EFFECTS OF THERMOPERIOD ON SEEDLING DEVELOPMENT IN *EUCALYPTUS OBLIQUA*

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ABSTRACT

High day and night temperatures (28°C) inhibited elongation of the mainstem but not branches in **Eucalyptus obliqua** L'Herit seedlings. Inhibition of stem growth was associated with amounts of growth inhibiting substances detected in the stem.

A wide thermoperiod with nightly chilling (28°/5°C and 24°/5°C day/night temperature) increased (a) branch number, (b) root/stem and root/shoot dry weight ratio and, (c) levels of cytokinin-like growth promoting substance. Results suggest that these environmental effects on branching and seedling development are mediated through effects on the amounts of growth promoting and inhibiting substances in the stem.

INTRODUCTION

Diurnal temperature fluctuations often exceed 20°C in inland areas of Australia but the significance of thermoperiod (i.e. day/night temperature variation) on eucalypt growth and development is largely unknown. For experimental convenience either an unnaturally narrow (e.g. 5°C) day/night temperature differential or constant temperature regimes are used to study seedling growth and development.

Previous work on the effects of thermoperiod on eucalypt branching is inconclusive. Scurfield (1961) found evidence that thermoperiod could influence branch number in several species of eucalypts but relied on moving plants from out-of-doors into heated glasshouses for the overnight period. Seedlings of *Eucalyptus regnans* F. Muell. grown by Cremer (1975) under a suboptimal (21°C) constant day temperature with the night temperatures varying from 10°C to 31°C showed a large increase in relative branching (i.e. dry weight of branches as percentage of stem weight).

In the present work, day and night temperatures were varied to cover the range from hot (28°C) days and nights to chilling (5°C) temperatures so as to vary the thermoperiod from 0°C to 23°C.

Environmental factors which influence the relative development of branches and main stem on a tree could affect sawlog production. Hence an understanding of the effects of environment on seedling form can be of importance particularly given the wide planting of *Eucalyptus* spp outside their natural range.

This study is part of a broader project aimed at elucidating the effects of environmental stresses on eucalypt development and physiology.

METHODS

Phytotron experiment

One month-old seedlings of *E. obliqua* were raised in plastic cups from seed collected from a single tree at Mawbana, Tasmania. These were allocated at random to one of seven temperature regimes (9 replicates per treatment) in naturally lit 'C' cabinets (Morse and Evans, 1962) inside the 'Ceres' phytotron (Robeson, 1966) at Canberra. The regimes were 28°/28°C, 28°/18°C, 28°/5°C, 24°/24°C, 24°/18°C, 24°/5°C and 10°/5°C. The seedlings were watered twice daily, once with standard Hoagland's nutrient solution and once with water; the natural daylength conditions were supplemented with incandescent light to provide a 14-hour photoperiod. Seedling total height, branch length, branch number and dry weights of the roots, stem, branches and leaves were determined after 2 months' growth under the above conditions.

Root temperatures were not monitored in this experiment, however, other (unpublished) data indicate that soil temperatures in the plastic cups may equilibrate up to 30 minutes after exposure to ambient temperature in the growth chambers and vary (±) by several degrees from air temperature.

Bioassays

Levels of growth-promoting and growth-inhibiting substances present in an extract (from 10g fresh weight) of inner bark and outer living wood of the lower stem of young *E. obliqua* seedlings were estimated as follows:—

Procedures for the methanolic extraction and thin layer chromatographic purification are described previously (Blake and Carrodus, 1970). Promotion and inhibition to growth of lettuce hypocotyls (a gibberellin assay; Franklin and Wareing, 1960) and radish cotyledons (a cytokinin assay; Letham, 1968) were used to assay levels of growth-promoting and inhibiting substances.

Hormone levels in the "cambial region" of the stem were assayed in an attempt to explain the release of axillary and accessory buds which, in very young seedlings, lie mainly on or adjacent to the outer stem.

RESULTS

Phytotron experiment

Temperature and thermoperiod affected height growth and branching as follows:

(i) a large day/night temperature differential with cold nights (28°/5°C and 24°/5°C) increased branch number (Table 1, Fig. 1). This increase in branching was associated with poor height growth and an increase in the root/stem and root/shoot dry-weight ratios. Conversely, seedlings showing optimal height growth (28°/18°C; 24°/24°C and 24°/18°C treatment) had few branches and lower root/stem and root/shoot dry-weight ratios by comparison with treatments providing a wider thermoperiod (Table 1).

(ii) High (28°C) night temperatures inhibited stem, but not branch, elongation compared with the 18°C night temperature, resulting in shorter plants with few, relatively long, branches.

TABLE 1—Effect of thermoperiod on height growth and branching in **Eucalyptus obliqua** seedlings

Day/night temperature	Thermo-period	Mean total height (cm)	Mean number of branches	Mean branch length (cm)	Dry-weight ratios root/stem	root/shoot
28°/28°C	0°C	6.8	4.6	7.0	1.1	0.1
28°/18°C	10°C	10.6	3.0	4.6	2.2	0.4
28°/5°C	23°C	4.0	11.4	2.6	12.2	1.8
24°/24°C	0°C	10.8	4.6	6.3	1.1	0.3
24°/18°C	6°C	11.5	4.0	5.3	4.2	1.0
24°/5°C	19°C	3.4	10.2	3.3	16.4	2.3
10°/5°C	5°C	1.9	0	0	2.9	0.9
L.S.D.*		3.3	3.3	1.6		

* Least Significant Difference P = 0.05%. A nonsignificant difference between two adjacent mean values is indicated by a line linking them.

FIG. 1—Effects of thermoperiod on development of **E. obliqua** seedlings. From left to right, seedlings from the 24°/5°C; 24°/18°C; 24°/24°C; 28°/5°C; 28°/18°C; 28°/28° C; and 10°/5°C treatments.

Bioassays

Significant amounts of growth-inhibiting substance(s), as estimated by the degree of inhibition in both bioassays, were detected in the high (28°/28°C) temperature treatment, but were undetected in assays of extracts of plants taken from the 28°/18°C and 28°/5°C treatments (Tables 2 and 3).

Significant and approximately equal quantities of gibberellin occurred in both the 28°/18°C and 28°/5°C treatments (0.04 μg/g fresh weight of gibberellic acid equiva-

TABLE 2—Effects of thermoperiod on stem gibberellin levels as estimated by the lettuce hypocotyl assay

Day/night temperature	Thermoperiod	Control[1] (mm)	Promotion[2] (mm)	Inhibition[2] (mm)
28°/28°C	0°C	24.0	0	20.0 (—4.0)
28°/18°C	10°C		31.0 (+7.0)	0
28°/5°C	23°C		32.0 (+8.0)	0
L.S.D.		±4.0		

[1] Lengths of hypocotyls grown on blank chromatographed plates.
Least significant differences (L.S.D., P = 0.01%) determined by a 't' test of the results of an analysis of variance.
[2] Lengths of hypocotyls grown on chromatograms of seedling extracts. Bracketed figure shows significant promotion/inhibition in the assay.

lents) while gibberellin was undetected in the 28°/28°C treatment (Table 2). Since plants showing strong apical dominance (28°/18°C treatment) and poor apical dominance (28°/5°C treatment) had approximately the same stem gibberellin content, branch formation did not appear to be correlated with amounts of gibberellin.

Significant cytokinin (2.15 μg/g fresh weight of kinetin equivalents) was detected in the 28°/5°C treatment, but not the 28°/18°C or 28°/28°C treatments (Table 3).

TABLE 3—Effects of thermoperiod on stem cytokinin levels as estimated by the radish cotyledon assay

Day/night temperature	Thermoperiod	Control[1] (mg $\times$ 10^{-1})	Promotion[2] (mg $\times$ 10^{-1})	Inhibition[2] (mg $\times$ 10^{-1})
28°/28°C	0°C	4.2	0	3.0 (-1.2)
28°/18°C	10°C		0	0
28°/5°C	23°C		6.8 (+1.6)	0
L.S.D.		±0.8		

[1] Weight of radish cotyledons grown on blank chromatographed plates.
Least significant difference (L.S.D. P = 0.01%) determined by a 't' test of the results of an anlysis of variance.
[2] Weight of radish cotyledons grown on chromatograms of seedling extracts. Bracketed figures indicate a significant promotion/inhibition in the assay.

DISCUSSION

Eucalypts are being widely planted under environmental conditions vastly different from those experienced in their natural range. Hence an understanding of the effects of environment on eucalypt growth and development may prevent costly failures.

A limiting effect of higher day and night temperatures on height growth may explain the observed failure of many eucalypt species from southern Australia when introduced into sub-tropical areas, for example, in Brazil (V. Moura, pers. comm.). Inhibition of stem elongation of *E. obliqua* seedlings grown at higher (28°/28°C) day/night temperatures was associated with an increase in amounts of growth inhibitor(s) in the stem (Tables 2 and 3), indicating a possible mechanism for this effect.

Sustained high (30°C) day and night temperatures similarly induced a quiescence in height growth of young *E. obliqua* seedlings, the induced thermodormancy being associated with a progressive increase in levels of growth inhibiting substances (Blake, 1976).

Thermoperiod exerted an important influence on seedling shape i.e. height growth relative to branching, suggesting that a wide thermoperiod with cold (5°) nights may lead to a bushy habit (Fig. 1, Table 1).

It was proposed (Brown, McAlpine and Kormanik, 1967) that the term "apical dominance" should refer to the inhibition to lateral bud growth occurring on currently elongating shoots, and "apical control" to the "physiological condition governing the excurrent or decurrent pattern of growth" i.e. tree form resulting from the relative growth of branches and main stem.

It is of interest that the physiological mechanism controlling branch formation (i.e. apical dominance) is different from that controlling subsequent elongation relative to the stem (i.e. apical control—*sensu* Brown, *et al.,* 1967). An increase in branch number was associated with higher concentrations of root-produced cytokinins and lower levels of growth-inhibiting substances. However maximum branch elongation relative to the stem was associated with higher inhibitor concentrations which reduced stem but not branch elongation.

Extrapolation of these results to other species should be approached with caution. However, a wide thermoperiod with nightly chilling increased the number of secondary branches in *E. diversicolor* F. Muell. seedlings (P. D. Stirling and T. J. Blake, unpublished).

Field observations (T. J. Blake, unpublished) indicate that *Eucalyptus grandis* W. Hill ex Maiden and *E. obliqua* show a bushy habit (due to an increase in the number of branches) when grown at altitudes higher than those normally encountered in their natural range. Similarly, Büsgen and Münch (1929) report a bushy habit for beech (*Fagus sylvatica*) grown at higher altitudes. The studies reported here suggest that an increase in branch number is associated with (a) a higher root/stem dry weight ratio, and (b) increased amounts of a cytokinin-like growth promoting substance.

A greatly increased root development (and corresponding decrease in stem growth) may increase the concentration of root-produced cytokinin in the stem. This may explain the increase in branch number resulting from wide day/night temperature differentials. The possible importance of root temperature to branching should therefore be considered in any further studies of this nature.

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