STUDIES ON THE EUCALYPT LIGNOTUBER

A thesis submitted in fulfilment
of the requirements for the degree of M.Sc.

in the

Department of Developmental Biology,
Research School of Biological Sciences,
Australian National University,
Canberra

by

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May 1983
Dedicated to Professor Denis J. Carr and Mrs. Stella G. M. Carr for their valuable contribution to our understanding of Australian eucalypts.
DECLARATION

I hereby declare that the work involved in this thesis was performed by the author. However, due recognition is given to Professor and Mrs. Carr for the ecological description of adaptations to fire of non-lignotuberous species of the *Lehmannianae* in Chapter 2, and for the concept that the lignotuber is a position-dependent organ in Chapter 6. No material herein has been submitted for any previous degree, diploma or publication except where due reference is made.

Roland Jahnke
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This work was initially carried out in the Department of Developmental Biology, RSBS and completed while I was in the Department of Neurobiology.

I sincerely thank Professor D. J. Carr for first suggesting the Eucalypt lignotuber as a study for an M.Sc. thesis. Having previously completed an honours thesis in marine algal pathology, I was somewhat reluctant to break into the immense field of Eucalyptology. However, with Professor Carr's vast reserves of information, I knew that he could re-educate me in the realm of plant science. For this I am particularly grateful and I hope that I will be able to continue his analytical tradition. To Mrs. S. G. M. Carr I give many thanks for her valuable suggestions and numerous discussions on problems related to this work. Working with two such renowned people has certainly been very stimulating and rewarding.

I would also like to thank Professor Brian Gunning who made his department available for my use throughout the course of this work. Many thanks go to Professor Adrian Horridge, Head of the Department of Neurobiology who allowed me ample time to complete this work when I was transferred to his department. He was indeed very lenient with me when his work got second priority.

I sincerely thank each of the following persons for their assistance: Dr. Michael Adena and his staff for being patient enough to teach me the use of statistics;
Dr. Chin Wong who assessed and advised me on my experimental procedures for the nutrient experiment; Dr. Pamela Warren-Wilson and Chris Lacey for helpful discussions on aspects of this thesis; staff of the seed section in the Forest Research Division, CSIRO, Yarralumla; Eric Hines, Lydia Milkovits and Kerrie Ruth for teaching me their technical expertise; David Sandilands and David Smith of the Computer Unit; and Rod Lamb and his staff of the Plant Culture section, who took care of hundreds of seedlings grown throughout the course of this study. Special thanks go to Mr. George Weston, Head of the Electron Microscopy Unit, and his staff who always made it possible for me to "get on with the job".

I would also like to thank Gillian Hines for the unnerving task of typing this thesis.

Finally, I thank my wife, Vanda who persisted with all the odd hours and who helped in the final collation of this thesis.

Thanks very much to all of you.

Roland S. Jahnke
SUMMARY

The initiation, development and function of the eucalypt lignotuber is re-investigated, paying particular attention to the previous experimental and developmental observations of Kerr and Chattaway. Chapter 2 contains an account of the role of the lignotuber in the survival of species of the *Lehmannianae* in their natural habitats. Other species of the same group, which lack lignotubers, survive alongside the lignotuberous ones due to alternative strategies, including precocious flowering and seed maturation, and retention of seed in capsules which remain closed until the parent plant is burnt or uprooted. Massive regeneration of these species from seeds results in densely crowded stands of pole-like plants which later thin out. In clearings the remaining plants achieve their mature branched form by outgrowth of epicormic buds.

Lignotuber development was studied in one natural group (*Lehmannianae*) of species and between species of various other taxonomic groups (Chapters 2 and 3 respectively). Initiation of a lignotuber is presaged by the appearance of a suite of accessory buds adaxial to the axillary bud, and sometimes by a similar group of buds abaxial to it. The course of lignotuber development at the cotyledonary nodes varies between species. At least four different modes of development can be distinguished (Chapter 3). In certain species lignotuber fail to
develop, despite the presence of a set of "upper" accessory buds. Two provenances of *E. camaldulensis*, differing in their capacity to form lignotubers, but both equipped with suitable suites of accessory buds and presumably, genes for lignotuber development, were experimentally treated to see whether mineral nutrition, known to affect lignotuber growth, increases the penetrance of lignotuber gene expression in the normally non-lignotuberous provenance (Chapter 4). Lignotubers did not develop in the normally non-lignotuberous (southern provenance) seedlings irrespective of the plane of nutrition.

Chapter 5 describes some preliminary and exploratory experiments on the lignotuber; the translocation and storage of photosynthate, studies involving reciprocal approach grafting of lignotuberous and non-lignotuberous seedlings to study the possible effects of the shoot on lignotuber development, surgical studies, involving amputation and ringbarking of the lignotuber to study dominance effects on its growth and the outgrowth of its buds, and finally a preliminary study of water flow through the lignotuber and the stem xylem enclosed in a laterally-fused pair of lignotubers.

In Chapter 6, the nature and definition of the lignotuber are treated, especially in relation to organs of vegetative reproduction in other Australian genera. Also dealt with are the implications of the physiological properties of the lignotuber in the water relations of lignotuberous species, especially mallees. Appendix A
provides a list (necessarily incomplete) of eucalypt seedlings observed to bear or not to bear lignotubers.
This thesis contains a number of chapters which are based directly on publications which have either appeared in print, are in press, have been submitted to a journal, or are in preparation for submission. Three of these are joint publications with D. J. and S. G. M. Carr, another with C. J. Lacey.

Because of the necessity to introduce the topics of these publications, some references to previously published work appear a number of times in the thesis, usually in the introductory part of each chapter or in the discussions.

The publications also stand on their own with regard to methods and materials and thus obviate the need for an introductory section of the thesis on materials and methods. The bibliographies pertaining to each chapter have been collected together to form a single bibliography of the thesis.
CHAPTER 1

INTRODUCTION

1.1 HISTORICAL

The lignotuber first came to general attention owing to the expansion of wheat growing into mallee areas around 1860-80. At first the land was cultivated between the mallee stumps (large lignotubers). To bring large acreages into cultivation the "stump jump plough" was invented so that field ploughing could be achieved. The first scientific account of the lignotuber was given at the AAS meeting (in Sydney) in 1898 by Professor Ralph Tate. He noted "the large inflation of the stem" in two-year old saplings of *E. rostrata*, (= *E. camaldulensis*), *E. leucoxylon* and *E. viminalis*, and described its persistence in mallees.

Nineteenth-century descriptions of eucalypts tended to dismiss or misinterpret the part of the tree or mallee now known as the lignotuber. Alan Cunningham (1817), Tenison-Woods (1862), and Mueller (1879-1884) wrote brief descriptions of mallees. For example, Mueller described mallees as "....many stems with one root". Ednie Brown (1882) describes the "root" (of Mueller's descriptions) as "....being apparently a kind of dwarf trunk, the stems of our description representing its branches". Howitt (1890)
described *E. gunnii*: "...in its dwarf form it has a large butt level with the ground of several feet in diameter, from which arise numerous shoots". These descriptions have one thing in common. They deal with an unusual plant feature but do not recognise it as an organ *sui generis*.

Hall (1914) showed somewhat more interest in the nature of the lignotuber in stating: "In nearly all the *Eucalyptus* seedlings, and also in the Angophoras, there is developed, especially if growth is checked, a small woody swelling in the stem at the point of attachment of the cotyledons. A number of buds will develop from this, and shoots start from them. If the growth of the seedling proceeds in the form of one main stem, this swelling is soon obliterated, but, in the mallee, these secondary shoots grow almost as quickly as the main stem, and so, instead of a tree in the ordinary sense, we have an enlarged rootstock, from which spring numerous stems, all more or less the same size". This information also provides insight on the role of the lignotuber in the origin of tree or mallee form.

The pathologist Erwin Smith (1911; reviewed by Fletcher and Musson, 1918) investigated the relationship of bacteria to plant disease, in particular "crown gall". The first experimental investigation of a possible pathological origin of the lignotuber was carried out by Clayton O. Smith, reported in 1912. The species used was *E. tereticornis*. Seedlings 1-2 m tall were inoculated and in some cases small swellings were produced at the site of
inoculation. Since in some experiments swellings were produced on the branches of small seedlings, it seems possible that these were crown galls. Smith does not claim them as lignotubers. Since seedlings of *E. tereticornis* usually have lignotubers it is possible that Smith's seedlings also developed cotyledon "swellings", i.e. lignotubers at the node. He attempted to isolate a pathogen but failed. However he still believed them to be pathogenic in origin. The work by C. O. Smith greatly influenced Fletcher and Musson who presented a paper: "On certain shoot-bearing tumours of Eucalypts and Angophoras, and their modifying influence on the growth habit of the plants", in 1918 to the Linnaean Society, N.S.W. Although convinced that bacteria were responsible for the "tumours" they found, they did no experiments and provided no supporting evidence for the hypothesis that bacteria are responsible for "tumours" in eucalypts. The following extract summarises most of their erroneous conclusions: "...organisms are confined to outgrowths, and the encircling tumours to which they give rise and probably do not invade the tissue of the seedlings. The tumours do not kill the seedlings or even seriously damage their tissues. They are a drag on the normal development of the plants, especially so when shoots do not develop and by interfering with the water supply, and also by their shoots preventing the development of normal branching. In the mallees, so much water is intercepted by the tumours, that the seedling stem is dwarfed, and, by the persistence of
the shoots, the growth-habit is permanently distorted, so that the plants are prevented from realising their potentialities as trees. The seedling-stem may possibly be sometimes crowded out and got rid of".

Their work is valuable for its informative description of the variation of lignotuber morphology within some groups of eucalypts. They provide many excellent photographs of lignotuber form.

It seems surprising that it took almost 150 years from the earliest descriptions of eucalypts for the lignotuber to be recognised and its development and function to be interpreted. It was not until 1925 that Lesley Kerr published evidence of the non-pathological nature of the lignotuber. She published the term "lignotuber" to describe woody swellings in the axils of the cotyledons in seedlings of species of the genus *Eucalyptus*. She made several attempts to produce lignotubers artificially by infecting the internode of a seedling using lignotuber extract. No lignotubers developed at the point of injection. In another experiment, sterilised seeds were germinated and grown under aseptic conditions. Lignotubers developed after the usual lapse of time. These observations rejected previous theories that lignotubers developed in response to pathogens.
1.2 LIGNOTUBER MORPHOLOGY

At present there are few reports of the initial morphological development of the lignotuber in seedlings.

Lignotubers develop in the axils of the cotyledons or in the axils of succeeding leaves. As many as 12 nodes of a single stem have been observed to bear lignotubers, i.e. 24 lignotubers (see Fletcher and Musson 1918, Plate XVIII, *E. eugenioides* and Plate X, *E. robusta*). Kerr states: "There are exceptional cases in which lignotubers occur either on the internodes after the ones in the axils of the cotyledon or one side of the stem only. In rare cases they develop on the hypocotyl below the cotyledons as shown in Pl. X, Fig. E. This frequently occurs in *E. corymbosa* and *E. exima*, but rarely in other species". By her own definition of the lignotuber, i.e. a structure which develops in the axils of the cotyledons and the following few leaf nodes, these observations are evidently incorrect. More likely she has observed mature seedlings in which the lower nodes would be difficult to discern, thus giving the appearance of lignotuber development on the internode or hypocotyl. As lignotubers enlarge at a node, they gradually fuse encircling the seedling stem. During further growth, the thickening of the stem may extend down the hypocotyl and incorporate the proximal part of the main root, forming a partly subterranean structure. Lignotubers which grow slowly and do not encircle the young stem may become less evident if the radial growth of the stem is rapid. Seedlings which possess obvious lignotubers may
develop into trees which bear no obvious traces of lignotubers. Kerr (1925) noted that in several instances mature trees of *E. hemiphloia* var. *microcarpa*, *E. sideroxylon* and *E. melliodora* possessed recognisable lignotubers. However she did not look for lignotuber buds which might indicate that the lignotuber was still capable of giving rise to shoots, nor did she present evidence of regeneration from such lignotubers after the main stem was destroyed.

In mallees the lignotuber persists throughout the life of the plant, increasing in size with age. The multi-stemmed lignotuber becomes partly subterranean affording protection of lignotuber buds from fire or insect damage. Mullette (1976) has measured a lignotuber of *E. gummifera* to be 75 m² in area. Evidently the continued growth of a mallee lignotuber represents a balance of dominance between the growth of a main stem (tree) and the growth of the lignotuber. Lacey (1983) has described the development of large plate-like lignotubers in *E. botryoides*. As death of the upper lignotuber surface occurred in response to harsh environmental factors, in particular fire, growth was restricted to the protected subterranean periphery of the lignotuber which could give rise to many successive generations of stems. Using radiocarbon dating techniques he reservedly estimated some decayed samples tested to be less than 200 years old. This compares favourably with Wellington*et al.* (1979) estimation of radiocarbon dating for *E. oleosa* lignotubers.
There exists considerable variability in the time taken for lignotuber initiation and subsequent development. This occurs not only between species but also within species. Kerr (1925) noted up to 12 pairs of lignotubers in one seedling of *E. numevoea* while another older seedling possessed only a single pair. She hypothesised that some edaphic factor influenced lignotuber development. She investigated nutritional effects of fertilizers on lignotuber size. Superphosphate considerably enhanced lignotuber development. Nitrates had less effect. This topic will be taken up again in Chapter 4. Previous results are summarised in Table 1.1.

Carter (1929) claimed to have shown experimentally (although no data are provided) that various factors hasten or retard lignotuber development. He also gave a list of species which do or do not develop lignotubers (see Appendix A). Karschon (1971) wrote that root competition in *E. camaldulensis* retarded seedling growth. This resulted in a greater persistence of the lignotubers in slow growing trees. Evidently active growth of seedlings reduces the rate of increase of lignotuber size.

Chattaway (1958a) discounted Kerr's suggestion that edaphic factors influence lignotuber formation. She suggested that "aberrations from the normal species behaviour are the result of working with a seed from unknown origin". Chattaway examined the anatomical structure of the cotyledonary node and succeeding leaf nodes in 47 species of *Eucalyptus*, 12 of which had an
Table 1.1 The effects of nutrition on lignotuber development

Kerr 1925: (i) superphosphate enhanced lignotuber formation;
(ii) nitrates had less effect.

Carter 1929: (i) direct sunlight hastens lignotuber development;
(ii) some fertilizers, especially phosphatidic, hasten appearance and increase the size of the lignotuber;
(iii) poor drainage and disease retard lignotuber development.

Beadle 1968: (i) high P and N levels restricted the development of the lignotuber

Weir (1969): (i) light intensity was negligible in influencing lignotuber development, so long as it was adequate for overall growth;
(ii) in *E. radiata*, the ratio of lignotuber size to seedling size decreased with an increase in the level of P from 1 ppm to 50 ppm, unless a high level of N (100 ppm) was present.

Mullette and Bamber (1978): (i) an increase in P levels stimulated lignotuber development in *E. gummifera* until levels became toxic.

Dell and Jones (1982): (i) P-deficient seedlings had a greater proportion of their biomass in lignotubers and higher lignotuber P concentrations than plants fertilized with phosphate.
accessory bud situated above the axillary bud at the cotyledonary node as well as an accessory bud situated below the axillary bud. Leaf nodes were described as having an accessory bud only below the axillary bud. Unfortunately Chattaway worked only with radial longitudinal sections through the cotyledonary node. She was therefore led to the conclusion that the presence of an upper accessory bud was a necessary requirement for lignotuber development.

1.3 LIGNOTUBER ANATOMY

Kerr (1925) described the anatomy of the lignotuber from transverse sections of 12-week old seedlings of *E. morrisii*. "Tracheae" (old term for xylem vessels; Esau 1977) were said to develop from rows of brick-shaped cambial elements. Broad bands of thick walled vessel elements, shorter and wider than those produced earlier in lignotuber formation, are formed between ray parenchyma. She named these "phloeotracheae", a term used by Benson (1910) to describe tracheids of *Exocarpus*. Kerr suggested that the phloeotracheae became contorted (her "phlebotracheae") due to active growth in the horizontal plane of the developing lignotuber. She also suggested that the lignotuber originates as a result of xylem proliferation from the vascular cambium. Carter (1929) confirmed Kerr's anatomical investigation by examining transverse sections of a two-year old *E. hemiphloia*
lignotuber. He divided the lignotuber into a "definite bark and wood cylinder" and described all the tissues Kerr had observed. Chattaway (1958a) investigated elements of the xylem of the lignotuber in *E. obliqua* and *E. maculata* using maceration techniques. The so-called "phloeoctracheae" (sic, not phlaeotracheae) were revealed to be tracheids and fibre-tracheids. She described them as being thick-walled with oval bordered pits and because they contained starch (found later to be incorrect), were held to have a storage function. All the tissue elements of the stem could be recognised in sections of lignotubers in a radial longitudinal section of an *E. obliqua* lignotuber. She noted that secondary xylem distortion (Plate 1, Fig. 4) was greatest "near the vascular strands by which the buds are connected with the central normal portion of the lignotuber". This was the first record of lignotuber bud vascular strands in the lignotuber. Unfortunately no attempt was made by Chattaway to describe the origin of these "vascular strands" nor their subsequent anatomical development during lignotuber bud growth. Weir (1969), Carrodus and Blake (1970) and Bamber and Mullette (1978) carried out anatomical investigations on the distribution and amount of storage tissue in the lignotuber relative to the stem and/or root. Weir (1969) found a slightly higher ratio (1.2 times) of xylem parenchyma (storage tissue) per unit area of xylem in the lignotuber of *E. radiata* than that observed in the adjacent stem. Carrodus and Blake (1970) carried out a similar investigation. They presented
a photograph (Plate 3, No. 4) showing the "normal arrangement of the stem" in *E. camaldulensis*. In fact they have only shown a region of secondary xylem tissue from the stem. They used *E. camaldulensis* since Jacobs (1955) had stated that this species does not develop a lignotuber. However, the seedling Carrodus and Blake chose for their investigation developed "a swelling on the stem near the ground". Evidently they were unaware that some populations of *E. camaldulensis* form lignotubers. Another figure (Plate 4, No. 5) shows the contorted arrangement of cells found in the woody part of an *E. obliqua* lignotuber. Unfortunately this figure is also not labelled and one photograph does not "show the proportion of ray to vascular tissue" to be found in a lignotuber.

Bamber and Mullette (1978) investigated the anatomy of the large mallee-like lignotubers of *E. gummifera*. They described the rhytidome, phloem, cambium, sapwood, heartwood and kino veins. They also described lignotuber bark anatomy, which was similar to bark anatomy of the tree. They found that the lignotuber differed from stem tissue only in quantitative aspects; the mean lignotuber fibre length is 0.69 mm compared to mature wood (1.21 mm) of *E. gummifera* and the proportion of axial parenchyma of the wood is greater (two times) and there is less expansion of axial parenchyma of the outer phloem than in stem tissue. An investigation of sapwood and heartwood showed vessel elements, fibre tracheids, axial and longitudinal parenchyma, and vasicentric tracheids. The vessels were
arranged in multiples either radially or in clusters. Axial parenchyma is arranged in paratracheal bands 1 to 3 cells wide (their Fig. 5). The rays are formed entirely of parenchyma cells 1 to 3 cells wide by 1 to 8 cells high (their Figs. 6 and 7). Axial parenchyma in the stem were not found in wide bands. They suggested that the increase in the proportion of axial parenchyma in the lignotuber (compared to the stem) was at the expense of supportive tissues, i.e. fibre and vasicentric tracheids.

Only Shimoya and Zunti (1973) have attempted to investigate changes in anatomy during the growth of the lignotuber. They examined the lignotuber in *E. saligna* at six months and two years. In transverse sections of six-month old lignotubers, at the cotyledonary node, they observed zones of parenchymatous tissue originating from the pith and extending "through the xylem". They state that "the origin of the tuberosity was traced to the pith region and this is an intensive phenomenon causing lysis, regression and wood tissue re-structuration, these bringing about pressure on the entire back tissue giving rise to tuberosity" (adopted from the English summary).

1.4 LIGNOTUBER FUNCTION

The lignotuber is considered to have a dual role:

(i) as a storage organ of food materials, in particular starch;

(ii) as a source of dormant buds for regeneration.
Kerr (1925) observed that tracheids and fibre tracheids (her phloeo-tracheae) and ray parenchyma were "packed full of food materials, mainly starch". She found that "when the seedlings are subjected to adverse or abnormal conditions which affect the plant's nutrition, the starch in the lignotubers diminishes. These food materials are rapidly replaced on the return of normal conditions of nutrition". These observations have not since been re-examined or confirmed. Jacobs (1955) considered the lignotuber to be a modified stem structure. He described shoot regeneration from the lignotuber: "if the mainstem of the tree is destroyed by fire or otherwise, the inhibition is removed and numerous new shoots may arise from the lignotuber and ultimately one of them will replace the mainstem, particularly in the case of young trees". Chattaway (1958a,b,c,) carried out anatomical investigations on lignotubers of *E. obliqua* and *E. maculata*. She confirmed Kerr's observations by describing storage tissues. She also described the "dormant buds" of the lignotuber.

Weir (1961) suggested that "the production of cellulose and lignin required to form these non-storage elements (i.e. tracheary elements and fibers) must represent a sink for a proportion of the total energy and photosynthate made by the seedlings. In this regard then a lignotuber must represent a much less efficient storage organ than other recognized plant storge organs such as the potato tuber". He also found that in vigorous growing
seedlings of *E. obliqua*, the lignotuber represented less than 2% of the total seedling weight. From this observation he presumed that "under conditions of normal growth the lignotuber is unlikely to be very important as a storage organ". However Weir concluded that "although the lignotuber has no major advantages as a storage organ in increasing the total storage capacity available to the normal seedling it may still have an important function in providing a readily available supply of carbohydrates for the buds located in the phloem and outer surface of the lignotuber".

Carrodus and Blake (1970) were also intrigued by the fact that "a large proportion of the lignotuber does not function as storage tissue". They did not observe the starch in tracheids or fibre tracheids reported by Chattaway (1958b). However starch was abundant in parenchymatous tissues of the rays and cortex. Soluble carbohydrates (glucose, fructose, sucrose) were extracted and identified in the cleared extract by paper chromatography and by reaction with location agents. In comparing the carbohydrate content of the tap-root, stem and lignotuber of young and more mature seedlings (no age given) of *E. obliqua*, they found that the ratio of starch held per unit dry weight of tap-root tissue to lignotuberous tissue increased with seedling maturity. Thus they concluded: "the lignotuber is no more efficient than the root or stem as storage tissue, and that its prime function is probably related to the large number of buds it
holds in a protected position". Bamber and Mullette (1978) showed (their Table 1) that wood of *E. gummifera* lignotuber had twice the proportion of storage tissue, i.e. axial and radial parenchyma, as stem wood and thus a larger potential for starch storage. They also note Carrodus and Blake's reference to Chattaway: "Chattaway (1958) described the presence of starch in tracheids and fibre tracheids". They say this is not correct and that she referred to these cells as "performing the function of storage tissue". Chattaway (1958b) however does state: "they are woody masses of storage tissue: fibres, tracheids and parenchyma cells are all packed full of starch, and ray cells contain protein also".

1.5 INHERITANCE OF THE LIGNOTUBER

Chattaway (1958a) proposed that variation in the presence or absence of lignotubers within eucalypt species comes from hybridization with other species. Pryor (1957) has investigated the genetic control of lignotuber occurrence. Open-pollinated progenies were raised from seeds obtained from trees said to be naturally-occurring hybrids between *E. gigantea* × *E. robertsonii*; *E. fastigata* × *E. pauciflora*; *E. fastigata* × *E. robertsonii*; and *E. camaldulensis* × *E. viminalis* (the first of each pair of species being congenitally without lignotubers). Obviously the identification of "naturally-occurring hybrids" poses some difficulty. Are they F₁ or of later generations? In
Venkatesh and Sharma (1979) made a comparison of the manipulated cross *E. tereticornis* (with lignotubers) x *E. grandis* (without lignotubers) with a putative natural hybrid said to be of the same parent species. Seedlings of the controlled cross apparently inherited the capacity to form lignotubers from the *E. tereticornis* mother. *E. gummifera* seeds were obtained by Mullette and Bamber (1978) "from a tree growing in open woodland and a mallee from open heath in the Royal National Park". Seedlings were harvested after one year's growth and the percentage of seedlings with lignotubers and the percentage of the shoot dry mass as lignotuber was recorded. Their results (their Table 2) show that 76% of the progeny of the mallee parent possessed lignotubers as compared to 59% of the parent tree progeny. Presence or absence of the lignotuber was determined by measurements of stem diameter at the cotyledonary node at six months age. The difference in shoot dry mass as lignotuber was also statistically significant when the mallee seedlings (8.3% of 163 plants) were compared to lignotuberous tree seedlings (5.8% of 143 plants). This type of experimentation reveals little of the inheritance of the lignotuber. Obviously manipulated crosses between lignotuberous eucalypts (whether tree or mallee) and non-lignotuberous eucalypts are essential in determining heritability of characters such as that of the capacity to form a lignotuber.
some cases they might be distinct new species with intermediate characters. Since the seeds owe their origin perhaps partly to selfing, partly to outcrossing (e.g. with one or other parent) the genetical information which can be obtained is difficult to interpret. In the *E. gigantea* × *E. robertsonii*, seedlings were said to be closely related to *E. gigantea* in character but that they also had lignotubers which are normally absent from pure *E. gigantea* (Plate ii, Fig. 1). In fact all crosses produced seedlings bearing lignotubers. He also suggests: "the fact that recombination is possible between the lignotubers of one species and morphological features of the other indicates that this character is heritable".

Pryor and Byrne (1969) investigated geographical variation in *E. camaldulensis*. They examined genetic variation including that of lignotuber frequency. Seedlings of northern provenances possessed a much higher frequency of lignotubers than those of southern provenances. The absence of lignotubers in most southern populations of *E. camaldulensis* was considered to be a "derived character due to a process suppressing their development". The nature of the "suppressing process" is not mentioned. They concluded that *E. camaldulensis* was one distinct species composed of a northern and a southern population which were "clearly distinguishable at their extremes". They suggested that further investigations would be required to elucidate the status of the total population as a single species or as a group of subspecies.
1.6 LIGNOTUBER TISSUE CULTURE

Aneja and Atal (1969) suggest that lignotubers may be valuable for the clonal propagation of highly uniform commercial seedlings. They investigated plantlet formation in tissue cultures from lignotubers of *E. citriodora*. They selected "tissues from the main aerial stem, from the lignotuber and from the root tips of germinated seeds". These were grown on a modified Murashige's inorganic salts medium. A granular mass of callus developed from lignotuberous tissues in 3-4 weeks, eventually producing buds which subsequently developed into plantlets which formed roots.

Lee and de Fossard (1974) failed to repeat this work, commenting that "the authors did not describe the medium used in sufficient detail". They therefore examined "the effects of various auxins (IAA, NAA, 2,4-D, NOA) and cytokinins (kinetin, BAP) on *in vitro* culture of stem and lignotuberous tissues of *E. bancroftii*". Although they successfully cultured calluses of lignotuber explants, possessing meristematic regions, groups of tracheary elements and parenchyma, they were unsuccessful in obtaining root and shoot regeneration from lignotuber tissue.

Bhatnagar and Joshi (1973) claimed to have carried out "vegetative propagation of *E. tereticornis* lignotubers". Pieces of two-year old lignotubers were soaked in IBA, IAA and IPA (50 ppm and 100 ppm) for 24 h. Controls were treated with distilled water, the treated pieces were
planted in a soil-sand mixture (50:50). IBA increased the rooting response (50-60% at 100 ppm compared to 10% in controls). New shoots and roots developed within two to three months. Unfortunately the validity of the conclusions are marred by the fact that only 10 seedlings were used per treatment.

1.7 LIGNOTUBER PHYSIOLOGY

Blake and Carrodus (1970) and Blake (1972) considered that "the large bud potential of the eucalypt lignotuber and its ability to survive defoliation and decapitation make it very useful for the study of apical dominance". Blake and Carrodus (1970) investigated endogenous inhibitor levels correlated to apical dominance in *E. obliqua* seedlings. Lignotuber bud sprouting is inhibited by some substance produced by the crown. This inhibition is removed when the mainstem of the seedling is destroyed. They detected a "sprouting inhibitor" in mature leaves and bark which is absent in juvenile leaves. Thin layer chromatography followed by a wheat embryo bio-assay, showed inhibitory activity in extracts from adult leaves (Rf 0.5-0.8) and lignotuber bark (Rf 0.5-0.7). They considered that "an inhibitory substance" present only in adult tissues is "correlated with the lack of complete apical dominance observed in very young seedlings". As adult leaves are produced, lignotuber bud growth is also dominated by crown control. In another experiment,
lignotuberous seedlings of *E. obliqua* were decapitated and the inhibitor levels compared with undecapitated control seedlings. Decapitation was shown to reduce the levels of inhibitory substances (*Rf* 0.5-0.7) in lignotuber bark to a non-significant level after two days. After a period of 10 days, the inhibitor was again detectable (their Fig. 2). In bio-assays, the inhibitory substance "was found to run at the same *Rf* on chromatograms as abscisic acid".

Attempts were made to purify and identify the inhibitory substances, but it did not behave as abscisic acid. Eluted *Rf* zones, 0.6-0.8, from paper chromatograms of a methanolic extract of lignotuber were applied, in lanoline paste, to decapitated *E. obliqua* seedlings. The extract "significantly inhibited sprouting after 17 days whereas abscisic acid (4 ppm) in lanoline paste had no effect (their Fig. 4) when compared to control plants which were decapitated and lightly smeared with lanoline paste".

Blake (1972) investigated seasonal variation of apical dominance and the effect of temperature variation in lignotubers of *E. obliqua*. He examined the "effect of mineral nutrient and light starvation on the sprouting of seven-day old decapitated lignotuberous seedlings (his Table 1)". The results are difficult to interpret since the average number of bud shoots per treatment would be biased towards those lignotubers with the greater number of buds. Nevertheless Blake concludes that plants subjected to "mineral deficiency showed a statistically insignificant increase in sprouting". He also showed that the number of
buds which developed on lignotubers varied seasonally; most buds developed during winter (his Fig. 2). This was correlated with a winter reduction in the amount of growth inhibitory substance (Rf 0.5-0.8) assayed using the wheat embryo bio-assay.

A 25°/15°C day/night temperature regime increased the number of buds compared with other treatments; 25°/20°, 22°/20°, 20°/15°C). Daylength (8, 11, 14 h) had no effect. The limitation of seven replicas per treatment makes suspect any conclusions drawn from these experiments.
CHAPTER 2

DEVELOPMENT OF THE LIGNOTUBER AND PLANT FORM
IN THE LEHMANNIANAE

2.1 INTRODUCTION

Carr and Carr (1980) have described a small group of closely-related species, the Lehmannianae Carr & Carr, five of which (including one as yet undescribed) possess lignotubers while the other three lack them. Those species which form lignotubers, *E. burdettiana* Blakely & Steedman, *E. lehmannii* (Schau.) Benth., *E. bennettiae* Carr & Carr and *E. talyuberlup* Carr & Carr and the undescribed species, have the potentiality to develop as mallees (many-stemmed shrubs) or as single-stemmed trees. In cultivation, *E. burdettiana*, which is always a mallee in its natural habitat, may form a small single-stemmed tree. The non-lignotuberous species, *E. megacornuta* C.A. Gardn. and *E. newbeyi* Carr & Carr are single-stemmed trees; *E. conferruminata* Carr & Carr forms a short-trunked tree or marlock with wide-spreading branches when grown in the open, but in young, dense, natural stands it grows as a pole-like tree. Of this group, species with and without lignotubers grow in close proximity in a number of sites in the area of occurrence, a narrow coastal strip in Western Australia, extending from near Albany eastwards to
Pt. Malcolm. The *Lehmannianae* therefore offers an opportunity to study the comparative morphology of seedling development in closely-related lignotuberous and non-lignotuberous species of the same or similar habitats, and to consider the ecological implications of the different plant forms of these species.

2.2 MATERIALS AND METHODS

Seeds of each species of the *Lehmannianae* were sown in vermiculite and the seedlings raised in a greenhouse in which the temperature ranged between 20 and 30°C. Each month for a year observations were made of the morphology of the potentially lignotuberous cotyledonary and succeeding nodes. Cotyledonary and other nodes were fixed for 2 h in 2% formaldehyde + 2.5% glutaraldehyde in phosphate buffer or (in some cases) overnight in 8% acrolein. Dehydration followed the procedure of Feder and O'Brien (1968). The material was embedded in glycol methacrylate and sectioned at 2 μm or it was embedded in paraffin and sectioned at 8 μm. Macrophotography of the cotyledonary nodes involved the use of a Zeiss Tessovar or a Wild photomacroscope. For scanning electron microscopy (SEM), selected nodes were dried by the critical point method, coated with gold in a Polaron E5000 coating unit and observed using an Hitachi HHS-2R 500 scanning microscope at 15kV. For preliminary SEM, the node, stripped of its cotyledon and axillary bud, was attached to
a stub with an adhesive, immersed in liquid nitrogen for 40 sec then introduced as quickly as possible into the specimen chamber.

2.3 RESULTS

2.3.1. Origin and development of the lignotuber

The mode of development of the lignotuber in *E. burdettiana* will be described first since it is typical and the material lends itself readily to macroscopy. In this species, lignotubers are formed at the cotyledonary node and at one or two nodes above. At two months several accessory buds appear above the axillary bud in the cotyledonary axil. At three months more accessory buds are usually visible, forming a crescent around the axillary bud (Figs. 2.1,.2,.3). They decrease in size from the centre of the crescent. Tangential longitudinal sections (Fig. 2.2) show a series of accessory buds borne on an upper crescent-shaped meristem, partly surrounding the axillary bud. The buds appear to form a dichasial branching system, the first occupying the centre of the crescent, the successive orders of branch buds placed laterally, the youngest at its tips. The lower accessory meristem is also extensive, forming a shallow crescent, but its buds are few and develop rather more slowly than the upper ones. If an upper accessory bud is damaged or removed during development, adjacent buds grow larger in compensation.
Figs. 2.1-3. *E. burdettiana*, cotyledonary node at c. two months. Scale markers, Figs. 1 & 2, 1 mm, Fig. 3, 2 mm.

Fig. 2.1. Scanning electron micrograph, the cotyledon (Cot) and axillary bud (A) cut off. The seedling stem and the paired leaf primordia of the upper accessory buds numbered in size sequence, bear emergent oil glands each equipped with a stellate hair.

Fig. 2.2. Tangential longitudinal section through a cotyledonary node. La, lower accessory bud meristem. The upper accessory buds are numbered in order of size (1, 2, 3) and sequence in a dichasial branching system.

Fig. 2.3. Cotyledonary node. Abbreviations as in Figs. 1 and 2.

Fig. 2.4. *E. talyuberlup*, scanning electron micrograph of a node about three months old. Abbreviations and scale marker as in Figs. 2.1 and 2.2.
The number of growing and visible upper accessory buds varies from five to eight, but the actual number of apical bud meristems, including those which are hidden in the axillary gap or are too small to be visible, is much larger. Growth of the axillary bud is usually of short duration and it abscises within about six months. The larger accessory buds also wither and abscise, having formed one or two pairs of rudimentary leaves, which fail to expand. About this time the beginning of lignotuber formation is seen as a semicircular wedge of tissue bearing the bud scars of the upper accessory buds and the meristem which produced them.

In further lignotuber development the axillary bud or its scar and that of the cotyledon become incorporated in the growing wedge, the thick edge of which is uppermost. At 12 months, two well-rounded lignotubers, each approximately 4 mm in diameter, have formed. Lignotuber buds, although inconspicuous, are present over their entire surface. In *E. talyuberlup*, at three months, one or two upper accessory buds are visible at the cotyledonary node (Fig. 2.4). Longitudinal radial sections reveal a series of accessory buds deep-seated in the axillary gap (Fig. 2.6). Unlike those of *E. burdettiana* the upper accessory buds tend to remain within the axillary gap during development. Both upper and lower accessory buds are present, the upper ones in greater profusion. At four months small lignotubers, 1-2 mm in diameter, are formed. In *E. bennettiaæ* and the as yet undescribed
Figs. 2.5 and 2.6. Longitudinal radial sections through cotyledonary nodes. Scale markers, 100 μm.

Fig. 2.5. *E. newbeyi* (non-lignotuberous). Fig. 2.6. *E. talyuberlup* (lignotuberous). Ua, upper accessory bud meristem. La, lower accessory bud meristem. A, axillary bud.
species, lignotubers develop at the cotyledonary node and at several successive nodes (Fig. 2.7). Within three months of germination, a crescent of upper accessory buds forms in the cotyledonary axil and within a further three months an upper lignotuberous swelling appears. During this time another swelling forms, abaxial to the axillary bud, and at about six months the axillary bud abscises. The upper and lower swellings then fuse. The same sequence takes place at the lignotuberous nodes above the cotyledonary node, but the number of upper accessory buds declines acropetally with node number, ultimately to only one. The lignotubers produced also tend to decline in size acropetally. Nodes above the last potentially lignotuberous node have no upper accessory buds, or only one, and have only the complement of lower accessory buds usual at intermediate and adult vegetative nodes.

In *E. lehmannii* there is a relatively poor development of the upper crescent of accessory buds, which form only one pair of rudimentary leaves and remain in this state during lignotuber development. At six months the lignotubers at the cotyledonary node are 1-2 mm in diameter. The cotyledonary lignotubers evidently may not fuse for several years, since the stem between initially maintains a rate of radial growth sufficient to keep them apart. Should the activity of the lignotuber cambium considerably overtake that of the stem cambium, as happens, e.g. if the seedling shoot dies back, the expanding lignotubers flare out sufficiently to meet, usually at the
mid-line of the stem (Fig. 2.8). In these lignotuberous species, the cotyledons and their axillary shoots abscise at about 3-6 months.

*E. megacornuta, E. newbeyi* and *E. conferruminata* do not form lignotubers (Figs. 2.19, 20, 21). At three months, median longitudinal sections of the cotyledonary node show in some instances traces of an upper accessory meristem and there is always a lower accessory meristem, together with a bud or buds. The upper meristem does not usually give rise to buds bearing leaf primordia, although a single such bud may occasionally be produced (e.g. in *E. newbeyi*, Fig. 2.5). Chattaway (1958c) found no upper accessory bud in the cotyledonary axil of "*E. lehmannii*" (= *E. conferruminata*), which she listed as a non-lignotuberous species.

2.3.2 Later development: the origin of the plant form

Subsequent development of the shoot and lignotubers depends on a number of factors which determine the continuance of growth of the main seedling axis and its branches. Good site conditions, involving an absence of prolonged drought, of destructive grazing or trampling, of root disturbance or fire, favour the continuation of growth of the original seedling axis. On a good site or in the greenhouse, some of the lower axillary buds in the non-lignotuberous species form rapidly-growing shoots which, unlike those of the lignotuberous species, soon become as long as the seedling shoot (Figs. 2.19, 2.20; cf. *E. bennettiae*, Fig. 2.7). However, these developed branches do not persist; they die and are shed within a year.
The seedling crown at between one and three years is a dome of shoots approximately equal in length. The axillary buds of these shoots rarely develop into long branches - most of the growth is by accessory buds. The lengths of the shoots in the seedling crown is 0.3-0.6 m, and as long as its shoot apex remains viable, growth of each shoot continues while temperatures are favourable. In the winter, the tips of the branches may be killed and they are replaced in the spring by outgrowths of accessory buds. Seedling and intermediate stages in early development may be recognised on the basis of leaf shape (Figs. 2.19, 2.20). At between 18 months and three years the plant seedling enters its adult stage, and the indefinite shoot growth of the seedling and intermediate stages, producing long branches which tend to keep on growing throughout the season, is replaced by a determinate shoot growth. Annual shoots are then produced, during a growth period of only 2-3 months. These shoots tend to be comparatively short (0.1-0.2 m).

Under such conditions and with shoot growth interrupted only by seasonal conditions, the lignotuberous species, *E. burdettiana* and *E. talyuberlup* produce small trees, usually less than 8 m, with relatively small lignotubers. In the habitat of *E. burdettiana*, on the steep seaward-facing slopes of East Mt. Barren and a soil derived from micaceous schist, the conditions are not conducive to continued growth of the main stem for more than a few years, and so it is invariably a small mallee
Fig. 2.7. *E. bennettiae*, seedling six months old, showing lignotubers which have already grown to surround the bases of axillary shoots at three nodes, including those (A) at the cotyledonary node. The arrow indicates the wedge-like profile of the young lignotuber. Scale, 0.01 m.

Figs. 2.8-11. *E. lehmannii*.

Fig. 2.8. Base of a plant of which the seedling shoot (SS) has died and been replaced by a shoot (RS) from one of the lignotubers. Lateral fusion of the two cotyledonary lignotubers is not yet completed. Scale, 0.01 m.

Fig. 2.9. Small mallee (Old Ongerup Road), showing multiple thin stems and the relatively large lignotuber. Scale, 0.1 m.

Fig. 2.10. The massive subterranean lignotuber of a mallee at Cape Riche. The arrow indicates a scale object (20 cent coin, 28 mm diameter).

Fig. 2.11. Large mallee near the estuary of the Pallinup River, with relatively massive trunks, 0.1 m diameter.
Figs. 2.12 and 2.13. *E. talyuberlup*.

Fig. 2.12. A spreading large mallee, 7.8 m tall in an open community at Tachalarup.

Fig. 2.13. A mallee with two main stems, c. 4 m tall, in a closed community near Talyuberlup, Stirling Range.

Figs. 2.14 and 2.15. A new, as yet undescribed, species of the *Lehmannianae* from Mt. Desmond.

Fig. 2.14. To show its common form as a single-stemmed tree, c. 4 m tall. The arrow indicates its lignotube.

Fig. 2.15. Close-up of the overground lignotuber of another specimen, with two main stems. Scale object, 20 cent coin.
with a relatively large lignotuber. The latter evidently arises by enlargement and concrescence of the lignotubers already formed on the seedling stem, together with secondary lignotubers formed on the bases of branches which grow from the lignotuber itself (Carr et al., 1982).

*E. lehmannii* is always a mallee or shrub in its natural habitats. Even on the best sites it is never found as a tree. Whether it could be induced in cultivation to grow as a single-stemmed tree is not known. It is possible that the original seedling stem is of definite duration and is replaced after a time by shoots from the lignotuber. The mature lignotuber is a relatively massive turnip-shaped structure which is almost entirely subterranean (Fig. 2.10). In the best sites (e.g. Pallinup River) the lignotuber supports trunks 0.1 m in diameter and 3-4 m tall (Fig. 2.11), but in poorer sites (e.g. Mt. Desmond near Ravensthorpe) the stems are no more than 3-4 cm in diameter and only 1-2 m tall (Fig. 2.9). *E. talyuberlup* is a small tree in many of the valley sites it occupies in the Stirling Ranges (Fig. 2.13) but on more exposed and drier slopes it develops as a mallee (Fig. 2.12).

The as yet undescribed species (from Mt. Desmond) has a large dome-shaped lignotuber which bears either a few small stems, or more frequently, a single stem which may be up to 6 m tall and 0.25 m in diameter (Figs. 2.14,15).

*E. conferruminata* is a tall tree of sandy gullies on the coast and islands. In one locality, Middle Mount Barren, Fitzgerald River National Park, a stand examined in
1979 occupied a dry, sandy creek bed, while the slopes leading down to it were occupied by *E. lehmanii*. At one point the two were separated by no more than 2 m. Some evidently old and spreading trees of *E. conferruminata* had been overturned by a torrent in the creek bed, and thus killed. Their capsules had released copious quantities of seeds and a dense pole stand of young *E. conferruminata* trees had grown up (Figs. 2.17,18). Excavations around the bases of these trees revealed no trace of lignotubers - the three or four main anchoring roots emerged horizontally from the base of the trunk (Fig. 2.21). The trees were judged to be about eight to 10 years old. They lacked any living developed lower branches - the first branches were at about 2-3 m above the ground. Already some of these young trees had fallen over or died, opening gaps in the canopy. On the trees surrounding, or in, such gaps, epicormic shoots had begun to develop, the lowest not more than 15 cm from the ground (Fig. 2.21). Similar pole-stage stands of *E. conferruminata* have been reported by Storr (1965) in gullies on Bald Island.

*E. conferruminata* is widely grown as a shelter-belt tree (Brockway, 1959). Given ample light from its seedling stages onwards, some of its lower branches continue to develop and do not abscise; they may ultimately become almost as long as the whole tree is high, producing, on the best sites, an initially pyramidal shape up to 10 m tall, borne on a short trunk which may be no more than 1 m long. Later, in the overmature stage, when height
Figs. 2.16-2.18. *E. conferruminata*.

Fig. 2.16. Overmature specimen, 14 m tall, in a hedge near Narrogin, W.A. The arrows indicate lower branches which have fallen under the weight of fruits.

Fig. 2.17. Pole-stage community at Middle Mt. Barren, Fitzgerald River National Park. In the centre, a relatively well-lit opening has appeared, due to the death and collapse of a number of stems. The arrows indicate the large fruits borne on short lateral shoots of the main stems. The trees are 5-6 m tall.

Fig. 2.18. Same community as Fig. 2.17, but on its edge. The sufficiency of light has allowed the growth and persistence of a number of branches from low on the main stem of a tree 3 m tall.
and its massive, curving branches.
growth and the growth of the lower branches has ceased and they have been shed, the upper branches may continue to extend and the pyramidal shape rounds off (Fig. 2.16). The dead trees in the Middle Mt. Barren gully were of this marlock (short-trunked tree) form, and one could therefore expect the final survivors of the dense population of pole-like young trees to achieve such a form by the outgrowth and continued growth of epicormic shoots from their lower trunks (Fig. 2.21).

*E. megacornuta* also grows in pole-like even-age stands, as on the slopes of Mt. Desmond near Ravensthorpe. In 1980, excavations about the bases of these trees revealed no trace of lignotubers. The trees were unbranched to about 2-3 m from the ground (Fig. 2.24). At other sites, on hills north of Ravensthorpe, old, open stands of *E. megacornuta* were observed to consist of large spreading trees with relatively massive, upwardly-curved limbs (Fig. 2.25). *E. megacornuta* is apparently unable to replace, by later outgrowth of epicormic shoots placed low on the trunk, the lowest branches lost by shading in its pole stage.

*E. newbeyi* is a tree of dense woodland in the Pallinup River Estuary and on the banks of the Susetta River, Fitzgerald National Park. No stands of young trees are known and consequently we have no knowledge of its capacity for regeneration of branches shaded out or lost by competition. Its mature form is not unlike that of trees of *E. talyuberlup*. It lacks the height of *E. megacornuta*, and its massive, curving branches.
Figs. 2.19 and 2.20. Seedlings, six months old, of *E. conferruminata* (Fig. 2.19) and *E. newbeyi* (Fig. 2.20). Note the long and relatively persistent axillary branches, as compared with *E. bennettiae* (Fig. 2.7) raised under the same conditions in a greenhouse. Scale, 0.1 m.

Figs. 2.21 and 2.22. *E. conferruminata*, at the same site as Fig. 2.17.

Fig. 2.21. Base of a pole-stage tree excavated to reveal the main horizontal roots. This tree was in a well-lit clearing, and epicormic shoots (asterisks) had begun to grow out from near the base of the trunk. Scale object, 20 cent coin.

Fig. 2.22. Portion of a pole-stage trunk about 2 m from the ground, showing the persistent fruits, the bases of their subtending lateral shoots having become overgrown by the main stem. Scale, 0.1 m.
Fig. 2.23. *E. conferruminata*. Branch of a large wind-thrown specimen cultivated at Albany, to show the proliferation of epicormic shoots. Scale, 0.1 m.

Figs. 2.24 and 2.25. *E. megacornuta*.

Fig. 2.24. Somewhat advanced pole-stage community at Mt. Desmond. Some trees, like the one arrowed in the foreground, had died and fallen leaving clearings. The standing tree (5.3 m tall) in the foreground is branched from just above 2 m.

Fig. 2.25. A mature tree (about 9 m tall) on a hill north of Ravensthorpe.
2.4 DISCUSSION

2.4.1 Development of the lignotuber

The work of Kerr (1925) and others established that some species of eucalypts congenitally form lignotubers while others do not. It was left to Chattaway, a third of a century later, to find out what morphological characters of the seedling determine this difference. In longitudinal radial sections through the cotyledonary nodes of seedlings of a number of species (Chattaway 1958,a,b,c) she observed that those which later formed lignotubers had two accessory buds, one above (i.e. adaxial to) the axillary bud and one below it (i.e. abaxial to it); non-lignotuberous species had only the lower accessory bud. This latter condition is that which is common at the older nodes of eucalypts generally (Cremer, 1972). Usually, however, more than one lower accessory bud is formed and they lie hidden in a groove between the insertion of the petiole and the axillary bud. Long after the petiole and its axillary bud have abscised, these accessory buds may persist and proliferate, and they may under certain circumstances give rise to epicormic shoots (Cremer, 1972). Chattaway's criterion appeared clear-cut until she later found that some non-lignotuberous species also may have both upper and lower accessory buds in the cotyledonary axils. These species included a provenance of *E. camaldulensis* (presumably a southern one since most northern provenances do form lignotubers), *E. astringens*, *E. diversicolor* and *E.*
*pilularis*. Chattaway did not explore the causal relationship between the presence of upper accessory buds and the development of the lignotuber itself. That there is such a relationship seems clear from this study of *Lehmanianae*. The size of the lignotubers formed appears to bear a relationship to the initial complement of upper accessory buds. This number usually declines acropetally from the cotyledonary node, as does the size of the lignotubers, although in *E. bennettiae* and the as yet unnamed species, the decline may not begin until nodes 3 to 5. The relationship between the number and vigour of the upper accessory buds and the initiation and development of the lignotuber thus takes a quantitative form. Whether nodes which have only a single upper accessory bud are in general incapable of forming lignotubers is not known, but it seems likely to be the case, since such nodes are sometimes located well above the last lignotuber-forming node on the seedling stem. The absence of lignotuber formation in species which lack a complement of developed upper accessory buds is, at least in the *Lehmannianae*, quite clear. This too argues a causal relationship between the two. Evidently in these species, as at higher nodes of lignotuberous species, the development to some degree of a meristem capable of producing buds in the upper accessory bud position or even the production by such a meristem of one or two buds is not sufficient in itself to sustain the formation of a lignotuber.
The branching of the upper accessory buds, initially apparently dichasial, continues as the surface on which descendant buds are displayed increases with enlargement of the lignotuber. We have seen no evidence of the production of endogenous lignotuber buds, as claimed by Chattaway (1958a).

2.4.2 Adaptations to fire of non-lignotuberous species

The lignotuber is evidently an organ of perennation and must be regarded as a morphological adaptation to uncertainty of the habitat, especially stress such as defoliation, prolonged drought or fire likely to result in death and loss of the overground parts of the plant. It is not an organ restricted to species of eucalypts of arid habitats as is often stated (e.g. by Kerr, 1925). Nor is it the only type of adaptation to the sort of stresses mentioned above available to eucalypt species, even in the same habitats. The consistently lignotuberous *E. lehmannii* grows side by side with the non-lignotuberous *E. megacornuta* at Mt. Desmond, with *E. conferruminata* at Middle Mt. Barren, and with *E. newbeyi* at the estuary of the Pallinup River. All these species are evidently adapted to these habitats. The non-lignotuberous species overcome the problem of the loss or death of all overground parts by retaining quantities of seeds in capsules which remain indehiscent while attached to the tree and releasing them on death of the shoot. This capacity to maintain the capsules closed while attached must depend on maintenance
of their water content, and argues the possibility, not yet demonstrated, of a slow transpiration stream through them, keeping them sufficiently hydrated to prevent dehiscence.

Another aspect of this adaptation is precocity in flower formation. Seedlings of *E. new beyi* raised in Canberra formed three generations of inflorescences within two years of germination. Seedlings of *E. conferruminata* also form flower primordia within 18 months of germination, and are known to produce viable seeds within five years (Brockway, 1959). The precocity of flowering plants of *E. cornuta* (= *E. conferruminata*) raised at Kew was mentioned by J.D. Hooker (Hooker, 1875). Many of the early inflorescences are produced on the main seedling stems (Fig. 2.22) so that even in the pole stage the fruits are retained on the stem, their peduncles often becoming embedded so that they appear pseudo-cauline. Therefore, so long as the incidence of disaster such as destructive fire is not more than about once a decade, regeneration of the stand is both possible and likely. On the other hand, the first production of flowers by the lignotuberous species such as *E. lehmannii* is postponed for many years. Seedlings of *E. lehmannii* had not flowered five years after seed sowing (R.W. Goodman, a resident of Albany, West Australia, personal communication). It also appears that seedlings of *E. bennettieae*, *E. talyuberlup* and the undescribed species do not produce flowers during the first two or three years of growth.
Although the "tree" species of the Lehmannianae lack lignotubers, they do not lack epicormic buds. This was attested by the copious production of epicormic shoots on trees of *E. conferruminata* cultivated near the airport at Albany, which were wind-thrown during a cyclone in 1976 (Fig. 2.23). It is from these epicormic buds that the mature form of the trees is derived, as the pole stage community thins out. Of course, a tree grown in isolation has the opportunity of developing this form *ab initio*, as is shown by cultivated specimens of *E. conferruminata* and *E. megacornuta*. The epicormic buds of these two species evidently cannot survive intense fire; this is attested by the even-aged stands of *E. megacornuta* at Mt. Desmond, the result of regeneration from seed after fire. Following the intense fires of early 1977 on Middle Island (Recherche Archipelago), regeneration of *E. conferruminata* took place entirely from seedlings (A.M. Hopkins, W.A. Wildlife Research Centre, Perth, personal communication).
CHAPTER 3

INITIATION, EARLY DEVELOPMENT AND ANATOMY
OF LIGNOTUBERS IN SOME SPECIES OF EUCALYPTUS

3.1 INTRODUCTION

Following Kerr's (1925) publication describing the origin, function and nature of lignotubers in *Eucalyptus*, Chattaway (1958 a,b) examined radial longitudinal sections of the cotyledonary nodes of 47 species in an attempt to understand why some form lignotubers and others do not. In contrast to the adult leaf nodes, in which there was always a lower accessory bud, abaxial to the axillary bud, the lignotuber-forming cotyledonary and succeeding nodes of seedlings were found to possess an upper accessory bud, adaxial to the axillary bud. Despite finding an upper accessory bud in the cotyledonary nodes of 12 species which do not form lignotubers, she concluded that it was the "necessary morphological structure" essential to the formation of a lignotuber. Chattaway describes lignotubers as arising first and developing to a greater size adaxially to the axillary bud. She made some preliminary investigations of the early anatomy and concluded that the lignotuber arose from "extension of the (stem) cambium into (the)...swelling...of the stem above the axillary bud group". She also concluded (as did Kerr, 1925) that some
of the buds of the lignotuber arise "endogenously from meristematic tissue in the cortical or phloem tissue of the lignotubers". A previous contribution (Chapter 2) showed that in the *Lehmannianae*, lignotuber development, as a formation of a wedge-shaped expansion of the underlying tissue, is preceded by the appearance of a crescent-shaped array of accessory buds, adaxial to the axillary bud. The further development of the lignotuber in that group of species was also described. Studies of lignotuber development in other species reveal a considerable diversity of at least the earliest stages of development, which shall be considered in this contribution. Examined also are the other matters raised by Chattaway, namely the origin of buds and aspects of the anatomy of the young lignotuber.

3.2 MATERIALS AND METHODS

Seedlings of the following species were raised in soil in a greenhouse at approximately 25°C.

Section *Exsertaria* of Pryor and Johnson, 1971

*Eucalyptus amplifolia*

*E. bancroftii*

*E. camaldulensis* (a northern, i.e. lignotuberous, provenance)

*E. morrisii*
Sub-genus *Corymbia*

*E. citriodora*

*E. eximia*

*E. intermedia*

*E. watsoniana*

*E. grandifolia*

Section *Transversaria*

*E. pellita*

*E. botryoides*

Section *Maidenaria*

*E. st.johnii*

*E. bicostata*

Section *Adnataria*

*E. leucoxylon* var. *rosea*

Observations of seedling development were made regularly over a period of six months and the further progress of lignotuber development was followed for a total period of a year or more. Stages in lignotuber development were recorded by macrophotography, using a Wild Photomacroscope, or by scanning electron microscopy (SEM). For the latter, excised cotyledonary nodes were either mounted fresh on a stub, immersed in liquid nitrogen for 40 sec and observed directly, or they were fixed in Karnovsky's fluid (1965), dehydrated through an acetone series, dried by the critical point method, coated with gold and examined in an Hitachi HHS-2R scanning electron microscope at 15 KV. For anatomical studies excised cotyledonary nodes were fixed overnight in Karnovsky's fluid and then embedded in glycolmethacrylate (Feder and O'Brien, 1968) or in araldite by standard procedures.
Sections 2 μm thick were cut and stained with a 2% solution of toluidine blue 0. Fluorescence of phloem sieve tubes was observed after staining sections with water soluble aniline blue (Edward Gurr) (0.05% w/v in 0.06 M phosphate buffer at pH 9.2). Controls were mounted in buffer solution. The specimens were examined in a Zeiss Photomicroscope III with a Phillips CS 200 WA mercury vapour lamp, and the fluorescence induced was observed using Zeiss UG1 as exciter filter and Zeiss 41 as the barrier filter.

3.3 RESULTS

3.3.1 Categories of early morphological development

In all cases the earliest evidence of lignotuber formation is the production, adaxial and/or abaxial to the axillary bud of the cotyledonary node, of a tangentially arranged series of accessory buds. These buds may remain concealed in the cleft (Chattaway, 1958a) occupied by the accessory meristem or extend precociously out of it. Subsequent development of the lignotuber in different species may follow one of at least four morphologically different pathways, illustrated schematically in Fig. 1 and here designated A-D.

Group A: The first type of early development is exemplified by the red-gums, E. amplifolia, E. camaldulensis, E. morrisii, E. bancroftii, by the
Fig. 3.1, A-D. Diagram of four patterns of lignotuber (L) initiation at a cotyledonary node in different species of *Eucalyptus*. C, scar of attachment of cotyledon; Ax, axillary bud; A, A', accessory buds, adaxial and abaxial, respectively, to the axillary bud; L, L', lignotuber.
Figs. 3.2-4. Cotyledonary nodes of seedlings six months old (except where stated). Lettering as in Fig. 1.

Fig. 3.2. *E. banarofitii*. Arrowheads, flakes of epidermis and cortical tissues. Scale, 1 mm.

Fig. 3.3. *E. morrisii*. Decussate arrangement of lignotubers at cotyledonary and first node. Scale, 4 mm.

Fig. 3.4. *E. citriodora*. Small degree of lignotuber development associated with upper accessory buds, but most related to lower accessory buds (arrowhead). Scale, 4 mm.
blue-gums, *E. st.johnii* and *E. bicostata*, and by *E. pellita*. A small swelling appears above the axillary bud (Figs. 3.2, 3.3); at first the upper accessory buds remain deep-seated in the cleft. At six months, seedlings show only minor lignotuber development at one or two nodes above the cotyledonary node. The axillary buds tend to persist at the lignotuberous nodes for about 12 months.

**Group B:** A second type of development is that followed by *E. citriodora*. The lignotuber arises within four months of germination as an abaxial swelling. Suites of accessory buds are developed both adaxially and abaxially to the axillary bud, but the latter appear to be those which most influence the early growth of the lignotuber (Fig. 3.4). These data are contrary to the drawing published by Chattaway (1958a). At four months clusters of lignotuber buds are already exposed on its surface. Some swelling takes place below the upper accessory buds, but its contribution to the formation of the lignotuber is minimal.

**Group C:** In two of the species studied, *E. leucoxylon* var. *rosea* and *E. pauciflora*, lignotubers develop about six months after germination. A group of upper accessory buds becomes conspicuous at about three months (Fig. 3.5), presaging lignotuber initiation as a crescentic swelling. After a further period, a separate swelling appears below the lower, abaxial suite of accessory buds. The two crescentic swellings then fuse laterally, enclosing the base of the axillary bud (Fig.
3.6). The axillary shoot may persist during this period but eventually abscises.

**Group D:** The bloodwoods, *E. watsoniana*, *E. intermedia* and *E. eximia*, exemplify a fourth pattern, characterized by the precocious development of the central upper and the central lower accessory bud preceding lignotuber formation. This is clearly apparent in three months old seedlings of *E. watsoniana* (Fig. 3.7). The axillary bud withers and abscises early in lignotuber formation. At six months (Fig. 3.8) the lignotuber is already well formed and bears accessory buds. In *E. grandifolia* (Fig. 3.9) the central upper accessory bud is usually better developed than the lower. The rapidity of these early events in *E. eximia* (Fig. 3.10) results in occlusion of the whole cotyledonary node by lignotuberous swellings within three months of germination, at which stage numerous bud primordia are evident on their surface.

**3.3.2 Later morphological development**

From these various beginnings, the later course of the morphology of lignotuber development also takes different pathways, to some extent dependent on external and other conditions affecting seedling growth. In *E. bicostata* seedlings, observed over a period of four years (Fig. 3.11), lignotubers develop only at the cotyledonary nodes; they are covered with bud primordia. The cotyledonary lignotubers do not fuse laterally around the stem but remain separated by continued stem expansion. In
Figs. 3.5-10. Cotyledonary nodes of seedlings six months old, except where stated. Lettering as in Fig. 1.

Fig. 3.5. *E. leucoxylon* var. *rosea*. Scale, 4 mm.

Fig. 3.6. Lignotubers (arrowheads) encircle axillary buds. Scale, 8 mm.

Fig. 3.7. *E. watsoniana*, three months old. The leaf primordia in this and Figs. 8-10 are decorated with numerous emergent oil glands. Arrows, scars of abscised axillary buds. Scale, 3 mm.

Fig. 3.8. *E. watsoniana*, eight months old. Arrows, scars of axillary buds. S, seedling stem. Accessory buds now separated by developing lignotuber. Scale, 8 mm.

Fig. 3.9. *E. grandifolia*, five months old. Upper accessory buds expanded, the axillary buds (arrows) have abscised, but the cotyledons persist. Scale, 4 mm.

Fig. 3.10. *E. eximia* (scanning electron micrograph). The whole cotyledonary node is enveloped in lignotuber growth. Individual lignotuber buds subtend (asterisks) additional swellings.
seedlings of the related *E. st.johnii* the bud clusters are especially prominent in 2-year old pot-grown seedlings (Fig. 3.13), and in addition to lateral fusion of the lignotubers, their downward growth unites those at adjacent nodes into a complex. In *E. bancroftii* (Fig. 3.12) later stages of lignotuber development are predominantly abaxial to the axillary bud, although the lignotuber was initiated adaxially to it (Fig. 3.2). Here again, there is no lateral fusion of the pairs of lignotubers around the stem. Under adverse conditions, and especially where the seedling stem dies and is replaced by the outgrowth of successive generations of shoots from the lignotubers, the lignotubers of adjacent nodes may become fused together to form a complex structure (Fig. 3.14). The thickening may extend downwards involving the hypocotyl and even the upper part of the main roots. The bulk of this complex may be added to by the formation and coalescence of secondary lignotubers on the lowermost nodes of shoots derived from lignotuber buds (as described in Carr *et al.*, 1982) (Fig. 3.15, 3.16).

3.3.3 Early anatomical development

Early anatomical development of the lignotuber is much more consistent than its early morphological development. In six months old seedlings of *E. bancroftii* the developing lignotuber (Fig. 3.17, cf. Fig. 3.2) consists initially of a mass of secondary xylem without any bud vascular strands, in accordance with its origin above
Figs. 3.11-16. Later morphology of lignotuber development.

Fig. 3.11. *E. bicostata*, four years old. The cotyledonary lignotubers have been kept apart by growth of the intervening stem. A branch developed from an axillary bud has persisted (arrow) and shows that in later stages the lignotuber grows most below the axillary bud. Note the buds dotted over the lignotuber surface. Scale, 15 mm.

Fig. 3.12. *E. bancroftii*. Similar to *E. bicostata* but lignotuber buds not prominent. Scale, 10 mm.

Fig. 3.13. *E. st.johnii*. The lobules are consistently associated with bud clusters. Lateral fusion of lignotubers at a node takes place within 18 months. In this example full fusion has been delayed by tissue damage (asterisk). Scale, 10 mm.
Fig. 3.14. *E. torelliana*, pot-grown four years old. The seedling stem (arrow) has survived but secondary stems have been formed from the lignotubers. Lignotuber thickening has extended downwards to the hypotocyl (asterisk) and proximal parts of the main roots. Scale, 10 mm.

Fig. 3.15. *E. watsoniana*, pot-grown, four years old. The bulk of the lignotuber has been derived from the coalescence of lignotubers at successive nodes (arrows). A replacement stem bears secondary lignotubers (asterisk). Scale, 15 mm.

Fig. 3.16. *E. ptysachocarpa*, pot-grown, four years old. The seedling stem (arrow) has been replaced by several stems (one bearing secondary lignotubers, asterisk) from the lignotuber, which has assumed an ellipsoidal form. As in Fig. 3.14, the hypocotyl and proximal parts of the main roots have swollen. Scale, 14 mm.
the suite of upper accessory buds (Fig. 3.2). Below it, the accessory bud strands connect to the tissues of the primary stem of the seedling. In *E. leucoxylon* var. *rosea* the symmetrical development of the upper and lower parts of the lignotuber around the axillary bud leads to lignotuber xylem masses surrounding the bud strands of the suites of upper and lower accessory buds (Figs. 3.18, 3.19). The bud strands connect through to the seedling primary stem tissues (Fig. 3.19). In transverse section through the young lignotuber of *E. botryoides*, its swollen form is seen to be produced by a cambium developed across the cotyledonary leaf gap, and contiguous at its outer margins with the stem cambium (Fig. 3.20). Once initiated, this lignotuber cambium produces xylem at a much greater rate than the stem cambium. The lignotuber xylem is different in texture and constitution from that produced by the stem cambium. The wedge of lignotuber xylem expands laterally as the lignotuber cambium itself broadens, by radial divisions of its initials. The lignotuber cambium eventually extends across the whole of the cotyledonary leaf gap and envelopes also the vascular traces of the axillary bud and cotyledon.

3.3.4 Lateral fusion of lignotubers: stem dominance and lignotuber dominance

Whether or not the pair of lignotubers at a single node will fuse laterally to enclose the stem xylem depends on the relative activity of the stem cambium and the
Figs. 3.17-21. Scanning electron micrographs of sections through developing lignotubers at cotyledonary nodes. Lettering as in Fig. 3.1, except where stated.

Fig. 3.17. *E. bancroftii*, median longitudinal section (MLS). P, pith. Scale, 1 mm.

Figs. 3.18-19. *E. leucoxylon* var. *rosea*, MLS. The vascular traces of accessory buds and axillary buds converge at the central region of the cotyledonary node. Arrows indicate upper and lower portions of lignotuber. Scale, 1 mm. Arrowhead indicates accessory bud.

Fig. 3.19. A lower accessory bud (lower LH side of Fig. 18) is enlarged. T, bud vascular trace; Ca, lignotuber cambium. Scale, 0.5 mm.

Figs. 3.20-21. *E. botryoides*, transverse sections (TS), showing the lignotuberous woody tissue (bounded by arrows) converging on the pith (P). Bud vascular strands (T) run right through the lignotuber tissue to buds (A). The asterisks mark the conjunction of lignotuber cambium with stem cambium. Scale, 1 mm. Note the differences between the wood of the lignotuber and the stem, the latter (Fig. 3.21) with numerous large vessels (arrows). Stem cortex (C) much wider than lignotuber cortex (C'). Scale, 0.5 mm.
lignotuber cambium. After a period of relatively high activity, during the initiation of the lignotuber, its cambium may settle down to a rate of activity more in balance with that of the stem cambium. This will result in persistence and growth of both the lignotubers and the intervening portions of stem (e.g. Figs. 3.11, 3.12). If the ratio of cambial activity is in favour of the lignotuber, however, the lateral extension of the two lignotubers may lead to their fusion around the stem (Fig. 3.22A). However, if the ratio of cambial activity is in favour of the stem, it will increase in girth more rapidly than the lignotubers, which may appear to regress as they become proportionally smaller (Fig. 3.22B). Fusion is a complex process; as the flanks of the two lignotubers approach one another initials of the stem cambium change the pace and type of their activity, adding to the diameter at about the same rate as the lignotuber cambium. The result of these changes is that the stem cambium produces a narrowing zone of xylem and of secondary phloem (Fig. 3.23), "filling in" between the two lignotubers.

Eventually the lignotuber cambia become linked laterally to form a complete cambial cylinder, enclosing the whole node. Either the remaining stem cambium initials disappear altogether or else they become converted into lignotuber cambial initials, producing xylem indistinguishable from that produced by the lignotuber cambium. The original stem xylem remains as a recognisable structure, enclosed within lignotuber tissue. Within a single species there is a
Figs. 3.22, A&B. Diagram showing alternative pathways for lignotuber development. (A) lignotuber dominance, (B) stem dominance; X, stem xylem; L, lignotuber xylem.

Figs. 3.23-24. *E. st.johnii*, two years old, pot-grown, TS through the cotyledonary node, to illustrate the alternative pathways of development of Fig. 3.22. S, stem xylem; L, lignotuber xylem; C, cortex. Scale, 4 mm.

Fig. 3.23. Showing lateral fusion of the lignotubers. The narrowing zone of intermediate xylem produced by the stem cambium is marked by asterisks. Arrowhead, bud trace

Fig. 3.24. Stem dominance, no lignotuber fusion.
Figs. 3.25-26. MLS to show anatomy of very young cotyledonary lignotubers.

Fig. 3.25. *E. bancroftii*. P, pith; Ca, lignotuber cambium; C', lignotuber cortex; C, stem cortex; Pr, primary xylem, part of cotyledonary strand; S, stem secondary xylem with numerous vessels; T, bud vascular strands. Other lettering as in Fig. 3.1. The lignotuber wood (Lx) is very regularly seriated but apparently devoid of vessel elements. A cork cambium (CC) has formed over the lignotuber and the primary cortex is exfoliating (arrows). Scale, 0.15 mm.

Fig. 3.26. *E. pellita*, MLS. A, accessory buds. Other lettering as in Fig. 25. The section passes through a number of bud vascular strands. Part of the cotyledonary vascular strand has been encountered (Pr, lower right). Scale, 0.2 mm.
degree of variability between seedlings in the expression of stem or lignotuber dominance, as is shown in Figs. 3.23, 3.24, in which the seedlings were of one batch grown under the same conditions. Translocation studies (in preparation) show that the phloem of the bud vascular strands (Fig. 3.32) and the internal stem phloem continues to function for over 18 months.

These different pathways of later development clearly reflect the relative dominance of activity of the stem cambium and that of the lignotuber cambium. In turn, these are governed to a great extent by conditions favouring or not favouring seedling stem growth: the former tend to prevent lateral lignotuber fusion and the assumption of a single-stemmed or tree habit; conditions unfavourable to stem growth (or repeated replacement of the seedling stem, especially from lignotuber buds) favour lignotuber fusion and the assumption of a multi-stemmed or mallee habit.

3.3.5 Anatomy of the tissues of the young lignotuber

One of the most striking features of the young lignotuber is the great regularity of its cambial activity (Fig. 3.25), to which it owes its relatively rapid initial expansion. This regularity continues except where the cambial derivatives are traversed by the vascular strands to the proliferating buds on the lignotuber surface (Figs. 3.28, 3.29). The rate of cell division in the lignotuber cambium does not appear to be the main or the only factor
governing the relatively greater rate of tissue formation, compared with that of the stem (Fig. 3.27). A greater rate of maturation of the cambial derivatives into xylem and phloem is also involved. The cambial initials are, however, more active in multiplicative (radial) division than are those of the stem cambium (also Fig. 3.28). The lignotuber xylem differs sharply from that produced from the stem cambium. Transverse sections of the lignotuberous node (Figs. 3.20, 3.21) show clearly that while the stem wood has numerous, large vessels, they are lacking or very few and small in the lignotuber wood. The fusiform initials of the lignotuber cambium produce an abundance of short tracheids with bordered pits (Fig. 3.26); the ray initials produce rays comparable with those in the stem wood (Fig. 3.28).

Where the lignotuber spreads above and below the node, it does so by conversion of the stem cambial initials into lignotuber cambial initials. Transverse sections of such regions (Fig. 3.28) reveal that the regular seriation of the xylem produced is uninterrupted (except around bud vascular strands, where there is some distortion) but that the activity of the cambium is greatly increased.

Chattaway (1958a) was of the opinion that bud strands themselves are not the centre of the "swirls" in the lignotuber xylem. While this may be true for individual "swirls" it is not true for groups of "swirls", like those shown in Fig. 3.29, in which the bud strands are sectioned transversely. Around these "swirls" the secondary
Figs. 3.27-29. *E. pellita*, sections of young lignotuber.

Fig. 3.27. LS to show the junction between stem cambium and lignotuber cambium. Compare the large number of divisions, but slow maturation, in the stem cambium (SC), with the more rapid maturation of cells derived from the lignotuber cambium (LC). Note also the cork cambium (CC) formed in the primary cortex of the lignotuber. Scale, 40 µm.

Fig. 3.28. TS above the cotyledonary gap. Stem cambium has been converted to lignotuber cambium but the regular seriation of the elements of the secondary wood continues uninterrupted, except in the vicinity of the vascular strands (arrows) of the upper accessory buds. Lettering as in Fig. 3.25. Scale, 0.4 mm.

Figs. 3.29. Tangential longitudinal section. Bud traces (T) surrounded by "swirls" of lignotuber xylem. Scale, 0.15 mm.
lignotuber tissue is quite regularly arranged (Fig. 3.28). Although the tissues of the bud strands differ little from the surrounding lignotuber tissue, so that the majority of their xylem is tracheidal, diligent search reveals protoxylem elements of the primary vascular strands in the form of a few spirally-thickened vessel elements (Figs. 3.30, 3.31). It is difficult to find phloem elements in these strands, even in very thin sections, but they can be observed by suitable techniques of fluorescence microscopy (Fig. 3.32).

The sudden expansion of the lignotuber outstrips the capacity of the cortical stem tissues to cover it. The cortex breaks up in flakes (Figs. 3.1, 3.26) and is replaced by tissues derived from the activity of a precociously developed cork cambium (Fig. 3.27). No evidence has been seen in these studies for the supposed endogenous origin of lignotuber buds. Where sections pass through a bud, but not through its vascular connection to the other buds strands (e.g. Figs. 3.19, 3.20), that connection is apparent in adjacent sections. The lignotuber buds arise, like the buds of epicormic shoots, by branching from existing buds, themselves descended from the accessory buds of the lignotuber-bearing node.

3.4 DISCUSSION

The data represent no more than those of an initial survey of the diversity of early development of the
Figs. 3.30-32. Details of the primary vascular elements of lignotuber bud strands.

Fig. 3.30. *E. pellita*. LS of a part of a cotyledonary vascular strand to show protoxylem elements (arrows). X, secondary xylem. P, pith. Inset, portion of LS lignotuber to show the location (arrow) of Fig. 3.30.

Fig. 3.31. *E. pellita*. Spirally thickened protoxylem elements (arrows) of lignotuber bud vascular strand. Scale, 40 μm.

Fig. 3.32. *E. st.johnii*. Fluorescence micrograph of LS lignotuber, stained with aqueous aniline blue, to show sieve elements (arrows) of lignotuber bud vascular strand. C, control section. Scale, 50 μm.
lignotuber and its early anatomy. Further and more extensive study is required to amplify and confirm these findings and would, no doubt, reveal other patterns of lignotuber initiation. Despite a suggestion of similarity of lignotuber initiation within some taxonomic groups, that is not true of *Corymbia*. In *E. perfoliata*, unlike the species more intensively studied here, lignotuber initiation begins as in our Group A, but its later growth is greater abaxially, while in *E. latifolia* and *E. haematoxylon*, early development appears to be like that of Group D, and in *E. jacobsiana*, from preliminary observations, development follows the pattern of *E. citriodora*. Chattaway's (1958a) hypothesis of the relationship between the presence of "an upper accessory bud" and the propensity to form a lignotuber is supported, but with considerable modification. The limitation imposed by restricting investigation to examination of radial longitudinal sections of cotyledonary nodes led her to find only one - presumably the largest, central one - of a suite of accessory buds. Such a suite may also be present abaxially to the axillary bud. The presence at a given node of but a single upper accessory bud, or a meristem capable of giving rise to such buds, is no guarantee of lignotuber formation at that node, even in lignotuberous species (Jahnke et al., 1983b). Chattaway's insistence on the determining role of the upper accessory bud led her to assume that, in all species, the lignotuber is initiated in association with it, above the axillary bud. Her diagrams
(Chattaway, 1958 a,b) show this, in particular in *E. leuкоxylon* and *E. citriodora*, in contrast to our findings. On the other hand, we do not disagree with her diagram of lignotuber initiation in *E. viminalis*, which corresponds with that in our Group A. Non-lignotuberous species which, according to Chattaway (1958a) possess an upper accessory bud at the cotyledonary node, actually have a suite of such buds (*E. camaldulensis*, a southern provenance, Jahnke *et al.*, 1983a; *E. pilularis*, D.J. Carr, unpublished observations). During a recent reconnaissance of sand-dune areas in the Myall Lakes National Park, C.J. Lacey, R.D. Johnston and D.J. Carr (unpublished) discovered clumps of *E. pilularis* stems borne peripherally on large (2 m diameter) plate-like lignotubers. Evidently the lignotuber, which is not formed in the seedling, can develop much later in the life of the plant.

Kerr (1925) and Chattaway (1958a) claim that buds are formed on the young lignotuber from its cortical or phloem tissues. This opinion is also shared by Shimoya and Zunti (1973) who found no buds on their lignotubers but refer to the possibility of their arising later (than two years) due to "external influences of genetic determinants". No evidence has been found of buds arising in the material examined other than from existing bud meristems. Lignotuber buds proliferate in the same manner as epicormic buds (Cremer, 1972). Neither the lignotuber cambium nor the lignotuber buds are replaced after excision. Amputation of a lignotuber leads only to the
formation of scar tissue (unpublished data). Eventually the wound is covered over in the usual way by tissue produced by a cambium regenerated from parenchyma contiguous with the existing stem cambium, but the regenerated cambium is not the lignotuber type.

Previous studies of the anatomy of seedling lignotubers have been published by Kerr (1925), Chattaway (1958a) and Shimoya and Zunti (1973). Macerated lignotuber tissue yielded xylem elements of the same type as those of the stem xylem, but considerably distorted, according to Chattaway (1958a). She gave no indication of the relative proportions of vessel members, fibres, tracheids or parenchyma cells. In "mature" (i.e. not seedling) lignotuber wood (E. gummifera) the proportions of all woody elements is the same as in the stem xylem, according to Bamber and Mullette (1978). However, in this study (and according to illustrations in Shimoya and Zunti (1973), in E. saligna) the wood of young lignotubers lacks vessels or they are very much smaller or less abundant than in the stem wood. Further quantitative, comparative studies of lignotuber and stem wood are needed.

Kerr (1925) mistook the bordered pits of tracheids for aleurone bodies, but showed that the starch stored in the lignotuber could be mobilised during starvation of seedlings. Chattaway (1958b) mistakenly claimed that "fibres, tracheids and parenchyma cells are all packed full of starch". This is borne out neither by the present studies nor by those of Carrodus and Blake (1970) who found
that starch is confined to the parenchyma. Carrodus and Blake (1970) de-emphasised the starch-storage role of the lignotuber, but Bamber and Mullette (1978) showed that there was a large difference between the percentage of axial parenchyma (and therefore putative starch-storing tissue) in "mature" lignotuber wood (47%) and that in stem wood (14-22%).

Shimoya and Zunti (1973) correctly identify the lignotuber cambium as developing from the large "rays" or parenchymatous gaps subtended by the cotyledons in the seedling primary vascular cylinder. On the other hand, their view of the origin of the lignotuber is unusual: this tract of tissue, they maintain, is "cleared of primary xylem by cell lysis. The cells "disappear" and the primary layers of cells remaining at the apparent limit of the parenchyma (of the pith) grow out obliquely as though forced by liquid pressure from below the affected area". This extraordinary concept is based on examination of thick (15-40 microns) transverse sections of *E. saligna* plants of two ages, six months and two years.

We have briefly outlined the process of lateral lignotuber fusion. The process is not at all well-understood and deserves special, intensive study. The width of the zone of wood contributed by the stem cambium steadily diminishes during the process of lateral fusion. This could come about either by loss of cambial initials or a progressive conversion of the flanking initials to lignotuber-type cambium, giving rise thereafter to tissue
characteristics of the lignotuber rather than of the stem. The possibility of a loss of cambial initials from the stem cambium, contributing to the narrowing of the zone between the approaching lignotubers, requires investigation. It has been shown by Bannan (e.g. 1951, 1953) and by Bannan and Bayly (1956) that during normal functioning of the stem cambium in Gymnosperms, some of its fusiform and ray initials cease to divide and either die or differentiate to xylem or phloem elements, i.e. there are continued losses of cambial initials, replaced, of course, by radial divisions of adjacent initials. Similar losses have been reported from Angiosperms (references in Philipson et al., "The Vascular Cambium" (1971)).

However, an explanation based on gradual conversion, brought about by a sort of homeogenetic induction from the flanks of the lignotuber cambium, of the stem cambium to lignotuber cambium during lateral fusion, is strongly supported by evidence (e.g. Fig. 3.28) of the downward and upward extension of the lignotuber away from the node itself.

Evidently the young lignotuber cambium and the stem cambium of the seedling are differentiated in that their products differ both quantitatively and qualitatively. Lateral fusion of the lignotubers is preceded by certain changes in the nature of the xylem produced by the diminishing stem cambium. This also may reflect the gradual conversion of stem type cambium initials to lignotuber type initials. Whether, in the case of stem
dominance, the converse can take place, *viz.*, conversion of what was lignotuber type cambium to stem type cambium, requires investigation. Most authors have taken the view that "in most species the lignotubers are overtaken by the normal growth of the stem and are no longer visible by the time the tree reaches the pole stage" (Chattaway, 1958b) but say nothing about what happens to the strips of lignotuber cambium, or whether the lignotubers present in the seedling stage continue to be present, however small. Observations of a tree, 10 m tall, of a northern provenance of *E. camaldulensis* planted in the grounds of the Australian National University, showed a sub-spherical lignotuber of 15 cm diameter, bearing a shoot and a root, and attached to the base of the tree by a slender, woody stalk. It would appear that, once lateral fusion of lignotubers has occurred, and the original stem tissue has become well enclosed in lignotuber tissue, conversion of the lignotuber cambium back to stem cambium, by homeogenetic induction from the stem cambium above (or below) the lignotuber, does not take place or is unlikely to do so. The downward growth of the lignotuber over an internode or over the hypocotyl involves conversion of stem cambium to a type of lignotuber cambium; the nature of the xylem produced in what has been called the "carrot-like" thickening of the hypocotyl (Figs. 3.14, 3.16) remains to be investigated.

The inverse relationship between lignotuber growth and the retention of the initial seedling shoot suggests an
interaction between dominance of the shoot and lignotuber expansion. It would appear that each time the previously existing shoot is replaced (either by one or by several shoots from the lignotuber itself) a stimulus is given to expansion of the lignotuber, not least by incorporation of secondary lignotubers formed at the basal nodes of the replacement shoots (Figs. 3.15, 3.16).

The consequences of complete lignotuber fusion around the base of the seedling stem are that the form of the resultant plant will be constrained to be that of a mallee or at best a small tree. On the other hand, zones of stem left between non-fused lignotubers may become more active than the lignotuber cambium, forcing the lignotubers apart; such an event is likely if a single shoot assumes dominance and a single-stemmed tree is formed. In the field, many species of bloodwoods (Corymbia) - all of which (contrary to Burbidge, 1952), as seedlings have lignotubers - adopt one or other habit, depending on the degree of adversity faced by their seedlings during the first few years of growth. Heard and Rance (1975) are worth quoting on this point. Writing of the cultivation of E. nesophila in Darwin, they say: "Provided the seedling does not go into a lignotuber stage, growth is rapid. If the plant does form a woody tuber, growth may be poor for several years, then suddenly it may shoot up to 2-3 m in a few months". Relative dominance of the stem or lignotuber may be important in other growth habits. Lacey (1974) has described the rhizomatous habit of bloodwoods and other
eucalypts in the Northern Territory, giving rise to extensive clones bearing leafy shoots < 2 m. Each such leafy shoot will develop a lignotuber complex at its base (Carr et al., 1982). Since the rhizomes themselves are derived from a lignotuber, they and their derivatives may be predisposed to lignotuber dominance. This may explain why it has proved difficult or impossible to establish trees from rooted shoots taken from rhizomatous populations (Lacey, pers. comm.).
LIGNOTUBER DEVELOPMENT AND GROWTH PARAMETERS IN
EUCALYPTUS CAMALDULENSIS (DEHNHL): EFFECTS
OF PHOSPHORUS AND NITROGEN LEVELS

4.1 INTRODUCTION

Whether or not lignotubers develop in eucalypt species is said to be dependent on nutritional factors, especially levels of phosphorus and nitrogen (Kerr, 1925; Beadle, 1968; Weir, 1969; Mullette, 1976; Mullette and Bamber, 1978).

Kerr (1925) found superphosphate to be more effective than nitrate in increasing the size of lignotubers in eucalypts which normally form them. Beadle (1954, 1966, 1968) proposed that, in some way, past soil phosphorus levels had directed evolutionary processes in certain Australian taxa and were primarily responsible for the delimitation of some plant communities in eastern Australia. He suggested that the lignotuber was an adaptation to soils of low fertility. In experiments briefly reported he claimed that "high" phosphorus and nitrogen levels restricted the development of lignotubers in E. oleosa, E. salinga and E. gummifera.

Weir (1969) tested the effect of light intensity using a factorial experiment containing "four levels of light intensity by two levels of phosphorus by three levels
of nitrogen". Light intensity was negligible in influencing lignotuber development, so long as it was adequate for overall growth. He found that in *E. radiata*, the ratio of lignotuber size to seedling size decreased with an increase in the level of phosphorus from 1 ppm to 50 ppm, unless a high level of nitrogen (100 ppm) was present. Mullette and Bamber (1978) found to the contrary that an increase in phosphorus levels stimulated lignotuber development in *E. gummifera*.

Kerr (1925) found that *E. diversicolor* failed to form lignotubers irrespective of the level of superphosphate or nitrate supplied in her treatments. The capacity to form a lignotuber is evidently inherited since some species are not known to have it. The genes for lignotuber formation appear to be dominant (Pryor, 1957; Pryor and Byrne, 1969; Barrett *et al.*, 1975; Venkatesh and Sharma, 1979). It is evident that in many species the penetrance of these genes is 100% and lignotubers are thus formed irrespective of a wide variety of conditions, climatic and edaphic. In other species the penetrance is not complete and the expression of lignotuber genes, i.e. lignotuber formation, may be considerably affected by environmental conditions. For example seedlings of some northern populations of *E. camaldulensis* always or nearly always form lignotubers, whereas those from more southerly populations rarely or never form them (Pryor and Byrne, 1969; Karschon, 1971). If nutritional levels affect the penetrance of lignotuber genes it is possible that this
might be demonstrated in *E. camaldulensis*. This was attempted in the nutrient experiment to be described. Levels of nitrogen and phosphorus were varied in view of the work reported above. The levels of N and P used are well within those cited in studies of red gum soils (e.g. Boomsma, 1950). The experiment involved supplying 1, 10 or 100 ppm of nitrogen or phosphorus to *E. camaldulensis* seedlings raised from seed for two provenances, a northern one reported as regularly forming lignotubers and a southern one reported not to form lignotubers (Pryor and Byrne, 1969; Karschon 1971). These two widely-variant provenances were chosen since Pryor and Byrne (1969) suspected the variation between groups of provenances to be influenced by different selection pressures. They proposed the absence of the lignotuber in eucalypts to be a derived character, since lignotubers were normally common throughout the genus, and that an unspecified lignotuber-suppressing mechanism operated in southern provenances. As our working hypothesis, the plane of mineral nutrition might affect the degree of suppression.

Since the experiment was terminated after five months, all the measurements and conclusions based on them are restricted to development within that period. However, the remaining plants were kept under observation for a further six months, without noticeable change to the conclusions drawn from the more rigorous data.
4.2 MATERIALS AND METHODS

The seeds (kindly supplied by the Division of Forest Research, CSIRO, Canberra) of *E. camaldulensis* were as follows:

(i) Northern provenance: seed collection number 12187, locality 8 km west of Irvinebank towards Petford, North Queensland. 145° 09', 17° 24', alt. 680 m. Aspect and slope, flat. Soil, red-brown clay-loams.

(ii) Southern provenance: seed collection number 10666, locality east side of Lake Albacutya, 5 km west of Yaapeet, Victoria. 142° 02', 35° 44', alt. 70-80 m. Aspect and slope, flat. Soil, dry lake bed, sand over grey clay, pH 6-6.5.

4.2.1 Growth Conditions

Five combinations of P and N levels were used (100P:100N; 100P:10N; 100P:1N; 10P:100N; 1P:100N, in ppm). The number of treatments was restricted by available greenhouse space. The number of seedlings in each treatment was 30. Seeds from each provenance were set to germinate in vermiculite on 10.10.1979 in a greenhouse, in which the temperature ranged from 25°C to 30°C during the day and was kept to about 20°C at night. Relative humidity varied between 40% and 60%. After 3-4 weeks when the first pair of leaves had unfolded, the seedlings were pricked out
and transplanted into plastic tubes, 10 cm (diameter), 20 cm (height) containing a light sandy loam (Bungendore, N.S.W., analysis as follows: NO$_3^-$ 13 ppm, NH$_4^+$ 26 ppm, total N 39 ppm; P extractable (Mehlich method, extraction with 0.25N H$_2$SO$_4$, 0.05N HCl) as PO$_4$ = 5 ppm. Nutrient application commenced 6.12.1979 when the seedlings had recovered from transplanting. Each tube contained approximately 1 kg of soil with a field capacity of 120 ml/kg soil. Nutrient solutions were initially supplied at the rate of 150 ml/week. On intervening days 200 ml of tap water was supplied. After two months, nutrient solutions were fed to the seedlings twice a week and, to avoid significant levels of nitrogen or phosphorus accumulating in the soil, water was applied twice on intervening days.

4.2.2 Nutrient Treatments

Complete nutrient solutions were based on Hewitt's nitrate-type nutrient solution (Hewitt and Smith, 1975, Tables 4.1, 4.2). From the stock solutions (Table 4.1) five treatment solutions were made up giving the required levels of N and P while levels of K, Ca and Mg were kept constant (Table 4.3).

4.2.3 Measurements

Nutrient application, measurements of seedling height, stem diameter and lignotuber diameter were terminated on 17.4.1980. Dry weight measurements were made on 20 seedlings from each treatment.
Height: Measurements of seedling height were taken monthly from the cotyledonary node to the apex of the plant. During growth of some of the seedlings it became difficult to distinguish the cotyledonary node. This was especially true of those which did not form lignotubers and the position of the cotyledonary node was judged subjectively in such plants. All the measurements were made to the nearest cm.

Branches: Branch number and branch length (above 35 mm) were recorded for each seedling.

Lignotubers: At the end of the experiment lignotuber occurrence and diameter (across the pair of lignotubers and the stem) were recorded. The measurements were made, to the nearest 0.1 mm, using a vernier caliper. The stem diameter above the lignotuber was subtracted from the stem-plus-lignotuber measurement.

Dry weight measurement: For each provenance, 20 seedlings from each nutrient treatment were separated into shoot and "washed" root material below the cotyledonary node or lignotuber. Lignotubers were excised, enclosed in labelled paper bags and dried in an oven at 100°C to a constant dry weight measured using an electronic balance.

Analysis of data: Statistical analyses were carried out using the GL1M3 computer package (Baker and Nelder, 1978). Lignotuber frequency, and branch number were statistically analysed using log-linear models for two-way contingency tables (Everitt, 1977). All other analyses were evaluated by analysis of variance. Dead seedlings were excluded from the analysis.
Table 4.1. Nutrient solutions based on Hewitt's nitrate-type solution

<table>
<thead>
<tr>
<th>Compound</th>
<th>Stock solutions g/l</th>
<th>(a)</th>
<th>(b)</th>
<th>(c)</th>
<th>(d)</th>
<th>(e)</th>
<th>(f)</th>
</tr>
</thead>
<tbody>
<tr>
<td>KNO₃</td>
<td>23.8</td>
<td>2.38</td>
<td>0.238</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ca(NO₃)₂.4H₂O</td>
<td>55.6</td>
<td>5.56</td>
<td>0.556</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MgSO₄.7H₂O</td>
<td></td>
<td></td>
<td></td>
<td>36.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NaH₂PO₄.2H₂O</td>
<td></td>
<td></td>
<td></td>
<td>50.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KCl</td>
<td>12.8</td>
<td>28.0</td>
<td>29.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CaCl₂</td>
<td>23.4</td>
<td>55.5</td>
<td>58.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[EDTA]₂FeNa</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>20</td>
</tr>
</tbody>
</table>

Table 4.2. Micronutrient solution

<table>
<thead>
<tr>
<th>Compound</th>
<th>Stock solution g/l (dilution supplied, 1 ml/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MnSO₄·H₂O</td>
<td>1.690</td>
</tr>
<tr>
<td>ZnSO₄·7H₂O</td>
<td>0.288</td>
</tr>
<tr>
<td>CuSO₄·5H₂O</td>
<td>0.250</td>
</tr>
<tr>
<td>H₃BO₃</td>
<td>3.092</td>
</tr>
<tr>
<td>Na₂MoO₄·2H₂O</td>
<td>0.121</td>
</tr>
<tr>
<td>NaCl</td>
<td>5.844</td>
</tr>
<tr>
<td>Co(NO₃)₂·6H₂O</td>
<td>0.058</td>
</tr>
</tbody>
</table>
Table 4.3. Final ionic concentrations used (ppm)

<table>
<thead>
<tr>
<th>Element</th>
<th>(N_{100}:P_{100})</th>
<th>(N_{10}:P_{100})</th>
<th>(N_{1}:P_{100})</th>
<th>(N_{100}:P_{10})</th>
<th>(N_{100}:P_{1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>100</td>
<td>10</td>
<td>1</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>P</td>
<td>100</td>
<td>100</td>
<td>10</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>K</td>
<td>160</td>
<td>160</td>
<td>160</td>
<td>160</td>
<td>160</td>
</tr>
<tr>
<td>C</td>
<td>160</td>
<td>160</td>
<td>160</td>
<td>160</td>
<td>160</td>
</tr>
<tr>
<td>Mg</td>
<td>36</td>
<td>36</td>
<td>36</td>
<td>36</td>
<td>36</td>
</tr>
<tr>
<td>Na</td>
<td>76</td>
<td>76</td>
<td>76</td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td>Cl</td>
<td>177</td>
<td>405</td>
<td>428</td>
<td>177</td>
<td>177</td>
</tr>
</tbody>
</table>

Table 4.4. Effects of applied N on growth parameters: data for the two provenances of *E. camaldulensis* combined

<table>
<thead>
<tr>
<th>Growth parameter</th>
<th>Level of N applied</th>
<th>Significance of differences (F&lt;sub&gt;2,7&lt;/sub&gt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(N_{1})</td>
<td>(N_{10})</td>
</tr>
<tr>
<td>Height (mm)</td>
<td>389</td>
<td>530</td>
</tr>
<tr>
<td>Branch length (mm)</td>
<td>73.4</td>
<td>85.8</td>
</tr>
<tr>
<td>Shoot dry weight (g)</td>
<td>1.48</td>
<td>3.01</td>
</tr>
<tr>
<td>Root dry weight (g)</td>
<td>1.29</td>
<td>1.69</td>
</tr>
<tr>
<td>Stem diameter (mm)</td>
<td>3.53</td>
<td>4.79</td>
</tr>
</tbody>
</table>
Table 4.5. Analysis of variance of mean height measurements: both provenances combined. P:N and residual sums of squares were pooled.

<table>
<thead>
<tr>
<th>Source of variance</th>
<th>df</th>
<th>ss</th>
<th>F(a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrogen</td>
<td>2</td>
<td>1195000</td>
<td>240***</td>
</tr>
<tr>
<td>Provenance</td>
<td>1</td>
<td>36660</td>
<td>14.7*</td>
</tr>
<tr>
<td>N x provenance</td>
<td>2</td>
<td>4677</td>
<td>0.94NS</td>
</tr>
<tr>
<td>Phosphorus:Nitrogen</td>
<td>2</td>
<td>1350</td>
<td></td>
</tr>
<tr>
<td>(a)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Residual</td>
<td>2</td>
<td>8603</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>9</td>
<td>1246000</td>
<td></td>
</tr>
</tbody>
</table>

Table 4.6. Percentage of seedlings of the northern provenance (12187) of *E. camaldulensis* with lignotubers in relation to mineral nutrition. (No seedlings from the southern provenance, 10666, formed lignotubers)

<table>
<thead>
<tr>
<th>Treatment, ppm</th>
<th>P₁₀</th>
<th>P₁₀₀</th>
</tr>
</thead>
<tbody>
<tr>
<td>N₁</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>N₁₀</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>N₁₀₀</td>
<td>66 (19/20)</td>
<td>79 (23/29)</td>
</tr>
</tbody>
</table>
4.3 RESULTS

This study emphasises the effects of P since the N supply evidently determines overall growth (Figs. 4.1, 2, 3). Tables 4.4, 5, 6, 7 summarise the measurements and Table 4.8 the results of the statistical analysis. Since the nitrogen supply strongly influences overall growth (Figs. 4.1 and 4.2), the effects of N\textsubscript{1} and N\textsubscript{10} have been examined for only the P\textsubscript{100} level of phosphorus, and P\textsubscript{1} and P\textsubscript{10} have been examined for only the N\textsubscript{100} level of nitrogen. Care has been exercised in interpreting the effects of these two variables, since there are too few data to detect any interactions between them. However, for all measurements except branch number, when an effect of nitrogen level is taken into account, there was no additional effect of phosphorus. Since the converse is not true the simplest explanation is that nitrogen does affect the measurements while phosphorus levels do not. Increased N levels were associated with increased height, branch length, stem diameter and root and shoot dry weights (Table 4.4) in both populations.

**Seedling height:** There is a significant height difference between the two populations (Table 4.5). There is no effect of P after fitting a separate mean for each nitrogen level (F\textsubscript{2, 2} = 0.16\textsuperscript{NS}) although the residual sum of the squares (ss) has few degrees of freedom. The residual ss was pooled with that for phosphorus and the effect of nitrogen was the same in both provenances (F\textsubscript{2, 4} = 0.94\textsuperscript{NS}). N\textsubscript{100} resulted in an average mean height of
Fig. 4.1 Seedlings of northern provenance (12187) five months old. Scale, 10 cm intervals. Effects of combinations of N and P levels.
Fig. 4.2. Seedlings of southern provenance (10666) five months old. Scale, 10 cm intervals. Effects of combinations of N and P levels.
1160 mm, while \( N_{10} \) and \( N_1 \) were 530 mm and 390 mm respectively. These average heights are significantly different, \( F_{2,4} = 240, p < 0.001 \), (Figs. 4.1). On average, seedlings of the southern provenance were 120 mm taller than those of the northern provenance (\( F_{1,4} = 14.7, 0.01 < p < 0.05 \)).

**Number and length of branches:** Branch number but not branch length is statistically different between the provenances. Plants of the southern provenance had the greater number of branches. For \( P_1 \), \( P_{10} \) and \( P_{100} \) at \( N_{100} \), branch number has been examined in some detail using log-linear analysis of a 2 x 3 contingency table. The branch number is different at each phosphorus level (\( \chi^2 = 21.7, p < 0.001 \)).

**Lignotuber frequency:** Lignotubers were formed only by plants of the northern provenance. The frequency of lignotubers was not constant with treatment (\( \chi^2 = 17.4, p < 0.001 \)) but ranged from 55% (16/29) for the \( N_{100}P_{100} \) treatment, to 93% (28/30) for the \( N_{10}P_{100} \) treatment (Table 4.6). At \( P_{100} \), the \( N_1 \) and \( N_{10} \) levels gave a high frequency of lignotubers, while high \( N_{100} \) levels gave only half the number (\( \chi^2 = 15.9, p < 0.001 \)). However, at \( N_{100} \) the frequency is similar for each phosphorus level (\( \chi^2 = 3.88, p > 0.10 \)). The stem plus lignotuber diameter is significantly higher in plants grown under a high nitrogen regime than in those under a low nitrogen regime (Fig. 4.3). Although the paucity of data and relatively small size differences preclude the attainment of statistical
Table 4.7. Lignotuber measurements in relation to level of nitrogen supplied at \( P_{100} \)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Level of nitrogen supplied</th>
<th>Significance of differences ((F_{2,2}))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( N_1 )</td>
<td>( N_{10} )</td>
</tr>
<tr>
<td>Stem + Lignotuber diameter (mm)</td>
<td>6.54</td>
<td>8.81</td>
</tr>
<tr>
<td>Lignotuber diameter (mm)</td>
<td>2.77</td>
<td>3.82</td>
</tr>
<tr>
<td>Dry weight of lignotuber (g)(^{(a)})</td>
<td>0.006</td>
<td>0.014</td>
</tr>
</tbody>
</table>

\(^{(a)}\) Correlation between lignotuber volume (cube of diameter/2) and weight was significant \((\text{diam}^3/\text{wt}, r = 0.7373, \text{wt/diam}^3, r = 0.98)\).
Table 4.8. Summary of analysis of variance of combined measurements of seedlings of both provenances of *E. camaldulensis* in relation to mineral nutrition

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Source of variance</th>
<th>N</th>
<th>P</th>
<th>Provenance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height</td>
<td>***</td>
<td>NS</td>
<td>NS</td>
<td>**</td>
</tr>
<tr>
<td>Shoot dry wt</td>
<td>***</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Root dry wt</td>
<td>***</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Stem diameter</td>
<td>***</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Stem + lignotuber diameter</td>
<td>**</td>
<td>NS</td>
<td>NS</td>
<td>- (a)</td>
</tr>
<tr>
<td>Lignotuber diameter</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>-</td>
</tr>
<tr>
<td>Lignotuber dry wt</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>-</td>
</tr>
<tr>
<td>Branch length</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Branch number</td>
<td>**</td>
<td>**</td>
<td>***</td>
<td></td>
</tr>
</tbody>
</table>

NS, not significant; * p < 0.05; ** p < 0.01; *** p < 0.001.

(a) No lignotubers formed in southern provenance.
Fig. 4.3. Portions of seedlings of northern provenance (12187) to show effects of levels of N and P on stem diameter and lignotuber growth.
significance of differences in lignotuber diameter or dry weight for plants grown under different nutrient regimes, there appears to be a trend towards an increase in dimensions in higher N levels with little effect of P levels (Table 4.7).

Lignotuber diameter and dry weight increased (but not significantly) with increasing levels of N, the increase in stem diameter was significant ($F_{2,2} = 143.1^{***}$, $p < 0.001$, Fig. 4.3).

4.4 DISCUSSION

4.4.1 Seedling Height

Levels of nitrogen and phosphorus, especially high N levels, play a significant role in determining seedling biomass. Seedling height is correlated with increases in shoot and root dry weight, branch length and stem diameter. These observations support previous conclusions on the effect of P and N levels on seedling growth reached by Weir (1969), Blake (1972), Mullette (1976) and Mullette and Bamber (1978). Seedlings of the southern provenance (10666) grew on average 120 mm taller than those of the northern provenance (12187), contrary to the prediction of Pryor and Byrne (1969) that the northern provenances would grow faster than the southern.
4.4.2 Number and Length of Branches

Increased nitrogen supply increases total branch length irrespective of provenance. At high levels of N, there was a significant increase in the number of branches with increased P, within and between the provenances. Branch number was higher in the southern population. This may reflect a difference in degree of apical dominance, and the related control of branching between these populations. The results support Blake's (1972) observations of a requirement for increased levels of P for maximum branching in E. obliqua seedlings.

4.4.3 Lignotuber Frequency

In the experiment, lignotubers were not formed in seedlings from the Lake Albacutya (southern) provenance, irrespective of the levels of phosphorus and nitrogen. This suggests three hypotheses:

(i) that the potential for lignotuber formation in this provenance is not directly influenced by P and N;
(ii) in this provenance recessive alleles of the genes for lignotuber development are present;
(iii) that dominant alleles are present but their expression is suppressed in the presence of other modifying genes, i.e. the degree of penetrance is low.

Pryor and Byrne (1969) for E. camaldulensis, Ladiges (1974) for E. viminalis and Kirkpatrick (1975) for
E. globulus have found considerable variation in seedling lignotuber development between different populations of these species.

Although P and N levels did not induce lignotuber development in the southern provenance, it does not preclude the possibility of other environmental factors affecting lignotuber formation. For example Kerr (1925) reported that shading to reduce sunlight levels retarded lignotuber growth in some eucalypt species. However, Weir (1969) found a negligible specific response of lignotuber development to various levels of light intensity. Daylength or day-night temperature might regulate lignotuber growth but light intensity is not likely to be an environmental limitation.

At high levels of P associated with low levels of N, the northern provenance of E. camaldulensis produces a high percentage of lignotuberous seedlings. At the highest level, $P_{100} N_{100}$ pm, there is a significant reduction in lignotuber frequency. This suggests that high P levels are directly involved in increasing lignotuber frequency only if the associated N levels are low. It may be that high N levels increase the rate of radial or secondary growth of the stem to such an extent that the lignotuber development is initiated, but the lignotuber remains morphologically inapparent. Such indistinct cases would lower the apparent lignotuber frequency. This may be one reason for the reported variability of lignotuber frequency between E. camaldulensis populations (Pryor and Byrne, 1969; Karschon, 1971).
4.4.4 Lignotuber Size

As expected there is a significant correlation between lignotuber volume and dry weight (Table 4.7). Lignotuber dry weight and diameter increase with nitrogen supply at constant high P level (Table 4.7), although, the increase just failed to reach statistical significance. In contrast, Weir (1969) found with *E. radiata* that there was, at a low level of P, a significant (p < 0.01) increase in lignotuber dry weight with increasing N levels. Weir found it "impractical" to excise the lignotubers from the stem, therefore his measurements on lignotuber dry weights were influenced by the amount of stem tissue between pairs of lignotubers. Because N had a significant effect on stem diameter in his experiments the data could be misleading. The same effect of increased N level on stem diameter was shown in the present study (Table 4.7, Fig. 4.3).

Previous authors (Kerr, 1925; Beadle, 1968; Weir, 1969; Mullette, 1976; Mullette and Bamber, 1978) found that high P levels affected lignotuber size. Mullette (1976) and Mullette and Bamber (1978) held that the decrease in lignotuber size with increasing P levels, claimed by Beadle (1968), was contrary to their own results. But Beadle (1968) used combinations of P with a constant high (180 ppm) N Level. Mullette and Bamber (1978) also applied a single level of nitrogen, as equal amounts of NH$_4^+$ and NO$_3^-$; even if N were supplied as ammonium nitrate, the nitrogen supply would not have been optimal for the growth of *E. gummifera*, according to the data of Moore and
Keraitis (1971). Mullette and Bamber (1978) concluded that high P levels did not cause a decrease in lignotuber size until the levels became toxic. While the data in Mullette and Bamber (1978) show a decrease in lignotuber frequency at $P_{100}$, no explanation is given for the apparent increase in lignotuber frequency from 100 ppm to 1000 ppm P. Evidently the number of "surviving plants" was so few as to result in an apparently greater percentage of lignotuberous seedlings than would have been observed had numbers of seedlings not died. Perhaps survival of seedlings was dependent on their having already formed a lignotuber. The data of Mullette and Bamber may be thus re-interpreted as showing a suppressing effect of high levels of P at less than toxic levels. Thus the criticism by Mullette and Bamber (1978) of Beadle's results is invalid. In fact Beadle's general conclusions (based on experiments which were never published in detail) are supported by the results of the present study.
CHAPTER 5

EXPERIMENTAL OBSERVATIONS

A study of the effects of nitrogen and phosphorus levels on the development of lignotubers in *E. camaldulensis* is reported in Chapter 4. Previous work on the effects of mineral nutrients on lignotuber development are also reviewed in that Chapter.

The experiments to be described in this Chapter must be regarded as merely preliminary explorations of the physiology of lignotubers. Apart from the hormonal studies of Blake and Carrodus (1970), few experimental observations have been made on lignotubers. Studies are reported below on translocation and storage in the seedling lignotuber and the movement of water through it; the influence of the shoot on the development of the lignotuber; and the effects of various surgical treatments on seedling lignotubers.

(A) PHOTOSYNTHATE TRANSLOCATION AND ACCUMULATION WITHIN THE LIGNOTUBER

5.1. INTRODUCTION

Studies of the movement of $^{14}$CO$_2$ assimilated in photosynthesis have shown *inter alia* that in seedlings of
Eucalyptus spp. both internal phloem and external phloem are utilised in downward translocation (Wilson and Bachelard, 1975; Zamski and Tsivion, 1977). At present the pathway of translocation of assimilates in the Eucalyptus lignotuber has not been investigated. This has been examined in the present study using autoradiographic techniques.

5.2 MATERIALS AND METHODS

A small phial containing a weighed amount of a mixture of Ba\(^{12}\)CO\(_3\) and Ba\(^{14}\)CO\(_3\) (solid), approximately 3 mg (37 Ci/mg), was tied to a leaf petiole of seedling plants of E. stjohnii (18 months old) or E. citriodora (30 months old). A plastic bag was placed over the shoot and securely tied around the stem, preventing the escape of labelled \(^{14}\)CO\(_2\). Each seedling was placed in full sunlight for 2 h before 1 ml of 10% lactic acid was injected into the barium carbonate through a rubber patch sealed to the plastic bag. The puncture was sealed with rubber solution. The plants were left in sunlight for at least 8 h. Residual \(^{14}\)CO\(_2\) was then absorbed by injecting 1 ml of alkali solution into the phial, terminating treatment. The lignotuber with stem portion was cut off and placed in a solution of dry ice in acetone (-70°C) for 15 min. The lignotuber was cut longitudinally or transversely into 5 mm pieces using a jeweller's saw. Rough surfaces were smoothed with a razor blade. The material was placed
smooth cut surface downwards on Ilford X-ray film and held in a special light-tight holder. Exposure took place at -10°C for 2 to 4 weeks. The X-ray film was then developed in a solution of Phenisol X-ray developer at 20°C for 5-8 min, fixed for 3 min and washed for 20 min. The autoradiographed cut surfaces of the pieces of lignotuber or stem were photographed using a Wild photomacroscope.

5.3 RESULTS

Conduction of assimilates in the lignotuber is shown to occur mainly in the secondary (external) phloem. Translocation continues to take place in the internal phloem for up to at least 18 months in *E. st.johnii* (Figs. 5.1a, 3a) and 30 months in *E. citriodora* (Fig. 5.2a). The bud traces originating from the primary xylem and phloem in the lignotuber of *E. st.johnii* remains visible for up to 18 months (Fig. 5.3a). These traces become labelled, indicating that they also remain functional for at least that time. No evidence was found of labelling of the older parts of the bud traces of lignotubers of 30 months old *E. citriodora*. The older, inner woody regions of the lignotuber were not labelled. However, there is evidence of storage of current assimilates in the woody tissue recently formed by the lignotuber cambium.
LEGENDS TO FIGURES (SECTON A)

Fig. 5.1 (a,b). *E. st.johnii*. Transverse section of 18 months old lignotuber (L) with $^{14}$C-labelled translocate in cortex (C') and pith (asterisk). Scale, 2 mm.

Fig. 5.2 (a,b). *E. citriodora*. Transverse section of 30-month old lignotuber with $^{14}$C in cortex and pith (arrow) of enclosed stem(s) which is also labelled (arrow). Recently formed tracts of bud traces (arrows) show no presence of $^{14}$C. Scale, 2 mm.

Fig. 5.3 (a,b). *E. st.johnii*. Longitudinal section through 18 months old lignotuber with $^{14}$C in cortex, pith (P) and bud traces (T). Scale, 2 mm.
5.4 DISCUSSION

5.4.1 Translocation in the internal phloem: Fukuda (1967) has reviewed the literature on translocation in relation to the internal phloem. Following the documentation of the internal phloem in *Cucurbita pepo* by Hartig (1854), Hanstein (1864) proposed that the phloem translocates nutrients both downwards and upwards. This hypothesis was much later confirmed by Biddulph (1956), Biddulph and Cory (1960, 1965) and Bonnemain (1965, 1980) using autoradiographic techniques. Bonnemain (1965) reported that in the *Solanaceae*, the internal phloem translocates upwards and the external phloem downwards. In other cases, the reverse may occur or the internal phloem may be involved in upward translocation and the external phloem in an upward and downward flow (for a general review see Fukuda 1967).

Results from this study show that the internal phloem continues to be functional, in both stem and lignotuber, for over 18 months in *E. st.johnii* and *E. citriodora*. In the younger lignotubers, bud traces are labelled all along their paths from the pith of the subtending stem; in the older lignotuber the internal phloem of the stem appears to continue functioning for up to 30 months after seed germination.

Although a few protophloem elements may be added initially to ones already present in the internal phloem of the primary stem (Lignier, 1886) there is no further production during secondary growth. This implies that the
protophloem sieve tubes of the internal phloem may remain functional for approximately two years.

5.4.2 Storage of current assimilates: Kerr (1925), Carter (1929), Jacobs (1955), Chattaway (1958a), Carrodus and Blake (1970) and Mullette and Bamber (1978) have considered the primary functions of the lignotuber to be storage and the provision of a continual supply of buds. However, Carrodus and Blake (1970) presented quantitative data on the amount of carbohydrates in lignotuber tissues. They concluded that there was no significant difference in starch content (percentage dry weight) between lignotuber tissue and stem tissue, storage in both cases being in the ray parenchyma. Bamber and Mullette (1978) disagreed, arguing that the lignotuber wood in *E. gummifera* has twice the amount of storage tissue (i.e. rays and vertical parenchyma) as the stem wood. They thus concluded that the lignotuber tissue has a larger potential for starch storage than the stem wood.

Although autoradiography shows the translocation pathway of photosynthates, there still remains a degree of uncertainty as to whether the stored $^{14}$C-labelled assimilate is indeed starch. *Iodine – KI* staining for starch indicates its presence in the lignotuber xylem and cortex (Fig. 5.3c), but not in the vascular bud traces originating from the pith (Fig. 5.3d). Tests of bud traces from several other species show that starch is absent from them. The translocated $^{14}$C assimilate, possibly sucrose, is eventually stored in the ray and vertical parenchyma as starch.
Fig. 5.3c. *E. st.johnii* lignotuber (3 a,b). Longitudinal median section stained with iodine in potassium iodide to show the distribution of starch (3). Bud trace (T), pith (P), C. x 6.

Fig. 5.3d. Portion of 5.3c, at a greater magnification, to show the localisation of starch in the xylem and its absence from the pith and bud vascular strands. C. x 14.
Current assimilates do not appear in older tissues of the lignotuber but are evidently stored in recently-formed cells produced by the lignotuber cambium. During periods of rapid lignotuber bud growth, storage material is probably mobilised from cells of the secondary cortex and xylem. Although in the lignotuber current photosynthate is initially stored in newly-formed tissues (cortex and xylem), this does not preclude the possibility of a slow exchange of current assimilates with older stored materials in the ray parenchyma.

Further work on older lignotubers is required to establish in them the dynamics of long-term starch storage, its mobilisation and the possibility of its depletion during lignotuber bud growth.

In this study, the secondary phloem of the older lignotuber is shown to be involved in translocation between the shoot and the root. As a corollary, the ringbarking of its lignotuber would effectively kill a mallee.

5.4.3 The lignotuber as a storage organ

Kerr (1925) found that when seedlings were subjected to adverse conditions the starch in the lignotuber diminished. The starch was said to be quickly replaced when seedlings resumed active growth. To test Kerr's observations, healthy 2-year old lignotuberous seedlings of *E. st.johnii* were placed in the dark. Control lignotubers were found to contain abundant starch at this stage. The experimental plants were supplied with water only, without nutrients. After one month, the main shoot
had died and numerous etiolated shoots had developed from each lignotuber. Sampled lignotubers were found to be devoid of starch (potassium-iodide test). When the seedlings were returned to sunlight, the shoots from the lignotubers developed green leaves and after one month starch was again evident in the lignotuber.

(B) SHOOT/ROOT INFLUENCE ON LIGNOTUBER DEVELOPMENT

5.1 INTRODUCTION

So far, investigations of factors influencing lignotuber development have been restricted to environmental ones such as mineral nutrition, light intensity and fire. To date there have been no investigations into the influence of the shoot or root on the capacity of a stem node to develop a lignotuber.

This can be tested by grafting a shoot (scion) of a normally non-lignotuberous seedling to the base (stock) of a compatible, potentially lignotuberous seedling and vice-versa, i.e. by reciprocal grafting.

Lignotuber development is usually initiated after the formation of accessory buds. Under normal environmental conditions, outgrowth of accessory buds is determined by shoot apical dominance. Blake and Carrodus (1970) found that lignotuber buds in *E. obliqua* appeared to be "prevented from developing by the presence of growth substances produced by the crown". This suggests that
accessory bud development might also be controlled to such an extent (complete dominance) that lignotuber initiation would be delayed or impossible. Anatomical investigations of the cotyledonary node of *E. camaldulensis* populations (a northern one forming lignotubers, and a southern one without lignotubers) showed that both upper and lower accessory buds developed in both. However, the lignotuberous species had the greater number. Since the inheritance of the lignotuber is genetically controlled, then perhaps apical dominance, which is generally held to be related to the production of hormones in the seedling shoot, is in part responsible for controlling lignotuber development.

This investigation involved seedlings of *E. camaldulensis* of two provenances and the closely related species *E. pellita* and *E. grandis* (Fig. 5.1). Anatomically, the structure of the cotyledonary node is very similar in both species of *E. camaldulensis* (Fig. 5.2a,b) and presumably any stock or scion influence on the expression of lignotuber genes should be observed in reciprocal grafts. *E. pellita* (lignotuber-bearing) and *E. grandis* (non-lignotuberous) are believed to be sufficiently closely related for stock or scion influences to be observed in reciprocal grafts.
5.2 MATERIALS AND METHODS

The graft partners were:

(i) *E. camaldulensis* 12187: lignotuberous (northern population); and *E. camaldulensis* 10666: non-lignotuberous (southern population);

and (ii) *E. pellita*: lignotuberous; and *E. grandis*: non-lignotuberous.

Seeds of each species were germinated in vermiculite, and after three weeks of growth were pricked out in pairs for reciprocal approach-graft treatment. Approach grafts were made when the seedlings were two months old and averaged 6 cm in height. A thin sliver of stem tissue was excised from adjacent stems, previously bound above and below the desired grafting regions. Plastic grafting tape was used to securely fix the graft faces of stem tissue.

*E. pellita* may form lignotubers up to the fourth or fifth leaf node, while *E. camaldulensis* 12187 develops lignotubers at the cotyledonary node and sometimes the first leaf node. Reciprocal approach-grafts were therefore made above the fourth leaf node in each species pair.

Grafted plants were placed under a mist spray on a greenhouse bench. At this stage, seedlings of *E. pellita* and *E. camaldulensis* 12187 showed no overt signs of lignotuber development. After the graft had taken, usually six weeks or more, one shoot and one root were decapitated from each pair so that the remaining individual possessed
its original basal shoot and root and a new shoot (scion), or vice-versa. The individuals were of two kinds: *E. pellita* on *E. grandis* and the reciprocal *E. grandis* on *E. pellita*. In addition, homografts (e.g. *E. pellita* on *E. pellita*) were made. Thus, the total number of treatment for each pair of species was four. The survival rate of the grafts was approximately 50%, reflecting the difficulty in approach-grafting very young seedlings, and the stresses imposed upon them.

Approach-grafts were made at intervals during the year until a final number of 30 replicate-grafted seedlings for each of eight treatments was available. The experiments commenced on 7.2.80 and observations were made over a two year period, long enough to ensure sufficient time for lignotuber development to be expressed, especially in normally non-lignotuberous species. Grafts were examined at intervals for proliferation of roots from basal stem callus. These and aerial roots from the non-grafted extension of the scion developed readily under moist conditions provided by the mist spray. They were removed as they formed.

5.3 RESULTS

5.3.1 *E. camaldulensis* reciprocal approach-grafts

(a) Scion 10666/Stock 12187:

Irrespective of the provenance of the shoot or root, lignotubers developed within four months at the
cotyledonary node and first leaf node of *E. camaldulensis* 12187. The time of first appearance and rate of lignotuber growth was comparable to that in the homografts of the same lignotuberous provenance. In some cases shoots developed from buds on the newly formed lignotuber. These were removed as they appeared, to prevent their interaction in lignotuber growth with that of the scion. Lignotubers were well developed after 16 months (Fig. 5.3).

(b) Scion 12187/Stock 10666:
Lignotubers did not develop in provenance 10666. However, it was noted that in several cases the scion, which still possessed the cotyledonary and successive leaf nodes, produced lignotubers, usually in about six months, on these lower nodes.

(c) Homografts:
Lignotubers developed in control approach-grafts of 12187. Homografts of 10666 produced no lignotubers.

5.3.2. *E. grandis* and *E. pellita* reciprocal approach-grafts

(a) *E. grandis* Scion/*E. pellita* Stock:
Lignotubers characteristically developed at lower nodes on the *E. pellita* stem. After 16 months, only one or two nodes bearing lignotubers could be observed (Fig. 5.4). No lignotubers developed on the stem of the *E. grandis* stock. Lignotuber development was similar to that in the homografts of *E. pellita* (Fig. 5.5).

(b) *E. pellita* Shoot (scion)/*E. grandis* Stock:
Lignotubers did not develop at the basal stem nodes
Fig. 5.1. Bases of seedlings (18 months) of non-lignotuberous *E. grandis* (G) and lignotuberous *E. pellita* (P). Scale, 14 mm.

Fig. 5.2 (a,b). Median longitudinal sections of cotyledonary nodes. Fig. 2a. Lignotuberous *E. camaldulensis* 1287 (4 months). Upper accessory buds (A), lower accessory bud (A'), axillary bud (Ax), cotyledon scar (cot). Scale, 0.5 mm.

Fig. 5.2b. Non-lignotuberous. *E. camaldulensis* 10666.

Fig. 5.3. 18 months old graft (asterisk) of *E. camaldulensis*, non-lignotuberous southern species (scion) (Ss) and lignotuberous northern species (stock) (Ns). Scale, 15 mm.

Fig. 5.4. 18 months old graft (asterisk) of non-lignotuberous *E. grandis* scion (Gs) onto a lignotuberous *E. pellita* stock (Ps). Scale, 5 mm.

Fig. 5.5. 18 months old homograft (asterisk) of *E. pellita*. Lignotuber (arrow). Scale, 5 mm.

Fig. 5.6. Two years old graft of *E. pellita* scion (Ps) on to *E. grandis* (Gs) stock. Lignotubers (arrows) still develop at *E. pellita* basal nodes. Scale, 10 mm.
of *E. grandis* stocks. However, if the *E. pellita* scion still retained a node which would normally produce a lignotuber in an untreated seedling, then a lignotuber developed there, despite the removal of the *E. pellita* root-system (Fig. 5.6).

(c) Homografts:

In the *E. pellita* control approach-grafts, lignotubers developed within four months (Fig. 5.5). They occurred not only on the stock, as in the stem of ungrafted seedlings, but also on the lower nodes of the scion, i.e. at those nodes belonging to either the cotyledonary or lower leaf nodes. No lignotubers developed on *E. grandis* homografts.

5.4 DISCUSSION

The results show that the capacity of a node to produce a lignotuber is neither induced nor suppressed by the shoot or root. Evidently the mechanism(s) which operates in determining lignotuber development is inherent in the lignotuberous nodes. Carr *et al.* (1982) suggested that the capacity to form lignotubers is related to "positional information" within the developing seedling. If this is the case, then one would expect the shoot or root to have little or no effect on the potential of a node to develop lignotubers. This concept is supported by the results of the experiment. However, this does not negate the possibility that shoot or root may influence the extent
or rate of lignotuber growth, as Kerr (1925) found that lignotuber development was dependent on the vigour of shoot growth.

(C) SURGICAL EXPERIMENTS ON SEEDLING LIGNOTUBERS

5.1 INTRODUCTION

According to Kerr (1925), "checking the growth by removal of the growing point (of the shoot) causes a considerable increase in size of the lignotubers, provided that there is a fair proportion of leaf area". This suggests a role for shoot apical dominance controlling the growth of the lignotuber. She also wrote: "if the lignotubers are excised they are rapidly formed again", but gave no details of any experiments performed to show this.

To test this, lignotubers at the cotyledonary or first node of *E. st.johnii* seedlings were surgically treated in various ways and their responses examined.

5.2 MATERIALS AND METHODS

The experiments were carried out in a greenhouse, using 18-month old seedlings of *E. st.johnii*. Their lignotubers, at the cotyledonary node and first leaf node were well developed although not yet showing signs of lateral fusion. Paired adjacent lignotubers were arbitrarily designated as "left" or "right" in relation to
the seedling stem. Experiments were commenced on 31.3.80 and measurements made on 12.4.81. However, observations were continued up to the end of 1982. Ten seedlings were used in each of the following treatments. Seedlings with lignotubers of average length throughout all treatments were selected to remain intact as controls. Basically three kinds of treatment were carried out:

(i) 4 mm wide ringbarking of one of a pair of lignotubers;
(ii) total removal of one or both paired lignotubers;
and (iii) excision of the stem cambium and outer bark layers between a pair of lignotubers.

Morphological observations were made, first weekly, then monthly as the change in lignotuber response to treatment became less apparent. Vernier calipers were used to measure the downward growth of the lignotuber (to the nearest 0.1 mm), parallel to the stem. Differences in initial and final lignotuber length measurements were measured and the significance of the differences from the controls assessed by t-test.

5.3 RESULTS

Measurements of lignotuber growth for each treatment and computed "t-values" are summarised in Table 5.1. At the end of the experiment, lignotubers from the control seedlings of *E. st.johnii* had grown 12 mm on average (Fig.
Table 5.1: Summary of lignotuber measurement for each treatment and computed t-test values (after one year)

<table>
<thead>
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<th>TREATMENT</th>
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</tr>
<tr>
<td>Ringbark</td>
<td></td>
</tr>
<tr>
<td>L=10</td>
<td>28</td>
</tr>
<tr>
<td>R=10</td>
<td>28</td>
</tr>
<tr>
<td>Stem bark excision</td>
<td>38</td>
</tr>
<tr>
<td>Lignotuber excision</td>
<td>28</td>
</tr>
</tbody>
</table>

n = number of lignotubers  
\( \bar{x} \) = mean length of lignotuber growth during experiment 
SD = standard deviation 
DF = degrees of freedom
5.1). Each lignotuber developed numerous bud clusters which gave rise to a lobulated appearance. All seedlings survived all treatments.

If the stem tissue, including the cambium, is excised from between a pair of lignotubers, the lignotubers significantly increase their rate of downward growth (Fig. 5.2). Following lignotuber-ringbarking, lignotuberous buds sprouted (within two weeks) adjacent to the lower side of the ringbark edge in the mid-region of the lignotuber (Fig. 5.3). Only in this region did lignotubers sprout. Within four weeks the buds withered and abscised as wound healing occurred. The upper portion of the ringbarked lignotuber appeared to develop very slowly. In several cases the lower portion of the ringbarked lignotuber failed to re-establish downward growth (Fig. 5.4). However, in most cases the lower portion of the ringbarked lignotuber maintained a growth rate comparable with intact control lignotubers, $t = 0.17, p > 0.05$. The opposite lignotuber, which had not been ringbarked, grew to an average size of 15 mm in approximately 12 months, which is significantly more than the controls (Fig. 5.5).

When only one lignotuber of a pair at the cotyledonary node is removed, there is an almost 2-fold increase in the downward growth of the remaining lignotuber compared to controls. There was xylem proliferation from the stem region pertaining to the excised lignotuber. Seedlings continue to survive if both lignotubers are removed. The wounds are healed over by tissue produced
Fig. 5.1. *E. st.johnii* (30 months). Control seedling bearing paired lignotubers at cotyledonary node. Scale, 6 mm.

Fig. 5.2. *E. st.johnii* (30 months). Seedling in which the tissues of the stem down to the cambium and bark wood have been removed from between paired lignotubers (arrows). Lignotubers bear many lobules (asterisks). Scale, 6 mm.

Fig. 5.3. *E. st.johnii* (19 months). Two weeks after ringbarking (arrow) bud sprouts on lower part of lignotuber (asterisk). Scale, 3 mm.

Fig. 5.4. *E. st.johnii* (30 months). Ringbarked lignotuber (arrow) has healed, and the lignotuber has expanded laterally but the lower portion has not continued to grow. The intact opposite lignotuber (asterisk) shows compensatory growth. Scale, 8 mm.

Fig. 5.5. *E. st.johnii* (30 months). Ringbarked lignotuber wound (arrow) of which the lower portion has continued to grow at a rate comparable to the opposite intact lignotuber. Scale, 6 mm.

Fig. 5.6. *E. st.johnii* (30 months). Wound healing response (asterisk). Tissue generated from the intact stem cambium has grown over the wounds left after both lignotubers were excised. Scale, 10 mm.
cambium has grown over the wounds left after both lignotubers were excised. Scale, 10 mm.
from the cambium (Fig. 5.6). When this experiment was
terminated, seedlings from this treatment were decapitated
above the remaining lignotuber to see if any lignotuber
buds were present. After four weeks, lignotuber bud
sprouting was still not evident. Microscopical examination
of the regenerated lignotuber tissue showed that there were
no buds present in the tissue over the wounds. This tissue
closely resembles lignotuber tissue, although derived from
overgrowth of the stem cambium.

5.4 DISCUSSION

If one pair of lignotubers is removed or ringbarked,
the downward growth of the remaining lignotuber
increases. This compensatory growth is most likely due to
the re-direction of nutrients previously shared by both
lignotubers to the remaining intact lignotuber. Excision
of the stem cambium between a pair of lignotubers delays
lateral fusion. However, this does not interfere with
translocation around the lignotuber nor with seedling
survival under these conditions. Following the ringbarking
of a lignotuber, several lignotuberous buds sprout adjacent
and centrally to the lower edge of the ringbark. One
explanation may be that shoot apical dominance over these
lignotubers buds is temporarily removed. However, pathways
for the expression of that dominance still exist or are
rapidly restored and further growth of the buds is
suppressed. One such pathway could be via the internal
LEGEND TO FIGURE (SECTION C)

Fig. 5.7. *E. st.johnii* (two years). Cambium and bark layers removed to show buds at centre of xylem proliferation (lobules). Specimen stained with an aqueous solution of Safranin O. C. x 5.
phloem, which is shown above to be still functional in 18-month old seedlings.

Lobulation of the lignotuber is presaged by the development of bud clusters. The relationship between the increased activity of the cambium and bud growth was first described by Priestley (1930). The characteristic lobulations of the *E. st.johnii* lignotuber (Fig. 5.7) owe their origin to locally increased cambial activity. The continued proliferation of buds at the lignotuber surface may provide an hormonal stimulus to cambial activity. Auxin, which is known to have a direct effect on the cambium, could be the hormone involved. This may also explain why the upper portion of a ringbarked lignotuber ceases to develop, due to its lack of proliferating buds.

Blake and Carrodus (1970) attempted to isolate a growth-inhibiting compound which reduced the sprouting of lignotuberous buds in decapitated seedlings. They extracted an inhibitor, which was only partially characterised, from mature leaves and bark of *E. obliqua* which was found to be absent in juvenile tissues of very young seedlings. So far, this work has not been repeated.

Kerr (1925) found that lignotubers regenerated after being excised. In similar studies on *E. st.johnii*, the regeneration of tissue appears to be a response to wounding. The tissue mainly constitutes xylem, which is differentiated from the remaining stem xylem; the swellings bear no lignotuber buds and their xylem is not traversed by vascular traces. The process whereby
regeneration once again re-establishes a continuous secondary vascular system is still to be anatomically investigated.

(D) WATER FLOW THROUGH THE SEEDLING LIGNOTUBER

According to Fletcher and Musson (1918), "in the Mallees, so much water is intercepted by the tumours (lignotubers), that the seedling stem is dwarfed; and, by the persistence of shoots, the growth habit is permanently distorted, so that the plants are prevented from realising their potentialities as trees". This is the first suggestion that the lignotuber has a role in the water relations of the shoot. To test this, 2-year old seedlings of *E. st.johnii*, bearing laterally fused lignotubers, were decapitated and the rate of water conductance through the lignotuber and stem was measured; a piece of the plant, consisting of a portion of stem together with the lignotuberous node and the hypocotyl, was inverted in a Scholander pressure bomb containing water dyed with Safranin O. A pressure of 1 atmosphere (approximately 100 K Pascals) was applied and the dyed water forced through the stem and lignotuber during a period of 5 min was collected in a vial and weighed. The process was repeated three times. This procedure was repeated at several pressures. The portion of stem was then cut off and used for water conductance measurements made at the same pressures as
before. The lignotuber was then sectioned transversely to reveal the extent of the cross-sectioned area of stained xylem, i.e. the pathway for the transport of water (Fig. 5.1). The cross-sectional area was related to the volume of stain collected in 5 min under a specific applied pressure and compared to similar measurements made for the stem xylem, for an equivalent cross-sectional area. Preliminary results suggest the flow of water through the lignotuber takes place almost entirely in the xylem of the stem, which became included within the fused lignotuber. If the hypocotyl of a seedling is cut and placed immediately in a solution of Safranin O and the shoot allowed to transpire for 4 h in daylight, stain appears not only in the stem xylem enclosed by the lignotuber but also in the lignotuber xylem itself (Fig. 5.2). This suggests that the lignotuber would, under normal conditions, offer a pathway of water flow but possibly a less effective one than the stem xylem.
LEGENDS TO FIGURES (SECTION D)

Fig. 5.1. *E. st.johnii* (18 months). Transverse section showing stem xylem stained with aqueous Safranin O under applied pressure in a Scholander bomb. Lignotuberous tissue did not transmit the stain. C. x 5.

Fig. 5.2. *E. st.johnii* (18 months). Transverse section showing uptake of Safranin O stain during transpiration of a seedling, cut below the lignotuber. C. x 5.
6.1 NATURE AND DEFINITION OF THE LIGNOTUBER

By definition a tuber is a storage organ derived from a stem or root, and is usually underground. If derived from a stem it will be equipped with a bud and will serve to vegetatively propagate the plant. Similarly, a lignotuber is by definition a woody tuber. It may function as a storage organ and enable the plant to regenerate after fire or a similar catastrophe. It may also have other functions which have not yet been described. For instance, in jarrah (*E. marginata*), it is evidently a temporary structure which serves in seedling establishment, perhaps up to the first 10 years of growth. Functioning primarily as a storage organ and a reserve of buds, it enables the root to travel down to permanent water. Following that event, one of the lignotuber shoots dominates and the plant develops into a tree. Thus, the lignotuber may only function in the seedling stage in certain tree species temporarily as an organ of establishment. The lignotuber is often incorrectly thought of as a subterranean structure. In fact, it is usually only partially underground, if at all. The lignotuber expands in all directions during early development and may
incorporate the hypocotyl, by the conversion of the hypocotyl cambium into lignotuber cambium. Its lateral expansion may give rise to the familiar bulbous base of a multi-stemmed mallee or a horizontal plate-like structure as described by Lacey (1983) for *E. botryoides* and by Mullette and Bamber (1978) for *E. gummifera*. On the other hand, in a number of species it is a transient structure which more or less disappears during or after seedling growth. Morphologically it is difficult to define since it is somewhat polymorphic.

Tubers or portions of tubers bearing a bud or buds can be used to propagate plants when excised and planted separately, but so far it has not proved possible to take a piece of a eucalypt lignotuber and from it propagate a new plant. One reason for this is that the lignotuber does not readily form roots. Lacey (1983) has described the formation of roots from the plate-like lignotuber structure of *E. botryoides*, but a similar large (> 2 m) structure found in *E. pilularis* (Lacey, Carr and Johnston, unpublished) does not form roots and is dependent on the original root system of its parent tree. Thus, the lignotuber is generally only functional while the original root system remains in contact. In certain species, new roots may form from the lignotuber, but in most cases new roots appear only at the bases of new shoots.

Lignotubers form only at the base of a plant or from shoots which arise at the base. There appears to be a particular region on the plant from which the lignotuber
can arise. This will be discussed under a separate heading (Section 6.5).

Certain criteria may now be proposed as being diagnostic of lignotubers. They are: woody, underground structures; form from shoots; store food materials; possess suppressed buds which have scale leaves and which are capable, when released from inhibition, of producing shoots. Lacey (unpublished) has recently described structures which develop at the basal nodes of *Notelaea longifolia* seedlings. Morphological and anatomical investigations (Lacey and Jahnke, in preparation) show that they are woody, underground stem-like organs which grow vertically downwards to a depth of up to 1 m. They store abundant starch in simple pitted parenchyma cells (Figs. 6.4, 5) and are equipped with superficial buds which produce scale leaves. These are evidently not rhizomes since they do not branch or spread out horizontally underground. They are initiated in the axils of the cotyledons and are preceded by a proliferation of buds (Fig. 6.1). Eventually, one of the two structures predominates and the other ceases to grow. The woody tuber produces numerous adventitious roots (Fig. 6.2). Buds at its apex develop scale leaves (Fig. 6.3). The scale leaves become separated from the original apical bud by intercalary growth, and the buds in their axils also develop. Bud clusters are thus formed. The buds remain dormant in the sense that they do not grow out. Those on the flanks of the tuber cease to produce scale leaves but the ones near the tip continue to do so
Fig. 6.1. Swollen cotyledonary node of *Nootka longifolia* bearing numerous buds (arrows). Scale, 2 mm.

Fig. 6.2. Field collected seedling of *N. longifolia*. Stem (S), root (R). Vertically descending lignotuber swelling (L) with adventitious roots (arrowheads). One of the paired woody swellings has aborted (asterisk). Scale, 10 mm.

Fig. 6.3. Apex of woody swelling with buds bearing scale leaves(s). Scale, 0.4 mm.

Fig. 6.4. Transverse section of woody swelling stained with I-KI. Cortex (C), pith (P), xylem (X). Scale, 1 mm.

Fig. 6.5. Scanning electron micrograph of pitted (arrows) tracheid containing numerous starch grains (S) with attached remnants of protoplasm (asterisk). Scale, 2 µm.
and thus give rise to new generations of buds. The network of vascular strands, which interconnect the buds, branch from previous strands which originated from the buds of the cotyledonary axil. When pieces of the tuber are excised and planted, new plants arise from them. Since many of the characteristics of these structures are like those of eucalypt lignotubers, they should be called lignotubers. Organs more resembling eucalypt lignotubers are known in many desert plants such as species of *Casuarina, Hakea* and *Banksia*. As yet, nothing is known about their development, physiology or capacity for vegetative reproduction.

6.2 LIGNOTUBER CAMBIUM AND STEM CAMBIUM

Anatomical studies show that the stem cambium is different from the lignotuber cambium, as is evident by differences in the constitution of the xylem produced. The lignotuber cambium appears to have very short fusiform initials which produce short, vertical, woody elements and a good deal of vertical parenchyma. Fibres in the lignotuber phloem are much longer than the woody xylem elements and appear to elongate intrusively. The seedling lignotuber also has a greater proportion of ray parenchyma in the xylem than stem wood. During early development there appears to be a degree of competition between the two kinds of cambium, which may result in either the stem cambium or the lignotuber cambium dominating the other. This has quite marked consequences for the habit of the
plant, since the seedling initially has the potential to become either a tree or a mallee. If the lignotuber cambium is favoured, the lignotubers may encircle the stem, producing a tendency towards the development of a mallee or at best a small tree. Since some species, particularly bloodwoods, can exist in the field as either mallees or trees, selection of plant form is determined by site qualities and the incidence of fire or insect attack. If the lignotuber is not formed or quickly regresses after early development, the seedling always develops into a tree. In other species the lignotuber is always dominant, and they are constrained to be mallees.

The encirclement of the stem by lateral fusion of the lignotubers is brought about by conversion of stem cambium into lignotuber cambium. The same process is involved in the downward growth of lignotubers over internodes or the hypocotyl. The conversion of one kind of cambium into another is in itself a process of considerable biological interest, and should be closely investigated. One possibility is that conversion takes place by homeogenetic induction from the flanks of the lignotuber cambium. This is not supported by the results of experiments in which the two lignotubers at a node are removed entirely. Following wound healing, lignotuberous wood (lacking buds) is produced by the former stem cambium in the complete absence of any lignotuber cambium.
6.3 THE RELATIONSHIP OF ACCESSORY BUDS TO LIGNOTUBER DEVELOPMENT

There is good evidence for a relationship between the presence of a constellation of accessory buds at a node and the initiation of a lignotuber at that node. In the absence of such a set of accessory buds, lignotubers are not formed. The size of the lignotuber bears some relationship to the initial number of accessory buds. For instance, in a lignotuberous species a lignotuber may fail to develop at nodes which have only one upper accessory bud. Contrary to this, there are cases where a set of accessory buds exist, but no lignotubers are developed. However, in one such species, *E. pilularis*, lignotubers may be formed when the plant is much older, suggesting that the potential to form a lignotuber remains and can be expressed long after the seedling stage.

In category A of the four classes of lignotuber formation (Chapter 3), the lignotuber is initiated above the suite of upper accessory buds and appears therefore to be independent of them. Furthermore, if the lignotuber is amputated, a substitute is formed without any buds (Chapter 5 (C)). No doubt the surgical removal of the accessory buds soon after seed germination would resolve the matter of the dependence of lignotuber formation on accessory bud development, but the difficulties of doing so are formidable.
6.4 LIGNOTUBER FUNCTION

6.4.1 **Storage:** Kerr (1925) showed that the lignotuber could serve as a storage organ containing reserves of starch which can be released and subsequently replenished. She accomplished this by starving lignotuberous seedlings and then replanting them, showing that starch disappeared from and then reappeared in the lignotuber. Observations (Chapter 5 (A)) on similarly treated seedlings of *E. st. johnii* confirm Kerr's conclusions. Dell and Jones (1983) claim that the lignotuber of *E. marginata* accumulates a high level of P compared to equivalent stem tissue when seedlings are grown in P-deficient soil. This may of course reflect only the greater proportion of living cells in the lignotuber, which contains proportionately more ray and vertical parenchyma than the stem xylem (Bamber and Mullette, 1978). It would be interesting to know the comparative P:N ratio (especially protein-N) of stem and lignotuber. Moreover, investigations to show whether or not the P stored in the lignotuber is mobile and capable of being used in promoting shoot growth have not been reported.

Storage of photosynthetic assimilates has been shown (Chapter 5 (A)) in the lignotuber. However, the chemical nature of the translocate and the rate of its conversion into starch in the lignotuber are still little known. If the lignotuber encircles the stem, then ultimately the secondary phloem of the lignotuber must be the pathway for translocation to it and to the root.
system. This function is evident when the phloem of the stem between a pair of developing lignotubers is excised. The seedling does not die because the lignotuber phloem functions in translocation. The experiments described in Chapter 5 (A) show that assimilates do reach the lignotuber and are stored, particularly in parenchyma recently formed from the lignotuber cambium.

Amputation experiments suggest that there is competition between lignotubers at a node for assimilates involved in growth. If one is damaged in any way the other one grows much more in compensation.

6.4.2 Water movement through the lignotuber:

Preliminary studies on water movement suggest that the seedling lignotuber may afford a greater resistance to water flow than the stem xylem (Chapter 5 (D)). If the latter is still functional, then water under pressure moves preferentially through the stem xylem. This would be especially important during the period following lateral fusion of the lignotubers at a node as further formation of stem xylem ceases. As the vessels of the stem xylem become aspirated or tylosed, water flow would be increasingly restricted to lignotuber tissue with its apparently high resistance. In seedlings, lignotuber wood differs from stem wood in consisting of short elements, mostly pitted tracheids; lignotuber wood appears to contain few or very small vessels (vide contra Chattaway, 1958a). The physiological implications of this are still unknown. Furthermore, it is not yet certain whether the xylem of
adult lignotubers is similarly constituted. There is, however, some evidence in support of the view that the constitution of adult lignotuber xylem might be such as to provide something of a barrier to water flow. If this were true it would explain why it is that:

(a) many desert mallees have "water-roots" in which, even during the hottest part of the day, a considerable amount of free water may be available in vessels of relatively large diameter;

(b) there appears to be a restriction on the height and trunk diameter which can be reached by mallees.

It has been reported that Neales (1983) has made measurements of water status and transpiration of bull mallee (*E. behriana*), a plant which tolerates conditions of extreme drought and high temperatures. He found a considerable negative water potential of leaves at dawn, as though water deficits resulting from a day of heavy transpiration could not be reduced during the night. This has led him to assume some sort of block in the supply of water from the soil to the leaves. This block could, of course, be due to the lignotuber. After measuring the water deficits and transpiration rates of foliage, he incised the bark and wood of the trunk and supplied water directly to it. This immediately relieved the leaf water deficit. He stated, "it could be that the famous Mallee
root is a form of contorted plumbing that offers a resistance to water flow in the tree thus keeping the leaves and branches normally under considerable water deficits". The evidence against such a possibility is weighty. The nature of the lignotuber wood is not unlike that of conifer wood, where no such water supply problems have been shown to arise. Moreover, in the chain of resistances to water flow from the soil to the air, the resistance offered by the flow of water in the wood of the trunk is known to be negligible in comparison with the resistance in the leaf-to-air pathway. There is, therefore, something of a paradox in the proposal that the lignotuber could offer a resistance to water movement of sufficient amount to control the transpiration and water deficits of the leaves.

6.5 THE EUCALYPT LIGNOTUBER: A POSITION-DEPENDENT ORGAN

A considerable body of observational and experimental evidence shows that the capacity to form a lignotuber may also be a property of shoots arising from lignotuber buds, rhizostolons and rhizomes. These phenomena may be explained by the assumption of a so-called "position effect" for the production of lignotubers in the sense that the term has been used for other position-dependent phenomena in plants (Olesen, 1978). One explanation of these phenomena is that plant cells contain some form of information specifying their position within
Fig. 6.6. Vegetative propagation of shoots of *E. ficifolia* from sterile culture of basal accessory meristems of a seedling. Scale, 5 mm.

Fig. 6.7. Lignotubers on the base of a propagated shoot of *E. ficifolia*. Scale, 5 mm.

Fig. 6.8. *E. stellulata*, "rhizo-stolons" bearing lignotubers at their nodes (asterisks). Scale, 10 mm.
the plant, more commonly referred to as "positional information". The idea that this mechanism might operate in eucalypt lignotubers (Carr et al., 1982) was expanded by examining propagated plantlets of *E. ficifolia* F. Muell. and *E. obtusiflora* D.C., derived from seedling nodes which, in the intact seedling, would eventually bear lignotubers (Fig. 6.6). Cultivated plantlets, grown under greenhouse conditions, produced lignotubers at successive basal nodes (Fig. 6.7). When reciprocal approach-grafts of lignotuberous and non-lignotuberous species are made, lignotubers always develop at the original, pre-determined lignotuberous nodes site, irrespective of the provenance of root or grafted shoot. If cuttings of *E. pellita* (lignotuberous) are made above the 8th stem node of a seedling and then propagated in soil, lignotubers do not develop at basal nodes, which apparently lack the required information. Thus the lignotuber is not merely a structure which can develop between stem and root. Previous authors (Fletcher and Musson, 1918; Kerr, 1925; Lacey, 1974; Lacey and Whelan, 1976) have described field observations which can now be understood in terms of a "position effect". The more recent work describes rhizomes of some tropical eucalypts of regions of the Northern Territory which are subject to annual burning. The rhizomes are subterranean, plagiotropic branches from the lignotuberous bases of trees, e.g. *E. porrecta* S.T. Blake, *E. ptychocarpa* F. Muell., *E. oligantha* Schau., which can give rise to aerial shoots which then develop lignotubers at their bases.
Gillison et al. (1980) have described "rhizo-stolons" of *E. moluccana* Roxb. spp. *moluccana* at Bungonia, N.S.W., which are also plagiotropic developing from lignotubers. Where rhizo-stolons touched the ground, lignotuberous tissue formed which had then rooted and produced clusters of shoots. Observations on *E. stellulata* Sieb. also show a similar pattern when stoloniferous shoots spread outwards from the bases of old trees in disturbed situations (Fig. 6.8). The above descriptions all provide evidence for the idea that the lignotuber is a position-dependent organ.
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Table of *Eucalyptus* species showing presence (+) or absence (-) of lignotubers at 6 or 12 months after seed germination, Wail Nursery, Victoria.

**Legend**
- (C) - adopted from Carter (1929)
- (K) - adopted from Kerr (1925)
- +? - time of lignotuber formation unknown (field plants)
- * - species known to always lack lignotubers

Note: Names referred to by some authors have been superseded, but have been left in the original form. Absence of lignotubers does not preclude the seedling from developing them later on.

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<td>megacarpa</td>
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<td>petraea sp. nov. pileata</td>
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E. redunda var. elata +6
regnans* (K)
regnans var. fastigata* (K)
E. saligna (K) +?
salubris -6
salmonophloia -6
ergentii -6
sepulcralis +6
shiressii -6
sideroxylon +6
siderophloia (C) +6
sieberi -6
spathulata -6
steedmanii -6
stoatei -12
stricklandii -12
sturgissiana +?

E. talyuberlup +6
tetragona +12
tetraptera -6
transcontinentalis -6
torquata -6

E. uncinata +6
urbrae -6
urnigera -6

E. viminalis +12
viridis +12

E. websterana -6
woodwardii -6

E. youngiana -6