EFFECT OF 6-BENZYLAMINOPURINE AND 1-NAPHTHYLACETIC ACID ON *IN VITRO* AXILLARY BUD DEVELOPMENT OF MATURE *ACACIA MELANOXYLON*

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Abstract Mature *Acacia melanoxylon* nodal stem pieces of one clone showed improved *in vitro* axillary bud development after a single treatment of 4.44 μM benzylaminopurine (BAP) alone, or in combination with 2.68 μM naphthylacetic acid (NAA), when compared to segments placed upon a medium without plant growth regulators. Control and NAA treatments alone produced shoots with fewer leaves and had fewer leaves exhibiting juvenile form. BAP treatments increased the formation of juvenile and mature leaves. Shoots did not maintain leaf number with serial transfer *in vitro* and did not root *in vivo* following 16 weeks *in vitro*.

INTRODUCTION

Interest has been shown in *A. melanoxylon* as a forest species as it has a rapid growth rate and is an attractive, fine grained timber suitable for furniture (1). At present New Zealand does not have any identified clones, i.e., with suitable stem form and disease resistance, but clonal selection has been carried out in Australia and South Africa. Tissue culture techniques may provide early amplification of limited explant material.

Juvenile *Acacia melanoxylon* grows well *in vitro*, showing shoot multiplication and spontaneous root production. Plantlets survive well upon transfer to glasshouse and nursery environments. They do not require plant growth regulators (PGR) during any phase of growth (2).

Mature nodal segments placed *in vitro* initially respond with axillary bud outgrowth and develop leaves with mature morphology, but after 6 to 8 weeks, leaves abscise leaving a stem unsuitable for rooting.

The addition of single doses of PGR as a pulse treatment, or in a sustained medium addition, often enhances growth of many plant species *in vitro*. Meyer and Van Staden (3) reported that 1.0 μM benzyladenine and 1 μM indole-acetic acid enhanced development of callus which gave rise to multiple bud formation in coppice material from *A. melanoxylon*.

The aim of our study was to obtain vigorous axillary bud outgrowth from nodal segments of mature *A. melanoxylon*. Nodal segments were maintained *in vitro* for 16 weeks in order to assess the long-term effect of a PGR pulse treatment.

METHODS

Plant material. Explants were taken from a single clone of *Acacia melanoxylon* which was imported as cuttings from South Africa in 1985. Rooted cuttings have been maintained as potted plants in the glasshouse. These were sprayed fortnightly with the

fungicides, Euparen at 0.4 g l^{-1} and Benlate at 2 g l^{-1} alternately. New lateral and terminal shoots (up to 30 cm) were cut into 5 to 10 mm nodal segments for *in vitro* studies.

Tissue disinfestation. The following procedure was used to disinfest explants:

1. Silwet 7607 (non-ionic organosilicone surfactant): 150 microlitres in 200 ml of sterile water for 20 min. with intermittent agitation.
2. Calcium hypochlorite (5% chlorine)/sterile water, (20:80) for 15 min.
3. Rinse in sterile water.
4. Hydrogen peroxide (100 vol.)/sterile water, 6:100 for 10 min.
5. Rinse in sterile water 2 times.

Nodal segments were placed on a proprietary Acacia Medium (AM) without any PGR. After 14 days, nodal segments were assessed for contamination and clean material was transferred to treatment media.

Treatment. Media used:

(a) AM
(b) AM plus 2.68 μM 1-naphthylaceticacid (NAA);
(c) AM plus 4.44 μM benzylaminopurine (BAP);
(d) AM plus 2.68 μM NAA+4.44 μM BAP.

Medium solidified with 1% Difco Bacto agar was poured into 600 ml Agee jars (100 ml/jar). Five stem segments were placed in each jar with 5 to 7 jars per treatment. Lids were clear plastic petri dish tops held in place with plastic film (Gladwrap) wrapped around the jar rim. After 4 weeks on the media, nodal segments were transferred to basal AM media. They were subsequently transferred to fresh AM media at 4 weekly intervals.

A 16-hour photoperiod was maintained with a photosynthetic photon flux density of 100–120 μmol $m^{-2}s^{-1}$ provided by fluorescent tubes. The *day* temperature was 21 to 25°C and the *night* temperature was 18 to 19°C.

Prior to each transfer, developing axillary shoots were assessed for leaf number and leaf morphology (mature phyllode and juvenile bipinate forms, Figures 1 and 2). Assessments were made of leaf abscission which occurred in all treatments. No stems were discarded from any treatment during the experiment. Experiment 1 had 4 transfers; experiment 2 had 2 transfers.

Data was analysed using ANOVA after performing a $\log_e (x + 20)$ transformation. A transformation was required as the distribution of results was skewed rather than normal due to the effect of abscission on numbers of leaves measured. Means were separated using the least significant difference test with = 0.01. The experiment was repeated once.

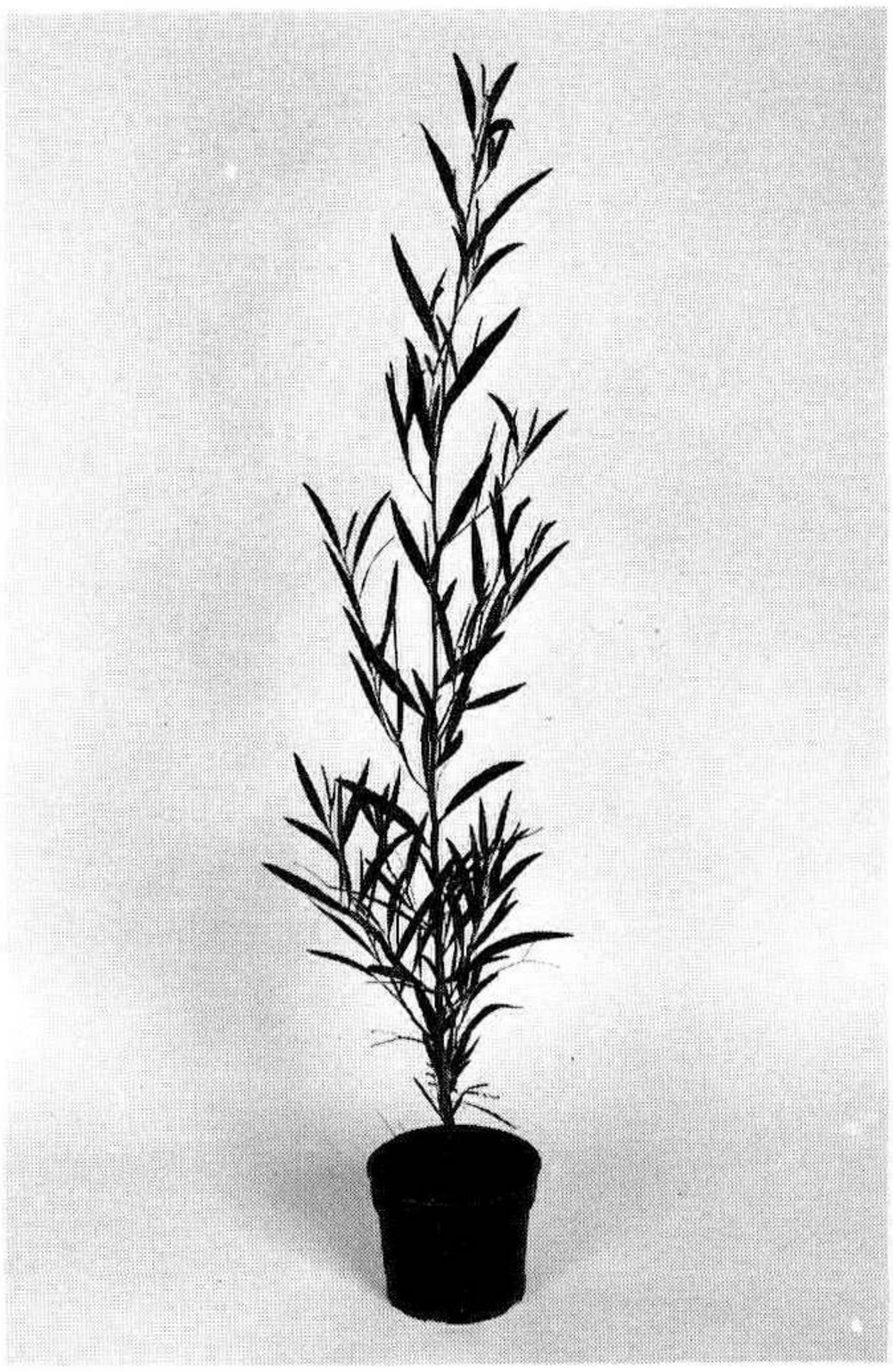

Figure 1. *Acacia melanoxylon* showing mature phyllode foliage

Figure 2. Juvenile bipinnate foliage *in vitro* from same clone as in Figure 1.

RESULTS

At the time stem pieces were first placed in culture, all leaves were excised, but each piece had an axillary bud. The axillary buds started to grow out while on AM media. When on AM plus PGR medium, leaf outgrowth was slow in Experiment 1, (Table 1). In subsequent transfers, the percentage of shoots showing leaf abscission *in vitro* was lower for PGR pulsed shoots than for controls. Although the number of stems with total of leaf abscission *in vitro* between T3 and T4 was much lower for BAP pulsed shoots in Experiment 1, the differences were not statistically significant. This was due to a large variability among replicates. Because of the increasing level of abscission with successive transfers in Experiment 1, fewer transfers were used in Experiment 2 before shoots were placed in a rooting environment.

In Experiment 2, only the stems treated with both NAA and BAP showed no leaf development at T1. At T2 only the controls and stems treated with only NAA showed abscission.

Table 1. *In vitro* leaf abscission in a mature clone of *Acacia melanoxylon*.

	Percent stems with no leaves			
	Transfer number[1]			
PGR pulse medium	T1	T2	T3	T4
Experiment 1				
AM[2]	6.7	3.3	16.7	43.3
AM + NAA[3]	33.3	0	13.3	26.7
AM + BAP[4]	20.0	0	8.0	16.0
AM + NAA + BAP	25.7	0	8.6	17.9
Experiment 2				
AM	0	33.3	—	—
AM + NAA	0	14.3	—	—
AM + BAP	0	0	—	—
AM + NAA + BAP	10.3	0	—	—

[1]Transfer number = number of 4 weekly transfers to AM following PGR pulse of 4 weeks on medium indicated. T1 = Time of transfer to AM from AM + PGR medium.
[2]AM—Acacia medium
[3]NAA—naphthylacetic acid, 2.68 μM
[4]BAP—benzylaminopurine, 4.44 μM

Treatment with PGR had significant effects on the stem mean leaf number over subsequent transfers (Table 2). In both experiments, BAP increased the formation of juvenile and mature leaf forms on the same stem piece.

A BAP pulse alone, or in combination with NAA, significantly increased the mean number of leaves per stem shown over successive transfers. This trend was reflected in both juvenile and mature leaves per stem, although the results were statistically significant only for juvenile leaves.

No root formation was seen in shoots from either experiment when they were subsequently placed in a non-sterile high humidity environment.

DISCUSSION

These experiments show clearly that a four week pulse with 4.44 μM benzylaminopurine had a sustained effect on leaf formation in subsequent transfers of a mature clone of *Acacia melanoxylon*. There also appeared to be reduced leaf abscission compared with the non-PGR control, even though this did not prove to be statistically significant due to the large variation among replicates. Since all shoots were of a single clone, genotypic variability can be excluded as a causative factor.

Although a clear cytokinin effect on leaf formation has been demonstrated (Figures 1 and 2), further work is necessary to define the optimum concentration and time of application so that shoots are of sufficiently high health to form rooted plants.

Table 2. Patterns of leaf formation *in vitro* in a mature clone of *Acacia melanoxylon*.

	Mean leaves per stem[1]				Mean leaves per stem[2]	
	Transfer Number					
PGR pulse medium	T1	T2	T3	T4	Juvenile	Mature
Experiment 1						
					(At T4)	
AM	2.1 na	2.8 na	2.3 na	1.8 a	1.5 a	1.7 a
AM + NAA	2.4 na	2.7 na	2.3 na	2.1 ab	1.8 a	1.6 a
AM + BAP	2.9 na	4.4 na	5.2 na	3.5 b	2.0 b	4.5 a
AM + NAA + BAP	3.6 na	4.2 na	5.0 na	3.5 b	3.5 b	2.0 a
Experiment 2						
					(At T2)	
AM	2.1 na	1.8 a[2]	—	—	0 a	1.8 a
AM + NAA	1.8 na	2.1 a	—	—	0 a	2.1 a
AM + BAP	1.6 na	3.9 b	—	—	2.1 b	3.1 a
AM + NAA + BAP	1.8 na	3.8 b	—	—	2.9 b	2.9 a

[1]Mean number of leaves per stem (excluding shoots with total leaf abscission). Values bearing the same subscript in each column for each experiment do not differ significantly at $p \geq 0.01$. na = not analysed.
[2]Significant at $p \geq 0.05$ for increased leaf number in the BAP treatments.

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