FLORAL INDUCTION IN

Eucalyptus nitens (DEANE & MAIDEN) MAIDEN

IN SOUTH AFRICA

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In fulfilment of the requirements for the degree of
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in
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ABSTRACT

Eucalyptus nitens (Deane & Maiden) Maiden is an important, commercial eucalypt planted predominantly for pulpwood in several southern hemisphere countries. In South Africa, the erratic and sparse flowering habit of *E. nitens* severely impedes genetic improvement and commercial seed production in the species. The comparatively abundant flower bud production at specific high altitude sites in the summer rainfall region suggested that cumulative cold may be implicated in the floral induction process. Series of field trials and semi-controlled environment trials were undertaken between 1996 and 2001 to investigate this. Three chill models were used to investigate whether winter temperature data can be related to *E. nitens* flower bud production.

In the field trials, not only was the relationship between winter chilling and subsequent flower bud crop investigated, but also the relationship between cumulative winter drought conditions and floral bud production. In the trials under semi-controlled environmental conditions, the effect of applied winter chilling on floral bud production and photosynthetic efficiency was investigated.

In the field trials, amount of accumulated winter chill, in conjunction with paclobutrazol treatment, was able to explain between 66 and 72 % of the variation in *E. nitens* flower bud production at four and five years after planting. Very high levels of accumulated winter chill (≥ 88 Chilling Portions (CPs) of the Dynamic Model) stimulated a high percentage of seedlings (25 - 50 %) and grafts (55 - 77 %) to produce flower buds. At low to moderate levels of winter chill (41 to 72 CPs), paclobutrazol application increased flower bud production significantly, but at high levels of winter chill (> 76 CPs) paclobutrazol had a negligible effect. Cumulative winter drought did not promote floral bud production.

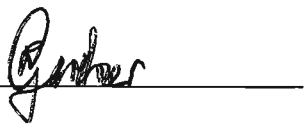
In the semi-controlled environment trials, cold suppressed vegetative growth and induced flowering in paclobutrazol-treated 18-month old grafted trees. Cold without paclobutrazol did not promote floral bud production. The results suggest that accumulated winter chill units (according to the Dynamic Model) are more effective than accumulated cold hours (hours below 5^0 C). A high number of cold hours (1366 hours) reduced photosynthetic efficiency, but did not induce flowering. Furthermore, photosynthetic efficiency remained high for the moderate cold treatments which did induce flowering, suggesting that stress is not correlated to flowering in *E. nitens*.

The results of the field and semi-controlled environment trial series suggest that precocity and floral productivity in *E. nitens* are under strong genetic control. Better accuracy in predicting flower bud crops in *E nitens* could probably be achieved by excluding genetic variability and

increasing the range of chilling conditions in such trials in future. The results indicate that future research should focus on the identification of optimum chilling (temperature) criteria for floral induction in *E. nitens*, the use evaporative cooling in seed orchards to reduce warm winter daytime conditions, the possible use of low-chill rootstocks, and the location of orchards as far south as possible in the winter rainfall region to achieve maximal exposure to temperatures which fulfil the chilling requirement of the species.

DECLARATION

I hereby declare that the research reported in this thesis is original and the result of my own investigations, except where acknowledged. This thesis has not, in its entirety or in part, been previously submitted to any University for degree purposes.

Signed: 

Robin A.W. Gardner

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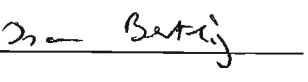
Signed: 

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LIST OF ABBREVIATIONS

ABA, Abscisic acid

AET, Actual evapotranspiration

BC (%), Increase in basal stem circumference, expressed as a percentage

b.s.c., Basal stem circumference (circumference of the stem measured immediately above the soil line)

CERU, Controlled environment research unit, University of Natal

CCWR, Computing Centre for Water Research, University of Natal

Cold Hour, One hour where the average temperature for that hour was less than 5 ^{0}Celsius

Conviron, Semi-controlled environmental conditions unit, e.g. a cold room or growth cabinet

CP, Chilling Portion, chill unit measured by the Dynamic Model

C.V. (%), Coefficient of variation (expressed as a percentage)

DPCU, Daily Positive Utah Chill Unit, chill unit measured by the Daily Positive Utah Chill Unit Model

ICFR. Institute for Commercial Forestry Research

ISCW, Institute for Soil, Climate and Water

LSD, Least significant difference

MAP, Mean annual precipitation

MAT, Mean annual temperature

PAR, Photosynthetically active radiation

PBZ, Paclobutrazol

PEA, Plant Efficiency Analyser

PET, Potential evapotranspiration

PGR, Plant growth regulator

r, Correlation coefficient

R^2, Regression "Coefficient of determination"

S.E.D., Standard error of the differences of the means

SSB, Soil field capacity

SSL, Current soil moisture level

SSM, Soil storage capacity

SWP, Soil wilting point

UCU, Utah Chill Unit, chill unit measured by the Utah Chill Model

CHAPTER 1

INTRODUCTION

1.1 Ecological distribution of *E. nitens*

Shining Gum (*Eucalyptus nitens* (Deane & Maiden) Maiden) is a tall forest tree which, in its natural habitat, grows on the slopes and mountain tops of high tablelands and coastal ranges in south-eastern Australia. The species occurs in several disjunct populations from the Dorrigo Plateau in New South Wales (about 30 ^{0} 23 ' S. latitude) to the central highlands of Victoria (38 ^{0} 00 ' S. latitude) (Brooker and Kleinig 1983, Chippendale 1988). Furthermore, the species occurs over a range of altitudes from 1500 m on the tablelands in the north, to 600 m in the Victorian coastal ranges (Eldridge et al. 1993). *Eucalyptus nitens* grows in tall open forest formations, and in many instances pure stands. Preferred soils are well-drained, acidic loams. Parent materials include basalt, granite schist, shale and sandstone (Boland et al. 1992, Chippendale 1988). The natural distribution of the species and locality of the major provenances is indicated in ***Figure 1.1.***

The climate of the majority of the areas in which *E. nitens* occurs is cool temperate, with mild summers and cool to cold winters. Mean maximum temperature of the hottest month ranges from 21 to 26 ^{0}C and mean minimum of the coldest month from -5 to - 2 ^{0}C . In winter, frosts are numerous and severe. Light to moderate snowfalls, possibly remaining on the ground for several days to a week or more at a time, occur in the winter months throughout most of the species habitat. *Eucalyptus nitens* is confined to areas with a mean annual precipitation of at least 750 mm, rising to well over 1000 mm at many locations. Rainfall distribution is more or less evenly spread throughout the year apart from the most northerly areas where rainfall has a distinct summer maximum. Months with less than 50 mm, even in the drier localities, are rare (Boland et al. 1992, Eldridge et al. 1993).

1.2 Taxonomical classification of *E. nitens*

The genus *Eucalyptus* belongs to the family *Myrtaceae* which comprises 90 genera and over 3000 species of woody plants (Penfold and Willis 1961, Chippendale 1988). About 500 eucalypt species are currently recognized (Pryor 1985). Other plants of commercial importance within the family *Myrtaceae* include *Psidium guajava* (edible guava) (Swart et al. 1991), *Melaleuca spp.* (paperbarks) (Boland et al. 1992), *Leptospermum spp.* (tea bushes) (Lawrence 2001) and *Syzigium*

spp. (waterberry trees) (Venter and Venter 1996).

Pryor and Johnson (1971) carried out one of the most comprehensive classifications of the eucalypts. These authors based their classification primarily on an appreciation of the genetic relationships between species, a classification system making use of ten genetically isolated subgenera. Each subgenus is divided into sections, series, subseries, superspecies, species and subspecies (Florence 1996). *E. nitens* is included in subgenus *Symphyomyrtus*, section *Maidenaria*, series *Viminales* and subseries *Globulinae*. Other species included in this subseries group include other commercially important forest plantation eucalypts such as the Southern Blue Gums (superspecies *Globulus*) and *E. cypellocarpa* (Mountain Grey Gum) (Pryor and Johnson1971). Although an alternative re-classification of the eucalypts, entailing substantial changes to the taxa levels and names assigned by Pryor and Johnson (1971), has been proposed by Brooker (2000), for historical reasons the classification system of Pryor and Johnson (1971) will be adopted in this dissertation.

1.3 Floral biology of *E. nitens*

1.3.1 Floral induction

According to O'Neill (1993), flowering can be separated into four sequential component processes: a) floral induction; b) transduction of the induced state to the meristem; c) floral evocation of the meristem, and d) organogenesis, the development of the flower.

Floral induction may be defined as "the processes required for evocation" (Bernier et al.1981) or "the events triggering the processes rendering a plant capable of flowering" (Bernier 1988). More specifically, *floral induction* entails the processes occurring at one site in a plant, such as the leaves, which lead to the production and translocation of a floral stimulus elsewhere in the same plant (Evans 1971, Aukerman and Amasino 1998).

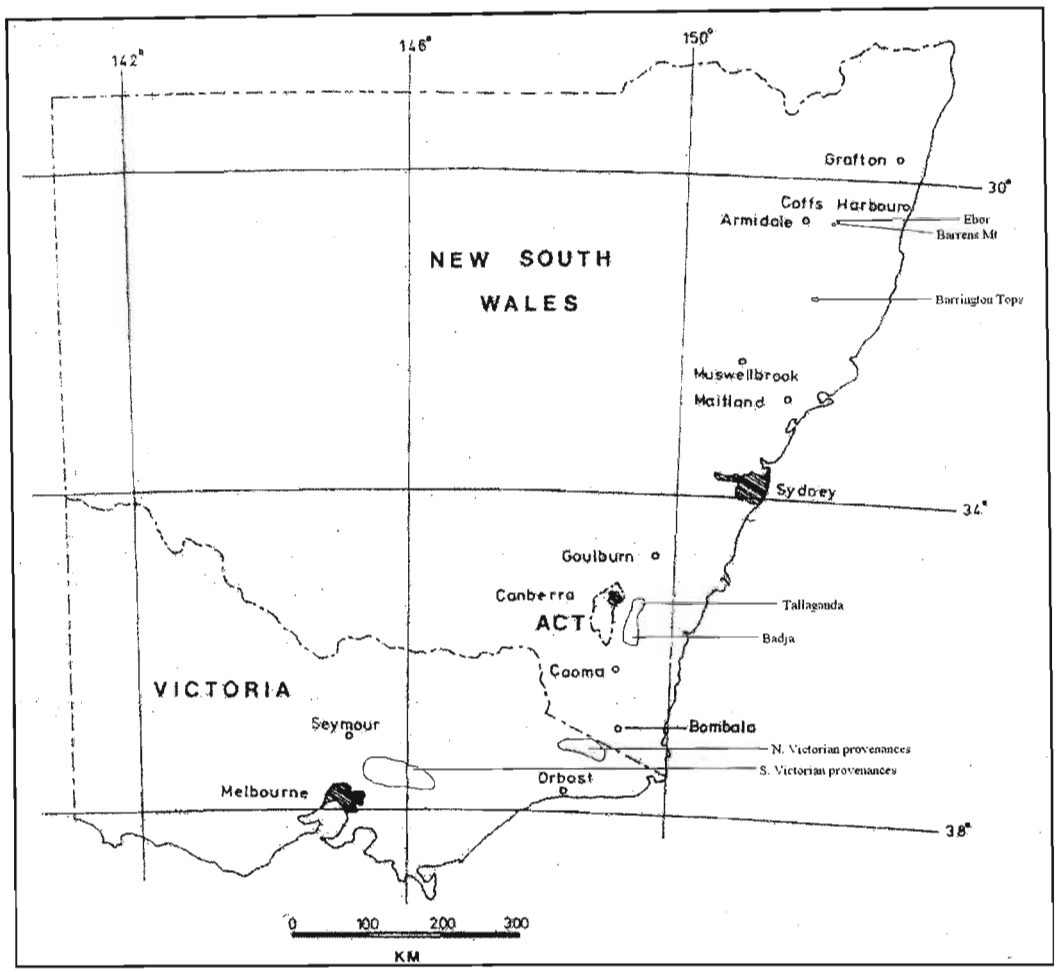


Figure 1.1 Map of the distribution of *E. nitens* with localities of the major provenances (adapted from Purnell and Lundquist (1986)).

Floral evocation may be defined as "the processes in the apex required for irreversible commitment to initiate flower primeordia" (Bernier et al.1981), or "the processes which occur in shoot apical meristems following perception of the translocatable stimulus" (Evans 1971). O'Neill (1993) described *floral evocation* as "the series of events in the development of the reproductive structures involving the expression of new genetic material and associated changes in metabolism following *induction*". Salisbury and Ross (1992) expand on this in that " in many species, arrival of the flowering stimulus leads to an immediate increase in mitotic activity, and nuclear size often increases, as does the size of the nucleolus".

It can therefore be said that the processes of *floral induction* and *evocation* are separated both spatially and temporally (O'Neill 1993).

Floral initiation was described by Bernier et al. (1981) as "the production by meristems of clearly

recognizable flower primordia and all preceding reactions that are required if flowers are to be initiated". In practice, *floral initiation* is recognized as the stage at which a reproductive apex can clearly be seen (microscopically) to differ morphologically from the vegetative apex by the comparative broadening and flattening of the reproductive apex of the former (Moncur 1988).

From the above definitions, it is clear that the terms *floral evocation* and *floral initiation* refer specifically to the inception of floral structures (individual inflorescences and flowers), whereas *floral induction* refers more to the induction processes occurring in macro structures such as leaves and shoots.

Although the ontogenetic stage of the plant is critical, environmental signals are key factors in determining the switch from vegetative to reproductive growth (Ting 1982, Davenport 1990). Phytohormones and nucleotides participate in the activation and derepression of the genes responsible for development of the flower primordia (Larcher 1995, Meilan 1997). Almost every aspect of the environment, particularly photoperiod, temperature and moisture stress, have been known to influence the flowering response (Gepts 1987, Thomas 1993).

1.3.1.1 Temperature

Winter chilling promotes floral induction in certain temperate eucalypts (Moncur 1992, Hasan and Reid 1995).

Moncur and Hasan (1994) and Moncur (1998) subjected potted *E. nitens* grafts to controlled environment conditions and various concentrations of paclobutrazol. These experiments showed that a period of cold is pre-requisite for floral induction in *E. nitens*.

Low temperatures are effective in promoting floral induction in several evergreen horticultural tree crops such as olive (*Olea europaea* L.) (Martin et al.1994), sweet orange (*Citrus sinensis* (L.) Osbeck) (Davenport 1990) and lychee (*Litchi sinensis* Sonn.) (Menzel 1983).

1.3.1.2 Soil moisture

There is some evidence that a period of low water status in autumn/early winter may predispose reproductively mature field-grown eucalypts to flower (Moncur 1998). The results of a trial with potted *E. globulus* seedlings suggested that water stress may play a complimentary role together with other promoting factors such as cold and anti-gibberellin treatments in the stimulation of

floral induction in the species (Hasan and Reid 1995). However, Moncur and Boland (2000) reported that flower-bud production did not occur in potted *E. nitens* grafts following subjection of the plants to various levels of soil moisture stress.

The effect of drought stress on flowering in citrus crops is well-documented (Davenport 1990, Krajewski and Rabe 1995). In lemon (*Citrus limon*), floral induction occurs within two weeks of imposing drought stress on orchards trees (Nir et al. 1972). Somewhat longer periods (four to five weeks) of cyclical drought conditions are necessary to induce flowering in lime (*Citrus latifolia*) (Southwick and Davenport 1986). In lychee (*Litchi sinensis*), ecological and experimental evidence suggests that floral initiation is promoted by water stress during the preceding autumn and winter (Menzel 1983).

Drought stress has been implicated in the stimulation of flower bud production in a range of conifers and broadleaf forestry tree species (Philipson 1990, Nilsen and Orcutt 1996).

1.3.1.3 Photoperiod

Precision of timing of developmental processes is of particular ecological significance for successful reproduction of plants (Larcher 1995). The role of photoperiod in stimulating a timeous floral response in a variety of crops (photoperiodic induction) has been intensively researched and is well documented (Salisbury and Ross 1992). Although *Eucalyptus* species produce flower buds in the leaf axils of new growth in spring (Tibbits 1989, Jones and van Staden 2001), under controlled environment photoperiod did not appear correlated to induction of flowering in two cold tolerant eucalypt species, namely *E. lansdowneana* (Moncur 1992) and *E. nitens* (Moncur and Hasan 1994).

1.3.2 Floral development and morphology

Most eucalypts are heteroblastic. The foliage morphology of "juvenile" and "adult" plants is not distinctly different in all heteroblastic species though. With respect to *E. nitens*, in all provenances the juvenile foliage is conspicuously different from the adult foliage in that the juvenile leaves are opposite, sessile, ovate, glaucous and discolorous, and the adult leaves are alternate, petiolate, lanceolate, green and concolorous (Pryor 1985, Boland et al.1992).
In most eucalypts, including *E. nitens* and its close relative *E. globulus*, under natural environmental conditions, flowers are produced on the spring flush of adult, reproductively mature trees. However, recent work on heteroblastic eucalypt species, such as these, has suggested that the

timing of vegetative phase change and that of first flowering are genetically (Jordan et al. 1999) and physiologically (Hasan and Reid 1995, Moncur and Boland 2000) independent.

1.3.2.1 Reproductive shoots

Eucalyptus trees produce naked buds in the axils of all leaves, the buds becoming macroscopically visible as each subtending leaf unfolds. Outgrowth of these newly formed buds can remain inhibited for one or more seasons due to apical dominance (Penfold and Willis 1961, Pryor and Johnson 1971).

Depending on circumstances, resting buds may give rise to "vegetative" or "reproductive" shoots. In the cool subtropical areas of South Africa and cool temperate areas of Chile and south eastern Australia, where *E. nitens* is grown commercially, outgrowth and development of "reproductive shoots" from resting buds coincides with the spring growth flush (Tibbits 1989, Gardner 2001*b*, Jones and van Staden 2001).

Floral initiation in *E. nitens* has not yet been intensively investigated in South Africa. In S.E. Australia (Canberra, A.C.T. and Tallaganda, N.S.W), floral initiation in *E. nitens* occurs in early spring (late August/ early September) (Moncur et al. 1994*a*). Worldwide little is known about the timing of inductive cold events for flowering in *E. nitens*. It is unclear as to whether cold as a floral stimulus occurs in one single inductive event, or cumulatively over a series of events (Moncur and Hasan 1994, Meilan 1997), although observations of flowering patterns in *E. nitens* in South Africa seem to indicate that the latter is more likely.

In a range of dicotyledonous crops researched, commitment to develop a floral apex did not occur within the shoot apical meristem, but rather the meristem formed either vegetative or reproductive structures depending on the signals received from leaves (Aukerman and Amasino 1998). It is likely that this phenomenon is applicable to temperate eucalypts such as *E. nitens*, as local experience has shown in the latter species, it is a common occurrence that not all buds produced along the length of "reproductive shoots" are flower buds (R. A. W. Gardner unpublished data). The early morphological stages of reproductive shoot development prior to anthesis in *E. nitens*, i.e. from resting bud to early outgrowth of shoots, are illustrated in ***Figure 1.2***. The later morphological stages of reproductive shoot development prior to anthesis, i.e. the ongoing development of umbels with involucral bracts remaining intact, till the stage where involucral bracts are shed, are illustrated in ***Figure 1.3.***

1.3.2.2 Inflorescences

The inflorescence in *E. nitens* is first discerned as a single bud in the axil of a newly developing leaf. All component incipient flower buds are enclosed by an involucre of bracts. As growth and expansion of the enclosed floral bud cluster takes place, the involucral bract is shed and the separate flower buds appear (Pryor 1985) (***Figure 1.3 b***). The inflorescence is axillary and simple, and consists of a condensed dichasial cyme. The bud pedicels are attached to a common peduncle forming an umbel (Pryor 1985). In *E. nitens* the umbel normally consists of seven flowers and is borne on an angular to slightly flattened peduncle (Boland et al. 1992). In most previous publications on *E. nitens* flowering research work, the inflorescence in the species is referred to as an "umbel", therefore, in all further discussion in this thesis, the same terminology will be used. In *E. nitens*, flower buds (umbels with involucral bracts still intact) normally become first visible to the naked eye around late October in South Africa, but this stage of flower bud development can occur through till March (late summer) depending on genotype and environmental factors such as spring heat units (Moncur and Boland 2000, Jones and van Staden 2001).

1.3.2.3 Flowers

Individual flowers in the umbel are sessile to shortly pedicellate and average about 7 x 3 mm in size. Fruits (woody capsules) are sessile, ovoid in shape and average 6 x 5 mm in size (Brooker and Kleinig 1983). Prior to anthesis the developing inner floral organs are protected by two conical bud caps (opercula). The outer (calycine) operculum consists of fused sepals, whereas the inner (corolline) operculum consists of fused petals (Pryor and Knox 1971, Pryor 1985). The outer operculum is usually shed well before anthesis, whereas the inner operculum is shed immediately prior to anthesis (Pryor 1985, Tibbits 1989).

1.4 Aim of the study

Eucalyptus nitens is one of the most important eucalypt species grown for sawlog and pulpwood production in south-eastern Australia and Tasmania (Pinkard and Beadle 1998), New Zealand (Gea et al. 1997) and Chile (Gardner 2001*b*). The species is the most important cold tolerant eucalypt species grown for pulpwood production in South Africa (Darrow 1996, Carlson et al. 2000). However, tree improvement and seed production efforts with *E. nitens* have been presented with problems over the years by certain aspects of the floral biology of the species. In its natural habitat

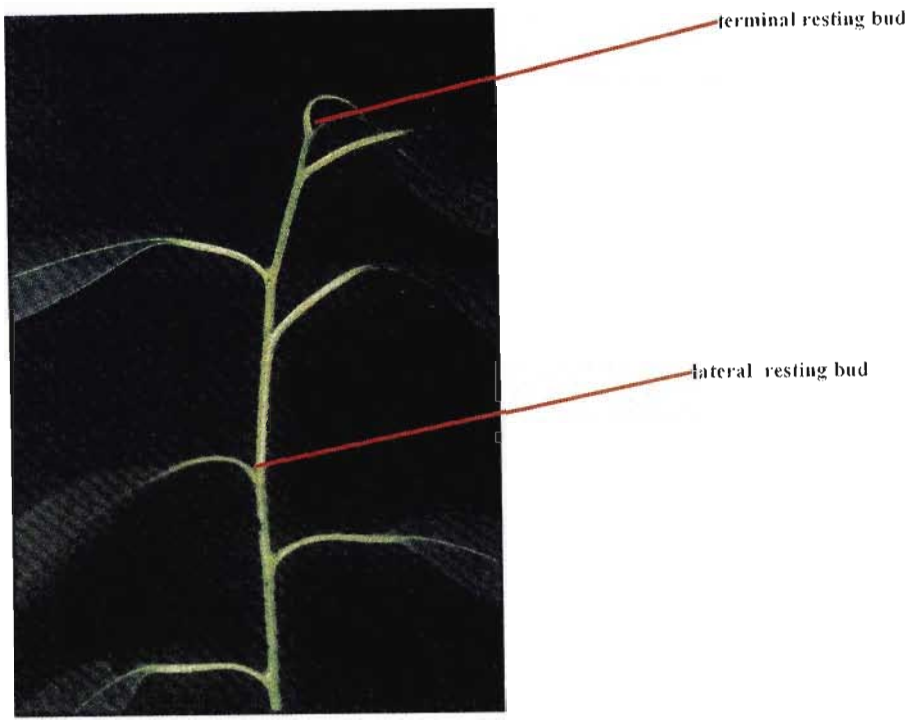
and/or in fertilised plantation conditions in Australia, *E. nitens* rarely produces flowers before the fifth year and any worthwhile seed crop prior to ten years (Reid et al.1995, Moncur and Boland 2000). In South Africa, *E. nitens* is renowned for its tardy, unreliable and shy flowering tendencies. Trees seldom produce flowers and any noticeable amount of seed before the age of 10-12 years (Poynton 1979, Eldridge et al. 1993, Swain and Chiappero 1998). Over the years, these characteristics of *E. nitens* have posed problems to breeders (slow generation turnover time) and commercial seed producers (insufficient and unreliable quantities of seed), both overseas and locally (Reid et al. 1995, Swain 2001, Swain and Gardner 2002).

Precocity in *E. nitens* appears to be under a high degree of genetic control, similar in this respect to its close relative *E. globulus*, though favourable environmental conditions are still of prime importance for promoting early flowering (Chambers et al.1997, Swain and Chiappero 1998, Jones 2002).

Vegetative propagation, either by macro- or micro-culture methods has proven difficult in *E. nitens*, and until this problem is overcome, seed remains the only viable option for planting programs (Eldridge et al.1993, Moncur and Boland 2000).

During the past decade, considerable progress has been made towards the development of a management system for the promotion of earlier and more abundant flowering and seed production in *E. nitens*. One of the most significant outcomes of the research during this period has been the identification of the triazole type plant growth retardant paclobutrazol ((2RS,3RS)-1-(4-chlorophenyl)-4,4-dimethyl-2-1,2,4-triazol-1-yl-pentan-3-ol) as a powerful floral stimulant in temperate eucalypts such as *E. globulus* and *E. nitens* (Moncur and Boland 2000, Williams et al. 1999). Precocious flowering in young trees and more regular and abundant flower and seed crops in adult trees of *E. nitens* as a result of paclobutrazol application, has significantly reduced the generation turnover period, and improved seed production in the species (Griffin et al. 1993, Williams et al. 1999). For this reason, paclobutrazol treatment has become an extremely popular tool in *E. nitens* breeding and seed production programmes worldwide. Regardless of paclobutrazol application though, a period of cold appears near obligatory for floral induction in *E. nitens* (Moncur and Hasan 1994, Moncur et al.1994*b*).

a.



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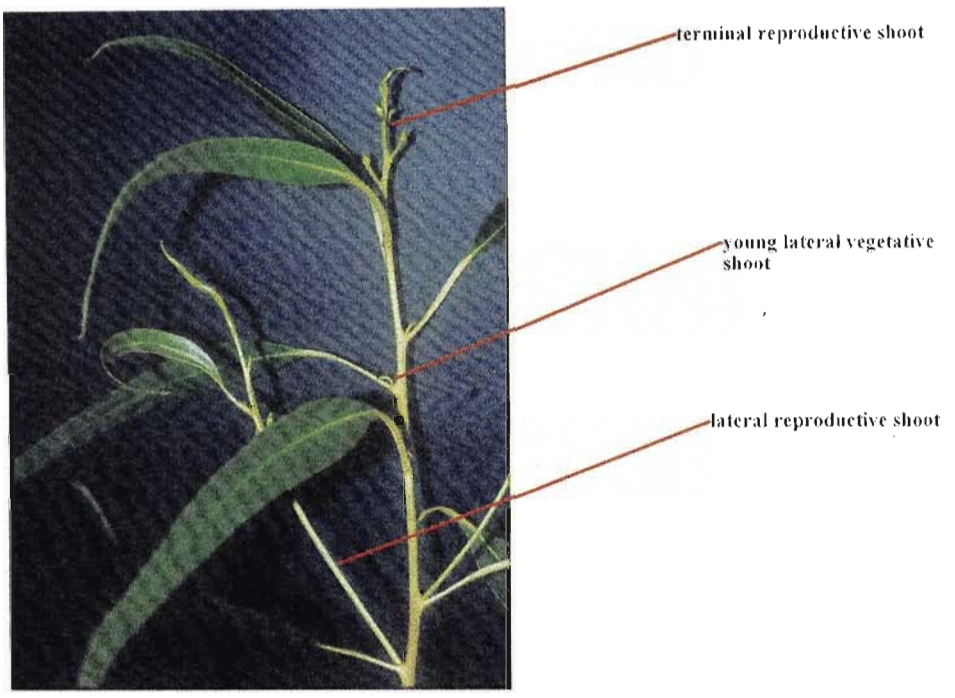
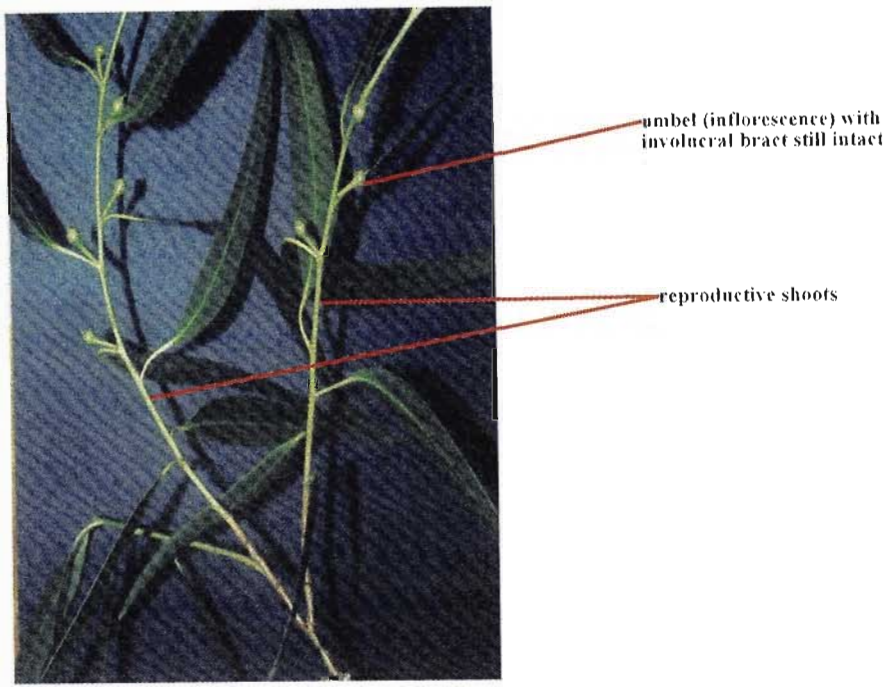


Figure 1.2 Early morphological stages of reproductive shoot development prior to anthesis in *E. nitens*: a. resting bud stage; b. early stages of outgrowth of vegetative and reproductive shoots.

a.



b.

Figure 1.3 Later morphological stages of reproductive shoot development prior to anthesis in *E. nitens*: a. ongoing development of reproductive shoots with involucral bracts still intact; b. shedding of involucral bracts, individual flower buds within umbels exposed.

In South Africa, due to apparently unfavourable climatic conditions for flowering, tree breeders and commercial seed producers are currently almost totally reliant on paclobutrazol application to obtain any significant amount of flowering and seed in *E. nitens* (Swain and Chiappero 1998, Swain and Gardner 2002). This practice, however, is far from the ideal, as apart from the high cost of the chemical, paclobutrazol persists actively in the soil for several years, the latter feature attracting increasing pressure from environmentalists (Reid et al.1995, Moncur 1998). It is thus highly desirable to investigate more economical and environmentally sustainable ways of stimulating seed production in *E. nitens*.

Floral initiation in *E. nitens* has yet to be intensively investigated in South Africa. In S.E. Australia (Canberra, A.C.T.), floral initiation in *E. nitens* occurs in late winter (August to early September) (Moncur et al. 1994*a*). Although winter cold has been identified as an important stimulus for floral induction, little is known about the timing of inductive cold events for flowering in *E. nitens*. It is unclear as to whether cold as a floral stimulus occurs in one single inductive event, or cumulatively over a series of events (Moncur and Hasan 1994, Meilan 1997). Observations of flowering patterns in *E. nitens* in South Africa tend to indicate that the latter is more likely. At certain sites in the summer rainfall area, trees of *E. nitens* seem to flower more precociously and consistently than usual. Personal experience indicates that these sites are characterized by high altitude (>1400 m.a.s.l.), exposed position (up-slopes and crests), susceptibility to light frosts, and generally a southwest to eastern aspect. Soil moisture availability at these sites is high all year round as a result of the combined effect of high mean annual precipitation (MAP) (> 950 mm), frequent summer mist, occasional winter snow and deep soils (> 1.0 m). Comparing these climatic conditions to those of neighbouring areas of similar latitude, altitude and available soil moisture, but where *E. nitens* does not flower regularly, suggests that uniform cool conditions in winter are more promotive of flowering in this species than extremely varying temperature conditions. Hence, a cumulative cold requirement may be implicated in floral induction in *E. nitens*.

Current *E. nitens* commercial seed orchards (and indeed the bulk of the plantations of the same species) in South Africa are located in "warm temperate" areas of the summer rainfall region where large day/night temperature amplitudes regularly occur during the winter months (May to September) (Schulze 1997, Swain and Gardner 2003). Daytime temperatures commonly exceed 20 ^{0}C whilst nighttime temperatures descend below -5 ^{0}C, which results in day/night amplitudes greater than 25 ^{0} C (Richardson and McMahon 1992). Such extreme temperature

conditions in winter are antagonistic towards chilling accumulation and bud endo-dormancy release in temperate fruit crops (Couvillon and Erez 1985). In contrast, "uniformly cool" conditions associated with cloudy and foggy weather in winter enhance chilling accumulation (Erez 2000).

Therefore, chill models used to quantify the effect of such winter chilling on bud dormancy release in temperate fruit crops (George and Erez 2000, Halgryn et al. 2001) could provide a useful tool to estimate floral bud production in *E. nitens*. In order to characterise "effective" cold for floral induction in *E. nitens*, this study evaluated three such chill models to determine whether chill units could account for the variability in *E. nitens* floral bud production. Information arising from such an investigation would not only add to the limited knowledge of optimal temperatures for floral induction in *E. nitens*, but may also assist in the siting of *E. nitens* seed orchards in South Africa. In addition, photosynthetic efficiency of cold-treated *E. nitens* plants and the relationship between drought and floral productivity in *E. nitens* were also examined to investigate whether flowering in *E. nitens* is plant stress related or not.

CHAPTER 2

GENERAL EXPERIMENTAL PROCEDURES

2.1 Field trials

A series of four field trials was established across a range of high altitude sites in the summer rainfall forestry belt during 1996. The essence of these trials was to subject *E. nitens* trees to a range of winter cold conditions, and investigate whether the resultant flower bud crops were related to amount of prior winter chilling received by the trees. Information derived from these trials may contribute towards the characterising of *E. nitens* cold requirement for floral induction, and ultimately assist in the siting of *E. nitens* seed orchards in South Africa.

2.1.1 Plant material

Seedlings and grafts of three *E. nitens* provenances previously selected to be the most suited for commercial pulpwood plantings in the summer rainfall regions of South Africa (Swain et al.1998, Swain 2001) were used in the trials. The origin of each provenance is briefly described in ***Table 2.1***.

Table 2.1 Australian origins of the *E. nitens* provenances and families in the field trials.

PROVENANCE	FAMILY	STATE	LATITUDE	LONGITUDE	ALTITUDE (m)
Barrens Mountain	32091	New South Wales	30° 25 '	152° 28 '	1505
Barrens Mountain	32097	New South Wales	30° 24 '	152° 29 '	1535
Barrington Tops	34838	New South Wales	31° 55 '	151° 30 '	1450
Tallaganda	37255	New South Wales	35° 54 '	149° 30 '	1290

The seedlings were produced as follows: In October 1995, four separate seedlots were sown and raised in planter trays in the ICFR Pietermaritzburg nursery. The seedlots were selected families from the three provenances listed in ***Table 2.1***, with each seedlot consisting of seed from a different, single mother tree in New South Wales, Australia.

The grafts were produced as follows: In August 1995, scions were cut from four different three year old grafted ramets in the Institute for Commercial Forestry Research (ICFR) clonal orchard at Lions River, KwaZulu-Natal (KZN), and grafted onto six month old, potted seedling rootstocks. The rootstocks were grown from South African open-pollinated orchard seed of mixed provenance background. The scions were grafted at a height of about 300 mm from bases of the seedlings (potting soil level). The above four ramets were originally produced by grafting scions from six year old "plus trees" of the same families as the seedlings. Seedlings and grafts of the same families were therefore half-siblings. At the time of cutting the scions from the three year old ramets, none of the latter had previously produced flower buds (R. A. W. Gardner unpublished data). In mid-January 1996, the surviving grafts were tallied, and trial layouts designed according to number of healthy grafts available for planting and blanking.

Prior to planting, all plants were raised in milled and composted pine bark as a growing medium. Seedlings were grown in polystyrene planter trays with 128 x 75 cm^3 individual compartments. Grafts were grown in 5 litre black polythene grow- bags.

2.1.2 Environmental conditions

To test the possible effect of chilling on flowering in *E. nitens*, a gradient in winter chilling was created by selecting trial sites at four separate localities in the summer rainfall forestry area based on altitude and latitude (***Table 2.2***). However, sites differed only slightly in daylength, with a maximum of 27 min difference in daylength between sites at the shortest day (21st June) and a maximum of 5 min difference in daylength between sites at the longest day (30th September) in the period of cold accumulation (Schulze 1997).
All trials were located at elevated positions in the landscape where diurnal temperature range would be at a minimum, ie. cold air on winter evenings would drain away freely and daytime maxima in winter would be reduced. Previous research suggested that sites in the summer rainfall area where *E. nitens* was known to flower best were those where high numbers of winter chill units were regularly logged (R. A. W. Gardner unpublished data). Winter chill was quantified using certain chill models used by the local deciduous fruit industry to predict winter rest completion of flower buds. A brief description of each of these models is provided in paragraph 2.4.2 below.

Based on mean annual temperature (MAT) and mean minimum temperature of the coldest

month, the sites chosen, from warmest to coldest, were Gowan Brae, Mossbank, Blyfstaanhoogte and Tentkop. The trial names are indicative of the plantations on which the trials were established. Site conditions for the four field trials are presented in ***Table 2.2***.

Table 2.2 Site conditions for the *E. nitens* field trials.

Trial name	Gowan Brae	Mossbank	Blyfstaanhoogte	Tentkop
District, province	Boston, KZN	Bulwer, KZN	Lydenberg, MPU	Maclear, EC
Planting date	21/02/1996	07/02/1996	26/04/1996	06/03/1996
Latitude	29° 37' 22"' S	29° 49' 08"' S	25° 10' 03"' S	30° 48' 30"' S
Longitude	30° 08' 52" E	29° 42' 20" E	30° 36' 40" E	28° 15' 35" E
Altitude (m)	1465	1680	1995	1920
Mean annual precipitation (mm) *	990	1105	1198	963
Mean monthly max (hottest month) (°C)	24,7	22,8	22,4	23,6
Mean monthly min (coldest month) (°C)	3,6	1,9	3,6	1,3
Mean annual temperature (°C) *	15,2	14,0	13,2	13,0
Soil classification:				
Soil form/family **	Magwa 1100	Magwa 1100	Magwa 1100	Hutton 1100
Parent material	Dolerite/ Shale	Dolerite/ Shale	Dolerite/ Shale	Dolerite
Soil depth (cm)	> 120	120	100	> 120

* Schulze (1997)

** Soil Classification Working Group (1991)

A description of the trial sites based on spatial temperature stratification is as follows: Gowan Brae was located in the Highland Cool Temperate physiographic forestry region (Kunz and Pallett 2000) near Boston in southern KwaZulu-Natal province at 1465 m altitude. This site, with a MAT of 15.2 ^{0} C, was at the upper MAT threshold for successful commercial planting of *E. nitens* in South Africa (Herbert 2000, Gardner 2001*a*).

Mossbank was located in the Highland Cool Temperate physiographic forestry region (Kunz and Pallett 2000), 50 kms south-west of Gowan Brae, closer to the Drakensberg mountain range in KwaZulu-Natal. This site, with an elevation of 1680 m and mean annual temperature of 14.0 ^{0} C, was chosen as an intermediate between Gowan Brae and Blyfstaanhoogte.

Blyfstaanhoogte was located in the Cool physiographic forestry region (Kunz and Pallett 2000) in the mountains above Sabie in eastern Mpumalanga province. This site, with an elevation of 1995 m and mean annual temperature of 13.2 ^{0} C, was the second coldest site.

Tentkop was located in the Cool physiographic forestry region (Kunz and Pallett 2000) in the high foothills of the southern Drakensberg mountain range near Rhodes in the Eastern Cape province. This site, with an elevation of 1920 m and a mean annual temperature of 13.0 ^{0} C was chosen as the most extreme cold site and at the lower MAT threshold for successful commercial planting of *E. nitens* in South Africa (Herbert 2000, Gardner 2001*a*).

In selecting sites, in addition to temperature criteria being employed, site history was also taken into consideration. Two of the trials, Gowan Brae and Blyfstaanhoogte, were sited where *E. nitens* seedlings in an ICFR species trial and a commercial plantation block had previously demonstrated precocity (flower buds produced at 5 years after planting) (Nixon pers. comm. 1995).

The site at Tentkop was chosen based on precocious flowering earlier observed in trees of *E. nitens* in a eucalypt species trial at Thaba Putsoa, Lesotho (Richardson 1985). In this trial, seedlings of *E. nitens* produced abundant flowers when 5 years old (Stanger pers. comm. 1995). Tentkop was therefore chosen as a South African summer rainfall area equivalent to the severely cold, apparently highly inductive site at Thaba Putsoa. Although the altitude of Tentkop was slightly lower than that of Blyfstaanhoogte, it's latitude was much higher.

All sites selected were "well-watered", i.e. mean annual precipitation (MAP) was at least 900 mm, and soil profiles were deep, i.e. soil depth was at least 100 cm. Therefore, sites were not normally drought-prone, as dry conditions would complicate the envisaged study of the effect of cold temperatures on flowering.

2.1.3 Chemical treatment

The suspension concentrate "Cultar®" (ICI Agrochemicals, formulation = 250 g paclobutrazol per litre), was used as the paclobutrazol treatment (PBZ). The choices of application rate, method and timing were based on those treatments found most successful and practical in earlier work carried out by Griffin et al.(1992), and Moncur et al.(1994*b*). Rate of application per tree was 0.25 g a.i. (ie. 1.0 ml "Cultar®") per centimetre basal stem circumference (b.s.c.). The suspension was applied as a soil drench during early April 1998, two years after planting. Date of application and soil moisture details for the paclobutrazol soil treatment are given in ***Table 2.3***. The soil moisture level in the upper the soil profile at each site was around 50 % of

field capacity. Mean tree heights for the four trials on the date of application are presented in *Table 3.4.*

To calculate the paclobutrazol dose for any given tree, b.s.c. was measured at the narrowest point along the stem between graft union (in the case of grafts) or root collar (in the case of seedlings) and the first lateral shoot (secondary branch).

The method of application for each tree was as follows:

1. A circle of 1.0 m radius of soil surface around each tree stem was raked free of organic litter.
2. The calculated amount of Cultar® was mixed vigorously in five litres of water.
3. The solution was evenly distributed onto the soil surface around the stem using a watering can. This method of application prevented any run-off.

Table 2.3 Details of paclobutrazol soil treatment in the field trials.

		% field moisture	
Trial	**Application date**	**A-horizon (depth in cm)**	**B-horizon (depth in cm)**
Gowan Brae	01/04/1998	55.3 (0 - 35)	60.8 (35 - 75)
Mossbank	02/04/1998	46.5 (0 - 40)	39.0 (40 - 120)
Blyfstaanhoogte	07/04/1998	55.0 (0 - 15)	58.2 (15 - 100)
Tentkop	07/04/1998	53.0 (0 - 30)	45.8 (30 - 120)

2.1.4 Trial layout

The number of available grafts of each "family" restricted the number of trial sites to four. The following treatments were applied in each trial:

PROPAGULE: seedling; graft.

FAMILY (provenance in parentheses): 32091 (Barren Mt.); 32097 (Barren Mt.); 34838 (Barrington Tops); 37255 (Tallaganda).

PBZ (paclobutrazol application): 0.00 g paclobutrazol/ cm b.s.c.; 0.25 g paclobutrazol/ cm b.s.c.

At each trial site a split plot design experiment was laid out. The experiment consisted of two whole-plots (0.00 g and 0.25 g paclobutrazol/ cm b.s.c.), each of these being further divided

into 24 sub-plots. The sub-plots each consisted of five trees, and were randomly assigned to different combinations of PROPAGULE x FAMILY. The total number of data trees at each trial site was 240, and trees were spaced 3.0 m x 3.0 m apart. The necessary buffer rows were incorporated around each trial. Details of the allocation of sub-plots to treatments are given in *Table 2.4*.

Table 2.4 Details of the allocation of sub-plots to treatments in the four field trials.

Treatment	0.00 g paclobutrazol/ cm b.s.c.		0.25 g paclobutrazol/ cm b.s.c.	
Family	Seedling	Graft	Seedling	Graft
32091	3 (15)*	3 (15)*	3 (15)*	3 (15)*
32097	3 (15)	3 (15)	3 (15)	3 (15)
34838	3 (15)	3 (15)	3 (15)	3 (15)
37255	3 (15)	3 (15)	3 (15)	3 (15)
Total	12 (60)	12 (60)	12 (60)	12 (60)

Note: *, in each cell in this column the total number of sub-plots, followed by total number of trees (in parentheses) are given.

2.1.5 Data collection

2.1.5.1 Tree growth measurements

Breast height diameter (dbh) and height was measured annually in April for the duration of the trials. Height was later deemed the most reliable indicator of tree growth for statistical analysis purposes, as the multi-stemmed nature of the plants, particularly in the case of the grafts, caused difficulties in accurately measuring dbh. Many of the trees at the highly frost-prone Tentkop site had dual or multi stems resulting from frost damage during the first two winters after planting.

2.1.5.2 Flowering assessments

Because strong growth was anticipated in the field trials during the observation period, a simple scoring system for rating umbel crops on trees was devised. Each tree was scanned for umbels and a score allocated on the following basis:

0 = no umbels

1 = very light crop; 25 % or less of the secondary laterals* bearing one or more umbels

2 = light crop; > 25 % - 50 % of secondary laterals bearing one or more umbels

3 = moderate crop; > 50 % - 75 % of secondary laterals bearing one or more umbels

4 = heavy crop; > 75% - 100 % of secondary laterals bearing one or more umbels

* secondary laterals were defined as branches originating from primary scaffold branches. Number of primary scaffold branches ranged from one (single stemmed plants) to several (multi-stemmed plants).

The rating of trees was carried out once annually from ground level. Observations were initially made with the naked eye, but from April 1999 it became necessary to use a suitable pair of binoculars for scanning upper portions of the tree crowns for umbels.

April was determined to be the most suitable month for annual flower crop assessment in *E. nitens* as, on average, the range of provenances and genotypes evaluated were most conspicuous at this time with the majority of involucral bracts shed and individual flower buds visible.

In South Africa, in early flowering genotypes individual flower buds are usually fully expanded in March as anthesis is about to commence. Umbel crop scoring becomes risky from about the end of April when abortion of flowers in early flowering genotypes commences due to non-pollination and/or the onset of inclement dry and cold weather particularly at high altitude sites.

2.1.5.3 Temperature measurements

Hourly temperature measurements were logged in all trials between April and October each year from establishment in 1996 to October 2000. In the winter rainfall deciduous fruit growing areas of South Africa chill unit accumulation commences in May, but in some instances, depending on location and year, as early as April (Matthee1982, Linsley-Noakes 1995, Schulze 1997). This is also the case at Pietermaritzburg, KwaZulu-Natal, where low chill peaches may be grown successfully under summer rainfall conditions (Allan and Burnett 1995). Calculations of chill units using hourly temperature data modelled from daily minimum and maximum temperatures for select high altitude sites (1200 - 1700 m altitude range) in the forestry areas of

KwaZulu-Natal revealed a similar trend (R. A. W. Gardner unpublished data). Hourly temperatures were modelled with a computer program requiring daily minimum temperature (MinT), daily maximum temperature (MaxT), latitudinal and longitudinal co-ordinates and daylength inputs and applying a sinusoidal-exponential daily heating-cooling wave (Linvill 1990, Linsley-Noakes et al. 1995).

The equipment used in the measurement of temperature is described in Chapter 2.3 below. In the case of Blyfstaanhoogte, due mainly to the distance of the site from the office, on a few occasions during the course of the trial logistical problems occurred with respect to data-logging hardware and maintenance of the temperature station. This necessitated later modelling of temperature data for certain periods. Daily minimum and maximum temperatures for the Blyfstaanhoogte site were modelled from daily minimum and maximum temperatures of a nearby ISCW (Institute for Soil, Climate and Water, Agricultural Research Council) weather station. Information pertaining to the modelling of Blyfstaanhoogte temperature data is presented in ***Appendix 1.***

2.2 Trials under semi-controlled conditions

Following the early appearance of flower buds on trees in the Tentkop field trial at two years after planting, two successive controlled environment experiments were undertaken to investigate whether similar amounts of chilling, applied "artificially" under semi-controlled environmental conditions, could induce potted *E. nitens* nursery-stock size trees to produce flower buds. The information from such experiments may not only contribute towards the understanding of how flowering is controlled in *E. nitens*, but also ultimately provide breeders with a simple, manipulative tool to assist decrease the generation turnover time in the species. The first pilot experiment (Experiment 1) was undertaken at the ICFR in Pietermaritzburg during winter 1999. Following limited success in this experiment, a second experiment (Experiment 2) was conducted during winter 2000. In either experiment, the treatments consisted of different amounts of winter cold accumulated between 01 April and 30 September each year, with artificially applied portion of the chilling treatments commencing in June after "natural" cold-hardening in an outdoor environment had been allowed to take place during April and May. A third experiment was carried out during 2001 to demonstrate the rate of uptake of paclobutrazol, when applied as a soil drench, in potted *E. nitens* grafts.

2.2.1 Plant material

2.2.1.1 Experiment 1

In September 1998, scions were cut from four and five year old grafted ramets of three selections in the ICFR grafted orchard at Lions River (KZN) and grafted onto 400 six-month old, potted rootstocks of unrelated "families". In early March 1999, stock was taken of all surviving grafts and a pot trial designed around the available number of healthy plants.

Grafts were grown in 5 litre black polyethylene growing bags filled with standard potting medium consisting of composted, milled pine-bark and fine river sand. Between 03 and 07 July 2000, plants were repotted into10 litre black polythene growing bags using the same potting medium formulation described for the 5 litre bags above.

The origins of the plant material used in this experiment are given in ***Table 2.5***.

Table 2.5 Origins of plant material used in *E. nitens* Experiments 1, 2 and 3.

Propagule type	Clone/ seedling no.	Family	Provenance	Latitude	Longitude	Altitude (m)
1999 experiment						
Graft (clone)	1	32098	Barrens Mt. (NSW)	30° 24 '	152° 29 '	1535
Graft (clone)	2	34838	Barrington Tops (NSW)	31° 55 '	151° 30 '	1450
Graft (clone)	3	37254	Tallaganda (NSW)	35° 54 '	149° 30 '	1290
Graft (clone)	4	37255	Tallaganda (NSW)	35° 54 '	149° 30 '	1290
2000 experiment						
Graft (clone)	5 [1]	34840	Barrington Tops (NSW)	31° 55 '	151° 30 '	1450
Graft (clone)	3	37254	Tallaganda (NSW)	35° 54 '	149° 30 '	1290
Seedling [2]	3	(37254)	(Tallaganda (NSW))	-	-	-

[1] common to Experiments 2 and 3

[2] seedlings were derived from open pollinated seed collected from a single ramet of Clone 3. Hence Clone 3 and Seedling 3 in Experiment 2 were half-siblings.

NSW, New South Wales State (Australia)

2.2.1.2 Experiment 2

Seedlings: In October 1999, three separate open-pollinated seedlots harvested from six-year old ramets of the same selections grafted in August 1999 at the Sappi Shaw Research Centre at Tweedie were sown and raised in planter trays in the ICFR nursery. In mid-January 2000, 100 seedlings of each selection were transplanted into 5-litre bags containing the same growing medium used for the grafts. From here on, the cultivation methods for the potted seedlings were identical to those described for the grafts above. In early March 2000, following the tally of the surviving grafts, Clone treatment no. 2 was replaced with Seedling treatment no. 3. Details of plant material used in Experiment 2 are given in ***Table 2.5***.

Grafts: In August 1999, scions were cut from four year old grafted ramets of three selections in the ICFR orchard at Lions River (KZN) and grafted onto 450 (150 per selection) six-month old, potted rootstocks of unrelated "families". In early March 2000, the surviving grafts were tallied and the experiment designed according to the number of available, healthy plants. Clone no. 2 which performed well and produced umbels in Experiment 1 was not included in Experiment 2 because of it's poor grafting take. Cultivation methods for grafts in Experiment 2 were identical to those in the previous experiment.

2.2.1.3 Experiment 3

In July 2000, scions were cut from four year old grafted ramets of *E. nitens* Clone no.5 (Barrington Tops provenance, Family 34830) (***Table 2.5***) in the ICFR orchard at Lions River and grafted onto 50 five-month-old potted rootstocks of an unrelated commercial bulk *E. nitens* seedlot. Cultivation techniques for this experiment were identical to those used in Experiments 1 and 2. On the 12 March 2001, a batch of eight healthy grafts of similar size and vigour were selected for the experiment and allocated permanent positions in a nursery row.

2.2.2 Environmental conditions

2.2.2.1 Experiment 1

Plants were subjected to various cold treatments by manipulating the duration of exposure to temperatures in the optimum range for chill accumulation of deciduous fruit crops (6 ^{0} to

13 ^{0} C) (Erez et al. 1990, Seeley 1996). However, the minimum temperature of the cold phases was lowered to 4 ^{0} C as a "stop" in vegetative growth seems to induce flowering in *E. nitens* (Moncur pers. comm. 1998). On average, temperatures below 5 ^{0} C are needed for this cessation to occur (Moncur and Hasan 1994).

Periods of cold were alternated with periods of moderate temperatures in an attempt to simulate natural daily temperature cycling, and the 1997 winter weather patterns at Tentkop. Exceptionally good flowering occurred in *E. nitens* at Tentkop following the particularly cold winter at this site during 1997. Furthermore, Erez et al. (1990) and Erez (2000) showed that winter chilling in deciduous fruit crops was enhanced by temperature cycling.

The experiment was carried out in three localities, namely the shadehouse at the ICFR nursery, a conviron (controlled environment unit) at the ICFR and at CERU (Controlled Environment Research Unit) at the Faculty of Agriculture, University of Natal, Pietermaritzburg. Each conviron could only accommodate 36 plants at any given time. The three localities were within 75 metres of each other.

Controlled chilling was applied in the convirons of the ICFR and CERU. At CERU, plants were housed by "day" in a growth room and by "night" in the cold room alongside. The reason for this was that the growth room could not achieve temperatures below 12.5 ^{0} C required for the "night" phase. Day- and night-length settings are referred to in more detail below in section "Temperature and day-length settings".

The control temperature treatment and outdoor temperature phases for all other treatments were applied in the ICFR nursery shadehouse using black shadenetting yielding 80% full sun conditions.

Maximum effort was made to equate environmental conditions in the two convirons as follows:

Lighting:

During June to September 1998, a pilot trial had been conducted in the ICFR conviron where no detrimental effects on plant growth and development, such as etiolation, from applied indoor conditions were noted. Therefore, lighting similar to that applied in the 1998 pilot trial was used in both the ICFR and the

CERU convirons. Lighting was once again provided by OSRAM L58W/77 "Fluora" tubes.

Light levels in the two convirons were equated to almost identical readings. Twenty separate PAR (photosynthetically active radiation) waveband readings were taken in each of the two facilities using a Sunfleck Ceptometer Model SF-80 (Decagon Devices Inc., Wichita, USA). Light banks in each facility were then adjusted to 100 $\mu mol.m^{-2}.s^{-1}$. Final readings were between 88.0 - 108.0 $\mu mol.m^{-2}.s^{-1}$ (average 95.5 $\mu mol.m^{-2}.s^{-1}$) in the ICFR conviron and between 94.0 - 114.0 $\mu mol.m^{-2}.s^{-1}$ (average 104.3 $\mu mol.m^{-2}.s^{-1}$) in the CERU conviron.

Temperature and day-length settings:

For the entire duration of the indoor treatments, applied light and temperature conditions were managed so that the "dark phase" was 15 mins out of synchrony with applied night-time cold. Day-light commenced each day 15 mins before heating was switched on to reach the selected warm phase temperature. Similarly, day-light ended 15 mins before cooling was switched on to reach the selected cold phase temperature. This should minimise shock to the plants during transition from warm and high light intensity conditions to cold dark conditions.

In the ICFR conviron the thermostat was generally able to control temperatures within 1^0 C of either side of a particular setting. Temperature threshold range was slightly narrower within the CERU conviron.

The schedule for Experiment 1 is diagrammatically presented in ***Figure 2.1***.

Watering, fertilisation and pest control:

No rigid daily watering schedule was followed as soil-water usage by plants was inversely related to air temperature, and temperature treatments were continually changing. However, aims were pursued meticulously with respect to the watering schedule; soil moisture levels were maintained as close as possible to field capacity (75 - 100%) to avoid water logging and possible drought stress.

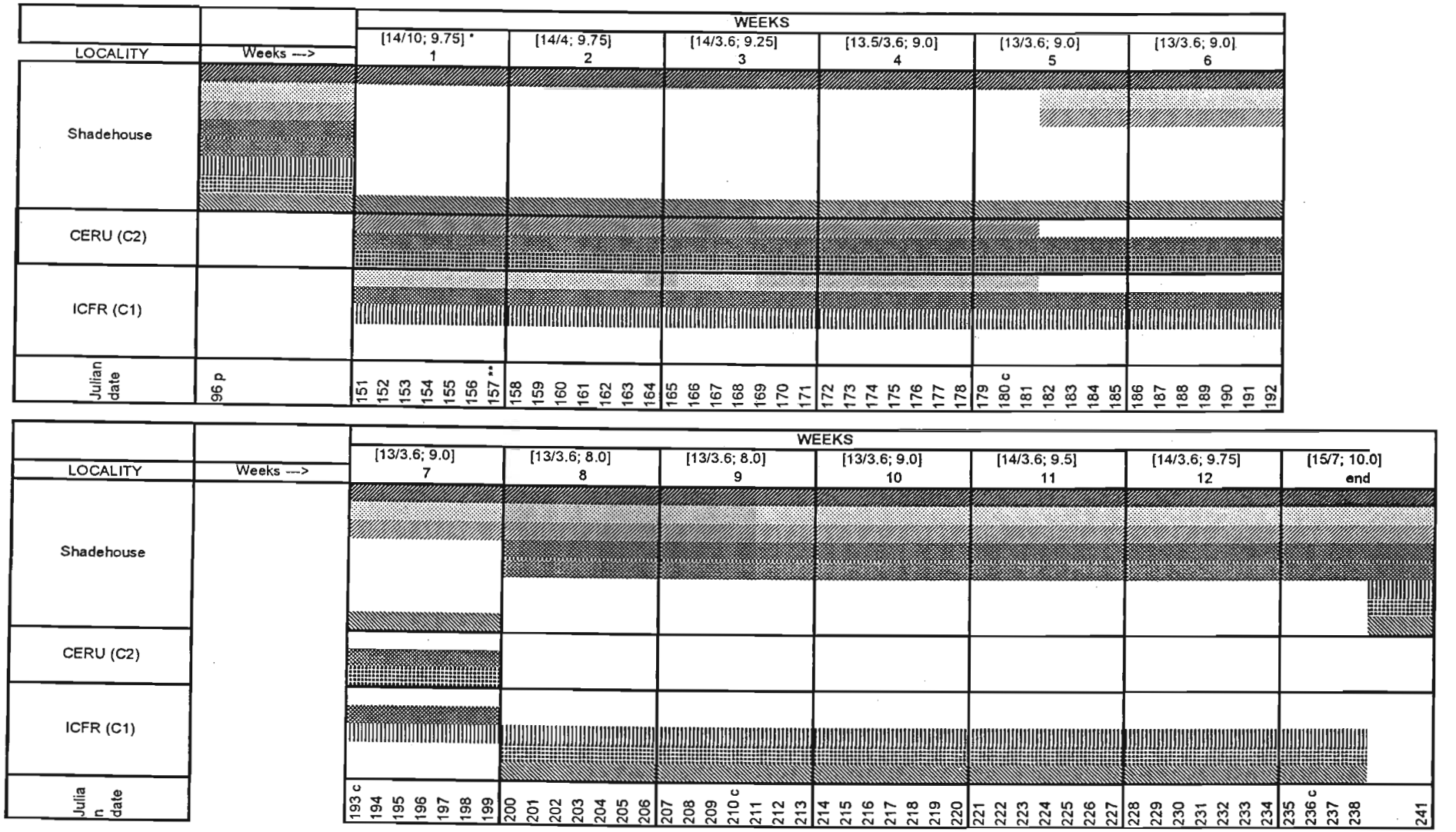


Figure 2.1. Schedule for Experiment 1.

In practice, plants in the conviron were watered twice a week on average with 300 ml distilled water per plant. Drip-trays were allowed to dry out for 2 days between watering. The watering schedule in the ICFR shadehouse was different to that in the conviron due to rainfall and the different transpo-evaporation pattern in the shadehouse.

The composted medium was pre-fertilised with a 2/3/1 mix of lime, super-phosphate and urea based on volume. From 01 April 1999 to the end of the experiment (February 2001), all plants were fertigated fortnightly with "Gromor Plant Food ®" (National Plant Food, Cato Ridge, South Africa) hydroponic mix. The strength of the fertigation solution was 1 g "Gromor Plant Food ®" per litre water. The solution was applied as a soil drench.
Plants were sprayed as necessary against insect pests.

2.2.2.2 Experiment 2

As success of Experiment 1 was limited, plants were subjected to a greater number of hours below 5 ^{0} C in Experiment 2. Thus, the main thrust of the cold treatments in this experiment was to apply similar temperature cycling as in Experiment 1, but to increase the number of hours below 5 ^{0} C particularly during the "daylight" phase.

The experiment made use of the ICFR shadehouse and the CERU conviron. Controlled temperature conditions were applied in a growth room (***Figure 2.2***) and a cold room at CERU.

Lighting:

In the ICFR shadehouse, 80 % full sun was effected using 20 % shadecloth. In the growth room, lighting was provided by OSRAM L58W/77 "Fluora" tubes. Twenty separate PAR (photosynthetically active radiation) waveband readings taken in the growth room using a Sunfleck Ceptometer Model SF-80 showed a range of 97.0 - 135.0 $\mu mol.m^{-2}.s^{-1}$ and an average PAR reading of 118.0 $\mu mol.m^{-2}.s^{-1}$.
In the coldroom, an overhead lighting structure was assembled and erected to provide very cold "daytime" conditions. The floor and sides of the coldroom were surfaced with 250 µmm thick white plastic sheeting in attempt to

conserve and distribute light as efficiently as possible within the cold-room. The maximum light levels which could be achieved in the cold room without hampering refrigeration ranged between 12.0 - 17.0 $\mu mol.m^{-2}.s^{-1}$, averaging 15.0 $\mu mol.m^{-2}.s^{-1}$.

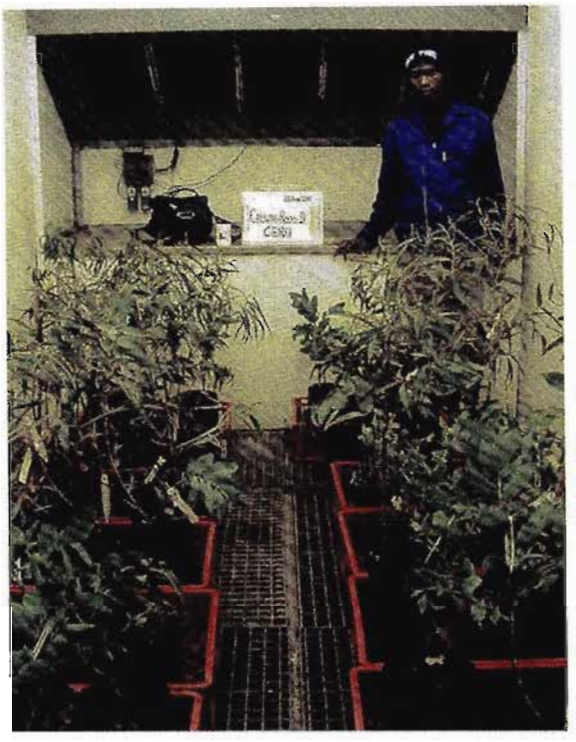


Figure 2.2 *Eucalyptus nitens* seedlings and grafts of Experiment 2 in CERU growth room.

Temperature and day-length settings:

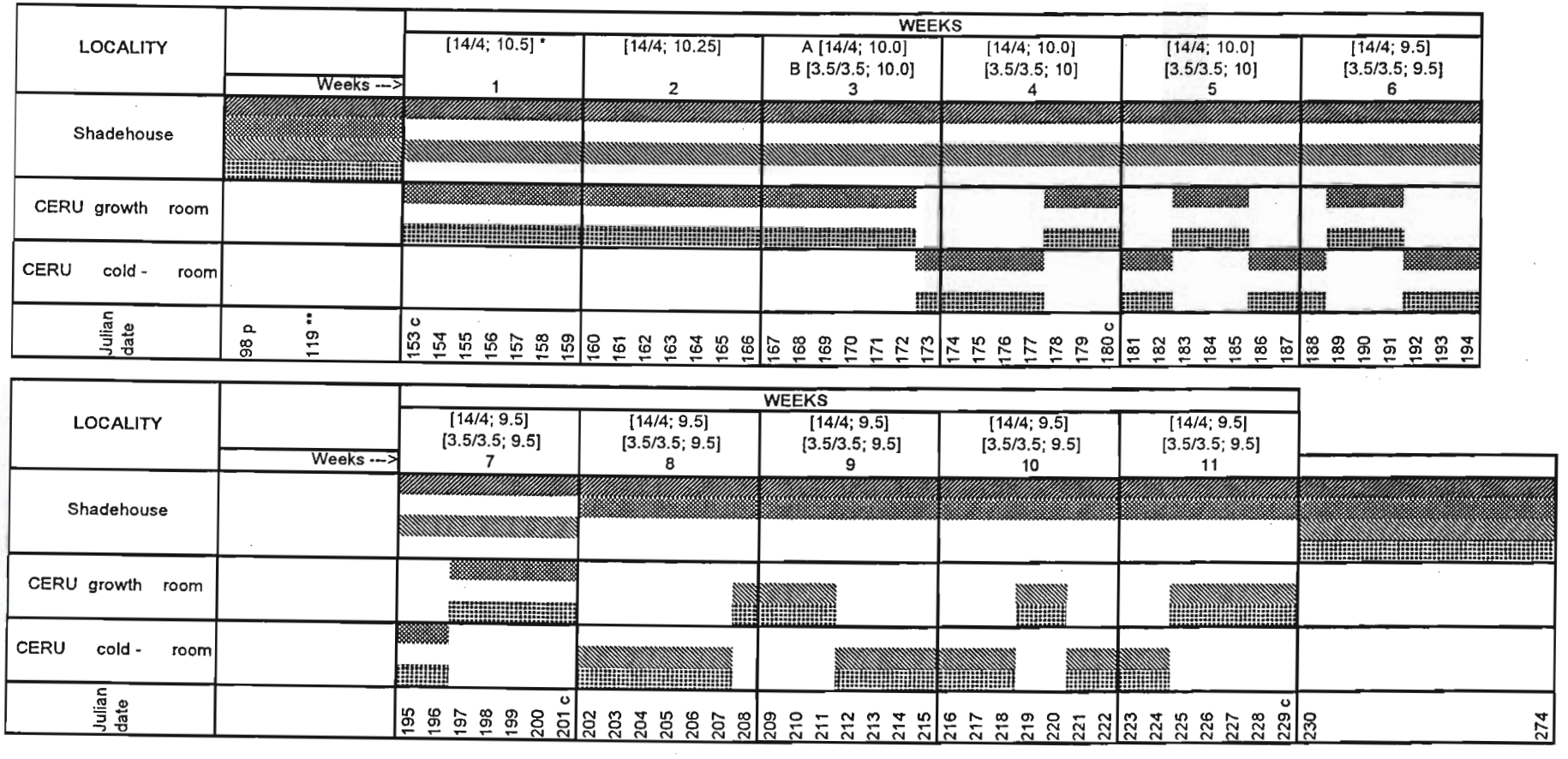
The cold room described above was used to apply temperatures below 5 ^{0} C. Unfortunately, no controlled environment facility of suitable size was available to provide temperatures ranging between 3 ^{0} and 13 ^{0} C.

As for Experiment 1, unsynchronous start and end times of lighting and temperature phases were applied to avoid excessive shock to the plants during transition from warm and high light intensity conditions to cold and dark conditions and vice versa.

The schedule for Experiment 2 is presented in ***Figure 2.3***.

Watering, fertilisation and pest control:

The conditions applied were identical to those in Experiment 1.



* [day/night temperature range (deg C); photoperiod (hrs)]
A = regime for first half of week
B = regime for second half of week
** commencement of chilling accumulation
p = paclobutrazol applied
c = chlorophyll fluorescence m/ments taken

Figure 2.3. Schedule for Experiment 2.

2.2.2.3 Experiment 3

The experiment made use of the ICFR shadehouse, where 80 % full sun conditions were achieved using 20 % shadecloth. Watering, fertilisation and pest control were identical to that described for the ICFR shadehouse in Experiment 1.

2.2.3 Chemical treatment

2.2.3.1 Experiments 1, 2 and 3

The timing of the paclobutrazol soil drench applications in controlled environment Experiments 1 and 2 was based on existing reports of previously described experiments with *E. globulus* and *E. nitens* using paclobutrazol soil drench treatments (England et al.1992, Moncur and Hasan 1994, Moncur et al.1994*b*).

In all three pot trials (Experiments 1, 2 and 3) paclobutrazol was applied to each plant at 0.25 g a.i. per centimetre stem circumference in 200 ml of water as a collar drench. In calculating the dose for each tree, stem circumference was measured at the narrowest point between graft union and first lateral in the case of grafts, or root collar and first lateral in the case of seedlings. Application dates were 06 April 1999, 07 April 2000 and 12 March 2001 for Experiments 1, 2 and 3 respectively. To avoid paclobutrazol treatment inaccuracies from possible leaching of the chemical from the growing medium, each plant bag was positioned in a suitable drip-tray from start to completion of the experiment.

2.2.4 Trial layout

2.2.4.1 Experiment 1

The following treatments were allocated to 64 plants: four grafted clones (refer ***Table 2.5***), five cold treatments (T1, T2, T3, T4 and T5) and two chemical treatments (control and paclobutrazol). The experiment consisted of a randomised complete blocks design, with four clones (grafts) acting as replicates. Because of limited space, cold treatments T1 (shadehouse control) and T5 consisted of eight plants each, half the number of plants that cold treatments T2, T3 and T4 each consisted of).

To solve the problem of uneven light distribution patterns within each conviron, plants were re-randomized regarding their position in the conviron at 08h00 each day. In the ICFR shadehouse plants were allocated positions in north-south orientated rows, and light distribution patterns were not as problematic. However, as time progressed, the smaller plants which had become stunted by paclobutrazol were prone to shading by taller plants and therefore plants were re-randomized in the nursery rows once a week from the beginning of May.

2.2.4.2 Experiment 2

The trial consisted of 72 plants in total (24 grafts of each of two selections and 24 seedlings of one open-pollinated seedlot) (***Table 2.5***). Four chilling temperature treatments were applied, with outdoor shadehouse conditions representing the control treatment. The experiment consisted of a randomised complete blocks design with three replicates. The re-randomization schedules for convirons and shadehouse used in Experiment 1 to solve lighting pattern differences, were again applied in Experiment 2.

2.2.4.3 Experiment 3

This experiment was not laid out statistically due to the shortage of suitably healthy plants at the time. On the 12 March 2001, eight healthy grafts of similar size and vigour were chosen for the experiment from a total batch of twenty-two surviving grafts. To four of these eight grafts selected randomly, paclobutrazol was applied. On each graft, three upright, actively-growing shoots were selected at about 120^{0} apart from each other around the stem axis for length measurements. Shoot no.1 on each plant faced north. Shoot length was always measured from shoot base (proximal end) to nodal ring of last unopened leaf (distal end). The re-randomization schedule for the shadehouse used in Experiment 1 to solve lighting pattern differences was applied in this experiment.

2.2.5 Data collection

2.2.5.1 Tree growth and flowering assessments

2.2.5.1.1 Experiment 1

To monitor growth and development of the different treatments, basal stem circumference was measured on the date of paclobutrazol application (06 April 1999) and at two other dates in 1999 using the measurement method described in section 2.2.3. In December 1999, March 2000 and March 2001, the trees were scored for number of umbels and number of flowers per umbel.

2.2.5.1.2 Experiment 2

Accurate measurement of stem circumferences of grafts in the previous experiment was difficult due to the small size and branched nature of the plants. Therefore tree height was measured in the second experiment. Heights were measured at four intervals during 2000/ 2001. Actual measurement dates were 14/04/2000, 17/08/2000, 22/12/2000 and 01/06/2001. During April and September 2001, plants were inspected for the presence of umbels.

2.2.5.1.3 Experiment 3

Weekly shoot length measurements were carried out from 05 March to 06 August 2001.

2.2.5.2 Temperature measurements

2.2.5.2.1 Experiments 1 and 2

Temperature measurements at the top of plants were taken during the winter months (April to September) each year. Outdoor temperatures in the ICFR shadehouse were measured at hourly intervals. Within the conviron facilities temperatures were measured at 15 minute intervals and then averaged to obtain hourly data. The instrumentation used to measure temperature is described in section 2.3 below.

2.2.5.3 Photosynthetic efficiency

2.2.5.3.1 Experiments 1 and 2

Photosynthetic efficiency (chlorophyll fluorescence) measurements were taken at the end of

each cold treatment using a "Plant Efficiency Analyser" (PEA) unit (Hansatech Instruments Ltd., Norfolk, UK). However, before proceeding with initial measurements in 1999, calibration experiments were carried out to obtain the optimum dark adaptation period and light intensity exposure.

2.3 Agro-meteorological instrumentation

2.3.1 Data loggers

Hobo® miniature data loggers (Onset Computer Corporation, Minnesota, USA) were used to take temperature readings in field and pot trials. Early version Hobo® - Temp loggers with relatively small memory storage capacity (1800 points, i.e. 75 days duration measuring at hourly intervals) were initially used and interchanging/downloading of loggers every two months was necessitated. This was logistically problematic especially regarding the sites distant from Pietermaritzburg. From 1998 on, however, the new H8 series Hobo® loggers with extended memory capacity (7944 points, i.e. 331 days duration at hourly intervals) were purchased and installed at all four sites.

According to Borsari (pers. comm. 2000), the accuracy of the Hobo loggers is determined by a combination of the component accuracy, the thermistor accuracy, and the temperature resolution. Temperature accuracy and resolution information and data for the Hobo H8 series temperature logger were provided by Onset Computer Corporation (Borsari pers. comm. 2000) and are presented in ***Appendix 2***. Although the H8 loggers are designed for optimum functioning between temperatures of - 20 ^{0} to + 70 ^{0}C, their optimal temperature for greatest accuracy in thermistor reading (0.68 ^{0}C) and temperature resolution (0.36 ^{0}C) occurs at 25 ^{0} C. The loggers are more cold sensitive than heat sensitive. At temperatures below - 10 ^{0}C the error rises above 0.9 ^{0}C (thermistor accuracy) and 0.54 ^{0}C (resolution). At + 40 ^{0}C the error is 0.74 ^{0}C (thermistor accuracy) and 0.45 ^{0}C (resolution).

2.3.2 Data logger housing structure

The problem of in-field theft and vandalism of agro-meteorological equipment prevented measurement of climatic data in all remote ICFR field trials for many years. In order to obtain accurate on-site temperature measurements in the flowering trials established in 1995, an alternative, robust structure was designed to house the miniature data loggers.

The structure consisted of a white-painted, steel fencing corner-pole which could be cemented into the ground. The steel pole was designed so that the pole head would ventilate well and air temperature within the head would be as close to outside air temperature as possible. The head should also be able to accommodate two loggers at a time, one serving as a backup. Other considerations were that the structure should be resistant to excessive entry of rain.

Within the cap (pole head), each logger was housed in a protective, plastic pill-box having six ventilator holes. The pill-boxes containing the loggers were positioned in the cap at 1,3 m above ground level. The logger poles were sited 15.0 m from the nearest tree on the northern side of each trial.

With respect to the pot trials, the pill-boxes containing the data loggers were fastened with masking tape to the stems of randomly chosen plants at a height of approximately two thirds the average plant height. The "Hobo" H8 series temperature logger and housing structures used in the flowering trials are illustrated in ***Figure 2.4***.

2.3.3 Comparison of "Hobo pole" air temperature to Stevenson Screen air temperature

Worldwide, the Stevenson Screen is regarded as the standard housing for meteorological thermometers (World Meteorological Organisation 1996). As an uncertainty existed as to whether air temperatures measured within the "Hobo pole" were similarly accurate to those measured in a Stevenson Screen, an experiment was carried out at the University of Natal, Agrometeorology section weather station at Pietermaritzburg during 2000 to investigate this.

A "Hobo pole" identical to those used in the field experiments was installed two metres away, on the northern side, from a standard Stevenson Screen. Four Hobo-Temp H8 series loggers were first checked against each other for measurement accuracy and then installed in the screen and pole during late March. Two loggers were installed in each housing, one logger being a backup in each instance. Within the screen, the loggers were suspended with wax-impregnated nylon string to hang 1.3 m above ground level. Data were collected from 01 April to 30 September 2000.

The following simple linear regression analyses were then carried using the statistical package Genstat® for Windows™ , Release 4.2 and the method described by McConway et al. (1999), as follows:

1. regression of Hobo pole daily minimum air temperature (PoleMinT) on Stevenson Screen daily minimum air temperature (ScrnMinT) (results in ***Appendix 3.1***).
2. regression of Hobo pole daily maximum air temperature (PoleMaxT) on Stevenson Screen daily maximum air temperature (ScrnMaxT) (results in ***Appendix 3.2***).

On average, PoleMinT was 1.54 ^{0}C cooler than ScrnMinT, and PoleMaxT was 2.0 ^{0}C warmer than ScrnMaxT. This meant that during the winter months, the daily Hobo pole air temperature range recorded was slightly wider than actual Stevenson Screen air temperature.

2.3.4 Comparison of Stevenson Screen air temperature to *E. nitens* resting bud temperature

To investigate whether a discrepancy between Stevenson Screen air temperature and the temperature of resting buds in the canopies of mature *E. nitens* trees existed, an experiment was carried out in summer during the months of November 2001 to February 2002 at the Shaw Research Centre at Tweedie. Information on actual resting bud temperature may assist later controlled chill work with *E. nitens*.

The experiment was carried out during the wet summer months at Tweedie when the bark of previous-season shoots was "slipping". During this active growth period, bark is easily separated from the woody cylinder (primary xylem) because walls of cells in the "cambial zone" are thin and easily ruptured (Weier et al. 1974, Hartmann et al. 1990). It was important that bark should be easily detachable from xylem to allow insertion of Hobo-logger thermo-sensors under the bark. Bark does not "slip" easily at Tweedie during the relatively dry months of April to September.

A Stevenson Screen and a Hobo logger pole were positioned about 4.0 metres from the canopy perimeter of the northern end of a row of mature six year old grafted ramets. Before commencement of the experiment, six Hobo-Temp series H8 loggers were checked against each other for similar accuracy of measurement. The loggers were then installed as follows:

1. All loggers were launched to take temperature readings at 15 minute intervals.
2. Two loggers were suspended inside the Stevenson Screen at 1.3 m above ground level using wax-impregnated nylon string.
3. Two loggers were installed in the logger pole.
4. Two loggers in water-tight pillboxes were fastened in the canopy of the second tree

from row-end at a height of 1.3 metres above ground level. The pillboxes were secured on the south-facing, shaded sides of upright-growing two year old shoots. One logger was positioned on the northern side of the tree canopy and the other on the southern side. In each case the thermal sensor wire of the logger was rigged to protrude externally through the pillbox wall. After securing the pillbox to the shoot, the thermo-sensor head was pushed about two centimetres through a vertical slit in the bark of a one-year-old shoot and positioned beneath the bark alongside a dormant bud on the furthest side away from the slit. The bark wound was immediately tied closed with wax-impregnated nylon string and sealed with a thin (watered-down) coating of anti-transpirant solution. The positioning of the "Hobo" temperature logger in the tree and underbark positioning of the logger thermo-sensor wire alongside a resting bud is illustrated in ***Figure 2.5***.

After 82 days of hourly data (logger memory capacity) were recorded, the Hobo loggers were retrieved from the site and data downloaded. The logger in the northern aspect of the tree was damaged by water leaking into the pillbox, hence no temperature data was available for this position in the tree. Tree resting bud temperature is thus represented by the bud temperature recorded in the southern portion of the tree canopy. Fifteen-minute interval data were converted to hourly data and daily maximum and minimum temperatures extracted as necessary. The following simple linear regression analyses were then carried using the statistical package Genstat® for Windows™ , Release 4.2 and the method described by McConway et al. (1999) as follows:

1. regression of *E. nitens* bud hourly temperature (BudHrly) on Stevenson Screen hourly air temperature (ScrnHrly) (results in ***Appendix 4.1***).
2. regression of *E. nitens* bud daily minimum temperature (BudMinT) on Stevenson Screen daily minimum air temperature (ScrnMinT) (results in ***Appendix 4.2***).
3. regression of *E. nitens* bud daily maximum temperature (BudMaxT) on Stevenson Screen daily maximum air temperature (ScrnMaxT) (results in ***Appendix 4.3***).
4. regression of Hobo pole daily minimum air temperature (PoleMinT) on Stevenson Screen daily minimum air temperature (ScrnMinT) (results in ***Appendix 4.4***).
5. regression of Hobo pole daily maximum air temperature (PoleMaxT) on Stevenson Screen daily maximum air temperature (ScrnMaxT) (results in ***Appendix 4.5***).

On average, daily minimum bud temperatures (BudMinT) were 0.33 ^{0}C cooler than daily minimum screen temperatures (ScrnMinT), and daily maximum bud temperatures (BudMaxT)

were 0.62 ^{0}C warmer than daily maximum screen temperatures (ScrnMaxT).

The regression of PoleMinT on ScrnMinT gave a somewhat different result to the 2000 winter experiment (refer paragraph 2.3.3). On average, pole daily minimum air temperatures (PoleMinT) were 0.6 ^{0}C cooler than screen daily minimum air temperatures (ScrnMinT), compared to PoleMinT being 1.54 ^{0}C cooler than ScrnMinT in the winter experiment.

The regression of PoleMaxT on ScrnMaxT gave similar results to those of the 2000 winter experiment at the University of Natal Agrometeorology section (refer paragraph 2.3.3 above). On average, pole daily maximum air temperatures (PoleMaxT) were 1.9 ^{0}C warmer than screen daily maximum air temperatures (ScrnMaxT).

2.4 Floral induction modelling

2.4.1 Soil moisture modelling

According to Moncur (1992) there is some circumstantial field evidence that a period of low soil water status in autumn/early winter can predispose eucalypts to flower. However, in a trial with potted *E. nitens* grafts where the plants were subjected to various soil moisture stress regimes, the latter factor did not stimulate flower-bud production (Moncur and Boland 2000).

Although drought stress in not reported to have any effect on floral induction in *E. nitens*, soil moisture stress is known to stimulate flowering in several other tree crops such as various *Citrus spp.* (Davenport 1990, Krajewski and Rabe 1995), lychee (*Litchi sinensis* Sonn.) (Menzel 1983) and certain conifers and broadleaf forestry tree species (Philipson 1990). It was therefore decided worthwhile to tentatively investigate whether there exists a correlation between soil moisture stress and floral induction in *E. nitens*. As soil moisture level was not monitored with any instruments in any of the trials during the entire period of the floral induction experiment, a simple soil moisture model, "WETNES", was chosen to estimate soil water content using precipitation and evaporation data.

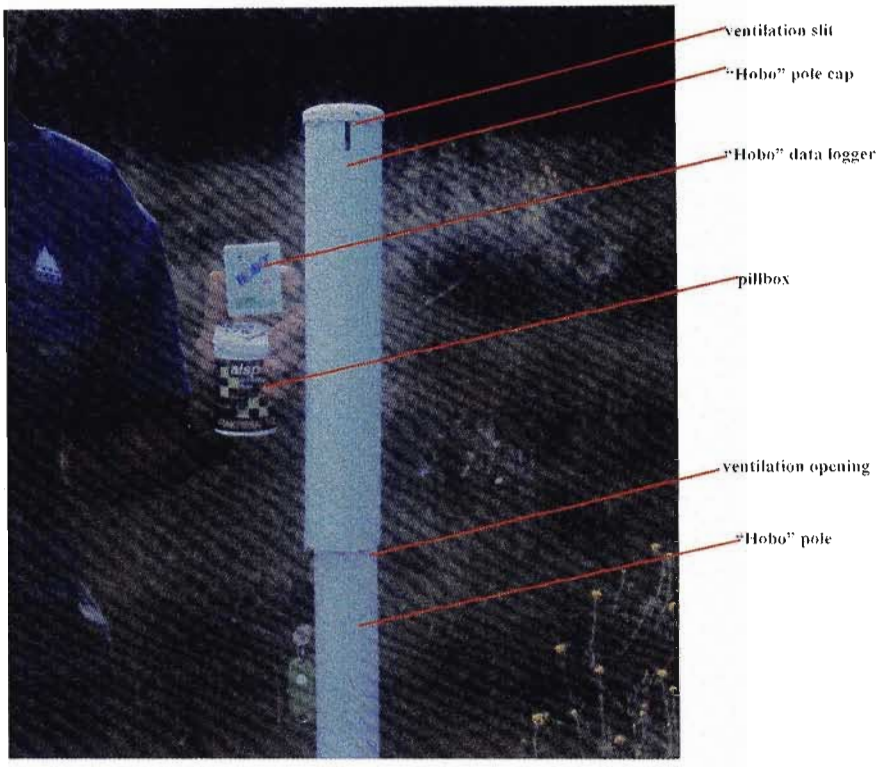


Figure 2.4 "Hobo" logger and housing structures used in *E. nitens* flowering trials.

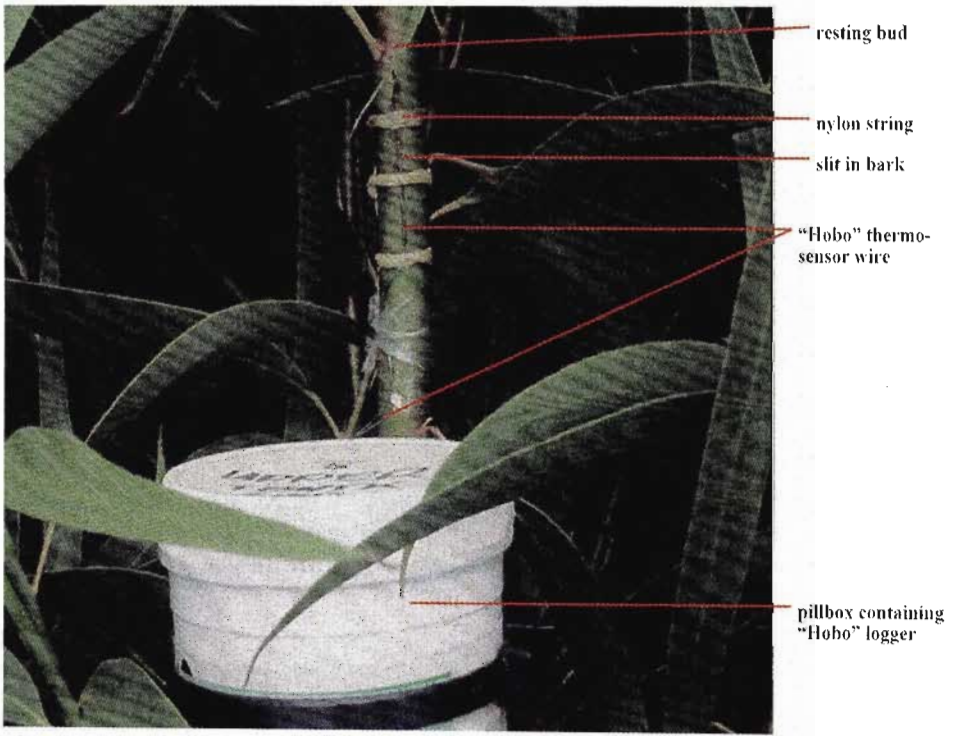


Figure 2.5 Securing method for "Hobo" temperature logger and sensor head in temperature calibration experiment at Shaw Research Centre, Tweedie.

2.4.1.1 "WETNES" soil moisture model

This soil water budgeting model was developed by the ICFR to simulate changing soil moisture conditions using limited data types. The aim was to develop a model for plantation managers which would function as a simple tool for simulating changing soil moisture conditions in their plantations on a continuous basis. The conceptual framework of WETNES and also the structures of individual models within the cascade are described in Roberts (1994*a* and *b*). Inputs into the model include daily rainfall (observed or stochastic), mean monthly potential evaporation data, land cover type and certain soils information.

2.4.2 Chill modelling

Low temperatures stimulate precocious and prolific flowering in certain temperate eucalypts (Moncur and Hasan 1994, Hasan and Reid 1995, Meilan1997). Winter chilling is also effective in inducing flowering in certain evergreen horticultural tree crops such as olive (*Olea europaea* L.) (Martin et al. 1994), sweet orange (*Citrus sinensis* (L.) Osbeck) (Davenport 1990) and lychee (Menzel 1983).

Deciduous fruit trees, however, require a certain amount of winter chilling for vegetative and flower-bud break and resumption of growth after winter dormancy (Fishman et al. 1987*a*, Linsley-Noakes and Allan 1994). Therefore, various models have been developed to estimate the onset of bud growth and end of dormancy according to the amount of winter chilling accumulated by deciduous fruit crops in temperate regions (Allan 1999).

There is a remarkable similarity between the bell-shaped temperature response curves for the vernalization of rye, endodormancy transition of apple seeds, and peach bud chilling (Hansel 1953, Schander 1995 and Erez and Lavee 1971, c.f. Seeley (1996)).

Salisbury and Ross (1992) drew attention to the similarities in mechanisms underlying the biological processes of dormancy release in buds of perennial woody plants, cold-moist stratification and dormancy breaking of seed, induction of underground storage organ formation and vernalization.

Fishman et al. (1987*b*) suggested that the chill model they had developed (later named the "Dynamic Model") to describe the role of low temperatures in breaking of bud dormancy could well be applicable to the analysis of mechanisms of the low-temperature responses mentioned by Salisbury and Ross (1992).

Chill models provide a means of calculating the number of chill units necessary for adequate rest completion of buds in different fruit species and cultivars. This information is mainly used in correct siting of orchards and for indication of where and when dormancy breaking chemicals should be applied during or after insufficiently cold winters.

One of the facets of this project was to test three well-known chill models used by local and overseas deciduous fruit industries, namely the Utah Chill Model, the Dynamic Model and the Daily Positive Utah Chill Unit Model (described below), for their suitability in predicting a cold-induced flowering response in *E. nitens*. The intention was to contribute towards the ongoing worldwide effort to develop a floral induction model for important temperate plantation eucalypts such as *E. nitens*.

Prior to the interrogation of logged "Hobo pole" temperature data using the models below, the necessary adjustments were applied to logged temperature data using the results of the calibration experiments described in sections 2.3.3 and 2.3.4 above.

2.4.2.1 Utah Chill Model

The Utah Chill Unit model for temperate zone endodormancy release of peach flower buds was introduced in 1974 (Richardson et al.1974). It has evolved through several interim versions and improvement is ongoing. Seeley (1996) provides a detailed description of the temperature response curve for estimating chill units and chill unit negation for the Utah Chill Model. Currently, the "Utah" chill unit is defined as 1.0 hrs at the optimum chilling temperature at the optimum chilling time. The model takes into account the negating effect of high temperatures on chill unit accumulation, assigning negative chill units to temperatures $\geq 16\ ^{0}$ C.

Local research has shown that this model is not suitable for areas in South Africa having high winter day temperatures ($> 20\ ^{0}$ C). The model was found to give inaccurate results at Robertson ($33\ ^{0}$ 49' S. latitude, 156 m altitude, winter rainfall region) and Ukulinga, Pietermaritzburg ($29\ ^{0}$ 40' S. latitude, 767 m altitude, summer rainfall region), both areas suited

to successful cultivation of low-chill peaches (Allan 1999). It is not unusual for negative winter totals of "Utah" chill units to be logged in Pietermaritzburg (Allan and Burnett 1995).

Nevertheless, the Utah Chill Model is popular for the accurate prediction of bud endodormancy release in medium to high-chill requiring peach cultivars in the temperate zones of several countries. As the eucalypt floral induction trials were all located at cool, high altitude sites, and not in sub-tropical areas, the Utah Chill Model was included in the investigation. "Utah" chill units will be referred to as UCUs in all further discussion in this thesis, according to the terminology defined by Allan (1999).

2.4.2.2 Dynamic Model

The Dynamic Model was developed along similar lines to the Utah Model, but is more sophisticated. The model not only takes into consideration the positive effect of cool temperatures and the negative effect of high temperatures on chilling accumulation that the Utah Model does, but also the positive effect that moderate temperatures have on chilling accumulation and the effect of chilling cycle length on negation by high temperatures (Fishman et al. 1987*a* and *b*).

The model assigns chill units, termed Chilling Portions (CPs), in whole numbers. When a critical level (1.0) or quantum of the "intermediate" is amassed, it is transferred, irreversibly, in a second step to a quantum (one portion) of the stable dormancy breaking factor. Once a CP has been formed it cannot be broken down (negated) by subsequent high temperatures (Allan 1999).

The temperature response curve for the Dynamic Model indicates that optimum chilling occurs at temperatures between 6 and 8 ^{0} C and zero chilling effect occurs at - 2 ^{0} C and 13 ^{0} C. Moderate day temperatures between 13 and 15 ^{0} C enhance the chilling effect of temperatures in the optimum range. Negative CPs are assigned at temperatures $\geq$20 ^{0} C. However, the degree of chilling negation decreases with cycle length (Fishman et al.1987*b*).

The model has proven to be equally accurate for areas with mild or cold winter climates (Linsley-Noakes et al.1995, Allan 1999). This flexibility may render the model suitable for the *E. nitens* floral induction investigations, as the climates at the trial sites in the series ranged from Cool to Highland Cool Temperate physiographic forestry conditions (Kunz and Pallett

2000).

2.4.2.3 Daily Positive Utah Chill Unit Model

The Daily Positive Chill Unit Model (DPCU), a modification of the Utah Chill Model, was proposed and developed by Linsley-Noakes (1995). The main problem with the Utah Chill Model was that good budbreak would occur in areas with mild winters despite the fact that negative amounts of UCU's had accrued.

Features of the Dynamic Model were used to modify the Utah Chill Model to allow more accurate prediction of winter chill accumulation in areas with mild winters (Linsley-Noakes et al. 1994). For instance, carry-over of negative units resulting from "detrimentally" high temperatures from one day to the next was eliminated. The proposal by Allan (1999) that Daily Positive Utah Chill Unit be abbreviated as DPCU will be adopted for all further discussion in this thesis.

CHAPTER 3

EFFECT OF WINTER CHILLING AND PACLOBUTRAZOL ON VEGETATIVE GROWTH AND FLORAL BUD PRODUCTION IN *E. NITENS*

3.1 Introduction

A range of environmental conditions and cultural techniques are known to influence floral induction in eucalypts (Moncur and Boland 2000). These include cold, water stress and pot size (environmental), and espalier pruning, tying branches, grafting, girdling, paclobutrazol and silviculture (cultural). Of these, cold and paclobutrazol appear to be the most effective treatments for temperate species such as *E. nitens* and *E. globulus* (Moncur and Boland 2000). Regardless of the application of paclobutrazol, a period of cold is prerequisite for floral induction in *E. nitens* (Moncur and Hasan 1994).

The effectiveness of cold, particularly when in combination with paclobutrazol treatment, in promoting floral induction in *E. nitens*, promises several possibilities in the field of floral manipulation, improved seed production and reduction in generation turnover time in the species. For an environmentally-friendly, management system for enhanced flowering and seed production in *E. nitens* to be optimised, however, a detailed characterization of the environmental stimuli of flowering in *E. nitens* is required (Reid et al. 1995, Moncur 1998).

Adequate winter cold alone is sufficient for the induction of flowering in *E. nitens* and *E. globulus* seedlings (Moncur and Hasan 1994, Williams et al.1999). In Australia, New Zealand and Chile, in most years, untreated (no artificial flowering enhancement technique applied) trees, sometimes as young as five years, in seedling and grafted *E. nitens* orchards produce acceptable flower bud crops depending on site, genotype and tree age (Moncur and Hasan 1994, Gardner 2001*b*, R. McConnochie pers. comm. 2001). In South Africa, untreated seedling and grafted trees in *E. nitens* seed orchards rarely flower before the age of eight years and only following the severest of winters at certain sites (Poynton 1979, Eldridge et al. 1993, Swain and Chiappero 1998).

In Australia, New Zealand and Chile, based on mean annual temperature (MAT), *E. nitens* is grown under cooler climatic conditions than in South Africa. Overall latitudinal bracket for these overseas plantations and orchards is 35.0 ^{0} to 46.0 ^{0} S., approximate MAT range being 8.0 ^{0} to 15.5 ^{0} C (Tibbits et al. 1997, INFOR 2002, McKinley et al. 2002). Winter rainfall climatic conditions in these areas cause diurnal temperatures during the winter months to be uniformly cool.

In South Africa, the *E. nitens* plantations and seed orchards are located between 25.6 ^{0} and 31.0 ^{0} South latitude and within an approximate mean annual temperature range of 13.0 ^{0} to 16.0 ^{0} C (R. Kunz unpublished data, Swain and Gardner 2002). Because of the summer rainfall climatic conditions, large day/night temperature variations with daytime temperature maxima in excess of 20 ^{0} C are common in these areas during the winter months (Richardson and McMahon 1992, Schulze 1997). Excessive daily temperature fluctuations during winter have an adverse effect on chilling accumulation in deciduous fruit tree crops (Richardson et al. 1974, Couvillon and Erez 1985). It is possible that this non-uniform chilling pattern during winter negatively affects the floral induction process in temperate eucalypts species such as *E. nitens*. If winter cold is a major limiting factor in floral induction in *E. nitens* in South Africa, it needs to be intensively investigated.

3.2 Materials and methods

The investigation into the effects of temperature and paclobutrazol on growth and flowering in *E. nitens* involved a series of four field trials established in 1996 (refer to section 2.1 for descriptions of site conditions and trial measurements), and three pot trials undertaken separately during 1999 to 2001 (refer to section 2.2 for description of trial layouts and measurements). In the field trials, temperature treatments were determined by site locality, whilst in the pot trials temperature treatments were determined by different levels of artificially applied cold (refer paragraphs 2.1.2 and 2.2.2, respectively, in Chapter 2 (General Experimental Procedures).

3.2.1 Statistical analyses

3.2.1.1 Field trials

3.2.1.1.1 General

The treatments applied are listed in paragraph 2.1.4 ("Trial layout"). Statistical analyses were performed to investigate the effect of site, propagule, family and paclobutrazol application on tree height and umbel score per reproductive tree.

Data were initially analysed using the method of Analysis of Variance (ANOVA), in Genstat® for Windows™, Release 4.2 (Lane and Payne 1996). The ANOVA analyses gave inefficient estimates of the standard errors for each site because of the unbalanced data (different numbers of missing trees at the different sites), and therefore the data were re-analysed using the method of restricted maximum likelihood (REML) analysis (Patterson and Thompson 1971, Lane and Payne 1996) in Genstat® for Windows, Release 4.2. The REML analysis outputs estimates of variance parameters taking into account the degrees of freedom used in estimating fixed effects, like those generated by ANOVA in balanced data sets, but are more accurate than the ANOVA-calculated values. Therefore, the tables of means with their standard errors generated in the REML analysis are more exact and preferable (Patterson and Thompson 1971; Lane and Payne 1996). Variance components were estimated using the mixed model analysis of variance (Steel and Torrie 1981).

In the **individual site** analyses for tree growth (height) and flowering ("%_trees" and "LFLW_2001"), the following statistical model was used to separate out the different variance components:

$$Y_{ijklm} = \mu + W_i + F_j + C_k + P_l + (FC)_{jk} + (FP)_{jl} + (CP)_{kl} + (FCP)_{jkl} + \epsilon_{ijklm}$$

where,

i = 1,2; j = 1,2,3,4; k = 1,2; l = 1,2; m = 1,2,3,4,5; and

Y_{ijklm} = observed response for the ijklmth tree,

μ = constant (test mean),

W_i = random effect of the ith plot (random whole plot error),

F_j = fixed effect of the jth FAMILY,

C_k = fixed effect of k^{th} PBZ treatment,

P_l = fixed effect of the l^{th} PROPAGULE treatment,

$(FC)_{jk}$ = fixed effect of the interaction between the j^{th} FAMILY and the k^{th} PBZ treatment,

$(FP)_{jk}$ = fixed effect of the interaction between the j^{th} FAMILY and the l^{th} PROPAGULE treatment,

$(CP)_{kl}$ = fixed effect of the interaction between the k^{th} PBZ treatment and the l^{th} PROPAGULE treatment,

$(FCP)_{jkl}$ = fixed effect of the interaction between the j^{th} FAMILY, the k^{th} PBZ treatment and the l^{th} PROPAGULE treatment, and

ϵ_{ijklm} = random effect (random experimental unit error).

In the **across-site** analyses for tree growth (height) and flowering ("%_trees" and "LFLW_2001"), the following statistical model was used to separate out the different variance components:

$$Y_{ijklmn} = \mu + S_i + F_j + C_k + P_l + (SF)_{ij} + (SC)_{ik} + (SP)_{il} + (FC)_{jk} + (FP)_{jl} + (CP)_{kl} + (SFC)_{ijk} + (SFP)_{ijl} + (FCP)_{jkl} + (SFCP)_{ijkl} + \epsilon_{ijkl} + \epsilon_{ijklm}$$

where,

i = 1,2,3,4; j = 1,2,3,4; k = 1,2; l = 1,2; m = 1,2,3,4,5; and

Y_{ijklmn} = observed response for the $ijklm^{th}$ tree,

μ = constant (test mean),

S_i = fixed effect of the i^{th} SITE,

F_j = fixed effect of j^{th} FAMILY,

C_k = fixed effect of the k^{th} PBZ treatment,

P_l = fixed effect of the l^{th} PROPAGULE treatment,

$(SF)_{ij}$ = fixed effect of the interaction between the i^{th} SITE and the j^{th} FAMILY,

$(SC)_{ik}$ = fixed effect of the interaction between the i^{th} SITE and the k^{th} PBZ treatment,

$(SP)_{il}$ = fixed effect of the interaction between the i^{th} SITE and the l^{th} PROPAGULE treatment,

$(FC)_{jk}$ = fixed effect of the interaction between the j^{th} FAMILY and the k^{th} PBZ treatment,

$(FP)_{jl}$ = fixed effect of the interaction between the j^{th} FAMILY and the l^{th}

PROPAGULE treatment,

$(CP)_{kl}$ = fixed effect of the interaction between the k^{th} PBZ treatment and the l^{th} PROPAGULE treatment,

$(SFC)_{ijk}$ = fixed effect of the interaction between the i^{th} SITE, the j^{th} FAMILY and the k^{th} PBZ treatment,

$(SFP)_{ijl}$ = fixed effect of the interaction between the i^{th} SITE, the j^{th} FAMILY and the l^{th} PROPAGULE treatment,

$(FCP)_{jkl}$ = fixed effect of the interaction between the j^{th} FAMILY, the k^{th} PBZ treatment and the l^{th} PROPAGULE treatment,

$(SFCP)_{ijkl}$ = fixed effect of the interaction between the i^{th} SITE, the j^{th} FAMILY, the k^{th} PBZ treatment and the l^{th} PROPAGULE treatment,

ϵ_{ijkl} = random effect (whole plot error), and

ϵ_{ijklm} = random effect (sub-plot error).

Multiple regression technique in Genstat® for Windows™ (McConway et al. 1999) was used to determine the relationship between winter chilling ("COLD_*x*") and the percentage of trees with umbels ("%_trees"), and between ("COLD_*x*") and umbel score per tree ("LFLW_2001").

The variate "COLD_*x*" consisted of chill units for the 2000 winter (winter preceding umbel crop assessment) calculated from temperature data using different *chill model* x *chill period* combinations. The different chill models and the reasons for their inclusion in the investigation are discussed in Chapter 3. Initially, twelve *chill model* x *chill period* combinations were tested in pilot regressions of umbel production on winter chilling amount. However, with all three chill models tested, no chill units accumulated during the month of October in any of the years. Therefore, only the two best *chill model* x *chill period* combinations in each chill model group, namely COLD_1 and COLD_2 (Dynamic Model), COLD_3 and COLD_4 (Utah Chill Model) and COLD_5 and COLD_6 (Daily Positive Utah Chill Unit Model), were retained for inclusion as explanatory variables in the multiple regressions. The final list of all response and explanatory variables included in the multiple regressions is presented in ***Table 3.1***.
To investigate the degree of relatedness between the different explanatory variables, particularly between the retained *chill model* x *chill period* combinations, separate correlation matrices were produced for seedlings and grafts using data for the percentage of trees producing umbels at five years (%_trees). The variate "%_trees" consisted of plot means in this analysis.

In the preliminary multiple regressions, various curves were fitted to the data. Because the linear curve resulted in the best fit on all occasions, multiple linear regression was used in all further analyses.

Table 3.1 Description of response and explanatory variables used in the multiple linear regression analyses.

Variate assessed	Abbreviation used in text	Description of variate
Response Variable (floral response)		
Umbel crop load	LFLW_2001	Umbel crop score per tree transformed to natural logarithmic value.
% trees with umbels	%_trees	Number of trees with one or more umbels in each plot (rep), expressed as a percentage of the total number of live trees in the plot
Explanatory Variable (chill model x chill period combination)		
Dynamic Model	COLD_1	Number of CPs [1] calculated for the period 01 Apr - 30 Sep 2000
Dynamic Model	COLD_2	Number of CPs [1] calculated for the period 01 May - 30 Sep 2000
Utah Chill Model	COLD_3	Number of UCUs [2] calculated for the period 01 Apr - 30 Sep 2000
Utah Chill Model	COLD_4	Number of UCUs [2] calculated for the period 01 May - 30 Sep 2000
Daily Positive Utah Chill Unit model	COLD_5	Number of DPCUs [3] calculated for the period 01 Apr - 30 Sep 2000
Daily Positive Utah Chill Unit model	COLD_6	Number of DPCUs [3] calculated for the period 01 May - 30 Sep 2000
Explanatory Variable (plant material and paclobutrazol treatments)		
Propagule type	PROPAGULE	1 = graft; 2 = seedling (refer section 2.1.1)
E. nitens family	FAMILY	Families 32091, 32097, 34838 and 37255 (refer *Table 2.1*)
Paclobutrazol application	PBZ	0 = 0.00 g paclobutrazol per cm basal stem circumference (b.s.c.); 1 = 0.25 g paclobutrazol per cm b.s.c.

[1] CP, Chilling Portion, the chill unit measured by the Dynamic Model (Fishman et al. 1987*a* and *b*).

[2] UCU, Utah Chill Unit, the chill unit measured by the Utah Chill Unit Model (Seeley 1996).

[3] DPCU, Daily Positive Chill Unit, the chill unit measured by the Daily Positive Utah Chill Unit Model (Linsley-Noakes 1995).

3.2.1.1.2 Effect of site and paclobutrazol application on tree height

The effect of site and paclobutrazol application on tree height was investigated via statistical analysis of five-year tree height data.

3.2.1.1.3 Effect of site and paclobutrazol application on percentage trees producing umbels

The effect of site and paclobutrazol application on *E. nitens* floral productivity was statistically investigated by examining the percentage of trees producing umbels at four and five years after planting. The variate "%_trees" represented the percentage of trees scored with one or more umbels in April 2000 or 2001. Prior to the analyses, all percentage data were angularly (square root scale) transformed, as the slightly varying number of surviving trees across the four sites could possibly bias the percentage (%_trees) estimate (Steel and Torrie 1981). Transformation of percentage data is recommended to normalize the residuals and homogeneity of the error variances (Gomez and Gomez 1984, Mead et al. 1993).

In the across-site analyses for either year, separate analyses were carried out for the two propagule types (seedlings and grafts) and PBZ treatments (0.00 g paclobutrazol per cm b.s.c. and 0.25 g paclobutrazol per cm b.s.c.), but families were pooled.

3.2.1.1.4 Effect of site and paclobutrazol application on umbel crop per tree

Individual tree umbel crop size was scored using the method described in paragraph 2.1.5.2 ("Flowering assessments"). The first significant crops of umbels produced by both seedlings and grafts occurred at five years after planting, and therefore these data were chosen for analysis. Due to the non-constant variances of the trials and the many zero counts in each trial data set, it was more appropriate to analyse on a logarithmic scale, and therefore the original data (x) were transformed to $\log_{10(x+1)}$ (Gomez and Gomez 1984, Mead et al. 1993). The variate "LFLW_2001", which represented the natural log of the individual tree umbel score at five years, was statistically analysed on a "per site" and "across-site" basis initially using standard ANOVA method, but later using REML analysis for the reasons discussed in paragraph 3.2.1.1.1.

3.2.1.1.5 Relationship between winter chilling and percentage trees producing umbels

Separate multiple linear regressions were carried out for seedlings and grafts because it was anticipated that ontogenetical variation may confound the results for "%_trees" in the former propagule type. The following statistical model was fitted in these regressions:

$$y = a + b_1 x_1 + b_2 x_2$$

where,

y = response variable,

a = intercept,

$x_{1\text{-}2}$ = explanatory variables, and

$b_{1\text{-}2}$ = linear regression coefficients.

In the case of either seedlings or grafts, the response variate "%_trees" consisted of pooled family data. This pooling was done to reduce within-trial variances due to non-consistent umbel bearing patterns across the different families within each propagule type. As a further output in the regressions, residual plots were carried out according to the procedures described by McConway et al.(1999) in Genstat® for Windows™. The plots of residuals against fitted values showed that the main assumptions for a valid regression analysis, which included homogeneity of variance and normality of distribution, were met. The residual plots also served to highlight abnormally large residuals which drew attention to potential outliers in the data. Such outliers were only removed from the explanatory data set if, on checking of the original data, it was clear that an obvious error had been made in data capture or that the data value was non-representative.

3.2.1.1.6 Relationship between winter chilling and umbel crop per tree

Multiple linear regressions were carried out with LFLW_2001as response variable, and *chill model* x *chill period* combination (COLD_*x*), propagule type (PROPAGULE) and paclobutrazol treatment (PBZ) as explanatory variables. PROPAGULE was included as an explanatory variable to statistically compare its effect against that of the two other explanatory variables (COLD_*x* and PBZ). The following statistical model was fitted in this set of regressions:

$$y = a + b_1 x_1 + b_2 x_2 + b_3 x_3$$

where,

y = response variable,

a = intercept,

x_{1-3} = explanatory variables, and

b_{1-3} = linear regression coefficients.

Families were again pooled for the analyses. Residual plots were performed, according to the procedures laid down by McConway et al.(1999) in Genstat® for Windows™, to check and ensure that the assumptions for a valid regression analysis were met, and to identify and correct any erroneous outliers in the observed data set.

3.2.1.2 Pot trials

3.2.1.2.1 Rate of uptake of paclobutrazol by potted *E. nitens* grafts

The data were not analysed statistically to establish the source and amount of variation within treatments for reasons given in paragraph 2.2.4 ("Trial layout"). Mean percentage increase in shoot length was plotted and curves fitted for either of the treatments. Percentage increase in shoot length was averaged for the 12 sample shoots in the "0.00 g paclobutrazol" and "0.25 g paclobutrazol per cm b.s.c." treatments for each measurement occasion.

3.2.1.2.2 Effect of temperature and paclobutrazol application on tree growth

Experiments 1 and 2 were both analysed as randomized complete block designs using ANOVA in Genstat® for Windows™, Release 4.2 (Lane and Payne 1996). In Experiment 1, data for the two localities were pooled as the environmental conditions at either locality were identical, and clones (grafts) were treated as blocks. In Experiment 2, clones and seedlings were kept separate, and the experiment consisted of three replicates. The statistical model may be represented as follows:

$$y_{ij} = \mu + \beta_i + \tau_j + \epsilon_{ij}$$

where,

y_{ij} = observed response for the i^{th} treatment in the j^{th} block,

μ = population mean,

β_i = mean response for the i^{th} block (replicate),

τ_j = treatment effect on response for the j^{th} treatment, and

ϵ_{ij} = random unit variation within a block.

3.2.1.2.3 Effect of temperature and paclobutrazol application on percentage trees producing umbels

The results of Experiments 1 and 2 did not warrant detailed statistical analyses. Means and percentages were calculated for Experiment 1 data.

3.3 Results and discussion

3.3.1 Field trials

3.3.1.1 Winter chill unit accumulation

All four sites accumulated the highest number of chill units, within the three years of observation (1998 to 2000), during 2000 (***Table 3.2***), when Blyfstaanhoogte accumulated the highest number of chill units of all sites (100.9 CPs; 2032 UCUs; 2108 DPCUs), recorded during the April to September period. Gowan Brae, Mossbank and Tentkop accumulated their lowest number of chill units during 1999. In this year, Gowan Brae accumulated the lowest number of chill units of all sites and years (42.8 CPs; 621 UCUs; 1005 DPCUs) (April to September). In all years and at all sites, both the Dynamic Model and the Daily Positive Utah Chill Unit Model began accumulating chill units during April, however, at Gowan Brae and Mossbank, the Utah Chill Model showed chill unit negation during April 1998 and 1999. It is well-documented that the Utah Model can give inaccurate estimations of winter chilling accumulation in low-chill deciduous fruit varieties in subtropical regions (Linsley-Noakes et al. 1994, Allan and Burnett 1995, Allan 1999).

Table 3.2 Chill units accumulated during the years 1998, 1999 and 2000 at the four trial sites.

Trial	Floral response year	Chill units					
		Chilling Portions (Dynamic Model)		Utah Chill Units (Utah Chill Model)		Daily Positive Utah Chill Units (DPCU Model)	
		COLD_1 *	COLD_2 ¶	COLD_3 *	COLD_4 ¶	COLD_5 *	COLD_6 ¶
Gowan Brae	1999	48.6	46.6	750	878	1096	1073
	2000	42.8	40.8	621	732	1005	967
	2001	59.3	51.7	1144	1073	1342	1204
	Mean	50.2	46.4	838	894	1148	1081
Mossbank	1999	60.5	58.5	1112	1108	1310	1248
	2000	55.3	51.3	918	934	1225	1142
	2001	80.0	70.8	1604	1414	1741	1531
	Mean	65.3	60.2	1211	1152	1425	1307
Blyfstaanhoogte	1999	72.0	65.0	1081	1119	1366	1272
	2000	96.5	88.4	1694	1601	1840	1688
	2001	100.9	89.1	2032	1836	2108	1897
	Mean	89.8	80.8	1602	1519	1771	1619
Tentkop	1999	84.5	79.5	1356	1314	1444	1344
	2000	81.5	71.6	1299	1193	1385	1228
	2001	87.8	76.0	1505	1261	1560	1302
	Mean	84.6	75.7	1387	1256	1463	1291

Note: the chill units presented for each floral response year above were calculated using temperature data of the previous winter.

* in this column, chill unit totals for the period 01 April to 30 September are presented.

¶ in this column, chill unit totals for the period 01 May to 30 September are presented.

3.3.1.2 Effect of site and paclobutrazol application on tree growth

A summary of the results from the across-site analysis of variance for height at five years, in which the Wald statistics and relevant calculated *F*-test values for fixed effects are given, is presented in ***Table 3.3.*** The Wald statistic produced in REML is a Chi-square statistic. In unbalanced designs, the *F* distribution is only approximate, the test becoming more accurate with increasing sample size. In practice, the observed *F* value (F_{calc}) for a particular fixed term is calculated by dividing the Wald statistic by the number of degrees of freedom for the term. F_{calc} is then compared with F_{table}, the latter being derived from the relevant table using the number of degrees of freedom of the same fixed term and for the error (SAS 1999, Genstat® for

Windows™ 2000).

Table 3.3 Wald statistics and calculated *F*-test values for fixed effects in the across-site REML analysis for tree height at five years.

Fixed term	Wald statistic	d.f.	F_{calc}
SITE	177.1	3	59.0**
FAMILY	3.7	3	1.23
PBZ	39.4	1	39.4**
PROPAGULE	37.4	1	37.4**
SITE x FAMILY	13.4	9	1.49
SITE x PBZ	23.2	3	7.73**
FAMILY x PBZ	0.7	3	0.23
SITE x PROPAGULE	6.2	3	2.07
FAMILY x PROPAGULE	3.1	3	1.03
PBZ x PROPAGULE	1.1	1	1.1
SITE x FAMILY x PBZ	10.9	9	1.21
SITE x FAMILY x PROPAGULE	11.4	9	1.27
SITE x PBZ x PROPAGULE	0.9	3	0.3
FAMILY x PBZ x PROPAGULE	3.7	3	1.23
SITE x FAMILY x PBZ x PROPAGULE	7.1	9	0.79

Note: **, significant at $p < 0.01$.

The *F*-test for SITE showed that highly significant ($p < 0.01$) differences existed between sites regarding tree height at five years after planting (***Table 3.3***). Mean tree height at two and five years after planting is presented in ***Table 3.4***. The sites ranked in order from highest to lowest mean tree height at five years were Gowan Brae (16.4 m), Mossbank (13.6 m), Blyfstaanhoogte (12.0 m) and Tentkop (10.2 m).

PROPAGULE (graft or seedling), PBZ and SITE x PBZ were also highly significant ($p < 0.01$) in the REML across-site analysis (***Table 3.3***). Mean tree heights for the SITE x PBZ interaction are given in ***Table 3.5***. Separate *t*-tests for the different sites established that paclobutrazol application significantly reduced tree height at Gowan Brae (the warmest site) and Tentkop (coldest site), but did not influence tree height at the two intermediate sites Mossbank and Blyfstaanhoogte. This may be indicative of compound stress, i.e. the stunting effect of paclobutrazol added to the stress already being incurred by the trees from growing conditions being either too warm (Gowan Brae) or too cold (Tentkop) for the particular tree species.

Table 3.4 Mean tree height at two and five years after planting in the four field trials.

SITE	Gowan Brae	Mossbank	Blyfstaanhoogte	Tentkop	S.E.D.
Mean tree height (m) at 2 years of age ¶ §	7.91*a*	6.53*b*	5.48*c*	3.26*d*	0.181 *
Mean tree height (m) at 5 years of age §	16.38*a*	13.64*b*	11.95*c*	10.18*d*	0.486 *

Note: *, significant at $p < 0.05$.

¶ Date of two year height measurement coincided with date of paclobutrazol application.

§ Within this row, values followed by the same letter are not significantly different ($p < 0.05$)

Table 3.5 Mean tree height at five years after planting for the SITE x PBZ interaction.

	PBZ treatment		
SITE	0.00 g paclobutrazol per cm b.s.c. §	0.25 g paclobutrazol per cm b.s.c. §	difference between means
Gowan Brae	18.11*a*	14.64*a*	3.47 *
Mossbank	14.28*b*	13.00*b*	1.28
Blyfstaanhoogte	11.93*c*	11.97*c*	0.04
Tentkop	12.16*c*	8.20*d*	3.96 *

Note: *, significant at $p < 0.05$.

§ Within this column, values followed by the same letter are not significantly different ($p < 0.05$)

3.3.1.3 Effect of site and paclobutrazol application on percentage trees producing umbels

The first umbels were recorded on grafts three years after planting (***Table 3.6***). The first noteworthy crops, however, were recorded in April 2000 and 2001 at four and five years after planting, respectively, and hence, statistical analyses of the percentage of trees producing umbels in April (%_trees) were only carried out on these (four and five year) data. In the separate analyses of variance for "untransformed" and "angularly transformed" five year percentage data (refer paragraph 3.2.1.1.3), the significance of results were similar. On closer inspection of the "untransformed" and "angularly transformed" data, it appeared that the angular transformation had not noticeably improved the homogeneity or additivity of the data. It was therefore decided to present the results of both "untransformed" and "angularly transformed" data (***Table 3.6***), but only to discuss the results of the former.

At each site, the percentage of trees bearing one or more umbels in April increased steadily for all treatments and in both propagule types each year from 1999 onwards (***Table 3.6***). With the exception of Blyfstaanhoogte, very few of the seedlings, whether untreated or paclobutrazol-

treated, produced flower buds before the age of five years. The number of seedling trees bearing umbels was at all sites and over all the years of assessment less than in grafted trees.

Table 3.6 Percentage of trees bearing one or more umbels at three, four and five years after planting.

Trial	Floral response year	Seedlings		Grafts		Chilling Portions for the period 01 May to 30 September (COLD_2)
		0.00 g PBZ / cm b.s.c.	0.25 g PBZ/ cm b.s.c.	0.00 g PBZ/ cm b.s.c.	0.25 g PBZ/ cm b.s.c.	
Gowan Brae	1999	0 (0)	0 (0)	8.53 (2.2)	11.97 (4.3)	46.6
	2000	0 (0)	22.87 (15.1)	10.47 (3.3)	17.05 (8.6)	40.8
	2001	16.32 (7.9)	32.96 (29.6)	19.46 (11.1)	30.98 (26.5)	71.7
Mossbank	1999	0 (0)	0 (0)	8.53 (2.2)	11.39 (3.9)	58.5
	2000	0 (0)	16.64 (8.2)	0 (0)	21.39 (13.3)	51.3
	2001	8.33 (2.1)	32.52 (28.9)	15.89 (7.5)	56.17 (69.0)	70.8
Blyfstaan-hoogte	1999	0 (0)	0 (0)	17.85 (9.4)	26.85 (20.4)	65.0
	2000	30.00 (25.0)	30.07 (25.1)	47.87 (55.0)	49.31 (57.5)	88.4
	2001	44.83 (49.7)	43.97 (48.2)	53.37 (64.4)	61.41 (77.1)	89.1
Tentkop	1999	0 (0)	0 (0)	21.39 (13.3)	14.18 (6.0)	79.5
	2000	0 (0)	13.44 (5.4)	0 (0)	42.82 (46.2)	71.6
	2001	27.35 (21.1)	25.33 (18.3)	28.73 (23.1)	48.33 (55.8)	76.0

Note: Because the percentage data were transformed for the ANOVA and regression analyses, untransformed percentages are given in parentheses for the reader's benefit. Chilling Portions (CPs) are included for the benefit of the reader. CPs presented for each floral response year were calculated using temperature data of the previous winter.

3.3.1.3.1 Umbel production at three years after planting

In the April 1999 flowering assessment (three year after planting) none of the seedlings were scored with umbels. However, a small percentage of grafts in both the control (nil paclobutrazol) and 0.25 g paclobutrazol per cm b.s.c. treatments did produce umbels at each site (***Table 3.6***). The highest percentage of grafts bearing umbels was recorded at Blyfstaanhoogte (10.7 %) followed closely by Tentkop (9.7 %). At Gowan Brae and Mossbank, where substantially less winter chilling accumulated (46.6 CPs and 58.5 CPs, respectively) than at the two afore-mentioned sites (Blyfstaanhoogte 65.0 CPs; Tentkop 79.5 CPs), 3.3 % and 3.1

% of the grafts produced umbels. At Gowan Brae, Mossbank and Blyfstaanhoogte, where accumulated winter chill figures ranged between 46.6 and 65.0 CPs, paclobutrazol application approximately doubled the percentage trees producing umbels recorded by the untreated grafts.

At Tentkop, the site accumulating the highest number of chill units during the 1998 winter (79.5 CPs; 1314.0 UCUs; 1344 DPCUs), a different trend in the response from paclobutrazol treatment was recorded. The percentage umbel-producing grafts for the control (nil paclobutrazol) (13.3 %) was double that of the paclobutrazol-treated grafts (6.0 %) (***Table 3.6***). A possible reason for this might be that the high amount of cold accrued at Tentkop during the1998 winter was sufficient to satisfy the cold requirement of the grafts for floral induction and no further floral stimulus was necessary.

The question remains, however, as to why fewer paclobutrazol-treated grafts produced umbels at Tentkop than at Blyfstaanhoogte during 1999 (6 % compared to 20.4 %) when Tentkop accumulated considerably more chilling than did Blyfstaanhoogte (79.5 CPs compared to 65.0 CPs). A possible explanation may be that the trees at Tentkop were more juvenile than at Blyfstaanhoogte. The severe winter conditions at Tentkop retarded tree vegetative growth and development particularly whilst trees were young. Both seedlings and grafts experienced considerable frost damage at this site each winter during the first two years following planting, whereas plants at the remaining three sites received no damage. The tables of tree height at five years (***Tables 3.4*** and ***3.5***) illustrate the slow growth rate which occurred at Tentkop relative to the remaining three sites. Although Moncur and Hasan (1994) found that no reversion to juvenility occurred in 6- and 18-month old paclobutrazol-treated *E. nitens* grafts, this phenomenon has been observed in similarly aged plants at the ICFR nursery in Pietermaritzburg (R. A. W. Gardner unpublished data). Paclobutrazol was possibly very effective at Blyfstaanhoogte
during 1998 because the cool conditions at the site were ideal for tree growth (***Table 3.5***), and trees were more reproductively mature at the time when inductive conditions occurred.

3.3.1.3.2 Umbel production at four and five years after planting

The results of the analyses of variance for the percentage trees which produced umbels at 4-years after planting at four trial sites are presented graphically in ***Figure 3.1***. The graph shows the results of the separate analyses of variance for the four different PROPAGULE (propagule type) x PBZ (paclobutrazol application) treatment combinations. As mentioned earlier

(paragraph 3.2.1.1.3), family data were pooled in these analyses.

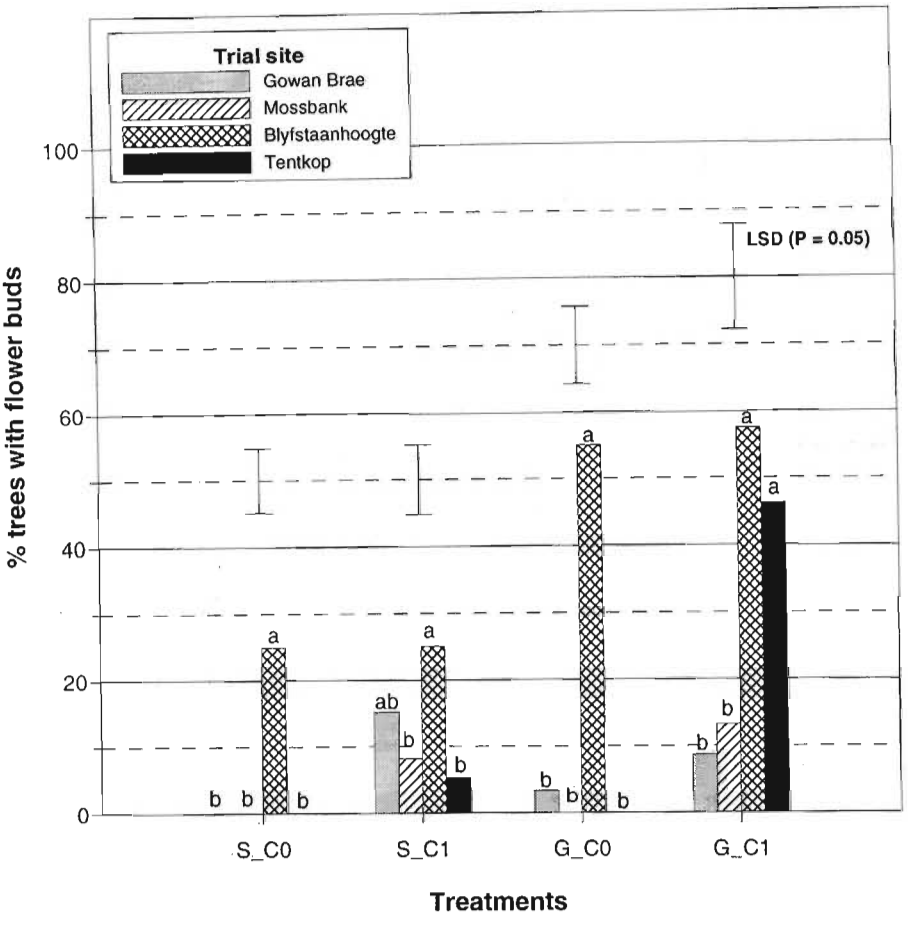


Figure 3.1 Percentage of trees with umbels at four different sites at four years after planting (S = seedling; G = graft; C0 = 0.00 g paclobutrazol/ cm b.s.c.; C1 = 0.25 g paclobutrazol/ cm b.s.c.). Within each treatment, values (bars) accompanied by the same letter do not differ significantly ($p = 0.05$). Whiskers indicate the LSD values ($p = 0.05$) for each treatment (bar group).

The Blyfstaanhoogte site recorded the highest percentage trees bearing umbels in each of the four PROPAGULE x PBZ treatment combinations. In the case of either seedlings or grafts at this site, the percentage trees bearing umbels was similar for control and paclobutrazol-treated trees (25 % and 25 % respectively in seedlings, and 55 % and 58 % respectively in the grafts).

A possible explanation for this is as follows:

Mean tree height at five years after planting (***Table 3.4***) shows that conditions at Blyfstaanhoogte were not particularly conducive to rapid growth. Gowan Brae and Mossbank

recorded mean heights of 16.4 m and 13.6 m respectively, whereas Blyfstaanhoogte recorded only 11.9 m. Although it was not clear as to why paclobutrazol had no effect on 5-year tree height at Blyfstaanhoogte (***Table 3.5***), the relatively slow growth at this site may have been associated with lowered gibberellin levels resulting from the particular site conditions. However, gibberellin levels were not monitored in the experiment, and therefore this is purely speculative. The mode of action of paclobutrazol in plants is well-documented (e.g. Graebe 1987). Paclobutrazol is known to reduce the level of endogenous gibberellins in a variety of plant species (Graebe 1987, Fletcher et al. 2000). In *E. nitens*, paclobutrazol application resulted in lowered gibberellin levels and reduced vegetative growth, and where lowered gibberellin levels were complemented with sufficient cold, floral induction was stimulated (Griffin et al 1992, Moncur and Hasan 1994, Williams et al. 1999). Chouard (1960) found a common property of all vernalization phenomena to be "... that concomitant growth may be either moderate or very slow, and that if it ceased completely, vernalization did not occur". If the environmental conditions at Blyfstaanhoogte resulted in slowed or reduced growth rate together with lowered gibberellin levels during winter 1999 and optimum winter chilling conditions for floral induction also occurred during this time, then the inductive effects of paclobutrazol treatment (via lowering of gibberellin levels) would quite likely have been masked.

For a high rate of flowering (relative to South African standards) to take place in young *E. nitens* trees, as did occur in both seedlings and grafts at Blyfstaanhoogte four years after planting, it is then likely that not only were gibberellin levels within the optimum range, but also the amount of accumulated winter chilling (88.4 CPs; 1601.0 UCUs) was close to the optimum for floral induction in the species. If gibberellin levels and winter chilling amount were at an optimum for floral induction, the question remains as to why not all plants produced umbels at Blyfstaanhoogte. A possible reason for this is that flowering in *E. nitens* appears to be under strong genetic control (Swain and Chiappero 1998, Jones et al. 2000). This suggestion is substantiated judging by flowering data for paclobutrazol-treated seedlings and grafts of the different "families" in the experiment presented in ***Tables 3.7*** and ***3.8***. Regarding genotype influence on flowering precocity, this appears most noticeable in the grafts, where the different "families" (clones of particular "families") differed significantly from one another on the basis of percentage trees producing umbels ("%_trees") at four years (***Table 3.8***). What was further noteworthy from these tables was the effect of propagule type on "family" performance. For instance, grafted "family" 32097 performed poorly compared to all other grafted treatments (20 % trees produced umbels) (***Table 3.8***), but the seedling "family" 32097, from which grafted

"family" 32097 was originally selected, was the top performing seedling "family" (27 % trees produced umbels) (***Table 3.7***). Alternatively, grafted family 34838 was the top performing clone (62 % of the trees produced umbels), whereas seedling family 34838 was the second poorest performing seedling family (8.3 % of the trees produced umbels). This is perhaps indicative of the genetical variation which evidently exists within families of similar provenances of *E. nitens*, based on historical observations of growth and flowering attributes (Swain 2001, Jones 2002).

Table 3.7 Percentage of paclobutrazol-treated seedlings which produced umbels at four years after planting at four trial sites. (* Values accompanied by the same letter do not differ significantly ($p = 0.05$)).

SEEDLING "FAMILY"		TRIAL SITE				
Family	Provenance	Gowan Brae	Mossbank	Blyfstaanhoogte	Tentkop	MEAN *
32097	Barren Mt	19.4	32.8	54.4	0.0	26.7 *a*
37255	Tallaganda	8.3	0.0	26.1	21.7	14.0 *b*
32091	Barren Mt	19.4	0.0	0.0	0.0	4.9 *b*
34838	Barrington Tops	13.3	0.0	0.0	0.0	8.3 *b*
MEAN	-	15.1	8.2	25.1	5.4	13.5
LSD "FAMILY" ($p = 0.05$)	10.53					
C.V. (%)	8.3					

The lack of floral response in grafts of the "nil paclobutrazol" PBZ treatment at Tentkop (Treatment G_C0) (***Figure 3.1***) compared to the 13 % response in grafts of the same PBZ treatment in 1999 when plants were a year younger (***Table 3.6***), appears to further confirm a minimum winter chilling requirement for floral induction in *E. nitens*. At Tentkop, less winter chilling accumulated during 1999 (71.6 CPs) than during 1998 (79.5 CPs). Although Tentkop recorded the lowest tree height growth at five years (10.18 m), and hence on average the slowest tree growth of all sites, the amount of winter chilling during 1999 may not have been sufficient to trigger a good flowering response in the trees during 2000.

Table 3.8 Percentage of paclobutrazol-treated grafts which produced umbels at four years after planting at four trial sites. (* Values accompanied by the same letter do not differ significantly ($p = 0.05$)).

GRAFTED ("FAMILY")		TRIAL SITE				
Family	Provenance	Gowan Brae	Mossbank	Blyfstaanhoogte	Tentkop	MEAN *
32097	Barren Mt	11.1	0.0	56.7	13.3	20.3 *c*
37255	Tallaganda	0.0	6.7	0.0	0.0	1.7 *d*
32091	Barren Mt	6.7	0.0	86.7	75.0	42.1 *b*
34838	Barrington Tops	16.7	46.7	86.7	96.7	61.7 *a*
MEAN	-	8.6	13.3	57.5	46.2	31.4
LSD "FAMILY" ($p = 0.05$)	15.36					
C.V. (%)	1.9					

Where grafts received paclobutrazol (Treatment G_C1) (***Figure 3.1***), Blyfstaanhoogte recorded the highest percentage trees with umbels (58 %) of all sites, though this was not significantly greater than Tentkop which recorded the second highest percentage trees with umbels (46 %). The ranking order of sites from lowest percentage trees producing umbels (9 % at Gowan Brae) to highest percentage producing umbels (58 % at Blyfstaanhoogte) matched the ranking order of sites from least winter chilling accrued (Gowan Brae 40.8 CPs) to most winter chilling accrued (Blyfstaanhoogte 88.4 CPs) (***Table 3.2***). Regressions to study the relationship between floral bud production and winter chilling were not carried out on the four year data, as regressions using five year data should give more reliable results as trees would be older and therefore more reproductively mature (Meilan 1997, Moncur 1998).

The results of the analyses of variance for the percentage trees which produced umbels at five years after planting are presented in ***Figure 3.2***. The graph shows the results of the separate analyses of variance for the four different PROPAGULE x PBZ treatment combinations. Again, family data were pooled in these analyses.

At five years after planting (April 2001), all treatments produced umbels. Although each site recorded a greater amount of winter chill accumulation during 2000 (prior to the 2001 crop) than in 1999 (prior to the 2000 crop) (***Table 3.2***), it is not clear what proportions of the generally better flowering response in 2001 were due to the colder winter conditions and

increased age of the trees. In the seedlings, it is likely that increased age was responsible for a large proportion of the increase in percentage flowering trees, as under "natural" conditions in South Africa, seedlings of *E. nitens* do not normally produce flower buds before the age of about eight years (Eldridge et al. 1993, Swain and Chiappero 1998). Each year of increased age would therefore be a step closer to reproductive maturity in the seedlings. In the grafts, however, it is less likely that increased age played a significant role, as by the start of the experiment the physiological age of all grafts was already more than eight years (refer section 2.1.1). The accuracy of the actual physiological age of the grafts in this experiment could be debated to some extent however, as reversion to juvenility following grafting cannot be ruled out. Although concrete documented evidence of the phenomenon of reversion to juvenility following grafting in *E. nitens* is still lacking, the transmittance of rootstock characteristics to scion following grafting, including temporary transferral of aspects of immaturity, is a well-known phenomenon in tree fruit crops (Janick and Moore 1975, Hartmann et al. 1990).

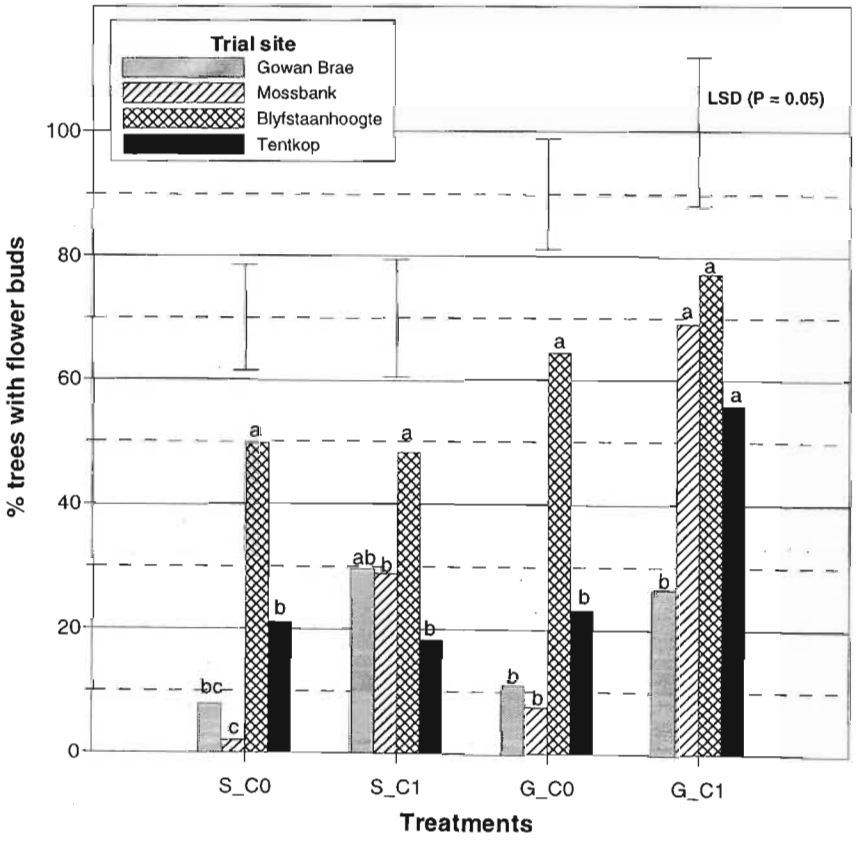


Figure 3.2 Percentage of trees with umbels at four different sites at five years after planting (S = seedling; G = graft; C0 = 0.00 g paclobutrazol/ cm b.s.c.; C1 = 0.25 g paclobutrazol/ cm b.s.c.). Within each treatment, values (bars) accompanied by the same letter do not differ significantly ($p = 0.05$). Whiskers indicate the LSD values ($p = 0.05$) for each treatment (bar group).

In the seedlings, paclobutrazol clearly appeared to provide the additional stimulus necessary for floral induction at the two warmest sites, Gowan Brae (51.7 CPs) and Mossbank (70.8 CPs) (***Figure 3.2***). At Gowan Brae the percentage trees with buds increased from 8 % to 30 % when paclobutrazol was applied, and at Mossbank the percentage flowering trees increased from 2 % to 29 %. On the other hand, at the sites accumulating the highest number of winter chill units during 2000 (***Table 3.2***), namely Tentkop (76 CPs) and Blyfstaanhoogte (89.1 CPs), paclobutrazol did not induce more trees to produce flower buds. The percentages control and paclobutrazol-treated seedlings which flowered were 21 % and 18 % for Tentkop, respectively, and 50 % and 48 % for Blyfstaanhoogte, respectively (***Figure 3.2***). This suggests that the amounts of winter chilling at Tentkop and Blyfstaanhoogte were sufficient to mask or substitute any trigger brought about by paclobutrazol application in the seedlings.

In the grafts, the effect of paclobutrazol at the two warmest sites, Gowan Brae and Mossbank, was as dramatic as in the seedlings at the same sites. At Gowan Brae the percentage five year old grafts with buds increased from 11 % to 27 % when paclobutrazol was applied, and at Mossbank the percentage grafts with buds increased from 8 % to 69 % (***Figure 3.2***).

At Gowan Brae 51.7 CPs were recorded, whilst the chill units for Mossbank (70.8 CPs) were similar to those for Tentkop (76.0 CPs) (***Table 3.2***). Therefore, the floral response at Mossbank was expected to be almost similar to that of Tentkop for all treatments. Indeed this was the case in all but one of the PROPAGULE x PBZ combinations, namely the graft/ 0.25 g paclobutrazol per cm b.s.c. combination (G_C1 treatment) (***Figure 3.2***), where Mossbank (69 %) scored an even higher percentage trees with buds than did Tentkop (56 %).

On the one hand, this deviation from the expected umbel production pattern may be indicative of particular genotype x environment interactions characteristic of eucalypt clones. For instance, a clone may have a specific optimum chilling (winter temperature) range for floral induction, and temperatures below this lower threshold might possibly be deleterious for floral induction and/or other aspects of growth. The small height of the paclobutrazol-treated trees at Tentkop (8.2 m) relative to those at Mossbank (13.0 m) (***Table 3.5***) may have meant that the trees at the former site were more prone to cold damage. Further research is needed to establish whether damage and stress from cold is deleterious to (or perhaps promotive of) flowering in *E. nitens*. It is still unclear as to whether the promotive effect of winter chilling regarding flowering is related to cold stress or not.

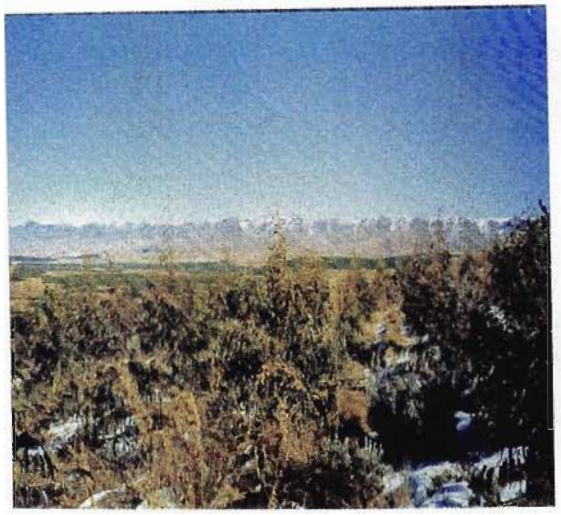
The fact that umbels were produced on several paclobutrazol-treated seedlings of Ebor provenance (Family 37656) and Tallaganda provenance (Family 37255) which were still entirely in juvenile leaf at the age of 45 months at Tentkop (***Figure 3.3***), suggests that trees at this site were severely stressed by site conditions. The soils at this site were deep (***Table 2.2***) and therefore it is unlikely that trees experienced any prolonged period of drought stress for the duration of the experiment. Severe scorching of the foliage of seedlings from cold was noted each year during mid-winter at this site, whereas no cold-damage symptoms were ever recorded at any of the other sites. The phenomenon of seedlings of *E. nitens* producing umbels on juvenile foliage is unusual (Pryor 1976, Boland et al. 1992) and has been documented in only one other instance (Moncur 1998). As in Moncur's observation, the umbels on the juvenile foliage at Tentkop consisted mostly of three flower buds each. However, at Tentkop, a further divergence from the norm (Boland et al. 1992) occurred. In several instances, in seedling trees of both provenances (Ebor and Tallaganda), paired inflorescences occurred. Both paired and unpaired inflorescences occurred in axillary and terminal positions on the shoots. The illustrations in ***Figure 3.3*** provide an insight into the latter flowering observations at Tentkop. Umbels were also produced on several paclobutrazol-treated seedlings of the Ebor (Family 37656 and the S.O. mix), Tallaganda (Family 37255), Barren Mountain (Family 32091) and Barrington Tops (Families 34832 and 34838) provenances on juvenile and intermediate foliage (Pryor 1976, Tibbits 1986) on the same trees, at 45 months at Tentkop.
On the other hand, the deviation from the expected result may be an indication of the lack of appropriateness of the chill models and/or *chill model* x *chill period* combinations used in this study.

3.3.1.4 Effect of site and paclobutrazol application on umbel crop per tree

3.3.1.4.1 Individual trial analyses

The separate ANOVAs of five-year umbel score data for the four different trials showed that a zero or constant variance across all trials was not attained. The estimates of variance (on a plot level) for each site from the subsequent REML analyses of variance are given in ***Table 3.9*** below. The variance at Gowan Brae was almost six times that of Blyfstaanhoogte or twice that of Tentkop. The high variance at Gowan Brae probably resulted from the canopies of several trees being partly damaged by vandalism during 1999 and thus being treated as "missing trees" in the data set. The high variance at Tentkop (more than twice that of Blyfstaanhoogte), most likely resulted from the damage to trees by strong winds typical to this exposed site.

a.



b.

c.

d.

Figure 3.3 Umbel production at Tentkop. a. 18 month-old *E. nitens* grafts at the Tentkop trial site, b. and c. umbels on juvenile foliage of 45-month old *E. nitens* seedlings during 2001. (b. 3-flowered umbels on seedling of Ebor provenance, Family 37656, c. paired inflorescences at terminal node in Tallaganda provenance, Family 37255, seedling), d. umbels on mature foliage of 45-month old *E. nitens* seedling, Ebor provenance, Family 37656).

Table 3.9 Estimated variance (on a plot mean level) in the REML analysis of variance for umbel score per tree (logarithmic transformation) at five years after planting (LFLW_2001).

SITE	Gowan Brae	Mossbank	Blyfstaanhoogte	Tentkop
VARIANCE	0.01776	0.00001	0.0035	0.00856

3.3.1.4.2 Across-site analysis

A summary of the results from the across-site REML analysis of variance for the variate LFLW_2001, including *F*-test values for the fixed effects, is presented in ***Table 3.10***.

The *F*-tests revealed that certain factors, alone or in combination with other factors, were highly significant. "SITE" was the only factor which was independently highly significant ($p < 0.001$) (***Table 3.10***). Mean transformed umbel scores ("LFLW_2001") for the different sites are presented in ***Table 3.11***. The sites ranked in order from lowest to highest umbel score per tree as follows: Gowan Brae (0.0967), Mossbank (0.2468), Tentkop (0.3018) and Blyfstaanhoogte (0.4515).

"FAMILY" was the only other independent factor which was significant ($p < 0.01$). The FAMILY x PROPAGULE interaction was highly significant ($p < 0.001$) (***Table 3.10***), and therefore this aspect should be discussed with particular reference to umbel productivity per tree (LFLW_2001). The mean umbel score per tree at five years after planting (logarithmic transformation) (LFLW_2001) for the FAMILY x PROPAGULE interaction (logarithmic transformation) is presented in ***Table 3.12.***

There was a noticeable re-ordering of families between the two different propagule types (as did occur in the case of "%_trees producing umbels" at four years after planting, which was discussed in paragraph 3.3.1.3.2). For instance, family 34838 ranked first amongst the grafts (LFLW_2001 = 0.6073), but ranked only third amongst the seedlings (LFLW_2001 = 0.1524). In the case of family 37255 there was a complete change in ranking order between the two propagule types. Family 37255 ranked poorest amongst the grafts (LFLW_2001 = 0.1569) but first amongst the seedlings (LFLW_2001 = 0.2404).

Table 3.10 Results of the across-site REML analysis for umbel score per tree (logarithmic transformation) at five years after planting (LFLW_2001).

Fixed term	Wald statistic	d.f.	F_{calc}
SITE	81.4	3	27.13 ***
FAMILY	19.7	3	6.57 **
PBZ	87.9	1	87.9
PROPAGULE	47.6	1	47.6
SITE x FAMILY	21.0	9	2.33
SITE x PBZ	11.6	3	3.87 *
FAMILY x PBZ	2.0	3	0.66
SITE x PROPAGULE	25.6	3	8.53 ***
FAMILY x PROPAGULE	59.6	3	19.87 ***
PBZ x PROPAGULE	12.4	1	12.4 ***
SITE x FAMILY x PBZ	13.7	9	1.52
SITE x FAMILY x PROPAGULE	31.9	9	3.54 *
SITE x PBZ x PROPAGULE	24.0	3	8.0 ***
FAMILY x PBZ x PROPAGULE	16.8	3	5.6 **
SITE x FAMILY x PBZ x PROPAGULE	13.1	9	1.46

Levels of significance: * denotes $p < 0.05$; ** denotes $p < 0.01$; *** denotes $p < 0.001$

Table 3.11 Mean umbel score per tree at five years after planting (logarithmic transformation) (LFLW_2001) for the four field trials.

SITE	Gowan Brae	Mossbank	Blyfstaanhoogte	Tentkop
LFLW_2001	0.0967	0.2468	0.4515	0.3018
Standard Error of the differences between the means (S.E.D.):			0.0403	

Table 3.12 Mean umbel score per tree at five years after planting (logarithmic transformation) (LFLW_2001) for the FAMILY x PROPAGULE interaction.

FAMILY (refer *Table2.1*)	Provenance	Seedling	Rank	Graft	Rank
32091	Barren Mountain	0.1132	4	0.4657	2
32097	Barren Mountain	0.2155	2	0.2423	3
34838	Barrington Tops	0.1524	3	0.6073	1
37255	Tallaganda	0.2404	1	0.1569	4
Standard Error of the differences between the means (S.E.D.):		0.05704			

These results reinforce existing knowledge of the presence of considerable within-family variation, regarding precocity, in the South African *E. nitens* breeding population (Swain 2001, Jones 2002). The results therefore emphasize the importance of including as wide a range of genetic material possible in future trials, if later inferences, particularly on a family level, are to be valid.

Regarding the highly significant ($p < 0.01$) "PBZ x PROPAGULE" interaction, a series of *t*-tests established that the only significant difference ($p < 0.05$) occurred between the "0.00 g paclobutrazol, seedling" and "0.025 g paclobutrazol, graft" treatments.

3.3.1.5 Relationship between winter chilling and percentage trees producing umbels

In the initial assessment of the data, separate correlation matrices were produced for seedlings and grafts as described in paragraph 3.2.1.1.1. The main purpose was to investigate the degree of relatedness of the different *chill model* x *chill period* combinations. Because the resultant correlation coefficients (r) for the explanatory variables were identical in the case of either propagule type, only the results of the correlation analysis for grafts is presented (***Table 3.13***). Correlation coefficients ranged from 0.809 for COLD_1 and COLD_6 (weakest correlation) to 0.999 for COLD_1 and COLD_2 (strongest correlation). Generally, exceptionally high correlations ($r > 0.98$) were found when the period of chill unit accumulation was extended from 01 May to 30 September to 01 April to 30 September.

Table 3.13 Correlation matrix for all explanatory variables investigated in the relationship between the percentage grafts producing umbels at 5 years (%_trees) and *chill model* x *chill period* combination (COLD_*x*) and paclobutrazol application (PBZ) (explanatory variable abbreviations as per ***Table 3.1***).

COLD_1	1.000							
COLD_2	0.999	1.000						
COLD_3	0.941	0.956	1.000					
COLD_4	0.874	0.894	0.984	1.000				
COLD_5	0.882	0.903	0.990	0.996	1.000			
COLD_6	0.809	0.835	0.959	0.992	0.989	1.000		
PBZ	0.000	0.000	0.000	0.000	0.000	0.000	1.000	
%_trees	0.667	0.672	0.682	0.668	0.664	0.640	0.527	1.000
	COLD_1	COLD_2	COLD_3	COLD_4	COLD_5	COLD_6	PBZ	%_trees

Note: correlation ***df*** = 22 (n - 2); $p < 0.05 = 0.404$; $p < 0.01 = 0.515$.

The Utah Chill Model based *chill model* x *chill period* combinations COLD_3 (April-September data) and COLD_4 (May - September data) performed best in the separate regressions for seedlings and grafts regarding percentage trees producing umbels (%_trees), although the Dynamic Model based combinations COLD_1 and COLD_2 gave only marginally less favourable results (***Table 3.14*** and ***Table 3.15***).

In the seedlings, the relationship between the percentage trees producing umbels (%_trees) and *chill model* x *chill period* combination was highly significant ($p < 0.001$) for all combinations tested, when paclobutrazol (PBZ) was applied. Of all the combinations tested, the Utah Chill Model 01 May - 30 September combination (COLD_4) with PBZ application accounted for the highest percentage variance (67.7 %) (R^2 value) (Campbell et al. 1981, McConway et al. 1999) (***Table 3.14***).

Table 3.14 Step-wise regression analyses for percentage *seedlings* which produced umbels at five years on *chill model* x *chill period* combination and paclobutrazol application (PBZ) (explanatory variable abbreviations as per ***Table 3.1***).

		COLD_1 PBZ	COLD_2 PBZ	COLD_3 PBZ	COLD_4 PBZ	COLD_5 PBZ	COLD_6 PBZ
Source	d.f.	m.s	m.s	m.s	m.s	m.s	m.s
Regression	2	2295.4 ***	2317.0 ***	2463.5 ***	2575.9 ***	2450.3 ***	2474.6 ***
Residual	21	129.1	127.1	113.1	102.4	114.4	112
Total	23	317.5	317.5	317.5	317.5	317.5	317.5
R^2		59.3	60	64.4	67.7	64	64.7
SED		11.4	11.3	10.6	10.1	10.7	10.6
Estimate of parameters							
Constant		-55.3	-55	-54	-56.2	-64.8	-60.6
COLD_x		0.658	0.746	0.03354	0.03928	0.0376	0.03998
PBZ		19.26	19.26	19.26	19.26	19.26	19.26
Parameter *t*-values (21 d.f.)							
Constant		-3.79 **	-3.84 ***	-4.23 ***	-4.62 ***	-4.37 ***	-4.38 ***
COLD_x		4.28 ***	4.35 ***	4.89 ***	5.35 ***	4.84 ***	4.93 ***
PBZ		4.15 ***	4.19 ***	4.44 ***	4.66 ***	4.41 ***	4.46 ***

Levels of significance: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

In the grafts, the relationship between percentage trees producing umbels (%_trees) and *chill model* x *chill period* combination was again highly significant for all combinations tested when PBZ was applied. Of all combinations tested, the Utah Chill Model 01 May - 30 September combination (COLD_3) with PBZ applied accounted for the highest percentage variance (71.8 %) (***Table 3.15***).

Overall, regarding the entire string of regressions carried out for percentage trees producing umbels (%_trees) against each different *chill model* x *chill period* combination (***Table 3.14*** and ***Table 3.15***), in the case of each combination the grafts accounted for a slighter higher percentage variance than the seedlings. The highest correlation between floral response (%_trees) and *chill model* x *chill period* combination was achieved in the grafts with the Utah Chill Model 01 April - 30 September combination (COLD_3) (71.8 % of the total variance accounted for).

Table 3.15 Step-wise regression analyses for percentage *grafts* which produced umbels at five years on *chill model* x *chill period* combination and paclobutrazol application (PBZ) (explanatory variable abbreviations as per ***Table 3.1***).

		COLD_1 PBZ	COLD_2 PBZ	COLD_3 PBZ	COLD_4 PBZ	COLD_5 PBZ	COLD_6 PBZ
Source	d.f.	m.s	m.s	m.s	m.s	m.s	m.s
Regression	2	7051.9 ***	7126.2 ***	7248.8 ***	7069.4 ***	7019.8 ***	6706.6 ***
Residual	21	258.3	251.2	239.5	256.6	261.3	291.2
Total	23	849	849	849	849	849	849
R^2		69.6	70.4	71.8	69.8	69.2	65.7
SED		16.1	15.8	15.5	16	16.2	17.1
Estimate of parameters:							
Constant		-108.6	-107.9	-101.8	-99.8	-119	-106.8
COLD_x		1.261	1.428	0.06146	0.0678	0.0675	0.0685
PBZ		30.07	30.07	30.07	30.07	30.07	30.07
Parameter *t*-values (21 d.f.):							
Constant		-5.26 ***	-5.36 ***	-5.47 ***	-5.19 ***	-5.31 ***	-4.79 ***
COLD_x		5.80 ***	5.93 ***	6.15 ***	5.83 ***	5.74 ***	5.24 ***
PBZ		4.58 ***	4.65 ***	4.76 ***	4.60 ***	4.56 ***	4.32 ***

Levels of significance: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

If the results for the *chill model* x *chill period* combinations were averaged according to parent chill model, the Utah Chill Model (based on COLD_3 and COLD_4) correlated best with the percentage trees producing umbels (%_trees) in the case of seedlings and grafts (66.1 % and 70.8 % respectively).

Where chill units were calculated using the Dynamic Model, a slightly better fit between winter chilling and the percentage trees producing umbels (%_trees) was found for the grafts when April temperature data were excluded. The Dynamic Model 01 April - 30 September chill period combination (COLD_1) explained 69.6 % of the total variance, whereas the Dynamic Model 01 May - 30 September combination (COLD_2) explained 70.4 % of the variance.

3.3.1.6 Relationship between winter chilling and umbel crop per tree

The Dynamic Model based *chill model* x *chill period* combinations, COLD_1 (April -

September data) and COLD_2 (May - September data), gave the best results for predicting umbel yield per tree with propagule type and paclobutrazol application (PBZ) included as explanatory variables.

The transformed ($\log_{10(x+1)}$) individual tree umbel scores for April 2001 ("LFLW_2001") are presented in ***Table 3.16***.

Table 3.16 Mean transformed (logarithmic) umbel scores at five years after planting ("LFLW_2001") for site, level of paclobutrazol application and propagule type.

Site	Level of paclobutrazol application	Propagule type		Accumulated chill units	
		Seedling	Graft	CPs	UCUs
Gowan Brae	0.00 g per cm b.s.c.	0.0390	0.0000	51.7	1073.0
	0.25 g per cm b.s.c.	0.1846	0.1633		
	Difference	0.1456	0.1633		
Mossbank	0.00 g per cm b.s.c.	0.0000	0.0805	70.8	1414.0
	0.25 g per cm b.s.c.	0.1428	0.7638		
	Difference	0.1428	0.6833		
Blyfstaanhoogte	0.00 g per cm b.s.c.	0.2311	0.4890	89.1	1836.0
	0.25 g per cm b.s.c.	0.4856	0.6002		
	Difference	0.2545 **	0.1112		
Tentkop	0.00 g per cm b.s.c.	0.1141	0.1766	76.0	1261.0
	0.25 g per cm b.s.c.	0.2455	0.6710		
	Difference	0.1314	0.4944		

S.E.D. for interaction and for same level of factor: 0.08067

Levels of significance: * denotes $p < 0.05$; ** denotes $p < 0.01$; *** denotes $p < 0.001$

Note: b.s.c., basal stem circumference

Correlation matrices for seedlings and grafts investigating the relatedness of the different *chill model* x *chill period* combinations gave almost identical results to those for "%_trees" (data not presented). All regressions were highly significant ($p < 0.001$). The highest correlation between umbel yield per tree ("LFLW_2001") and *chill model* x *chill period* combination was achieved using the Dynamic Model, i.e. COLD_1 (Dynamic Model 01 April to 30 September) and COLD_2 (01 May to 30 September), where either of these, together with explanatory variables PROPAGULE and PBZ, accounted for 65.3 % of the total variance (***Table 3.17***).

Table 3.17 Step-wise regression analyses for transformed (logarithmic) umbel scores at five years (LFLW_2001) on *chill model* x *chill period* combination, propagule type and paclobutrazol application (explanatory variable abbreviations as per ***Table 3.1***).

		COLD_1 PROPAGULE PBZ	COLD_2 PROPAGULE PBZ	COLD_3 PROPAGULE PBZ	COLD_4 PROPAGULE PBZ	COLD_5 PROPAGULE PBZ	COLD_6 PROPAGULE PBZ
Source	d.f.	m.s.	m.s.	m.s.	m.s.	m.s.	m.s.
Regression	3	0.4543 ***	0.4548 ***	0.4435 ***	1.2842 ***	0.4283 ***	0.4107 ***
Residual	28	0.0223	0.0222	0.0234	0.7021	0.0251	0.0269
Total	31	0.0641	0.064	0.0641	1.9863	0.0641	0.0641
R^2		65.3	65.3	63.4	60.9	60.9	58.0
SED		0.149	0.149	0.153	0.158	0.158	0.164
Estimate of parameters:							
Constant		- 0.526	- 0.516	- 0.446	- 0.416	- 0.536	- 0.444
COLD_x		0.00828	0.00932	0.00038	0.00041	0.00041	0.00040
PROPAGULE		- 0.1885	- 0.1885	- 0.1885	- 0.1885	- 0.1885	- 0.1885
PBZ		0.2690	0.2690	0.2690	0.2690	0.2690	0.2690
Parameter *t*-values:		28 d.f.	28 d.f.	28 d.f.	28 d.f.	28 d.f.	28 d.f.
Constant		- 2.86 **	-2.84 **	- 2.50 *	- 2.25 *	- 2.58 *	- 2.17 *
COLD_x		4.74 ***	4.75 ***	4.47 ***	4.10 ***	4.10 ***	3.70 ***
PROPAGULE		- 3.57 **	-3.58 **	-3.48 **	-3.37 **	- 3.37 **	-3.25 **
PBZ		5.10 ***	5.11 ***	4.97 ***	4.81 ***	4.81 ***	4.64 ***

Levels of significance: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

The poorest fitting *chill model* x *chill period* combination (in combination with PROPAGULE and PBZ) was the Daily Positive Utah Chill Unit Model 01 May to 30 September combination (COLD_6), accounting for only 58 % of the total variance.

A series of *t*-tests was carried out to explore the role of the different explanatory variables in each multiple regression. *Chill model* x *chill period* combination (COLD_*x*), PBZ and PROPAGULE were all highly significant ($p < 0.01$) and thus highly influential in each regression. The high significance of the partial regression coefficients "PBZ" and "PROPAGULE" in all regressions indicated that significant differences existed between the 0.00 g and 0.25 g paclobutrazol/ cm b.s.c. treatments in the case of PBZ, and between seedling and graft in the case of PROPAGULE.

3.3.1.7 Summary of regression results

The most noteworthy aspect of the regression results was that, in conjunction with one another, the variables COLD_*x* and PBZ were able to explain between 66 and 72 % of the variation in *E. nitens* floral bud production (represented by the percentage grafts producing flower buds at five years after planting) (***Table 3.15***). However, these results should be seen in the context that the high level of variance accounted for may be, in part, due to the fact that, in each case, the explanatory variable COLD_*x* consisted of only four unique points. Of all *chill model* x *chill period* combinations tested, the combination COLD_2 (Dynamic Model, May - September temperature data) appeared best-suited for the purpose of predicting flower bud crops in *E. nitens* under summer rainfall South African conditions, although this combination's superiority over all other combinations was not proven to be statistically significant. Overall, the difference in performance of the six *chill model* x *chill period* combinations was fairly minimal.

3.3.2 Pot trials

3.3.2.1 Rate of uptake of paclobutrazol by potted *E. nitens* grafts

Difficulty was experienced in accurately measuring the small weekly change in shoot length (between one and two millimetres per week), and this may have contributed towards noticeable week-to-week fluctuations apparent in the actual data sets for either treatment (***Figure 3.4***). Where large fluctuations between weeks occurred, the growth measurements for "0.00 g paclobutrazol/ cm b.s.c." and "0.25 g paclobutrazol/ cm b.s.c." did appear to fluctuate more or less synchronously. These spurts and declines in growth in either treatment were most likely due to the non-constant outdoor environmental conditions in the shadehouse, where plants were subjected to the natural fluctuations in weather patterns. Because plants were maintained in a continuous well-watered state by the automated nursery watering system, changes in air temperature according to changing weather conditions were the most likely cause of the non-uniform growth patterns in the grafts.

Second degree polynomial curves were fitted to the data sets of both "0.00 g paclobutrazol/ cm b.s.c." and "0.25 g paclobutrazol/ cm b.s.c." treatments in their plots against time. In the "nil-paclobutrazol" grafts, according to the fitted line, shoot growth continued for the duration of the experiment, with the lowest point in growth rate being recorded during the last week of June (0.33 % weekly increase in shoot length). In the treated (0.25 g paclobutrazol/ cm b.s.c.) grafts,

zero shoot growth occurred for a three week period between mid-June and the 07 July. In the non-treated plants, growth rate only dropped below 1 % in the first week of June, whereas in the treated plants this occurred as early as the first week of May. In both treatments, shoot extension began around the end of the first week in July. This most probably related to increasing soil temperature which is normal for this time of the year in the ICFR Pietermaritzburg nursery (R. A. W. Gardner unpublished data).

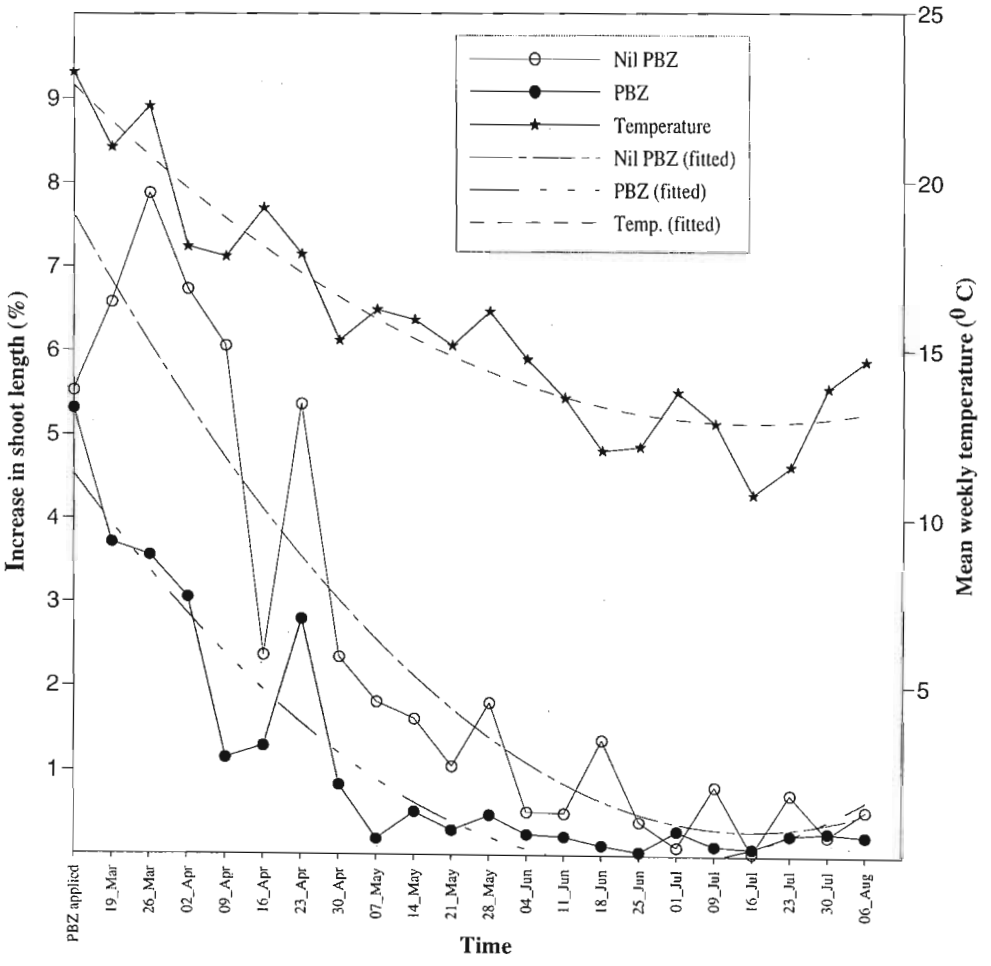


Figure 3.4 Percentage increase in shoot length in control (0.00 g paclobutrazol/ cm b.s.c.) (Nil PBZ) and 0.25 g paclobutrazol/ cm b.s.c. (PBZ) treated *E. nitens* grafts, and mean weekly air temperature during the experiment (Experiment 3).

According to the fitted curves, the shoot growth rate of paclobutrazol-treated *E. nitens* grafts was less than that of non-treated grafts throughout the experiment (***Figure 3.4***). Shoot growth in either paclobutrazol treatment steadily declined from the time of first measurement (one week before date of paclobutrazol application) towards winter. Temperature was the most likely factor responsible for progressive change in shoot growth rate in either the control (0.00 g paclobutrazol/ cm b.s.c.) or the 0.25 g paclobutrazol/ cm b.s.c. treated grafts during the experiment (***Figure 3.4***). It appears that photoperiod does not significantly influence floral induction in temperate eucalypts such as *E. nitens* (Moncur 1992, Moncur and Hasan 1994).

In experimental work where different paclobutrazol treatments were applied to field-grown *E. nitens* and *E. globulus* seedlings, it was concluded that paclobutrazol has to be present in meristems (in either of these two species) prior to the commencement of the natural cycle of floral initiation, if a significant increase in the number of flower buds produced is to be achieved (Griffin et al. 1993). Floral initiation in *E. nitens* takes place during late winter (August to early September) under Canberra, Australia conditions (Moncur et al. 1994*a*). It is likely that a similar timing for floral initiation in *E. nitens* (i.e. late winter) is applicable to South Africa.

It was hoped that data from Experiment 3 would clearly demonstrate the rapidity of uptake of paclobutrazol by potted *E. nitens* plants when PBZ is applied via collar drench method in late summer in South Africa. In all three experiments under controlled conditions, paclobutrazol was applied using the collar drench method. A sudden divergence between the growth curves for untreated and paclobutrazol-treated grafts was not evident in the plotted data (***Figure 3.4***). However, in all years a decrease in leaf size and internode length was clearly visible by mid-May in all paclobutrazol-treated plants (including seedlings in Experiment 2), eight weeks following the paclobutrazol application. It is possible that if shoot extension measurements had commenced earlier, perhaps one or two months before paclobutrazol application date, an initial sudden difference in growth rate between the two treatments would have been more pronounced. The growth rate in the treated compared to non-treated plants was distinctly slower. As grafts were "force fed" paclobutrazol, this being achieved by drying plants off prior to application, uptake may have been so rapid that suppression in growth occurred would have occurred within a week. This may explain the 3 % difference in growth rate between paclobutrazol-treated and untreated plants one week after paclobutrazol was applied.
In summary, evidence presented in the preceding sections suggests that paclobutrazol uptake by potted *E. nitens* grafts in the Pietermaritzburg nursery during late summer (March) is rapid,

effects of the chemical being evident in the plants within two weeks, on condition the paclobutrazol is applied according to the methods used in the experiment (described in sections 2.2.2 and 2.2.3). It is highly likely, therefore, that paclobutrazol was present within the meristems of paclobutrazol-treated plants of Experiments 1 and 2 before the onset of winter chilling accumulation at the beginning of May.

3.3.2.2 Effect of temperature and paclobutrazol application on tree growth

The results of the field trials showed that using the Dynamic Model (Fishman et al. 1987*a* and *b*) with chill accumulation between May - September was the most suitable of all *chill model* x *chill period* combinations tested for the prediction of floral response in field grown *E. nitens* seedlings and grafts from preceding winter chill units (paragraphs 3.3.1.4 and 3.3.1.5). Linsley-Noakes and Allan (1994) reported the Dynamic Model to be equally accurate for estimating winter chilling accumulation in dormant peach buds in areas with mild or severe winters. Therefore, in both 1999 and 2000 pot trials, accumulated winter chilling (Chilling Portions (CPs), Erez et al. 1990) was calculated from temperature data logged between 01 May and 30 September using the Dynamic Model.

Regarding the cold treatments in Experiment 2, similar amounts of winter chilling (CPs) were applied but the numbers of hours where temperatures remained below 5 ^{0} C were increased by approximately 50 % (***Table 3.18***).

Table 3.18 Total number of accumulated Chilling Portions and hours below 5 ^{0} C in the different treatments in *E. nitens* controlled environment Experiments 1 and 2.

	EXPERIMENT 1						**EXPERIMENT 2**			
	Cold treatment						**Cold treatment**			
	T1	**T2**	**T3**	**T4**	**T5**		**T1**	**T2**	**T3**	**T4**
CPs * (1999)	7	25	37	63	32	**CPs * (2000)**	16	45	59	30
Cold Hours ** (1999)	164	402	598	938	502	**Cold Hours ** (2000)**	256	866	1366	752
CPs * (2000)	16	16	16	16	16	**CPs * (2001)**	18	18	18	18
Cold Hours ** (2000)	256	256	256	256	256	**Cold Hours ** (2001)**	178	178	178	178

* CPs, Chilling Portions (chill units calculated by the Dynamic Model (Fishman et al. 1987*a* and *b*)) for the period 01 May - 30 September.

** Cold Hours, hours below 5 ^{0} C for the period 01 May - 30 September.

3.3.2.2.1 Experiment 1

The chilling schedule for Experiment 1 is diagrammatically presented in ***Figure 2.1***, and the numbers of accumulated chilling units (CPs) and hours below 5^0 C (Cold Hours) for the different cold treatments are given in ***Table 3.18***. From the latter table it can be seen that regarding temperature, environmental conditions in the ICFR and CERU convirons were well equated.

Winter chilling accumulation in the shadehouse (T1 cold treatment) commenced on 06 June (Julian day 157) and ended on 19 September (Julian day 262), a total of 7 chilling portions (CPs) and 164 hours below 5^0 C (Cold Hours) being logged for this treatment. After 26 August, the date on which the final controlled chill treatments (T4 and T5) were terminated and all plants were finally positioned back in the shadehouse (***Figure 2.1***), only 1 CP accumulated in the latter venue. No visible symptoms of cold stress or etiolation appeared at any stage during any of the cold treatments.

The second winter of the experiment (2000) was much colder and a total of 16 CPs and 256 Cold Hours were accumulated in the shadehouse (all cold treatments) between the 28 April and 21 September. In both years, winter Cold Hours began accumulating later and ceased accumulating sooner than CPs (***Table 3.18***).

Treatment T4 accumulated the most chill units (63 CPs) and was characterised by suppressed basal circumference (BC) growth, whilst paclobutrazol increased the BC (***Table 3.19***). In untreated trees, an increment in BC was inversely related to accumulated winter chilling amount. The two coldest treatments, T3 (37 CPs) and T4 (63 CPs), recorded the lowest increases in BC (34.3 % and 36.7 %, respectively), compared to the shadehouse control (7 CPs, 63.0 % BC increment).

3.3.2.2.2 Experiment 2

The chilling schedule for this experiment is presented in flow-chart form in ***Figure 2.3***, and the numbers of accumulated chilling units (CPs) and Cold Hours for the different cold treatments are given in ***Table 3.18***.

Winter chilling accumulation in the shadehouse (T1 cold treatment) began on 28 April 2000 (Julian day 119) and ended on 21 September (Julian day 264). A total of 16 CPs and 256 cold hours were logged during this period. After 16 August (Julian day 229), the date on which the last cold treatments (T3 and T4) ended and all plants were finally positioned back in the shadehouse (***Figure 2.3***), only 5 CPs accumulated in the shadehouse during the period 17 August to 21 September.

No cold stress symptoms were noted in any of the grafts for the entire duration of the experiment. However, the foliage of seedlings in the maximum cold treatment (T3) did develop cold stress symptoms (slight purpling of leaf lamina and necrosis of leaf margins) from the beginning of the ninth week of the controlled condition phase. On relocation of these seedlings to the shadehouse on 16 August, all plants showed strong signs of recovery within five weeks, as indicated by the substantial amount of new fully-expanded, healthy-coloured foliage which had developed on the plants by this time.

During the second winter following the initial controlled cold treatments (2001), 18 CPs were logged in the shadehouse between 24 May (start of chill unit accumulation) and 28 September (end of chill unit accumulation). Cold Hours commenced and ceased accumulating earlier (06 May and 01 September, respectively), with 178 Cold Hours being recorded in total (***Table 3.18***).

Cold suppressed plant growth rate (***Table 3.20***). By the end of the controlled environment phase (16 August; Julian day 229), trees in the maximum cold treatment (T3) had a significantly lower height than trees of other cold treatments. Paclobutrazol suppressed growth rate on an individual and propagule mean basis. In the analysis of the cold x paclobutrazol treatment interaction it was found that growth rate was significantly suppressed by paclobutrazol in all cold treatments except for the maximum cold treatment, T3.

Following paclobutrazol application, differing growth responses occurred in Experiments 1 (basal stem circumference measured) and 2 (plant height measured). In the case of Experiment 1, paclobutrazol application resulted in a substantially higher growth increase over the duration of the experiment (although not significant at the $p < 0.05$ level), whereas in Experiment 2, paclobutrazol application resulted in a significantly ($p < 0.05$) lower growth increase. This appears as a contradiction of the effect of paclobutrazol in the two trials. It is well documented that the most pronounced morphological effect of triazoles such as paclobutrazol on plants is a reduction in height (Fletcher et al. 2000). This results primarily from the overall reduction in

internode length resulting from lowered endogenous gibberellin levels (Sterret 1988, Hasan and Reid 1995). Although there appears to be no previous reports on triazole application causing an increase in basal stem circumference in eucalypts, it is possible the phenomenon of stem widening in the *E. nitens* plants in Experiment 1 merely resulted from the stems becoming more compact as a result of the progressive shortening of internodes after the paclobutrazol application. In retrospect, it was probably not wise to use basal stem circumference as an indicator of plant growth in a paclobutrazol application experiment, as was the case in Experiment 1.

3.3.2.3 Effect of temperature and paclobutrazol application on umbel production

3.3.2.3.1 Experiment 1

The results of the umbel scores for March 2000 and February 2001are presented in ***Table 3.21***. The first umbels became visible in early December 1999 and scoring was first carried out in March 2000. Following the cold treatments applied during 1999, only three grafts produced umbels. The plants that produced umbels were: one graft of Clone 1 which had been subjected to the T3 cold treatment, and two grafts of Clone 2 subjected to the T4 cold treatment. All grafts were previously treated with paclobutrazol at 0.25 g a.i. per cm b.s.c..Too few trees produced umbels to warrant an analysis of variance for the data. Following the natural (shadehouse) winter conditions during 2000, the same two grafts of Clone 2, as well as one graft of Clone 2 from the previous years T1 (shadehouse control)/ 0.25 g paclobutrazol/ cm b.s.c. treatment produced umbels (February 2001 assessment).

3.3.2.3.2 Experiment 2

All plants were inspected for the presence of umbels in December 2000 and March 2001. No umbels were visible on any of the plants on these assessment dates.

Paclobutrazol treated, precocious *E. nitens* grafted clones in commercial forestry company breeding arboreta in KwaZulu-Natal produce light to moderate crops of flower buds (and ensuing capsule and seed crops) following exposure to winter chill amounts of 50 or more CPs (R. A. W. Gardner , unpublished data). The results of the field trials seem to suggest that 71 or more CPs are necessary to stimulate flowering in 20 % or more of five year old, paclobutrazol-treated *E. nitens* grafts. Pietermaritzburg (29 ^{0} 40 ' S. latitude, 820 m altitude, 20.9 ^{0}C mean

annual temperature) and its immediate vicinity is unsuitable for the commercial planting of *E. nitens* because of the sub-tropical climate of the area. The mild winter conditions are also unfavourable for floral induction in *E. nitens*. In the ICFR, between about 10 and 20 CPs accumulate each winter. Under natural climatic conditions in the nursery (full sun and 80 % full sun), neither paclobutrazol-treated nor untreated potted *E. nitens* grafts produced flower buds over a period of five years (Stanger 1992, R. A. W. Gardner unpublished data).

In Experiments 1 and 2, cold (***Table 3.18***), without paclobutrazol applied, did not stimulate the production of flowers in one or two year old potted grafts or seedlings of *E. nitens* (***Table 3.21***). Following two levels of applied cold (37 CPs and 63 CPs), flowers were produced by paclobutrazol-treated grafts, namely Clone 1 (Barren Mt. provenance) and Clone 2 (Barrington Tops provenance). Similar chilling amounts were not successful in stimulating floral bud production in three other clones, Clone 3 (Tallaganda provenance), Clone 4 (Tallaganda) and Clone 5 (Barrington Tops), in the 1999 or 2000 experiments, whether plants were treated with paclobutrazol or not. These results reinforce existing knowledge that flowering precocity in *E. nitens* is under strong genetic control (Swain and Chiappero 1998, Jones et al. 2000).

Paclobutrazol treated clones which produced flower buds in March 2000 following the controlled 1999 winter chill, again produced buds in the following year (2001) after the low winter chilling amount (16 CPs) logged during 2000. This suggests that paclobutrazol application results in a lowering of the cold requirement for floral induction in trees of *E. nitens*. As paclobutrazol is known to reduce the gibberellin levels in plants, which is positively correlated to the ontogenetic stage of development (Day and Gould 1998, Moncur 1998), it is likely to also reduce the chilling requirement for floral induction in years subsequent to its application. This, in addition to a probable flowering precocity, may explain why one paclobutrazol-treated plant of Clone 2, which received the T1 (shadehouse control) treatment (7 CPs accumulated) during 1999 and produced no flower buds in the year thereafter, produced flower buds following the 2000 winter when a similarly low amount of winter chilling (16 CPs) had again accumulated. On the other hand, a possible promotive effect of stress on floral induction, resulting from root restriction or low soil water status, as reported for potted *E. globulus* seedlings (Hasan and Reid 1995), is unlikely, as plants were repotted into larger containers during early July in 2000 and maintained in a well-watered state for the entire two-year duration of the experiment.

The complete lack of floral response in Clone 3 (Tallaganda provenance) and Clone 5 (Barrington Tops provenance) in Experiment 2 (0.00 g paclobutrazol or 0.25 g paclobutrazol / cm b.s.c.) to any of the cold treatments applied, suggests that floral induction in *E. nitens* is not proportionally dependent on cessation in vegetative growth effected by temperatures below 5 ^{0} C (***Table 3.20***). The similarities between the vernalization response in annual plants, the rest-breaking response to winter chilling in deciduous fruit and the floral induction response in certain subtropical fruit and temperate eucalypts to winter chilling were discussed earlier in paragraph 2.4.2. Chouard (1960) stated that one of the common properties of all vernalization phenomena is that the growth concomitant to floral initiation by vernalization should be "either moderate or very slow", but conditions resulting in a dormant stage do not allow vernalization to occur.

Cold suppressed growth, with maximum cold (T3, 59 CPs, 1366 Cold Hours) recording a significantly ($p < 0.001$) lower growth rate than all other cold treatments (***Table 3.20***). The minimal 5.6 % increase in tree height over four months for the maximum cold treatment (T3) compared to the growth increases recorded by the other three cold treatments, illustrated the significant inhibitory effect of extreme cold on vegetative growth. In the cold x paclobutrazol interaction, paclobutrazol significantly ($p < 0.001$) suppressed height growth in all cold treatments besides the maximum cold treatment (T3). However, none of the treatments or treatment combinations in this experiment appeared to stimulated floral induction.

In Experiment 1, the maximum cold treatment T4 (63 CPs, 938 Cold Hours), which produced flower buds, recorded the lowest growth rate of all cold treatments (40.5 %) (***Table 3.19***). The second coldest treatment T3 (37 CPs, 598 Cold Hours), which also produced flower buds, recorded second lowest growth rate (51.6 %).

In contemplating the lack of floral response to the cold conditions applied in Experiment 2 relative to the promising results achieved in Experiment 1, it should be reiterated that the cold conditions applied in the two experiments were quite different, based on total Cold Hours, although similar on the basis of applied Chilling Portions (***Table 3.18***). Nilsen and Orcutt (1996) drew attention to the significant effect which temperature can have on the quantity and type of gibberellins synthesised in plants. Low temperatures often cause the synthesis of specific gibberellins depending on plant species and temperature. It is therefore possible that certain gibberellins were present at high, inhibitive (of flowering) levels in plants of the maximum cold (T3) treatment in Experiment 2 at a time critical in the floral induction process.

Table 3.19 Effect of paclobutrazol and cold treatment on growth increment of 8-month-old potted *E. nitens* grafts in Experiment 1.

TREATMENT			0.25 g paclobutrazol/ cm b.s.c.	0.00 g paclobutrazol/ cm b.s.c.	Mean
			BC (%) [1]	BC (%) [1]	BC (%) [1]
COLD	CPs	Cold Hours	26 Aug (238 [2])	26 Aug (238 [2])	26 Aug (238 [2])
T1	7	164	57.1	63.0	60.1
T2	25	402	79.9	55.3	67.6
T5	32	502	56.6	49.5	53.1
T3	37	598	69.0	34.3	51.6
T4	63	938	44.2	36.7	40.5
Mean			61.4	47.8	54.6

ANOVA

	F probability	LSD ($p = 0.05$)
Source	26 Aug	26 Aug
COLD	0.005	14.2
Paclobutrazol (P)	0.004	9.0
COLD x P	0.050	20.1
C.V. (%)	37.0	

[1] percentage increase in basal circumference between 06 April (date paclobutrazol applied) and 26 August (end of controlled chilling treatments) (refer ***Figure 2.1***)

[2] Julian date

Nevertheless, it would be speculative to suggest that either the lack of vegetative growth or an excess of any particular gibberellin caused by excessive numbers of cold hours applied in Experiment 2, was the direct and sole cause of the lack of flowering response. Besides genetic differences, it is highly likely that other factors besides cold are also involved in the triggering of floral initiation.

Table 3.20 Effect of paclobutrazol and cold treatment on growth increment of 9-month-old potted *E. nitens* grafts and seedlings in Experiment 2.

TREATMENT			% increase in height [1]								
			0.25 g paclobutrazol / cm b.s.c.				0.00 g paclobutrazol / cm b.s.c.				
COLD	CPs	Cold Hours	Clone 5	Clone 3	Seedling 3	Mean (Paclobutrazol)	Clone 5	Clone 3	Seedling 3	Mean (Control)	Mean (COLD treatment)
T1	16	256	1.39	0.00	1.63	1.01	42.41	2.76	39.36	28.18	14.59
T4	30	752	3.88	1.23	5.48	3.53	23.17	8.63	31.29	21.03	12.28
T2	45	866	5.00	0.00	4.29	3.10	31.26	5.20	21.27	19.25	11.17
T3	59	1366	0.49	7.80	1.59	3.29	12.20	4.20	7.23	7.88	5.59
Mean			2.69	2.26	3.25	2.73	27.26	5.20	24.79	19.08	10.91

ANOVA

Source	*F* probability	LSD ($p = 0.05$)
COLD	< 0.001	3.69
Paclobutrazol (P)	< 0.001	2.61
Propagule (Pg)	< 0.001	3.20
COLD x P	< 0.001	5.22
COLD x Pg	< 0.001	6.39
Paclobutrazol x Pg	< 0.001	4.52
COLD x P x Pg	0.028	9.04
C.V. (%)	11.4	

[1] percentage increase in height between 07 April (paclobutrazol application date) and 16 August 2000 (end of controlled chilling treatments)

Table 3.21 Effect of paclobutrazol * and cold treatment on umbel production in *E. nitens* grafted clones (Clones 1 and 2) **, in Experiment 1.

Cold treatment	Grafts [1]	% laterals [2]	Umbels [3]	Buds [4]	Grafts [1]	% laterals [2]	Umbels [3]	Buds [4]
March 2000 assessment					**February 2001 assessment**			
Clone 1					**Clone 1**			
T1	0/1	0	0	0	0	0	0	0
T2	0/2	0	0	0	0	0	0	0
T3	1/2	11.1	2.0	6.5	0	0	0	0
T4	0/2	0	0	0	0	0	0	0
T5	0/1	0	0	0	0	0	0	0
Clone 2					**Clone 2**			
T1	0/1	0	0	0	1/2	10.0	4	7
T2	0/2	0	0	0	0	0	0	0
T3	0/2	0	0	0	0	0	0	0
T4	2/2	34.7	18.0	4.4	2/2	10.5	2.0	4.0
T5	0/1	0	0	0	0	0	0	0

* only paclobutrazol-treated plants produced umbels

** only Clones 1 and 2 produced umbels. Clones 3 and 4 did not produce umbels for the entire duration of the experiment

[1] Number of grafts out of two with buds

[2] Percentage primary laterals with umbels

[3] Mean number of umbels per graft

[4] Mean number of individual flower buds per umbel

CHAPTER 4

EFFECT OF TEMPERATURE AND PACLOBUTRAZOL ON PHOTOSYNTHETIC EFFICIENCY IN *E. NITENS*

4.1 Introduction

Lichtenthaler (1996) defined plant stress as "any unfavourable condition or substance that affects or blocks a plant's metabolism, growth or development". Salisbury and Ross (1992) suggested that "any change in environmental conditions resulting in plant response being less than the optimum" could be considered stressful.

4.1.1 Cold stress in plants

At low temperatures, all metabolism is reduced, the uptake of water and nutrients is restricted, less biosynthesis takes place, assimilation is reduced and growth stops (Nilsen and Orcutt 1996). The more frequent, the longer, and the colder the periods of low temperatures, the more damaging are the consequences for the plant (Larcher and Neuner 1989).

The first detectable result of low temperatures is the cessation of cytoplasmic streaming, which is a phenomenon directly dependent on energy supplied by respiratory processes and on the availability of high-energy phosphate (Larcher 1995). The term "high-energy" is actually a misnomer, since many bonds of different organic compounds in the cell contain more energy than that found in the bond of phosphate with the rest of the molecule in ATP (adenosine triphosphate). Rather, it is the readily transferable nature of the last phosphate group in ATP which plays a vital role in cytoplasmic streaming (Salisbury and Ross 1992). Impairment of photosynthesis soon follows, which is detectable at an early stage by gas-exchange measurements and by in vivo chlorophyll fluorometry (Larcher 1995).

4.1.2 Detection of cold stress in plants by chlorophyll fluorescence determination

The functioning of photosystem II (PSII) is the most sensitive indicator of environmental stress in plants (Adams et al. 1990, Ball et al. 1994). Most stress factors, even if they do not directly affect the composition of the photosynthetic apparatus or its functions, will ultimately affect the

photosynthetic process. Under non-stressed conditions, about 80 to 90 % of the absorbed light energy will be dissipated from excited chlorophyll *a* (Chl*) via photosynthetic quantum conversion, whereas de-excitation by heat emission (about 5 to 15 %) and the red + far-red chlorophyll fluorescence (0.5 to 2 %) are much lower. Under stress, the photosynthetic quantum conversion declines, and correspondingly heat emission and chlorophyll fluorescence increase considerably (Salisbury and Ross 1992, Lichtenthaler 1996). Sudden or extreme cold can damage PSII, resulting in lower quantum utilization and a lower assimilation yield (photoinhibition) (Salisbury and Ross 1992, Larcher 1995).

Determination of chlorophyll fluorescence has been used successfully as a tool for early indication of cold stress in agricultural crops such as maize (Hetherington et al. 1983) and green peppers (Lurie et al. 1994) and in forestry crops such as conifers (Lindgren and Haellgren 1993, Sutinen et al. 2000) and broadleaves, e.g. eucalypts (Ball et al. 1991).

One of the most widely used fluorescence parameters used in plant stress studies is the ratio F_v /F_m (variable/maximum fluorescence), measured during the dark to light transition in healthy, dark-adapted leaves, F_v is the difference between the fluorescence level when the plastoquinone electron acceptor pool (Q_A) is transiently fully reduced (F_m) and the fluorescence level when Q_A is fully oxidised (F_o). Öquist and Wass (1988) describe the chlorophyll fluorescence response curve in detail, and define F_v /F_m ratio as a quantitative indicator of reductions in the photon yield of O_2 evolution from intact leaves, when exposure to high light levels under favourable temperatures and water status results in photoinhibition. In brief, F_v /F_m ratio is a quantitative measure of the photochemical efficiency of photosystem of PSII (Demmig and Björkman 1987). This ratio of F_v /F_m is typically around 0.83 in healthy leaves (Björkman and Demmig 1987) while ratios below 0.6 indicate transient damage to PSII (photoinhibition) and hence the stress level of the plant.

In most of the early studies of chlorophyll fluorescence, measurements were taken at temperatures of 77 K (temperature of liquid nitrogen) where plant material is deep frozen. The purpose of this was to stop all biochemical but not photochemical activity, so the fluorescence measured was from PSII uninfluenced by further electron transport, i.e. what is now known a F_m (maximum fluorescence). Modern equipment making use of advanced electronics developed over the past two decades, now employs the generation of a very short, bright (saturating) flash of light, and the resultant fluorescence is taken as F_m (Pammenter pers. comm. 2002).

The objective of this investigation was therefore to investigate if floral initiation in *E. nitens* is triggered by cold stress or if the cold period preceding floral development is simply a "non-stress" trigger of growth cessation/ retardation which would be enhanced by paclobutrazol

4.2 Materials and methods

In both 1999 and 2000 potted *E. nitens* experiments (Experiments 1 and 2, respectively), chlorophyll fluorescence measurements were taken from all plants just before the end of each controlled cold treatment, to investigate whether a reduction in photosynthetic efficiency had occurred as a result of the treatment. A "Plant Efficiency Analyser microprocessor" (PEA) (Hansatech Instruments Limited, Norfolk, United Kingdom) was used for the chlorophyll fluorescence measurements (refer ***Figure 4.1***). Sensor illumination in the particular PEA model (Firmware Version DEV004.8, Analyser Version 3.02) is provided by an array of six high intensity light emitting diodes (LEDs) which are focussed onto the leaf surface to provide even illumination over the exposed area of the leaf (4 mm diameter). The LEDs provide red light of a peak wavelength of 650 nm, which is readily absorbed by the chloroplasts of the leaf. The maximum light (illumination) intensity at the leaf surface is approximately 3000 μmol. m^{-2}. s^{-1} (Hansatech 1997).

The timings of the PEA measurements carried out in potted Experiments 1 and 2 are indicated in ***Figures 2.1*** and ***2.3*** respectively. The manufacturer-recommended methodology for measuring leaf chlorophyll fluorescence with the PEA was adhered to. Plants were maintained in a well-hydrated state for the entire duration of the experiment (Chapter 2 "Materials and methods") and so water-deficit was not a confounding factor in the cold stress investigation.

The optimum "dark adaptation period" and "light exposure intensity" for *E. nitens* were established using potted grafts at the ICFR nursery on the 28th June 1999. This information was used to calibrate the PEA unit for all further chlorophyll fluorescence measurements in the *E. nitens* experiments. Prior to the commencement of PEA measurements in Experiment 1, pilot tests were carried out to briefly investigate possible within-plant variation in chlorophyll fluorescence readings and sources of this measurement error. The nett results of this investigation were an overall high consistency of within-plant chlorophyll fluorescence readings, with leaf quality (damaged vs. undamaged) and age (young vs. over-mature) being the main sources of measurement error (R. A. W. Gardner unpublished data). The conclusion was reached that, with careful choice of leaves, chlorophyll fluorescence measurement of only one

leaf per plant (using a PEA) was necessary to obtain a representative reading for each plant. However, to ensure minimal experimental error arose from the latter-mentioned sources, on each measurement occasion in Experiments 1 and 2 PEA readings were taken from two leaves per plant. The criteria applied for the selection of leaves for measurements were as follows: a shoot must be well lit all day, choice of the second, fully-expanded leaf from the shoot terminal, the adaxial leaf surface must be orientated towards the sun, the leaf must be free of damage, defect, excessive waxiness and dirt. These criteria were meticulously adhered. If the readings of two leaves on the same plant differed noticeably, the lower reading of the two was discarded and a further leaf selected and measured (after the required period of dark adaptation) as a check for measurement error. Measurements commenced at 11h30 in the nursery and at 12h30 in the convirons when plants should be fully quenched (Krause and Weis 1991, Salisbury and Ross 1992), as "daylight" hours indoors and outdoors commenced at 06h45 on average.

4.3 Statistical design and analyses

The layouts and statistical analyses for Experiments 1 and 2 were described in sections 2.2.4 ("Trial layout") and 3.2.1 ("Statistical analyses") respectively. Each data point (F_v/F_m reading) in the analyses was obtained by averaging the two F_v/F_m readings of the two leaves on each plant.

4.4 Results and discussion

Using an exposure time of 1 second and 50 % of the maximum light intensity (1500 $\mu mol^{-2}. s^{-1}$), a peak in the ratio F_v/F_m was reached at a "dark adaptation period" of 34 minutes (***Appendix 5***). Using the same exposure time (1 second) and a dark adaptation period of 34 minutes, a peak in F_v/F_m ratio was reached at a "light intensity" reading of 1800 $\mu mol^{-2}. s^{-1}$ (***Appendix 6***). These optimal readings for dark adaptation period and light intensity were therefore used in all further PEA measurements, namely those taken in Experiments 1 and 2.

Figure 4.1 "Plant Efficiency Analyser" (PEA) with leaf-clips in place on *E. nitens* grafts in shadehouse in Experiment 1.

Observations made during the "PEA calibration experiment" reinforced that F_v/F_m ratio is a good method for the early detection of stress in plants. During initial plant calibration measurements, low F_v/F_m readings (below 0.700) were encountered in three randomly positioned plants. As the plants appeared totally normal and healthy, repeat measurements were taken, though F_v/F_m readings remained low. The plants were sidelined from the calibration tests and monitored over the weeks. Only about three weeks after the PEA measurements did the first signs of abnormal colouring appear in the foliage of one of the plants. Soon after this, all three plants began deteriorating, eventually dying about two months after the first unhealthy symptoms appeared. The cause of the mortality appeared to be incompatibility between rootstock and scion in all cases. Therefore, F_v/F_m reading appears to be a means of early detection of graft incompatibility, which may be a useful tool for eliminating "faulty" plants in the planning and pre-planting stages of experiments and orchards.

In Experiment 1, F_v/F_m did not fall below 0.80 for any of the treatments at any time of

measurement (***Table 4.1***). On the 24 August (Julian day 236), the day before the maximum cold treatment (T4, 63 CPs, 938 Cold Hours) (***Table 3.19***) ended (***Figure 2.1***), F_v/F_m ratio for the latter treatment (0.8507) was significantly higher than that of all other cold treatments (7 to 37 CPs, 164 to 598 Cold Hours).

In Experiment 2, on the 16 August (Julian day 229), the day before the maximum cold treatment (T3, 59 CPs, 1366 Cold Hours) (***Table 3.19***) ended (***Figure 2.3***), the photosynthetic efficiency of plants in the latter treatment (F_v/F_m ratio 0.7681) was significantly lower than that of plants in all other cold treatments (***Table 4.2***). Nevertheless, this F_v/F_m figure showed that the cold applied in the T3 treatment was not severe enough to cause photoinhibition. Paclobutrazol alone did not appear to significantly affect chlorophyll fluorescence throughout the experiment, as on all measurement occasions this treatment was non-significant. Paclobutrazol in combination with cold (COLD x P interaction) was significant at the $p < 0.05$ level on the 28th June (during fourth week of experiment), though F_v/F_m of all treatments remained high (above 0.82).

As stress is considered to be a major factor in flowering response (Bernier et al. 1981, Thomas 1993), the hypothesis that cold-induced stress promotes flowering in *E. nitens* was investigated. Analysis of the chlorophyll fluorescence data as a stress indicator from Experiment 1 (***Table 4.1***) suggests that this is not the case. F_v/F_m remained high (> 0.80) for all treatments and treatment combinations throughout the experiment. Even on an individual plant basis, the F_v/F_m levels of grafts which produced flower buds in the March 2000 assessment in Experiment 1 (***Table 3.21***) , did not at any time show F_v/F_m levels lower than 0.80. On the contrary, the data suggested that the warm 1999 winter conditions in the shadehouse (T1, control) led to a reduction in photosynthetic efficiency, and hence were less favourable for growth and non-conducive to floral induction in *E. nitens* (paragraph 3.3.2.3 and ***Table 3.21***). Furthermore, the extreme cold conditions of the T3 treatment in Experiment 2 (***Table 3.19***) significantly reduced F_v/F_m (0.77) relative to that of all other cold treatments (***Table 4.2***), but did not induce flowering (Section 3.3.2.3 and ***Table 3.21***). This suggests that, firstly, a threshold may exist regarding the cumulative number of hours below $5\ ^0$ C (Cold Hours) which the particular genotypes of *E. nitens* can tolerate before the conditions are perceived as stressful, and secondly, that cold-induced stress does not play a positive role in the induction of flowering in the species. This is substantiated by the non-significant effect of the interaction between cold treatment and propagule (genotype) on the F_v/F_m ratio (***Table 4.2***). Furthermore, based on the chlorophyll fluorescence data, paclobutrazol in combination with any other factor did not appear to significantly affect plant stress in *E. nitens*. This is surprising, as triazoles such as

paclobutrazol significantly enhance chilling resistance in a number of crops, seemingly by way of increasing the abscisic acid (ABA) levels in plants (Fletcher et al. 2000).

Although the results suggest that cold stress does not play a promotive role in floral induction in *E. nitens*, further research is needed to confirm this. The plants in the experiments represented a narrow range of *E. nitens* genetic material from a summer rainfall climatic zone in Australia (New South Wales). *Eucalyptus nitens* genotypes from further south in Victoria, Australia, are adapted to markedly different sets of environmental conditions (e.g. winter rainfall distribution) to those further north, and therefore may react differently to winter cold.

Also, further work is needed to establish whether stress caused by the range of environmental factors historically implicated in the promotion of flowering in other crops, for example drought and radiation, can play a positive role in the induction of flowering in *E. nitens*. As yet, there is no conclusive evidence of any other environmental factor besides cold playing a role in the stimulation of floral induction in *E. nitens*.

Table 4.1 Effect of paclobutrazol and cold treatment on growth increment and leaf chlorophyll fluorescence of 8-month-old potted *E. nitens* grafts in Experiment 1.

TREATMENT			0.25 g paclobutrazol / cm b.s.c.					0.00 g paclobutrazol / cm b.s.c.					Mean				
			BC (%) [1]	Fv/Fm ratio				BC (%) [1]	Fv/Fm ratio				BC (%) [1]	Fv/Fm ratio			
COLD	CPs	Cold Hours	26 Aug (238 [2])	29Jun (180)	12Jul (193)	29Jul (210)	24Aug (236)	26 Aug (238 [2])	29Jun (180)	12Jul (193)	29Jul (210)	24Aug (236)	26 Aug (238 [2])	29Jun (180)	12Jul (193)	29Jul (210)	24Aug (236)
T1	7	164	57.1	0.8138	0.8065	0.8122	0.8122	63.0	0.8256	0.8231	0.844	0.8356	60.1	0.8197	0.8148	0.8281	0.8239
T2	25	402	79.9	0.8211	0.8374	0.8394	0.8394	55.3	0.8339	0.8374	0.8365	0.8365	67.6	0.8275	0.8374	0.8379	0.8379
T5	32	502	56.6	0.8428	0.839	0.8302	0.8312	49.5	0.8305	0.8393	0.8505	0.8412	53.1	0.8366	0.8391	0.8404	0.8362
T3	37	598	69.0	0.8336	0.8354	0.8416	0.8405	34.3	0.8368	0.8329	0.8320	0.8320	51.6	0.8352	0.8341	0.8368	0.8363
T4	63	938	44.2	0.8396	0.8349	0.8514	0.8494	36.7	0.8309	0.8304	0.8502	0.8520	40.5	0.8353	0.8326	0.8508	0.8507
Mean			61.4	0.8302	0.8306	0.8350	0.8346	47.8	0.8315	0.8326	0.8426	0.8395	54.6	0.8309	0.8316	0.8388	0.8370

ANOVA

	F probability					LSD ($p = 0.05$)				
Source	26 Aug	29Jun	12Jul	29Jul	24Aug	26 Aug	29Jun	12Jul	29Jul	24Aug
COLD	0.005	0.01	< 0.001	0.004	< 0.001	14.2	0.009	0.01	0.0111	0.0083
Paclobutrazol (P)	0.004	0.638	0.533	0.033	0.066	9.0	-	-	0.0071	-
COLD x P	0.050	0.017	0.235	0.002	0.003	20.1	0.0128	-	0.0158	0.0117
C.V. (%)	37.0	0.9	0.8	0.5	0.7					

[1] percentage increase in basal circumference between 06 April (date paclobutrazol applied) and 26 August (end of controlled chilling treatments) (refer ***Figure 2.1***)

[2] Julian date

Table 4.2 Effect of paclobutrazol and cold treatment on leaf chlorophyll fluorescence of 9-month-old potted *E. nitens* grafts and seedlings in Experiment 2.

			Fv/Fm ratio											
TREATMENT			0.25 g paclobutrazol / cm b.s.c.				0.00 g paclobutrazol / cm b.s.c.				Mean			
COLD	CPs	Cold Hours	01 Jun (153 [2])	28Jun (180)	19Jul (201)	16Aug (230)	01 Jun (153 [2])	28Jun (180)	19Jul (201)	16Aug (230)	01 Jun (153 [2])	28Jun (180)	19Jul (201)	16Aug (230)
T1	16	256	0.8122	0.8432	0.8403	0.8335	0.8064	0.8253	0.8331	0.8264	0.8093	0.8343	0.8367	0.8300
T4	30	752	0.7926	0.8304	0.8174	0.8105	0.8221	0.8412	0.8293	0.8120	0.8073	0.8358	0.8234	0.8112
T2	45	866	0.7978	0.8361	0.8258	0.8232	0.8146	0.8320	0.8303	0.8334	0.8062	0.8341	0.8281	0.8283
T3	59	1366	0.8172	0.8228	0.8124	0.7586	0.8029	0.8330	0.8287	0.7776	0.8101	0.8279	0.8206	0.7681
Mean			0.8049	0.8331	0.8240	0.8064	0.8115	0.8329	0.8304	0.8124	0.8082	0.8330	0.8272	0.8094

ANOVA

	F probability				LSD ($p = 0.05$)			
Source	01Jun	28Jun	19Jul	16Aug	01Jun	28Jun	19Jul	16Aug
COLD	0.977	0.409	0.113	< 0.001	-	-	-	0.0256
Paclobutrazol (P)	0.344	0.942	0.199	0.513	-	-	-	-
Propagule (Pg)	0.239	0.084	0.067	0.139	-	-	-	-
COLD x P	0.104	0.019	0.357	0.758	-	0.0143	-	-
COLD x Pg	0.098	0.577	0.185	0.218	-	-	-	-
Paclobutrazol x Pg	0.034	0.467	0.569	0.926	0.0239	-	-	-
COLD x P x Pg	0.650	0.524	0.471	0.633	-	-	-	-
C.V. (%)	0.4	0.1	0.4	1.1				

[2] Julian date

CHAPTER 5

EFFECT OF DROUGHT STRESS ON FLORAL BUD PRODUCTION IN *E. NITENS*

5.1 Introduction

Drought stress has been known to predispose certain *Eucalyptus* species to flower under certain sets of field conditions (Moncur 1998). There is some evidence that soil moisture stress, in conjunction with paclobutrazol and cold, can play a complimentary role in the stimulation of flowering in temperate eucalypt species (Hasan and Reid 1995). In the latter experiment with 30-month old potted *E. globulus* seedlings, water stress was not purposely applied, but rather unavoidably and repetitively during the few winter months prior to the appearance of flower buds in spring due to the restrictive size of the plant containers (Hasan and Reid 1995). Eighteen month old potted *E. nitens* grafts were subjected to various levels of soil moisture stress, but the latter treatment failed to induce flower buds in the plants (Moncur and Boland 2000).

Floral initiation occurs in *E. nitens* during early spring following a cold winter (Moncur and Hasan 1994). Overall, the available evidence seems to suggest that the induction of flowering in temperate eucalypt species such as *E. nitens* and *E. globulus* is plant stress related.

Data from the *E. nitens* field trials provided an opportunity to tentatively investigate the relationship between soil moisture level and floral induction in *E. nitens*, the objective being to establish whether low soil water status may have played a stimulative role regarding floral induction in *E. nitens*. If this was the case, then the effect of drought would have confounded the results of the investigations regarding the effect of winter chilling and paclobutrazol on floral induction in trees in the field trials.

The timing of floral initiation in *E. nitens* in South Africa has not yet been established. Moncur et al. (1994*a*) found that floral initiation in mature *E. nitens* trees at Tallaganda State Forest (New South Wales) occurred during early spring (mid-August to early September), 6 - 8 weeks before flower buds were first visible to the naked eye. In South Africa, on average, the flower buds (umbels) of early flowering genotypes of *E. nitens* become first visible to the naked eye

during early November (Jones and van Staden 2001, R. A. W. Gardner unpublished data). If it is assumed that the time interval between floral initiation and buds first visible in South Africa is similar to that of Tallaganda State Forest (6 - 8 weeks), then environmental conditions after the end of September would not be implicated in the floral induction process in South Africa. As discussed earlier in paragraph 1.3.2.1, no local knowledge exists about the timing of inductive event/s responsible for influencing resting buds to later give rise to reproductive shoots in *E. nitens*.

In this investigation into the possible effect of drought on floral induction in *E. nitens*, a hypothesis was tested that severity of drought during the winter months, i.e. during the period 01st May to 30th September, was proportionally related to spring flower bud (umbel) production.

Initially the intention was to carry out this investigation on three seasons data, but it was later decided to limit the study to only one season (2000/2001) for the following reasons:

1. Of the rainfall data accessed for the years1997 - 2000, a highly reliable daily rainfall data set was only available for 2000.
2. Best flower bud production occurred in the trials during summer 2000/2001, when trees were oldest (four and a half years) and nearer to full reproductive maturity

5.2 Materials and methods

5.2.1 Soil moisture modelling

The original research proposal did not intend to study the effects of drought stress on flowering and hence no soil-moisture readings were taken during the flowering study.

However, certain soil information (depth and texture of upper (A) and lower (B) soil horizons) and daily rainfall data, the minimum data needed to run the soil water budgeting model WETNES (refer Section 2.1.1), were available as follows:

1. Soil samples were taken during detailed inspections of soil profiles in the trials during April 1997. Soil-textures were determined from these samples by the ICFR analytical laboratories.
2. Sets of daily rainfall figures from four suitable weather stations near to the four trials were accessed from three institutions, namely the Computing Centre for Water Research (CCWR, University of Natal), the Institute for Soil Climate and Water (ISCW,

Agricultural Research Council) and North East Cape Forests (NECF). In two cases, daily rainfall for actual trial sites (Gowan Brae and Mossbank) were approximated by applying correction factors (derived from altitude and spatial co-ordinate differences between donor station and actual trial site) supplied by CCWR to donor station figures. Main criteria for choice of suitable donor stations were nearness of donor station to actual trial site, and reliability and completeness of data sets.

Details of the rainfall data donor stations are presented in ***Table 5.1***.

Table 5.1 Details of the rainfall data donor stations representing the four trial sites.

	DONOR STATION DETAILS						
TRIAL SITE	**Station name**	**Institution ***	**Latitude (S)**	**Longitude (E)**	**Altitude (m)**	**Distance from trial site (km)**	**Applied correction factor (rainfall)**
Gowan Brae	Cedara College	ISCW	29 ° 32 '	30 ° 17 '	1100	12.2	0.9561
Mossbank	Bulwer	ISCW	29 ° 49 '	29 ° 46 '	1480	6.2	1.1530
Blyfstaanhoogte	Long Tom Potato Research	ISCW	25 ° 08 '	30 ° 37 '	2040	4.5	1.0000
Tentkop	Elands Heights	NECF	30 ° 49 '	28 ° 16 '	1820	12.0	1.0000

* ISCW = Institute for Soil Climate and Water; NECF = North East Cape Forests, Eastern Cape.

Outputs from the model included daily estimations of "potential evapotranspiration" (PET), "actual evapotranspiration" (AET) and soil water content. WETNES was run for the period 01 January 1998 to 31 December 2000. For the Blyfstaanhoogte trial, WETNES was only run for the period 01 January 1998 to 30 September 2000 due to a dearth of reliable rainfall data available for the last three months of 2000.

The ratio of actual evapotranspiration to potential evapotranspiration (AET/PET ratio) represents the balance between water supply (available soil moisture dependant on rainfall and storage in the soil profile) and demand (evapotranspiration), and is therefore a useful index to rate growing conditions at any given time based on available soil water supply. Dunin and Aston (1984) and Kunz (1995) both found the AET/PET ratio a useful indicator of stressful growing conditions in natural forest and plantation forestry situations respectively.

Furthermore, following the results of a case study involving evapotranspiration patterns in a wild stand of eucalypts, Dunin and Aston (1984) drew attention to the significance of the point at which 40 % of the range between soil wilting point (SWP) and field capacity (SSB) is reached. They concluded that as a soil profile dries out and this particular point is reached, AET/PET ratio suddenly drops away from 1. Therefore, a decline in AET/PET ratio is directly proportional to a deterioration in growing conditions due to drought stress (Dunin and Aston 1984). Later workers such as Landsberg and Gower (1997) also highlight the significance of this threshold. In certain instances, in further discussion in this report, the point at which 40 % of the range between SWP and SSB is reached will be referred to as the "SP40 threshold".

The AET/PET ratio was calculated for each day during winter (01 May to September), and the number of days on which the AET/PET ratio was < 1.0 (i.e. days on which soil moisture level equalled or was less than the SP40 threshold) expressed as a percentage of the total number of days for the same period. "Winter drought severity" was therefore represented by the percentage days between 01 May and 30 September when soil moisture levels equalled or descended below the SP40 threshold.

5.2.2 Umbel production

During April 2001, each individual tree within each of the field trials was assessed for the presence of umbels and allocated a flowering score according to the methodology described in paragraph 2.1.5.2.

5.2.3 Statistical analyses

5.2.3.1 General

The relationship between "winter drought severity" and umbel production was investigated using the method of multiple regression analysis in the statistical package Genstat® for Windows™, Release 4.2 (McConway et al. 1999).

Two relationships were explored, between "winter drought severity" ("**DRY_2000**") and the percentage trees producing umbels (%_trees), and between "**DRY_2000**" and umbel yield (umbel score) per tree transformed to the natural logarithmic value ("**LFLW_2001**") (the reasons for the transformation of values was explained in paragraph 3.2.1.1.4). In the

regressions, "**COLD**" (winter chilling) was included as an explanatory variable to investigate a possible interactive effect of drought and cold on floral induction. The significant relationship between winter chilling and floral bud production was highlighted in Chapter 3. As explained in paragraph 3.2.1.1.4, the variate "**COLD**" consisted of chill units for the 2000 winter calculated from winter temperature data using six different *chill model* x *chill period* combinations ("**COLD_1**" to "**COLD_6**").

The list of all response and explanatory variables included in the regressions is presented in ***Table 5.2.*** To investigate the degree of relatedness between the different explanatory variables, separate correlation matrices were produced for seedlings and grafts using data for the percentage of trees producing umbels at five years (%_trees). The variate "%_trees" consisted of replicate (plot) means in this analysis.

5.2.3.1.1 Relationship between winter drought severity and percentage trees producing umbels

In the analyses, propagule type ("PROPAGULE") was not included as a separate explanatory variable, but rather separate regressions were carried out for seedlings and grafts (clones) due to the vast differences in growth rate and precocity of these two propagule types. As mentioned earlier, seedlings and grafts of the same treatment number were a maximum of 50 % related.

In the case of either seedlings or grafts, the response variate "%_trees" consisted of pooled family data. This pooling was done to reduce within-trial variances due to non-consistent umbel bearing patterns across the different families in each propagule type. Replicates within each trial were kept separate.

5.2.3.1.2 Relationship between winter drought severity and umbel crop per tree

Multiple linear regressions were carried out with LFLW_2001 as response variable, and winter drought severity (DRY_2000), *chill model* x *chill period* combination (COLD_*x*), propagule type (PROPAGULE) and paclobutrazol application (PBZ) as explanatory variables. PROPAGULE was included as an explanatory variable to investigate the effect of plant type on the outcomes of the regressions. Families were pooled in these analyses.

Table 5.2 Description of all response and explanatory variables used in the multiple linear regression analyses in the drought study.

Variate assessed	Abbreviation used in text	Description of variate
Response Variable (floral response)		
Umbel crop load	LFLW_2001	Umbel crop score per tree transformed to natural logarithmic value.
% trees with umbels	%_trees	Number of trees with one or more umbels in each plot (rep), expressed as a percentage of the total number of live trees in the plot
Explanatory Variable (winter drought severity and chill model x chill period combination)		
Winter drought severity	DRY_2000	Number of days between 01 May - 30 September 2000 on which SP40 threshold was reached, expressed as a percentage of the total number of days for this period
Dynamic Model	COLD_1	Number of CPs [1] calculated for the period 01 Apr - 30 Sep 2000
Dynamic Model	COLD_2	Number of CPs [1] calculated for the period 01 May - 30 Sep 2000
Utah Chill Model	COLD_3	Number of UCU's [2] calculated for the period 01 Apr - 30 Sep 2000
Utah Chill Model	COLD_4	Number of UCU's [2] calculated for the period 01 May - 30 Sep 2000
Daily Positive Utah Chill Unit model	COLD_5	Number of DPCU's [3] calculated for the period 01 Apr - 30 Sep 2000
Daily Positive Utah Chill Unit model	COLD_6	Number of DPCU's [3] calculated for the period 01 May - 30 Sep 2000
Explanatory Variable (plant material and plant growth regulator treatment)		
Propagule type	PROPAGULE	1 = graft; 2 = seedling (refer section 2.1.1)
E. nitens family	FAMILY	Families 32091, 32097, 34838 and 37255 (refer *Table 2.1*)
Plant growth regulator (PGR)	PBZ	0 = no PGR (control); 1 = 0.25 g paclobutrazol per cm b.s.c. applied as a soil drench in April 1998

[1] CP = Chilling Portion, the chill unit measured by the Dynamic Model (Fishman et al. 1987*a* and *b*).

[2] UCU = Utah Chill Unit, the chill unit measured by the Utah Chill Unit Model (Seeley 1996).

[3] DPCU = Daily Positive Chill Unit, the chill unit measured by the Daily Positive Utah Chill Unit Model (Linsley-Noakes 1995).

In the case of either relationship explored, a string of separate regressions were carried out with "DRY_2000" in combination with each of the six *chill model* x *chill period* combinations.

5.3 Results

5.3.1 Winter drought severity

Actual daily rainfall and computed daily soil moisture levels for the four sites for the period 01

January 1998 to 31 December 2000 (01 January 1998 to 30 September 2000 in the case of Blyfstaanhoogte) are presented in ***Figure 5.1***. The summer rainfall peaks and extended periods when soil moisture levels were low in winter at each site (***Figure 5.1***) clearly demonstrate the summer rainfall climatic nature of the region.

Computed daily soil moisture levels and AET/PET ratios, together with field capacity (SSB), wilting point (SWP) and SP40 threshold, for the four sites during the period 01 January 1998 to 31 December 2000, are presented in graphical form in ***Figure 5.2***.

The percentage days during winter (between 01 May - 30 September) with AET/PET ratio below 1.0 at the four sites in 1998, 1999 and 2000 are presented in ***Table 5.3***.

Table 5.3 Percentage days during winter (between 01 May - 30 September) with AET/PET ratio below 1.0 at the four sites in 1998, 1999 and 2000.

	YEAR		
TRIAL SITE	**1998**	**1999**	**2000**
Gowan Brae	100.0	100.0	100.0
Mossbank	93.5	82.4	42.5
Blyfstaanhoogte	100.0	75.8	89.5
Tentkop	66.7	90.2	48.4

5.3.2 Relationship between winter drought severity and percentage trees producing umbels

In the initial assessment of the data, separate correlation matrices were produced for seedlings and grafts as described in paragraph 5.2.3.1. The main purpose was to investigate the degree of relatedness of the different explanatory variables. As the resultant correlation coefficients (r) for the explanatory variables were identical in the case of either propagule type, only those for the grafts are presented (***Table 5.4***). The correlation coefficients (r values) ranged from -0.030 for DRY_2000 and COLD_5 (weakest correlation), to -0.253 for DRY_2000 and COLD_1 (strongest correlation). Although the Dynamic Model based combinations COLD_1 (April - September temperature data) and COLD_2 (May - September data) gave the best, albeit negative, correlations with DRY_2000 (- 0.253 and - 0.244 respectively), these two explanatory

variables were nevertheless weakly correlated. The correlations between the various *chill model* x *chill period* combinations were discussed in paragraph 3.3.1.5.

Table 5.4 Correlation matrix for all explanatory variables investigated in the relationship between the percentage grafts producing umbels at 5 years (%_trees) and *chill model* x *chill period* combination (COLD_*x*), paclobutrazol application (PBZ) and severity of winter drought (DRY_2000).

COLD_1	1.000								
COLD_2	0.999	1.000							
COLD_3	0.941	0.956	1.000						
COLD_4	0.874	0.894	0.984	1.000					
COLD_5	0.882	0.903	0.990	0.996	1.000				
COLD_6	0.809	0.835	0.959	0.992	0.989	1.000			
PBZ	0.000	0.000	0.000	0.000	0.000	0.000	1.000		
DRY_2000	- 0.253	- 0.244	- 0.103	0.048	- 0.030	0.087	0.000	1.000	
%_trees	0.667	0.672	0.682	0.668	0.664	0.640	0.527	- 0.018	1.000
	COLD_1	COLD_2	COLD_3	COLD_4	COLD_5	COLD_6	PBZ	DRY_2000	%_trees

Note: correlation $df = 22$ (n - 2); $p < 0.05 = 0.404$; $p < 0.01 = 0.515$.

The summarised results of the string of multiple linear regressions carried out for percentage trees producing umbels (%_trees) in the *seedlings* are presented in ***Table 5.5***. The regression of flowering (%_trees) on drought severity (DRY_2000) and paclobutrazol treatment (PBZ) showed that the explanatory variables significantly ($p < 0.01$) influenced the percentage reproductive trees. However, only 31.9 % of the total variance was accounted for. Subsequent *t*-tests revealed that PBZ played a highly significant ($p < 0.01$) role in the regression but that cumulative drought conditions (DRY_2000) had no effect.

In the regressions where the different winter chill combinations (COLD_1 to COLD_6) were included as explanatory variables together with drought (DRY_2000) and paclobutrazol application (PBZ), COLD_4 (Utah Chill Model with units calculated for May to September) gave the best result (not all the results for the different regressions are presented). Although a total of 72.8 % of the variance was accounted for by the explanatory variables DRY_2000, COLD_4 and PBZ, subsequent *t*-tests showed that COLD_4 and PBZ treatment were highly significant ($p < 0.001$) but winter drought (DRY_2000) was non-significant (***Table 5.5***).

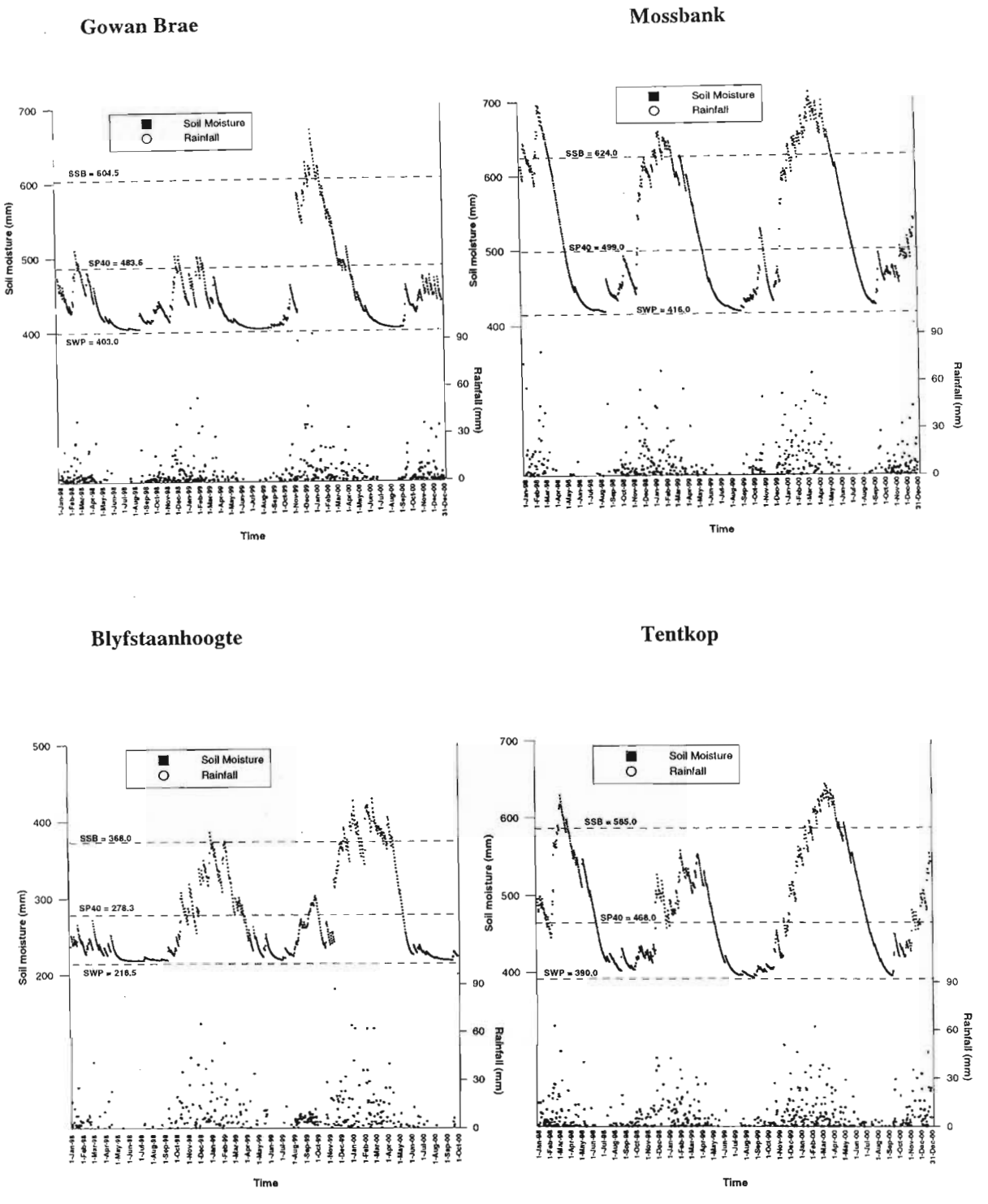


Figure 5.1 Daily rainfall (mm) and soil moisture (mm) levels at the four trial sites for the period January 1998 to December 2000.

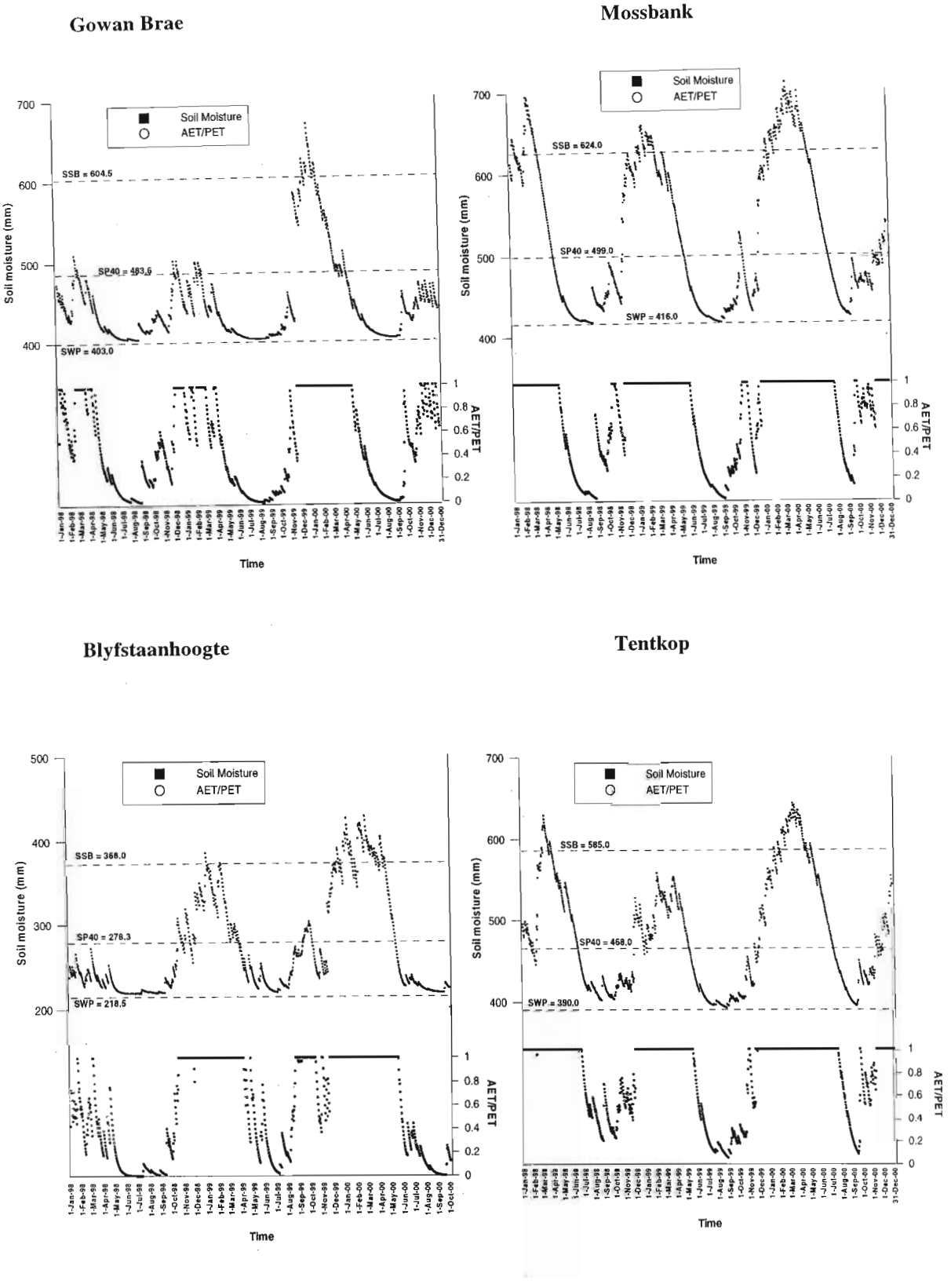


Figure 5.2 Daily soil moisture (mm) levels and AET/PET ratios at the four trial sites for the period January 1998 to December 2000.

Table 5.5 Summary of the analysis of variance carried out for the step-wise multiple linear regression on percentage *seedlings* which produced umbels at five years (%_trees).

	COLD_4 PBZ		PBZ DRY_2000		COLD_4 PBZ DRY_2000	
SOURCE	**d.f.**	**m.s**	**d.f.**	**m.s.**	**d.f.**	**m.s**
Regression	2	2575.9 ***	2	1379.7 **	3	1857.96 ***
Residual	21	102.4	21	216.3	20	86.41
Total	23	317.5	23	317.5	23	317.48
R^2		67.7		31.9		72.8
SED		10.1		14.7		9.3
Estimate of parameters:						
Constant		- 56.2		- 14.6		- 66.9
COLD_4		0.03928		n/a		0.03856
PBZ		19.26		19.26		19.26
DRY_2000		n/a		0.188		0.1677
Parameter t-values:	21		21		20	
Constant		- 4.62 ***		- 1.15		- 5.49 ***
COLD_4		5.35 ***		n/a		5.71 ***
PBZ		4.66 ***		3.21 **		5.08 ***
DRY_2000		n/a		1.57		2.21

Levels of significance: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

The summarised results of the string of regressions carried out for the percentage trees producing umbels (%_trees) in the *grafts* are presented in ***Table 5.6***. The regression of flowering (%_trees) on drought severity (DRY_2000) and paclobutrazol application (PBZ) was significant ($p < 0.05$) though only 20 % of the total variance was accounted for. Subsequent *t*-tests showed once again (as in the case of the seedlings) that PBZ played a highly significant ($p < 0.01$) role in the regression but drought (DRY_2000) had an insignificant effect.

In the regressions where the different winter chill combinations (COLD_1 to COLD_6) were included as explanatory variables together with drought (DRY_2000) and paclobutrazol application (PBZ), COLD_1 (Dynamic Model with units calculated for April to September) gave the best result (not all the results for the different regressions are presented). Although a total of 70.9 % of the variance was accounted for by the explanatory variables DRY_2000,

COLD_1 and PBZ, subsequent t-tests showed that COLD_1 and PBZ treatment were highly significant ($p < 0.001$) but severity of winter drought (DRY_2000) was non-significant (***Table 5.6***).

5.3.3 Relationship between winter drought severity and umbel crop per tree

The summarised results of the multiple linear regressions carried out for umbel crop per tree (seedlings and grafts pooled) (LFLW_2001) are presented in ***Table 5.7***. The regression of umbel crop per tree (LFLW_2001) on winter drought severity (DRY_2000), propagule type (PROPAGULE) and paclobutrazol application (PBZ) was highly significant ($p < 0.001$) yet only 38 % of the total variance was accounted for. Subsequent *t*-tests showed that PBZ was highly significant ($p < 0.001$) and PROPAGULE significant ($p < 0.05$) in the regression, but drought was had no effect.

In the regressions where the different winter chill combinations (COLD_1 to COLD_6) were tried together with drought (DRY_2000), propagule type (PROPAGULE) and paclobutrazol application (PBZ) as explanatory variables, COLD_1 (Dynamic Model with units calculated for April to September) gave the best result. Although 64.3 % of the total variance was accounted for in this combination of COLD_1 with DRY_2000, COLD_1, PROPAGULE and PBZ as explanatory variables, subsequent *t*-tests showed that PBZ, PROPAGULE and COLD_1 were all highly significant ($p < 0.01$), but that drought did not have a significant effect on flowering (***Table 5.7***), as was the case in all previous regressions in this section.

5.4 Discussion

The above regressions of umbel production in five year old *E. nitens* on severity of winter drought (DRY_2000), whether separately or in combination with the explanatory variables PROPAGULE (seedling or graft), PBZ (0.00 g paclobutrazol or 0.25 g paclobutrazol per cm b.s.c.) and COLD (different *chill model* x *chill period* combinations), revealed that drought played an insignificant role in floral induction.

Based on the total number of days between 01 May - 30 September ("winter"), Gowan Brae was the most drought-stressed site during this period in all three years assessed (1998,1999 and 2000) (***Figure 5.2*** and ***Table 5.3***). On each day during "winter" in these years, AET/PET ratio at Gowan Brae was below 1.0.

The percentage "drought" days (days when AET/PET ratio < 1.0) during winter 2000 and floral bud production data for April 2001 provided an ideal opportunity to investigate for any relationship between drought stress and floral response, as during 2000 a wide variation in percentage "drought stress" days occurred across the sites (***Table 5.3***) and trees were at their oldest physiological age at the final floral crop assessment in April 2001.

Table 5.6 Summary of the analysis of variance carried out for the step-wise multiple linear regression on percentage *grafts* which produced umbels at five years (%_trees).

	COLD_1 PBZ		PBZ DRY_2000		COLD_1 PBZ DRY_2000	
SOURCE	**d.f.**	**m.s.**	**d.f.**	**m.s.**	**d.f.**	**m.s.**
Regression	2	7051.9 ***	2	2715.1 *	3	4859.7 ***
Residual	21	258.3	21	671.3	20	247.4
Total	23	849	23	849	23	849
R^2		69.6		20.9		70.9
SED		16.1		25.9		15.7
Estimate of parameters:						
Constant		- 108.6		- 3.8		- 127.8
COLD_1		1.261		n/a		1.338
PBZ		30.07		30.1		30.07
DRY_2000		n/a		- 0.02		0.184
Parameter t-values:	21		21		20	
Constant		- 5.26 ***		- 0.17		- 5.22 ***
COLD_1		5.80 ***		n/a		6.08 ***
PBZ		4.58 ***		2.84 **		4.68 ***
DRY_2000		n/a		- 0.1		0.181

Levels of significance: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Table 5.7 Summary of the analysis of variance carried out for the step-wise multiple linear regression on transformed umbel score at five years after planting (LFLW_2001).

	COLD_1 PROPAGULE PBZ		DRY_2000 PROPAGULE PBZ		COLD_1 DRY_2000 PROPAGULE PBZ	
SOURCE	**d.f.**	**m.s.**	**d.f.**	**m.s.**	**d.f.**	**m.s.**
Regression	3	0.4543 ***	3	0.2915 ***	4	0.3422 **
Residual	28	0.0223	28	0.0397	27	0.0229
Total	31	0.0641	31	0.0641	31	0.0641
R^2		65.3		38.0		64.3
SED		0.149		0.199		0.151
Estimate of parameters:						
Constant		- 0.526		0.206		- 0.583
COLD_1		0.00828		n/a		0.00852
DRY_2000		n/a		- 0.00075		0.00055
PROPAGULE		- 0.1885		- 0.1885		- 0.1885
PBZ		0.2690		0.2690		0.2690
Parameter t-values:	28		28		27	
Constant		- 2.86 **		- 1.13		- 2.66 *
COLD_1		4.74 ***		n/a		4.65 **
DRY_2000		n/a		- 0.53		0.50
PROPAGULE		- 3.57 **		- 2.68 *		- 3.58 **
PBZ		5.10 ***		3.82 ***		5.05 ***

Levels of significance: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

During 2000, Gowan Brae recorded the highest percentage "drought stress" days (100 %) followed by Blyfstaanhoogte (89.5 %), Tentkop (48.4 %) and Mossbank (42.5 %) (***Table 5.3***). Based on the percentage of non-paclobutrazol-treated (0.00 g paclobutrazol per cm b.s.c.) grafts (rather than the physiologically younger seedlings) which produced umbels at five years (April 2001 assessment), Gowan Brae recorded significantly ($p < 0.05$) less trees with umbels (11.1 %) than Blyfstaanhoogte (64.4 %) (***Figure 3.2***). Regarding the percentage paclobutrazol-treated (0.25 g paclobutrazol per cm b.s.c.) grafts which produced umbels at five years (treatment G_C1), Gowan Brae again recorded a significantly lower percentage (26.5 %) than Blyfstaanhoogte (77.1 %). If drought stress is an important environmental factor promoting floral induction in *E. nitens*, then one would expect the percentage trees producing umbels at

Gowan Brae in April 2001 to have been at least equal to that at Blyfstaanhoogte, as Gowan Brae was as drought stressed as Blyfstaanhoogte during the winter months in 2000. However, this was not the case.

It is well-documented that triazoles such as paclobutrazol increase drought-resistance in a wide range of plants including both herbaceous and woody perennials. The mechanisms of increased drought resistance include the reduction of transpiration rate, elevation of antioxidant activity and a transient increase in ABA levels (Fletcher et al. 2000). Assuming that paclobutrazol does alleviate drought stress in *E. nitens*, it is likely that the control (0.00 g paclobutrazol/ cm b.s.c.) grafts would have been more drought-stressed than the paclobutrazol-treated (0.25 g paclobutrazol/ cm b.s.c.) grafts at the different sites on days when AET/PET ratio dropped below 1.0 during 2000. Furthermore, if drought stress does indeed promote floral induction in *E. nitens*, this should have been reflected by a greater flowering response in the non-treated (0.00 g paclobutrazol/ cm b.s.c.) trees than in the paclobutrazol-treated (0.25 g paclobutrazol/ cm b.s.c.) trees. However, this was not the case at any of the sites (***Figure 3.2***).

The trends evident in the data suggest that winter drought-stress does not significantly promote umbel production in *E. nitens*. However, certain downfalls of the WETNES model exist and will be briefly discussed. The model WETNES uses characteristics of the soil profile such as soil-depth, organic carbon content and soil-texture to calculate outputs such as soil wilting point (SWP), soil moisture level (SSL) and soil water storage capacity (SSM) (Roberts 1994*a* and *b*). It does not take into account the possibility of further sources of groundwater in the weathering or non-weathered rock layers below the soil profile.

Root systems of mature eucalypts, however, have been known to penetrate to great depths in their search for water, though the extent of this depends on factors such as tree species (Turnbull et al. 1993), geology and the degree of weathering and temperature of the underlying parent material (Stone and Kalisz 1990).

In a soil water balance study carried out under fast growing *Eucalyptus* plantation conditions on deep sandy soils in Brazil, Soares and Almeida (2001) found that during the dry season, from April to August, an upward water flux of about 1 mm day $^{-1}$ by capillary movement from below the root zone occurred. However, this kept the soil water storage in that zone to only about 15 % of the storage capacity which was only just sufficient to prevent complete stomatal closure in the nine year old trees.

In eucalypts most of the total root mass consists of fine roots, and the bulk of the root system is located in the upper section of the soil profile (Florence 1996).

The four *E. nitens* experimental sites were located at high altitude, and characterised by the frequent occurrence of mist in summer. As trees were also only five years old at the time of final assessment in April 2001, it is reasonable to suggest that a large proportion of the fine root mass would have been present in the upper (A- and B-) soil horizons (Soil Classification Working Group 1991) at this age. Therefore, it is likely that the WETNES model was suited to this particular task.

Soil moisture levels at all four sites during 2000 were high till around mid-May (***Figure 5.1***) and were at their lowest levels by early to mid- August. Moncur (1998) reported that a period of low water status in autumn/early winter induced field-grown mature trees of *E. viminalis* to flower in France, and that summer moisture stress (in association with high light intensity) stimulated floral initiation in *E. diversicolor* in Western Australia. Such low soil moisture conditions, however, did not occur at any of the local experimental sites at similar times of the year.

CHAPTER 6

GENERAL DISCUSSION AND CONCLUSIONS

The main goals pursued in this project were an improved understanding of "effective" cold for floral induction in *E. nitens* and an exploration into the feasibility of using controlled cold as a manipulative tool for floral induction in *E. nitens* in South Africa.

Although the mechanisms controlling floral induction in eucalypts are not well understood (Moncur 1998), quite understandably there has been little urgency to do so over the years in early and prolific flowerers such as the sub-tropical *E. grandis*. The latter is the most important commercial eucalypt species in South Africa, and genetic improvement and commercial seed production programmes for this species are highly advanced (Eldridge et al. 1993, Pierce and Verryn 2000). However, in South Africa, sites with altitude > 1200 m and/or MAT < 16.5 ^{0} C are unsuited to *E. grandis*, and in these areas cold-tolerant species such as *E. nitens* are the preferred species, and thus play an important role in South African short rotation forestry (Herbert 2000, Swain and Gardner 2003). The flowering controls in subtropical eucalypt species and provenances may be quite different to the controls in temperate eucalypts. Pryor (1976) reported no indication of photoperiodic regulation of flowering in *E. tereticornis*, latitudinally speaking the most widely occurring eucalypt species (8 ^{0} S. to 38 ^{0} S. latitude (Boland et al. 1992)). A flowering response of certain eucalypts to daylength has been described by Paton (1978); however, this author stated that they are relative insensitive long-day plants.

Some progress has recently been made in identifying the flowering controls in *E. nitens*. Although the species may be insensitive to daylength, a period of cold appears pre-requisite for floral induction (Moncur and Hasan 1994). However, neither has the actual amount of cold required been determined, nor is it known if cold is the sole trigger of floral induction.

By investigating the relationship between winter chill units and floral bud production data, this research has demonstrated four important points:
Firstly, it is possible to calculate the potential **chilling requirement** of *E. nitens* by using either the Utah Model, the Dynamic Model or the Daily Positive Utah Chill Unit Model. Secondly, flower induction does not appear to result from one single chill event, but from a cumulative

chilling process. Thirdly, by using the Dynamic Model to calculate cumulative winter chilling, floral bud productivity of *E. nitens* grafts can be fairly accurately predicted for summer rainfall sites in South Africa, if the level of paclobutrazol application is also included as an explanatory factor. Fourthly, under "natural" environmental conditions, between 80 and 95 CPs (Chilling Portions) appear necessary to optimally satisfy the chilling requirement for floral bud initiation in *E. nitens* northern provenances.

This chilling requirement indicated for *E. nitens* floral induction (80 to 95 CPs) is high compared to the amount required to effect dormancy release in most deciduous fruit tree species. The chill requirements for the latter range from about 12 CPs for low-chill peaches to about 70 CPs for high-chill apples and sweet cherries (Erez 2000). Although the amount of Chilling Portions necessary for floral induction in olive (*Olea europea* L.), a temperate broadleaf evergreen tree crop, is uncertain, Hackett and Hartmann (1967) calculated that 800-1000 hours below 8 ^{0} C were required for floral induction in this species, although the precise amount depends on specific cultivar.

Planting a trial at an even colder location than Tentkop, the coldest site used in this study, as well as comparing the chill accumulation data of this site with data from sites where *E. nitens* flowers regularly, might result in a more accurate determination of the chilling requirement of the species. Preliminary investigations relating air temperature data for 1998 to 2000 of Chilean *E. nitens* seed orchards to actual floral bud break showed that the amount of winter chill accumulated at these sites between May and September ranged between 66 and 88 CPs for a "warm" site, and between 90 and 95 CPs and 98 and 105 CPs for two "cold" sites. Flowering at the warm site rated "average to good" and at the two cold sites "consistently good" (R.A.W. Gardner, unpublished data). This coincides very well with the modelled cold requirement for *E. nitens* of 80 to 95 CPs.

Moncur and Hasan (1994) suggested that the flowering response of *E. nitens* to successive periods of cold may result from a gradual destruction of a flowering inhibitor. The results confirm this suggestion. Best flowering performance of *E. nitens* was recorded at Blyfstaanhoogte, which accumulated the highest number of chill units in 1999 and 2000. However, judged by MAT, Tentkop is a colder site than Blyfstaanhoogte. If the destruction of this flowering inhibitor occurs only under certain circumstances and these are similar to those for chilling accumulation, the idea of a gradual destruction of a flowering inhibitor could explain why the highest number of trees with umbels (***Table 3.6***) as well as the highest umbel

crop per tree was achieved in Blyfstaanhoogte in 2000 and 2001. Certain - or all - of the juvenile characteristics of the seedling rootstocks imposed an inability to flower onto the grafted tree. However, even these trees were not fully mature yet, otherwise 100 % flowering should have been achieved in 2001 after the exposure to 88 CPs at Blyfstaanhoogte.

On the other hand, the lack of chilling in 1988 (65 CPs) seems to have been the reason behind the poor flowering in 1999 in the grafted trees at Blyfstaanhoogte. In 2001, less than 3/4 of the grafted trees produced umbels, possibly indicating that the chilling amount of 89.1 CPs did not entirely meet the chill requirement of the *E. nitens* trees assessed, and that the actual requirement may be closer to the upper end of the calculated range of 80 to 95 CPs.

Alternatively, these grafted trees were still too juvenile to flower, even though the rootstocks were six years old. Most likely, the transfer of a juvenile signal from the seedling rootstock onto the adult scion, as described a few decades ago (Janick and Moore 1975, Couvillon et al. 1984), elicited this response.

Paclobutrazol, possibly due to its action as a gibberellin biosynthesis inhibitor, has been found to reduce plant height in a variety of woody plants (Sterrett 1985). The application of 0.25 g paclobutrazol per cm b.s.c. significantly reduced tree height. Tree height was similarly significantly reduced by cold (***Table 3.5***). As paclobutrazol treatment has been found to be obligatory for early flower induction in *E. nitens*, it seems that both plant growth regulator (paclobutrazol) treatment and exposure of trees to low temperatures act similarly in inducing flowering by way of slowing down growth. The mechanism by which these two treatments reduce growth appears to be different, as paclobutrazol cannot replace the exposure to cold and the two treatments appear to have a cumulative effect on flowering (***Table 3.6***). Paclobutrazol has not been found to result in a plant stress but rather in an optimisation of plant metabolism (Fletcher et al. 2000), while exposure to cold might reduce plant growth as a typical stress response. In the controlled environment experiments, high numbers of hours below 5 ^{0} C did not stimulate floral induction in *E. nitens,* but did lead to cold stress (based on chlorophyll fluorescence measurement) of plants. Although a few of the grafts in Experiment 1 did produce umbels following the higher winter chilling treatments, the low overall number of plants in the trials (due to space limitations) and numbers of plants which flowered prevented any meaningful regression analyses between winter chill units and floral response being undertaken. Nevertheless, the results suggested that floral bud production in *E. nitens* promoted by winter chilling treatment is not a plant stress response.

Eucalyptus nitens is known to be an inherent shy flowerer following the onset of reproductive maturity (Moncur et al. 1994*a*, Moncur et al. 1994*b*). A range of cultural techniques including pruning and training techniques, girdling, grafting and paclobutrazol application (Moncur and Boland 2000) and/or nitrogen fertilizer application (Williams et al. 2003) can increase flower and seed production in *E. nitens*. Regardless of any of these treatments though, a period of winter cold is prerequisite for floral induction in the species (Moncur and Hasan 1994). The results of this project not only confirm the findings of Moncur and Hasan (1994), but also indicate a specific chilling requirement for floral induction in *E. nitens*. As yet, there is no conclusive evidence linking floral bud production in *E. nitens* to drought conditions, or indeed to any other pronounced stress condition. The results of this research indicate that the most important environmental factor to be considered in the choice of sites for *E. nitens* seed orchards in South Africa is "amount of accumulated winter chill". Other essential growing requirements of the species, such as adequate available soil moisture, nutrition and soil drainage, should also be met.

Eucalyptus nitens is an inherent shy flowering species (Boland et al. 1980, Eldridge et al. 1993). Whilst "optimal" siting of *E. nitens* orchards for adequate winter chilling may substantially improve the consistency and magnitude of resulting flower and seed crops in South Africa, the fact that flowering traits are strongly related to genotype in *E. nitens* (Moncur 1998, Swain and Chiappero 1998, Williams et al. 1999, Jones 2000) is an inescapable fact.

Recommended future research

In the summer rainfall region of South Africa, sites providing the apparent desirable level of winter chilling (80 to 95 CPs) are generally limited to remote, mountainous country, and are not easily accessible. Thus, the location of *E. nitens* orchards at suitable sites in this region poses practical problems with orchard management and research functions. Possible solutions for improving flowering in local *E. nitens* seed orchards include the use of evaporative cooling in winter to increase the level of accumulated winter chill, and the use of precocious, low-chill rootstocks. Alternatively, it may be necessary to locate seed orchards of this species further south in the winter rainfall region, an area characterized by high chill accumulation. To test these concepts, the following research is recommended:

Evaporative cooling: Allan (1976) demonstrated how overhead sprinkling could reduce shoot and foliage temperatures in macadamias (*Macadamia integrifolia*) by up to 13 ^{0} C on warm

winter days in Pietermaritzburg. At Cedara and Tweedie in the Natal Midlands (1160 m and 1100 m, respectively) where the major timber companies have established their research centres, preliminary analysis of temperature data (ISCW- supplied data) indicated that accumulated winter chilling ranges between 42 and 64 CPs, based on the warm 2001 winter and exceptionally cold 1997 winter. Reductions in foliage temperatures in *E. nitens* on warm winter days via evaporative cooling, similar to that reported for macadamia (Allan 1976), may substantially improve winter chilling and elevate the number of chill units to above the apparent necessary threshold (80 to 95 CPs). This method of satisfying the chilling requirement of *E. nitens* for floral bud production appears to be the most practical, and perhaps economical method of the three suggested above, and therefore it would be worthwhile focussing concerted research effort in this direction in future.

Low-chill rootstocks: The issue of influence of rootstock on scion phenotypic expression was not dealt with in preceding chapters, nor as a matter of interest, in most, if not all, previous publications on floral induction in *E. nitens*. The transmission of rootstock phenotypical characteristics to scion following grafting in woody crops is a well-known phenomenon (Janick and Moore 1975, Hartmann et al. 1990). Transferred characteristics may include chilling requirement and precocity (Couvillon et al. 1984, Du Plooy and Van Huysteen 2000, Erez 2000). Although *E. nitens* does not propagate easily vegetatively (Eldridge et al. 1993), it would be worthwhile investigating whether the precocity and chilling requirement of specific clones can be transmitted to tardy flowerers via rootstock to scion transmission. Similarly, the feasibility of applying dwarfing rootstocks to control scion growth should also be investigated.

Location of production orchards in winter rainfall region: High chill areas of the South Western Cape may provide the degree of winter chilling necessary for *E. nitens* flower production. This region is located further south between 34 ^{0} and 35 ^{0} S latitude, and winter chill unit figures in excess of 80 CPs are typical to many areas (Linsley-Noakes 1995). Seed orchard sites could probably be located in areas far more accessible and congenial to essential orchard activities where chilling requirement for floral bud initiation is still met. One possible complication to this concept pertains to the topic of natural outcrossing. In the summer rainfall region of South Africa, *E. nitens* is predominantly winter flowering (April to October with peak in July) (Jones and Van Staden 2001), although flowering can extend to early December in certain genotypes at cold, high altitude sites (R.A.W. Gardner, unpublished data). There is still uncertainty as to what the major insect pollination vectors are. Although the success of controlled pollinations is unlikely to be affected, winter rainfall conditions may negatively affect

natural outcrossing and subsequent seedset (Sanhueza pers. comm. 2002). Furthermore, the costs of managing *E. nitens* production seed orchards in the South Western Cape could be prohibitive, as orchards would have to be managed from a distance (KwaZulu-Natal research centre) as well as locally (SW Cape contractor). However, it is anticipated that the bulk of these would be once-off costs mainly relating to seed orchard establishment activities and personnel (contractor) training. It is therefore recommended that one or two mini-orchards, consisting of a sample range of "South African"*E. nitens* material, be established at suitable sites in the South Western Cape to investigate the flowering performances of the trees under the winter rainfall climatic conditions.

Improved accuracy of prediction of chilling models: A better accuracy to predict *E. nitens* floral bud production using chilling models is necessary before commercial *E. nitens* orchards can be "optimally" sited. On the one hand, this could be achieved by reducing within-family variation in an experimental orchard, for example by using the seed of one select family to produce the seedlings and/or grafts for the trials. Considerable within-family variation in precocity exists within the South African *E. nitens* breeding population (Swain 2001, Jones 2002). Although as much as 72 % of the variation in floral bud production of mature grafts was explained by accumulated winter chilling amount and level of paclobutrazol application, the fact that the regressions depended on only four unique points with respect to the variable "COLD_*x*" may have inflated the amount of variation accounted for. To verify these results, and to enable more confident prediction of floral productivity according to site conditions, a further trial series involving a large number of sites (and associated chill unit data points) should be undertaken.

Floral productivity spatial mapping: Accurate determination of the "floral chill requirement" of a limited range of "South African" *E. nitens* genotypes will pave the way for preliminary mapping of sites according to floral productivity. Prerequisite is the mapping of South African sites according to accumulated chill units on a scale much finer than that presented by Schulze (1997). It may be feasible to initially carry out this mapping on a limited, experimental basis, i.e. for target areas in South Africa. It should be possible to determine mean number of Chilling Portions (CPs) for a specific site using Geographic Information System (GIS) technology, by extrapolating CPs from mapped data such as altitude, aspect and relief, if the resolution of spatial data for the specific areas is fine enough (Schulze pers. comm. 2003).

Manipulation of timing of anthesis: Although success was achieved in manipulating flower buds at an early stage (time) with paclobutrazol/temperature/grafting, buds could not be

stimulated to appear any other time of the year besides spring as occurs naturally in the field (Moncur pers. comm. 2003). As it would be highly beneficial to breeders if the timing of anthesis (flowering window) could be manipulated, it would be challenging to investigate this aspect under controlled conditions. To facilitate such research, prior knowledge of the low temperature requirements for floral induction in certain clones would be prerequisite.

Optimal temperature criteria: Currently, little is known about the optimal temperature criteria for floral induction in *E. nitens*. The chill models evaluated in this study have complex constructions, but in general, premium chilling values are assigned to temperatures within a relatively narrow range, i.e. between about 1.5 and 10 ^{0} C (Fishman et al. 1987*a* and *b*, Linsley-Noakes 1995, Seeley 1996). The results of the investigations suggest a commonality between the temperature regimes promoting accumulation of chill units and rest completion in deciduous fruit trees, and temperatures which promote floral induction in *E. nitens*. However, subtle differences undoubtedly exist between the biological processes, and the method of calculating chill unit accumulation in *E. nitens* should be tailored to suit the particular crop. For example, in olive (*Olea europea* L.), a temperate broadleaf evergreen tree crop requiring winter chilling for floral induction (Hartmann and Whisler 1975), it was established that the temperature criteria for this process differ somewhat from those for optimum chilling accumulation in deciduous fruit. Optimum chilling accumulation in olives occurs at temperatures fluctuating in a diurnal sine wave pattern from a minimum of 2 ^{0} C to a maximum of 15 ^{0} C on either side of the cardinal temperature (12.5 ^{0} C) (Hartmann and Opitz 1980). Similarly, there is a need to establish the optimal temperature criteria for floral induction in *E. nitens*. An accurate, environmentally friendly floral induction management strategy can only be assembled for *E. nitens* for South Africa once this information is available.

Plant growth hormones: Further research into the metabolic changes brought about by paclobutrazol application as well as cold treatment should be undertaken to clarify whether a certain hormonal homeostasis, brought about either by "natural ageing" or by paclobutrazol, is the prerequisite for flowering in *E. nitens*, whilst a certain amount of accumulated winter chilling is the actual trigger.

Drought/ flowering relations: The results of the research carried out in this thesis suggest that plant stress, resulting from the application of cold or drought conditions does not stimulate floral bud production in *E. nitens*. However, as was discussed earlier in Chapter 5, the investigation of the relationship between drought and floral bud production in *E. nitens* only

dealt with one aspect of drought, i.e. cumulative "drought stress days" between May and September. Therefore, the results do not conclusive dispel the possibility that drought, under certain circumstances, may promote floral bud production in *E. nitens*. Jackson and Sweet (1972) stated that "... a period of rest brought about by some form of stress, either by low temperatures or low levels of available water, is considered a prerequisite for floral induction in many woody perennials". A flowering response from drought conditions may depend on a number of separate events and/or the timing and interval of such events. Thus, it would be worthwhile testing a variety of such treatments under controlled conditions. Combinations worth testing would include the intentional application of drought conditions during autumn/early winter at varying intensity, duration and degree of suddenness.

LITERATURE REFERENCES

Adams III, W.W., B. Demmig-Adams, K. Winter. and U. Schreiber. 1990. The ratio of variable to maximum chlorophyll fluorescence from photosystem II measured in leaves at ambient temperature and at 77K, as an indicator of the photon yield of photosynthesis. Planta.180:166-174.

Allan, P. 1976. Evaporative cooling and the alleviation of plant water stress: a proposed experiment on macadamia trees. Hort. Report No.1. Dept. Hort. Sci., Univ. Natal, Pietermaritzburg. pp 3-6.

Allan, P. 1999. Measuring winter chilling in areas with mild winters. Deciduous Fruit Grower/ Sagte Vrugte Boer. 49(10):1-8.

Allan, P. and M.J.I. Burnett. 1995. Peach production in an area with low winter chilling. J. S. Afr. Soc. Hort. Sci. 5(1):15-18.

Ashton, D.H. 1975. Studies of flowering behaviour in *Eucalyptus regnans* F. Muell. Australian Journal of Botany. 23:399-411.

Aukerman, M.J. and R.M. Amasino. 1998. Floral Induction and Florigen. Cell. 93:491-494.

Ball, M.C., V.S. Hodges and G.P. Laughlin. 1991. Cold-induced photoinhibition limits regeneration of snow gum at treeline. Funct. Ecol. 5:663-668.

Ball, M.C., J.A. Butterworth, J.S. Roden, R. Christian, and J.J.G. Egerton. 1994. Applications of chlorophyll fluorescence to forest ecology. Aus. J. Plant Physiol. 22:311-319.

Bernier, G., J. Kinet and R.M. Sachs. 1981. The Physiology of Flowering (Vol I). CRC Press, Florida. pp 76-124.

Bernier, G. 1988. The control of floral evocation and floral morphogenesis. Ann. Review Plant Physiol. 39:175-219.

Björkman, O. and B. Demmig. 1987. Photon yield of O_2 evolution and chlorophyll fluorescence characteristics at 77K among vascular plants of diverse origins. Planta. 170:489-504.

Boland, D.J., M.I.H. Brooker and D.A. Kleinig. 1980. *Eucalyptus* seed. CSIRO, Canberra. pp 90-91.

Boland, D.J., M.I.H. Brooker, G.M.Chippendale, N. Hall, B.P.M. Hyland, R.D. Johnston, D.A. Kleinig, and J.D. Turner. 1992. Forest trees of Australia. CSIRO Publishing, Victoria, Australia. pp 450-573.

Borsari, P. 2000. Personal communication. Onset Computer Corporation, Minnesota, USA.

Brooker, M.I.H. 2000. A new classification of the genus *Eucalyptus* L'Her. (Myrtaceae). Australian Systematic Botany. 13:79-148.

Brooker, M.I.H. and D.A. Kleinig.1983. Field Guide to the Eucalypts. South-eastern Australia (Vol. 1). Inkata Press, Melbourne. pp 201-203.

Campbell, R.C. 1981. Statistics for Biologists. 2nd ed. Cambridge University Press, London. 385 pp.

Carlson, C., T. Swain and S. Soko. 2000. Preliminary investigation into nutritional differences between shy and early flowering families of *Eucalyptus nitens*. ICFR Bulletin Series No. 4/2000. ICFR, Pietermaritzburg. p 2.

Chambers, P.G.S., B.M. Potts and P.A. Tilyard.1997. The genetic control of flowering precocity in *Eucalyptus globulus* ssp. *globulus*. Silv. Genet. 46(4):207-213.

Chippendale, G.M.1988. *Eucalyptus, Angophora (Myrtaceae), Flora of Australia 19.* Aus. Govt. Printing Services, Canberra. p 350.

Chouard, P. 1960. Vernalization and it's relation to dormancy. Ann. Rev. Plant Physiol. 11:191-238.

Couvillon, G.A. and A. Erez. 1985. Effect of level and duration of high temperatures on rest in

the peach. J. Amer. Soc. Hort. Sci. 110:579-581.

Couvillon, G.A., N. Finardi, M. Magnani and C. Freire. 1984. Rootstock influences chilling requirement of "Rome Beauty" apple in Brazil. HortScience. 19(2):255-256.

Darrow, W.K. 1996. Species trials of cold-tolerant eucalypts in the summer-rainfall zone of South Africa: Results at six years of age. ICFR Bulletin Series No. 9/96. ICFR, Pietermaritzburg. p 36.

Davenport, T.L. 1990. Citrus flowering. Hort. Reviews. 12:349-408.

Davis, T.D. and E.A. Curry. 1991. Chemical regulation of vegetative growth. Crit. Rev. Plant. Sci. 10:151-188.

Day, J.S. and K.S. Gould. 1998. Adventitious root initiation, plasticity, and response to plant growth regulator treatments of seedling, juvenile, and adult *Eleocarpus hookerianus* plants. N. Z. J. Bot. 36(3): 97-114.

Demmig, B. and O. Björkman. 1987. Comparison of the effect of excessive light on chlorophyll fluorescence (77K) and photon yield of O_2 evolution in leaves of higher plants. Planta.171:171-184.

Dunin, F.X. and A.R. Aston.1984. The development and proving of models of large scale evapotranspiration: an Australian study. Agric. Water Management. 8:305-323.

Du Plooy, P. and P. van Huysteen. 2000. Effect of BP1, BP3 and Quince A rootstocks, at three planting densities, on precocity and fruit quality of "Forellle" pear (*Pyrus communis* L.). S. Afr. J. Plant Soil, 17(2):57-59.

Eldridge, K.G., J. Davidson, C. Harwood and G. van Wyk.1993. Eucalypt Domestication and Breeding. Oxford University Press, New York. pp 114-122.

England, N.F., J.E. Espejo, P.M. Rojas and A.R. Griffin. 1992. Effects of paclobutrazol application on height growth and bud production of 3 and 5 year old *E. nitens* after one year. *In* Proc. IUFRO/ AFOCEL symposium " Mass production technology for genetically improved fast

growing forest tree species 14-18 September 1992", Bordeaux, France. pp 211-217.

Erez, A., S. Fishman, G.C. Linsley-Noakes and P. Allan. 1990. The dynamic model for rest completion in peach buds. Act. Hortic. 276:165-175.

Erez, A. 2000. Temperate Fruit Crops in Warm Climates. Kluwer Academic Publishers, Netherlands.

Evans, L.T.1971. Flower induction and the florigen concept. Ann. Rev. Plant Physiol. 22:365-394.

Fishman, S., A. Erez and G.A. Couvillon. 1987*a*. The temperature dependence of dormancy breaking in plants: mathematical analysis of a two-step model involving a cooperative transition. J. Theoretical Biol. 124:473-483.

Fishman, S., A. Erez and G.A. Couvillon. 1987*b*. The temperature dependence of dormancy breaking in plants: computer simulation of processes studied under controlled temperatures. J. Theoretical Biol. 126:309-321.

Fletcher, R.A., A. Gilley, N. Sankhla and T. D. Davis. 2000. Triazoles as plant growth regulators and stress protectants. Hort. Reviews. 24:55-138.

Florence, R.G. 1996. Ecology and Silviculture of Eucalypt Forests. CSIRO Publishing, Victoria, Australia.

Gardner, R.A.W. 2001*a*. Site-species interaction studies with cold-tolerant eucalypts at high altitudes in South Africa. *In* Proceedings of the IUFRO symposium " Developing the Eucalypt of the Future", Valdivia, Chile, 10-15 Sept. 2001. Proceedings on CD-ROM only.

Gardner, R.A.W. 2001*b*. Report on the IUFRO International Symposium "Developing the Eucalypt of the Future". ICFR Bulletin Series No. 21/2001. ICFR, Pietermaritzburg.

Gea, L.D., R. McConnochie and N.M.G. Borralho. 1997. Genetic parameters for growth and wood density traits in *Eucalyptus nitens* in New Zealand. N. Z. J. For. Sci. 27(3):237-238.

Genstat® for Windows™. 5th ed. Release 4.2. Copyright 2000. Lawes Agricultural Trust, Rothamstead Experimental Station, UK.

George, A.P. and A. Erez. 2000. Stone fruit species under warm subtropical and tropical climates. Temperate fruit crops in warm climates. Ed. A. Erez. Kluwer Academic Publishers, Netherlands. pp 231-265.

Gepts, P. 1987. Characterizing plant phenology: growth and development scales. Plant growth modelling for resource management. Vol. II. Quantifying plant processes. Eds. K. Wisiol and J.D. Hesketh. CRC Press, Inc., Florida. pp 4-6.

Gomez, A.K. and A.A. Gomez. 1984. Statistical Procedures for Agricultural Research. 2nd ed. John Wiley & Sons, New York.

Graebe, J.E. 1987. Gibberellin biosynthesis and control. Ann. Rev. Plant Physiol. 38:419-465.

Griffin, A.R., P. Whiteman, T. Rudge, I.P. Burgess and M.W. Moncur.1993. Effects of paclobutrazol on flower-bud production and vegetative growth in two species of *Eucalyptus*. Can. J. For. Res. 23:640-647.

Halgryn, P.J., K.I. Theron and N.C. Cook. 2001. Genotypic response to chilling period of apple buds from two Western Cape localities. S. Afr. J. Plant and Soil. 18(1):21-27.

Hansatech. 1997. Operating Instructions for Plant Efficiency Analyser Advanced Fluorescence Analysis. Issue No. 1.0. Hansatech Instruments Ltd., Norfolk, United Kingdom.

Hartmann, H.T. and I. Porlingis. 1957. Effect of different amounts of winter chilling on fruitfulness of several olive varieties. Bot. Gazette. 119:102-104.

Hartmann, H.T. and J.E. Whisler. 1975. Flower production in olive as influenced by various chilling temperature regimes. J. Amer. Soc. Hort. Sci. 100(6):670-674.

Hartmann, H.T. and K.W. Opitz.1980. Olive production in California. Leaflet 2474, Univ. of Calif. Div. of Agric. Sci., Davis.

Hartmann, H.T., D.E. Kester and F.T. Davies.1990. Plant propagation: principles and practices. 5th ed. Prentice-Hall, New Jersey, USA.

Hasan O. and J.B. Reid. 1995. Reduction in generation time in *Eucalyptus globulus*. Plant Growth Regul. 17(1):53-60.

Herbert, M.A. 2000. Site requirements and Species Matching: Eucalypts and Wattles. SAIF Forestry Handbook. Ed. D.L. Owen. Vol.1. SAIF, Pietermaritzburg. pp 85-94.

Hetherington, S., R.M. Smillie, A.K. Hardacre and H.A. Eagles.1983. Using chlorophyll fluorescence *in vivo* to measure the chilling tolerance of different populations of maize. Aus. J. Plant Physiol. 10:247-256.

Hodgson, L.M. 1975. Some aspects of reproductive behaviour in *Eucalyptus grandis* (Hill) Maiden. D.Sc. thesis, University of Pretoria, South Africa. pp 24-28.

Thesis Ref.

INFOR, 2002. Requerimientos ecológicos Eucalyptus nitens. http://www.infor.cl/webinfor/pw-sistemagestion/pt_02/Txt/ReqEcol/RENIT.htm

Jackson, D.I. and G.B. Sweet. 1972. Flower initiation in temperate woody plants. A review based largely on the literature of conifers and deciduous fruit trees. Hortic. Abstracts. 42:9-24.

Janick, J. and J.N. Moore. 1975. Advances in fruit breeding. Purdue Univ. Press, Ind., USA. pp 3-37.

Jones, W.R., 2002. Breeding systems of some cold tolerant *Eucalyptus* species. MSc thesis, University of Natal, South Africa. pp 49-75.

Jones, W.R., C.R.E. Clarke and J. van Staden. 2000. Understanding the breeding system of cold tolerant *Eucalyptus* species and its impact on seed production. *In* Proc. of the IUFRO symposium "Forest Genetics for the Next Millenium", Durban, 8-13 Oct. 2000. ICFR, Pietermaritzburg. pp 146-150.

Jones, W.R. and J. van Staden. 2001. The reproductive sequences of four important commercial *Eucalyptus* species in South Africa. *In* Proc. of the IUFRO symposium " Developing the

Eucalypt of the Future", Valdivia, Chile, 10-15 Sept. 2001. Proceedings on CD-ROM only.

Jordan, G.J., B.M. Potts and R.J.E. Wiltshire. 1999. Strong, independent, quantitative genetic control of the timing of vegetative phase change and first flowering in *Eucalyptus globulus* ssp. *globulus* (Tasmanian Blue Gum). 83:179-187.

Krajewski, A.J. and E. Rabe. 1995. Citrus flowering: A critical evaluation. J. Hortic. Sci.. 70(1): 357-374.

Krause, G.H. and E. Weis. 1991. Chlorophyll fluorescence and photosynthesis: The Basics. Ann. Rev. Plant Physiol. and Plant Mol. Biol. 42:313-349.

Kunz, R.P. 1985. Site Classification and GIS. ICFR Annual Research Report. ICFR, Pietermaritzburg. pp 317-332.

Kunz, R. and R. Pallett. 2000. A stratification system based on climate and lithology for locating commercial forestry permanent sample plots. ICFR Bulletin Series No. 01/2000. ICFR, Pietermaritzburg. pp. 3-6.

Landsberg, J.J. and S.T. Gower. 1997. Application of physiological ecology to forest management. Academic Press, San Diego. pp. 89-124.

Lane, P.W. and R.W. Payne. 1996. Genstat® for Windows™, an introductory course. Lawes Agricultural Trust, Rothamstead Experimental Station, UK.

Larcher, W. 1995. Physiological Plant Ecology. Springer: New York. pp 294-295.

Larcher, W. and G. Neuner. 1989. Cold-induced sudden reversible lowering of *in vivo* chlorophyll fluorescence after saturating light pulses. A sensitive marker for chilling susceptibility. Plant Physiol. 89:740-742.

Lawrence, B.M. 2001. Progress in essential oils. Perfumer and Flavorist. 26:2-39.

Lichtenthaler, H.K. 1996. Vegetation stress: and introduction to the stress concept in plants. Plant Physiol. 148:4-14.

Lindgren, K. and J.E. Haellgren.1993. Cold acclimation of *Pinus contorta* and *Pinus sylvestris* assessed by chlorophyll fluorescence. Tree Physiol. 13(1):97-106.

Linsley-Noakes, G.C. 1995. Temperature as a factor in nectarine production in the Western Cape. PhD thesis, University of Natal, South Africa.

Linsley-Noakes, G.C. and P. Allan. 1994. Comparison of two models for the prediction of rest completion in peaches. Scientia Hortic. 59:107-113.

Linsley-Noakes, G.C., P. Allan and G. Matthee. 1994. Modification of rest completion prediction models for improved accuracy in South African stone fruit orchards. J. S. Afr. Soc. Hort. Sci. 4(1):13-15.

Linsley-Noakes, G.C., M. Louw and P. Allan. 1995. Estimating daily positive Utah chill units from maximum and minimum temperatures. J. S. Afr. Soc. Hort. Sci. 5(1):19-24.

Linvill, D.E. 1990. Calculating chilling hours and chill units from daily maximum and minimum temperature observations. HortScience. 25(1):14-16.

Lurie S., R. Ronen and S. Meier. 1994. Determining chilling injury induction in green peppers using non-destructive, pulse amplitude modulated fluorometry. HortScience. 119:59-62.

Martin, C., L. Ferguson and V.S. Polito. 1994. Flowering, Pollination, Fruiting, Alternate Bearing, and Abcission. Olive Production Manual. Eds. L. Ferguson, G.S. Sibbett and G.C. Martin. Univ. Calif. Press, USA. pp 51-52.

Matthee, G.W. 1982. Koue-eenhede in die wintereëngebied van Suid-Afrika. Tegniese Mededeling No. 177. Depart. Landbou en Visserye, Pretoria.

McConnochie, R. M. 2001. Personal communication. NZFRI Ltd., Rotorua, New Zealand.

McConway, K.J., M.C. Jones and P.C. Taylor. 1999. Statistical modelling using Genstat®. Oxford University Press Inc., New York. pp 65-292.

McKinley, R.B., C.J.A. Shelbourne, C.B. Low, B. Penellum and M.O. Kimberley. 2002.

Comparison of wood properties of young Eucalyptus nitens, E. globulus and E. maidenii in Northland, New Zealand. N. Z. J. For. Sci. In press.

Mead, R., R.N. Curnow and A.M. Hasted. 1992. Statistical methods in agriculture and experimental biology. 2nd ed. Chapman & Hall, London.

Meilan, R. 1997. Floral induction in woody angiosperms. New Forests. 14:179-202.

Menzel, C.M.1983. The control of floral initiation in lychee: a review. Scient. Hortic. 21:201-215.

Moncur, M.W. 1992. Effect of low temperature on floral induction of *Eucalyptus lansdowneana* F. Muell. & J. Brown subsp. *lansdowneana.* Aus. J. Bot. 40:157-167.

Moncur, M.W. 1998. Personal communication. CSIRO Forestry and Forest Products, Canberra, Australia.

Moncur, M.W. 1998. Hastening Seed Production: A Tool for Increasing the Rate of Genetic Improvement in Eucalypt Species. *In* Tree Improvement: Applied Research and Technology Transfer. Ed. S. Puri. Science Publishers, Inc., New Hampshire, USA. pp 81-93.

Moncur, M.W. 2003. Personal communication. CSIRO Forestry and Forest Products, Canberra, Australia.

Moncur, M.W. and O. Hasan. 1994. Floral induction in *Eucalyptus nitens* (Deane and Maiden) Maiden. Tree Physiol. 14:1303-1312.

Moncur, M.W., F.C. Hand and N.G. Ramsden. 1994*a*. Environmental and cultural effects on flowering and seed production of plantation grown *Eucalyptus nitens*. Division of Forestry. CSIRO, Canberra.

Moncur, M.W., G.F. Rasmussen and O. Hasan.1994*b*. Effect of paclobutrazol on flower-bud production in *Eucalyptus nitens* espalier seed orchards. Can. Jnl. For. Res. 24:46-49.

Moncur, M.W. and D.J. Boland. 2000. Production of genetically improved *Eucalyptus nitens*

seed for reforestation. Aus. For. 63(3): 211-217.

Nilsen, E.T. and D.M. Orcutt. 1996. The physiology of plants under stress. John Wiley & Sons, Inc., New York. pp 337-514.

Nir, I., R. Goren and B. Leshem. 1972. Effects of water stress, gibberellic acis and 2-chloroethyltrimethyl-ammonium chloride (CCC)on flower differentiation in 'Eureka' lemon trees. J. Amer. Soc. Hortic. Sci. 97:774-778.

Nixon, K. M. 1995. Personal communication. Institute for Commercial Forestry Research, Pietermaritzburg, South Africa.

Öquist, G. and R. Wass. 1988. A portable, microprocessor operated instrument for measuring chlorophyll fluorescence kinetics in stress physiology. Physiologia Plantarum. 73:211-217.

O'Neill, S.D. 1993. Changes in gene expression associated with floral induction and evocation. The Molecular Biology of Flowering. Ed. B.R. Jordan. CAB International. pp 69-92.

Pammenter, N.W. 2002. Personal communication. School of Life and Environmental Sciences, University of Natal, Durban, South Africa.

Paton, D.M. 1978. *Eucalyptus* physiology. 1. Photoperiodic responses. Aus. J. Bot. 26:633-642.

Patterson, H.D. and R. Thompson.1971. Recovery of inter-block information when block sizes are unequal. Biometrika. 58:545-554.

Penfold, A.R. and J.L. Willis. 1961. The Eucalypts Botany, Cultivation, Chemistry and Utilization. Interscience Publishers, New York. p 74.

Philipson, J.J. 1990. Prospects for enhancing flowering of conifers and broadleaves of potential silvicultural importance in Britain. Forestry. 63(3):223-240.

Pierce, B.T. and S.D. Verryn. 2000. Five year results of a site by clone interaction trial series of *Eucalyptus grandis* in the summer rainfall areas of South Africa. *In* Proc. of the IUFRO symposium "Forest Genetics for the Next Millenium", Durban, 8-13 Oct. 2000. ICFR,

Pietermaritzburg. pp 86-191.

Pinkard, E.A. and C.L. Beadle. 1998. Effects of green pruning on growth and stem shape of *Eucalyptus nitens* (Deane and Maiden) Maiden. New Forests. 15:108-109.

Poynton, R.J. 1979. Tree Planting in Southern Africa. Vol. 2. The Eucalypts. DEA, Pretoria. pp 532-536.

Pryor, L.D., 1976. The Biology of Eucalypts. Institute of Biology's Studies in Biology no. 61. Edward Arnold Publishers (Ltd.), London.

Pryor, L.D.1985. *Eucalyptus*. CRC Handbook of Flowering, Volume II. Ed. A.H. Halevy. CRC Press, Florida, USA. pp 476-482.

Pryor, L.D. and L.A.S. Johnson. 1971. A Classification of the Eucalypts. Australian National University, Canberra.

Pryor, L.D. and R.B. Knox. 1971. Operculum development and evolution in eucalypts. Aus. J. Bot. 19:143-171.

Purnell, R.C. and J.E. Lundquist. 1986. Provenance variation of *Eucalyptus nitens* on the Eastern Transvaal Highveld in South Africa. S. Afr. For. J. 138:23-31.

Reid, J.B., O. Hasan, M.W. Moncur and S. Hetherington. 1995. Paclobutrazol as a Management Tool for Tree Breeders to Promote Early and Abundant Seed Production. *In* Proc. of the IUFRO symposium " Eucalypt plantations: Improving Fibre Yield and Quality, 19-24 February 1995", Hobart. CRC for Temperate Hardwood Forestry, Tasmania. pp 293-298.

Richardson, E.A., S.D. Seeley and D.R. Walker. 1974. A model for estimating the completion of rest for "Redhaven" and "Elberta" peach trees. HortScience. 9:331-332.

Richardson, D.M. and J.P. McMahon. 1992. A bioclimatic analysis of *E. nitens* to identify potential planting regions in southern Africa. S. Afr. J. Sci. 88:380-387.

Richardson, K.F. 1985. Five year results of species and provenance trials with cold-resistant

eucalypts in Lesotho. S. Afr. Forestry J. 135:43-52.

Roberts, P.J.T. 1994*a*. Stochastic silviculture - Part 2: A description of the conceptual structure of models WETNES, DEATH and TREGRO. ICFR Bulletin Series No. 4/1994. ICFR, Pietermaritzburg.

Roberts, P.J.T. 1994*b*. Variation in soil moisture availability in South African plantations: initial results of parameter sensitivity tests of model WETNES. ICFR Bulletin Series No. 8/1994. ICFR, Pietermaritzburg.

Salisbury, F.B. and C.W. Ross. 1992. Plant Physiology. 4th ed. Wadsworth Publishing Co., California. pp 485-503.

Samish, R.M. 1954. Dormancy in woody plants. Ann. Rev. Plant Physiol. 5:183-203.

SAS, 1999. SAS® System for Windows©. Proprietary Software Release 8.2. SAS Institute Inc., Cary, NC, USA.

Sanhueza, R.P. 2002. Personal communication. Departemento Mejoramiento Genético, Forestal Y Agricola Monteaguila S.A., Los Angeles, Chile.

Savage, M.J. 2000. Personal communication. School of Applied Environmental Sciences, University of Natal, Pietermaritzburg, South Africa.

Schönau, A.P.G. and R.A.W. Gardner. 1991. Eucalypts for colder areas in Southern Africa. *In* Proc. of the IUFRO symposium "Intensive Forestry: The role of Eucalypts 2-6 Sept. 1991", Durban. Ed. A.P.G. Schönau. Vol. 1:467-479.

Schulze, R.E. 1997. South African Atlas of Agrohydrology and -Climatology. Report TT 82/96. Water Research Commission, Pretoria.

Schulze, R.E. 2003. Personal communication. School of Bioresources, Engineering and Environmental Hydrology, University of Natal, South Africa.

Seeley, D.S. 1996. Modelling climatic regulation of bud dormancy. Plant Dormancy. Ed. G.A.

Lang. CAB International, Oxford, UK. pp 361-376.

Snedecor, G.W. and W.G. Cochran. 1956. Statistical methods applied to experiments in agriculture and biology. Iowa State College Press, Iowa.

Soares, J.V. and A.C. Almeida. 2001. Modeling the water balance and soil water fluxes in a fast growing Eucalyptus plantation in Brazil. J. Hydrol. 253:130-147.

Soil Classification Working Group. 1991. Soil classification - A taxonomic system for South Africa. Department of Agricultural Development, Pretoria.

Southwick, S.M. and T.L. Davenport. 1986. Characterisation of water stress and low temperature effects on flower induction in citrus. Plant Physiol. 81:26-29.

Stanger, T. K. 1992. A1. Genetic Improvement of Eucalypts. ICFR Annual Research Report 1992. ICFR, Pietermaritzburg. p 5.

Stanger, T. K. 1995. Personal communication. Sappi Forests, Shaw Research Centre, Tweedie, South Africa.

Steel, R.G.D. and Torrie, J.H. 1981. Principles and procedures of statistics. A biometrical approach. McGraw-Hill International Book Co., Singapore. pp 76-238.

Sterrett, J.P. 1985. Paclobutrazol: A promising growth inhibitor for injection into woody plants. J. Amer. Soc. Hort. Sci. 110:4-8.

Sterret, J.P. 1988. XE-1019: Plant response, translocation and metabolism. J. Plant Growth Regul. 7:19.

Stone, E.L. and P.J. Kalisz.1990. On the maximum extent of tree roots. For. Ecol. Management. 46:159-102.

Sutinen, M.L., T. Rep, S. Sutinen, H. Lasarov, L. Alvila and T.T. Pakkanen. 2000. Physiological changes in *Pinus sylvestris* needles during early spring under sub-arctic conditions. For. Ecol. Management.135:217-228.

Swain, T. 2001. ICFR Tree improvement Project: Status Report. *Eucalyptus nitens*. ICFR Bulletin Series No. 5/2001. ICFR, Pietermaritzburg. pp 3-5.

Swain, T. and C. Chiappero. 1998. Collection of improved *E. nitens* seed from ICFR seed orchards. ICFR Newsletter May 1998. ICFR, Pietermaritzburg. pp 7-10.

Swain, T., C. Chiappero and R.A.W. Gardner. 1998. Final measurements of six ICFR *E. nitens* provenance/progeny trials in the summer rainfall region of South Africa. ICFR Bulletin Series No. 5/1998. ICFR, Pietermaritzburg. pp 6-17.

Swain, T. and R.A.W. Gardner. 2002. Use of site-species matching and genetic gain to maximise yield - a South African example. Proc. of "International Symposium on Eucalypts Plantations; Guangzhou/Zhaoqing, Guandong, P.R. China, Sept 1- 6, 2002".

Swart, W.J., Conradie, E. and M.J. Wingfield. 1991. *Cryphonectria cubensis*, a potential pathogen of *Psidium guajava* in South Africa. European Journal of Forest Pathology. 21:6-7.

Thomas, B. 1993. Internal and External Controls on Flowering. The molecular biology of flowering. Ed. B.R. Jordan. CAB International, Oxford, UK. pp 5-13.

Tibbits, W.N. 1986. Frost Resistance in Eucalyptus nitens (Deane and Maiden) Maiden. Phd. Thesis. Dept. Botany, University of Tasmania.

Tibbits, W.N. 1989. Controlled Pollination studies with Shining Gum (*Eucalyptus nitens* (Deane & Maiden) Maiden). Forestry. 62:111-126.

Tibbits, W.N., D.B. Boomsma and S. Jarvis. 1997. Distribution, biology, genetics, and improvement programs for *Eucalyptus globulus* and *E. nitens* around the world. *In* Proc. "24th Biennial Southern Tree Improvement Conference, 9-12 June 1997". Eds. T. White, D. Huber and G. Powell. Southern Tree Improvement Committee, Orlando, Florida. pp 1-15.

Ting, I.P. 1982. Plant Physiology. Addison-Wesley, Inc., Canada. pp 568-569.

Turnbull, C.R.A., D.E. McLeod, C.L. Beadle, D.A. Ratkowsky, D.C. Mummery and T. Bird.

1993. Comparative early growth of *Eucalyptus* species of the subgenera Monocalyptus and Symphyomyrtus in intensively managed plantations in southern Tasmania. Aus. For. 56:276-286.

Venter, F. and J-A. Venter. 1996. Making the most of indigenous trees. Briza Publications, Pretoria. pp 244-245.

Weier, T.E., C.R. Stocking and M.G. Barbour.1974. Botany: An introduction to plant biology. 5th ed. J.Wiley & Sons, New York.

Williams, D.A., J.J. Ross, J.B. Reid and B.M. Potts. 1999. Response of *Eucalyptus nitens* seedlings to gibberellin biosynthesis inhibitors. Plant Growth Regul. 27:125-129.

Williams, D.A., B.M. Potts and P. Smethurst. 2003. Promotion of flowering in *Eucalyptus nitens* by paclobutrazol was enhanced by nitrogen fertilizer. Can. J. For. Res. 33:74-81.

World Meteorological Organisation (WMO). 1996. Guide to meteorological instruments and method of observation. 6th Edition. WMO, Geneva.

APPENDICES

APPENDIX 1 Information pertaining to the modelling of Blyfstaanhoogte temperature data.

Appendix 1.1 Periods for which Blyfstaanhoogte data modelled

Year	Period
1996	5 Apr - 08 May
1996	22 July - 04 Aug
1997	01 Aug - 30 Sept
1998	15 Apr - 22 July
2000	12 Jun - 30 Sept

Appendix 1.2 Details of donor ISCW weather station

Station I.D.:	Long Tom, No. 555/188LO
Magisterial district:	Lydenburg, Mpumalanga
Co-ordinates:	Lat: 25.1333 ^{0} S.; Long: 30.6167 ^{0} E.
Altitude:	2118 m
Orientation:	3.6 kms N. of Blyfstaanhoogte site
Temperature sensor type:	max and min thermometer checked against thermo-hygrograph

Appendix 1.3 Regression of Blyfstaanhoogte Hobo pole daily minimum temperature (y) on Long Tom Pass Stevenson Screen daily minimum temperature (x)

Analysis of variance

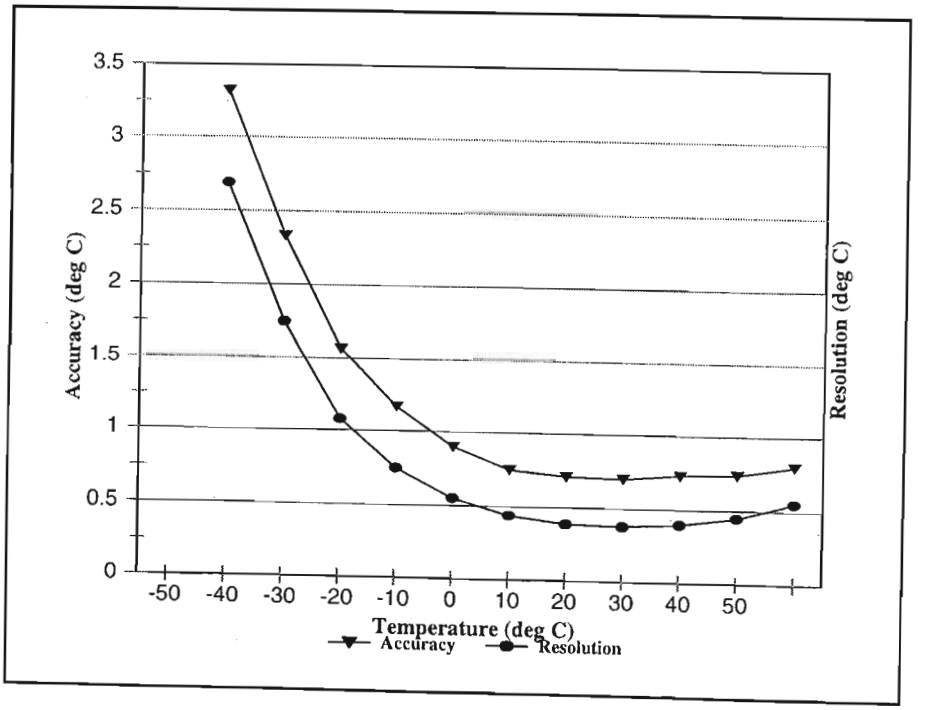
Source	d.f.	s.s.	m.s.	v.r.	*F* pr.
Regression	1	1285.8	1285.81	1090.55	< 0.001
Residual	174	205.2	1.179	-	-
Total	175	1491.0	8.520	-	-

Percentage variance accounted for:	88.2			
S.E.D.	1.09			
Estimates of parameters:	estimate	s.e.	*t* (174)	*t* pr.
Constant	- 0.365	0.197	-1.85	0.066
LongMinT	0.8921	0.0270	33.02	< 0.001
Fitted equation:	y = - 0.365 + 0.8921x			

Appendix 1.4 Regression of Blyfstaanhoogte Hobo Pole daily maximum temperature (y) on Long Tom Pass Stevenson Screen daily maximum temperature (x).

Analysis of variance					
Source	d.f.	s.s.	m.s.	v.r.	*F* pr.
Regression	1	2990.3	2990.312	1245.64	< 0.001
Residual	175	420.1	2.401	-	-
Total	176	3410.4	19.377	-	-
Percentage variance accounted for:	87.6				
S.E.D.	1.55				
Estimates of parameters:	estimate	s.e.	*t* (175)	*t* pr.	
Constant	- 0.072	0.072	0.540	- 0.13	0.895
LongMaxT	1.1350	0.0322	35.29	< 0.001	
Fitted equation:	y = - 0.072 + 1.1350x				

APPENDIX 2 Temperature accuracy and resolution of Hobo H8 series temperature logger (Borsari pers. comm. 2000).



APPENDIX 3 Results of the temperature calibration experiment carried out at the Agro-Meteorological weather station, University of Natal, Pietermaritzburg, during 2000.

Appendix 3.1 Regression of Hobo pole daily minimum air temperature (PoleMinT) (y) on Stevenson Screen daily minimum air temperature (ScrnMinT) (x)

Analysis of variance

Source	d.f.	s.s.	m.s.	v.r.	*F* pr.
Regression	1	2742.94	2742.94	13314.1	< 0.001
Residual	181	37.29	0.21	-	-
Total	182	2780.23	15.28	-	-

Percentage variance accounted for:	98.7			
S.E.D.	0.454			
Estimates of parameters:	estimate	s.e.	*t* (181)	*t* pr.
Constant	- 2.1142	0.0862	- 24.54	< 0.001
ScrnMinT	1.06680	0.00925	115.39	< 0.001
Fitted equation:	y = - 2.1142 + 1.0668x			

Appendix 3.2 Regression of Hobo pole daily maximum air temperature (PoleMaxT) (y) on Stevenson Screen daily maximum air temperature (ScrnMaxT) (x)

Analysis of variance

Source	d.f.	s.s.	m.s.	v.r.	*F* pr.
Regression	1	3527.6	3527.57	6237.53	< 0.001
Residual	181	102.4	0.5655	-	-
Total	182	3629.9	19.95	-	-

Percentage variance accounted for:	97.2			
S.E.D.	0.752			
Estimates of parameters:	estimate	s.e.	*t* (181)	*t* pr.
Constant	0.888	0.316	2.81	0.006
ScrnMaxT	1.0477	0.0133	78.98	<0.001
Fitted equation:	y = 0.888 + 1.0477x			

APPENDIX 4 Results of the 2002 temperature calibration experiment carried out at the Sappi Shaw Research Centre, Tweedie.

Appendix 4.1 Regression of *E. nitens* bud hourly temperature (BudHrly) (y) on Stevenson Screen hourly air temperature (ScrnHrly) (x).

Analysis of variance

Source	d.f.	s.s.	m.s.	v.r.	*F* pr.
Regression	1	40565.7	40565.7	143421.6	< 0.001
Residual	1667	471.5	0.28	-	-
Total	1668	41037.2	24.60	-	-

Percentage variance accounted for:	98.9			
S.E.D.	0.532			
Estimates of parameters:	estimate	s.e.	*t* (1667)	*t* pr.
Constant	- 0.8521	0.0538	-15.82	< 0.001
ScrnHrly	1.03466	0.00273	378.71	< 0.001
Fitted equation:	y = - 0.8521 + 1.03466x			

Appendix 4.2 Regression of *E. nitens* bud daily minimum temperature (BudMinT) (y) on Stevenson Screen daily minimum air temperature (ScrnMinT) (x).

Analysis of variance

Source	d.f.	s.s.	m.s.	v.r.	*F* pr.
Regression	1	198.81	198.81	3159.85	< 0.001
Residual	68	4.28	0.063	-	-
Total	69	203.09	2.943	-	-

Percentage variance accounted for:	97.9			
S.E.D.	0.251			
Estimates of parameters:	estimate	s.e.	*t* (68)	*t* pr.
Constant	- 0.404	0.260	- 1.55	0.125
ScrnMinT	1.0053	0.0179	56.21	< 0.001
Fitted equation:	y = - 0.404 + 1.0053x			

Appendix 4.3 Regression of *E. nitens* bud daily maximum temperature (BudMaxT) (y) on Stevenson Screen daily maximum air temperature (ScrnMaxT) (x).

Analysis of variance					
Source	d.f.	s.s.	m.s.	v.r.	*F* pr.
Regression	1	1896.26	1896.26	1956.7	< 0.001
Residual	68	65.9	0.9691	-	-
Total	69	1962.16	28.4372	-	-
Percentage variance accounted for:	96.9				
S.E.D.	0.984				
Estimates of parameters:	estimate	s.e.	*t* (68)	*t* pr.	
Constant	-1.055	0.609	-1.73	0.088	
ScrnMaxT	1.0677	0.0241	44.23	< 0.001	
Fitted equation:	y = - 1.055 + 1.0677x				

Appendix 4.4 Regression of Hobo pole daily minimum air temperature (PoleMinT) (y) on Stevenson Screen daily minimum air temperature (ScrnMinT) (x)

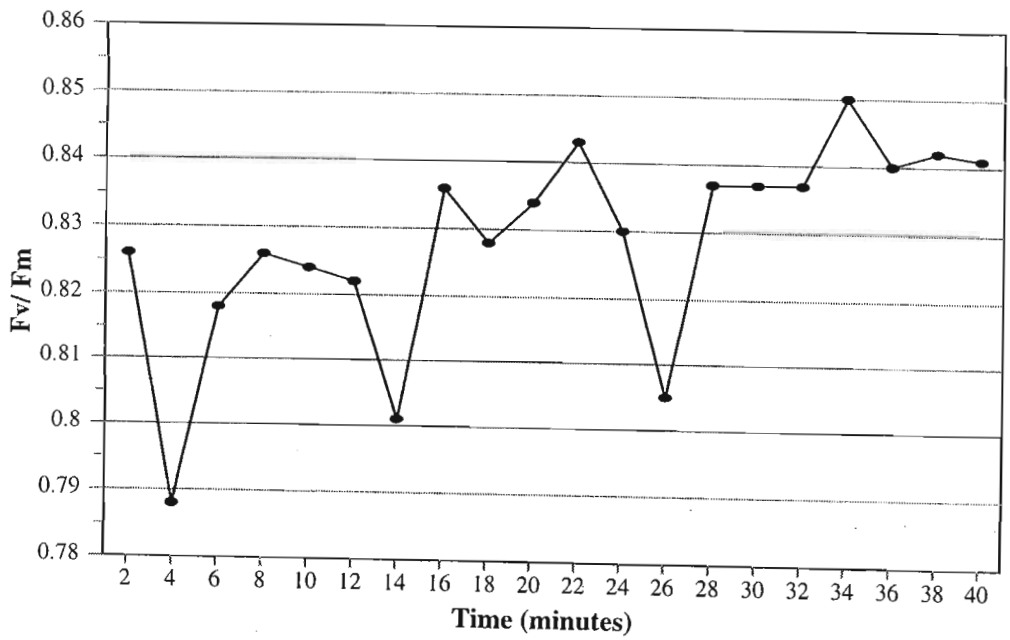
Analysis of variance					
Source	d.f.	s.s.	m.s.	v.r.	*F* pr.
Regression	1	172.74	172.74	2749.17	< 0.001
Residual	68	4.27	0.063	-	-
Total	69	177.02	2.565	-	-
Percentage variance accounted for:	97.6				
S.E.D.	0.251				
Estimates of parameters:	estimate	s.e.	*t* (68)	*t* pr.	
Constant	0.306	0.260	1.18	0.244	
ScrnMinT	0.9371	0.0179	52.43	< 0.001	
Fitted equation:	y = 0.306 + 0.9371x				

Appendix 4.5 Regression of Hobo pole daily maximum air temperature (PoleMaxT) (y) on Stevenson Screen daily maximum air temperature (ScrnMaxT) (x).

Analysis of variance

Source	d.f.	s.s.	m.s.	v.r.	*F* pr.
Regression	1	1956.0	1956.0	4222.97	< 0.001
Residual	68	31.5	0.46	-	-
Total	69	1987.49	28.80	-	-
Percentage variance accounted for:	98.4				
S.E.D.	0.681				
Estimates of parameters:	estimate	s.e.	*t* (68)	*t* pr.	
Constant	- 0.235	0.421	- 0.56	0.579	
ScrnMaxT	1.0844	0.0167	64.98	< 0.001	
Fitted equation:	y = - 0.235 + 1.0844x				

APPENDIX 5 F_v / F_m values as affected by increasing dark adaptation period (Exposure time 1 second, light intensity 1500 µmol $^{-2}$. s $^{-1}$).



APPENDIX 6 F_v / F_m value in response to varying light intensity (Exposure time 1 second, dark adaptation period 30 minutes).