RURAL INDUSTRIES RESEARCH
& DEVELOPMENT CORPORATION

Pollen Analysis of Eucalypts

in Western Australia

A report for the Rural Industries Research and Development Corporation

by Rob Manning

May 2001

RIRDC Publication No 01/53
RIRDC Project No DAW-91A

ISBN 0 642 0 642 582750
ISSN 1440-6845

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Project no. DAW-91A

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Published in May 2001
Printed on environmentally friendly paper by Canprint

Foreword

Pollen is the primary source of protein, fats, vitamins and minerals in the diet of the honey bee *(Apis mellifera).*

Newly emerged adults from the pupal stage of brood rearing must consume pollen during the first 10 days after emergence. Without this pollen, the hypopharyngeal glands that are responsible for producing food (worker jelly) for larval honey bees remain underdeveloped and non-functional.

When honey bees are maintained on pollens that are marginal in nutritive value because one or more amino acids are sub-optimal for honey bee longevity, brood rearing still continues, but body weights decline. The life span of honey bee is also shortened and the ability to feed young larvae can rapidly diminish, ultimately affecting the whole function of the beehive.

The results of this study of the nutritive value of six Western Australian pollens (Eucalyptus accedens, Eucalyptus diversicolor, Eucalyptus marginata, Eucalyptus patens, Eucalyptus wandoo, and Corymbia (Eucalyptus) calophylla) will highlight to beekeepers the necessary adoption of supplementary feeding of honey bees using artificial feedstuffs or quality pollens. This will subsequently maximise the performance of honey bees and as a consequence improve beekeeper incomes.

This report, a new addition to RIRDCs diverse range of almost 700 research publications, forms part of our Honey bee R & D program which aims to improve the productivity and profitability of the Australian beekeeping industry. The project focuses on the program's key strategy of improving hive husbandry and management practises for increased profitability and touches on another key strategy of identifying therapeutic values of specific floral species.

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Peter Core
Managing Director
Rural Industries Research and Development Corporation

Acknowledgements

Funded by the Rural Industries Research and Development Corporation ($10,500) and Dairy and Apiculture program, Agriculture WA ($22,066) and in-kind contributions (trapping and collection of pollens) by the following beekeepers.

Beekeepers (in alphabetical order) are sincerely thanked for their contribution to this project:


Harry East


Colin Fleay


Brian Higgs


Ron Jasper


Jim Ligman


Rod Pavy


Wayne Ridley


Phil Sammut


John Silcock


John Springall


Roger Tschabotar

Frank Jankovic
Rob Wallace
Jim Wallace
Steve Kutasi

The Chemistry Centre of WA and Animal Health Laboratories (AHL), Agriculture WA carried out pollen analyses. In particular, I thank Maeve Harvey and Malcolm McGrath (AHL) and David Harris and Bruce Youngberg from the WA chemistry.

Stoneville Research Station Cherry-Beetube experiment,
December 1998

11th July 1937 to 10th May 2000

One of the pioneers of commercial honey bee pollination and first to commercialise the "Beetube" pollination system in Western Australia.

This project dedicated to the memory of John Silcock

Thanks for all your help, your friendship and support of this project (and many others)

Abbreviations

S.E. = Standard error.

lipid = oil or fat

gram (g): 1000 g = 1 kilogram (kg)

microgram (g): 1,000,000 g = 1 gram

AFB = American foulbrood

c. = about/approximately

ns = not sampled.

parts per million (ppm) = mg/kg

milligram (mg): 1000 mg = 1 gram.

ml (millilitre): 1000 mls = 1 litre

EFB = European foulbrood

% = percent

Executive Summary

- Three Western Australian eucalypt pollens were deficient in one or more amino acids that would affect the performance of the honey bee.

Common name	Species	Amino acid in deficiency
Powderbark	*Eucalyptus accedens*	*Iso-leucine*
Karri	*Eucalyptus diversicolor*	*Nil*
Jarrah	*Eucalyptus marginata*	*Iso-leucine & Histidine*
Forest blackbutt	*Eucalyptus patens*	*Nil*
Wandoo	*Eucalyptus wandoo (all flower forms)*	*Iso-leucine*
Redgum	*Corymbia calophylla*	*Nil*

- Only one species, jarrah had a crude protein level below the minimal 20% required by honey bees [as defined by de Groot (1953)].

- Fat (lipid) level in eucalypt pollens was highest in karri pollen and lowest in jarrah pollen. But when compared with European species such as canola the fat levels of eucalypts were very low.

- The fat content of all pollens tested was dominated by C-18 fatty acids, particularly the antimicrobial linoleic acid (35.7 to 48%). The only exception was oleic acid, which was the dominant fatty acid at *c*. 22% in all three flowering forms of wandoo.

- Besides linoleic acid, redgum pollen was also dominant in two other fatty acids that have demonstrative antimicrobial activity: myristic and linolenic acid.

- Of the minerals, potassium was in the greatest amount (mg/kg) followed by phosphorus and sulphur.

- Comparisons with average amino acid levels in Italian pollens (once the natural food of the Italian bee used in Western Australian beekeeping) showed that our 'best' pollen, redgum, was at the lower end of the Italian pollen range for nine amino acids. The eucalypts had characteristically higher concentrations of proline in their pollens than Italian plants.

- There are differences in amino acid composition within the same species in different areas. Wandoo showed north to south i.e. latitude differences in amino acid composition and redgum showed differences between the coastal plain and pollen from trees growing along the Darling escarpment, inland from the coast.

Contents

1. Introduction

Western Australia has the largest number of endemic eucalyptus species identified in Australia (Chippendale and Wolf, 1981). Nectar (as honey) and pollen from these eucalypts play an important part in the health and sustainability of both the beekeeping industry and wildlife.

Little is known of the chemical composition of Western Australian eucalypt pollens, apart from the published work by Bell *et al.* (1983) and Spadek and Chambers (1987) for two species, *Corymbia calophylla* (redgum) and *Eucalyptus marginata* (jarrah). These analyses were for dried pollen packed for human consumption.

Bee collected pollen is a relatively recent product. It is derived from the use of special pollen traps fitted to beehives. These traps were developed in the early 1970s in Canada, with one being developed in Western Australia in 1973. Pollen traps have undergone constant modification ever since and an export market for Western Australian pollen has developed.

The market for honey bee collected pollen has become quite diverse and demand can be quite strong from a number of areas. Most pollen, being highly nutritious, is sold for human consumption or used as a 'feedback' inside beehives when pollen flows from native plants are of poor quality for continued bee breeding. This stimulus from feeding back pollen to bees is important to maintain productive hives in times when resources (nectar and pollen) are poor, often caused by adverse environmental conditions. Other developing markets for pollen are as a protein component in the manufacture of bumblebee pollination colonies worldwide. In 1999, production of bumblebee colonies was estimated to be 600,000 requiring 100 to 150 tonnes/pollen/year for the glasshouse tomato industry (van Doom, 2000). Homoeopathic 'apitherapics' and cosmetic industries also use pollen in some of the products.

Whilst the market for Western Australian pollen seems to be large, albeit poorly developed, the nutritive value of many Western Australian plant pollens still remains unknown. Without this knowledge, it is difficult to know how they might affect the honey bee's physiology and whether or not, the information could enhance the marketability of pollen for human consumption and improve feedback products to beekeepers. Those 'unanalysed' pollens that may have nutritional deficiencies (many of which have been identified in eastern States) cannot be manipulated to provide optimum levels for honey bee nutrition for the benefit of the beekeepers enterprise.

Most Western Australian pollen could be termed *"organic"* as it is collected from endemic native plant species distant from industrial and urban areas. Most of the trapped pollen is sourced from plant species that grow in relatively large homogeneous areas e.g. Jarrah/redgum (but flower at distinct times of the year) and wandoo. In these areas, pollen production is derived almost from a single species. If any of the species are deficient in an amino acid, then any physiological problem should be soon evident. This contrasts to the over-wintering areas of the northern coastal plain where numerous species contribute to the pollen that is trapped. With the variety of species flowering when beekeepers use this area, there is the expectation that any nutritional disorders will be diminished.

Beekeepers have noticed beehive populations severely decline after apiaries have been placed in winter wandoo country, whilst those placed on redgum and over-wintered on the coastal plain have remained in good condition. Some have also noticed that when apiaries are placed in jarrah country (October to December/January) they perform differently on the same sites when redgum flowers

(February to March). Large areas of brood comb are often seen packed out with jarrah pollen, which severely restricts the area, the queen bee has to lay eggs.

This project examined six commercially important beekeeper-targeted species: *Eucalyptus accedens* (powderbark), *E. diversicolor* (karri), *E. marginata* (jarrah), *E. patens* (forest blackbutt) and three flowering varieties (Winter, Spring and Summer) of *E. wandoo* (wandoo) for their protein composition, fatty acid, mineral profile and (limited) vitamin content. This is the first time in Australia that the lipid fraction of pollen has been analysed for fatty acids. Fatty acids such as linoleic acid were dominant in most of the pollens studied. Fatty acids such as linoleic have antimicrobial activity which can inhibit the growth of bee diseases such as European foulbrood (EFB) and American foulbrood (AFB) (Feldlaufer *et al*., 1993). Analysis of pollens, especially the fatty acid component may have possible importance in understanding the ecology of EFB and AFB diseases under Australian field conditions, apiary management and migratory relocations. Relocation and access to forest areas where good flows of pollens with high antimicrobial activity could be relevant for disease management (i.e. natural biocides). Access might become a problem for some beekeeping operations as more land is declared for reserves and land managers may restrict access.

Redgum pollen (*Corymbia calophylla*) was used as a base from which to measure differences between the other species. According to de Groot's (1953) data, redgum pollen does not exhibit any amino acid deficiency that would be detrimental to the health of the honey bee. From beekeeper observations the pollen provides excellent build-up of beehive strength. It was on this basis that redgum was used as the 'base-line' pollen, in other words as a minimum standard to which the other pollen species were measured against.

If any one of the ten essential amino acids is missing from honey bee diets, a substantial decrease in body weight of honey bees occurred within 14 days (Figure 1). However, all amino acids are always present in pollens but at various concentrations. If concentrations fall below minimum requirements (determined by de Groot, 1953) a reduction in body weight will again occur. This will decrease the lifespan of the honey bee which will result in a corresponding reduction in hive products (honey and pollen) and therefore beekeeper incomes.

The lipid fraction of eucalypt pollen, usually comprising less than 2% in pollen is examined for fatty acids for the first time in Australia. Chemical analysis of pollens from *Eucalyptus accedens, E. diversicolor, E. patens* and *E. wandoo* are published for the first time.

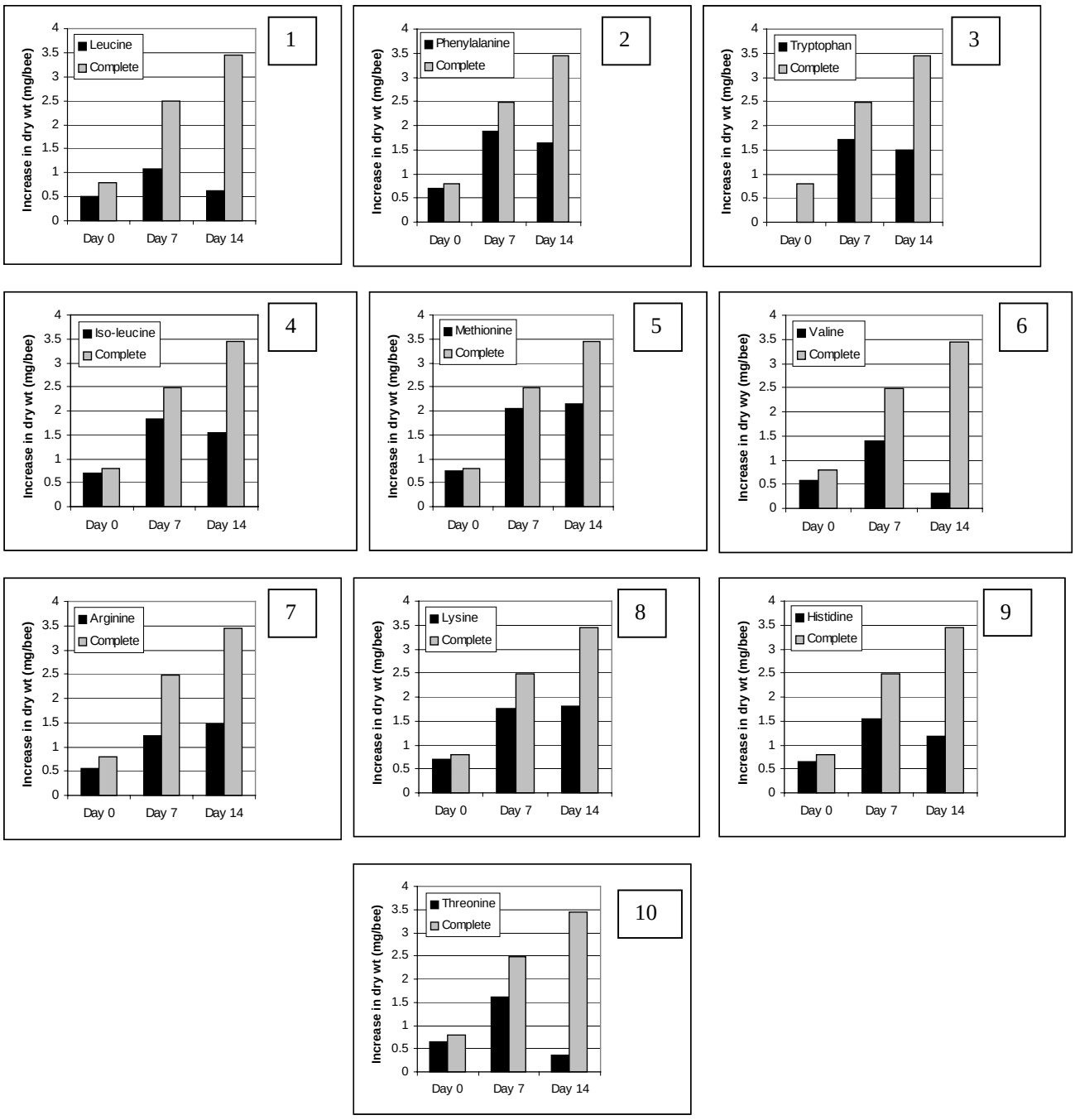


Figure 1 The effect of removing a single amino acid from a complete feed formula designed by de Groot (1953). The ten amino acids shown here are termed as essential because they affect honey bee development by reducing body weight when in deficiency. All other amino acids are determined as being 'non-essential' except for alanine, which can reduce honey bee body weight when removed from the diet.

For all of the feeding trials conducted by de Groot (1953) the feedstuff with one missing amino acid caused a decrease in body weight at day 14. The exception is methionine (Fig 1-5) where it remained similar to body weights at day 7 and arginine where body weights increased slightly. The complete feed formula when fed to honey bees increased honey bee body weights in all tests. From the graphs above, body weights of honey bees are more sensitive to three amino acids: leucine, valine and threonine than the other seven essential amino acids.

Redrawn from de Groot (1953).

1.2 Objectives

Provide a protein, mineral, fatty acid and vitamin analysis for six commercially important species of eucalypt.

1.3 Methodology

Honey bee collected pollens were trapped and collected by a number of beekeepers. The fresh samples were frozen until analysis by two laboratories. Pollen collection was made at the time of peak flows in order to maximise pollen purity (see Fig 2). Some of the pollen collections were mixed species that required sorting prior to analysis. Both laboratories conducted standard tests for amino acids, minerals, fatty acids and vitamin determinations. Analyses conformed to international standards and both laboratories were accredited to international standards (NATA).

Comparisons were made between the ten essential amino acids: methionine, lysine, iso-leucine, phenylalanine, arginine, histidine, tryptophan, leucine, threonine, valine and the minimum values required by honey bees as determined by de Groot (1953). Essential and non-essential amino acids, fatty acids, minerals and vitamins (limited because of expense) are also compared to the redgum pollen profile.

Pollen collection

Eucalyptus accedens (Powderbark)
Described by Smith (1969) as...*a tree from 9 to 20 metres high with smooth white to orange-red bark where the surface is powdery. Occurs on laterite outcrops on high ground in the wandoo forests northwards from Pingelly. Flowering occurs from December to March.*

Pollen was collected from an area east of Perth (Fig 3). Only two samples were collected from this flow in 1998. In February 1999 and 2000 unfavourable environmental conditions made further collection impossible.

Eucalyptus diversicolor (Karri)
One of the tallest trees in the world that grows in a relatively restricted area of the south west of the State. Described by Smith (1969) as..*occuring on deep loamy soils on hill sides and valleys, flowering throughout the year but honey flows commence from December to April.*

Pollen was collected from a restricted area north of Walpole (Fig 3). Four samples were collected in April 1999.

Eucalyptus marginata (Jarrah)
The principal timber tree and a unique species whose honey rarely granulates. Honey flows can be erratic. Described by Smith (1969) as …*a straight tall tree common throughout the higher rainfall areas of the south west of the State on laterite gravels. Flowering commences in September through to January according to district.*

Pollen was collected east of Perth (Fig 3). Five samples were collected from 1998 to 2000.

Eucalyptus patens (Forest blackbutt)

It is an important source of honey and pollen and builds strong hives going into a marri (*Corymbia calophylla*) flow in February/March. Described by Smith (1969) as *..large tree with deeply fissured rough bark. Occurs on deep sandy moist soils, usually near rivers and creeks in jarrah forests. Flowering begins in November and continues through to February.*

Pollen was collected from an area east of Perth (Fig 3). Only three samples were collected by beekeepers that utilised this flow in 1999. In 1998 and 2000 unfavourable environmental conditions made collection from apiary sites impossible.

Eucalyptus wandoo (Wandoo)
Described by Smith (1969) as *..a tree 15 to 31 metres high with widely spread branches, white bark with grey patches. Occurs in valleys in the eastern parts of the jarrah forest on clay soils and generally to the east of the jarrah forest as far as the 38 mm mean rainfall isohyet. Flowering north of the Avon Valley takes place from March to June (known as winter wandoo). Flowering south to Wandering occurs in spring to early summer (known as spring wandoo) and a further flowering south of Wandering occurs from January to February (known as summer wandoo). At one time the most important source of honey. Massive areas containing this species were cleared from the better soils for agriculture. The remaining areas, mostly in forest reserves or private property are less dependable for honey production and good flowering is extremely difficult to predict.* Beekeepers can move bees onto the winter wandoo after marri *(C. calophylla)* when flowering years coincide.

Winter wandoo pollen was collected from an area north of Perth (Fig 3 & 4). Nine samples were collected over a wide area, in a north-south direction.

Spring wandoo pollen was collected from an area south east of Perth (Fig 3). Two samples were collected.

Summer wandoo pollen was collected from an area south east of Perth (Fig 3). Two samples were collected.

Corymbia calophylla (Marri/Redgum)
Described by Smith (1969) as *..a medium to large tree with wide spreading thick branches. The bark is rough and flaky with resinous exudate frequently being released on older trees. Occurs in the jarrah and karri forest on light sandy soils and along the coastal plain. Flowering occurs from February to March though individual plants can be in flower in July, December and January.*

Pollen was collected from an area south east of Perth (Fig 3). Nine samples were collected. The name "redgum" will be used instead of "marri" throughout the text as it is the preferred name in use by beekeepers.

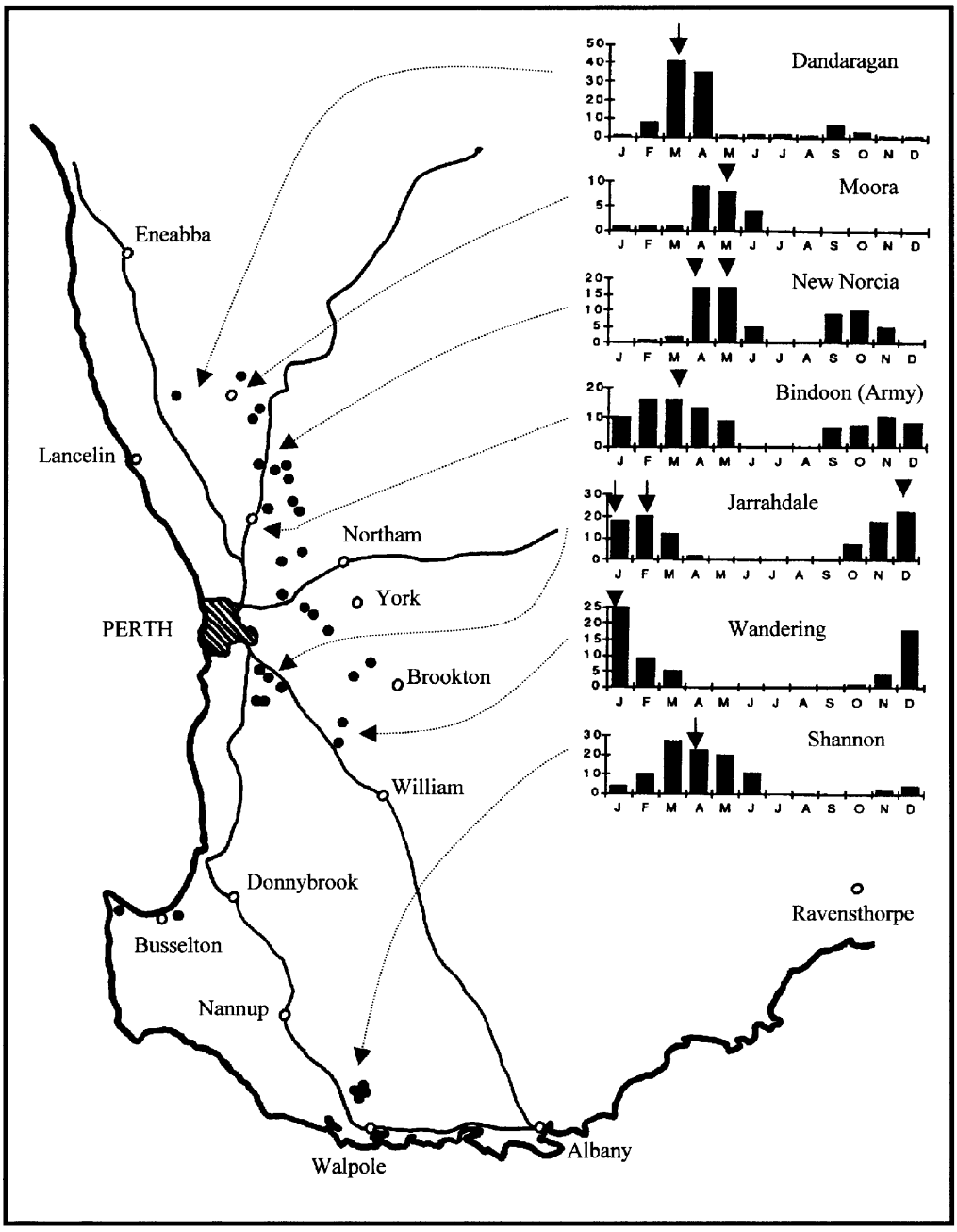


Figure 2 Flowering periods for some of the areas where pollen was collected. Pollen was collected at peak periods (see arrows above frequency graphs) when it would almost be considered to be derived from a single species i.e. 'pure' allowing the analyses to be as accurate as possible for each of the species.

The frequency graphs were sourced from Manning (1992).

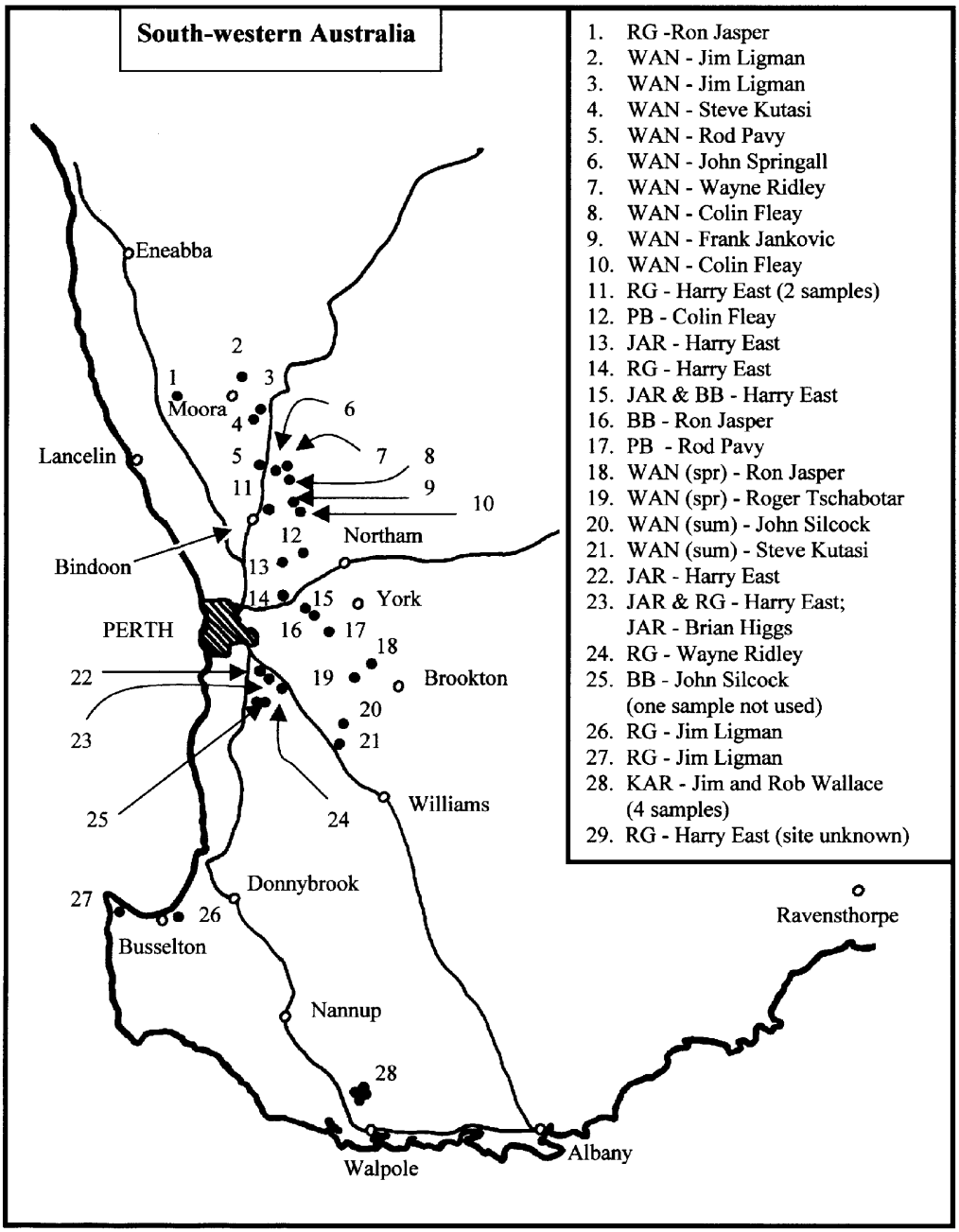


Figure 3 Collection sites of 36 samples of pollen analysed in this project.

KEY:
BB = forest blackbutt, JAR = jarrah, KAR = karri, PB = powderbark, RG = redgum, WAN = winter wandoo, WAN(spr) = spring wandoo and WAN(sum) = summer wandoo.

2. Results

There were laboratory differences in the analysis of the amino acid, cystine and fatty acids. The results for cystine are identified as to which laboratory they were tested. Some of the fatty acids were re-analysed so that the results were from the one laboratory.

The results for powderbark, spring wandoo, summer wandoo and forest blackbutt are only indicative as they were limited in their comparison with redgum by the low sample number. Nine samples from a range of locations were regarded as the minimum for a reliable comparison.

2.1 Eucalyptus accedens (Powderbark)

2.1.1 Amino Acid profile

The amino acid profile of powderbark pollen is shown in Table 1. Based upon de Groot (1953), the amino acid, iso-leucine was, on average, below the minimum concentration required by honey bees to maintain body weight. For all amino acids, redgum (*C. calophylla*) had higher concentrations than powderbark, except arginine.

Amino acid	***E. accedens***	***C. calophylla***	**de Groot (1953)** minimum level
Cystine	0.34 ± 0.02*	0.40 ± 0.01 (n=4)* 1.01 ± 0.05 (n=5)**	
Aspartic acid	2.04 ± 0.09	2.23 ± 0.07	
Methionine	0.48 ± 0.00	0.61 ± 0.03	0.3
Threonine	0.79 ± 0.05	0.96 ± 0.03	0.6
Serine	0.96 ± 0.04	1.17 ± 0.03	
Glutamic acid	2.45 ± 0.13	2.69 ± 0.08	
Proline	2.61 ± 0.24	3.45 ± 0.12	
Glycine	1.01 ± 0.05	1.27 ± 0.04	
Alanine	1.04 ± 0.03	1.38 ± 0.11	
Valine	1.01 ± 0.04	1.28 ± 0.05	0.8
Iso-leucine	**0.79 ± 0.04**	1.07 ± 0.05	**0.8**
Leucine	1.42 ± 0.06	1.82 ± 0.05	0.9
Tyrosine	0.59 ± 0.02	0.78 ± 0.03	
Phenylalanine	0.84 ± 0.00	1.11 ± 0.04	0.5
Lysine	1.24 ± 0.08	1.88 ± 0.11	0.6
Histidine	0.49 ± 0.03	0.85 ± 0.09	0.3
Arginine	1.99 ± 0.03	1.90 ± 0.06	0.6
Tryptophan	0.29 ± 0.03	0.58 ± 0.08 (n=4)	0.2
Protein	23.6 ± 0.60	26.7 ± 0.21	20
Sample number	2	9	

Table 1 A comparison of amino acids (% ± S.E.) in pollen from *Eucalyptus accedens* and *Corymbia calophylla*. Deficiencies shown in bold type.
Note: * & ** from two different laboratories: *Lab1; ** Lab2.

Most of the essential amino acids of powderbark pollen have to be increased to match those of redgum: methionine (27.1%), threonine (21.5%), valine (26.7%), iso-leucine (35.4%), leucine (28.2%), phenylalanine (32.1%), lysine (51.6%), histidine (73.5%) and tryptophan (100%).

A comparison between redgum and powderbark showed that the average protein concentration differed by 3.1%, redgum being higher at 26.7% (Table 1). Protein of powderbark pollen has to be increased by 13.1% to match redgum.

2.1.2 Fatty acid profile

Overall, powderbark pollen had higher concentrations of C-16, C-20, C-22 and C-24 fatty acids than redgum. Redgum was higher in C-14 and C-18 fatty acids (Table 2).

Powderbark had an average of 15.9% more total fat than in redgum pollen. Powderbark had higher percentages of palmitic, stearic, oleic, linoleic and arachidic fatty acids than redgum. Redgum had higher concentrations of myristic (nearly 4 x higher) and linolenic acids (3 x higher) (Table 3).

Powderbark pollen required 272.5% more myristic and 210% more linolenic fatty acids to match redgum pollen.

Fatty acid	***E. accedens***	***C. calophylla***
C-14	0.80 ± 0.10	2.98 ± 0.32
C-16	23.1 ± 0.60	17.8 ± 1.69
C-18	64.1 ± 1.10	68.0 ± 1.51
C-20	5.95 ± 0.05	3.96 ± 0.42
C-22	3.15 ± 0.75	1.46 ± 0.52
C-24	1.85 ± 0.05	1.13 ± 0.45

Table 2 Fatty acid (% ± S.E.) in lipid from *Eucalyptus accedens* compared with *Corymbia calophylla* pollen.

Fatty acid	***E. accedens***	***C. calophylla***
Total fat (% of pollen)	1.02 ± 0.04	0.88 ± 0.07
Myristic (C-14)	0.80 ± 0.10	2.98 ± 0.32
Palmitic (C-16)	22.7 ± 0.55	17.4 ± 1.75
Stearic (C-18)	7.1 ± 0.10	4.54 ± 0.51
Oleic (C-18)	15.8 ± 2.20	15.0 ± 1.35
Linoleic (C-18)	37.1 ± 1.60	35.7 ± 3.59
Linolenic (C-18)	4.10 ± 0.60	12.7 ± 0.85
Arachidic (C-20)	3.95 ± 0.45	2.02 ± 0.27
Sample number	2	9

Table 3 Dominant fatty acids (% ± S.E.) in lipid from *Eucalyptus accedens* compared with *Corymbia calophylla* pollen.

2.1.3 Mineral profile

Powderbark pollen had higher levels of sodium and calcium than redgum (Table 4). To be equivalent to redgum pollen, minerals would have to be increased: boron (71.8%), copper (32.7%), zinc (57.8%), phosphorus (31.3%), potassium (28.6%), magnesium (80%) and sulphur (15.4%). Iron and manganese were similar for both pollen species.

Mineral	***E. accedens***	***C. calophylla***
Boron	11.0 ± 0.00	18.9 ± 1.16

Copper	16.5 ± 0.50	21.9 ± 0.95
Iron	116.5 ± 23.5	124.4 ± 9.15
Manganese	33.5 ± 4.50	35.8 ± 3.28
Zinc	50.0 ± 1.00	78.9 ± 2.43
Phosphorus	0.32 ± 0.00	0.42 ± 0.01
Potassium	0.42 ± 0.00	0.54 ± 0.01
Sodium	0.02 ± 0.01	0.01 ± 0.00
Calcium	0.10 ± 0.00	0.06 ± 0.00
Magnesium	0.05 ± 0.00	0.09 ± 0.00
Sulphur	0.26 ± 0.00	0.30 ± 0.01
Sample number	2	9

Table 4 Mineral content of pollen (± S.E.) from *Eucalyptus accedens* compared with *Corymbia calophylla*.
Boron to Zinc in mg/kg; Phosphorus to Sulphur in %.

2.1.4 Vitamin profile

Powderbark pollen had a higher average concentration of ascorbic acid than redgum. A single sample for the rest of the redgum vitamins does not permit a comparison, but are included for interest (Table 5).

Vitamin	***E. accedens***	***C. calophylla***
Ascorbic Acid 'C'	76.5 ± 10.5	64.3 ± 32.1
Thiamine	2.80 ± 0.20	1
Riboflavin	0.61 ± 0.07	0.62
Niacin	1.00 ± 0.00	3.5
Pyridoxine	0.32 ± 0.00	0.4
Folic Acid	2.15 ± 0.15	2
Sample number	2	'Vit C' = 4, rest = 1

Table 5 Vitamin content of pollen (± S.E.) from *Eucalyptus accedens* compared with *Corymbia calophylla*.
Vitamin C in mg/kg; Rest of vitamins in mg/100g.

2.2 Eucalyptus diversicolor (Karri)

2.2.1 Amino Acid profile

The amino acid profile of karri pollen is shown in Table 6. Every amino acid, apart from cystine and proline was highest in redgum pollen. Most of the essential amino acids of karri pollen have to be increased to match those of redgum: methionine (5.2%), threonine (11.6%), valine (15.3%), iso-leucine (18.9%), leucine (21.3%), phenylalanine (16.8%), lysine (9.3%), histidine (23.2%) and arginine (72.7%).

A comparison between redgum (*C. calophylla*) and karri showed the average protein concentration differed by 3.3%, redgum being higher at 26.7% (Table 6). To match the protein content of redgum pollen, karri pollen protein levels have to be increased by 14%.

Amino acid	***E. diversicolor***	***C. calophylla***	**de Groot (1953)** minimum level
Cystine	1.22 ± 0.04**	0.40 ± 0.01 (n=4)* 1.01 ± 0.05 (n=5)**	
Aspartic acid	1.91 ± 0.05	2.23 ± 0.07	
Methionine	0.58 ± 0.04	0.61 ± 0.03	0.3
Threonine	0.86 ± 0.02	0.96 ± 0.03	0.6
Serine	1.03 ± 0.02	1.17 ± 0.03	
Glutamic acid	2.07 ± 0.07	2.69 ± 0.08	
Proline	3.84 ± 0.08	3.45 ± 0.12	
Glycine	1.06 ± 0.03	1.27 ± 0.04	
Alanine	1.11 ± 0.03	1.38 ± 0.11	
Valine	1.11 ± 0.03	1.28 ± 0.05	0.8
Iso-leucine	0.90 ± 0.03	1.07 ± 0.05	0.8
Leucine	1.50 ± 0.04	1.82 ± 0.05	0.9
Tyrosine	0.69 ± 0.02	0.78 ± 0.03	
Phenylalanine	0.95 ± 0.03	1.11 ± 0.04	0.5
Lysine	1.72 ± 0.05	1.88 ± 0.11	0.6
Histidine	0.69 ± 0.02	0.85 ± 0.09	0.3
Arginine	1.10 ± 0.01	1.90 ± 0.06	0.6
Tryptophan	ns	0.58 ± 0.08 (n=4)	0.2
Protein	23.4 ± 0.62	26.7 ± 0.21	20
Sample number	4	9	

Table 6 A comparison of amino acids (% ± S.E.) in pollen from *Eucalyptus diversicolor* with *Corymbia calophylla*.
Note: * & ** from two different laboratories: *Lab1; ** Lab2; ns = not sampled.

2.2.2 Fatty acid profile

Overall, karri was higher in C-16, C-18, C-22 and C-24 fatty acids (Table 7) whilst redgum was highest in C-14 and C-20.

Karri had an average of 115.9% more total fat in pollen than redgum (Table 8). Karri was highest in palmitic, oleic, and linoleic fatty acids. Redgum had a higher concentration of myristic, stearic, linolenic and arachidic fatty acids than karri (Table 8).

Karri pollen required 684.2% more myristic, 68.2% more stearic 98.4% more linolenic and 9.2% more arachidic fatty acids to match redgum pollen.

Fatty acid	*E. diversicolor*	*C. calophylla*
C-14	0.38 ± 0.05	2.98 ± 0.32
C-16	19.4 ± 0.92	17.8 ± 1.69
C-18	73.4 ± 0.64	68.0 ± 1.51
C-20	3.48 ± 0.23	3.96 ± 0.42
C-22	1.98 ± 0.57	1.46 ± 0.52
C-24	1.23 ± 0.31	1.13 ± 0.45

Table 7 Fatty acid (% ± S.E.) in lipid from *Eucalyptus diversicolor* compared with *Corymbia calophylla* pollen.

Fatty acid	*E. diversicolor*	*C. calophylla*
Total fat (% in pollen)	1.90 ± 0.09	0.88 ± 0.07
Myristic (C-14)	0.38 ± 0.05	2.98 ± 0.32
Palmitic (C-16)	19.1 ± 0.92	17.4 ± 1.75
Stearic (C-18)	2.70 ± 0.18	4.54 ± 0.51
Oleic (C-18)	16.3 ± 1.01	15.0 ± 1.35
Linoleic (C-18)	48.0 ± 0.39	35.7 ± 3.59
Linolenic (C-18)	6.4 ± 0.47	12.7 ± 0.85
Arachidic (C-20)	1.85 ± 0.03	2.02 ± 0.27
Sample number	4	9

Table 8 Dominant fatty acids (% ± S.E.) in lipid from *Eucalyptus diversicolor* compared with *Corymbia calophylla* pollen.

2.2.3 Mineral profile

Karri pollen was higher in concentration of sodium and calcium than redgum pollen (Table 9). To match redgum pollen, mineral concentrations have to be increased: boron (18.1%), copper (137.3%), iron (42.2%), potassium (22.7%), magnesium (80%) and sulphur (20%). Concentrations of manganese, zinc and phosphorus were similar for both species.

Mineral	*E. diversicolor*	*C. calophylla*
Boron	16.0 ± 1.41	18.9 ± 1.16
Copper	9.23 ± 0.60	21.9 ± 0.95
Iron	87.5 ± 6.61	124.4 ± 9.15
Manganese	33.5 ± 1.44	35.8 ± 3.28
Zinc	72.8 ± 9.59	78.9 ± 2.43
Phosphorus	0.40 ± 0.01	0.42 ± 0.01
Potassium	0.44 ± 0.01	0.54 ± 0.01
Sodium	0.02 ± 0.00	0.01 ± 0.00
Calcium	0.13 ± 0.00	0.06 ± 0.00
Magnesium	0.05 ± 0.00	0.09 ± 0.00
Sulphur	0.25 ± 0.01	0.30 ± 0.01
Sample number	4	9

Table 9 Mineral content of pollen (% ± S.E.) from *Eucalyptus diversicolor* compared with *Corymbia calophylla*.
Boron to Zinc in mg/kg; Phosphorus to Sulphur in %.

2.2.4 Vitamin profile

No Karri pollen was analysed for vitamins.

2.3 Eucalyptus marginata (Jarrah)

2.3.1 Amino Acid profile

The amino acid profile of jarrah is shown in Table 10. Every amino acid in jarrah was lower in concentration than redgum pollen. Amino acid deficiencies were found, particularly iso-leucine and histidine but the threonine concentration was also marginal.

Most of the essential amino acids of jarrah pollen have to be increased to match those of redgum: methionine (52.5%), threonine (39.1%), valine (32%), iso-leucine (40.8%), leucine (37.9%), phenylalanine (37%), lysine (37.2%), histidine (214.8%), arginine (108.8%) and tryptophan (45%).

A comparison between redgum and jarrah showed that the average protein concentration differed by 7%, redgum being highest at 26.7%. Average jarrah protein concentrations (19.7%) were below recommended minimum levels determined by de Groot (1953). The jarrah protein levels need to be increased by 35.5% to match redgum.

Amino acid	***E. marginata***	***C. calophylla***	**de Groot (1953)** minimum level
Cystine	0.61 ± 0.09 **	0.40 ± 0.01 (n=4)* 1.01 ± 0.05 (n=5)**	
Aspartic acid	1.44 ± 0.03	2.23 ± 0.07	
Methionine	0.40 ± 0.01	0.61 ± 0.03	0.3
Threonine	0.69 ± 0.02	0.96 ± 0.03	0.6
Serine	0.94 ± 0.05	1.17 ± 0.03	
Glutamic acid	1.77 ± 0.04	2.69 ± 0.08	
Proline	3.12 ± 0.20	3.45 ± 0.12	
Glycine	1.08 ± 0.07	1.27 ± 0.04	
Alanine	0.96 ± 0.04	1.38 ± 0.11	
Valine	0.97 ± 0.04	1.28 ± 0.05	0.8
Iso-leucine	**0.76 ± 0.04**	1.07 ± 0.05	**0.8**
Leucine	1.32 ± 0.04	1.82 ± 0.05	0.9
Tyrosine	0.52 ± 0.04	0.78 ± 0.03	
Phenylalanine	0.81 ± 0.03	1.11 ± 0.04	0.5
Lysine	1.37 ± 0.05	1.88 ± 0.11	0.6
Histidine	**0.27 ± 0.12**	0.85 ± 0.09	**0.3**
Arginine	0.91 ± 0.07	1.90 ± 0.06	0.6
Tryptophan	0.4 (n=1)	0.58 ± 0.08 (n=4)	0.2
Protein	**19.7 ± 0.18**	26.7 ± 0.21	**20**
Sample number	5	9	

Table 10 A comparison of amino acids (% ± S.E.) in pollen from *Eucalyptus marginata* and *Corymbia calophylla*. Deficiencies shown in bold.
Note: * & ** from two different laboratories: *Lab1; ** Lab2.

2.3.2 Fatty acid profile

Overall, jarrah was highest in C-16 and C-22 fatty acids than redgum. Redgum being highest in C-14, C-18, C-20 and C-24 fatty acids (Table 11).

Jarrah had an average of 33% less total fat in pollen than redgum (Table 12). Jarrah had higher concentrations of palmitic and linoleic fatty acids. Redgum had higher levels of myristic (5 x higher), stearic, oleic, linolenic (3 x higher) and arachidic fatty acids.

Jarrah pollen required 380.7% more myristic, 10.7% more stearic 217.5% more linolenic and 26.3% more arachidic fatty acids to match redgum pollen. Oleic fatty acid concentrations were similar for both species.

Fatty acid	***E. marginata***	***C. calophylla***
C-14	0.62 ± 0.17	2.98 ± 0.32
C-16	30.1 ± 1.10	17.8 ± 1.69
C-18	62.3 ± 3.44	68.0 ± 1.51
C-20	2.61 ± 0.67	3.96 ± 0.42
C-22	1.64 ± 0.34	1.46 ± 0.52
C-24	0.70 ± 0.70	1.13 ± 0.45

Table 11 Fatty acid (% ± S.E.) in lipid from *Eucalyptus marginata* compared with *Corymbia calophylla* pollen.

Fatty acid	***E. marginata***	***C. calophylla***
Total fat	0.59 ± 0.17	0.88 ± 0.07
Myristic (C-14)	0.62 ± 0.17	2.98 ± 0.32
Palmitic (C-16)	29.6 ± 1.21	17.4 ± 1.75
Stearic (C-18)	4.10 ± 0.48	4.54 ± 0.51
Oleic (C-18)	14.6 ± 2.36	15.0 ± 1.35
Linoleic (C-18)	39.7 ± 5.66	35.7 ± 3.59
Linolenic (C-18)	4.00 ± 1.26	12.7 ± 0.85
Arachidic (C-20)	1.60 ± 0.15	2.02 ± 0.27
Sample number	5	9

Table 12 Dominant fatty acids (% ± S.E.) in lipid from *Eucalyptus marginata* compared with *Corymbia calophylla* pollen.

2.3.3 Mineral profile

Jarrah pollen was highest in concentrations of manganese, potassium and calcium than redgum pollen (Table 13). To match redgum pollen, mineral concentrations have to be increased: boron (21.2%), copper (48%), iron (86.2%), zinc (65.1%) phosphorus (23.5%) and sulphur (57.9%).

Mineral	*E. marginata*	*C. calophylla*
Boron	15.6 ± 1.12	18.9 ± 1.16
Copper	14.8 ± 0.49	21.9 ± 0.95
Iron	66.8 ± 10.9	124.4 ± 9.15
Manganese	50.6 ± 4.27	35.8 ± 3.28
Zinc	47.8 ± 1.24	78.9 ± 2.43
Phosphorus	0.34 ± 0.00	0.42 ± 0.01
Potassium	0.57 ± 0.01	0.54 ± 0.01
Sodium	0.01 ± 0.00	0.01 ± 0.00
Calcium	0.13 ± 0.01	0.06 ± 0.00
Magnesium	0.09 ± 0.00	0.09 ± 0.00
Sulphur	0.19 ± 0.00	0.30 ± 0.01
Sample number	5	9

Table 13 Mineral content of pollen (± S.E.) from *Eucalyptus marginata* compared with *Corymbia calophylla*.
Boron to Zinc in mg/kg; Phosphorus to Sulphur in %.

2.3.4 Vitamin profile

A single jarrah pollen sample is shown for comparison with redgum. Single samples for the rest of the vitamins do not permit a true comparison, but are included for interest (Table 14).

Vitamin	*E. marginata*	*C. calophylla*
Ascorbic Acid 'C'	72	64.3 ± 32.1
Thiamine	ns	1
Riboflavin	ns	0.62
Niacin	ns	3.5
Pyridoxine	ns	0.4
Folic Acid	ns	2
Sample number	1	'Vit C' = 4, rest = 1

Table 14 Vitamin content of pollen (± S.E.) from *Eucalyptus marginata* compared with *Corymbia calophylla*.
Vitamin C in mg/kg; Rest of vitamins in mg/100g. ns = not sampled.

2.4 Eucalyptus patens (Forest Blackbutt)

2.4.1 Amino Acid profile

The amino acid profile of blackbutt is shown in Table 15. Blackbutt pollen had higher concentrations of proline and tryptophan amino acids and had relatively similar concentrations for most other amino acids to redgum. Notable exceptions being histidine and arginine, where if they were to match redgum levels they would have to be increased by 30.8 and 26.7% respectively.

The protein level of Blackbutt was 26.3%, which was almost identical to redgum pollen. A comparison between redgum and blackbutt showed that the average protein levels differed by only 0.4%.

Amino acid	*E. patens*	*C. calophylla*	de Groot (1953)

			minimum level
Cystine	0.62 ± 0.30*	0.40 ± 0.01 (n=4)* 1.01 ± 0.05 (n=5)**	
Aspartic acid	2.07 ± 0.10	2.23 ± 0.07	
Methionine	0.60 ± 0.05	0.61 ± 0.03	0.3
Threonine	0.95 ± 0.04	0.96 ± 0.03	0.6
Serine	1.18 ± 0.05	1.17 ± 0.03	
Glutamic acid	2.67 ± 0.21	2.69 ± 0.08	
Proline	3.78 ± 0.38	3.45 ± 0.12	
Glycine	1.23 ± 0.08	1.27 ± 0.04	
Alanine	1.23 ± 0.07	1.38 ± 0.11	
Valine	1.21 ± 0.04	1.28 ± 0.05	0.8
Iso-leucine	1.00 ± 0.06	1.07 ± 0.05	0.8
Leucine	1.76 ± 0.09	1.82 ± 0.05	0.9
Tyrosine	0.75 ± 0.02	0.78 ± 0.03	
Phenylalanine	1.08 ± 0.03	1.11 ± 0.04	0.5
Lysine	1.72 ± 0.07	1.88 ± 0.11	0.6
Histidine	0.65 ± 0.09	0.85 ± 0.09	0.3
Arginine	1.50 ± 0.12	1.90 ± 0.06	0.6
Tryptophan	0.70 ± 0.10 (n=2)	0.58 ± 0.08 (n=4)	0.2
Protein	26.3 ± 0.90	26.7 ± 0.21	20
Sample number	3	9	

Table 15 A comparison of amino acids (% ± S.E.) in pollen from *Eucalyptus patens* and *Corymbia calophylla*.
Note: * & ** from two different laboratories: *Lab1; ** Lab2.

2.4.2 Fatty acid profile

Blackbutt had higher concentrations of C-16 and C-24 fatty acids, whilst redgum had higher levels of C-14, C-18, C-20 and C-22 fatty acids (Table 16).

Blackbutt pollen had an average of 53.4% more fat than redgum pollen and had higher concentrations of palmitic, oleic and linoleic fatty acids. Redgum was higher in myristic (8 x higher), stearic, linolenic (> 2 x higher) and arachidic fatty acids (Table 17).

Blackbutt pollen required 705.4% more myristic, 76.7% more stearic, 115.3% more linolenic and 64.2% more arachidic fatty acids to match concentrations in redgum pollen.

Fatty acid	***E. patens***	***C. calophylla***
C-14	0.37 ± 0.20	2.98 ± 0.32
C-16	23.0 ± 1.04	17.8 ± 1.69
C-18	66.9 ± 3.16	68.0 ± 1.51
C-20	3.93 ± 0.66	3.96 ± 0.42
C-22	1.33 ± 0.18	1.46 ± 0.52
C-24	1.27 ± 0.37	1.13 ± 0.45

Table 16 Fatty acid (% ± S.E.) in lipid from *Eucalyptus patens* compared with *Corymbia calophylla* pollen.

Fatty acid	***E. patens***	***C. calophylla***

Total fat	1.35 ± 0.33	0.88 ± 0.07
Myristic (C-14)	0.37 ± 0.20	2.98 ± 0.32
Palmitic (C-16)	22.9 ± 1.07	17.4 ± 1.75
Stearic (C-18)	2.57 ± 0.18	4.54 ± 0.51
Oleic (C-18)	17.2 ± 5.20	15.0 ± 1.35
Linoleic (C-18)	41.2 ± 8.56	35.7 ± 3.59
Linolenic (C-18)	5.90 ± 0.85	12.7 ± 0.85
Arachidic (C-20)	1.23 ± 0.39	2.02 ± 0.27
Sample number	3	9

Table 17 Dominant fatty acids (% ± S.E.) in lipid from *Eucalyptus patens* compared with *Corymbia calophylla* pollen.

2.4.3 Mineral profile

Blackbutt pollen was highest in concentrations of potassium, sodium and calcium than redgum pollen (Table 18). To match redgum pollen, mineral concentrations have to be increased: boron (45.4%), copper (13.5%), iron (21.4%), zinc (13.9%), magnesium (12.5%) and sulphur (11.1%). Phosphorus and manganese concentrations were similar for both species.

Mineral	***E. patens***	***C. calophylla***
Boron	13.0 ± 1.73	18.9 ± 1.16
Copper	19.3 ± 0.48	21.9 ± 0.95
Iron	102.5 ± 12.1	124.4 ± 9.15
Manganese	36.0 ± 2.48	35.8 ± 3.28
Zinc	69.3 ± 5.31	78.9 ± 2.43
Phosphorus	0.40 ± 0.03	0.42 ± 0.01
Potassium	0.60 ± 0.04	0.54 ± 0.01
Sodium	0.02 ± 0.00	0.01 ± 0.00
Calcium	0.07 ± 0.01	0.06 ± 0.00
Magnesium	0.08 ± 0.00	0.09 ± 0.00
Sulphur	0.27 ± 0.01	0.30 ± 0.01
Sample number	3	9

Table 18 Mineral content of pollen (± S.E.) from *Eucalyptus patens* compared with *Corymbia calophylla*.
Boron to Zinc in mg/kg; Phosphorus to Sulphur in %.

2.4.4 Vitamin profile

Blackbutt pollen had a lower level of ascorbic acid when compared to redgum. A single sample for the rest of the vitamins does not permit a true comparison, but are included for interest (Table 19).

Vitamin	*E. patens*	*C. calophylla*
Ascorbic Acid 'C'	21	64.3 ± 32.1
Thiamine	ns	1
Riboflavin	ns	0.62
Niacin	ns	3.5
Pyridoxine	ns	0.4
Folic Acid	ns	2
Sample number	2	'Vit C' = 4, rest = 1

Table 19 Vitamin content of pollen (± S.E.) from *Eucalyptus patens* compared with *Corymbia calophylla*.
Vitamin C in mg/kg; Rest of vitamins in mg/100g. ns = not sampled.

2.5 Eucalyptus wandoo (Wandoo, Whitegum)

2.5.1 Amino Acid profile

The profile of amino acids of winter wandoo are shown in Table 20 with comparisons to both its spring and summer flowering types. The protein level of winter wandoo was lower by 5.6 to 8% than either spring or summer wandoo. With the exception of cystine and arginine, all amino acids are lower in concentration than in either spring or summer wandoo pollens, though arginine levels are higher in the summer than the winter flowering variety. Only one amino acid, iso-leucine was lower than the minimum level found by de Groot (1953) in all three wandoo varieties.

Every amino acid, apart from cystine (which was at the same level) was highest in redgum pollen. Most of the essential amino acids of winter wandoo pollen have to be increased to match those of redgum: methionine (32.6%), threonine (35.2%), valine (37.6%), iso-leucine (48.6%), leucine (41.1%), phenylalanine (48%), lysine (60.7%), histidine (93.2%), arginine (16.6%) and tryptophan (75.8%).

A comparison between redgum and wandoo showed that the average protein levels differed by 5.1%, redgum being the highest at 26.7% (Table 20). Protein levels in winter wandoo have to be increased by 22.5% to match redgum pollen.

Amino acid	Winter Wandoo	Spring Wandoo	Summer Wandoo	*C. calophylla*	de Groot (1953) minimum level
Cystine	0.40 ± 0.02*	0.32 ± 0.03*	0.34 ± 0.00*	0.40 ± 0.01 (n=4)* 1.01 ± 0.05 (n=5)**	
Aspartic acid	1.69 ± 0.03	1.78 ± 0.09	1.91 ± 0.05	2.23 ± 0.07	
Methionine	0.46 ± 0.01	0.50 ± 0.02	0.53 ± 0.03	0.61 ± 0.03	0.3
Threonine	0.71 ± 0.01	0.77 ± 0.05	0.82 ± 0.04	0.96 ± 0.03	0.6
Serine	0.90 ± 0.02	0.95 ± 0.05	1.00 ± 0.03	1.17 ± 0.03	
Glutamic acid	2.23 ± 0.04	2.39 ± 0.20	2.54 ± 0.10	2.69 ± 0.08	
Proline	2.52 ± 0.06	2.65 ± 0.09	2.67 ± 0.05	3.45 ± 0.12	
Glycine	0.92 ± 0.02	1.06 ± 0.06	1.14 ± 0.04	1.27 ± 0.04	
Alanine	0.97 ± 0.02	0.99 ± 0.06	1.07 ± 0.05	1.38 ± 0.11	
Valine	0.93 ± 0.02	0.96 ± 0.09	1.02 ± 0.04	1.28 ± 0.05	0.8
Iso-leucine	**0.72 ± 0.02**	**0.76 ± 0.06**	**0.77 ± 0.03**	1.07 ± 0.05	**0.8**
Leucine	1.29 ± 0.02	1.43 ± 0.09	1.52 ± 0.06	1.82 ± 0.05	0.9
Tyrosine	0.54 ± 0.01	0.60 ± 0.05	0.62 ± 0.05	0.78 ± 0.03	
Phenylalanine	0.75 ± 0.02	0.85 ± 0.08	0.90 ± 0.05	1.11 ± 0.04	0.5
Lysine	1.17 ± 0.02	1.43 ± 0.09	1.49 ± 0.06	1.88 ± 0.11	0.6
Histidine	0.44 ± 0.02	0.46 ± 0.04	0.48 ± 0.02	0.85 ± 0.09	0.3
Arginine	1.63 ± 0.05	1.58 ± 0.08	1.69 ± 0.03	1.90 ± 0.06	0.6
Tryptophan	0.33 ± 0.03	0.60 ± 0.00	0.50 ± 0.10	0.58 ± 0.08 (n=4)	0.2
Protein	21.8 ± 0.42	23.1 ± 0.55	23.7 ± 0.75	26.7 ± 0.21	20
Sample number	9	2	2	9	

Table 20 Amino acid (% ± S.E.) in pollen from *Eucalyptus wandoo* flowering types: winter, spring and summer and *C. calophylla*. Deficiencies shown in bold type.
Note: * & ** from two different laboratories: *Lab1; ** Lab2.

2.5.2 Fatty Acid profile

Fatty acid chains C-16, C-20, C-22, C-23 and C-24 were highest in winter wandoo whilst C-14 and C-18 chain fatty acids were highest for redgum pollen (Table 21).

The fatty acid profile of winter wandoo is shown in Table 22 with comparisons to both its spring and summer flowering types. The winter wandoo had the same oil level in pollen as the spring wandoo but higher than the summer flowering species. Winter wandoo had higher levels of palmitic, oleic, linolenic and linoleic fatty acids than either the spring or summer wandoo types. Spring wandoo had higher concentrations of myristic and stearic fatty acids whilst summer wandoo had a higher concentration of arachidic fatty acid.

Winter wandoo had 47.7% more total fat in pollen than redgum. All fatty acids with the exception of myristic, linoleic and linolenic fatty acid, were highest in pollen from winter wandoo than redgum. The average linolenic level in redgum pollen was 10 x greater than winter wandoo (Table 22).

Wandoo pollen required 893.3% more myristic, 19.4% more linoleic and 876.9% more linolenic fatty acid to match levels in redgum pollen

Fatty	Winter	Spring	Summer	*C.*

acid	Wandoo	Wandoo	Wandoo	*calophylla*
C-14	0.30 ± 0.10	1.20 ± 0.20	0.00 ± 0.00	2.98 ± 0.32
C-16	21.9 ± 1.30	18.4 ± 0.40	19.0 ± 2.65	17.8 ± 1.69
C-18	60.2 ± 3.66	47.0 ± 1.20	42.2 ± 1.65	68.0 ± 1.51
C-20	9.16 ± 1.55	11.3 ± 1.40	12.8 ± 1.05	3.96 ± 0.42
C-22	1.53 ± 0.17	2.90 ± 0.10	3.85 ± 0.25	1.46 ± 0.52
C-24	3.09 ± 0.88	4.30 ± 0.10	5.60 ± 1.50	1.13 ± 0.45

Table 21 Fatty acid (% ± S.E.) from *Eucalyptus wandoo* flowering types: winter, spring and summer and *C. calophylla* pollen.

Fatty acid	Winter Wandoo	Spring Wandoo	Summer Wandoo	*C. calophylla*
Total fat	1.30 ± 0.07	1.30 ± 0.08	1.28 ± 0.09	0.88 ± 0.07
Myristic (C-14)	0.54 ± 0.03	0.85 ± 0.05	0.80 ± 0.20	2.98 ± 0.32
Palmitic (C-16)	22.3 ± 0.53	24.5 ± 0.90	23.4 ± 2.65	17.4 ± 1.75
Stearic (C-18)	5.69 ± 0.21	4.80 ± 0.60	4.60 ± 0.00	4.54 ± 0.51
Oleic (C-18)	13.8 ± 1.35	12.7 ± 0.50	12.7 ± 1.50	15.0 ± 1.35
Linoleic (C-18)	46.7 ± 0.58	45.0 ± 3.15	47.9 ± 1.15	35.7 ± 3.59
Linolenic (C-18)	1.80 ± 0.29	1.65 ± 0.25	1.45 ± 0.05	12.7 ± 0.85
Arachidic (C-20)	3.86 ± 0.14	3.55 ± 0.65	3.40 ± 0.10	2.02 ± 0.27
Sample number	9	2	2	9

Table 22 Dominant fatty acids (% ± S.E.) in lipid from *Eucalyptus wandoo* flowering types: winter, spring and summer and *C. calophylla* pollen.

2.5.3 Mineral profile

The mineral profile of winter wandoo pollen is shown in Table 23 with comparisons to both its spring and summer flowering types. The winter wandoo had the highest iron and lowest boron content in pollen than either the spring or summer wandoo. The summer variety had the highest copper and manganese values whilst the spring variety had the higher zinc concentration in pollen. All other values of phosphorus, potassium, sodium, calcium, magnesium and sulphur were similar for all three varieties of wandoo.

Winter wandoo pollen was highest in levels of iron, sodium and calcium when compared to redgum (Table 23).

To match redgum pollen, mineral concentrations have to be increased: boron (81.7%), copper (48%), manganese (39.8%), zinc (52.9%), magnesium (80%), phosphorus (31.3%), potassium (28.6%) and sulphur (30.4%).

Mineral	Winter wandoo	Spring wandoo	Summer wandoo	*Corymbia calophylla*
Boron	10.4 ± 0.17	13.0 ± 0.00	12.5 ± 1.50	18.9 ± 1.16
Copper	14.8 ± 0.55	14.0 ± 0.00	17.5 ± 0.50	21.9 ± 0.95
Iron	181.4 ± 38.9	120.0 ± 20.0	145.0 ± 35.0	124.4 ± 9.15
Manganese	25.6 ± 1.02	25.0 ± 1.00	35.5 ± 0.50	35.8 ± 3.28
Zinc	51.6 ± 1.17	61.5 ± 8.50	49.0 ± 1.00	78.9 ± 2.43
Phosphorus	0.32 ± 0.01	0.35 ± 0.01	0.38 ± 0.02	0.42 ± 0.01
Potassium	0.42 ± 0.01	0.45 ± 0.00	0.46 ± 0.04	0.54 ± 0.01
Sodium	0.03 ± 0.00	0.04 ± 0.00	0.02 ± 0.00	0.01 ± 0.00
Calcium	0.09 ± 0.00	0.10 ± 0.01	0.10 ± 0.01	0.06 ± 0.00
Magnesium	0.05 ± 0.00	0.06 ± 0.01	0.06 ± 0.01	0.09 ± 0.00
Sulphur	0.23 ± 0.01	0.23 ± 0.01	0.25 ± 0.01	0.30 ± 0.01
Sample number	9	2	2	9

Table 23 Mineral content of pollen (± S.E.) from *Eucalyptus wandoo* flowering types: winter, spring and summer and *C. calophylla*.
Boron to Zinc in mg/kg; Phosphorus to Sulphur in %.

2.5.4 Vitamin profile

The vitamin profile of winter wandoo is shown in Table 24 with a comparison to both its spring and summer flowering types. The number of samples collected have probably influenced the result, but nevertheless, winter wandoo showed a higher ascorbic acid concentration than either spring or summer flowering species. Individual samples are quite variable for ascorbic acid (vitamin C).

Winter wandoo pollen had a higher concentration of ascorbic acid than redgum. A single sample for the rest of the vitamins does not permit a true comparison, but are included for interest.

Vitamin	Winter wandoo	Spring wandoo	Summer wandoo	*Corymbia calophylla*
Ascorbic Acid 'C'	206.5 ± 69.4	44.0 ± 24.0	102.0 ± 28.0	64.3 ± 32.1
Thiamine	3.40 ± 0.04	ns	ns	1
Riboflavin	0.65 ± 0.02	ns	ns	0.62
Niacin	2.25 ± 0.42	ns	ns	3.5
Pyridoxine	0.20 ± 0.00	ns	ns	0.4
Folic Acid	19.8 ± 1.27	ns	ns	2
Sample number	4	2	2	'Vit C' = 4, rest = 1

Table 24 Vitamin content of pollen (± S.E.) from *Eucalyptus wandoo* flowering types: winter, spring and summer and *C. calophylla*.
Vitamin C in mg/kg; Rest of vitamins in mg/100g. ns = not sampled.

2.6 Corymbia (Eucalyptus) calophylla (Marri, Redgum)

Redgum pollen had the highest concentration of:

Myristic and linolenic fatty acids

Minerals: boron, copper, zinc, phosphorus, magnesium and sulphur

Crude protein

Amino acids: aspartic acid, methionine, threonine, glutamic acid, glycine, alanine, valine, iso-leucine, tyrosine, leucine, phenylalanine, lysine and histidine

than any other pollens analysed in this project.

2.7 North-south differences in the chemical composition of winter wandoo pollen

Statistical analyses separated two distinct groups of wandoo. The northern group of plants had a number of amino acids that were significantly different ($P<0.05$) than the southern group of wandoo (Fig 4, Table 25).

Amino acid	North Group	South Group	P-value (0.05)
Aspartic Acid	1.77 ± 0.02	1.65 ± 0.02	P=0.019*
Methionine	0.49 ± 0.01	0.45 ± 0.01	P=0.018*
Threonine	0.75 ± 0.02	0.70 ± 0.01	P=0.008*
Serine	0.93 ± 0.03	0.88 ± 0.02	P=0.153
Glutamic acid	2.34 ± 0.04	2.17 ± 0.03	P=0.012*
Proline	2.62 ± 0.14	2.48 ± 0.05	P=0.276
Glycine	0.97 ± 0.02	0.90 ± 0.01	P=0.003*
Alanine	1.02 ± 0.02	0.94 ± 0.01	P=0.014*
Valine	0.99 ± 0.03	0.90 ± 0.00	P=0.002*
Iso-leucine	0.77 ± 0.02	0.69 ± 0.01	P=0.004*
Leucine	1.37 ± 0.02	1.26 ± 0.02	P=0.005*
Tyrosine	0.55 ± 0.01	0.53 ± 0.02	P=0.434
Phenylalanine	0.79 ± 0.03	0.73 ± 0.02	P=0.122
Lysine	1.21 ± 0.03	1.16 ± 0.02	P=0.118
Histidine	0.48 ± 0.04	0.42 ± 0.01	P=0.072
Arginine	1.69 ± 0.06	1.60 ± 0.06	P=0.374
Crude protein	22.6 ± 0.12	21.4 ± 0.56	P=0.192
Fat (lipid)	1.22 ± 0.15	1.34 ± 0.07	P=0.440
N	3	6	

Table 25 Statistical analysis of the chemical composition of pollen from a northern group of wandoo compared with a southern group of trees. (Cystine and tryptophan have been excluded). *Amino acids are significantly different at P= 0.05.

Of the 16 amino acids compared there were significant differences between nine amino acids including five essential fatty acids. Overall, all amino acids were lowest in pollen from the southern group of wandoo but lipid (fat) was higher than northern trees. Nutritionally, the northern group of wandoo had a better quality of pollen despite crude protein levels not being significantly different between the two groups.

2.8 Coastal plain redgum pollen compared to inland (hills) redgum pollen

Trees located on the coastal plain (Dandaragan, Busselton and Cape Naturalist) had a number of amino acids in the pollen that were significantly different ($P<0.05$) when compared to redgum located further inland from the coastal plain (see Map - Fig 3, Table 26).

Amino acid	Inland (hills)	Coastal	P-value (0.05)
Aspartic Acid	2.38 ± 0.06	2.03 ± 0.06	P=0.007*
Methionine	0.65 ± 0.04	0.56 ± 0.01	P=0.112
Threonine	1.01 ± 0.03	0.88 ± 0.01	P=0.013*
Serine	1.24 ± 0.03	1.07 ± 0.03	P=0.011*
Glutamic acid	2.74 ± 0.08	2.54 ± 0.16	P=0.261
Proline	3.63 ± 0.11	303 ± 0.27	P=0.228
Glycine	1.34 ± 0.06	1.16 ± 0.02	P=0.058
Alanine	1.57 ± 0.16	1.15 ± 0.04	P=0.103
Valine	1.37 ± 0.06	1.16 ± 0.04	P=0.060
Iso-leucine	1.16 ± 0.06	0.98 ± 0.07	P=0.118
Leucine	1.92 ± 0.07	1.7 ± 0.02	P=0.063
Tyrosine	0.83 ± 0.04	0.72 ± 0.03	P=0.087
Phenylalanine	1.19 ± 0.05	1.00 ± 0.01	P=0.029*
Lysine	2.05 ± 0.15	1.69 ± 0.09	P=0.149
Histidine	1.02 ± 0.1	0.66 ± 0.06	P=0.049*
Arginine	1.9 ± 0.07	1.84 ± 0.15	P=0.657
Crude protein	26.8 ± 0.20	26.5 ± 0.58	P=0.582
Fat (lipid)	0.93 ± 0.13	0.85 ± 0.08	P=0.680
N	5	3	

Table 26 Statistical analysis of pollen from coastal redgum trees compared with inland redgum.
(Cystine and tryptophan have been excluded). *Amino acids are significantly different at P=0.05. The location of the collection of one pollen sample was unknown.

Of the sixteen amino acids in Table 26, only 5 showed significant differences with three of these being from the 'essential' group of amino acids. Overall, pollen protein from coastal trees had lower percentages of amino acids and lipids (fats) than inland redgum. The quality of redgum pollen is therefore better from inland apiaries located in the hills on heavier soil types.

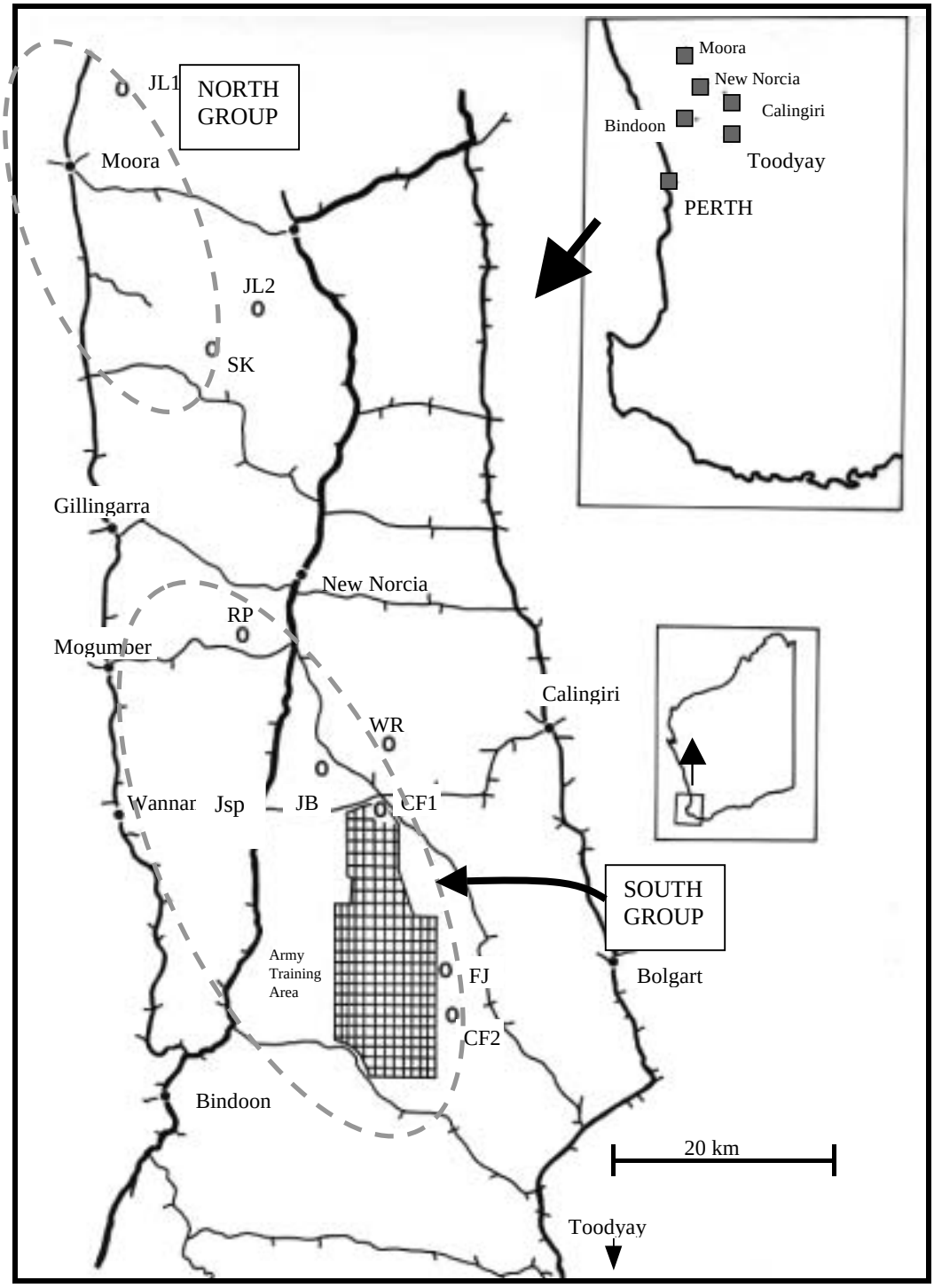


Figure 4 Pollen collection sites of winter flowering wandoo (*Eucalyptus wandoo*), April-May 1998.

2.9 Comparison of jarrah and redgum with previous published pollen analyses

Amino acids

The comparison between previously published data on redgum *(C. calophylla)* and jarrah *(E. marginata)* are shown in Tables 27 and 28 respectively. The comparisons were confounded by the differences in treatments of the pollens prior to the analyses being carried out.

Amino acid	***C. calophylla*** Bell *et al.* (1983)[1]	***C. calophylla*** Spadek & Chambers (1987)[2]	***C. calophylla***	**de Groot (1953)** minimum level
Cystine	0.55	0.47	0.40 ± 0.01 (n=4)* 1.01 ± 0.05 (n=5)**	
Aspartic acid	1.86	2.84	2.23 ± 0.07	
Methionine	0.76	0.58	0.61 ± 0.03	0.3
Threonine	1.07	1.08	0.96 ± 0.03	0.6
Serine	1.04	1.52	1.17 ± 0.03	
Glutamic acid	1.71	2.82	2.69 ± 0.08	
Proline	1.87	4.03	3.45 ± 0.12	
Glycine	0.84	1.21	1.27 ± 0.04	
Alanine	0.88	1.35	1.38 ± 0.11	
Valine	1.07	1.52	1.28 ± 0.05	0.8
Iso-leucine	1.12	1.13	1.07 ± 0.05	0.8
Leucine	1.45	2.15	1.82 ± 0.05	0.9
Tyrosine	0.97	ns	0.78 ± 0.03	
Phenylalanine	1.16	1.27	1.11 ± 0.04	0.5
Lysine	1.01	1.77	1.88 ± 0.11	0.6
Histidine	0.90	0.72	0.85 ± 0.09	0.3
Arginine	1.64	2.26	1.90 ± 0.06	0.6
Tryptophan	0.31	ns	0.58 ± 0.08 (n=4)	0.2
Protein	27.9	27.6	26.7 ± 0.21	20
Sample number	unknown	unknown	9	

Table 27 Amino acid (% ± S.E.) in pollen from *C. calophylla* compared to other published data.
Note: * & ** from two different laboratories: *Lab1; ** Lab2; ns = not sampled.
[1]already processed for human consumption (ie partially dried) prior to analyses. Samples collected in 1978. mg/g N converted to %. [2]samples dried by forced air at less than 45°C. g/16g N converted to %.

Pollens from both published accounts were air-dried before analyses (i.e. packed for human consumption) whereas the pollens from this project were 'wet' (i.e. fresh from the pollen traps) prior to testing. Some drying would have occurred over the few days the pollen was in the trap prior to being collected and during delivery to Agriculture WA.

Air drying of pollen, according to results in Spadek and Chambers (1987) affected the concentration of several amino acids. After forced air-drying of capeweed *(Arctotheca calendula)* and Paterson's curse *(Echium plantagineum)* pollen (5 samples) at 45°C, lysine concentration decreased by 19%, histidine by 12% and proline by 28%. Valine concentration increased by 17%.

Of jarrah and redgum pollen, lysine was the only amino acid to follow the expected decline from values determined from fresh pollen samples. The other amino acids (for both published accounts) contradict each other even though both had been thoroughly dried when compared to the 'wet' samples measured in this project.

Amino acid	***E. marginata*** Bell *et al.* (1983) [1]	***E. marginata*** Spadek & Chambers (1987) [2]	***E. marginata***	**de Groot (1953)** minimum level
Cystine	0.39	0.32	0.61 ± 0.09 **	
Aspartic acid	1.55	1.69	1.44 ± 0.03	
Methionine	0.57	0.43	0.40 ± 0.01	0.3
Threonine	0.92	0.73	0.69 ± 0.02	0.6
Serine	0.9	1.01	0.94 ± 0.05	
Glutamic acid	1.5	1.71	1.77 ± 0.04	
Proline	1.51	3.65	3.12 ± 0.20	
Glycine	0.75	0.81	1.08 ± 0.07	
Alanine	0.83	0.97	0.96 ± 0.04	
Valine	1.05	0.95	0.97 ± 0.04	0.8
Iso-leucine	0.88	**0.73**	**0.76 ± 0.04**	**0.8**
Leucine	1.16	1.36	1.32 ± 0.04	0.9
Tyrosine	ns	ns	0.52 ± 0.04	
Phenylalanine	1.03	0.87	0.81 ± 0.03	0.5
Lysine	0.82	1.20	1.37 ± 0.05	0.6
Histidine	0.92	0.43	**0.27 ± 0.12**	**0.3**
Arginine	1.00	1.06	0.91 ± 0.07	0.6
Tryptophan	0.36	ns	0.4 (n=1)	0.2
Protein	20.6	**19.7**	**19.7 ± 0.18**	**20**
Sample number	unknown	unknown	5	

Table 28 A comparison of amino acids (% ± S.E.) in pollen from *Eucalyptus marginata* compared with other published data. Deficiencies shown in bold type.
Note: * & ** from two different laboratories: *Lab1; ** Lab2; ns = not sampled.
[1]already processed for human consumption (ie partially dried) prior to analyses. Samples collected in 1978. mg/g N converted to %. [2]samples dried by forced air at less than 45°C and probably collected in 1986. g/16g N converted to %.

Minerals

The comparison of mineral concentration between those published by Bell *et al.* (1983) and fresh collected pollens shows some variability. Nonetheless, the mineral profile of each species showed distinct differences that could be used to separate species. The minerals calcium, zinc and copper showed significant differences (Table 29).

Minerals	***E. marginata*** Bell *et al.* (1983) [1]	***E. marginata***	***C. calophylla*** Bell *et al.* (1983)[1]	***C. calophylla***

Potassium	4653	5700	4627	5400
Phosphorus	3467	3400	3869	4200
Calcium	1123	1300	581	600
Sodium	931	100	865	100
Magnesium	776	900	880	900
Zinc	46	48	66	79
Copper	14	15	25	22
Manganese		51		35
Sulphur		1900		3000
Boron		16		19

Table 29 A comparison of minerals (mg/kg) in pollen from *Eucalyptus marginata* and *Corymbia calophylla* compared with published data (% in Table 13 converted to mg/kg).
[1]already processed for human consumption (ie partially dried) prior to analyses. Samples collected in 1978.

2.10 Comparison of redgum and winter wandoo with published pollen analyses from Italy

Commercial honey producers in Western Australia use the Italian honey bee (*Apis mellifera ligustica*) in their operations. The most "recent" importation from Italy, in the 1970s was extensively released to commercial beekeepers. The Italian honey bees have evolved with Italian plant species so therefore a comparative study would be useful in placing eucalypt pollens in context.

Bosi and Ricciardelli d'Albore (1975) measured amino acid profiles of honey bee collected pollen from 29 species of Italian plants. They averaged the values measured from all species which have been graphed (Fig 5). Of interest, is that for some Italian plant species, amino acids in pollen such as methionine, threonine, histidine and tryptophan are at near minimum concentrations required by honey bees. Generally, average concentrations are well above minimum requirements particularly for those amino acids such as valine and iso-leucine that are in low concentration in many eucalypt species.

The comparison suggests that redgum pollen could be higher in aspartic acid, iso-leucine and phenylalanine with slightly more valine and leucine in its pollen. The high proline concentration in redgum pollen significantly exceeded that of all 29 Italian plant species. Redgum came closest to Italian amino acid averages for lysine, alanine, glycine, tyrosine and serine (Fig 5).

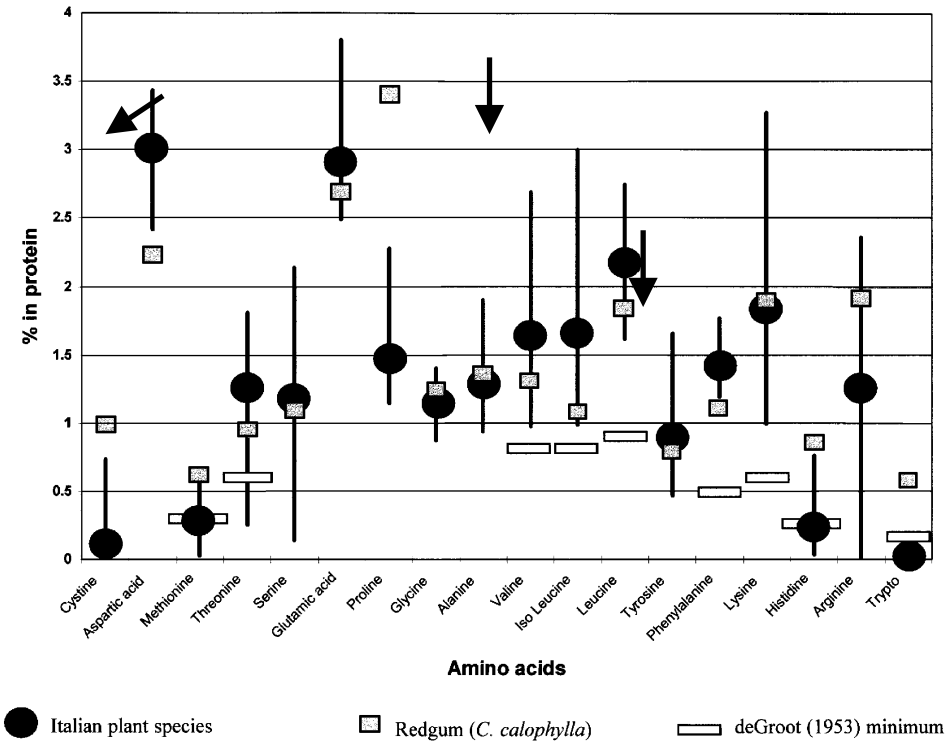


Figure 5 Comparison between the pollen profile of 29 Italian plant species and redgum. The amino acids from redgum that are, at or below, the minimum ranges of Italian plant pollens especially those arrowed.

From this study, winter wandoo was the poorer pollen when compared with redgum. But when compared to the averaged amino acid profile of Italian plant pollens, winter wandoo is at the extreme lower end of the Italian pollen range for 10 amino acids (Fig 6) with iso-leucine being below the minimum requirement required by honey bees. This perhaps best illustrates the need for beekeepers who work this nectar flow to supplementary feed their bees additional protein to optimise the life span of their honey bees.

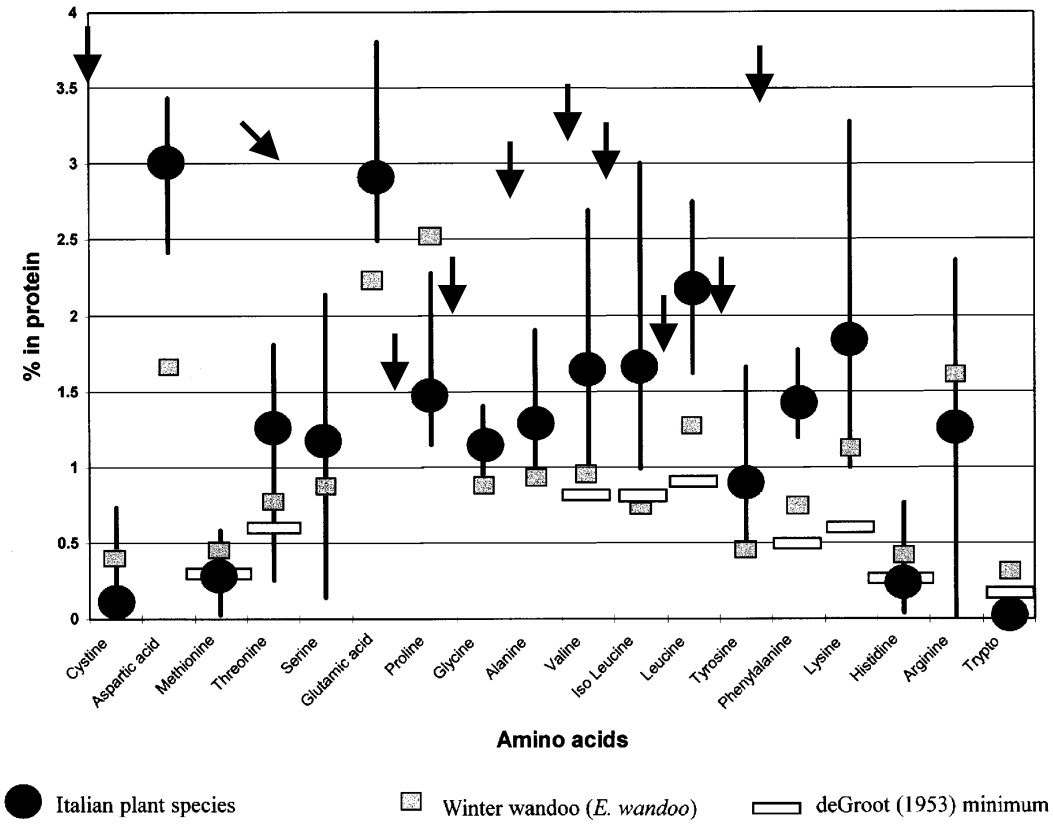


Figure 6 Comparison between the pollen profile of 29 Italian plant species and winter wandoo. The amino acids from winter wandoo that are, at or below, the minimum ranges of Italian plant pollens especially those arrowed.

3. Discussion

3.1 Amino acids

The analyses showed that redgum (*C. calophylla*), karri (*E. diversicolor*) and forest blackbutt (*E. patens*) were able to supply an ample concentration of amino acids for honey bee nutrition. Though comparisons with Italian flora pollens indicate that even these pollens could benefit from an additional source of a number of amino acids. The three other eucalypt species studied showed deficiencies in one or more amino acids that would affect body weights of honey bees and consequently the efficient production of hive products such as honey and pollen.

Powderbark (*E. accedens*) showed a deficiency in iso-leucine. Jarrah (*E. marginata*) pollen showed deficiencies in iso-leucine and histidine with borderline levels of threonine. In this respect, jarrah was very similar to pollen from pilliga box (*E. piligaensis),* an eastern States species. Honey bee colonies never 'collapse' after jarrah as they do after a flow from pilliga box. A reason for this is probably to do with the much higher protein level for jarrah (19.7%) than for pilliga box (16.6%), but it could also be that a forest blackbutt or redgum flow (both excellent pollens) usually occur following a jarrah flowering. Jarrah pollen protein is below the minimum 20% threshold determined by de Groot (1953) and all wandoo varieties showed deficiencies in iso-leucine.
The iso-leucine deficiency may be the cause of the problems that beekeepers experience after a wandoo flow (over the winter period) where AFB is apparently more prevalent and a significant decline in beehive population occurs. Honey bees on karri flows over winter have been known to abscond from beehives leaving boxes of honey and no brood. The analysis of karri pollen (protein, minerals and fatty acids) does not provide an explanation for this phenomenon. The jarrah flow with its apparent poorer nutrition status does not have the same perceived problem by beekeepers though the honey bees tend to pack-out the brood area with pollen which subsequently will cause a 'collapse' in bee numbers in the hive further down the track. With the recent outbreak of chalkbrood *(Ascophaera apis)* in Western Australia, one beekeeper has noticed that following a jarrah flow, a load of his bees were on dearth conditions for 2 to 3 weeks prior to being placed on a redgum flow, about 80% of his hives had chalkbrood. Another load that had a continuous source of nutrition right up to being placed on redgum showed no signs of chalkbrood (G. Kealley pers com). His best apiary had become his worst apiary.

The iso-leucine deficiency that occurred in pollen from powderbark, jarrah and wandoo are similar to that recorded for many other eucalypt species: *E. transcontinentalis* (Rayner and Langridge 1985), *E. piligaensis, E. albans, E. crebra, E. dealbata, E. andrewsii var campanulata, E. acmenoides, E. umbra, E. melanophloia,* and *E. planchoniana* (Stace, undated), *E. dumosa* (Somerville, 2000) with many other eucalypt species having borderline concentrations to that recommended by de Groot (1953).

After studying de Groot's (1953) data (see Fig 1), the amino acid deficiencies found in jarrah, wandoo and powderbark suggest that on these flows, there would be some body weight loss of honey bees. This would lead to honey bees having a shorter lifespan by either being weakened physically or by being susceptible to diseases such as *Nosema apis*. Kleinschmidt and Kondos (1976) showed that when high nosema spore counts coincided with poor quality or insufficient supply of pollen, the average lifespan of honey bees declined. Standifer *et al*. (1960) also

demonstrated that longer honey bee lifespan could be achieved with different protein concentrations from various protein sources in honey bee diets. Queen bees raised on winter wandoo in March tended to have shorter lifespan than those raised on redgum (T. Geer pers comm.) his observations are supported by a survey of eleven Western Australian beekeepers in 1991 which noted that queen bees bred on a redgum flow were preferred by industry (Hornitzky *et al*., 1991).

Apart from pollens effect on lifespan, different quality pollens can affect the development of the hypopharyngeal glands of nurse bees that produce worker jelly - the only food source for bee larvae. Standifer *et al*. (1970) showed that pollen containing 13.8% crude protein fed to nurse bees (at 1, 5 or 10% of a pollen substitute) resulted in slower development of hypopharyngeal glands than pollens with crude protein levels of 20.3%. The use of a pollen substitute with a crude protein content of 43.5% stimulated considerably more development than either the two pollen species. Higher protein levels will allow for higher percentages of amino acids to be present in concentrations above the minimum requirements found by de Groot (1953).

For those species that have amino acid deficiencies, it is recommended to artificially feed honey bees using ingredients such as soyflour or yeast. These are either mixed with irradiated pollen (a supplement) or mixed with sugar syrup/dry sugar (a substitute) to increase the concentrations of iso-leucine and many of the other amino acids at the same time. Soyflour contains about 2% iso-leucine and brewer's yeast contains 2.2 to 3.1% (Evans, 1985; Long, 1961) which are 2.5 and 2.8 to 3.9 times greater than the required minimum (0.8%, de Groot 1953). Torula yeast has a greater amount than brewer's yeast at 3.7%.

The species that did not exhibit any amino acid deficiencies (forest blackbutt and karri) had large differences in individual amino acid levels when compared to redgum pollen. Karri had about 73% less in arginine and forest blackbutt had about 31 and 27% less in histidine and arginine respectively than redgum pollen. Arginine, whilst not limiting in karri pollen (according to de Groot, 1953) had the second lowest concentration (jarrah being lower) of species measured. It is an important amino acid as Herbert *et al*. (1970) found. In Herbert's experiments using dandelion (species not given) the pollen was deficient in tryptophan and phenylalanine. They fortified artificial diets (that included 10% dandelion pollen) with these two amino acids with the result that hatched larvae never developed beyond the 2 day old stage even though the diet was readily consumed. When the artificial diet (with 10% dandelion pollen) was fortified with arginine only, the bees reared brood for 3 generations. Arginine in the pollen was not in deficiency but was considered borderline. Therefore the supplementation of arginine to bees hived on karri or jarrah flows could be of considerable advantage to beekeepers and those preparing feedstuffs.

The eucalypts have a characteristically high level of proline in pollen when compared with Italian plant ranges (Figs 5, 6). Crailsheim and Leonhard (1997) showed that proline was the predominant amino acid in worker haemolymph (*viz* blood) and it comprised of 50% of the total amino acids in newly-emerged bees and up to 80% in bees from the third day on. They also showed that foragers collected after flights had lower concentrations of proline in their haemolymph than 22 day-old bees collected from the hive. All other amino acid concentrations remained basically the same. Of all the species studied, the wandoos and powderbark had the lowest concentrations of proline (2.52 to 2.67%) whilst karri, forest blackbutt and redgum had the highest at 3.84, 3.78 and 3.45% respectively.

3.2 Fatty acids

The pollen from eucalypt species examined had lipid contents which ranged from 0.59% (jarrah) to 1.9% (karri). A large number of fatty acids were found in the lipid fraction of pollen. The main fatty acids were myristic (C-14), palmitic (C-16), stearic (C-18), oleic (C-18:1), linoleic (C-18:2), linolenic (C-18:3) and arachidic (C-20). There were smaller amounts of behenic (C-22) and nervonic (C-24) fatty acids[1].

Fatty acids play an important role in honey bee nutrition as they do for humans, but they have another extremely important attribute: antimicrobial activity. The fungal pathogen of honey bees, chalkbrood (*Ascophaera apis*) has been shown to cause a decline in the incidence of another honey bee disease, European foulbrood (EFB - *Melissococcus pluton*) in the USA (USDA, 1993). The fungus inhibited EFB by producing a fatty acid called linoleic (Feldlaufer *et al*., 1993b). Further tests showed that linoleic acid also inhibited the spore-forming honey bee disease, American foulbrood (AFB - *Paenibacillus larvae larvae*) (Feldlaufer *et al*., 1993a). Both linoleic and linolenic acids are potent inhibitors of bacteria. In humans, for example, tests have showed that linoleic acid inhibited the etiologic agent of the disease chronic gastritis and peptic ulcer, *Helicobacter pylori* (Petschow *et al*., 1996).

Spore germination of *Clostridium botulinum*, *Clostridium sporogenes* and *Bacillus cereus* were inhibited by linolenic acid and less so by linoleic acid (Ababouch *et al.*, 1992). The minimum inhibitory concentration (the lowest concentration of fatty acid that showed total inhibition of colony formation) of linolenic acid was 75 to 100 g/ml (depending upon bacteria species). Ababouch *et al.* (1992) also showed that the minimum inhibitory concentration of linoleic acid was 100 g/ml and for myristic acid it was greater than 150 g/ml. The free fatty acids linoleic and linolenic have salts known as sodium linoleate and sodium linolenate which have an inhibitory effect at concentrations as low as 30-50 g/ml and 10-40 g/ml respectively (Ababouch *et al.*, 1992).

Palmitic, stearic, arachidic and erucic acids only showed partial inhibition at concentrations as high as 150 g/ml acid (Ababouch *et al.*, 1992) whereas Feldlaufer *et al.* (1993a) showed that these saturated fatty acids were inactive against AFB (see Fig 7).

Research by Greceanu and Enciu (1976) found that pollen (originating from Romania) inhibited microbes (of animal origin) such as *Bacillus coli* and two forms of *Salmonella*. *Staphylococcus aureus* and *Bacillus anthracis* were moderately sensitive to pollens. Of interest was that *Bacillus cereus* was resistant to pollen used in Greceanu and Enciu (1976) whereas in Ababouch *et al*., (1992) it was sensitive to linolenic, linoleic and myristic acids. Clearly the Romanian pollen had differences in concentrations of these fatty acids.

If for example, pollen was stored at 0.1 g of pollen/cell (i.e. about 10 pellets), and using the average linoleic acid concentration per gram of eucalypt pollen (4.50 mg/g, Appendix 1), the concentration would be therefore be 450 g in 0.1g pollen. From Feldlaufer *et al.* (1993a) we know that 250 g of linoleic acid had a 68 mm inhibition zone on agar plates and so it seems possible that the higher linoleic acid levels in eucalypt pollens could have a similar inhibitory effect. The average concentration of linolenic acid in 0.1 g of eucalypt pollen was 48 g, though the average concentration in redgum reached 106 g in 0.1 g of pollen.

[1] There were many other fatty acids in very low concentrations in pollen present within these groups. These have not been recorded here.

Pollens from all eucalypts tested except the spring and summer wandoo were highest in linoleic acid - the fatty acid that has been documented to inhibit AFB (Fig 7) and EFB. Interestingly, redgum had another two fatty acids: myristic and linolenic acids in much higher concentrations than the other five eucalypt species studied. This suggests that redgum pollen could have a higher antimicrobial activity than other pollens.

Anecdotal evidence supports the view that redgum could be a natural biocide for AFB. An old saying within the industry told to me by K. West said, *"when the redgum flowered there would not be any disease (AFB) around for awhile (i.e. 10 to 12 months)".*

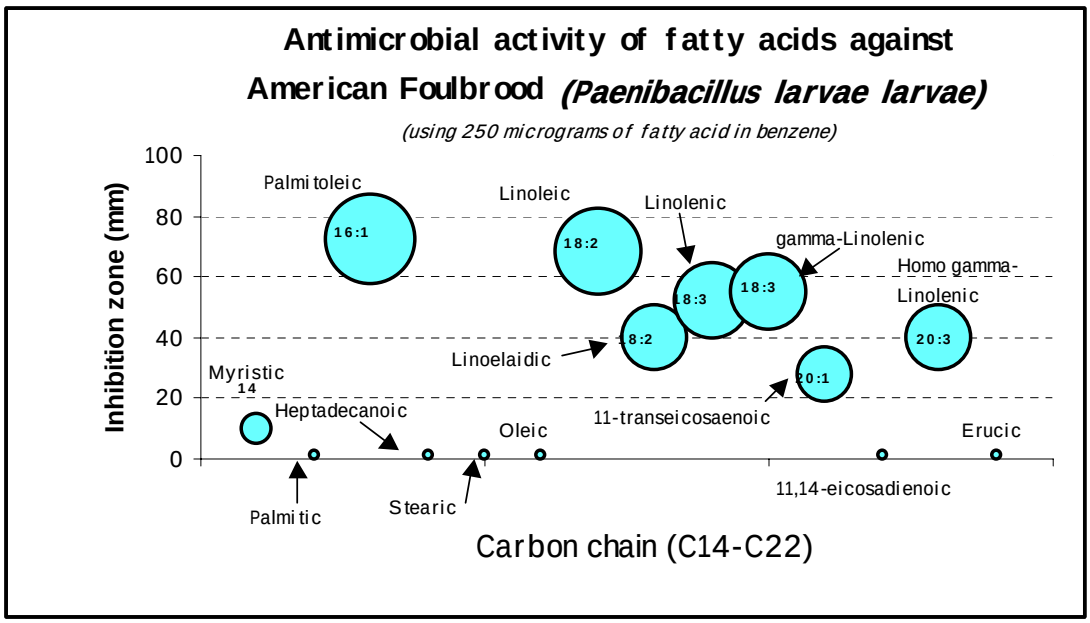


Figure 7 Graph redrawn from data in Feldlaufer *et. al.* (1993a) *Apidologie* **24**: 95-99. The circles represent a visual relationship of each fatty acid as to its inhibitory effect against American foulbrood.

Based upon the lipid content in the pollen, karri has nearly twice that of redgum and its higher linoleic acid concentration (than that of redgum) gives it a linoleic acid volume about three times higher than redgum. The linolenic volume also becomes similar to that of redgum. Therefore karri pollen could be considered 'a superior pollen' to redgum in its antimicrobial capacity.

Eucalypt pollens are low in lipids when compared to European/Mediterranean plants where honey bees evolved. Pollens from *Brassica napus* (canola) have fat levels of 5.9 to 7.3% (Somerville, 2000; Manning unpublished), *Lupinus* spp 1.7 to 3% (Somerville, 2000; Manning unpublished), and *Prunus dulcis* (almond) 1.9 to 2.7% (Somerville, 2000). Similarly, where *Apis mellifera capensis* has evolved alongside *Arctotheca calendula* (capeweed) in southern Africa its fat content of pollen is much higher than eucalypts at 2.1 to 3.4% (Manning unpublished; Somerville, 2000). There are many more pollens with lipid contents exceeding 8% and Standifer (1966) documents the following: red clover *(Trifolium pratense* 14.4%), lucerne *(Medicago sativa* 8.5%), apple (*Malus* spp 10.4%) - all of which are present in Australia.

The fatty acid profile of a single sample of canola (cv Karoo) has shown it to have 3x more myristic acid and 1.6x more linolenic acid but 2.6 x less linoleic acid than redgum (Manning, unpublished). The fat content of the canola pollen is also 6.7x greater than redgum. The higher

myristic and linolenic acids together with linoleic acid could make canola pollen a more powerful biocide than redgum. Again, anecdotal evidence seems to support this view - over a three-year period of regularly placing beehives on canola (in this case about 1,000) the incidence of AFB had virtually disappeared from all apiaries (K. West pers comm.). This is not to say that the disease won't re-establish itself sometime in the future as the AFB spores are only inhibited from germinating by the fatty acids.

If fatty acids are always present i.e. there are pollen flows or stored pollen within the hive, then its possible that some level of inhibition is always taking place. This could help keep the AFB spore number below the threshold that is required for the disease to develop in larvae. This threshold number is not very high especially for one day-old larvae. Chen *et al*. (1997), in laboratory tests, found that the LD_{50} (when 50% of the bees you are studying die) for one day-old larvae was 21 spores/larvae, whilst for a 95% kill some 445 spores/larvae are required. Two day-old larvae required some 45,000 spores/larvae to kill 37.2% of larvae and three day-old larvae were not at all susceptible at the same dose. In the field, with the normal flow of nectar and pollen into hives, Goodwin *et al*. (1994) demonstrated that colonies fed 50,000 and 500,000 spores did not develop AFB symptoms. Even when five hives were fed a 5,000,000 spore dose, 4 of the 5 hives only showed a light AFB infection (3 to 24 cells) after 30 days. The resistance of bees to succumb to high spore counts may be, in part, due to the fatty acids present in pollens.

Further testing of pollen and pollen species combinations for their inhibitory effect on bacteria using disc diffusion assay system such as those by Feldlaufer *et al*. (1993a) could confirm whether certain pollen flows or combinations of flows are beneficial to honey bees in the biological control of honey bee diseases. Further testing could also help market pollen to the cosmetic and food industry. Products that contain naturally occurring antibacterial agents (as pollens) could be made into innovative products and niche markets found.

This new finding that eucalypt pollen fats are dominated by linoleic fatty acid should be exploited so consumers can be aware of another benefit from consuming pollen. As a polyunsaturated fatty acid, linoleic acid helps lower the ratio of low density lipoproteins (LDLs) to high density lipoproteins (HDLs). The LDLs (known as the "bad" lipoprotein) carry fats such as cholesterol from our liver to our cells whilst HDLs carry cholesterol from our cells to our liver to be excreted as bile into the intestine (Erasmus, 1996).

By supplementary feeding of beehives, greater amounts of these fatty acids can be incorporated into the diet of honey bees. This can be achieved through additives such as torula yeast (though deficient in methionine, tyrosine and cystine (Long, 1961) and various oils such as linseed (probably never tested), grape seed oil (probably never tested), soy or safflower oil (Table 30) or actually adding the fatty acid (s).

Some 'experimentation' with fatty acids is currently taking place in Western Australia with anecdotal evidence showing favourable results. But this evidence really needs to be supported by a scientific approach to make it valid even though it is theoretically feasible.

Fatty acid source	% fat	% linoleic	% linolenic acid

		acid	
Torula yeast	6.4	49.7	4.4
Grape seed oil	100	46 to 71	0
Linseed oil	100	10 to 25	30 to 60
Canola oil	100	12 to 24	1 to 10
Safflower oil	100	57 to 59	0
Soybean oil	100	50 to 60	5 to 9
Hemp seed oil	100	54 to 70	15 to 22

Table 30 Some examples of sources of linoleic and linolenic fatty acids (Long 1961). Some of these may not be attractive to honey bees despite having large amounts of the two fatty acids.

3.3 Minerals

Mineral nutrition of plants can influence the quantity and quality of pollen that can influence mineral concentrations in honey bees. The most abundant minerals in honey bees are phosphorus and potassium (Herbert, 1997) which are also dominant in pollen (see Table 31, 32). Manganese is present in unusually high concentrations in adult honey bees with a range from 102 to 270 mg/kg (Nation and Robinson, 1971).

Minerals	**Adult worker bees[1]**	**Maryland (USA) pollen**	**Florida (USA) pollen**	***E. globulus* (USA) pollen**	***E. wandoo (winter)* pollen**	***C. calophylla* pollen**
Potassium	6675	2933	7440	6800	4200	5400
Phosphorus	ns	ns	ns	4900	3200	4200
Calcium	390	1067	1560	1700	900	600
Magnesium	1010	683	1770	900	500	900
Iron	114	83	58	26	181	124
Zinc	108	72	320	ns	52	79
Sodium	987	70	184	ns	300	100
Manganese	186	35	85*	ns	26	35
Copper	22.3	16	12	ns	15	22
Sulphur	ns	ns	ns	ns	2300	3000
Boron	ns	ns	ns	ns	10	19

Table 31 A comparison of minerals (mg/kg) in pollen from *Eucalyptus wandoo* and *Corymbia calophylla* compared with the mineral composition of adult worker bees and published data from:
Maryland (USA) pollen: Herbert and Shimanuki (1978)
Florida (USA) mixed pollen: Nation and Robinson (1971); *Mn reads as 848 mg/kg in their paper - probably in error.
E. globulus (USA) pollen: Todd and Bretherick (1942).
[1]Data averaged from autumn and spring data in Nation and Robinson (1971).
(% in Tables 13 & 23 for wandoo and redgum are converted to mg/kg for this table; ns = not sampled).

Compared to pollens from the USA, wandoo and redgum differ in a number of minerals. Within the eucalypt family of plants, mineral concentrations also vary (Table 31). The mineral profile of worker bees tends to differ markedly in sodium, manganese and calcium compared with pollen.

Research done by Herbert and Shimanuki (1978) on feeding various percentages of pollen ash into synthetic diets showed that by using 1% ash in a 454g patty (i.e. 4.6 g of ash) brood rearing reached its highest value of 4529 cm^2 at 10 weeks after 1.26 kg of synthetic diet was consumed. After recalculation of their pollen ash mineral concentrations, the amount of mineral in 1% ash added to the synthetic feed can be seen to be similar to pollen values in Table 32, particularly those of redgum. Recalculation of Herbert and Shimanuki (1978) results also allow some insight to *"how much is too much"* as far as minerals are concerned (Table 32).

Minerals	**Herbert and Shimanuki (1978)**	***E. wandoo (winter)***	***C. calophylla***	**Artificial feed to honey bees at 1% of feed[1]**	**High honey bee mortality via artificial feed[2]**
Potassium	2933	4200	5400	4218	>33287
Phosphorus	ns	3200	4200	ns	ns
Calcium	1067	900	600	1532	>12088
Magnesium	683	500	900	980	>7732
Iron	83	181	124	120	>944
Zinc	72	52	79	101	>799
Sodium	70	300	100	101	>799
Manganese	35	26	35	51	>399
Copper	16	15	22	23	>182
Sulphur	ns	2300	3000	ns	ns
Boron	ns	10	19	ns	ns

Table 32 A comparison of minerals (mg/kg) in pollen from *Eucalyptus wandoo* and *Corymbia calophylla* compared with published east coast USA (Maryland) data and requirements for an artificial feed that maximised brood production (recalculated from pollen ash values given by Herbert and Shimanuki, 1978).
[1]4.6g pollen ash (burnt at 550^{0}C) from pollen (column 1) was added to 208g (18 Amino acids) + dry sugar made up to 454 g in a blender and 50ml of 10 water soluble vitamins were added to form patties. Each patty was added to a nucleus hive that was free of honey and pollen, containing 400 g of newly emerged honey bees.
[2]Using >36.3g (>8%) pollen ash in the above mix produced less brood than the control in the experiment.
(% in Tables 13 & 23 for wandoo and redgum are converted to mg/kg for this table; ns = not sampled).

High concentrations of pollen ash (minerals) may cause brood laying to be restricted when added to feeds at >8% of total weight of feedstuff (Table 32). Waller *et al.* (1972) when studying the high potassium content of onion nectar determined that high levels of potassium had some deterrent effect on honey bee visits to onion flowers. Therefore too much potassium in artificial feeds could cause the consumption rate of the diet to be poor. Onion nectar potassium levels have been documented to range from 5347 to 6914 mg/kg (Hagler, 1990) which are higher than levels in redgum or the 1% ash level added to supplementary feeds, shown in Table 32.

Nation and Robinson (1971) recommended a mineral mixture for honey bees containing 5000 mg/kg potassium, 200 mg/kg sodium and 1000 mg/kg magnesium and calcium. The potassium and magnesium concentration is similar to redgum. On the basis of the tested ash at 1%, redgum could be enhanced with additional calcium. Karri and jarrah pollens have calcium levels of 1300 mg/kg, which were similar to the optimal concentration in the 1% ash experiment.

Concentrations between 100 and 200 mg/kg of sodium in a mineral supplement promoted brood rearing but sodium could be the culprit in increased honey bee mortality and less sealed brood when concentrations exceed these values. Herbert and Shimanuki (1977) fed 1653 mg/kg of sodium in synthetic diets with the result that no brood rearing occurred. Only after adding a double dose of vitamins did the honey bees produce sealed brood but this was still, only 11.5% of the brood area, when honey bees were fed pollen. Both winter and spring wandoo had sodium concentrations higher (300 and 400 mg/kg) than in the 1% ash experiments by Herbert and Shimanuki (1978) that had high brood production. The high levels of sodium in wandoo pollen could be the result of rising salinity in its environment due to land clearing.

Compared with many foods, pollen has high levels of minerals (Table 33) but because it is consumed in such small amounts compared to the other foods its importance is low for human consumption. But if taken on a regular basis it would be a beneficial supplement to those people on vegetarian diets where pollen could provide a source of minerals, particularly from the trace elements like Iron, zinc, manganese and copper.

3.4 Vitamins

The complete analyses of pollens for vitamins was constrained by high laboratory fees (and hence by the projects budget) but nevertheless a number of vitamins were measured. The exact dietary requirements of vitamins are difficult to determine, especially for fat-soluble vitamins (A, D, E & K), because microorganisms in the digestive tract may produce many of the vitamins (Herbert *et al*. 1985). But when vitamins are restricted from the diet of honey bees, vitamin concentrations in the body mass rapidly diminish. Haydak (1954) showed that in just 20 days bees fed only carbohydrate lost 50% of their body Thiamine. Haydak and Vivino (1943) found that vitamin content (Thiamine, riboflavin, pyridoxine, niacin, pantothenic acid and ascorbic acid) of honey bees generally decreased with age.

Vitamin C (ascorbic acid) concentration ranged from 21 to 207 mg/kg was similar to the measured levels found in honey bee prepupae body mass (64.5 to 103.5 mg/kg) by Herbert *et al*. (1985) despite the bees being fed up to 2000 mg/kg vitamin C. This high level of vitamin C in diets produced significantly more bees to sealed stage than bees fed 500 mg/kg or no vitamin C (control). Therefore vitamin levels in supplementary feeds using eucalypt pollen could be further enriched with vitamin C without any detrimental effect. This is supported by the analysis of pollens from Maryland (USA) which showed vitamin C concentrations can naturally reach 1943 mg/kg (Herbert *et al*. 1985).

Muss (1987) showed vitamin C concentrations in two eucalypt species ranged from 600 to 659 mg/kg which were much higher than the pollen concentration recorded for this project. Other species such as capeweed had 417 mg/kg, Paterson's curse 506 mg/kg and Navel orange *(Citrus sinensis)* with 914 mg/kg vitamin C.

It is known that vitamin C is very unstable when exposed to sunlight and air and undergoes rapid oxidation upon storage. With this in mind the above comparisons may be not be useful as Herbert *et al*. (1985) analysed pollens within the week of collection whilst for this project the period between collection and analysis was much longer. Herbert *et al*. (1985) measured the vitamin C concentration of fresh pollen up to 1 week old (346 to 401 mg/kg) and found the concentration decreased to 53.8 mg/kg after being frozen for one year.

Food	**K**	**P**	**Ca**	**Mg**	**Fe**	**Zn**	**Na**	**Mn**	**Cu**
Soy flour (ff)	20300	6400	2400	2900	91	32	140	29	31
Wheat bran	11600	12000	1100	5200	129	162	280	90	13
Almonds	7800	5500	2400	2700	30	32	140	17	10
Redgum pollen	**5400**	**4200**	**600**	**900**	**124**	**79**	**100**	**35**	**22**
Banana	4000	280	60	340	3	2	10	4	1
Chicken roast	3100	2100	90	240	8	15	810	0.3	1.2
Whiting (fried)	2900	2300	430	290	6	-	1800	-	-
Potato (boiled)	2500	280	50	120	3	1	90	1	0.6
Chops (grilled)	2300	1400	70	170	12	21	610	0.2	1.7
Mango	1800	160	120	130	7	1	20	3	1.2
Milk (pasteur.)	1400	920	1150	110	0.5	4	550	-	-
Eggs (raw/boil)	1300	2000	570	120	19	13	1400	-	0.8
Carrots (boiled)	1200	170	240	30	4	1	500	1	0.1
Apple (raw)	1200	110	40	50	1	1	30	1	0.2
Honey	510	170	50	20	4	9	110	3	0.5

Table 33 Comparison of redgum pollen *(Corymbia calophylla)* mineral profile (mg/kg) with other foods. Data (modified) for other foods taken from McCance and Widdowson (1992).
K = potassium; P = phosphorus; Ca = calcium; Mg = magnesium; Fe = iron; Zn = zinc; Na = sodium; Mn = manganese and Cu = copper; ff = full fat.

Another complicating factor is that copper rapidly oxidises ascorbic acid to dehydroascorbic acid (which still has some vitamin C activity). Herbert *et al*. (1985) showed that within a week, a loss of between 1 and 50% of ascorbic acid in a diet at a concentration of 2000 mg/kg occurred through the addition of 1% minerals containing copper sulphate. This was probably due to this oxidation process. A similar loss of between 7 and 76% occurred in diets fortified with 1000 mg/kg ascorbic acid. Copper concentration was highest in redgum pollen and this may have had some influence on its vitamin C concentration.

Thiamine concentrations ranged from 10 to 34 mg/kg; Riboflavin 6.1 to 6.5 mg/kg; Niacin 1 to 35 mg/kg; Pyridoxine 2 to 4 mg/kg and Folic acid 20 to 22 mg/kg.

4. Implications

Disease management
The analysis of pollen for fatty acids has shown a dominance of linoleic acid which has been shown by other researchers to inhibit the growth of spores of AFB and EFB bacteria. The interest here is, to what extent does this natural biocide have in the ecology of these diseases within the beehive? Further research should be undertaken in this area.

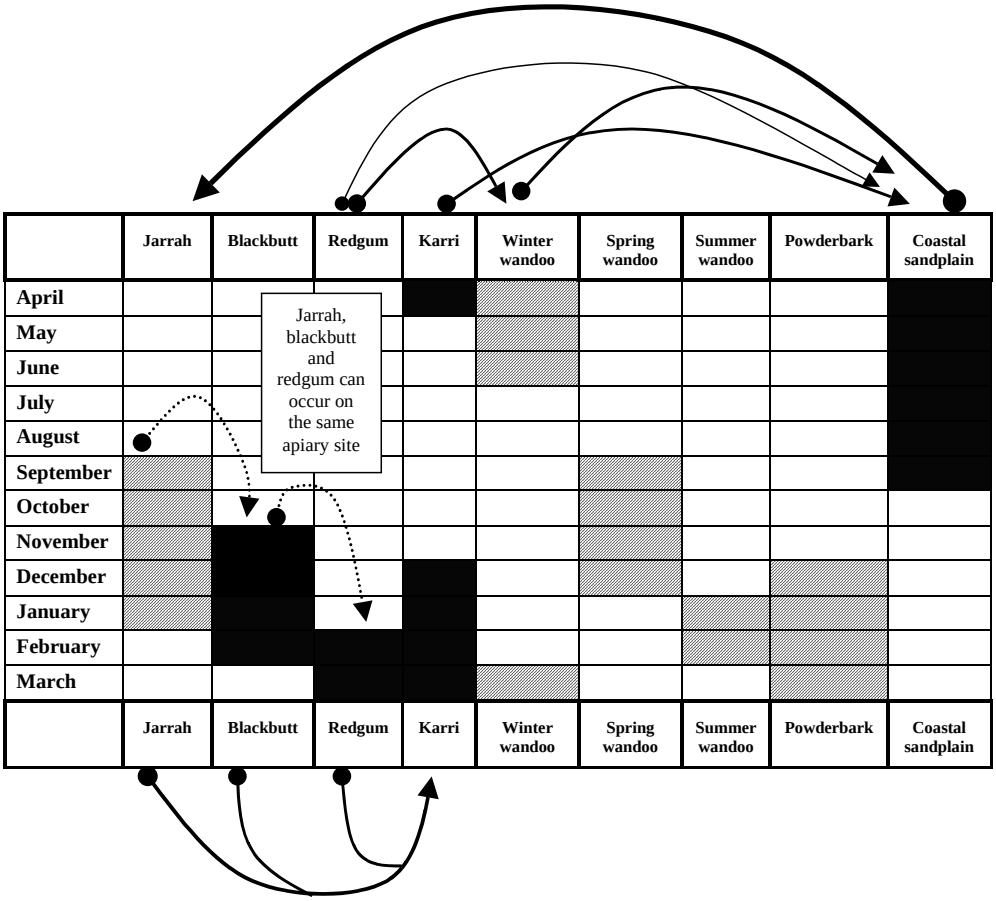
Access to forest (eucalypt) resources
Current saturated levels of apiary sites across many forest systems in (Western) Australia restrict many beekeepers from entering areas where nutrition from some eucalypt species may be better than others or where there are nutritional differences within the same species geographically. Newer beekeepers generally operate in nutritionally poorer areas ie a reduced source of quality pollen (esp. those with high C-18 fatty acids). Could this see a higher incidence of AFB and EFB (which is not present in WA) in this group of beekeepers? Differences in amino acids were found in winter wandoo in a north-south gradient that indicate the southern areas of wandoo are nutritionally poorer and therefore honey bees from colonies in these areas may shorten lifespan and generally harvest less honey and pollen over time. Restricted land access and relocation of apiary sites by land managers could also influence the nutrition status of beehives.

Logging
Silvicultural prescriptions for various forest types affect the nutrition supply for honey bees and other insects. Man-influenced dominance of single species forestry restricts the diversity of pollens available and influences the source of nutrition for honey bees and other insects. In the case of a jarrah forest the pollen source is nutritionally poor for honey bees and probably is so for a number of native animals. Prescribed burning in spring in these forests also assists in reducing the floral diversity of the resource and therefore the proteins available to honey bees within a forest system.

Probably due to extensive logging and its associated changes to the karri forest ecosystem, karri pollen has been extremely difficult to obtain in commercial quantities because of the poor flowering of this species since the late 1960s.

Redgum is the major contributor to the commercial pollen harvest in Western Australia with supply largely biennial. In forest management systems for timber, redgum has not been a desirable species and is extensively logged for the wood chip market especially in jarrah and karri forests. This reduction of redgum could cause a nutritional imbalance in the forest ecosystem.

Supplementary feeding on what flows?
Some beekeepers move onto summer wandoo (also known as whitegum) following a jarrah flow and both flows are deficient in one or more amino acids which should, over time, show signs of a decline in bee numbers. If the redgum fails or it’s an 'off' year (the species is biennial flowering) and honey bee colonies are not relocated onto good over-wintering sandplain (heath) country following the redgum flow then colony populations will sharply decrease. A number of scenarios (Fig 8 to 11) follow that can be used by beekeepers to be proactive in supplementary feeding of their beehives when a flowering sequence of eucalypts involves mainly powderbark, wandoo or jarrah.



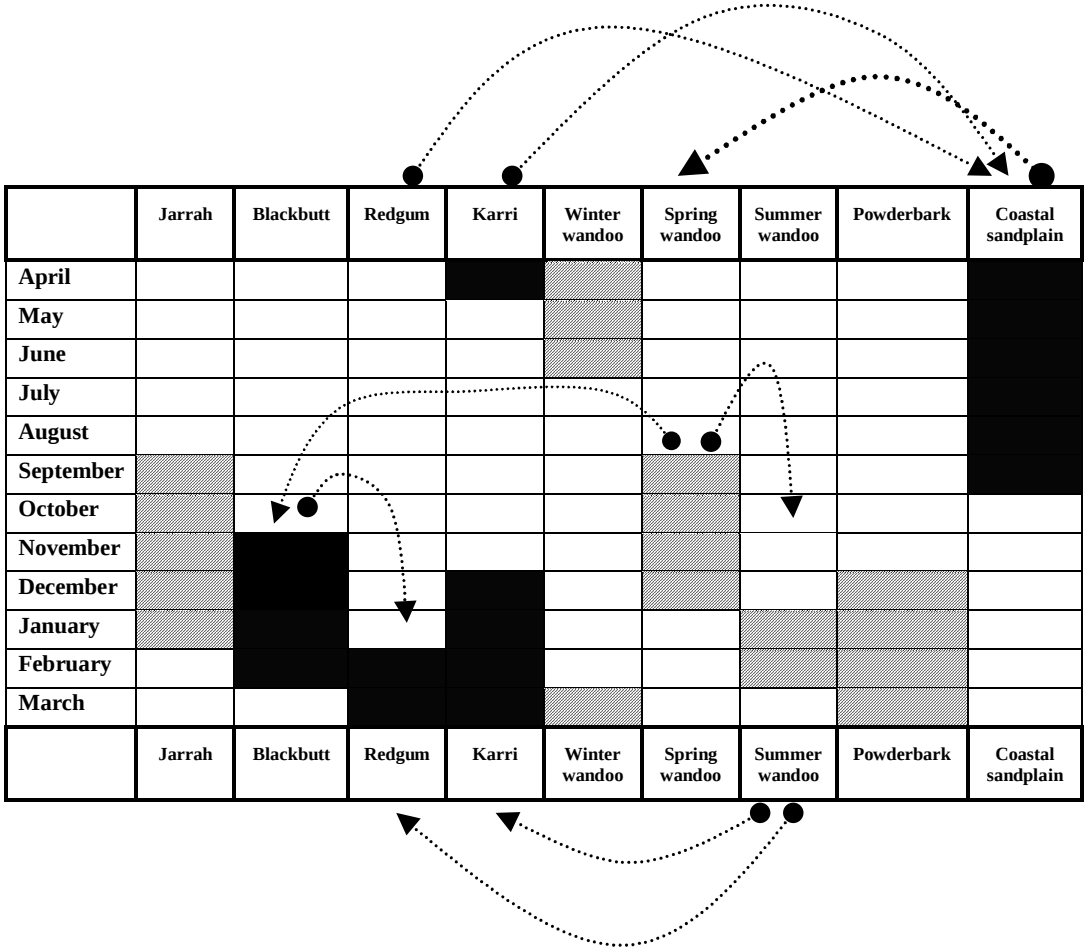
THE HIGHEST AND MOST EFFICIENT PRODUCTIVE FLOW

(JARRAH followed by BLACKBUTT followed by REDGUM followed by winter WANDOO)

Figure 8 A normal cycle of nectar flows utilised by beekeepers. Shifting an apiary from the coastal sandplain to jarrah, which could be followed, by a shift to blackbutt and/or redgum. A relocation of the apiary into karri forest at any stage (i.e. from jarrah, blackbutt or redgum) is also possible. Following these flows, beekeepers could move their bees from redgum directly to the coastal sandplain (if there is no winter wandoo) or from karri to sandplain or from winter wandoo to sandplain. Karri forests can contain jarrah and redgum.

GREY AREAS: Nutritionally poor pollen flows for honey bees.

Note: there are other species that for the purpose of this project have not been included. Coastal sandplain has been included because it is the major resource used by beekeepers annually. Some pollens from coastal sandplain species are nutritionally poor e.g. Mayflower *(Leucopogon).*

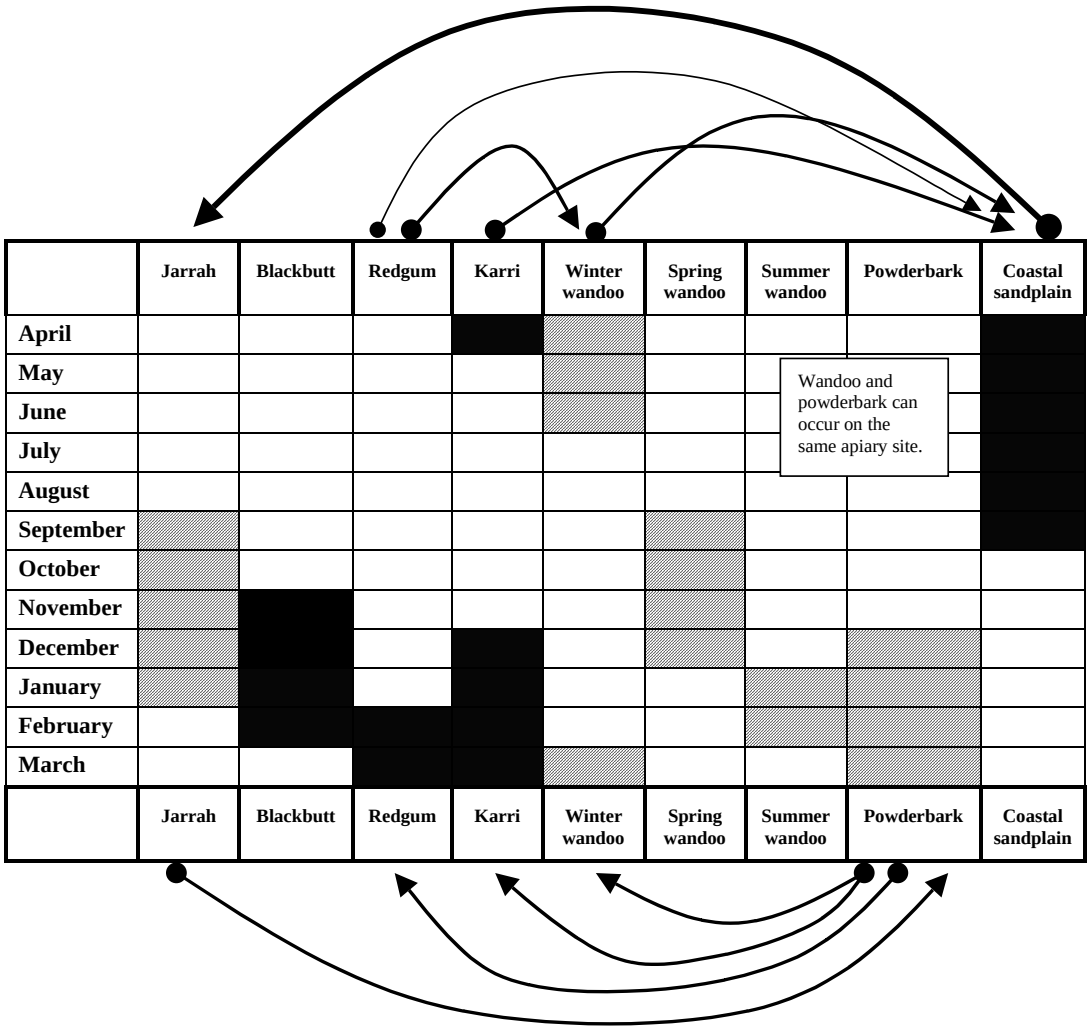


NO JARRAH FLOW

Figure 9 A second scenario for normal cycle of nectar flows utilised by beekeepers. Shifting an apiary from the coastal sandplain to spring wandoo that could be followed by a shift to summer wandoo. A move to blackbutt could also be possible then onto redgum. A relocation of the apiary into karri forest is also possible (which can contain redgum). Following these flows, beekeepers could move their bees from redgum directly back to the coastal sandplain (if there is no winter wandoo) or from karri to sandplain.

Hatched areas = Nutritionally poor pollen flows for honey bees.

Note: there are other species, which for the purpose of this project have not been included. Coastal sandplain has been included because it is the major resource used by beekeepers annually. Some pollens from coastal sandplain species are nutritionally poor e.g. Mayflower *(Leucopogon).*

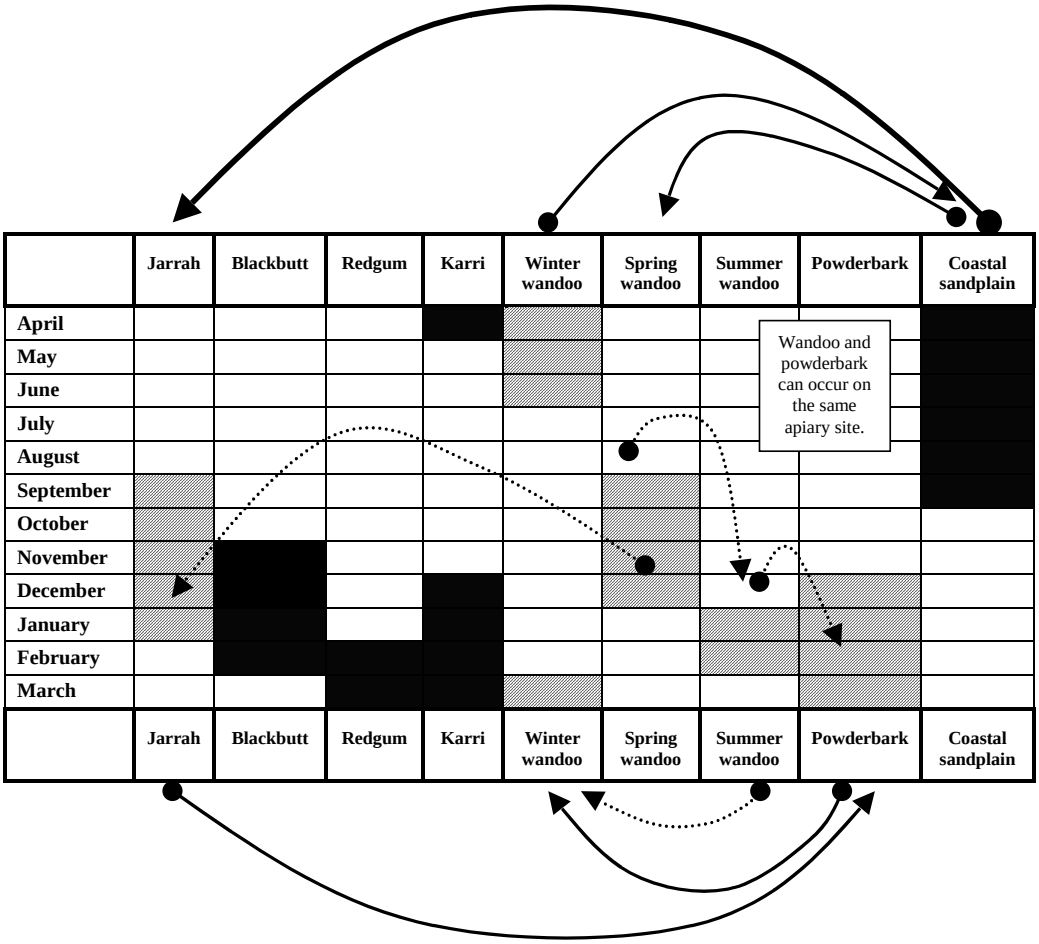


UTILISATION OF POWDERBARK

Figure 10 A third scenario for normal cycle of nectar flows utilised by beekeepers. Shifting an apiary from the coastal sandplain to jarrah, which could be followed by shift to powderbark. Following these flows, beekeepers could move their bees from redgum or karri or winter wandoo directly back to the coastal sandplain. Karri forests can contain jarrah and redgum. Beekeepers have noticed that it is not a "good move" to shift from powderbark to winter wandoo.

GREY AREAS: Nutritionally poor pollen flows for honey bees.

Note: there are other species, which for the purpose of this project have not been included. Coastal sandplain has been included because it is the major resource used by beekeepers annually. Some pollens from coastal sandplain species are nutritionally poor e.g. Mayflower *(Leucopogon).*



THE WORST POSSIBLE SITUATION

(No forest blackbutt, redgum or karri)!

Figure 11 A fourth scenario for normal cycle of nectar flows utilised by beekeepers. A move from the coastal sandplain to jarrah that could be followed by powderbark and or winter wandoo OR a move from sandplain to spring wandoo which could be followed by summer wandoo or late jarrah. Powderbark can occur on or near the same apiary sites as wandoo.

GREY AREAS: Nutritionally poor pollen flows for honey bees.

Note: there are other species, which for the purpose of this project have not been included. Coastal sandplain has been included because it is the major resource used by beekeepers annually. Some pollens from coastal sandplain species are nutritionally poor e.g. Mayflower *(Leucopogon).*

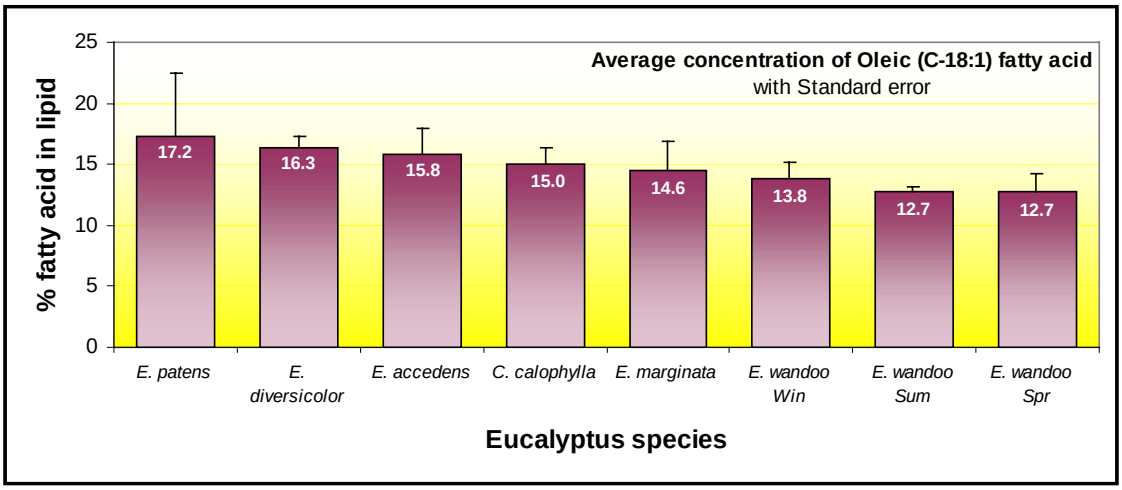
5. Recommendations

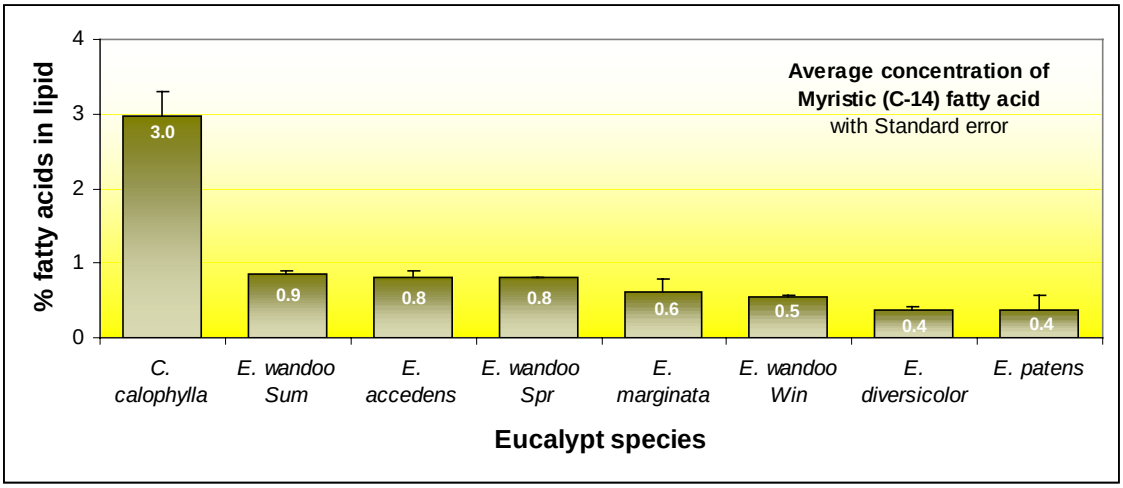
Future research on the chemical composition of pollens should analyse for the fatty acid and mineral components of pollen. All pollens that have had amino acid profiles determined should be re-examined for minerals and fatty acids.

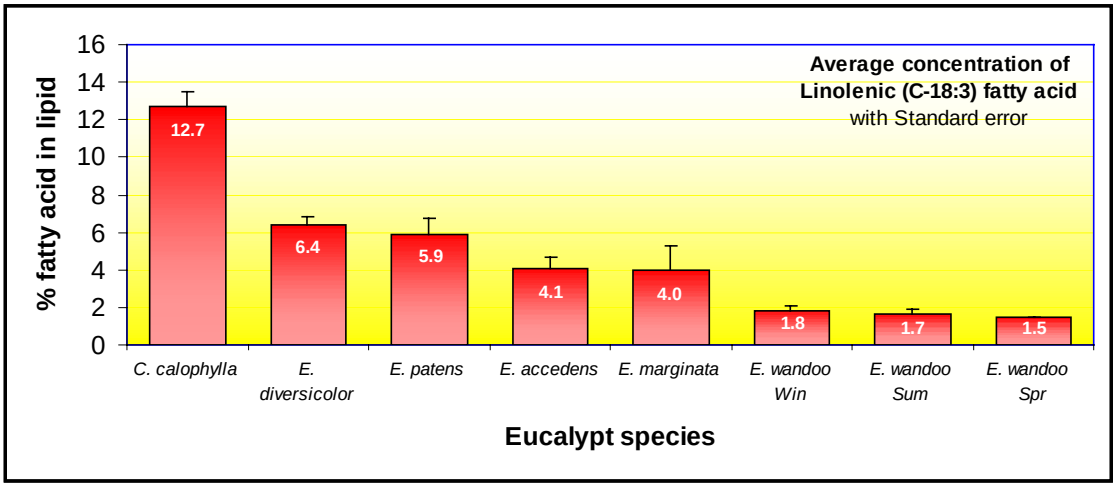
Further research work should be undertaken into the antimicrobial activity of pollen against bee diseases such as AFB and EFB. Testing could be considered for bacteria such as *Staphylococcus* species in wound infections and skin diseases. Addition of specific pollens to antibacterial honeys could improve their known effect against bacteria.

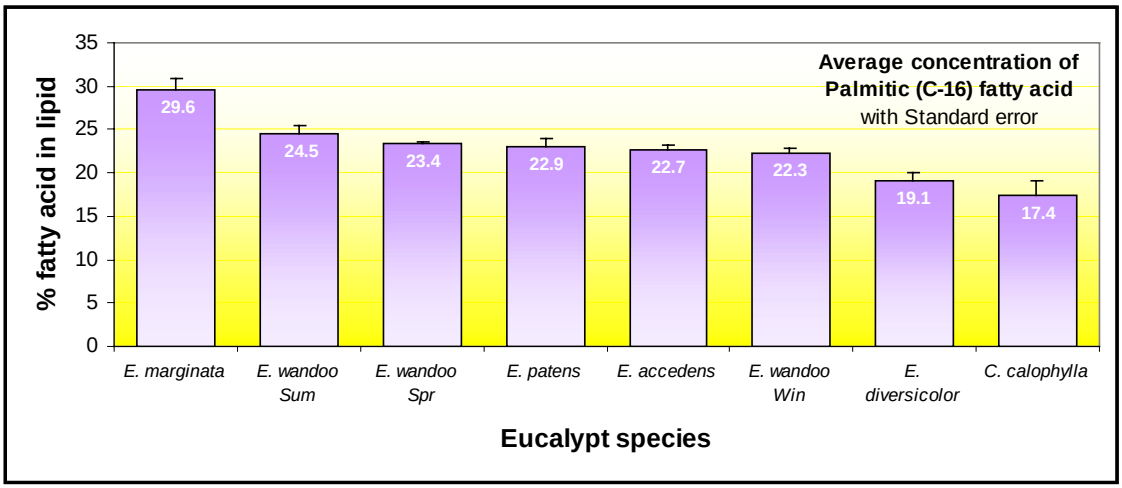
6. Graphic Summary

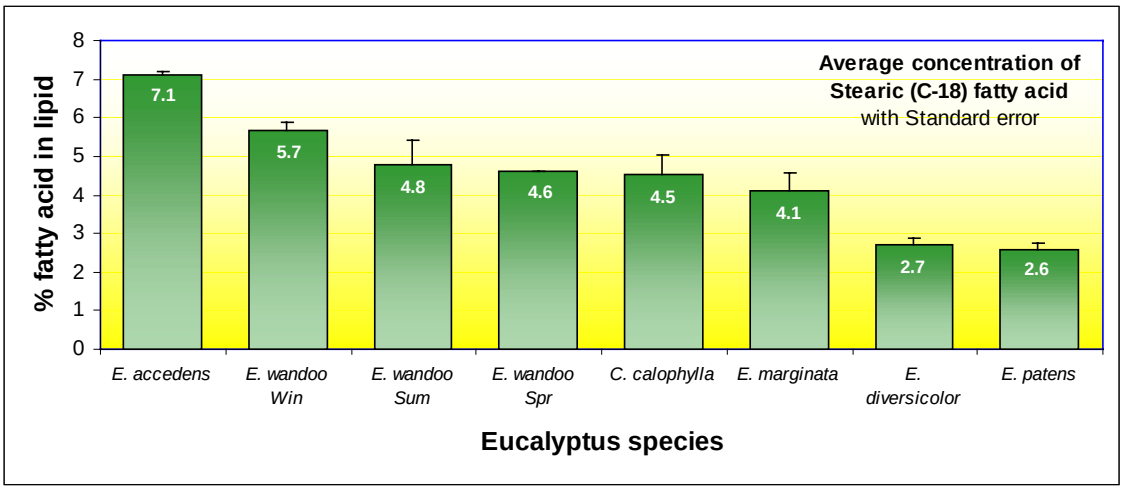
Fatty acids, minerals, crude protein, amino acids and lipid (fat).
(Graphs for cystine and tryptophan are excluded)

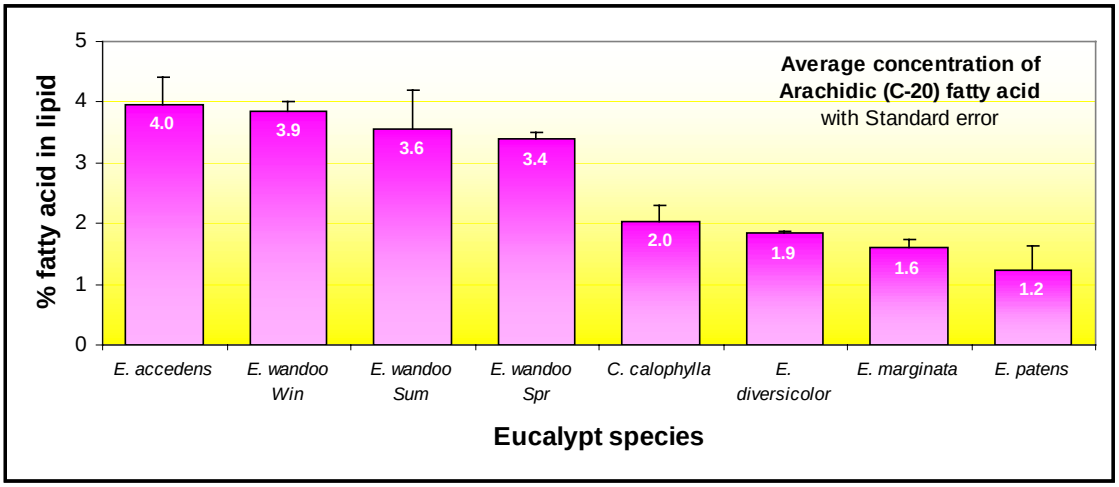


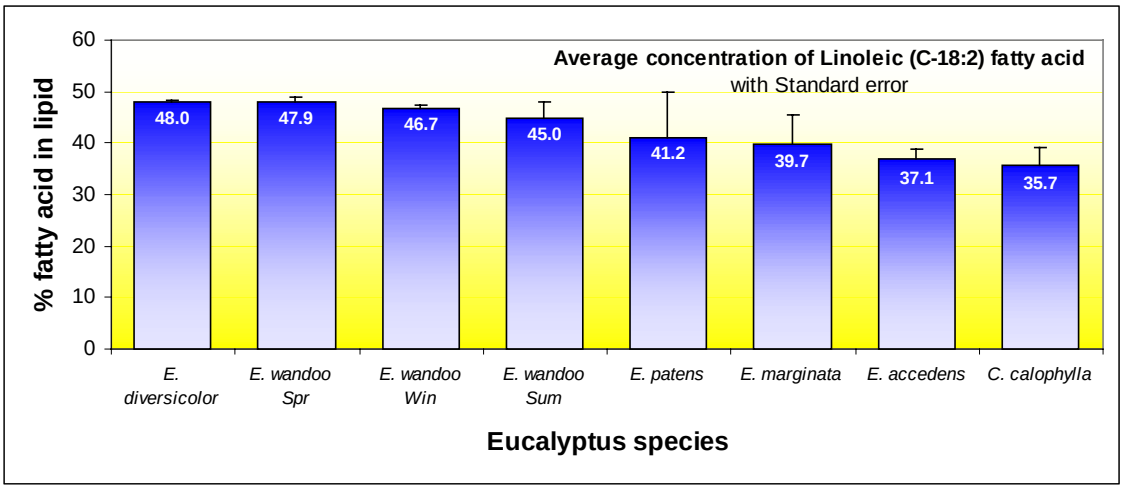


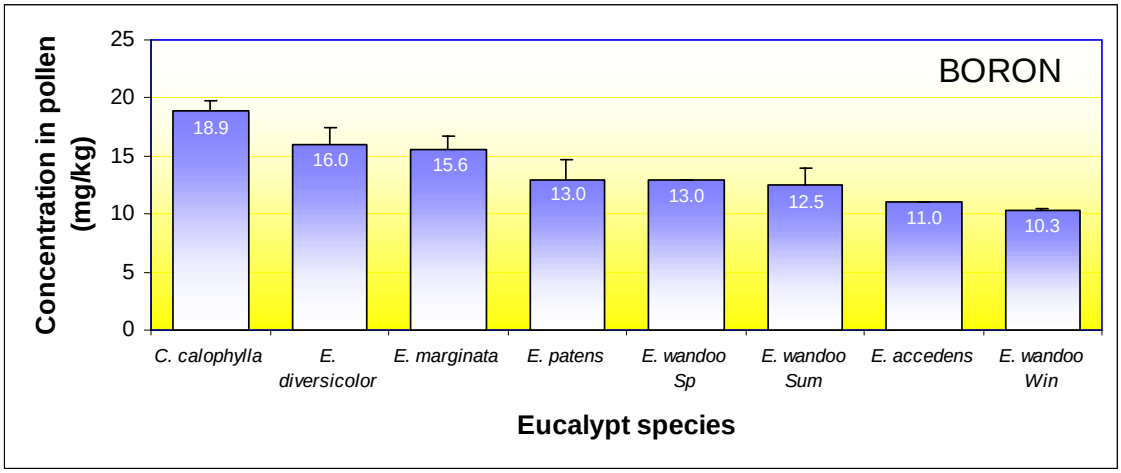

Average concentration of
Linolenic (C-18:3) fatty acid
with Standard error
% fatty acid in lipid
0
2
4
6
8
10
12
14
16
12.7
6.4
5.9
4.1
4.0
1.8
1.7
1.5
C. calophylla
E. diversicolor
E. patens
E. accedens
E. marginata
E. wandoo Win
E. wandoo Sum
E. wandoo Spr
Eucalypt species

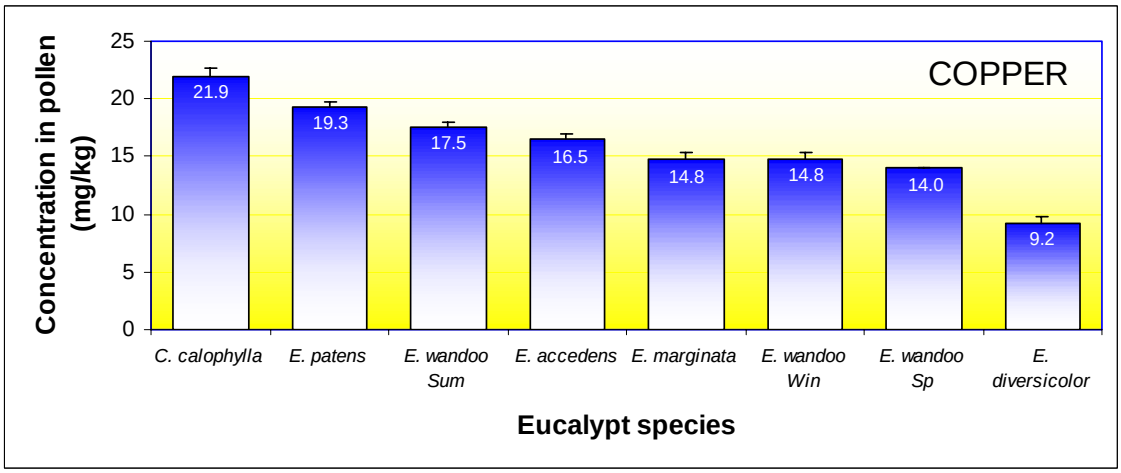

Average concentration of
Palmitic (C-16) fatty acid
with Standard error
% fatty acid in lipid
0
5
10
15
20
25
30
35
29.6
24.5
23.4
22.9
22.7
22.3
19.1
17.4
E. marginata
E. wandoo Sum
E. wandoo Spr
E. patens
E. accedens
E. wandoo Win
E. diversicolor
C. calophylla
Eucalypt species

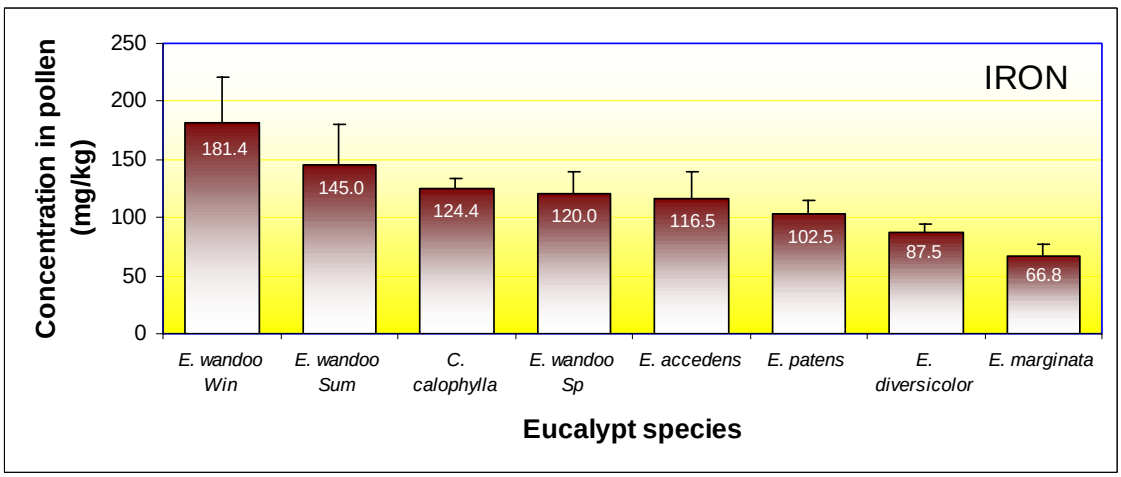

Average concentration of
Stearic (C-18) fatty acid
with Standard error
% fatty acid in lipid
0
1
2
3
4
5
6
7
8
7.1
5.7
4.8
4.6
4.5
4.1
2.7
2.6
E. accedens
E. wandoo Win
E. wandoo Sum
E. wandoo Spr
C. calophylla
E. marginata
E. diversicolor
E. patens
Eucalyptus species

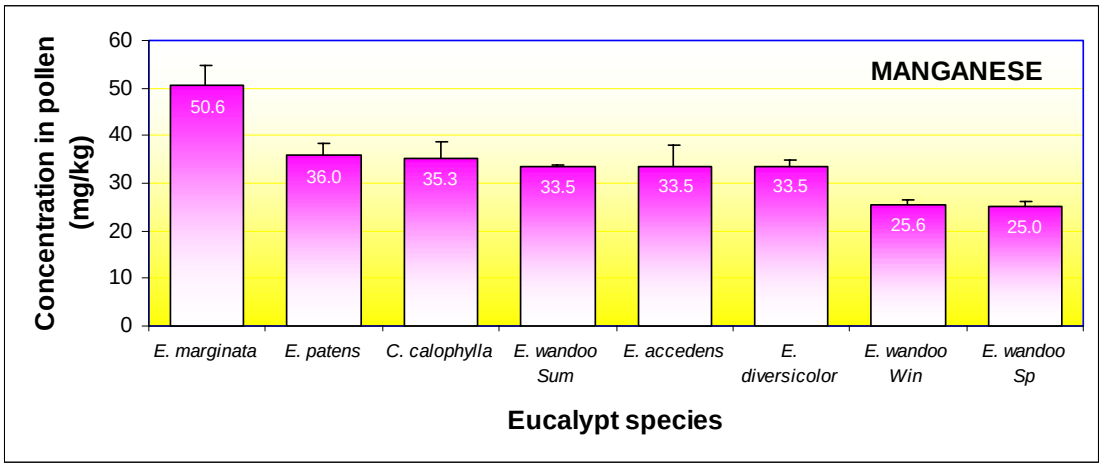

Average concentration of
Arachidic (C-20) fatty acid
with Standard error
% fatty acid in lipid
0
1
2
3
4
5
4.0
3.9
3.6
3.4
2.0
1.9
1.6
1.2
E. accedens
E. wandoo Win
E. wandoo Sum
E. wandoo Spr
C. calophylla
E. diversicolor
E. marginata
E. patens
Eucalypt species

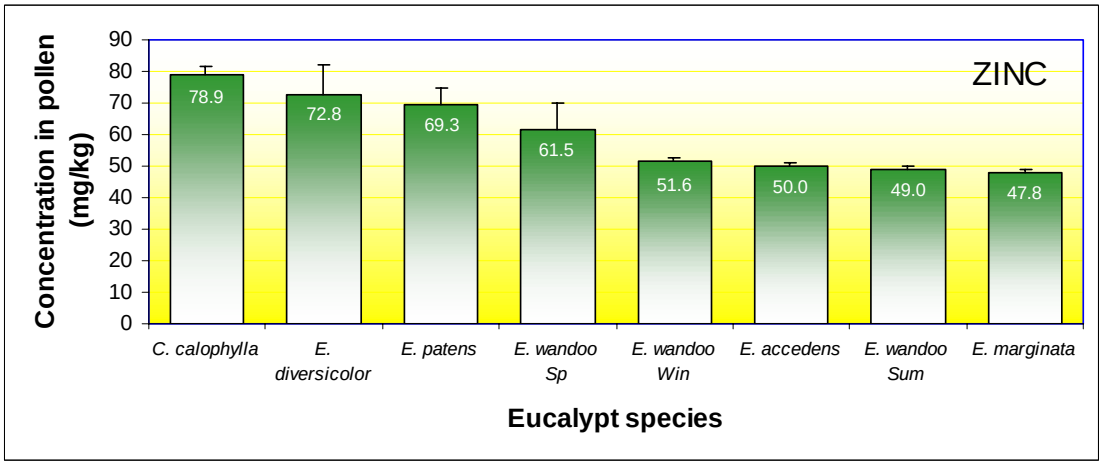

Average concentration of Linoleic (C-18:2) fatty acid
with Standard error
% fatty acid in lipid
60
50
40
30
20
10
0
48.0
47.9
46.7
45.0
41.2
39.7
37.1
35.7
E. diversicolor
E. wandoo Spr
E. wandoo Win
E. wandoo Sum
E. patens
E. marginata
E. accedens
C. calophylla
Eucalyptus species

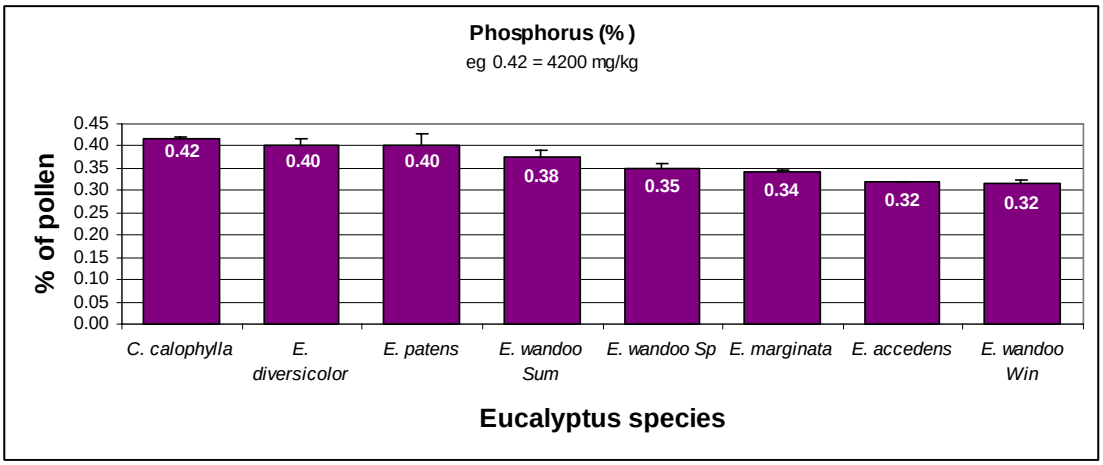

BORON
Concentration in pollen (mg/kg)
25
20
15
10
5
0
18.9
16.0
15.6
13.0
13.0
12.5
11.0
10.3
C. calophylla
E. diversicolor
E. marginata
E. patens
E. wandoo Sp
E. wandoo Sum
E. accedens
E. wandoo Win
Eucalypt species

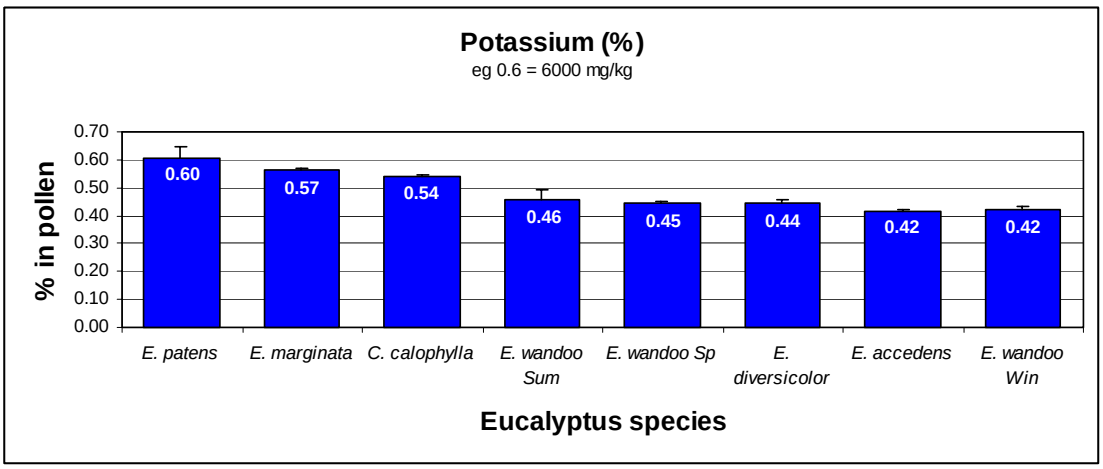

COPPER
Concentration in pollen (mg/kg)
25
20
15
10
5
0
21.9
19.3
17.5
16.5
14.8
14.8
14.0
9.2
C. calophylla
E. patens
E. wandoo Sum
E. accedens
E. marginata
E. wandoo Win
E. wandoo Sp
E. diversicolor
Eucalypt species

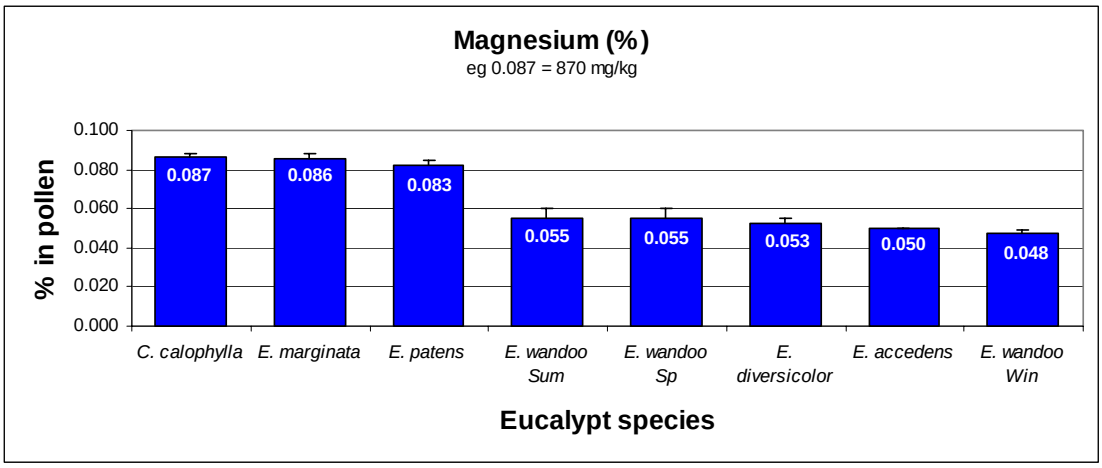

IRON
Concentration in pollen (mg/kg)
250
200
150
100
50
0
181.4
145.0
124.4
120.0
116.5
102.5
87.5
66.8
E. wandoo Win
E. wandoo Sum
C. calophylla
E. wandoo Sp
E. accedens
E. patens
E. diversicolor
E. marginata
Eucalypt species

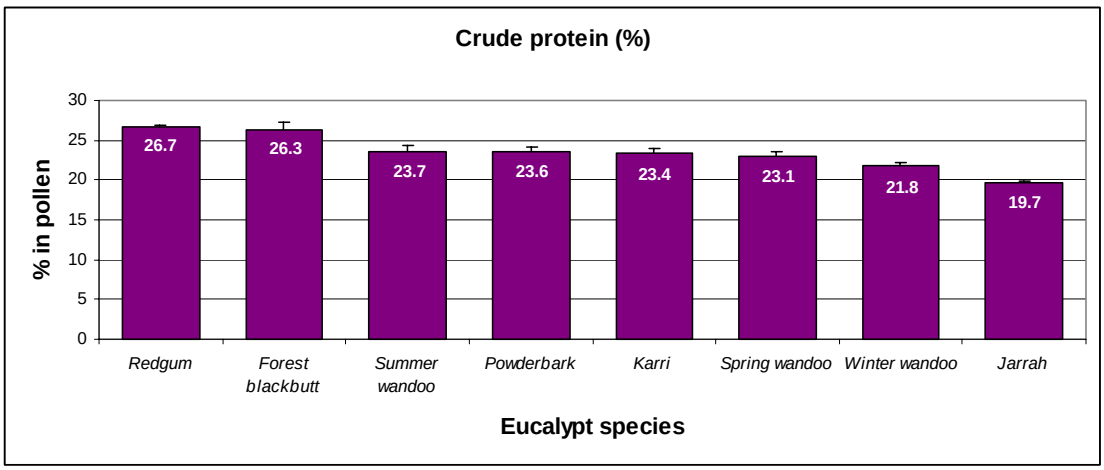

MANGANESE
Concentration in pollen (mg/kg)
60
50
40
30
20
10
0
50.6
36.0
35.3
33.5
33.5
33.5
25.6
25.0
E. marginata
E. patens
C. calophylla
E. wandoo Sum
E. accedens
E. diversicolor
E. wandoo Win
E. wandoo Sp
Eucalypt species

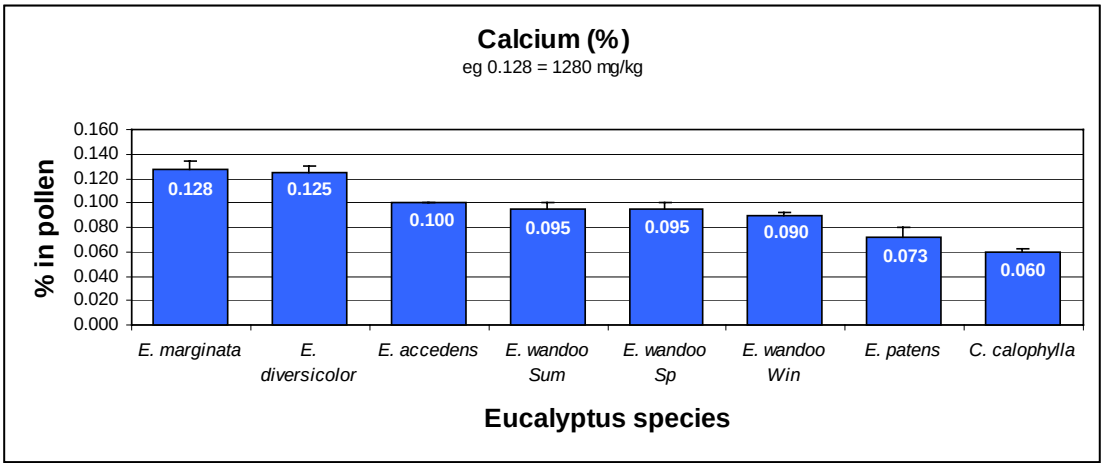

ZINC
Concentration in pollen (mg/kg)
90
80
70
60
50
40
30
20
10
0
78.9
72.8
69.3
61.5
51.6
50.0
49.0
47.8
C. calophylla
E. diversicolor
E. patens
E. wandoo Sp
E. wandoo Win
E. accedens
E. wandoo Sum
E. marginata
Eucalypt species

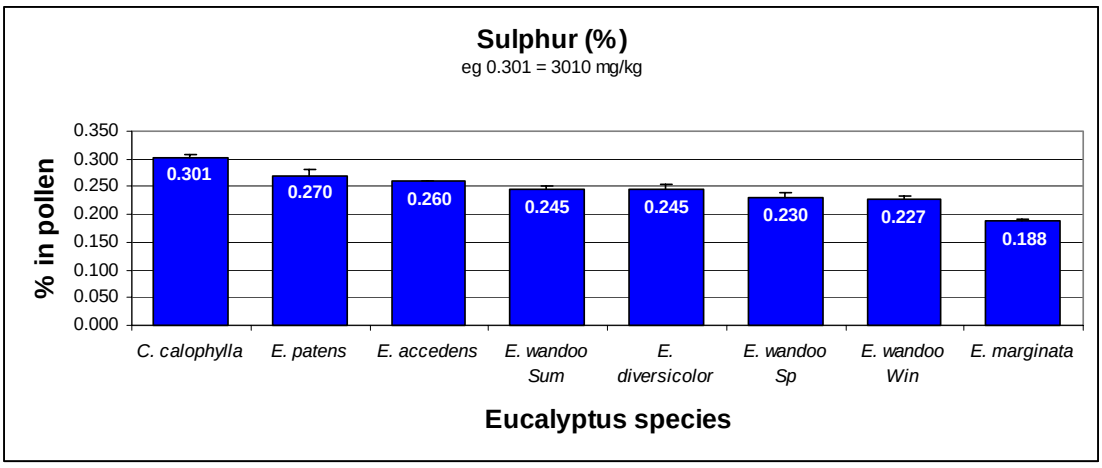

Phosphorus (%)
eg 0.42 = 4200 mg/kg
% of pollen
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
0.42
0.40
0.40
0.38
0.35
0.34
0.32
0.32
C. calophylla
E. diversicolor
E. patens
E. wandoo Sum
E. wandoo Sp
E. marginata
E. accedens
E. wandoo Win
Eucalyptus species

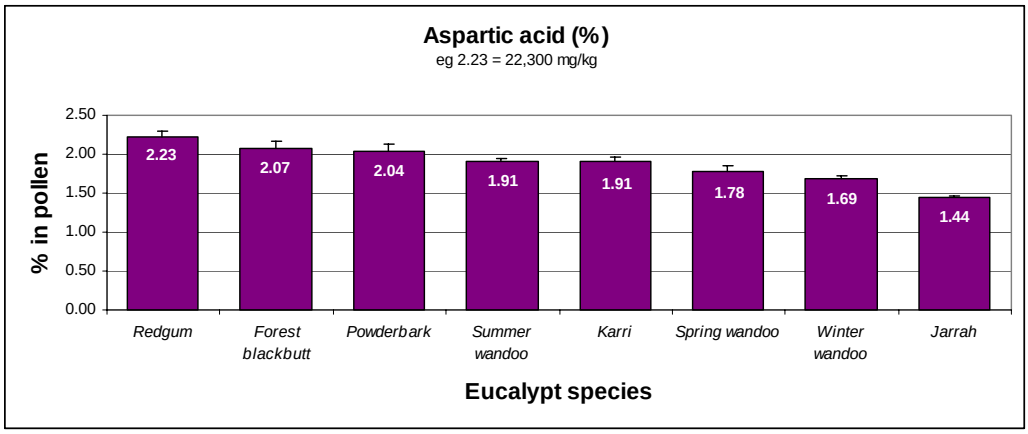

Potassium (%)
eg 0.6 = 6000 mg/kg
% in pollen
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
0.60
0.57
0.54
0.46
0.45
0.44
0.42
0.42
E. patens
E. marginata
C. calophylla
E. wandoo Sum
E. wandoo Sp
E. diversicolor
E. accedens
E. wandoo Win
Eucalyptus species

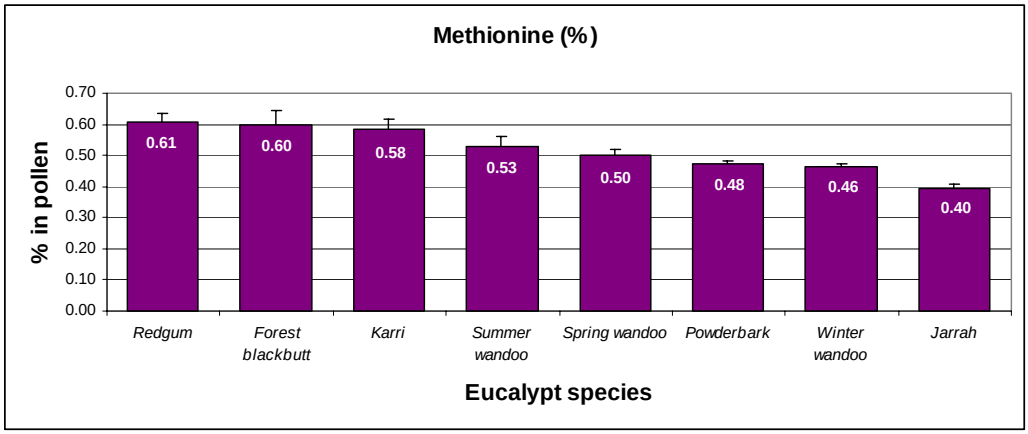

Magnesium (%)
eg 0.087 = 870 mg/kg
% in pollen
0.100
0.080
0.060
0.040
0.020
0.000
0.087
0.086
0.083
0.055
0.055
0.053
0.050
0.048
C. calophylla
E. marginata
E. patens
E. wandoo Sum
E. wandoo Sp
E. diversicolor
E. accedens
E. wandoo Win
Eucalypt species

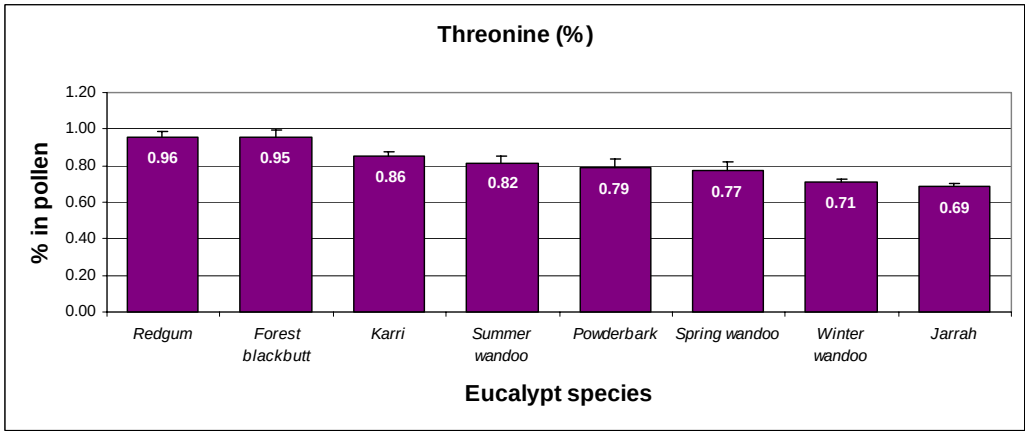

Crude protein (%)
% in pollen
30
25
20
15
10
5
0
26.7
26.3
23.7
23.6
23.4
23.1
21.8
19.7
Redgum
Forest blackbutt
Summer wandoo
Powderbark
Karri
Spring wandoo
Winter wandoo
Jarrah
Eucalypt species

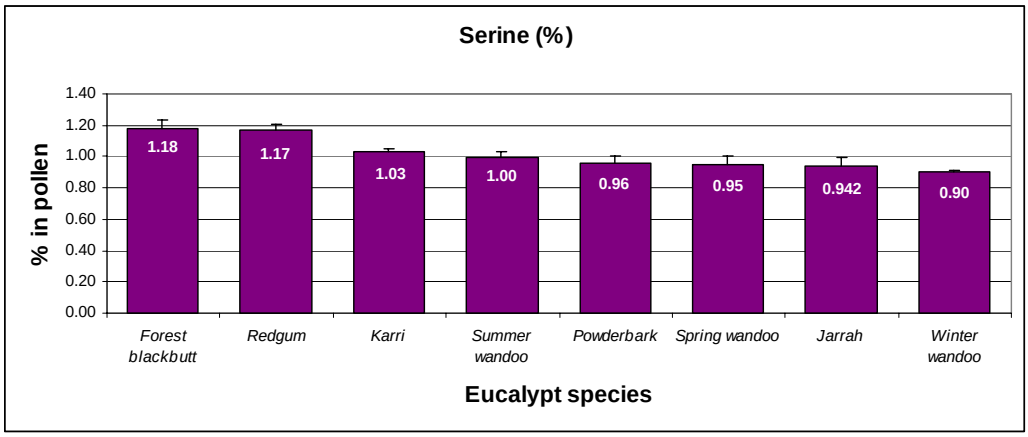

Calcium (%)
eg 0.128 = 1280 mg/kg
% in pollen
0.160
0.140
0.120
0.100
0.080
0.060
0.040
0.020
0.000
0.128
0.125
0.100
0.095
0.095
0.090
0.073
0.060
E. marginata
E. diversicolor
E. accedens
E. wandoo Sum
E. wandoo Sp
E. wandoo Win
E. patens
C. calophylla
Eucalyptus species

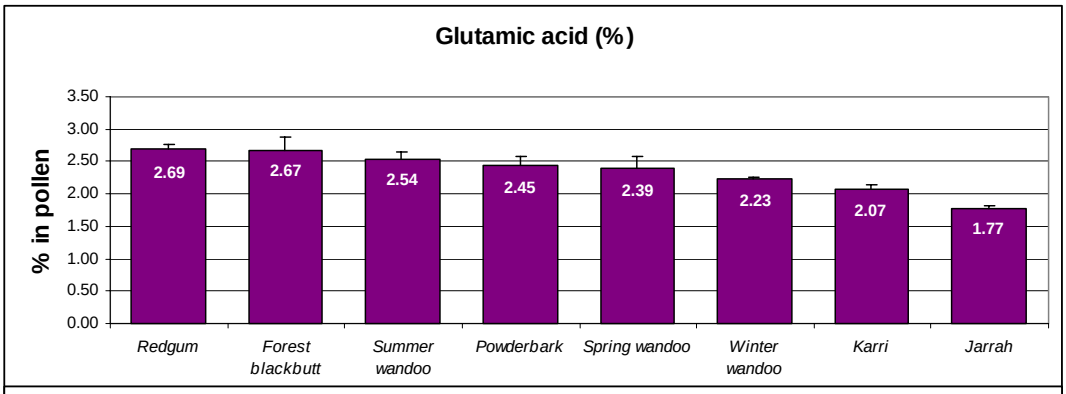

Sulphur (%)
eg 0.301 = 3010 mg/kg
% in pollen
0.350
0.300
0.250
0.200
0.150
0.100
0.050
0.000
0.301
0.270
0.260
0.245
0.245
0.230
0.227
0.188
C. calophylla
E. patens
E. accedens
E. wandoo Sum
E. diversicolor
E. wandoo Sp
E. wandoo Win
E. marginata
Eucalyptus species

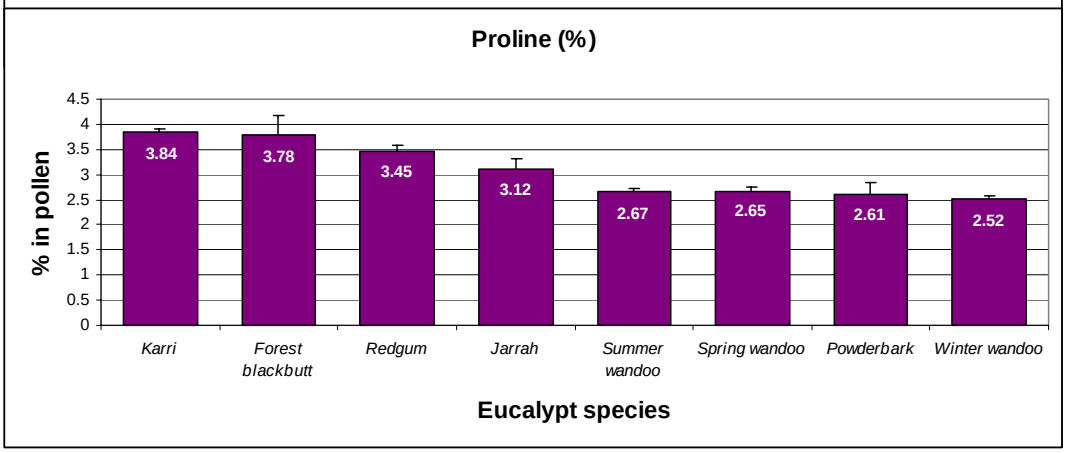

Aspartic acid (%)
eg 2.23 = 22,300 mg/kg
% in pollen
2.50
2.00
1.50
1.00
0.50
0.00
2.23
2.07
2.04
1.91
1.91
1.78
1.69
1.44
Redgum
Forest blackbutt
Powderbark
Summer wandoo
Karri
Spring wandoo
Winter wandoo
Jarrah
Eucalypt species

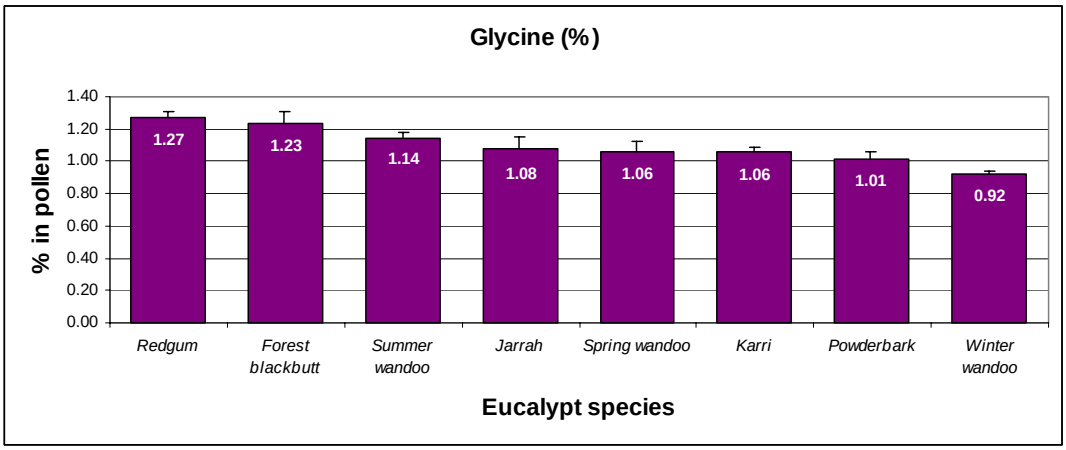

Methionine (%)
% in pollen
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
0.61
0.60
0.58
0.53
0.50
0.48
0.46
0.40
Redgum
Forest blackbutt
Karri
Summer wandoo
Spring wandoo
Powderbark
Winter wandoo
Jarrah
Eucalypt species

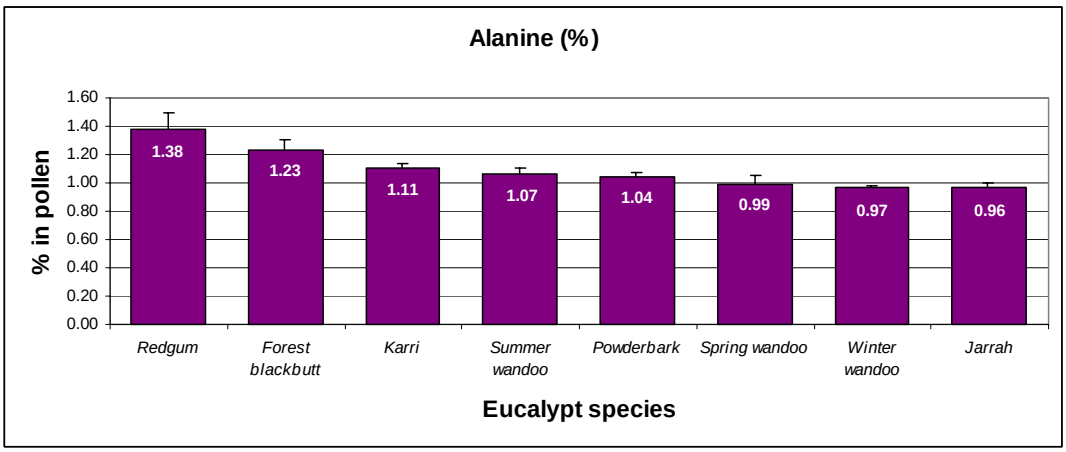

Threonine (%)
% in pollen
1.20
1.00
0.80
0.60
0.40
0.20
0.00
0.96
0.95
0.86
0.82
0.79
0.77
0.71
0.69
Redgum
Forest blackbutt
Karri
Summer wandoo
Powderbark
Spring wandoo
Winter wandoo
Jarrah
Eucalypt species

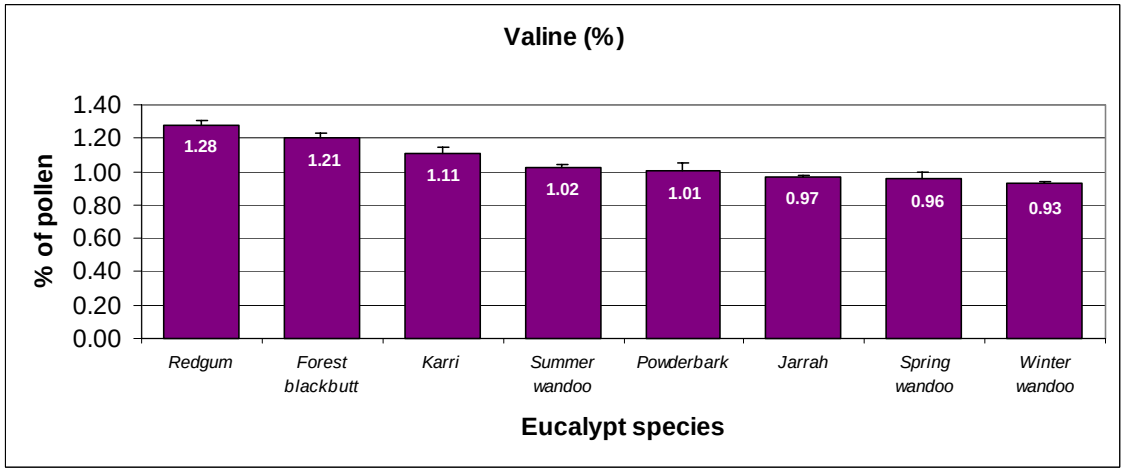

Serine (%)
% in pollen
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
1.18
1.17
1.03
1.00
0.96
0.95
0.942
0.90
Forest blackbutt
Redgum
Karri
Summer wandoo
Powderbark
Spring wandoo
Jarrah
Winter wandoo
Eucalypt species

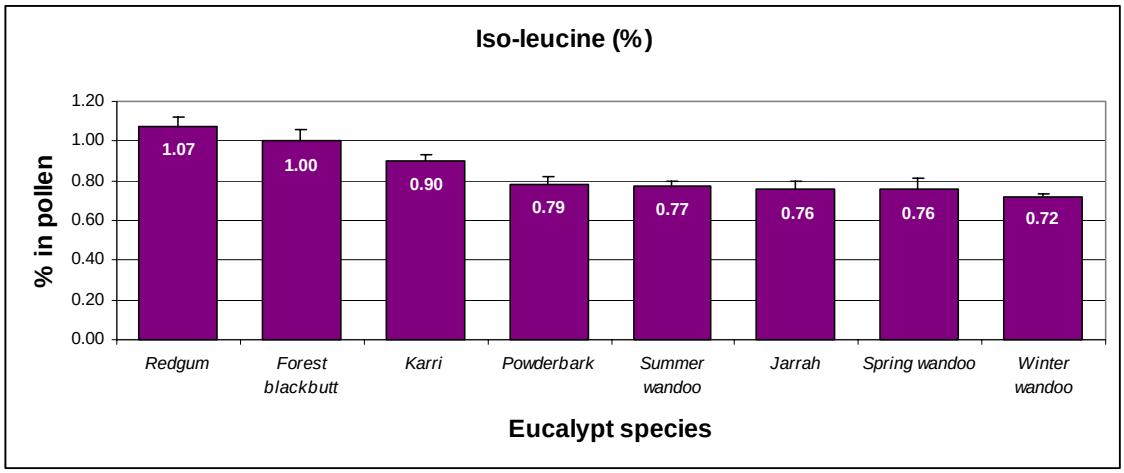

Glutamic acid (%)
% in pollen
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
2.69
2.67
2.54
2.45
2.39
2.23
2.07
1.77
Redgum
Forest blackbutt
Summer wandoo
Powderbark
Spring wandoo
Winter wandoo
Karri
Jarrah

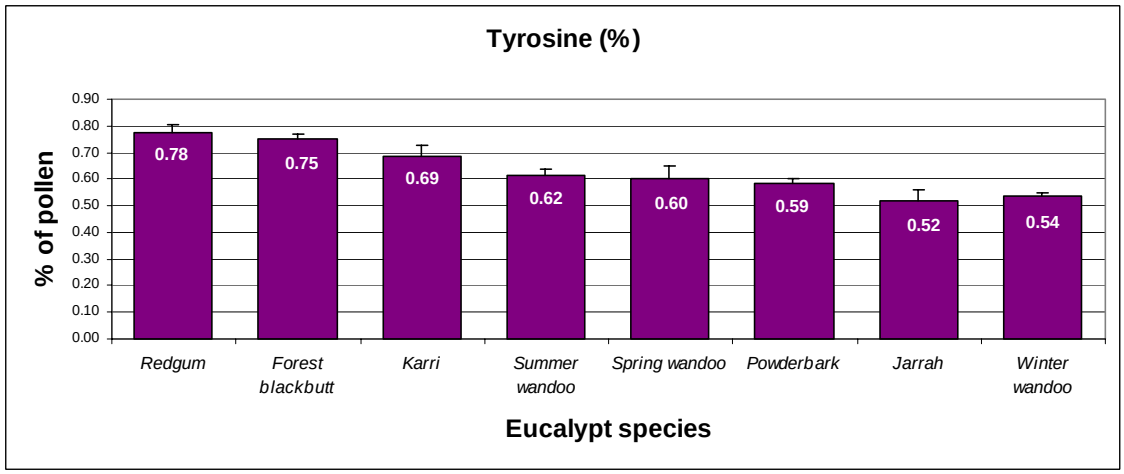

Proline (%)
% in pollen
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
3.84
3.78
3.45
3.12
2.67
2.65
2.61
2.52
Karri
Forest blackbutt
Redgum
Jarrah
Summer wandoo
Spring wandoo
Powderbark
Winter wandoo
Eucalypt species

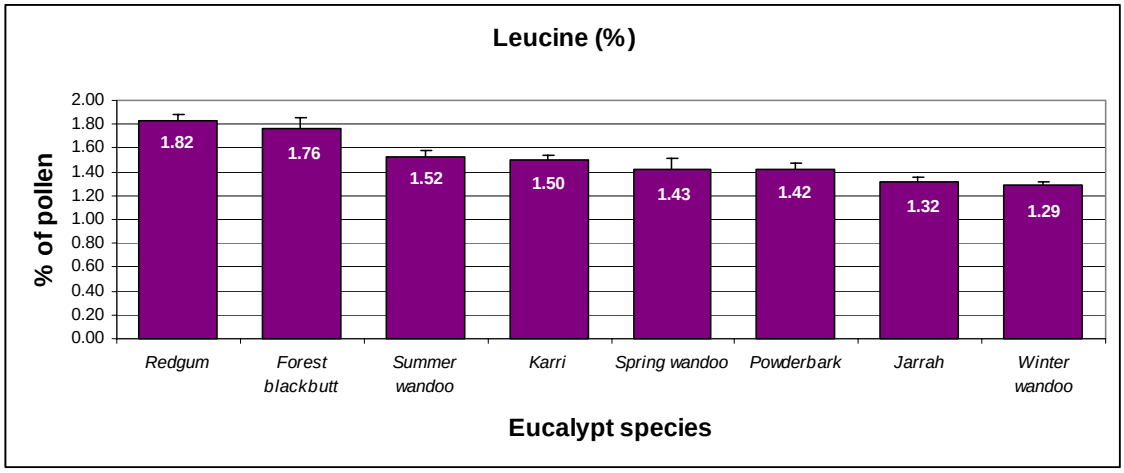

Glycine (%)
% in pollen
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
1.27
1.23
1.14
1.08
1.06
1.06
1.01
0.92
Redgum
Forest blackbutt
Summer wandoo
Jarrah
Spring wandoo
Karri
Powderbark
Winter wandoo
Eucalypt species

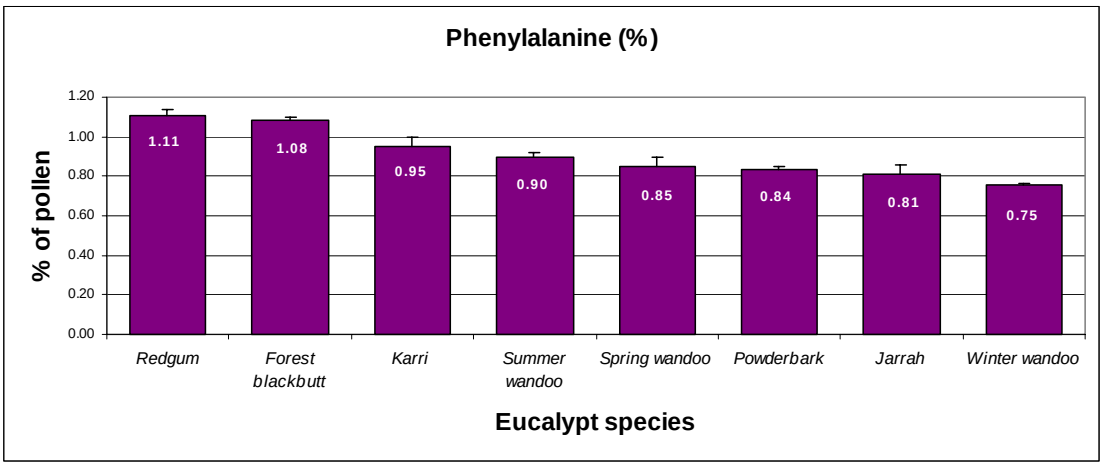

Alanine (%)
% in pollen
1.60
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
1.38
1.23
1.11
1.07
1.04
0.99
0.97
0.96
Redgum
Forest blackbutt
Karri
Summer wandoo
Powderbark
Spring wandoo
Winter wandoo
Jarrah
Eucalypt species

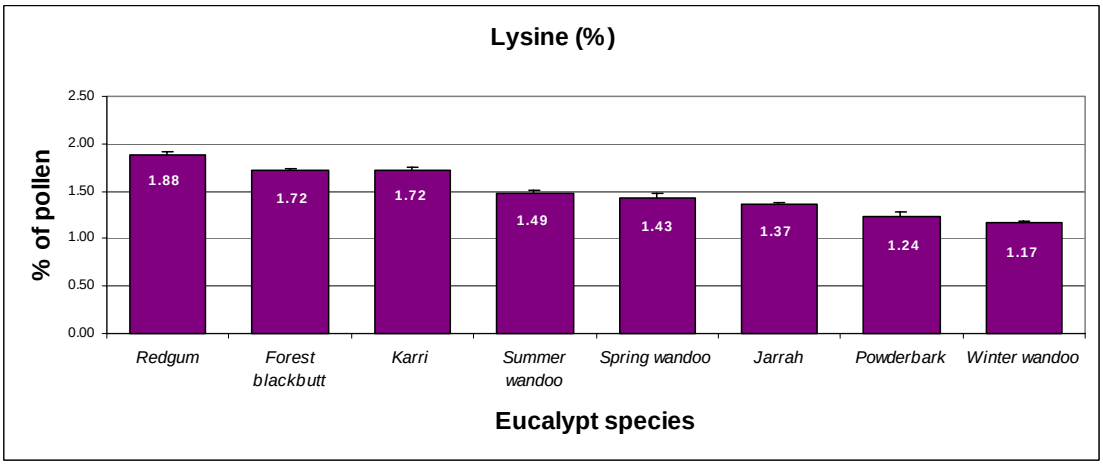

Valine (%)
% of pollen
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
1.28
1.21
1.11
1.02
1.01
0.97
0.96
0.93
Redgum
Forest blackbutt
Karri
Summer wandoo
Powderbark
Jarrah
Spring wandoo
Winter wandoo
Eucalypt species

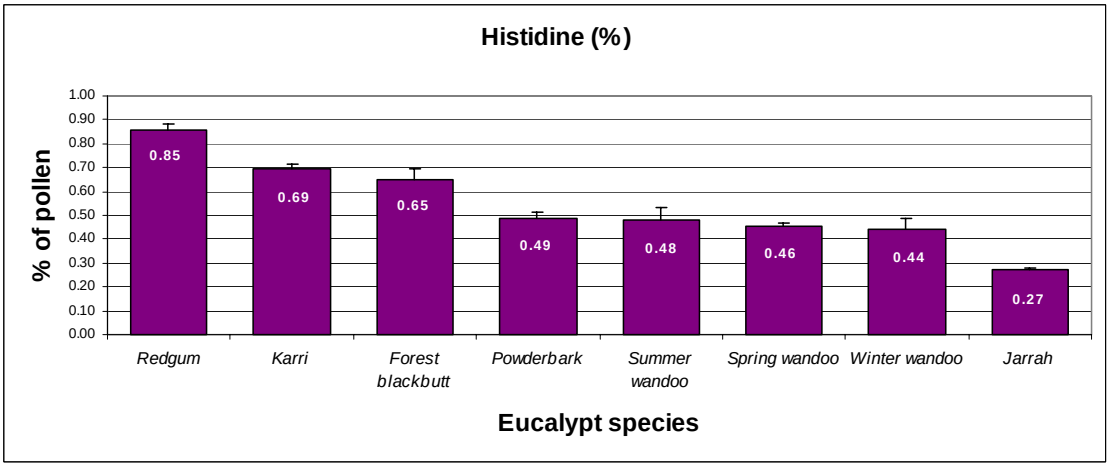

Iso-leucine (%)
% in pollen
1.20
1.00
0.80
0.60
0.40
0.20
0.00
1.07
1.00
0.90
0.79
0.77
0.76
0.76
0.72
Redgum
Forest blackbutt
Karri
Powderbark
Summer wandoo
Jarrah
Spring wandoo
Winter wandoo
Eucalypt species

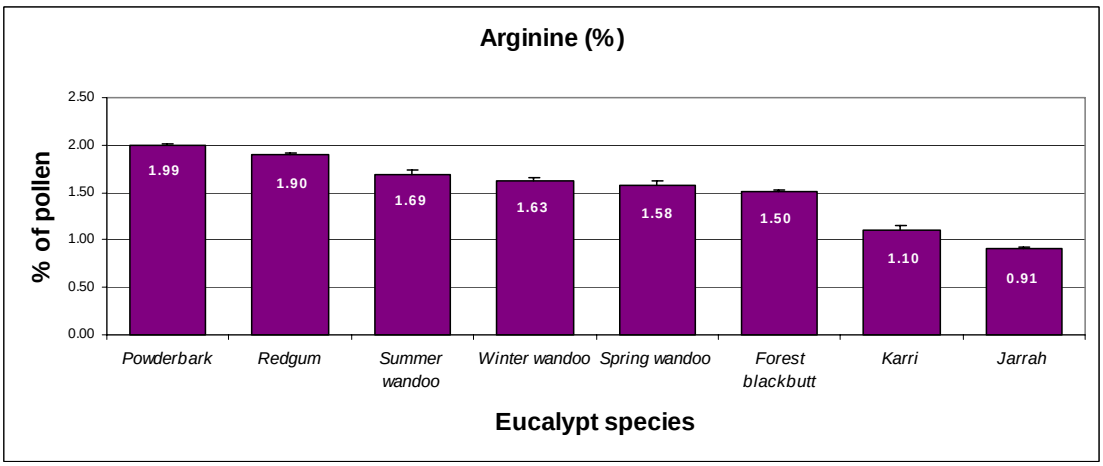

Tyrosine (%)
% of pollen
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
0.78
0.75
0.69
0.62
0.60
0.59
0.52
0.54
Redgum
Forest blackbutt
Karri
Summer wandoo
Spring wandoo
Powderbark
Jarrah
Winter wandoo
Eucalypt species

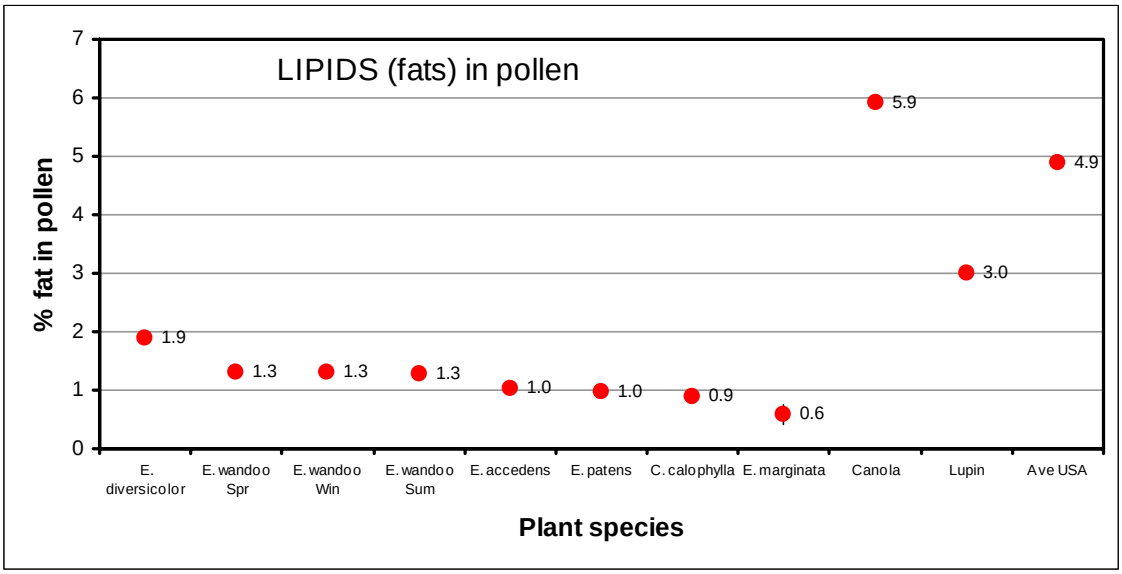

Leucine (%)
% of pollen
2.00
1.80
1.60
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
1.82
1.76
1.52
1.50
1.43
1.42
1.32
1.29
Redgum
Forest blackbutt
Summer wandoo
Karri
Spring wandoo
Powderbark
Jarrah
Winter wandoo
Eucalypt species


Phenylalanine (%)
% of pollen
1.20
1.00
0.80
0.60
0.40
0.20
0.00
1.11
1.08
0.95
0.90
0.85
0.84
0.81
0.75
Redgum
Forest blackbutt
Karri
Summer wandoo
Spring wandoo
Powderbark
Jarrah
Winter wandoo
Eucalypt species


Lysine (%)
% of pollen
2.50
2.00
1.50
1.00
0.50
0.00
1.88
1.72
1.72
1.49
1.43
1.37
1.24
1.17
Redgum
Forest blackbutt
Karri
Summer wandoo
Spring wandoo
Jarrah
Powderbark
Winter wandoo
Eucalypt species


Histidine (%)
% of pollen
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
0.85
0.69
0.65
0.49
0.48
0.46
0.44
0.27
Redgum
Karri
Forest blackbutt
Powderbark
Summer wandoo
Spring wandoo
Winter wandoo
Jarrah
Eucalypt species


Arginine (%)
% of pollen
2.50
2.00
1.50
1.00
0.50
0.00
1.99
1.90
1.69
1.63
1.58
1.50
1.10
0.91
Powderbark
Redgum
Summer wandoo
Winter wandoo
Spring wandoo
Forest blackbutt
Karri
Jarrah
Eucalypt species


LIPIDS (fats) in pollen
% fat in pollen
Plant species
0
1
2
3
4
5
6
7
1.9
1.3
1.3
1.3
1.0
1.0
0.9
0.6
5.9
4.9
3.0
E. diversicolor
E. wandoo Spr
E. wandoo Win
E. wandoo Sum
E. accedens
E. patens
C. calophylla
E. marginata
Canola
Lupin
Ave USA

7. References

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8. Appendix 1: linoleic acid (mg/g)

Species	Linoleic acid (C-18:2)
Forest blackbutt	5.81 ± 4.1
Spring wandoo	5.75 ± 0.17
Winter wandoo	5.41 ± 0.22
Karri	5.00 ± 0.44
Summer wandoo	4.78 ± 0.03
Jarrah	3.67 ± 0.91
Powderbark	2.83 ± 0.11
Redgum	2.77 ± 0.38
Average (mg/g)	4.50 (= 450 g in 0.1g pollen)

9. Appendix 2: linolenic acid (mg/g)

Species	Linolenic acid (C-18:3)
Redgum	1.06 ± 0.07
Forest blackbutt	0.82 ± 0.20
Karri	0.68 ± 0.10
Jarrah	0.36 ±0.10
Powderbark	0.31 ± 0.05
Winter wandoo	0.22 ± 0.04
Summer wandoo	0.18 ± 0.03
Spring wandoo	0.17 ± 0.01
Average (mg/g)	0.48 (= 48 g in 0.1g pollen)

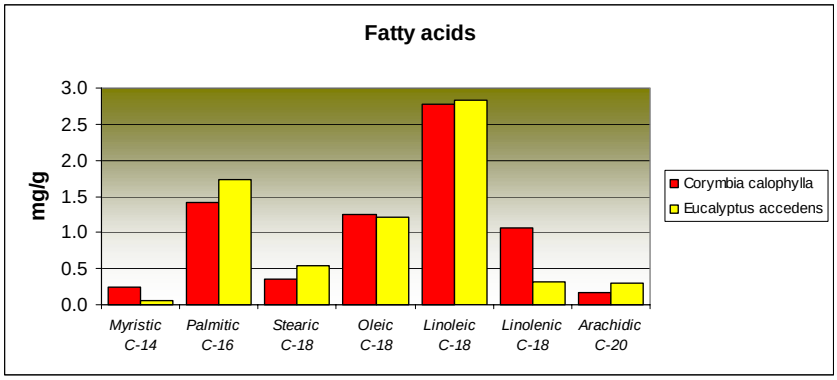
Powderbark Pollen

(Eucalyptus accedens)

A tree to 20 m that grows on laterite high ground in wandoo forests. Valuable source for pollen and nectar from December to March.

Amino acid profile

Powderbark amino acid levels are lower than for redgum *(Corymbia calophylla)*. Protein: 23.6% which is 3.1% lower than redgum (26.7%).

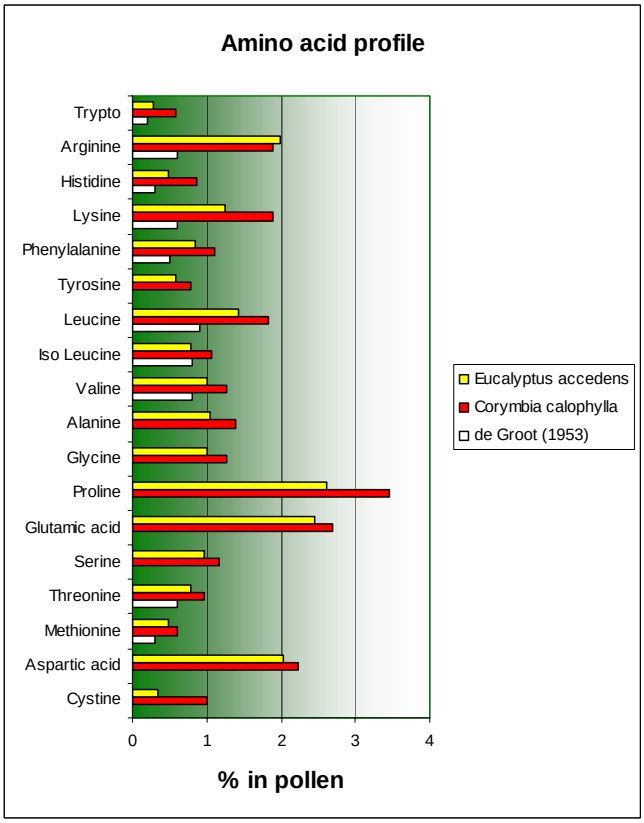


Amino acid deficiency

There is an amino acid deficiency in iso-leucine which will affect the performance of honey bees.

Fatty acid profile

Powderbark has slightly higher levels of linoleic acid compared with redgum. Lower in linolenic acid and myristic acid. Arachidic acid is higher than redgum. Lipid (fat) is 1.2 times greater than redgum (1.02% vs 0.88%).



Mineral profile

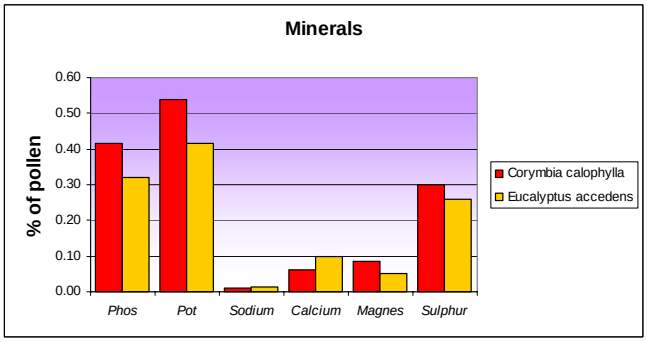
Powderbark pollen is higher in sodium and calcium than redgum pollen. The trace elements are all lower in concentration than redgum pollen.

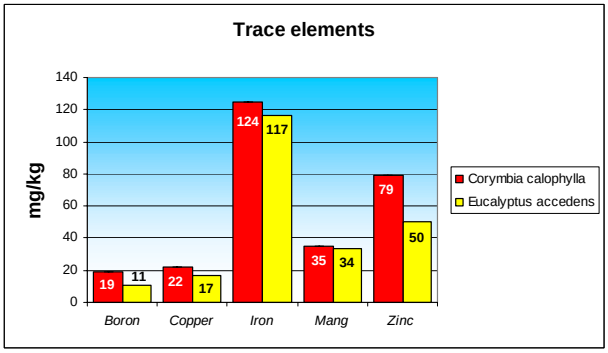
Adjustment for maximum honey bee performance

Powderbark pollen will have to be supplemented with additional protein to boost iso-leucine levels. Redgum pollen could be fed within a patty or as crushed powder.

Market for human consumption

Powderbark pollen per kilogram has less of everything in it besides the fat level, so it perhaps should be mixed with higher grade pollens to ensure 'value for money' nutrition.





Karri Pollen

(Eucalyptus diversicolor)

One of the tallest trees in the world, restricted to a relatively small area of the south west of Western Australia on hill sides and valleys. Valuable source for pollen and nectar from December to April.

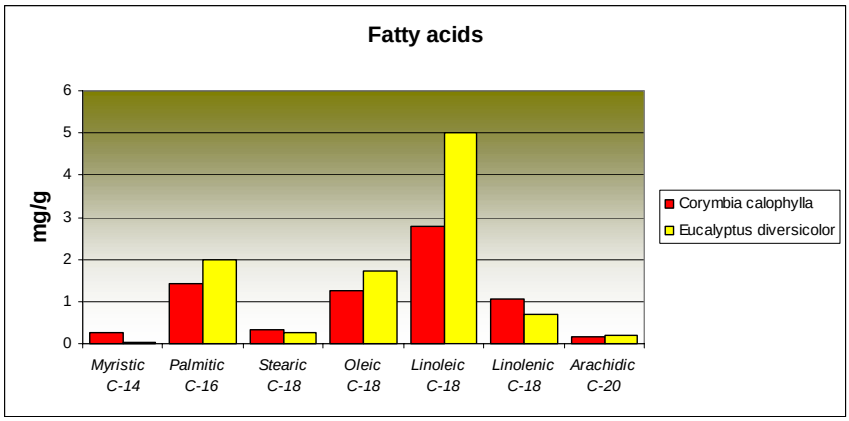
Record nectar flows were once recorded from this species with the last heavy flows being produced in the late 1960s.

Amino acid profile

Karri amino acid levels are lower than for Redgum *(Corymbia calophylla)*. Protein: 23.4% which is 3.3% lower than redgum (26.7%).

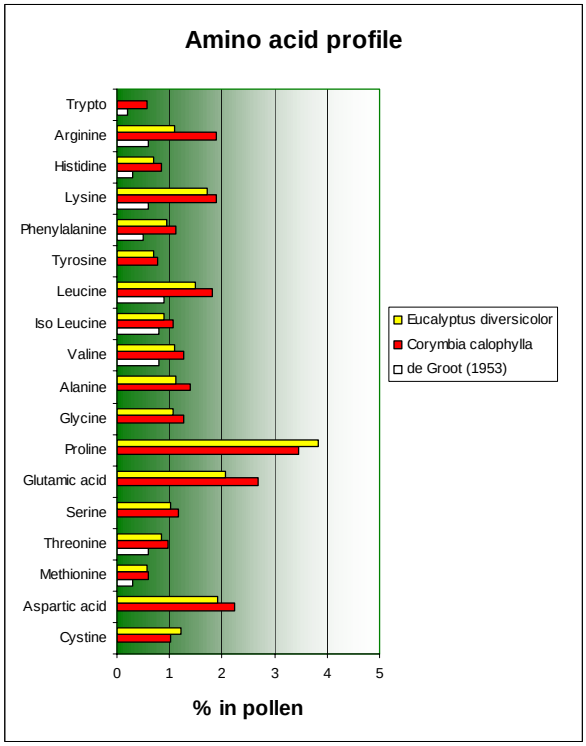
Amino acid deficiency

Karri pollen is not deficient in any amino acids that might affect bees but it could be borderline in iso-leucine.



Fatty acid profile

Karri pollen is high in linoleic acid compared with Redgum. Lower in linolenic acid and myristic acid. Arachidic acid is similar to redgum. Lipid (fat) is 2.2 times greater than redgum (1.90% vs 0.88%).



Mineral profile

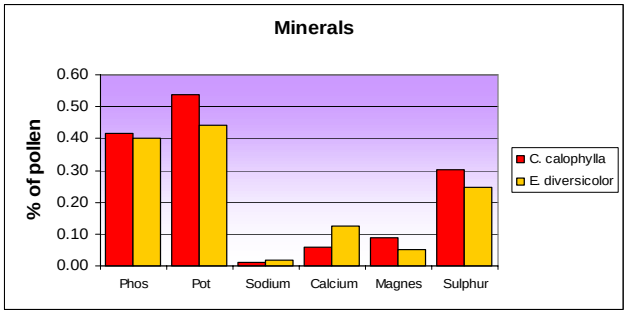
Karri pollen is lower in minerals except sodium and calcium. All trace elements are lower than for redgum pollen especially copper.

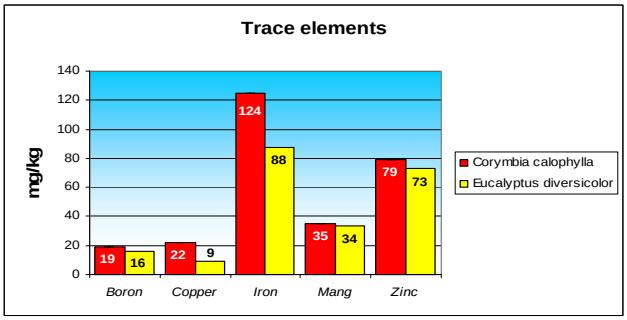
Adjustment for maximum honey bee performance

Karri pollen could be adjusted with additional protein which would alleviate the possible problem with iso-leucine.

Market for human consumption

Karri pollen is as attractive as redgum pollen. It's slightly higher fat content, higher concentration of linoleic acid would make it more attractive than redgum pollen.





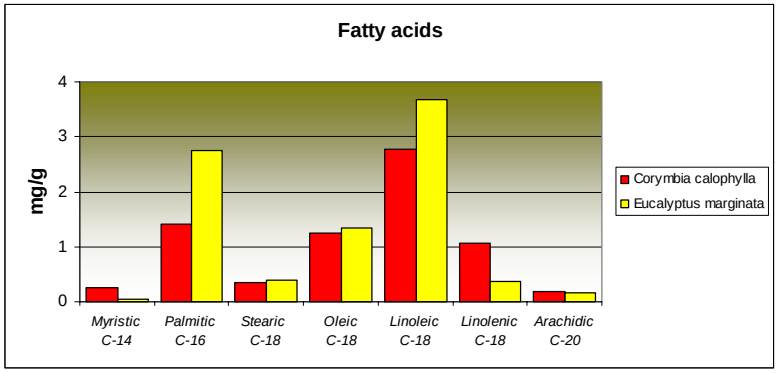
Jarrah Pollen

(Eucalyptus marginata)

The principle timber tree and a unique species whose honey rarely granulates. A common species on laterite gravels. Valuable source for pollen and nectar from September to January.

Amino acid profile

Jarrah amino acid levels are lower than for Redgum *(Corymbia calophylla)*. Protein: 19.7% which is 7% lower than redgum (26.7%).

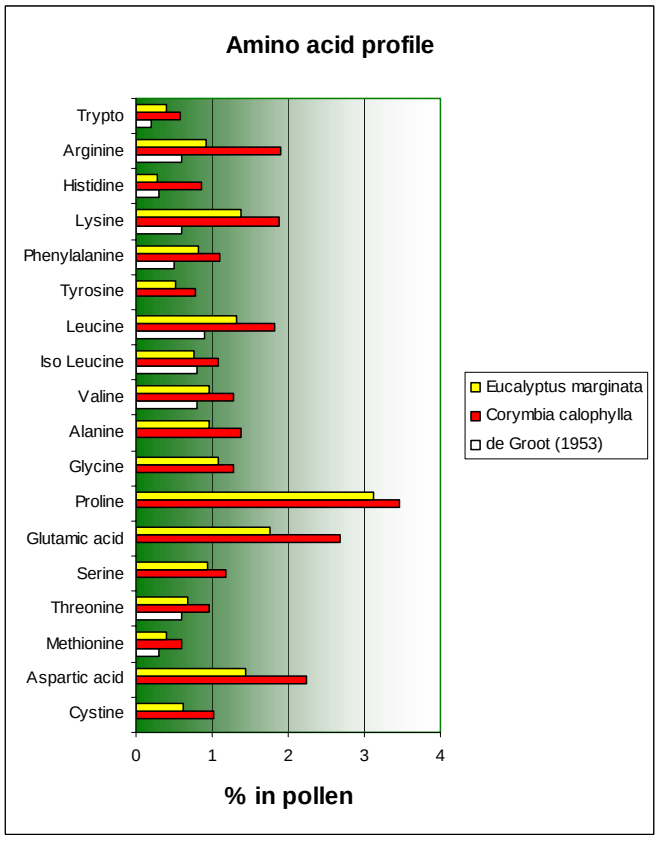


Amino acid deficiency

Overall protein is below the optimal 20% level for honey bees. Histidine and iso-leucine are deficient and will affect honey bee performance.

Fatty acid profile

Jarrah has a higher linoleic acid level compared with redgum. Lower in linolenic acid, myristic acid and arachidic acid. Lipid (fat) is very low and less than redgum (0.59% vs 0.88%).



Mineral profile

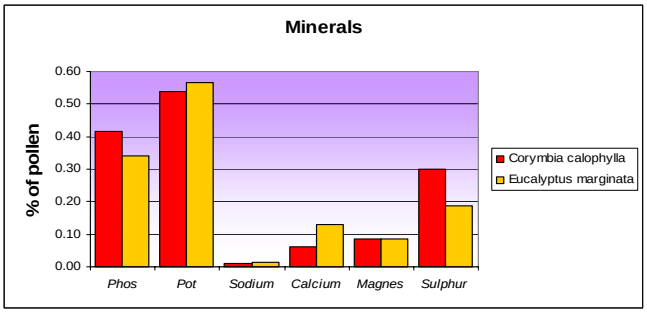
Jarrah pollen is higher in potassium (0.57% = 5700 mg/kg) compared with redgum. It is also higher in calcium and sodium. In trace elements, jarrah is only higher in manganese when compared to redgum.

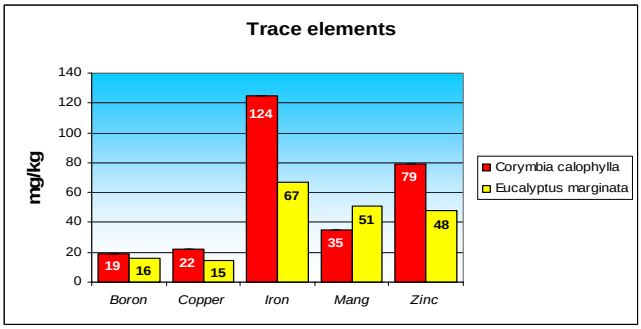
Adjustment for maximum honey bee performance

Jarrah pollen will have to enriched by additional protein either from artificial feedstuffs or by redgum pollen which could be fed within a patty or as crushed powder.

Market for human consumption

Jarrah pollen is as attractive as redgum pollen. It's similar fat content, higher concentration of linoleic acid would make it more attractive than redgum pollen. Its bright yellow colour is characteristic.





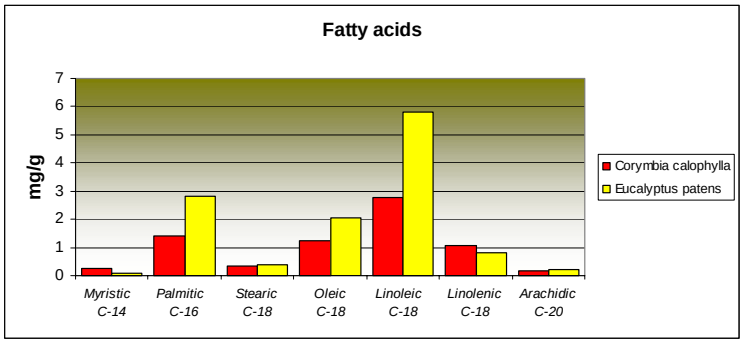
Forest Blackbutt Pollen

(Eucalyptus patens)

Widespread, growing along valley slopes near rivers and creeks in jarrah forest. Valuable source of pollen and nectar from November to February. Builds strong hive populations going into a redgum flow (Feb/Mar).

Amino acid profile

Forest blackbutt amino acid levels are similar to redgum *(Corymbia calophylla)*. Protein: 26.3% which is slightly lower than for redgum (26.7%).

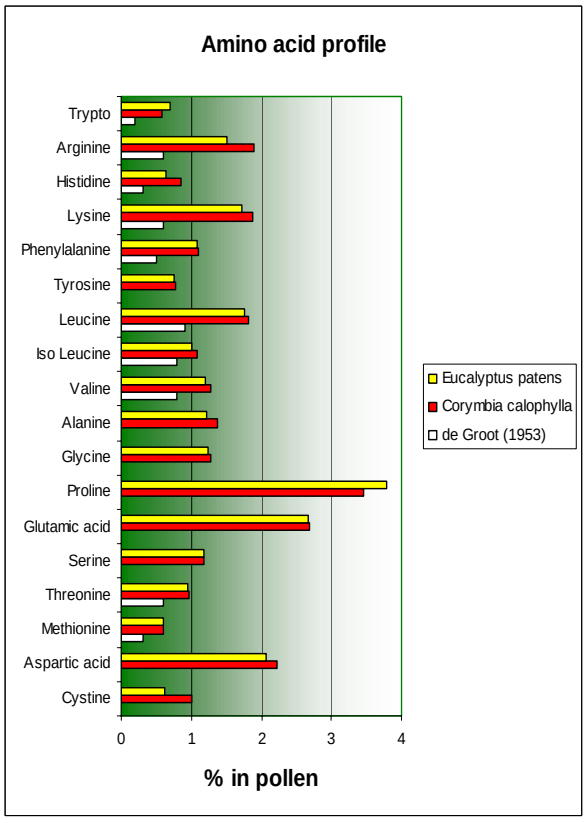


Amino acid deficiency

There are no apparent amino acid deficiencies in blackbutt pollen that would affect honey bees.

Fatty acid profile

Lipid (fat) in pollen is 1.5 times greater than redgum (1.35% vs 0.88%). Forest blackbutt has a higher linoleic acid compared with redgum and is lower in Linolenic acid and myristic acid. The amount of arachidic acid is slightly higher.



Mineral profile

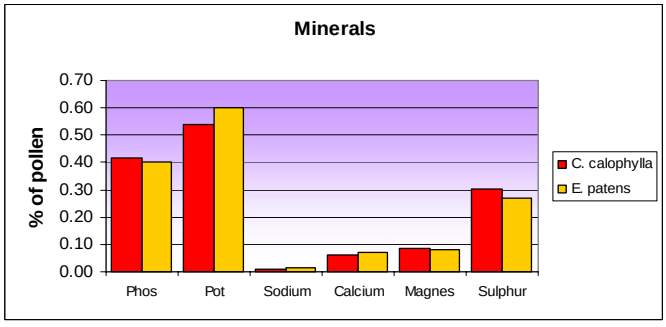
Mineral levels in forest blackbutt pollen are similar to redgum pollen, but higher in potassium (0.6% = 6000 mg/kg). The trace element profile is either the same or contains lesser amounts of elements than redgum pollen. Blackbutt pollen is higher in iron (103 mg/kg).

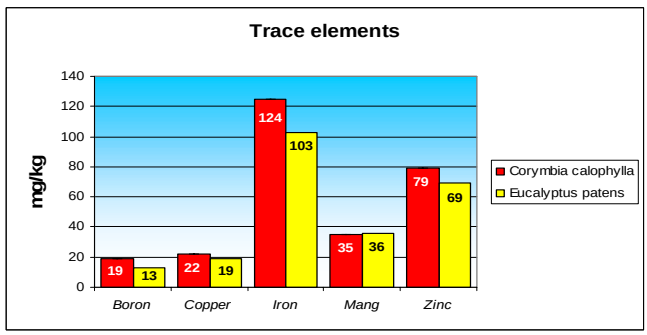
Adjustment for maximum honey bee performance

Forest blackbutt pollen will not need to be adjusted to maximise the performance of honey bees.

Market for human consumption

Forest blackbutt pollen should be similar as redgum pollen. It's slightly higher fat content with higher concentration of linoleic acid would make it more attractive than redgum pollen.



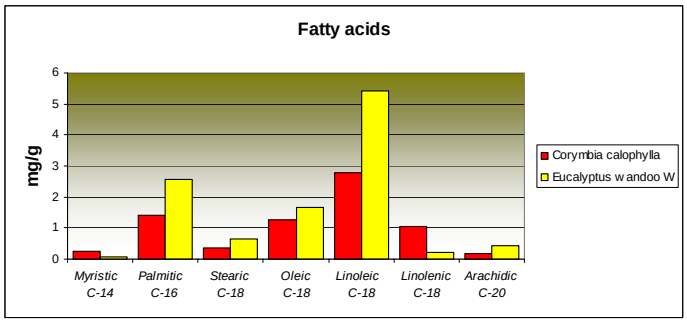


Winter Wandoo Pollen

(Eucalyptus wandoo)

Widespread in valleys in the eastern parts of the jarrah forest. Valuable source of pollen and nectar provided beehives are supplemented with more protein.

Three flowering forms known: Winter wandoo (Mar-June) north of the Avon Valley; Spring wandoo (Sept-Dec) Avon Valley to Wandering and Summer wandoo (Jan-Feb) south of Wandering.

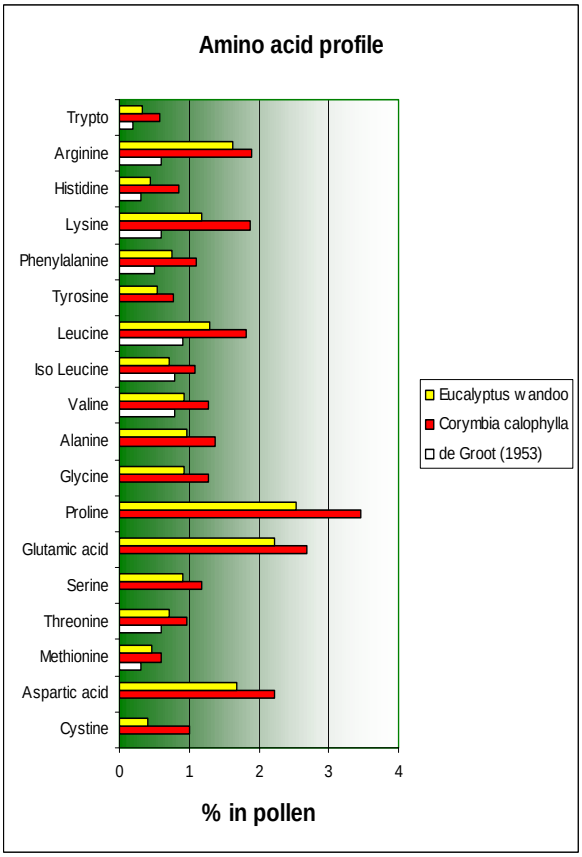


Amino acid profile

Winter wandoo amino acid levels are much lower than redgum *(Corymbia calophylla)*. Protein: 21.8% which is 4.9% lower than for redgum (26.7%).

Amino acid deficiency

There is an iso-leucine amino acid deficiency in wandoo pollen that would affect honey bees.



Fatty acid profile

Lipid (fat) in pollen is 1.5 times greater than redgum (1.3% vs 0.88%). Wandoo has a high linoleic acid compared with redgum and is much lower in Linolenic acid and myristic acid. The amount of arachidic acid is higher.

Mineral profile

Mineral levels in wandoo pollen are lower than redgum pollen, but are higher in sodium and calcium. The trace element profile is also lower than redgum pollen except for a much higher iron content (181 mg/kg).

Adjustment for maximum honey bee performance

Wandoo pollen will need to be adjusted to maximise the performance of honey bees by addition of iso-leucine via soyflour or redgum/blackbutt or karri pollen.

Market for human consumption

Wandoo pollen is as attractive as redgum pollen. It's slightly higher fat content, higher concentration of linoleic acid and high iron concentration (for women) would make it more attractive than redgum pollen.