Effect of winter chilling and paclobutrazol on floral bud production in *Eucalyptus nitens*

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Flower bud production of *Eucalyptus nitens* is comparatively abundant at specific high altitude sites in the South African summer rainfall region. This suggests that cumulative cold may be implicated in the floral induction process. Therefore, three chill models were used to investigate whether winter temperature data can be related to *E. nitens* flower bud production at sites differing in chill accumulation. Amount of accumulated winter chill, in conjunction with paclobutrazol treatment, was able to explain between 66 and 72% of the variation in *E. nitens* flower bud production. Although the potential chilling requirement of *E. nitens* can be calculated with similar accuracy by each of the chill models tested, the Dynamic Model performed best under the particular range of experimental conditions. At low to moderate levels of winter chill (43 to 81 Chilling Portions (CPs) of the Dynamic Model), paclobutrazol application hastened first flowering and increased the percentage reproductive trees by between 5–27% in the case of seedlings, and between 2–62% in the case of grafts. At high levels of winter chill (>87 CPs), paclobutrazol had a negligible effect on the percentage reproductive trees in the case of either seedlings or grafts. At four and five years after planting, very high levels of accumulated winter chill (96 CPs) stimulated a high percentage of seedlings (25–50%) and grafts (55–64%) to produce flower buds. Considerable variation in precocity and chilling requirement (for floral induction) is evident within the South African *E. nitens* breeding population. A better accuracy in relating *E. nitens* floral bud production to amount of prior winter chilling could be achieved by further experimentation involving fewer genotypes and a wider range of chilling conditions.

**Introduction**

*Eucalyptus nitens* (Deane and Maiden) is an important, fast growing eucalypt planted commercially in several southern hemisphere countries (Tibbits et al. 1997). The species is well adapted to the range of environmental conditions encountered at cold, high altitude sites in the summer rainfall region of South Africa (Swain and Gardner 2003), and is, therefore, currently the most favoured eucalypt for commercial pulpwood production in such areas (Clarke 2000, Little et al. 2002). The erratic and sparse flowering tendency of *E. nitens* has hampered genetic improvement and commercial seed production efforts of this species (Reid et al. 1995). In South Africa, seedling and grafted trees rarely flower before the age of ten years (Eldridge et al. 1993, Swain and Chiappero 1998), and personal experience has shown that, even then, flowering only occurs at select high altitude sites following exceptionally cold winters. In other southern hemisphere countries (Australia and Chile), *E. nitens* flowers far more consistently and prolifically (Moncur and Hasan 1994, Gardner 2001).

During the past decade, considerable progress has been made towards identifying the triggers controlling flowering in *E. nitens* and developing a management system for enhancing flower and seed production in the species (Moncur and Boland 2000). A range of environmental conditions and cultural techniques have been implicated in the induction of flowering in eucalypts (Moncur and Boland 2000), yet, until now, cold and the triazole-type plant growth retardant paclobutrazol ((2RS,3RS)-1-(4-chlorophenyl)-4,4-dimethyl-2,1,2,4-triazol-1-yl-pentan-3-ol) appear to be the most effective treatments in temperate species such as *E. nitens* and *E. globulus* (Moncur and Boland 2000, Williams et al. 2003). Other possible stimuli for floral induction, such as photoperiod, have been found to play no role in the induction of flowering, neither in the temperate ornamental eucalypt *E. lansdowneana* nor in the commercial timber species *E. nitens* (Moncur 1992, Moncur and Hasan 1994). Pryor (1976) reported no indication of photoperiodic regulation of flowering in *E. tereticornis*, – a species with
the highest latitudinal range among eucalypts (8°S to 38°S latitude (Boland et al. 1992)). Drought stress is known to stimulate floral induction in a range of evergreen tree crops including lychee (*Litchi sinensis* Sonn.) (Menzel 1983) and *Citrus* spp. (Krajewski and Rabe 1995), and in certain conifers and broadleaf forestry tree species (Philipson 1990). However, drought stress has not been conclusively linked to floral induction in temperate eucalypt species, including *E. nitens* (Moncur 1992, Moncur and Boland 2000).

Although a period of cold appears obligatory for floral induction in *E. nitens*, paclobutrazol treatment, in conjunction with sufficient winter cold, can significantly enhance floral bud production and subsequent seed crop size (Moncur and Hasan 1994, Williams et al. 1999). For this reason, paclobutrazol treatment has become an extremely popular tool in *E. nitens* breeding and seed production programmes worldwide. In South Africa, due to apparently unfavourable climatic conditions for flowering, tree breeders and commercial seed producers are currently almost completely reliant on paclobutrazol application to obtain a significant amount of flower and seed production in *E. nitens* (Swain and Chiappero 1998, Swain and Gardner 2002). This practice, however, is far from the ideal, as, apart from the high cost of the chemical, paclobutrazol persists actively in the soil for several years, the latter feature attracting increasing pressure from environmentalists to discontinue such practice (Reid et al. 1995, Moncur 1998). It is thus highly desirable to investigate more economical and environmentally sustainable ways of stimulating seed production in *E. nitens*.

Conditions favouring floral induction in *E. nitens* have yet to be intensively investigated in South Africa. In SE Australia (Canberra, ACT) floral initiation in *E. nitens* occurs in late winter (August to early September) (Moncur et al. 1994a). Although winter cold has been identified as an important stimulus for floral induction, little is known about the timing of inductive cold events for flowering in *E. nitens*. It is unclear as to whether cold acts as a floral stimulus in one single inductive event or cumulatively over a series of events (Moncur and Hasan 1994, Meilan 1997). Observations of flowering patterns in *E. nitens* in South Africa tend to indicate that the latter is more likely. At certain high altitude, exposed sites in the summer rainfall forestry belt, trees of *E. nitens* flower more consistently and profusely than at nearby sites of similar annual total precipitation, altitude, aspect, soil type and soil depth. Both ‘good flowering’ sites and ‘poor flowering’ sites occur above 1 400 metres above mean sea level (amsl) and are not drought-prone due to high mean annual precipitation (MAP) (>950mm) and deep soils (>1.0m). Our experience indicates that the main environmental difference between the two site types pertains to the degree of topographical relief and associated winter day/night temperature amplitudes. The ‘good flowering’ sites are always in exposed positions on crests or southwest to east-facing up-slopes where air drainage is good and winter day/night temperature amplitudes are low, whereas the ‘poor flowering’ sites occur on flatter terrain where winter day/night temperature amplitudes are relatively high. This phenomenon suggests that uniform cool conditions in winter are more promotive of flowering in *E. nitens* than extremely varying temperature conditions. Hence, a cumulative cold requirement may be implicated in floral induction in *E. nitens*.

In South Africa, currently *E. nitens* commercial seed orchards (and indeed the bulk of the plantations of this species) are located in ‘warm temperate’ areas of the summer rainfall region, between latitudes 25.5 and 31°S, where large day/night temperature amplitudes regularly occur during the winter months (May to September) (Schulze 1997, Swain and Gardner 2003). Winter daytime temperatures commonly exceed 20°C whilst night-time temperatures descend below –5°C, resulting in day/night amplitudes exceeding 25°C (Richardson and McMahon 1992). Such extreme temperature conditions in winter are antagonistic towards chilling accumulation and bud endo-
dormancy release in temperate fruit crops (Couvillon and Erez 1985). In contrast, ‘uniformly cool’ conditions associated with cloudy and foggy weather in winter enhance chilling accumulation (Erez 2000).

Therefore, chill models used to quantify the effect of such winter chilling on bud dormancy release in temperate fruit crops (George and Erez 2000, Halgren et al. 2001) could provide a useful tool to estimate floral bud production in *E. nitens*. In order to characterise ‘effective’ cold for floral induction in *E. nitens*, this study evaluated three such chill models to determine whether accumulated winter chilling was related to *E. nitens* floral bud production. Information arising from such an investigation would not only add to the limited knowledge of optimal temperatures for floral induction in *E. nitens*, but may also assist in the siting of *E. nitens* seed orchards.

**Material and Methods**

A series of four field trials was established across a range of high altitude sites in the summer rainfall forestry belt during 1996. The main aim of the series was to subject *E. nitens* trees to a range of winter cold conditions and to investigate whether flower bud production was related to amount of winter chilling received by the trees during the previous winter. In the process, the suitability of each of the three different chill models could be evaluated. The effects of ‘propagule type’ (seedling or graft), ‘*E. nitens* family’ and ‘paclobutrazol application’ on tree growth and floral bud production were also determined, as these were treatments relevant to then-current *E. nitens* seed orchard practices in South Africa (Swain et al. 1998, Jones 2002).

**Plant material**

Propagules used to establish trees for the experiments were derived from three *E. nitens* provenances, the origins of which are briefly described in Table 1. The seedling trees were produced as follows: In October 1995, four separate seedlots were sown and raised in a nursery. The seedlots were four selected families from the three provenances listed in Table 1, each seedlot consisting of seed from a different, single mother tree in a natural stand in New South Wales, Australia.

The grafted trees were produced as follows. In August 1995, scions were cut from four different three-year-old
A suspension of Cultar® (formulation 250g.l⁻¹ paclobutrazol; ICI Agrochemicals) was applied as a soil drench during early April 1998 at a rate of 0.25g a.i. per cm b.s.c.. Method and timing were based on treatments reportedly successful in inducing flowering in *E. nitens* and *E. globulus* (Griffin et al. 1992, Moncur et al. 1994b). B.s.c. represented the circumference at the narrowest point along the stem between graft union (in the case of grafts) or root collar (in the case of seedlings) and first primary lateral (branch). The paclobutrazol dose for each tree was dispersed in 2 litres of water and applied evenly to the soil surface in a 1.0m radius around the base of each tree.

### Environmental conditions

To test the effect of winter chilling on floral bud production in *E. nitens*, a gradient in chilling was created by selecting trial sites at four separate localities based on altitude and latitude (Table 2). However, sites varied only slightly in daylength, with a maximum of 27min difference in daylength between sites at the shortest day (21st June) and a maximum of 5min difference in daylength between sites at the longest day (30th September) of the cold accumulation period (Schulze 1997). Mean annual temperature (MAT) of the warmest site, Gowan Brae (15.2°C), was below the upper threshold for optimum commercial planting of *E. nitens* in South Africa (16.0°C), whereas that for the coldest site, Tentkop (12.6°C), was well below the lower threshold (14.0°C) (Swain and Gardner 2003) (Table 2). The other two sites were Mossbank (MAT 14.0°C) and Blyfstaanhoogte (MAT 13.2°C). Although the altitude of Blyfstaanhoogte and Tentkop were similar (1995 and 1920 metres amsl, respectively), the latter was a colder site as it was located further south (Table 2). All sites were similar in aspect (SE- to SW-facing) and exposure (mid-slope), and prone to light to moderate frosts in winter. Although the sites were all located in the summer rainfall area where rainfall distribution has a distinct summer maximum, drought conditions were highly unlikely to occur at these sites as MAP was high (>950mm), summer mists frequent and soils deep (>1.0m). Therefore, daylength as well as drought as floral induction stimuli, as reported for a number of woody angiosperms (Meilan 1997) including certain eucalypts (Moncur and Boland 2000), could be excluded.

### Paclobutrazol treatment

A suspension of Cultar® (formulation 250g.l⁻¹ paclobutrazol; ICI Agrochemicals) was applied as a soil drench during early April 1998 at a rate of 0.25g a.i. per cm b.s.c.. Method and timing were based on treatments reportedly successful in inducing flowering in *E. nitens* and *E. globulus* (Griffin et al. 1992, Moncur et al. 1994b). B.s.c. represented the circumference at the narrowest point along the stem between graft union (in the case of grafts) or root collar (in the case of seedlings) and first primary lateral (branch). The paclobutrazol dose for each tree was dispersed in 2 litres of water and applied evenly to the soil surface in a 1.0m radius around the base of each tree.

### Trial layout

At each trial site a split plot design experiment was laid out. Each experiment consisted of two whole-plots (0.0g and 0.25g paclobutrazol/cm b.s.c.) which were further divided into 24 sub-plots. The sub-plots each consisted of five trees, and were randomly assigned to different combinations of ‘propagule’ x ‘family’. Trees were spaced 3.0m x 3.0m apart. The necessary buffer rows were incorporated around each trial. Details of the allocation of treatments to sub-plots are given in Table 3.

### Data collection

#### Tree growth measurements

Tree height was measured once annually in April from 1998 to 2001.

#### Floral assessments

In South Africa, Jones and van Staden (2001) found that at a cool, medium altitude (MAT 16.7°C, 1 100 a.m.s.l) site in the KZN Midlands, newly emerged inflorescences first become visible to the naked eye in early November, while anthesis commences only four months later in March (Jones and van Staden 2001). Personal experience has shown that in late flowering genotypes at very cold sites, newly emerged umbels may first become visible only in early January. However, in the months immediately following this emergence, development and swelling of the individual flower buds in the umbels progresses rapidly as a result of the warm summer conditions (Moncur et al. 1994a), even though anthesis eventually only commences in October. Therefore, the presence of umbels was evaluated during April each year between 1998 and 2001. At this time, the majority of involucral bracts were shed, leaving the individual buds in the umbels exposed. Individual tree umbel crop size scores were allocated as follows: 0 = no umbels; 1 = very light crop, 25% or less of the secondary laterals bearing one or more umbels (‘secondary laterals were defined as branches

### Table 1: Australian origins of the *Eucalyptus nitens* provenances and families in the field trials

| Provenance      | Family | State               | Latitude | Longitude | Altitude (metres amsl*) |
|-----------------|--------|---------------------|----------|-----------|-------------------------|
| Barren Mountain | 32091  | New South Wales     | 30°25'   | 152°28'   | 1 505                   |
| Barren Mountain | 32097  | New South Wales     | 30°24'   | 152°29'   | 1 535                   |
| Barrington Tops | 34838  | New South Wales     | 31°55'   | 151°30'   | 1 450                   |
| Tallaganda      | 37255  | New South Wales     | 35°54'   | 149°30'   | 1 290                   |

*amsl, above mean sea level
| Trial name       | Gowan Brae | Mossbank | Blyfstaanhoogte | Tentkop     |
|-----------------|------------|----------|-----------------|-------------|
| Planting date   | 21/02/1996 | 07/02/1996 | 26/04/1996      | 06/03/1996  |
| Latitude        | 29° 38'22"S | 29°49'08"S | 25°10'03"S      | 30°48'30"S  |
| Longitude       | 30°08'52"E  | 29°42'20"E | 30°36'40"E      | 28°15'35"E  |
| Altitude (metres amsl*) | 1 465 | 1 680 | 1 995 | 1 920 |
| Mean annual precipitation (mm)** | 990 | 1 105 | 1 995 | 1 920 |
| Mean monthly max (hottest month) (°C)** | 24.7 | 22.8 | 22.4 | 23.6 |
| Mean monthly min (coldest month) (°C)** | 3.6 | 1.9 | 3.6 | 1.3 |
| Mean annual temperature (°C)# | 15.2 | 14.0 | 13.2 | 12.8 |
| Soils           | Dolerite/ Shale | Dolerite/ Shale | Dolerite/ Shale | Dolerite/ Shale |
| Taxonomy***     | Humic Ferralsol | Humic Ferralsol | Humic Ferralsol | Humic Ferralsol |
| Parent material | Dolerite/ shale | Dolerite/ shale | Dolerite/ shale | Dolerite/ shale |
| Depth (m)       | >1.2 | 1.2 | 1.0 | >1.2 |

* amsl, above mean sea level  
** long-term mean (Schulze 1997)  
*** (FAO-UNESCO 1974)  
# six-year mean for the period 1996 to 2001

Table 2: Site conditions for the *Eucalyptus nitens* field trials

| Trial name       | Gowan Brae | Mossbank | Blyfstaanhoogte | Tentkop     |
|-----------------|------------|----------|-----------------|-------------|
| Planting date   | 21/02/1996 | 07/02/1996 | 26/04/1996      | 06/03/1996  |
| Latitude        | 29° 38'22"S | 29°49'08"S | 25°10'03"S      | 30°48'30"S  |
| Longitude       | 30°08'52"E  | 29°42'20"E | 30°36'40"E      | 28°15'35"E  |
| Altitude (metres amsl*) | 1 465 | 1 680 | 1 995 | 1 920 |
| Mean annual precipitation (mm)** | 990 | 1 105 | 1 995 | 1 920 |
| Mean monthly max (hottest month) (°C)** | 24.7 | 22.8 | 22.4 | 23.6 |
| Mean monthly min (coldest month) (°C)** | 3.6 | 1.9 | 3.6 | 1.3 |
| Mean annual temperature (°C)# | 15.2 | 14.0 | 13.2 | 12.8 |
| Soils           | Dolerite/ Shale | Dolerite/ Shale | Dolerite/ Shale | Dolerite/ Shale |
| Taxonomy***     | Humic Ferralsol | Humic Ferralsol | Humic Ferralsol | Humic Ferralsol |
| Parent material | Dolerite/ shale | Dolerite/ shale | Dolerite/ shale | Dolerite/ shale |
| Depth (m)       | >1.2 | 1.2 | 1.0 | >1.2 |

* amsl, above mean sea level  
** long-term mean (Schulze 1997)  
*** (FAO-UNESCO 1974)  
# six-year mean for the period 1996 to 2001

originating from the primary stem/s); 2 = light crop, >25%–50% of secondary laterals bearing one or more umbels; 3 = moderate crop, >50%–75% of secondary laterals bearing one or more umbels; 4 = heavy crop, >75%–100% of secondary laterals bearing one or more umbels.

Because it was logistically impossible to assess the crop size of each individual tree in all trials at five years after planting – due to the height of the trees (Table 7) – only two out of three replicates were assessed for this variate ('CROP_2001') (refer to Table 4 for a description of the latter variate).

**Temperature measurements**

Hourly temperature measurements were recorded in the trials during the winter months (April to September) each year. At altitudes above 1300m in the summer rainfall area, winter chill units begin accumulating in May, but at very cold sites in particularly cold years, chill unit accumulation can begin as early as April (Linsley-Noakes 1995, Schulze 1997).

**Calculation of chill units**

In general, the three chill models tested, namely the Utah Model (Richardson et al. 1974, Seeley 1996), Dynamic Model (Fishman et al. 1987a and b) and Daily Positive Utah Chill Unit Model (Linsley-Noakes et al. 1994), assign premium chilling values to temperatures within a relatively narrow range, i.e. between about 1.5 and 10°C. However, they differ regarding the assignment of chilling values to temperatures within the chill-effective range.

The Utah Model assigns one chill unit (Utah Chill Unit (CU)) for every hour when temperatures are between 2.5 and 9.1°C, half a unit for every hour between 1.5 and 2.4 or between 9.2 and12.4°C, and zero units for every hour where temperatures are below 1.4 or between 12.5 and 15.9°C. Negative chill units are assigned to temperatures 16.0°C (Seeley 1996). The model has been used to accurately predict budbreak of temperate fruit crops in temperate zones, but is known to give inaccurate winter chilling estimations in areas experiencing mild winters (Linsley-Noakes and Allan 1994, Allan and Burnett 1995).

In the Dynamic Model, chilling is accumulated in certain portions. Once a distinct amount of chilling has occurred, this portion is ‘fixed’ and counts towards the chilling requirement, even when warm temperatures prevail thereafter. Therefore, chilling accumulation depends on the timing of exposure to temperatures in a cycle. This complex model not only takes into account the negating effect of high day temperatures, but also recognises the positive effect of moderate temperatures in the chilling cycle. Although one Chilling Portion (CP) is accumulated fastest at temperatures between 6.0 and 8.0°C, moderate day temperatures (13.0 to 15.0°C) enhance the chilling effect of temperatures in the optimum range (Fishman et al. 1987a and b, Erez et al. 1990). The model assigns negative chill units to temperatures 20°C, although the degree of chilling negation decreases with the cycle length. The model has proven to be equally accurate for areas with mild as well as cold winter climates (Linsley-Noakes and Allan 1994, Allan et al. 1995).

The Daily Positive Utah Chill Unit Model, a modification of the Utah Model, uses features of the Dynamic Model to modify the Utah Model to allow more accurate prediction of winter chilling accumulation in areas with mild winters (Linsley-Noakes et al. 1994). Although the time to form one CP, under favourable temperatures, varies from about 26 to 40h, Linsley-Noakes et al. (1994) proposed that there would be less inaccuracy in assuming that chilling negation by high temperatures is limited to a 24h period. Therefore, the carry-over of negative chill units resulting from ‘detrimentally’ high temperatures from one day to the next is deleted.

**Statistical analyses**

Statistical analyses were performed to investigate the effect of site, propagule, family and paclobutrazol application on tree height, percentage of trees with umbels, and umbel score per reproductive tree at five years after planting.

Variance components were estimated using the mixed model analysis of variance (Steel and Torrie 1981) utilising
restricted maximum likelihood analyses (Patterson and Thompson 1971, Lane and Payne 1996) in Genstat® for Windows, Release 4.2. Prior to analysis, angular and log$_10$(x + 1) transformations of the raw data were undertaken for the percentage of trees with umbels (variate ‘TREES_2001’) and umbel score per reproductive tree (variate ‘CROP_2001’), respectively, to normalise the residuals and homogeneity of the error variances (Gomez and Gomez 1984, Steel and Torrie 1981). For the across-site analyses, trees were treated as nested within plots, and plots nested within sites. F-tests, using F-values calculated from computed Wald-statistics, were used to determine which factors and interactions were significant (Lane and Payne 1996).

Multiple regression analysis in Genstat® for Windows™ (McConway et al. 1999) was used to determine the relationship between accumulated winter chilling (‘CHILL’) and the percentage of trees with umbels (TREES_2001), and between CHILL and umbel score per tree (CROP_2001). Residual plots were performed in Genstat® for Windows™ according to the procedures described by McConway et al. (1999), whereby the necessary checks were made to ensure that the assumptions for a valid regression analysis were not violated. A description of all response and explanatory variables used in the regression analyses is presented in Table 4. The variate ‘CHILL’ consisted of chill units for the 2000 winter (winter preceding 2001 umbel crop assessment) calculated using different chill model x chill period combinations (Table 4). Separate multiple linear regressions were carried out for seedlings and grafts because it was anticipated that ontogenetical variation may confound the floral productivity results of the former propagule type. Various regression models were evaluated, but in all cases the linear regression model provided the best fit between explanatory and response variates.

**Results**

**Winter chilling accumulation**

The highest numbers of chill units in all three years (1998 to 2000) were accumulated during 2000 (floral response year 2001) at all four sites (Table 5). Blyfstaanhoogte

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**Table 3:** Details of the allocation of sub-plots to treatments in the four field trials

| Family | Seedling | Propagule | Graft | Seedling | Propagule | Graft |
|--------|----------|-----------|-------|----------|-----------|-------|
| 32091  | 3 (15)*  | 3 (15)*   | 3 (15)*| 3 (15)   | 3 (15)    | 3 (15) |
| 32097  | 3 (15)   | 3 (15)    | 3 (15)| 3 (15)   | 3 (15)    | 3 (15) |
| 34838  | 3 (15)   | 3 (15)    | 3 (15)| 3 (15)   | 3 (15)    | 3 (15) |
| 37255  | 3 (15)   | 3 (15)    | 3 (15)| 3 (15)   | 3 (15)    | 3 (15) |
| Total  | 12 (60)  | 12 (60)   | 12 (60)| 12 (60)  | 12 (60)   | 12 (60) |

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

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

| Variate assessed | Abbreviation used in text | Description of variate |
|------------------|---------------------------|------------------------|
| Umbel crop load  | CROP_2001                 | Umbel crop score per tree transformed to natural logarithmic value |
| % trees with umbels | TREES_2001               | Number of trees with one or more umbels in each plot, expressed as a percentage of the total number of live trees in the plot |
| Explanatory variable (CHILL) (chill model x chill period combination) | | |
| Dynamic Model | CP-1                      | Number of CPs* calculated for the period 01 Apr–30 Sep 2000 |
| Dynamic Model | CP-2                      | Number of CPs calculated for the period 01 May–30 Sep 2000 |
| Utah Chill Model | CU-1                    | Number of CUs¶ calculated for the period 01 Apr–30 Sep 2000 |
| Utah Chill Model | CU-2                    | Number of CUs calculated for the period 01 May–30 Sep 2000 |
| Daily Positive Utah Chill Unit Model | DPCU-1                | Number of DPCUs# calculated for the period 01 Apr–30 Sep 2000 |
| Daily Positive Utah Chill Unit Model | DPCU-2                | Number of DPCUs calculated for the period 01 May–30 Sep 2000 |
| Explanatory variable (plant material and paclobutrazol treatments) | | |
| Propagule type | PROPAGULE                 | 1 = graft; 2 = seedling |
| Paclobutrazol application | PBZ                  | 0 = 0.00 g paclobutrazol per cm basal stem circumference (b.s.c.) (control); 1 = 0.25 g paclobutrazol per cm b.s.c. |

*CP, Chilling Portion, the chill unit measured by the Dynamic Model (Erez et al. 1990).
¶CU, Utah Chill Unit: the chill unit measured by the Utah Chill Unit Model (Seeley 1996).
#DPCU, Daily Positive Chill Unit: the chill unit measured by the Daily Positive Utah Chill Unit Model (Linsley-Noakes 1995).
accumulated the highest number of chill units of all sites (100.9 CPs/2 032 CUs/2 108 DPCUs), recorded during the period April to September (CP-1; CU-1; DPCU-1). Gowan Brae, Mossbank and Tentkop accumulated the lowest number of chill units during 1999 (floral response year 2000). In this year, Gowan Brae accumulated the lowest number of chill units of all sites and years (42.8 CPs/621 CUs/1 005 DPCUs) (April to September), demonstrating that it is not only the warmest site chosen based on MAT, but also on chill units. On the other hand, the coldest site (Tentkop), with respect to MAT, did not accumulate the highest number of chill units during 1999 and 2000 (floral response years 2000 and 2001), but the second coldest site (Blyfstaanhoogte) did. In all years and at all sites, both Dynamic Model and Daily Positive Utah Chill Unit Model began accumulating chill units in April whereas the Utah Model did not. At Gowan Brae and Mossbank, only the Utah Model showed chill unit negation during April 1998 and 1999 (floral response years 2000 and 2001), whilst at Blyfstaanhoogte the Utah Model only showed chill unit negation during April in 1998 (floral response year 1999).

### Table 5: Chill units accumulated during the years 1998, 1999 and 2000 at the four trial sites

| Trial                | Chilling Portions (Dynamic Model) | Chill units (Utah Chill Model) | Daily Positive Utah Chill Units (DPCU Model) |
|----------------------|----------------------------------|--------------------------------|---------------------------------------------|
|                      | CP-1*                             | CP-1**                        | DPCU-1*                                    |
|                      | CP-2*                             | CU-1*                          | DPCU-2*                                    |
|                      |                                  | CU-2*                          |                                             |
| Gowan Brae 1999      | 48.6                              | 46.6                           | 750                                         |
|                      |                                   |                                | 878                                         |
|                      |                                   |                                | 1 096                                       |
| 2000                 | 42.8                              | 40.8                           | 621                                         |
|                      |                                   |                                | 732                                         |
|                      | 59.3                              | 51.7                           | 1 144                                       |
|                      |                                   |                                | 1 073                                       |
| 2001                 | 50.2                              | 46.4                           | 838                                         |
|                      |                                   |                                | 894                                         |
|                      | Mean                              | 50.2                           | 1 108                                       |
|                      |                                   |                                | 1 248                                       |
| Mossbank 1999        | 60.5                              | 58.5                           | 1 112                                       |
|                      |                                   |                                | 1 108                                       |
| 2000                 | 55.3                              | 51.3                           | 918                                         |
|                      |                                   |                                | 934                                         |
|                      | Mean                              | 56.0                           | 1 104                                       |
|                      |                                   |                                | 1 248                                       |
| Blyfstaanhoogte 1999 | 72.0                              | 65.0                           | 1 081                                       |
|                      |                                   |                                | 1 119                                       |
| 2000                 | 96.5                              | 88.4                           | 1 694                                       |
|                      |                                   |                                | 1 601                                       |
|                      | Mean                              | 91.8                           | 1 592                                       |
|                      |                                   |                                | 1 711                                       |
| Tentkop 1999         | 84.5                              | 79.5                           | 1 356                                       |
|                      |                                   |                                | 1 314                                       |
| 2000                 | 81.5                              | 71.6                           | 1 299                                       |
|                      |                                   |                                | 1 193                                       |
|                      | Mean                              | 83.5                           | 1 301                                       |
|                      |                                   |                                | 1 358                                       |
|                      |                                   |                                | 1 385                                       |
|                      |                                   |                                | 1 228                                       |

Effect of site, propagule type, family and paclobutrazol application on tree height

Statistical evaluation revealed that the factors SITE, PBZ, PROPAGULE and the SITE x PBZ interaction exerted a highly significant (P < 0.01) effect on tree height at five years after establishment (Table 6).

In the interaction of SITE x PBZ, tree height diminished as MAT decreased (Table 7). At the warmest site, Gowan Brae, trees grew tallest, whether paclobutrazol-treated or non-treated. In untreated trees, mean tree height was reduced by one third, by six metres, with a reduction in MAT of the sites by only 2.6°C (Table 7). Between the second coldest site, Blyfstaanhoogte, and the coldest site, Tentkop, no significant difference in tree height of untreated trees was found. However, comparing tree height between sites for the paclobutrazol-treated trees revealed significant differences between all four sites, with a reduction of tree height by 44% between the warmest and the coldest site.

Separate t-tests (Steel and Torrie 1981) for each site showed that paclobutrazol application significantly (p < 0.05) reduced tree height at the warmest and coldest sites (Gowan Brae and Tentkop respectively), but not at the two intermediate sites (Mossbank and Blyfstaanhoogte) (Table 7).
**Floral bud production**

**Effect of site, propagate type, family and paclobutrazol application on floral bud production**

The first umbels were recorded on grafted trees in 1999, three years after planting (Table 8). On seedling trees, however, no umbels were found prior to 2000 (four years after planting), and then, only on paclobutrazol treated trees. Blyfstaanhoogte was, however, an exception, as floral production was evident on both propagule types during 2000, whether treated with paclobutrazol or not. At all sites, the percentage of seedling trees bearing one or more umbels increased steadily from 1999 to 2001, whether paclobutrazol had been applied or not.

A similar trend was found for the grafted trees, with the exception of 2000, when at Tentkop and Mossbank no trees with umbels were recorded, although in the previous year umbels were found on 13.3% and 2.2% of the trees, respectively. With the exception of Blyfstaanhoogte, very few seedling trees, whether untreated or paclobutrazol-treated, produced flower buds before five years (floral response year 2001). At all sites in all years of assessment, the number of seedling trees bearing umbels was less than that of grafted trees (Table 8). By five years, all seedlings at all sites had reached the adult vegetative phase, as indicated by the morphological characteristics of the foliage. (Pryor (1976) provides a detailed insight into the heteroblastic nature of eucalypts). Generally though, the colder the site (based on MAT), the less advanced was the state of transition from juvenile to adult foliage in the seedlings.

At Tentkop, at four years, four out of 15 paclobutrazol-treated seedlings of family 37255 (Tallaganda provenance) (Table 1) produced umbels whilst still vegetatively juvenile. One year later, the same trees, as well as several other paclobutrazol-treated seedlings of families 32091 (Barren Mt.) and 34838 (Barrington Tops), produced umbels on vegetatively juvenile portions of tree crowns (Gardner 2003). The phenomenon of flowers being produced by vegetatively juvenile plants is not an uncommon feature of eucalypts (Pryor 1985), though in species such as E. nitens and E. globulus, this phenomenon appears rare and highly circumstantial (Moncur 1998, Williams et al. 1999).

Umbel score per tree at five years after planting (CROP_2001) was significantly (P < 0.01) influenced by site (SITE), either alone or in combination with certain other factors (Table 9). The sites ranked from lowest to highest umbel score per tree (logarithmic transformation) were Gowan Brae (0.0967), Mossbank (0.2468), Tentkop (0.3018) and Blyfstaanhoogte (0.4515). The FAMILY x PROPAGULE interaction was also highly significant (P < 0.01) (Table 9), with a change in rank ordering of families between the two propagules taking place. Family 34838 (refer Table 1) ranked first amongst the grafts with 0.6073 umbels per tree, but ranked only third amongst the seedlings with 0.1524 umbels per tree. Family 37255 ranked poorest amongst the grafts with 0.1569 umbels per tree but first amongst the seedlings with 0.2404 umbels tree. These results reinforce existing knowledge of the presence of considerable within-family variation, on the basis of precocity, in the South African E. nitens breeding population (Swain 2001, Jones 2002).

**Table 7: Mean tree height (metres) at five years of age for the SITE x PBZ interaction**

| SITE          | 0.00g paclobutrazol per cm b.s.c. ‡ | 0.25g paclobutrazol per cm b.s.c. ‡ | difference between means | MAT (°C) |
|---------------|--------------------------------------|--------------------------------------|--------------------------|----------|
| Gowan Brae    | 18.11a‡                              | 14.64a‡                              | 3.47**                   | 15.2     |
| Mossbank      | 14.28b                               | 13.00b                               | 1.28                     | 14.0     |
| Blyfstaanhoogte | 11.93c                              | 11.97c                               | 0.04                     | 13.2     |
| Tentkop       | 12.16c                               | 8.20d                                | 3.96**                   | 12.6     |

‡ within this column, values followed by the same letter are not significantly different (P < 0.05)
** significant at P < 0.05
years, 96.5 and 100.9 CPs preceded substantial percentages of trees producing umbels (25 to 50% of the seedlings and 55 to 64% of the grafts), regardless of the application of paclobutrazol. Similarly, at Tentkop, the second coldest site, in 1999 when trees were only three years old, 84.5 CPs preceded a higher percentage of untreated grafts producing umbels (13.3%) than paclobutrazol-treated grafts (6.0%) (Table 8). One year later at the same site, a slightly lower accumulated winter chill amount of 81.5 CPs preceded a zero floral response in untreated grafts whereas 46.2% of paclobutrazol-treated trees produced umbels. Generally, at low to moderate levels of winter chill (> 42 < 82 CPs), paclobutrazol-treated trees recorded markedly higher percentages of trees producing umbels than non-paclobutrazol treated trees (Table 8).

### Discussion

By investigating the relationship between accumulated winter chilling and floral bud production our research has demonstrated four important points (described below).

Firstly, it is possible to calculate the potential cold (winter chilling) requirement of *E. nitens* by using the Utah Model, the Dynamic Model or the Daily Positive Utah Chill Unit Model. However, the Dynamic Model performed best under the range of conditions tested. Secondly, flower induction does not seem to result from a single chill event, but from a cumulative process. Thirdly, by using the Dynamic Model, floral bud production in *E. nitens* grafts, on the basis of chill accumulation and paclobutrazol application, can be predicted with a fairly high degree of accuracy. Fourthly, the amount of chilling necessary for uniform flowering in *E. nitens* ranges between 85 to 101 CPs. This chilling requirement is relatively high compared to that for promotion of budbreak in the range of popular deciduous fruit types. The range for the latter runs from about 12 CPs for low-chill peaches to about 70 CPs for high-chill apples and sweet cherries (Erez 2000). Although chilling portions necessary for floral induction in olive (*Olea europea* L.), a temperate broadleaf evergreen tree crop, have not been calculated, Hackett and Hartmann (1963) calculated that approximately 18h50 below 7.2°C were required, with a slight variation depending on olive cultivar.

Planting a trial at an even ‘colder’ (based on chill unit accumulation) location than Blyfstaansthoogte, – the ‘coldest’ site used in this study, – as well as comparing the chill accumulation data of this site with data from sites where *E. nitens* flowers regularly, might result in a more accurate determination of the chilling requirement of the species. Preliminary investigations relating air temperature data for 1998 to 2000 of Chilean *E. nitens* seed orchards to subsequent floral bud production showed that the amount of winter chill accumulated at these sites between April and September ranged between 70 and 89 CPs for a ‘mild’ site.
and between 100 and 104 CPs and 108 and 119 CPs for two 'cold' sites. Floral bud and seed production at the mild site rated 'average to good' and at the two cold sites 'consistently good' (R.A.W. Gardner, unpublished data). This coincides very well with the modelled cold requirement for E. nitens of 85 to 101 CPs.

In the summer rainfall region of South Africa, forestry sites with such high levels of winter chill (85 to 101 CPs) are generally limited to remote, mountainous country, and are, hence, not easily accessible. Thus, the location of E. nitens orchards at suitable sites in this region poses practical problems with orchard management and research functions. A possible solution would be to employ evaporative cooling during winter in existing orchards. Allan et al. (1994) demonstrated how overhead sprinkling could reduce macadamia (Macadamia integrifolia) foliage temperatures by up to 16°C and kiwifruit (Actinidia chinensis) bud temperatures by up to 10°C on warm winter days in KwaZulu-Natal, South Africa. Similar reductions in E. nitens foliage temperatures on 'problematic' warm winter days could be achieved by this method of evaporative cooling. This practice may increase the total accumulated winter chilling amount above the critical threshold. The selection of genotypes of E. nitens that require lower chilling factors — thereby adapting the species to warmer growing locations — and/or the use of precocious/low-chilling-requiring rootstocks may also be possible solutions to this problem for South African as well as overseas orchards. Transmittance of precocious/ chilling-requiring characteristics from rootstock to scion is a phenomenon commonly exploited in temperate fruit trees (Couvillon et al. 1984, Du Plooy and Van Huyssteen 2000).

A higher degree of accuracy in predicting floral bud production is necessary before future E. nitens commercial seed orchards can be sited with confidence. This could probably be achieved following further experimentation involving fewer genotypes and a far wider range of chilling conditions than in the current trial series. The results of the multiple linear regressions should be seen in the context that the high level of variance may be partly due to the fact that, in each case, the explanatory variable 'CHILL' consisted of only four unique points.

Some progress has recently been made in identifying the flowering controls in E. nitens. Although the species may be insensitive to daylength, a period of cold appears to be a pre-requisite for floral induction (Moncur and Hasan 1994). However, the actual amount of cold required has also been determined, nor is it known if cold is the sole trigger of floral induction. Moncur and Hasan (1994) suggested that the flowering response of E. nitens to successive periods of cold may result from the gradual destruction of a flowering inhibitor. Our results confirm such a suggestion. Best flowering performance of E. nitens was recorded at Blyfsaanaanhoogte, which accumulated the highest number of chill units in 1999 and 2000. However, judged by MAT, Tentkop is a colder site than Blyfsaanaanhoogte. If the destruction of this flowering inhibitor occurs only under certain circumstances and these are similar to those for chilling accumulation, the idea of a gradual destruction of a flowering inhibitor could explain why the highest number of trees with umbels (Table 8), as well as the highest umbel crop per tree, was achieved in Blyfsaanaanhoogte in 2000 and 2001. Certain — or all — of the juvenile characteristics of the seedling rootstocks imposed an inability to flower onto the grafted tree, otherwise 100% flowering should have been achieved in 2001 after the exposure to 88 CPs at Blyfstaanhoogte.

On the other hand, the lack of chilling in 1998 (72 CPs) seems to have been the reason behind the poor flowering in 1999 in the grafted trees at Blyfsaanaanhoogte. In 2001, only about 2/3 of the grafted trees produced umbels, possibly indicating that the chilling amount of 100.9 CPs did not
entirely meet the chilling requirement of these \textit{E. nitens} trees, and that the actual requirement may be even higher. Alternatively, these grafted trees were still too juvenile to flower regularly, even though the rootstocks were six years old. The transfer of a juvenile signal from a juvenile rootstock onto an adult scion has been described for \textit{E. x trabutii} by Siniscalco and Pavolettoni (1988).

Recent work on floral induction via chemical means in \textit{E. globulus} and \textit{E. nitens} has suggested that the timing of vegetative phase change and first flowering in these two species are independent and can be separately manipulated (Hasan and Reid 1995, Moncur 1998). Only at Tentkop did vegetatively-juvenile seedling trees (paclobutrazol-treated) produce umbels. This was recorded in 2000 and 2001 following exposure of the trees to 81.5 and 87.8 CPs, respectively (Table 5). In these years, no vegetatively-juvenile seedling trees of similar families and/or paclobutrazol treatment produced umbels at Blyfstaanhoogte following exposure to the even higher amounts of accumulated winter chilling of 96.5 and 100.9 CPs, respectively. Thus, it would appear that neither cumulative winter chill, nor paclobutrazol application, nor both combined, are the key flowering trigger in vegetatively-juvenile \textit{E. nitens} seedlings.

Williams \textit{et al.} (1999) reported that 24-month-old paclobutrazol-treated, vegetatively-juvenile \textit{E. nitens} seedlings failed to produce flower buds when grown under outside climatic conditions that were conducive to flowering in nearby mature \textit{E. nitens} trees, and similar to those conditions that were successful for containerised \textit{E. globulus} seedlings (Hasan and Reid 1995). Any one, or a combination, of the minimal differences which undoubtedly occurred between the environmental and/or cultural conditions applied in the experiments of Williams \textit{et al.} (1999) and Hasan and Reid (1995) and Moncur (1998) could have led to the relatively poor flowering result in the former experiment. However, the most glaring difference appears to have pertained to container size, with the trees in the Williams \textit{et al.} (1999) experiment most likely having experienced far less root constriction. Apart from the amount of accumulated winter chilling, a major difference between the environmental conditions of Tentkop and Blyfstaanhoogte pertained to the severity of winter conditions. The radiation frosts occurring at Tentkop were far more frequent and severe than at Blyfstaanhoogte, and, by the end of each winter, the shorter, paclobutrazol-treated seedlings at the former site displayed substantial frost-scorch of the canopy peripherals.

The results of the overseas and local work therefore suggest that it is likely that plant stress resulting from an environmental factor such as cold is the key flowering trigger in vegetatively immature \textit{E. nitens} plants.

Paclobutrazol – possibly due to its action as a gibberellin biosynthesis inhibitor – has been found to reduce plant height in a variety of woody plants (Sterrett 1985). The application of 0.25g paclobutrazol per cm b.s.c. significantly reduced tree height. Tree height was similarly significantly reduced by cold (Table 7). As paclobutrazol treatment has been found to enhance early flower induction in \textit{E. nitens} (Moncur 1998, Williams \textit{et al.} 2003), it seems that both plant growth regulator (paclobutrazol) treatment and exposure of trees to low temperatures, act in a similar manner in inducing flowering. Because paclobutrazol cannot replace exposure to cold, and the two treatments appear to have a cumulative effect on flowering (Table 8), it is speculated that the mechanism by which these two treatments reduce growth is different. This is underlined by the fact that paclobutrazol application does not seem to result in plant stress, but rather in an optimisation of plant metabolism (Fletcher \textit{et al.} 2000), while exposure to cold might reduce plant growth as a typical stress response.

Further research into the metabolic changes brought about by paclobutrazol application as well as cold treatment should be undertaken to clarify whether a certain hormonal homeostasis, brought about either by ‘natural ageing’ or by paclobutrazol, is the prerequisite for flowering in \textit{E. nitens}, while the exposure to 85 to 101 CPs is the actual flowering trigger of this species.

\textbf{References}

Allan P, Burnett MJ (1995) Peach production in an area with low winter chilling. \textit{Journal of the Southern African Society for Horticultural Sciences} 5: 15–18

Allan P, Cullis NA, Savage MJ, Lightbody KE (1994) Effects of evaporative cooling on macadamia and kiwifruit. \textit{Journal of the Southern African Society for Horticultural Sciences} 4: 16–20

Allan P, Linsley-Noakes GC, Matthee GW, Rufus G (1995) Winter chill models in a mild subtropical area and effects of constant 6°C chilling on peach bud break. \textit{Acta Horticulturae} 409: 9–17

Boland DJ, Broker MIH, Chippendale GM, Hall N, Hyland BPM, Johnston RD, Kleingia NA, Turner JD (1992) \textit{Forest Trees of Australia}. CSIRO Publishing, Victoria, Australia, pp 450–573. ISBN 0–643–05423–5

Clarke CRE (2000) Wood and pulp properties of four New South Wales provenances of \textit{Eucalyptus nitens} grown on a warm and a cold site in South Africa. \textit{Appita Journal} 53: 231–236

Couvillon GA, Erez A (1988) Effect of level and duration of high temperatures on rest in the peach. \textit{Journal of the American Society for Horticultural Science} 110: 579–581

Couvillon GA, Finardi N, Magnani M, Freire C (1984) Rootstock influences chilling requirement of ‘Rome Beauty’ apple in Brazil. \textit{HortScience} 19: 255–256

Du Plooy P, Van Huysteen P (2000) Effect of BP1, BP3 and Quince A rootstocks, at three planting densities, on precocity and fruit quality of ‘Forelle’ pear (\textit{Pyrus communis} \textit{L}.). \textit{South African Journal of Plant and Soil} 17. 57–59

Eldridge KG, Davidson J, Harwood C, Van Wyk G (1993) \textit{Eucalypt Domestication and Breeding}. Oxford University Press, New York, pp 114–122. ISBN 0–19–854149–X

Erez A (2000) Bud dormancy: phenomenon, problems and solutions in the tropics and sub-tropics. In: Erez A (ed) \textit{Temperate Fruit Crops in Warm Climates}. Kluwer Academic Publishers, Netherlands, pp 17–48. ISBN 0–412–63290–X

Erez A, Fishman S, Linsley-Noakes GC, Allan P (1990) The dynamic model for rest completion in peach buds. \textit{Acta Horticulturae} 276: 165–175
Pietermaritzburg, South Africa
Swain T, Chiappero C (1998) Collection of improved E. nitens seed from ICFR seed orchards. ICFR Newsletter, May 1998. ICFR, Pietermaritzburg, South Africa
Swain T, Gardner RAW (2002) Use of site-species matching and genetic gain to maximise yield — a South African example. In: Run-Peng Wei, Daping Xu (eds) Proceedings of the International Symposium Eucalyptus Plantations: Research, Management and Development, Guangzhou, China, 1–6 September, 2002. World Scientific, Singapore, pp 174–179
Swain T, Gardner RAW (2003) A summary of current knowledge of cold tolerant eucalypt species (CTEs) grown in South Africa. ICFR Bulletin Series No. 3/2003. ICFR, Pietermaritzburg, South Africa
Swain T, Chiappero C, Gardner RAW (1998) Final measurements of six ICFR E. nitens provenance/progeny trials in the summer rainfall region in South Africa. ICFR Bulletin Series No. 5/1998. ICFR, Pietermaritzburg, South Africa
Tibbits WN, Boomsma DB, Jarvis S (1997) Distribution, biology, genetics, and improvement programs for Eucalyptus globulus and E. nitens around the world. In: White T, Huber D, Powell G (eds) Proceedings of the 24th Biennial Southern Tree Improvement Conference, 9–12 June, 1997. Southern Tree Improvement Committee, Orlando, Florida, pp 1–15
Williams DA, Ross JJ, Reid JB, Potts BM (1999) Response of Eucalyptus nitens seedlings to gibberellin biosynthesis inhibitors. Plant Growth Regulation 27: 125–129
Williams DA, Potts BM, Smethurst PJ (2003) Promotion of flowering in Eucalyptus nitens by paclobutrazol was enhanced by nitrogen fertilizer. Canadian Journal of Forest Research 33: 74–81