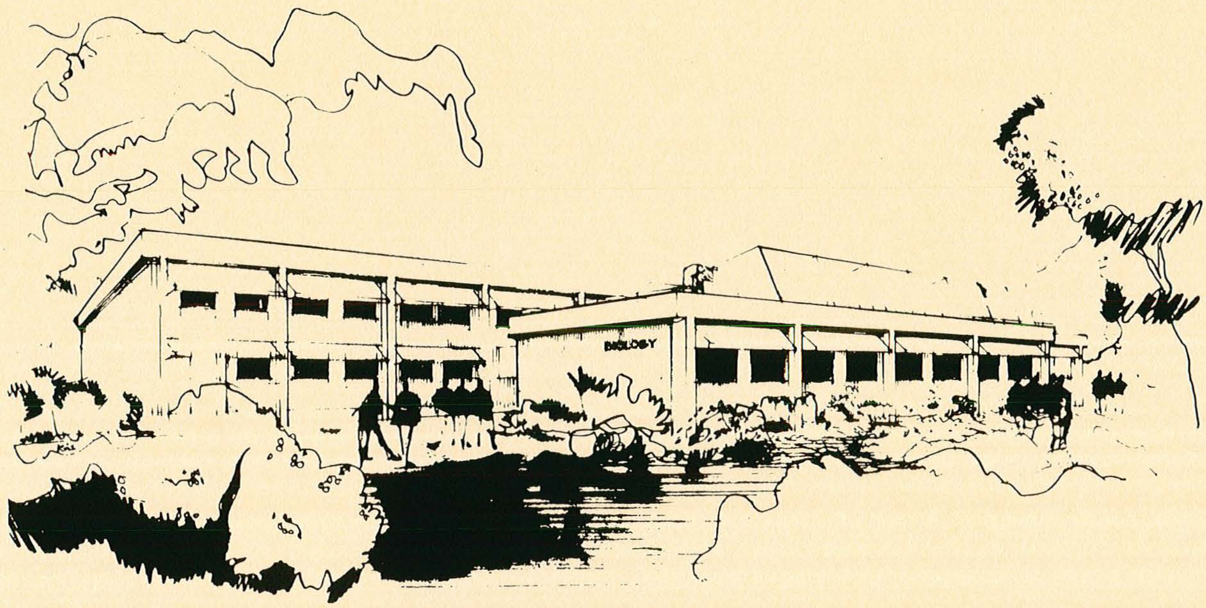
curtin
University of Technology
Western Australia

School of Environmental Biology

GRASSTREE PROJECT MEETING

Report to Department of Conservation and Land Management



Wendy Colangelo (Editor)

July 1998

28 pp.

Grasstree Project Meeting

Report to Department of Conservation and Land Management

Wendy Colangelo (Editor)

July 1998

28 pp.

TABLE OF CONTENTS

GRASSTREE PROJECT MEETINGS

Wednesday 24 June, 9.00 am in Biology Building, 311:118 (Boardroom) and (as necessary) Wednesday 8 July.

Agenda for 24 June

9.00 am	Introduction and announcement of Grasstree Project Management Committee	Byron Lamont
9.15 am	Background to the study and validation of the technique	David Ward
9.45 am	Mineral nutrient distribution relative to fire and season	Chantal Burrows
10.15 am	Mineral nutrient cycling in relation to fire	Roy Wittkuhn
10.30 am	Break	
11.00 am	Histological aspects	Wendy Colangelo (nee Leppard)
11.15 am	What stable isotypes can tell us	Perry Swanborough
New Projects:		
11.45 am	1. Biomass and water cycling (morphology, phenology) relative to fire and season 2. Population Biology	Byron Lamont
12.00 noon	3. Minesite rehabilitation	John Koch
12.10 noon	Lunch	
1.00 pm	Discussion	
3.00 pm	Finish	

Announcement of Grasstree Project Management Committee

Members include:

Byron Lamont (Chair)	Curtin University
Neil Burrows (deputy Rick Sneeuwjagt)	CALM
Lachi McCaw	CALM
David Ward	CALM
Jonathan Majer	Curtin University

Curtin University & Dept of CALM

Outline of Presentation to Grasstree Project Meeting by David Ward

Wednesday 24 June 1988, 9.15 to 9.45 am

A sequence of overheads was presented with discussion of:

1. Fire is a major process in the natural systems of south-western Australia, linking plants, animals, soils, nutrition, Aboriginal studies etc. Grasstree research can help us to understand past fire regimes, and this is of importance for conservation.

2. The potential size and age of grasstrees, illustrated by a 5 metre specimen from Augusta golf course, hence their potential as historical records.

3. The flammability of grasstree thatch. Most grasstrees will burn when an area is burnt, with the exception of very tall ones.

4. When the top of a recently burnt grasstree is ground off, a black band of leafbases is revealed at the junction of the live and dead leaves. All evidence so far suggests that this is the result of some green leaves burning.

5. A cleaned grasstree stem to show the method of ageing and fire dating.

6. A cleaned stem showing a typical fire pattern for the current era in the jarrah forest, i.e. fires at about 10 year intervals.

7. A cleaned stem showing the typical fire pattern for the pre-European era in the jarrah forest, i.e. fires at about 3 year intervals.

8. A map of the south-west, showing the jarrah forest with fifty sample points. At each point, several grasstrees were cleaned, and the fire history reconstructed.

9. A graph, derived from the above sample points, of fire frequency since 1750.

10. An example of a letter written in 1846 by John Corbett Singleton, an Irish magistrate at Dandalup. This was part of a revealing correspondence between the Governor, senior public servants, and magistrates on the subject of bushfires lit by Aborigines. This correspondence led to the first Bushfire Act of 1847, which aimed, through public flogging and imprisonment on Rottnest Island, to discourage the Aborigines from firing the bush for hunting. This was followed up by handouts of flour and blankets by the Government to compensate for the loss of meat and skins to the Aborigines.

11. A summary of the main points made by Singleton. These are a) that the coastal plain near Pinjarra was burnt biennially b) that the fires were lit by Nyoongars in windy conditions c) that the fires killed many native animals d) that the grazing for European stock was best the year after a fire e) that setting fire to hollow trees was a common method of catching possums, and caused many fires.

12. Two graphs of Aboriginal prisoner numbers on Rottnest 1830-1930, suggesting, *prima facie*, a decline in traditional burning, or Nyoongar population, or both, in the south-west. This needs treating with caution until further research can be done into the possible effects on Rottnest prisoner numbers of the opening of regional prisons, summary local floggings, police numbers and distribution etc.

13. An outline of the John Forrest National Park Project on fire & landform. The first year (1998) will be spent mainly on research into the social, including Aboriginal, history of the park; the second year (1999) on the natural history of the park; and the third year (2000) on integrating these two aspects with the fire history reconstructed from grasstree fire marks.

14. A suggestion for a new technique of grinding the tops off grasstrees, so enabling more accurate observation and measurement of needle growth rates, longevity, and emigration. This should provide fundamental information on the growth of grasstrees, which may be useful, for example, in nutrient studies.

15. A picture of the intriguing spiral phyllotaxy of grasstrees, which can be mimicked by a mathematical model combining the pentagram of Pythagoras (*ca.* 500 B.C.), the spiral of Archimedes (*ca.* 287 BC-212 BC), and the sequence of Fibonacci of Pisa (*ca.* 1180-1250). This model might make a suitable paper for the journal *Biometrika*, and will also help with a fundamental understanding of grasstree growth. Further, the *spira mirabilis* of Jacques Bernouilli (1654-1705) will be investigated for relevance (this may necessitate funding of a trip to Basel in Switzerland for careful examination of this spiral, which is reputedly engraved on his tombstone...)

ACTION STATEMENT

Over the coming year, David Ward to:

1. Continue work on John Forrest National Park study.
2. Write paper on a mathematical model of spiral phyllotaxy for Biometrika.
3. Write paper on Nyoongar population decline for an historical journal.
4. Resubmit paper (with Byron) on grasstree flowering probability to J. Roy. Soc. WA.
5. Supply Byron with extra graphs for Intecol conference at Florence, July 1998.
6. Help Chantal to get extra samples from the Amphion stem for chemical analysis.
7. Help Wendy to obtain any samples she may need for histological analysis.
8. Clean grasstrees at Eneabba with Byron, to see if past fire frequency matches with mathematical model of Ulrich based on *Banksia* flowering.
9. In co-operation with Roy and Perry, explore applications of the grasstree top grinding technique for understanding grasstree needle growth, longevity etc.
10. Opportunistically collect specimens of wasps, bees, bardis, scorpions, cockroaches etc. which seem to have intimate associations with grasstrees (for Jon Majer)

-oOo-

Grasstrees as bioindicators of the present and historical chemical environment

Summary of presentation by Chantal Burrows

A new technique for ageing grasstrees and determining their fire history was examined in this study. It was concluded that paired light and dark brown coloured bands along the length of *Xanthorrhoea preissii* Endl. stems were consistent with annual growth cycles, and that additional black bands were formed during the passage of fire. The coloured bands were composed of closely packed persistent leaf bases. By assessing the number of pairs of light and dark browns bands, and the frequency of black bands, it was possible to calculate the frequency of fire during the last 100 years. An overall decrease in the frequency of fire was revealed over this period. Fire frequencies in the 1890's and 1900's ranged from 3 to 4 fires per decade, this decreased to 0 to 1 per decade in the 1970's and 1980's.

The nutrient composition of leaf bases within each successive colour band along three *X. preissii* stems was analysed in an attempt to reveal long-term patterns of nutrient cycling. The innermost and outermost cm sections of the remnant leaf bases were analysed separately and usually showed different patterns, the innermost highlighting fire effects and the outermost seasonal effects. The leaf base concentrations of S, K, B, Mn, Zn and N trended downwards with the passage of time, while Ca remained relatively static. Statistical analyses confirmed significant, but temporary, increases in tissue concentrations of Ca, Mn and Zn after fire. The leaf base concentrations of Mg decreased temporarily in response to fire. Significant winter/spring peaks in the leaf base concentrations of Ca, Mg and Mn were revealed. A significant summer/autumn peak in S concentrations was recorded. No consistent pattern of variation was displayed for the concentrations of Fe and Cu within the remnant leaf bases. The concentrations of P and Na did not consistently fluctuate above trace levels. It was concluded that the distribution of certain nutrients may be used to identify and confirm the existence of annual growth fluctuations and fire events, and to reveal nutrient cycling patterns over an extended time period.

Mineral Nutrient Cycling in Relation to Fire

Summary of presentation by Roy Wittkuhn

Tentative Project Title:

Mineral Nutrition of *Xanthorrhoea preissii* in the south-west of Western Australia in relation to season, fire and soil fertility.

Introduction

The work of Chantal Burrows in the mineral content of dead leaf bases on the trunks of *Xanthorrhoea preissii* has shown great promise in verifying the ageing technique of the plants proposed by (Ward 1996), who adapted a technique proposed by (Lamont and Downes 1979). My contribution to the Grasstree Project is to expand on a lot of what Chantal has done, and to try and show and explain patterns of nutrient distribution in the grasstree. This involves the analysis of living tissue as well as continuing with the analysis of dead leaf bases.

As I am in the early stages of my PhD, this chapter contains many questions, thoughts and ideas that I believe must be considered when addressing the nutrition of *X. preissii.*

Environmental Factors Influencing the Mineral Nutrition of *X. preissii*

There are environmental factors that influence the mineral nutrition of the grasstree, and may influence the distribution and allocation of nutrients within the plant. The three predominant environmental factors that will influence the nutrition of grasstrees are season, soil fertility and fire. These factors will interact and affect mineral form and availability in the soil for plant uptake. It is likely that they also affect the internal cycling of nutrients in *X. preissii.*

Seasonal Factors

The mediterranean climate of the south-west of Western Australia has a distinct wet and dry season, with winter/spring the wet season and summer/autumn the dry. Water availability in the shallow soil is greatest over the wet season, and this means that there is likely to be greater nutrient uptake over this period than in the dry season.

The growth patterns of *X. preissii* in drought conditions are not known in great detail, and this is one of the objectives of this study. Questions that I believe need to be addressed during the course of my PhD and that are related to the influence of season on mineral nutrition are:

1. is there significant nutrient uptake over the drought period (summer/autumn)?
2. is there significant leaf growth and initiation during the drought, and do those leaves initiated during the winter/spring have higher nutrient concentrations than those initiated during summer?
3. is there retranslocation of nutrients from dying leaves over the drought, and if so, to which parts of the plant do the nutrients go?

Soil Fertility

The soils in which *X. preissii* grow have a low fertility in comparison with soils around the world (Lamont 1983). Most native species have adapted strategies to survive in such a nutrient impoverished environment, but little is known about *X. preissii.*

Individual plants can live for hundreds of years (Lamont and Downes 1979; Ward 1996), and this raises questions of the mechanisms for nutrient uptake. How do these plants cope in the poor soils? Although it is unlikely that I will study root anatomy or morphology, I may conduct fertilisation experiments to provide some clues on nutrient uptake. If another PhD student begins next year, they may study root morphology which may link in with my work.

Fire

X. preissii is one of the first species to resprout after fire (Baird 1977). Fire also seems to stimulate flowering of the grasstree, with the inflorescence produced in the first season following a burn (Baird 1977; Lamont and Downes 1979). With these observations come many questions:

1. is there a flush of growth of leaves after fire, and if so, what is the source of the nutrients that permits the flush? Is the flush from within the soil, or does it come from stored nutrient within the plant? In order to answer this, I will compare rates of leaf growth and nutrient concentrations between burnt and unburnt plants.
2. is there any difference in nutrient concentrations of newly initiated leaves and roots in burnt plants compared to unburnt plants?
3. is it the increase in soil nutrient availability after fire that permits the production of the inflorescence in *X. preissii*? It may be internal nutrient reserves, or starch that is stored in the caudex.

Interactions

The environmental factors outlined above all interact to influence the nutrition of *X. preissii*. Season affects water availability in the soil, and since mineral nutrients are absorbed by plants in the aqueous phase, season affects soil fertility. Season will affect the intensity of fires. Prescribed burning by CALM is conducted either in spring or autumn. Spring burns are generally cooler than autumn burns because the burn is conducted at the end of winter when vegetation and litter have relatively high water contents. Fire affects the nutrient status of the soil. Fire mineralises many organic nutrients, making them directly available for plant uptake (Adams and Attiwill 1986; Bauhus *et al.* 1993). It also volatilises nutrients such as nitrogen, removing them from the nutrient cycle (Hingston *et al.* 1980/1981).

Interactions such as those outlined above must be considered when addressing many of the questions proposed under each section.

Following is a brief outline of the factors that I believe need consideration when looking at the plant in isolation.

Plant Component Parts

Leaves and Leaf Bases

In light of Chantal Burrows' work on the mineral content of leaf bases, living tissue from leaves and leaf bases is important to consider in an attempt to understand mineral accumulation and movement. This may help to verify the ageing technique developed by (Ward 1996).

I have begun an experiment to observe the differences in leaf nutrition with respect to fire and season. I am looking at two environments:
1) Darling Plateau (laterite)
2) Sand over limestone near Yanchep.

I have burnt the Darling Plateau site already, and am waiting on a few days of fine weather and good winds to burn at Yanchep. This is supposed to be an autumn burn, and although late, I will push ahead with it, since it may still provide some results and will allow me to trial several tagging and collection techniques. I will be observing the growth and nutrition over time since fire, comparing control (unburnt) sites with burnt sites. I will also conduct spring burns for a comparison, and will also burn in the next autumn to get a hotter and more typical autumn prescribed burn.

The monitoring will continue for approximately three years, during which time I will take leaf samples and analyse living blades and bases for nutrient contents.

Stem (caudex)

I believe that the inner stem or caudex may show some seasonal patterns of mineral distribution, since the caudex contains many scattered vascular bundles which transport nutrients through the plant. If nutrient uptake during the dry season is low (summer/autumn), then nutrient content may also be lower. I will need to take samples from the caudex at different times of the year, and analyse for nutrient content.

Roots

The roots may show the same pattern as the stem if there is less nutrient uptake over the dry period. I will need to distinguish between deeper roots that would function mainly as water pumps, and lateral surface roots that would be involved in both water and nutrient uptake. I will probably work closely with the other PhD student on the root morphology and water uptake efficiency to help explain nutrient patterns that may be evident.

Inflorescence

The inflorescence is a very fast growing compared to other parts of the plant, and must be a huge drain on the plant from a nutrient perspective.

The main question that I want to ask is: where does the nutrient come from to allow the growth of such a large organ? I would also like to know whether there is any retranslocation of nutrients back to the plant after seed set.

Dead Leaf Skirt

The dead leaf skirt can be very long and thick on plants that have not been burned for a long period of time. It appears to be an adaptive feature to give the plant every chance of being burned when a fire passes through; thus giving it a better chance of flowering.

From a nutrient perspective, however, I wonder if the skirt acts as a "slow-release fertiliser" for some of the nutrients such as nitrogen and phosphorus. I will be collecting the dead skirt from sites with varying fuel load ages to determine if there is a close relationship between time since burn and (a) mass of dead leaves in the skirt; and (b) total nutrient content within the skirt. I have begun planning an experiment to determine whether nutrients leach out of the dead leaves.

Conclusion

This report has consisted of questions as well as thoughts and ideas that I believe need to be addressed as part of my topic on the mineral nutrition of *X. preissii*. I am in the process of devising methodology for the various aspects of the project, so that each part can be discussed in isolation as well as in relation to other aspects.

References

Adams, M. A. and Attiwill, P.M. (1986). "Nutrient cycling and nitrogen mineralization in eucalypt forests of south-eastern Australia. II: Indices of nitrogen mineralization." Plant and Soil **92**: 341-362.

Baird, A. M. (1977). "Regeneration after fire in King's Park, Perth, Western Australia." Journal of the Royal Society of Western Australia **60**(1): 1-22.

Bauhus, J., Khanna, P.K. and Raison, R.J. (1993). "The effect of fire on carbon and nitrogen mineralization and nitrification in an Australian forest soil." Australian Journal of Soil Research **31**: 621-639.

Hingston, F. J., Dimmock, G.M. and Turton, A.G. (1980/1981). "Nutrient Distribution in a jarrah (*Eucalyptus marginata* Donn ex Sm.) ecosystem in south-west Western Australia." Forest Ecology and Management **3**: 183-207.

Lamont, B. (1983). Strategies for maximizing nutrient uptake in two mediterranean ecosystems of low nutrient status. Ecological Studies: Mediterranean-Type Ecosystems. Eds: F. J. Kruger, Mitchell, D.T. and Jarvis, J.U.M. Berlin, Springer-Verlag. **43**.

Lamont, B. B. and Downes, S. (1979). "The longevity, flowering and fire history of the grasstrees *Xanthorrhoea preissii* and *Kingia australis*." Journal of Applied Ecology **16**: 893-899.

Ward, D. (1996). Reconstructing pre-European fire history in south-west Australian forests. 13th International Conference on Fire and Forest Meteorology, Lorne, Victoria, Australia.

Histological Aspects of Grasstree Project

Summary of presentation by Wendy Colangelo

My part in the Grasstree project is to determine the anatomical basis and origin of the difference in colouration between the cream, brown and dark brown bands seen on the leafbases.

David Ward has suggested the compound responsible for the dark colouration in the fireband tó be lapachol. Lapachol was found to be present in the vascular bundle of the dark leaf base when isolated.

It is therefore my aim to section leaf bases from the three different bands to determine a difference in colouration at the light microscope level and if accumulation occurs for example in the vascular bundles.

Xanthorrhoea leaves are crowded in a terminal rosette, narrowly linear and rhombic in cross-section. The leaf bases remain on the trunk after the narrow blade has fallen.

Little work has been done on the anatomy of leaf bases in Grasstrees. However, Staff and Waterhouse (1981) published this LM photo of a transverse section of *Xanthorrhoea preissii* leaf.

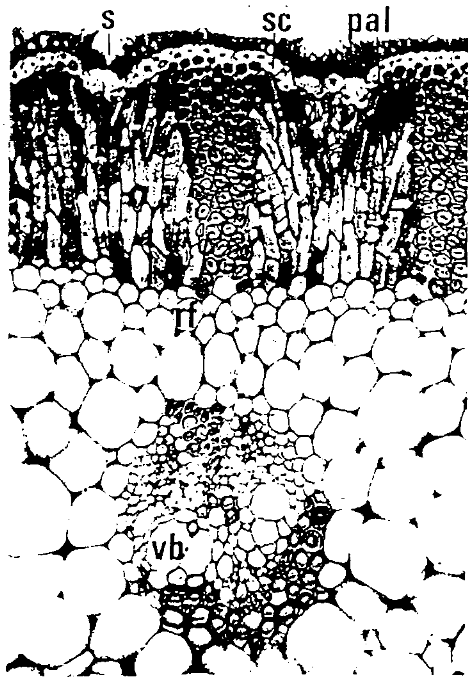


Figure 1. Transverse section of *X. preissii* leaf showing vascular bundle and reaction fibres (Mag x160) . From Staff and Waterhouse (1981).

Figure 1 shows a sunken stomata, reaction fibres in sclerenchyma girders (whose role it is to re-orientate the leaves) and the vascular bundle. A distinguishing characteristic of Xanthorrhoea is a cross-section of the irregularly scattered vascular bundles shows the xylem is V-shaped with 2 phloem groups present, each situated at the end of either xylem arm.

Staff and Waterhouse made no mention of their histological technique so I decided to trial various techniques.

In my first trial, I attempted to section fresh leaf bases using a sledge microtome. This cuts sections approximately 10 microns thick. This failed as the leaf base when cut created shavings rather than a section.

Next, I tried embedding leaf bases in wax. 5 x 1cm sections taken from the 3 different colour bands were embedded in wax. The duration of this trial was 1 week.

Unfortunately, the wax did not penetrate the resinous exterior of the leaf base so when I attempted to cut 10 micron sections, a whole was left in the wax in the shape of the specimen.

After these initial trials, I conferred with Dr John Kuo Physics UWA, Dr Stewart Chew Bio-Med Science and Lydia Kupsky Curtin University. All three agreed that using a resin GMA glycol methacrylate suitable for light microscopy was my most likely method for successfully embedding the leaf bases so that sections may be cut.

However, even though I have from John Kuo a protocol for tissue fixation, dehydration, infiltration and embedding for botanical tissue, I need to adapt this to suit the Grasstree leaf base which will require trial and error.

I am currently performing my first trial using GMA. I have taken three leaf bases; one from a fireband,one cream (winter/spring) and one brown (summer/autumn).

They were cut into 5 x 1cm sections than cut in 1/2 longitudinally to reduce the size of the specimen and hasten infiltration.

The material is noticeably dry so I reduced John Kuo's dehydration protocol by doing only 2 full days in 2 different ethanols rather than his 4 different ethanols for 3 days each.

On Day 3 the samples were placed in GMA and left for 5 days agitating in a fumehood.

On Day 7 and 14, the GMA was replaced with fresh GMA, vacuumed to aid infiltration and left agitating for another week.

On Day 21, I embedded 2 samples, one from the cream band and one from the brown band. I was not able to embed a section from the fireband as all sections were still floating indicating enough air had not been removed. The samples will need to be vacuumed until they are properly infiltrated and than embedded for sectioning.

Since initiating this first trial in GMA I have found an earlier paper written by Staff(1974). Staff was the first to report the finding of reaction fibres in the leaves of the arborescent monocotelydon *Xanthorrhoea australis.* Reaction fibres occur predominantly near the abaxial surface of the leaves in a position just above the broadened leaf bases. Their role is to orientate the leaf for efficient photosynthetic activity.

More importantly, Staff describes his method of sectioning Xanthorrhoea. It is important to note Staff's sample area is in the region marked "x" on Figure below. Fresh frozen sections were cut 10 microns thick using a sledge microtome. This method is worthy of a trial.

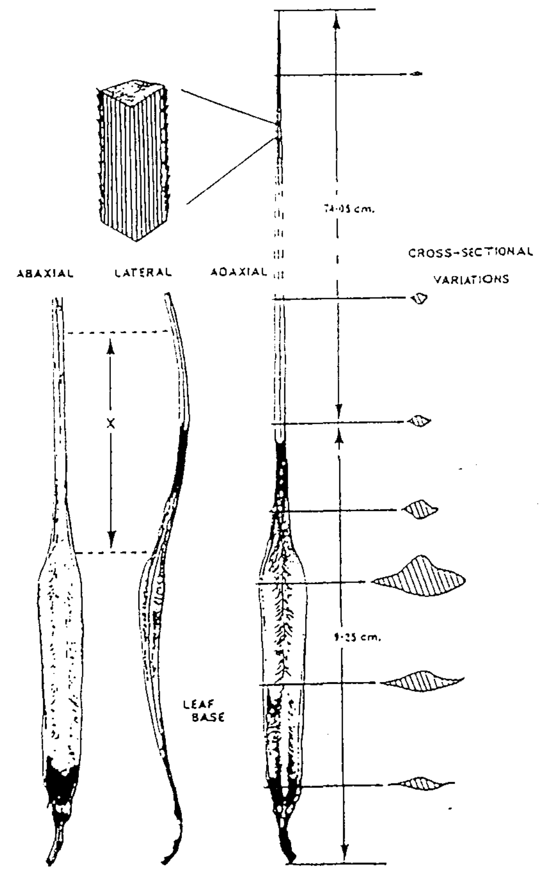


Figure 2. Diagram of mature *X.australis* leaves illustrating differences in morphology and dimensions between leaf base and photosynthetic portion of the leaf. "X" denotes Staff's area of study.

In summary, I will continue with the GMA trial until embedding of all sections is completed and also attempt to cut frozen sections. Once I have established a successful technique for leaf base sectioning, the extension of its use into different facets of the grasstree project will be possible.

References

Staff, I.A. and Waterhouse, J.T. (1981). The Biology of Arborescent Monocotyledons, with Special Reference to Australian Species. In the Biology of Australian Plants, Chapter 8; 216-257 (eds. J.S. Pate and A.J. McComb) University of Western Australia Press, Western Australia.

Staff, I.A. (1974) The Occurrence of Reaction Fibres in *Xanthorrhoea australis* R.Br. Protoplasma, **82**, 61-75.

What stable isotopes can tell us.

Summary of presentation by Perry Swanborough, Grasstree Research Group, School of Environmental Biology, Curtin University of Technology.

We are all aware that CO_2, at about 0.035% by volume of the atmosphere, is an important atmospheric component, being the carbon source for sunlight-driven photosynthetic carbon fixation which is the fundamental energy source for the biosphere. However, we are usually less aware that the biospheric CO_2 pool is a mixture of several different stable and unstable (i.e. radioactive) isotopes. For example, carbon in the biospheric pool exists as three different isotopes. The most common one is ^{12}C (about 98.89% of atmospheric CO_2), which coexists with ^{13}C (another stable isotope, about 1.11%) and traces of ^{14}C, a radioactive isotope familiar to us as the one commonly used to date organic material in radiocarbon dating. Due to the variation in mass of CO_2 molecules comprised of these different isotopes, many biospheric processes favour $^{12}CO_2$ over the slower-diffusing heavier alternatives. An example of this which has potentially important implications for environmental science and ecology is the discrimination favouring $^{12}CO_2$ over $^{13}CO_2$ by photosynthetic carbon fixation (Farquhar *et. al.*, 1982). This discrimination has two components. Physical diffusion discrimination against $^{13}CO_2$ occurs when carbon dioxide diffuses from the atmosphere through leaf stomata and intercellular spaces to the carboxylation sites within leaf chloroplasts. A further chemical discrimination process favours $^{12}CO_2$ at the carboxylation sites in the chloroplast matrix where reduction of CO_2 to ribulose bisphosphate (the first step in the so-called "dark reactions" of photosynthesis) is catalysed by ribulose bisphosphate carboxylase (rubisco).

Given that photosynthetic processes discriminate against ^{13}C, it follows that discrimination will vary with rate changes in these processes. In the northern hemisphere, ^{13}C concentration has been observed to oscillate seasonally. The oscillation correlates with seasonal growth rate oscillation as photosynthetic rates vary. Superimposed on the seasonal oscillation is a consistent long-term decline in the ^{13}C relative concentration as large-scale combustion of ^{13}C-depleted fossil fuels, which are products of past photosynthesis, continues (Flanagan and Ehleringer, 1998).

An encouraging observation for us in the southern hemisphere is the correlation of isotopic ratio in cellulose of *Kingia australis* R.Br. with the annual temperature oscillation. This demonstrates that studies of isotopic ratios in plant material can potentially allow us to make reliable inferences about past growing conditions. With records of past occurrences of fire, we can potentially correlate fire occurrence with vegetation growth rates inferred from isotopic discrimination measurements.

Two points about isotopic discrimination are relevant to how it is expressed. First, determination of isotopic discrimination within a plant is integrative, i.e. the result obtained is the net consequence of discrimination over the entire prior history of the plant (or plant component), and therefore cannot be associated with a particular moment in time as other kinds of measurements (eg. net photosynthetic gas exchange rate measurements) usually are. Second, photosynthetic isotopic discrimination occurs relative to the air source present during the discrimination process. Given that the isotopic composition of the atmosphere varies seasonally and over the long term, and is also influenced by local conditions (eg. high photosynthetic rates within a rainforest canopy near midday may locally raise the relative concentration of ^{13}C in the surrounding air), it is necessary to determine isotopic discrimination relative to a reliable standard. Therefore, ^{13}C discrimination is calculated relative to the Pee-Dee Belemnite (PDB) standard, a geological carbonate deposit with a highly homogeneous composition of carbon isotopes. The result for ^{13}C discrimination is given as the ^{13}C concentration difference (in parts per thousand) between the sample and PDB (Griffiths, 1991).

Isotopic discrimination measurements are expressed by the quantities δ or Δ (Farquhar *et. al.*, 1982) :

$$\Delta = a + (b - a) * p_i/p_a \qquad (1)$$

where a is the coefficient of diffusion discrimination (4.4‰), b is the coefficient of carboxylation discrimination(27‰), and p_i/p_a is the ratio of intercellular CO_2 to external CO_2 partial pressure.

Δ is a positive quantity, with increasingly positive values representing increasing discrimination against ^{13}C.

Alternatively,

$$\delta = (a - b) * p_i/p_a - a \qquad (2)$$

where the components are as given above.

δ is a negative quantity, with increasingly negative values representing increasing discrimination against ^{13}C.

The relationship between plant growth rate and isotopic discrimination may only be potentially definable, as isotopic discrimination is a direct consequence of photosynthetic processes, but the net growth rate of a whole plant is not exclusively a function of the leaf photosynthetic rate. The rate of dry matter addition to a whole plant is more comprehensively defined by the net photosynthetic rate(s) of the leaves integrated over the total plant leaf area. It follows that a plant with a modest-sized crown of strongly photosynthesizing foliage might not accumulate dry matter at a rate exceeding that of a plant with much more leaf area photosynthesizing at a lower rate.

However, a physiological attribute which appears to be more strongly related to isotope discrimination is the ratio of net assimilation rate to transpiration, or water use efficiency (WUE)(Farquhar and Richards, 1984). At high WUE, a relatively high rate of carbon assimilation is accompanied by relatively low stomatal conductance. Under these conditions, p_i is drawn down by high assimilation, and is not readily replenished from the atmosphere due to the high stomatal resistance to movement of external air into the leaf. Consequently, the p_i/p_a ratio in the intercellular space of the leaf is low. It is readily seen from equation (1) above that under these conditions (high WUE), Δ is relatively low. As WUE declines, it is similarly apparent that Δ increases as p_i/p_a increases (Evans *et. al.*, 1986). If we define discrimination by δ instead of Δ, and accordingly refer to equation (2), it can be readily seen that low WUE (i.e. high p_i/p_a) corresponds to a highly negative δ value which becomes less negative as WUE improves.

The relationship between WUE and isotope discrimination has been applied ecologically to a range of *Nothofagus* species (Read and Farquhar, 1991). Although interpretation of isotope discrimination results can be complicated by the effects of a range of physiological processes (eg. discrimination associated with translocation of carbon compounds between tree components), a clear conclusion was that *Nothofagus* species in winter rainfall regions tend to have high ^{13}C discrimination, indicating lower WUE compared with *Nothofagus* species in other regions.

Biospheric discrimination between isotopes of elements other than carbon may also be of use in environmental science and ecology (eg. Donovan and Ehleringer, 1994; Pendall and Leavitt, 1996). Water molecules comprising heavy isotopes of hydrogen and/or oxygen diffuse more slowly than the common lightest alternative ($^1H\ ^1H\ ^{16}O$), so high rates of evaporation and transpiration can result in an increasing concentration of heavy water molecules in plant tissue and soils. Monitoring the isotopic composition of water in plants and soils therefore may be useful for determining long-term changes in precipitation, transpiration and evaporation (Griffiths, 1991).

References

Donovan, LA and Ehleringer JR (1994). Water stress and use of summer precipitation ia a Great Basin shrub community. *Funct. Ecol.* **8**: pp 289-297.

Evans, JR; Sharkey, TD; Berry, JA and Farquhar, GD (1986). Carbon isotope discrimination measured concurrently with gas exchange to investigate CO_2 diffusion in leaves of higher plants. *Aust. J. Plant Physiol.* **13**: pp 281-292.

Farquhar, GD; O'Leary MH and Berry JA (1982). On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. *Aust. J. Plant Physiol.* **9**: pp 121-137.

Farquhar, G and Richards, RA (1984). Isotopic composition of plant carbon correlates with water-use efficiency of wheat genotypes. *Aust. J. Plant Physiol.* **11**: pp 539-552.

Flanagan, LB and Ehleringer, JR (1998). Ecosystem-atmosphere CO_2 exchange: interpreting signals of change using stable isotope ratios. *TREE* **13**: pp 10-14.

Griffiths, H (1991). Applications of stable isotope technology in physiological ecology. *Funct. Ecol.* **5**: pp 254-269.

Pendall, E and Leavitt S (1996). Stable isotopic tracers of water flux through pinyon pines across a climatic transect. *Suppl. Bull. Ecol. Soc. Am.* **77.**

Read, J and Farquhar, G (1991). Comparative studies in *Nothofagus* (Fagaceae). I. Leaf carbon isotope discrimination. *Funct. Ecol.* **5**: pp 684-695.

PhD project proposal

Biomass and water cycling (morphology, phenology) among grasstrees relative to fire and season

1. Do the seasonal/annual band widths vary?

   Can this be related to:
   - a) plant age?,
   - b) incidence of fire?,
   - c) incidence of flowering?,
   - d) seasonal/annual variations in rainfall?,
   - e) changes in land use?,
   - f) presence of *Phytophthora cinnamomi*?,
   - g) parrot damage?

2. Does number of leaves produced vary seasonally/ annually?

   Can leaf production be related to band width (by counting leaf bases)?

   Does leaf production vary with:
   - a) plant age (count leaf bases per band),
   - b) incidence of fire?,
   - c) incidence of flowering?
   - d) water availability (sever roots, build up rims of soil and irrigate to varying extents and times of year),
   - e) overhead cover (apply shadecloth of varying densities over burnt/unburnt plants, plants under/outside tree cover),
   - f) nutrient availability (as for c but apply nutrients) - is this more appropriate for Roy?,
   - g) leaf removal, simulating parrot herbivory.

3. Does total/individual leaf mass vary seasonally/annually?

Do inflorescences grow at the expense of leaves and caudex in terms of biomass? (Where does energy for inflorescence growth come from?)

Does leaf mass production vary with those factors under 2 above?

4. Does water content of leaves and inflorescences vary during their development?

Do water potential and stomatal conductance/transpiration vary seasonally/annually?

Can these changes in water status be related to variations in growth rates?

5. Can variations in stable isotopes of carbon (and possibly oxygen) be related to all of the above? - Perry may wish to do some of these tasks independently and share others that fit in to particular questions above with the PhD student.

PhD project proposal

Population biology of grasstrees in conservation reserves

A new technique for ageing grasstrees (Xanthorrhoea spp.) and determining their fire history has been developed by David Ward, a senior research scientist with CALM and a senior visiting fellow at Curtin. The technique is under refinement and testing at Curtin University under the supervision of Prof Byron Lamont in a three year program (1998-2000) funded by the Australian Research Council (Strategic Partnership with Industry - Research and Training Program) and CALM. The technique is based on seasonal changes in colour banding of the leaf bases retained on the plants for up to 250 years or more, and blackening of certain leaf bases as a result of fire. Two PhD students are at present funded by this program - one examining nutrient cycling in grasstrees as revealed by this technique, and another examining biomass and water cycling. An area not at present covered, and of great interest to CALM, is the population biology of grasstrees in relation to fire. It is proposed that this project address the following questions at several reserves administered by CALM (John Forrest, Yanchep and Walyunga National Parks):

1. Is there a relationship between height and age as determined by the Ward technique?
2. Do plants (or parts of plants) of equivalent age in the same population have the same fire history?

3. Is there an environmental explanation for plants that show different growth rates (outliers) within and between populations?
4. What is the height/age structure of populations in different vegetation types and reserves?
5. What is the pattern of seed production, and seedling establishment and recruitment, after fire in different seasons?
6. What is the fate of burnt grasstrees? - are small or large (branched) plants more likely to die? How does this compare with (long) unburnt plants?
7. Can population modelling be used to determine under what fire regime deaths would balance out recruits?

Xanthorrhoea spp establishment, survival and growth in rehabilitated bauxite mines

Summary of presentation by John Koch

Proposed Honours Project 1999

Supervisor: Byron Lamont
Associate Supervisor: John Koch (ALCOA)
Project assigned to Jennifer Richardson

Grasstrees (*Xanthorrhoea preissii* and *X.gracilis*) are dominant floristic and structural components of the northern jarrah forest. Seed is relatively plentiful and germination and field establishment rates are high. However, there appears to be high mortalities of seedlings in rehabilitated bauxite mines in the years following initial establishment.

This project aims to:

1. Determine field establishment rates of these two species.

2. Determine survival (mortality) rates from 1 year to approximately 9 years.

3. Identify factors affecting this survival rate (eg. competition, grazing, drought etc.)

4. Measure growth rates and plant morphology changes over this time period.

5. Examine root growth and morphology.

Alcoa has three dedicated permanent study sites, which contain grasstrees of known ages. These are from 1980, 1986 and 1998. The 1998 plots have known numbers of seeds of *X.preissii* and *X.gracilis* in separate plots. There are also more than 100 permanent vegetation plots where grasstree numbers have been recorded on two occasions and which could be remonitored to provide additional data points. These range from 1990 to 1998.

Grasstree mortality rates could be correlated with other vegetation parameters which have been measured in these plots (density and cover of all plant species).

Anatomical studies would be carried out to determine morphological development. Glasshouse growth studies may be performed.

Alcoa would provide support for the study, which would cover costs such as travel, consumables, photocopying etc. This would be approximately $2,000-$3,000 per year.

The project would formally commence in February, 1999 however some preliminary studies could commence in spring 1998.

Summary of General Discussion

Comments from John Fox on Chantal's Honour's Thesis that may need attention, noting his comment that it was **"a splendid piece of work".**

1. More replication (for what purpose?)
Within the technique validation section an anomaly between the stem fluctuations and the colour bands was revealed on the Amphion stem. Further replication from within this site will be completed to determine whether this was indeed an anomaly or a site effect.
Chantal's thesis involved nutrient analysis from 3 plants costing approximately $5,000 per plant. Cost is a determining factor and therefore further replication is not viable at this stage. However, Roy will be completing nutrient analysis of stems from other sites within his project.

2. Inadequate explanation of why one colour should represent one (particular) season
David Ward mentioned that undulations of the stem were first reported by Lamont and Downes (1979) and it was hypothesized that they reflect seasonal growth. Ward has suggested that this seasonal affect is also displayed by the coloured bands,the cream bands depict winter/spring growth while the brown bands show summer/autumn growth. So far there is no physical proof of this assumption. We need to determine the chemical basis for the difference in colouration. Long-term monitoring of leaf formation and longevity would also solve this problem which will be addressed by the 2 PhD projects.

David Ward and Gerard Van Didden (1997) reported the presence of lapachol in the vascular bundles in the fireband using gas chromatography and mass spectrometry analysis. Black leaf bases were found to contain some green pigment probably chlorophyll, suggesting that lapachol is formed when fire kills green leaves. A question that needs to be addressed is - Does the colour move up and down the leaf? Wendy may gain an insight into this in the histological analysis of leaf bases from the three different coloured bands.

David noted that in summer a large proportion of. *X preissii* leaves die more than for the remainder of the year. The rate of leaf senescence is also faster during the summermonths. In the Biomass Project we need to determine leaf dimensions, i.e.length and width. Colour - sample living leaves at intervals to detect change in colour in different parts of the leaf throughout the year.

Roy will be using paint to mark leaf tips. David suggested cutting 1/2 of *X.preissii* head in the field, paint and see how leaves grow over 3 years. Verifying from inside in an outward direction.

Byron postulates that the difference in colouration may be due to resin. Where does the resin come from? Is there a resin layer or resin glands? Check Staff (1974).The nutrient study showed that the first and fifth cm sections of the leafbase store different concentrations of nutrients. The histological analysis may provide an explanation for this eg.showing a difference in the amount of storage tissue. For example, Ca accumulates in leaves throughout their life as once deposited Ca is not retranslocated as it is immobile. Whiteness of leafbase implies little chemical (Byron). Winter/spring leaves die slowly, so chemical "x" may be pulled back gradually during this delayed death. David suggested time and therefore speed of death may affect colour. Roy will paint leaves and will be able to know when the majority of leaf deaths occur (summer or winter/spring).

3. When fire creates a black band presumably it is superimposed on 1 or other of the bands?
David noted that fire intensity depends on the skirt thatch thickness of the grasstree, hence affecting the width of the darkened fireband and accounting for fireband thickness variation. Leaf longevity was suggested to be approximately 3 years. This needs to be quantified.

It needs to be confirmed that one black band is equal to one summer. In the Gyngoorda block, Chantal found that the black bands were equivalent of 1 summer by cross-referencing with the available fire records. However, this may not always be the case. When leaf longevity is known, the period of growth represented by one black band can be revealed.

Lachi noted that CALM would be performing experimental summer burns this summer with variable intensities and our involvement is possible. Simultaneous fires will be lit with areas of fuel loads up to 16-20 years. Observing firebands from varying fire intensities could be a very interesting sideline.

4. Is there evidence that leaf initiation is continuous?
(John Pate wanted to know for how long the leaf keeps putting on mass). This will be covered in Roy's work in biomass accumulation.

5. Inflorescence production is such a major event that he expects it to affect leaf morphology and colour.
Byron noted a growth spurt at time of inflorescence causes a summer flush of colour. Roy will study a control site versus burnt to determine if there is a post-fire growth flush and if inflorescence occurs. Leaves will be ground to reveal the colour bands. Summer flush- white related to growth of leaves rather than time of year.

6. Nutrient cycling patterns should be greatly affected by inflorescence/infructescence production (including loss of nutrients in seeds and old spikes).
Agree but Roy to work out how to monitor.

7. Why should the Amphion stem differ in seasonal colour pattern?
Amphion is different - do we remove as an anomaly? This is a long unburnt area, will the second sample from Amphion show the same pattern? Another plant has been harvested to determine whether it is a site effect or an anomaly.

Amphion vs Gyngoorda - grind tops to see how they differ in growth.

8. You only assume that the black bands correspond to fire.
David noted that the presence of firebands was carefully cross referenced with fire history. Known fires corresponded to black bands, therefore it is confirmed that the blackbands are the result of the passage of fire.

9. The contrasting band widths at the three locations can be correlated with mean annual rainfall.
David mentioned that the UWA was interested in collaborative carbon dating study. The Amphion block has the highest growth rate and highest rainfall. Perry noted the higher the C13:C12 ratio the greater the band width which will be varified in his project. The three specimens used by Chantal are available for varification.

Byron noted that when grasstrees flower,there is a change in leaf placement to the side to accomodate the flower stalk. The leaves become more compact and smaller on the inside to allow this shift. This was to be covered in Mark's project so won't be covered until he is replaced.

10. Changes in nutrient cycling actually requires total nutrient content of each band and stem equivalent.
This will be addressed in Roy's project.

11. What is leaf longevity (in relation to fire effects)?
This was Mark's topic but will now be covered in Roy's work, this is of fundamental importance.

12. Do successive fires continue to erode the leaf bases? (if so, this would contribute to the pattern).
Yes, they do as noted by Drummond in 1847 quoted David. David varified that Chantal's sampling technique was sound and constant.

13. The taller the plant. the less likely it is to get burnt, and the fewer the black bands?
David noted that fire sometimes does miss the taller plants. A regression of height of plant and fire is significant. However, firebands were correlated with CALM fire history. Fire history would be more likely to be correct or underestimated rather than overestimated. Length of thatch skirt is important, time since last burn will affect fire intensity and also if long skirt will burn on tall plant. Cross-referencing between plants through the age-classes (and therefore height classes) will compensate for any tall specimens that were missed by the fire.

Byron Lamont:
Can the method be used to detect changes in nutrient acquisition patterns over time? (take unburnt site: only useful if pattern cannot be explained by leaching)

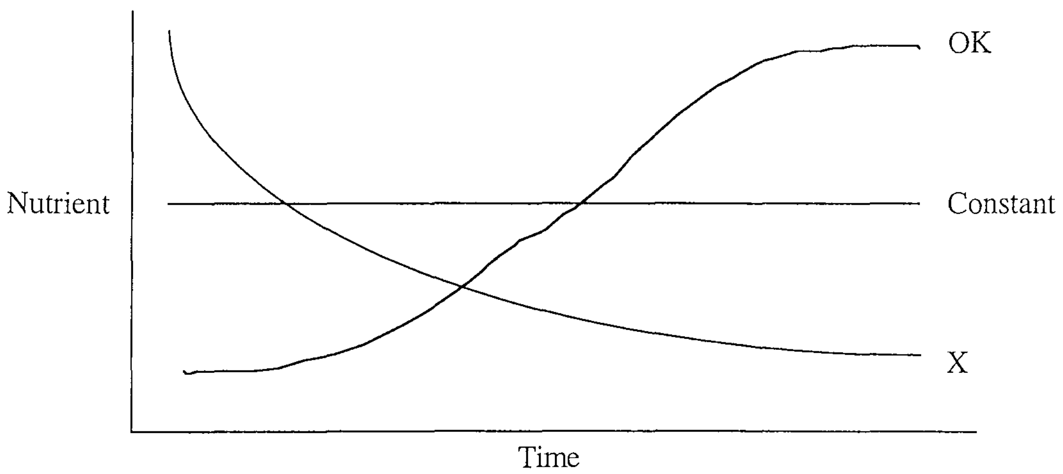


Figure 1. General trends in nutrient concentration within grasstree leaf bases over time.

See over 100 years whether a change in nutrient cycling relates to land use. We need a control, perhaps a 60 year unburnt site. Line "x" shows nutrient levels dropping, leached over time.If nutrients increase over time this cannot be due to leaching but a change in fire frequency. More frequent fires results in an increase in uptake of nutrients. Method to show nutrient recycling. Roy's project can detect responses to fire and season but not long term changes.

Assessors' Comments on Grant Application (mean from 6 assessors of 90.3%)

1. Fire history of John Forrest National Park - consider a Landsat history back to 1973? or work in an area that is "meticulously documented".

David Ward is confident that records of fire history of the John Forrest National Park are both accurate and comprehensive.

2. How will you deal with fire patchiness?
By cross-referencing of plants, patchiness will be identified by sampling

3. Rather than pruning roots to simulate drought, consider placing plastic on ground for 12 months.
This is not feasible as the plastic would act as a mulcher and cause numerous changes to the microclimate and microenvironment.

4. The large number of variables may make it difficult/impossible to account for the fire history of individual plants, making it difficult to generalize.
The comprehensive records of John Forrest National Park verifies the course of fires.

5. The application lacks details on sampling designs, statistical analysis - null hypotheses needed, spatial designs.
Sampling is limited to grasstree occurrence, using the plant centred method. Results will determine statistical analysis required.

What to do about the vacant PhD project?

1. Defer appointment until January,1999?

2. Try locally over next 2-3 weeks and start person while Byron Lamont is away?

3. Offer to one of the previous applicants? (the most promising is a Chinese researcher)

Abstract for Intecol Conference, Symposium on long-term dynamics of arid and semi-arid ecosystems, at Florence, Italy in July 1998. (to be delivered by Prof B. Lamont)

Grasstrees as bioindicators of human-induced events over the last 250 years in seasonally arid Australia

David Ward[1], Byron Lamont[2] and Chantal Burrows[2]

[1]Research Centre, Department of Conservation and Land Management, Hayman Rd, Como WA 6152, Australia
[2]School of Environmental Biology, Curtin University of Technology, PO Box U1987, Perth WA 6001, Australia

We have developed a technique for ageing grasstrees (*Xanthorrhoea* spp) based on alternating colour banding patterns among the leaf bases, which correspond to seasonal fluctuations in stem diameter along the trunk first reported by Lamont and Downes (1979). The advantage of this new approach is that it is quicker, individual plants need not be harvested, and suitable tissue for analysis is accessible on much older stems. In addition, we have shown that fire-stimulated flowering is not a reliable surrogate for fire history as it is age (height) dependent and spike remnants are lost through secondary growth. However, young leaf bases are blackened by fire and show up at intervals along the full length of the trunk. Initial results for eucalypt forest/woodland in southwestern Australia indicate frequent burning by Aborigines in the period 1750-1860 (2-4 fires per decade) with a gradual decline to the last 30 years of 0-1 fire per decade. These gradual changes are sometimes punctuated by sudden drops coinciding with outbreaks of measles among the Aborigines, or changes in land management practices or policies of the colonialists. The wooded areas with lower rainfall tended to burn more often. There is also a strong landscape element in drier woodlands, with lowland flats and valley floors (where Aborigines and game congregated) much more frequently burnt than the ridges and uplands. The level of the immobile nutrient calcium in the leaf bases is much higher during spring than autumn growth, with a marked jump in concentration immediately above the black rings which declines with time. The method holds promise of exploring the role of fire in nutrient and water cycling; detecting changes in climate and the forest canopy through long-term changes in the carbon isotope ratios, and changes in the water status of the soil through changes in band width and deuterium concentrations; identifying advent of the major root diseasé, *Phytophthora cinnamomi,* through a sudden reduction in band width; and describing the impact of humans on plant community composition and structure through analysis of pollen caught between the leaf bases. Computer modelling of such complex interactions would create quite a challenge. We would welcome cooperative work with other scientists on any of these topics.

Burrows[1], Chantal L., Lamont[1], Byron B. and Ward[2], David

[1]School of Environmental Biology, Curtin University of Technology, Perth, Western Australia
[2]Research Centre, Department of Conservation and Land Management, Perth, Western Australia

GRASSTREES AS BIOINDICATORS OF THE PAST AND PRESENT CHEMICAL ENVIRONMENT IN EUCALYPT FOREST, WESTERN AUSTRALIA

The nutrient content of the remnant leaf bases of grasstrees (*Xanthorrhoea* spp.), located in eucalypt forest near Perth, Western Australia, in relation to the passage of fire and seasonal variation were studied. A new technique of ageing grasstrees was implemented, providing a time scale for the nutrient analyses and a record of the passage of fire. Summer and winter nutrient concentrations for every year from 1890 to 1987 were recorded. Consistent trends in concentrations of some nutrients in the remnant leaf bases were observed. Ca showed significant differences seasonally (putative winter-spring vs summer-autumn) and after the passage of fire. A winter/spring maximum in the Ca concentration of leaf bases was recorded within each year. Ca concentration dropped below pre-fire levels at the time of fire, then increased rapidly over the next few growing seasons, before gradually returning to pre-fire levels. Mg concentrations dropped within six months after the passage of fire and began to recover to pre-fire levels after a further six months had passed. Mn concentrations increased to a distinct peak at the time of fire and then rapidly returned to pre-fire levels. The concentration of more mobile K decreased along the stem as the leaf base age increased. The effects on the ecosystem of decreasing rates of nutrient cycling over the last 100 years, associated with declining fire frequencies, are discussed.