Adventitious root formation in cuttings of *Backhousia citriodora* F. Muell: 1 Plant genotype, juvenility and characteristics of cuttings

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Abstract

Backhousia citriodora is a commercially valuable Australian woody species that has a reputation for being recalcitrant in forming adventitious roots from cuttings. A study was carried out to determine if maturation and plant genotype influenced rooting. It also tried to establish if genotypic differences in rooting ability were related to characteristics of the cutting material. The rooting of cuttings in *B. citriodora* declines after maturation and is strongly influenced by genotype. The cutting characteristics of actively growing axillary buds, wide stems and mature leaves are associated with rooting and survival but not related to genotype. Furthermore, the 8 to 24 weeks required by *B. citriodora* to form roots from cuttings makes it difficult to distinguish between the characteristics that increase rooting and those characteristics that enhance survival. A subsequent disbudding experiment demonstrated that axillary buds per se have an inhibitory effect on rooting. This suggests that the presence of actively growing axillary buds are an indication of overall growth and condition of the stock plant unrelated to the formation of adventitious rooting. The effects of other cutting characteristics on rooting are also discussed.

Key words: vegetative propagation, cuttings, genotype, maturation, axillary buds,

Introduction

Backhousia citriodora has a reputation for being difficult-to-root from cuttings taken from mature stock plants. Although, a number of growers have successfully propagated *B. citriodora* from juvenile material (House *et al.*, 1996, Lake, 1998) and from some genotypes (House *et al.*, 1996, Lake, 1998, P. Lewis personal communication 1999) cuttings can remain unchanged for months before forming roots. This suggests the formation of adventitious roots in cuttings from *B. citriodora* is limited to juvenile material and after maturation to some genotypes.

Paton *et al.* (1970) demonstrated the loss of rooting ability in *Eucalyptus grandis* was correlated with the number of nodes from the base. Rooting was highly unlikely above node 15. Rooting potential, a juvenile characteristic can be preserved at the base of the plant in juvenile tissue and used in the vegetative propagation of difficult to root mature plants (Huang, 1993, Eldridge *et al.*, 1994). A comparison of rooting success between material taken from juvenile and mature *B. citriodora* stock plants will determine if physiological age affects rooting of cuttings.

Genotypic differences in rooting have been documented in a number of woody Australian species, *Persoonia virgata* (Bauer, 1999), *Acacia baileyana* (Schwarz *et al.*, 1999) and *Eucalyptus resinifera* (McComb and Wroth, 1986). In *B. citriodora,* rooting success has been strongly associated with provenance (Chen, 1997).

The ability to form adventitious roots has also been associated with the characteristics of the cutting material (Hartmann *et al.*, 1997). However, the effect of cutting characteristics on the formation of roots varies from species to species (Harrison-Murray and Knight, 1997). It is not known if the characteristics of cuttings are associated with genetic differences in rooting ability or simply an expression of growth and the condition of the stock plant. Among the characteristics identified to influence the ability of cuttings to form adventitious roots are; size and age of leaves (Kawase, 1972, Wilson, 1993), presence of active buds (Fadl and Hartmann, 1967a, Howard, 1968), proximity of nodes to the base of the cutting (Hansen, 1986), size of the stem (Howard, 1991, Wilson, 1994) and position of cutting on the stock plant (Howard and Ridout, 1991). In *B. citriodora,* leaves frequently senesced and cuttings with soft leaf tissue failed to form roots (Henderson, 1998).

The purpose of this study was to determine if maturation of the stock plant and plant genotype influence the rooting of *B. citriodora* cuttings. It also aims to identify characteristics of cuttings that influence rooting and if they are related to genotypic differences in rooting ability.

Materials and methods

Collection and handling of plant material

Plant material was collected from either container-grown stock plants or from established trees, located at University of Queensland Gatton campus (UQG). Plant material 10-20 cm in length was transported to the propagation house at the UQG nursery within 2 hours. The plant material was washed for 10 min in an aqueous solution of sodium hypochlorite (600 µg/ml chlorine) and rinsed in tap water. Cuttings were dipped in 4000 µg/ml IBA for 10 s then inserted into cell trays containing a medium of peat/perlite/vermiculite (1:1:1 by volume). Cuttings were inserted into cell trays containing a medium of peat/perlite/vermiculite (1:1:1 by volume). Trays were placed on heated benches with controls set at 24 °C in a propagation glasshouse. Water was delivered in a mist at 2min intervals for 8s. air temperatures in the glasshouse were maintained at 22 – 29 °C, with 71-100% relative humidity. Light intensity was reduced to 22% of ambient sunlight and day-length is 12h ± 1h throughout the year

Experiment 1. To identify characteristics of genotypes that determines the survival and rooting of cuttings.

A completely randomised experiment was conducted with 108 sources of mature plant material to evaluate the effect of genotype on the ability of cuttings to form adventitious roots. All plant material came from 2-yearold container grown-plants over 2m in height. The characteristics of each genotype were simultaneously related to their influence on rooting. Five cuttings were taken from each of the mature plants in autumn (April 1999), except where insufficient material existed. Cuttings were assessed for the formation of roots after 3, 12, 17, 24 and 28 weeks.

The effect of genotype on root formation was repeated in spring (October 1999), with 79 of the original mature stock plants. Ten cuttings from each stock plant were assessed for rooting after 17 and 24 weeks.

Cuttings were characterised based on each of the following 5 pairs of characteristics:

- Presence or absence of an actively growing axillary bud. Axillary buds were judged to be active if they were longer than 1 mm and not active if they were less than 1 mm in length.
- Width of the stem. Cuttings were classified according to the diameter of their internodes. The internodes of thin cuttings were < 3 mm in diameter and those of wide cuttings ≥ 3 mm in diameter.
- Presence or absence of a node near the base of the stem. Nodes were considered to be near the base of the cutting if the stem length below the node was < 20 mm.
- Whether leaves were either immature or mature. Leaves were deemed to be immature if they were light green in colour, not fully expanded and soft. In contrast, mature leaves were dark green in colour, fully expanded and firm.
- Whether leaves were entire or reduced in area by 50%. Leaves were reduced by carefully cutting across the widest part of the leaf blade, perpendicular to the midrib.

Genotypic differences in rooting percentage and association between each of the characteristics and rooting percentage were evaluated using Chi-square test (SAS, 1998). A logistic regression model, *genmod* procedure was used to identify differences in rooting success between autumn and spring (SAS, 1998) and between the characteristics of genotypes. An overall test of significance used Chi-square analysis (SAS 1998). In all experiments, direct analysis was carried out on the raw binary data, thus avoiding the need for transformation. Significance was assumed whenever $P<0.05$

Experiment 2. To identify influences of active axillary buds on rooting.

The effect of active axillary buds on rooting was determined through a comparison of cuttings with or without axillary buds removed. An additional treatment involved wounding the stem internode of cuttings with active buds to check if wounding *per se* affected rooting (Howard, 1968). Ten cuttings per treatment were taken from each of 5 genotypes, making a total of thirty-cuttings/stock plant. Cuttings were taken from selected stems with active buds during early autumn (March 2001) when stock plants were actively growing and flowering is unlikely to effect rooting success (Kibbler *et al.*, 2002a). Stock plants that had an increase in the length of the selected stems over the 3 weeks prior to the experiment were judged to be actively growing. Axillary buds were judged to be active if they were larger than 5 mm. The experiment was repeated in late winter (Aug 2001) when both stem and axillary buds were inactive. Axillary buds were removed by carefully cutting out the bud and surrounding tissue. In the wound treatment, a similar sized section was removed from the stem internode of cuttings with axillary buds.

A logistic regression model, *genmod procedure* was used to identify differences in rooting success for axillary bud experiments (SAS, 1998). Correlations between rooting success and growth, represented by the increase in stem length, were determined using a general linear model (SAS, 1998).

Experiment 3. Effect of maturation on rooting.

The ability to form adventitious roots was compared between mature and juvenile stock plants. Twenty juvenile tip cuttings taken from seedlings were compared with 27 mature, semi-hardwood cuttings from 9 mature genotypes, all maintained at UQG nursery. The seedlings were grown from seed collected from the same 9 mature genotypes.

A second experiment compared juvenile and mature cuttings from the same tree. The tree was located in Birkdale nursery, in southeastern Queensland. Over 5 m high, the tree is believed to have been grown from seed (B. McGeogh personal communication 2000). Twenty cuttings were taken from the crown of the tree (mature) and 12 cuttings were taken from shoots grown from epicormic buds near the base of the tree (juvenile). A second group of 11 juvenile cuttings were taken from plants successfully propagated from the same source the previous year (February 2000). Cuttings from the previous year were maintained in 175cm pots in a greenhouse at UQG plant nursery. To maintain their juvenility, cuttings were hedged to 20cm above the top of the pot (Chen, 1997).

To determine that juvenility and not the position of the cutting on the stock plant affected rooting, 10 mature plants, propagated from cuttings and over 2m in height were sampled. For each of these 10 plants, five cuttings taken from the base of the plant were compared with five cuttings taken from the apex.

Chi-square test (SAS, 1998) was used to test for differences in rooting percentages between material taken from juvenile and mature stock plants.

Results

Experiment 1. To identify characteristics of genotypes that determines the survival and rooting of cuttings.

Cuttings continued to form roots up to 24 weeks, with the majority of the rooting taking place between 12 and 17 weeks (Table 1).

Table 1.

There were significant differences between *B. citriodora* genotypes in rooting percentages (Table 2). The percentage of rooted cuttings ranged from 0 to 100%. In 40 of the genotypes over the 2 experiments, rooting success was 50% or less. Although there was a significant increase in rooting during spring, some genotypes had their highest rooting in autumn. This was shown by the interaction between genotype and time ($P<0.001$, Table 2).

Table 2.

There was no association between any characteristic and the ability of genotypes to form roots. At the end of week 17, only the presence of active buds and mature leaves significantly influenced rooting of cuttings (Table 3). Fadl and Hartmann (1967b) suggested that the activity of buds is an indication of overall growth unrelated to the formation of roots. An investigation whether active buds influence rooting or is a concomitant indication of active growth was the objective of the next experiment. In the period between week 17 and week 24, a large percentage of cuttings with immature leaves (17%) and wide stems (10%) formed roots. However, survival of the cuttings was significantly increased by one of each pair of characteristics. No roots formed in cuttings with senesced leaves.

Table 3.

Experiment 2. To identify influences of active axillary buds on rooting.

In both autumn and winter, removal of axillary buds significantly promoted earlier rooting of cuttings. The increase in rooting lasted longer in winter compared to autumn. After 16 weeks in winter and 12 weeks in autumn there was no significant difference in rooting between treatments. Although axillary buds and surrounding tissue were removed, new buds were visible on the lower petioles by week 16. Overall, rooting success was higher across all treatments in autumn compared to winter (Fig 1).

Fig 1

It was observed that, during the colder months, growth decreased and internodes were shorter which resulted in each cutting having more nodes and axillary buds compared to periods of active growth in the warmer months (Plate 1).

Plate 1.

Experiment 3. Effect of maturation on rooting.

Cuttings taken from juvenile stock plants formed roots faster than cuttings taken from mature stock plants. This was true whether comparing juvenile and mature material from a range of stock plants (Table 4a) or from the same tree (Table 4b). After 12 weeks there was no significant difference between cuttings randomly selected from mature and juvenile plants (Table 4a) whereas mature and juvenile material taken from the same tree were significantly different (Table 4b).

Table 4

There was no difference in rooting between cuttings taken from the base of the mature plants, propagated from cuttings and those taken from the apex. Thus demonstrating that juvenility and not the position of the cutting on the stock plant affect rooting.

Discussion

The formation of adventitious roots in *Backhousia citriodora* is strongly influenced by genotype and the decline in rooting capacity after maturation of the stock plant. Although not related to genotype, the cutting characteristics of actively growing axillary buds, wide stems and mature leaves had a significant association with rooting and survival.

The 8 to 24 weeks required by *B. citriodora* cuttings to form roots means selection of cutting material with characteristics that prolong survival is an advantage. The long period required to form adventitious roots also makes it difficult to distinguish between characteristics of cuttings that enhance survival and those characteristics that increase rooting. Cuttings with reduced mature leaves, stems wider than 3 mm with a node within 20 mm of the base and collected from shoots with active axillary buds at the time of collection, had significantly more chance of surviving. Of these characteristics, actively growing axillary buds, wide stems and mature leaves had significant associations with rooting.

The presence of active axillary buds showed a positive effect on the rooting of cuttings after 16 weeks. However, results from the subsequent disbudding experiments suggest that, whereas the activity of axillary buds are an indication of overall growth and condition of the stock plant, axillary buds *per se* have an inhibitory effect on rooting. The speed of rooting was increased after the removal of both active (autumn) and inactive (winter) axillary buds. This demonstrates that rooting is inhibited by the presence of axillary buds and this response is not dependant on the activity of the bud. In winter, the effect of removing the axillary bud lasted longer compared to the same treatment in autumn. One explanation is the slower development of replacement buds in winter. Once new axillary buds are formed they may slow or inhibit the formation of adventitious roots. The increase in rooting cannot be accounted for by wounding *per se*. This is in contrast to *Prunus cerasifera*, where wounding did stimulate rooting of cuttings (Howard, 1968). The results are consistent with the hypothesis that rooting inhibitor(s) accumulate in the bud (Fadl and Hartmann, 1967b, Howard, 1968, Kibbler *et al.*, 2002b) or root formation is inhibited by the presence of some bud factor (Durand-Creswell, 1977).

Twenty-four weeks after cuttings were struck, significantly more cuttings with wide stems formed roots compared to cuttings with thin stems. Wide stems also increased the ability of *B. citriodora* cuttings to survive but this may not be a characteristic that directly increases rooting. In *Prunus insititia* wide-stemmed cuttings were less susceptible to death (Howard, 1994) but had lower rooting compared to thin-stemmed cuttings (Howard and Ridout, 1991).

The characteristics of mature and cut leaves reduce transpiration and prevent desiccation (Hartmann *et al.*, 1997). Immature leaves in particular, with their thinner epidermis and lack of secondary thickening (Esau, 1976) are more susceptible to desiccation than mature leaves. Cuttings with mature leaves had the added advantage of forming roots faster than cuttings with immature leaves. One explanation is the difference in the use of resources between mature and immature leaves. Immature leaves are net importers and may compete for resources with developing adventitious roots (Fischer and Hansen, 1977). In mature leaves, starch content tends to reach a maximum (Donnelly, 1977) and they are net exporters of resources. The importance of leaves for rooting was demonstrated when no roots formed on cuttings after the senescence of both leaves. This suggests that leaves either stimulate rooting (Reuveni and Ravivi, 1981) or increase survival of the cutting (Wilson, 1994). Manipulating the environment to prevent senescence of leaves may increase rooting.

The ability to form adventitious roots from cuttings is reduced once *B. citriodora* becomes mature. Early rooting was significantly increased through continual pruning of juvenile stock plants maintained under glasshouse conditions. This is consistent with juvenility being maintained in *B. citriodora* stock plants through continual pruning Chen (1997). However F.C. Wise (personal communication, 2000) reported that in *Pinus taeda.* L. continual pruning may only delay maturation and rooting ability may still decrease over time. Chen (1997) suggested that over a 5-year period maturation caused the decline in rooting success of *B. citriodora* plants maintained in a juvenile state by hedging.

Juvenility and genotype are critical factors in the formation of roots in *B. citriodora* from cuttings. Juvenile plants and some mature genotypes can provide cuttings of which 100% formed roots. But the 8 to 24 weeks cuttings need to survive before they form roots is not commercially viable. Unrelated to mature genotypes, the characteristics of active axillary buds, wide stems and mature leaves also influenced rooting. Whether, as demonstrated by the inhibitory effect of axillary buds per se, the characteristics may either be an indication of overall growth and condition of the stock plant or increase survival.

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Table 1

Number of weeks from initiation of cuttings	Number of rooted cuttings (% of total cuttings) cumulative
3	0
12	0
17	163 (29 %)
24	202 (36 %)
28	202 (36 %)

Table 2

Geno-type	Autumn	Spring	Geno-type	Autumn	Spring	Geno-type	Autumn	Spring
.	%	%	.	%	%	.	%	%
A	20	20	B31	20	20	B65	60	70
B1	20	70	B32	40	20	B66	80	30
B2	20	40	B35	40	50	B67	20	50
B3	40	60	B36	0	60	B69	40	20
B4	20	50	B37	40	30	B70	80	70
B6	20	80	B38	60	90	B71	20	100
B7	0	30	B39	60	70	B72	40	50
B8	20	30	B41	0	60	B74	0	70
B9	60	60	B42	80	80	B75	80	0
B11	20	70	B43	20	30	B76	20	30
B12	40	40	B44	20	40	B79	40	20
B13	0	70	B45	40	40	B81	40	60
B14	60	50	B46	60	40	B82	20	30
B15	100	60	B48	0	40	B85	0	70
B17	40	70	B49	0	50	B100	20	50
B18	80	50	B52	60	60	B101	40	0
B19	20	30	B54	0	80	B102	20	20
B20	40	60	B55	20	60	B103	10	0
B22	20	40	B57	60	30	B104	100	40
B23	80	50	B58	20	20	B105	10	30
B24	20	40	B59	80	80	B106	0	0
B25	20	50	B60	40	40	B107	0	20
B26	40	50	B61	60	30	B108	20	50
B28	0	40	B62	60	60	B109	90	90
B29	80	80	B63	40	80	C	13	13
B30	40	50	B64	20	80			
Mean % rooted: autumn = 35%: spring = 48% (P<0.0085)								

Table 3

	Week 17		Week 24			
Characteristic (No. of cuttings with the characteristic)	Percentage of rooted cuttings	Level of significance	Percentage of rooted cuttings	Level of significance	Percentage of cuttings that survived	Level of significance
Cut leaf (519)	29		37		86	
Entire leaf (45)	24	0.492	24	0.098	64	0.001*
Mature leaf (489)	31		36		86	
Immature leaf (75)	15	0.003*	32	0.460	69	0.001*
Node at base (430)	29		36		88	
No node at base (134)	28	0.706	35	0.838	70	0.001*
Active bud (279)	35		45		87	
Bud not active (285)	23	0.001*	27	0.001*	81	0.027*
Wide stem (249)	31		41		90	
Thin stem (315)	27	0.346	32	0.024*	79	0.001*

Table 4

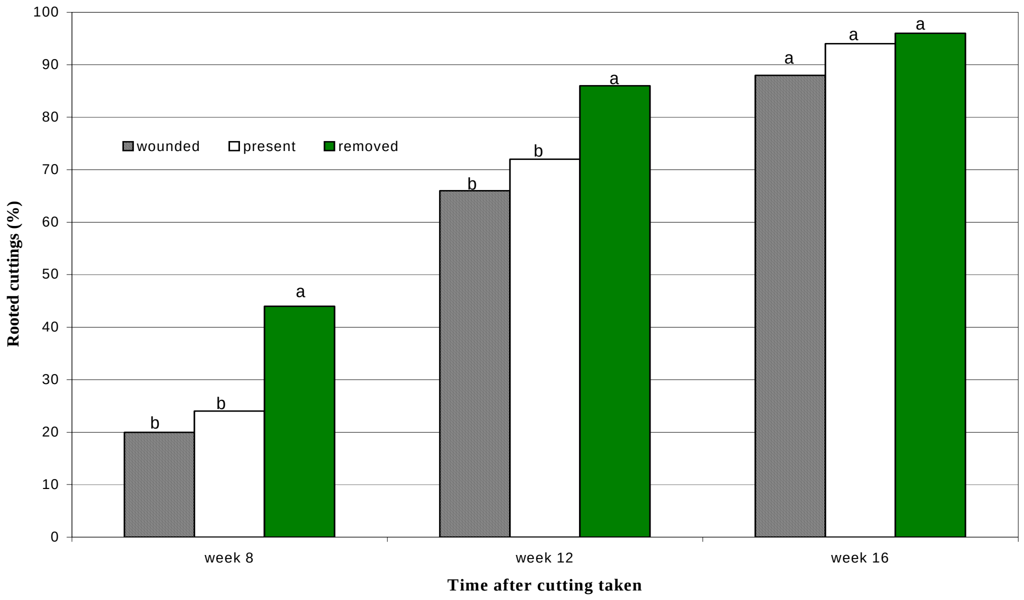
(a)

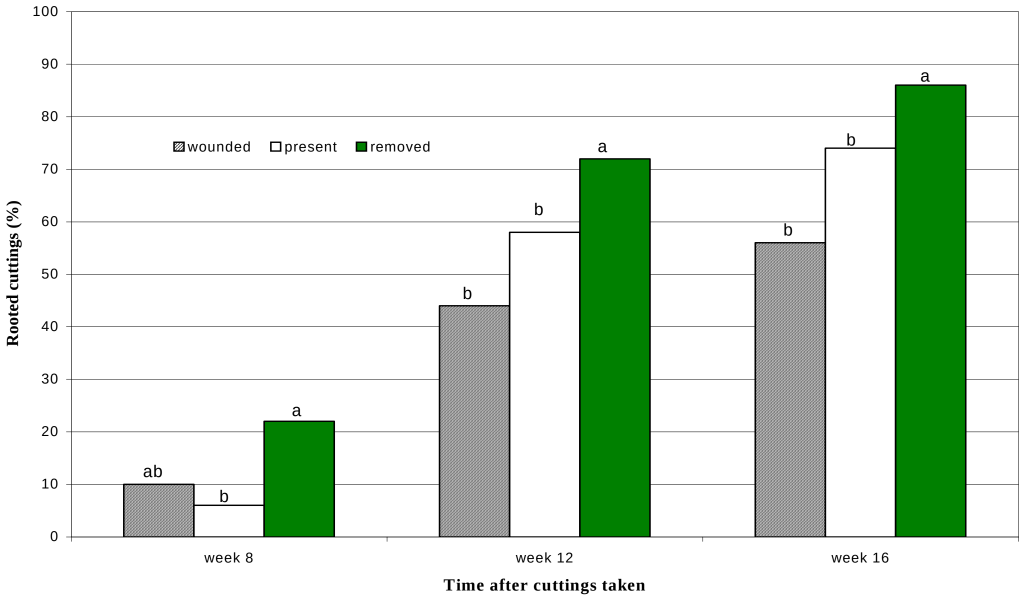
Weeks after propagation	Mature (%)	Juvenile (%)
Week 4	0 b	65 a
Week 8	22 b	80** a
Week 12	78 a	80 a
Week 16	85 a	80 a
Week 24	85 a	80 a

(b)

Weeks after propagation	Mature (%)	Juvenile (%)	Juvenile* (2000) (%)
Week 8	5 c	58 b	100 a
Week 12	40 b	92 a	100 a
Week 16	40 b	92 a	100 a
Week 24	40 b	92 a	100 a

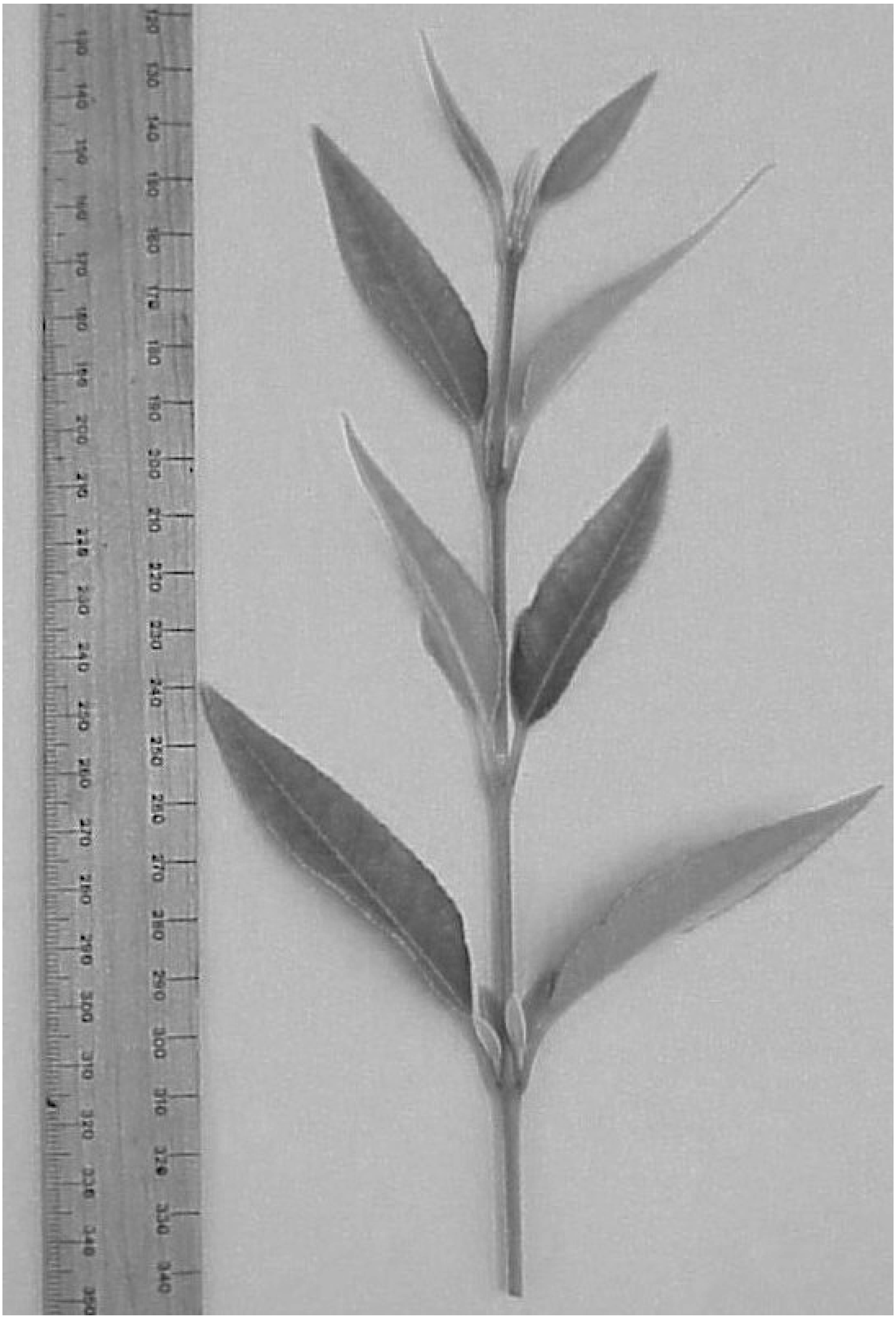
(a) autumn





(b) winter

Figure 1



(a)

(b)

Plate.1 comparison of internodal distances on cuttings in (a) summer (b) winter.

Table 1. Formation of adventitious roots in *Backhousia citriodora* cuttings over time from a total of 564 cuttings. Commenced autumn (April 1999).

Table 2 Rooting success of *Backhousia citriodora* genotypes from cuttings taken 24 weeks after striking cuttings, in autumn (April 1999) and spring (October 1999).

Table 3 Paired comparisons of the characteristics of *B. citriodora* cuttings that produced adventitious roots at 17 and 24 weeks. * Significant differences between paired characteristics.

Table 4 Comparison of rooting ability of cuttings from *Backhousia citriodora* taken from (a) a random selection of mature stock plants and juvenile seedlings (commenced February 2000) (b) juvenile and mature material from the same tree (commenced January 2001). *Cuttings taken from juvenile material propagated the previous year 2000. ** Cuttings either rooted or dead. Different subscript letters signify differences within rows.

Figure 1 Rooting success of *Backhousia citriodora* cuttings with axillary buds compared to cuttings with their axillary buds removed or the stem internodes wounded during (a) autumn (b) winter. Columns with different letters are significantly different (n=50).