# Final Report on the Australian Flora Foundation grant

# Stirlingia latifolia Establishment: 1994/96



Aileen Reid and Dr Alison Fuss Horticultural Industries Branch, Department of Agriculture, Western Australia 1 June 2005

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# Summary

*Stirlingia latifolia* is one of the most important crops currently bushpicked for export yet the horticulture and biology of this crop is still poorly understood. Knowledge of the flowering biology of *S. latifolia* helps us understand why so few "bobbles" are produced in relation to the apparently large number of flowers. Studies of growth and flowering have shown how important fire is in regeneration of this species, but pruning and clipping trials have failed to duplicate this response. In pot trials, *S. latifolia* responds to applications of nitrogen, potassium and phosphorus and it is likely that a significant part of the growth response following fire is due to a renewed supply of nutrients.

For propagation the best cutting material is from new sprouting material of *Stirlingia latifolia* following a fire or perhaps other disturbance. Or better still use *in vitro* shoots, preferably unrooted as the number of roots per propagule following auxin treatment appears to be superior to *in vitro* rooted shoots and much better than conventional cuttings. Clonal differences may have a bearing on success with this species and further testing on different populations of *Stirlingia latifolia* would be advisable before commitment to procedures followed in this study.

Much of the research conducted to date and reported here has focussed on *S. latifolia* as a member of the Proteaceae and also as a typical resprouter. The implications of these findings are discussed in the context of *S. latifolia* as a potential row crop and quality export floral product.

# Introduction

Two aspects of *Stirlingia* establishment were examined, firstly field production, and secondly propagation.

# **A. Field Production**

The following paper is presented as a report on this section of the project, but also includes recent work on *Stirlingia* by other researchers, as well as that carried out under the auspices of the AFF funded project.

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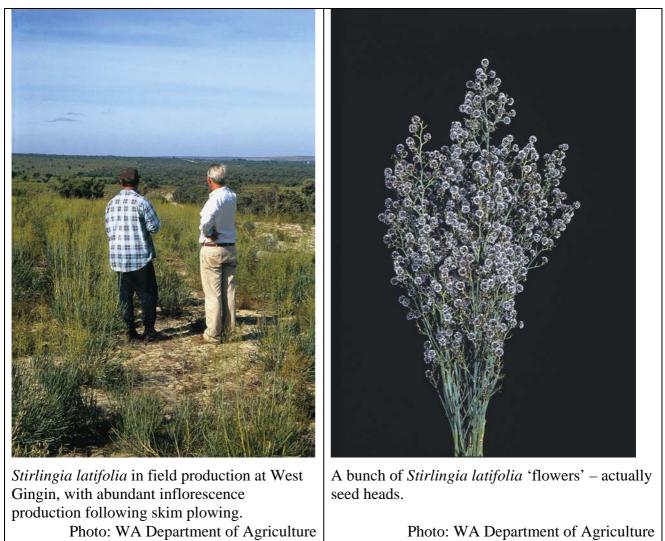
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## Introduction

*Stirlingia latifolia* is one of the most important crops currently bushpicked for export (Burgman and Hopper, 1982) with in excess of 2.9 million stems with a value of about \$348,000 picked each year. While the species is relatively abundant in its natural situation, urban encroachment is steadily reducing the extent of its habitat. Thus, there has been increasing interest in cultivating this crop in the same manner as banksias and waxflower. Germination from seed is generally poor and vegetative propagation difficult. Tissue culture has become the usual method of propagation.



Several researchers have studied *S. latifolia* in the context of its family the Proteaceae and also as a typical resprouter. Although this work is largely biological or ecological in its nature, it provides us with a deeper understanding of the responses of the crop in cultivation and helps indicate future avenues for research. For example, many growers do not realise that inflorescences of *S. latifolia* contain both bisexual heads with hermaphroditic and male flowers, and male only flower heads. Only the bisexual heads are able to produce the characteristic "bobbles" (technically fruits) that constitute the 'inflorescence' of the final product. Because of this lack of understanding it has been wrongly thought that flower abortion is a major problem in the crop.

The criteria for determining stem quality (Table 1) in *S. latifolia* do however, have some specific requirements in terms of bobble positioning and unfortunately, since they are on the periphery of the inflorescence, are more prone to desiccation by winds and frost damage and thus do regularly abscise. In cultivation, this problem could be solved with the use of windbreaks.

This report brings together most of the research carried out on *S. latifolia* so far, and shows the implications of this work for potential row cropping situations.

## Table 1. Criteria for Stirlingia latifolia stem quality

- 1. The stem must be picked when the 'bobbles' are silvery, ie fully mature. If the stems are picked too early, dye is not taken up and the ovaries are still visible as black dots in the centre of the bobble.
- 2. 'Bobbles' must be present on all key terminal positions, ie the presence of these determines the inflorescence shape.
- 3. There must be a good number of 'bobbles' and these must be distributed evenly so they are visible individually.
- 4. Branch angles should not be too wide.
- 5. Stems should be > 60 cm and not too thick relative to the length.
- 6. 'Bobbles' must be full, ie at least 6 fruits should be present otherwise the clusters are too small or lopsided.

## Flowering biology of Stirlingia

*S. latifolia* is an andromonoecious species, that is, it has separate male and bisexual flowers on the same plant. The inflorescence is an open panicle of clusters comprising mixed groups of male and hermaphroditic flowers, usually towards the ends of the inflorescence branches, and male clusters, generally lower down the branches.



Effect of poor seed set on *Stirlingia* bunches. Photo: WA Department of Agriculture

Flowers are relatively simple with a single perianth, the lobes of which reflex at anthesis to reveal the upright anthers coherent around the style below the stigma in hermaphroditic flowers. The anthers subsequently, either spontaneously, or after manipulation, spring outwards, projecting pollen above the flower. The gynoecium has a single ovule. Nectaries are absent. Almost all hermaphroditic flowers produce fruits. However, the overall seed set (especially when male flowers are included) is very low, often only two to three percent (Bowen, 1991; Ladd and Wooller, 1996).

*S. latifolia* is wind pollinated and, although feral honeybees actively forage pollen from this species, this affects neither pollen deposition nor seed set. Over a two hour period more than 100 feral bees were observed to visit *S. latifolia* flowers during full flowering compared with only four to nine native insects. Experiments conducted to compare pollination of *S. latifolia* in insect free enclosures with that in the open field showed no significant differences in pollination rates. Over 95% of plants in both areas contained pollen tubes. Thus, despite high rates of insect visitation, the evidence strongly suggests that *S. latifolia* is wind pollinated (Ladd and Wooller, 1996).

A comparison of panicles on plants at a range of locations with a range of histories of disturbance shows an interesting pattern. While the proportion of bisexual clusters remains relatively constant, as the history of disturbance becomes more severe and more recent, total numbers of bisexual clusters increase dramatically. Plants that had not been burnt for 30 years have an average number of 132 flower clusters per panicle, 22 of those being bisexual clusters. By comparison, much smaller plants disturbed by bulldozer raking 18 months previously, averaged 517 clusters of which 86 were mixed clusters - that is four times as many (Figure 1). From a commercial point of view, the latter are eminently more marketable.

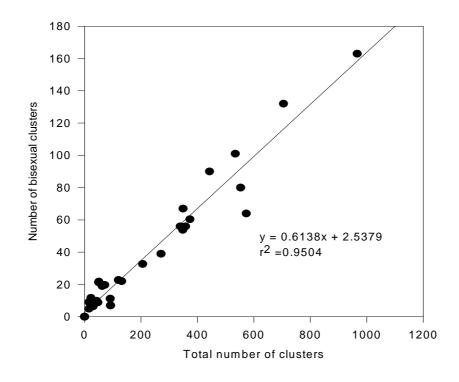


Figure 1. The relationship between the number of mixed clusters and total number of clusters for *S. latifolia* panicles from a range of sites.

#### Seed germination and early growth

As with many species of southwestern Australia, natural populations of *S. latifolia* rely on fire for germination of new individuals (Bell *et al.*, 1993). Post-fire conditions provide a favourable environment for young seedlings in terms of reduced competition and increased availability of nutrients in the soil (Stock and Lewis 1986; Lamont *et al.*, 1993). Germination of *S. latifolia* seeds takes place during late winter/early spring following a summer or autumn burn. However, viable seed production in this species tends to be extremely low. In one study only three percent of seeds sampled from one population were found to carry healthy embryos and a count of new seedlings to adult plants 12 months post-fire revealed only 0.25 to 0.5 recruits per parent plant (Bowen, 1991).

Shoot growth of *S. latifolia* following germination is slow with most of the growth occurring in the spring and summer (August to February) (Bowen, 1991). Under natural conditions, an individual will be on average only 6 cm tall (stem length only) and weigh approximately 2 grams (dry weight) by the time it has reached three years of age (Bowen, 1991). This slow shoot growth can be viewed as the result of a high deployment of resources to the roots, resulting in a low shoot: root ratio of approximately one in both juveniles and adults (Bowen and Pate, 1993). There are two major reasons for this pattern of resource allocation. The first is due to the fact that a significant quantity

of energy is directed towards the extension of a main tap root towards the water table. The onset of the dry summer months, about six months after germination, poses a real threat to young seedlings over 90% of which fail to survive the first season (Pate, unpublished data) and a priority exists to locate a reliable long term source of water as quickly as possible (Bowen and Pate, 1991). Indeed, the roots of *S. latifolia* may reach the water table within the first year of its life (Bowen, 1991), enabling it to tap the ground water supply and persist independently of rainfall (Dodd *et al.*, 1984).

Secondly, and possibly more expensive in terms of shoot growth, *S. latifolia* diverts significant amounts of photosynthates from its shoots to starch storage in its roots. The root surface is covered with small closely-spaced spherical outgrowths (tubercles) which together comprise a 'secondary cortex' on all but the finest roots. These cortical swellings are established within the first few years of growth and consist of highly vacuolate storage parenchyma which contains large quantities of starch (Pate et al., 1990; Bowen and Pate 1993). It has been demonstrated that these starch reserves play a vital role in providing an energy source for the initiation and growth of emergent shoots following defoliation such as in the event of fire (Bowen and Pate, 1993), allowing this species to be an extremely successful resprouter (Bell *et al.*, 1984).

## **Response to fire**

Evidence indicates that *S. latifolia* relies to some extent on fire for flowering, to initiate seed germination, and also for the general revitalisation of plant growth following a relatively long firefree period. The shoots are completely destroyed by fire and the plant regenerates rapidly by producing numerous shoots at or below the soil surface from its lignotuber. Reserves of starch in the root and lignotuber are utilised during such recovery and it has been shown that, in the initial 2-5 month period after fire, levels of stored starch fall by 50-75 % (Bowen and Pate, 1993). Once new shoots have been established, starch reserves are gradually replenished, although pre-fire levels of root starch are not achieved until inflorescence growth has been completed and flowering has taken place 1.5-2 years after the fire. In essence then, the complete recovery process of *S. latifolia* takes only two years, a major factor in the ability of the species to survive and, in many situations, become dominant in the understorey of very frequently burnt woodland (Bowen and Pate, 1993).

Sit e No	Location and fire history	Panicl e weight (g)	Vegetati ve weight (g)	Stem length (cm)	No. bisexu al cluster s	No. flowering stems/pla nt
1	Regans Ford, not burnt for 30	2.3	6.9	53.3	6.7	1.0
	years					
2	Regans Ford, burnt Jan 1993	12.4	24.5	102.8	60.9	14.5
3	Mt Adams, burnt 1971	1.7	3.5	45.4	8.9	0.4
4	Mt Adams, burnt 1985 and Jan	5.7	13.6	74.7	33.0	31.3
	1993					
5	Mt Adams burnt 1971 and Jan 1993	7.5	16.8	83.8	39.5	28.3

Table 2. Mean flowering data for *S. latifolia* in September 1994 from sites with different burning histories (Lamont and associates, unpublished data).

Seedlings of *S. latifolia* can recover from fire as young as one to two years of age. However, postfire flowering does not take place until the plant has reached reproductive maturity (possibly up to 10 years of age) (Bowen, 1991). Flowering in *S. latifolia* is an event strongly triggered by fire with little flower production between fires (George, 1984). Initiation of the inflorescence takes place in the first post-fire growing season and flowers open the following spring about 18 months after a summer burn and 12 months after a spring burn (Bowen and Pate, 1993). For example a comparison of inflorescence production in populations of *S. latifolia* with variable fire histories by Lamont and associates (unpublished data) showed a marked contrast between the number of inflorescences produced 18 months after a summer burn (sites 2, 4, and 5; Table 2) compared with plants that had not been burnt for a number of years (sites 1 and 3; Table 2). Stems of marketable quality could only be harvested from the recently burnt sites while inflorescences produced in sites 1 and 3 (23 and 30 years post-fire) were grossly inferior in terms of both yield and quality (Table 2).

In addition, the time of year in which the plants are burnt appears to influence the intensity of production of resprouting shoots and the proportion of these shoots which bear an inflorescence. Bowen (1991) studied four populations of S. latifolia, two burnt earlier in the year (summer/autumn) and two burnt later in the year (spring) and found that individuals burnt in summer or autumn resprouted on average 6-7 shoots from their lignotuber, of which 90% bore inflorescences. In comparison, those burnt in spring produced 4.6 shoots per plant of which only 40-60% bore inflorescences. The compounding effect of these differences resulted in striking differences in the total number of inflorescences produced. For example, of 800 plants of all ages scored in each population, those recovering after summer and autumn burns produced between 5000 and 5800 inflorescence bearing shoots the next flowering season, compared with between 1500 and 2500 for those burnt in spring. Furthermore, plants burnt earlier in the year produced significantly longer inflorescence-bearing shoots than did those burnt later in the year and thus represented a larger proportion of the total shoot biomass. A reasonable explanation for such differences in flowering output is the length of recovery period and thus the availability of resources for inflorescence production. Plants burnt in summer have a complete year of vegetative growth before initiating inflorescences, whereas those burnt in spring have only five months of vegetative growth before commencement of reproduction (Bowen and Pate, 1993).

Once the rapid phases of vegetative and reproductive growth have taken place, *S. latifolia* produces little additional shoot material in the remaining interval between fires and rarely flowers again before the next fire. An example of this can be seen by comparing the low vegetative weight of plants which have not been burnt for many years compared with plants burnt 18 months before weight measurements (Lamont and associates, 1996; Table 2; Bowen and Pate, 1993). Dead remains of reproductive shoots and leaves falling from the plant in the interval between fires builds up to form a skirt of litter around the base of the plant. This dead plant material is likely to provide an ideal substrate for the complete combustion of the any living shoot material in the event of fire, thus encouraging stimulation of the recovery process.

An interesting aspect of the recovery of the species to fire is that ecotypes of certain habitats (eg Yanchep), are single top rooted and non-suckering, and recruit largely, if not exclusively, from seed. Other ecotypes, (eg several populations in Kings Park) form clonal populations over a wide area by root suckering from their extensive roots. Recruitment from seed has not been observed in such clones (Pate, unpublished data).

The development of effective propagation methods for *S. latifolia* may therefore depend on the utilisation of material from the appropriate ecotype.

# **Response to mechanical manipulation**

Reid and Fuss (unpublished data) studied the effect of various mechanical management techniques on *S. latifolia* on a private property at Gingin in an area which had regrowth following an attempt to clear the land for agricultural production. Three management techniques were trialled in 1993 (Table 3). In one paddock one area (Area 'A'), was both bulldozer raked and then skim ploughed

whereas a second area (denoted 'B'), was only bulldozer raked. An adjacent paddock (area 'C') was not heavily wooded and was only skim ploughed in 1993.

June 1990	Virgin banksia bushland was bulldozed and chained.
August 1990	Burnt.
September/October 1991	S. latifolia harvested for the first time.
October 1992	S. latifolia flowered again, but not harvested because of insufficient
	stems.
April 1993	Some areas bulldozer raked, some skim ploughed and other
	combinations of the two. Attempted burn unsuccessful because of
	insufficient fuel.
November 1993	Regeneration after raking and ploughing obvious.
May 1994	Flowering stems budding up.

Table 3. Timing of management techniques used on the Gingin property.

Peak production of flowering stems occurred in 1994 (Table 4). Surprisingly, there was no significant difference in the number of marketable stems per plant from areas A, B or C. In the following year, stem production dropped significantly in all areas by an average of 96.9%. This response was similar to that following fire disturbance noted by Lamont and associates above.

 Table 4. Total numbers of flowering stems from plots with three different histories of disturbance.

Plot	m	g stems per 10 m x 10 plot eplicate plots)	Number of flowering stems in 1995 as a percentage of previous year		
	1994	1995			
Α	376.7	10.3	2.8		
В	183.3	5.3	3.0		
С	487.7	17.0	3.2		

Stem quality also declined over the two years of observation (Table 5). The highest proportion of marketable stems was harvested in 1994 for area C and was significantly different to area A but not area B.

In both years, stem lengths from treatment C were significantly shorter than those from the other two treatments (1994, p<0.001 and 1995, p<0.05) (Table 5.). There was no difference in stem diameter, bloom length, or number of clusters for either 1994 or 1995. Bloom width for area B was significantly greater than that for areas A and C in 1995.

Plot	Year	Stem length (cm)	Stem diameter (mm)	Inflorescen ce length (cm)	Inflorescen ce width (cm)	No. of heads	% marketable
A	1994	67.3	5.00	32.2	9.7	36.3	9.6
	1995	56.5	5.2	22.5	8.1	38.8	3.8
В	1994	69.6	5.2	30.7	10.0	37.9	15.1
	1995	62.6	5.7	26.1	10.9	48.7	16.7
C	1994	62.0	5.2	30.0	9.6	35.4	20.6
	1995	49.6	4.8	18.5	9.6	41.8	2.8

Table 5. Summary of characteristics of flowering stems harvested from sites at Gingin with three different histories of disturbance over two consecutive years.

The major cause of rejection of inflorescences by pickers or exporters was deficiencies in cluster numbers or their positioning in both years (up to 53% in area A) of the study. Area C had a significantly lower rejection rate from cluster deficiencies. There was no significant difference in the rate of rejection from length (ie stems <60 cm) or clusters in combination with length.

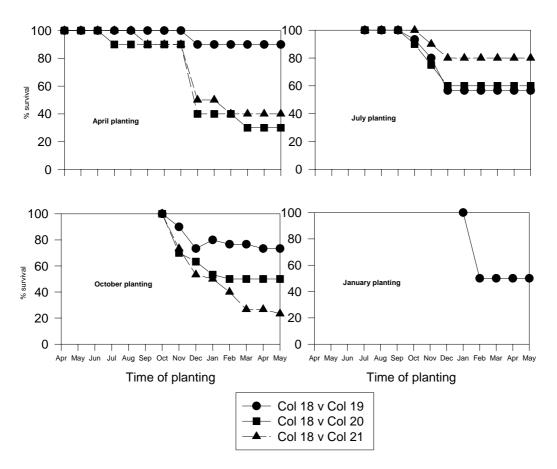
Thus despite the production of up to 49,000 stems valued at \$5,852 per hectare being present, up to \$4,646 (79%) per hectare was lost due to rejection. If the development of new agronomic and/or management techniques could overcome this and the accompanying problem of production decline as is demonstrated in 1995 the potential gain is in the order of over \$9,000 per hectare over two years.

# **Response to cultivation**

# Establishment

In stark contrast to the extremely slow rate of growth from seed in its natural situation (Bowen, 1991), tissue cultured *S. latifolia* plants are capable of reaching one metre in one year from an autumn planting and will normally send up one flower spike which will flower the following spring (18 months from planting) (Reid and Fuss, unpublished data). Winter (July) plantings will also flower at the same time. From these trials it appears as though spring plantings will also flower but at a later date.

The rate of survival from an April planting was superior to July, October or January plantings (Figure 2.). Plant size as reflected by pot type also has a significant effect on survival. Plants from peat pots (mean height 65 mm) and tubes (mean height 96 mm) showed the poorest rate of survival. More mature plants, also planted out from tubes but with an average height of 189 mm established far better and showed far greater resilience to attack by pests, particularly snails (Figure 2). The large number of deaths over all treatments in summer was the result of rabbits digging up plants while trying to access water from drippers.



# Figure 2. Percentage survival of *S. latifolia* planted out at four times of the year from three pot types.

# Pruning

The effect of pruning on *S. latifolia* was examined by Reid and Fuss on plants in a managed stand of regrowth (Table 3). Shoot lengths one year from pruning from plants cut back to ground level were significantly greater at 19.3 cm compared with 5.5 cm for unpruned plants and 8.2 cm for plants pruned back to 50% of their foliage height. However the growth response from these clipping trials is somewhat less than that after a burn and it is evident from these that clipping alone cannot substitute for a burn.

Lamont and associates (unpublished) performed clipping trials where plants were pruned back to ground level at various times of the year and then assessed for flower production in the following year. Although some plants cut back in spring 1994 or January 1995 did flower in the following spring, the numbers of flowering stems per plant and their length were inferior. Even for those plants cut back 18 months earlier, stem length was generally less than 60 cm and the number of bisexual clusters insufficient to constitute a marketable stem.

# Nutrition

Despite the fact that *S. latifolia* is a proteaceous species, nutrition experiments have shown a clear response to fertiliser, especially phosphorus. Over a range of 0 to 120 mg phosphorus (applied as superphosphate) per kilogram of soil, growth peaked dramatically at 40 mg/kg. The results for nitrogen and potassium were less consistent over the experimental range but between 10 to 60 mg potassium (applied as potassium sulfate) /kg soil and 20 to 80 mg nitrogen (applied as ammonium nitrate) /kg soil, growth was greater than that of the control plants.

# The future

For *Stirlingia* to become a profitable crop, several things must happen. Pinching methods to encourage branching at an early age would be desirable, to increase the number of stems per plant in the first and subsequent years of flowering. Limited trials to date indicate that it is possible to obtain more stems in the first year without sacrificing marketability. Early branching would also provide added stability to the plants which tend to topple easily under the weight of only a single flowering stem.

Selection work in the field has shown that a number of pure bud colours exist in *S. latifolia*. Thus the current market for the species in bud could be expanded by the use of single colour lines such as yellow, orange and red. Bicolour buds with combinations of yellow, orange and red might also provide variety and interest.



Orange, red and yellow forms of *Stirlingia latifolia*, photographed at late bud stage. Photo: WA Department of Agriculture

Whilst much of the work reported in this paper is of a biological or ecological nature, it is obvious from the foregoing account, that this species should have a high priority for research of a horticultural nature, especially in terms of how best to optimise production of good flowering populations in natural stands or in cultivation.

# References

Bowen, B. J., 1991. Fire response within the family Proteaceae: A comparison of plants displaying the seeder and resprouter mode of recovery: PhD thesis, Univ. West. Aust., Perth.

Bowen, B. J. and Pate, J. S., 1991. Adaptations of S.W. Australian Members of the Proteaceae: Allocation of Resources During Early Growth. Proceedings of the International Protea Associations Sixth Biennial Conference. 6: 289-301.

Bowen, B. J. and Pate, J. S., 1993. The significance of root starch in post-fire shoot recovery of the resprouter *Stirlingia latifolia* R. Br. (Proteaceae): Annals of Botany, 65:585-601.

Bell, D. T., Hopkins, A. J. M. and Pate, J. S., 1984. Fire in the kwongan: *In* Kwongan: Plant Life of the Sandplain. Eds. J. S. Pate and J. S. Beard, 178-204, University of Western Australia Press, Nedlands.

Bell, D. T., Plummer, J. A. and Taylor S. K., 1993. Seed germination ecology in southwestern Australia. Botanical Review 59: 24-73.

Burgman M. A. and Hopper, S. D., 1982. The Western Australian wildflower industry. Department of Fisheries and Wildlife of Western Australia, Report 53.

Dodd, J., Heddle, E. M., Pate, J. S. and Dixon, K. W., 1984. Rooting patterns of sandplain plants and their functional significance. *In*, Kwongan: Plant Life of the Sandplain. Eds. J.S. Pate and J.S. Beard, University of Western Australia Press, Nedlands.

George, A. S., 1984. An Introduction to the Proteaceae of Western Australia. Kangaroo Press, Australia.

Lamont, B. B., Witkowski, E. T. F. and Enright, N. J., 1993. Post-fire microsites: safe for seeds, unsafe for seedlings. Ecology, 74: 501-512.

Ladd, P. G. and Wooller, S. J., 1996. Explaining variation in pollination and seed set in an andromonoecious genus of the proteaceae. Paper presented at the 7th International Pollination Symposium, Lethbridge, Canada in June 1996.

Pate, J. S., Froend, R. H., Bowen, B. J., Hansen, A. and Kuo, J., 1990. Seedling growth and storage characteristics of seeder and resprouter species of mediterranean-type ecosystems of S.W. Australia. Annals of Botany, 65: 585-601.

Recher, H. F. and Christensen, P. E., 1981. Fire and the evolution of the Australian biota: *In* Ecological Biogeography of Australia. Ed. A. Keast, 137-162, Dr W. Junk Publishers, The Hague, Boston, London.

Stock, W. D. and Lewis, O. A. M., 1986, Soil nitrogen and the role of fires as a mineralizing agent in a South African coastal fynbos ecosystem: Journal of Ecology, 74: 317-328.

# B. Propagation of Stirlingia latifolia.

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### Introduction

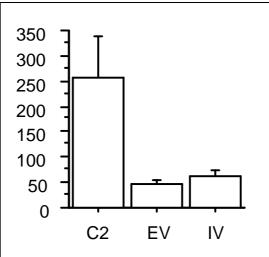
This report details progress made in defining the best propagation method for producing clonal plants of *Stirlingia latifolia*. The aim was to test conventional cuttings against *in vitro* produced plants and produce some basic morphological data to help formulate a better understanding of growth, particularly with regard to root production in the early stages of propagule development.

### Materials and methods

Cuttings were taken from plants regenerating after a fire, giving the best available cutting material. Earlier trials with material from unburnt plants did not produce any rooted cuttings. *In vitro* plants were either rooted *in vitro* then acclimatised and transplanted to soil or transferred to soil as unrooted plantlets. Auxin containing gel was used to stimulate rooting in cuttings and unrooted *in vitro* plantlets. *In vitro* rooted plants were induced to form roots on a standard rooting medium. All propagules were placed in a standardised potting mixture and humidity controlled during the first two to three weeks using vented plastic canopies. After propagules were seen to be rooting canopies could be removed. Number and length of roots, fresh and dry weights of whole plants, shoots and roots of all propagules were taken after 7 weeks. Data was analysed using a statistical program.

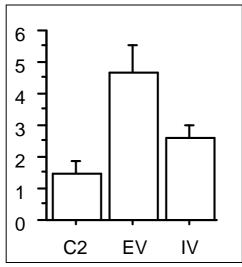
## Results

Cuttings began as larger propagules than *in vitro* propagules, hence fresh and dry top weights were much higher at the end of the experiment. Dry weights tops of *in vitro* rooted and unrooted plantlets were approximately the same (Figure 1). The number of roots per plantlet was higher with starting material of unrooted *in vitro* microshoots (*extra vitrum*) than either *in vitro* rooted shoots or standard cuttings (Figure 2). The average lengths of roots per plantlet was also highest in starting material of unrooted microshoots than for cuttings or *in vitro* rooted plants (Figure 3). The dry weights of roots of plants of cuttings and unrooted *in vitro* shoots as starting material are similar, but the dry weight of roots of *in vitro* rooted shoots is highest although highly variable (Figure 4). Root induction of cuttings varied from a best of 25 - 64 %, while 100% of unrooted *in vitro* shoots produced roots after auxin treatment. Over 80% of *in vitro* shoots initiated roots on root induction medium.

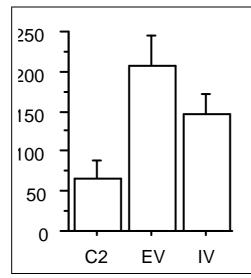


**Figure 1.** Dry weight of shoots per established propagule from cuttings (C2), *extra vitrum* rooted microshoots (EV) and *in vitro* rooted shoots (IV) of *Stirlingia latifolia*.

Y axis indicates the dry mass in mg of shoots per propagule. Standard errors of means are indicated.

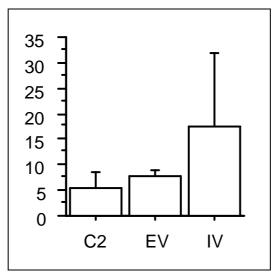


**Figure 2.** Number of roots per established propagule from cuttings (C2), *extra vitrum* rooted microshoots (EV) and *in vitro* rooted shoots (IV) of *Stirlingia latifolia*. Y axis indicates the number of roots per propagule. Standard errors of means are indicated.



**Figure 3.** Length of roots per established propagule from cuttings (C2), *extra vitrum* rooted microshoots (EV) and *in vitro* rooted shoots (IV) of *Stirlingia latifolia*.

Y axis indicates the mean length in mm of roots per propagule. Standard errors of means are indicated.



**Figure 4.** Dry weight of roots per established propagule from cuttings (C2), *extra vitrum* rooted microshoots (EV) and *in vitro* rooted shoots (IV) of *Stirlingia latifolia*. Y axis indicates the dry mass in mg of roots per propagule. Standard errors of means are indicated.

#### Discussion

Several interesting results have emerged from the data. The first is that the type of cutting material is a key factor in achieving success with cuttings of Stirlingia latifolia. Old growth material is virtually useless as cutting material with zero strike rate while young soft growth following a fire is capable of initiating up to 60% rooted cuttings, although there is considerable variability in strike rates in this trial. The second is that root induction with in vitro produced material is far superior to cutting material. Stirlingia shoots rooted in vitro or extra vitrum also root more consistantly compared to cuttings. The third factor is that although the dry weight of roots from in vitro rooted shoots is higher than for in vitro shoots rooted extra vitrum, the number of roots and length of roots per propagule is considerably less, indicating that fewer, thicker roots are produced *in vitro*, in contrast to more numerous but thinner roots (with less biomass) produced on microcuttings. The roots produced extra vitrum are more normal in appearance i.e. thinner with numerous roots hairs and branching while in vitro roots tend to be thicker and root hairs although present tend to be gummed up by residual agar clinging to the roots after transplanting. Such problems have been noted with other species and rooting microcuttings extra vitrum has been suggested as a means for overcoming these problems. Extra vitrum rooting appears to be a viable alternative with the material of Stirlingia latifolia as tested. Earlier work by Bunn and Dixon (1992) on micropropagation of S. latifolia also successfully used an extra vitrum method for establishment of unrooted microcuttings in soil.

## Conclusions

Based on this study the choice is to use cutting material from new sprouting material of *Stirlingia latifolia* following a fire or perhaps other disturbance. Or better still use *in vitro* shoots, preferably unrooted as the number of roots per propagule following auxin treatment appears to be superior to *in vitro* rooted shoots and much better than conventional cuttings. Clonal differences may have a bearing on success with this species and further testing on different populations of *Stirlingia latifolia* would be advisable before commitment to procedures followed in this study.

## References

Bunn E, Dixon KW. (1992). Micropropagation of *Stirlingia latifolia* (Proteaceae), an important cut flower from Western Australia. HortScience 27(4):368.