four-flush shoot, with no shoots flowering on a three-flush shoot. Similar to this study, no inflorescences initiated on three- or four-flush shoots, but rather on a shoot with a minimum of five flushes. It appears to be an exception should ôPink Iceô, a vigorous cultivar, initiate inflorescences on a shoot with less than five flushes.

For *Protea* óCarnivalô, Hoffman (2006) described a minimum stem diameter requirement of 7 mm for floral initiation to take effect óout of seasonô. In this study the stem diameter differed significantly between the autumn and spring floral initiation systems in April 2008 (Table 13), which emphasizes the minimum vegetative requirement of *Protea*. All the shoots that initiated inflorescences on the autumn flush had a mean stem diameter of 7.89 ± 0.3 mm, and a minimum requirement of 7.6 mm, whilst all shoots that initiated inflorescences on the subsequent spring flush had a stem diameter of less that 7.6 mm during April 2008 when shoots where selected. Therefore it seems as if *Protea* ôPink Iceô required at least a 7.6 mm stem diameter to allow inflorescence initiation. This value is higher than recorded for óCarnivalô, but could be due to the vigorous growth habit of *Protea* ôPink Iceô.

Autumn initiated inflorescence development required approximately 500 GDH more than spring initiated inflorescences (Table 15). The additional GDH required could be linked to the larger inflorescences as well as additional dry mass observed in the autumn flush inflorescences compared to spring flush inflorescences. The GDH accumulation period also differed significantly between initiation systems. The autumn flush inflorescences took three

months (on average 100 days) longer to accumulate sufficient GDH compared to the spring flush inflorescences (Table 15). The faster growth rate of spring initiated inflorescences could probably be linked to increased temperatures during the spring flush inflorescence growth period. In addition, changes in relative humidity and soil moisture also contribute seasonal difference.

Inflorescence width growth on the spring flush followed a linear growth curve, whereas inflorescences width growth on the autumn flush followed an exponential growth curve. This is comparable to what Gerber *et al*. (2001) found for *Protea* ôSylviaô on spring and autumn flushes, respectively.

This study provides evidence that inflorescences developing under cooler, milder climates may be larger and assimilate more dry mass compared to inflorescences developing under warmer and drier climates. Inflorescences subtended by the autumn flush also developed at a slower rate. This was most likely due to lower, more moderate winter temperatures. Higher spring and summer temperatures increased the development rate of inflorescences subtended by the spring flush. Even so, they were generally smaller in size with a lower number of bracts, florets and receptacle dry mass (Table 14b). Increased temperatures coinciding with the elongation and maturation of the spring flush could possibly reduce cell division which might result in decreased flower size of spring inflorescences.

In future, smaller inflorescences under elevated temperatures may be expected for a particular cultivar within the same production area. This was previously observed when inflorescence size and appearance from cooler and warmer areas were compared (G. Nieuwoudt, pers. comm., 2008). *Protea* ôPink Iceô, derived from the same clonal propagation material, but produced in a warmer production area were noted to be distinctly smaller, with a slightly bronze sheen and had a dark purple discolouration noticeable on the inside of the tips of the inner bracts compared to the larger more shiny pink inflorescences typical of cooler production areas (G. Nieuwoudt, pers. comm., 2008). These colour variants of ôPink Iceô from warmer production areas is not favoured by the export market, therefore resulting in lower prices.

Autumn initiated inflorescences were harvested approximately a month earlier than inflorescences subtended by the spring flush. As this is of significant commercial importance, further studies should aim to increase and favour the number of these shoots with inflorescences initiated in autumn with such advanced flowering times. Focus should be placed on the characterisation of shoots that initiate autumn inflorescences, together with developing pruning regimes in combination with growth regulator application, to promote

inflorescence initiation on the autumn flush (Hoffman, 2006). Inflorescences that initiate on the autumn flush develop during the cooler season; should temperatures rise, the development rate of ôPink Iceô initiated on the spring flush might decrease significantly if the number of days with temperatures exceeding 35°C during summer increases, compared to the current situation. At temperatures above 35°C photosynthetic rates decline significantly for most plants and also in *Protea*. In addition to slower growth rates, multiple buds and calluses formed in *Macadamia* at 35°C which later desiccated (Trochoulias & Lahav, 1983). Multiple buds sprouting from lateral positions in ôPink Iceô could further delay the harvest time of spring flush inflorescences in warm production areas compared to autumn flush inflorescences.

The selection of new prospective, suitable production areas for *Protea* cultivation should take into consideration the change in flowering time, as well as morphological differences that may change the current product specifications. The need for evaporative cooling when temperatures exceed 30 to 35°C may become a reality in warmer production areas when the aim is to produce a product of consistently high quality. However, evaporative cooling is costly and in addition, predictions are that water available for agricultural use, especially in the Western Cape, will come under increasing pressure (Louw & Callaway, 2008).

5.5 References

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Table 11. Time of pruning and inflorescence initiation of *Protea* ôPink Iceô in the óoff blockô of a biennial bearing system with the focus on shoots which initiate inflorescences on the autumn or spring flush.

Flush subtending inflorescence	2007	2008			2009		
	July	May	Aug/Sept	Oct/Nov	Dec/Feb	Mar/Apr	July
Autumn	Prune	Inflorescence initiation and development			Harvest		Prune
Spring	Prune		Spring flush	Inflorescence initiation and development		Harvest	Prune

Table 12. Gas exchange capacity derived from A/c_i curves as recorded on 30 October 2008 on the terminal flush of *Protea* ôPink Iceô of flowering shoots, where inflorescences initiated either on the autumn or spring flush (n=4).

Flush subtending the inflorescence	Modelled A_{sat} (µmol m^{-2} s^{-1})	Compensation point	Apparent carboxylation efficiency	Predicted R_{day} (µmol m^{-2} s^{-1})
Autumn	44.8 a[z]	73 a	0.157 a	10.5 a
Spring	38.2 b	72 a	0.132 a	9.1 a
		Pr>F		
	0.0221	0.9710	0.2729	0.5645

[z]Treatments with different letters differ significantly at LSD, P<0.05.

Table 13. Baseline shoot length, stem diameter and whole shoot leaf area of shoots, which subsequently flowered on either the autumn or spring flush, measured on 10 April 2008, prior to inflorescence initiation.

Flush subtending the inflorescence	Number of shoots	Shoot length (cm)	Stem diameter[x] (mm)	Whole shoot leaf area (cm^2)
Autumn	7	70.0 ns[z]	7.89 a[y]	375.1 a
Spring	23	70.3	7.48 b	322.8 b
Pr>F		0.9133	0.0463	0.0055

[z] Nonsignificant

[y] Treatments with different letters differ significantly at LSD, P<0.05.

[x] Measured at the upper intercalation between the terminal and subterminal flush.

Table 14. Vegetative (a) and reproductive (b) characteristics measured at the final harvest of *Protea* 'Pink Ice' shoots where inflorescences initiated terminally on either the autumn or spring flush.

(a)

Subtending flush	Harvest date ± SE days	Stem diameter (mm)	Stem length (cm)	Stem dry mass (g)	Leaf dry mass (g)	Leaf area (cm^2)
Autumn	31 Jan ± 10 a[z]	10.6 b	82.8 b	47.0 ns[y]	48.6 b	1876 b
Spring	8 Mar ± 3 b	11.6 a	97.1 a	52.4	60.9 a	2428 a
			Pr>F			
	<0.0001	0.0087	<0.0001	0.1402	0.0005	0.0002

[z] Treatments with different letters differ significantly at LSD, P<0.05.

[y] Nonsignificant

(b)

Subtending flush	Inflorescence width (mm)	Inflorescence length (mm)	Dry mass (g)			
			Total	Bracts	Florets	Receptacle
Autumn	59.4 a[z]	103.7 ns	36.4 a	12.0 a	18.1 a	6.49 a
Spring	55.0 b	102.0	30.7 b	10.1 b	15.2 b	5.43 b
		Pr>F				
	0.0480	0.3425	0.0074	0.0044	0.0408	0.0121

[z] Treatments with different letters differ significantly at LSD, P<0.05.

[y] Nonsignificant

Table 15. The accumulated growing degree hours (GDH) for inflorescence development (initiation to anthesis) at various base temperatures (°C) are indicated. The number of days from inflorescence initiation to harvest ± SE is shown for *Protea* 'Pink Ice' plants in two field production areas, grown under five different temperature regimes. An upper temperature limit of 35°C was set. Values in **bold** specify the base temperature with the lowest %CV. Mean, standard deviation (SD) and coefficient of variation (%CV) are reported.

Production Area	Cultivation conditions	Period	Days from inflorescence initiation to anthesis	Base temperature (T_b)						
				6°C	7°C	8°C	**9°C**	10°C	11°C	12°C
Stellenbosch	Floraland commercial	14-Nov-08 – 24-Mar-09	130 ± 5	50439	47582	44725	**41868**	39012	36163	33322
Stellenbosch	Floraland warming - Ambient	05-Nov-08 – 14-Mar-09	124 ± 3	44654	41737	38820	**35903**	32987	30076	27185
Stellenbosch	Floraland warming - Ambient+2.9°C	28-Oct-08 – 01-Mar-09	131 ± 5	48009	45266	42523	**39780**	37037	34294	31553
Hopefield	Arnelia commercial autumn	01-Jun-08 – 31-Jan-09	245 ± 14	56832	51350	46074	**41010**	36162	31538	27200
Hopefield	Arnelia commercial spring	01-Nov-08 – 08-Mar-09	130 ± 4	44608	41695	38782	**35872**	32971	30086	27228
			Mean	48908	45526	42185	**38887**	35634	32431	29298
			SD	5063	4101	3339	**2837**	2634	2704	2934
			%CV	10.35	9.01	7.92	**7.29**	7.39	8.34	10.01

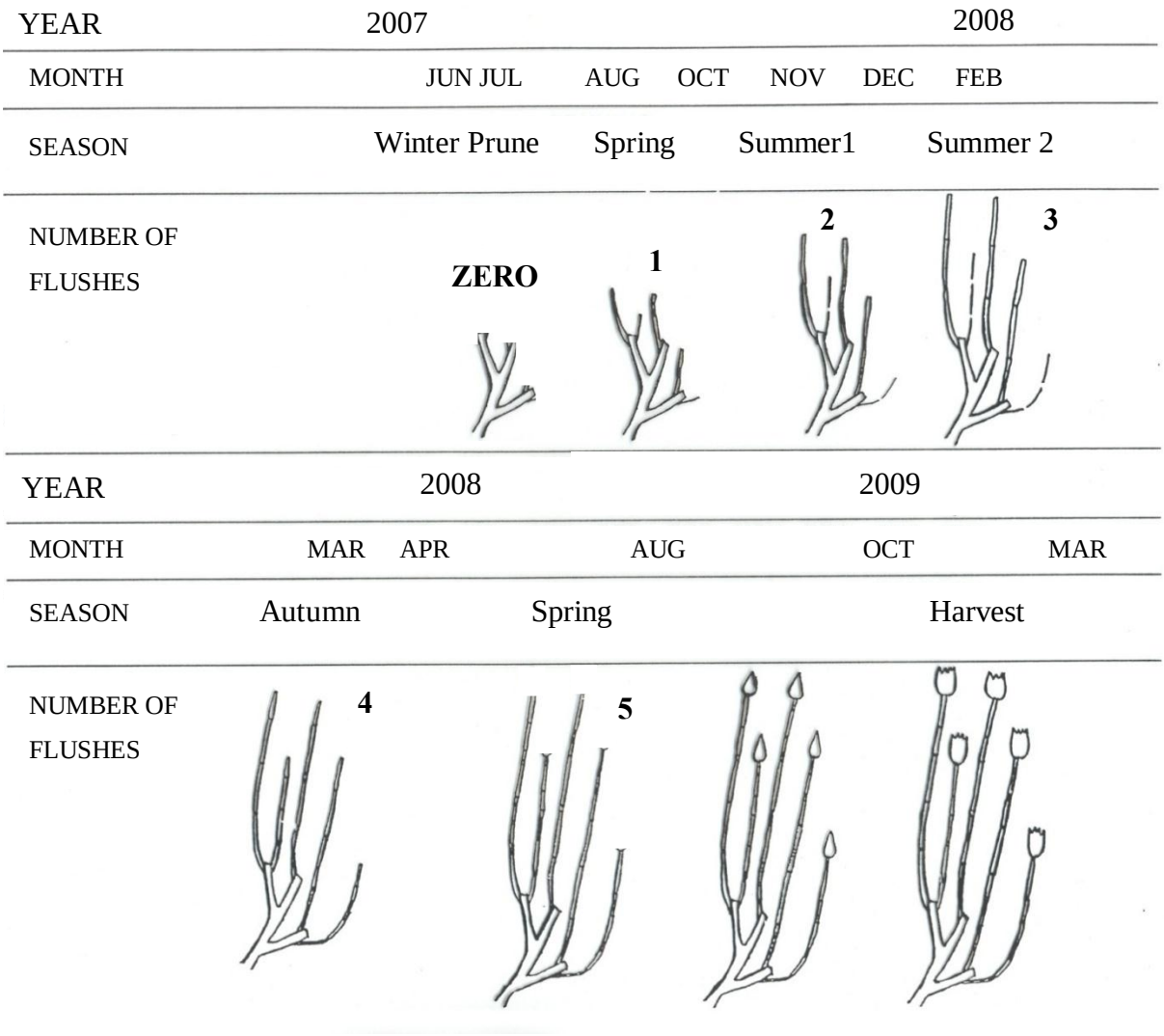


Fig. 48. Visual representations of Protea shoot growth, inflorescence initiation and development on the spring flush after pruning according to in a biennial bearing system. Only one of the alternating blocks, for example the óon blockô is shown. The óon blockô was therefore harvested in 2007 and again in 2009, whilst the óoff blockôwas harvested in 2008.

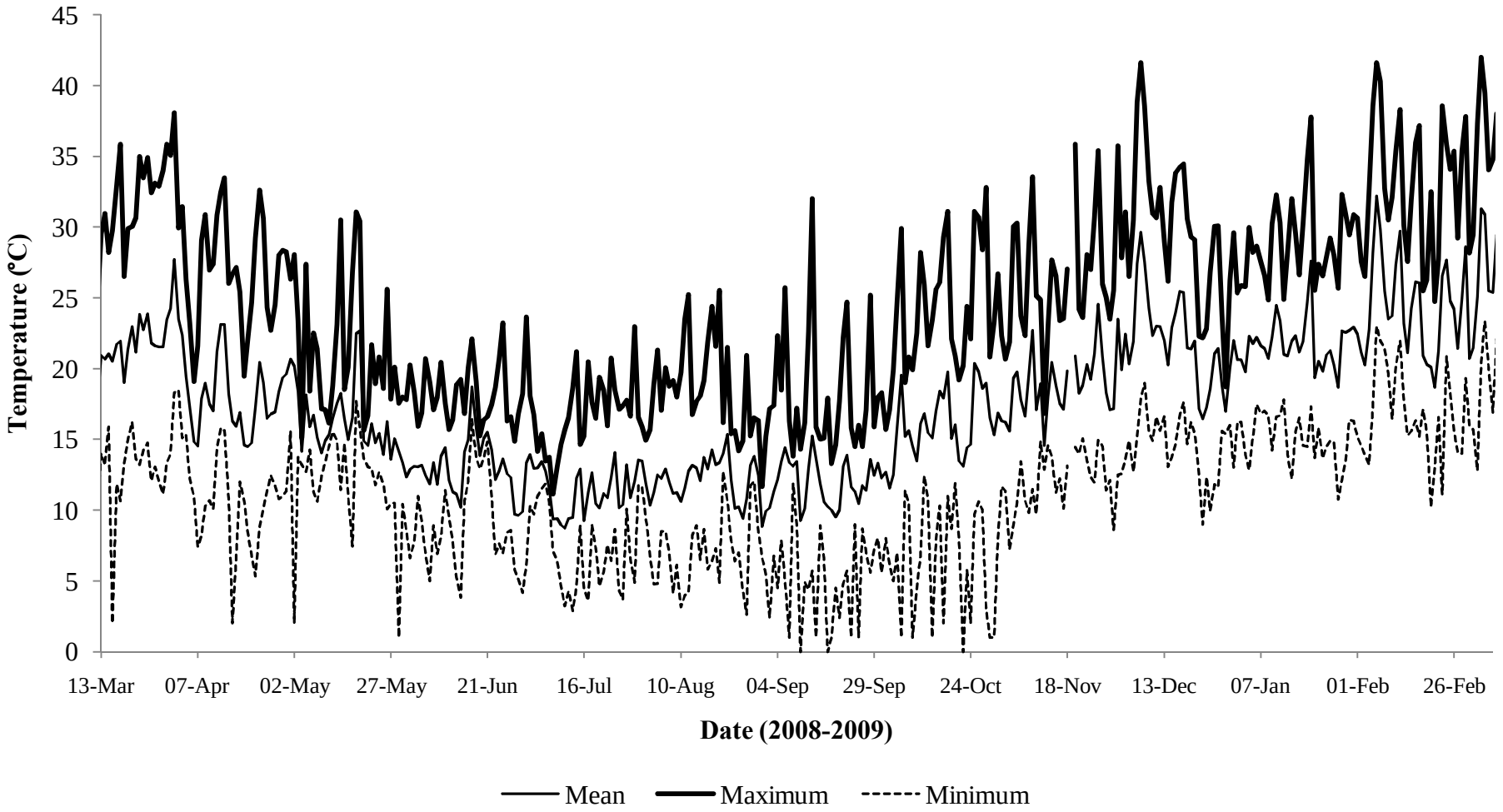


Fig. 49. Daily mean, maximum and minimum temperatures from March 2008 to March 2009 obtained from the Agricultural Research Council (ARC) weather station at Koperfontein, 10km from Arnelia farm, Hopefield.

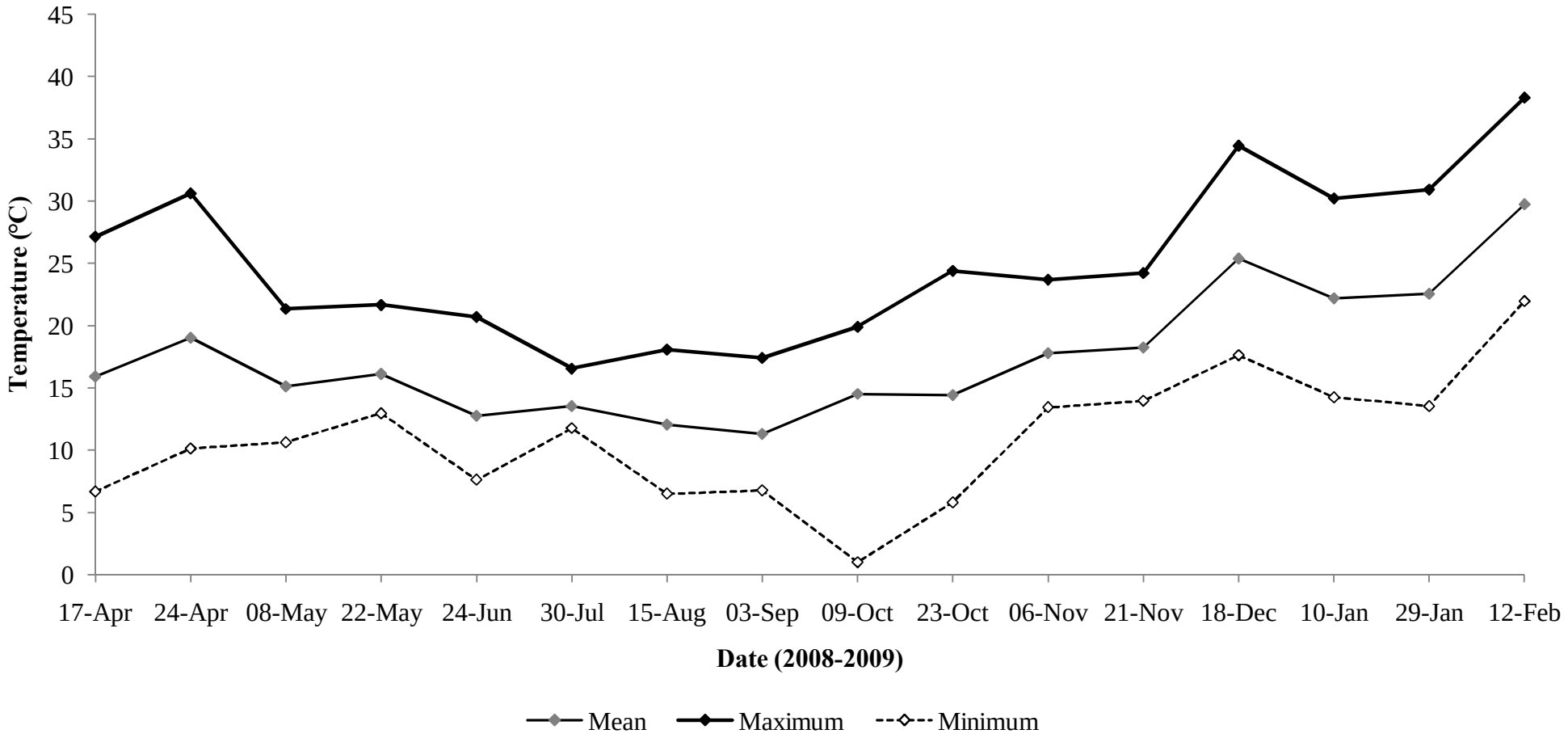


Fig. 50. Mean, maximum and minimum temperatures of the specific days on which spot gas exchange were recorded on six-year-old *Protea* ópink Iceô. Data were obtained from the Agricultural Research Council (ARC) weather station at Koperfontein, 10 km from Arnelia farm, Hopefield.

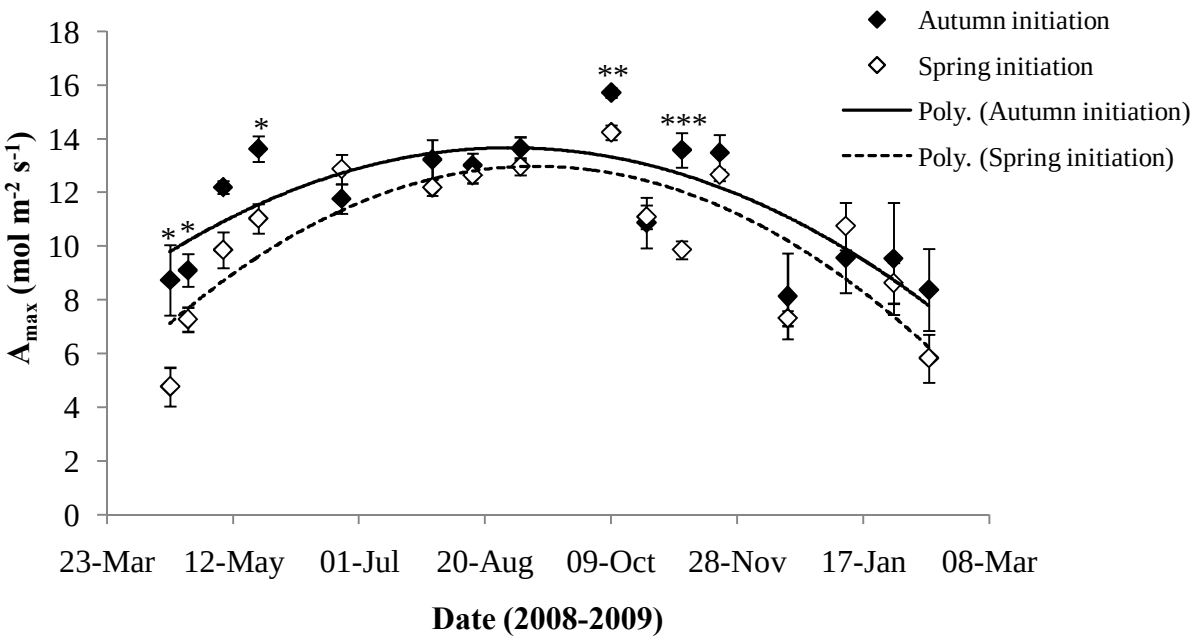


Fig. 51a. Maximum rate of light-saturated net CO_2 assimilation (A_{max}) ± SE for six-year-old *Protea* 'Pink Ice' from April 2008 to February 2009 on shoots which initiated inflorescences terminally on the autumn or spring flush.

* Significantly different at P<0.05; ** Significantly different at P<0.01; *** Significantly different at P<0.001.

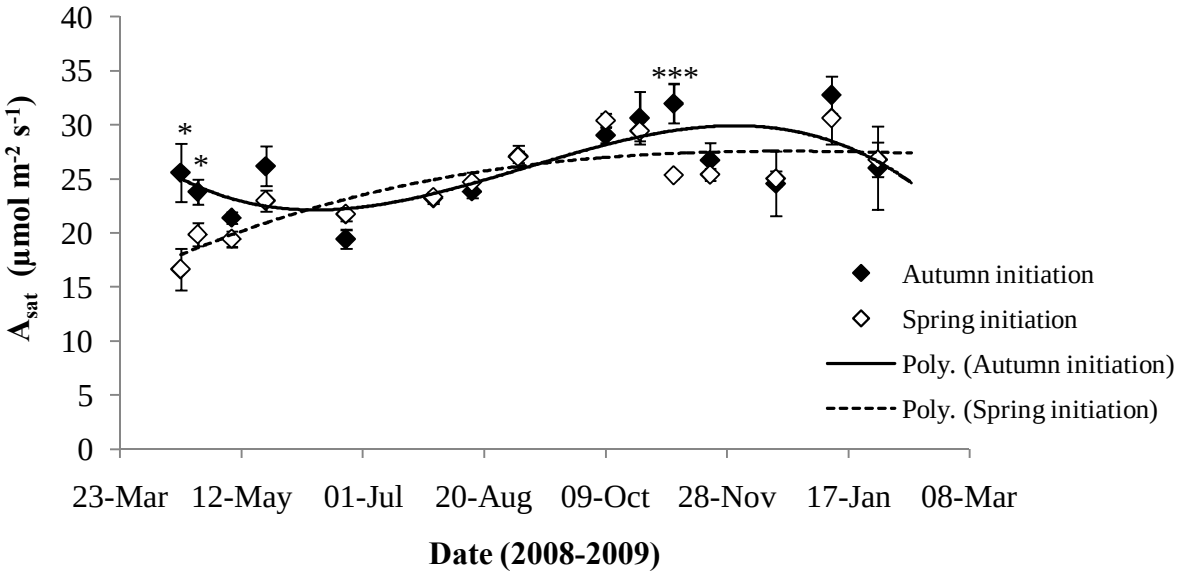


Fig. 4b. Light- and CO_2-saturated rate of net CO_2 assimilation (A_{sat}) ± SE for six-year-old *Protea* 'Pink Ice' from April 2008 to February 2009 on shoots which initiated inflorescences terminally on the autumn or spring flush.

* Significantly different at P<0.05; ** Significantly different at P<0.01; *** Significantly different at P<0.001.

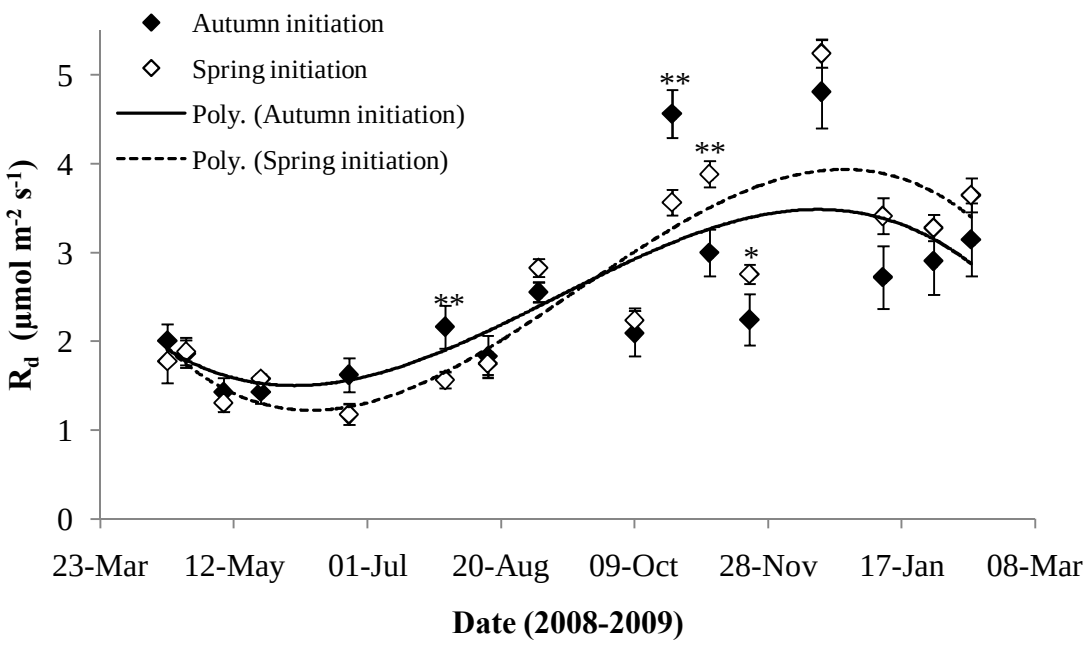


Fig. 4c. Dark respiration rate (R_d) ± SE for six-year-old *Protea* 'Pink Ice' from April 2008 to February 2009 on shoots which initiated inflorescences terminally on the autumn or spring flush.

* Significantly different at P<0.05; ** Significantly different at P<0.01; *** Significantly different at P<0.001.

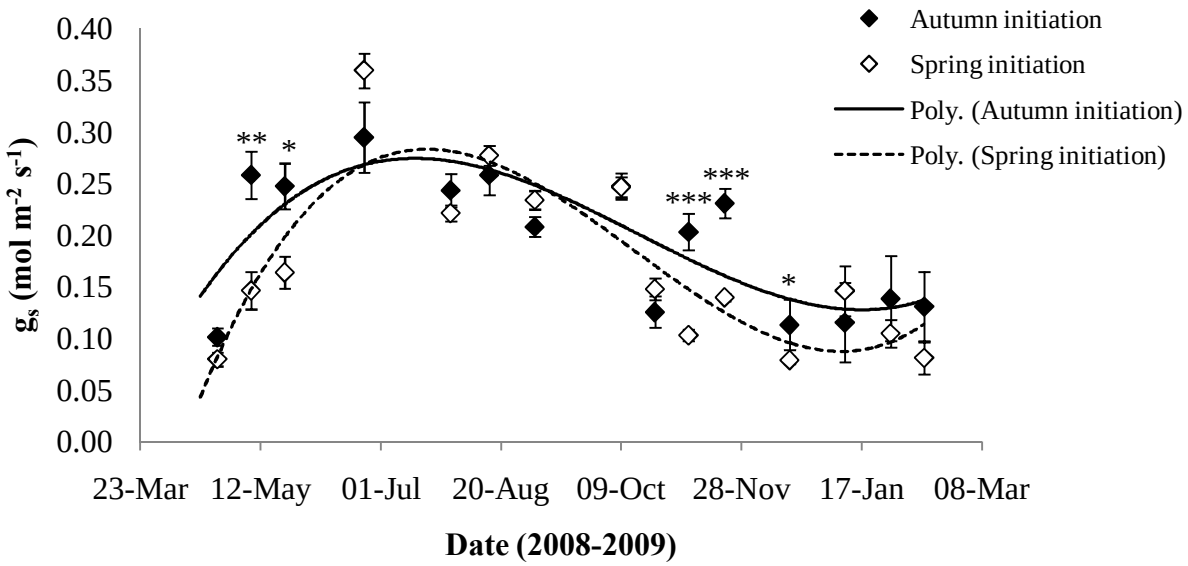


Fig. 4d. Stomatal conductance (g_s) ± SE for six-year-old *Protea* 'Pink Ice' from April 2008 to February 2009 on shoots which initiated inflorescences terminally on the autumn or spring flush.

* Significantly different at P<0.05; ** Significantly different at P<0.01; *** Significantly different at P<0.001.

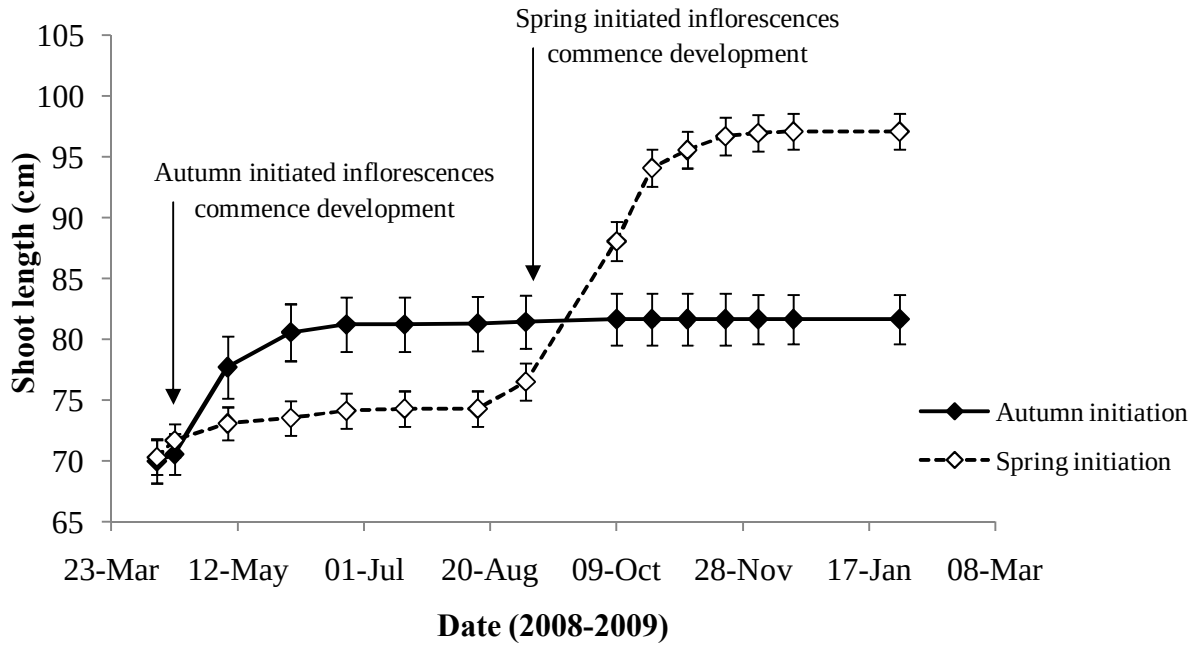
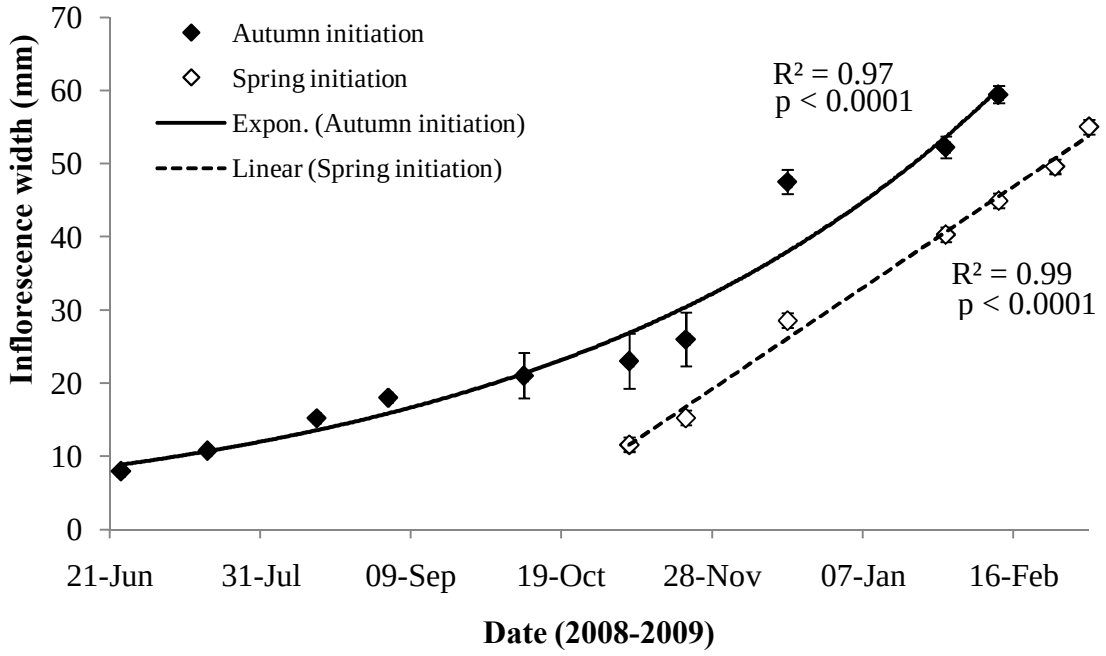


Fig. 52. Vegetative shoot elongation of six-year-old *Protea* ôPink Iceô from April 2008 to January 2009 for shoots which initiated inflorescences terminally on either the autumn or spring flush.

a)



b)

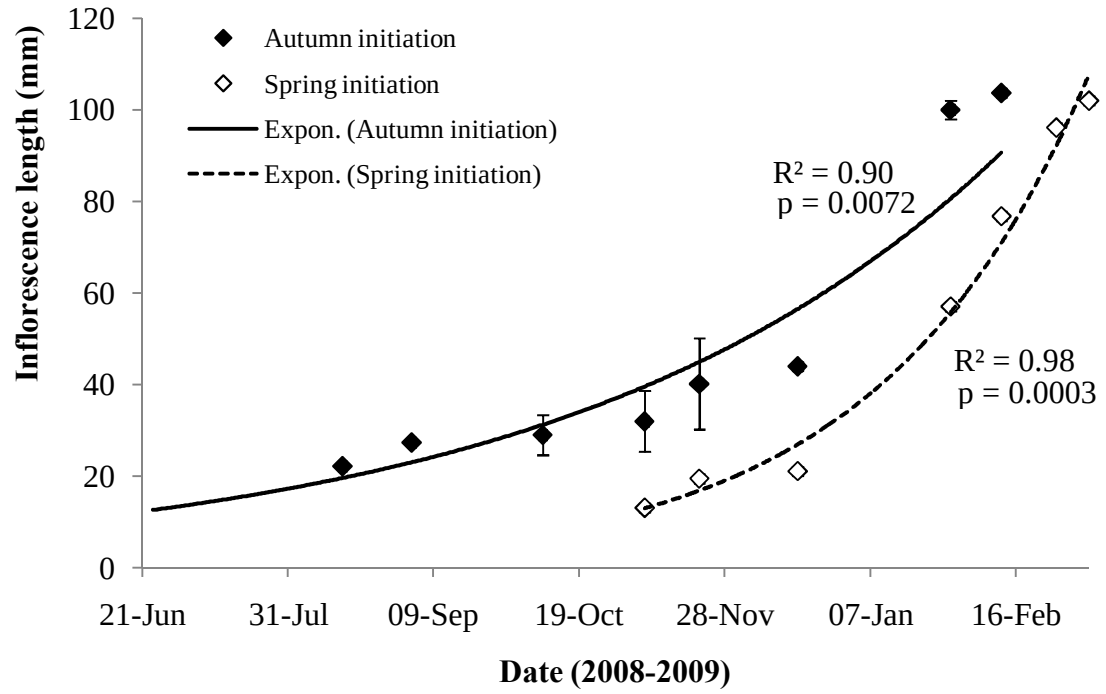
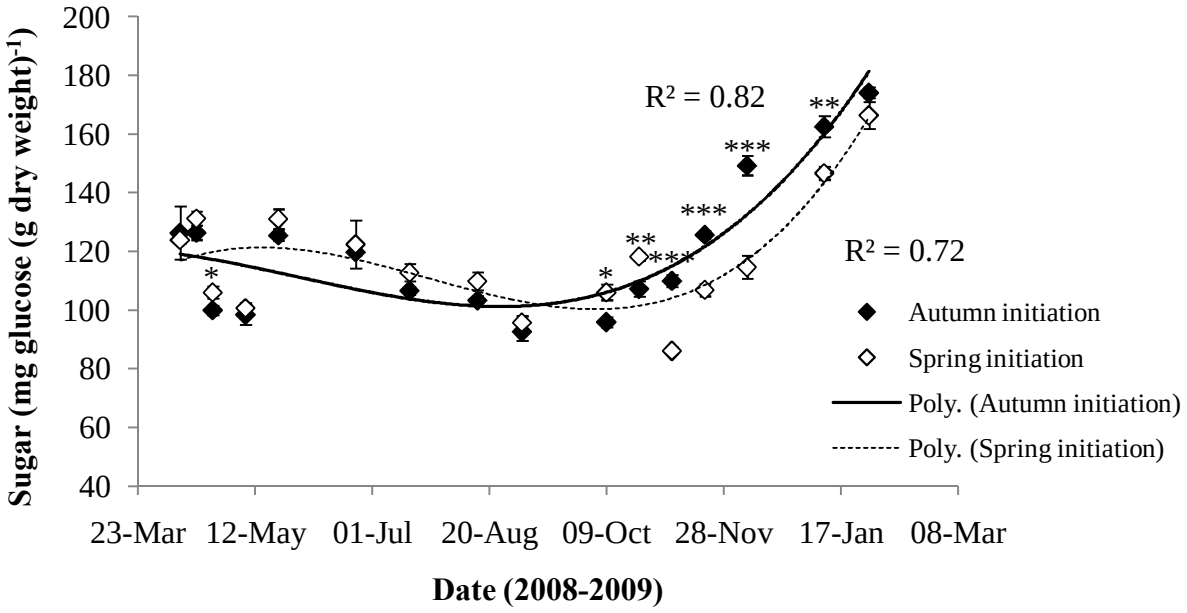


Fig. 53. Inflorescence (a) width ± SE and (b) length ± SE of six-year-old plants of *Protea* ôPink Iceô initiated terminally on either the autumn or spring flush.

a)



b)

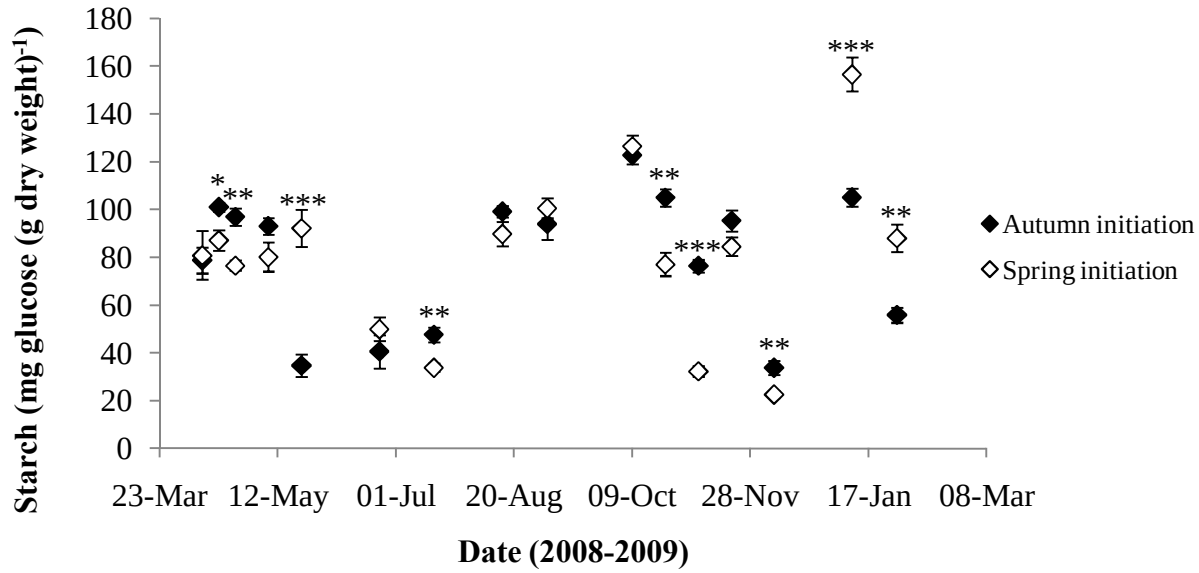


Fig. 54. The (a) total soluble sugar ± SE and (b) starch ± SE content expressed in glucose equivalents per gram dry mass in mature leaves of the terminal flush of shoots of six-year-old *Protea* 'Pink Ice', sampled from April 2008 to January 2009.

* Significantly different at P<0.05; ** Significantly different at P<0.01; *** Significantly different at P<0.001.

6. Seasonal patterns of gas exchange and leaf carbohydrate concentration of *Protea* cv. Pink Ice

Abstract

In spite of six decades of *Protea* cultivation very little research has focussed on carbohydrate fluctuations and gas exchange and how they are affected by seasonal temperatures and associated climatic shifts. As production areas in South Africa are expanding to warmer areas and global temperatures rise as a result of climate change, it becomes increasingly important to predict the response of crops to elevated temperatures. However, prior to modelling plant adaptation to warming, the physiology of flowering in *Protea* needs to be understood. A trial was established in a commercial *Protea* ôPink Iceô orchard outside Stellenbosch, and vegetative and reproductive growth, physiology, gas exchange and carbohydrate trends were observed for one year. The timing of phenological spring budbreak as well as gas exchange patterns were compared to data from the warming experiments (Chapter 3, 4). The optimum temperature of net CO_2 assimilation rate (T_{opt}) was higher than mean monthly temperatures depending on the time of the year, but in general, tracked seasonal temperatures closely. Net CO_2 assimilation rate (A_{max}) and stomatal conductance were the highest in mid-spring (September to October 2008). Leaf starch concentrations together with leaf dry mass of the terminal autumn flush increased during the winter and spring of 2008 until the cessation of spring flush growth extension, whilst total soluble sugar concentration decreased accordingly. This observed accumulation of starch in the period prior to floral initiation may act as an important signal in floral induction, but also floral expression and may well form part of a proposed minimum vegetative requirement that underpins floral initiation in *Protea*. Leaf starch and sugar concentrations started low in the newly formed spring flush, but increased steadily as the flush matured. At maturity, the total soluble sugar concentration decreased, probably to sustain the sink activity generated by the developing inflorescence. With future warming, supra-optimal temperatures may reduce rates of CO_2 assimilation. This in effect will lower starch levels to below a threshold level, in the leaves of the terminal flush, on which an inflorescence might have initiated under ambient temperatures. Such reduced starch levels may then result in fewer inflorescences initiating per plant and ultimately a reduction in the number of stems available for export.

6.1 Introduction

Despite 60 years of *Protea* cultivation (Coetzee & Littlejohn, 2001) and the ever increasing popularity of *Protea* as a commercial landscaping plant and cutflower, research on cultivated *Protea* has been limited. Harvest distributions and peaks of *Protea* cultivars in several areas may be obtained from individual production records and could be used to compare warmer to cooler years within an particular production system. However, information on the timing of spring budbreak, total soluble sugar and starch as well as gas exchange trends during the course of a season is not as readily available.

Greenfield *et al*. (1995) investigated seasonal changes of carbohydrates in the bark and wood of *Protea* óCarnivalô. Gerber *et al*. (2001a) recorded the total carbohydrate content in mature tissues (leaves and stems) at six intervals in *Protea* óLady Diô, whilst total leaf and stem carbohydrates were sampled on five occasions in *Protea* óSylviaô (Hettasch *et al*., 2001). However, there is no data available on carbohydrate trends in *Protea* óPink Iceô. Regular sample dates during the course of the entire season of óPink Iceô would provide greater insight into the leaf sugar and starch trends prevalent in *Protea* and their role prior to and at the switch to flowering.

Earlier studies on gas exchange measurements mostly focussed on selected fynbos species in their natural habitat. Mooney *et al*. (1983) recorded basic photosynthetic characteristics of four *Protea* species. Von Willert *et al.* (1989) examined leaf water relations and daily variation in the photosynthetic capacity of various fynbos species, including that of *Protea laurifolia*, in the Cedarberg region, South Africa. Similarly, Richardson & Kruger (1990) studied a range of indigenous mountain flora including two *Protea* species (*P. nitida* and *P. repens*). Van der Heyden & Lewis (1989) recorded seasonal variation of photosynthetic capacity and water use of five species, including *P. laurifolia*. Photosynthetic response to irrigation, seasonal temperature and irradiance was also investigated using the same species mentioned above (Van der Heyden & Lewis, 1990), but measurements were only taken on a single day in November and February respectively. In a more recent study, Smart (2005) studied floral induction of a commercially cultivated *Protea* hybrid, óCarnivalô (*P. compacta* x *P. neriifolia*) and measured total shoot photosynthesis of shoots with inflorescences in varying developmental stages. However, no seasonal gas exchange information is available for óPink Iceô and other cultivated *Protea* selections or hybrids, especially in relation to temperature.

Protea cultivation in South Africa is seen as a lucrative alternative to conventional fruit crops, with relative low inputs costs with which to diversify existing agricultural activities. In addition, areas under cultivation with Proteaceae within South Africa are continuously being expanded to new regions outside the traditional Mediterranean-climate cultivation zones. There are no scientific guidelines of which cultivars are best suited to these new areas. As there is currently a growing concern of the effect that climate change will have on the distribution range of fynbos and the impact of potential extinction of already threatened red data species (Rebelo, 2003) in the Cape Floral Region (CFR), fewer resources are channelled towards horticultural research of commercially utilized fynbos species.

The main objective of this study was to monitor and characterize seasonal baseline gas exchange, along with vegetative and reproductive seasonal growth patterns of an important commercial *Protea* cultivar, 'Pink Ice'. Total leaf soluble sugar and starch concentrations as well as dry mass fluctuations were tracked over the season. These physiological parameters mentioned above were linked to the reproductive phenology of inflorescence initiation and development under current climatic conditions. Phenological events that are highly temperature dependant such as the timing of spring flush growth was also monitored.

For the South African Fynbos industry to remain truly competitive in a global floricultural industry, where production factors and environmental conditions are often highly controlled and the biology of the floral crop greatly understood, it would be essential to have a greater understanding of the signals that control flowering, as well as how it is regulated by the environment, in particularly temperature. Better control of the phenology and flowering times in *Protea* would promote strategies for maximized production to satisfy exports markets.

6.2 Materials and methods

Plant material: This monitoring trial was performed on *Protea* 'Pink Ice', managed in an annual cropping system, at Floraland-Etshwaleni farm, Stellenbosch, South Africa (33°54'S; 18°48'E). The *Protea* cv. Pink Ice plants (*P. compacta* R. Br x *P. susannae* Phill.) were planted in a north-south row direction, in a five row system, spaced 1.5 x 1.5 m within rows with a 3 m service way. The soil was classified as a sandy soil. The farm dam was situated directly above the orchard and overflow water saturated the soil during winter and spring.

Plants within well drained rows were therefore selected. No irrigation was provided from August to October 2008. Irrigation during February 2009 amounted to 20.7ă water per plant per week, where in all other months the plants received 6.9ă per plant per week. Fertiliser applied by the producer comprised of Kelpak® (Kelp Products (Pty) Ltd, www.kelpak.com) as a foliar spray in December 2008, with follow up sprays in January and February 2009. K-max® (Gouws and Scheepers (Pty) Ltd, www.plaaskem.co.za/label/ KMAX.pdf) was applied once in March 2009. In June 2008, 5 to 10g K_2SO_4 per plant was applied. One four- or five-flush shoot per plant was randomly selected on 13 May 2008 on fifteen eight-year-old plants.

Gas exchange: The gas exchange measurements were taken with a LI-6400 Portable Photosynthesis System (Li-Cor, Lincoln, Nebraska, USA). Spot gas exchange measurements and photosynthetic temperature response curves previously described in Chapter 3.2 were performed. During the course of the experiment measurements were always performed at a photosynthetic photon flux (PPFD) of 2000 µmol m^{-2} s^{-1}. The PPFD was standardized to eliminate variation in light intensity, caused by the sun movement and cloud cover.

Spot gas exchange measurements included the maximum rate of light-saturated net CO_2 assimilation (A_{max}), the maximum rate of light- and CO_2-saturated net CO_2 assimilation (A_{sat}), dark respiration rate (R_d) and stomatal conductance (g_s). Spot gas exchange measurements were taken four weekly on a single mature leaf from a terminal flush on each tagged shoot. In 2008 on 4 May, 13 June, 11 July, 7 August, 11 September and 13 October measurements were taken on the autumn flush. On 10 November 2008, only the new spring flush was used, but as it was considered to still be immature, all measurements were repeated on the sub-terminal autumn flush on 18 November 2008. On 10 December 2008 a complete set of spot gas exchange measurements were taken on both the autumn and spring flush, whereas on 6 January, 4 February and 19 March 2009 only the spring flush was used as by then it had matured sufficiently.

Photosynthetic temperature response curves were performed to establish the optimum temperature for net CO_2 assimilation rate (T_{opt}). Five temperature curves, using one mature leaf from the terminal flush on each of five tagged shoots, were done on 5 June, 1 July, 5 August, 11 September, 13 October, 10 November, 10 December 2008 and 6 January, 4 February and 19 March 2009.

Pre-trial data collection, vegetative and reproductive development and harvest: Pre-trial measurements were taken on 13 May 2008 and included shoot length, shoot diameter at the upper intercalation between the terminal and sub-terminal flush, number of flushes from the bearer and numbers of leaves per flush. Budbreak was monitored regularly from 7 August to 11 September 2008. Shoot growth and reproductive development was measured every four weeks. Tagged shoots were harvested at a commercial ready harvest stage from 11 March to 8 April 2009.

At harvest, shoots were cut at the basal flush against the bearer. Shoots were separated into the respective flushes, whilst a flush was separated into the stem, leaves and the inflorescence. Vegetative measurements included final shoot length, stem diameter at the upper intercalation between the terminal and sub-terminal flush, number of flushes and flush length, number of leaves per flush, total leaf area per flush (measured using a Portable Area Meter, Li-3000A, Li-Cor, Lincoln, Nebraska, USA), and fresh mass of individual flush stems and leaves. Inflorescences were analysed by measuring the final length and width of the inflorescence as well as the fresh and dry mass of the inflorescence (bracts, florets and receptacle separately) for each individual inflorescence.

The approximate timing of phenological stages of both flowering and non-flowering shoots of ôPink Iceô in an annual cropping system is shown in Fig. 1 and represents the current climate scenario. Temperature increases to optimum levels as well as supra-optimal levels are also indicated in Fig. 55 with data from two other separate experiments (Chapter 4). Optimal temperatures for production can be defined as the highest number of stems harvested at the most desirable time for export, whereas under supra-optimal temperatures production decreases and the time of harvest shifts to times of lower demand from export countries.

Leaf total soluble sugar and starch concentration: Concurrent with gas exchange measurements 25 leaves were harvested at random from the terminal flush of shoots comparable to marked shoots. The subterminal or autumn flush was harvested from May to October 2008, with the terminal or spring flush harvested from November 2008 to February 2009. Oven-dried (Forced circulation incubator, FSIE 16, Labcon (Pty) Ltd, Roodepoort, South Africa) samples were milled in multiples of five (thus n = 5) and stored at -20°C until sugar and starch analysis could be performed. At harvest both the autumn (sub-terminal) and spring (terminal) flush were oven-dried, milled separately, and stored at -20°C. Total soluble

sugars were extracted by means of an 80% ethanol extraction method described by Allen *et al*. (1974) and Hamid *et al*. (1985). Starch was hydrolysed to glucose using an acetic acid buffer method and amyloglucosidase enzyme (Hamid *et al*., 1985). Final sugar and starch concentrations were obtained by means of an adapted spectrophotometric method (Dische, 1962), also described by Reed *et al*. (2004) where anthrone was used as a colorimetric agent. The absorbance of glucose was read with a Cary 50, Bio UV-visible spectrophotometer (Varian, Varian Australia Pty Ltd., Victoria, Australia) at 620 nm against a blank consisting of deionised water and anthrone. Sugar and starch concentrations are presented as glucose equivalents.

Climatic data: Hourly air temperatures, as well as daily minimum and maximum temperatures were obtained from the closest automatic weather station at the Agricultural Research Council (ARC), Nietvoorbij outside Stellenbosch.

Data analysis: Temperature curves were plotted in Microsoft® Office Excel® 2007. A polynomial trendline was fitted and the turning point was used to calculate the T_{opt} value. Differences between the spring and autumn flush in leaf total soluble sugar and starch concentration at harvest were compared by analysis of variance (ANOVA) using the PROC GLM procedure (SAS Institute Inc., 2003). Mean separation was conducted according to Tukeyôs test (Pr Ò0.05).

6.3 Results

Gas exchange: Spot gas exchange measurements showed the highest rate of light-saturated net CO_2 assimilation rates (A_{max}) (> 12 µmol m^{-2} s^{-1}) in spring from 11 September to 13 October 2008 in the autumn flush (Fig. 56a). These high values also coincided with the highest stomatal conductance (g_s) values obtained (Fig. 56d). Light- and CO_2-saturated net CO_2 assimilation rates (A_{sat}) were high in late autumn (14 May 2008) (> 25 µmol m^{-2} s^{-1}), decreased during winter months and then increased again from 11 September to 19 November 2008 in the autumn flush (Fig. 56b). Dark respiration (R_d) increased progressively to a maximum on 10 December 2008 in the autumn flush (Fig. 56c). The spring flush started on lower values compared to the autumn flush for all parameters measured (Fig. 56). From

January 2009 onwards, A_{max} (Fig. 56a) and g_s (Fig. 56d) remained approximately constant, whilst A_{sat} (Fig. 56b) increased and R_d (Fig. 56c) decreased until 19 March 2009.

T_{opt} values were constantly higher than the mean monthly temperature for the corresponding part of the season, especially from June to September 2008 (Fig. 57). T_{opt} decreased in early spring and reached maximum values during December 2008. In general, T_{opt} tracked ambient temperatures closely.

Vegetative and reproductive development; harvest: The dormant winter period (mid-June to August 2008) during which no or very little visible shoot growth was observed, was followed by spring budbreak. Budbreak occurred from early August to mid-September 2008 (Fig. 58), signalling the onset of spring flush growth from August to December 2008 (Fig. 59). No further shoot growth was detected once inflorescence initiation commenced.

Inflorescence width increase followed a linear trend (Fig. 60a), whilst inflorescence length increase was more exponential (Fig. 60b). The harvest dated from 11 March to 8 April 2009. Parameters measured at harvest are presented in (Table 16).

Sugar and starch: Total soluble sugar concentrations in the autumn flush remained relatively constant throughout winter (~120 mg glucose (g dry weight)$^{-1}$), to decrease in spring (Fig. 61). The spring flush displayed lower sugar concentrations with the first observable date in November 2008 and increased to 110 mg glucose (g dry weight)$^{-1}$ in November 2008, but increased throughout summer until January 2009, followed by a decrease in early February.

Starch concentrations varied greatly throughout the season (Fig. 61). In the autumn flush, starch concentrations progressed from low concentrations in autumn and early winter throughout late winter (July 2008) and spring (August to October 2008) to peak at the end of October 2008 at 180 mg glucose (g dry weight)$^{-1}$ as the sugar concentration decreased correspondingly. Starch levels were very low (~ 30 mg glucose (g dry weight)$^{-1}$) at the start of the spring flush (10 November 2008), but increased to ~ 60 mg glucose (g dry weight)$^{-1}$ toward autumn (March 2009).

Similarly, individual leaf dry mass increased simultaneously with the increase in starch from July to October 2008 in the autumn flush. Leaf dry mass of the spring flush started off low in November 2008, although comparable to the leaf dry mass of the autumn flush in May 2008, but increased significantly during summer towards the end of January 2009 (Fig. 61).

The spring flush leaves had a significantly greater leaf area compared to the autumn flush (Fig. 62). At harvest, the autumn (sub-terminal) flush had significantly higher leaf sugar content compared to the terminal spring flush, and both flushes showed higher levels in relation to the values obtained in the preceding season. However, starch concentrations were similarly low in both flushes (Fig. 63).

6.4 Discussion

Phenological events, such as the timing of spring budbreak, together with the optimum temperature for net CO_2 assimilation (T_{opt}) were found to be highly temperature dependant. In this study, spring budbreak extended over a five-week period and was closely linked to mean weekly air temperatures (Fig. 58). As a result of generally low mean temperatures during the week of 27 August 2008, the increase in percentage budbreak from the previous week was very small, but as temperatures improved the following week, the increase in budbreak percentage accelerated (Fig. 58). On 7 August 2008, 25% of shoots has reached budbreak (Fig. 58), whilst on that same day and experimental site, 50% of shoots has reached budbreak in the adjacent infra-red warming experiment under ambient+2.9°C (Chapter 4.3.1) (Fig. 55). In the same particular area spring budbreak may therefore take place earlier in warmer years to advance as temperatures increase as a result of global warming. Badeck *et al.* (2004) also found that with increasing temperature spring budbreak may be promoted together with earlier flowering in middle and higher latitudes.

Similarly, warm seasonal temperatures during summer caused a rise in the optimum temperature for net CO_2 assimilation rate (T_{opt}) (Fig. 57). This acclimation was not unexpected as it is known that particularly perennial evergreen shrubs and trees, such as oleander and various *Citrus* species, similar to *Protea* experience major seasonal climatic fluctuations, are considered to be more plastic and therefore acclimates readily to seasonal temperature changes (Hall, 2001). During the course of this study, T_{opt} for ôPink Iceô was found to be between 1 and 9°C higher than the monthly mean temperature depending on the season. Similar results were found in the photosynthetic temperature responses of *Eucalyptus* in Australia, where T_{opt} increased for *E. globulus* from 17 to 23°C as mean daily temperatures increased from 7 to 16°C, whereas T_{opt} for *E. nitens* increased from 14 to 20°C as mean daily temperatures increased from 7 to 19°C during a 9-month observation period (Battaglia *et al*., 1996).

When comparing T_{opt} obtained in the ôPink Iceô monitoring trial to the greenhouse-based warming experiment in Chapter 3.3.2, T_{opt} of plants grown in the greenhouse reached much higher values under warming, even exceeding 30°C during summer, whilst in the field T_{opt} values rarely exceeded 25°C. This indicates that *Protea* have to a large extent the capacity to acclimate to warming. However, when comparing absolute values of the rate of net CO_2 assimilation (A_{max}) and stomatal conductance (g_s) from the greenhouse-based warming experiment (Chapter 3.3.2) to the monitoring trial, the rates were lower in the greenhouse indicating reduced amounts of fixed carbon.

Proteas are known to be major water spenders (Stock *et al*., 1992). During very hot days in summer the evaporative demand probably exceeds water uptake by roots. The lowered leaf turgor pressure would cause stomatal closure, contributing to the observed lowered net CO_2 assimilation rates (Fig. 2) and greatly impact on carbohydrate levels and floral induction.

Leaf sugar levels decreased from August to November 2008 in the autumn flush (Fig. 7). This trend can be explained by the considerable increase in respiration (Fig. 56c) together with higher ambient temperatures at spring budbreak (Fig. 57). However, starch levels in the autumn flush increased throughout winter and remained high during spring budbreak, with start of spring flush growth, even surpassing the sugar levels (Fig. 61). This period of starch accumulation coincided with the highest rate of net CO_2 assimilation and maximum stomatal conductance during the season (Fig. 56a). The high rate of net CO_2 assimilation and favourable environmental conditions lead to an excess of assimilates, which was then available for storage. Total carbohydrate content of mature leaves as measured by Gerber *et al*. (2001a) in ôLady Diô displayed a similar pattern in terms of increased total carbohydrates from August to November, but starch levels remained low, whilst sugar increased significantly. This different pattern in sugar and starch accumulation in ôLady Diô could possibly be attributed to different sampling and analysis methods or to cultivar differences. However, dry mass accumulation of ôLady Diô and ôPink Iceô exhibited a similar trend (Fig. 7), as total carbohydrates, sugar and starch increased during a comparable period.

The leaf carbohydrates, most likely starch, are probably an underlying mechanism which is pivotal to flower induction. Hettasch *et al*. (2001) found ôSylviaô leaves to be a far more important source of total sugars than stems. When ôCarnivalô shoots were defoliated 40 days before spring budbreak or earlier, the defoliated shoots did not flower (Gerber *et al*., 2002), indicating the importance of leaves. According to Gerber *et al*. (2002) and Hoffman

(2006) inflorescence initiation takes place at the start of spring flush elongation, before which floral induction would have taken place. A threshold leaf starch or sugar level in mature leaves on the over-wintering flush may be critical for floral initiation to take effect. This hypothesis supports a threshold or minimum vegetative requirement in *Protea* to allow floral initiation. ‘Sylvia’ has the ability to flower all year round, but prefers to flower on the spring flush (Gerber *et al*., 2001b), with carbohydrates again possibly being involved. In subtropical crops with a flushing growth habit, such as mango or lychee, it has been found that a dormant period as induced by mild water stress or a cooler period usually precedes the transition to the reproductive phase as maturation of the vegetative flushes is required for effective floral initiation (Wilkie *et al*., 2008). Again this translates to the acquisition of a minimum concentration of starch or sugar as an apparent prerequisite for floral induction. In *Banksia*, a member of the Proteaceae, assimilate supply is also critical for floral initiation (Rieger & Sedgley, 1996). Therefore, the preference of ‘Pink Ice’ to flower on the spring flush could possibly be due to the accumulation of starch during winter (Fig. 61) linked to decreased plant activity, and not directly as a result of lower temperatures during winter. Carbohydrate assimilation may also be critical for floral initiation in *Protea* in the tropics, as *Protea* plants have the ability to flower at the equator where little variation in seasonal temperatures occur (R. Criley, pers. comm., 2009).

At harvest, leaf starch levels of both the spring and autumn flushes returned to approximately 40 mg glucose (g dry mass)$^{-1}$ (Fig. 63). However, the spring flush had significantly less leaf sugar content at harvest compared to the autumn flush. The mature spring flush mainly supply the inflorescences with carbohydrates, whilst only a fraction of carbohydrates from the autumn flush is allocated to the inflorescence, with most photosynthates from the autumn flush allocated basipetally (Smart, 2005).

Commercial ‘Pink Ice’ can be managed in a biennial pruning system, with two blocks in alternating phases (Chapter 5). Following pruning during June/July shoots produce spring, one to three summer and autumn flushes. A second spring flush is produced the following year on which the inflorescence is borne. Interestingly, for ‘Pink Ice’ produced in an annual system, shoot growth also extend over the course of two years, but growth is not synchronised. Shoots in various growth stages is therefore present in an annual management system. Subsequent shoot and inflorescence growth under current climatic conditions, together with possible future warming scenarios is represented in Fig. 55.

Finally, when comparing the data collected in this trial (current climate) to possible future scenarios where warming may lead to more optimal temperatures (field verification experiment, Chapters 2, 4) or become supra-optimal (greenhouse-based warming experiment, Chapters 2, 4) changes in the time of spring budbreak and the spring flush extension may cause changes in harvest times (Fig. 55). Continuous flushing at very high temperatures may shift floral initiation to first or second summer flushes, further extending the harvest period. More critical to the production of 'Pink Ice', high temperatures may reduce the amount and size of inflorescences produced (Chapter 4.3.1).

The data collected showed that temperature plays a critical role in *Protea* phenology and gas exchange. For successful protea production, producers should carefully choose 'Pink Ice' cultivation areas and align other cultivars to areas suitable to their requirements.

6.5 References

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Table 16. Vegetative and reproductive characteristics (± SE) of flowering shoots (n = 15) recorded at harvest of *Protea* 'Pink Ice' grown in a commercial orchard, Stellenbosch.

Shoot characteristics	Average	SE
Vegetative		
Stem diameter (mm)	14.8	± 5.8
Whole shoot leaf area (cm^2)	1270	± 90.5
Leaf area ratio ($cm^2\ g^{-1}$)	22.0	± 0.8
Dry mass		
Whole shoot leaf (g)	29.1	± 1.7
Whole shoot stem (g)	28.3	± 1.9
Reproductive		
Inflorescence dry mass (g)	25.4	± 0.7
Bracts dry mass (g)	9.0	± 0.2
Receptacle dry mass (g)	3.6	± 0.2
Florets dry mass (g)	12.8	± 0.4

Temperature regimes		February – March	April-May	June-July	August	September	November - December	January	February - March	April-May
Current	**Year 1**	Harvest	Autumn flush from **axillary** postion	Dormant winter	Spring budbreak	Spring flush extension	1st summer flush	2nd summer flush	Possible 3rd summer flush	
	Year 2		Autumn flush from **terminal** position	Dormant winter	Spring budbreak	Spring flush extension	Inflorescence initiation and development on spring flush		Harvest	
Optimal warming	**Year 1**	Harvest	Autumn flush from **axillary** postion	Dormant winter	Spring budbreak	Spring flush extension	1st summer flush	2nd summer flush	Possible 3rd summer flush	
	Year 2 Scenario 1		Autumn flush from **terminal** position	Dormant winter	Spring budbreak	Spring flush extension	Inflorescence initiation and development on spring flush		Harvest	
	Year 2 Scenario 2		Autumn flush from **terminal** position	Inflorescence initiation and development on autumn flush				Advanced harvest		
Supra-optimal warming	**Year 1**	Harvest	Autumn flush from **axillary** postion	Dormant winter	Spring budbreak	Spring flush extension	1st summer flush	2nd summer flush	Possible 3rd summer flush	
	Year 2 Scenario 1		Autumn flush from **terminal** position	Dormant winter	Spring budbreak	Spring flush extension	Inflorescence initiation and development on spring flush		Harvest	
	Year 2 Scenario 2		Autumn flush from **terminal** position	Dormant winter	Spring budbreak	Spring flush extension	1st summer flush	Inflorescence initiation and development on summer flush	Delayed harvest	
	Year 2 Scenario 3		Autumn flush from **terminal** position	Dormant winter	Spring budbreak	Spring flush extension	1st summer flush	2nd summer flush	Inflorescence initiation and development on summer flush	Late harvest
	Year 2 Scenario 4		Autumn flush from **terminal** position	Dormant winter	Spring budbreak	Spring flush extension	1st summer flush	2nd summer flush	Continued summer and autumn flushing	Remains vegetative
	Year 3 Scenario 4		Autumn flush from **terminal** position	Dormant winter	Spring budbreak	Inflorescence initiation and development on spring flush			Advanced harvest	

Fig. 55. A presentation of possible *Protea* ôPink Iceô crop production scenarios during the course of an entire production season, including the current climate, as well as future increased warming to an optimum- and supra-optimal warming levels. From March to July the sequence of events is similar for all three scenarios, but differences become evident with the onset of spring budbreak and onwards.

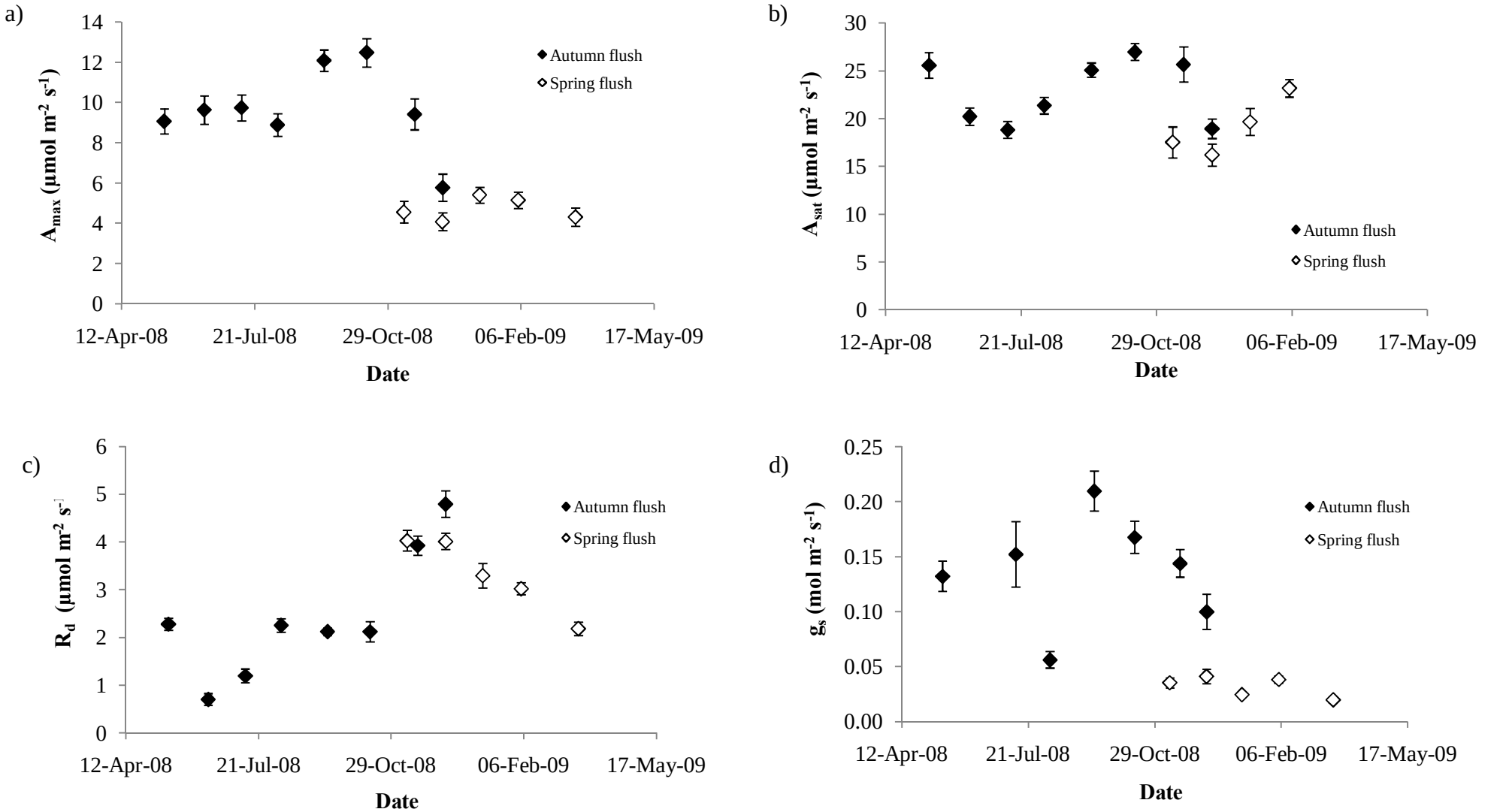


Fig. 56. Measurements of spot gas exchange, a) maximum rate of light-saturated net CO_2 assimilation (A_{max}), b) maximum rate of light- and CO_2-saturated net CO_2 assimilation (A_{sat}), c) dark respiration rate (R_d) and d) stomatal conductance (g_s), taken from May 2008 to February 2009 on the mature leaves of the terminal flush of *Protea* 'Pink Ice' within a commercial orchard, Stellenbosch. Data presented as mean values ± SE (n = 15).

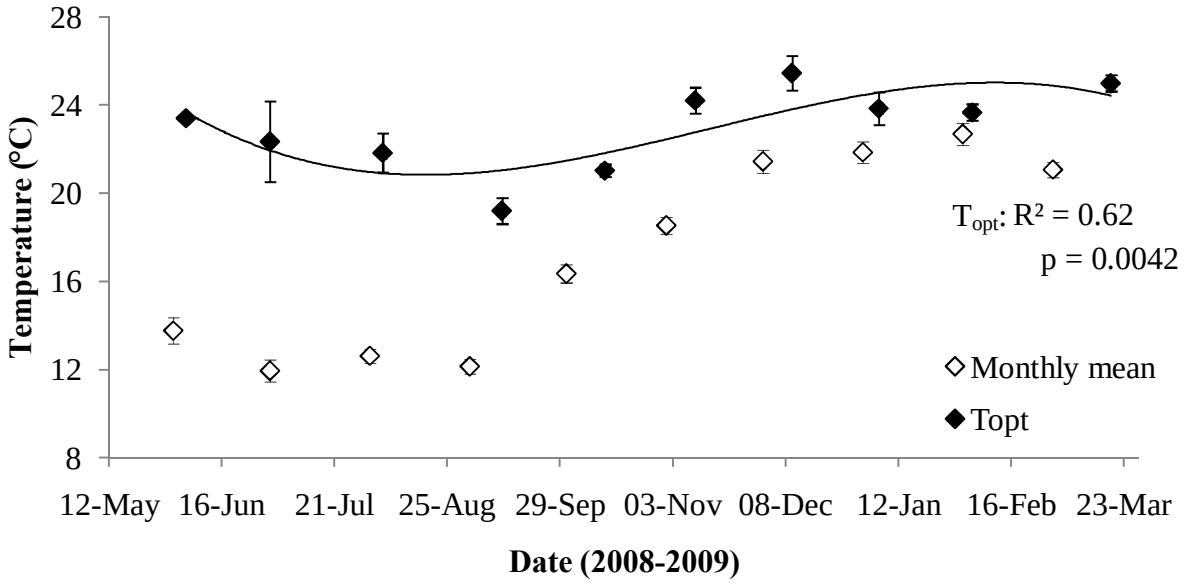


Fig. 57. The optimum temperature for the maximum rate of light-saturated CO_2 assimilation ± SE (n = 5) (T_{opt}) from May 2008 to March 2009 in mature leaves of the terminal flush of *Protea* õPink Iceô as grown in a commercial orchard, Stellenbosch. Monthly mean temperatures from May 2008 to March 2009 are also presented.

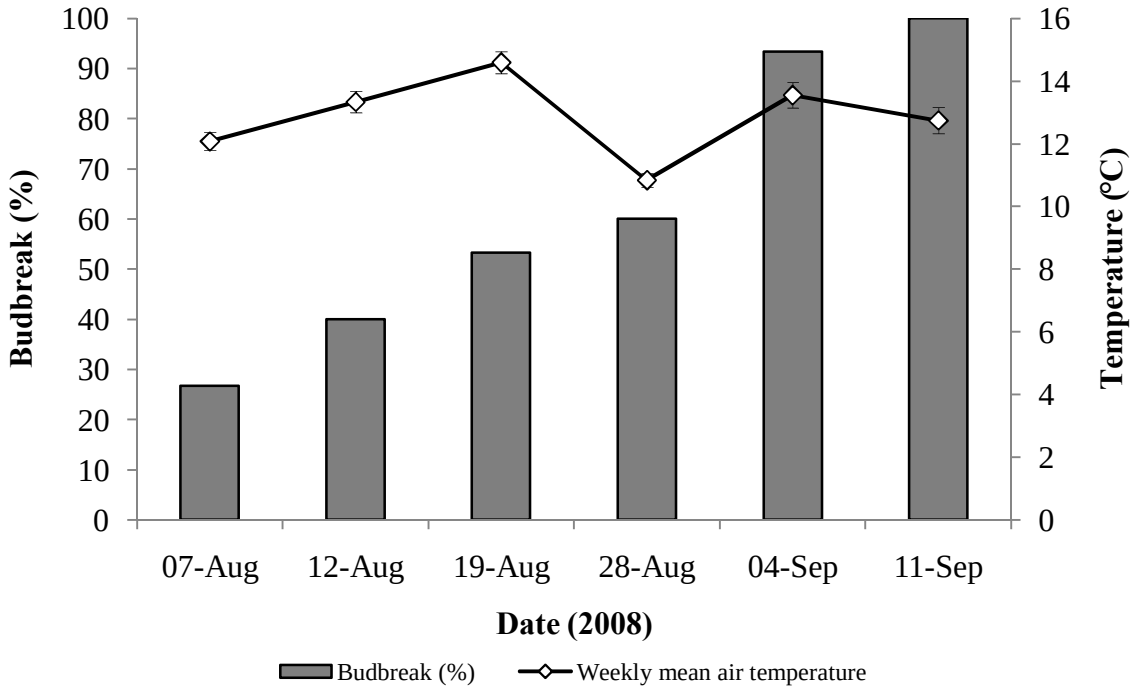


Fig. 58. Accumulative percentage budbreak distribution of *Protea* õPink Iceô (n = 15) grown in a commercial orchard, Stellenbosch, from August to September 2008, together with mean weekly air temperatures for the same period.

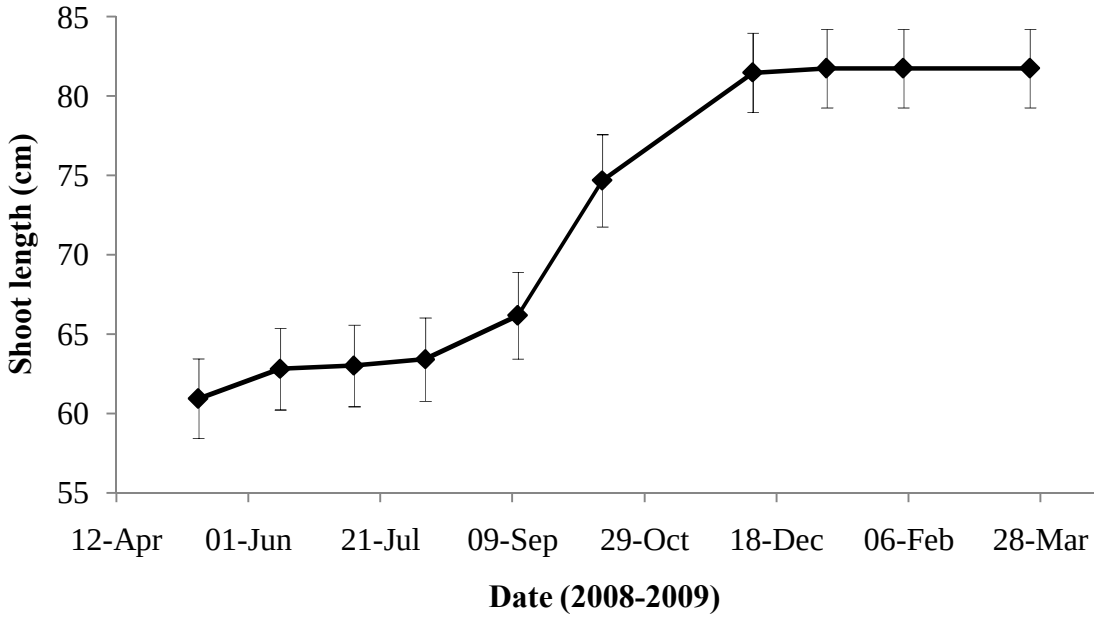
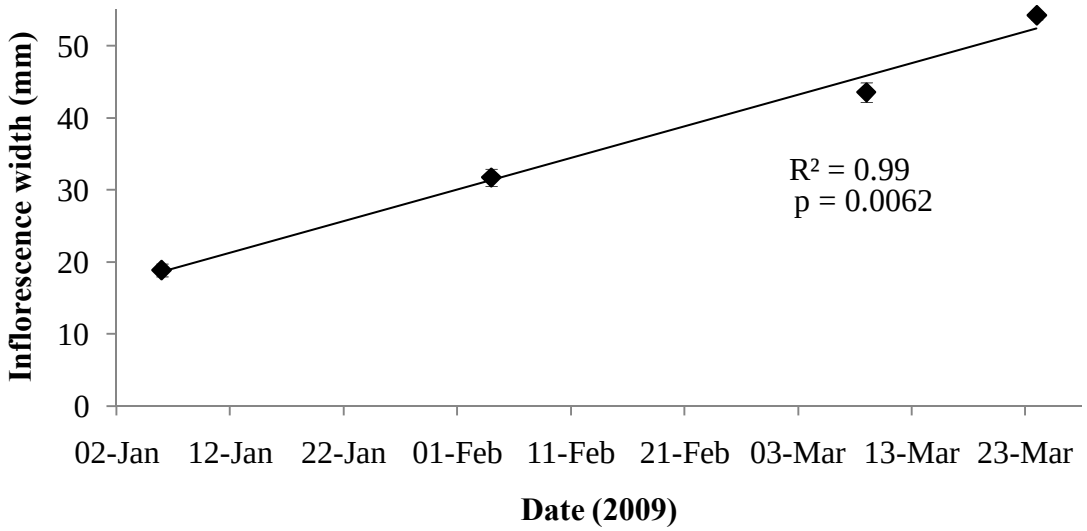


Fig. 59. Shoot length ± SE (n = 15) of *Protea* ôPink Iceô grown in a commercial orchard, Stellebosch, and measured from May 2008 to harvest in March 2009. The progression from spring budbreak to inflorescence anthesis required 215 ± 2 days.

a)



b)

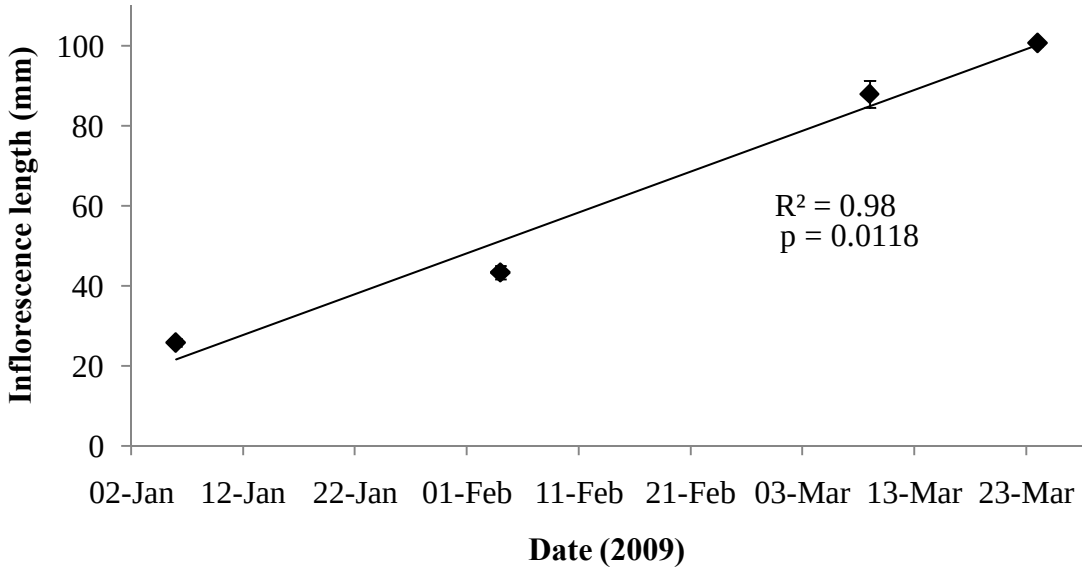


Fig. 60. The infloresence a) width increase ± SE and b) length increase of *Protea* ôPink Iceô(n = 15) grown in a commercial orchard, Stellenbosch, and measured from January 2009 until commercial harvest.

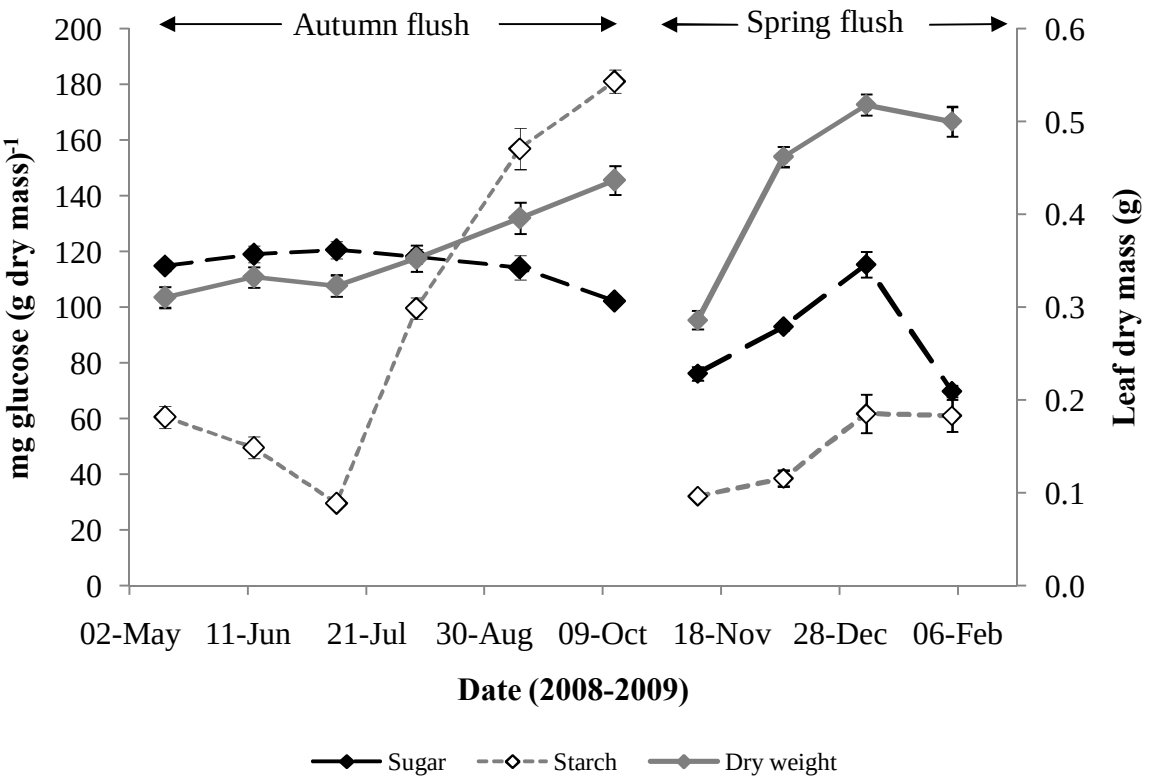


Fig. 61. Total soluble leaf sugar and starch concentration as glucose equivalents ± SE, and leaf dry mass ± SE (n = 5) determined in *Protea* ôPink Iceô mature leaves harvested from either the autumn or spring flush during the course of the season.

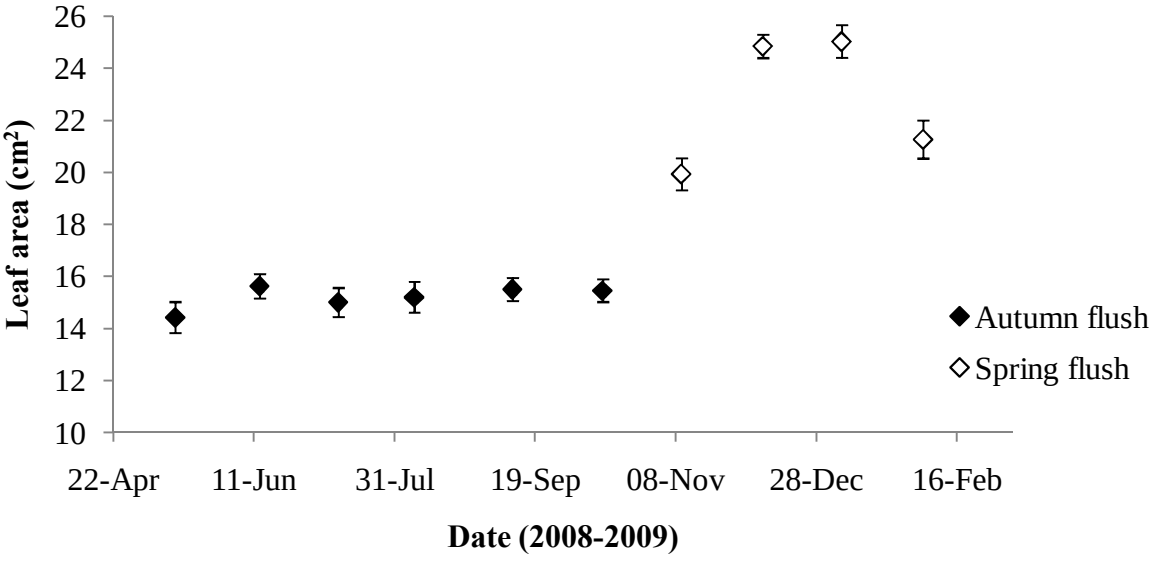


Fig. 62. Individual leaf area ± SE (n = 25) of *Protea* ôPink Iceô leaves sampled from either the autumn or spring flush on shoots comparable to experimental shoots throughout the season.

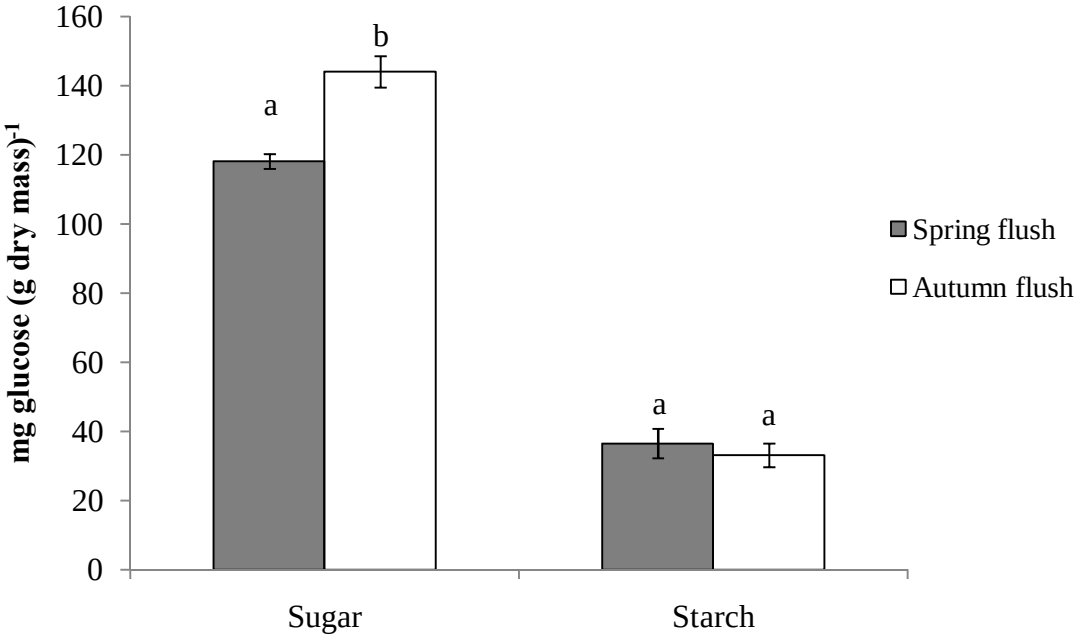


Fig. 63. The leaf sugar and starch concentration measured in glucose equivalents ± SE (n = 15) at harvest of the autumn (subterminal) and terminal spring (subtending the inflorescence) flush of *Protea* ôPink Iceô. Means with the same letter within graphs are not significantly different according to Tukeyôs test (Pr Ò0.05).

7. Integrated discussion

Fynbos crops are becoming increasingly popular as landscaping, cutflower and foliage plants. *Protea* cultivation is seen as a lucrative alternative or additional crop with relatively low input costs by existing temperate fruit crop producers in various climatic regions, who are using it to diversify existing agricultural activities. In addition, areas under cultivation with Proteaceae within South Africa are continuously being expanded to new regions outside the traditional Mediterranean-type climate cultivation zone commonly associated with *Protea*. Global temperatures are rising as a result of increased CO_2 emissions caused largely by human fossil-fuel-based activities. This could have several as yet unknown economic and production implications for the South African floriculture industry including the indigenous Proteaceae sector.

However, as insufficient scientific information on the effect of climatic factors on *Protea* gas exchange, growth and development is available, it is difficult to predict and make recommendations on the suitability and adaptability of existing cultivars to these new areas of cultivation, each with their unique temperature regimes and climatic and soil conditions. Furthermore, the vegetative requirements for flowering in *Protea*, in terms of the number of flushes and minimum stem diameter, are only slightly better understood than the role of temperature and carbohydrates in floral induction and initiation.

In this study several aspects of *Protea* ôPink Iceô growth and development were investigated and compared under ambient and elevated temperatures. These included gas exchange, vegetative growth, reproductive development and carbohydrate concentration fluctuations during both the dormant and growth seasons. The existence of possible carbohydrate signals inductive to flowering was peripherally explored.

Flowering of perennial plants in general is complex and *Protea* ôPink Iceô is no exception. Source-sink competition between vegetative and reproductive growth was observed at high temperatures, with elevated temperatures preferentially promoting vegetative growth. Accelerated, continuous and additional vegetative flushing interfered with flush maturation. In subtropical crops such as mango, lychee and citrus a period of low temperature or induced drought is required prior to reproductive flushes. In *Protea*, which appears to share certain flushing traits with these crops, a minimum level of shoot dry mass is apparently required prior to inflorescence initiation. A threshold carbohydrate level present in the terminal, autumn flush on overwintering shoots may possibly play an inductive and

signalling function for inflorescence initiation to proceed on the spring flush. If carbohydrate levels, most likely represented by starch, does not reach the required threshold level floral induction and initiation does not occur. This situation would manifest in very thin and weak vegetative shoots or, as shown in this thesis, under conditions supra-optimal warming.

In this study, higher, but not supra-optimal temperatures increased the growth potential of *Protea* ôPink Iceô plants, thus increasing their sink capacity. This likely caused a source to sink limitation as the source could not supply the required assimilates, as shown by the constant (area-basis) or declining (mass-basis) assimilation rates with increasing temperature. The minimum vegetative requirement for flowering could not be met; carbohydrates accumulated slowly to support vegetative growth, which continued without a rest period between flushes. No flush maturation was possible. This then resulted in fewer as well as smaller inflorescences produced per plant.

In many plants, during high temperature and high vapour pressure deficit conditions, leaf transpiration rate may exceed the rate of water uptake by the roots, even though available soil water is ample. Subsequent stomatal closure is sometimes not effective to ensure maintenance of leaf pressure potential (turgor). This condition is defined as physiological drought and it is suggested that it may explain some of the responses to high temperature reported in this thesis. *Protea* ôPink Iceô may adapt to elevated temperatures by increasing leaf densities (as shown by the increasing leaf mass per leaf area), cell wall thickness and sclerenchymatic tissue in order to resist daytime turgor loss. This scenario is supported by the fact that dry mass allocation patterns were altered, as more dry mass was allocated to the roots and leaves than to stems and inflorescences at elevated temperatures. Future studies should include measurements of diurnal leaf water relations.

Depending on the extent of warming in a specific production area, temperatures may initially become more optimal for *Protea* farming. Production under elevated, more optimal temperatures would result in earlier spring budbreak and also earlier flowering, as was seen in the field verification experiment. For a cooler region, such as Stellenbosch, optimal temperature appeared to be ambient+3°C. With further warming, ambient+4°C and above, temperatures may become supra-optimal (as seen in the greenhouse experiment). Earlier spring budbreak would still occur and only a small percentage of shoots would flower on the spring flush as continuous vegetative flushing would shift inflorescences initiation to later in the season on summer flushes or shoots may even stay vegetative. The result would be a reduction in the number of inflorescences produced and a more extended harvest period. Both

these outcomes would have serious commercial and economic implications. For the producer, management strategies involving the exogenous applications of cytokinin growth regulator treatments or pruning to shift flowering dates are becoming important, and in warm areas such as Hopefield, experiments with evaporative cooling at temperatures above 30°C could be very valuable, provided there is sufficient available water.

Fynbos vegetation is commonly associated with Table Mountain Sandstone soils, which are sandy, nutrient-poor soils. The main limitation to the photosynthetic capacity is, therefore, believed to be nitrogen-related. To ensure sufficient shoot growth, nitrogen fertiliser regimes should be investigated under warming. Will additional nitrogen applications aid shoot growth, maturation and flowering under supra-optimal warming or cause excessive shoot growth which will lead to non-flowering shoots?

In this study plants were grown under irrigation, but this is not the case in many commercial farms, where the flower developmental period coincides with the dry season. It would be important to further study possible interaction between warming and sub-optimal water relations on *Protea* physiology and performance. Water available for agricultural purposes is predicted to decrease, especially in the Western Cape, South Africa. Serious droughts, similar to that experienced currently in the southern Cape, will force producers of fynbos crops to reassess whether to irrigate or not, as well as irrigation quantity and timing.

Fortunately, *Protea* ôPink Iceô appeared to have good acclimation potential to increased temperatures similar to other Mediterranean-climate sclerophylls grown in areas with cool, wet winters and warm, dry summers. The optimum temperature for net CO_2 assimilation rate (T_{opt}) increased during summer, elevating the heat tolerance of these plants, with respect to photosynthesis, and then decreased during the cooler months. However, the absolute rates of net CO_2 assimilation (A_{max}) declined during the warm summer months, reducing the amount of available carbohydrates. The reduction is probably only significant under supra-optimal temperatures when stomatal closure becomes a problem. Therefore, if future temperature increases can be limited to 1-2°C, the gas exchange capacity of *Protea* should not suffer major reductions, especially in cooler areas.

Under conditions of future warming, ôPink Iceô would probably be produced more succesfully in cooler areas as it is a vigorous cultivar, whilst warmer productions areas might be more suitably to slower growing cultivars.

Further studies should include comparisons between a vigorous cultivar such as ôPink Iceô and a slower growing cultivar, possibly ôCarnivalô, to observe any differences in acclimation potential, flushing growth habit and flowering under elevated temperatures.

The baseline seasonal data collected may serve as a guide or reference for future studies, particularly when comparing different production areas varying in climate, consecutive years or cultivars, with a focus on flower size, timing of spring budbreak, shoot growth and gas exchange behaviour of *Protea*. There is scope to extend future research evaluate to the number of flowers harvested per plant in cooler versus warmer areas and to relate this information to changes in production that may be caused by future climatic warming.