OPTIMIZATION OF FLOWER AND SEED CROP PRODUCTION IN TEMPERATE *EUCALYPTUS* ORCHARDS IN SOUTH AFRICA THROUGH SITE SELECTION AND ENVIRONMENTAL MANIPULATION

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PREFACE

The research work contained in this thesis was carried out by the candidate while based at the Institute for Commercial Forestry Research in Pietermaritzburg, South Africa, under the supervision of Professor Isa Bertling and Professor Michael J Savage of the University of KwaZulu-Natal.

The contents of this work have not been submitted in any form to another university and, except where the work of others is acknowledged in the text, the results reported are due to investigations by the candidate.

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Professor Isa Bertling

Supervisor

Date: 12 March 2015

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Professor Michael J Savage

Co-Supervisor

Date: 12 March 2015

DECLARATION: PLAGIARISM

I, **Robin A W Gardner**, declare that:

(i) the research reported in this dissertation, except where otherwise indicated or acknowledged, is my original work;

(ii) this dissertation has not been submitted in full or in part for any degree or examination to any other university;

(iii) this dissertation does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons;

(iv) this dissertation does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:

a) their words have been re-written but the general information attributed to them has been referenced;

b) where their exact words have been used, their writing has been placed inside quotation marks, and referenced;

(v) where I have used material for which publications followed, I have indicated in detail my role in the work;

(vi) this dissertation is primarily a collection of material, prepared by myself, published as journal articles or presented as a poster and oral presentations at conferences. In some cases, additional material has been included;

(vii) this dissertation does not contain text, graphics or tables copied and pasted from the Internet, unless specifically acknowledged, and the source being detailed in the dissertation and in the References sections.

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Date: 12 March 2015

PUBLICATIONS AND PRESENTATIONS FROM THIS THESIS

Peer-reviewed publications

Gardner RAW, Savage MJ, Bertling I. 2011. Supplementing winter chilling in *Eucalyptus nitens* orchards using overhead irrigation: infrastructure, instrumentation and plant-atmosphere environmental effects. *ICFR Bulletin Series* No. 07/2011, Institute for Commercial Forestry Research, Pietermaritzburg.

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Gardner RAW, Bertling I, Savage MJ. 2013. Overhead sprinkling increased winter chilling and floral bud production in *Eucalyptus nitens*. *Southern Forests* 75(4): 199-212.

Gardner RAW, Bertling I, Savage MJ. 2014. Effect of temperature and paclobutrazol on *Eucalyptus nitens* reproductive phenology: a study in the summer rainfall area of South Africa (submitted to *South African Journal of Botany* 2/09/2014).

Gardner RAW, Bertling I, Savage MJ. 2014. Defining site requirements for maximal floral bud production in cold-tolerant eucalypts in South Africa: Final report on a *Eucalyptus nitens / E. smithii* site x flowering interaction trial series. *ICFR Bulletin Series* No. 03/2014, Institute for Commercial Forestry Research, Pietermaritzburg.

Gardner RAW, Bertling I, Savage MJ, Naidoo S. 2014. Investigating optimal site conditions for flower bud production in *Eucalyptus smithii* orchards in South Africa (submitted to *Australian Forestry* 13/12/2014)

Germishuizen I, Gardner RAW. 2014. A tool for identifying potential *Eucalyptus nitens* seed orchard sites based on climate and topography. *Southern Forests* (In press: DOI 10.2989/20702620.2014.984554).

Conference presentations

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ABSTRACT

Temperate eucalypts are an important part of the commercial forestry landscape in South Africa, comprising approximately 50% of the total *Eucalyptus* planted area. The majority of the commercial temperate eucalypts grown in South Africa are reticent, shy flowerers, and subsequently erratic seed producers. Disadvantages associated with sub-optimum (inconsistent and sparse) flowering in *Eucalyptus* orchards include decreased levels of out-crossing and compromised quantity and (genetic) quality of the seed produced. Genotype, physiological age and a range of environmental factors are known to influence flower bud production in temperate eucalypts. To date, winter cold and paclobutrazol (PBZ), a plant growth regulator, remain the most effective treatments for encouraging early and prolific flowering in temperate eucalypts. Disadvantages associated with the use of PBZ in the outdoor environment include the toxicity and recalcitrant nature and persistence of the chemical in soils, the high cost of PBZ and its orchard application, and the need to re-apply the chemical approximately every five years.

The main aim of this study was to provide a practical solution to the problem of shy flowering and seed crop production in important temperate *Eucalyptus* species in South Africa. The study focused on investigating key environmental factors associated with optimum flower bud production in temperate eucalypts. The resultant data were used to achieve optimization of flower bud production in temperate eucalypts in the summer rainfall forestry areas of South Africa, via informed site selection and/or manipulation of the environmental conditions. A subsidiary aim of the project was to lessen the dependency on PBZ for achieving satisfactory flowering levels in temperate eucalypts via improved site-orchard matching and environmental manipulation. The key objectives in the study included definition of summer rainfall area site conditions for maximal floral bud production in two important species, viz. *E. nitens* and *E. smithii*, investigation of the effects of optimum flowering environmental conditions on post-initiation floral development and seed maturation in temperate eucalypts, and development of a method for supplementing winter cold and increasing flower bud production in orchards located at marginal winter chilling sites.

For the undertaking of the field trial component, a reliable method of accurately measuring and recording *Eucalyptus* bud temperature at high elevation, remote summer rainfall sites in South Africa was required. A robust structure for housing the Hobo ® miniature loggers, termed “Hobo pole”, was designed and progressively modified for this purpose. Calibration tests were conducted to investigate the relationship between *E. nitens* bud temperature (BudT), Hobo pole air temperature (HoboAT) and screen air temperature (ScrnAT). In mid-winter, BudT on HoboAT gave the highest R^2 value (0.99) and lowest SE value (0.49 ^oC) of all regressions. In mid-summer, BudT on HoboAT together with BudT on ScrnAT gave the highest R^2 value (0.98). Bud winter chill units calculated from modelled bud temperature data were suitably accurate. It was concluded that the use of loggers having greater temperature measurement accuracy may reduce Hobo pole air temperature measurement error even further.

Two separate flowering field trial series, one *E. nitens* and the other *E. smithii*, were established across a range of high elevation (> 1550 m asl), cool temperate (13.5 to 16.0 ^oC mean annual temperature (MAT)) sites within the summer rainfall area. The interactive effects of a range of climate and landscape factors and paclobutrazol application on floral bud production in either species were investigated. The main objective was to broadly define temperate eucalypt site requirements for optimal floral bud production. Within the applied elevation and MAT ranges, of all landform factors, slope aspect had the greatest influence on floral bud production in both species. South-west, south and west-facing slopes were highly promotive of floral bud production, regardless of whether PBZ was applied or not. On sites selected for optimal floral bud production in *E. nitens*, *E. smithii* was a more prolific flowerer. *Eucalyptus smithii* showed a substantially lower chill requirement and greater responsiveness to PBZ for floral bud production than *E. nitens*. *Eucalyptus smithii* orchards situated in low landscape positions within the above elevational and MAT ranges, particularly those at high elevation (> 1800 m asl), cold (MAT < 14.0 ^oC) sites, carry a high risk of being severely damaged by frost and snow. Based on these findings, it is crucial to manage seed orchards of the various temperate eucalypt species differently. The results of the investigations indicated that, through careful site selection, the dependency on PBZ to achieve satisfactory floral bud crop production in temperate eucalypts may be substantially reduced.

To provide a practical solution to the problem of shy-flowering in temperate eucalypt breeding orchards due to inconsistent and/or insufficient winter chilling, supplementing winter chilling via evaporative cooling (overhead sprinkling) was investigated in the high chill requiring species *E. nitens*. The trial was conducted at Mondi's Mountain Home Research Centre near Hilton in KwaZulu-Natal. Treatments included three levels of sprinkling, two levels of PBZ and two grafted clones (prolific flowerer, shy-flowerer). Sprinkling reduced *E. nitens* daytime bud temperatures by as much as 16.2 ^{o}C on warm, dry winter days. During the relatively cold (2009) and warm (2010) winters, sprinkling increased chilling accumulation by 44% and 72%, respectively. In 2009, in the absence of PBZ, sprinkling resulted in a higher percentage trees of either clone producing umbels (flower buds) compared with the control. In the warmer 2010 winter, sprinkling again increased flowering, with the number of flowering shoots and umbels per tree being significantly higher than the control at $p < 0.05$. In both, 2009 and 2010, PBZ showed a strong additive effect to winter chilling on *E. nitens* floral bud production. The *E. nitens* clone x chilling x PBZ flowering interaction was complex, and therefore warrants more detailed investigation in future. Over three years, no negative affects of intermittent overhead sprinkling over a period of four months in (May to August) in winter on tree health were observed. It was concluded that evaporative cooling offers a practical method of supplementing winter chilling and optimizing floral bud production in high chill requiring temperate eucalypt species. The importance of this technology is likely to increase in view of the predicted ongoing climate warming.

To investigate the effects of PBZ and environmental conditions deemed highly promotive of flower bud production on post-initiation reproductive development in temperate eucalypts, the reproductive phenology of a single species, *E. nitens*, was monitored across four sites in KwaZulu-Natal over 2.4 years. The sites differed predominantly in elevation, with the lowest site being the maximum chilling treatment block in the evaporative cooling trial at Mountain Home (1133 m asl), and the highest being the field trial at Willowmere near Underberg (1708 m asl). Regardless of PBZ

application, there was a distinct trend across sites of anthesis being delayed (by at least 120 days) and anthesis duration being shortened (by at least 80 days) as elevation increased from the lowest to highest elevation. At Netherby3 (1678 m asl), a top-ranking field trial site on the basis of umbel crop production, anthesis was delayed by 33 days. The general effect of paclobutrazol application across sites was a decrease in anthesis duration of between 17 and 34%, from lowest to highest elevation. Although based on a narrow range of *E. nitens* genetic material, the observed trends in the study should be taken note of by local temperate eucalypt tree breeders and seed producers. At optimum temperate eucalypt flower bud production sites in South Africa, the levels of out-crossing and capture of genetic gain and variation may be substantially compromised. Further research, entailing a wider range of temperate eucalypt genetic material and investigating important orchard-related factors such as pollination and gene flow, is advocated.

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CHAPTER 1

Introduction

Rationale for the research

The majority of the temperate eucalypts used in commercial forestry are difficult to propagate vegetatively (Jones et al. 2000, Le Roux and Van Staden 1991), and therefore, to date, seedlings have been used for establishing the majority of temperate eucalypt plantations in South Africa. Inter-specific hybrids between the sub-tropical *E. grandis* and temperate *E. nitens* have met with a good degree of success in South Africa (Zwolinsky and Bayley 2001). High rooting capability conferred by *E. grandis* has resulted in the majority of these hybrids being deployed into the commercial plantation environment as rooted cuttings (Mokotedi et al. 2000). Of the total temperate eucalypt plantation area in South Africa, only 14% comprises clonal material (R Gardner unpublished data 2014). The industry is therefore largely dependent on the production of locally-improved, high (genetic) quality seed for establishing the local temperate eucalypt plantations (Eldridge et al. 1993).

A barrier to local production of sufficient quantities of temperate eucalypt seed for plantation establishment is the shy-flowering and erratic seed production habit of the most important temperate eucalypt species. In South Africa, seedling trees of species such as *E. nitens*, *E. smithii*, *E. dunnii* and *E. badjensis* rarely flower before the age of eight years, and even then, the floral bud and seed crops produced are sparse and erratic (Jones 2002, Swain and Gardner 2003). Genotype, physiological age and hormonal status of plants, and a range of environmental factors, are known to influence flower bud production in temperate eucalypts (Meilan 1997, Moncur and Boland 2000, Williams et al. 2003). To date, winter cold and paclobutrazol (a plant growth regulating chemical) remain the most effective treatments for encouraging flowering in temperate eucalypts (Hamilton et al. 2008, Moncur and Hasan 1994). In the absence of sufficient winter cold, paclobutrazol (PBZ) treatment is relatively ineffective. In South Africa and abroad, PBZ is used almost routinely as an orchard management tool, i.e. for assisting vegetative and reproductive growth control, in temperate eucalypts (Gardner et al. 2013, Hamilton et al. 2008). Disadvantages associated with the use of PBZ include the chemical's recalcitrant nature and persistence in soils (Fletcher et al. 2000, Jackson et al. 1996) and the high cost of its application in orchards. There is a need to develop a method of lessening the dependency on PBZ for achieving satisfactory flowering and seed crop production in temperate eucalypt species.

During the early 1990s, in the temperate eucalypt growing areas of South Africa, it was noted that non paclobutrazol-treated trees of *E. nitens* and *E. dunnii* flowered more prolifically and consistently at exposed, uniformly cool sites in the landscape, rather than low-lying sites typically prone to high diurnal temperature amplitudes in winter (Gardner 2003). This prompted an initial investigation (carried out between 1996 and 2001) into the relationship between winter chilling and flower bud production in temperate eucalypts with *E. nitens* as the test species. This investigation, based on a semi-controlled environment experiment and four field trials, yielded initial insight into *E. nitens* winter chilling requirement for floral induction, and the interaction between chilling, paclobutrazol and *E. nitens* floral bud production (Gardner 2003, Gardner and Bertling 2005). Interestingly, where trees were exposed to very high levels of winter chilling, *E. nitens* flowered equally as well in PBZ-treated and non-treated trees.

Objectives of the study

Due mainly to the restricted number of field sites, treatments and replicates applied in the 1996 to 2001 *E. nitens* flowering trial series, the results emanating from this research were fairly coarse, allowing only tentative guidelines to be drawn up. The information yielded was not detailed enough to allow accurate prediction of optimal *E. nitens* orchard sites within the South African forestry landscape. There remained a need to carry out further research as follows:

- More comprehensive investigation of the interactive effect of winter chilling and PBZ on floral bud production in temperate eucalypts.
- Definition of site conditions, including landscape and climatic factors, for optimal flower bud production in temperate eucalypt orchards within the summer rainfall

forestry area. This investigation should include at least two species evaluated over a far wider range of site conditions.

- The effect of the optimal flower bud production orchard site conditions on post-initiation reproductive development through to seed maturity.

Furthermore, the research centers of the major forestry companies are mostly located at low elevation (< 1200 m asl) sites within the summer rainfall region, where winter conditions are relatively warm and non-conducive to floral induction and initiation in high-chill requiring temperate eucalypt species such as *E. nitens* and *E. smithii* (Gardner 2003, Gardner and Bertling 2005, Jones 2002). The risk of annual floral bud and seed crop failure due to warm winter conditions is set to increase in accordance with predicted climate change (Linkosalo et al. 2009, Warburton and Schulze 2008). There was a need to explore a means of reducing the risk of insufficient winter chilling in high value, temperate eucalypt breeding orchards located at the research centers. Therefore, the following research was proposed:

- Development of a practical method of supplementing winter chilling to increase the consistency and abundance of flowering in temperate eucalypt breeding orchards located at marginal chilling sites.

Outline of thesis structure

Chapter 1 of the thesis discusses the rationale behind the research undertaken.

Chapter 2 is a Literature Review, covering all the important aspects relating to the research undertaken.

Chapter 3 is the first of the empirical chapters. It discusses the development of a method for monitoring air temperature at remote, high elevation sites in the summer rainfall area. The equipment designed, tested and adapted was essential to the site x PBZ x temperate eucalypt flowering interaction field trial research undertaken. The results of a calibration experiment, where the relationship between air temperature and *E. nitens* bud temperature was investigated, are also discussed.

Chapter 4 discusses *E. nitens* field research conducted across thirteen high altitude sites within the summer rainfall forestry area. Previous research and observations suggested that *E. nitens* has the highest cold requirement for floral bud production of all locally important temperate eucalypt species (Gardner 2003, Jones et al. 2000). Hence, it was important to include *E. nitens* as one of the species in the investigation. The main objective of the research was to define *E. nitens* orchard site requirements, on the basis of climatic and landscape factors, for optimal (consistent and prolific) flower bud production.

Chapter 5 discusses *E. smithii* field research conducted across thirteen high altitude sites within the summer rainfall forestry area. The methodology applied was almost identical to that carried out in the *E. nitens* project described in Chapter 4. *Eucalyptus smithii* produces the highest quality pulpwood of all commercial temperate eucalypts planted in South Africa. Previous research and observations indicated that *E. smithii* is a shy-flowering species, having a high cold requirement for floral bud production (Gardner 2003, Jones et al. 2000). Hence, it was important to include *E. smithii* as one of the species in the “definition of site conditions for optimal (consistent and prolific) flower bud production in temperate eucalypt species” investigation.

The research conducted and described in Chapter 6 was aimed at investigating whether sites optimal for floral bud production in temperate eucalypt species are also optimal for post-floral initiation reproductive growth, particularly production of high (genetic) quality seed. This investigation focused on a single species, viz. *E. nitens*, mainly due to resource constraints.

The final chapter, Chapter 7, provides a brief discussion and conclusion based on the work carried out. Recommendations for further research are also presented.

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CHAPTER 2

Literature Review

The genus *Eucalyptus*

Taxonomical classification

The genus *Eucalyptus* belongs to the family Myrtaceae. The name *Eucalyptus* is derived from the Greek words ‘eu’, meaning ‘well’, and ‘calyptos’, meaning ‘covered’ (Chippendale 1988). The term ‘well-covered’ refers to the reproductive organs in the flower bud being protected by an operculum (cap) prior to anthesis (Pryor 1976). Since the first publishing of the species name in 1788 by Charles-Louis L’Héritier de Brutelle (*E. obliqua*), hundreds of species and infraspecific taxa have been described, with about 900 being currently recognized (Boland et al. 2006). Pryor and Johnson’s (1971) comprehensive classification of the eucalypts became the benchmark for all eucalypt taxonomists and researchers. These authors based their classification primarily on an appreciation of the genetic relationships between species. Effectively, the genus was divided into seven subgenera, and these into sections, series, subseries, superspecies, species and subspecies (Brooker 2000, Florence 1996). The subgenera are effectively genetically isolated groups, which has important practical implications for disciplines such as tree breeding, genetics and conservation ecology (Boland et al. 2006). Brooker (2000) proposed a new classification of the eucalypts, based largely on Pryor and Johnson’s classification of 1971, but with a number of alterations based on new information emerging since the latter classification. Certain of the latter proposed changes to the Pryor and Johnson (1971) classification have gained acceptance, whilst others not (Boland et al. 2006). For the purpose of this thesis, the classification system of Brooker (2000) will be adopted.

Natural distribution

Virtually all eucalypts occur naturally only in Australia, with only two species endemic outside Australia (Indonesia and New Guinea), viz. *E. urophylla* and *E. deglupta*

(Boland et al. 2006). The latitudinal distribution of the eucalypts is approximately 9 ^{o}N (*E. deglupta*) to 44 ^{o}S (*E. obliqua* and other species) (Williams and Brooker 1997). The eucalypts occur naturally across most of Australia, their form ranging from short shrubs to tall forest trees depending on the environmental conditions to which they have adapted (Pryor 1976).

Eucalypts in South Africa

The first eucalypts were introduced into South Africa in 1828. These were in the form of nine *E. globulus* seedlings established in the Governor's Garden in Cape Town by then Governor of the Cape Colony, Sir Lowry Cole (Showers 2010). Since this time, many other *Eucalyptus* taxa have been introduced into South Africa for testing and/or commercial planting in both sub-tropical and temperate areas of the country (Poynton 1979). The development of the gold mining industry on the Witwatersrand from the early 1900s onwards was the major driver of the expansion of *Eucalyptus* plantings in South Africa (Darrow 1984). *Eucalyptus grandis*, mainly due to its rapid growth and remarkable adaptability to widely ranging climatic and edaphic conditions, established itself as the major commercial timber species in South Africa by the mid-20th century (Schönau 1991).

The majority of the gold (and later diamond) mines were located in high elevation (> 1200 m asl) areas of the inland plateau however, and there was an ongoing need to identify eucalypt species suitably adapted to the severe winter frosts and drought conditions typical to these areas (Poynton 1979, Schönau and Gardner 1991). During these early times, the terms "hard gums", "cold-resistant eucalypts" and "cold-tolerant eucalypts" were colloquially used in South Africa to describe these cold-hardy eucalypts (Beard 1958, Darrow 1984, Gardner and Swain 1996). *Eucalyptus* species finding initial favor due to high levels of cold-hardiness and suitability for mining timber (high strength and durability of wood) included *E. bridgesiana*, *E. dalrympleana, E. elata, E. fastigata*, *E. macarthurii*, *E. maidenii*, *E. sideroxlyon* and *E. viminalis* (Beard 1958, Nixon 1983). Over the past four decades, several other eucalypt species, e.g. *E. dunnii, E. fraxinoides, E. nitens* and *E. smithii*, emerged as popular alternatives to the original "hard gums" (Schönau and Gardner 1991, Schönau and Purnell 1987). The significant developments in the pulp and paper industry in South Africa and associated expansion of plantation forestry into cold highland areas of western KwaZulu-Natal and northern Eastern Cape during the early 1980s, added significant impetus to the search for alternative cold-hardy eucalypt species (Gardner 2001, Swain and Gardner 2002).

Internationally, and indeed in Australia, to date "temperate eucalypt" is the preferred term used to describe eucalypt species originating from temperate forest communities in the latter country (Boland et al. 2006, Florence 1981, Wardell-Johnson et al. 1997). Therefore, for the purpose of this thesis, the term "temperate eucalypt" will be used in preference to "cold-tolerant eucalypt" in all further discussion. Temperate eucalypts are an important part of the current commercial forestry landscape in South Africa. Of the total *Eucalyptus* plantation area (515 000 ha), about 50% comprises temperate species (Forestry South Africa 2013). The positive attributes of the current commercial temperate eucalypt species are numerous. These include good adaptation to cold climatic conditions (rapid vegetative growth, frost and snow tolerance, etc) and highly desirable wood and pulping properties (Gardner 2001, Swain and Gardner 2003).

Eucalyptus nitens

Taxonomical classification

Eucalyptus nitens (Deane & Maiden) Maiden (Shining Gum), is included in subgenus Symphyomyrtus, section Maidenaria, subsection Euryotae, series Globulares (Brooker 2000). Other species included in this series include the commercially important Southern Blue Gums, *E. bicostata, E. globulus* and *E. maidenii*.

Natural distribution

Eucalyptus nitens occurs naturally 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 eastern New South Wales (about 30^{0} 23.0 ' S latitude) to the central highlands of Victoria (38^{0} 0.0 ' S latitude) (Brooker and Kleinig 1983, Chippendale 1988). Shining Gum occurs over a range of elevations, from about 1600 m asl in the north, to 600 m asl in the Victorian coastal ranges (Boland et al. 2006, 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. 2006, Chippendale 1988).

The climate of the majority of the areas in which *E. nitens* occurs is cool temperate, with mild summers and cool to cold winters (Poynton 1979). Mean maximum temperature of the hottest month ranges from 19 - 27 ^{0}C, mean minimum of the coldest month ranges from -3 to -4 ^{0}C and mean annual temperature (MAT) ranges from 5 - 15 ^{o}C (Jovanovic and Booth 2002). 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 (Boland et al. 2006). *Eucalyptus nitens* is confined to areas with a mean annual precipitation (MAP) of at least 550 mm, rising to over 2000 mm at certain locations (Boland et al. 2006, Jovanovic and Booth 2002). 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.

Significance of *Eucalyptus nitens*

Eucalyptus nitens is an important eucalypt species grown for commercial wood production in several countries around the world (Hamilton et al. 2008, Tibbits et al. 1997). The species is the most important of all commercial temperate eucalypts grown in South Africa, mainly due to its high levels of cold, frost and snow tolerance, and excellent pulping properties (Gardner 2001, Swain et al. 2014). The results of a recent survey showed that commercial plantations of *E. nitens* currently cover approximately 45 000 ha or 17.5% of the temperate eucalypt plantation forestry area in South Africa (Forestry South Africa 2013, R Gardner unpublished data 2014). Hybrids between *E. nitens* and the sub-tropical *E. grandis* have met with substantial success in South Africa over the past two decades (Gardner 2007, Zwolinsky and Bayley 2001). Clones of these hybrids tend to out-perform either parent species at sites in the warm temperate forestry zone where the conditions are too cold for successful *E. grandis* cultivation and/or too warm for optimal *E. nitens* growth (Smith et al. 2005, Zwolinsky and Bayley 2001).

Approximately 27 000 ha of the total *Eucalyptus* plantation area in South Africa is currently planted to *E. grandis* x *E. nitens* and *E. nitens* x *E. grandis* clonal material (R Gardner unpublished data 2014).

Eucalyptus nitens in South Africa

In South Africa, *E. nitens* is planted commercially in high elevation areas of the summer rainfall forestry regions. The approximate altitudinal and latitudinal ranges of the current *E. nitens* commercial plantings in South Africa are 1350 to 1900 m asl and 25.7 to 31.3 ^{0} S, respectively (R Gardner unpublished data 2014). The current recommended summer rainfall area climatic boundaries for optimum *E. nitens* vegetative growth are between 14 ℃ and 16 ℃ MAT, and > 825 mm MAP (Smit h et al. 2005, Swain and Gardner 2003). Research has shown that overall the central and northern provenances of *E. nitens* are best adapted for commercial pulpwood production in South Africa (Purnell and Lundquist 1986, Swain et al. 2013b).

Eucalyptus nitens floral morphology

Inflorescence

The inflorescence in *E. nitens* is first discerned as a single bud (hereafter termed “inflorescence bud”) in the axil of a newly developing leaf (Pryor and Johnson 1971). The unit inflorescence consists of a condensed dichasial cyme, generally referred to as ‘umbel’ or ‘umbellaster’ (Pryor 1976). 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. It is customary to refer to the flower as a “bud” until the stage of opercula shed, after which it is referred to as a “flower” (Potts and Gore 1995). The sessile flower buds are attached to a common peduncle, forming an umbel (Brooker and Kleinig 1983). In *E. nitens*, the umbel is normally 7-flowered and is borne on an angular to slightly flattened peduncle (Boland et al. 2006).

Flowers and fruits

The individual flower buds within the umbel average about 7 mm (long) by 3 mm (wide) in size (Brooker and Kleinig 1983). Prior to anthesis, the developing inner floral organs are protected by two conical bud caps (opercula), the outer operculum consisting of fused sepals (calycine/ sepaline operculum) and the inner operculum consisting of fused petals (corolline/ petaline operculum) (Pryor 1976, Pryor and Knox 1971). The sepaline operculum is shed at a relatively early stage of bud development, whereas the petaline operculum is shed shortly before commencement of anthesis (Ladiges et al. 1995, Potts and Gore 1995, Pryor 1985, W Jones unpublished data 2014). Fruits (woody capsules) are sessile, ovoid in shape and approximately 7 x 5 mm in size (Brooker and Kleinig 1983). The valves (three to four) extend to rim level or are slightly exserted.

Eucalyptus smithii

Taxonomical classification

Eucalyptus smithii (R.T. Baker), Gully Gum, is included in subgenus Symphyomyrtus, section Maidenaria, sub-section Euryotae, series Compactae (Brooker 2000). The only other species included in the latter series is *E. badjensis* (Big Badja Gum), also of commercial forestry importance in South Africa.

Natural distribution

Eucalyptus smithii occurs naturally over a range of habitats in rolling country in south eastern Australia. The species is found primarily on lower hillslopes, beside streams and at the edges of swamps, where the soils never dry out completely (Boland et al. 1991). *Eucalyptus smithii*'s distribution is from the south-eastern end of the central tablelands in New South Wales (about 33 ^{0} 35.0 ' S latitude) southwards to south-west and eastern Victoria (about 37 ^{0} 34.0 ' S latitude) (Brooker and Kleinig 1983, Chippendale 1988). Gully Gum occurs over a range of elevations, from 1150 m asl in the north to 50 m asl in the south (Poynton 1979). Preferred soils are clay-loams or deep sandy loams over clays derived from sedimentary or volcanic parent material (Boland et al. 2006, Chippendale 1988). The climate of the majority of the areas in which *E. smithii* occurs is cool temperate to sub-humid (Poynton 1979). Mean maximum temperature of the hottest month ranges from 20 - 27 ^{0}C, mean minimum of the coldest month ranges from -3 to -7 ^{0}C and MAT ranges from 7 – 17 ^{o}C (Jovanovic and Booth 2002). Regular, moderate frosts and light snowfalls occur during the winter months at the inland sites (Boland et al. 2006). *Eucalyptus smithii* is confined to areas with a MAP of at least 610 mm, but this rises to about 1930 mm at certain locations (Jovanovic and Booth 2002, Poynton 1979). Rainfall is more or less uniformly distributed throughout the year with months recording less than 50 mm being rare.

Significance of *E. smithii*

Eucalyptus smithii is an important eucalypt species grown predominantly for essential oil production in various parts of the world (Clarke et al. 2008). 1,8-Cineole is the major component in the essential oil (Boland et al. 1991). In countries such as South Africa, China and Australia *E. smithii* is predominantly grown for pulpwood production due to the excellent pulping properties of its wood (Arnold et al. 2004, Swain and Gardner 2002). The wood of the species ranks second to *E. globulus* on the basis of eucalypt pulpwood quality (Norris 2014). Due to the fairly high density of the wood, *E. smithii* is also highly suitable for wood-chip exporting for pulping purpose (Clarke et al. 1999, Gardner 2001). The results of a recent survey showed that commercial *E. smithii* plantations currently cover approximately 22 000 ha or 8.3% of the temperate eucalypt plantation forestry area in South Africa (Forestry South Africa 2013, R Gardner unpublished data 2014).

Eucalyptus smithii in South Africa

In South Africa, *E. smithii* is planted commercially in high elevation areas of the summer rainfall forestry belt (Gardner 2001). The species tolerates light to moderate frosts and snowfalls (Gardner and Swain 1996). The approximate altitudinal and latitudinal ranges of the current *E. smithii* commercial plantings in South Africa are 1050 to 1650 m asl and 25.5 to 30.5 ^{0} S, respectively (R Gardner unpublished data 2014). The current recommended summer rainfall area climatic boundaries for optimum *E. smithii* vegetative growth are between 15 ℃ and 17 ℃ MAT, and > 825 mm MAP (Gardner 2006, Smith et al. 2005). Research has shown that the northern and central provenances of *E. smithii*, e.g. Wombeyan Road and Tallaganda, respectively, in New South Wales, are best adapted for commercial pulpwood production in the summer rainfall area (Swain and Gardner 2002, Swain and Gardner 2003).

Eucalyptus smithii floral morphology

Inflorescence

The inflorescence in *E. smithii* is first discerned as a single bud (hereafter termed "inflorescence bud") in the axil of a newly developing leaf (Pryor 1985). 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. The inflorescence is axillary and simple, consisting of a condensed dichasial cyme (Pryor 1985). The pedicellate flower buds are attached to a common peduncle, forming an umbel (Brooker and Kleinig 1983). In *E. smithii*, the umbel is normally 7-flowered and is borne on an angular to slightly flattened peduncle (Boland et al. 2006).

Flowers and fruits

The individual flower buds within the umbel average about 7 mm x 4 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 operculum consisting of fused sepals (calycine/ sepaline operculum) and the inner operculum consisting of fused petals (coralline/ petaline operculum) (Pryor 1976, Pryor and Knox 1971). The sepaline operculum is shed at a relatively early stage of bud development, whereas the petaline operculum is shed shortly before commencement anthesis (Ladiges et al. 1995, Potts and Gore 1995, Pryor 1985, W Jones unpublished data 2014). The capsules are pedicellate, ovoid to subglobular or campanulate in shape and approximately 7 x 7 mm in size (Brooker and Kleinig 1983). The valves (usually three) are exserted.

Temperate eucalypt flowering and seed production

Reproductive growth and development

Most eucalypts are heteroblastic (Ashton 1975, Pryor 1985). In *E. nitens* and *E. smithii*, the juvenile foliage is conspicuously different from adult foliage (Brooker and Kleinig 1983). In most temperate eucalypts, including *E. nitens* and *E. smithii*, flowers are produced on the adult foliage spring flush of reproductively mature trees (Pryor 1985). The results of recent work on *E. nitens* and *E. globulus* suggest that the timing of vegetative phase change and that of first flowering in temperate eucalypts are both physiologically (Gardner and Bertling 2005, Hasan and Reid 1995) and genetically (Jordan et al. 1999) independent. Genotype, physiological age and hormonal status of plants, and a range of environmental factors, are known to influence flower bud production in temperate eucalypts (Meilan 1997, Moncur and Boland 2000, Williams et al. 2003).

Biological restraints to flower and seed production

In South Africa, the main positive attributes of the commercial temperate eucalypt species are good adaptation to the cold, high elevation climatic conditions and favourable wood and pulping properties (Gardner 2001, Swain and Gardner 2003). A major negative though, is the shy-flowering and erratic seed production tendencies of the majority of the temperate eucalypt species (Chambers et al. 1997, Swain and Gardner 2002). Genotype, physiological age and hormonal status of plants, and a range of environmental factors, are known to influence flower bud production in temperate eucalypts (Meilan 1997, Moncur and Boland 2000, Williams et al. 2003). Seedling trees of important species such as *E. badjensis*, *E. dunnii*, *E. nitens* and *E. smithii* rarely flower before the age of eight years, and even then, the annual floral bud crops produced are sparse and erratic (Jones 2002, Swain and Gardner 2003, T-L Swain unpublished data 2014). The problem appears to lie not only in the lengthy juvenile phase, but also the exacting environmental requirements for triggering of flowering of the temperate eucalypt species (Hamilton et al. 2008, Jordan et al. 1999, Moncur and Hasan 1994). Reticent and inconsistent flowering are indeed a major hindrance to genetic improvement and commercial seed production in important temperate eucalypt species (Moncur et al. 1994, Reid et al. 1995, Swain and Gardner 2002). Over the past three decades, considerable temperate eucalypt reproductive biological research has been undertaken around the world, aimed at improving flowering and seed crop production in important temperate species such as *E. globulus* and *E. nitens* (Hamilton et al. 2008, Moncur and Boland 2000, Potts et al. 2007).

Environmental effects on flowering

To date, winter cold and paclobutrazol (a plant growth regulating chemical) remain the most effective treatments for encouraging flowering in temperate *Eucalyptus* species (Hamilton et al. 2008, Hetherington and Jones 1990, Meilan 1997, Moncur 1992).

Temperature

Cold winter conditions appear pre-requisite for floral bud production in temperate eucalypts (Gardner 2003, Moncur 1992, Moncur and Hasan 1994). Amount of accumulated winter chilling correlated well with the ensuing floral bud crop in *E. nitens* (Gardner and Bertling 2005). *Eucalyptus nitens* appears to have a minimum winter chilling requirement of ≈ 40 CP (Chilling Portion, the chill unit of the Dynamic Model (Fishman et al. 1987, Erez and Fishman 1998)) for floral induction, regardless of whether paclobutrazol has been applied or not (Gardner and Bertling 2005). In South Africa, the climatic conditions of areas in which the temperate eucalypt commercial plantations and seed orchards are located offer inconsistent, apparently often insufficient, winter chilling for floral bud production in temperate eucalypts (Gardner and Bertling 2005, Schulze and Maharaj 2007). Sites suitable for rapid vegetative growth and timber production may not necessarily be suitable for flowering and seed production. In high elevation (> 1200 m asl) areas of the summer rainfall forestry belt, temperate eucalypts such as *E. fraxinoides, E. nitens* and *E. smithii* have been observed to produce flower buds more prolifically and consistently at exposed, rather than low-lying, sites (Gardner 2003).

Soil moisture

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). 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. In an experiment conducted across four high elevation sites in South Africa, cumulative drought stress days through winter did not correlate significantly with *E. nitens* floral bud crop in the ensuing summer (Gardner 2003).

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). *Eucalyptus* species produce flower buds in the leaf axils of new growth in spring (Tibbits 1989, Jones and van Staden 2001), which suggests that floral initiation may be linked to photoperiod. However, Moncur (1992) and Moncur and Hasan (1994) found that, in contrast to many tree genera, floral development in temperate eucalypts is not day-length dependent.

Paclobutrazol

Over the past two decades, paclobutrazol ((2RS,3RS)-1-(4-chlorophenyl)-4,4-dimethyl-2-1,2,4-triazol-1-yl-pentan-3-ol), a chemical plant growth regulator, has been used almost routinely in temperate eucalypt seed orchards for assisting vegetative growth control and promoting precocious and abundant flowering (Williams et al. 2003, Hamilton et al. 2008). Paclobutrazol, a triazole derivative, interferes with the biosynthesis of gibberellin by inhibiting the oxidation of ent-kaurene to ent-kaurenoic acid (Graebe 1987, Hedden and Graebe 1985). Paclobutrazol, hereafter referred to as “PBZ”, does not replace winter chilling in the floral induction process in temperate eucalypts (Gardner 2003, Gardner and Bertling 2005). Rather, the chemical appears to act in an additive manner together with other inductive factors (Moncur and Hasan 1994, Hasan and Reid 1995, Meilan 1997). Major negatives associated with the use of PBZ include its high cost, recalcitrant nature, and persistence in soils (Jackson et al. 1996). For the latter two reasons, the chemical is not popular with environmentalists (Reid et al. 1995, Fletcher et al. 2000). Clearly, there is a need to develop a method of lessening the dependency on PBZ for achieving satisfactory flowering and seed crop production in temperate eucalypt species in South Africa.

Pollination and associated orchard problems

In eucalypts, nectar production appears to be a function of the epidermal cells lining the floral cup between the staminal ring and the base of the style (Davis 1968). The breeding system of *Eucalyptus* appears to be well adapted for pollen-mediated gene flow (Pryor 1976). In South Africa, eucalypts are a major source of nectar for honey production (Johannsmeier and Mostert 2001). In the summer rainfall area, in contrast to the prolific flowering and nectar producing sub-tropical *E. grandis*, temperate eucalypt species cultivated for commercial pulpwood production (including *E. nitens* and *smithii*) are generally poor flowerers and unsuitable for commercial honey production (Gardner 2004). Local eucalypt breeders and seed producers rely predominantly on insect cross-pollination of small-flowered species such as *E. nitens* and *E. smithii* (Eldridge et al. 1993, Swain et al. 2013a). In Australia, Hingston et al. (2004) found that *E. nitens* flowers seemed to be consistently unattractive to honeybees. In South Africa, flies and ants appear to be the predominant visitors to *E. nitens* and *E. smithii* flowers (Jones 2002, R Gardner unpublished data 2013). Inter-tree (cross-) pollination success can be affected by an number of factors, including presence of pollination vectors, distance between trees/ flowers, difference in flowering times between genotypes and difference in flowering intensity (House 1997, Moncur and Boland 2000).

The timing and duration of anthesis among genotypes within an orchard affects the degree of out-crossing and genetic makeup of the resultant seed crop (Kang et al. 2004, Stoehr et al. 1998). The genetic gain from seed orchards where sub-optimal flowering levels, synchronicity between genotypes and cross-pollination occur is at risk of being severely compromised (Griffin and Cotterill 1988, Swain et al. 2013a). In species such as *E. nitens* where geitonogamy is known to occur (Tibbits 1989), orchard-related problems include reduced capsule set, seed yield and seed viability (Moncur and Boland 2000, Williams et al. 1999). Although perusal of the published literature revealed scant information on *E. smithii* pollination biology, a similar problem may be present in orchards of the species.

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CHAPTER 3

Development of a method for monitoring *Eucalyptus* bud temperature at remote, high elevation forestry sites in the summer rainfall area of South Africa

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Abstract

Temperature is a key environmental trigger of flowering in many temperate woody plants. Over the past two decades, considerable site x *Eucalyptus* flowering interaction research has been undertaken in high elevation (> 1100 m asl) areas of the summer rainfall forestry regions in South Africa. A reliable method of accurately measuring and recording *Eucalyptus* bud temperature in field trials located at high elevation remote sites was required. Utilization of traditional methods for the purpose was not viable, due to the significant risk of data and meteorological equipment loss posed by vandalism, theft and severe weather. Between 1996 and 2004, a robust structure for housing the miniature Hobo® temperature logger (hereafter termed “Hobo pole”) was designed. During 2009 and 2010, a calibration experiment was conducted to investigate the relationship between *E. nitens* bud temperature (BudT), Hobo pole air temperature (HoboAT) and screen air temperature (ScrnAT). In the simple linear regression analyses for BudT on HoboAT, BudT on ScrnAT and ScrnAT on HoboAT, R^2 values were generally greater and SE values lower for mid-winter data than for mid-summer data. In mid-winter, BudT on HoboAT gave the highest R^2 value (0.99) and lowest SE value (0.49 ^{o}C) of all regressions. In mid-summer, BudT on HoboAT together with BudT on ScrnAT gave the highest R^2 value (0.98). On the basis of SE, BudT on ScrnAT (0.77 ^{o}C) ranked first, with BudT on HoboAT (0.79 ^{o}C) ranking second. Although these results could be seen as extremely promising, the degree of accuracy of representation of bud temperature by Hobo pole air temperature will likely only suffice for certain applications. Bud winter chill units calculated from modelled bud temperature data were suitably accurate. The use of loggers having greater temperature measurement accuracy, would likely contribute to a reduction in Hobo pole air temperature measurement error.

Keywords: chill unit, *Eucalyptus nitens*, flowering, remote weather station, temperate eucalypt, temperature logger, thermocouple

Introduction

The monitoring and measuring of climate variables in forestry field research trials in South Africa poses a major challenge. The trials are often in remote locations, and, based on experience, vandalism and/or theft of meteorological equipment poses a significant risk of data loss. Furthermore, in the case of temperate *Eucalyptus* site x flowering interaction research, trials are frequently located at high elevation (> 1200 m asl) sites in mountainous areas, where weather conditions are typically extreme. Frequent visits to such sites for equipment maintenance and downloading of data are often impractical, due to difficult road access and time and resource constraints. The potential costs of damage to/ loss of equipment are also considerable.

The South African Atlas of Climatology and Agrohydrology (Schulze 2007) is a comprehensive source of South African climate information. However, the data is fairly coarse, being based on 1 minute by 1 minute (~ 1.7 x 1.7 km) grids derived from historical data from weather stations distributed across the country. The data are therefore only suitable for use on a macro-climatic scale. Geographical factors such as latitude, elevation and distance from sea/ ocean typically affect the macro-climate of particular areas. On a finer micro-climatic scale, topographical factors such as aspect, slope and relief can exert major influences on air, soil and plant foliage temperatures (Schulze and Horan 2007, Sharma et al. 2010). Air temperature commonly exerts a major influence on reproductive growth and development in temperate woody perennials (Meilan 1997, Tooke and Battey 2010). Winter cold is a key environmental trigger of floral induction in temperate eucalypts (Moncur and Hasan 1994, Moncur and Boland 2000, Gardner et al. 2013). The flowering stimulus is generally perceived in the leaves of temperate plants (Bernier 1988) and subsequently translocated to the apical meristems (Aukerman and Amasino 1998, Tremblay and Colasanti 2006). Following induction, in temperate evergreen crops such as domestic olive (*Olea europea*), buds can undergo conditioning according to influences, both internal and external (environmental), in their path towards irreversible commitment to the formation of reproductive structures (Fabbri and Benelli 2000, Jordan 2006). Little is known about the precise physiological processes implicated in temperate eucalypt flowering.

Progress has been made in quantifying the different levels of winter chilling associated with floral induction and initiation of important temperate eucalypts such as *E. nitens* and *E. smithii* (Gardner and Bertling 2005, Gardner et al. 2013, Gardner et al. 2014). Agricultural chill models, such as the Dynamic Model (Fishman et al. 1987, Allan 2004), require hourly temperature data for accurate calculation of chill units (Erez et al. 1990, Seeley 1996). For the purpose of conducting temperate *Eucalyptus* site-flowering interaction research in South Africa, there was a need to develop a reliable and secure method of measuring and recording *Eucalyptus* bud temperature, at remote, high elevation forestry sites within the summer rainfall area.

In 1996, the Institute for Commercial Forestry Research (ICFR) established a series of *E. nitens* site x flowering interaction trials across a range of high elevation (> 1450 m asl), cool temperate (mean annual temperature ≤16 ^{o}C; Smith et al. 2005) sites in the summer rainfall area. There was a need to measure and record air and bud temperature in each of the trials. The Hobo® -Temp loggers (Onset Computer Corporation, Bourne, USA) had previously demonstrated suitability (adequate robustness, accuracy and memory capacity) for measurement and recording of air temperature and relative humidity via conventional methods (loggers housed in Stevenson Screens) in secure orchards located at research centres (Gardner 2003). The use of the Hobo thermistors for direct measurement of *Eucalyptus nitens* bud temperatures in high elevation field trials was found to be impractical. The thermistor measurements were sufficiently accurate, but the safety of the loggers and data was severely compromised due to the typical severity of weather (hail- and snow-storms) and risk of equipment theft at such sites (Gardner 2003). For the purpose of housing the Hobo loggers on-site in the 1996-established field trials, in the same year the ICFR designed an initial robust structure and embarked upon experimenting with a few different prototypes of the latter. In 1999, the Hobo H8 Family loggers were introduced. With the establishment of a further more extensive site x eucalypt flowering interaction trial series in 2003/2004 (Gardner et al. 2014), an improvement to the Hobo logger housing structure was carried out during 2004. This facilitated increased accuracy of representation of actual air temperature, without comprising the robustness of the logger housing. This particular design was used successfully in the monitoring of air temperature in all further ICFR site x flowering interaction research trials.

We describe the specifications of the 2004-improved Hobo logger housing (hereafter termed “Hobo pole”). The results of an investigation into the relationship between Hobo pole air temperature, *E. nitens* bud temperature and radiation screen (hereafter termed “screen”) air temperature, and the relationship between chill units derived from the three temperature variables, are also discussed.

Material and methods

Hobo pole design specifications

The 2004 Hobo pole basic design specifications are illustrated (Figure 1). The entire housing was constructed out of 1 mm-thick, mild steel. The exterior of the Hobo pole was painted with glossy white enamel paint.

On-site Hobo pole and Hobo logger installation specifications

The Hobo pole was installed upright in an open (un-shaded), grassed area alongside the northern boundary of each research block (Figure 1). On installation, the base plate was covered with a 100 mm-deep layer of poured concrete (15 kg dry weight), and this layer covered with topsoil to match the level of the surrounding soil surface. The Hobo logger was housed within a PVC pill-box within the cap of the Hobo pole, at a height of 1.3 m above ground level (WMO 2008). The Hobo pole and cap was cleaned and re-painted as necessary on a biannual basis.

Bud and air temperature calibration experiment

This experiment was conducted in and alongside an *E. nitens* grafted breeding seed orchard at the Mondi Mountain Home Research Centre in KwaZulu-Natal (29°34.070’ S; 30°16.467’ E; elevation 1133 m; mean annual air temperature (MAT_Air) 16.2 ℃). The orchard was protected by a covering of 20% shade rated (80% solar irradiance transmittance) hail-netting. Screen air temperature was measured at 1.3 m above ground level using a HygroClip® S3 probe (Rotronic AB, Bassersdorf, Switzerland) mounted in a 6-plate gill radiation shield supported on a white-painted wooden pole. The HygroClip probes were connected to a CR1000 datalogger and AM16/32 Channel Multiplexer (Campbell Scientific Inc., Logan, USA). Hobo pole air temperature was measured and logged using the procedure described previously. Thermistor accuracy and resolution data for the Hobo H8 series temperature logger are presented in Figure 2 (Borsari pers. comm.[1]). At 0 ^{o}C and 20 ^{o}C thermistor temperature, Hobo H8 temperature logger measurement accuracy (including sensor resolution error) was 0.75 ^{o}C and 0.70 ^{o}C, respectively. The Hobo and HygroClip logger poles were positioned 2.0 m apart in an open (un-shaded), grassed area alongside the northern boundary of the orchard. Within the orchard, bud temperatures were measured in two three-year old grafted *E. nitens* trees at 1.3 m above ground using copper-constantan thermocouple (TC) sensors, constructed from 24-gauge (insulated) wire, connected to the datalogger system. The calibration differences between the TCs and HygroClip were negligible. In each tree, one TC was inserted in a shoot located on the north side of the tree and the other in a shoot on the south side of the tree. Each TC was inserted beneath the bark within the cambial layer alongside an axillary bud located on the south-facing side of an actively growing, mature (woody) lateral shoot. The logged temperatures of the four buds were averaged to represent *E. nitens* bud temperature. The measured trees were situated approximately 10 m from one another and the Hobo and HygroClip logger poles. Each tree was surrounded by two buffer rows of *E. nitens* grafted trees of similar age. Bud temperature, Hobo pole air temperature and screen air temperature were logged on a 2-min interval basis, between 1 April 2009 and 30 September 2010. These data were later averaged to hourly temperature data. Chill units were calculated from hourly temperature data using the Dynamic Model, which assigns chill units termed Chilling Portions (CP) (Erez and Fishman 1998).

Statistical analysis

Simple linear regression analyses using Genstat® (2008) were carried out to determine the relationship between *E. nitens* bud temperature, Hobo pole air temperature and

[1] Borsari P. 2006. Onset Computer Corporation, Bourne, USA. www.onsetcomp.com

screen air temperature, and the relationship between chill units derived from the three temperature variables.

Results

Over a five year period of continuous recording of Hobo air temperature at 13 trial sites across the summer rainfall temperate forestry area, minimal problems were encountered with the functioning of the equipment (Gardner et al. 2014). At one site, in two consecutive years, the Hobo pole cap lock was broken and the loggers stolen. The vandalism problem at the particular site was eventually solved by semi-permanently welding the cap onto the pole upright. Missing data were patched using measured data from a nearby Hobo logger and pole.

Bud and air temperature calibration experiment

During 2009, mean hourly Hobo pole air temperature (14.77 ℃) and mean hourly screen air temperature (15.53 ℃) were 0.6 ℃ lower and 0.16 ℃ higher than mean hourly *E. nitens* bud temperature (15.37 ℃), respectively (Table 1) . During 2010, the differences were similar to 2009, with mean hourly Hobo pole air temperature (15.81℃) and mean hourly screen air temperature (16.57 ℃) b eing 0.62 ℃ lower and 0.14 ℃ higher than mean hourly bud temperature (16.43 ℃), respectively. Based on screen air temperature, the mean hourly temperature for 2009 (15.53 ℃) was more relevant to the MAT range of the 2003/2004 field trial series (13.8 - 15.5 ℃) (Gardner et al. 2014) than the mean hourly temperature for 2010 (16.57 ℃).

During mid-winter 2009 and mid-summer 2009/2010, Hobo pole air mean daily maximum temperatures (18.63 ℃ and 23.48 ℃, respec tively) and screen air mean daily maximum temperatures (18.35 ℃ and 23.41 ℃) were lower than *E. nitens* bud mean daily maximum temperatures (18.96 ℃ and 25.21 ℃) (Table 1). However, in the same mid-winter and mid-summer periods, Hobo pole air mean daily maximum temperatures were closer to bud mean daily maximum temperatures than were screen air mean daily maximum temperatures.

During mid-winter 2009 and mid-summer 2009/2010, Hobo pole air mean daily minimum temperatures (4.42 ℃ and 13.0 ℃, respectively) were lower than *E. nitens* bud mean minimum temperatures (5.32 ℃ and 14.15 ℃), whereas screen air mean daily minimum temperatures (6.64 ℃ and 14.18 ℃) were higher than bud mean minimum temperatures (Table 1). During winter, Hobo pole air mean daily minimum temperature was closer to bud mean daily minimum temperature than was screen air mean daily minimum temperature, but in mid-summer, screen air mean daily minimum temperature was closer to bud mean daily minimum temperature than was Hobo air mean daily minimum temperature (Table 1).

During mid-winter 2009, Hobo pole air mean diurnal temperature range (14.21 ℃) differed from that of *E. nitens* bud (13.64 ℃) by 0.57 ℃, whereas screen air mean diurnal temperature range (11.71 ℃) differed from that of bud by 1.93℃ (Table 1). A similar trend was observed in mid-summer 2009/2010, where Hobo pole air mean diurnal temperature range (10.48 ℃) differed from that of *E. nitens* bud (11.06 ℃) by 0.58 ℃, and screen air mean diurnal range (9.23 ℃) differed from bud by 1.83 ℃. The mean diurnal temperature waves for mid-winter 2009 and mid-summer 2009/ 2010 (Figures 3 and 4, respectively) illustrated seasonal effects regarding the relationships between *E. nitens* bud temperature, Hobo pole air temperature and screen air temperature. During mid-winter 2009, Hobo pole air mean daily maximum and minimum temperatures were on average 0.33 ℃ and 0.9 ℃ lower than bud mean daily maximum and minimum temperatures. In mid-summer, Hobo pole air mean daily maximum and minimum temperatures were on average 1.73 ℃ and 1. 16 ℃ lower than *E. nitens* bud mean daily maximum and minimum temperatures. Alternatively, during mid-winter 2009, screen air mean daily maximum and minimum temperatures were on average 0.61 ℃ lower and 1.32 ℃ higher than bud mean daily maximum and minimum temperatures, whereas during mid-summer, screen air mean daily maximum and minimum temperatures were 1.8 ℃ and 0.03 ℃ higher than *E. nitens* bud mean daily maximum and minimum temperatures. Thus, for increased calibration accuracy, separate models would need to be developed for winter and summer temperature data. A window period of a few weeks could be used for this purpose.

The results of simple linear regression analyses (including best fit models) for *E. nitens* bud temperature on Hobo pole air temperature, *E. nitens* bud temperature on screen air temperature, and screen air temperature on Hobo pole air temperature during mid-winter (2009) and mid-summer (2009/2010) are presented (Figures 5 to 10). In all three of the latter relationships, higher percentage variances were accounted for during mid-winter than in mid-summer (Table 2). In mid-winter, the regression of bud temperature on Hobo pole air temperature produced the highest R^2 value (0.99) of all regressions. In mid-summer, the regressions of bud temperature on Hobo pole air temperature and bud temperature on screen air temperature produced the highest R^2 value of 0.98.

Accumulated winter (April to September) chill units were calculated for *E. nitens* bud temperature, Hobo pole air temperature and screen air temperature at Mountain Home during 2009 and 2010 (Figure 11). According to 2009 and 2010 winter CP totals for bud temperature (54.8 CP and 39.6 CP), Hobo pole air temperature (55.3 CP and 41.6 CP) and screen air temperature (56.6 CP and 39.6 CP), the 2009 winter was markedly cooler than the 2010 winter (Table 3). In 2009, Hobo pole air temperature and screen air temperature accumulated 0.5 CP and 1.8 CP more than bud temperature (0.91% and 3.3% respective differences) (Table 3 and Figure 11). During the warmer 2010 winter, Hobo pole air temperature began accumulating chill units 13 days prior to chill unit accumulation commencement by bud temperature and screen air temperature on 24 April (Table 3). The additional 3.4 CP accumulated by Hobo pole air temperature in this 13-day period were carried through the end of winter total (41.6 CP).

Discussion

Based on the minimal problems encountered in-field over a period of five years, the Hobo logger/Hobo pole combination appears to offer one practical method of monitoring *Eucalyptus* bud temperature at remote, high elevation forestry sites in South Africa.

Bud and air temperature calibration

The lower temperature measurement accuracy capability of the Hobo H8 logger compared to that of the HygroClip should be considered in all deductions concerning Hobo logger temperature data. On the basis of accuracy of measurement, the Hobo pole yielded satisfactory results in the bud and air temperature calibration experiment.

The greatest disparity between Hobo pole air and screen air temperatures occurred during nighttime. Mean daily minimum temperatures for Hobo pole air were slightly lower than those for screen air, in both the winter and summer months. The most likely reason for this was the greater thermal conductivity of the metal Hobo pole compared to that of air. The disparity between Hobo pole air and *E. nitens* bud mean daily temperature minima was substantially less than that between Hobo pole air and screen air mean daily temperature minima. Substituting the steel material of the Hobo pole structure with plastic or wood material would likely reduce the bias between Hobo pole air and screen air temperature (WMO 2008). However, based on experience, the safety of the housed meteorological equipment and stored data would be substantially compromised.

Based on the 2009 mid-winter and 2009/2010 mid-summer data, Hobo pole air mean daily maximum and minimum temperatures and diurnal temperature ranges were closer to the same temperature criteria for *E. nitens* bud than were screen air mean daily maximum and minimum temperatures and diurnal temperature ranges (Table 1). During mid-winter, Hobo pole air mean hourly temperature correlated perfectly with bud mean hourly temperature, whereas screen air mean hourly temperature did not. During mid-summer, both Hobo pole and screen air temperatures (15.45 °C and 15.96 °C) underestimated bud mean hourly temperature (18.98 °C) by fairly large margins (Table 1). The developed seasonal regression equations provide a means of improving the accuracy of *E. nitens* bud and screen air temperature data modelled from Hobo pole air temperature data. All regression analyses carried out in this study utilized hourly temperature data calculated from 2-min interval data in order to increase the accuracy of the developed relationships. Hourly Hobo pole air temperature data calculated from coarser interval temperature data may yield a less accurate result, and this would need investigating, so that corrections can be made where necessary.

Based on Mountain Home 2009 winter temperature data, total CP (chill units of the Dynamic Model) modelled for Hobo pole air temperature were closer to that for *E. nitens* bud temperature than for screen air temperature (Table 3). A possible reason for this was the smaller winter diurnal temperature ranges of screen air temperature compared to that for Hobo pole air and *E. nitens* bud temperature, as indicated by the hourly means for 2009 and 2010 (Table 1). High daily temperature maxima and diurnal temperature amplitudes during winter are generally associated with chilling negation and lowered levels of chilling accumulation (Couvillon and Erez 1985, Seeley 1996), particularly in areas with mild winters (Erez 2000). This is further indicative of the importance of using bud temperature, rather than air temperature, in studies such as this. The additional 3.4 CP accumulated by Hobo pole air temperature (41.6 CP total) over that accumulated by bud or screen air temperature (39.6 CP total) during 2010 winter is possible further indication that the Hobo pole method of monitoring bud temperature is most suitable for high elevation (> 1200 m asl) sites in the summer rainfall temperate eucalypt forestry areas.

Conclusions

A practical method of monitoring *Eucalyptus* bud temperature with a satisfactory level of accuracy at remote, high elevation sites in the summer rainfall forestry areas of South Africa was developed. The Hobo pole/ logger combination developed demonstrated a high level of robustness, i.e. the ability to withstand inclement weather and general lack of vulnerability to vandalism. Through application of the separate temperature regression models developed for winter and summer temperature data, Hobo pole air temperature has the ability to serve as a surrogate to bud temperature. The use of miniature loggers having greater temperature measurement accuracy would likely contribute to a reduction in Hobo pole air temperature measurement error and therefore improved estimates of bud temperature.

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Table 1: Mean daily maximum, daily minimum, diurnal range and hourly *E. nitens* bud, Hobo pole air and screen air temperatures for 2009 and 2010 at Mountain Home.

	Annual[1]			Mid-winter[2]			Mid-summer[3]		
2009	BudT	HoboAT	ScrnAT	BudT	HoboAT	ScrnAT	BudT	HoboAT	ScrnAT
Mean daily maximum (°C)	23.21	22.42	21.87	18.96	18.63	18.35	25.21	23.48	23.41
Mean daily minimum (°C)	9.75	8.90	10.57	5.32	4.42	6.64	14.15	13.00	14.18
Mean diurnal range (°C)	13.46	13.52	11.30	13.64	14.21	11.71	11.06	10.48	9.23
Mean hourly (°C)	15.37	14.77	15.53	11.25	11.25	12.59	18.98	15.45	15.96
2010									
Mean daily maximum (°C)	24.58	24.16	23.22	19.83	19.60	19.25	25.28	24.02	23.21
Mean daily minimum (°C)	10.62	9.66	11.42	5.10	3.45	6.06	13.97	13.40	14.64
Mean diurnal range (°C)	13.96	14.50	11.80	14.73	16.15	13.19	11.31	10.62	8.57
Mean hourly (°C)	16.43	15.81	16.57	11.41	10.53	11.96	18.50	17.87	18.36

[1] Means for the entire year (1 January to 31 December)

[2] Means for the period 7 June to 5 July

[3] Means for the periods 7 December 2009 to 4 January 2010 (termed 2009) and 8 December 2010 to 5 January 2011 (termed 2010)

BudT = *Eucalyptus nitens* bud temperature; HoboAT = Hobo pole air temperature; ScrnAT = screen air temperature

Table 2: R-squared (R^2) values and estimates of the standard error of observation (SE) from the 2009 mid-winter and 2009/2010 mid-summer hourly *Eucalyptus nitens* bud, Hobo pole air and screen air temperature simple linear regressions.

Response/ Explanatory variable	Mid-winter[1]		Mid-summer[2]	
	R^2	SE (°C)	R^2	SE (°C)
BudT on HoboAT	0.99	0.49	0.98	0.79
BudT on ScrnAT	0.98	0.67	0.98	0.77
ScrnAT on HoboAT	0.98	0.72	0.97	0.86

[1]The period 7 June 2009 to 5 July 2009
[2]The period 7 December 2009 to 4 January 2010
BudT = *Eucalyptus nitens* bud temperature; HoboAT = Hobo pole air temperature; ScrnAT = screen air temperature

Table 3: Chilling Portion (CP) accumulation for the variables *Eucalyptus nitens* bud temperature, Hobo pole air temperature and screen air temperature at Mountain Home during 2009 and 2010.

	BudT		HoboAT		ScrnAT	
	Commence[1]	Total[2]	Commence[1]	Total[2]	Commence[1]	Total[2]
Year	CP	CP	CP	CP	CP	CP
2009	21 April	54.8	21 April	55.3	21 April	56.6
2010	24 April	39.6	11 April	41.6	24 April	39.6

[1]Date of commencement of chill unit accumulation
[2]Total CPs accumulated between the date of commencement and 30 September each year
BudT = *Eucalyptus nitens* bud temperature; HoboAT = Hobo pole air temperature; ScrnAT = screen air temperature

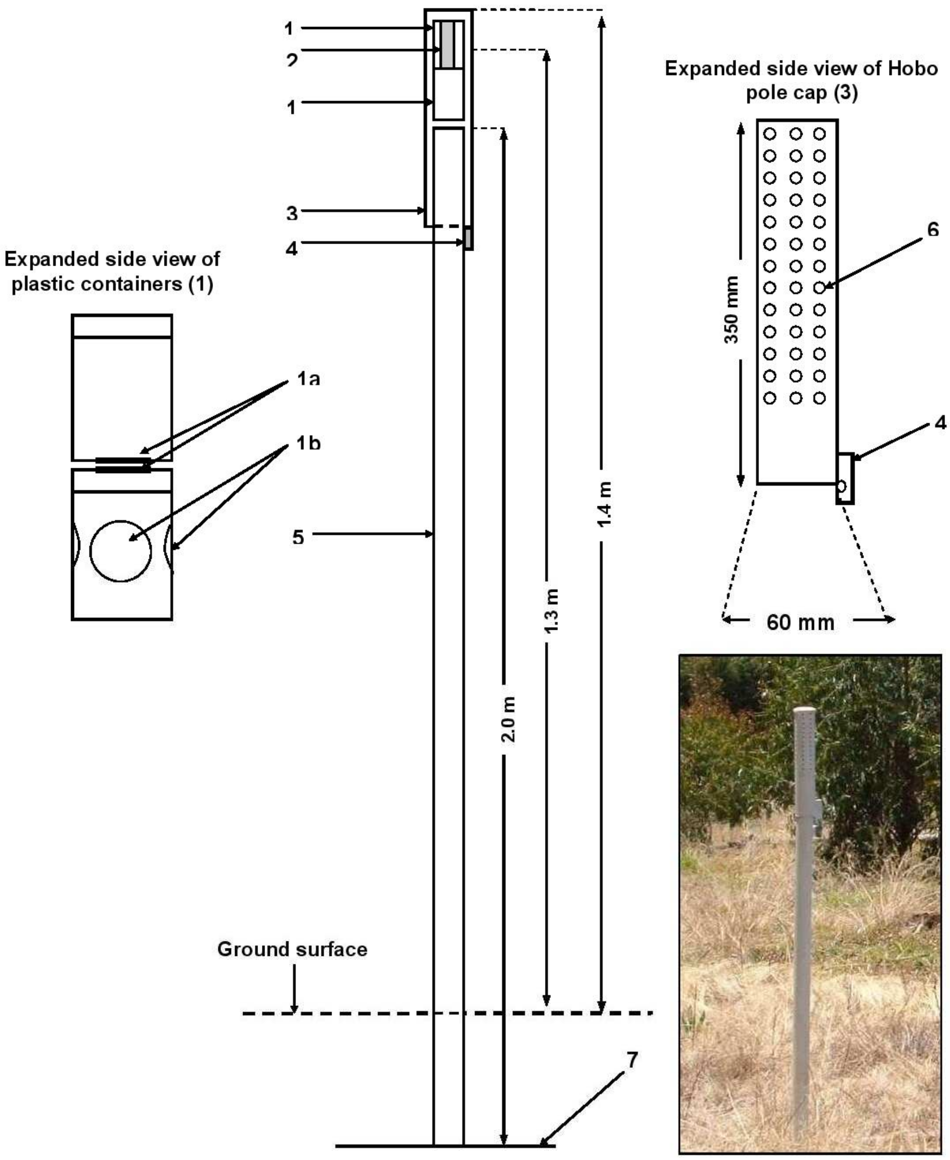

Figure 1: Basic design specifications (not to scale) of the structure, termed "Hobo pole", used to house the Hobo H8 series miniature temperature loggers. [1 = PVC (0.8 mm thick) cylindrical container with screw-top, 80 mm high x 50 mm diameter (Ø); 1a = horizontal circular apertures, 25 mm Ø; 1b = four vertical circular apertures, 25 mm Ø; 2 = Hobo H8 series logger; 3 = Hobo pole "cap"; 4 = cap locking pin; 5 = Hobo pole main upright, 50 mm outside Ø; 6 = ventilator holes, 15.6 mm x 24.8 mm apart, 9 mm Ø; 7 = Hobo pole base plate, 200 mm x 200 mm]. Insert shows positioning of Hobo pole alongside field trial block

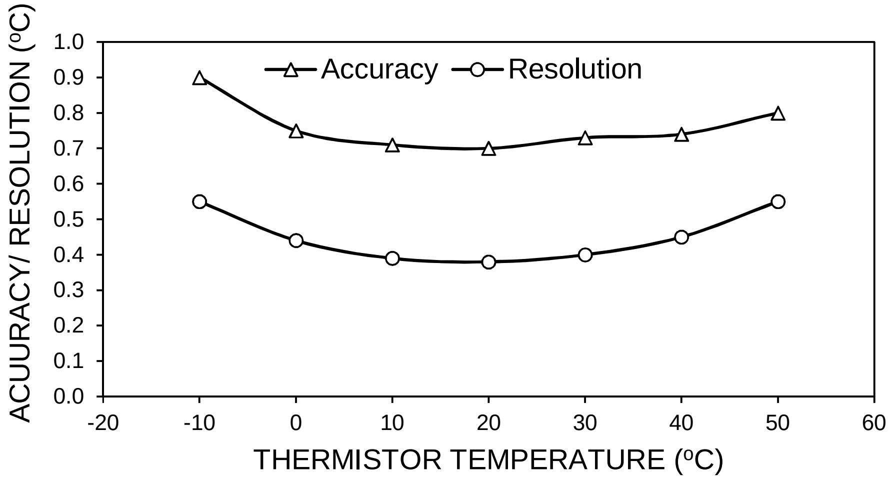

Figure 2: Temperature accuracy and resolution of Hobo H8 series miniature temperature logger

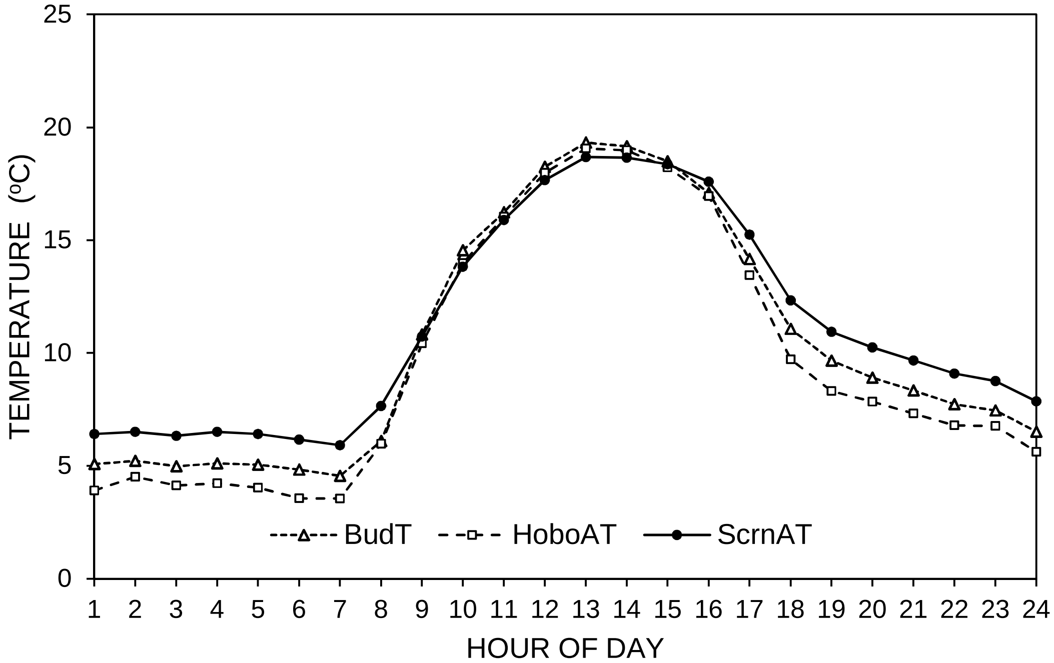

Figure 3: Mean mid-winter (7 June to 5 July) diurnal temperature variation at Mountain Home during 2009. BudT= *E. nitens* bud temperature; HoboAT = Hobo pole air temperature; ScrnAT = screen air temperature. Mean sunrise and sunset times for this period were 06:51 and 17:05, respectively (SAAO 2013)

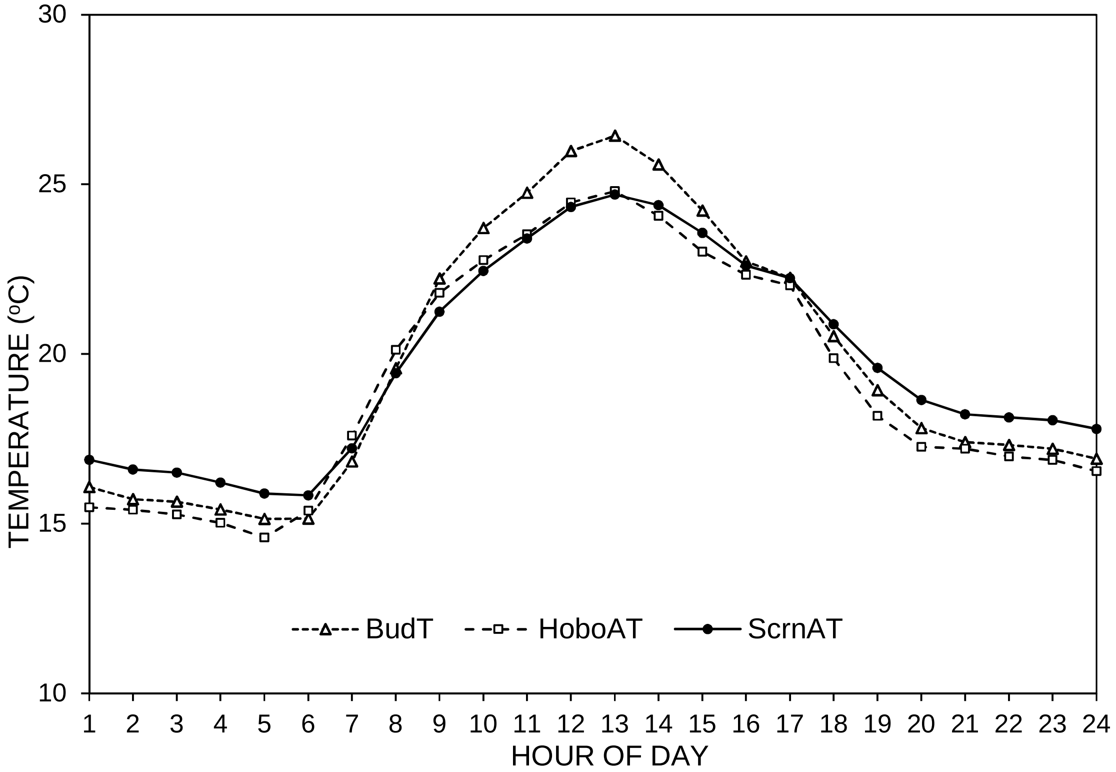

Figure 4: Mean mid-summer (7 December 2009 to 4 January 2010) diurnal temperature variation at Mountain Home. BudT = *E. nitens* bud temperature; HoboAT = Hobo pole air temperature; ScrnAT = screen air temperature. Mean sunrise and sunset times for this period were 04:52 and 18:57, respectively

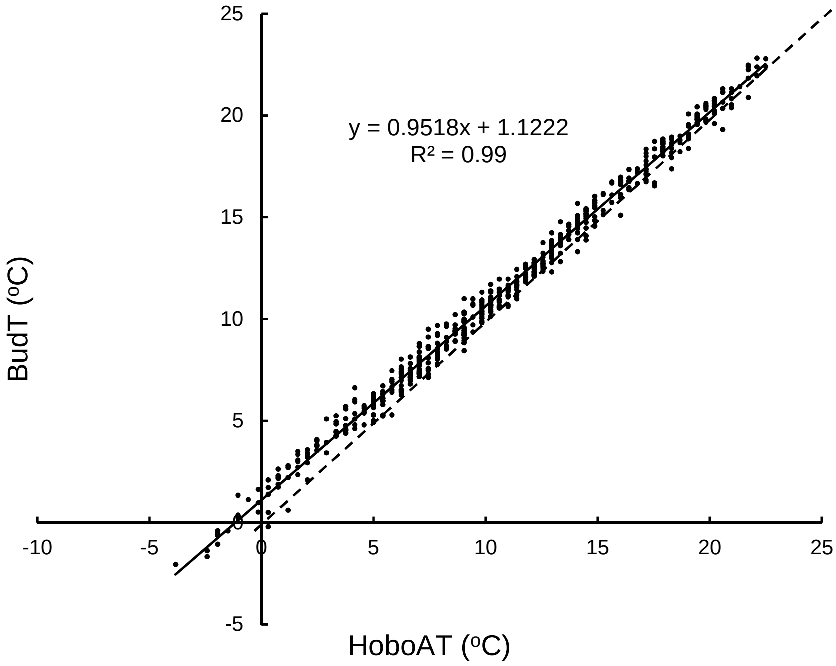

Figure 5: Relationship between *E. nitens* bud temperature and Hobo pole air temperature during mid-winter 2009 (7 June to 5 July) at Mountain Home. BudT = *E. nitens* bud temperature; HoboAT = Hobo pole air temperature. Temperatures were logged on a 2-min interval basis and these data averaged to hourly data and then plotted. Dashed line represents 1:1 relationship

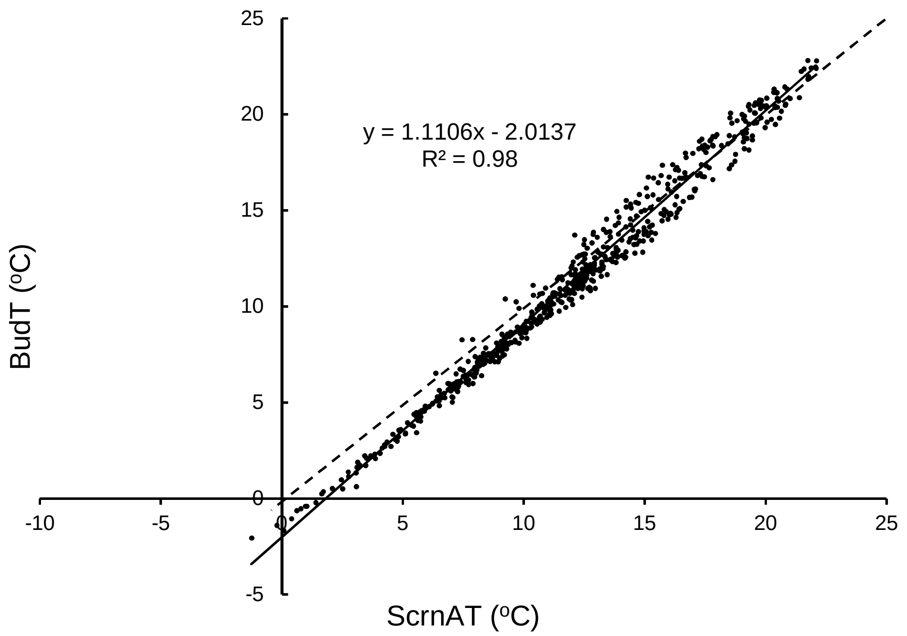

Figure 6: Relationship between *E. nitens* bud temperature and screen air temperature during mid-winter 2009 (7 June to 5 July) at Mountain Home. BudT= *E. nitens* bud temperature; ScrnAT = screen air temperature. Temperatures were logged on a 2-min interval basis. These data were averaged to hourly data and then plotted. Dashed line represents 1:1 relationship

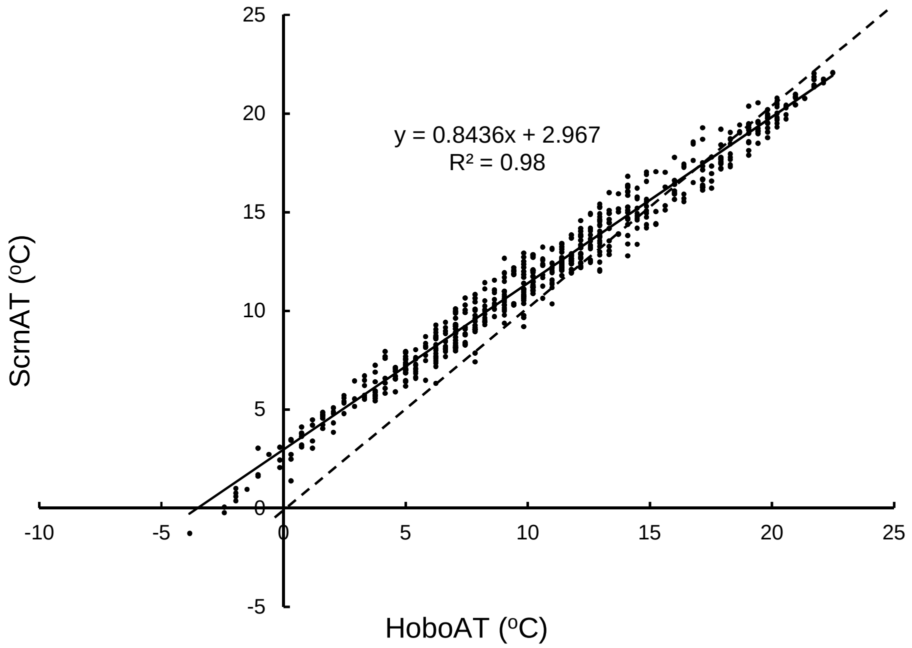

Figure 7: Relationship between screen air temperature and Hobo pole air temperature during mid-winter 2009 (7 June to 5 July) at Mountain Home. ScrnAT= screen air temperature; HoboAT = Hobo pole air temperature. Temperatures were logged on a 2-min interval basis. These data were averaged to hourly data and then plotted. Dashed line represents 1:1 relationship

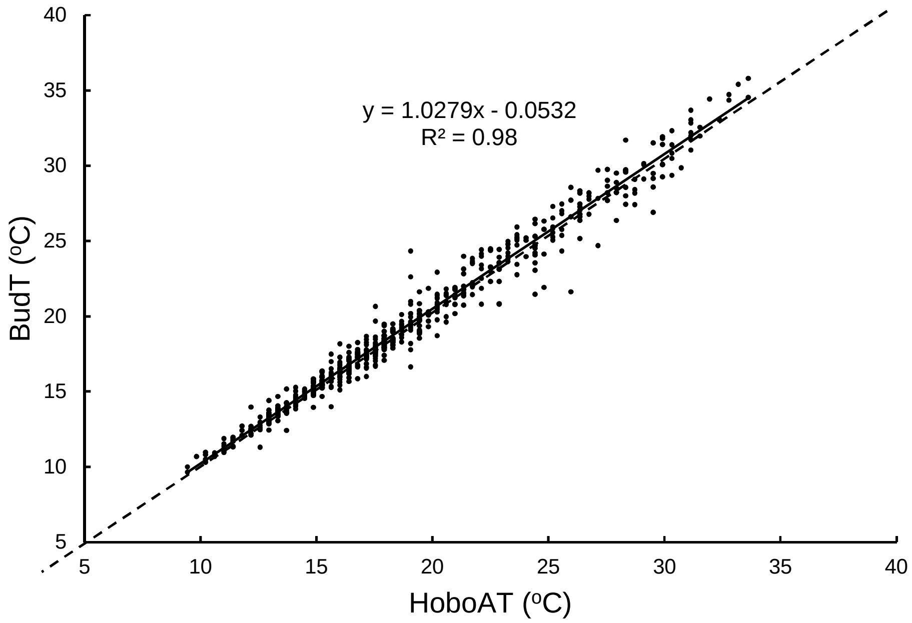

Figure 8: Relationship between *E. nitens* bud temperature and Hobo pole air temperature during mid-summer 2009/2010 (7 December to 4 January) at Mountain Home. BudT = *E. nitens* bud temperature; HoboAT = Hobo pole air temperature. Temperatures were logged on a 2-min interval basis. These data were averaged to hourly data and then plotted.Dashed line represents 1:1 relationship

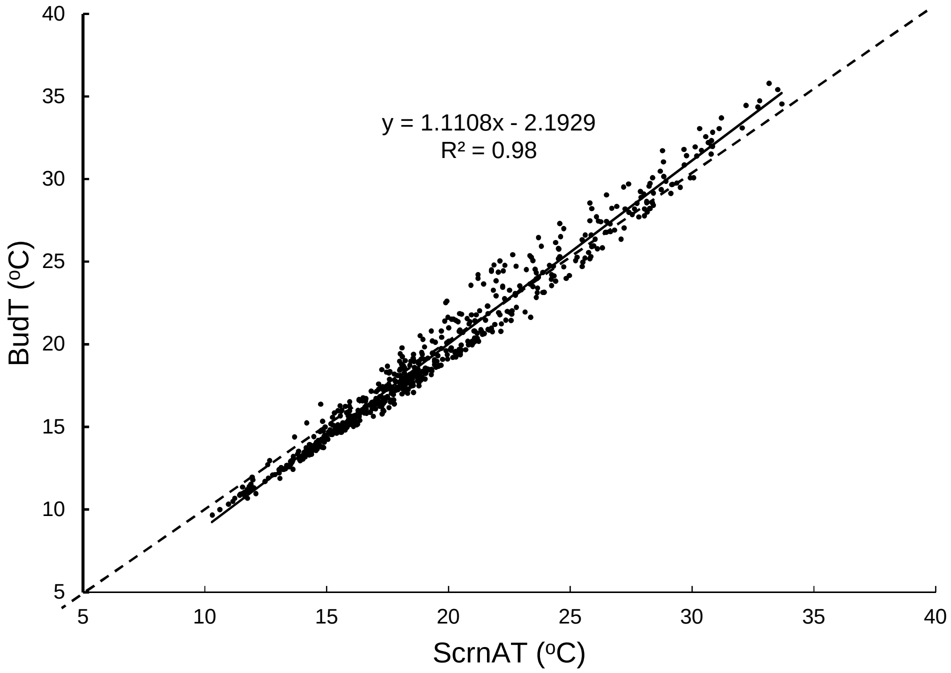

Figure 9: Relationship between *E. nitens* bud temperature and screen air temperature during mid-summer 2009/2010 (7 December to 4 January) at Mountain Home. BudT = *E. nitens* bud temperature; ScrnAT = screen air temperature. Temperatures were logged on a 2-min interval basis. These data were averaged to hourly data and then plotted. Dashed line represents 1:1 relationship

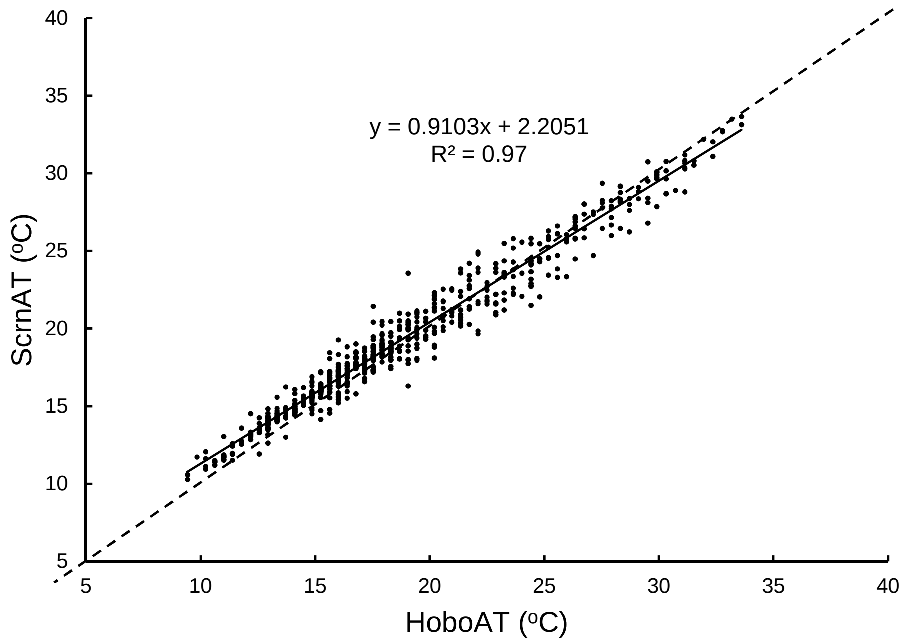

Figure 10: Relationship between screen air temperature and Hobo pole air temperature during mid-summer 2009/2010 (7 December to 4 January) at Mountain Home. ScrnAT = screen air temperature; HoboAT = Hobo pole air temperature. Temperatures were logged on a 2-min interval basis. These data were averaged to hourly data and then plotted. Dashed line represents 1:1 relationship

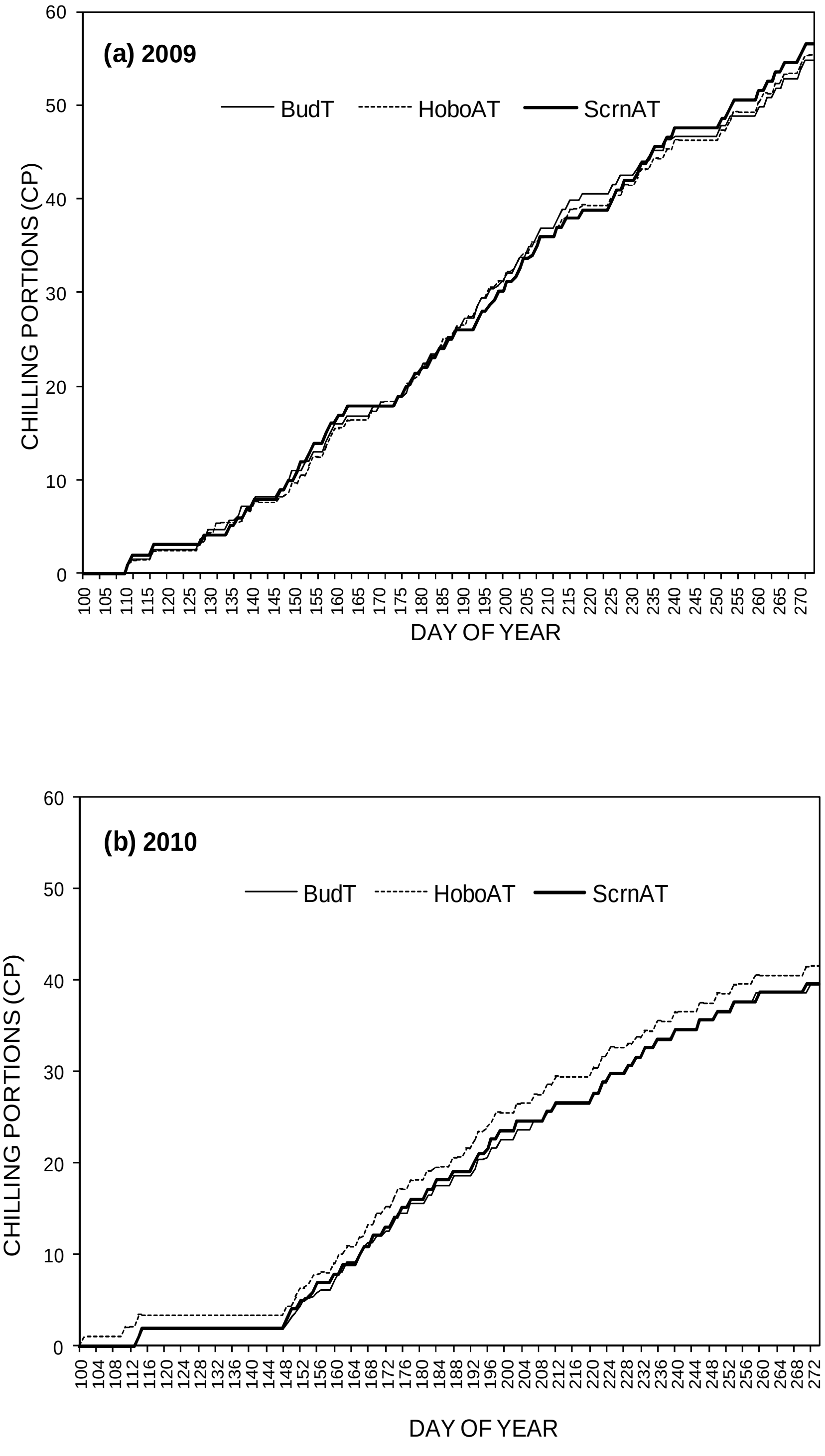

Figure 11: Accumulation of Chilling Portions (CP) for *E. nitens* bud temperature, Hobo pole air temperature and screen air temperature at Mountain Home during (a) 2009 and (b) 2010. BudT= CP for *E. nitens* bud temperature; HoboAT = CP for Hobo pole air temperature; ScrnAT = CP for screen air temperature

CHAPTER 4

Defining site requirements for maximal floral bud production in *Eucalyptus nitens* in South Africa

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Abstract

In 2003/ 2004, a series of *Eucalyptus nitens* flowering trials were established across a range of high elevation sites within the summer rainfall forestry areas of South Africa. The interaction between a range of climate and landform factors and paclobutrazol application on *E. nitens* floral bud production was investigated. The main aim was to define *E. nitens* site requirements for optimal floral bud crop production. At five years, paclobutrazol application reduced *E. nitens* tree height (mean of four grafted clones) by 30%. Regardless of whether PBZ was applied or not, *E. nitens* floral bud production varied markedly across sites, in both fifth and sixth crop years. Of all landform factors, slope aspect had the greatest influence on *E. nitens* floral bud crop production. Within the applied elevation and MAT ranges (> 1550 m asl and ≤ 15.5 ℃, respectively), south-west, south and west-facing slopes (in order of decreasing effectiveness) exerted a strong positive effect on *E. nitens* floral bud production, regardless of whether PBZ was applied or not. The specific environmental factors associated with the positive reproductive growth response in *E. nitens* to these slope aspects remain to be determined. The results of the investigations indicated that, through careful site selection, the dependency on paclobutrazol to achieve satisfactory floral bud crop production in *E. nitens* can be substantially reduced.

Keywords

Chill models, cold-tolerant eucalypt, *E. nitens,* floral induction, flowering, seed orchard, temperate eucalypt, winter chilling

Introduction

Eucalyptus nitens is an important species planted for commercial wood production in the cool temperate, summer rainfall forestry areas of South Africa (Smith et al. 2005, Swain et al. 2014). The species is renowned for high levels of cold, frost and snow tolerance, and excellent wood and pulping properties (Gardner 2001, Swain and Gardner 2003). *Eucalyptus nitens* is difficult to propagate vegetatively, and to date all commercial plantings of the species have comprised seedlings. The South African forestry industry is highly dependent on the production of high quality, locally-bred *E. nitens* seed for the purpose. Seedling trees of *E. nitens* rarely flower before the age of eight years, and even then, annual floral bud crops produced are sparse and erratic (Jones 2002, Swain and Gardner 2003). The latter traits hinder *E. nitens* genetic improvement and commercial seed production (Moncur and Boland 2000, Swain and Gardner 2002).

Eucalyptus nitens flowering controls are not fully understood. Neither photoperiod nor drought stress exerted a noticeable effect on *E. nitens* floral induction (Gardner 2003, Hasan and Reid 1995, Moncur 1998). A certain period, or amount, of winter cold is a pre-requisite for the species to produce flower buds (Moncur and Hasan 1994, Williams et al. 2003). In South Africa, *E. nitens* was noticed to flower more prolifically and consistently at exposed, rather than low-lying, sites in the high elevation (> 1200 m asl) areas where the species is grown commercially (Gardner 2003). The sites suitable for *E. nitens* timber production are not necessarily those suitable for floral bud and seed crop production in the same species (Gardner et al. 2013).

Eucalyptus nitens has a minimum winter chilling requirement of approximately 40 Chilling Portions (CP, the unit of the Dynamic Model (Erez and Fishman 1998, Fishman et al. 1987)) for floral induction (Gardner and Bertling 2005, Gardner et al. 2013). *Eucalyptus nitens* genotypes appear to differ in chilling requirement for floral bud production, both across and within provenance (Gardner 2003, Gardner et al. 2013). This phenomenon resembles the genotypical/ cultivar chilling requirement differences for endodormancy release occurring in temperate fruit crops (George and Erez 2000, Powell et al. 1986, Seeley 1996). In *E. nitens*, paclobutrazol (PBZ) has the potential to significantly reduce time to first flowering in seedlings and increase overall flower and seed crop production (Potts et al. 2007, Williams et al. 2003), depending on whether trees have been exposed to sufficient winter cold or not. PBZ is highly promotive of flowering in *E. nitens* at medium to high winter chilling levels, i.e. between 55 and 90 CP (Gardner and Bertling 2005, Gardner et al. 2013). PBZ is used almost routinely in temperate eucalypt seed orchards for the promotion of precocious and abundant flowering (Gardner et al. 2013, Hamilton et al. 2008). Disadvantages associated with the use of PBZ include the recalcitrant nature and persistence of the chemical in soils (Fletcher et al. 2000, Jackson et al. 1996), and the high cost of its application. Therefore, investigating ways of lessening the dependency on PBZ to achieve satisfactory flowering and seed crop production in *E. nitens* is warranted.

During the summer of 2003/ 2004, an *E. nitens* site x PBZ x flowering interaction trial series was established across a wide range of high elevation, high chill temperate eucalypt forestry sites in the summer rainfall area of South Africa. The main objective was to explore the interactive effect of a range of climate and landscape factors (particularly those associated with air temperature) and PBZ on *E. nitens* floral bud production. The main aim was to define the site requirements for maximal floral bud production in *E. nitens*. Such information would allow future informed siting of *E. nitens* orchards, and possibly lead to a reduced dependency on PBZ to achieve satisfactory flowering and seed production levels in the same species. Furthermore, the data could be used in the development of a GIS (Geographical Information System) tool for the prediction of optimal *E. nitens* flowering sites in the summer rainfall area.

This paper discusses the outcomes of the research carried out between 2003 and 2011.

Material and methods

The trial series was established across 12 sites within the summer rainfall forestry region, between December 2003 and April 2004.

Site conditions

The latitudinal range of trial sites covered almost the entire span of the summer rainfall temperate eucalypt forestry belt, i.e. from 29°54' S in the south near Maclear, to 25°02' S in the north near Sabie (Table 1). The primary site selection criteria were mean annual air temperature (MAT) between 13.5 and 16.0 ℃, elevation of more than 1550 and less than 1850 m (asl) and soil depth ≥ 0.8 m. In South Africa, the altitudinal and MAT criteria for optimum *E. nitens* vegetative growth is 1350-1900 m and 14-16 ℃ (Smith et al. 2005, Swain and Gardner 2003). Water stress is known to stimulate flower bud production in a number of woody angiosperms (Davenport 1990, George and Erez 2000, Meilan 1997), and therefore drought stress was minimized as a factor in floral bud production by selecting sites with high mean annual precipitation (MAP) (> 840 mm) and uniformly deep soils (> 0.8 m) (Table 1) (Darrow 1994, Schönau and Grey 1987). Within the above MAT and altitudinal confines, sites were selected for maximum likelihood of high levels of annual winter chilling, i.e. sites with high levels of exposure, relative relief and steepness of slope (Gardner and Bertling 2005, Schulze 2007a, Schulze and Horan 2007). The altitudinal range of this trial series (1568-1828 m) was substantially narrower than that of the 1996 trial series (1465-1995 m), although the MAT ranges were similar (13.8-15.5 ℃ versus 13.6-15.2 ℃) (Gardner and Ber tling 2005). Most of the sites selected were within the optimal *E. nitens* vegetative growth range (Smith et al. 2005, Swain and Gardner 2003). Following trial establishment, regular uniform weed control was carried across all sites on a biannual basis.

Plant material

In August 2003, scions from four genotypes (ICFR breeding selections) (Table 2) were grafted onto six-month old *E. nitens* seedlings grown from South African improved commercial seed. The scions were collected from reproductively mature (nine years or older) grafted ramets in the ICFR and Sappi clonal seed orchards (CSOs) at Mountain Home and Tweedie, respectively. *Eucalyptus nitens* was represented by two (scion) provenances forming part of the South African breeding populations (Swain and Gardner 2002). Each provenance was represented by two (scion) genotypes differing in flowering potential (Table 3).

Experimental design and treatments

A 2 x 4 factorial experiment consisting of 32 grafted trees, with treatments replicated twice, was laid out as a split plot design (Gomez and Gomez 1984). "PBZ application" (PBZ0 = nil PBZ applied (control), PBZ1 = PBZ soil drench applied) was assigned as whole plot Factor A, and "Clone (scion genotype)" (four individual clones) as sub-plot Factor B (Table 3). Each experimental unit consisted of two trees (2-tree contiguous plot) with both trees being measured. Trees were spaced 4 m (across slope) x 5 m apart. Due to the sloped nature of most of the sites, even though PBZ is known to have low mobility in soils (Jackson et al. 1996, Reid et al. 1995), the whole plots (PBZ treatments) were laid out across the slope to exclude possible contamination of the PBZ0 treatment plots by PBZ from the PBZ1 plots. Each experiment was surrounded by two buffer rows of grafted trees of similar species.

Paclobutrazol treatment

The PBZ treatment was applied during late summer 2006 (March-April), approximately two years after trial establishment. A suspension of Avocet® (formulation 250 g L^{-1} PBZ, Fine Agrochemicals Ltd, Whittington, UK) was applied as a soil drench at a rate of 0.025 g a.i. per mm basal stem circumference (BSC) (Gardner and Bertling 2005, Gardner et al. 2013). The latter measurement was taken at the narrowest point between graft union and lowest primary lateral (branch) on the scion. Each calculated PBZ dose was dispersed in 5.0 L water and applied evenly to the soil surface in a 1.0 m radius around the base of the tree. Prior to application, a 150 mm high "wall" was created around each tree at about 1.25 m radius to prevent possible run-off of the PBZ / water suspension due to steepness of slope or heavy rain within the ensuing few days.

Data collection

Air temperature measurement

Air temperature was measured on an hourly basis at each of the experimental sites, from time of establishment to time of completion of the experiments. Because of the risk of vandalism and/ or theft of meteorological equipment at the remote trial localities, a robust data logger housing (hereafter termed "Hobo pole") was used to house the

Hobo® H8 series miniature temperature loggers (Onset Computer Corporation, Bourne, USA). The Hobo pole was designed, tested and refined by the ICFR over a period of eight years, between 1996 and 2004 (Gardner et al. 2014). At each site, the Hobo pole was positioned in a non-shaded, grassed area 10 m from the northern boundary (outer surround row) of the trial. Calibration curves developed were used to model hourly radiation screen (hereafter termed “screen”) air temperature data from hourly Hobo pole air temperature data (refer Chapter 3).

Chill unit calculation

Of three popular models tested, the Dynamic Model (Erez and Fishman 1998, Fishman et al. 1987) quantified *E. nitens* winter chilling accumulation most accurately across a range of South African summer rainfall temperate eucalypt plantation forestry sites (Gardner and Bertling 2005). The Dynamic Model gives a more accurate account of the effectiveness of winter chilling in areas with mild winters than the Utah Model (Linsley-Noakes and Allan 1994, Richardson et al. 1974). However, the Daily Positive Utah Chill Unit Model, a modification of the Utah Model, showed a high degree of accuracy in calculating deciduous fruit tree crop chilling accumulation in warmer growing areas of South Africa (Linsley-Noakes et al. 1994). The latter model assigns Daily Positive Utah Chill Units (DPCUs), and long-term mean DPCUs for May to September are available on a 1 minute by 1 minute grid basis for the whole of South Africa (Schulze 2007a). Comparison of the results obtained for screen air temperature data measured at Mountain Home over three seasons (2008 - 2010) using the Dynamic and the Daily Positive Utah Chill Unit Models indicated that the ratio between the two (CP:DPCU) is approximately 1:17 (R. Gardner, unpublished data, 2014). In the main deciduous fruit-growing areas of South Africa, winter chill units generally begin accumulating in May (Schulze and Maharaj 2007a). However, in the main temperate eucalypt plantation forestry areas of the country which are located predominantly in the summer rainfall region, chill units generally begin accumulating in April (Gardner 2003, Gardner et al. 2013). Winter daily CP and DPCU totals were calculated for each site using modelled hourly screen air temperature data (Gardner et al. 2014).

Vegetative growth measurement

Tree height was measured on trial planting anniversary date each year following trial establishment for five years.

Reproductive growth assessment

A flowchart illustrating the sequence of events pertaining to *E. nitens* annual floral bud crop production and assessments between 2004 and 2010 is presented in Figure 1. Floral bud abundance was assessed on an individual tree basis in February and May/ June of each year. In the summer rainfall area, *E. nitens* inflorescence buds emerge between October and late January, the timing of emergence depending on long- and short-term environmental conditions (R. Gardner, unpublished data, 2014). By mid-May/ early June, the majority of the involucral bracts are shed and the umbels (inflorescences) with their individual flower buds in pre-anthesis or early anthesis stage are at their most conspicuous. Individual tree floral bud crop was estimated using the following scoring system: 0 = no umbels; 1 = very light crop, 25% or less of the secondary laterals bearing one or more umbels (secondary laterals defined as branches originating from primary stems); 2 = light crop, between 26 and 50% of secondary laterals bearing one or more umbels; 3 = moderate crop, between 51 and 75% of secondary laterals bearing one or more umbels; 4 = heavy crop, between 76 and 100% of secondary laterals bearing one or more umbels (Gardner and Bertling 2005).

Statistical analysis

Vegetative and reproductive growth assessments

Statistical analyses were performed to investigate the effect of site, PBZ and clone on tree height at five years and the fifth and sixth floral bud crops. As illustrated in Figure 1, the fifth and sixth floral bud crops were those initiated in 2008 and 2009 (respectively) and scored in 2009 and 2010 (respectively). The reasons why the previous years' floral bud crops were not included in the analyses are as follows:

- The possible confounding effects of trial establishment differences, e.g. the different planting and blanking dates, on analytical results were likely to be avoided

- The possible negative effect of transmission of juvenile (reproductive immaturity) signal from seedling rootstock to reproductively mature scion on flowering potential of the scion would likely be minimized (Gardner and Bertling 2005, Siniscalco and Pavelettoni 1988).

The Chamisso site was excluded from the analysis of height growth (at five years) but included for the analysis for floral bud crop (fifth and sixth crops). The main reason was that the outer surround row at the site was planted to *E. nitens* seedlings due to a shortage of planting material at time of establishment and blanking. By four years after planting, the vigorous growth of the seedlings exerted a significant negative impact on the height growth of the immediate neighbouring (inner surround) row of grafted trees, and minimal negative effect on the height growth of the first row of data (grafted) trees (R. Gardner, unpublished data, 2010). Chamisso was a valuable component of the site x flowering interaction trial series as it was one of only two sites located in the far south (Table 1). It was therefore decided to include the site for the fifth and sixth floral bud crop analyses, but not for the fifth year height analyses.

The effects of PBZ and clone (scion genotype) on tree height and floral crop load were explored in individual trial analyses using restricted maximum likelihood (REML) analysis in GenStat® (2012) (Payne et al. 2012). Fixed effects were specified as PBZ, clone and the PBZ by clone interaction. Fisher's protected least significant difference test was used to compare main effect and interaction means at the 5% level (Steel and Torrie 1981).

The interactive effect of site, PBZ and clone on tree height and floral crop score was investigated using REML meta-analysis in GenStat® (2012). REML meta analysis produces estimates of means and variances, which are more accurate than that of a combined Analysis of Variance (ANOVA) (Patterson and Thompson 1971, Robinson 1987) as separate residual terms per site are utilised, and not a pooled residual over all sites. The fixed effects were specified as site, PBZ and clone and all their interactions. For the across-site analyses, trees were treated as nested within plots and plots within sites. All assumptions for valid REML analyses were satisfied.

Correlation analyses were carried out in GenStat® (2012) to investigate the degree of relatedness between selected environmental factors, PBZ and the sixth floral bud crop. The main objective was to investigate the relatedness of the different explanatory variables, particularly those climate-related. For the purpose of the correlation analyses, variate “FLW” (floral bud crop) represented average floral crop score for clones over replicates in the sixth year after planting.

Multiple linear regression analyses in GenStat® (2012) were used to investigate the relationships between selected climatic and landform factors, PBZ application and *E. nitens* fifth and sixth floral bud crops. The landform factors described in Table 4 were fitted into the statistical models as factors, and therefore handled as dummy variables in the analyses. Preliminary multiple regressions established that the linear model most accurately fitted the relationship between the environmental and floral response data. Separate sets of multiple linear regressions were then carried out, for the fifth and sixth crops, and with and without PBZ included as a treatment. The response and explanatory variables included in the multiple linear regression analyses are described in Tables 4 and 5.

Results

Effect of site, PBZ and clone on tree height

A summary of the results of the across-site REML analyses for tree height at five years, including calculated *F*-test values for the fixed effects, is presented in Table 6. Site, PBZ, clone and the PBZ x site and clone x site interactions were all highly significant ($p < 0.01$). The PBZ x clone and site x PBZ x clone were both non-significant ($p = 0.757$ and $p = 0.411$, respectively). On average (across-site), PBZ application reduced tree height by 30.4%, i.e. from 10.84 m to 7.54 m ($p < 0.001$) (Table 7).

Mean five year tree heights for the site x PBZ interactions are presented in Figure 2, and ranked according to height for the control (PBZ0) trees in Table 8. PBZ is known to retard vegetative growth in various woody perennials, including *Eucalyptus* (Fletcher et al. 2000), therefore the mean heights of the PBZ0 (control) trees, rather than those of the PBZ1 (PBZ-treated) trees, are a more accurate reflection of site effect on vegetative growth of *E. nitens*. The sites with highest tree growth (Table 8) did not coincide with sites having the highest MAP figures (Table 1). The three top-ranking sites for tree height growth, Wyntoun (13.81 m), Netherby1 (13.19 m) and The Peak (12.81 m), ranked only 7th, 3rd and 4th for MAP (905 mm, 948 mm and 929 mm, respectively). To the contrary, the site ranking lowest for tree height at five years, Gilboa (7.46 m), ranked 2nd highest for MAP (957 mm). The regression of *E. nitens* five year height on MAP (Schulze and Lynch 2007) yielded an R^2 value of 0.002 (p = 0.901) (data not presented). Based on five year height, no clear linear relationship between MAP and vegetative growth was observed.

Similarly, no clear trend between MAT and height growth was evident (Tables 1 and 8, respectively). Virtually the entire MAT range of the trial series (13.8 - 15.5 ℃) applied to the three top-ranking sites for height, viz. Wyntoun (13.8 m, 15.0 ℃), Netherby1 (13.2 m, 14.1 ℃) and The Peak (12.8 m, 15.5 ℃). However , the lowest mean tree height was recorded at the site having the lowest MAT, i.e. Thoresway (8.1 m, 13.8 ℃). The regression of *E. nitens* five year height on MAT (Schulze and Maharaj 2007b) yielded an R^2 value of 0.029 (p = 0.615) (RAW Gardner, data not presented).

Separate *t*-tests were carried out for each site, to compare height growth of PBZ0 (control) trees against that of PBZ1 (PBZ-treated) trees. PBZ significantly reduced tree height at all but one site, viz. Netherby1 (Table 8). PBZ had the greatest growth retarding effect at Willowmere (6.6 m (60%) height reduction), the 2nd coldest site in the series (MAT 14.1 ℃), and at Thoresway (4.7 m (58%) height reduction), the coldest site in the series (MAT 13.8 ℃). The two sites where PB Z had the least effect, viz. Netherby1 (zero height reduction) and Netherby3 (1.0 m (10%) height reduction) were also at the lower end of the series MAT range (14.1 ℃).

Regarding mean clonal effect on tree height at five years, clone EN08 (Ebor provenance, moderate flowerer) outperformed all other clones at 10.81 m (p < 0.001) (Tables 3 and 9). Clone EN47 (Barrington Tops, moderate flowerer) recorded the lowest mean tree height at 8.13 m. There were highly significant ($p < 0.001$) site x clone interactions for height at five years. The scion genotypes (clones) included in the trial series were a relatively narrow sample of the South African breeding and commercial planting stock, therefore a detailed discussion on the relative performance of the different clones across sites is not warranted. Mean five-year height across clones was a useful indicator of the relative growth potential of the different sites in the series. No visible signs of graft incompatibility were present in any of the plants throughout the duration of the trials, and therefore it appears unlikely that this phenomenon could have confounded the results for tree height growth. Summaries of the results of the separate REML analyses for *E. nitens* tree height at five years for the individual sites are presented in Appendix 1.

Effect of site, PBZ and clone on umbel production

In all years assessed (2008 to 2010), the new season's flower buds emerged over a period of about three months, from early October to late December. The first, sparse umbel crops were recorded in several of the *E. nitens* trials during May/ June 2006, approximately 28 months after trial establishment. The first significant umbel crops were scored in May/ June 2007. Summaries of the results of the across-site REML analyses for *E. nitens* fifth and sixth floral bud crops (initiated 2008 and 2009), including the calculated *F*-test values for fixed effects, are presented in Tables 10 and 11, respectively. In the PBZ0 (control) treatment, mean umbel production (across clones and sites) increased dramatically from year five (2008) to year six (2009) (114% increase) (Table 12). In the PBZ1 treatment (PBZ applied), only a slight increase in mean umbel production from fifth to sixth year was recorded (16%).

In the fifth year (2008), all *E. nitens* treatments and their interactions, except PBZ x clone and site x PBZ x clone, were highly significant for umbel crop score ($p < 0.001$) (Table 10) in the across-site REML analysis. The site x PBZ x clone interaction was significant at $p = 0.025$. PBZ application almost tripled umbel production, elevating mean umbel crop score from 0.57 (PBZ0) to 1.61 (PBZ1) (Table 12).

Mean fifth year (2008) umbel crop scores for *E. nitens* site x PBZ interactions are illustrated in Figure 3, and ranked according to umbel crop scores for PBZ0 in Table 13. PBZ is known to stimulate floral bud production in *Eucalyptus* species (Fletcher et al. 2000). Therefore umbel crop scores for *E. nitens* non-PBZ-treated trees (PBZ0 treatment) were a more accurate representation of the true effect of site on reproductive growth. In the control (PBZ0) treatment, Netherby3 ranked first for umbel production (crop score 1.508), significantly ($p < 0.05$) outperforming all other sites in this regard (Table 13). In the same PBZ treatment (PBZ0), Thoresway (1.071), the second best site, significantly outperformed the remaining two treatments in the upper tertile, viz. Willowmere (0.821) and In De Diepte (0.817) ($p < 0.05$).

Separate *t*-tests were carried out for each of the *E. nitens* trials comparing mean fifth year umbel crop production for the PBZ0 (control) and PBZ1 (PBZ-treated) trees (Table 13). The level of significance for difference in umbel production between PBZ0 and PBZ1 treatments varied considerably across sites. PBZ application significantly increased umbel production at nine of twelve (66.7%) sites ($p < 0.05$). At five of these nine sites, the positive effect of PBZ application on umbel production was highly significant ($p < 0.001$).

Regarding mean clonal effect on *E. nitens* fifth year umbel crop, clone EN47 (Barrington Tops, moderate flowerer) outperformed all other clones at 1.772 ($p < 0.001$), irrespective of PBZ level (Table 14). The site x clone interaction for fifth year umbel crop was significant ($p < 0.001$) (Table 10). The summaries of the results of the separate REML analyses for *E. nitens* fifth year umbel crop scores for the individual sites are presented in Appendix 2. Clone (mean of PBZ treatments) was significant ($p < 0.05$) at seven out of twelve sites (Appendix 2). The significant ($p < 0.05$) site x PBZ x clone interaction evident in the results of the across-site REML analysis (Table 10) was further investigated by means of the individual site REML analysis. The results of the latter indicated that the clone effect (mean of PBZ treatments) was significant ($p < 0.05$) at seven out of twelve sites (Appendix 2). The scion genotypes (clones) included in the trial series represent a narrow sample of the South African breeding and commercial planting stock, therefore a detailed discussion on the relative performance of the different clones across sites is not warranted.

In the sixth crop year (2009), with the exception the PBZ x clone x site interaction (significant at $p < 0.05$), all treatments and their interactions were highly significant ($p < 0.001$) for umbel crop score (Table 11). PBZ application caused a 53% increase in mean (across-site) umbel crop production in *E. nitens*, elevating mean umbel crop score from 1.22 (PBZ0) to 1.86 (PBZ1) (Table 12).

Mean sixth year umbel crop scores for *E. nitens* site x PBZ interactions are presented in Figure 4, and ranked according to umbel crop scores for the control (PBZ0) treatment in Table 15. Regarding the non PBZ-treated (PBZ0) trees, in the sixth year Netherby3 again ranked first for umbel production (crop score 1.943) (Table 15). Tweefontein (1.818), Thoresway (1.756) and Willowmere (1.631) were the only other sites ranking within the upper tertile, although these did not differ apart significantly ($p < 0.05$). In summary, regarding the control (PBZ0) treatment, Netherby3, Thoresway and Willowmere were the only three sites ranking within the upper tertile for umbel crop score in both the 2008 and 2009 floral crop years (Tables 13 and 15).

Mean sixth year (2009) umbel crop scores of control trees were compared to those of PBZ1 (PBZ-treated) trees for the different sites (Table 15). The effect of PBZ application on sixth year umbel crop score was generally less distinct than in the previous (fifth) crop year. The levels of significance for the difference in umbel crop score between control and PBZ1 treatments ranged substantially across sites, from non-significant (seven out of 12 sites) to highly significant ($p < 0.001$) (three out of 12 sites).

Regarding mean clonal effect on sixth year umbel crop score, clone EN47 (Barrington Tops, moderate flowerer) again outperformed all other clones at 2.413 ($p < 0.001$) (Table 14). The umbel crop score of lowest ranking treatment, clone EN08 (Ebor, moderate flowerer) (1.088), was less than half that of clone EN47. A closer investigation of the clonal differences for umbel production through individual site REML analyses revealed that the clonal effect was significant ($p < 0.05$) at half of the twelve sites, whilst the PBZ x clone interaction was significant (p < 0.05) at only three of the twelve sites (Appendix 3).

Relationship between site factors, PBZ and umbel production

The results of the correlation analyses for the range of environmental factors, PBZ application and *E. nitens* sixth year (2009) floral bud crops, are presented in Table 16. The correlations between the different chill model x chill period combinations were all highly significant (p < 0.01) (Table 16). Across the *E. nitens* sites, correlations ranged from 0.863 (CP_2 with DPCU_2) to 0.996 (DPCU_1 with DPCU_2). CP_1 (Dynamic Model, April to September) correlated highly with DPCU_1 (Daily Positive Utah Chill Unit Model, April to September) at 0.900. MAT (mean annual temperature) was moderately negatively correlated with LAT (-0.639), CP_1 (-0.598) and CP_2 (0.577) (p < 0.01). There was a moderate positive correlation between PBZ and FLW in *E. nitens* (r = 0.447). The correlations between FLW and all other factors were weak and insignificant.

Summaries of the results of the multiple linear regressions yielding the highest R^2 values and significance in each set (fifth and sixth year umbel crops, with and without PBZ treatment) are presented in Table 17. ASPECT was consistently the most influential environmental explanatory variable across the suite of regression analyses carried out. In both fifth and sixth crop years, the relationships between explanatory and response variables were generally noticeably weaker where PBZ was included as an explanatory variable than where it was not (Table 17).

In the linear models pertaining to control (PBZ0) trees only, in the fifth year (2008), 81% of the variance was accounted for by MAT and ASPECT (R^2 = 0.814, p < 0.05) (Table 17). The south-west (SW) aspect exerted the strongest positive influence on FLW5 (fifth year umbel crop) (p < 0.01), followed by the west (W) and south (S) aspects at p < 0.05 The impacts of the ASPECT categories were measured relative to that for the north (N) aspect category, the latter being closest to the mean umbel crop score for the particular explanatory variable. Mean hourly air temperature for 2008 (MAT) was strongly negatively related to umbel crop (FLW5) in the same year ($p < 0.05$). With respect to the sixth year (2009) umbel crop, 96% of the variance was accounted for by MAT, SLOPE and ASPECT ($R^2 = 0.961$, $p < 0.01$). South (S) aspects exerted the greatest positive effect on umbel production ($p < 0.01$), with all other aspects exerting relatively minor positive or negative influences on crop response. Steep (ST) slopes exerted the strongest positive effect on umbel production (significant at $p < 0.01$), with moderate (MO) to very gentle (VG) slopes exerting the greatest negative effects on umbel production ($p < 0.01$ and $p < 0.05$, respectively). The impacts of the slope categories were measured relative to that for the gentle (GE) slope category, the latter being closest to mean umbel crop response for SLOPE. Again, mean hourly air temperature for 2009 (MAT) was significantly negatively related to umbel production in the same year (FLW6) ($p < 0.05$).

In the *E. nitens* linear models where PBZ was included as an explanatory variable, in the fifth umbel crop year (2008), a maximum of 47% of the variance could be accounted for by the best combination of explanatory variables, i.e. PBZ, MAT and ASPECT ($R^2 = 0.474$, $p < 0.05$) (Table 17). In the same model, PBZ exerted the strongest and most significant positive influence ($p < 0.001$) on umbel crop. All other factors were non-significant at $p \geq 0.05$. In the sixth crop year (2009), 32% of the variance was accounted for by PBZ, CP_1 and ASPECT ($R^2 = 0.319$, $p = 0.06$). South-west (SW) aspect followed by south (S) aspect categories exerted the strongest positive effects on umbel crop production (both significant at $p = 0.05$). PBZ was the only other explanatory variable in the model showing a significant (and positive) relationship with sixth year umbel crop ($p < 0.05$).

Discussion

Suitability of chill models

Chilling requirements for plant physiological processes such as dormancy completion and floral bud induction differ across species (Naor et al. 2003), ecotypes (Ghelardini et al. 2009, Myking and Heide 1995), cultivars within-species (De Melo-Abreu et al. 2004, Fabbri and Benelli 2000) and bud types within-cultivar (Erez 2000). Furthermore, the optimum temperature criteria for chilling accumulation has been established for relatively few species/ crops, and limited cultivars/ genotypes within species. The temperatures at which chilling accumulation begins are likely to be species- (and genotype within-species) specific (Erez pers. comm.[2]). Regarding chilling accumulation, Naor et al. (2003) found that apple responded to lower temperatures than peach. This is not surprising, considering apple (*M. sylvestris*) originates from, and is generally planted at, higher latitudes than peach (Janick and Moore 1996). In contrast to this, Myking and Heide (1995) found that high latitude ecotypes of *Betula pendula* and *B. pubescens* had lower chilling requirements for bud dormancy release than low latitude ecotypes of the same species. They hypothesized that the low latitude ecotypes of either species have adapted to a milder and more variable winter climate by developing greater dormancy stability involving both a longer chilling requirement and a higher base temperature for active vegetative growth.

Of the two chill models evaluated in the field trial series, the Dynamic Model again demonstrated greater suitability than the Daily Positive Utah Chill Unit Model for the particular purpose, i.e. quantification of winter chilling across a wide range of summer rainfall temperate *Eucalyptus* forestry site conditions in South Africa (Gardner and Bertling 2005). Luedeling et al. (2011) pointed out the main shortcomings of the agricultural chill models in use today, including the Dynamic Model, is that they are purely empirical, and are not based on a functional understanding of plant physiology. The models likely need some degree of fine-tuning in order to adapt them to each particular plant species and/or genotype for increased accuracy of chilling quantification. The effectiveness of temperatures, and the duration of exposure to these on the floral

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[2]Erez A. 2013. Volcani Center, ARO, Bet-Dagan, Israel

bud induction and differentiation processes in the key commercial temperate eucalypt species in South Africa, such as *E. nitens* and *E. smithii*, warrant further investigation, i.e. if a more thorough understanding of the accumulation of chilling, and the effect of chilling on floral bud production in the different species is to be acquired.

Effect of site and PBZ on tree height

Mean height of control (PBZ0) trees were more indicative of the true effect of site on tree vegetative growth than mean height of PBZ1 trees. Based on mean tree height for the control treatment at five years, the relationship between (long-term) MAP and height was not strong (Tables 1 and 8). The deep soil conditions (≥ 1.0 m) at each of the sites may have played an ameliorative role in preventing soil water shortages from developing, particularly during the dry winter months, at the lower rainfall sites in the series during the first five years after planting (Darrow 1994, Schönau and Grey 1987). Analysis of the relationship between actual MAP (mean for 2004 to 2009) and five year tree height and may have yielded a somewhat different result. Soil water availability is commonly the most limiting growth factor for plantation stands at any site (Louw 1999, Schönau and Grey 1987). Similarly, the relationship between MAT and five-year height growth of control trees was not strong. The site recording the second lowest tree height at five years (Thoresway) coincided with the site having the lowest MAT in the series (13.8℃). However, the site recording the absolute lowest tree height in this species (Gilboa) was at the other end of the MAT scale at 15.31 ℃ (Tables 1 and 8).

Thus, according to *E. nitens* five-year mean height data, vegetative growth did not appear to be linearly related to either MAP or MAT across the range of site conditions. It is postulated that other environmental factors such as those relating to micro-topography, e.g. slope aspect and steepness, and genetic factors such as frost and heat tolerance of the different scion genotypes, may have exerted a stronger effect on tree height growth over the first five years. It was unlikely that soil nutrient content differences impacted on the results. Soil sampling and nutrient analyses carried out in 2012 at each of the sites revealed no significant deficiencies for any of the elements (and organic carbon) commonly associated with tree vegetative growth (R Gardner, unpublished results). Furthermore, graft incompatibility was discounted as a possible cause of confounding five-year tree height growth measurements, both within and across trial sites, due to the absence of incompatibility symptoms in any of the trees at the five year measurement.

PBZ application significantly reduced mean tree height in *E. nitens* (30.4% reduction) (Table 7), as would be expected (Hetherington and Jones 1990). The site x PBZ interaction showed a general trend of the growth suppressive effect of PBZ being most evident at sites having both low MAP and low MAT. However, this was not always the case. For example, at Gilboa, the site with second highest MAP and MAT values (957 mm and 15.31 ℃, respectively), a large percentage height growth reduction occurred as a result of PBZ application. As referred to earlier, with respect to the PBZ0 treatment, Gilboa recorded the lowest tree height growth of all sites for *E. nitens.* The varying effect of PBZ application on tree height growth at five years across 11 sites did not relate well to MAP and MAT data for the sites (Table 1). Possibly, long-term data for the key climatic variables did not adequately represent actual site conditions that occurred over the period in which the trials were conducted. The micro-topographies of the trial sites may have contributed to the effect. Topographical factors such as aspect, steepness of slope and relative relief are known to influence air, soil and plant canopy temperatures (Dahlgren et al. 2007, Sader 1986, Schulze 2007b, Schulze and Horan 2007).

Effect of site, PBZ and clone on umbel production

Regardless of whether PBZ was applied or not, mean umbel production in *E. nitens* varied markedly across sites, in both the fifth and sixth crop years (Tables 13 and 15). This indicated the existence of important differences in environmental conditions across the range of sites in each of the years. Environmental conditions can exert a strong influence on floral induction and development in temperate eucalypts (Moncur et al. 1994, Potts et al. 2007).

The increase in mean umbel production from fifth to sixth crop year differed substantially for *E. nitens* control (PBZ0) trees (Table 12). The sixth year umbel crop more than doubled that of the fifth year (increase of 144%) even though winter chilling increased by only 2.6 CP (3.1%). This suggested that some factor other than winter chilling was responsible for the substantial increase in floral bud production between the fifth and sixth crop years. The scions used in the production of the *E. nitens* grafted propagules for the trials were collected from reproductively mature trees, but the rootstock propagules were grown from seed and thus were only 5.5 and 6.5 years old at the time of floral initiation in the scions in the fifth and sixth crop years (Moncur and Hasan 1994, Moncur et al. 1994). In the South African *E. nitens* breeding populations, open grown seedlings rarely produce flower buds before the age of eight years if not treated with PBZ, and even from this age onwards, flower bud crops are sparse (Jones 2002, Swain and Gardner 2003). The fifth and sixth year crop results for PBZ0 treatment suggest that stage of reproductive maturity of the seedling rootstocks, or possibly climatic differences between 2008 and 2009, or both of these, may have been responsible for the marked difference in reproductive performances between the two years. Temporary setback of reproductive maturity of adult scions by transmission of a juvenile signal from reproductively immature seedling rootstocks has been reported for fruit tree crops (Pliego-Alfaro and Murashige 1987) and *Eucalyptus* (Gardner and Bertling 2005, Gardner et al. 2013, Siniscalco and Pavellettoni 1988). Given the observed increase in *E. nitens* floral bud production between fifth and sixth crop year, a longer-term study aimed at investigating the effect of rootstock physiological age on scion (reproductive) phenotypic expression would appear to be worthwhile.

Mean umbel production across sites and clones in the PBZ1 treatment was at least double that of the control treatment on an annual basis (Appendices 2 and 3). The ability of PBZ application to significantly increase flowering in temperate eucalypts, given favourable environmental conditions, is well documented (Hasan and Reid 1995, Meilan 1997, Moncur and Hasan 1994). A general reduction in the enhancing effect of PBZ on floral bud production occurred from the fifth to the sixth crop year (Tables 13 and 15). As trees were older, and the accumulated winter chilling was generally greater across all sites in the sixth crop year (2009) compared to the fifth year (2008), the most likely cause of this overall drop off in the difference in umbel production between the two PBZ treatments was lowered levels of active PBZ within the trees of the PBZ1 treatment in the sixth crop year (2009). The PBZ doses were applied in autumn (March/ April) 2006, therefore the results imply that a drop-off in the floral stimulatory effect of PBZ in temperate eucalypt orchard trees can be expected from the fourth winter after application. Moncur (1998) reported soil-applied PBZ exerting a positive effect on floral bud production in *E. nitens* espalier orchards for at least five years. Gardner et al. (2013) reported a rapid drop-off in the vegetative growth-retarding effect of soil-applied PBZ in overhead irrigated, potted (organic growing medium) *E. nitens* grafts, from 12 months after application. Therefore, where the success of a commercial temperate eucalypt seed production enterprise is dependent on PBZ application for achieving a consistent supply of high (genetic) quality seed, the need to re-apply PBZ to orchards approximately every five years should be taken into consideration.

Assessment of the clonal effect on *E. nitens* mean umbel production showed that the floral productivities of the different clones were significantly influenced by both site and PBZ. The response of the different *E. nitens* clones to PBZ application varied substantially across sites, although Clone EN47 (Barrington Tops, moderate flowerer) performed consistently well across all sites in both years where the clone effect was significant. These results confirm that the interaction between environment (site), PBZ and genotype regarding floral induction in *E. nitens* is complex, and tend to support existing evidence that environment and PBZ act independently of one another in the *E. nitens* floral induction process (Gardner and Bertling 2005, Gardner et al. 2013, Moncur and Hasan 1994). However, cognisance should be taken of the fact that a significant site by PBZ interaction was observed in both the fifth and sixth years.

Relationship between site factors, PBZ and umbel production

The results of the correlation analyses at five years (Table 16) indicated several important points (described below). Firstly, none of the environmental factors individually correlated highly with umbel crop, suggesting multiple linear regression analysis was warranted in order to investigate the significance of different combinations of explanatory variables. Secondly, PBZ correlated moderately with umbel crop in *E. nitens*. Thirdly, the strongest inter-chill model correlation was between the CP_1

(Dynamic Model, April to September) and DPCU_1 (Daily Positive Utah Chill Unit Model, April to September) combinations. Fourthly, MAT (mean hourly temperature) had a fairly high negative correlation with CP_1.

In the multiple regressions, environmental variables played a significant role in accounting for the percentage variance (Table 17). The sites for the *E. nitens* field trials were all selected for maximum likelihood of a high level of annual winter chilling, based on knowledge of the effects of latitude, altitude and certain topographical factors on air and soil temperatures (Sharma et al. 2010, Schulze and Horan 2007) and winter chilling accumulation (Erez et al. 1990, Schulze and Maharaj 2007a). Due to the availability of suitable sites at the time of trial establishment, the variations in landform factors (landform classes) could not be replicated equally. Rather, these were applied on a fairly *ad hoc* basis. Hence this should be taken into consideration when interpreting the results of the regressions. The results of the regressions where PBZ was excluded as an explanatory variable are more indicative of the true effect of site on floral bud production. The results of the trial series indicated that, in *E. nitens*, sites with sufficiently low MAT and southerly (S) to south-westerly (SW) slope aspects were most conducive to floral bud production (high umbel crop scores). Where PBZ was applied, southerly (S) to south-westerly (SW) slope aspects in combination with PBZ were again the most positively influential environmental factors on floral bud production in both years.

Regarding the positive effect of southerly slope aspects on floral bud production, what remain unclear are the portions of positive floral responses that are due to factors such as air, bud and soil temperature, soil water level and solar irradiance at these sites. Such factors are typically responsible for variations in plant vegetative and reproductive growth across sites (Dahlgren et al. 2007, Granger and Schulze 1977, Sader 1986, Schulze 2007b, Sharma et al. 2010). In the field trial series, even though deep soil conditions were selected for all trials, to reduce the chance of pronounced soil water deficits from developing during the course of the trials, soil water levels in the topsoil horizons would most likely have declined to some degree during the relatively dry winter months. However, in the *E. nitens* semi-controlled environment and field trial investigations undertaken by the ICFR between 1996 and 2001, neither low temperature stress nor drought stress significantly influenced floral bud production in the particular species (Gardner 2003). These findings support existing reports that soil water deficit does not play a significant positive role in *E. nitens* floral induction (Hamilton et al. 2008, Moncur and Boland 2000).

In Southern Hemisphere countries such as South Africa, south-facing slope aspects are generally cooler with respect to both soil and air temperature, resulting in slower plant growth rates occurring on southerly, compared to northerly, aspects (Granger and Schulze 1977, Schulze 2007b). A similar phenomenon was evident in the current trial series at Netherby in the KwaZulu-Natal Midlands. In the PBZ0 treatment, significantly lower mean tree heights were recorded at five years on the south-facing slope (Netherby3 trial) compared to the north-facing slope (Netherby1 trial) (Table 8). In addition, in both fifth and sixth crop years, Netherby3 significantly ($p < 0.05$) outperformed Netherby1 on the basis of umbel crop production (Tables 13 and 15). *Eucalyptus nitens* appears to require an extended period of slowed, rather than a cessation of, vegetative growth during winter to initiate flower buds (Gardner 2003). The results of the 2003/ 2004 *E. nitens* field trial series tend to concur with this. The cool air, soil and foliage temperatures, and moist soil conditions, all features commonly associated with sites on southerly slope aspects in the Southern Hemisphere (Bale et al. 1998, Schulze 2007b, Sharma et al. 2010), may in part be responsible for providing the cold, relatively stress-free growing conditions during the winter months that are required by *E. nitens* for floral induction. At inland sites in the winter and uniform rainfall areas in South Africa, because of the more frequent cloud cover and associated increased chilling during the winter months (Schulze and Maharaj 2007a), aspect may not be such an important criterion as it is in the summer rainfall temperate forestry areas. However, decreased solar irradiance associated with increased cloud cover, and increased number of rainy days during the winter months may exert negative effects on other aspects of *E. nitens* reproductive growth and development (Moncur and Boland 2000). These site x *E. nitens* reproductive development dynamics all warrant investigating.

Conclusions

New information that will assist the accurate siting of *E. nitens* orchards in the South African summer rainfall area was derived from the research. The data produced lends itself to the development of a GIS tool for identifying potential *E. nitens* seed orchard sites based on climate and topography. The results suggested that, through careful site selection, the dependency on PBZ to achieve satisfactory levels of flowering and seed crop production in *E. nitens* can be considerably reduced. In areas where *E. nitens* winter chilling requirement for floral induction (based on air temperature) is met, south-west, south and west-facing slope aspects (in order of decreasing effectiveness) have a significant additive effect on floral bud production. The specific environmental factors associated with the positive *E. nitens* reproductive growth effect of these slope aspects remain to be determined. Further research is needed to elucidate these findings. Environmental conditions conducive to floral bud production in *E. nitens* may not necessarily favour *E. nitens* reproductive growth and development in the post-floral bud emergence phase. This aspect warrants urgent investigating.

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Table 1: Site details for the *Eucalyptus nitens* flowering trials

Trial name	In De Diepte	Wyntoun	Gilboa	The Peak	Tweefontein	Netherby1	Netherby2	Netherby3	Willowmere	Blair Athol	Thoresway	Chamisso
Locality:												
Province	MPU	MPU	KZN	KZN	KZN	KZN	KZN	KZN	KZN	KZN	EC	EC
Latitude	25°02' S	26°12' S	29°14' S	29°15' S	29°15' S	29°39' S	29°38' S	29°38' S	29°51' S	29°52' S	30°50' S	30°54' S
Longitude	30°44' E	30°44' E	30°17' E	30°09' E	30°13' E	29°38' E	29°38' E	29°38' E	29°26' E	29°37' E	28°13' E	28°11' E
Elevation (m)	1828	1733	1595	1629	1588	1688	1700	1678	1708	1568	1809	1686
Climatic factors:												
MAP (mm)[1]	1241	905	957	929	842	948	948	948	914	843	908	904
MAT (ºC)[2]	14.5	15.0	15.3	15.5	15.1	14.1	14.1	14.1	14.1	14.6	13.8	14.1
MAC (DPCU)[3]	962.1	784.8	764.8	727.0	811.0	865.3	865.3	865.3	863.2	799.8	1078.2	951.4
Edaphic factors:												
Soil form and series[4]	Magwa 1100	Clovelly 1200	Kranskop 1200	Magwa 1200	Magwa 1200	Inanda 1200	Magwa 1100	Kranskop 1200	Kranskop 1200	Magwa 1200	Kranskop 1100	Magwa 1200
Soil unit[5]	Haplic Ferralsol	Haplic Ferralsol	Haplic Acrisol	Haplic Acrisol	Haplic Acrisol	Haplic Acrisol	Haplic Ferralsol	Haplic Acrisol	Haplic Acrisol	Haplic Acrisol	Haplic Ferralsol	Haplic Acrisol
Soil depth (m)	1.0	1.0	> 1.2	> 1.2	> 1.2	> 1.2	1.0	> 1.2	> 1.2	> 1.2	> 1.2	> 1.2
Landform elements:												
Aspect[6]	NW	S	N	SE	N	N	E	SW	E	E	E	NE
Slope[6]	VS	VG	VG	VG	GE	MO	GE	ST	GE	MO	GE	MO
Relief[6]	H	L	H	H	VL	VH	VH	H	VL	L	H	L

MAP = Mean annual precipitation; MAT = Mean annual temperature; MAC = Mean annual chill units; DPCU = Daily Positive Utah Chill Units
MPU = Mpumalanga, KZN = KwaZulu-Natal, EC = Eastern Cape
[1] Schulze and Lynch (2007)
[2] Schulze and Maharaj (2007b)
[3] Schulze and Maharaj (2007a)
[4] Soil Classification Working Group (1991)
[5] IUSS Working Group (2006)
[6] Refer to Table 5 for landform elemental class description

Table 2: Details of the origins of the *Eucalyptus nitens* scion genotypes represented in the field trials

Clone No.	Origins			
	Provenance	**Latitude (S)**	**Longitude (E)**	**Altitude (m asl)**
EN08	Ebor, NSW	30°23' S	152°27' E	1400
EN35	Barrington Tops, NSW	31°55' S	151°30' E	1450
EN47	Barrington Tops, NSW	31°55' S	151°30' E	1450
EN55	Ebor, NSW	30°23' S	152°27' E	1400

NSW = New South Wales, Australia

Table 3: Allocation of the treatments in the *Eucalyptus nitens* split-plot design experiments

	Treatment	**Treatment level description**
Factor A:	**PBZ soil application**	
Level 1	PBZ0	Nil PBZ (control)
Level 2	PBZ1	PBZ soil drench applied
Factor B:	**Clone (scion genotype)**	**Provenance, flowering potential***
Level 1	EN08	Ebor, moderate
Level 2	EN35	Barrington Tops, shy
Level 3	EN47	Barrington Tops, moderate
Level 4	EN55	Ebor, shy

PBZ = Paclobutrazol
* Flowering potential rating derived from ICFR historical orchard records

Table 4: Description of all response and explanatory variables used in the multiple linear regression analyses

Variate assessed	**Abbreviation used in text**
Response variables:	
Fifth year mean umbel crop score per tree (floral buds initiated 2008)[1]	FLW5
Sixth year mean umbel crop score per tree (floral buds initiated 2009)[1]	FLW6
Explanatory variables:	
Cultural factors:	
Paclobutrazol soil application	PBZ
Climatic factors:	
Latitude (°S)	LAT
Altitude (m asl)	ALT
Annual mean hourly air temperature for year of floral bud initiation (°C)[2]	MAT
Accumulated Chilling Portions (CP)[3] for the period 01 April to 30 September in year of floral bud initiation	CP_1
Accumulated Chilling Portions (CP) for the period 01 May to 30 September in year of floral bud initiation	CP_2
Accumulated Daily Positive Utah Chill Units (DPCU)[4] for the period 01 April to 30 September in year of floral bud initiation	DPCU_1
Accumulated Daily Positive Utah Chill Units (DPCU) for the period 01 May to 30 September in year of floral bud initiation	DPCU_2
Landform factors:	
Slope aspect	ASPECT
Slope steepness	SLOPE
Relative relief	RELIEF

[1] Site mean for umbel crop score
[2] Air temperature modeled from hourly Hobo pole air temperature data
[3] The chill units assigned by the Dynamic Model (Erez and Fishman 1998)
[4] The chill units assigned by the Daily Positive Utah Chill Unit Model (Linsley-Noakes et al. 1994)

Table 5: Description of the landform factors and classes included in the multiple linear regressions

Factor and class	Class abbreviation	Class definition
Slope aspect[1] :		**Average**
North	N	North-facing
East	E	East-facing
South	S	South-facing
West	W	West-facing
North East	NE	North East-facing
South East	SE	South East-facing
South West	SW	South West-facing
North West	NW	North West-facing
Slope steepness[2]:		**Average (upper boundary)**
Level	LE	0º 20’ (0º 35’)
Very gently inclined	VG	1º (1º 45’)
Gently inclined	GE	3º (5º 45’)
Moderately inclined	MO	10º (18º)
Steep	ST	23º (30º)
Very steep	VS	37º (45º)
Relative relief[2]:		
Very low	VL	9 – 30 m
Low	L	31 – 90 m
High	H	91 – 300 m
Very high	VH	300 - 600 m

[1] Compass bearing
[2] Adapted from McDonald et al. (1984)

Table 6: Wald-statistics and calculated *F*-test values for fixed effects in the *Eucalyptus nitens* across-site REML analyses for tree height at five years

Fixed term	Wald statistic	d.f.	*F* statistic	*F* prob
SITE	865.53	10	81.65	<0.001
PBZ	368.3	1	368.3	<0.001
CLONE	100.79	3	33.6	<0.001
SITE.PBZ	115.03	10	10.85	<0.001
SITE.CLONE	86.05	30	2.69	<0.001
PBZ.CLONE	1.18	3	0.39	0.757
SITE.PBZ.CLONE	61.42	31	1.09	0.411

PBZ = Paclobutrazol
d.f. = Degrees of freedom

Table 7: Effect of PBZ treatment on *Eucalyptus nitens* tree height at five years

	PBZ treatment		
	PBZ0	PBZ1	Difference[1]
Mean	10.84*a*	7.54*b*	3.30***
SED	0.14	0.14	-

PBZ0 = No PBZ applied (control)
PBZ1 = PBZ applied to soil in March/ April 2006
SED = Standard error of the differences between means
[1] Difference between means (ns = not significant, * = significant at $p < 0.05$, ** = significant at $p < 0.01$, *** = significant at $p < 0.001$)

Table 8: Mean *Eucalyptus nitens* tree height at five years for the SITE x PBZ interactions, ranked according to height for the PBZ0 treatment

	PBZ treatment		
SITE[1]	PBZ0[#]	PBZ1[#]	Difference[2]
Wyntoun	13.81*a*	9.51*b*	4.29***
Netherby1	13.19*b*	13.26*a*	0.07ns
The Peak	12.81*b*	8.49*c*	4.32***
Blair Athol	11.23*c*	7.11*d*	4.12***
Willowmere	11.09*c*	4.48*e*	6.61***
In De Diepte	10.88*cd*	8.90*c*	1.98**
Netherby3	10.56*d*	9.56*b*	1.01*
Tweefontein	10.53*d*	7.20*d*	3.33***
Netherby2	9.89*e*	7.26*d*	2.64***
Thoresway	8.13*f*	3.45*f*	4.68***
Gilboa	7.46*g*	3.74*f*	3.73***
SED	0.45	0.45	-

PBZ0 = Nil PBZ (control)
PBZ1 = PBZ applied to soil in March/ April 2006
SED = Standard error of the differences between means
[1] Sites ranked according to height for the PBZ0 treatment
[2] Difference between means (ns = not significant, * = significant at $p < 0.05$, ** = significant at $p < 0.01$, *** = significant at $p < 0.001$)
[#] Within this column, values followed by the same letter do not differ significantly ($p < 0.05$)

Table 9: Effect of clone on *Eucalyptus nitens* tree height at five years of age

Clone no.	**Provenance**	**Height (m)[#]**
EN08	Ebor	10.81*a*
EN35	Barrington Tops	9.25*b*
EN55	Ebor	8.58*c*
EN47	Barrington Tops	8.13*c*
Mean	-	9.19

[#] Within this column, values followed by the same letter do not differ significantly from each other ($p < 0.001$)

Table 10: Wald statistics and calculated *F*-test values for fixed effects in the *Eucalyptus nitens* across-site REML analyses for fifth year umbel crop score

Fixed term	Wald statistic	d.f.	*F* statistic	*F* prob
SITE	94.45	11	8.07	<0.001
PBZ	139.98	1	139.98	<0.001
CLONE	90.43	3	30.14	<0.001
SITE.PBZ	125.53	11	10.73	<0.001
SITE.CLONE	96.2	33	2.74	<0.001
PBZ.CLONE	7.39	3	2.46	0.063
SITE.PBZ.CLONE	57.72	33	1.63	0.025

PBZ = Paclobutrazol
d.f. = Degrees of freedom

Table 11: Wald statistics and calculated F-test values for fixed effects in the *Eucalyptus nitens* across-site REML analyses for sixth year umbel crop score

Fixed term	Wald statistic	d.f.	*F* statistic	*F* prob
SITE	125.19	11	10.7	<0.001
PBZ	81.44	1	81.44	<0.001
CLONE	238.66	3	79.55	<0.001
SITE.PBZ	109.07	11	9.33	<0.001
SITE.CLONE	174.02	33	4.93	<0.001
PBZ.CLONE	15.54	3	5.18	0.002
SITE.PBZ.CLONE	57.88	33	1.64	0.024

PBZ = Paclobutrazol
d.f. = Degrees of freedom

Table 12: Effect of PBZ treatment on *Eucalyptus nitens* fifth and sixth year umbel crop scores

	PBZ treatment		
	PBZ0	PBZ1	Difference[1]
Fifth year (2008)			
Mean	0.57*a*	1.61*b*	1.05***
SED	0.09	0.09	-
Sixth year (2009)			
Mean	1.22*a*	1.86*b*	0.64***
SED	0.09	0.09	-

PBZ0 = Nil PBZ (control)
PBZ1 = PBZ applied to soil in March/ April 2006
SED = Standard error of the differences between means
[1] Difference between means (ns = not significant, * = significant at $p < 0.05$, ** = significant at $p < 0.01$, *** = significant at $p < 0.001$)

Table 13: Mean *Eucalyptus nitens* fifth year umbel crop scores for the SITE x PBZ interactions, ranked according to umbel crop score for the PBZ0 treatment

	PBZ treatment		
SITE[1]	PBZ0[#]	PBZ1[#]	Difference[2]
Netherby3	1.508*a*	1.304*f*	0.204ns
Thoresway	1.071*b*	1.554*de*	0.483ns
Willowmere	0.821*c*	2.054*c*	1.233***
In De Diepte	0.817*c*	1.433*ef*	0.616*
Wyntoun	0.633*cd*	2.179*c*	1.546***
Tweefontein	0.571*de*	1.679*d*	1.108***
Chamisso	0.446*def*	0.742*h*	0.296ns
Netherby1	0.258*fg*	0.867*gh*	0.609*
Blair Athol	0.196*g*	2.992*a*	2.796***
Gilboa	0.196*g*	1.054*g*	0.858**
The Peak	0.133*g*	2.429*b*	2.296***
Netherby2	0.133*g*	1.054*g*	0.921**
SED	0.226	0.226	-

PBZ0 = Nil PBZ (control)
PBZ1 = PBZ applied to soil in March/ April 2006
SED = Standard error of the differences between means
[1] Sites ranked according to umbel crop score for the PBZ0 treatment
[2] Difference between means (ns = not significant, * = significant at $p < 0.05$, ** = significant at $p < 0.01$, *** = significant at $p < 0.001$)
[#] Within this column, values followed by the same letter do not differ significantly ($p < 0.05$)

Table 14: Effect of clone on *Eucalyptus nitens* fifth and sixth year umbel crop scores

Fifth year (2008)			Sixth year (2009)		
Clone no.	Provenance	Umbel crop score[#]	Clone no.	Provenance	Umbel crop score[#]
EN47	Barrington Tops	1.772*a*	EN47	Barrington Tops	2.413*a*
EN08	Ebor	0.938*b*	EN35	Barrington Tops	1.380*b*
EN55	Ebor	0.832*b*	EN55	Ebor	1.275*bc*
EN35	Barrington Tops	0.813*b*	EN08	Ebor	1.088*c*
Mean	-	1.089	Mean	-	1.539

[#] Within this column, values followed by the same letter do not differ significantly ($p < 0.001$)

Table 15: Mean *Eucalyptus nitens* sixth year umbel crop scores for the SITE x PBZ interaction, ranked according to umbel crop score for the PBZ0 treatment

	PBZ treatment		
SITE[1]	PBZ0[#]	PBZ1[#]	Difference[2]
Netherby3	1.943*a*	2.557*c*	0.614*
Tweefontein	1.818*ab*	1.932*de*	0.114ns
Thoresway	1.756*ab*	1.744*efg*	0.012ns
Willowmere	1.631*b*	1.807*ef*	0.176ns
In De Diepte	1.253*c*	1.497*g*	0.244ns
Wyntoun	1.193*c*	2.619*bc*	1.426***
Netherby2	1.193*c*	1.619*fg*	0.426ns
The Peak	0.943*d*	3.057*ab*	2.114***
Chamisso	0.881*d*	0.619*i*	0.262ns
Blair Athol	0.818*de*	3.119*a*	2.301***
Gilboa	0.631*ef*	0.619*i*	0.012ns
Netherby1	0.568*f*	1.119*h*	0.551*
SED	0.247	0.247	-

PBZ0 = No PBZ applied (control)
PBZ1 = PBZ applied to soil in March/ April 2006
SED = Standard error of the differences between means
[1] Sites ranked according to umbel crop score for the PBZ0 treatment
[2] Difference between means (ns = not significant, * = significant at $p < 0.05$, ** = significant at $p < 0.01$, *** = significant at $p < 0.001$)
[#] Within this column, values followed by the same letter do not differ significantly ($p < 0.05$)

Table 16: Correlation matrix for selected explanatory variables included in the regressions between environmental factors, PBZ and *Eucalyptus nitens* floral crop response (sixth year umbel crop score).
[Critical values for Pearson's r (df = 22): $p < 0.05 = 0.404$; $p < 0.01 = 0.515$]

LAT	1.000								
ALT	-0.430	1.000							
MAT	-0.639	0.053	1.000						
CP_1	0.365	-0.319	-0.598	1.000					
CP_2	0.444	-0.385	-0.577	0.987	1.000				
DPCU_1	0.174	-0.211	-0.380	0.900	0.873	1.000			
DPCU_2	0.183	-0.241	-0.329	0.879	0.863	0.996	1.000		
PBZ	N/A	N/A	N/A	N/A	N/A	N/A	N/A	1.000	
FLW	-0.146	-0.023	-0.116	0.132	0.098	0.118	0.097	0.447	1.000
	LAT	ALT	MAT	CP_1	CP_2	DPCU_1	DPCU_2	PBZ	FLW

LAT = South latitude in degrees
ALT = Altitude in metres
MAT = Mean hourly (modeled) screen air temperature for 2009
CP_1 = Accumulated Chilling Portions (CP) for the period 01 April-30 September 2009
CP_2 = Accumulated Chilling Portions (CP) for the period 01 May-30 September 2009
DPCU_1 = Accumulated Daily Positive Utah Chill Units (DPCU) for the period 01 April-30 September 2009
DPCU_2 = Accumulated Daily Positive Utah Chill Units (DPCU) for the period 01 May-30 September 2009
PBZ = Paclobutrazol soil treatment (PBZ0 or PBZ1)
FLW = *Eucalyptus nitens* sixth year umbel crop score
N/A = Not applicable

Table 17: Summary of the results of the multiple linear regression analyses for *Eucalyptus nitens* fifth and sixth year floral bud crops on environmental factors and PBZ treatment

	FLW5				FLW6			
		MAT		**PBZ**		**MAT**		**PBZ**
				MAT		**SLOPE**		**CP_1**
		ASPECT		**ASPECT**		**ASPECT**		**ASPECT**
SOURCE	**d.f.**	**m.s.**	**d.f.**	**m.s.**	**d.f.**	**m.s.**	**d.f.**	**m.s.**
Regression	6	0.2865*	7	1.2756*	8	0.2970**	7	0.9703
Residual	5	0.0318	16	0.3217	3	0.0085	16	0.3821
Total	11	0.1707	23	0.6120	11	0.2183	23	0.5611
R^2		0.814		0.474		0.961		0.319
SED		0.178		0.567		0.092		0.618
Estimate of parameters:								
Constant		5.59		5.48		5.90		-2.44
PBZ				1.083				0.656
MAT		-0.395		-0.394		-0.341		
CP_1								0.035
SLOPE MO						-0.679		
SLOPE ST						0.472		
SLOPE VG						-0.725		
SLOPE VS						-0.032		
ASPECT E		0.028		0.197		-0.140		0.577
ASPECT SE		-0.252		0.467		0.190		0.754
ASPECT S		0.651		0.994		0.801		1.373
ASPECT SW		1.115		0.584		0.000[1]		1.507
ASPECT W		0.609		0.484		0.000[2]		0.772
Parameter *t*-values:	5		16		3		16	
Constant		3.77*		1.64		7.42**		-1.41
PBZ				4.68***				2.60*
MAT		-3.56*		-1.58		-5.43*		
CP_1								1.89
SLOPE MO						-8.95**		
SLOPE ST						3.91*		
SLOPE VG						-5.71*		
SLOPE VS						-0.24		
ASPECT E		0.21		0.65		-1.76		1.73
ASPECT SE		-1.22		1.01		1.44		1.48
ASPECT S		2.84*		1.93		5.84**		2.32*
ASPECT SW		5.41**		1.26		na[2]		2.78*
ASPECT W		2.92*		1.03		na[3]		1.35

PBZ = Paclobutrazol soil treatment (levels described in Table 3)
MAT = Mean hourly (modeled) screen air temperature for the year 2008 (FLW5) or 2009 (FLW6)[1]
CP_1 = Accumulated Chilling Portions (CP) for the period 01 April-30 September 2009[1]
SLOPE = Slope steepness categories (described in Table 5)
ASPECT = Slope aspect categories (described in Table 5)
FLW5 = *Eucalyptus nitens* fifth year (2008) umbel crop score
FLW6 = *Eucalyptus nitens* sixth year (2009) umbel crop score
[1]Calculated from modeled screen air temperature
[2] Parameter could not be included in model as aliased with parameter SLOPE ST
[3] Parameter could not be included in model as aliased with parameter SLOPE VS
* = significant at $p < 0.05$, ** = significant at $p < 0.01$, *** = significant at $p < 0.001$)

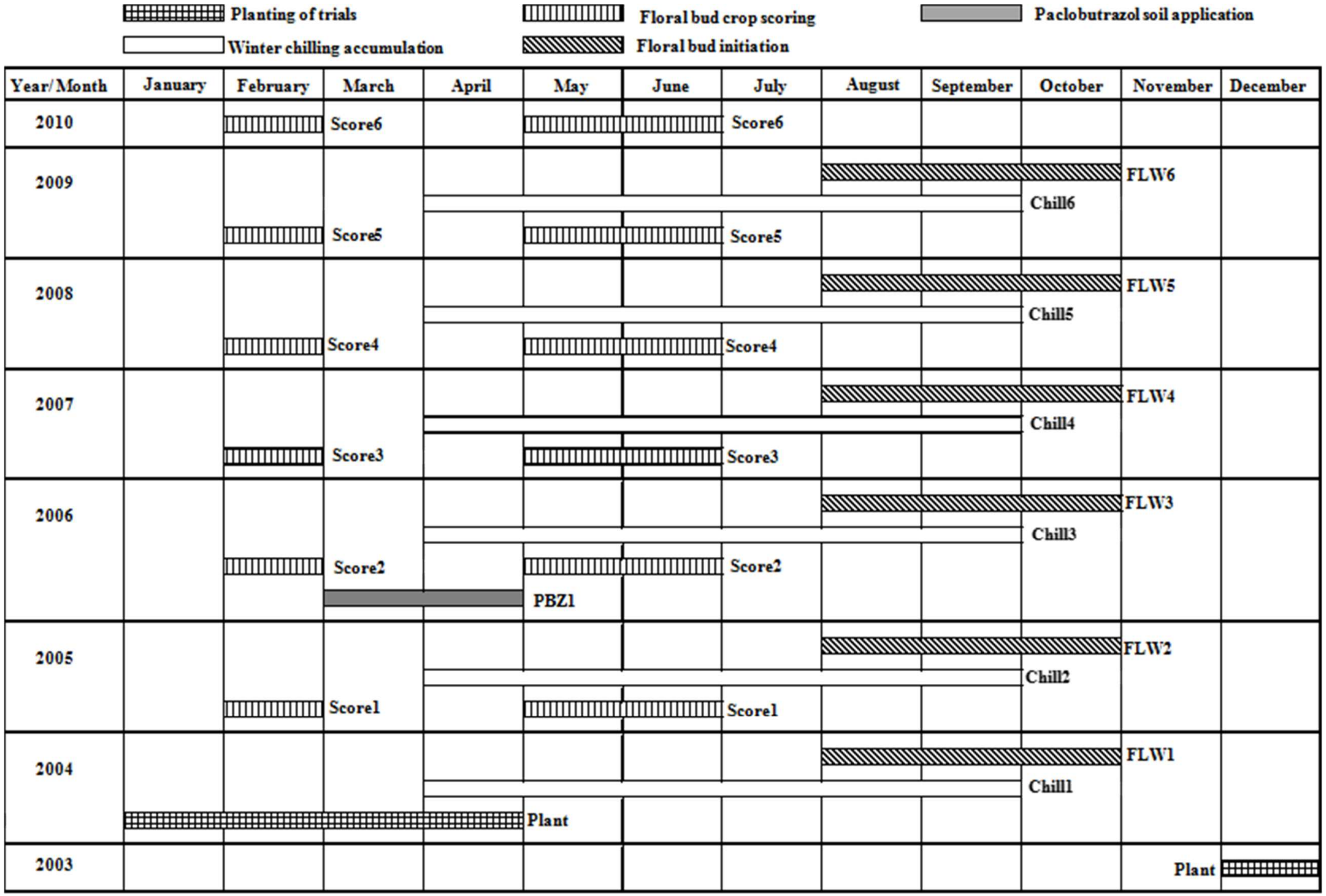

Figure 1: Sequence of events associated with *E. nitens* annual floral bud crop production between 2004 and 2010

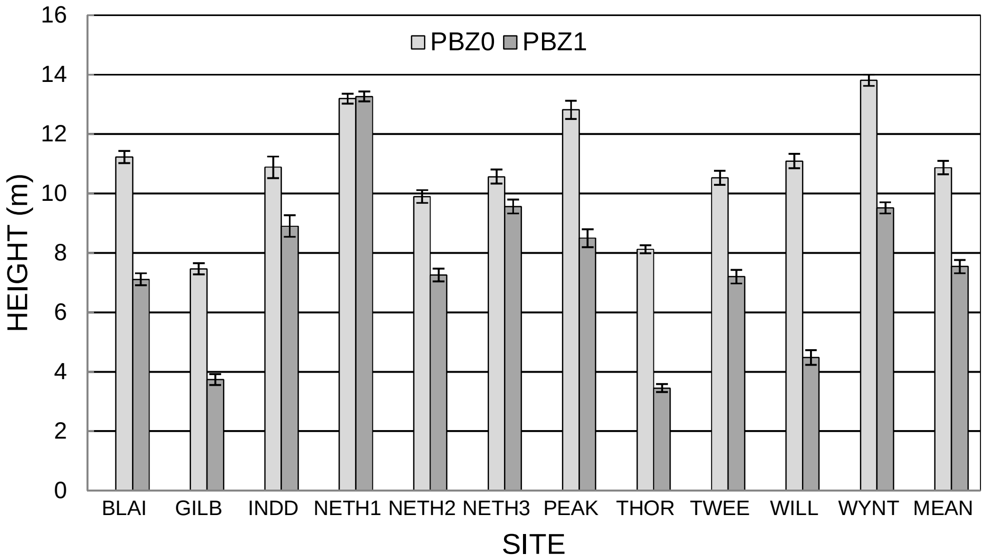

Figure 2: *Eucalyptus nitens* mean tree heights for PBZ treatments in the individual trials in the series at five years of age (2009). The trials are ranked alphabetically from left to right, with the across-site mean at the extreme right. PBZ0 = Nil PBZ (control), PBZ1 = PBZ applied to the soil in March/ April 2006. Error bars represent the standard error (SE) of the predicted mean

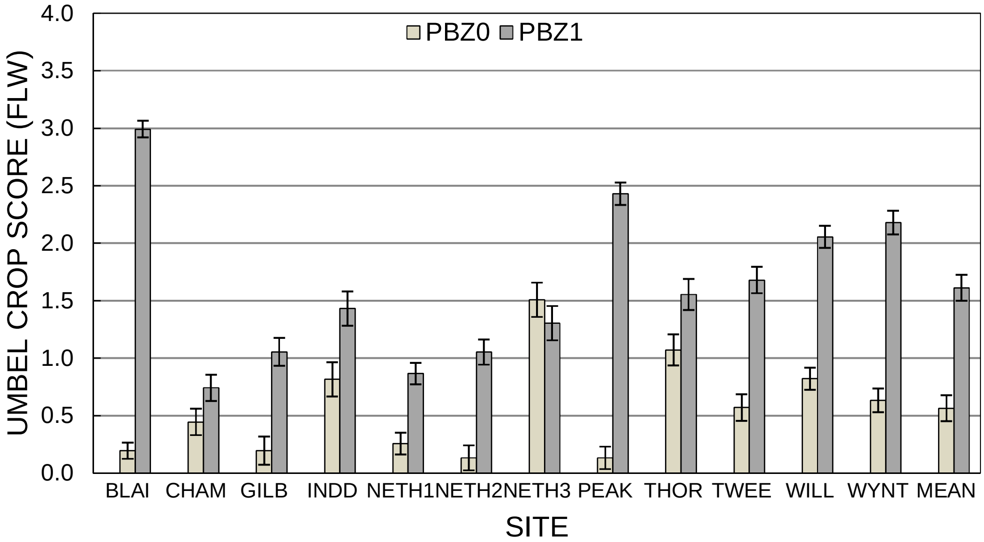

Figure 3: *Eucalyptus nitens* mean fifth year (2008) umbel crop score for PBZ treatments in the individual trials in the series. The trials are ranked alphabetically from left to right, with the across-site mean at the extreme right. PBZ0 = Nil PBZ (control), PBZ1 = PBZ applied to the soil in March/ April 2006. Error bars represent the standard error (SE) of the predicted mean

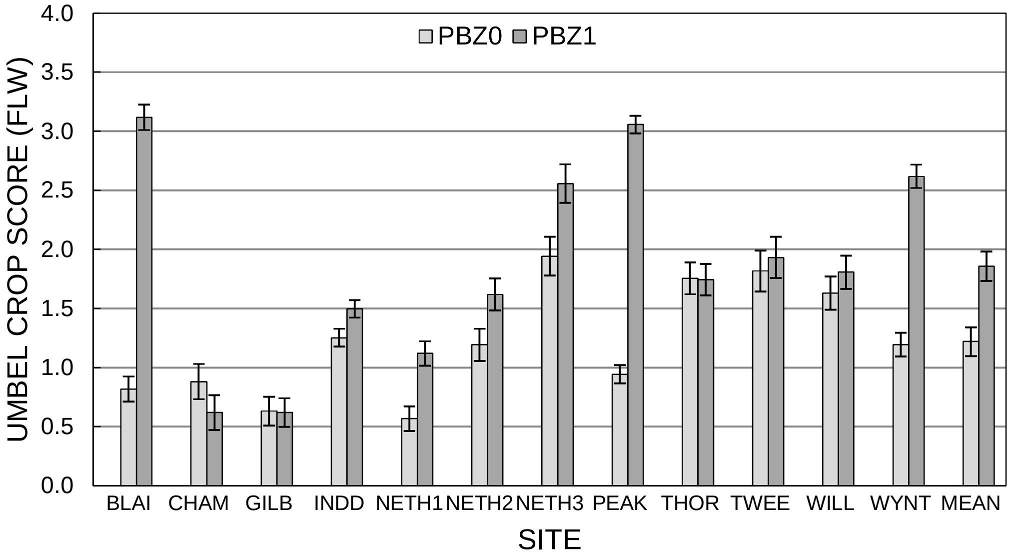

Figure 4: *Eucalyptus nitens* mean sixth year (2009) umbel crop score for PBZ treatments in the individual trials in the series. The trials are ranked alphabetically from left to right, with the across-site mean at the extreme right. PBZ0 = Nil PBZ (control), PBZ1 = PBZ applied to the soil in March/ April 2006. Error bars represent the standard error (SE) of the predicted mean

CHAPTER 5

Defining site requirements for maximal floral bud production in *Eucalyptus smithii* in South Africa

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Abstract

During the summer of 2003/ 2004, an *E. smithii* flowering field trial series was established across a range of high elevation (> 1550 m asl), cool temperate (13.5 to 16.0 ^{o}C mean annual temperature (MAT)) sites in the South African summer rainfall forestry regions. The interactive effect of a range of climatic and landscape factors and paclobutrazol application on *E. smithii* floral bud (umbel) production was investigated. The main aim was to define *E. smithii* site requirements for optimal (consistent and prolific) floral crop production.

Paclobutrazol application reduced mean tree height in the *E. smithii* grafted clones by 20% at five years, and at least doubled the mean (across-site) umbel production in the same clones in the fifth and sixth crop years. At some of the sites, where the environmental conditions were apparently highly inductive of flowering in *E. smithii*, excessive production of umbels and reduction of vegetative growth resulted. The study demonstrated that, through careful site selection, the dependency on paclobutrazol to achieve satisfactory umbel production levels in reproductively mature *E. smithii* trees can be substantially reduced. In the absence of paclobutrazol application, high elevation (> 1700 m asl), cold (MAT ≤ 14.5 ^{o}C) sites were generally the most productive on the basis of *E. smithii* umbel crop. However, orchards situated in low landscape positions within the elevational and MAT ranges applied in the trial series, and those at high elevation (> 1800 m asl), cold (MAT < 14.0 ^{o}C) sites carry a high risk of being severely damaged by frost.

Keywords

Cold-tolerant eucalypt, *E. smithii*, floral induction, flowering, seed orchard, site-species matching, winter chilling

Introduction

Eucalyptus smithii, Gully Gum, is an important tree species grown predominantly for essential oil production in various parts of the world (Clarke et al. 2008). In countries such as South Africa, Australia and China, *E. smithii* is grown mainly for commercial pulpwood production due to the species' excellent fibre productivity and quality (Arnold et al. 2004, Clarke et al. 1999). In South Africa, the species has shown moderate cold-, drought-, frost- and snow-tolerance (Darrow 1994, Gardner and Swain 1996, Schönau and Gardner 1991). Optimum tree growth is achieved at high elevation (> 1100 m asl), moderately cool (15 ^{o}C to 17 ^{o}C mean annual temperature) sites within the summer rainfall forestry area (Swain and Gardner 2002, Gardner 2007). *Eucalyptus smithii* plantations cover approximately 22 000 ha, or 8.3%, of the temperate eucalypt plantation forestry area in South Africa (Forestry South Africa 2013, R Gardner unpublished data 2014). The species is difficult to propagate vegetatively (Jones et al. 2000), and thus, to date, seedlings are used in the establishment of the *E. smithii* commercial plantations. The South African forestry industry is largely dependent on the production of locally-improved *E. smithii* seed for plantation use. Seedlings of *E. smithii* rarely flower before the age of eight years, and even then, annual floral bud crops of the species are sparse and erratic (Jones 2002). The latter traits hinder *E. smithii* genetic improvement and commercial seed production (Swain and Gardner 2002).

Eucalyptus smithii flowering controls are not well understood. Cold winter conditions appear to be pre-requisite for floral bud production in temperate eucalypts (Moncur and Hasan 1994, Williams et al. 2003, Gardner and Bertling 2005). To date, cold and paclobutrazol (a plant growth regulating chemical) have proven to be the most effective treatments for encouraging flowering in the commercial temperate eucalypt species (Gardner et al. 2013, Hamilton 2008, Swain and Gardner 2002). In temperate eucalypts, depending on whether trees have received sufficient winter cold or not, paclobutrazol (PBZ) has the potential to significantly reduce time to first flowering in seedlings and increase overall flower and seed crop production (Potts et al. 2007, Williams et al. 2003).

In South Africa, in the high elevation (> 1200 m asl) summer rainfall forestry areas, temperate eucalypts such as *E. fraxinoides*, *E. nitens* and *E. smithii* have been observed to produce flower buds more consistently and prolifically at exposed, rather than low-lying, sites (Gardner 2003). Considerable environment x PBZ x flowering interaction research was carried out in an attempt to quantify *E. nitens* cold requirement for floral induction. Of all agricultural chill models evaluated, the Dynamic Model (Fishman et al. 1987) performed best over the range of high elevation site conditions (Gardner and Bertling 2005). The results indicated that *E. nitens* has a minimum winter chilling requirement of 40 CP (Chilling Portion, the unit of the Dynamic Model; Erez and Fishman 1998) for floral induction (Gardner and Bertling 2005, Gardner et al. 2013). At medium to high winter chilling levels, i.e. between 55 and 90 CP, PBZ was highly promotive of flowering in *E. nitens* (Gardner et al. 2013, Gardner et al. 2014). *Eucalyptus smithii* chilling requirement for floral induction remains to be investigated.

In South Africa, and indeed abroad, PBZ is used almost routinely as an orchard management tool for controlling vegetative growth and enhancing floral bud and seed crop production in important temperate species such as *E. globulus* (Hasan and Reid 1995, Potts et al. 2007), *E. nitens* (Gardner et al. 2013, Hamilton et al. 2008) and *E. smithii* (Jones et al. 2000, Jones and Van Staden 2001). Disadvantages associated with the reliance on PBZ for achieving early and prolific flowering and seed production in temperate eucalypt orchards include the chemical's recalcitrance and persistence in soils (Fletcher et al. 2000, Jackson et al. 1996) and the high financial cost of the necessary repetitive applications of PBZ (Gardner et al. 2013). Investigating ways of lessening the dependency on PBZ to achieve satisfactory flowering and seed crop production in *E. smithii* is well-warranted.

During the summer of 2003/ 2004, an *E. smithii* site x PBZ x flowering interaction trial series was established across a range of high elevation, high chill temperate forestry sites in the summer rainfall forestry area. The main objective was to investigate the interactive effect of climate and landscape factors, particularly those associated with air temperature, and PBZ on *E. smithii* floral bud production. The main goal was to derive a set of data that would assist the selection of sites for optimal (consistent and prolific) flower bud production in *E. smithii*. This article discusses the outcomes of the investigation.

Material and methods

The trial series was established across 12 sites within the summer rainfall forestry region, between December 2003 and March 2004.

Site conditions

The latitudinal range of trial sites covered almost the entire span of the summer rainfall temperate eucalypt forestry belt, i.e. from 30°53’ S in the south near Maclear (Eastern Cape), to 25°02’ S in the north near Sabie (Mpumalanga) (Table 1). The primary site selection criteria were mean annual air temperature (MAT) between 13.5 and 16.0 °C, elevation between 1550 and 1850 m asl and soil depth ≥ 0.8 m. Some of the sites selected for the trials were colder and/or at higher elevations than those recommended for optimum *E. smithii* vegetative growth (Swain and Gardner 2002, Gardner 2007) (Table 1). Water stress is known to stimulate flower bud production in a number of woody angiosperms (Davenport 1990, George and Erez 2000, Meilan 1997), therefore drought stress was minimized as an inductive factor by selecting sites with both high mean annual precipitation (MAP) (> 840 mm) and uniformly deep soils (> 0.8 m) (Table 1) (Darrow 1994, Schönau and Grey 1987). Within the above MAT and elevation boundaries, sites were selected for maximum likelihood of high levels of chilling accumulating in winter, i.e. those with moderate to high levels of exposure and relative relief (Gardner and Bertling 2005, Schulze 2007a, Schulze and Horan 2007). From date of trial establishment, uniform weed control was carried out across all trial sites on a biannual basis.

Plant material

In August 2003, scions from four genotypes (ICFR breeding selections) (Table 2) were grafted onto six-month old *E. smithii* seedlings grown from South African improved commercial seed. The scions were collected from reproductively mature (nine years or older) grafted ramets in the ICFR and Sappi clonal seed orchards (CSOs) at Mountain Home and Tweedie (KwaZulu-Natal midlands), respectively. *Eucalyptus smithii* was represented by two (scion) provenances forming part of the South African *E. smithii* breeding population (Swain and Gardner 2002). Each provenance was represented by two (scion) genotypes differing in flowering propensity according to ICFR records (Table 3).

Experimental design and treatments

A 2 x 4 factorial experiment consisting of 32 grafted trees, with treatments replicated twice, was laid out as a split plot design (Gomez and Gomez 1984). “PBZ application” (PBZ0 = nil PBZ applied (control), PBZ1 = PBZ soil drench applied) was assigned as whole plot Factor A, and “Clone (scion genotype)” (four individual clones) as sub-plot Factor B (Table 3). Each experimental unit consisted of two trees (2-tree contiguous plot) with both trees being measured. Due to the sloped nature of most of the sites, even though PBZ is known to have low mobility in soils (Jackson et al. 1996, Reid et al. 1995), the whole plots (PBZ treatments) were laid out across the slope to exclude contamination of the PBZ0 treatment plots by PBZ from the PBZ1 plots. Each experiment was surrounded by two buffer rows of grafted trees of similar species.

Paclobutrazol treatment

The PBZ treatment was applied during late summer 2006 (March-April), approximately two years after trial establishment. A suspension of Avocet® (formulation 250 g L^{-1} PBZ, Fine Agrochemicals Ltd, Whittington, UK) was applied as a soil drench at a rate of 0.025 g a.i. per mm basal stem circumference (BSC) (Gardner and Bertling 2005, Gardner et al. 2013). The latter measurement was taken at the narrowest point between graft union and lowest primary lateral (branch) on the scion. Each calculated PBZ dose was dispersed in 5.0 L water and applied evenly to the soil surface in a 1.0 m radius around the base of the tree. Prior to application, a 150 mm high “wall” was created around each tree at about 1.25 m radius to prevent possible run-off of the PBZ/ water suspension due to steepness of slope or heavy rain within the ensuing few days.

Data collection

Air temperature measurement

Air temperature was measured on an hourly basis at each of the experimental sites, from time of establishment to time of completion of the experiments. Because of the risk of vandalism and/ or theft of meteorological equipment at the remote trial localities, a robust data logger housing (hereafter termed “Hobo pole”) was used to house Hobo® H8 series miniature temperature loggers (Onset Computer Corporation, Bourne, USA). At each site, the Hobo pole was positioned in a non-shaded, grassed area, 10 m from the northern boundary (outer surround row) of the trial. The Hobo pole was designed, tested and refined over a period of eight years, between 1996 and 2004 (Gardner et al. 2014). Temperature calibration curves developed by Gardner et al. (2014) were used to model hourly radiation screen (hereafter termed “screen”) air temperature data from hourly Hobo pole air temperature data (refer Chapter 3), for the period March 2004 to February 2011.

Chill unit calculation

Of three popular models tested, the Dynamic Model (Erez and Fishman 1998, Fishman et al. 1987) quantified *E. nitens* winter chilling accumulation most accurately across a range of South African temperate eucalypt plantation forestry sites (Gardner and Bertling 2005). The Daily Positive Utah Chill Unit Model, a modification of the Utah Model (Linsley-Noakes and Allan 1994, Richardson et al. 1974) used by the South African deciduous fruit industry, assigns Daily Positive Utah Chill Units (DPCUs). Long-term mean DPCUs for May to September are available on a 1 minute by 1 minute grid basis for the whole of South Africa (Schulze 2007a). In the main deciduous fruit-growing areas of South Africa, winter chill units generally begin accumulating in May (Schulze and Maharaj 2007a). However, in the temperate eucalypt plantation forestry areas of the country, which are located predominantly in the summer rainfall region, chill units generally begin accumulating in April (Gardner 2003, Gardner et al. 2013). Based on research carried out over three winters, between 01 April and 30 September in 2008, 2009 and 2010 in KwaZulu-Natal, the ratio between CP and DPCU is approximately 1:17 (R Gardner, unpublished data, 2014). CP and DPCU daily totals between April and

September each year, for the period 2004 to 2010, were calculated using modelled hourly screen air temperature data (Gardner et al. 2014).

Vegetative growth measurement

Tree height was measured on trial planting anniversary date of each year following trial establishment for five years.

Reproductive growth assessment

A flowchart illustrating the sequence of events pertaining to *E. smithii* annual floral bud crop production and assessments between 2004 and 2010 is presented in Figure 1. Floral bud abundance was assessed on an individual tree basis in February and May/ June of each year. In the summer rainfall area, *E. smithii* inflorescence buds emerge between October and late January, the timing of emergence depending on both long- and short-term environmental conditions (R. Gardner, unpublished data, 2014). By mid-May/ early June, the majority of the involucral bracts are shed and the umbels (inflorescences) with their individual flower buds in pre-anthesis or early anthesis stage are at their most conspicuous. Individual tree floral bud crop was estimated using the following scoring system: 0 = no umbels; 1 = very light crop, 25% or less of the secondary laterals bearing one or more umbels (secondary laterals defined as branches originating from primary stems); 2 = light crop, between 26 and 50% of secondary laterals bearing one or more umbels; 3 = moderate crop, between 51 and 75% of secondary laterals bearing one or more umbels; 4 = heavy crop, between 76 and 100% of secondary laterals bearing one or more umbels (Gardner and Bertling 2005).

Statistical analysis

Vegetative and reproductive growth assessments

Statistical analyses were performed to investigate the effect of site, PBZ and clone on tree height at five years and the fifth and sixth floral bud crops. As illustrated in Figure 1, the fifth and sixth floral bud crops were those initiated in 2008 and 2009, respectively, and scored in 2009 and 2010, respectively. The reasons why the previous years' floral bud crops were not included in the analyses are as follows:

- The possible confounding effects of trial establishment differences, e.g. the different planting and blanking dates, on analytical results were likely to be avoided
- The possible negative effect of transmission of juvenile (reproductive immaturity) signal from seedling rootstock to reproductively mature scion on flowering potential of the scion would likely be minimized (Gardner and Bertling 2005, Siniscalco and Pavelettoni 1988).

The effects of PBZ and clone (scion genotype) on tree height and floral crop load were explored in individual trial analyses using restricted maximum likelihood (REML) analysis in GenStat® (2012) (Payne et al. 2012). Fixed effects were specified as PBZ, clone and the PBZ by clone interaction. Fisher's protected least significant difference test was used to compare main effect and interaction means at the 5% level (Steel and Torrie 1981).

The interactive effect of site, PBZ and clone on tree height and floral crop score was investigated using REML meta-analysis in GenStat®. REML meta analysis produces estimates of means and variances which are more accurate than that of a combined Analysis of Variance (ANOVA) (Patterson and Thompson 1971, Robinson 1987), as separate residual terms per site are utilised and not a pooled residual over all sites. The fixed effects were specified as site, PBZ and clone and all their interactions. For the across-site analyses, trees were treated as nested within plots and plots within sites. All assumptions for valid REML analyses were satisfied.

Correlation analyses were carried out in GenStat® to investigate the degree of relatedness between selected environmental factors, PBZ and the sixth floral bud crop. The main objective was to investigate the relatedness of the different explanatory variables, particularly those that were climate-related. For the purpose of the correlation analyses, variate "FLW" (floral bud crop) represented average floral crop score for clones over replicates in the sixth year after planting.

Multiple linear regression analyses in GenStat® were used to investigate the relationships between selected climatic and landform factors, PBZ application and *E.*

smithii fifth and sixth floral bud crops. The landform factors described in Table 4 were fitted into the statistical models as factors, and therefore handled as dummy variables in the analyses.Preliminary multiple regressions established that the model most accurately fitted the relationship between the environmental and floral response data. Separate sets of multiple linear regressions were then carried out, for the fifth and sixth crops, and with and without PBZ included as a treatment. The response and explanatory variables included in the multiple linear regression analyses are described in Tables 4 and 5.

Results

At Thoresway and Chamisso (Eastern Cape) and Tweefontein (KwaZulu-Natal), sites all highly frost-prone due to their low-lying landscape situations, all *E. smithii* trees were killed by frost seven months after the initial planting, and again during winter following the February 2005 re-planting. Therefore, these three sites were excluded from all growth data analyses.

Effect of site, PBZ and clone on tree height

A summary of the results of the across-site REML analyses for tree height at five years, including calculated *F*-test values for the fixed effects, is presented in Table 6. Site and PBZ, and all interactions implicating site, PBZ and clone were highly significant ($p < 0.01$). The clonal effect was non-significant ($p = 0.846$). PBZ application reduced mean tree height in *E. smithii* by 20.7%, i.e. from 10.98 m to 8.71 m) ($p < 0.01$) (Table 7).

Mean five year tree heights in the *E. smithii* site x PBZ interaction are presented in Figure 2, and ranked according to height for the control (PBZ0) trees in Table 8. PBZ is known to retard vegetative growth in various woody perennials, including *Eucalyptus* (Fletcher et al. 2000), thus mean heights of the control trees, rather than those of PBZ-treated (PBZ1) trees, are a more accurate reflection of site effect on vegetative growth of *E. smithii*. The sites with highest tree growth (Table 8) did not coincide with sites having the highest MAP figures (Table 1). The three top-ranking sites for height, Netherby1 (14.73 m), Wyntoun (13.64 m) and Netherby3 (12.76 m), ranked only 3rd, 6th and 3rd for MAP (948 mm, 905 mm and 948 mm, respectively). The site ranking lowest for tree height at five years, Netherby2 (7.00 m), also ranked 3rd for MAP (948 mm). The regression of *E. smithii* five year height on MAP (Schulze and Lynch 2007) yielded an R^2 value of 0.089 (p = 0.436) (data not presented). Based on five year height, no clear linear relationship between MAP and vegetative growth was observed.

Similarly, no clear trend between MAT and height growth was evident (Tables 1 and 8, respectively). The site producing the tallest trees (Netherby1, 14.7 m) and shortest trees (Netherby2, 7.0 m) were both at the lower end of the MAT range (14.1 ℃). The regression of *E. smithii* five year height on MAT (Schulze and Maharaj 2007a) yielded an R^2 value of 0.052 (p = 0.554).

Separate *t*-tests were carried out for each site, to compare height growth of PBZ0 (control) trees against that of PBZ1 (PBZ-treated) trees. PBZ significantly reduced tree height at all but one site, viz. The Peak (Table 8). PBZ had the greatest growth retarding effect at Willowmere (5.5 m, or 47%, height reduction), a site at the lower end of the MAT range (14.1 ℃), and Blair Athol (4.5 m, or 40% , height reduction), a moderately cold site in the series (MAT 14.6 ℃). The two site s where PBZ had the least effect, viz. The Peak (0.7 m, or 7%, height reduction) and In De Diepte (1.5 m, or 17%, height reduction) were at either end of the MAT range (15.5 ℃ for The Peak, 14.5℃ for In De Diepte) (Table 1).

The site x clone interaction for height at five years was highly significant (p < 0.001). The four clones (scion genotypes) included in the trial series represented a narrow sample of the South African breeding and commercial planting stock. Therefore, a detailed discussion on the relative performance of the different clones across sites is not warranted. The *E. smithii* clonal mean for height at five years is a useful indicator of the relative growth potential of each of the 11 sites. No visible signs of graft incompatibility were present in any of the plants throughout the duration of the trials. Thus it seems unlikely that incompatibility between scion genotype and rootstock confounded the results for tree height. Summaries of the results of the separate REML analyses for *E.*

smithii tree height at five years for the individual sites in the series are presented in Appendix 4.

Effect of site, PBZ and clone on umbel production

In all years assessed (2008 to 2010), the new season's flower buds emerged over a period of about three months, from early October to late December. The first, sparse umbel crops were recorded in several of the *E. smithii* trials during May/ June 2006, approximately 28 months after trial establishment. The first significant umbel crops were scored in May/ June 2007, 40 months after trial establishment. Summaries of the results of the across-site REML analyses for *E. smithii* fifth and sixth floral bud crops (initiated 2008 and 2009), including the calculated *F*-test values for fixed effects, are presented in Tables 9 and 10, respectively. In the PBZ0 (control) treatment, mean umbel production (across clones and sites) increased dramatically from year five (2008) to year six (2009) (47% increase) (Table 11). In the PBZ1 treatment, a slight decrease in mean umbel production from fifth to sixth year was recorded (-6.7%).

In the fifth year (2008), site, PBZ and site x PBZ interaction were all highly significant ($p < 0.001$), and clone x site interaction significant at $p = 0.034$ (Table 9). Clone and PBZ x clone and PBZ x clone x site interactions were all non-significant at $p \leq 0.05$. Paclobutrazol application more than doubled umbel production, increasing mean umbel crop score from 1.24 (PBZ0) to 2.88 (PBZ1) (Table 11).

Mean fifth year (2008) umbel crop scores in the *E. smithii* site x PBZ interaction are presented graphically in Figure 3, and ranked according to umbel crop scores for the PBZ0 treatment in Table 12. PBZ is known to stimulate floral bud production in *Eucalyptus* species (Fletcher et al. 2000), therefore umbel crop scores for *E. smithii* non-PBZ-treated trees (PBZ0 treatment) were more indicative of the site effect on reproductive growth. In the control (PBZ0) treatment, Netherby3 ranked first for umbel production (crop score 2.062), significantly ($p < 0.05$) outperforming all other sites in this regard (Table 12). Willowmere also performed well, ranking second at 1.687 and significantly ($p < 0.05$) outperforming all other sites except Netherby3 (2.062) and Netherby1 (1.437). Separate *t*-tests were carried out for each of the *E. smithii* trials comparing mean fifth year umbel crop production for the control and PBZ1 trees (Table 12). The levels of significance for the difference in umbel production between the PBZ0 and PBZ1 treatments ranged markedly across sites. PBZ application significantly increased umbel production at all nine sites ($p < 0.05$). At six of the nine sites, the positive effect of PBZ on umbel crop was highly significant ($p < 0.001$).

Mean clonal effect on fifth year umbel crop was non-significant ($p = 0.512$) (Table 9). The site x clone interaction for fifth year umbel crop was significant at $p < 0.05$ (Table 9). Summaries of the results of the separate REML analyses for *E. smithii* fifth year umbel crop scores for the individual sites are presented in Appendix 5. Clone was significant ($p < 0.05$) at only one out of nine sites, viz. Willowmere ($p < 0.01$). The scion genotypes (clones) included in the trial series are a relatively narrow sample of the South African breeding and commercial planting stock, therefore a detailed discussion on the relative performance of the different clones across sites was not warranted.

In the sixth year (2009), site and PBZ were the only treatments to significantly ($p < 0.001$) influence umbel crop score (Table 10). The effects of clone, and the interactions between site, PBZ and clone, were all non-significant ($p \leq 0.05$). PBZ increased mean (across-site) umbel crop production by 48%, elevating mean umbel crop score from 1.82 (control) to 2.69 (PBZ1) (Table 11).

Mean sixth year umbel crop scores in the *E. smithii* site x PBZ interaction are presented graphically in Figure 4, and ranked according to umbel crop scores for control treatment in Table 13. In the control (PBZ0) treatment, Netherby2 ranked first for umbel production (crop score 2.5), significantly ($p < 0.05$) outperforming all other sites except Willowmere (2.438), the second most productive site (Table 13).
Willowmere, one of the coldest (MAT 14.1°C) and highest elevation (1708 m asl) sites evaluated (Table 1), was the only site to rank within the top third sites for umbel production in both fifth and sixth crop years (Tables 12 and 13). Separate *t*-tests were carried out for each of the nine sites comparing the sixth year umbel crop scores of the control trees against that of the PBZ1 trees. The effect of PBZ application on sixth year umbel crop score was generally less distinct than in the previous (fifth) crop year (Table

13). The levels of significance for the difference in umbel crop score between PBZ0 and PBZ1 treatments ranged substantially, from non-significant (two out of nine sites) to moderately significant ($p < 0.01$) (five out of nine sites).

The effect of clone and all interactions involving clone were all non-significant in the across-site REML analyses for sixth year umbel crop (Table 10). In the REML analyses for individual sites for the same variate, clone was significant ($p < 0.05$) at one of the sites, viz. Netherby2. The results for the individual REML analyses of sixth year umbel crop scores are presented in Appendix 6.

Relationship between site factors, PBZ and umbel production

The results of the correlation analyses for the range of environmental factors, PBZ application and *E. smithii* sixth year floral bud crop are presented in Table 14. The correlations between the different chill model x chill period combinations were all highly significant ($p < 0.01$) (Table 14). Correlations ranged from 0.862 (CP_2 versus DPCU_2) to 0.995 (DPCU_1 with DPCU_2). CP_1 (Dynamic Model, April to September) correlated highly with DPCU_1 (Daily Positive Utah Chill Unit Model, April to September) at 0.889. Mean annual temperature (MAT) was moderately negatively correlated with LAT (-0.580), CP_1 (-0.719) and CP_2 (-0.691) ($p < 0.01$). A moderate positive correlation existed between PBZ and FLW ($r = 0.645$). The correlations between FLW and all other factors were weak and insignificant.

Summaries of the results of multiple linear regressions yielding the highest R^2 values and significance in each set (fifth and sixth year umbel crops, with and without PBZ treatment) are presented in Table 15. ASPECT was consistently the most influential environmental explanatory variable across the suite of regressions. The analyses implicating fifth crop year data generally yielded substantially higher R^2 values than those implicating sixth crop year data (Table 15).

In the models pertaining to control (PBZ0) trees, in the fifth year (2008), 92% of the variance was accounted for by MAT and ASPECT ($R^2 = 0.924$, $p = 0.06$) (Table 15, FLW5a). The south-west (SW) aspect exerted the strongest positive effect on umbel crop (refer FLW5a) of all ASPECT categories, although this was only significant at p = 0.22. South-east (SE) aspect was significantly negatively related to umbel crop production ($p < 0.05$). The impacts of the ASPECT categories were measured relative to that for north (N) ASPECT category, the latter being closest to mean umbel crop score for ASPECT. In the sixth crop year (Table15, FLW6a), 75% of the variance was accounted for by DPCU_1 and RELIEF ($R^2 = 0.753$, $p < 0.05$). The very low (VL) RELIEF category exerted the greatest positive effect on umbel crop production (FLW6) ($p < 0.05$), with the remaining RELIEF categories exerting relatively minor positive or negative influences on umbel crop. Daily Positive Utah Chill units for the period April to September 2009 (DPCU_1) demonstrated a significant ($p < 0.05$) but negative relationship with umbel crop production.

Where PBZ was included as an explanatory variable, in the fifth year 79% of the variance was accounted for by PBZ and ASPECT ($R^2 = 0.794$, $p < 0.001$) (Table 15, FLW5b). South-west (SW) followed by west (W) aspects ($p < 0.05$ and $p = 0.05$, respectively), showed strong positive relationships with fifth year umbel crop. The relationship between PBZ and FLW5 was highly significant ($p < 0.001$). In the sixth crop year (Table 15, FLW6b), 61% of the variance was accounted for by PBZ and ASPECT ($R^2 = 0.610$, $p < 0.01$). None of the ASPECT categories were particularly dominant in the relationship with umbel crop, although east (E) aspect was the highest positively correlated category ($p = 0.09$) and south-east (SE) aspect the highest negatively correlated category ($p = 0.63$) with FLW6b. Again, the relationship between PBZ and sixth year umbel crop (FLW6b) was highly significant ($p < 0.001$).

Discussion

Effect of site and PBZ on tree height

Mean height of the control (PBZ0) trees were more indicative of the true effect of site on tree vegetative growth than mean height of the PBZ1 trees. Based on mean tree height for control trees at five years, the relationship between MAP and height was not strong (Tables 1 and 8). The deep soil conditions (≥ 1.0 m) at each of the sites may have played an ameliorative role in preventing soil water shortages from developing, particularly during the dry winter months, at the lower rainfall sites in the series (Darrow 1994, Owens 1995). Analysis of the relationship between five year tree height and actual MAP (mean for the period 2004 to 2009) may have yielded a somewhat different result. Soil water availability is the most common growth-limiting factor in *Eucalyptus* stands (Louw 1999, Schönau and Grey 1987). Similarly, the relationship between MAT and five-year height growth of the control (PBZ0) trees was not strong. Two sites at the lower end of the MAT range, viz. Netherby1 (MAT 14.1 ℃) and Netherby2 (14.1 ℃), produced mean tree heights at the opposite ends of the height range (14.7 m and 7.0 m, respectively). According to *E. smithii* five-year mean height data, vegetative growth did not appear to be linearly related to either MAP or MAT across the range of site conditions. It is postulated that other environmental factors, such as those relating to micro-topography, e.g. slope aspect and steepness, and genetic factors such as frost and heat tolerance of the different scion genotypes, may have exerted a stronger effect on tree height growth over the first five years. It was unlikely that soil nutrient content differences impacted on the results. Soil sampling and nutrient analyses carried out at each of the sites during 2012 revealed no significant deficiencies for any of the elements (and organic carbon) typically associated with *Eucalyptus* vegetative growth (R Gardner unpublished data 2012). Furthermore, graft incompatibility was excluded as a cause of confounding five-year tree height growth measurements, both within and across trial sites, due to the absence of incompatibility symptoms in any of the trees during the five years after planting.

Paclobutrazol application significantly reduced mean tree height in *E. smithii* (20.7% reduction) (Table 7) as would be expected (Hetherington and Jones 1990). The SITE x PBZ interaction showed a general trend of the growth suppressing effect of PBZ being most evident at sites having both low MAP and low MAT figures. However, this was not always the case. For example, at Gilboa, the site with the second highest MAP and MAT values (957 mm and 15.31 ℃, respectively), a l arge percentage height growth reduction occurred as a result of PBZ application. As referred to earlier, with respect to the PBZ0 treatment, Gilboa recorded the second lowest tree height growth of all sites. The varying effect of PBZ application on five-year tree height growth across the nine sites did not relate well to MAP and MAT data for the sites (Tables 1 and 8). Possibly, long-term data for the key climatic variables did not adequately represent actual site conditions that occurred over the period in which the trials were conducted. The micro-topographies of the trial sites may have contributed to the effect. Topographical factors such as aspect, steepness of slope and relative relief are known to influence air, soil and plant canopy temperatures (Dahlgren et al. 2007, Sader 1986, Schulze 2007b, Schulze and Horan 2007).

Effect of site, PBZ and clone on umbel production

Regardless of whether PBZ was applied or not, in both the fifth and sixth crop years mean umbel production varied markedly across sites (Tables 12 and 13). This indicated the existence of important differences in environmental conditions across the range of sites in each of the years. Environmental conditions are known to exert a strong influence on floral induction and development in temperate eucalypts (Moncur et al 1994, Potts et al. 2007).

In the control (PBZ0) treatment, between the fifth and sixth crop years a marked (47%) increase in mean (across site) umbel production occurred (Table 11), even though the mean (across site) increase in winter chilling of 5.2% (from 81.5 CP to 85.7 CP), was minimal. This suggested that some factor other than winter chilling was responsible for the substantial increase in floral bud production between the fifth and sixth crop years. The scions used in the production of the *E. smithii* grafted propagules for the trials were collected from reproductively mature trees, but the rootstock propagules were grown from seed. Thus, at the likely time of initiation of the fifth and sixth year floral bud crops, the seedling rootstocks were only 5.5 and 6.5 years old. In South Africa, open grown non-PBZ treated *E. smithii* seedlings rarely produce flower buds before the age of eight years and even thereafter flower bud crops are typically sparse (Jones 2002, Swain and Gardner 2003). The increase in floral bud crop from fifth to sixth crop year suggested that the stage of reproductive maturity of the seedling rootstocks, or possibly climatic differences between 2008 and 2009, or both of these, may have been responsible for the marked difference in reproductive performance between the two years. Temporary setback of reproductive maturity of adult scions by transmission of a juvenile signal from reproductively immature seedling rootstocks has been reported for fruit tree crops (Pliego-Alfaro and Murashige 1987) and *Eucalyptus* (Gardner and Bertling 2005, Gardner et al. 2013, Siniscalco and Pavellettoni 1988).

The high levels of floral bud production in non-PBZ treated (PBZ0) trees at certain of the sites in both crop years, e.g. Netherby3, Willowmere, Netherby1 and In De Diepte, was highly encouraging. These were the only sites with umbel scores above the across-site mean for PBZ0 in both years (Tables 11, 12 and 13). The most common environmental criteria across these sites appeared to be low MAT (≤ 14.5 ^{o}C) and high elevation (≥ 1678 m asl) (Table 1), though it is possible that such factors played an interactive role together with one or more other environmental factors in stimulating floral bud production in *E. smithii*. The effects of the highly inductive site conditions (on the basis of floral bud production) on other post-initiation aspects of reproductive growth in *E. smithii*, such as anthesis timing and duration, and rate of capsule and seed development, warrant investigating. Moncur et al. (1994) reported a three-week delay in *E. nitens* anthesis commencement coinciding with an elevational increase of 672 m in Tasmania.

Mean umbel production across sites and clones in the PBZ1 treatment was at least double that of the control (PBZ0) treatment on an annual basis (Appendices 5 and 6). The ability of PBZ application to significantly increase flowering in temperate eucalypts, given favourable environmental conditions, is well documented (Hasan and Reid 1995, Meilan 1997, Moncur and Hasan 1994). Moncur (1988) reported soil-applied PBZ exerting a positive floral stimulatory effect on *E. nitens* espalier-grown trees for a minimum of five years. In the *E. smithii* trials, a general reduction in the positive effect of PBZ on floral bud production was observed between fifth and sixth crop year (Tables 12 and 13). As trees were older and levels of winter chilling generally higher across sites during the sixth crop year, the most likely cause of the overall drop-off in difference in umbel production between the two PBZ treatments was lowered levels of active PBZ within PBZ-treated trees during the 2009 winter. The PBZ doses were applied in autumn (March/April) 2006 and therefore the results imply that a drop-off in the floral stimulatory effect of PBZ in temperate eucalypt orchards can be expected from the fourth winter after application. In South Africa, where the success of a commercial temperate eucalypt seed production enterprise is dependent on PBZ application to achieve a consistent abundant supply of improved seed, the need to re-apply the chemical approximately every five years needs to be taken into consideration in the economics of the operation. The cost of the chemical alone needed to treat one average orchard of young (9 yr old), elite (grafted) *Eucalyptus* trees (@ 200 trees per ha and mean breast height diameter = 30 cm) is approximately R 17 100 (±US$1480) (R Gardner unpublished data 2014).

Regarding clonal effect on mean umbel production, the general lack of significance of difference regarding floral crop production amongst the four *E. smithii* clones, within or across-site, and PBZ-treated or not, in both years (Tables 9 and 10) suggested that the environmental conditions applied in the trial series were close to optimal for *E. smithii* floral induction.

At sites where PBZ application caused the greatest reductions in tree height (Table 8) as well as the greatest increases in floral bud crops (Tables 12 and 13) over the control, e.g. Willowmere and Blair Athol, excessive bearing was noted in more than 33.3% of the PBZ treated trees. This phenomenon was not clone-specific (R Gardner unpublished data 2013). Possibly, in both the 2008 and 2009 winters, *E. smithii'*s chilling requirement for floral induction was adeqately met or even far exceeded, and under such conditions the additional floral stimulatory effect of PBZ resulted in over-bearing of certain scion/ rootstock genotype combinations. If so, possibly the ideal approach in future would be to apply the PBZ doses in two split-applications, particularly at sites where high levels of winter chilling are anticipated to occur on an annual basis. Negative consequences commonly associated with excessive flowering in temperate eucalypt seed orchard trees include reduced tree vigour, increased self-pollination, in-breeding and capsule abortion rates, and lowered genetic quality of seed (Eldridge et al. 1993, Moncur 1998, Moncur and Boland 2000, Reid et al. 1995). The merits of split-dose PBZ applications for controlling flower and seed crop production in commercial temperate eucalypts remain to be investigated in South Africa.

Relationship between site factors, PBZ and umbel production

The results of the correlation analysis for environmental factors, PBZ and *E. smithii* sixth year floral crop (Table 14) gave three main indications. Firstly, none of the environmental factors individually correlated highly with *E. smithii* sixth year umbel crop score, suggesting multiple linear regression analysis was warranted in order to investigate the significance of the different explanatory variable combinations. Secondly, PBZ correlated moderately with *E. smithii* umbel crop. Thirdly, the strongest inter-chill model correlation was between CP_1 (Dynamic Model, April to September) and DPCU_1 (Daily Positive Utah Chill Unit Model, April to September). Fourthly, MAT (mean annual temperature) showed a fairly high negative correlation with CP_1.

In the multiple regressions, environmental variables played a significant role in accounting for percentage variance (Table 15). The sites in the *E. smithii* trial series were all selected for high levels of annual winter chilling, based on knowledge of the effects of latitude, elevation and certain topographical factors on air and soil temperatures (Schulze and Horan 2007, Sharma et al. 2010) and chill unit databases for South Africa (Schulze and Maharaj 2007b). Due to the limited number of sites available at the time of trial establishment, the variations in landform factors (landform classes) could not be replicated equally. Rather, these were applied on a fairly *ad hoc* basis. Hence this should be taken into consideration when interpreting the results of the regressions.

The results of the regressions where PBZ was excluded as an explanatory variable are more indicative of the true effect of site on floral bud production. In the fifth crop year, low MAT and southerly (S) to south-westerly (SW) slope aspects were highly promotive of flowering, but in the sixth year there was some indication that sites with low levels of accumulated DPCU and very low relative relief were more conducive to floral bud production. Where PBZ was applied, southerly (S) to south-westerly (SW) slope aspects were again the most dominant positively influential environmental factors on floral bud production in both years. Regarding the positive effect of more southerly slope aspects on *E. smithii* floral bud production, what remains unclear are the portions of the positive floral responses due to environmental factors such as air, bud and soil temperature, soil water level and solar irradiance. Such factors have been known to substantially influence plant vegetative and reproductive growth, as well as species composition (Austin et al. 1983, Granger and Schulze 1977, Moore et al. 1993), over a range of environments (Dahlgren et al. 2007, Sader 1986, Schulze 2007b, Sharma et al. 2010).

In the *E. smithii* field trials, even though all sites were selected to have deep soils to prevent any major soil water deficit from developing at any stage through the lifespan of the trials, soil water levels in the topsoil horizons would most likely have declined to some degree during the drier winter months. In an earlier field trial series, neither low temperature nor drought stress significantly enhanced floral bud production in *E. nitens* grafts or seedlings (Gardner 2003). In South Africa, *E. smithii* has demonstrated high levels of drought tolerance at cool temperate (MAT ≤ 16 ºC) sites in the summer rainfall area (Darrow 1994, Gardner 2007, Smith et al. 2005). Based on earlier investigations undertaken on *E. nitens*, indications are that soil water deficit does not play a stimulatory role in floral bud production in either *E. nitens* or *E. smithii*. Thus it seems unlikely that minor soil water deficits, such as those that may have occurred in the topsoil horizons during winter, could have played a significant positive role in *E. smithii* floral bud crop production.

In Southern Hemisphere countries such as South Africa, south-facing slope aspects are generally cooler with respect to both soil and air temperature, resulting in slower plant growth rates occurring on southerly compared to northerly aspects (Granger and Schulze 1977, Schulze 2007b). A similar phenomenon was noted at Netherby plantation in the KwaZulu-Natal midlands. In the *E. smithii* control (PBZ0) treatment, in the fifth crop year, significantly lower mean tree heights ($p < 0.05$) were recorded in the trial on the south-facing slope (Netherby3) compared to the trial on the north-facing slope (Netherby1) (Table 8). In the same crop year, the two sites, both in the top three for umbel crop production (Table 12), differed significantly ($p < 0.05$) for the same variate, with Netherby3 (2.062) outperforming Netherby1 (1.437). In the following (sixth) crop year, the difference between the two sites on the basis of umbel crop production was neglible and non-significant ($p = 0.05$) (Table 13). The particular pattern of vegetative and reproductive growth across the two sites suggests the following:

- *E. smithii's* chilling requirement for floral induction is more consistently met when exposed to the environmental conditions associated with south-facing slopes in the summer rainfall area
- Slowed vegetative growth during the winter months may be a key factor in *E. smithii* floral induction process

The results of semi-controlled environment and field trial research carried out by the ICFR during the period 1996 to 2001 tended to suggest that cold, moist, stress-free growing conditions during winter favour *E. nitens* floral bud production (Gardner 2003). In the summer rainfall area of South Africa, such environmental conditions are typically associated with sites located on south-west facing slopes and having deep (> 1.0 m) soil profiles (Bale et al. 1998, Louw 1999, Schulze 2007b). In the winter and uniform rainfall areas, because of the more frequent cloud cover and associated increased chilling during the winter months (Schulze and Maharaj 2007b), aspect may not play such an important role as it does in the summer rainfall area. Decreased solar irradiance due to increased cloud cover during winter and spring may negatively affect temperate eucalypt vegetative and reproductive growth (Bell and Williams 1997, House 1997, Moncur 1992), and this would need investigating.

Conclusions

The research yielded new information that will be invaluable in assisting tree breeders and commercial seed producers select sites for *E. smithii* orchards where optimum floral crop production is aimed at. Plant-soil-atmospheric environmental factors associated with the highly inductive slope aspects need closer investigating. Increased knowledge of the key environmental triggers and associated plant physiological processes implicated in floral induction in temperate eucalypts may increase the ability to manipulate flower and seed crop production in high value species such as *E. smithii*. The value of methods of manipulating temperate eucalypt flowering may increase substantially in future if the current climate warming scenario continues at the predicted rate.

The application of PBZ generally resulted in a significant improvement in *E. smithii* floral crop yield. However, in the same species, PBZ in combination with highly inductive environmental conditions carries a high risk of excessive flowering and capsule production and compromised genetic quality of seed, as well as significantly decreased vegetative growth. The study demonstrated that through careful selection of sites within the high elevation, summer rainfall forestry landscape in South Africa, the dependency on PBZ to achieve satisfactory levels of flower bud production in *E. smithii* may be substantially reduced. This is an important finding, due not only to the negative environmental connotations associated with the use of triazoles in the outdoor environment, but also the temporary nature of PBZ's effectiveness in the stimulation of flowering. The investigation and application of other enabling horticultural technologies, such as the use of low-chill, precocious rootstocks, could lead to a further reduction in the dependency on PBZ to improve temperate eucalypt orchard economics. In the absence of PBZ application, high elevation (> 1700 m asl), cold (MAT ≤ 14.5 ^{o}C) sites were generally the most productive on the basis of *E. smithii* umbel crop. However, within the elevational and MAT ranges applied in the trial series (1550 to 1850 m asl, and 13.5 to 16.0 ^{o}C, respectively), caution needs to be exercised in selecting sites for *E. smithii* orchards. Based on our experience, the siting of *E. smithii* orchards in low landscape positions within the above elevational and MAT confines, or at high elevation (> 1800 m asl) sites in cold (MAT < 14.0 ^{o}C) areas carries with it a high risk of trees being severely damaged and/or killed by frost. The effects of the environmental conditions of highly inductive sites (on the basis of floral bud production) on various aspects of *E. smithii* post-initiation reproductive growth, such as anthesis timing and duration and rate of capsule and seed development, remain to be investigated.

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Table 1: Site details for the *Eucalyptus smithii* flowering trials

Trial name	In De Diepte	Wyntoun	Gilboa	The Peak	Tweefontein	Netherby1	Netherby2	Netherby3	Willowmere	Blair Athol	Thoresway	Chamisso
Locality:												
Province	MPU	MPU	KZN	KZN	KZN	KZN	KZN	KZN	KZN	KZN	EC	EC
Latitude	25°02' S	26°12' S	29°14' S	29°15' S	29°15' S	29°39' S	29°38' S	29°38' S	29°51' S	29°52' S	30°50' S	30°54' S
Longitude	30°44' E	30°44' E	30°17' E	30°09' E	30°13' E	29°38' E	29°38' E	29°38' E	29°26' E	29°37' E	28°13' E	28°11' E
Elevation (m)	1828	1733	1595	1629	1588	1688	1700	1678	1708	1568	1809	1686
Climatic factors:												
MAP (mm)[1]	1241	905	957	929	842	948	948	948	914	843	908	904
MAT (°C)[2]	14.5	15.0	15.3	15.5	15.1	14.1	14.1	14.1	14.1	14.6	13.8	14.1
MAC (DPCU)[3]	962.1	784.8	764.8	727.0	811.0	865.3	865.3	865.3	863.2	799.8	1078.2	951.4
Edaphic factors:												
Soil form and series[4]	Magwa 1100	Clovelly 1200	Kranskop 1200	Magwa 1200	Magwa 1200	Inanda 1200	Magwa 1100	Kranskop 1200	Kranskop 1200	Magwa 1200	Kranskop 1100	Magwa 1200
Soil unit[5]	Haplic Ferralsol	Haplic Ferralsol	Haplic Acrisol	Haplic Acrisol	Haplic Acrisol	Haplic Acrisol	Haplic Ferralsol	Haplic Acrisol	Haplic Acrisol	Haplic Acrisol	Haplic Ferralsol	Haplic Acrisol
Soil depth (m)	1.0	1.0	> 1.2	> 1.2	> 1.2	> 1.2	1.0	> 1.2	> 1.2	> 1.2	> 1.2	> 1.2
Landform elements:												
Aspect[6]	NW	S	N	SE	N	N	E	SW	E	E	E	NE
Slope[6]	VS	VG	VG	VG	GE	MO	GE	ST	GE	MO	GE	MO
Relief[6]	H	L	H	H	VL	VH	VH	H	VL	L	H	L

MAP = Mean annual precipitation; MAT = Mean annual temperature; MAC = Mean annual (long-term) chill units; DPCU = Daily Positive Utah Chill Units
MPU = Mpumalanga, KZN = KwaZulu-Natal, EC = Eastern Cape
[1] Schulze and Lynch (2007)
[2] Schulze and Maharaj (2007b)
[3] Schulze and Maharaj (2007a)
[4] Soil Classification Working Group (1991)
[5] IUSS Working Group (2006)
[6] Refer to Table 5 for landform elemental class description

Table 2: Details of the origins of the *Eucalyptus smithii* scion genotypes represented in the field trials

Clone No.	Origins			
	Provenance	Latitude (S)	Longitude (E)	Elevation (m asl)
ES01	Wombeyan Road, NSW	34°21' S	150°12' E	700
ES06	Tallaganda, NSW	35°23' S	149°37' E	950
ES71	Tallaganda, NSW	35°23' S	149°37' E	950
ES74	Wombeyan Road, NSW	34°21' S	150°12' E	700

NSW = New South Wales, Australia

Table 3: Allocation of the treatments in the *Eucalyptus smithii* split-plot design experiments

	Treatment	Treatment level description
Factor A:	**PBZ soil application**	
Level 1	PBZ0	Nil PBZ (control)
Level 2	PBZ1	PBZ soil drench applied
Factor B:	**Clone (scion genotype)**	**Provenance, flowering potential***
Level 1	ES01	Wombeyan Road, moderate
Level 2	ES06	Tallaganda, moderate
Level 3	ES71	Tallaganda, shy
Level 4	ES74	Wombeyan Road, shy

PBZ = Paclobutrazol
* Flowering potential rating derived from ICFR historical orchard records

Table 4: Description of all response and explanatory variables used in the multiple linear regression analyses

Variate assessed	Abbreviation used in text
Response variables:	
Fifth year mean umbel crop score per tree (floral buds initiated 2008)[1]	FLW5
Sixth year mean umbel crop score per tree (floral buds initiated 2009)[1]	FLW6
Explanatory variables:	
Cultural factors:	
Paclobutrazol soil application	PBZ
Climatic factors:	
Latitude (^{o}S)	LAT
Altitude (m asl)	ALT
Annual mean hourly air temperature for year of floral bud initiation (^{o}C)[2]	MAT
Accumulated Chilling Portions (CP)[3] for the period 01 April to 30 September in year of floral bud initiation	CP_1
Accumulated Chilling Portions (CP) for the period 01 May to 30 September in year of floral bud initiation	CP_2
Accumulated Daily Positive Utah Chill Units (DPCU)[4] for the period 01 April to 30 September in year of floral bud initiation	DPCU_1
Accumulated Daily Positive Utah Chill Units (DPCU) for the period 01 May to 30 September in year of floral bud initiation	DPCU_2
Landform factors:	
Slope aspect	ASPECT
Slope steepness	SLOPE
Relative relief	RELIEF

[1] Site mean for umbel crop score
[2] Air temperature modeled from hourly Hobo pole air temperature data
[3] The chill units assigned by the Dynamic Model (Erez and Fishman 1998)
[4] The chill units assigned by the Daily Positive Utah Chill Unit Model (Linsley-Noakes et al. 1994)

Table 5: Description of the landform factors and classes included in the multiple linear regressions

Factor and class	Class abbreviation	Class definition
Slope aspect[1] :		**Average**
North	N	North-facing
East	E	East-facing
South	S	South-facing
West	W	West-facing
North East	NE	North East-facing
South East	SE	South East-facing
South West	SW	South West-facing
North West	NW	North West-facing
Slope steepness[2]:		**Average (upper boundary)**
Level	LE	0^o 20' (0^o 35')
Very gently inclined	VG	1^o (1^o 45')
Gently inclined	GE	3^o (5^o 45')
Moderately inclined	MO	10^o (18^o)
Steep	ST	23^o (30^o)
Very steep	VS	37^o (45^o)
Relative relief[2]:		
Very low	VL	9 – 30 m
Low	L	31 – 90 m
High	H	91 – 300 m
Very high	VH	300 - 600 m

[1] Compass bearing
[2] Adapted from McDonald et al. (1984)

Table 6: Wald-statistics and calculated *F*-test values for fixed effects in the *Eucalyptus smithii* across-site REML analyses for tree height at five years

Fixed term	Wald statistic	d.f.	*F* statistic	*F* prob
SITE	127.13	8	15.05	<0.001
PBZ	29.17	1	29.17	0.008
CLONE	0.81	3	0.27	0.846
SITE.PBZ	142.17	8	16.83	<0.001
SITE.CLONE	85.38	24	3.34	<0.001
PBZ.CLONE	21.09	3	7.03	<0.001
SITE.PBZ.CLONE	84.74	24	3.32	<0.001

PBZ = Paclobutrazol
d.f. = Degrees of freedom

Table 7: Effect of PBZ treatment on *Eucalyptus smithii* tree height at five years

	PBZ treatment		
	PBZ0	PBZ1	Difference[1]
Mean	10.98*a*	8.71*b*	2.27**
SED	0.27	0.27	-

PBZ0 = Control
PBZ1 = PBZ applied to soil in March/ April 2006
SED = Standard error of the differences between means
[1] Difference between means (ns = not significant, * = significant at $p < 0.05$, ** = significant at $p < 0.01$, *** = significant at $p < 0.001$)

Table 8: Mean *Eucalyptus smithii* tree height at five years for the SITE x PBZ interactions, ranked according to height for the PBZ0 treatment

	PBZ treatment		
SITE[1]	PBZ0[#]	PBZ1[#]	Difference[2]
Netherby1	14.73*a*	10.52*a*	4.21***
Wyntoun	13.64*b*	10.46*a*	3.19**
Netherby3	12.76*c*	9.60*b*	3.16**
Willowmere	11.59*d*	6.13*d*	5.46***
Blair Athol	11.36*d*	6.86*c*	4.50***
The Peak	9.69*e*	8.98*b*	0.71ns
In De Diepte	9.29*ef*	10.83*a*	1.54*
Gilboa	8.78*f*	5.36*e*	3.43***
Netherby2	7.00*g*	9.64*b*	2.64**
SED	0.66	0.66	-

PBZ0 = Nil PBZ (control)
PBZ1 = PBZ applied to soil in March/ April 2006
SED = Standard error of the differences between means
[1] Sites ranked according to height for the PBZ0 treatment
[2] Difference between means (ns = not significant, * = significant at $p < 0.05$, ** = significant at $p < 0.01$, *** = significant at $p < 0.001$)
[#] Within this column, values followed by the same letter do not differ significantly ($p < 0.05$)

Table 9: Wald statistics and calculated *F*-test values for fixed effects in the *Eucalyptus smithii* across-site REML analyses for fifth year umbel crop score

Fixed term	**Wald statistic**	**d.f.**	***F* statistic**	***F* prob**
SITE	31.28	8	3.71	<0.001
PBZ	200.44	1	200.44	<0.001
CLONE	2.31	3	0.77	0.512
SITE.PBZ	25.9	8	3.07	0.004
SITE.CLONE	43.2	24	1.69	0.034
PBZ.CLONE	4.35	3	1.45	0.230
SITE.PBZ.CLONE	21.31	24	0.84	0.686

PBZ = Paclobutrazol
d.f. = Degrees of freedom

Table 10: Wald statistics and calculated F-test values for fixed effects in the *Eucalyptus smithii* across-site REML analyses for sixth year umbel crop score

Fixed term	Wald statistic	d.f.	*F* statistic	*F* prob
SITE	58.53	8	6.94	<0.001
PBZ	54.28	1	54.28	<0.001
CLONE	5.51	3	1.84	0.142
SITE.PBZ	14.84	8	1.76	0.097
SITE.CLONE	31.47	24	1.23	0.227
PBZ.CLONE	0.07	3	0.02	0.995
SITE.PBZ.CLONE	25.88	24	1.01	0.453

PBZ = Paclobutrazol
d.f. = Degrees of freedom

Table 11: Effect of PBZ treatment on *Eucalyptus smithii* fifth and sixth year umbel crop scores

	PBZ treatment		
	PBZ0	PBZ1	Difference[1]
Fifth year (2008)			
Mean	1.24*a*	2.88*b*	1.64***
SED	0.93	0.93	-
Sixth year (2009)			
Mean	1.82*a*	2.69*b*	0.88***
SED	0.10	0.10	-

PBZ0 = Nil PBZ (control)
PBZ1 = PBZ applied to soil in March/ April 2006
SED = Standard error of the differences between means
[1] Difference between means (ns = not significant, * = significant at $p < 0.05$, ** = significant at $p < 0.01$, *** = significant at $p < 0.001$)

Table 12: Mean *Eucalyptus smithii* fifth year umbel crop scores for the SITE x PBZ interactions, ranked according to umbel crop score for the PBZ0 treatment

	PBZ treatment		
SITE[1]	PBZ0[#]	PBZ1[#]	Difference[2]
Netherby3	2.062*a*	3.188*bc*	1.126**
Willowmere	1.687*b*	3.125*c*	1.438***
Netherby1	1.437*bc*	2.125*e*	0.688*
In De Diepte	1.312*c*	3.438*ab*	2.126***
Blair Athol	1.188*cd*	3.500*a*	2.312***
Gilboa	1.187*cd*	2.000*e*	0.813*
Netherby2	1.000*de*	2.812*d*	1.812***
Wyntoun	0.875*e*	2.750*d*	1.875***
The Peak	0.437*f*	3.021*cd*	2.584***
SED	0.276	0.276	-

PBZ0 = Nil PBZ (control)
PBZ1 = PBZ applied to soil in March/ April 2006
SED = Standard error of the differences between means
[1] Sites ranked according to umbel crop score for the PBZ0 treatment
[2] Difference between means (ns = not significant, * = significant at $p < 0.05$, ** = significant at $p < 0.01$, *** = significant at $p < 0.001$)
[#] Within this column, values followed by the same letter do not differ significantly ($p < 0.05$)

Table 13: Mean *Eucalyptus smithii* sixth year umbel crop scores for the SITE x PBZ interaction, ranked according to umbel crop score for the PBZ0 treatment

	PBZ treatment		
SITE[1]	PBZ0[#]	PBZ1[#]	Difference[2]
Netherby2	2.500*a*	3.312*a*	0.812**
Willowmere	2.438*a*	3.250*a*	0.812*
In De Diepte	2.063*b*	2.188*de*	0.125ns
Netherby1	2.000*b*	2.000*e*	0.000ns
Netherby3	1.937*bc*	2.937*b*	1.000*
Gilboa	1.875*bc*	2.625*c*	0.750**
Wyntoun	1.687*cd*	2.750*bc*	1.063**
Blair Athol	1.500*d*	2.875*bc*	1.375**
The Peak	0.375*e*	2.312*d*	1.937***
SED	0.286	0.286	-

PBZ0 = No PBZ applied (control)
PBZ1 = PBZ applied to soil in March/ April 2006
SED = Standard error of the differences between means
[1] Sites ranked according to umbel crop score for the PBZ0 treatment
[2] Difference between means (ns = not significant, * = significant at $p < 0.05$, ** = significant at $p < 0.01$, *** = significant at $p < 0.001$)
[#] Within this column, values followed by the same letter do not differ significantly ($p < 0.05$)

Table 14: Correlation matrix for selected explanatory variables included in the regressions between environmental factors, PBZ and *Eucalyptus smithii* floral crop response (sixth year umbel crop score).
[Critical values for Pearson's *r* (*df* = 22): $p < 0.05 = 0.404$; $p < 0.01 = 0.515$]

LAT	1.000								
ALT	-0.784	1.000							
MAT	-0.580	0.351	1.000						
CP_1	0.478	-0.257	-0.719	1.000					
CP_2	0.544	-0.347	-0.691	0.991	1.000				
DPCU_1	0.343	-0.076	-0.538	0.889	0.876	1.000			
DPCU_2	0.360	-0.106	-0.480	0.866	0.862	0.995	1.000		
PBZ	N/A	N/A	N/A	N/A	N/A	N/A	N/A	1.000	
FLW	0.112	0.135	-0.135	-0.146	-0.147	-0.168	-0.191	0.645	1.000
	LAT	**ALT**	**MAT**	**CP_1**	**CP_2**	**DPCU_1**	**DPCU_2**	**PBZ**	**FLW**

LAT = South latitude in degrees
ALT = Elevation in metres
MAT = Mean hourly (modeled) screen air temperature for 2009
CP_1 = Accumulated Chilling Portions (CP) for the period 01 April-30 September 2009
CP_2 = Accumulated Chilling Portions (CP) for the period 01 May-30 September 2009
DPCU_1 = Accumulated Daily Positive Utah Chill Units (DPCU) for the period 01 April-30 September 2009
DPCU_2 = Accumulated Daily Positive Utah Chill Units (DPCU) for the period 01 May-30 September 2009
PBZ = Paclobutrazol soil treatment (PBZ0 or PBZ1)
FLW = *Eucalyptus smithii* sixth year umbel crop score
N/A = Not applicable

Table 15: Summary of the results of the multiple linear regression analyses for *Eucalyptus smithii* fifth and sixth year floral bud crops on environmental factors and PBZ treatment

		FLW5a		**FLW5b**		**FLW6a**		**FLW6b**
		MAT ASPECT		**PBZ ASPECT**		**DPCU_1 RELIEF**		**PBZ ASPECT**
SOURCE	**d.f.**	**m.s.**	**d.f.**	**m.s.**	**d.f.**	**m.s.**	**d.f.**	**m.s.**
Regression	6	0.2880	6	2.3418***	4	0.6922*	6	1.0322**
Residual	2	0.0167	11	0.1960	4	0.0977	11	0.1897
Total	8	0.2202	17	0.9534	8	0.3950	17	0.4871
R^2		0.924		0.794		0.753		0.610
SED		0.129		0.443		0.313		0.436
Estimate of parameters:								
Constant		10.34		0.864		6.08		1.688
PBZ				1.646				0.875
MAT		-0.653						
DPCU_1						-0.00269		
RELIEF VL						1.874		
RELIEF L						-0.549		
RELIEF VH						0.669		
ASPECT E		-0.433		0.531				0.521
ASPECT SE		-1.277		0.065				-0.781
ASPECT S		-0.173		0.125				0.094
ASPECT SW		0.335		0.938				0.313
ASPECT W		-0.114		0.688				0.000
Parameter *t*-values:	2		11		4		11	
Constant		4.43*		3.53**		5.48**		7.01***
PBZ				7.89***				4.26***
MAT		-3.87						
DPCU_1						-4.11*		
RELIEF VL						4.40*		
RELIEF L						-1.80		
RELIEF VH						2.47		
ASPECT E		-2.73		1.86				1.85
ASPECT SE		-6.75*		0.17				-2.07
ASPECT S		-1.00		0.33				0.25
ASPECT SW		1.76		2.44*				0.83
ASPECT W		-0.71		1.79				0.00

* = significant at $p < 0.05$, ** = significant at $p < 0.01$, *** = significant at $p < 0.001$
PBZ = Paclobutrazol soil treatment (levels described in Table 3)
MAT = Mean hourly (modeled) screen air temperature for the year 2008 (FLW5) or 2009 (FLW6)
DPCU_1 = Accumulated Daily Positive Utah Chill Units (DPCU) for the period 01 April-30 September 2009[1]
RELIEF = Relative relief categories (described in Table 5)
ASPECT = Slope aspect categories (described in Table 5)
FLW5a = *Eucalyptus smithii* fifth year (2008) umbel crop score regressions, PBZ treatment excluded as an explanatory variable
FLW5b = *Eucalyptus smithii* fifth year (2008) umbel crop score regressions, PBZ treatment included as an explanatory variable
FLW6a = *Eucalyptus smithii* sixth year (2009) umbel crop score regressions, PBZ treatment excluded as an explanatory variable
FLW6b = *Eucalyptus smithii* sixth year (2009) umbel crop score regressions, PBZ treatment included as an explanatory variable
[1]DPCU modelled from hourly Hobo pole air temperature data

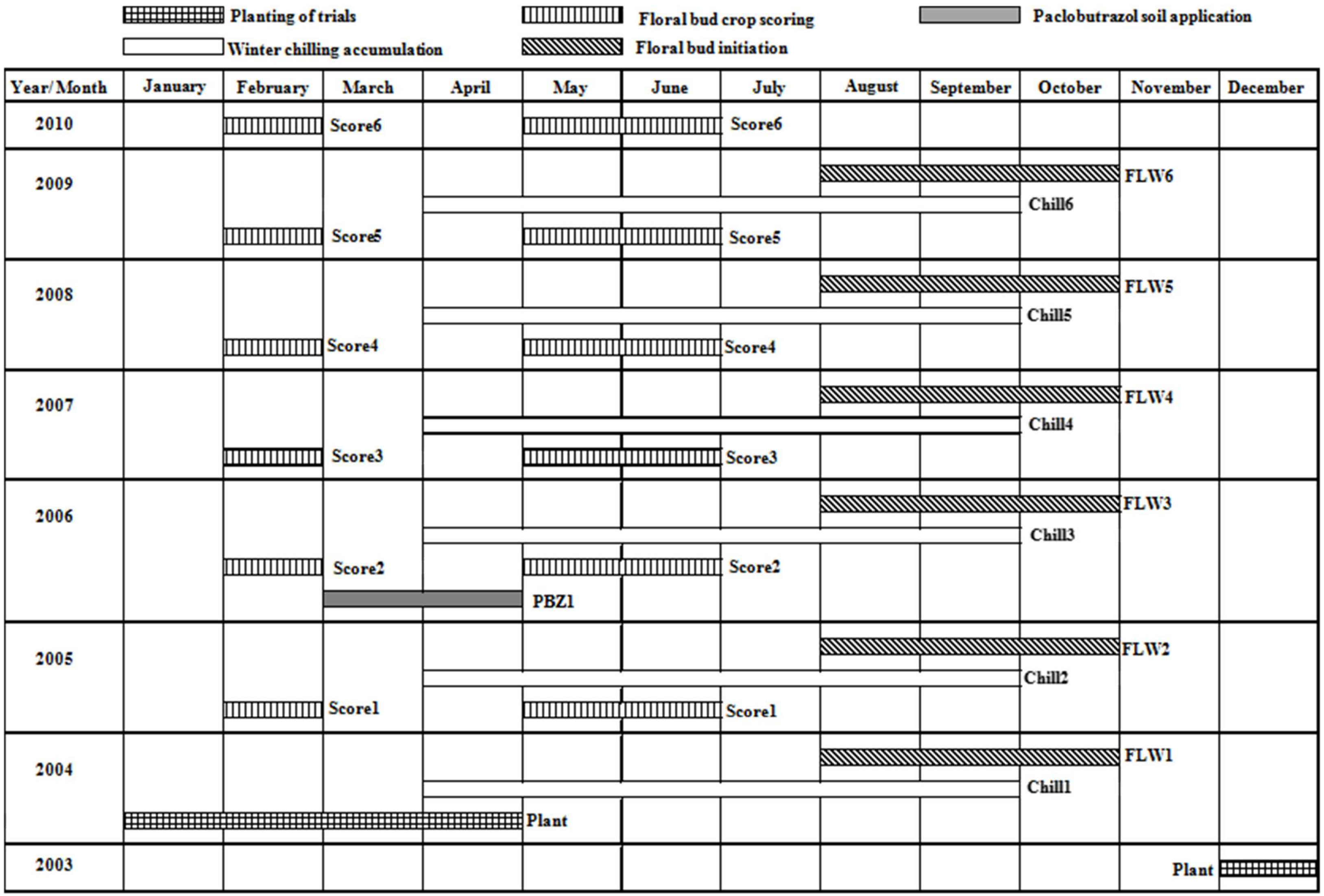

Figure 1: Sequence of events associated with *E. smithii* annual floral bud crop production between 2004 and 2010

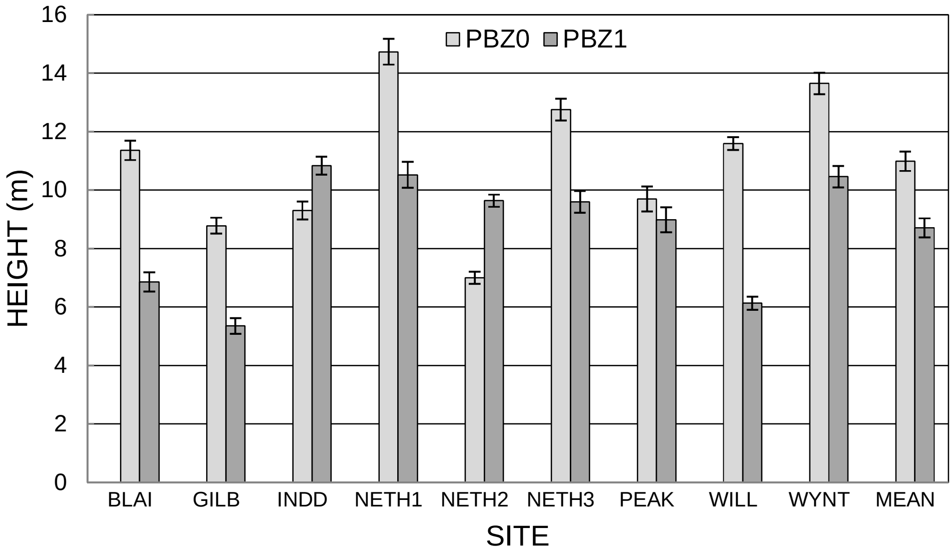

Figure 2: *Eucalyptus smithii* mean tree heights for PBZ treatments in the individual trials in the series at five years of age (2009). The trials are ranked alphabetically from left to right, with the across-site mean at the extreme right. PBZ0 = Nil PBZ (control), PBZ1 = PBZ applied to the soil in March/ April 2006. Error bars represent the standard error (SE) of the predicted mean

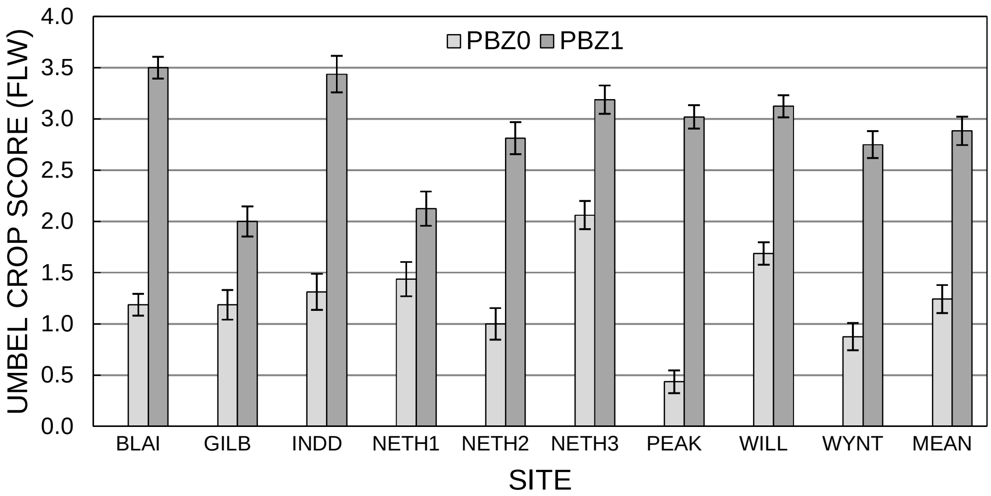

Figure 3: *Eucalyptus smithii* mean fifth year (2008) umbel crop score for PBZ treatments in the individual trials in the series. The trials are ranked alphabetically from left to right, with the across-site mean at the extreme right. PBZ0 = Nil PBZ (control), PBZ1 = PBZ applied to the soil in March/ April 2006. Error bars represent the standard error (SE) of the predicted mean

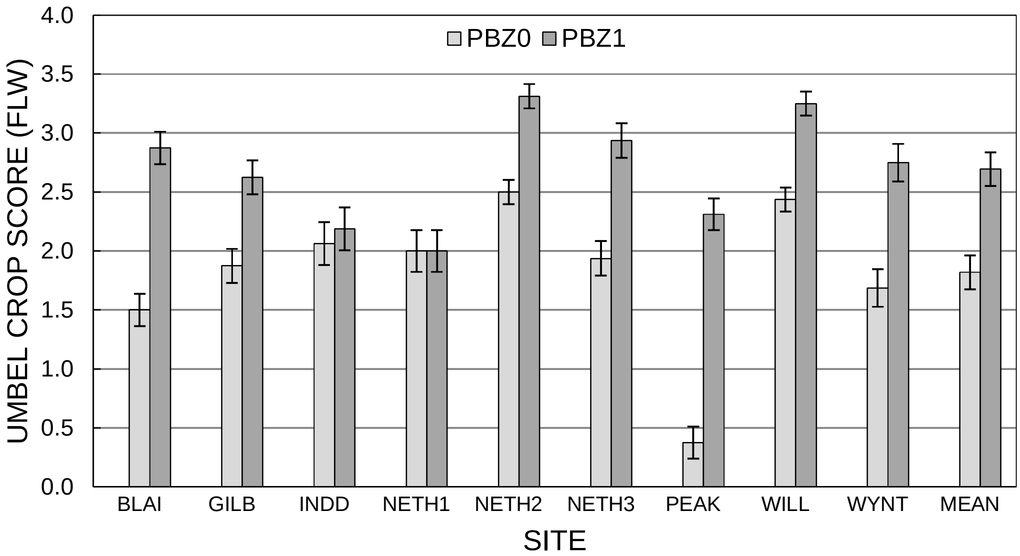

Figure 4: *Eucalyptus smithii* mean sixth year (2009) umbel crop score for PBZ treatments in the individual trials in the series. The trials are ranked alphabetically from left to right, with the across-site mean at the extreme right. PBZ0 = Nil PBZ (control), PBZ1 = PBZ applied to the soil in March/ April 2006. Error bars represent the standard error (SE) of the predicted mean

CHAPTER 6

Overhead irrigation increased winter chilling and floral bud production in *Eucalyptus nitens*

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Abstract

Eucalyptus nitens requires a sufficiently cold winter to produce flower buds. In areas in South Africa where *E. nitens* commercial plantations as well as breeding and production seed orchards are located, winter chilling is often insufficient for floral bud initiation. Hence, under such conditions, *E. nitens* floral bud and seed crops are poor and inconsistent. The local industry is almost entirely dependent on paclobutrazol (PBZ) applications for encouraging flowering in *E. nitens* seed orchards. Between 2008 and 2010, an experiment was conducted to investigate the potential of overhead irrigation (sprinkling) as a means of supplementing winter chilling to improve floral bud production in *E. nitens.* The treatments included three levels of sprinkling (nil, 10-week duration, 16-week duration), two levels of PBZ (nil, 0.025 g a.i. per mm basal stem circumference) and two grafted clones (prolific flowerer, shy-flowerer). Sprinkling reduced *E. nitens* daytime bud temperatures by as much as 16.2 ^{o}C on warm, dry winter days. In 2009 (cold winter) and 2010 (warm winter), sprinkling increased chilling accumulation by 44% and 72% (nil versus maximum sprinkling), respectively. In 2009, in the absence of PBZ, sprinkling resulted in a higher percentage trees of either clone producing umbels (flower buds) compared with the control. In the warmer 2010 winter, sprinkling again increased flowering, with the number of flowering shoots and umbels per tree being significantly higher than the control at $p < 0.05$. In both, 2009 and 2010, PBZ showed a strong additive effect to winter chilling on *E. nitens* floral bud production. The *E. nitens* clone x chilling x PBZ flowering interaction was complex, and warrants more detailed investigation in future. Overhead sprinkling offers a practical method of supplementing winter chilling and improving floral bud production in high chill requiring temperate eucalypt species such as *E. nitens*.

Keywords: chill modelling, evaporative cooling, global warming, insufficient chilling, paclobutrazol, seed orchard

Introduction

Eucalyptus nitens is an important temperate eucalypt cultivated for commercial pulpwood production in South Africa (Van den Berg and Stanger 2007, Velilla et al. 2007). The species is typically planted at high elevation (> 1300 m asl) sites within the summer rainfall forestry areas (Swain and Gardner 2003, Smith et al. 2005). The breeding and production seed orchards of the species are also located within these areas. The reticent and erratic flowering habit of *E. nitens* poses major challenges to tree breeders and commercial tree seed producers (Reid et al. 1995, Moncur and Boland 2000, Hamilton et al. 2008). In South Africa, *E. nitens* plantation-grown and/or seed orchard seedling trees rarely flower before the age of eight years, and annual floral bud and seed crops are typically sparse (Jones 2002, Swain and Gardner 2003). An added factor hampering flower and seed crop production in *E. nitens* is the species' high cold requirement for floral initiation (Moncur and Hasan 1994, Moncur 1998).

In most years, *E. nitens* cold requirement for floral bud production is not adequately met at South African seed orchard sites (Gardner 2003). The frequent sparse flower and seed crops that follow "warm" winters are a major hindrance to local temperate eucalypt tree improvement and seed production efforts. Increased global warming is likely to exacerbate the problem (Linkosalo et al. 2009, Warburton and Schulze 2008). Floral induction in *E. nitens* likely results from a cumulative chill process rather than a single chill event (Moncur and Hasan 1994, Gardner and Bertling 2005). A strong correlation exists between accumulated winter chilling and ensuing *E. nitens* floral bud crop (Gardner 2003, Gardner and Bertling 2005). Over the past half-century, several agricultural models have been developed for the quantification of winter chill. These models are based predominantly on the results of pioneering experiments where the effectiveness of temperatures, and the duration of exposure to the particular temperatures, on a range of cold-dependent plant physiological processes such as vernalization and endodormancy release of seeds and buds were established (Seeley 1996). The chill accumulation models in use today are purely empirical and based on the results of controlled environment and field experiments, rather than on a functional understanding of plant physiology (Luedeling et al. 2011).

The Dynamic Model (Fishman et al. 1987, Erez and Fishman 1998) is accurate in areas with either mild or cold winters (Allan 2008, Luedeling and Brown 2011). The chill unit that this model assigns is termed a Chilling Portion (CP) (Erez and Fishman 1998). Research in South Africa indicated that the minimum winter chilling requirement for floral bud production in *E. nitens* is approximately 40 CPs (Gardner 2003, Gardner and Bertling 2005). At less than 90 CPs, only 20% of reproductively mature trees can be expected to produce umbels (flower buds), but at sites where more than 95 CPs are accumulated, more than 50% of the trees can be expected to produce umbels. The relationship between winter chill units and *E. nitens* floral bud production (percentage trees producing umbels) does not appear to be linear. Rather, a minimum winter chilling threshold seems to exist for the majority of trees to be stimulated into producing umbels.

Where the plant growth regulator paclobutrazol (PBZ) is applied, the minimum chilling requirement to achieve 50% trees producing umbels can be lowered to about 80 CPs (Gardner 2003, Gardner and Bertling 2005). Similarly, where PBZ is applied, to achieve 20% trees producing umbels the chilling requirement can be lowered to about 70 CPs. Worldwide, PBZ is used almost routinely in *E. nitens* orchards for assisting vegetative growth control and promoting precocious and abundant flowering (Williams et al. 2003, Hamilton et al. 2008). However, PBZ application is relatively ineffective in the absence of a period of sufficient winter cold (Moncur and Hasan 1994, Williams et al. 1999).

In the South African temperate eucalypt plantation forestry areas, sites that have the high winter chilling levels needed by *E. nitens* for satisfactory flowering and seed set are restricted to fairly remote, highland areas distant from the main forestry research centres (Gardner 2004). The location of breeding and production seed orchards at such sites is inconvenient from a breeding and orchard management perspective and increases risks and financial costs. Therefore, investigating more practical and economically viable methods of winter chilling supplementation for improved flower and seed crop production in high value temperate eucalypt orchards is warranted.

During 1999 and 2000, a controlled environment experiment was carried out by the Institute for Commercial Forestry Research (ICFR) to investigate the relationship between artificially applied winter chilling and floral bud production in *E. nitens* (Gardner 2003). The facilities consisted of an artificially-lit growth cabinet and a cold room. Applied winter chilling significantly increased floral bud production, but the method did not hold particular promise for future *Eucalyptus* seed production efforts. This was due to impracticalities such as the inherent large size of the plants, the limited space and high running costs of the growing rooms and the high maintenance requirements of plants growing in such indoor environments. Therefore, an outdoor experiment was conducted between 2008 and 2010 to investigate the feasibility of using overhead irrigation (sprinkling) to cool plants, supplement winter chilling and increase floral bud production in *E. nitens* orchard trees. Intermittent overhead sprinkling has been used successfully in a number of temperate fruit crops for a variety of purposes, including the reduction of bud temperature and improvement in winter dormancy break in deciduous fruit crops (Gilreath and Buchanan 1979, Allan and Hattingh 1998, Allan 2004) and the manipulation of flowering and fruit ripening dates in both deciduous and evergreen crops (Matthews and Magein 1996, Allan et al. 1994, Iglesias et al. 2005).

The main objective of the current study was to investigate the feasibility of using overhead sprinkling to increase winter chilling and improve floral bud production in *E. nitens* orchard trees in South Africa. If the technique was successful in *E. nitens*, it may hold promise for improving flower and seed crop production in other important, yet inconsistent flowering, temperate eucalypt species in similar climates with marginal winter chilling.

Material and methods

Experimental site

This chilling response study was conducted between 2008 and 2010 using potted, grafted *E. nitens* trees at the Mondi Mountain Home Research Centre near Hilton, KwaZulu-Natal, South Africa (29º 34.070' S; 30º 16.467' E), having an elevation of 1133 m asl and long-term mean annual precipitation (MAP) and air temperature (MAT) of 924 mm and 16.2 ºC, respectively (Schulze 2007). The site was selected for marginality of floral bud production in *E. nitens*, close access to electrical power and irrigation water supply and adequate exposure and slope to facilitate free air movement across the site.

In South Africa, establishment of *E. nitens* plantations is not recommended for areas where altitude is less than 1300 m asl and/or MAT exceeds 16.0 ºC, due to the increased likelihood of poor tree growth and infection by diseases such as *Mycosphaerella* leaf blotch (Swain and Gardner 2003, Smith et al. 2005, Hunter et al. 2008). Based on air temperature data for the period 2001 to 2010 (De Nysschen pers. comm.[3]), mean annual accumulated winter chilling for the nearby Cedara Experimental Station (29º 32.515' S; 30º 15.896' E; 1066 m asl), 3.0 km to the north of Mountain Home, is 52 CPs. This amount of chilling can be considered marginal for reproductive growth in *E. nitens* (Gardner and Bertling 2005).

Plant material

In September 2006 scions from two *E. nitens* genotypes (ICFR breeding selections) were grafted onto six-month old *E. nitens* seedlings grown from South African commercial orchard seed. The scions were collected from two 11-year old grafted ramets in the ICFR Gowan Brae *E. nitens* Clonal Seed Orchard (CSO). In February 2007, six months after grafting, the plants were transplanted into 100 L (0.6 m diameter) black polythene plant bags containing a 1:1:1:1 mixture (by volume) of coir, perlite, pine-bark and coarse river-sand. The potted plants were then positioned on 0.6 m x 0.6 m concrete slabs spaced 2.0 m diagonally apart. The trees received alternate, fortnightly soil drench applications of Gromor Plant Food® (3:1:3 (37) + microelements) (National

[3] De Nysschen G. 2011. Institute for Soil, Climate and Water, ARC, Pretoria, South Africa

Plant Food cc, Cato Ridge, RSA) and Nutriplex™ (1:1:6 (38) + microelements) (Omnia, Johannesburg, RSA) hydroponic fertiliser. The applications consisted of 1.5 g Gromor Plant Food® or 10 g Nutriplex™ per litre water per plant. The nutritional status of the trees was monitored on a quarterly basis throughout the year, and any developing elemental deficiencies corrected accordingly via foliar application. Tree height was restricted to 1.5 m by tying down emerging dominant shoots to between 30º and 45º above the horizontal on a fortnightly basis.

Experimental design and treatments

The choice of design was determined by the available plant material, site dimensions and practicalities relating to the overhead sprinkling treatments and associated infrastructure. A 3 x 2 x 2 factorial experiment, consisting of 120 grafted trees, with treatments replicated five times was laid out as a split-split plot design (Gomez and Gomez 1984). "Sprinkling" (0 = nil sprinkling (control), 1 = controlled intermittent sprinkling for 10 weeks, 2 = controlled intermittent sprinkling for 16 weeks) was assigned as main-plot factor and “PBZ soil application” (PBZ 0 = control, PBZ 1 = soil drench applied) and "Scion genotype” (Clone 1, Clone 2) as sub-plot factors (Table 1). Each experimental unit consisted of two trees, both trees being measured. Due to practicalities associated with the application of the sprinkling treatments, the whole plots were laid out in three separate blocks across the site. During winter, panels of white 80% weave plastic netting, each 2.0 m high by 2.5 m wide, were erected between sprinkling treatments to prevent contamination of the Sprinkle 0 treatment trees by sprinkled droplets from the Sprinkle 1 and Sprinkle 2 treatments during sprinkler ON intervals. In addition, two isolation (non-measured) trees were positioned between sprinkling treatments within rows to add a further measure of protection against sprinkling contamination and possible confounding of growth effects resulting from the sprinkling treatments.

Paclobutrazol treatment

A suspension of Cultar® (formulation 250 g L^{-1} PBZ, Syngenta (Pty) Ltd, Johannesburg, RSA) was applied as a soil drench on two separate occasions, viz. 12 November 2007 and 03 May 2010, at a rate of 0.025 g a.i. per mm basal stem circumference (BSC). The latter measurement was taken at the narrowest point between graft union and first primary lateral (branch). Each calculated PBZ dose was mixed in 1.0 L water and poured onto the surface of the growing medium in a circle around the tree stem, midway between stem and plant bag perimeter. The reason for the second PBZ application was that the vegetative growth-suppressing effect of the initial PBZ application had substantially diminished by early 2009 (indicated by the diminishing difference in BSC) (Figure 1).

Infrastructure and instrumentation

The experiment was established under a pitched roof anti-hail shelter incorporating 20% shade (80% solar irradiance transmittance) rated black polythene hail-netting. The plant rows and irrigation lines were orientated approximately North-South (330^{o}-150^{o}).

A CR1000 Logger/Controller in tandem with AM16/32 Channel Multiplexer (Campbell Scientific Inc., Logan, USA) was used to schedule and control the irrigation (watering and overhead sprinkling) systems and the agro-meteorological instrumentation. The logger/ controller was programmed to measure every 30 s and average/convert and store these data as 2 min interval data. A simple 220 V centrifugal pump was used to maintain a water pressure of between 250 to 350 kPa during irrigation intervals. Watering was carried out via four peg drippers per plant fed from one 4 L h^{-1} button dripper. The frequency of watering was scheduled to maintain 75 to 100% field capacity of the potting soil. Soil water content (m^3 m^{-3}) was monitored in one non-sprinkled *E. nitens* "dummy" plant on a 2 min basis throughout the year using two Echo EC20 Soil Moisture Probes (Decagon Devices Inc., Pullman, USA). During occasional excessively hot and dry periods, the need for supplementary watering was indicated by the measured soil water content and irrigation supplied by the manual opening of solenoid valves. Furthermore, the irrigation schedule was updated every two months to accommodate seasonal alterations in weather.

Installation of the overhead sprinkling system was completed mid-June 2008 and controlled intermittent sprinkling first commenced on 7 July of the same year. In the subsequent years (2009 and 2010), overhead sprinkling began in May once the following climatic criteria were regularly met: nighttime minimum air temperature of 12 ^{o}C or lower; daytime maximum air temperature ≤ 26 ^{o}C; daytime minimum RH ≤ 25%. According to the Dynamic Model (Fishman et al. 1987), chilling will accumulate when nighttime temperatures are 12 ^{o}C or lower and daytime temperatures remain below 19 ^{o}C (Erez and Fishman 1998). The overhead sprinkling system utilised hanging Gulf Micro Sprinklers (Agriplas (Pty) Ltd, Cape Town, RSA) comprising coarse-droplet, short-range spinners with wetting radius of ca. 1.5 m and nominal flow rate of 30 L h^{-1} at 150 kPa. Sprinkling was controlled by the data-logger and switched on when mean *E. nitens* bud temperature within the sprinkled block reached 16 ^{o}C and switched off after 5 min sprinkling, i.e., the sprinkling OFF periods were of variable duration whereas the ON periods were consistently 5 min to ensure all plants became fully wet. Two durations of sprinkling were evaluated: 10 weeks and 16 weeks.

Rainfall outside the anti-hail shelter was measured using a Rain Collector II rain gauge (Davis Instruments Corporation, Hayward, USA) connected to a Hobo® Pendant Event Logger (Onset Computer Corporation, Bourne, USA). Rainfall plus sprinkled water within the anti-hail shelter was measured with a Rain-o-matic Professional Rain Gauge (Pronamic®, Rinkoebing, Denmark). Air temperature and RH were measured in the center of the sprinkled block and outside the anti-hail shelter using HygroClip® S3 probes (Rotronic® AB, Bassersdorf, Switzerland) mounted in 6-plate radiation shields. Wind direction and speed were measured at sprinkler height within the anti-hail shelter using an R.M. Young Company (Traverse City, USA) Model 03001 Wind Sentry.

Eucalyptus nitens “bud temperature” was measured by inserting the tip of fine copper-constantan thermocouple (TC) wire immediately beneath the bark and positioning it alongside a developing axillary bud located 250 to 300 mm below the tip of an actively growing lateral shoot (Savage and Allan 1997, Gardner 2003). Bud temperatures were measured on the northern and southern sides of each of two spatially separated sample trees per sprinkling treatment, thus employing a total of 12 TCs. The TCs remained in place throughout the year but were re-positioned early April each year before commencement of the winter sprinkling period to accommodate past and present changes in tree canopy architecture. Prior to the initial installation of the TC cables in the experiment in June 2008, the accuracy of all TCs was checked against each other and a mercury thermometer over a range of temperatures (0.0 to 26.0 ^{o}C) in a laboratory. Furthermore, prior to commencement of controlled intermittent sprinkling in 2008, 2009 and 2010, mean bud temperature in each of the sprinkling treatment blocks was investigated to identify and rectify any incorrectly positioned TCs, e.g. those not sufficiently shaded by foliage. This ensured that the bud temperature measurement methodology and accuracy was similar across the different sprinkling treatment blocks.

Data collection

Photosynthetic photon flux density

During September 2010, photosynthetic photon flux density (PPFD) across the experimental area was investigated using a Sunfleck Ceptometer Model SF-80 (Decagon Devices Inc., Pullman, USA). Statistical analysis of the data revealed that PPFD did not differ significantly for sprinkling treatments (R Gardner unpublished data 2012).

Vegetative growth and floral assessments

Fortnightly measuring of BSC and monitoring for symptoms of foliar diseases was carried out from mid-September 2007 to the end of December 2010. For these purposes all trees were measured and assessed. It was anticipated that the intermittent sprinkling of *E. nitens* foliage over extended periods in winter could result in significant outbreaks of eucalypt foliar and root diseases such as *Mycosphaerella* leaf blotch and *Phytophthora* root-rot. Stem/ shoot internode length is a good indicator of vegetative growth rate, particularly in experiments implicating PBZ treatments (Davis et al. 1988, Fletcher and Hofstra 1988). However, in the Mountain Home experiment, BSC was elected as the preferred indicator of *E. nitens* vegetative growth due to the anticipated lengthy duration of the experiment, high measurement frequency, large number of treatments and the concern that manipulation of the tree canopies (water-shoot control) may affect the accuracy of shoot length (SL) measurements. Shoot length was measured fortnightly between 06 April 2010, one month before the second PBZ application, and 06 December 2010 to monitor the rapidity of uptake of PBZ after the second application of the chemical (03 May 2010) had taken place. For this purpose, four actively-growing secondary shoots distributed equally around the tree canopy periphery (eastern, southern, western and northern sides) were tagged and measured during the eight month period. Due to logistical constraints, shoot growth was only monitored in trees of one scion genotype, viz. Clone 2. Flowering shoot and umbel counts per tree (all trees) were carried out during mid-December in 2008, 2009 and 2010.

Statistical analysis

The effects of sprinkling, clone and PBZ on BSC and shoot length increment and number of flowering shoots and umbels per tree in 2009 and 2010 were investigated using the method of restricted maximum likelihood (REML) analysis (Robinson 1987, Lane and Payne 1996) in GenStat® (2008). Prior to analysis, flowering shoot and umbel count data were logarithmically ($\log_{10}(x + 1)$) transformed to normalize the residuals and homogeneity of the error variances (Steel and Torrie 1981, Gomez and Gomez 1984).

Results

Plant-atmosphere environmental effects

Mean daytime (07:00 to 18:00) wind direction and speed within the anti-hail shelter for 2009 and 2010 sprinkling periods were 260.1^{o}/1.34 km h^{-1} and 253.8^{o}/1.06 km h^{-1}, respectively. The absolute maximum daytime wind speeds reached during the 2009 and 2010 sprinkling periods were 17.3 km h^{-1} and 15.6 km h^{-1}, respectively. Thus, daytime wind directions and wind speeds within the anti-hail shelter during the 2009 and 2010 sprinkling periods were not conducive to contamination of trees in the non-sprinkled (control) blocks by sprinkled droplets and/or cooled air originating from the sprinkled blocks.

Prior to the commencement of sprinkling in 2009 (13 May) and 2010 (14 May), *E. nitens* diurnal bud temperatures were similar across the three sprinkling treatment blocks (Figure 2). Over the 14-day pre-sprinkling calibration period, nighttime bud temperatures (mean of 2009 and 2010) were almost identical but daytime maximum bud temperatures differed by as much as 1.6 ^{o}C across sprinkling treatments. Ambient nighttime air temperatures within and outside the anti-hail shelter were almost identical, but were approximately 1.0 ^{o}C warmer than mean nighttime bud temperatures. A possible reason for the latter was the conductive heat losses from plant canopy to ground through the damp growing medium. Daytime air temperature maxima within the anti-hail shelter were on average 1.0 ^{o}C higher than the air temperature maxima outside the anti-hail shelter. This was possibly due to the solar energy absorbed by the black anti-hail netting being transmitted to the air within the anti-hail shelter, and not being able to dissipate freely to the outside air due to restriction in air movement imposed by the hail-netting. Daytime bud temperature maxima were between 0.01 and 1.5 ^{o}C higher than outside air temperature maxima.

Overhead sprinkling substantially reduced *E. nitens* bud temperature over that of control bud, open air and shaded air, for the majority of time during the 2009 and 2010 sprinkling periods (Table 2). Mean daytime (07:00 to 18:00) sprinkled bud temperature reductions over that of control bud, open air and shaded air temperatures during the 2009 and 2010 sprinkling periods were 3.0, 3.2 and 2.6 ^{o}C and 4.1, 3.8 and 3.1 ^{o}C, respectively. The highest maximum daytime sprinkled bud temperature reductions over control bud, open air and shaded air temperatures occurred during the relatively warm 2010 winter (16.2, 15.5 and 13.6 ^{o}C, respectively). On typical warm, cloudless winter days when the overhead sprinklers were intermittently operating between about 09:30 and 16:00, the temperature patterns for sprinkled bud and outside wet bulb thermometer followed similar trends (Figure 3).

Mean daily temperature maxima for shaded air, open air, *E. nitens* control and *E. nitens* sprinkled buds during the 2010 sprinkling period were 20.3, 21.0, 21.8 and 15.9 ^{o}C, respectively. The absolute daily temperature maxima for the same variables in the same year were 31.7, 32.0, 32.0 and 16.3 ^{o}C, respectively. Regardless of the 2010 winter being warmer than the 2009 winter, in both years the greatest bud temperature reductions from sprinkling occurred during the late August - early September period, when daytime RH was lowest and ambient air and control bud temperature maxima were highest.

In 2008, 2009 and 2010, *E. nitens* buds commenced accumulating winter chilling on 08 April, 21 April and 24 April, respectively, and outside (full sun) air temperature conditions showed a similar trend in the same years (Table 3). In 2009 and 2010, when overhead sprinkling was applied for the full season (110- and 116-day periods, respectively), 4.7 CPs and 1.8 CPs accumulated in the periods prior to commencement of overhead sprinkling in May (Figure 4). In all three years, overhead sprinkling substantially increased bud chilling accumulation over that of control trees (Table 3). In 2008, when the overhead sprinkling system was operational for only half the winter (59-day period), sprinkling was able to increase control bud chilling level by 40.5% from 50.1 CPs (control) to 70.4 CPs. In 2009, sprinkling increased control bud chilling from 55.0 CPs (control) to 79.0 CPs (43.6%) and in 2010 from 39.6 to 68.0 CPs (71.7%). In all three years, total accumulated chilling for the control buds was similar to that for outside (full sun) air temperature conditions, differing by a maximum of 1.4 CPs in 2008. During the relatively warm 2010 winter, accumulated chilling for open air and control bud were identical. Further investigations are needed to substantiate this phenomenon.

Vegetative growth response

Paclobutrazol rapidly suppressed tree growth following both, the 2007 and 2010 PBZ treatments (Figure 1). In the maximum winter chilling (Sprinkle 2) block, following the initial (12 November 2007) PBZ application, a maximum difference in BSC between the PBZ-treated (PBZ 1) and control (PBZ 0) trees of 8.8 mm (13.1%) was reached about six months after application in late April 2008. From thereon, the difference in BSC between PBZ 1 and PBZ 0 trees steadily diminished over a seven-month period till late summer 2008, following which the difference in BSC between the two PBZ treatments remained fairly constant till re-application of PBZ in May 2010 (Figure 1). From April 2008, canopies of the PBZ-treated trees remained substantially more compact and required less water-shoot control than those of the control trees.

In 2009, there was a significant ($p < 0.05$) PBZ x Clone interaction effect for percentage BSC increment over winter (Table 4). In Clone 1, mean percentage BSC increment for PBZ 1 trees was 2.0% less than that of PBZ 0 trees. Conversely, in Clone 2, mean BSC increment of PBZ 1 trees was 2.1% greater than that of PBZ 0 trees. In 2010, the effect of the PBZ x Clone interaction on BSC increment was non-significant ($p < 0.05$). However, the effect of PBZ on percentage BSC increment (mean of two clones) was significant ($p < 0.05$) and highly significant ($p < 0.01$) on percentage shoot length increment in Clone 2 (SL was not measured in Clone 1) (Table 4). Mean percentage BSC increment of the PBZ 1 trees was 2.4% less than that of the PBZ 0 trees, whereas mean percentage SL increment of the PBZ 1 Clone 2 trees was 30.8% less than that of the PBZ 0 Clone 2 trees.

From the establishment of the *E. nitens* plants *in situ* at Mountain Home in November 2007 through till December 2010, no noteworthy foliar, stem or root disease symptoms were observed in the experimental trees.

Reproductive growth response

Number of trees producing umbels

The first umbels were recorded on five trees in November 2008, 21 months after establishment *in situ* at Mountain Home. Three of the five trees had been PBZ-treated and subjected to the maximum chilling amount of 70 CPs (Sprinkle 1).

In 2009, 23% of the total number of plants (120) produced umbels (Table 5). One and a half times as many Clone 1 plants produced umbels as those of Clone 2 (16/60 versus 11/60). In the PBZ 0 treatment with Sprinkle 0, 55 CPs occurred (Table 3) resulting in a poor flowering response i.e. ≤ 10% of the plants of either clone produced umbels (Table 5). At the moderately high chilling levels of 70 and 79 CPs (Sprinkle 1 and Sprinkle 2 treatments), Clone 1 produced a moderate flowering response, i.e., 30% trees produced umbels in either case. At these chilling levels (70 and 79 CPs) less than 20% of the Clone 2 plants produced umbels. This suggested Clone 2 may have a higher chilling requirement for floral bud production than Clone 1.

Application of PBZ (PBZ 1 treatment) had a positive effect on flowering of Clone 1 and Clone 2 at the moderately low chilling level of 55 CPs (Sprinkle 0) (30% trees producing umbels in either clone). At the moderately high chilling levels of 70 and 79CPs (Sprinkle 1 and Sprinkle 2), PBZ application had a minimal additive effect on the number of trees producing umbels in Clone 1, but in Clone 2, the application of the chemical increased the number of flowering trees substantially, from 0 to 30% (Table 5). On the basis of percentage trees producing umbels, the 2009 results suggested that not only is Clone 2 an inherent less prolific flowering genotype than Clone 1, but the former clone may have a substantially higher chilling requirement for floral bud production than Clone 1.

In 2010, 32% of the total number of plants produced umbels following a winter when the accumulated chilling in each of the three sprinkling treatments was substantially lower than that of the previous year (Sprinkle 0 = 40 CPs, Sprinkle 1 = 53 CPs, Sprinkle 2 = 68 CPs) (Tables 3 and 5). Three times as many Clone 1 plants produced umbels as those of Clone 2 (29/60 versus 9/60) in the 2010 season.

In the PBZ 0 treatment, at the low chilling level of 40 CPs (Sprinkle 0) no flowering was observed in either clone (Table 5). In Clone 1, the supplementary 13 and 28 CPs provided in Sprinkle 1 and Sprinkle 2 (total chilling 53 CPs and 68 CPs, respectively) increased the number of flowering trees by 40% and 20%, respectively. Clone 1's flowering response to the moderately low chilling level of 53 CPs in 2010 (Sprinkle 1, 40% trees producing umbels) contrasted strongly with the clone's response to the similar chilling level of 55 CPs (Sprinkle 0, 0% trees producing umbels) in 2009. This suggested that age may have had an influential effect on the reproductive growth of the grafted trees in the experiment. In Clone 2, the moderately low chilling levels of 53 and 68 CPs (Sprinkle 1 and Sprinkle 2) in 2010 produced poor flowering responses, with ≤ 10% plants producing umbels in either case.

PBZ application (PBZ 1) showed a strong positive effect regarding flowering in either clone across all levels of winter chilling (Table 5). In Clone 1, at the low chilling level of 40 CPs the application of PBZ markedly increased the percentage trees producing umbels, from nil to 40%. With the supplementary 13 and 28 CPs provided in Sprinkle 1 and Sprinkle 2 (total chilling 53 CPs and 68 CPs, respectively), PBZ application more than doubled the percentage trees with umbels in Clone 1 (90% and 100%, respectively). This suggested that PBZ application resulted in Clone 1's cold requirement for flowering being considerably lowered. In Clone 2, PBZ application had a mild positive effect on flowering across all three chilling levels (24% mean increase in percentage trees producing umbels). At any of the chilling levels, PBZ was not able to stimulate more than 30% of the trees to produce umbels. This suggested Clone 2 genotype may have a high chilling threshold that needs surpassing before flowering can be triggered, and/or the clone is an inherent shy-flowering genotype.

Number of flowering shoots and umbels produced per tree

In 2009, neither sprinkling, PBZ nor clone had a significant ($p > 0.05$) effect on the number of flowering shoots or number of umbels produced per tree (Table 5). Nevertheless, the significance of the sprinkling x PBZ x clone interaction on number of umbels per tree ($p = 0.093$) far exceeded that of all other treatments and their interactions regarding the same reproductive variable. In 2010, sprinkling exerted a significant effect ($p < 0.05$) on number of flowering shoots and number of umbels produced per tree (Table 5). PBZ, clone, and sprinkling x clone, PBZ x clone and sprinkling x PBZ x clone interactions were all highly significant ($p < 0.01$) for number of flowering shoots and number of umbels produced per tree. The only treatment or interaction not exerting a significant effect on either reproductive attribute was the sprinkling x PBZ interaction.

Discussion

Plant-atmosphere environmental effects

The maximum temperature reduction in *E. nitens* buds achieved through sprinkling at Mountain Home, viz. 16.2 ^{o}C in 2010 (Table 2), was of similar magnitude to that reported for sprinkled winter-dormant kiwi-fruit (*Actinidia deliciosa*) buds (ca. 10.0 ^{o}C) (Allan et al. 1994) and macadamia (*Macadamia integrifolia*) sunlit leaves (16 to 18 ^{o}C) (Allan et al*.* 1994, Savage et al.1997). At Mountain Home, the greatest bud temperature reductions took place around midday when RH figures were at their lowest and evaporative cooling rates (of *E. nitens* foliage) likely highest (refer daytime hourly wet bulb temperature pattern in Figure 3). This daytime evaporative cooling pattern concurred with that observed for macadamia (Allan et al. 1994) and apple (*Malus domestica*) (Iglesias et al. 2002). It generally became impractical to operate the overhead sprinkling system beyond the first week of September due to persistent excessively high night- and daytime bud temperatures coupled with increasingly higher daytime RHs.

Eucalyptus nitens buds began accumulating winter chilling at Mountain Home well before the dates of overhead sprinkling initiation in 2009 (13 May) and 2010 (14 May) (Table 3). High daytime RH values at Mountain Home prior to these dates each year was the main climatic factor hampering the cooling system and accumulation of chill units. At drier and less humid sites further inland, initiation of sprinkling in April and accumulation of additional chilling may be possible. At higher altitudes and/or more southerly latitudes than Mountain Home, lower winter daytime air temperatures between April and September would likely result in substantially less sprinkler "ON" time and total irrigation water being necessary to maintain daytime foliage temperatures below 16 ^{o}C (Allan et al. 1994). The additional chill units accrued in April and September would increase total winter chilling accumulation considerably, possibly further enhancing floral bud and seed crop production.

In orchards where overhead sprinkling is employed, climate and canopy density and spatial dimension are the major determinants of the actual water volumes needed to achieve similar or greater levels of supplementary winter chilling (Evans 1999, Wand et al. 2005). Over the past two decades, the effectiveness of different sprinkler nozzle types and overhead sprinkling application rates for fruit crops have been investigated fairly extensively. For macadamia and table grape (*Vitis vinifera*), mini-sprinklers that wet 360^{o} simultaneously with small droplets were more effective than rotating impact-type sprinklers that produce large drops of water and take longer to wet all plant parts (Savage et al*.* 1997, Allan and Hattingh 1998). For apples, higher application rates reduced fruit temperatures better than lower rates (Evans 1999); rapid wetting of apple foliage and fruit followed by evaporation of water directly from the fruit surface was more effective in reducing fruit temperatures and resulted in water conservation. In windy areas, large droplet size and closer spacing of sprinkler nozzles were increasingly necessary for adequate penetration of the canopy and uniform wetting of foliage. In the relatively protected (low wind speed) conditions under the hail-netting at Mountain

Home, the particular mini-sprinklers functioned efficiently. In open air (not hail-net protected) orchards, higher wind speeds would likely necessitate the employment of coarser-droplet sprinklers to achieve a similar efficiency of plant foliage wetting.

Vegetative growth response

A likely reason for PBZ significantly affecting tree growth (percentage increase in BSC) in 2010, but not in 2009, is the contrasting response of Clone 1 and Clone 2 trees to PBZ application in 2009 (Table 4). Mean percentage BSC increment for PBZ 1 trees was less than that of PBZ 0 trees in both, 2009 and 2010. For Clone 2, in 2009 the mean percentage BSC increment of PBZ 1 trees was 2.1% greater than that of PBZ 0 trees, but in 2010 the mean percentage BSC increment for PBZ 1 trees was 2.7% less than that of PBZ 0 trees. A possible explanation for the contrasting vegetative growth responses of the two different genotypes to PBZ treatment in 2009 is that an inherent difference in cold resistance between the two *E. nitens* genotypes existed. PBZ is known to increase cold stress resistance and even enhance vegetative growth under cold conditions in a number of herbaceous and woody crops (Fletcher et al. 2000). The likely low levels of PBZ present in the foliage of PBZ 1 trees during 2009, as indicated by the minimal growth suppressive effect of PBZ from December 2008 onwards (Figure 1), may have resulted in the relatively cold 2009 winter conditions (across all sprinkling treatments) negatively impacting more on the vegetative growth of the lesser cold stress resistant genotype. Based on the BSC increment data, Clone 1 appeared to be less cold stress resistant than Clone 2. Under the substantially warmer winter conditions of 2010, when trees were one year older and scions vegetatively more mature, and PBZ levels in the PBZ 1 trees high due to the second PBZ application, the cold stress resistance enhancing effect of PBZ would have been masked to a greater extent in Clone 1. *Eucalyptus nitens* seedlots of Barrington Tops provenance have previously demonstrated superior cold and frost resistance to those of Ebor provenance in field trials in South Africa (Darrow 1983, Nixon 1983, Purnell and Lundquist 1986). Variation in cold resistance between provenance, and among genotypes within provenance, has been reported for temperate eucalypt species such as *E. nitens* (Tibbits and Hodge 2003, Hamilton et al. 2008).

Sprinkling alone did not significantly ($p \leq 0.05$) affect vegetative growth rate (as indicated by percentage increase in BSC and/or SL) in either 2009 or 2010. This suggested that it was unlikely that overhead sprinkling caused total or partial leaching of PBZ from the growing medium of PBZ 1 trees in the Sprinkle 1 and Sprinkle 2 treatments. The PBZ applied to the growing medium was taken up rapidly by trees soon after application resulting in an almost immediate effect on BSC (Figure 1). To encourage rapid uptake of water plus PBZ dose, immediately prior to each PBZ application the growing medium was maintained in a slightly dry state. This was followed by controlled conservative irrigation for a two-day period. PBZ is known to be relatively immobile in soil and bound mainly by organic matter (Lever 1986, Davis et al. 1988). The high proportion of organic matter in the growing medium at Mountain Home would have further contributed to the prevention of loss of PBZ into the environment outside the plant growing bags.

Soil application of PBZ remains a highly successful method of achieving rapid and sustained vegetative growth suppression and enhanced floral bud and seed crop production in *Eucalyptus* orchard trees (Williams et al. 2003, Hamilton et al. 2008). With an evidently increasing worldwide trend towards more intensively planted and managed commercial eucalypt orchards, the dependency on plant growth regulating chemicals (PGRs), such as PBZ, to achieve the necessary levels of growth control is likely to increase (Moncur 1998, Potts et al. 2007, Gardner and Oscroft 2009). However, the recalcitrant nature of triazoles, such as PBZ, and their persistence in soil (Jackson et al. 1996) remains problematic regarding their use in the outdoor environment. Thus, it is prerogative to investigate alternative methods to achieve similar vegetative and reproductive growth control. These would include improvement in application technique (e.g. better targeting) of PGRs (Fletcher et al. 2000), development and testing of PGRs with less negative environmental impact (Meilan 1997), environmental manipulation (Moncur 1998) and the use of inductive and semi-dwarfing rootstocks and containerized orchards for breeding purpose (Gardner and Bertling 2005).

The lengthy periods of wet, humid conditions imposed on the *E. nitens* trees in the Sprinkle 1 and Sprinkle 2 treatments in three successive winters did not result in any problematic foliar, stem or root disease symptoms manifesting in the experiment. The high tolerance of the specific clones to persistent high humidity winter conditions may in part be explained by the climates of the areas of origin of the particular scion genotypes. Ebor and Barrington Tops both experience a summer rainfall climate, with fairly high MAPs (1232 mm and 1963 mm, respectively). They also have moist winters (May to September) with monthly rainfall totals generally above 60 mm on average (Australian Government Bureau of Meteorology 2013).

Reproductive growth response

Tree age appeared to exert a strong influence on the reproductive growth of the trees in the experiment. The percentage trees producing umbels increased almost linearly over time, from the date of establishment *in situ* at Mountain Home to end of the experiment. PBZ was applied in early November 2007, and, based on the fortnightly vegetative growth measurements PBZ was still present in the foliage of the treated trees by onset of winter 2008. However, following winter 2008, only five out of 120 trees produced umbels, and, of these, three had been treated with PBZ and experienced a moderately high winter chilling amount of 70 CPs. This suggests that the reproductive maturity of the scions was temporarily set back by transmission of a juvenile signal from the reproductively immature seedling rootstock to the scion (Pliego-Alfaro and Murashige 1987, Siniscalco and Pavolettoni 1988). Establishment of the exact magnitude and duration of reversion to partial reproductive immaturity (juvenility) in the *E. nitens* scions was not within the scope of this experiment. If the reversion (to reproductive juvenility) was still present in the scions during 2010, then it is possible that the transmitted juvenile effect would have lessened even further with time, predisposing an even higher number of trees to produce umbels, given adequate winter chilling.

The timing of vegetative phase change and first flowering are genetically independent in *E. globulus* ssp. *globulus* (Jordan et al. 1999). Flowering in the juvenile phase has been induced chemically, using PBZ, without any effect on vegetative phase change in *E. globulus* (Hasan and Reid 1995). Evidence exists that the timing of vegetative phase change and first flowering is independent and able to be manipulated separately in *E. nitens* (Moncur 1998, Gardner and Bertling 2005). In a series of field experiments, four-year old PBZ-treated *E. nitens* seedlings, still entirely in juvenile leaf, produced floral buds following particularly cold winter conditions (85 CPs accumulated) (Gardner 2003). However, this same phenomenon did not occur in non-PBZ-treated seedlings of the same seedlot at the same site, or in the same half-sib related seedlings at warmer sites in the experimental series in the same year, whether treated with PBZ or not. In the case of *E. nitens*, it would appear that severe cold, possibly high levels of winter chilling, and PBZ act together in an additive manner enabling the timing of vegetative phase change and first flowering to be manipulated independently. Griffin et al. (1993) and Williams et al. (1999) suggested that a large difference in the ability to induce precocious flowering exists between the two closely related temperate species *E. globulus* and *E. nitens*. Based on our results, we propose that the major difference between *E. globulus* and *E. nitens* in this regard lies in a difference in cold, possibly chilling, requirement for floral induction between the two species.

Scion genotype (clone) had a strong effect on flowering propensity; the results for 2009 and 2010 not only confirmed a large difference in floral bud production between the two clones, but also suggested that a substantial difference in winter chilling requirement for floral bud production existed between the two genotypes. This was clearly borne out by the strongly contrasting floral responses of the two clones to either PBZ treatment in 2010 (Table 5). In 2009, the significance of the sprinkling x PBZ x clone interaction on number of umbels per flowering tree ($p = 0.093$) over that of all other treatments and interactions in 2009 added strength to the suggestion that the interaction between winter chilling, PBZ and genotype is a complex one, and that winter chilling and PBZ tend to act more powerfully in tandem, rather than singularly, in the stimulation of flowering in temperate eucalypt species such as *E. nitens* (Moncur and Hasan 1994, Hasan and Reid 1995). The floral bud production trends of either clone at Mountain Home reinforced the argument that, as for temperate fruit species (Hartmann and Whisler 1975, Powell et al. 1986), reproductively mature *E. nitens* genotypes have individual winter chilling thresholds which must be surpassed before the particular chilling-dependent plant physiological responses (floral bud initiation in the case of *E. nitens*) can be triggered (Gardner and Bertling 2005). This chilling threshold may, to a certain extent, be lowered by the application of PBZ (Hasan and Reid 1995, Meilan 1997).

The results of local field research, implicating a fairly wide range of *E. nitens* provenance material and chilling requirements, suggested that, on average, depending on whether PBZ is applied or not, ≈70 or 90 CPs are necessary to achieve a mild flowering response (20% of reproductively mature trees producing umbels) (Gardner 2003, Gardner and Bertling 2005). According to four years Mountain Home air temperature data (2007 to 2010), the mean annual winter chilling for the area is about 48 CPs. Under such marginal winter chilling conditions, the supplementary 26 CPs achieved through overhead sprinkling (mean of 2009 and 2010 CPs) (Table 3) would increase total chilling to 74 CPs and could be expected to result in a mild flowering response, i.e. a maximum of 20% reproductively mature *E. nitens* trees producing umbels, with or without the application of PBZ. Based on the clonal means for PBZ-treated and non-treated trees in the Sprinkle 2 treatment at Mountain Home during 2010 (PBZ 1 = 6/10 trees, PBZ 0 = 1.5/10 trees), the total winter chilling amount of 68 CPs resulted in 60% or 15% of reproductively mature trees producing umbels, depending on whether PBZ was applied or not (Tables 3 and 5). The difference in reproductive response of PBZ-treated trees in the field trials and the Mountain Home experiment to a winter chilling amount of ≈70 CPs was marked, i.e. 20% and 60% trees producing umbels, respectively. The most likely reason for this was that the range of genetic material in the Mountain Home experiment was particularly narrow (two clones), and the exceptionally high floral response of Clone 1 (100% of PBZ-treated trees producing umbels) inflated the clonal mean. In future controlled environment investigations where a greater range of chilling levels might be applied, real time monitoring of chilling accumulation to determine the exact timing of sprinkler shutdown for each specific chilling treatment would be advisable. This would facilitate far more accurate quantification of chilling requirement for flowering.

Conclusions

Overhead sprinkling increased winter chilling accumulation and floral bud production in *E. nitens* without negatively affecting tree health. Greater bud temperature reductions and winter chilling gains through sprinkling occurred with increasingly warmer winter conditions. Thus the technique has good potential for improving floral bud and seed crop production in high chill requiring temperate eucalypt species such as *E. nitens* in areas with inadequate or unreliable winter chilling. With increased global warming, the usefulness of overhead sprinkling as a means of supplementing winter chilling and increasing the consistency of floral bud production in temperate eucalypt species such as *E. nitens* is likely to increase. The cooling system needs to be tested on a wider range of clones and on a larger scale in an open air environment. Here, a wider range of chilling treatments and genotypes should be tested in order to gain a more accurate understanding of *E. nitens* chilling requirement for floral bud production.

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Table 1: Allocation of treatments in the *E. nitens* split-split-plot design overhead sprinkling experiment at Mountain Home

	Treatment	Description of treatment levels
Factor A:	Sprinkling	
Level 1	Sprinkle 0	Nil sprinkling (control)
Level 2	Sprinkle 1	Minimum duration (sprinkling for ≈10 weeks)
Level 3	Sprinkle 2	Maximum duration (sprinkling for ≈16 weeks)
Factor B:	PBZ soil application	
Level 1	PBZ 0	Nil PBZ (control)
Level 2	PBZ 1	PBZ soil drench applied
Factor C	Scion genotype	
Level 1	Clone 1	*E. nitens* Ebor provenance (NSW), prolific flowerer
Level 2	Clone 2	E. nitens Barrington Tops provenance (NSW), shy flowerer

NSW = New South Wales (Australia)

Table 2: Mean daytime (07:00 to 18:00) *E. nitens* bud temperature reductions in winter as a result of overhead sprinkling in the experiment at Mountain Home. Controlled intermittent sprinkling in winter was carried between 13/05/2009 and 31/08/2009, and 14/05/2010-7/09/2010

Year	Daytime hours[1]	Percentage of daytime[2] (%)			Mean temperature reduction[3] (ºC)			Temperature reduction variance[4] (ºC)			Maximum temperature reduction[5] (ºC)		
		SprklBT < CtrlBT	SprklBT < OutAT	SprklBT < ShdAT	SprklBT < CtrlBT	SprklBT < OutAT	SprklBT < ShdAT	SprklBT < CtrlBT	SprklBT < OutAT	SprklBT < ShdAT	SprklBT < CtrlBT	SprklBT < OutAT	SprklBT < ShdAT
2009	1332	92.0	90.1	92.2	3.0	3.2	2.6	8.4	6.6	4.3	12.7	12.2	11.8
2010	1404	81.3	87.0	92.4	4.1	3.8	3.1	15.8	12.5	7.5	16.2	15.5	13.6
Mean	1368	86.7	88.6	92.3	3.6	3.5	2.9	12.1	9.6	5.9	14.5	13.9	12.7

[1] Total number of daytime (07:00 to 18:00) hours during the winter controlled intermittent sprinkling periods
[2] Percentage daytime hours in the sprinkling period where SprklBT < CtrlBT, OutAT or ShdAT
[3] Mean daytime reduction of sprinkled bud temperature (SprklBT) over that of control bud (CtrlBT), open air (OutAT) or shaded air (ShdAT).
[4] Variance for daytime reduction of sprinkled bud temperature (SprklBT) over that of control bud (CtrlBT), open air (OutAT) or shaded air (ShdAT)
[5] Maximum reduction of daytime sprinkled bud temperature (SprklBT) over that of control bud (CtrlBT), open air (OutAT) or shaded air (ShdAT)
SprklBT = Mean bud temperature of sprinkled trees; CtrlBT = Mean bud temperature of control (non-sprinkled) trees; OutAT = Mean air temperature outside the anti-hail structure (open air); ShdAT = Mean air temperature within the anti-hail structure (shaded air)

Table 3: Effect of sprinkling treatment on *E. nitens* bud chilling accumulation in the experiment at Mountain Home

Year	Commencement date		Sprinkle 0		Sprinkle 1		Sprinkle 2		OutAT	
	Chilling (CP) accumulation	Overhead sprinkling	Sprkl days[1]	CP total[2]	Sprkl days[1]	CP total[2]	Sprkl days[1]	CP total[2]	Sprkl days[1]	CP total[2]
2008	08th April	07th July	0	50.1 (45.3)	59	70.4 (65.6)	n/a	n/a	n/a	48.7 (44.7)
2009	21st April	13th May	0	55.0 (52.0)	75	70.0 (67.0)	110	79.0 (76.0)	n/a	56.6 (53.6)
2010	24th April	14th May	0	39.6 (37.8)	64	53.1 (51.2)	116	68.0 (66.2)	n/a	39.6 (37.6)
Mean	-	-	0	48.2 (45.0)	66	64.5 (61.3)	113‡	73.5‡ (71.1‡)	n/a	48.3 (45.3)

Sprinkle 0 = Nil sprinkling (control); Sprinkle 1 = Minimum duration sprinkling; Sprinkle 2 = Maximum duration sprinkling
CP = Chilling Portion of the Dynamic Model (Erez and Fishman 1998)
OutAT = Air temperature in full sun conditions outside the anti-hail shelter
[1] Total length of the annual controlled intermittent sprinkling period (in days)
[2] Total CPs accumulated between 1 April and 30 September (total CPs for 1 May to 30 September are given in parentheses for the reader's benefit)
n/a = Not applicable
‡ Mean of two values only

Table 4: Effect of PBZ and sprinkling on the vegetative growth of two *E. nitens* clones in the experiment at Mountain Home

Treatment	PBZ 1			PBZ 0		
	BSC increment (%)		SL increment (%)	BSC increment (%)		SL increment (%)
	Oct2009[1]	Oct2010[2]	Oct2010[3]	Oct2009[1]	Oct2010[2]	Oct2010[3]
Clone 1						
Sprinkle 0	12.7	9.8	na	14.4	8.5	na
Sprinkle 1	14.1	6.8	na	13.4	12.1	na
Sprinkle 2	10.6	9.1	na	15.8	11.5	na
Mean	12.5	8.6	na	14.5	10.7	na
Clone 2						
Sprinkle 0	15.7	10.9	33.9	11.7	13.2	74.8
Sprinkle 1	14.5	9.2	na	13.7	10.7	na
Sprinkle 2	14.8	7.7	40.9	13.2	12.1	61.6
Mean	15.0	9.3	37.4‡	12.9	12.0	68.2‡
Mean (PBZ)	13.7	8.9	37.4‡	13.7	11.3	68.2‡

ANOVA

	F probability		
Source	Oct2009[1]	Oct2010[2]	Oct2010[3]
Sprinkle	0.958	0.838	0.766
PBZ	0.984	0.024	0.003
Clone	0.658	0.284	na
Sprinkle x PBZ	0.409	0.270	0.332
Sprinkle x Clone	0.955	0.437	na
PBZ x Clone	0.036	0.781	na
Sprinkle x PBZ x Clone	0.322	0.277	na

Sprinkle 0 = Nil sprinkling (control); Sprinkle 1 = Minimum duration sprinkling; Sprinkle 2 = Maximum duration sprinkling
PBZ 0 = Nil PBZ applied (control)
PBZ 1 = PBZ applied to growing medium on 12/11/2007 and 3/05/2010
BSC = Basal stem circumference
SL = Shoot length
[1] Percentage increase in BSC between 6/04/2009 and 5/10/2009
[2] Percentage increase in BSC between 6/04/2010 and 5/10/2010
[3] Percentage increase in SL between 6/04/2010 and 5/10/2010
[4] Mean of two values only
na = Not available

Table 5: Effect of PBZ and accumulated winter chilling on the reproductive growth of two *E. nitens* clones in the experiment at Mountain Home. The annual flowering assessments were carried out on 14/12/2009 and 14/12/2010

Treatment	2009[#]						2010[#]					
	PBZ 0			PBZ 1			PBZ 0			PBZ 1		
	Plants[1]	Shoots[2]	Umbels[3]	Plants[1]	Shoots[2]	Umbels[3]	Plants[1]	Shoots[2]	Umbels[3]	Plants[1]	Shoots[2]	Umbels[3]
Clone 1												
Sprinkle 0	0/10	0.000	0.000	3/10	0.994	1.326	0/10	0.000	0.000	4/10	0.947	1.485
Sprinkle 1	3/10	1.586	2.367	3/10	0.545	0.715	4/10	0.942	1.190	9/10	18.238	39.039
Sprinkle 2	3/10	0.406	0.432	4/10	1.457	1.805	2/10	0.232	0.232	10/10	29.440	61.838
Mean	2.0/10	0.539	0.691	3.3/10	0.964	1.238	2.0/10	0.338	0.393	7.7/10	9.460	17.439
Clone 2												
Sprinkle 0	1/10	0.330	0.398	3/10	1.391	1.878	0/10	0.000	0.000	3/10	1.834	2.127
Sprinkle 1	0/10	0.000	0.000	3/10	1.093	1.856	0/10	0.000	0.000	3/10	0.765	1.176
Sprinkle 2	2/10	0.521	0.582	2/10	0.456	0.557	1/10	0.149	0.284	2/10	0.710	0.935
Mean	1.0/10	0.265	0.303	2.7/10	0.939	1.340	0.3/10	0.048	0.087	2.7/10	1.046	1.361
Mean (PBZ)	1.5/10	0.395	0.485	3.0/10	0.951	1.288	1.2/10	0.184	0.230	5.2/10	3.630	5.605

ANOVA

	F probability			
	2009		2010	
Source	Shoots[2]	Umbels[3]	Shoots[2]	Umbels[3]
Sprinkle	0.957	0.865	0.022	0.013
PBZ	0.151	0.141	<0.001	<0.001
Clone	0.587	0.652	<0.001	<0.001
Sprinkle x PBZ	0.605	0.647	0.168	0.135
Sprinkle x Clone	0.439	0.501	0.002	0.003
PBZ x Clone	0.596	0.470	0.001	<0.001
Sprinkle x PBZ x Clone	0.125	0.093	0.009	0.009

[#] The original flowering data were $\log_{10}(x + 1)$ transformed. In this table, back transformed data are presented for the benefit of the reader
PBZ 0 = Nil PBZ applied (control)
PBZ 1 = PBZ applied to growing medium on 12/11/2007 and 3/05/2010
[1] Number of plants out of 10 that produced umbels
[2] Mean number of flowering shoots per plant
[3] Mean number of umbels per plant
Sprinkle 0 = Nil sprinkling (control); Sprinkle 1 = Minimum duration sprinkling treatment; Sprinkle 2= Maximum duration sprinkling treatment

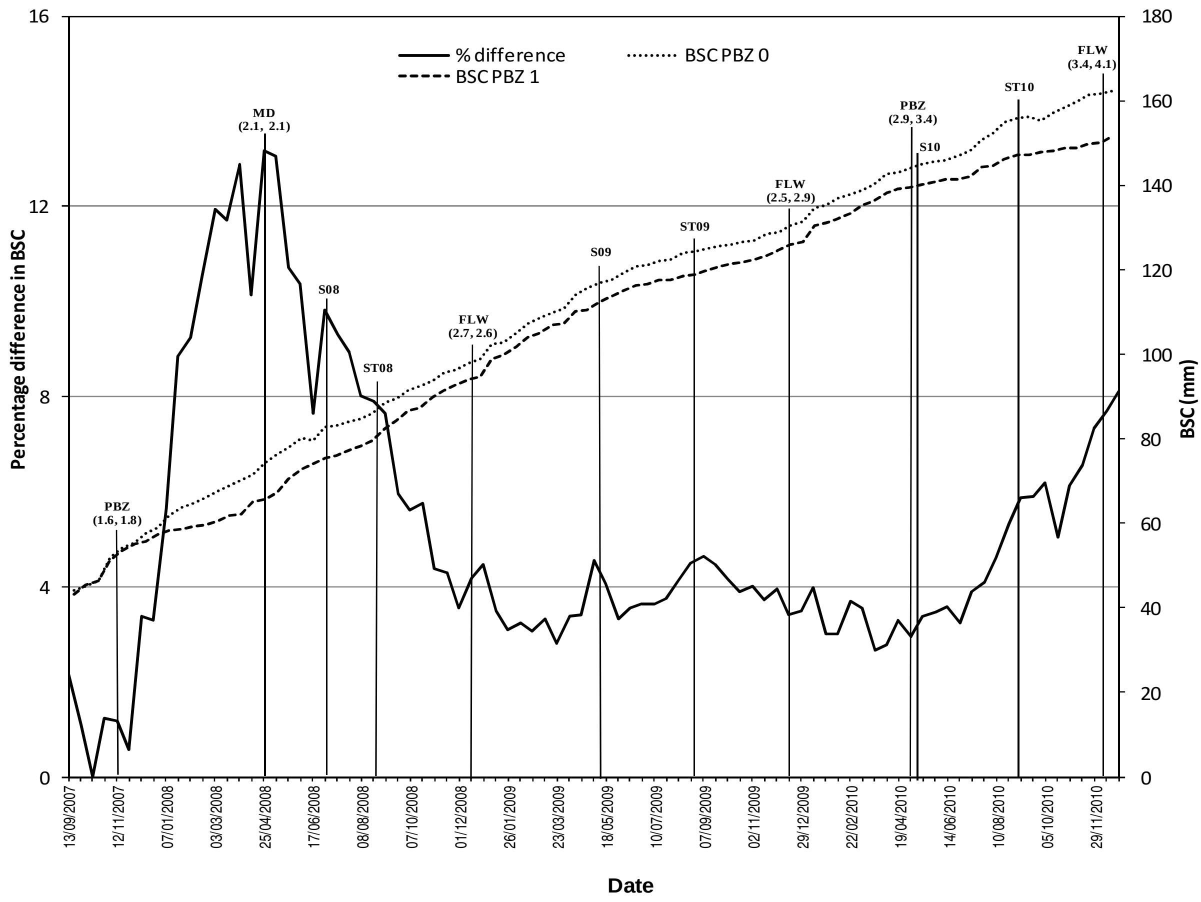

Figure 1: The effect of PBZ on the vegetative growth of *E. nitens* grafted trees in the maximum duration sprinkling (Sprinkle 2) treatment at Mountain Home over time. The basal stem circumferences (BSC) of the PBZ 1 (PBZ-treated) and PBZ 0 (control) trees are indicated by dotted lines. The percentage difference between the two (((BSC PBZ 0 – BSC PBZ 1)/BSC PBZ1)*100) is indicated by the solid line. Standard errors for BSC measurements of PBZ 0 and PBZ 1 trees are shown in parenthese (in respective order) at certain stages through the trial duration as follows: PBZ = PBZ application, MD = maximum difference in BSC between PBZ 0 and PBZ 1 trees after the 1st PBZ application, FLW = floral crop assessment. The annual sprinkling period initialization and cessation dates are also indicated in the graph as follows: S08 = initialization of 2008 sprinkling period, ST08 = cessation of 2008 sprinkling period, S09 = initialization of 2009 sprinkling period, ST09 = cessation of 2009 sprinkling period, S10 = initialization of 2010 sprinkling period, ST10 = cessation of 2010 sprinkling period

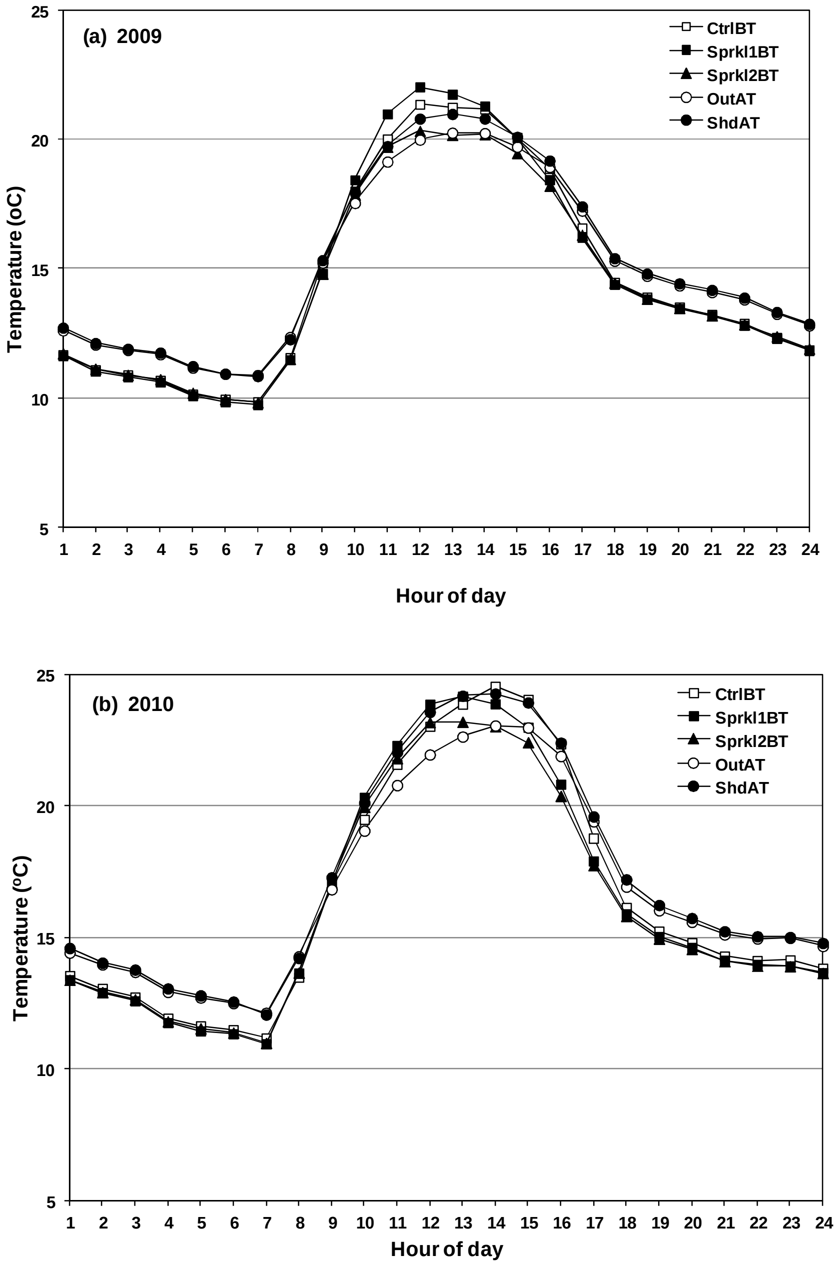

Figure 2: Mean diurnal variation in *E. nitens* bud and air temperatures over a period of 14 days (29 April to 12 May) prior to the initialization of controlled intermittent sprinkling in (a) 2009 and (b) 2010 at Mountain Home. CtrlBT = mean bud temperature in the control (Sprinkle 0) treatment; Sprkl1BT = mean bud temperature in the minimum duration sprinkling (Sprinkle 1) treatment; Sprkl2BT = mean bud temperature in the maximum duration sprinkling (Sprinkle 2) treatment; OutAT = air temperature outside the anti-hail shelter (open air); ShdAT = air temperature within the anti-hail shelter (shaded)

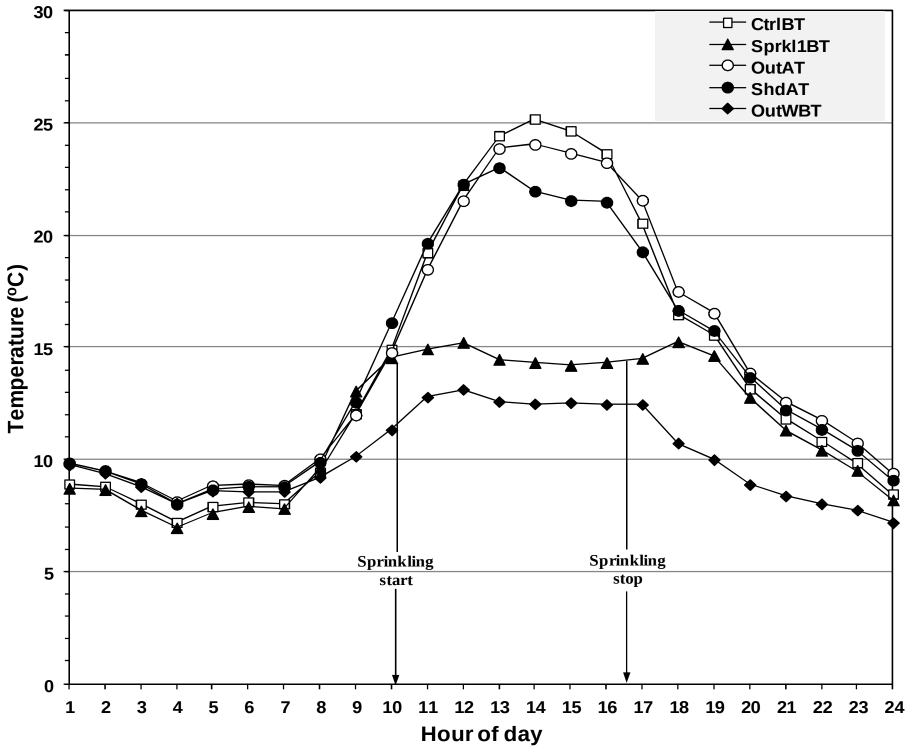

Figure 3: Effect of overhead sprinkling on *E. nitens* bud and air temperatures on a typical winter day (26 July 2010) at Mountain Home. CtrlBT = mean bud temperature in the control (Sprinkle 0) treatment; Sprkl1BT = mean bud temperature in the minimum duration sprinkling (Sprinkle 1) treatment; OutAT = air temperature outside the anti-hail shelter (open air); ShdAT = air temperature within the anti-hail shelter (shaded); OutWBT = wet bulb temperature outside the anti-hail shelter (open air)

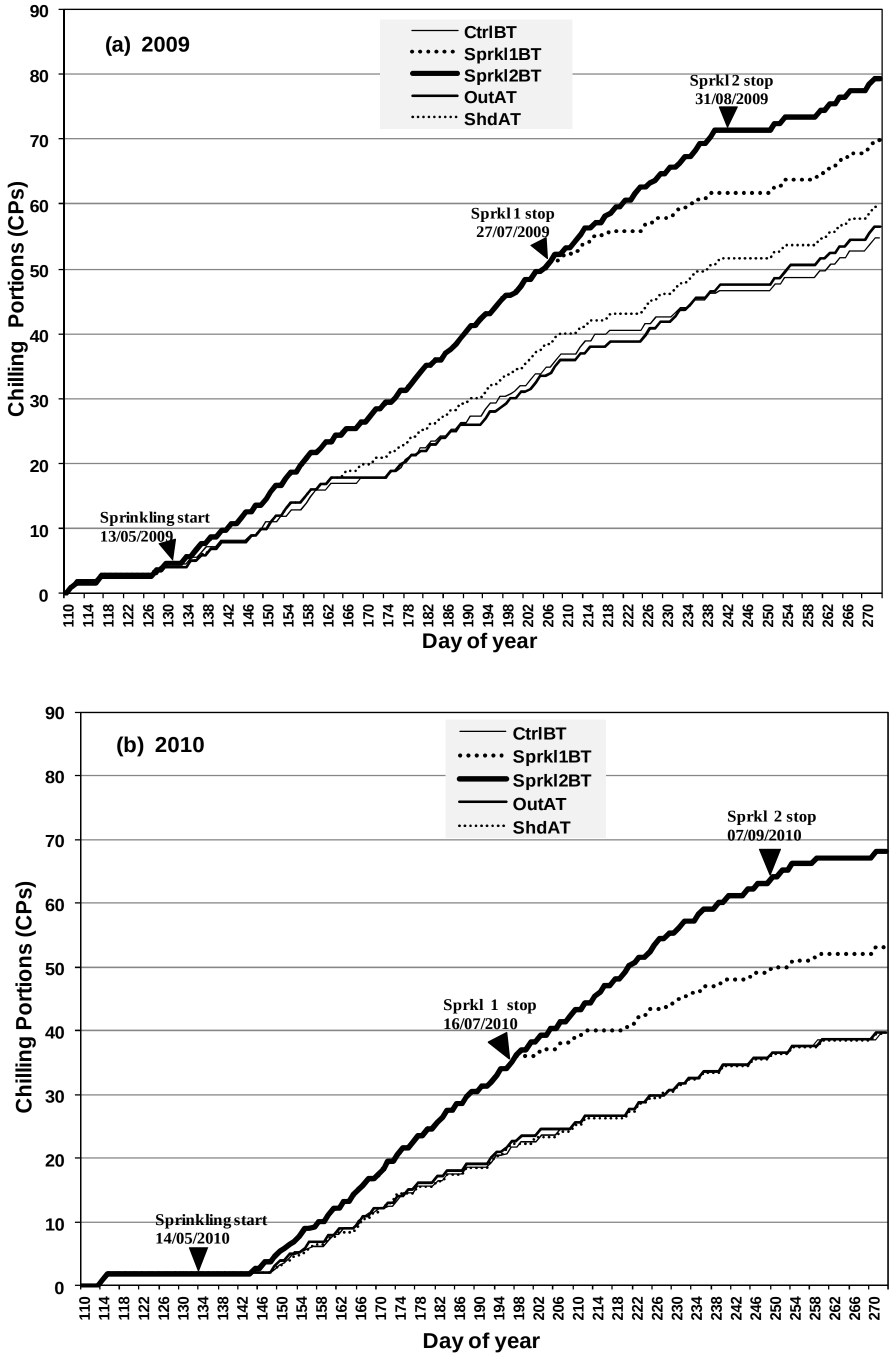

Figure 4: Accumulating Chilling Portions for *E. nitens* bud and air temperatures measured at Mountain Home, during (a) 2009 and (b) 2010. CtrlBT = mean bud temperature in the control (Sprinkle 0) treatment; Sprkl1BT = mean bud temperature in the minimum sprinkling duration (Sprinkle 1) treatment; Sprkl2BT = mean bud temperature in the maximum duration sprinkling (Sprinkle 2) treatment; OutAT = air temperature outside the anti-hail shelter (open air); ShdAT = air temperature within the anti-hail shelter (shaded)

CHAPTER 7

Effect of temperature and paclobutrazol on *Eucalyptus nitens* reproductive phenology: a study in the summer rainfall area of South Africa

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ABSTRACT

Eucalyptus nitens is an important, yet shy-flowering, temperate tree species planted for commercial pulpwood production in the summer rainfall forestry areas of South Africa. To initiate flower buds, *E. nitens* requires a cold winter followed by warm conditions. Paclobutrazol is commonly applied in *E. nitens* orchards to encourage precocious and prolific flowering. In South Africa, *E. nitens* produces flower buds most prolifically at sites located on cold slopes in high elevation (> 1550 m asl), cool temperate (mean annual air temperature ≤ 16.0 ^{o}C) areas. We investigated the effect of these environmental conditions and paclobutrazol application on the reproductive phenology of *E. nitens* across four sites in KwaZulu-Natal province between November 2008 and March 2011.

With or without paclobutrazol application, a distinct trend across sites of anthesis being delayed (by at least 120 days) and anthesis duration being shortened (by at least 80 days) as elevation increased from 1133 to 1708 m asl was evident. At Netherby3 (1678 m asl), a top-ranking site on the basis of *E. nitens* floral bud crop production, anthesis was delayed by 33 days. Generally, the effect of paclobutrazol application across sites was a decrease in anthesis duration of between 17 and 34%, from lowest to highest elevation. Although the study was based on a narrow range of *E. nitens* genetic material, the observed trends should be taken note of by local temperate eucalypt tree breeders and seed producers. At optimum temperate eucalypt flower bud production sites in South Africa, the levels of out-crossing and capture of genetic gain and variation may be substantially compromised.

***Keywords*:**
Floral initiation
Flowering
Seed orchard
Temperate eucalypt
Thermal time
Winter chilling

***Abbreviations*:** BFV, inflorescence buds first visible; BSC, basal stem circumference; CP, Chilling Portion; MAP, mean annual precipitation; MAT, mean annual temperature; PBZ, paclobutrazol

1. Introduction

Eucalyptus nitens is the most widely planted temperate eucalypt species in South Africa. The species has exceptional cold, frost and snow tolerance (Darrow, 1996; Gardner and Swain, 1996) and desirable pulping properties (Gardner, 2001; Swain and Gardner, 2003) making it highly suitable for commercial plantings in high elevation (> 1300 m asl) areas of the summer rainfall forestry regions (Swain et al., 2014). *Eucalyptus nitens*‘ shy flowering and seed producing tendencies hamper the species' breeding and commercial seed production programmes (Williams et al., 2003). Mature individuals rarely produce flower buds before the age of eight years, and even then the flower and seed crops are sparse (Eldridge et al., 1993; Gardner and Bertling, 2005). The plant growth regulator paclobutrazol (PBZ) has proven to be a valuable tool for temperate eucalypt orchard management (Hamilton et al., 2008). Applications of the chemical not only assist with vegetative growth control, but under favourable environmental conditions can also advance the onset of flowering in seedlings and increase floral bud crop production (Williams et al., 2003). No negative carryover effects as a result of PBZ application have been noted with respect to pollen and seed produced from *E. nitens* trees treated with the chemical (Griffin et al., 1993; Moncur 1998). However, abnormally low flower bud numbers within umbels of PBZ-treated *E. nitens* seedlings still in juvenile leaf occur fairly commonly (Gardner, 2003; Moncur, 1998). Applications of PBZ are expensive and need to be repeated periodically (Gardner et al., 2013, 2014). The chemical's relative persistence and immobility in soil (Fletcher et al., 2000) and toxicity to a range of soil microflora (Jackson et al., 1996; Silva et al., 2003) make it a non-ideal field tool from an environmental conservation perspective (Meilan, 1997; Moncur, 1998). When unaccompanied by sufficient winter cold, however, PBZ is ineffective in the stimulation of flowering in *E. nitens* (Hasan and Reid, 1995; Williams et al., 1999a; Gardner et al., 2013).

Over the past two-and-a-half decades, considerable reproductive biological research has been carried out aimed at improving flowering and seed crop production in important temperate eucalypt species such as *E. globulus* and *E. nitens* (Hamilton et al., 2008; Potts et al., 2007). In temperate plants, the flowering process is attuned to seasons through environmental cues, particularly photoperiod and temperature (Tooke and Battey, 2010). In *E. nitens*, neither photoperiod nor drought stress appear to play an influential role in the floral induction process (Gardner, 2003; Hasan and Reid, 1995; Moncur, 1998; Moncur and Boland, 2000). A period of winter cold seems obligatory for *E. nitens* floral induction (Moncur and Hasan, 1994; Williams et al., 1999a). Environmental conditions favouring temperate eucalypt vegetative growth do not necessarily favour floral induction in the same species (Moncur, 1998; Moncur and Boland, 2000). In South Africa, *E. nitens* chilling requirement for floral bud production is mostly not met in areas where the commercial plantations and seed orchards are situated (Gardner, 2004b; Gardner et al., 2014). *Eucalyptus nitens* has a minimum winter chilling requirement of approximately 40 Chilling Portions (CP, the chill unit of the Dynamic Model (Erez and Fishman, 1998; Fishman et al., 1987)) for floral induction (Gardner et al., 2013, 2014; Gardner and Bertling, 2005). A minimum of 90 CPs appears necessary to pre-dispose mature non-PBZ-treated trees to achieve maximum flower bud production. *Eucalyptus nitens* floral induction response in the intermediate chilling range (> 40 < 90 CP) appears largely genotype-, possibly provenance-, dependent, with respect to chilling requirement (Gardner, 2003; Gardner et al., 2013, 2014).

Extensive field research carried out over the past two decades in South Africa resulted in the identification of key climatic and landform parameters influencing *E. nitens* floral bud production (Germishuizen and Gardner, 2014; Gardner et al., 2014). In the summer rainfall area, *E. nitens* produces flower buds most prolifically and consistently at sites located on cold (south-west to west-facing) slopes in high elevation (> 1550 m asl), cool temperate (mean annual air temperature (MAT_Air) ≤ 16.0 ^{o}C) areas (Gardner et al., 2014). Nevertheless, even at these sites, the periodic deviations from mean climatic conditions, e.g. unusually warm winter conditions, negatively impact on *E. nitens* floral bud and seed crop production. Furthermore, increased seasonal weather extremes associated with global warming are likely to exacerbate this situation in the future (Linkosalo et al., 2009; Luedeling et al., 2011). Therefore, a previous investigation into the feasibility of using evaporative cooling to apply controlled winter chilling to improve the consistency of flower bud production in *E. nitens* orchards located at marginal chilling sites was undertaken between 2007 and 2011 (Gardner et al., 2013). In two consecutive years, controlled overhead sprinkling substantially increased winter chilling and flower bud production in non-PBZ-treated *E. nitens* grafts. PBZ application produced a strong additive effect in combination with winter chilling on *E. nitens* floral bud production.

Environmental conditions favouring *E. nitens* floral induction and initiation may not necessarily favour *E. nitens* reproductive development in the post-floral initiation phase (Hamilton et al., 2008). Sites typically experiencing cold winter conditions required for prolific flower bud production in *E. nitens* may not necessarily be conducive to rapid flower, capsule and seed development (Moncur et al., 1994). In temperate south-eastern Australia, the time taken for *E. nitens* flower buds to develop from bud emergence to seed maturity stage differed by more than 12 months between two sites similar in latitude, but differing in elevation and mean annual temperature, with the highest elevation and coldest site of the two taking longest (Moncur and Boland, 2000). In *E. nitens*, vegetative growth ceased at temperatures below 5 ^{o}C (Moncur and Hasan, 1994). Accumulated daily heat sums (degree days above 4 ^{o}C), rather than total number of days, was a fairly reliable predictor of the time taken from (inflorescence) buds first visible (BFV) stage to commencement of anthesis in *E. nitens*, and from BFV to seed maturity stage in the same species (Moncur and Boland, 2000).

In this study, the main aim was to investigate the effects of PBZ treatment and the site conditions deemed optimal for *E. nitens* flower bud production in the summer rainfall area on flower and fruit development and seed maturation in the same species. The timing of *E. nitens* floral initiation relative to chilling accumulation was explored since such knowledge is likely to be useful in further molecular biological investigations pertaining to *E. nitens* floral induction and development.

2. Materials and methods

2.1. Semi-controlled environment (potted) trial

In August 2006, scions from two different *E. nitens* genotypes (ICFR breeding selections) were grafted onto six-month-old *E. nitens* seedlings grown from South African commercial orchard seed. In February 2007, six months after grafting, the plants were transplanted into 100 L (0.6 m diameter) black polythene bags containing commercial growing medium. The potted plants were then positioned 2.0 m apart at an experimental site at Mondi Mountain Home Research Centre, Hilton, KwaZulu-Natal (Table 1). The site was considered marginal for *E. nitens* floral bud production on the basis of mean annual winter chilling (Gardner et al., 2013), and, therefore ideal for evaluating the effect of supplementary chilling on *E. nitens* floral bud production. The original experimental layout, infrastructure and instrumentation were comprehensively described by Gardner et al. (2013). All 40 trees of two clones within the maximum winter chilling treatment block were used in this study on *E. nitens* reproductive development and floral phenology.

Provision of supplementary winter chilling during the months of May to August 2007 was achieved by evaporatively cooling the trees using controlled intermittent overhead sprinkling. Two levels of PBZ treatment were applied, PBZ0 (nil PBZ) and PBZ1 (PBZ applied as a soil drench). For the PBZ1 treatment, a suspension of Cultar® (formulation 250 g L^{-1} PBZ, ICI Agrochemicals) was applied on two separate occasions (12 November 2007 and 03 May 2010) at a rate of 0.025 g a.i. per mm basal stem circumference (BSC). BSC was measured at the narrowest point between graft union and first lateral (scion) branch. Two scion genotypes were included: Clone 1 (Ebor provenance, prolific flowerer) and Clone 2 (Barrington Tops provenance, moderate-shy flowerer). The frequency of watering was scheduled to maintain soil water level at between 75% and 100% field capacity throughout the year.

Air and *E. nitens* bud temperatures were measured at 1.3 m above ground level on a 30 s interval basis throughout each year using the methodology described by Gardner et al. (2013), and mean hourly data calculated for use in chill and heat unit modelling. Bud temperatures were measured on the northern and southern sides of each of two spatially separate sample trees (approximately15 m apart) in the maximum winter chilling block and the mean of these four values calculated (refer Chapter 6).

2.1.1. Floral initiation and early development

During late April 2010, only the 24 trees of *E. nitens* Clone 1 from the maximum winter chilling treatment block which had produced inflorescences in the preceding floral crop season were selected for the study. Twelve of these trees had been treated with PBZ. On each tree, one healthy secondary branch was flagged at each of the four aspects (east, south, west and north) around the tree canopy. On a fortnightly basis, between 05 May and 01 November 2010, one actively growing shoot apex per secondary branch was collected from each flagged branch. On each sampling occasion, the four shoot apices per tree were bulked and fixed in formalin acetic acid alcohol (FAA; 10 ml formaldehyde: 5 ml acetic acid: 50 ml ethanol: 35 ml water) for a minimum of 48 hours. During the first week of November 2010, samples of recently emerged (within the past 48 h) inflorescence buds were also collected and stored separately in FAA. In mid-December 2010 and mid-January 2011, all 24 trees from which the shoot apex samples were collected were inspected for the presence of inflorescence buds. Stored shoot apices from the three PBZ0 trees that had produced the greatest number of reproductive shoots (current season shoots having one or more inflorescence buds) by mid-January 2011, as well as three trees that had produced no inflorescence buds by the same date, were retained for the microscopy investigation.

The total number of samples for light microscopy (LM) was reduced by selecting samples according to level of accumulated winter chilling. Eight bulked samples from trees that produced inflorescence buds in 2010 were selected at intervals of approximately 10 CP (as calculated from bud temperature), from 1.8 CP to 73.2 CP (Table 2). For samples from trees that did not produce inflorescence buds in 2010, three intervals spread roughly across the entire 2010 winter chilling range were selected (controls). Thus, a total of 132 samples (3 trees x 4 apices x 11 chill unit intervals) were subjected to LM. Standard LM sample preparation and sectioning procedures were carried out (Baker, 1958; Johansen, 1940). Sections were studied using a Zeiss Axio Imager M2 light microscope, and micrographs captured using a Zeiss AxioCam MRm monochrome digital camera (Carl Zeiss Microscopy GmbH, Göttingen, Germany).

2.1.2. Flower and fruit development phenology

Floral bud and capsule development were tracked in all 40 trees (Clones 1 and 2, PBZ0 and PBZ1) in the maximum winter chilling treatment block on a fortnightly basis over one floral bud crop year, i.e. from beginning October 2009 to end January 2011. On each assessment occasion, the presence of four key *E. nitens* reproductive developmental stages (Table 3 and Fig. 1) was scored, and the commencement and ending dates of each stage estimated. In *E. nitens*, depending on weather conditions, dehiscence of anthers and pollen dispersal typically commences within one to two days of the inner operculum being shed (Gardner, unpublished results; Tibbits, 1989). For the purpose of this study, commencement of anthesis, i.e. commencement Stage 3 (Table 3), was designated as that stage where the inner operculum of any one flower within the umbel was shed, leaving the filaments and stigma exposed. This stage of floral development is clearly distinguishable in *E. nitens*, a species with relatively small flower buds (Tibbits, 1989; Williams et al., 1999b). Typically, most pollen is shed on the fourth day after the (designated) commencement of anthesis, and it is around this time that stigmas are most receptive (Tibbits, 1989).

2.2. *Field trials*

In August 2003, scions from four different *E. nitens* genotypes (ICFR breeding selections, i.e. Clones 2-5) were grafted onto six-month-old *E. nitens* seedlings grown from South African commercial orchard seed. Two of the clones, Clone 1 (Ebor provenance, prolific flowerer) and Clone 2 (Barrington Tops provenance, moderate-shy flowerer), were identical to the two clones applied in the semi-controlled environment (potted) trial at Mountain Home.In March-April 2004, a series of 13 site x PBZ x flowering interaction trials were established across a range of high elevation/ high chill summer rainfall sites within the optimum range for *E. nitens* growth (elevation ≥ 1 350 m asl; mean annual air temperature (MAT_Air) 14-16 ^{o}C; mean annual precipitation (MAP) ≥ 840 mm; Gardner et al., 2014; Smith et al., 2005; Swain and Gardner, 2003). Water stress is known to stimulate flower bud production in a number of temperate woody angiosperms (George and Erez, 2000; Meilan, 1997), and, therefore, drought stress was minimized as a factor in the stimulation of flowering by selecting all sites having high MAP (> 840 mm) and uniformly deep soils (> 0.8 m) (Owens, 1995; Schönau and Grey, 1997). Tree spacing in the trials was 4 m x 5 m. Two levels of PBZ were applied, PBZ0 (nil PBZ) and PBZ1 (PBZ applied as a soil drench). For the PBZ1 treatment, a suspension of Cultar® was applied during March 2006, at a rate of 0.025 g a.i. per mm BSC. Air temperature was measured at each site on an hourly basis throughout the year from trial inception to termination using Hobo® temperature loggers (Onset Computer Corporation, Bourne, USA) according to the methodology described by Gardner et al. (2014). Equations developed in a temperature calibration experiment at the Mountain Home experimental site were used to model *E. nitens* bud temperature from hourly air temperature data for each site.

2.2.1. Flower and fruit development phenology

During November/ December 2009, four Clone 2 trees bearing newly emerged inflorescence buds were selected at each of the eight sites for the study. The reason for selecting the Clone 2 for the study was that in earlier environment x flowering interaction research, the particular clone demonstrated consistent and prolific flowering when grafted onto seedlings and subjected to high levels of accumulated winter chilling (Gardner, 2003; Gardner, unpublished results). At each site, two of the four trees had been PBZ-treated (PBZ1 treatment) and the other two not (PBZ0 treatment). On each tree, two healthy primary branches, one each on the north and south aspects of the canopy, bearing recently emerged (within the past 2-3 days) inflorescence buds were selected and flagged. Floral bud and capsule development was tracked in all 32 *E. nitens* trees (one clone x two PBZ levels x 8 sites x 2 replicates) on a fortnightly basis, from November 2009 through till end of March 2011 (one floral crop season). The two replicates of Clone 2 were from different replicates of the main plots. On each assessment occasion, the presence of four key *E. nitens* reproductive developmental stages (Table 3, Fig. 1), and estimates of the commencement and end dates of the particular stages, were recorded.

During the second year of the survey (2010), the assessments were reduced to the three best overall *E. nitens* flowering sites in KwaZulu-Natal based on floral crop production (refer Chapter 4). Site details for the three sites are presented in Table 1.

2.3. Seed maturity

For both the Mountain Home potted trial and the KwaZulu-Natal field trials, the “seed maturity” stage was defined as that stage at which 70% seed germination was reached (Moncur et al., 1994). From completion of anthesis, ripening capsules were collected fortnightly on a random basis from the pertinent treatments. The capsules were dried and the seed and chaff extracted and stored in labeled sealed polythene bags at 5 ^{o}C for a minimum of one month. Germination tests and counts were carried out according to the protocols described by Boland et al. (1980).

2.4. Thermal time calculations

Thermal time (^{o}Cd), also known as heat units or degree days (Bonhomme, 2000; Schulze and Maharaj, 2007a; Tsimba et al., 2013), accumulated between key *E. nitens* reproductive growth stages was calculated for trees in both the evaporative cooling and field trial experiments. Daily thermal time (TT) was calculated as: TT = T_{mean} - T_{base}, where T_{mean} = daily mean temperature calculated as ((T_{max} - T_{min}) /2), T_{max} = daily maximum temperature, T_{min} = daily minimum temperature and T_{base} = base temperature for *E. nitens* vegetative growth, i.e. 4 ^{o}C (Moncur and Hasan, 1994).

3. Results

3.1. Semi-controlled environment (potted) trial

3.1.1. Floral initiation

The vegetative apical meristem is enclosed by developing leaf primordia (Fig. 2A). An increase in the size of *E. nitens* Clone 1 apical meristems was first observed on 6 September 2010 (Fig. 2B), by which stage the trees had been exposed to 63 CP (Table 2). At this point in time, bracteate inflorescence buds were also observed developing spirally around the growing point (Fig. 2C). Due to the sampling intervals being one month apart, and inflorescence buds in early stage of development already evident on 06 September, apical meristem size increase in Clone 1 likely began at some point between 10 August (51 CP accumulated) and 6 September 2010 sampling dates (Table 2).

3.1.2. Early development of reproductive buds

Newly emerging *E. nitens* Clone 1 inflorescence buds (Fig. 1A) first became visible to the naked eye (BFV stage) during the second week of October 2010. By this stage, a thermal time of 472 ^{o}Cd had passed since an increase in apical meristem size was first noted in Clone 1 on 6 September 2010 (Table 4). The timing of BFV in Clone 1 was approximately six weeks earlier than for mature *E. nitens* trees in a natural stand at Tallaganda State Forest, New South Wales, Australia (35^{o} 30' S; 149^{o} 32' E; elevation 1000 m asl; MAT_Air 15.6 ^{o}C; Australian Government Bureau of Meteorology, 2014; Moncur et al., 1994). At Mountain Home, by late October 2010, individual flower buds within the umbels were elongated, and well-defined (Fig. 2D). By early November the same year, approximately three weeks after BFV, the differentiation of floral organs was well underway (Fig. 2E).

3.1.3. Flower and fruit development phenology

The first floral bud crops were produced by trees in both PBZ0 and PBZ1 treatments in early November 2008 (Gardner et al., 2013). Based on observations carried out over three crop seasons (October 2008 to March 2011), new season's inflorescence buds

(Fig. 1A) emerged over a period of 14 weeks, from beginning of October at the earliest, through to early January at the latest, regardless of PBZ treatment. The clonal effect regarding timing of inflorescence bud emergence (BFV stage) was fairly consistent across years and PBZ treatments. In both 2009 (Table 5) and 2010 (data not presented), Clone 1 buds emerged at least two weeks prior to those of Clone 2. An interaction occurred between PBZ and clone regarding timing of BFV stage. In the PBZ0 treatment, inflorescence buds of Clone 1 trees emerged 27 and 28 days earlier than those of Clone 2, in 2009 and 2010, respectively. In the PBZ1 treatment, inflorescence buds of Clone 1 trees emerged 16 and 17 days earlier than those of Clone 2 in the same respective years.

On average (2008 to 2011), bract shed (Fig. 1C) commenced 10-11 weeks after BFV stage, i.e. late December (Table 5), regardless of clone or PBZ treatment.

Anthesis commenced during April 2010 (Fig. 1E), approximately six months after BFV, across clone and PBZ treatments (Table 5). The effect of clone on timing of anthesis commencement date was negligible (Table 5), but a slight delay in commencement of anthesis was observed in the PBZ1 treatment compared to that in the control (PBZ0) treatment. Mean (across-clone) timing of anthesis commencement for PBZ0 trees (14 April 2010) was 11 days prior to that of PBZ1 trees (25 April 2010). Clone 2 anthesis commenced 12 days earlier in PBZ0 treatment than in PBZ1 treatment, i.e. 12 April and 24 April, respectively (Table 5).

Regarding duration of anthesis, the across-clone means for PBZ0 and PBZ1 treatments were similar (133 and 132 days, respectively) (Table 6). In the PBZ0 treatment, the two clones differed considerably with respect to anthesis duration, with Clone 1 and Clone 2 recording four and five months for this period, respectively. A clone x PBZ interaction was evident, with anthesis duration in Clone 1 being approximately three weeks shorter in PBZ0 treatment than in PBZ1 treatment, and anthesis duration in Clone 2 being approximately three weeks longer in PBZ0 treatment than in PBZ1 treatment (Table 6).

Mean (across-clone) time taken between BFV and seed maturity was 32 days/ 456 ^{o}Cd less in PBZ0 treatment than in PBZ1 treatment (Table 6). The least time taken between BFV and seed maturity stage (374 days, 4736 ^{o}Cd) occurred in the non-PBZ-treated Clone 2 treatment trees (Table 6). In the same clone, PBZ application (PBZ1) increased the time taken between earliest BFV and seed maturity by 46 days/ 641 ^{o}Cd to 15 months/ 5377 ^{o}Cd. Twelve of the 46 days were possibly carried over from the earlier inflorescence bud emergence date of PBZ0 trees during spring 2009 (Table 5).

3.2. Field trials

3.2.1. Flower and fruit development phenology

Based on observations carried out over two crop seasons (November 2009 to March 2011) in Clone 2, new season's inflorescence buds (Fig. 1A) emerged over a period of approximately 14 weeks at each of the sites, i.e. from late November through to early March, regardless of PBZ treatment.

Apart from Willowmere, commencement of bract shed (Fig. 1C) in Clone 2 occurred later in PBZ0 trees than in PBZ1 trees (Table 7). In the PBZ0 treatment, timing of bract shed ranged from early March (Willowmere) through to late May (Tweefontein), i.e. on average 21 weeks after BFV stage. In the PBZ1 treatment, bract shed ranged from beginning January (Tweefontein) through to early March (Willowmere), i.e. on average 10 weeks after BFV.

Regardless of PBZ treatment, earliest and latest Clone 2 anthesis commencement dates occurred at the lowest and highest elevation sites of the three, viz. Tweefontein (1588 m asl) and Willowmere (1708 m asl), respectively (Tables 1 and 7). Mean (across-site) Clone 2 anthesis commencement date was nine days earlier in the control than in the PBZ1 treatment (Table 7). At Willowmere, the highest elevation site of the three, anthesis commencement date was similar in both PBZ treatments (30 September). At Tweefontein, the lowest elevation site of the three, anthesis commenced slightly later in PBZ0 treatment compared to PBZ1 treatment (19 September versus 12

September). At Netherby3, the intermediate site (Table 1), anthesis commenced 33 days earlier in the control (20 August) than the PBZ1 treatment (22 September).

Mean (across-site) duration of anthesis for PBZ0 and PBZ1 treatments were 82 days (≈ 12 weeks) and 72 days (≈10 weeks), respectively. These durations were both considerably shorter than Mountain Home mean (across-clone) anthesis durations for PBZ0 and PBZ1 (19 weeks in either treatment) (Tables 6 and 8), and considerably shorter than Mountain Home Clone 2 anthesis duration (21 and 18 weeks for PBZ0 and PBZ1, respectively). In both PBZ0 and PBZ1 treatments, the KwaZulu-Natal field sites where the shortest and longest anthesis durations occurred (Table 8) were those located at the highest and lowest elevations respectively, namely Willowmere (51 and 48 days) and Tweefontein (98 and 105 days) (Table 1).

Mean (across-site) time taken between BFV and seed maturity stages in Clone 2 was 23 days/ 357 ^{o}Cd greater in PBZ0 treatment than in PBZ1 treatment (Table 8). The least time taken between BFV and seed maturity (413 days, 4576 ^{o}Cd) occurred in PBZ1 treatment at Tweefontein, the lowest elevation site of the three field trials (Tables 1 and 8). At the same site, trees in the control treatment took on average 40 days/ 630 ^{o}Cd longer than trees in the PBZ1 treatment to reach seed maturity. At the highest elevation field trial site (Willowmere), there was minimal difference between the control trees (437 days, 5310 ^{o}Cd) and PBZ-treated trees (442 days, 5405 ^{o}Cd). In the absence of PBZ application, the greatest time taken between BFV and seed maturity occurred at Netherby3 (464 days, 5850 ^{o}Cd).

4. Discussion

4.1. Timing of floral initiation

Eucalyptus nitens floral initiation was investigated in non-PBZ treated trees of one prolific flowering genotype (Clone 1, Ebor provenance) in the maximum winter chilling treatment block at Mountain Home. The size increase in *E. nitens* apical meristems observed during the first week of September indicated that the processes relating to floral evocation, including changes in gene expression and associated metabolisms (O'Neill, 1993; Meilan, 1997) began some days, possibly weeks, prior to this particular point in time. The findings, although based on a single genotype, resulted from an experiment where winter chilling was applied on a controlled basis. Bud temperature was measured continuously on a 30 sec basis, and the 51 and 63 CP accumulated by the 10 August and 6 September (Table 2) respectively, represented actual amount of chilling to which the sampled trees had been exposed. Paclobutrazol may influence timing of floral induction, evocation and initiation, but this was not investigated in the Mountain Home trial. Perez-Barraza et al. (2000) reported that a soil application of PBZ resulted in 45-day advancement of the timing of commencement of inflorescence initiation in mango (*Mangifera indica*). A perusal of the published literature suggested that the effect of PBZ on timing of the latter events remains to be investigated in temperate eucalypt species such as *E. nitens*.

4.2. Timing of inflorescence bud emergence

The earlier timing of inflorescence bud emergence (BFV) at sites at the warm end of *E. nitens* growth range in South Africa (MAT_Air > 16.0 ^{o}C) (Smith et al., 2005), i.e. beginning October to early January (Mountain Home and Tweedie), compared to that at sites at the cooler end of the species growth range (MAT_Air ≤ 15.5.0 ^{o}C) and where conditions were deemed favourable for *E. nitens* floral bud production (Gardner et al., 2014), i.e. late November to early March (e.g. Tweefontein and Netherby3), appeared to be carried through to anthesis onset and seed maturity stage (Tables 5 and 7). The earlier bud emergence at warmer sites potentially offers breeders and/or seed producers a means of shortening *E. nitens* breeding and/ or seed production cycles to some extent, particularly in environments where adequate heat and soil water levels conducive to rapid post-emergence reproductive growth and development are typically present (Luedeling et al., 2013; Moncur and Hasan, 1994; Owens, 1995).

4.3. Timing of bract shed

At Canberra, Australia (latitude 35°18' S; elevati on 550 m asl; MAT_Air 13.1 ℃), bract shed occurs 6-8 weeks following BFV in early December (Moncur and Boland, 2000). At

Tallaganda State Forest (Australia), with similar latitude but substantially higher elevation and mean annual temperature than Canberra (35°30' S; 1000 m asl; MAT_Air 15.6 ℃), bract shed is delayed until October, 9-10 months following BFV in late December. A similar effect of elevation on bract shed timing was observed in non-PBZ treated Clone 2 trees across the four South African sites. At the low elevation, warm Mountain Home site, bract shed commenced 10-11 weeks following BFV in late October-early November (Table 1). At the cooler KwaZulu-Natal high altitude field trial sites which were of fairly similar latitude to Mountain Home, bract shed commenced on average 21 weeks after BFV in late November-early March.

4.4. Timing of anthesis

Timing of flowering in temperate trees is generally regulated by mechanisms which act to ensure that flower emergence occurs during suitable conditions (Powell et al., 1986; Tooke and Battey, 2010). Seasonal shifts in flowering times of *E. nitens* genotypes occur, though differences in relative flowering times between genotypes remain fairly consistent across seasons (Jones, 2002; Moncur et al., 1994; Tibbits, 1989). In South Africa, *E. nitens* provenance differences on the basis of time of year when anthesis commences are a common phenomenon. In PBZ-treated, grafted *E. nitens* orchard trees at Tweedie, KwaZulu-Natal province (elevation 1100 m asl; MAT 16.7 ^{o}C), genotypes of northern New South Wales (NSW) provenances Ebor, Barren Mountain and Barrington Tops consistently commenced flowering later than grafted clones of the southern NSW provenances Tallaganda and Badja (Jones and Van Staden, 2001).

In the four *E. nitens* trials (one semi-controlled environment and three field trials) located across southern KwaZulu-Natal, of all treatments applied, environment exerted the most pronounced effect on timing of anthesis. In Clone 2, regardless of PBZ treatment, the commencement of anthesis was delayed by four months when elevation increased from 1133 m asl (Mountain Home) to 1588 m asl (Tweefontein), and by a further month when elevation increased to 1708 m asl (Willowmere) (Tables 1, 5 and 7). A similar trend was observed in non-PBZ treated *E. nitens* planted stands in south-eastern Australia, where trees at lower elevations consistently flowered earlier and for longer than those at higher elevations (Barbour et al., 2006; Moncur and Boland, 2000;

Williams, 2000). In Tasmania, an increase in elevation from 40 m asl to 712 m asl showed a consistent annual delay of about 21 weeks in the commencement of *E. nitens* flowering (Moncur et al., 1994).

At lower elevation (≤ 1200 m asl) temperate eucalypt forestry sites in KwaZulu-Natal (e.g. Mountain Home and Tweedie), *E. nitens* flowers predominantly through the winter months (Table 5; Gardner, unpublished results; Jones and Van Staden, 2001). At sites such as these, the severities of winter climatic risk factors such as low temperature, frost and snow are low, with such factors rarely posing a threat to *E. nitens* young trees, flowers and fruit (Gardner and Swain, 1996; Kunz and Gardner, 2001; Smith et al., 2005). At the higher elevation (> 1550 m asl) temperate eucalypt forestry sites in KwaZulu-Natal where environmental conditions have been rated as optimum for *E. nitens* floral bud production (Gardner et al., 2014) (e.g. Tweefontein, Netherby3 and Willowmere), *E. nitens* flowers from late July onwards (Table 7; Jones and Van Staden, 2001). At such high elevation sites, the severities of the winter climatic risk factors are generally high, posing a substantial threat to *E. nitens* young trees, flowers and fruit.

In Clone 2, the mean effect of PBZ observed across the four trial sites was an 11-day delay in anthesis commencement in the PBZ1 treatment (Tables 6 and 7). The substantial (33-day) delay in anthesis commencement that occurred in PBZ-treated trees at Netherby3 is cause for concern. Based on the results of the comprehensive *E. nitens* site x genotype x flowering interaction trial series stratified across a wide range of high elevation sites within the summer rainfall forestry area of South Africa, Netherby3 was the top-performing site on the basis of provision of near optimal environmental conditions for consistent floral bud production in the species (Germishuizen and Gardner, 2014; Gardner et al., 2014). If the anthesis delaying effect of PBZ proves a general phenomenon across the range of genotypes comprising the South African *E. nitens* base breeding population (Swain et al., 2013b), the negative implications could be substantial. In south-eastern Australia, a marked delay in *E. nitens* anthesis commencement (non-PBZ treated trees) was accompanied by a significant condensing of anthesis period, and extended time between floral bud emergence and seed maturity (Moncur et al., 1994). The observed anthesis delaying effect of PBZ treatment in *E.*

nitens Clone 2 at Netherby3 in 2010 suggests there may be potential to utilize PBZ application as a manipulative tool for synchronizing flowering in asynchronous flowering genotypes within the orchard. This could be advantageous to temperate eucalypt breeders and commercial seed producers in South Africa. In this case, PBZ treatment would likely need to be applied in a more precise manner, e.g. via foliar application (Hetherington et al., 1991; Williams et al., 1999a). Manipulation of timing and duration of anthesis using PBZ applications has been reported for a number of horticultural tree crops, including peach (Erez, 1986), sweet cherry (Looney and McKellar, 1987), pistachio (Porlingis and Voyiatzis, 1993) and mango (Perez-Barazza et al., 2000).

4.5. Duration of anthesis

A trend of decreasing anthesis duration with increasing elevation, regardless of PBZ treatment, was observed across the four KwaZulu-Natal sites (Tables 1, 6 and 8). If only the three field trial sites were considered, in both the PBZ0 and PBZ1 treatments there was a considerable difference in Clone 2 anthesis duration between lowest and highest elevation sites, viz. Tweefontein and Willowmere (47 days and 57 days, respectively). If the Mountain Home site was included in the comparison, the difference in Clone 2 anthesis duration between the lowest and highest elevation sites increased to 99 days and 80 days for PBZ0 and PBZ1 treatments, respectively. A similar trend was reported for non-PBZ treated *E. nitens* trees in natural and/or planted stands located across a wide range of sites in south-eastern Australia, where trees at lower elevations consistently flowered for longer than those at higher elevations (Barbour et al., 2006; Moncur and Boland, 2000; Williams, 2000).

The trend towards decreasing anthesis duration with increasing elevation in the local *E. nitens* trials is cause for concern. In South Africa, temperate eucalypt tree breeders and commercial seed producers rely predominantly on insects for effecting cross-pollination within orchards of small-flowered species such as *E. nitens* (Eldridge et al., 1993; Swain et al., 2013a). Decreased duration of anthesis is generally accompanied by decreased chance of flowering synchronicity among parents within an orchard (Funda et al., 2009; Lindgren and Mullin, 1998). The timing of anthesis onset and duration of anthesis among genotypes within an orchard is critical (El-Kassaby et al., 1984; Stoehr et al., 1998). Sub-optimal flowering synchronicity and out-crossing among genotypes can severly compromise capture of genetic gain within *Eucalyptus* orchards (Griffin and Cotterill, 1988; Swain et al., 2014; Varghese et al., 2009). Notwithstanding *E. nitens* habits of (partial) protandry (Moncur and Boland 2000; Tibbits, 1989) and varying levels of self-incompatibility (Pound et al., 2003), the species (geitonogamous) self-pollinating nature predisposes *E. nitens* to common orchard-related problems such as reduced capsule set, seed yield and seed viability, and nursery-related problems such as increased abnormality and mortality of developing seedlings (Moncur and Boland, 2000; Tibbits, 1989; Williams et al., 1999b). In the summer rainfall area of South Africa, optimum (satisfactory) flower bud production in *E. nitens* is generally only achieved in those orchards sited on cold slopes in high elevation (> 1550 m asl), cool temperate (MAT ≤ 16.0 ^{o}C) forestry areas (Gardner et al., 2014; Germishuizen and Gardner, 2014). The fact that *E. nitens* is inherently shy-flowering further elevates the importance of achieving maximal duration of anthesis in all genotypes within an orchard (Hamilton et al., 2008; Swain et al., 2013a).

The effect of PBZ application on anthesis duration across the four KwaZulu-Natal sites in 2010 deserves comment. In Clone 2, the mean trend across sites was a 13-day reduction in anthesis duration where PBZ was applied (Tables 1, 6 and 8). There was one exception, viz. Tweefontein, the lowest elevation of the three field trial sites, where anthesis duration in PBZ1 trees (105 days) was seven days greater than that for non-PBZ treated trees (98 days). It is difficult to speculate what the particular reason(s) for this exception was, as several key climatic variables commonly associated with both vegetative and reproductive tree growth, e.g. rainfall and available soil water (Moncur and Hasan, 1994; Owens, 1995), were not measured on-site during the course of the trials. Any of these factors, either singularly or in combination with one another, may have played an interactive role, together with PBZ, in influencing reproductive development during the particular developmental phase. Where only the three field trial sites were considered, on average, non-PBZ treated trees flowered for 10 days longer than PBZ-treated trees (Table 8). The marked reduction in *E. nitens* Clone 2 anthesis duration where PBZ treatment was applied, at both Mountain Home (22 day reduction) and Netherby3 (33 day reduction) (Tables 6 and 8), is cause for concern. As discussed above, reduction in anthesis duration decreases the chance of flowering synchronicity among parents within orchards (Lindgren and Mullin, 1998; Funda et al., 2009), pre-disposing tree breeders and seed producers to seed production and seed quality problems.

The effect of PBZ treatment on anthesis duration differed across clones at Mountain Home (Table 6). In Clone 1, anthesis duration in the control treatment (116 days) was 20 days shorter than that in the PBZ1 treatment (136 days). In Clone 2, anthesis duration in the control treatment (150 days total) was 22 days longer than that in the PBZ1 treatment (128 days). Possibly, anthesis duration was confounded by differences in umbel abundance between clones in either PBZ treatment in the same year, either wholly or in part. In the same crop year (2009), umbel abundance did not differ statistically between clones or for PBZ x clone interaction (Gardner et al., 2013), thus the latter possibility seems unlikely.

The results of our study indicate that the interaction between environment, PBZ, genotype and timing and duration of anthesis in *E. nitens* warrants closer investigation. Such an investigation should not be limited to *E. nitens*, but also include all other locally important temperate eucalypts of which the breeding and production orchards are typically located at high elevation (> 1200 m asl) sites in South Africa. Particularly in the case of important, yet shy-flowering temperate eucalypt species such as *E. nitens, E. smithii, E. dunnii* and *E. badjensis*, the effect of the high altitude orchard environmental conditions on insect pollinator dynamics deserves investigating. Due to inherent scarcity of flowers, pollen and nectar, these eucalypt species are renowned for their relative unattractiveness to important pollination vectors such as honey bee, *Apis mellifera* (Gardner, 2004a; Johannsmeier and Mostert, 2001).

4.6. Time from inflorescence bud emergence to seed maturity

Fastest time taken (in days) from BFV to seed maturity was recorded in control (PBZ0) Clone 2 trees at Mountain Home (374 days, Table 6). This was 63 days faster than the time taken by the best-performing field trial control, Willowmere (437 days, Table 8). Netherby3, with its high elevation and steep-sloped, south-west facing disposition within the landscape (Table 1), provided the most optimum environmental conditions for *E. nitens* flower bud production of all 13 sites evaluated (Gardner et al., 2014). However, on the basis of time taken from BFV to seed maturity in the PBZ0 treatment (464 days), the site ranked relatively poorly compared to the lowest elevation Mountain Home (374 days) and highest elevation Willowmere (437 days) sites. The results suggest that in situations where the use of PBZ is not an option, but where rapid generation turnover is required, there may be merit in utilizing a semi-controlled environment orchard system situated at low elevation (< 1200 m asl), warm (MAT_Air 16.0 – 16.5 ºC) KwaZulu-Natal site, similar to that implemented at Mountain Home (Table 1). The trees at Mountain Home were grown in containers, and it remains to be established whether trees grown in open soil and subjected to similar evaporative cooling for the fulfilling of winter chilling requirement would perform similarly on the basis of floral bud production and rate of flower and fruit development. Across the field trial sites, the time taken from BFV to seed maturity in the control treatment did not correlate to elevation (Table 8). Furthermore, the thermal time taken from BFV to seed maturity in the control treatment varied across sites by as much as 12.3% (644 ºCd). Hence, it is probable that some other environmental factors(s), possibly in addition to elevation and/or thermal time, exerted a strong influence on *E. nitens* reproductive development in the BFV to seed maturity phase. Photoperiod was an unlikely factor, as daylength differed across the KwaZulu-Natal trial sites by a maximum of eight minutes, on both the shortest and longest days of the year (SAAO, 2014).

Where PBZ was applied (PBZ1) at Mountain Home, the time between BFV and seed maturity in Clone 2 increased from 374 to 420 days (Table 6). The latter time was similar to that for the KwaZulu-Natal field trial PBZ1 mean (428 days) (Table 8). On average, the time taken from BFV to seed maturity across the field trials decreased from 451 to 428 days. The only exception was at Willowmere, the highest elevation site of the three, where a slight (5 day) increase in time from BFV to seed maturity was recorded. Across the field sites, the general trend in the PBZ1 treatment was a decrease in time between BFV and seed maturity as elevation increased.

Of all four KwaZulu-Natal trials evaluated, an increase in the time taken (days and thermal time) between BFV and seed maturity through application of PBZ occurred only at the lowest and warmest Mountain Home site (MAT_Bud 15.3 ^{o}C) and highest and second coolest Willowmere site (MAT_Bud 13.2 ^{o}C) (Tables 1, 6 and 8). The increase in time was greater at Mountain Home (46 days, 641 ^{o}Cd) than at Willowmere (5 days, 95 ^{o}Cd). It is difficult to speculate what the particular reason was for this difference in magnitude of *E. nitens* Clone 2's response to PBZ across the two sites, as PBZ is known to interact with a wide range of environmental factors regarding its effect on plant vegetative and reproductive growth (Davis et al., 1988). One possibility is that the environmental conditions at Mountain Home provided far more stressful growing conditions for *E. nitens* than those at Willowmere, and PBZ in combination with the Mountain Home environmental conditions slowed *E. nitens* post-emergence reproductive growth even further. At Mountain Home, although drought was not a risk factor as soil water levels were maintained at between 75% and 100% field capacity throughout the year, mean annual air temperature (MAT_Air 16.2 ^{o}C) was close to the upper MAT boundary for successful *E. nitens* vegetative growth (MAT_Air 16.5 ^{o}C) (Jovanovic and Booth, 2002; Smith et al., 2005). At Mountain Home, during winter when the evaporative cooling system was operational, daytime maximum temperatures were similarly low as the KwaZulu-Natal high elevation field trial sites (Gardner et al., 2013; Gardner, unpublished results). However, during the summer months, the average daily air temperatures at Mountain Home were substantially higher than at the KwaZulu-Natal field sites. The high summer temperatures at Mountain Home (in comparison to those at the high elevation sites) may have been responsible for encouraging rapid reproductive growth between time of BFV and seed maturity in control trees (Table 6), but in combination with PBZ the same high temperatures may have exerted a negative effect on reproductive growth rate in *E. nitens*.

Soil temperatures were never monitored at Mountain Home or in the KwaZulu-Natal field trials. It is possible that mean daytime soil temperatures within the plant bags at Mountain Home were substantially higher than those in the upper and lower soil profiles at the high elevation field trial sites, particularly during the summer months when the evaporative cooling system at Mountain Home was not operational. Again, these high root and canopy temperatures in combination with the growth-retarding effect of PBZ may have been responsible for significant slowing of *E. nitens* post-emergence reproductive growth at Mountain Home. Paclobutrazol has been known to increase plant resistance to high temperature stress (Fletcher and Hofstra, 1988). Experiments with wheat suggested that triazole-induced heat stress protection may be a result of increased efficiency of free radical scavenging systems (Fletcher et al., 2000).

4.7. Thermal time approach

To investigate whether accumulated heat units related more strongly than number of days to rate of reproductive development between BFV and seed maturity in *E. nitens* (Moncur and Boland, 2000), thermal time over one seed crop cycle was calculated for the range of *E. nitens* orchard sites in KwaZulu-Natal. Where PBZ was not applied (PBZ0), on the basis of thermal time between BFV and seed maturity in the particular *E. nitens* material tested there was a noticeable lack of similarity (> 12% < 60% difference) between Mountain Home (clonal mean 4848 ^{o}Cd), KwaZulu-Natal high elevation trials (mean 5455 ^{o}Cd) and Australian sites (mean 3396 ^{o}Cd) trial sites (Tables 6 and 8; Moncur and Boland, 2000). Where PBZ was applied (PBZ1), in the particular *E. nitens* material tested there was a strong similarity (4% difference) between Mountain Home (clonal mean 5304 ^{o}Cd) and the KwaZulu-Natal high elevation trials (mean 5098 ^{o}Cd), but a noticeable dissimilarity (38% and 43% difference) between either of the latter and the Tweedie site (clonal mean 7293 ^{o}Cd) (Tables 6 and 8; Jones and Van Staden, 2001). This raises doubt as to the usefulness and/or applicability of the thermal time approach in explaining *E. nitens* reproductive growth and development rate. Photoperiod cannot be excluded as a possible causal factor in the substantially differing thermal time for *E. nitens* BFV to seed maturity phase between the South African and Australian survey sites, even though daylength differed by a maximum of ≈30 min on both the shortest and longest days of the year (SAAO, 2014).

Difference in *E. nitens* genetic material across trial groups was a possible source of discrepancy on the basis of thermal time taken between BFV and seed maturity stages. However, even where a single scion genotype, Clone 2, was evaluated across the range of KwaZulu-Natal high elevation sites, there was still substantial variation in thermal time between BFV and seed maturity (12% and 18% for PBZ0 and PBZ1, respectively) (Tables 6 and 8). A further possible source of discrepancy across trial groups and sites may be the difference in origin of temperature data. At Mountain Home, *E. nitens* foliage was evaporatively cooled during the winter months and therefore bud temperature, rather than air temperature, was measured (Gardner et al., 2013) and these data utilized in calculating daily heat sums. However, in the non-evaporatively cooled (control) plots at Mountain Home, *E. nitens* bud temperature was found to correlate closely with radiation screen air temperature (R^2 = 0.98) in both the summer and winter months (Gardner et al., 2014). Regarding all other local and Australian sites and trials, actual and/or modeled standard air temperature (WMO, 2008) data were utilized in the daily heat sum calculations (Gardner et al., 2014; Jones, 2002; Moncur et al., 1994).

In the Mountain Home maximum winter chilling block, the (bud) temperature measurements and thermal time calculations for two different *E. nitens* clones (Clone 1 and Clone 2) growing at two different PBZ treatment levels (PBZ0 and PBZ1) were precise. However, in the monitored 2009/2010 crop cycle, substantial differences in thermal time for BFV to seed maturity stage were observed between clones within each PBZ treatment, and between PBZ treatments (clonal means). Therefore, apart from genetics, some environmental factor(s) other than, but possibly in combination with, daily heat sums, exerted an influence on *E. nitens* post bud emergence reproductive growth and development at the particular site. Bonhomme (2000) re-iterated that plant reproductive development rate can rarely be quantified by thermal time alone. Apart from air and canopy temperature (Dahlgren et al., 2007; Luedeling et al., 2013; Tooke and Battey, 2010), plant/soil/atmospheric environmental factors commonly associated with vegetative and reproductive growth and development in temperate woody perennials include solar irradiance (Granger and Schulze, 1997; Schulze, 2007), soil water availability (Owens, 1995; Louw, 1999) and soil and root temperature (Lahti et al., 2005; Lopushinsky and Max, 1990).

5. Conclusions

To our knowledge, this is the first attempt at investigating the interactive effect of environment and PBZ on *E. nitens* reproductive phenology in South Africa. The observed trends, although based on a narrow range of *E. nitens* genetic material, provide a strong indication that both disadvantages and opportunities may be associated with the use of PBZ and the locating of orchards at high elevation/ high chill sites to achieve prolific floral bud production. These need to be explored more fully, i.e. by investigating a broader range of genetic material over a wider range of high elevation sites. The locating of *E. nitens* orchards at more moderate elevation (< 1400 m asl) sites where adequate winter chilling for floral bud production is provided through careful site selection and/or winter chilling supplementation via "artificial" means such as evaporative cooling, may provide the necessary degree of climatic amelioration. Under such orchard site conditions, the risk of damage to *E. nitens* young trees, flowers and fruit and seed crop loss by inclement winter weather might be minimized, and anthesis duration, level of out-crossing and genetic quality of seed quality maximized. The relative dearth of knowledge that exists in South Africa regarding high elevation orchard environment x temperate eucalypt flowering x insect pollinator dynamics needs urgent attention. Such information is necessary to facilitate the future designing of elite (clonal) temperate eucalypt orchard layouts in South Africa.

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Table 1
Site details for the *E. nitens* flowering trials in KwaZulu-Natal.

Trial name	Mountain Home[1]	Netherby3[2]	Tweefontein[2]	Willowmere[2]
Latitude	29°34' S	29°38' S	29°15' S	29°51' S
Longitude	30°17' E	29°38' E	30°13' E	29°26' E
Elevation (m asl)	1133	1678	1588	1708
Climatic factors:				
MAP (mm)[3]	924	948	842	914
MAT_Air (°C)[4]	16.2	14.1	15.1	14.1
MAT_Bud (°C)[5]	15.3	13.9	13.0	13.2
MAC_Air (CP)[6]	48.3	76.0	93.2	86.8
MAC_Bud (CP)[7]	73.5	80.0	93.5	88.5
Soil form and series[8]	na	Kranskop 1200	Magwa 200	Kranskop 1200
Soil unit[9]	na	Haplic Acrisol	Haplic Acrisol	Haplic Acrisol
Soil depth (m)	na	> 1.2	> 1.2	> 1.2
Landscape factors:				
Aspect[10]	NE	SW	N	E
Slope[11]	Moderate	Steep	Gentle	Gentle

CP = Chilling Portion, the chill unit of the Dynamic Model (Erez and Fishman, 1998).
na = Not applicable.
[1]Potted trial, maximum chilling treatment block.
[2]Field trial.
[3]Long term mean annual precipitation (Schulze and Lynch, 2007).
[4]Long term mean annual air temperature (Schulze and Maharaj, 2007b).
[5]Mean annual *E. nitens* bud temperature for the period 2008 to 2010. Except for Mountain Home, bud temperature data were modeled from hourly Hobo-logger data.
[6]Mean annual accumulated winter (April to September) CP for the period 2008 to 2010. Except for Mountain Home, screen air temperature data were modeled from hourly Hobo-logger data.
[7]Mean annual accumulated winter (April to September) CP for the period 2008 to 2010. Except for Mountain Home, bud temperature data were modeled from hourly Hobo-logger data.
[8]Soil Classification Working Group (1991).
[9]IUSS Working Group (2006).
[10]Compass bearing.
[11]McDonald et al. (1984).

Table 2
Details of the *E. nitens* shoot apex samples investigated.

Sample collection date	Day of year	Accumulated CP Bud[1]	Accumulated CP Air[1]	Inflorescence buds[2]
17/05/2010	137	1.8	2.0	No
17/05/2010	137	1.8	2.0	Yes
14/06/2010	165	14.1	8.9	Yes
28/06/2010	179	23.5	16.1	Yes
12/07/2010	193	32.0	20.1	Yes
26/07/2010	207	41.3	24.6	No
26/07/2010	207	41.3	24.6	Yes
10/08/2010	222	50.7	27.6	Yes
06/09/2010	249	63.1	35.6	Yes
01/11/2010	305	73.2	44.7	No
01/11/2010	305	73.2	44.7	Yes

CP= Chilling Portion of the Dynamic Model (Erez and Fishman, 1998)
Bud = CP calculated from *E. nitens* bud temperatures in the maximum winter chilling block (Gardner et al., 2013)
Air = CP calculated from radiation screen air temperatures in the maximum winter chilling block (Gardner et al., 2013)
[1]CP began accumulating on 24 April 2010
[2]Inflorescence buds initiated in the spring of 2010

Table 3
Description of *E. nitens* reproductive stages scored[#].

Stage	Description of reproductive stage
1	Inflorescence bud with involucral bract fully intact
2	Involucral bract shed and individual flower buds within the umbel fully exposed. Opercula of individual flower buds fully intact.
3	Two-layered operculum shed from one or all of the individual flower buds within umbel leaving anthers and stigma exposed.
4	Anthesis completed in all flowers within the umbel. Stamens withered and/or abscised and capsule ripening underway.

[#]Modification of a scoring system devised by Jones and Van Staden (2001)

Table 4
Accumulated winter chilling and thermal time during the early reproductive development stages of *E. nitens* Clone 1 at Mountain Home during 2010.

	Meristems[1]		BFV[2]		Floral organs[3]	
Temperature variable	CP	ºCd	CP	ºCd	CP	ºCd
AirT	35.4	0	40.4	479	44.5	786
BudT	63.1	0	69.2	472	73.2	786

[1]Apical meristem size increase first detected (6 September 2010).
[2]New season's inflorescence buds first became visible to the naked eye (BFV stage, 10 October 2010).
[3]Individual flower buds appear (microscopically) as finger-like structures, with floral organs initiated (5 November 2010).
AirT = Air temperature within maximum chilling treatment block.
BudT = Mean *E. nitens* bud temperature within maximum chilling treatment block.
CP = Total Chilling Portions accumulated since 24 April 2010 when the first CP was irreversibly fixed.
ºCd = Thermal time taken since date apical meristem size increase first observed (6 September 2010).

Table 5
Floral phenology of two *E. nitens* clones grown at two levels of paclobutrazol application in the maximum winter chilling treatment block at Mountain Home during 2009/ 2010.

Treatment	BFV	Bract	Anthesis1	Anthesis2	Seed
PBZ0					
Clone 1	02/10/2009	22/12/2009	16/04/2010	10/08/2010	30/10/2010
Clone 2	30/10/2009	28/12/2009	12/04/2010	09/09/2010	08/11/2010
PBZ1					
Clone 1	02/10/2009	26/12/2009	26/04/2010	09/09/2010	17/11/2010
Clone 2	18/10/2009	23/12/2009	24/04/2010	30/08/2010	12/12/2010

PBZ0 = Nil paclobutrazol applied (control).
PBZ1 = Paclobutrazol applied to growing medium on 12/11/2007 and 3/05/2010.
BFV = Earliest date emerging inflorescence buds visible to the naked eye.
Bract = Earliest date of total shedding of involucral bracts.
Anthesis1 = Date anthesis commenced.
Anthesis2 = Date anthesis ceased.
Seed = Earliest date mature seed available.

Table 6
Number of days and thermal time (in parentheses) between key *E. nitens* phenological events at Mountain Home during 2009/ 2010. Thermal time (^{o}Cd) was calculated from *E. nitens* bud temperature.

Treatment	Anthesis1 to Anthesis2	BFV to Seed
PBZ0		
Clone 1	116 (925)	393 (4960)
Clone 2	150 (1212)	374 (4736)
Mean	133 (1069)	384 (4848)
PBZ1		
Clone 1	136 (1019)	411 (5232)
Clone 2	128 (949)	420 (5377)
Mean	132 (984)	416 (5304)

PBZ0 = Nil paclobutrazol applied (control).
PBZ1 = Paclobutrazol applied to growing medium on 12/11/2007 and 3/05/2010.
BFV = Earliest date emerging inflorescence buds became visible to the naked eye.
Anthesis1 = Date anthesis commenced.
Anthesis2 = Date anthesis ceased.
Seed = Earliest date mature seed available.

Table 7
Floral phenology of *E. nitens* Clone 2 grown at two levels of paclobutrazol (PBZ) application at three high elevation sites in KwaZulu-Natal over one floral crop season.

Treatment	BFV	Bract	Anthesis1	Anthesis2	Seed
PBZ0					
Netherby3	02/12/2009	19/05/2010	20/08/2010	24/11/2010	11/03/2011
Tweefontein	25/11/2009	24/05/2010	19/08/2010	25/11/2010	21/02/2011
Willowmere	02/12/2009	10/03/2010	30/09/2010	20/11/2010	12/02/2011
PBZ1					
Netherby3	30/11/2009	10/02/2010	22/09/2010	24/11/2010	03/02/2011
Tweefontein	26/11/2009	01/01/2010	12/08/2010	25/11/2010	13/01/2011
Willowmere	02/12/2009	10/03/2010	30/09/2010	17/11/2010	17/02/2011

PBZ0 = Nil paclobutrazol applied (control).
PBZ1 = Paclobutrazol applied to soil in March 2006.
BFV = Earliest date emerging inflorescence buds visible to the naked eye.
Bract1 = Earliest date of total shedding of involucral bracts.
Anthesis1 = Date anthesis commenced.
Anthesis2 = Date anthesis ceased.
Seed = Earliest date mature seed available.

Table 8
Number of days and thermal time (in parentheses) between key phenological events for one *E. nitens* clone (Clone 2) grown at two levels of paclobutrazol (PBZ) application at three high elevation sites in KwaZulu-Natal, over one floral crop season. Thermal time (ºCd) calculated from modeled *E. nitens* bud temperature.

Treatment	Anthesis1 to Anthesis2	BFV to Seed
PBZ0		
Netherby3	96 (1211)	464 (5850)
Tweefontein	98 (1100)	453 (5206)
Willowmere	51 (659)	437 (5310)
Mean	82 (990	451 (5455)
PBZ1		
Netherby3	63 (800)	430 (5314)
Tweefontein	105 (1153)	413 (4576)
Willowmere	48 (614)	442 (5405)
Mean	72 (856)	428 (5098)

PBZ0 = Nil paclobutrazol applied (control).
PBZ1 = Paclobutrazol applied to soil in March 2006.
BFV = Earliest date emerging inflorescence buds visible to the naked eye.
Anthesis1 = Date anthesis commenced.
Anthesis2 = Date anthesis ceased.
Seed = Earliest date mature seed available.

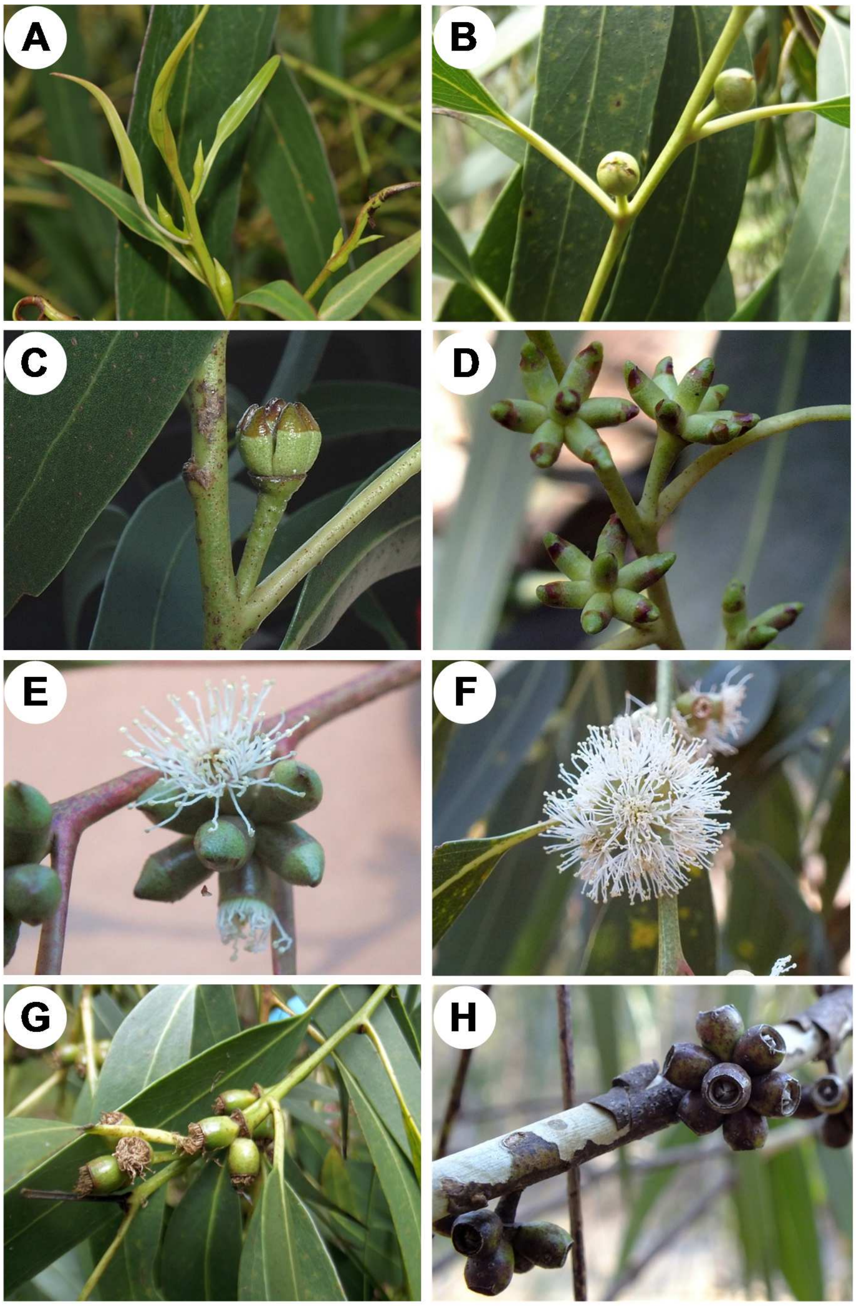

Fig. 1. Flower and fruit development in *E. nitens*. (A) Emerging inflorescence buds (early Stage 1, Table 3). (B) Early stage of involucral bract shedding (late Stage 1). (C) Involucral bract shed, leaving individual flower buds within the umbel exposed (early Stage 2). (D) Seven-flowered umbels immediately prior to anthesis (late Stage 2). (E) Commencement of anthesis (early Stage 3). (F) Inflorescence in full bloom (late Stage 3). (G) End of anthesis, fruit (capsule) development commenced (early Stage 4). (H) Ripening capsules (late Stage 4).

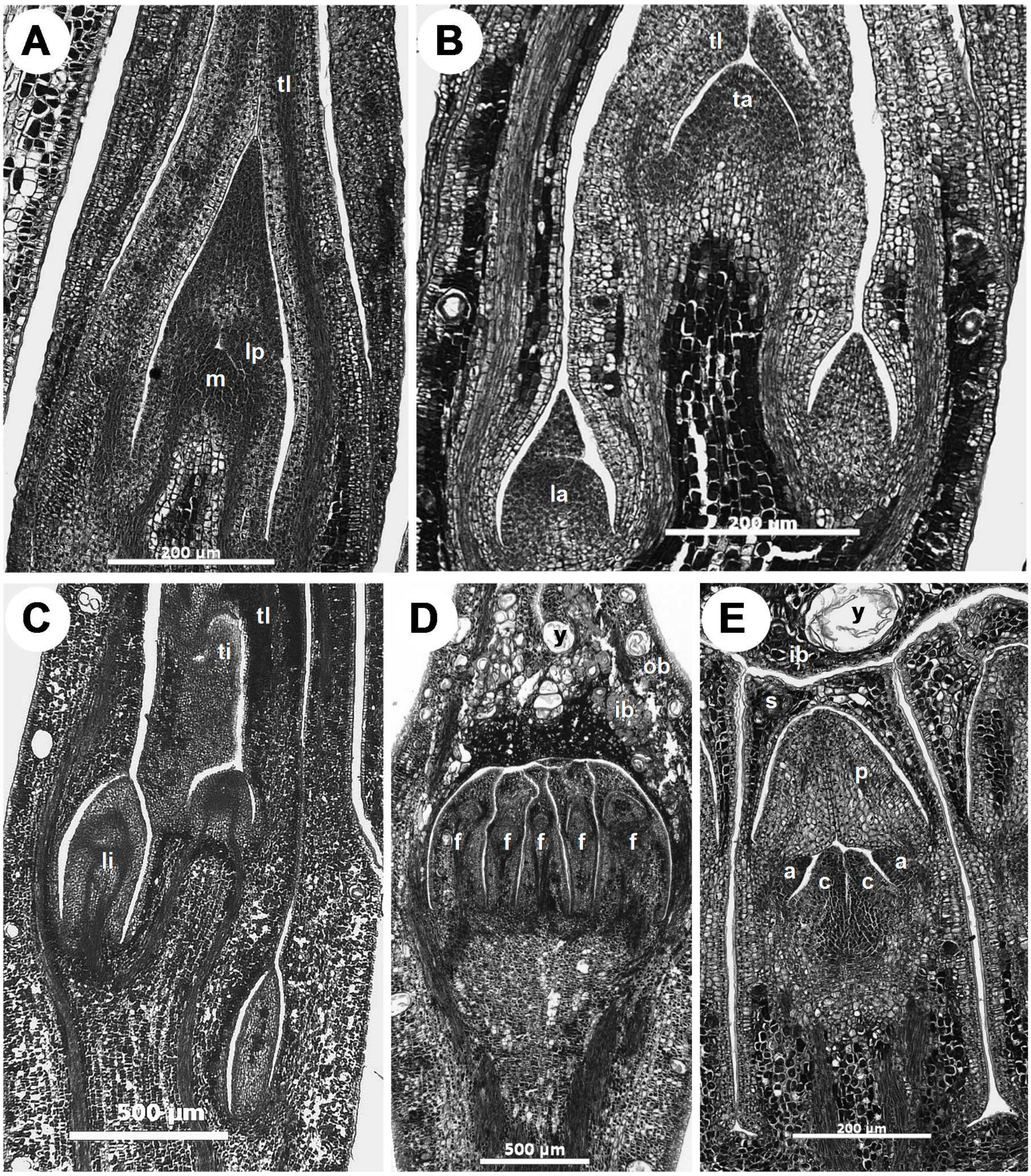

Fig. 2. Floral initiation and development in *E. nitens.* Longitudinal sections of (A) vegetative shoot apex, showing apical meristem (m), leaf primordium (lp) and developing terminal leaf (tl); (B) early stage of apical meristem broadening, showing lateral apex (la), terminal apex (ta) and developing terminal leaf (tl); (C) inflorescence buds developing spirally around the growing point, with lateral inflorescence bud (li), terminal inflorescence bud (ti) and developing terminal leaf (tl); (D) inflorescence bud approximately three weeks after the BFV stage, with individual flower bud/s (f) at pre-organ differentiation stage, inner bract (ib), outer bract (ob) and oil gland (y); and (E) individual flower bud with floral organ differentiation underway, showing stamen initials (a), carpel initials (c), petaline operculum (p), sepaline operculum (s), inner bract (ib) and oil gland (y).

CHAPTER 8

CONCLUSIONS AND RECOMMENDATIONS FOR FURTHER RESEARCH

Introduction

Temperate (cold-tolerant) eucalypt species and hybrids comprise approximately 50% of the total planted *Eucalyptus* area in South Africa (Forestry South Africa 2014). Due to their high levels of cold, frost and snow tolerance, as well as favorable pulping properties, temperate species such as *E. badjensis*, *E. benthamii*, *E. dunnii*, *E. nitens*, *E. macarthurii* and *E. smithii* are the most popular choice for high elevation (> 1100 m asl) commercial plantings in frost- and snow-prone areas of the summer rainfall forestry region (Schönau and Gardner 1991, Gardner 2001, Swain and Gardner 2002). Cuttings of temperate eucalypts generally do not root well, and therefore, to date, the majority of the temperate eucalypt plantations in South Africa have been established with seedlings (Zwolinsky and Bayley 2001). This practice seems set to continue, and therefore the local industry is dependent on the production of locally-improved seed for the establishment of temperate eucalypt plantations. The majority of the commercial temperate species in South Africa are reticent flowerers (Swain and Gardner 2002), for reasons genetic and environmental (Gardner 2004). This hinders breeding and commercial seed production pertaining to the species (Eldridge et al. 1993). Temperate eucalypts appear to have a minimum winter chilling requirement for floral bud production (Gardner and Bertling 2005). This has proven problematic in the summer rainfall plantation areas of South Africa, where winter chilling levels are notoriously inconsistent and insufficient for satisfactory floral bud production in the higher chill requiring *Eucalyptus* species such as *E. nitens*. Climate warming seems set to increase the extent of the problem (Warburton and Schulze 2008, Luedeling et al. 2009). For the past two and a half decades, the local industry has depended on paclobutrazol (PBZ) soil treatment to encourage flowering and seed crop production (Jones 2002, Gardner and Bertling 2005, Gardner et al. 2013). For reasons explained in previous chapters of this thesis, the use of paclobutrazol in the outdoor environment is an unsatisfactory practice (Jackson et al. 1996, Fletcher et al. 2000).

Aims and objectives

The research facets of this PhD project represent a combined effort aimed at not only improving flower and seed crop production in temperate eucalypts through informed site selection and environmental manipulation, but also lessening the dependency on PBZ. Substantial progress was made in achieving this goal. Scope exists for further research, aimed at investigating some of the findings of the PhD project in more detail.

Challenges and opportunities

Species representation

Of the six main temperate eucalypt species currently grown for commercial pulpwood production in the summer rainfall area (refer above), those most prone to shy-flowering are *E. badjensis, E. benthamii, E. dunnii, E. nitens* and *E. smithii*. According to a recent survey carried out by the author, the combined land area currently planted to the latter four species represents about 60% of the total temperate *Eucalyptus* plantation area (approximately 264 000 ha) (Forestry South Africa 2014, R Gardner unpublished data 2014). Due to the nature of the research in this PhD project, as well as resource constraints, the challenge was that not all of the shy-flowering temperate eucalypts could be included in the investigations. Two species, *E. nitens* and *E. smithii*, were selected for use as test species in the investigations for the following reasons:

- Based on field observations during the early 1990s, *E. nitens*, *E. fraxinoides* and *E. smithii* appeared to be most reticent seed producers and have the highest cold requirements for flower bud production of all then-current commercial eucalypts
- During the planning phase of the 2003/ 2004 site x PBZ x flowering field trial series, due to the commercial importance of *E. nitens* and *E. smithii*, the request

of the funding companies was to include the latter two species in the investigations

The research findings emanating from this PhD project should be applied tentatively to other shy-flowering temperate eucalypt species. It is recommended that, firstly, the necessary tests be conducted to evaluate the extent to which the recommendations for *E. nitens* and *E. smithii* apply to species such as *E. badjensis, E. benthamii* and *E. dunnii.* Secondly, regarding each of the species already investigated, i.e. *E. nitens* and *E. smithii*, it would be worthwhile exploring the flowering response of a wider range of genetic material to the recommended environmental conditions.

Bud and air temperature measurement

One of the major challenges of the PhD project research, particularly with respect to the field trial component, was the need to develop a practical and suitably accurate method of monitoring air and bud temperature "on site" in remote, high elevation areas of the summer rainfall forestry regions. Temperature is one of the key environmental factors implicated in various aspects of temperate eucalypt flowering, including floral induction and initiation (Moncur and Hasan 1994, Hasan and Reid 1995). The available infrastructure and instrumentation at the Mountain Home *E. nitens* overhead sprinkling trial presented the ideal opportunity to investigate the relationship between bud, screen air and Hobo pole air temperature. This opportunity was siezed, the necessary investigations carried out, and a set of calibration curves developed. The four year old *E. nitens* grafts utilized in the study were all in adult leaf, and care was taken to position the thermocouples (TCs) alongside buds in the canopy periphery without exposing the TC sites to direct sunlight. The question remains as to the relevance of the temperature calibration equations to temperate eucalypt species other than *E. nitens*. Eucalypt species differ in leaf morphology, canopy architecture and canopy density to various extents (Pryor 1976, Brooker and Kleinig 1983, Florence 1996). Due to the careful positioning and maintenance of TCs within the canopy peripheries, although unlikely, it is possible that the inter-specific differences in adult leaf morphology and canopy architecture may affect the relevance of the developed temperature calibration equations which were based on *E. nitens*. Therefore, although not priority in the opinion of the author, it would be worthwhile investigating the relationship between the same three temperature variables in each of the above-mentioned shy-flowering temperate eucalypt species.

Species growth responses to PBZ and environmental conditions

The *E. nitens* and *E. smithii* site x PBZ x flowering interaction field trial series yielded valuable new information that has assisted the siting of orchards of these species. Utilizing combined data from the 1996-established and the 2003/ 2004 *E. nitens* site x PBZ x flowering interaction trial series, an additional output (at the request of the funding companies) was the development of a computerized GIS tool based on climatic and topographical factors to assist forestry planners identify potential *E. nitens* orchard sites in the summer rainfall area (Germishuizen and Gardner 2014). The results of the 2003/ 2004 *E. nitens* and *E. smithii* trial series highlighted the fact that species can differ substantially in winter chilling requirement for floral induction and reproductive growth response to PBZ application. *Eucalyptus smithii* showed a lower chill requirement and greater responsiveness to PBZ than *E. nitens*. At sites optimum (highly inductive) for *E. nitens* flower bud production:

- PBZ-treated trees of *E. smithii* are prone to production of excessive flowers and capsules and experiencing die-back of bearing branches, probably as a result of nutrient competition and/or depletion
- PBZ-treated trees of *E. smithii* trees are prone to increased risk of selfing due to the excessive flower crops produced
- Trees of *E. smithii* are at risk of frost and snow damage

Within the recommended climatic and topographical specifications for establishment of temperate eucalypt orchards for optimal flower bud production (based on the field research in this project), species need to be treated differently, according to both tolerance to climate risk factors such as frost and snow, and reproductive growth response to PBZ application. In order to accrue additional data needed for fine-tuning of the orchard site x temperate species matching specifications, it is recommended that test orchards of shy-flowering temperate species such as *E. badjensis, E. benthamii* and *E. dunnii* be established over ranges of carefully selected high elevation sites, to monitor reproductive and vegetative growth responses to site factors and PBZ application over several years. Species such as *E. badjensis, E. benthamii* and *E.*

dunnii differ substantially on the basis of frost- and snow-tolerance (Gardner and Swain 1996, Gardner 2001).

Environment x plant growth dynamics

The results of the *E. nitens* controlled environment and field trial research carried out by the ICFR between 1996 and 2001 suggested that temperate eucalypts require an extended period of slowed vegetative growth provided by cool, stress-free growing conditions during winter to initiate flower buds (Gardner 2003, Gardner and Bertling 2005). The results of the 2003/ 2004 *E. nitens* and *E. smithii* field trial series tended to concur with this. One of the key outcomes of the latter was the strong positive effect of south-facing slope aspects on floral bud production. In South Africa, and indeed other Southern Hemisphere countries, south-facing slopes are generally associated with cooler soil, air and foliage temperatures and slower plant growth rates, compared to those on north-facing slopes (Granger and Schulze 1977, Bale et al. 1998, Schulze 2007, Sharma et al. 2010).

At Netherby plantation in KwaZulu-Natal (2003/ 2004 *E. nitens* and *E. smithii* field trial series), two sites of similar elevation, MAT and MAC but differing vastly in slope aspect, viz. Netherby1 (1688 m asl, 14.1 ^{o}C, 865 DPCU, N-facing) and Netherby3 (1678 m asl, 14.1 ^{o}C, 865 DPCU, SW-facing) differed substantially in floral bud production over two floral crop seasons. In general, non-PBZ treated trees of *E. nitens* and *E. smithii* produced substantially more flower buds (umbels) at Netherby3 than at Netherby1. This suggests a strong possibility that soil and/or or root environmental factors, possibly together with plant canopy factors, are implicated in temperate eucalypt floral induction. Root-zone temperatures have been known to influence a range of reproductive growth aspects in woody tree crops, including root growth and nutrient uptake, induction, bud dormancy, development and phenology (Menzel and Simpson 1994, O'Hare 2004, Greer et al. 2005, Lahti et al. 2005). It would be worthwhile investigating the interaction between the particular inductive environmental conditions and floral induction on the basis of plant physiological aspects such as assimilate and hormonal balances and proteomics. Such a study could be undertaken over two to three floral crop years in reproductively mature research orchards. Information gained from such an investigation could contribute substantially towards the ability to manipulate flower and seed crop production in temperate eucalypts under controlled conditions. Such technology would be useful in temperate eucalypt breeding and hybridization, and be highly applicable to elite breeding orchards situated at research centres where natural environmental conditions are generally marginal for floral induction in commercial temperate eucalypts.

Effect of optimal floral bud production site conditions on flower and fruit development

The results of the research work described in Chapter 7 indicated that the interaction between environment, PBZ, genotype and post-floral initiation reproductive development warrants more intensive investigation. The main objectives of the recommended further work would be:

- To investigate the effect of optimum flowering site conditions (cold slopes in high elevation areas of the summer rainfall forestry regions) on important orchard-related factors such as flower abundance, timing and duration of anthesis, pollination (pollen abundance and range and pollination vector diversity and activity), capsule and seed development, and genetic quality of the seed produced.
- To investigate ideal sites for temperate eucalypt orchards based on both floral crop abundance and seed production (quantity and quality).

For the purpose of such an investigation, a fairly wide range of genetic material (grafted elite selections) of at least two species should be evaluated across three to four temperate sites ranging in elevation within the summer rainfall area of South Africa. Apart from elevation, MAT and MAC, all other environmental factors should remain similar. The results of the reproductive phenological research work also indicated that sites with cold winters but warm spring and summer conditions (maximum chilling treatment block at Mountain Home) are more conducive to rapid capsule and seed development, and possibly more suitable for commercial seed production, than those at higher elevations (optimum flowering field trials) in KwaZulu-Natal. This confirms an earlier proposition that there may be merit in establishing test orchards of high-chill requiring commercial *Eucalyptus* species, such as *E. nitens*, in southern areas of the Western Cape (Gardner 2003, Barrington 2006). Although most parts of the Western

Cape receive abundant heat units (Schulze and Maharaj 2007), the test sites would need to be carefully selected for sufficiently cold winters (≥ 80 CP).

Application of rootstocks

The problem of the reproductive maturity of scions being temporarily set back by transmission of a juvenile signal from reproductively immature seedling rootstocks was briefly discussed in Chapter 6. The influence of rootstock on scion phenotypic expression following grafting is a well-known phenomenon in woody crops (Janick and Moore 1996, Hartmann et al. 2011). Transferred positive characteristics include increased reproductive precocity, lowered chilling requirement and reduced vegetative growth (Du Plooy and Van Huysteen 2000, George and Erez 2000). The use of rootstocks appears to hold several opportunities for temperate *Eucalyptus* seed orchards (Adejumo 2014), and this warrants further investigation.

Climate change

Should climate change and warming continue as predicted (Warburton and Schulze 2008, Linkosalo et al. 2009), the importance of the findings and relevance of the technologies developed within this PhD project may be set to increase substantially.

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APPENDICES

Appendix 1: Results of *Eucalyptus nitens* individual site REML analyses of variance for height at five years after planting

Site		Clone 8	35	47	55	PBZ mean[2]
Blair Athol	PBZ0	12.4	11.7	9.4	11.4	11.2a
	PBZ1	8.1	5.9	7.3	7.2	7.1b
	Clone mean[1]	10.2	8.8	8.3	9.3	-
F-pr		PBZ = 0.001		Clone = 0.129		PBZ.Clone = 0.161
SE		0.43		0.58		0.83
Site		Clone 8	35	47	55	PBZ mean[2]
Gilboa	PBZ0	8.2	6.9	6.7	8.1	7.5a
	PBZ1	5.6	4.6	2.4	2.3	3.7b
	Clone mean[1]	6.9a	5.8ab	4.6b	5.2b	-
F-pr		PBZ = < 0.001		Clone = 0.030		PBZ.Clone = 0.098
SE		0.37		0.53		0.75
Site		Clone 8	35	47	55	PBZ mean[2]
In de Diepte	PBZ0	14.0	9.6	9.9	10.1	10.9a
	PBZ1	13.0	10.4	6.2	6.1	8.9a
	Clone mean[1]	13.5a	10.0b	8.0b	8.1b	-
F-pr		PBZ = 0.063		Clone = 0.003		PBZ.Clone = 0.303
SE		0.72		1.02		1.44
Site		Clone 8	35	47	55	PBZ mean[2]
Netherby 1	PBZ0	15.3	12.2	10.0	14.1	12.9a
	PBZ1	14.3	14.0	10.2	14.6	13.3a
	Clone mean[1]	14.8a	13.1b	10.1c	14.4ab	-
F-pr		PBZ = 0.435		Clone = < 0.001		PBZ.Clone = 0.269
SE		0.35		0.49		0.69
Site		Clone 8	35	47	55	PBZ mean[2]
Netherby 2	PBZ0	12.1	9.3	8.4	9.8	9.9a
	PBZ1	8.8	7.7	5.8	6.7	7.3b
	Clone mean[1]	10.4a	8.5b	7.1b	8.3b	-
F-pr		PBZ = 0.013		Clone = 0.005		PBZ.Clone = 0.741
SE		0.49		0.63		0.88
Site		Clone 8	35	47	55	PBZ mean[2]
Netherby 3	PBZ0	14.0	11.7	9.3	7.3	10.6a
	PBZ1	11.5	9.5	8.7	8.6	9.6a
	Clone mean[1]	12.8a	10.6b	9.0bc	7.9c	-
F-pr		PBZ = 0.273		Clone = < 0.001		PBZ.Clone = 0.185
SE		0.58		0.69		0.98

PBZ0 = No PBZ applied (control)
PBZ1 = PBZ applied to soil in March/ April 2006
F-pr = *F*-probability value
SE = Standard error of predicted mean
[1] Within this row, values followed by the same letter do not differ significantly from each other (level of significance given in Table)
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Appendix 1 (contd.): Results of *Eucalyptus nitens* individual site REML analyses of variance for height at five years after planting

Site		Clone 8	35	47	55	PBZ mean[2]
The Peak	PBZ0	14.7	12.6	11.4	12.6	12.8a
	PBZ1	11.0	8.9	6.9	7.3	8.5b
	Clone mean[1]	12.8a	10.7ab	9.1b	9.9b	-
F-pr		PBZ = < 0.001		Clone = 0.030		PBZ.Clone = 0.881
SE		0.60		0.85		1.20
Site		Clone 8	35	47	55	PBZ mean[2]
Thoresway	PBZ0	7.8	8.8	7.1	8.9	8.1a
	PBZ1	4.7	2.8	3.9	2.4	3.5b
	Clone mean[1]	6.2	5.8	5.5	5.6	-
F-pr		PBZ = < 0.001		Clone = 0.604		PBZ.Clone = 0.006
SE		0.27		0.38		0.54
Site		Clone 8	35	47	55	PBZ mean[2]
Tweefontein	PBZ0	11.4	12.2	10.9	7.7	10.5a
	PBZ1	6.0	8.1	6.9	7.9	7.2b
	Clone mean[1]	8.7	10.1	8.9	7.8	-
F-pr		PBZ = < 0.001		Clone = 0.119		PBZ.Clone = 0.036
SE		0.47		0.66		0.93
Site		Clone 8	35	47	55	PBZ mean[2]
Willowmere	PBZ0	11.8	9.4	12.2	11.0	11.1a
	PBZ1	5.3	5.0	4.2	3.5	4.5b
	Clone mean[1]	8.5	7.2	8.2	7.2	-
F-pr		PBZ = < 0.001		Clone = 0.365		PBZ.Clone = 0.189
SE		0.67		0.71		1.01
Site		Clone 8	35	47	55	PBZ mean[2]
Wyntoun	PBZ0	16.6	12.9	13.3	12.4	13.8a
	PBZ1	11.4	9.4	8.0	9.2	9.5b
	Clone mean[1]	14.0	11.1	10.7	10.8	-
F-pr		PBZ = < 0.001		Clone = < 0.001		PBZ.Clone = 0.395
SE		0.42		0.54		0.77

PBZ0 = No PBZ applied (control)
PBZ1 = PBZ applied to soil in March/ April 2006
F-pr = *F*-probability value
SE = Standard error of predicted mean
[1] Within this row, values followed by the same letter do not differ significantly from each other (level of significance given in Table)
[2] Within this column, values followed by the same letter do not differ significantly from each other (level of significance given in Table)

Appendix 2: Results of *Eucalyptus nitens* individual site REML analyses of variance for fifth year umbel crop score (floral buds initiated in 2008 and scored in 2009)

Site		Clone 8	35	47	55	PBZ mean[2]
Blair Athol	PBZ0	0.000	0.000	0.500	0.250	0.187a
	PBZ1	2.750	2.250	3.750	3.250	3.000b
	Clone mean[1]	1.375bc	1.125c	2.125a	1.750ab	-
F-pr		PBZ = < 0.001		Clone = 0.005		PBZ.Clone = 0.299
SE		0.133		0.188		0.265
Site		Clone 8	35	47	55	PBZ mean[2]
Chamisso	PBZ0	0.000	0.500	0.500	0.750	0.438
	PBZ1	0.250	0.250	2.000	0.500	0.750
	Clone mean[1]	0.125	0.375	1.250	0.625	-
F-pr		PBZ = 0.334		Clone = 0.100		PBZ.Clone = 0.193
SE		0.224		0.317		0.448
Site		Clone 8	35	47	55	PBZ mean[2]
Gilboa	PBZ0	0.000	0.000	0.750	0.029	0.188
	PBZ1	1.693	0.750	0.750	1.057	1.063
	Clone mean[1]	0.832	0.375	0.750	0.543	-
F-pr		PBZ = 0.078		Clone = 0.766		PBZ.Clone = 0.351
SE		0.248		0.335		0.474
Site		Clone 8	35	47	55	PBZ mean[2]
In de Diepte	PBZ0	0.750	0.500	2.000	0.000	0.813
	PBZ1	1.250	1.250	3.250	0.000	1.437
	Clone mean[1]	1.000b	0.875b	2.625a	0.000b	-
F-pr		PBZ = 0.151		Clone = 0.002		PBZ.Clone = 0.767
SE		0.298		0.421		0.595
Site		Clone 8	35	47	55	PBZ mean[2]
Netherby 1	PBZ0	0.051	0.000	1.000	0.000	0.250
	PBZ1	0.776	0.250	2.500	0.000	0.875
	Clone mean[1]	0.413b	0.125b	1.750a	0.000b	-
F-pr		PBZ = 0.091		Clone = < 0.001		PBZ.Clone = 0.191
SE		0.211		0.263		0.372
Site		Clone 8	35	47	55	PBZ mean[2]
Netherby 2	PBZ0	0.000	0.000	0.000	0.500	0.125a
	PBZ1	0.000	0.500	1.500	2.250	1.063b
	Clone mean[1]	0.000b	0.250b	0.750ab	1.375a	
F-pr		PBZ = 0.005		Clone = 0.019		PBZ.Clone = 0.166
SE		0.215		0.304		0.430
Site		Clone 8	35	47	55	PBZ mean[2]
Netherby 3	PBZ0	1.000	1.221	2.250	1.529	1.500
	PBZ1	1.558	1.000	1.692	1.000	1.312
	Clone mean[1]	1.279	1.110	1.971	1.265	-
F-pr		PBZ = 0.724		Clone = 0.429		PBZ.Clone = 0.761
SE		0.356		0.437		0.618

PBZ0 = No PBZ applied (control)
PBZ1 = PBZ applied to soil in March/ April 2006
F-pr = *F*-probability value
SE = Standard error of predicted mean
[1] Within this row, values followed by the same letter do not differ significantly from each other (level of significance given in Table)
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Appendix 2 (contd.): Results of *Eucalyptus nitens* individual site REML analyses of variance for fifth year umbel crop score (floral buds initiated in 2008 and scored in 2009)

Site		Clone 8	35	47	55	PBZ mean[2]
The Peak	PBZ0	0.000	0.000	0.500	0.000	0.125a
	PBZ1	2.615	1.250	3.500	2.385	2.437b
	Clone mean[1]	1.308b	0.625c	2.000a	1.192bc	-
F-pr		PBZ = < 0.001		Clone = 0.004		PBZ.Clone = 0.069
SE		0.233		0.260		0.368
Site		Clone 8	35	47	55	PBZ mean[2]
Thoresway	PBZ0	0.000	0.250	2.750	1.250	1.063
	PBZ1	0.750	1.750	1.000	2.750	1.563
	Clone mean[1]	0.375b	1.000ab	1.875a	2.000a	
F-pr		PBZ = 0.191		Clone = 0.015		PBZ.Clone = 0.015
SE		0.263		0.372		0.525
Site		Clone 8	35	47	55	PBZ mean[2]
Tweefontein	PBZ0	0.500	0.249	1.250	0.251	0.563a
	PBZ1	1.750	2.500	2.000	0.500	1.688b
	Clone mean[1]	1.125	1.374	1.625	0.376	-
F-pr		PBZ = 0.022		Clone = 0.073		PBZ.Clone = 0.203
SE		0.230		0.324		0.458
Site		Clone 8	35	47	55	PBZ mean[2]
Willowmere	PBZ0	0.500	0.750	1.250	0.750	0.813a
	PBZ1	2.047	2.250	2.750	1.203	2.063b
	Clone mean[1]	1.274	1.500	2.000	0.976	-
F-pr		PBZ = 0.008		Clone = 0.063		PBZ.Clone = 0.395
SE		0.216		0.269		0.381
Site		Clone 8	35	47	55	PBZ mean[2]
Wyntoun	PBZ0	1.250	0.000	1.304	0.000	0.625a
	PBZ1	3.162	2.000	3.750	0.000	2.188b
	Clone mean[1]	2.206a	1.000b	2.527a	0.000c	-
F-pr		PBZ = 0.009		Clone = < 0.001		PBZ.Clone = 0.014
SE		0.266		0.289		0.409

PBZ0 = No PBZ applied (control)
PBZ1 = PBZ applied to soil in March/ April 2006
F-pr = *F*-probability value
SE = Standard error of predicted mean
[1] Within this row, values followed by the same letter do not differ significantly from each other (level of significance given in Table)
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Appendix 3: Results of *Eucalyptus nitens* individual site REML analyses of variance for sixth year umbel crop score (floral buds initiated in 2009 and scored in 2010)

Site		Clone 8	35	47	55	PBZ mean[2]
Blair Athol	PBZ0	0.250	1.203	1.250	0.547	0.812a
	PBZ1	2.726	2.500	3.774	3.500	3.125b
	Clone mean[1]	1.488	1.851	2.512	2.024	-
F-pr		PBZ = 0.002		Clone = 0.131		PBZ.Clone = 0.258
SE		0.225		0.300		0.424
Site		Clone 8	35	47	55	PBZ mean[2]
Chamisso	PBZ0	0.000	1.750	0.750	1.000	0.875
	PBZ1	0.250	0.250	1.500	0.500	0.625
	Clone mean[1]	0.125	1.000	1.125	0.750	-
F-pr		PBZ = 0.552		Clone = 0.349		PBZ.Clone = 0.269
SE		0.293		0.415		0.586
Site		Clone 8	35	47	55	PBZ mean[2]
Gilboa	PBZ0	0.481	0.250	1.750	0.019	0.625
	PBZ1	0.736	1.250	0.500	0.015	0.625
	Clone mean[1]	0.608	0.750	1.125	0.017	-
F-pr		PBZ = 1.000		Clone = 0.160		PBZ.Clone = 0.147
SE		0.241		0.333		0.471
Site		Clone 8	35	47	55	PBZ mean[2]
In de Diepte	PBZ0	0.752	0.251	3.999	0.000	1.250
	PBZ1	0.499	1.751	3.749	0.000	1.500
	Clone mean[1]	0.626b	1.001b	3.874a	0.000c	-
F-pr		PBZ = 0.217		Clone = < 0.001		PBZ.Clone = 0.021
SE		0.145		0.205		0.289
Site		Clone 8	35	47	55	PBZ mean[2]
Netherby 1	PBZ0	0.250	0.250	1.750	0.000	0.563
	PBZ1	0.750	0.500	2.500	0.750	1.125
	Clone mean[1]	0.500b	0.375b	2.125a	0.375b	-
F-pr		PBZ = 0.061		Clone = < 0.001		PBZ.Clone = 0.913
SE		0.203		0.286		0.405
Site		Clone 8	35	47	55	PBZ mean[2]
Netherby 2	PBZ0	1.000	0.000	1.500	2.250	1.188
	PBZ1	0.250	1.750	2.000	2.500	1.625
	Clone mean[1]	0.625c	0.875bc	1.750ab	2.375a	-
F-pr		PBZ = 0.378		Clone = 0.010		PBZ.Clone = 0.173
SE		0.321		0.396		0.560
Site		Clone 8	35	47	55	PBZ mean[2]
Netherby 3	PBZ0	0.750	1.500	3.000	2.500	1.938
	PBZ1	2.750	1.750	2.250	3.500	2.563
	Clone mean[1]	1.750	1.625	2.625	3.000	-
F-pr		PBZ = 0.184		Clone = 0.120		PBZ.Clone = 0.210
SE		0.323		0.456		0.645

PBZ0 = No PBZ applied (control)
PBZ1 = PBZ applied to soil in March/ April 2006
F-pr = *F*-probability value
SE = Standard error of predicted mean
[1] Within this row, values followed by the same letter do not differ significantly from each other (level of significance given in Table)
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Appendix 3 (contd.): Results of *Eucalyptus nitens* individual site REML analyses of variance for sixth year umbel crop score (floral buds initiated in 2009 and scored in 2010)

Site		8	Clone 35	47	55	PBZ mean[2]
The Peak	PBZ0	1.250	0.000	2.500	0.000	0.937a
	PBZ1	3.000	2.500	4.000	2.750	3.062b
	Clone mean[1]	2.125b	1.250c	3.250a	1.375c	-
F-pr		PBZ = < 0.001		Clone = < 0.001		PBZ.Clone = 0.107
SE		0.140		0.198		0.280
Site		8	Clone 35	47	55	PBZ mean[2]
Thoresway	PBZ0	0.250	1.500	3.500	1.750	1.750
	PBZ1	0.500	1.750	1.750	3.000	1.750
	Clone mean[1]	0.375b	1.625a	2.625a	2.375a	-
F-pr		PBZ = 1.000		Clone = 0.001		PBZ.Clone = 0.054
SE		0.260		0.368		0.520
Site		8	Clone 35	47	55	PBZ mean[2]
Tweefontein	PBZ0	1.500	1.250	2.750	1.750	1.812
	PBZ1	1.500	3.250	2.500	0.500	1.937
	Clone mean[1]	1.500	2.250	2.625	1.125	-
F-pr		PBZ = 0.798		Clone = 0.141		PBZ.Clone = 0.144
SE		0.342		0.484		0.685
Site		8	Clone 35	47	55	PBZ mean[2]
Willowmere	PBZ0	1.500	1.750	2.000	1.250	1.625
	PBZ1	1.054	2.250	2.750	1.196	1.812
	Clone mean[1]	1.277	2.000	2.375	1.223	-
F-pr		PBZ = 0.695		Clone = 0.099		PBZ.Clone = 0.659
SE		0.321		0.393		0.556
Site		8	Clone 35	47	55	PBZ mean[2]
Wyntoun	PBZ0	1.500	0.500	2.500	0.250	1.188a
	PBZ1	2.750	3.500	3.250	1.000	2.625b
	Clone mean[1]	2.125ab	2.000b	2.875a	0.625c	-
F-pr		PBZ = < 0.001		Clone = < 0.001		PBZ.Clone = 0.022
SE		0.193		0.272		0.385

PBZ0 = No PBZ applied (control)
PBZ1 = PBZ applied to soil in March/ April 2006
F-pr = *F*-probability value
SE = Standard error of predicted mean
[1] Within this row, values followed by the same letter do not differ significantly from each other (level of significance given in Table)
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Appendix 4: Results of *Eucalyptus smithii* individual site REML analyses of variance for height at five years after planting

Site		Clone 1	Clone 6	Clone 71	Clone 74	PBZ mean[2]
Blair Athol	PBZ0	12.0	12.1	10.1	11.3	11.4a
	PBZ1	7.9	6.8	5.7	7.1	6.9b
	Clone mean[1]	9.9	9.4	7.9	9.2	-
F-pr		PBZ = 0.006		Clone = 0.451		PBZ.Clone = 0.953
SE		0.67		0.91		1.28
Site		1	6	71	74	PBZ mean[2]
Gilboa	PBZ0	10.6	3.8	9.1	11.6	8.8a
	PBZ1	4.3	5.6	5.5	6.0	5.4b
	Clone mean[1]	7.4a	4.7b	7.3a	8.8a	-
F-pr		PBZ = < 0.001		Clone = 0.005		PBZ.Clone = 0.003
SE		0.51		0.73		1.03
Site		1	6	71	74	PBZ mean[2]
In de Diepte	PBZ0	6.2	8.9	13.1	9	9.3a
	PBZ1	11.3	9.9	11.8	10.4	10.8a
	Clone mean[1]	8.7b	9.4b	12.4a	9.7b	
F-pr		PBZ = 0.217		Clone = 0.018		PBZ.Clone = 0.065
SE		0.77		0.90		1.28
Site		1	6	71	74	PBZ mean[2]
Netherby 1	PBZ0	14.5	14.1	15.9	14.5	14.7a
	PBZ1	8.4	11.2	12.3	10.2	10.5b
	Clone mean[1]	11.4	12.7	14.1	12.3	-
F-pr		PBZ = 0.002		Clone = 0.495		PBZ.Clone = 0.819
SE		0.87		1.24		1.75
Site		1	6	71	74	PBZ mean[2]
Netherby 2	PBZ0	5.2	9.1	6.0	7.6	7.0a
	PBZ1	12.4	11.5	9.6	5.1	9.6b
	Clone mean[1]	8.8ab	10.3a	7.8bc	6.3c	-
F-pr		PBZ = < 0.001		Clone = < 0.001		PBZ.Clone = < 0.001
SE		0.39		0.55		0.78
Site		1	6	71	74	PBZ mean[2]
Netherby 3	PBZ0	8.5	15.1	14	13.4	12.8a
	PBZ1	15.4	8.2	8.1		9.6a
	Clone mean[1]	11.9	11.7	11.6.71	10.1	
F-pr		PBZ = 0.141		Clone = 0.949		PBZ.Clone = < 0.001
SE		1.26		1.21		1.71

PBZ0 = No PBZ applied (control)
PBZ1 = PBZ applied to soil in March/ April 2006
F-pr = *F*-probability value
SE = Standard error of the predicted mean
[1] Within this row, values followed by the same letter do not differ significantly from each other (level of significance given in Table)
[2] Within this column, values followed by the same letter do not differ significantly from each other (level of significance given in Table)

Appendix 4 (contd.): Results of *Eucalyptus smithii* individual site REML analyses of variance for height at five years after planting

Site		Clone 1	6	71	74	PBZ mean[2]
The Peak	PBZ0	11.5	10.8	6.4	10.1	9.7a
	PBZ1	7.8	12.2	8.7	7.3	9.0a
	Clone mean[1]	9.6	11.5	7.5	8.7	-
F-pr		PBZ = 0.633		Clone = 0.131		PBZ.Clone = 0.209
SE		0.99		1.23		1.74
Site		Clone 1	6	71	74	PBZ mean[2]
Willowmere	PBZ0	10.5	12.4	12.2	11.3	11.6a
	PBZ1	6.0	6.1	5.7	6.8	6.1b
	Clone mean[1]	8.2	9.3	9.0	9	-
F-pr		PBZ = < 0.001		Clone = 0.651		PBZ.Clone = 0.500
SE		0.42		0.60		0.85
Site		Clone 1	6	71	74	PBZ mean[2]
Wyntoun	PBZ0	14.5	13.5	11.5	15.1	13.6a
	PBZ1	14.0	3.5	11.3	13.0	10.5a
	Clone mean[1]	14.3a	8.5b	11.4ab	14.1a	
F-pr		PBZ = 0.005		Clone = 0.002		PBZ.Clone = 0.007
SE		0.72		1.02		1.45

PBZ0 = No PBZ applied (control)
PBZ1 = PBZ applied to soil in March/ April 2006
F-pr = *F*-probability value
SE = Standard error of the predicted mean
[1] Within this row, values followed by the same letter do not differ significantly each other (level of significance given in Table)
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Appendix 5: Results of *Eucalyptus smithii* individual site REML analyses of variance for fifth year umbel crop score (floral buds initiated in 2008 and scored in 2009)

			Clone			
Site		1	6	71	74	PBZ mean[2]
Blair Athol	PBZ0	1.250	1.250	0.750	1.500	1.188a
	PBZ1	2.500	3.500	4.000	4.000	3.500b
	Clone mean[1]	1.875	2.375	2.375	2.750	-
F-pr		PBZ = < 0.001		Clone = 0.257		PBZ.Clone = 0.157
SE		0.212		0.300		0.424
			Clone			
Site		1	6	71	74	PBZ mean[2]
Gilboa	PBZ0	1.500	0.750	2.000	0.500	1.188a
	PBZ1	1.250	1.750	2.000	3.000	2.000a
	Clone mean[1]	1.375	1.250	2.000	1.750	-
F-pr		PBZ = 0.059		Clone = 0.558		PBZ.Clone = 0.101
SE		0.290		0.410		0.580
			Clone			
Site		1	6	71	74	PBZ mean[2]
In de Diepte	PBZ0	2.000	1.938	1.000	0.312	1.313a
	PBZ1	3.250	3.500	3.250	3.750	3.437b
	Clone mean[1]	2.625	2.719	2.125	2.031	-
F-pr		PBZ = 0.026		Clone = 0.741		PBZ.Clone = 0.423
SE		0.430		0.522		0.738
			Clone			
Site		1	6	71	74	PBZ mean[2]
Netherby 1	PBZ0	1.500	1.482	1.268	1.500	1.437a
	PBZ1	1.482	2.518	1.250	3.250	2.125a
	Clone mean[1]	1.491	2.000	1.259	2.375	-
F-pr		PBZ = 0.229		Clone = 0.356		PBZ.Clone = 0.488
SE		0.355		0.480		0.679
			Clone			
Site		1	6	71	74	PBZ mean[2]
Netherby 2	PBZ0	1.250	0.500	0.500	1.750	1.000a
	PBZ1	3.250	3.250	2.250	2.500	2.812b
	Clone mean[1]	2.250	1.875	1.375	2.125	-
F-pr		PBZ = < 0.001		Clone = 0.515		PBZ.Clone = 0.460
SE		0.309		0.438		0.619
			Clone			
Site		1	6	71	74	PBZ mean[2]
Netherby 3	PBZ0	2.500	2.500	1.500	1.750	2.063a
	PBZ1	3.500	3.000	3.250	3.000	3.187b
	Clone mean[1]	3.000	2.750	2.375	2.375	-
F-pr		PBZ = 0.008		Clone = 0.615		PBZ.Clone = 0.726
SE		0.277		0.392		0.554

PBZ0 = No PBZ applied (control)
PBZ1 = PBZ applied to soil in March/ April 2006
F-pr = *F*-probability value
SE = Standard error of predicted mean
[1] Within this row, values followed by the same letter do not differ significantly from each other (level of significance given in Table)
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Appendix 5 (contd.): Results of *Eucalyptus smithii* individual site REML analyses of variance for fifth year umbel crop score (floral buds initiated in 2008 and scored in 2009)

Site		Clone 1	6	71	74	PBZ mean[2]
The Peak	PBZ0	0.750	0.000	0.750	0.250	0.437a
	PBZ1	3.378	2.750	3.500	2.329	2.989b
	Clone mean[1]	2.064	1.375	2.125	1.290	-
F-pr		PBZ = 0.001		Clone = 0.141		PBZ.Clone = 0.847
SE		0.282		0.324		0.458
Site		Clone 1	6	71	74	PBZ mean[2]
Willowmere	PBZ0	0.750	2.500	1.500	2.000	1.688a
	PBZ1	2.000	3.750	3.000	3.750	3.125b
	Clone mean[1]	1.375b	3.125a	2.250ab	2.875a	-
F-pr		PBZ = < 0.001		Clone = 0.002		PBZ.Clone = 0.928
SE		0.218		0.308		0.436
Site		Clone 1	6	71	74	PBZ mean[2]
Wyntoun	PBZ0	1.250	1.500	0.250	0.500	0.875a
	PBZ1	3.343	2.750	2.500	2.407	2.750b
	Clone mean[1]	2.297	2.125	1.375	1.453	-
F-pr		PBZ = 0.007		Clone = 0.207		PBZ.Clone = 0.759
SE		0.318		0.382		0.540

PBZ0 = No PBZ applied (control)
PBZ1 = PBZ applied to soil in March/ April 2006
F-pr = *F*-probability value
SE = Standard error of predicted mean
[1] Within this row, values followed by the same letter do not differ significantly from each other (level of significance given in Table)
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Appendix 6: Results of *Eucalyptus smithii* individual site REML analyses of variance for sixth year umbel crop score (floral buds initiated in 2009 and scored in 2010)

Site		Clone 1	6	71	74	PBZ mean[2]
Blair Athol	PBZ0	1.750	1.481	0.769	2.000	1.500a
	PBZ1	2.347	3.500	2.750	2.903	2.875b
	Clone mean[1]	2.049	2.409	1.760	2.451	-
F-pr		PBZ = 0.029		Clone = 0.508		PBZ.Clone = 0.433
SE		0.310		0.398		0.563
Site		Clone 1	6	71	74	PBZ mean[2]
Gilboa	PBZ0	2.000	2.200	2.050	1.250	1.875a
	PBZ1	2.252	1.998	2.750	3.500	2.625a
	Clone mean[1]	2.126	2.099	2.400	2.375	-
F-pr		PBZ = 0.233		Clone = 0.874		PBZ.Clone = 0.126
SE		0.391		0.432		0.611
Site		Clone 1	6	71	74	PBZ mean[2]
In de Diepte	PBZ0	2.000	1.477	2.500	2.273	2.063a
	PBZ1	2.750	1.477	2.023	2.500	2.187a
	Clone mean[1]	2.375	1.477	2.261	2.386	-
F-pr		PBZ = 0.862		Clone = 0.513		PBZ.Clone = 0.831
SE		0.481		0.545		0.770
Site		Clone 1	6	71	74	PBZ mean[2]
Netherby 1	PBZ0	2.250	1.750	1.250	2.750	2.000a
	PBZ1	1.843	1.157	2.000	3.000	2.000a
	Clone mean[1]	2.046	1.454	1.625	2.875	-
F-pr		PBZ = 1.000		Clone = 0.191		PBZ.Clone = 0.766
SE		0.409		0.5169		0.7309
Site		Clone 1	6	71	74	PBZ mean[2]
Netherby 2	PBZ0	2.750	1.750	2.250	3.250	2.500a
	PBZ1	3.750	3.500	2.250	3.750	3.312b
	Clone mean[1]	3.250ab	2.625bc	2.250c	3.500a	-
F-pr		PBZ = 0.010		Clone = 0.022		PBZ.Clone = 0.205
SE		0.206		0.291		0.411
Site		Clone 1	6	71	74	PBZ mean[2]
Netherby 3	PBZ0	2.250	1.443	2.057	2.000	1.938a
	PBZ1	3.834	2.500	2.500	2.916	2.938a
	Clone mean[1]	3.042	1.972	2.278	2.458	-
F-pr		PBZ = 0.123		Clone = 0.332		PBZ.Clone = 0.803
SE		0.349		0.431		0.6098

PBZ0 = No PBZ applied (control)
PBZ1 = PBZ applied to soil in March/ April 2006
F-pr = *F*-probability value
SE = Standard error of predicted mean
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Appendix 6 (contd.): Results of *Eucalyptus smithii* individual site REML analyses of variance for sixth year umbel crop score (floral buds initiated in 2009 and scored in 2010)

Site		Clone 1	6	71	74	PBZ mean[2]
The Peak	PBZ0	0.500	0.250	0.000	0.750	0.375a
	PBZ1	2.750	2.250	2.750	1.500	2.312b
	Clone mean[1]	1.625	1.250	1.375	1.125	-
F-pr		PBZ = < 0.001		Clone = 0.814		PBZ.Clone = 0.313
SE		0.269		0.380		0.538
Site		Clone 1	6	71	74	PBZ mean[2]
Willowmere	PBZ0	1.500	3.250	2.250	2.750	2.438a
	PBZ1	3.000	3.250	3.250	3.500	3.250b
	Clone mean[1]	2.250	3.250	2.750	3.125	-
F-pr		PBZ = 0.009		Clone = 0.087		PBZ.Clone = 0.334
SE		0.203		0.286		0.405
Site		Clone 1	6	71	74	PBZ mean[2]
Wyntoun	PBZ0	2.750	1.500	1.500	1.000	1.687a
	PBZ1	2.592	2.750	3.000	2.658	2.750a
	Clone mean[1]	2.671	2.125	2.250	1.829	-
F-pr		PBZ = 0.106		Clone = 0.523		PBZ.Clone = 0.466
SE		0.386		0.461		0.652

PBZ0 = No PBZ applied (control)
PBZ1 = PBZ applied to soil in March/ April 2006
F-pr = *F*-probability value
SE = Standard error of predicted mean
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