Patterns of decay within stems in a regrowth *Eucalyptus* forest

John Francis Wilkes

BSc.(For.), Grad. Dip. Sci. [ANU]

Thesis submitted for the degree of Doctor of Philosophy

at the

Australian National University

July 1984

Other than where acknowledged this thesis is my own original work

John Wilkes

.....

John Wilkes

Acknowledgements

With much gratitude I acknowledge the valued advice of my supervisors Dr W.A. Heather (of the Department of Forestry at this University) and Dr W.E. Hillis (CSIRO, Division of Chemical and Wood Technology, Melbourne). My comradeship with Dr Heather, and his encouragement, have helped enormously. I also thank the Department of Forestry for making facilities and equipment available for this investigation, and my wife Gail, for her help in many ways.

Arrangement of Thesis

As provided under the Rules [Courses and Degrees (Degree of Doctor of Philosophy)] of the Australian National University, the six experimental chapters of this thesis are presented as manuscripts of a series of closely related papers intended for publication in the wood science journal 'Holzforschung'. These chapters are in a form requested by, or acceptable to, the Journal. At the time of submission of this thesis the first three scripts (Chs. 1-3) had been accepted for publication, and the contribution to these manuscripts of the editors and referees of Holzforschung is gratefully acknowledged. The remaining chapters were submitted to the Journal in July 1984.

Where appropriate, other components of the thesis are adapted to this method of presentation. Thus for example:

- The 'Reference List' contains only those works referred to in the 'Preface' or 'General Discussion', since each experimental chapter is accompanied by a separate literature section.
- The 'Preface' replaces a 'General Introduction' since the experimental work is introduced adequately in appropriate manuscripts.
- Larger areas of speculative discussion are necessarily restricted to a final 'General Discussion' chapter.
- Tables and Figures are located at the end of the relevant chapter.
- Hierarchical subdivisions are not used.

Contents

Addendum and Corrigenda at rear

List of Tables

List of Figures

Abstract

A series of studies examined certain host attributes potentially influencing the extent and patterns of decay within stems of four *Eucalyptus* spp. [*E. bancroftii* (Maid.) Maid., *E. dealbata* A. Cunn. ex Schau., *E. macrorhyncha* F. Muell. ex Benth. and *E. sideroxylon* A. Cunn. ex Woolls] which dominated a 40 yr old, dry sclerophyll, regrowth forest. Bole dissections revealed that volumes of decay were greatest in *E. bancroftii* and *E. dealbata*, and minimal in *E. sideroxylon*. Deterioration was normally restricted to heartwood, within which barrier zones e.g. kino veins, formed previously at the cambium as a response to injury, frequently limited the centrifugal progression of decay. Additional to this influence of barrier zones was a trend, most obvious in *E. sideroxylon* and *E. dealbata*, of increasing resistance to heartrot from inner to outer heartwood.

Variation, both between species and radially within stems, in the resistance of normal heartwood tissues to decay *in vitro* (soil-jar test) was loosely associated with the observed variation in resistance of the tissues to heartrot *in vivo*, presumably reflecting some form of causal relationship. This was supported by a study of included sapwood showing that such tissue, which is highly susceptible to deterioration in living trees, had a low natural decay resistance *in vitro* (ascribed to the reduced occurrence of fungitoxic phenolic extractives).

When properties (including basic density, moisture content, pH, mineral concentrations, content of methanol-soluble extractives, and resistance to decay *in vitro*) of clear, infected (natural heartrot) and artificially injured heartwood were compared, little evidence of tissue responsiveness was detected. Visual examination of the injured and infected tissues also suggested nonreactiveness of heart-

wood; hence it was concluded that this factor does not play a significant role in directing the progression of heartrot in the eucalypts. In contrast, living sapwood was found to be dynamically responsive, and the spread of discolouration and microorganisms from sites of mechanical injury, and from regions of heartrot, was very limited in this tissue. An increased resistance to decay *in vitro*, content of polyphenols and frequency of tyloses within and/or adjacent to injured sapwood suggested antibiotics and vessel plugging as agents of the highly effective compartmentalisation of deterioration in this tissue.

Studies of the anatomy and chemistry of artificially induced barrier zones in the eucalypts failed to elucidate the characteristic(s) which is responsible for limiting microbial invasion. The continuing effectiveness of the barrier zones, even in the absence of living cells (following incorporation in heartwood), and over a wide variation in wood anatomy, cell wall lignification and gross polyphenol content indicated that these four features are probably not functionally important properties of many of the barriers.

The complementary roles of these various factors (the natural decay resistance of tissues, protective responses of differentiated tissues, and barrier zones) as part of an integrated defense system of the host are examined.

Preface

As pressures for use of the agriculturally productive areas of the world continue to rise, it is increasingly important to avoid 'drains' on the productivity of those areas set aside primarily for wood production. Tree decay is one such drain; enormous volumes of potential roundwood and sawn timber are lost annually through tree decay in Australian eucalypt forests (Heather 1962; Wilkes pers. obs.), although this loss has not been accurately quantified. Apart from the direct loss of timber which is actually decayed, there is wastage of sound wood either attached to defective sections after log breakdown, or left in the forest in very defective trees. The economic impact of decay is augmented by additional costs incurred in harvesting and processing stems containing decay e.g. in the falling, snigging and hauling of defective material to the point of processing; in modification of log breakdown sequences to accommodate defects; in disposal of defective wood; and in sorting and grading processed timber.

Because in virgin forests the extent of decay often increases with tree size (age), a view has persisted that tree decay in *Eucalyptus* is only a problem associated with the overmaturity of virgin forests. However, there is now ample evidence that regrowth and plantation stems of a number of species may be highly defective (Da Costa 1973; Edwards 1973; Davidson 1974; Gadgil and Bawden 1981; Wilkes 1981). Despite this, many Australian forest managers appear to accept tree decay as a 'fact of life', and knowledge of fundamental aspects of the problem is very limited e.g. little is documented on infection of eucalypt stems by decay causing organisms, the rate of spread of decay within the trees, and the factors determining this rate of spread and patterns of decay.

In this work, just one aspect of decay in *Eucalyptus* is examined - the attributes of host stems affecting the extent and patterns of regions of decay which develop from wounds i.e. assuming that microorganisms have access to a stem, what are some of the properties of the host which determine where, and how quickly, deterioration progresses within the stem? Intuitively, and on the basis of work with deciduous hardwoods (see Wagener and Davidson 1954; Shigo 1979a; Wilkes 1982a), host attributes of three types are likely to be particularly relevant: the natural decay resistance of bole tissues; the ability of differentiated tissues to respond protectively; and the ability of the cambium to react protectively. Thus the primary aim of these studies was to ascertain the importance of each of these three factors in determining the extent and patterns of heartrot within certain eucalypt stems. A secondary aim was to gain some understanding of any protective responses of the boles to injury and infection i.e. to lay a foundation for further study in this area.

The 40 yr old dry sclerophyll forest examined was selected because it: contained a range of eucalypt species; was of known age and history; supported considerable volumes of tree decay; contained coppice material (see Chs. 2, 4); and was to be cleared for agricultural purposes. The location of this forest is shown in Fig. 1. Table 1 summarises various details, including the physiography, of the study area.

Since the nomenclature of the eucalypts is at present in a state of flux, conventional names, essentially as given by Pryor and Johnson (1971), are used in this study. Thus for example, while Brooker and Kleinig (1983) have recently entitled the taxa studied in detail in this series as *Eucalyptus bancroftii* (Maid.) Maid., *E. dealbata*

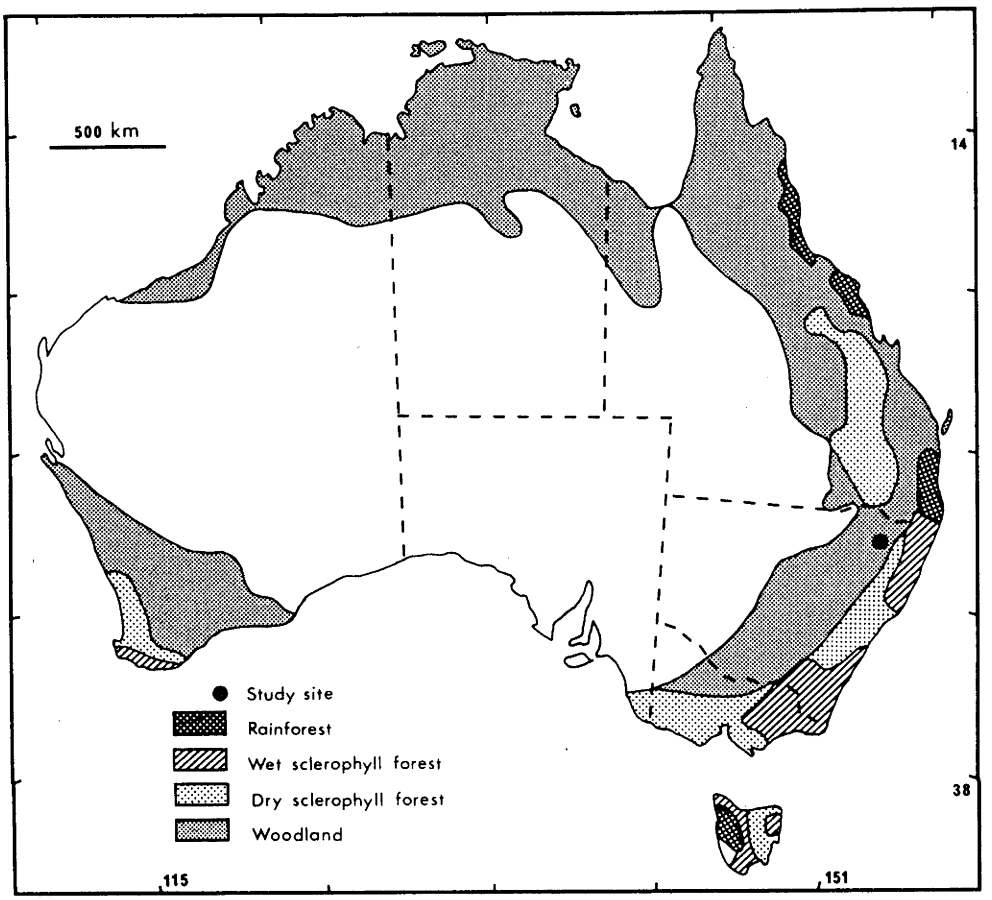
A. Cunn. ex Schau. var. *dealbata*, *E. macrorhyncha* F. Muell. ex Benth. subsp. *macrorhyncha*, and *E. sideroxylon* A. Cunn. ex Woolls subsp. *sideroxylon*, the varietal and subspecific divisions are not used here.

Table 1. Details of the study area

Factor	Description/details
Location	8 km SE of Inverell, NE New South Wales, Australia ca. 30°S, 151°E.
Altitude	ca. 800 m
Climate (Inverell)	Mean maximum temperature: January 28.9 ; July 14.9°C Mean minimum temperature: January 16.3 ; July 3.1°C Mean rainfall: 783 mm, on 89 days, per annum
Soils	Largely podzolic duplex granite: 0-30 cm, grey sandy loam; 30+ cm, yellow-grey clay; small areas of deep red-brown basalt
Topography	Undulating, slope usually < 5° All aspects represented
Extent	ca. 50 ha
Vegetation/ history	Area cleared ca. 1940-1945, but allowed to regrow almost immediately. Eucalypts have grown as seedlings or from lignotubers and stumps. Vegetation now constitutes a 'dry sclerophyll forest' ['open forest' - tallest stratum 10-30 m with 30-70% projective foliage cover (Specht 1970)] Overwood: predominantly *Eucalyptus* (8 spp. - see Ch. 1) Black cypress pine (*Callitris endlicheri* (Parl.) F.M. Bailey) also present Overbark diameters at breast height ca. 15-30 cm Understorey: relatively sparse. Predominantly wattle (*Acacia* spp.) and tea tree (*Leptospermum* spp.), 1-3 m high Area logged lightly over past 20 yrs No major fires

Fig. 1. Location of the study area, and dominant forest types of Australia. Shaded areas indicate regions in which the various forest types are most common. 'Rainforest', 'wet sclerophyll forest' and 'dry sclerophyll forest' are here considered synonymous with 'closed forest' [projective foliage cover of tallest stratum 70-100%], 'tall open forest' [tallest stratum > 30 m high, cover 30-70%] and 'low open forest/open forest' [5-30 m, 30-70%] respectively. The projective foliage cover of trees in woodland is < 30%. Note that the study forest is in a predominantly woodland area.

Source: Hall et al. 1970; Specht 1970.


500 km
14
38
115
151
Study site
Rainforest
Wet sclerophyll forest
Dry sclerophyll forest
Woodland

CHAPTER 1. *PATTERNS OF NATURAL DECAY*

Host attributes affecting patterns of decay in a regrowth eucalypt forest I. Patterns of natural decay

by J. Wilkes

Department of Forestry, Australian National University,
G.P.O. Box 4, Canberra, A.C.T. 2601 Australia

Summary

The patterns of stem decay occurring in a regrowth eucalypt forest were examined by extensive dissection of ca. 100 trees of each of four species (*Eucalyptus bancroftii, E. dealbata, E. macrorhyncha* and *E. sideroxylon*). Brown cubical rot and white pocket rot were the major types of deterioration, with the causal organisms entering through branch stubs, basal wounds and trunk wounds of insect origin. Decay was normally confined to heartwood suggesting a responsiveness of living sapwood to injury and infection. In contrast, no evidence was found of active compartmentalisation of decay in heartwood. The longitudinal extension of rots was usually greatest near the pith, centrifugal development frequently being constrained by barrier zones (usually kino veins) presumably formed in response to wounding of the cambium. Additional to this influence

of barrier zones was a trend, most obvious in *E. dealbata* and *E. sideroxylon*, of decreasing susceptibility to heartrot from inner to outer heartwood. This pattern possibly reflected a centrifugal increase in the natural decay resistance of heartwood tissues. Included sapwood was highly susceptible to deterioration *in viva*

Keywords: Tree decay, decay patterns, barrier zone, reaction zone, compartmentalisation of decay, decay resistance, *Eucalyptus*

Introduction

It has been suggested that the patterns of decay in living trees result largely from the responses of the tree to wounding and microbial invasion (Shigo 1979; Shortle 1979a, b). These responses fall into two categories: the reactions of differentiated tissues present in the tree at the time of wounding; and those of the cambium. In the case of sapwood, the area of the former response is often referred to as a 'reaction zone' (Shain 1979), which serves to wall-off or compartmentalise deterioration. Hence the configuration of regions of discolouration and decay in sapwood reflects the effectiveness of this compartmentalising process in the various directions within the tree (Shigo and Marx 1977; Shortle 1979b). Any reaction of heartwood tissues is likely to be passive (Shigo and Hillis 1973), and thus variation in the natural decay resistance of heartwood (as can be assessed *in vitro*) is possibly an important factor governing the rate and direction of microbial progression *in vivo* (Da Costa 1973; Wilkes 1982a, b).

The reaction of the cambium serves both to produce callus over the wound, and to form a sheath of abnormal cells, a 'barrier zone' (Shigo and Marx 1977), largely impervious to microorganisms. This zone confines organisms invading through a wound to tissues extant at the time of wounding. Hence it acts as a reserve defence mechanism, backing-up the response systems of differentiated tissues. In eucalypts, a portion of the barrier zone may be macroscopically obvious as a kino vein (Shigo and Hillis 1973).

The influence of these factors on patterns of decay in *Eucalyptus* is largely unknown. The present paper reports a study on the

configuration of regions of decay in standing trees in a dry sclerophyll forest. This investigation was a prerequisite to the subsequent examination of attributes of the host trees which influence the progression of decay within stems in this particular forest.

Materials and Methods

The regrowth forest studied, situated in northern New South Wales, contained *E. albens* Benth. (white box), *E. bancroftii* (Maid.) Maid. (Bancroft's red gum), *E. dealbata* A. Cunn. ex Schau. (tumbledown red gum), *E. globoidea* Blakely (white stringybark), *E. goniocalyx* F. Muell. ex Miq. (long-leaved box), *E. macrorhyncha* F. Muell. ex Benth. (red stringybark), *E. melliodora* A. Cunn. ex Schau. (yellow box) and *E. sideroxylon* A. Cunn. ex Woolls (red ironbark) in both mixed and single species stands. The forest was ca. 40 yrs old and consisted of stems of both seedling and coppice origin, 5-20 m tall, with diameters at breast height over bark usually in the range 15-30 cm. The forest had been logged lightly in recent years.

Over a four week period, some 100, randomly selected trees of each of *E. bancroftii*, *E. dealbata*, *E. macrorhyncha* and *E. sideroxylon* were felled and dissected on site. These trees encompassed the spatial and size distributions of each species. Previous studies in the forest (e.g. Wilkes 1983) suggested that such a sample would give a reliable indication of the patterns of decay characterising the selected species in this location. Many of the trees had branch stubs, decaying parent stumps or logging wounds, suspected to indicate sites of internal decay. Stems and larger branches appearing likely to be defective were sawn in the transverse and/or

axial diametrical planes through the external indicator of defect. Internal defects were described, measured and photographed. The box species were virtually free of microbially related defect and hence were excluded from the study.

Results

The most common type of defect of microbe origin was decay - either brown cubical rot or white pocket rot. The white rot was usually more extensive in individual stems, although the overall incidence of brown rot was greater (Table 1). Discolouration around heartrot was usually very limited, the tissues being a darker red-brown colour for ca. 2 mm transversely and up to 2 cm longitudinally beyond the boundaries of decay. In all species examined, a grey-brown discolouration occurred in the sapwood immediately (< 1-5 cm) above and below open wounds, but this was largely obscured when such tissues were incorporated into the relatively dark coloured heartwood. Visually obvious wetwood was not encountered.

The type and extent of decay was largely dependent on tree species (Table 1). *E. bancroftii* and *E. dealbata* were highly susceptible to heartrot [> 70% of trees contained decay]; that in *E. bancroftii* usually constituted a brown, and that in *E. dealbata* generally a white rot. *E. macrorhyncha* contained intermediate volumes of decay [30% of trees] - usually the brown rot. Decay was uncommon in *E. sideroxylon*.

Decay was associated with above-ground wounds of three types:

- Basal injuries. Logging damage and the above-ground union of coppice stems with parent stumps were common infection courts.

- Branch stubs. In many cases, branches which attained a diameter exceeding 2-3 cm before death were not shed effectively, and decay organisms often gained entry through the dead stubs.

- Insect bole wounds. In *E. bancroftii*, and to a lesser extent *E. dealbata*, decay (mainly brown rot) was associated with many of the tunnels of boring larvae, especially those of the longicorn borer, *Phoracantha semipunctata* F.

Decay was normally confined to the heartwood region of the bole although in the gums, when virtually the entire heartwood cross-section at a particular level was decaying, the adjacent sapwood became darkly discoloured, and decayed to a limited extent (Fig. 1). Thus the heartrot fungi required a considerable inoculum potential to invade uninjured sapwood.

Barrier zones substantially influenced decay patterns, frequently restricting decay (and to some extent discolouration) to the inner and middle heartwood (Fig. 2). Often the zones were apparent as kino veins or as darker, red-brown surfaces < 1 mm wide, but even in the absence of these, the position of the cambium at the time of wounding formed the outer boundary of many regions of decay. The barrier zones also restricted the inward progression of rot where a tree had received multiple wounds, and the resulting series of separate decay zones produced a ring-rot effect.

After removal of bark, the typical basal wound, the exposed sapwood died rapidly and a light-coloured, dry region became included in the bole when the wound had been overgrown. Such an inclusion was extremely susceptible to stem rot (Fig. 3), and often appeared to give heartrot fungi the inoculum potential required to invade heartwood tissues centripetally. Similarly, sapwood present internal to a kino vein at the time of vein formation, usually failed to darken to the normal heartwood colour when eventually included in heartwood. In all species investigated, these regions of 'included sapwood' were commonly in an advanced state of decay (Figs. 4-6). This effect was particularly obvious in *E. macrorhyncha* which produced relatively wide veins (2-5 mm).

The death or breaking of branches resulted in barrier zone formation, with or without the production of kino veins. The absence of distinct growth rings in these eucalypts precluded branch and stem ageing, and thus estimation of the exact location of the less obvious branch stub barrier zones. However, the boundary of any decay present internal to a branch stub was usually abrupt and contiguous with the stub extremities (Figs. 7, 8). Where a dead stub became included in the stem, kino was normally deposited between the stub and adjacent stem tissues formed subsequent to branch death (Figs. 8-10). Decay organisms did not usually spread from the stub through this kino to the bordering regions not protected by (i.e. outside) the original barrier zone which formed when the branch died.

The extent of barrier zone formation depended largely on the dimensions of wounds. Thus, where cambial injury was slight e.g. an insect tunnel, decay associated with the wound commonly extended to positions from which an 'escape' into tissues produced post-wounding

was possible. The effectiveness of barrier zones was also diminished when borers, especially the longicorn larvae, tunnelled through barrier zones and regions of decay; the latter often progressed along the holes, thus escaping the confines of the zones (Fig. 8).

Barrier zones constituted a surface of physical weakness, and hence ring shake was common, being particularly obvious in drying samples. This did not directly affect patterns of decay *in situ*, although ring shake and internal decay were associated with most instances of ray shake encountered (Fig. 3). Discolouration and decay progressed outwards along these ray shakes which occasionally extended into the sapwood.

Within the confines of barrier zones, apparent random spread of discolouration and decay in localised areas was common e.g. white pocket rot was sometimes present in small groups of pockets, separated by several cm of visually clear wood. On a broader scale, the occurrence and vertical extension of heartrot was usually at a maximum near the pith in all species. This trend reflected, in part, the cumulative risk of infection of the older, inner heartwood tissues, the greater age of some of the rots in this region of the bole, and the influence of barrier zones. However, radial variation in susceptibility to heartrot was obvious also in *E. dealbata* and *E. sideroxylon* (Table 2). In *E. sideroxylon*, the decay which occurred in inner heartwood failed to progress into outer heartwood, and deterioration did not spread from wounds or regions of included sapwood in the outer heartwood of this species. Decays in the outer heartwood of *E. dealbata* were usually very limited in extent. This radial differential in susceptibility to stem decay was much less pronounced in *E. bancroftii* and *E. macrorhyncha* (Table 2), as when

not confined by barrier zones, many of the decays in the inner regions of the stems progressed centrifugally into the outer heartwood. The vertical extension of such decays in outer heartwood was often considerable e.g. 30-50 cm.

Discussion

Eucalypt trees of regrowth and plantation forests frequently contain large volumes of heartrot relative to slower grown stems of a similar size in virgin areas (Edwards 1973; Wilkes 1982a). As reported here for a regrowth forest, branch stubs (e.g. Gadgil and Bawden 1981), insect wounds (e.g. Edwards 1973) and other forms of bark damage (e.g. Davidson 1974) are common infection courts in *Eucalyptus* plantations. It is likely that the extensive deterioration in some of these forests reflects both the abundance of infection courts and rapid spread of decay within stems.

Injured and infected tissues are compartmentalised (walled-off) in the heartwood of certain deciduous hardwoods (Shigo 1979; Shigo and Shortle 1979). However, the general lack of intense discolouration around either the white or brown rots, and the absence of clearly delineated boundaries of many decayed areas suggests a lack of comparable responsiveness in heartwood tissues of these eucalypts. This agrees with findings for *E. microcorys* F. Muell. (Wilkes 1982b; Wilkes and Heather 1983). Nevertheless, it is possible that the heartwoods of the species under study react, in ways not macroscopically obvious, to injury and the presence of microbes, and thus influence the establishment and progression of heartrot.

The discolouration of sapwood bordering regions of decay, and the exclusion of decay from living sapwood of the eucalypts, indicate a capacity of the living portions in the boles to respond to microbial invasion. Such a responsiveness of sapwood has been recorded for many genera (Shain 1979; Shortle 1979b). Presumably, in the absence of protective reactions, living sapwood tissues could be extensively invaded by microbes possessing some parasitic ability. Zones of eucalypt sapwood killed rapidly after removal of bark were readily decayed, probably reflecting the high levels of food reserves and/or the near absence of fungistatic extractives in sapwood (Scheffer and Cowling 1966; Hillis 1971).

In these eucalypts, the production of barrier zones by the cambium, as a response to wounding, may be more important in reducing decay volumes than local responses of either the heartwood or sapwood. It is not known whether the eucalypt barrier zones act as chemical or mechanical barriers to the movement of microorganisms. Kino, although abundant in some zones, is apparently absent from others which are equally effective barriers, suggesting that this polyphenol is either not particularly fungitoxic/fungistatic or is not the sole chemical barrier within the zones. The formation of a kino vein results in the potential sacrifice (deterioration) of the region of pseudo-sapwood subsequently included in the heartwood. This production of included sapwood under the barrier zones of certain eucalypts, previously noted by Hillis (1962), is consistent with the belief that heartwood extractives are formed at the heartwood-sapwood boundary largely from carbohydrate precursors translocated inwards from the sapwood or cambium (Hillis 1971) - the zone would tend to block such centripetal movement of carbohydrates, or more generally, of any 'heartwood-inducing substance' (Bamber 1976).

In the heartwood of *E. microcorys*, natural decay resistance resulting from the presence of fungitoxic extractives is probably an important factor controlling the extent and patterns of heartrot (Wilkes 1982b). The present work suggests that the same may apply to these other eucalypts e.g. the tendency for decay to progress rapidly in the inner relative to the outer heartwood of *E. dealbata* and *E. sideroxylon* is likely to correspond with a gradient of increasing natural decay resistance from inner to outer heartwood (Rudman 1964). Possibly also, the differences in rot volumes between the species can be attributed, in part, to differences in natural decay resistance of the heartwoods. Thus regardless of the wound type, *E. sideroxylon*, reputedly containing highly durable heartwood (Thornton et al. 1981), supported little decay relative to the other, presumably less durable species.

Conclusions

Within the stems of the eucalypt species studied, decay fungi do not spread totally at random; rather, three basic properties of tree boles possibly govern decay volumes and patterns:

- variations in the natural decay resistance within the bole heartwood;
- localised protective responses of differentiated tissues, particularly those of the sapwood; and
- barrier zones resulting from cambial activity.

Acknowledgement

The advice of Dr W.A. Heather is greatly appreciated.

References

Bamber, R.K. 1976. Heartwood, its function and formation. Wood Sci. Technol. 10, 1-8.

Da Costa, E.W.B. 1973. Natural durability and heart rots. 16th For. Prod. Res. Conf. (Aust.), Topic 1/15, 2pp.

Davidson, J. 1974. Decayed wood in living trees of *Eucalyptus deglupta* Blume. Papua New Guinea Dept. Forests, Trop. For. Res. Note SR 18, 31pp.

Edwards, D.W. 1973. Defects of fast-grown eucalypts in New South Wales, Australia. Proc. IUFRO Div. 5 Meet. 2, S. Africa. pp.256-270.

Gadgil, P.D. and A.D. Bawden. 1981. Infection of wounds in *Eucalyptus delegatensis*. N.Z. J. For. Sci. 11, 262-270.

Hillis, W.E. 1962. The distribution and formation of polyphenols within the tree. In: Hillis, W.E. (ed). Wood extractives and their significance to the pulp and paper industries. Academic Press, New York. pp.59-131.

Hillis, W.E. 1971. Distribution, properties and formation of some wood extractives. Wood Sci. Technol. 5, 272-289.

Rudman, P. 1964. Durability in the genus *Eucalyptus*. Aust. For. 28, 242-257.

Scheffer, T.C. and E.B. Cowling. 1966. Natural resistance of wood to microbial deterioration. Annu. Rev. Phytopathol. 4, 147-170.

Shain, L. 1979. Dynamic responses of differentiated sapwood to injury and infection. Phytopathology 69, 1143-1147.

Shigo, A.L. 1979. Tree decay - an expanded concept. U.S. Dept. Agric. Forest Service, Agric. Info. Bull. No. 419, 72pp.

Shigo, A.L. and W.E. Hillis. 1973. Heartwood, discolored wood, and microorganisms in living trees. Annu. Rev. Phytopathol. 11, 197-222.

Shigo, A.L. and H.G. Marx. 1977. Compartmentalization of decay in trees. U.S. Dept. Agric. Forest Service, Agric. Info. Bull. No. 405, 73pp.

Shigo, A.L. and W.C. Shortle. 1979. Compartmentalization of discolored wood in heartwood of red oak. Phytopathology 69, 710-711.

Shortle, W.C. 1979a. Compartmentalization of decay in red maple and hybrid poplar trees. Phytopathology 69, 410-413.

Shortle, W.C. 1979b. Mechanisms of compartmentalization of decay in living trees. Phytopathology 69, 1147-1151.

Thornton, J.D., N.E.M. Walters and I.W. Saunders. 1981. A field test of the natural durability of the heartwood of Australian

timbers in ground contact. 20th For. Prod. Res. Conf. (Aust.), Topic WP 5, 10pp.

Wilkes, J. 1982a. Stem decay in deciduous hardwoods - an overview. Aust. For. 45, 42-50.

Wilkes, J. 1982b. Pattern and process of heartrot in *Eucalyptus microcorys*. Aust. For. 45, 51-56.

Wilkes, J. 1983. Use of the Shigometer technique to detect decay in eucalypts. Aust. For. 46, 35-38.

Wilkes, J. and W.A. Heather. 1983. Influence of wood extractives on fungi invading the stems of *Eucalyptus microcorys*. Holzforschung 37, 163-166.

Table 1. Incidence, type and extent of decays occurring in four *Eucalyptus* spp. in a dry sclerophyll forest

Tree species	Number of trees dissected	Number of trees containing: Brown cubical rot only	White pocket rot only	Both rot types	Average number of distinct regions of decay per tree[a]	Mean vertical extension of regions of decay (cm) Brown cubical rot	White pocket rot
E. bancroftii	100	72	7	5	2.8	28	45
E. dealbata	98	12	56	4	1.4	33	99
E. macrorhyncha	110	28	4	1	0.6	27	47
E. sideroxylon	95	4	5	0	0.1	14	26

a Areas of decay separated by less than 5 cm of visually clear wood at any point were regarded as one region unless obviously otherwise

Regions of decay confined to a single branch stub or included sapwood, and those extending < 5 cm vertically, have been excluded from the table

Table 2. Radial variation in susceptibility to heartrot in four *Eucalyptus* spp.

Tree species	Development of decay from wounds[a] in:		Spread of decay from included sapwood in:		Progression of decay from inner to outer heartwood[c]
	Inner heartwood[b]	Outer heartwood[b]	Inner heartwood	Outer heartwood	
E. bancroftii	✓	✓	✓	✓	✓
E. dealbata	✓	?	✓	?	?
E. macrorhyncha	✓	✓	✓	✓	✓
E. sideroxylon	?	-	?	-	-

Coding: ✓ commonly occurred; ? sometimes occurred; - did not occur

a Decays confined to individual branch stubs or included sapwood are not considered

b Inner heartwood, outer heartwood - the inner and outer halves (by distance) of the heartwood respectively

c In the absence of barrier zones

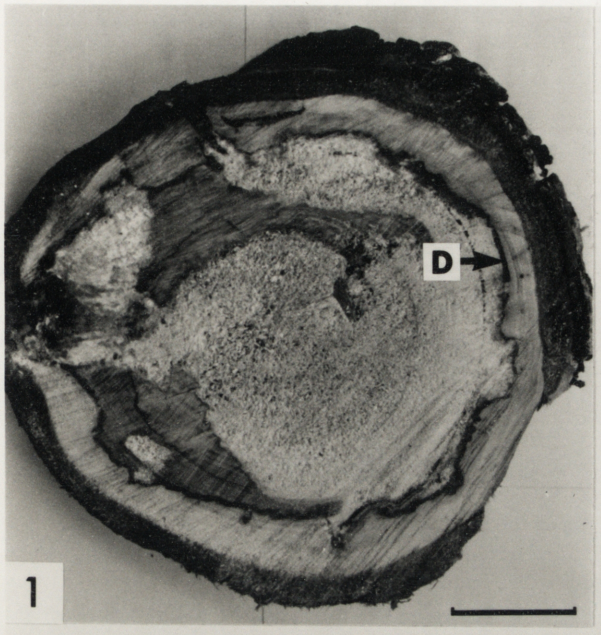
Fig. 1. Extensive white pocket rot in *E. dealbata*. The decay has been largely restricted to heartwood, a narrow band of intense discolouration (D) existing between decaying heartwood and adjacent sapwood.

Fig. 2. *E. dealbata* white pocket rot internal to a kino vein (KV). The rot external to the zone (top) has resulted from a relatively recent branch wound.

Fig. 3. Trunk wound partly overgrown on *E. dealbata*. The dead sapwood (SW) and tissues near the pith are partially decayed. A large ray shake is evident in the inner and middle heartwood.

Fig. 4. Cross-section of *E. sideroxylon* 20 cm above a basal wound. The sapwood present internal to the cambium at the time of wounding has been included in the heartwood, and subsequently has decayed.

[Figs. 1-4 : scale bar = 5 cm].


D
1


KV
2


SW
3


4

Fig. 5. *E. sideroxylon* in longitudinal section showing white pocket rot (R) development in included sapwood internal to a kino vein (KV) formed in response to a basal wound (W).

Fig. 6. Decay in *E. dealbata*, having escaped a branch stub barrier zone (BZ), has preferentially developed internal to a small kino vein (KV).

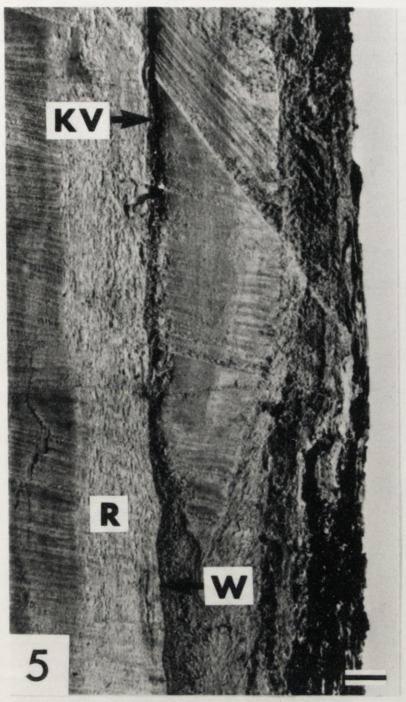
Fig. 7. Broken branch on *E. bancroftii* supporting incipient decay (I) inward from a barrier zone (BZ) which has formed as a result of the break. The stub of a cleanly shed branch is visible (bottom).

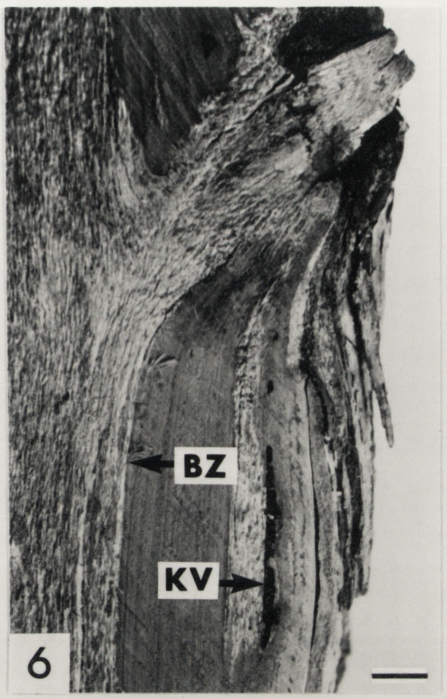
Fig. 8. Decayed branch stub in *E. bancroftii*. Wood boring larvae have permitted decay to move through a barrier zone (BZ). Kino (K) has been deposited between the dead stub and newly forming tissues.

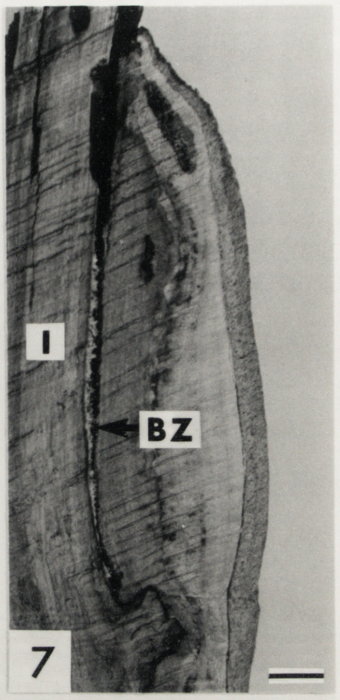
Fig. 9. *E. dealbata* showing the deposition of kino (K) about a stub, neither shed effectively nor grown over. Decay organisms have gained entry through this stub.

Fig. 10. Large sound *E. bancroftii* stub surrounded by kino (K), an outward extension of the barrier zone (BZ).

[Figs. 5-10 : scale bar = 1 cm].

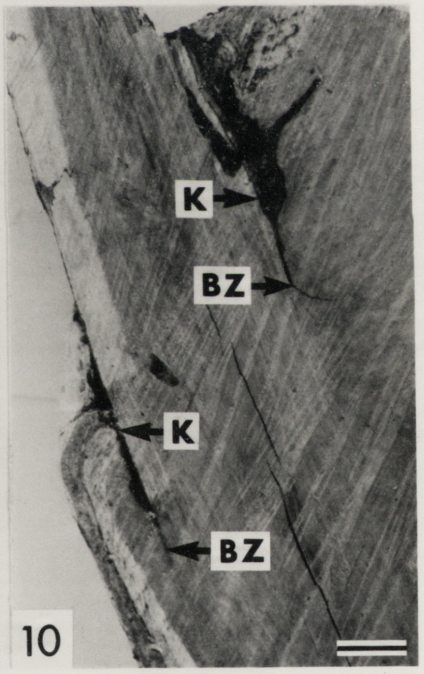

KV
R
W
5


BZ
KV
6


I
BZ
7


K
BZ
8


K
9


K
BZ
K
BZ
10

CHAPTER 2. *THE RESISTANCE OF HEARTWOOD TO DECAY* IN VITRO

Host attributes affecting patterns of decay in a regrowth eucalypt forest II. The resistance of heartwood to decay *in vitro*

by J. Wilkes

Department of Forestry, Australian National University,
G.P.O. Box 4, Canberra, A.C.T. 2601 Australia

Summary

Blocks cut from the inner and outer heartwood of 40 yr old stems of four eucalypt species were assessed for resistance to decay by six fungi using a modified soil-jar technique. After an incubation period of 10 weeks, average weight losses, for inner and outer heartwood respectively, were: *Eucalyptus macrorhyncha* 8.5, 10.1%; *E. bancroftii* 9.4, 7.8%; *E. dealbata* 7.9, 4.3%; and *E. sideroxylon* 7.1, 3.6%. The species which showed the greatest difference, between inner and outer heartwood, in resistance to decay *in vitro* (*E. dealbata, E. sideroxylon*) are those characterised by the most pronounced radial variation in resistance to heartrot *in viva*. However, variation between tree species in the rate of extension of heartrots does not correspond closely with variations in natural

decay resistance *in vitro*, suggesting that additional factors influence the development of heartrot in the field. The decay resistance of outer heartwood tissues in large (fast grown) and small (slow grown) trees did not differ significantly ($P = 0.05$), indicating that growth rate does not appreciably influence the volumes of heartrot in the study species through a direct effect on tissue decay resistance.

Keywords: Decay resistance, tree decay, decay patterns, growth rate, *Eucalyptus*

Introduction

Due to complicating factors including variability in the number of infection courts on different trees/species, and the influence of time since wounding, the natural decay resistance of heartwood tissues (as assessed *in vitro*) may not relate closely to the amount of heartrot in a stem at a particular time (Wagener and Davidson 1954; Highley and Kirk 1979). However, the rate of progression of heartrot in trees could be influenced by tissue decay resistance, and thus decay patterns would be similarly affected i.e. providing barrier zones (Shigo and Marx 1977) were not encountered, decay would progress most rapidly through those regions of the stem possessing least natural resistance to decay. In Part I of this series it was hypothesised that variation in tissue decay resistance may influence the patterns of rot occurring in a defective, regrowth eucalypt stand, and partly explain differences in rot volumes between species. This hypothesis is examined further here. The effect of rate of growth of the tree on heartwood decay resistance is examined since controversy persists as to whether rapidly grown eucalypts may be unusually prone to heartrot because of low natural decay resistance of the heartwood (Wilkes 1982).

Materials and Methods

A disc 5 cm thick was cut at breast height from a large (25-30 cm diameter at breast height over bark - dbhob) and a small (15-20 cm dbhob) tree in five, 40 yr old coppice clumps of each of four eucalypt species [*Eucalyptus bancroftii* (Maid.) Maid. (Bancroft's red gum), *E. dealbata* A. Cunn. ex Schau. (tumbledown red gum), *E.*

macrorhyncha F. Muell. ex Benth. (red stringybark), and *E. sideroxylon* A. Cunn. ex Woolls (red ironbark)]. From each disc, six blocks 10 x 15 x 10 mm (radial, tangential, longitudinal), were sawn from the outer heartwood (10-20 mm from the heartwood-sapwood boundary). Another six blocks of the same dimensions but orientated radially, were cut 15-30 mm from the pith of a further stem, selected at random, in each of the coppice clumps. The use of coppice material in this way allows elucidation of any rate of growth (and radial position) effects with limited replication, since the trees within a clump are genetically matched.

Blocks were subjected to decay using a variant of the wood-block/soil-jar technique (Leutritz 1946). Jars, 375 ml capacity, were one third filled with a sandy loam forest soil, and the moisture content of the latter raised to 110% of field capacity. Three filter papers (Whatman No. 54, 5.5 cm), soaked in a nutrient solution containing 0.2% (w/w) asparagine, 0.1% KH_2PO_4, 0.05% $MgSO_4 7H_2O$ and small amounts of iron, zinc, manganese, biotin and thiamine (Lilly and Barnett 1951), were used as the feeder strip on the soil surface. After sterilisation of the jars, strips were inoculated centrally with a culture of one of the test fungi. The organisms selected comprised three brown rot fungi [*Coniophora olivacea* (Fr. ex Pers.) Karst. DFP 1779, *Trametes lilacino-gilva* (Berk.) Lloyd DFP 1109 and an organism, tentatively assigned to the genus *Piptoporus*, isolated from and apparently the primary cause of the brown rot encountered during the stem dissection study (Wilkes 1985)], and three white rot species [*Coriolus (Polyporus) versicolor* (L. ex Fr.) Quel. DFP 2666, *Fomes lividus* (Kalch.) Sacc. DFP 7904 and *Phellinus badius* (Berk.) G.H. Cunn., the latter causing most of the white pocket rot in trees in the study area]. After the fungi covered the

feeder strips, gas (propylene oxide) sterilised blocks, oven dried to a constant weight, were allocated to the bottles in a stratified random fashion such that one of the six blocks from each tree was placed on a culture of each fungal species, three blocks per jar. Three blocks from the outer heartwood of each of two pole-sized stems of *E. delegatensis* R.T. Bak., a species with nondurable heartwood (Kloot 1965; Da Costa 1979), were similarly cultured on each of the test fungi. Three blocks of each tree species, including *E. delegatensis*, were also placed in jars prepared as detailed but without fungal inoculum (controls). The jars were incubated in the dark at 25°C in a humidity controlled (85-95% R.H.) chamber for 10 weeks. Blocks were then scraped free of adhering soil and mycelium, oven dried to a constant weight, and the percentage weight loss calculated. These calculated values were corrected for weight changes not attributable to microbial activity by reference to the control blocks.

Results (other than those for *E. delegatensis*), variously transformed, were subjected to three way analysis of variance (ANOVA) [fungal species x tree species x block position/tree size] using SPSS (Nie et al. 1975) and the data tested for normality, homoscedasticity and autocorrelation using GLIM (Baker and Nelder 1978) programs. For each fungal species - tree species combination, weight losses in blocks from outer heartwood of large and small trees were also compared using a paired *t* test [2 tree growth rates, 5 coppice clumps].

Results

The ANOVA results (Table 1) suggest that all three main factors were important determinants of weight losses in the blocks, although the significant (P = 0.05) first order interactions indicate that exceptions exist in the various trends. The mean per cent weight losses for each fungal species - tree species - block position combination (Table 2) can be compared using a single least significant difference value calculated from the ANOVA results.

1. Block position effect. For species other than *E. macrorhyncha*, per cent weight loss due to decay by a particular fungus was often significantly higher in inner, as compared to outer heartwood (Table 2). This difference was sometimes pronounced in *E. dealbata* and *E. sideroxylon*. In *E. macrorhyncha*, the mean weight loss for outer heartwood was slightly greater than that for inner tissues, due mainly to the relatively limited attack of inner heartwood blocks by *C. olivacea* and *F. lividus*.

2. Tree species effect. Differences between tree species in block weight losses were relatively small for the inner heartwood position (most juvenile wood), the range of means being 7.1% in *E. sideroxylon* cf. 9.4% in *E. bancroftii* (Table 2). For some of the test organisms, per cent weight losses in inner heartwood blocks were as great in *E. sideroxylon* as in *E. bancroftii* and/or *E. macrorhyncha*. However, at the outer heartwood position, interspecific differences were pronounced e.g. for the large trees, 3.6% mean weight loss in *E. sideroxylon* cf. 10.6% in *E. macrorhyncha*. These differences were substantially independent of test fungus, and consequently 'tree species' accounted for a large proportion of the variation in the

weight loss data (Table 1). Resistance to decay of the reference timber (*E. delegatensis*), was minimal in comparison with that of the other four species [mean weight losses: 36% cf. < 11% respectively (Table 2)].

3. Fungal species effect. For comparable wood blocks, particularly those from inner heartwood, *T. lilacino-gilva*, *P. badius* and the *Piptoporus* sp. often caused significantly more decay than the other organisms, with *F. lividus* inducing relatively small weight losses (Table 2). Thus rot type (white, brown) was not an important and consistent determinant of the extent of block deterioration.

Irrespective of the fungal species - tree species combination, weight losses in the outer heartwood blocks did not differ significantly between the large and small stems.

Discussion

Heartwood durability ratings have apparently not been assigned to *E. bancroftii* and *E. dealbata*. However, the results in Table 2 suggest that these two gums have a natural resistance to decay greater than that of *E. macrorhyncha*, but lower than that of *E. sideroxylon*. The heartwoods of the latter two species have been rated as moderately durable [service life in ground contact: 8-15 yrs (Tamblyn 1966)], and very highly durable [> 25 yrs] respectively (Kloot 1965; Thornton et al. 1981). Da Costa (1979), in examining the resistance of a wide range of eucalypt heartwoods to decay under laboratory conditions, ranked *E. macrorhyncha* in an intermediate position. All four species have appreciable resistance to decay in comparison with the nondurable *E. delegatensis* (Table 2).

If decay in standing trees was affected by the natural decay resistance of tissues, it could be expected that: (1) variation in decay resistance within trees would influence the location and shape of decays within the stems; and (2) variation in decay resistance between species would be reflected in corresponding interspecific variation in the extent of heartrots in the field.

1. In many eucalypt species decay resistance increases from inner to outer heartwood (Rudman 1966; Da Costa 1975). This pattern is evident for *E. sideroxylon* and *E. dealbata* (Table 2), which accords with the pronounced radial variation in resistance to heartrot observed previously in these species e.g. in the study forest, heartrot occurs only in the inner heartwood of *E. sideroxylon*, and is particularly restricted in the outer heartwood tissues of *E. dealbata* (Wilkes 1985). Conversely, in *E. macrorhyncha*, weight losses in blocks from the inner and outer heartwood are often similar; this is consistent with the high susceptibility to heartrot of both inner and outer heartwood in this species.

2. The interspecific differences in decay resistance *in vitro* are at a maximum in the relatively mature tissues of outer heartwood (Table 2). Here again there is some evidence of a relationship between decay *in vitro* and heartrot *in viva*. Thus the low per cent weight loss in blocks from the outer heartwood of *E. dealbata* and *E. sideroxylon* corresponds with the limited development of heartrot in such tissues, as opposed to the its appreciable extension in comparable regions of *E. bancroftii* and *E. macrorhyncha* (Wilkes 1985). However, at the inner heartwood position, the differences between species in decay *in vivo* do not always reflect interspecific

variation in natural decay resistance e.g. the exceptionally rapid extension of white pocket rot in the inner regions of standing *E. dealbata* (Wilkes 1985) is not parallelled by an unusually low resistance of this heartwood to decay, even by *P. badius*, the organism which causes the white pocket rot in the field (Table 2). Further, the *Piptoporus* sp., which progresses slowly in the inner heartwood of living trees of *E. sideroxylon*, degrades such tissues relatively rapidly *in vitro*.

It has been suggested that in some living trees, heartwood tissues can, by their response to wounding or the presence of microorganisms, exclude rot or minimise its spread (Shigo and Shortle 1979; Wilkes 1982). If certain tissues in the study species can respond in this way, any relationship between rot volumes and tissue decay resistance would be weakened. Other factors not operative in decay tests *in vitro*, but likely to influence the progression of heartrot fungi such as *P. badius* and *Piptoporus* in standing trees, include tissue aeration (Highley and Kirk 1979; van der Kamp et al. 1979) and the activities of nondecay, pioneer microbes (Bourchier 1961; Basham 1966; Shigo 1967).

P. badius and *Piptoporus*, which cause heartrot in the study forest, produced greater mean weight losses in blocks than three of the four standard decay test fungi (Table 2). Such decay capacities could contribute to the prevalence of both these organisms in the forest. However, the common occurrence of *P. badius* and *Piptoporus* as causes of heartrot in *E. dealbata* and *E. bancroftii* respectively (Wilkes 1985), cannot be explained in terms of any differential capacity of the two fungi to decay wood blocks of the two heartwoods *in vitro*. The infection court possibilities available to each

fungus in each tree species could be important here e.g. *E. bancroftii* is frequently attacked by the longicorn borer *(Phoracantha semipunctata* F.) which appears to 'transmit' the *Piptoporus* sp. more commonly than *P. badius* (Wilkes 1985).

In agreement with the present results (Table 2), studies on other species have shown a negligible influence of rate of growth on the resistance of heartwood tissues to deterioration *in vitro* (Da Costa et al. 1961; Rudman 1966). However, in the four eucalypts under study, the size of the juvenile core, as defined by radial variation in basic density, is positively correlated with growth rate (Wilkes 1984). Much of this core is likely to have a low resistance to decay (Rudman 1966), and thus (assuming some association between decay *in vitro* and that *in vivo*) rapid growth could result in a greater volume of heartrot in a fast than in a slow grown tree of a particular size. Rapid growth may also result in a high incidence of stem wounds e.g. branch shedding may be poor in vigorously growing eucalypts (Jacobs 1955), contributing further to the development of heartrot in the forest.

Conclusions

The natural decay resistance of heartwood tissues in the eucalypts studied appears to partially control the extent and patterns of heartrot in the standing trees. The decay resistance is little affected by rate of growth *per se*.

Acknowledgement

The advice of Dr W.A. Heather is greatly appreciated.

References

Baker, R.J. and J.A. Nelder. 1978. Generalised Linear Interactive Modelling. The GLIM System, Release 3. Royal Statistical Society, England.

Basham, J.T. 1966. Heart rot of jack pine in Ontario II. Laboratory studies on the pathogenicity and interrelationships of the principal heartwood-inhabiting fungi. Can. J. Bot. 44, 849-860.

Bourchier, R.J. 1961. Laboratory studies on microfungi isolated from the stems of living lodgepole pine, *Pinus contorta* Dougl. Can. J. Bot. 39, 1373-1385.

Da Costa, E.W.B. 1975. Natural decay resistance of wood. In: Liese, W. (ed). Biological transformation of wood by microorganisms. Proc. 2nd Int. Cong. Plant Pathol., Minneapolis. pp.103-117.

Da Costa, E.W.B. 1979. Comparative decay resistance of Australian timbers in accelerated laboratory tests. Aust. For. Res. 9, 119-135.

Da Costa, E.W.B., P. Rudman and F.J. Gay. 1961. Relationship of growth rate and related factors to durability in *Tectona grandis*. Emp. For. Rev. 40, 308-319.

Highley, T.L. and T.K. Kirk. 1979. Mechanisms of wood decay and unique features of heartrots. Phytopathology 69, 1151-1157.

Jacobs, M.R. 1955. Growth habits of the eucalypts. For. Timb. Bur. Aust., Canberra. 262pp.

van der Kamp, B.J., A.A. Gokhale and R.S. Smith. 1979. Decay resistance owing to near-anaerobic conditions in black cottonwood wetwood. Can. J. For. Res. 9, 39-44.

Kloot, H. 1965. Revised strength grouping system. CSIRO (Aust.), For. Prod. Newsl. No. 324, 4pp.

Leutritz, J. 1946. A wood soil contact culture technique for laboratory study of wood-destroying fungi, wood decay and wood preservation. Bell Syst. Tech. J. 25, 102-135.

Lilly, V.G. and H.L. Barnett. 1951. Physiology of the fungi. McGraw-Hill, New York. 427pp.

Nie, H.H., C.H. Hull, J.G. Jenkens, K. Steingrenner and D.H. Bent. 1975. Statistical Package for the Social Sciences. 2nd edn. McGraw-Hill, New York. 675pp.

Rudman, P. 1966. The causes of variations in the natural durability of wood: inherent factors and ageing and their effects on resistance to biological attack. In: Becker, G. and W. Liese (eds). Holz und Organismen. Int. symp., Berlin-Dahlem. pp.151-162.

Shigo, A.L. 1967. Successions of organisms in discoloration and decay of wood. Int. Rev. For. Res. 2, 237-299.

Shigo, A.L. and H.G. Marx. 1977. Compartmentalization of decay in trees. U.S. Dept. Agric. Forest Service, Agric. Info. Bull. No. 405, 73pp.

Shigo, A.L. and W.C. Shortle. 1979. Compartmentalization of discolored wood in heartwood of red oak. Phytopathology 69, 710-711.

Tamblyn, N. 1966. Natural durability ratings. CSIRO (Aust.), For. Prod. Newsl. No. 335, 1p.

Thornton, J.D., N.E.M. Walters and I.W. Saunders. 1981. A field test of the natural durability of the heartwood of Australian timbers in ground contact. 20th For. Prod. Res. Conf. (Aust.), Topic WP 5, 10pp.

Wagener, W.W. and R.W. Davidson. 1954. Heart rots in living trees. Bot. Rev. 20, 61-134.

Wilkes, J. 1982. Stem decay in deciduous hardwoods - an overview. Aust. For. 45, 42-50.

Wilkes, J. 1984. The influence of rate of growth on the density and heartwood extractives content of eucalypt species. Wood Sci. Technol. 18, In Press.

Wilkes, J. 1985. Host attributes affecting patterns of decay in a regrowth eucalypt forest I. Patterns of natural decay. Holzforschung, Submitted.

Table 1. Analysis of variance of wood block weight losses

Source of variation	Degrees of freedom	Mean square	F ratio[a]
Fungal species	5	197	30.9
Tree species	3	469	73.5
Block position	2	140	21.9
Interactions[b]	61	28	4.4
Residual	288	6.4	
Total	359	17	

a All F values significant (P = 0.05)

b All two way interactions are significant individually. Three way interaction not significant

Data not transformed

Table 2. Mean per cent weight losses in wood blocks cut from the inner and outer heartwood of four *Eucalyptus* spp. when exposed to each of six decay fungi for 10 weeks

Tree species	Tree size/ block position[a]	Fungus						
		Fomes lividus	*Coniophora olivacea*	*Coriolus versicolor*	*Phellinus badius*	*Trametes lilacino-gilva*	*Piptoporus* sp.	Mean[b]
E. sideroxylon	I	1.9	7.4	3.6	5.2	12.5	11.9	7.1
	L, O	2.1	4.1	3.2	3.6	5.3	3.5	3.6
	S, O	2.0	3.3	2.9	3.3	5.2	4.2	3.5
E. dealbata	I	0.8	8.1	4.9	9.5	11.9	12.2	7.9
	L, O	4.8	4.4	5.3	5.2	4.4	2.8	4.5
	S, O	2.6	4.6	4.5	4.6	4.9	3.6	4.1
E. bancroftii	I	2.4	7.2	10.4	14.3	7.9	14.4	9.4
	L, O	4.6	7.7	7.3	9.7	7.5	10.0	7.8
	S, O	4.3	8.1	7.7	9.5	7.0	10.1	7.8
E. macrorhyncha	I	3.9	4.6	9.0	10.0	12.1	11.4	8.5
	L, O	9.8	11.2	10.8	11.7	11.0	9.3	10.6
	S, O	7.5	8.2	11.0	11.1	9.4	9.8	9.5
Mean[b]		3.9	6.6	6.7	8.1	8.3	8.6	
E. delegatensis	O	36.3	17.2	30.4	38.2	48.2	47.6	36.3

a L - large trees, S - small trees, I - inner heartwood, O - outer heartwood

b Statistical comparison of row and column means is inappropriate since interactions occur between the factors see (Table 1)

Least significant difference (P = 0.05) for comparison between any two cell means - 3.1 (*E. delegatensis* excluded)

Each value in the table body the mean of five replicates (six for *E. delegatensis*)

CHAPTER 3. *THE RESPONSES OF HEARTWOOD TO INJURY AND INFECTION*

Host attributes affecting patterns of decay in a regrowth eucalypt forest III. The responses of heartwood to injury and infection

by J. Wilkes

Department of Forestry, Australian National University,
G.P.O. Box 4, Canberra, A.C.T. 2601 Australia

Summary

A range of wood properties were determined on tangential courses through isolated regions of brown cubical rot or white pocket rot, and also through artificially injured tissues, in the heartwoods of *Eucalyptus bancroftii*, *E. dealbata*, *E. macrorhyncha* and *E. sideroxylon*. Changes detected in several of the properties during the heartrot process from clear to discoloured to decayed tissue [appreciable decreases in basic density, moisture content and levels of extractives; small increases in concentrations of potassium, calcium and manganese] can be better explained as consequences of the activities of invading microorganisms, rather than as a protective response of the host. Six months after heartwood was wounded *in situ* by drilling holes through stems, resistance to decay, fungitoxicity of hot water-soluble extracts, and several other properties of the

'injured' wood immediately above and below the holes had altered little, again suggesting a lack of responsiveness of the heartwood tissues. Hence, protective reactions of heartwood of the host appear to be of little significance in determining the extent and patterns of heartrot in the eucalypts.

Keywords: Wood properties, heartwood, heartrot, wounding, tissue responsiveness, tree decay, decay patterns, *Eucalyptus*

Introduction

Despite the absence of living cells, heartwood tissues in certain tree species are claimed to react to wounding and the presence of microorganisms, so restricting the progression of discolouration and decay (Shigo 1979, 1983; Shigo and Shortle 1979). Thus, when variation in the natural decay resistance of heartwood tissues offered only a partial explanation for the differing extents and patterns of heartrot within trees of four species in a regrowth eucalypt forest (Wilkes 1985a), it was suggested that variation in the ability of the heartwoods to respond protectively to injury and microbial invasion could be important in this context. This possibility is investigated in a study of changes, both during decay and following injury, in wood properties including moisture content, pH, level of extractives and mineral concentration. Such properties have commonly been shown to alter markedly when the responsive sapwoods of both conifers (Shain 1971; Hart et al. 1975; Coutts 1976) and hardwoods (Shigo and Sharon 1970; Tattar et al. 1971; Shortle 1979) are injured and/or invaded by microorganisms.

Materials and Methods

Natural heartrot

Forty yr old trees of *Eucalyptus bancroftii* (Maid.) Maid. (Bancroft's red gum), *E. dealbata* A. Cunn. ex Schau. (tumbledown red gum), *E. macrorhyncha* F. Muell. ex Benth. (red stringybark) and *E. sideroxylon* A. Cunn. ex Woolls (red ironbark), which showed external evidence of decay, were felled and cross-cut repeatedly.

Where possible, a disc, ca. 5 cm thick and containing an isolated pocket of advanced decay, ca. 2-3 x 2-3 cm in cross-section and situated in the middle or outer heartwood, was collected from a fallen tree. The decay pockets selected did not appear to be associated with barrier zones. An equal replication, for each species, of discs which fulfilled these rot pocket criteria proved impractical e.g. decay is rarely situated in middle or outer heartwood in *E. sideroxylon* in this forest (Wilkes 1985b). Although the proportion of discs containing brown cubical rot or white pocket rot varied between the species, at least one disc containing each rot type was obtained for each species (Table 1). Within 2 hrs of cutting, the discs were individually wrapped in black plastic and stored at 4°C.

Approximately one week later, certain properties were determined on wood blocks located on a circumferential course through each decay pocket, following the general sampling technique of Wilkes and Heather (1982a). This form of sampling was chosen since a radial course could lead to confusion between natural radial variation in wood properties and changes in the latter associated with heartrot (Wilkes and Heather 1982b). In each disc, five blocks, ca. 1 cm^3, were sawn from wood in each of three visually recognisable classes of tissue deterioration - clear, incipient decay (including any small areas of the red-brown discoloured tissues), and advanced decay. The categories were differentiated subjectively, advanced decay being very soft, and incipient decay slightly weaker (resistance to penetration of a knife) and/or darker than clear wood. Tissues in a state of incipient decay were located immediately adjacent to the pocket of advanced decay, while clear wood samples were at least 5 cm removed from such areas.

Microbial isolations were attempted to partially verify the subjective classification of wood condition. Splinters ca. 2 x 2 x 5-8 mm were removed from one block representing each stage of tissue deterioration in each disc, and cultured at 25°C in darkness for four weeks on basidiomycete agar (Taylor 1974) [10 chips] or malt-yeast agar (Shigo and Sharon 1970) [20 chips].

The mass, green and oven dry (105°C), and the swollen volume as obtained by water displacement (Haygreen and Bowyer 1982), were recorded for a sample block from each position in each disc to allow calculation of basic density [g(oven dry) cm^{-3}(green)] and moisture content [%; g cm^{-3}(green)]. The remaining three blocks at each position were air dried and ground through a 0.8 mm screen. Of the sawdust produced, ca. 1 g was extracted for 8 hrs in a soxhlet (4 cycles per hr) with methanol, typically a solvent for the majority of eucalypt heartwood extractives (Da Costa and Rudman 1958; Rudman and Da Costa 1958, 1961). The weight loss of this dust was regarded as methanol-soluble extractives [expressed mg cm^{-3}(green)]. The pH of the filtrate of a solution, consisting of freshly distilled water (12 ml) and a further 0.5 g of the sawdust (Stamm 1961), left to stand for 15 hrs, was taken using an Anax meter. Flame emission and atomic absorption spectrophotometry were used to determine concentrations [µg cm^{-3}(green)] of sodium, potassium, calcium, magnesium and manganese when 0.3 g of sawdust was digested in 5 ml of hydrochloric acid and 15 ml of nitric acid.

For each wood property, data were subjected to three way analysis of variance [4 tree species x 2 rot types x 3 wood conditions].

Injured heartwood

For each of the four eucalypt species under study, eight healthy trees with diameters at breast height over bark of ca. 25 cm were wounded in mid summer by drilling a 16 mm diam hole through the stem 1 m above the ground level. After six months, trees were felled and a stem bolt, 20 cm long, was cut to centrally include the drill hole.

Following cold storage (4°C, 1-2 weeks), the bolts were cross-cut flush with the top and bottom of the drill hole, and again 1 cm above and below the hole, producing two discs. From these discs, side (circumferentially) matched blocks, 2 x 1 x 1 cm (2 cm radially), were split from the outer heartwood - one from tissues immediately above or below the drill hole ('injured' heartwood), and one from adjacent clear tissues, at the same level but ca. 3 cm tangentially removed from the first block. A faint tan discolouration was usually present in injured heartwood, at least in those tissues adjoining the drill hole. Four pairs of blocks (2 discs x 2 radii) were produced from each tree.

Three of these pairs of blocks were used to determine wood properties and microbial condition as outlined previously. Also, combined sawdust samples (6 g) representative of clear and injured tissues in each species were extracted for 2 hrs with 100 ml of boiling water. Solutions were evaporated or diluted to an extractives concentration of 10 mg ml^{-1} (1%), and following the addition of 1.0% glucose and 0.3% asparagine, were dispensed to 10 McCartney bottles, 5 ml per bottle. The units were sterilised (105°C dry heat for 1 hr) and inoculated with a 2 mm disc of a 10 day culture of *Coriolus (Polyporus) versicolor* (L. ex Fr.) Quel. [strain DFP 2666] or

Trametes lilacino-gilva (Berk.) Lloyd [DFP 1109] on malt agar. After incubation at 25°C for 14 days, cultures were harvested and weighed air dry on tared filter papers.

The final pair of blocks were subjected to decay by *T. lilacino-gilva* in a soil-jar test as detailed by Wilkes (1985a). The blocks in each pair were assigned to a single jar. Loss in weight [%] was the measure of decay susceptibility.

For each tree species, the effect of wounding on wood properties was assessed by paired *t* analyses [8 trees, 2 wood conditions]. Student-*t* tests were used to compare mean values of culture weight obtained in the bioassay.

Results

Natural heartrot

Microbes failed to develop from chips removed from tissues classified as clear. Bacteria were apparently absent from deteriorating tissues also, but decay (hymenomycetous) and sometimes nondecay fungi were isolated from all regions classified as incipient or advanced decay.

Rot type alone, and interactions of rot type with tree species and tissue status were not significant ($P = 0.05$) determinants of variation in any of the wood properties. Thus the data were summarised according to tree species and wood condition only (Table 2). Basic density, moisture content and the concentration of methanol-soluble extractives of tissues decreased significantly in

the transition from clear to incipient decay [mean decreases: 0.07 g cm^{-3}, 7%, 36 mg cm^{-3} respectively], and again during the incipient-advanced decay stage [0.28 g cm^{-3}, 9%, 33 mg cm^{-3}]. Concentrations of the minerals potassium, calcium and manganese increased significantly during the deterioration process [mean total increases: 17, 280, 13 µg cm^{-3} respectively]. Alterations in pH and levels of sodium and magnesium were not significant (Table 2).

Injured heartwood

Clear tissues were free of microorganisms, while discoloured heartwood supported numerous species of fungi, mainly non-Hymenomycetes. Bacteria were not isolated.

Within all tree species, moisture content was significantly ($P = 0.05$) lower [mean decrease: 9%], and potassium levels higher [mean increase: 75 µg cm^{-3}], in tissues adjacent to drill holes than in comparable clear wood. Other wood properties, including resistance to decay by *T. lilacino-gilva* (Table 3), altered little after injury. Absolute values of the properties other than resistance to decay were similar to those presented for clear tissues in Table 2. Growth of both *C. versicolor* and *T. lilacino-gilva* in nutrient solution was retarded significantly by the presence of extractives, but this effect was similar for injured and clear tissues (Table 3).

Discussion

In the sapwood of many tree species including *Picea abies* (L.) Karst. (Shain 1971) and *Pinus taeda* L. (Shain 1967), changes in certain

properties of wood [e.g. increases in mineral, moisture and extractives levels, and tissue pH], may occur during discolouration well in advance of fungal penetration, suggesting a responsiveness of tissues (Shain 1979). Since some of these changes e.g. the accumulation of various extractives, may retard microbial invasion, the responses have been regarded as protective in nature (Shain and Hillis 1971; Shain 1979). In contrast, fungal invasion often precedes visible signs of deterioration in heartwood (Wagener and Davidson 1954; Boyce 1961), and in the heartrots studied here, the recorded changes in wood properties (Table 2) occurred only in tissues already invaded by microbes.

Alterations which occur in wood properties as tissues are actually degraded may be a result of tissue response and/or a consequence of microbial activity. Thus the substantial reduction in contents of materials soluble in methanol, both early and later in the deterioration process in all species (Table 2), could reflect the activities of microbes able to utilise or degrade extractives (Shortle et al. 1971; Shortle and Cowling 1978). Active conduction of water through fungal mycelia (Peterson and Cowling 1973) could account for the reduced moisture content of decaying tissues both in the eucalypts examined here (Table 2), and in *E. microcorys* F. Muell. (Wilkes 1982), despite the production of water as cell wall substance is metabolised. Similarly, the increases in levels of potassium, calcium and manganese (Table 2), often being small in absolute terms [e.g. 10-30 $\mu g\ cm^{-3}$ for potassium cf. 400-3500 $\mu g\ cm^{-3}$ in *Acer rubrum* L. (Tatter et al. 1972; Safford et al. 1974)], could reflect the activities of organisms which can accumulate and translocate minerals (Shortle and Shigo 1973; Piirto and Wilcox 1978).

Thus the results of the study on natural heartrot can be explained without postulating a responsiveness of eucalypt heartwood i.e. evidence of an antagonistic response is lacking. In the stem wounding study the trees were harvested six months after injury in an attempt to limit the influence of invading microorganisms on wood properties. The similarity in properties of clear and injured heartwood apparently indicates the attainment of this objective, and also, the absence of a host response to wounding. The lower moisture content, and higher levels of potassium, in injured than in clear heartwood, presumably result from desiccation of tissues exposed to air, and the activities of pioneer microbes, respectively.

If heartwood were to respond protectively to injury or microbial invasion, thus minimising the spread of microorganisms within a stem (Shigo and Shortle 1979), the response would probably be based on the production of antibiotic substances. Such antibiotics could be produced through the conversion of one class of extractives to another, a process possibly mediated by naturally occurring heartwood enzyme systems. In such an event, total extractives levels might alter only marginally. However, the fungitoxicity of hot water-soluble extractives, and tissue decay resistance (presumably reflecting the quality and quantity of extraneous materials within the wood), do not alter significantly after injury in the eucalypt heartwoods (Table 3), again suggesting the absence of a protective response.

Conclusions

The results suggest that the heartwoods of the four eucalypt species are unresponsive to injury and microbial invasion. Consequently,

variations in the extent and patterns of heartrot within the eucalypts (Wilkes 1985b) probably do not relate to variation in some dynamic, protective capacity of heartwood tissues.

Acknowledgement

The advice of Dr W.A. Heather is greatly appreciated.

References

Boyce, J.S. 1961. Forest pathology. McGraw-Hill, New York. 572pp.

Coutts, M.P. 1976. The formation of dry zones in the sapwood of conifers I. Induction of drying in standing trees and logs by *Fomes annosus* and extracts of infected wood. Eur. J. For. Path. 6, 372-381.

Da Costa, E.W.B. and P. Rudman. 1958. The causes of natural durability in timber I. The role of toxic extractives in the resistance of tallowwood *(Eucalyptus microcorys* F. Muell.) to decay. Aust. J. Biol. Sci. 11, 45-57.

Hart, J.H., J.F. Wardell and R.W. Hemingway. 1975. Formation of oleoresin and lignans in sapwood of white spruce in response to wounding. Phytopathology 65, 412-417.

Haygreen, J.G. and J.L. Bowyer. 1982. Forest products and wood science. Iowa State Uni. Press, Ames. 495pp.

Peterson, C.A. and E.B. Cowling. 1973. Influence of various initial moisture contents on decay of Sitka spruce and sweetgum sapwood by *Polyporus versicolor* in the soil-block test. Phytopathology 63, 235-237.

Piirto, D.D. and W.W. Wilcox. 1978. Critical evaluation of the pulsed-current resistance meter for detection of decay in wood. For. Prod. J. 28, 52-57.

Rudman, P. and E.W.B. Da Costa. 1958. The causes of natural durability in timber II. The role of toxic extractives in the resistance of silvertop ash *(Eucalyptus sieberiana* F. Muell.) to decay. CSIRO (Aust.), Div. For. Prod. Technol. Pap. No. 1, 8pp.

Rudman, P. and E.W.B. Da Costa. 1961. The causes of natural durability in timber IV. Variation in the role of toxic extractives in the resistance of durable eucalypts to decay. Holzforschung 15, 10-15.

Safford, L.O., A.L. Shigo and M. Ashley. 1974. Gradients of cation concentration in discolored and decayed wood of red maple. Can. J. For. Res. 4, 435-440.

Shain, L. 1967. Resistance of sapwood in stems of loblolly pine to infection by *Fomes annosus*. Phytopathology 57, 1034-1045.

Shain, L. 1971. The response of sapwood of Norway spruce to infection by *Fomes annosus*. Phytopathology 61, 301-307.

Shain, L. 1979. Dynamic responses of differentiated sapwood to injury and infection. Phytopathology 69, 1143-1147.

Shain, L. and W.E. Hillis. 1971. Phenolic extractives in Norway spruce and their effects on *Fomes annosus*. Phytopathology 61, 841-845.

Shigo, A.L. 1979. Tree decay - an expanded concept. U.S. Dept. Agric. Forest Service, Agric. Info. Bull. No. 419, 72pp.

Shigo, A.L. 1983. Tree defects: a photo guide. U.S. Dept. Agric. Forest Service, Gen. Tech. Rep. NE-82, 167pp.

Shigo, A.L. and E.M. Sharon. 1970. Mapping columns of discolored and decayed tissues in sugar maple, *Acer saccharum*. Phytopathology 60, 232-237.

Shigo, A.L. and W.C. Shortle. 1979. Compartmentalization of discolored wood in heartwood of red oak. Phytopathology 69, 710-711.

Shortle, W.C. 1979. Compartmentalization of decay in red maple and hybrid poplar trees. Phytopathology 69, 410-413.

Shortle, W.C. and E.B. Cowling. 1978. Interaction of live sapwood and fungi commonly found in discolored and decayed wood. Phytopathology 68, 617-623.

Shortle, W.C. and A.L. Shigo. 1973. Concentrations of manganese and microorganisms in discolored and decayed wood in sugar maple. Can. J. For. Res. 3, 354-358.

Shortle, W.C., T.A. Tattar and A.E. Rich. 1971. Effects of some phenolic compounds on the growth of *Phialophora melinii* and *Fomes connatus*. Phytopathology 61, 552-555.

Stamm, A.J. 1961. Three methods of determining the pH of wood and paper. For. Prod. J. 11, 310-312.

Tattar, T.A., A.L. Shigo and T. Chase. 1972. Relationship between the degree of resistance to a pulsed electric current and wood in progressive stages of discoloration and decay in living trees. Can. J. For. Res. 2, 236-243.

Tattar, T.A., W.C. Shortle and A.E. Rich. 1971. Sequence of microorganisms and changes in constitutents associated with discoloration and decay of sugar maples infected with *Fomes connatus*. Phytopathology 61, 556-558.

Taylor, J.B. 1974. Biochemical tests for identification of mycelial cultures of Basidiomycetes. Ann. Appl. Biol. 78, 113-123.

Wagener, W.W. and R.W. Davidson. 1954. Heart rots in living trees. Bot. Rev. 20, 61-134.

Wilkes, J. 1982. Pattern and process of heartrot in *Eucalyptus microcorys*. Aust. For. 45, 51-56.

Wilkes, J. 1985a. Host attributes affecting patterns of decay in a regrowth eucalypt forest II. The resistance of heartwood to decay *in vitro*. Holzforschung, Submitted.

Wilkes, J. 1985b. Host attributes affecting patterns of decay in a regrowth eucalypt forest I. Patterns of natural decay. Holzforschung, Submitted.

Wilkes, J. and W.A. Heather. 1982a. The association of wood properties and resistance to a pulsed electric current in tallowwood. Aust. For. Res. 12, 55-62.

Wilkes, J. and W.A. Heather. 1982b. Detection of decay with a pulsed-current resistance meter, and radial variation in some wood properties in tallowwood. Aust. For. Res. 12, 63-70.

Table 1. Tree species and rot types sampled

Tree species	Number of discs containing: White pocket rot	Brown cubical rot	Total
Eucalyptus bancroftii	2	6	8
E. dealbata	4	2	6
E. macrorhyncha	1	4	5
E. sideroxylon	3	1	4
			23

Table 2. Changes in the properties of heartwood tissues in four *Eucalyptus* spp. during microbial deterioration

Tree species	Wood condition	Wood property								
		Basic density ($g\ cm^{-3}$)	Moisture content ($g\ cm^{-3}$ /%)	Methanol extracts ($mg\ cm^{-3}$)	pH	Mineral concentration ($\mu g\ cm^{-3}$)				
						Na	K	Ca	Mg	Mn
E. bancroftii	C[a]	0.65	0.55/85	74	3.10	19	78	260	250	17
	I	0.57	0.40/70	36	3.30	19	88	430	300	24
	A	0.38	0.24/63	23	3.28	9	86	730	280	23
E. dealbata	C	0.78	0.45/58	96	3.57	5	12	18	8	5
	I	0.72	0.42/58	74	3.53	15	31	170	25	9
	A	0.35	0.14/40	16	3.60	4	40	320	35	33
E. macrorhyncha	C	0.68	0.48/71	94	3.24	2	12	0	0	1
	I	0.61	0.39/64	58	3.07	3	24	0	5	1
	A	0.39	0.20/51	30	3.02	5	32	100	21	6
E. sideroxylon	C	0.86	0.39/45	119	3.64	1	6	130	4	3
	I	0.79	0.33/42	66	3.47	7	18	310	21	13
	A	0.40	0.16/40	24	3.39	8	19	230	16	16
Mean for all trees[b]	C	0.73	0.48/66	92	3.35	9	34	120	90	8
	I	0.66	0.39/59	56	3.34	12	47	250	120	13
	A	0.38	0.19/50	23	3.33	7	51	400	110	21
LSD (P=0.05)		0.05	0.04/03	16	NS	NS	13	80	NS	7

LSD Least significant difference

NS Not significant

a C - clear, I - incipient decay, A - advanced decay

b Each value the mean of 23 replicates. For replication within individual species, see Table 1

Table 3. Resistance to decay, and fungitoxicity of hot water-soluble extracts, of injured and clear heartwood from four *Eucalyptus* spp.

Tree species	Wood condition	Susceptibility to decay by *Trametes lilacino-gilva*[a] (% weight loss)	Dry weight (mg) of 14 day fungal cultures produced in nutrient solution containing wood extracts[b]	
			Coriolus versicolor	*Trametes lilacino-gilva*
E. bancroftii	Clear	6.4	6.6	6.4
	Injured	6.0	6.5	6.5
E. dealbata	Clear	3.4	3.0	4.5
	Injured	3.2	2.9	5.1
E. macrorhyncha	Clear	6.5	6.6	10.3
	Injured	6.6	6.6	9.0
E. sideroxylon	Clear	2.7	3.9	4.1
	Injured	3.4	4.3	4.4

a Blocks incubated for eight weeks in a wood-block/soil-jar test

b Solutions (5 ml) contained extracts (1%), asparagine (0.3%) and glucose (1.0%)
Growth in control (extractives free) solutions: *C. versicolor* 11.3 mg; *T. lilacino-gilva* 14.2 mg. These values are significantly (P = 0.05) greater than all corresponding values in the table

Values of 'susceptibility to decay' the mean of eight replicates. Culture weights the mean of five replicates

In no case do the corresponding values for clear and injured tissues differ significantly

CHAPTER 4. *THE RESPONSES OF SAPWOOD TO INJURY*

Host attributes affecting patterns of decay in a regrowth eucalypt forest IV. The responses of sapwood to injury

by J. Wilkes

Department of Forestry, Australian National University,
G.P.O. Box 4, Canberra, A.C.T. 2601 Australia

Summary

Six months after holes were drilled into stems of *Eucalyptus baneroftii, E. dealbata, E. macrorhyncha* and *E. sideroxylon*, certain properties of discoloured sapwood above and below the wounds were compared with those of clear tissues. During discolouration, as parenchyma cells died and became devoid of starch and fats, most wood properties either altered little [e.g. tissue pH, the proportion of hot water-soluble extractives constituted by phenols, and concentrations of potassium were largely unaffected], or varied in ways not obviously indicative of a protective response by the host [e.g. moisture content and extractives levels often decreased]. However, the resistance to decay *in vitro* by *Trametes lilacino-gilva* was greater in discoloured than in clear sapwood, and in 'clear' tissues adjoining the discoloured regions, tyloses were abundant and the content of polyphenols high. Possibly the tyloses and certain broad spectrum phenolic antibiotics were agents of the highly effective compartmentalisation of the columns of deterioration, which extended only ca. 2-5 cm from the holes. The compartmentalisation was not influenced appreciably by tree vigour or wood anatomy.

Keywords: Compartmentalisation of decay, tissue responsiveness, sapwood, wounding, wood properties, tree decay, decay patterns, *Eucalyptus*

Introduction

In the living trees of many species which form heartwood, the sapwood is more resistant to stem decay than is the heartwood (Shain 1979). The sapwood, although largely devoid of those extractives which confer natural decay resistance in heartwood (Scheffer and Cowling 1966), contains living parenchyma cells and is believed to respond actively to injury and microbial invasion (Hillis and Inoue 1968; Shain 1971; Shortle 1979). Such a responsiveness may limit the development of deterioration in the stem subsequent to wounding of the sapwood, and further, prevent heartrot organisms from progressing centrifugally into the sapwood. Unless first killed by other means such as removal of bark, the sapwood of the eucalypts examined in this series (Wilkes 1985a), and that of *Eucalyptus deglupta* Blume (Davidson 1974), is rarely extensively invaded by microbes. This paper reports the responsiveness to wounding of the sapwood of certain eucalypts.

Materials and Methods

Sample collection

Four pairs of ca. 40 yr old coppice stems were selected from each of the four species under study in this series viz. *E. bancroftii* (Maid.) Maid. (Bancroft's red gum), *E. dealbata* A. Cunn. ex Schau. (tumbledown red gum), *E. macrorhyncha* F. Muell. ex Benth. (red stringybark) and *E. sideroxylon* A. Cunn. ex Woolls (red ironbark). While a considerable difference in diameter at breast height over bark (15-20, 25-30 cm) occurred between individuals of a pair of coppice stems, unhealthy trees e.g. those with small crowns or

supporting large amounts of mistletoe, were excluded. In mid summer, during a relatively normal growing season, a hole 16 mm in diameter was drilled to the outer heartwood at breast height in the four cardinal directions in each of the chosen stems. After six months, a 30 cm bolt, centred at breast height, was removed from each stem, wrapped in plastic and retained at 4°C for the 1-2 weeks prior to laboratory sampling.

Distribution of discolouration

The extension of regions of discolouration (stain) in the radial-longitudinal plane through drill holes orientated in the east/west direction in the standing trees was recorded. Subsequently the samples were cut transversely at varying distances from the drill holes to reveal the shape of discolouration columns in cross-section. For the purpose of photographing regions of discolouration, several *E. melliodora* A. Cunn. ex Schau. (yellow box) stems, wounded as above, were felled and dissected, and appropriate sections sanded smooth when air dry. The areas of discolouration in *E. melliodora* were of a similar shape to those in the study species, but were darker, and hence more suitable for black and white photography.

Anatomy

Longitudinal and transverse surfaces (produced above) were cleaned with a scalpel and examined at 40X magnification to assess the frequency and distribution of tyloses, and the degree of discolouration of the various cell types. For discoloured wood, the percentage of vessels (n = 200) containing tyloses visible on a transverse face bisecting each column, was noted. The percentage volumes of parenchyma (vertical and ray) and vessels in a 15 µm

cross-section cut from the middle sapwood above and below each area of discolouration were determined using a point-count technique with a projection microscope (Wilkes and Abbott 1983). The outside tangential diameters of 80 randomly selected vessels [S.E. of mean < 5%] in each section were measured (100X) employing an eyepiece micrometer. Since the eucalypts studied are diffuse porous and contain vertical parenchyma only in diffuse and paratracheal forms, the exact radial positioning of the thin sections was of little consequence.

Histochemistry

Freehand, radial-longitudinal thin sections cut from various positions in clear and discoloured wood were stained, using the techniques of Jensen (1962), in either:

- an aqueous solution of potassium-iodide/iodine (2.0, 0.2%) - black colouring indicating the presence of starch;
- a 70% ethanol solution saturated with Sudan black B - residual black colouration after differentiation with 50% ethanol indicating the occurrence of fats, oils, waxes, free fatty acids and/or phospholipids; or
- a 1% aqueous solution of ferric chloride - blue or black colouring in this case suggesting the presence of polyphenols.

Blocks, ca. 3 x 3 x 10 mm (10 mm longitudinally), of clear and discoloured tissues were placed in 1% triphenyl tetrazolium chloride (TTC) overnight. Parenchyma cells showing pink colouration on sectioning of the samples were regarded as actively metabolising i.e. living (Wardell and Hart 1970).

Wood properties and microbial condition

Four pairs of blocks, each ca. 1 x 1 x 2 cm (2 cm radially), were cut from the sapwood adjacent to the two holes on the north-south axis in each tree. Pairs consisted of a discoloured block from immediately above or below a drill hole, and a side-matched clear sample (3 cm circumferentially removed). Following the methods of Wilkes (1985b), tissue density, moisture content and microbial condition [the presence or absence of decay (hymenomycetous) and nondecay organisms] were determined using one block pair. The resistance to decay by *Trametes lilacino-gilva* (Berk.) Lloyd DFP 1109, of a further pair, was assessed in a six week soil-jar decay test (Wilkes 1985c), while the remaining samples were ground for determination of pH and mineral levels (Wilkes 1985b), and extractives content. The latter was taken as the percentage loss in weight when 1.0 g of sawdust was held in a porous tissue bag in boiling water for 2 hrs. The proportion of phenols in the hot water-soluble materials was assessed by the Folin-Denis technique (Swain and Hillis 1959).

Statistical analysis

For each species, tylosis abundance and the anatomical properties were correlated against the vertical extent of discolouration employing least squares linear regression. Changes in wood properties during the discolouration were assessed statistically using the t test for paired observations [8 trees, 2 wood conditions]. This test was used also to compare such changes, and the vertical extent of discolouration, between the small and large trees of each species [4 tree pairs, 2 trees/pair]. The effect of rate of growth on the anatomy of these eucalypt species was not analysed in detail here since it has been examined previously (Wilkes and Abbott 1983).

Results

Distribution of discolouration

Drill holes had rarely healed completely, and in all species, sapwood tissues immediately above and below the holes were discoloured grey-brown (Figs. 1, 2). In this area, rays and tyloses were commonly a prominent red-brown colour. A tangential extension of discolouration from the holes of 1-2 mm contrasted with vertical extensions which increased from 1-5 cm in the outer sapwood extant at wounding, to 1.5-7 cm near the heartwood-sapwood boundary. The vertical extent of discolouration in the mid sapwood region was significantly ($P = 0.05$) greater in red stringybark [mean: 43 mm] than in the other eucalypts [means: 20-25 mm] (Table 1), but did not differ significantly between the large and small stems of any species. The basipetal and acropetal extensions of stain were of similar size and general appearance. Sapwood formed after wounding had not discoloured.

Anatomy and discolouration

While tyloses were plentiful in most areas of discoloured sapwood (Table 1), particularly those distal to the wounds, their frequency reached a maximum, with the structures often packing vessels, in 'clear' tissues adjoining regions of discolouration. This region of abundant tyloses extended 1-3 mm tangentially beyond the boundaries of discolouration; the axial extension varied between 5 and 10 mm in *E. macrorhyncha*, and between 15 and 50 mm in the other species. As in discoloured wood, tyloses in this area were often a red-brown colour, in which case vessel lines were pronounced on clean longitudinal surfaces. Clear wood further removed from the discolouration contained few tyloses.

In species other than *E. sideroxylon*, the length of columns of discolouration was significantly and negatively associated with the abundance of tyloses in discoloured wood (r^2 = 0.52-0.61, Table 1). However, correlations between the extent of discolouration and percentage volume of vessels (r^2 = 0.01-0.36), percentage volume of parenchyma (r^2 = 0.06-0.31) and mean vessel diameter (r^2 = 0.02-0.12) were not significant.

Histochemistry

The ferric chloride stain typically gave a stronger (darker) reaction for parenchyma cells (in sections) from normal, than from discoloured sapwood. However, the most intense staining occurred in tissues from a 'marginal zone' extending ca. 1 mm tangentially and 2-5 mm axially beyond the discoloured sapwood i.e. into the zone of abundant tyloses (Figs. 1, 2). The coloured tyloses in discoloured and adjacent tissues blackened in the presence of iron. Starch grains were plentiful in (the parenchyma of) normal sapwood, less common in the marginal zone of high polyphenol content, and rare in discoloured tissues. While the stain for fats was invariably weak, the occurrence of these materials appeared to follow the pattern of starch. The TTC test indicated that living cells were present only in tissues which had not discoloured.

Wood properties

In most species, moisture content (exception *E. sideroxylon*) and levels of extractives (exception *E. dealbata*) were significantly lower in discoloured than clear wood [mean decreases: 10-16%, 6-9 mg cm^{-3} respectively] (Table 2). Susceptibility to decay by *T. lilacino-gilva* was consistently greater in clear [mean weight losses:

9.0-15.8%] than in stained [5.5-9.0%] tissues. Discoloured sapwood contained significantly elevated [increase: 20-260 μg cm^{-3}] concentrations of magnesium (all species), calcium (three species) and manganese (*E. dealbata* and *E. macrorhyncha*). Basic density, pH, the proportion of the water-soluble matter constituted by phenols, and concentrations of sodium and potassium varied little and/or inconsistently between the two types of tissue (Table 2). Changes associated with injury in any wood property did not differ significantly between the small and large stems of a particular species.

Microbial condition

Nondecay (nonhymenomycetous) fungi, bacteria, and occasional decay fungi and actinomycetes, were isolated from the samples of discoloured sapwood, but not from other tissues.

Discussion

Six months after wounding, regions of discolouration above and below drill holes in the sapwood of these *Eucalyptus* spp. failed to exceed ca. 5 cm in length in the middle sapwood (Table 1); columns frequently extend ca. 10-50 cm from bore holes in deciduous hardwoods (Hepting et al. 1949; Toole and Grammage 1959; Armstrong et al. 1981). Since areas of discolouration associated with older injuries to sapwood of the eucalypts are also less than ca. 5 cm in length (Wilkes 1985a), it seems that such deterioration does not extend appreciably prior to inclusion in heartwood. This is supported by the finding that the abundance of tyloses is consistently at a maximum adjacent to, but not within, the columns of stained tissue

i.e. the discolouration does not envelop the zone of intense tylosis formation. It thus appears that the volume of deterioration is determined largely by inherent wood properties or initial responses of the host to injury and infection, and does not reflect a degree of success of microbial invasion.

In a *Populus* hybrid (Eckstein et al. 1979) and *Betula papyrifera* Marsh. (Bauch et al. 1980), discolouration spreads most extensively in trees containing large vessel volumes and/or vessels of large diameter. Possibly the absence of such a relationship here (Table 1) reflects an overriding influence of tyloses; the height of columns of discolouration is negatively correlated (sig. P = 0.05, three of the four species) with the abundance of tyloses in discoloured wood, and discolouration is most extensive in *E. macrorhyncha*, the species in which fewest tyloses are formed. The 'sealing off' of injured or infected sapwood by tyloses (e.g. Klein 1923; Zycha 1948) would depend substantially on the physiological condition of ray parenchyma cells e.g. it is likely that the greater extension of discolouration in inner relative to outer sapwood may be partly a consequence of the ageing of parenchyma cells in normal sapwood (Sucoff et al. 1967; Kile and Wade 1975), reducing the rapidity and frequency of tylosis formation after wounding. Thus the influence of wood anatomy on volumes of discolouration in the eucalypt sapwoods is probably secondary to that of cell physiology.

The withdrawal of food reserves as sapwood tissues die slowly is well documented (e.g. Wardell and Hart 1970; Shigo and Hillis 1973; Worrall and Parmenter 1982). Presumably the majority of such foods would be soluble in hot water, and thus the lower quantities of these materials in discoloured than clear sapwood would contribute to the

parallel trend in extractives content evident in certain of the eucalypts (Table 2). It is also possible that in the eucalypts studied, as in *Malus* (Kile and Wade 1975), some of the extraneous substances present in discoloured sapwood e.g. the materials darkening ray cells and tyloses, could be largely unextractable, further reducing the measured extractives content of this tissue.

The increased natural decay resistance of discoloured tissue, recorded here (Table 2) and elsewhere for certain deciduous hardwoods (Hossfeld et al. 1957; Hart and Johnson 1970; Kile and Wade 1975), may indicate the production of antibiotics by the host, although other possibilities exist e.g. it could reflect the absence of foods readily assimilated by microbes, impedance to fungal penetration by tyloses, and/or the effects of microbes present in discoloured wood *in vivo* (Kile and Wade 1975). Indeed, neither the total nor phenolic extractive contents are increased in the discoloured sapwood of the study eucalypts (Table 2). Possibly, as wounded sapwood tissues die in these species, antimicrobial extractives are produced, but these are subsequently utilised or altered by the microbes resident in discoloured wood (Siegle 1967; Shortle et al. 1971). However, it seems more likely that the regions of discoloured sapwood examined were tissues killed relatively rapidly after injury, preventing the formation of large quantities of extraneous material. Such a production of extractives has apparently occurred in the marginal zone which is 'sheltered' from the external environment by the abundant tyloses. Natural decay resistance is probably at a maximum in this zone (see Hossfeld et al. 1957; Shain 1971; Shortle 1979).

The discolouration of sapwood of these eucalypts is not accompanied by marked increases in moisture content, levels of potassium and pH (Table 2), such as may occur preceding or during deterioration in deciduous hardwoods (Good et al. 1955, 1968; Hart 1968; Shigo and Sharon 1970; Safford et al. 1974). While changes in these properties may not be essential for effective compartmentalisation of deterioration in either the eucalypts or the deciduous hardwoods, they serve as useful indicators of a host response in the latter woods. The increased contents of calcium, magnesium and/or manganese in discoloured sapwood, both in certain of these eucalypts (Table 2), and in other hardwoods such as *Acer* (Good et al. 1955; Safford et al. 1974), could be of host or microbial origin (see Wilkes 1985b).

The resistance of living tissues to microbial deterioration *in vivo* is probably a multifactor phenomenon (Kuć and Shain 1977; Hart 1981). Of the factors considered here, only tyloses and polyphenolic compounds would seem likely to be significant in the defence system of the eucalypts. The functions of these two agents could be complementary. The abundant tyloses present in the marginal zone between clear and discoloured wood may, as suggested for wound gums (Kile and Wade 1975), directly (e.g. by physical obstruction) and/or indirectly (e.g. by limiting movement of gases and liquids) resist the longitudinal progression of infection, and so act as 'Wall 1' in the model proposed to explain compartmentalisation of decay in trees (Shigo and Marx 1977). Spread of microbes in the transverse direction in the eucalypt sapwoods is possibly restricted by antimicrobial phenols produced in parenchyma cells e.g. a concentration of such compounds in rays would retard tangential progression of deterioration. These protective responses of the

eucalypt parenchyma are apparently little affected by tree vigour, contrasting with other observations (Shigo and Hillis 1973; Armstrong et al. 1981) suggesting that trees growing rapidly are capable of more effective compartmentalisation than those of lower vigour.

Conclusions

It is likely that the effective compartmentalisation of discolouration and infection in the sapwood of living trees of four eucalypt species (Wilkes 1985a) reflects, in part, the production of antibiotic materials and tyloses by host parenchyma cells dying in response to injury and/or microbial invasion.

Acknowledgements

The advice of Drs W.A. Heather and W.E. Hillis is appreciated.

References

Armstrong, J.E., A.L. Shigo, D.T. Funk, E.A. McGinnes and D.E. Smith. 1981. A macroscopic and microscopic study of compartmentalization and wound closure after mechanical wounding of black walnut trees. Wood and Fiber 13, 275-291.

Bauch. J., A.L. Shigo and M. Starck. 1980. Wound effects in the xylem of *Acer* and *Betula* species. Holzforschung 34, 153-160.

Davidson, J. 1974. Decayed wood in living trees of *Eucalyptus deglupta* Blume. Papua New Guinea Dept. Forests, Trop. For. Res. Note SR 18, 31pp.

Eckstein, D., W. Liese and A.L. Shigo. 1979. Relationship of wood structure to compartmentalization of discolored wood in hybrid poplar. Can. J. For. Res. 9, 205-210.

Good, H.M., J.T. Basham and S.D. Kadzielawa. 1968. Respiratory activity of fungal associations in zones of heart rot and stain in sugar maple. Can. J. Bot. 46, 27-36.

Good, H.M., P.M. Murray and H.M. Dale. 1955. Studies on heartwood formation and staining in sugar maple, *Acer saccharum* Marsh. Can. J. Bot. 33, 31-41.

Hart, J.H. 1968. Morphological and chemical differences between sapwood, discolored sapwood, and heartwood in black locust and Osage orange. For. Sci. 14, 334-338.

Hart, J.H. 1981. Role of phytostilbenes in decay and disease resistance. Annu. Rev. Phytopathol. 19, 437-458.

Hart, J.H. and K.C. Johnson. 1970. Production of decay-resistant sapwood in response to injury. Wood Sci. Technol. 4, 267-272.

Hepting, G.H., E.R. Roth and B. Sleeth. 1949. Discolorations and decay from increment borings. J. For. 47, 366-370.

Hillis, W.E. and T. Inoue. 1968. The formation of polyphenols in trees - IV. The polyphenols formed in *Pinus radiata* after *Sirex* attack. Phytochem. 7, 13-22.

Hossfeld, R.L., J.C. Oberg and D.W. French. 1957. The appearance and decay resistance of discolored aspen. For. Prod. J. 7, 378-382.

Jensen, W.A. 1962. Botanical histochemistry. Freeman and Co., San Francisco. 408pp.

Kile, G.A. and G.C. Wade. 1975. *Trametes versicolor* on apple II. Host reaction to wounding and fungal infection and its influence on susceptibility to *T. versicolor*. Phytopath. Z. 82,1-24.

Klein, G. 1923. Zur Ätiologie der Thyllen. Z. Botan. 15, 417-439.

Kuć, J. and L. Shain. 1977. Antifungal compounds associated with disease resistance in plants. In: Siegel, M.R. and H.D. Sisler (eds). Antifungal compounds. Vol. 2. Marcel Dekker, New York. pp.497-535.

Safford, L.O., A.L. Shigo and M. Ashley. 1974. Gradients of cation concentration in discolored and decayed wood of red maple. Can. J. For. Res. 4, 435-440.

Scheffer, T.C. and E.B. Cowling. 1966. Natural resistance of wood to microbial deterioration. Annu. Rev. Phytopathol. 4, 147-170.

Sucoff, E., H. Ratsch and D.D. Hook. 1967. Early development of wound-initiated discoloration in *Populus tremuloides* Michx. Can. J. Bot. 45, 649-656.

Swain, T. and W.E. Hillis. 1959. The phenolic constituents of *Prunus domestica* I. The quantitative analysis of phenolic constituents. J. Sci. Food Agric. 10, 63-68.

Toole, E.R. and J.L. Grammage. 1959. Damage from increment borings in Bottomland hardwoods. J. For. 57, 909-911.

Wardell, J.F. and J.H. Hart. 1970. Early responses of sapwood of *Quercus bicolor* to mechanical injury. Can. J. Bot. 48, 683-686.

Wilkes, J. 1985a. Host attributes affecting patterns of decay in a regrowth eucalypt forest I. Patterns of natural decay. Holzforschung 38, In Press.

Wilkes, J. 1985b. Host attributes affecting patterns of decay in a regrowth eucalypt forest III. The responses of heartwood to injury and infection. Holzforschung 38, In Press.

Wilkes, J. 1985c. Host attributes affecting patterns of decay in a regrowth eucalypt forest II. The resistance of heartwood to decay *in vitro*. Holzforschung 38, In Press.

Wilkes, J. and D. Abbott. 1983. Influence of the rate of tree growth on the anatomy of eucalypt species. Appita 37, 231-232.

Worrall, J.J. and J.R. Parmenter. 1982. Formation and properties of wetwood in white fir. Phytopathology 72, 1209-1212.

Zycha, H. 1948. Über die Kernbildung und verwandte Vorgänge in Holz der Rotbuche. Forstw. Cbl. 67, 80-109.

Table 1. The association between the vertical extent of discolouration and other properties of the wounded sapwood of four *Eucalyptus* spp.

Tree species	Extent of discoloura-tion[a]	Wood property			
		Abundance of tyloses[b]	Proportions of elements (% vol.)		Vessel diameter
	(mm)	(%)	Vessels	Parenchyma	(μ)
E. bancroftii	25±8[c]	88 (0.61-)[d]	15 (0.25+)	29 (0.06-)	102 (0.11-)
E. dealbata	20±6	83 (0.52-)	20 (0.36+)	18 (0.19-)	107 (0.12+)
E. macrorhyncha	43±9	54 (0.60-)	18 (0.01-)	16 (0.31-)	115 (0.02-)
E. sideroxylon	23±6	95 (0.41-)	16 (0.11-)	15 (0.09+)	114 (0.07+)

a Vertical extension in the middle sapwood. Average of upward and downward extensions from two drill holes per tree

b Percentage of vessels showing tyloses on clean transverse faces bisecting discolouration columns

c 95% confidence interval

d Values in brackets are coefficients of determination (r^2) when wood properties are regressed against vertical extent of discolouration. Values > 0.50 significant (P = 0.05). +,- indicates positive or negative correlation

Each value (not bracketed) the mean for eight trees

Table 2. Properties of discoloured and clear sapwood of four *Eucalyptus* spp.

Tree species	Wood condition	Wood property										
		Basic density ($g\ cm^{-3}$)	Moisture content (%)	Hot water extracts ($mg\ cm^{-3}$)	Phenols (% of water extracts)	pH	Decay susceptibility[a] (% weight loss)	Mineral concentration ($\mu g\ cm^{-3}$)				
								Na	K	Ca	Mg	Mn
E. bancroftii	Clear	0.59	88*	53*	46	4.82	15.8*	15	910	200*	200*	85
	Discoloured	0.57	72	45	43	4.63	9.0	17	650	330	270	78
E. dealbata	Clear	0.69	55*	52	41	4.82	9.0*	36	760	260*	120*	50*
	Discoloured	0.70	44	56	39	4.73	6.0	29	760	520	330	130
E. macrorhyncha	Clear	0.59	73*	37*	34	4.75	9.6*	41	480	120*	58*	26*
	Discoloured	0.60	63	31	38	4.55	5.6	35	390	140	170	51
E. sideroxylon	Clear	0.77	40	47*	33	4.88	9.6*	22	740	330	77*	90
	Discoloured	0.77	39	38	35	4.78	5.5	21	910	370	250	98

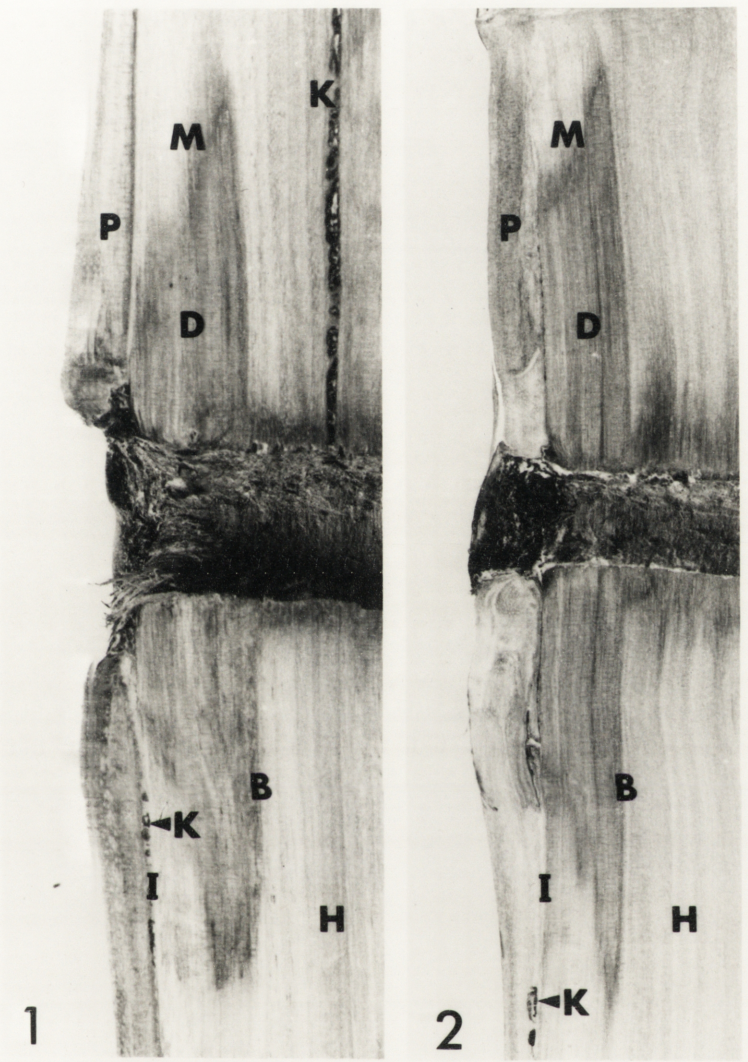
* Wood property significantly (P = 0.05) different between clear and discoloured tissues

a Blocks incubated for six weeks on cultures of *Tramestes lilacino-gilva* in a wood-block/soil-jar test

Each value the mean of eight replicates

Figs. 1 and 2. Discolouration associated with injury in *E. melliodora* stems. P - post-wounding tissues (have tended to darken during drying - clear in freshly cut wood); I - position of cambium at time or injury; D - discoloured sapwood; M - marginal zone (tyloses abundant, content of polyphenols increased); B - heartwood-sapwood boundary; H - heartwood; K - kino veins [either present prior to the artificial injury (top), or produced in response to the injury (bottom)]. Loose-fitting dowels have prevented wound closure in these particular trees. [Drill wounds 16 mm diam].

Fig. 1 - 1.2X
Fig. 2 - 0.8X


K
M
P
D
B
K
I
H
1
M
P
D
B
I
H
K
2

CHAPTER 5. *BARRIER ZONES*

Host attributes affecting patterns of decay in a regrowth eucalypt forest V. Barrier zones

by J. Wilkes

Department of Forestry, Australian National University,
G.P.O. Box 4, Canberra, A.C.T. 2601 Australia.

Summary

Anatomical and chemical characteristics of barrier zones, formed at the bole cambium in response to artificial injury, were examined in *Eucalyptus bancroftii, E. dealbata, E. macrorhyncha* and *E. sideroxylon*. Immediately (< 3 cm) above and below the eight month old drill wounds, zones usually contained abundant, undifferentiated parenchyma cells, while vessels, fibres and identifiable rays were absent. Regions of the undifferentiated cells had often collapsed, and the resulting cavities filled with kino. Barrier zone tissues, which usually included some living cells, were often lowly lignified, and in the absence of kino, had a hot water-soluble extractives content normal for outer sapwood. In both barrier zone and control tissues, phenols comprised ca. 30-40% of the soluble matter, which was not markedly toxic to *Trametes lilacino-gilva* in nutrient solution. Possible sources of effectiveness of the zones, in both sapwood and heartwood, are examined.

Keywords: Barrier zone, kino vein, compartmentalisation of decay, wounding, tree decay, decay patterns, *Eucalyptus*

Introduction

A barrier zone is a thin sheath of abnormal tissue, produced by the cambium of a root, stem or branch in response to injury or infection; it restricts microbes to tissues extant when the cambium is affected (Moore 1978; Shigo and Tippett 1981a; Tippett and Shigo 1981a). Morphologically, the zone may vary e.g. in eucalypts, in contrast to most other genera, the barrier is often macroscopically visible as a kino vein. However, in all cases reported, the zone is effective in limiting the centrifugal progression of discolouration and decay, providing it is not disrupted e.g. by the tunnels of boring insects (Shigo 1966; Wilkes 1985). Despite this importance, the barrier zones of *Eucalyptus* have received little attention; indeed even in those northern hemisphere woods studied in more detail e.g. *Acer* and *Juglans*, the exact source(s) of effectivness of the barriers is not well understood. In this study on regrowth eucalypts, certain anatomical and chemical properties of barrier zones are examined because of the important influence of these zones on patterns of decay within the stems (Wilkes 1985).

Materials and Methods

In January, during a summer of approximately average rainfall, five randomly selected, 40 yr old stems of each of two gum (smooth) barked species [Bancroft's red gum - *E. bancroftii* (Maid.) Maid. and tumbledown red gum - *E. dealbata* A. Cunn. ex Schau.], and two full (rough) barked species [red stringybark - *E. macrorhyncha* F. Muell. ex Benth. and red ironbark - *E. sideroxylon* A. Cunn. ex Woolls] in the second growth forest were injured. At breast height, a 16 mm

diam hole was drilled into the outer sapwood on both the northern and southern sides of each stem. A further hole was drilled into the eastern side of the boles, 2 m above ground level. Eight months later the trees were felled and sample bolts, cut to include the wounds, were stored at 4°C for the 1-3 days prior to use.

Scanning electron microscopy

From each bolt cut at breast height, blocks ca. 5 x 5 x 100 mm (100 mm longitudinally) were sawn from tissues above and below, or to the side of, one drill hole, such that the cambium formed a tangential face of the samples. Most of the blocks were softened by boiling for four days in a 50% glycerol solution, then sliced in the transverse or radial-longitudinal directions at varying distances from the drill hole. A few blocks, which contained obvious kino veins, were not boiled, but split longitudinally, either radially or tangentially, down the region of the vein. Small blocks were stub mounted, dried at 60°C, coated with 20 nm of gold, and examined under a scanning electron microscope (Cambridge Stereoscan 180) operating at 30 kV.

Histochemistry

Blocks ca. 5 x 5 x 10 mm (10 mm axially), positioned to include any wound response barrier zone, were cut from tissues adjacent to the second drill hole in each bolt taken at breast height. Transverse sections (15-20 μm) were microtomed from these and stained for polyphenols [1% aqueous ferric chloride (Jensen 1962)], or for lignin [both the phloroglucinol (Jensen 1962) and safranin/fast-green (Berlyn and Miksche 1976) techniques]. The remnant of each block, still relatively fresh, was placed overnight in a 1% triphenyl tetrazolium chloride solution and subsequently examined for the presence of living cells (pink colouration) in barrier zone tissues.

Analysis of extractives

Using a scalpel, ca. 0.3 g (air dry) of thin shavings were cut from the light coloured band of barrier zone tissue, 0.5-1 mm wide radially, situated immediately (< 3 cm) above and below the drill wound inflicted at the 2 m level. To avoid the sampling of kino veins, it was sometimes necessary to select tissues further removed from the hole. A control sample was prepared from apparently comparable tissues, situated at the same level but to the opposite side of the stem. Shavings were extracted for 2 hrs in 10 ml of boiling water, washed onto tared filter papers, and reweighed. The content of phenols in the extracts was assessed using the Folin-Denis method (Swain and Hillis 1959). Extractives solutions were evaporated to a concentration of 2 mg (dry matter) ml^{-1} i.e. 0.2%, and supplemented with 0.3% asparagine and 1.0% glucose. Five ml of each solution was transferred to a McCartney bottle, sterilised with dry heat (105°C, 1 hr), and inoculated with a 2 mm disc of malt agar supporting actively growing mycelium of the brown rot fungus *Trametes lilacino-gilva* (Berk.) Lloyd, strain DFP 1109. After 14 days, cultures were harvested from these solutions, and from others free of extractives (controls), and weighed air dry on filter papers.

For each tree species, paired t tests were used to compare data for barrier zone and control tissues (2 tissue types, 5 trees).

Results

Scanning electron microscopy

Barrier zone anatomy varied considerably within and between species e.g. in *E. dealbata* and *E. macrorhyncha*, the wound usually resulted

in the formation of a kino vein, while in other species the response was less obvious. However, in all stems the initial observable reaction of the cambium to wounding was the production of undifferentiated (often isodiametric) parenchyma cells of large cross-sectional area (Figs. 1, 2). The width of this band of 'traumatic parenchyma' (Skene 1965) decreased with distance from the wound, being up to 3 mm wide (radially) immediately adjacent to the hole, but not distinguishable 10 cm above or below the wound (Fig. 3), or 3 cm to either side. In *E. dealbata* and *E. macrorhyncha*, portions of the wider bands of parenchyma had collapsed to form a system of circumferentially anastomosing lacunae (Figs. 4, 5) which usually narrowed centrifugally. In unboiled samples, these cavities were filled with kino (Fig. 5), as were the lumens of many of the adjacent parenchyma cells which had maintained structural integrity e.g. those within rays or constituting the bridges across the veins (Figs. 6, 7).

Vessels were absent from the regions of undifferentiated parenchyma, while external to such areas e.g. 1 mm radially, and within less obvious zones, vessel size and frequency were often reduced relative to those in normal tissue (Figs. 1, 3). Vessels internal to the barrier zones, particularly those within 2-3 cm of drill holes i.e. in discoloured sapwood, were often heavily tylosed (Fig. 3). Tyloses were normally sparse in post-wounding tissues. Apparently normal rays transgressed the less distinct barrier zones (Fig. 3), and although ray cells were not distinguishable in regions of traumatic parenchyma (Fig. 2), the same ray was commonly recognisable on both sides of a kino vein. Fibrous elements produced subsequent to wounding closely resembled those internal to the barrier zones. Barrier zone anatomy was similar above and below wounds.

While fungi and bacteria were observed in sapwood internal to the barrier zones and adjacent to drill holes (discoloured wood), tissues within the zone, and external to it, appeared sterile. Fungal mycelia were frequently observed in intimate contact with kino to the inner side of veins (Fig. 8).

Histochemistry

In comparison with surrounding tissues, barrier zones were most commonly lowly lignified but of approximately 'normal' polyphenol content, although for each of these properties, intraspecific variability was pronounced. Kino stained intensely in the presence of iron. Living cells were usually present in the zones.

Analysis of extractives

No significant (P = 0.05) difference occurred between barrier zone and control tissues, in either the content of hot water-soluble matter [ca. 6-8%], or the percentage component of phenols in these extractives [ca. 30-40%] (Table 1). At the concentration tested, the water-soluble matter from both types of tissue usually failed to influence significantly the growth of *T. lilacino-gilva* in nutrient solution [mean culture weights: 11-14 mg] (Table 1).

Discussion

With increasing distance from the wound, the cambial response in both the eucalypts and other woods [e.g. *Acer* (Rier and Shigo 1972); *Quercus* (Phelps and McGinnes 1977)] diminishes rapidly in the lateral, but more slowly in the vertical, direction. Possibly a similar mechanism controls barrier zone formation in most, or all,

tree species; Smith (1980) has suggested that a localised hormonal imbalance may occur in the vascular cambium after wounding.

Skene (1965) has detailed the stages of formation of kino veins at the cambium of *E. obliqua* L'Herit. Fully developed kino veins in this species, and those in *E. camaldulensis* Dehnh. (Day 1959) and *E. maculata* Hook. (Jacquiot and Hervet 1954), closely resemble those described here. Also in agreement with the present observations, vessel size and/or frequency are commonly reduced in the barrier zones of deciduous hardwoods e.g. *A. rubrum* L. (Mulhern et al. 1979; Bauch et al. 1980) and *Q. coccinea* Muenchh. (Kandeel and McGinnes 1970), while in the zones of many genera, including *Juglans* (Phelps and McGinnes 1980; Smith 1980), *Liquidamber* (Moore 1978) and *Ulmus* (Shigo and Tippett 1981b; Tippett and Shigo 1981a), the quantity of parenchyma increases. However, the hypertrophy of ray cells, characteristic of the barrier zones of genera such as *Acer* (Sharon 1973; Mulhern et al. 1979; Bauch et al. 1980), contrasts with the presence in eucalypts of apparently normal rays in the smaller zones, and the absence of recognisable rays from kino veins. The initiation of new rays in relatively narrow barrier zones in the stems (Bauch et al. 1980) and roots (Tippett and Shigo 1981b) of certain tree species is not an obvious feature of the zones in eucalypts.

Studies of barrier zones have generally failed to differentiate those characteristics which have a protective significance from others which lack such a function. Examples highlight this situation:

1. The increased or qualitatively altered lignification of barrier zone tissues recorded for certain species [e.g. *A. rubrum*, *Betula papyrifera* Marsh. (Bauch et al. 1980); *J. nigra* L. (McGinnes

et al. 1971); *L. styraciflua* L. (Moore 1978)] is not likely to be important in the long term resistance of barrier zones to penetration by microbes such as white rot decay fungi (see Kirk 1971). In the trees examined here, lignification of cell walls in the barrier zones was frequently minimal eight months after wounding, but microorganisms present in discoloured sapwood adjacent to drill holes failed to invade post-wounding tissues.

2. The significance of the inclusions of extraneous materials within barrier cells (Gerry 1921; McGinnes 1975; McGinnes et al. 1977; Tippett and Shigo 1981b) is unknown. Kino is not toxic to certain fungi (Fig. 8), and is absent from many effective barrier zones in eucalypts, suggesting that it is not of primary importance in determining the resistance of the barriers to penetration by microbes.

3. While the anatomy of barrier zones has been commonly studied, the influence of wood anatomy and/or density on decay resistance is typically minor and transient (Southam and Ehrlich 1943; Kurmar 1971); in durable woods the influence is very much secondary to that of extractives. Thus it is unlikely that the effectiveness of barrier zones is primarily dependent on unusual gross structural characteristics of zone tissues. Indeed, Moore (1978) has noted that barrier zones may be effective at distances from wounds beyond which any anatomical response is detectable.

The eucalypt barrier zones studied did not contain abnormally large quantities of phenols or other extraneous material soluble in water (Table 1). The staining for polyphenols gave a similar result. Further, the materials extractable in hot water generally had little effect on the growth of *T. lilacino-gilva* in nutrient solution.

Possibly, in the sapwood of *Eucalyptus*, living cells within barrier zones produce minute quantities of antibiotics in localised areas i.e. at the point of contact by microbes. Alternatively, Tippett and Shigo (1981a) suggest that living elements of barrier zones may limit the centrifugal development of deterioration by restricting the outward diffusion of toxic materials produced by fungi or dying host tissues. Regardless of such possibilities, only those organisms with some parasitic ability could penetrate a zone of living tissue. The absence of living cells from barrier zones included in heartwood would presumably preclude an active protective response of the regions; hence their effectiveness (e.g. Wilkes 1985) is attributable to other factors. Perhaps antimicrobial agents, such as toxins or enzymes, are deposited in barrier zones either cumulatively over the years prior to heartwood formation, or mainly during the sapwood-heartwood transition, such that an elevated concentration of these materials within heartwood zones would partially compensate for the absence of living (responsive) cells.

Conclusions

Barrier zones in the eucalypts studied have anatomical and chemical features in common with, and distinct from, the zones of other hardwood species. The cause(s) of effectiveness of the regions as barriers to decay in eucalypt stems (Wilkes 1985) is speculative.

Acknowledgements

The advice of Dr W.A. Heather is appreciated.

References

Bauch, J., A.L. Shigo and M. Starck. 1980. Wound effects in the xylem of *Acer* and *Betula* species. Holzforschung 34, 153-160.

Berlyn, G.P. and J.P. Miksche. 1976. Botanical microtechnique and cytochemistry. Iowa State Uni. Press, Ames. 326pp.

Day, W.R. 1959. Observations on eucalypts in Cyprus I. The character of gum veins and anatomical indications for their origin. Emp. For. Rev. 38, 35-44.

Gerry, E. 1921. American storax production: results of different methods of tapping red gum trees. J. For. 19, 15-24.

Jacquiot, C. and N. Hervet. 1954. Contribution à l'étude des "poches à kino" des *Eucalyptus*. Rev. Path. Vég. Ent. Agric. Fr. 33, 199-207.

Jensen, W.A. 1962. Botanical histochemistry. Freeman and Co., San Francisco. 408pp.

Kandeel, S.A. and E.A. McGinnes. 1970. Ultrastructure of ring shake in scarlet oak (*Quercus coccinea*, Muench). Wood Sci. 2, 171-178.

Kirk, T.K. 1971. Effects of microorganisms on lignin. Annu. Rev. Phytopathol. 9, 185-210.

Kumar, S. 1971. Causes of natural durability in timber. J. Timb. Dev. Assoc. India. 18, 1-15.

McGinnes, E.A. 1975. Response of black walnut to environmental mishap. Annu. Rep. Northern Nut Growers Assoc. 66,102-107.

McGinnes, E.A., C.I-J. Chang and K.Y-T. Wu. 1971. Ring shake in some hardwood species: the individual tree approach. J. Polymer Sci. (Part C - symposia) 36, 153-176.

McGinnes, E.A., J.E. Phelps, P.S. Szopa and A.L. Shigo. 1977. Wood anatomy after tree injury - a pictorial study. Uni. Missouri Columbia, Agric. Exp. Stn. Res. Bull. No. 1025, 35pp.

Moore, K.E. 1978. Barrier-zone formation in wounded stems of sweetgum. Can. J. For. Res. 8, 389-397.

Mulhern, J., W. Shortle and A. Shigo. 1979. Barrier zones in red maple: an optical and scanning microscope examination. For. Sci. 25, 311-316.

Phelps, J.E. and E.A. McGinnes. 1977. Anatomical responses to basal injury in white and black oak. Wood Sci. 10, 15-21.

Phelps, J.E. and E.A. McGinnes. 1980. Discolorations and streaks in black walnut - a microscopic evalution. In: Bauch, J. (ed). Natural variations of wood properties. Proc. IUFRO Working Party S.5.01-02, All Div. 5 Conf., Oxford. pp.99-111.

Rier, J.P. and A.L. Shigo. 1972. Some changes in red maple, *Acer rubrum*, tissues within 34 days after wounding in July. Can. J. Bot. 50, 1783-1784.

Sharon, E.M. 1973. Some histological features of *Acer saccharum* wood formed after wounding. Can. J. For. Res. 3, 83-89.

Shigo, A.L. 1966. Decay and discoloration following logging wounds on northern hardwoods. U.S. Dept. Agric. Forest Service, Res. Pap. NE-47, 43pp.

Shigo, A.L. and J.T. Tippett. 1981a. Compartmentalization of decayed wood associated with *Armillaria mellea* in several tree species. U.S. Dept. Agric. Forest Service, Res. Pap. NE-488, 20pp.

Shigo, A. and J.T. Tippett. 1981b. Compartmentalization of American elm tissues infected by *Ceratocystis ulmi*. Plant Disease 65, 715-718.

Skene, D.S. 1965. The development of kino veins in *Eucalyptus obliqua* L'Herit. Aust. J. Bot. 13, 367-378.

Smith, D.E. 1980. Abnormal wood formation following fall and spring injuries in black walnut. Wood Sci. 12, 243-251.

Southam, C.M. and J. Ehrlich. 1943. Decay resistance and physical characteristics of wood. J. For. 41, 666-673.

Swain, T. and W.E. Hillis. 1959. The phenolic constituents of *Prunus domestica* I. The quantitative analysis of phenolic constituents. J. Sci. Food Agric. 10, 63-68.

Tippett, J.T. and A.L. Shigo. 1981a. Barrier zone formation: a mechanism of tree defence against vascular pathogens. IAWA Bull. 2, 163-168.

Tippett, J.T. and A.L. Shigo. 1981b. Barriers to decay in conifer roots. Eur. J. For. Path. 11, 51-59.

Wilkes, J. 1985. Host attributes affecting patterns of decay in a regrowth eucalypt forest I. Patterns of natural decay. Holzforschung, In Press.

Table 1. Content and properties of water-soluble extractives in barrier zone and control tissues of the sapwood of four *Eucalyptus* spp.

Tree species	Tissues[a]	Content of hot water soluble matter (%)	Phenols (% of soluble matter)	Dry weight of 14 day *Trametes lilacino-gilva* cultures produced in solutions containing wood extracts[b] (mg)
E. bancroftii	Control	7.8	37	12.6
	Barrier zone	8.5	35	11.6*
E. dealbata	Control	7.1	41	14.0
	Barrier zone	6.9	35	13.7
E. macrorhyncha	Control	6.2	29	12.6
	Barrier zone	6.4	32	12.7
E. sideroxylon	Control	6.8	28	11.4*
	Barrier zone	7.1	29	11.3*

[a] Barrier zone and control tissues situated on opposite sides of the stems

[b] Solutions (5 ml) contained extracts (0.2%), asparagine (0.3%) and glucose (1.0%)

Growth in control (extractives free) solution 13.8 mg - significantly (P = 0.05) different from values marked *

All values the mean of five replicates

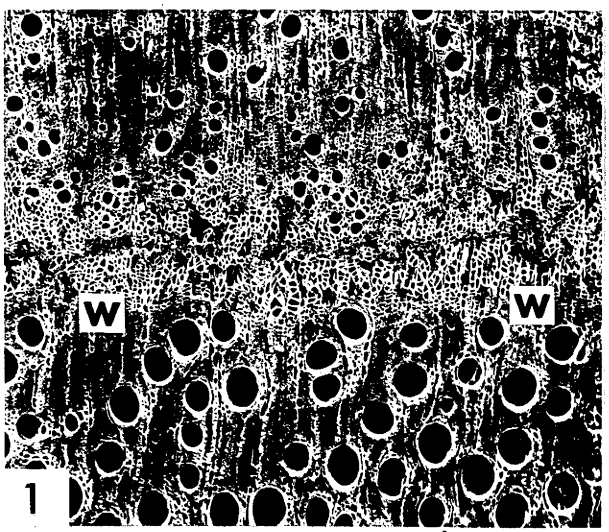
In no case do the corresponding values for barrier zone and control tissues differ significantly

Fig. 1. Undifferentiated parenchyma in *E. sideroxylon*, produced after wounding when the cambium was at position W. Sample cut 1 cm above wound. Post barrier zone tissue to top. [Transverse section (T.S.), 45X].

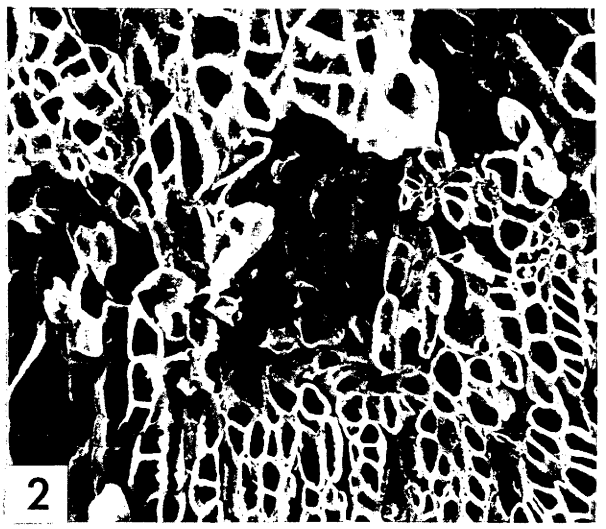
Fig. 2. Traumatic parenchyma in *E. bancroftii*. [T.S., 200X]

Fig. 3. *E. macrorhyncha* barrier zone (BZ) 10 cm above a wound. Zone is difficult to distinguish except for a reduction in vessel size. Pre-wounding tissue (bottom) heavily tylosed. [T.S., 120X].

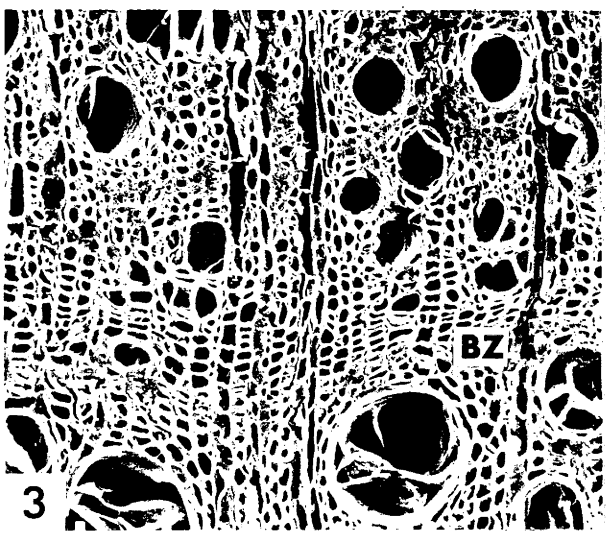
Fig. 4. Kino vein in *E. macrorhyncha*. Parenchyma bridges (PB) extend radially between cavities (formerly) filled with kino. Post barrier zone tissue to top. [T.S., 20X].



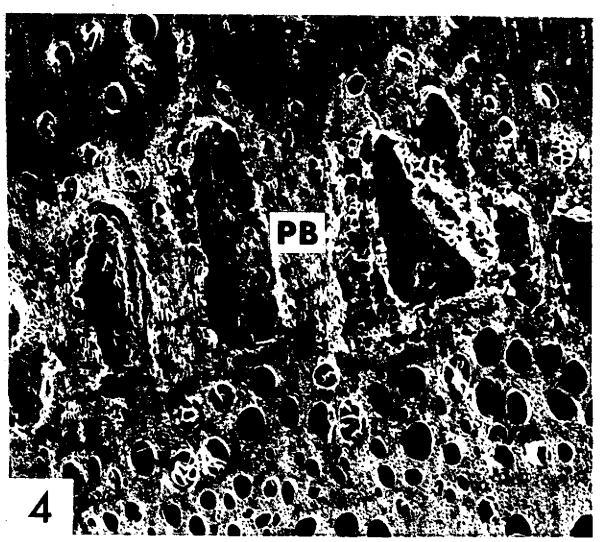
1



2



3



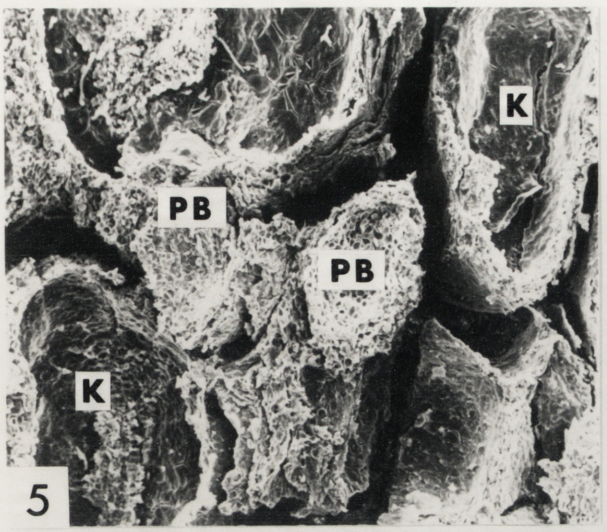
4

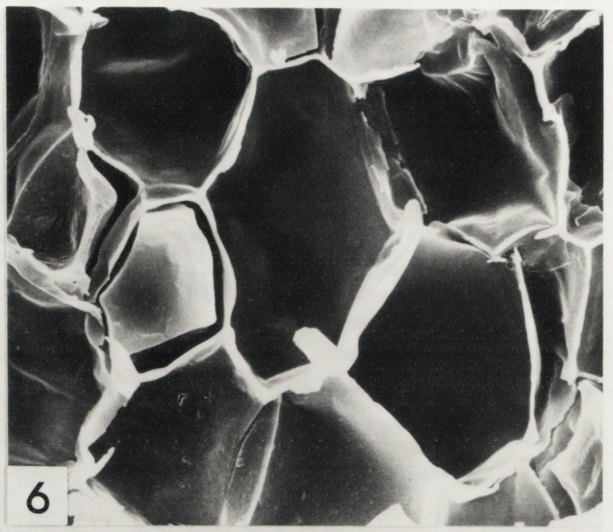
Fig. 5. Longitudinal surface obtained when a kino vein of *E. macrorhyncha* was split tangentially. Parenchyma bridges (PB) are surrounded by masses of kino (K), which have shrunken on drying. [25X].

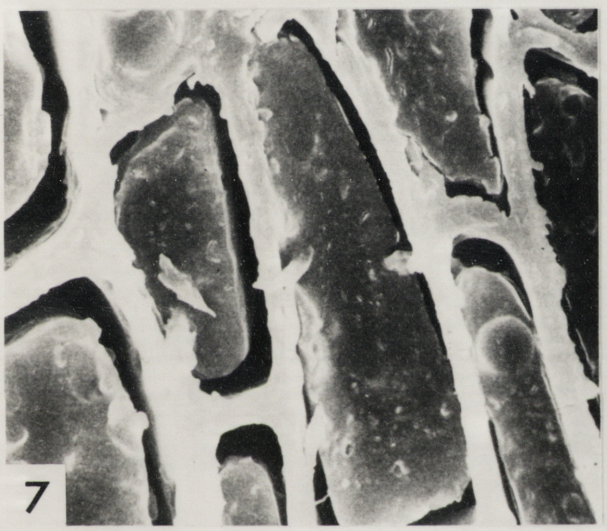
Fig. 6. A parenchyma bridge shown in Fig. 5 at 800X. Kino (or a like substance) is present in one of the cells.

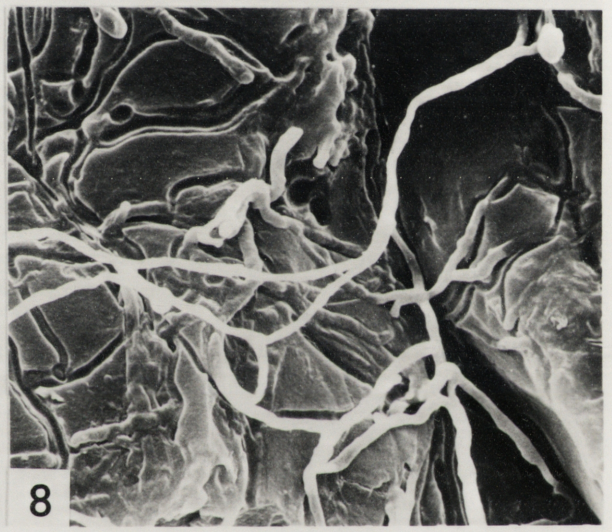
Fig. 7. Kino filling cells in a parenchyma bridge of an *E. dealbata* kino vein. [T.S., 850X].

Fig. 8. Microbe(s) growing in intimate contact with kino in *E. dealbata*. [1000X].


K
PB
PB
K
5


6


7


8

CHAPTER 6. *INCLUDED SAPWOOD*

Host attributes affecting patterns of decay in a regrowth eucalypt forest VI. Included sapwood

by J. Wilkes

Department of Forestry, Australian National University,
G.P.O. Box 4, Canberra, A.C.T. 2601 Australia

Summary

In *Eucalyptus macrorhyncha*, kino veins, produced at the cambium in response to injury, often disrupt the developmental processes of heartwood such that the bulk of sapwood internal to the vein at the time of formation of this barrier zone retains its light colour after inclusion in heartwood. In five stems which contained a distinct region of this 'included sapwood' in the middle heartwood, susceptibility to decay *in vitro* by *Trametes lilacino-gilva* and certain other wood properties of probable relevance to microbial deterioration were assessed for included sapwood, comparable heartwood and normal sapwood. While included sapwood was somewhat less susceptible to decay *in vitro* than normal sapwood, it decayed rapidly in comparison with heartwood. This low natural decay resistance, which was ascribed to a reduced occurrence of fungitoxic phenolic extractives, presumably accounts for the rapid degradation of the pseudo-sapwood *in viva*. Evidence is presented to suggest that kino veins obstruct the centripetal translocation of carbohydrate precursors to heartwood phenols.

Keywords: Included sapwood, kino vein, barrier zone, heartwood formation, wood properties, tree decay, decay patterns, *Eucalyptus*

Introduction

In many species of *Eucalyptus*, sapwood internal to a kino vein forming at the cambium may not darken appreciably on inclusion in heartwood, and conventionally this tissue is referred to as 'included sapwood' (Dadswell and Hillis 1962). Similar regions of included sapwood have been observed internal to other types of abnormal tissue and/or circumferential shakes in conifer species (Frey-Wyssling 1938; Frey-Wyssling and Bosshard 1959; McGinnes et al. 1969). In living trees of the eucalypts examined in this series, such sapwood decays readily, resulting in significant variation from the 'normal' patterns of heartrot (Wilkes 1985a). The present study of *E. macrorhyncha* F. Muell. ex Benth. (red stringybark), a species which produces prominent kino veins, was undertaken to further characterise the included sapwood in relation to its susceptibility to heartrot *in vivo*.

Materials and Methods

In the second growth forest some 40 yrs of age, ca. 50 *E. macrorhyncha* trees were felled in May and the stems dissected to locate kino veins and regions of included sapwood. These were described, measured and photographed in both the radial longitudinal and transverse planes. Stem samples appropriate for the laboratory investigation contained, within the middle heartwood region, a band of cream coloured included sapwood, 10-12 mm wide (radially), situated immediately internal to a visually distinct (> 1 mm wide) kino vein which extended at least 4 cm circumferentially and 10 cm axially. Stems eccentric in cross-section were rejected as likely

to contain tension wood. Five sample biscuits, 15-20 cm in diameter under bark and 10-12 cm in length, were debarked and air dried.

In the laboratory, the biscuits were split diametrically (Fig. 1), and eight, 1 cm thick 'discs' cross-cut from the resulting section which contained the kino vein. On the radius which passed through the centre of the kino vein, six positions were marked on each disc: 1 - inner heartwood (ca. 2 cm from the pith); 2 - inner included sapwood of intermediate colour; 3 - outer included sapwood of a light colour; 4 - heartwood immediately (< 5 mm) external to the kino vein; 5 - outer heartwood (ca. 1 cm from the heartwood-sapwood boundary); and 6 - the central sapwood (Fig. 1). Comparable positions were located on a radius (control) at 90° to that through the vein. A block ca. 10 x 4 x 30 mm (axial, radial, circumferential) was dissected from each of the 12 positions in seven of the discs. Several wood properties were determined using these blocks:

Disc 1. Basic density (mass - oven dry; volume - water displaced when swollen) was assessed for each position.

Discs 2,3. For these discs, each block was bisected radially and the resulting samples cultured for six weeks with *Trametes lilacino-gilva* (Berk.) Lloyd in soil-jar decay chambers (Wilkes 1985b). On the basis of the earlier study (Wilkes 1985b), it was assumed that *T. lilacino-gilva* would give results broadly representative of several decay fungi.

Discs 4,5. A transverse face of each block was sanded using a fine abrasive paper to provide a 0.4 g sample of wood dust for starch determination (Humphreys and Kelly 1961).

Discs 5-7. Blocks from corresponding positions in these discs were grouped and ground to pass a 0.8 mm screen. Approximately 1.2 g of the sawdust from each position was extracted with methanol, and subsequently with 0.1N sodium hydroxide (Rudman and Da Costa 1961). Total extractive was computed as the sum of the methanol- and dilute alkali-soluble materials. The proportion of phenols in the methanol extract was assessed using the Folin-Denis technique (Swain and Hillis 1959). Crude methanol extracts were chromatographed on Whatman No. 1 paper employing the two dimensional system, and chromogenic sprays, described by Hart and Hillis (1974).

Disc 8. (Not dissected). The occurrence of tyloses in vessels at the various positions was noted on examining a cleaned surface of the disc magnified 40X.

For each wood property, data for corresponding positions on the kino vein and control radii were compared using analysis of variance. Interpositional variation for each radius was similarly analysed.

Results

Field observations

Visually distinguishable included sapwood occurred internal to virtually all kino veins in heartwood, regardless of vein dimensions. Thus, some regions of the pseudo-sapwood extended less than 1 cm tangentially (Figs. 2, 3), while others formed a complete ring within the heartwood. Where a tree contained no heartwood [i.e. was less than ca. 5-8 yrs old] at the time of formation of a

kino vein which largely or totally encircled the stem, a core of included sapwood was left surrounding the pith (Fig. 3). The inner boundary of included sapwood was not usually distinct, as the colour of tissues graded from cream-brown (near the kino vein) to the red-brown of normal heartwood. The radial thickness of most of the bands of lighter coloured tissue was ca. 8-12 mm (Figs. 2, 3), contrasting with a thickness of normal sapwood of ca. 15 mm. Only occasionally did the length or circumferential width of a region of included sapwood exceed that of the associated kino vein. In cross-section, the sides of the included regions frequently followed radii contiguous with the tangential extremities of the veins (Fig. 3).

Considerable variation in colour occurred between regions of included sapwood internal to apparently similar kino veins (Fig. 2). However, included sapwood was usually most distinct (of lightest colour) when associated with wide kino veins e.g. those > 2 mm radially. Where parenchyma bridges were absent from a 'kino vein' i.e. where a 'kino pocket' occurred, included sapwood was invariably a very light colour in freshly cut wood. As wood dried, normal heartwood became a lighter colour while included sapwood tended to darken slightly, such that the two tissue types were less distinct and the contrast was insufficient for black and white photography.

Laboratory study

On the control radius i.e. in normal tissues, basic density (Table 1), and the methanol-soluble and total extractives contents (Fig. 4), increased significantly ($P = 0.05$) between inner (Position 1) and outer (Position 5) heartwood. While other wood properties did not vary significantly in the radial direction within this normal

heartwood, the transition from sapwood to heartwood resulted in a significant change in each of the wood features assessed.

In comparison with control tissues 90° (5-8 cm) circumferentially removed, the included sapwood immediately adjoining the kino veins (Position 3) had a significantly lower unextracted (but not extracted) wood density and resistance to decay by *T. lilacino-gilva* (Table 1). The methanol and total extractive contents, and percentage phenol in the methanol extracts were also lower in this included sapwood (Fig. 4). Such differences between control samples and the inner, darker coloured included sapwood on the kino vein radius (Position 2) were less pronounced, with only resistance to decay reduced significantly. Immediately external to the kino veins (Position 4) the methanol-soluble extractives content was significantly increased relative to control tissues. Quantities of materials removed in alkali (after pre-extraction with methanol) and the content of starch did not vary appreciably between radii. For all of these quantitative wood features, included (Position 3) and normal (Position 6) sapwoods differed significantly. Tyloses were abundant in included sapwood and heartwood, but uncommon in sapwood.

Paper chromatography separated the methanol extracts from both heartwood and included sapwood into 12 components, six of which were pronounced e.g. gallic acid and ellagic acid (cochromatography). Only gallic and ellagic acids were prominent in the extracts of normal sapwood, although three other fractions were located.

Discussion

Since, in *Eucalyptus*, the absolute values and normal patterns of radial variation in most of the wood properties examined are well established (e.g. Rudman and Da Costa 1961; Bamber and Humphreys 1965; Hillis 1978; Wilkes and Heather 1983), these aspects are not discussed further.

The significantly (P = 0.05) lower resistance to decay of included sapwood than normal heartwood in a comparable position (Table 1), could account substantially for the excessive degrade of this pseudo-sapwood in the standing trees (Wilkes 1985a). Since the natural decay resistance of the heartwood of moderately durable eucalypts (e.g. *E. macrorhyncha*) is conferred primarily by antimicrobial extractives (Rudman 1964), the lower resistance of included sapwood to decay by *T. lilacino-gilva* is probably explained largely by the reduced quantity of extractives in this tissue (Fig. 4). Also, the lower phenolic content of the methanol-soluble extractives of included sapwood indicates that these differed qualitatively from those in normal heartwood; this difference was not detectable with the chromatographic system used (developed to separate phenolic fractions). Such qualitative variation could contribute further to the reduced natural decay resistance of included sapwood, particularly if the nonphenolic materials are readily utilised as food by microorganisms. The virtual absence of starch [a potential source of nutrition for fungi (Hulme and Shields 1970)], and the abundant tyloses [a possible hindrance to microbial invasion (Hösli and Osuský 1978)], clearly do not confer appreciable natural decay resistance to the included sapwood of *E. macrorhyncha* (Table 1).

The starch content of *E. macrorhyncha* sapwood [8-9 mg cm^{-3} - ca. 1.5% (Table 1)] was probably at a seasonal minimum at the time of sampling i.e. late autumn (Bamber and Humphreys 1965; Humphreys and Humphreys 1966), but it greatly exceeds that recorded for included sapwood and heartwood (ca. 1 mg cm^{-3}). The widely reported loss of starch from parenchyma cells preceding or during heartwood formation (e.g. Chattaway 1952; Frey-Wyssling and Bosshard 1959; Parameswaran and Bauch 1975), may reflect use of this storage material, either directly or indirectly, in the synthesis of phenols (Wardrop and Cronshaw 1962; Hillis 1977). The extraction procedures employed would have removed virtually all 'food' materials from the sapwood; hence the higher content of extraneous matter in heartwood than sapwood (Fig. 4) suggests the importance of continuing centripetal translocation of carbohydrate along rays in the production of phenols at the heartwood-sapwood boundary (Hillis 1977). Kino cavities and the disorganisation of cells within the parenchyma bridges of kino veins (Skene 1965), could disrupt this movement, and so explain the reduced levels of extractives in the included sapwood. Possibly, centripetal migration of carbohydrate present internal to the cambium at the time of wounding accounts for both the tendency towards higher levels of extractives in the inner than outer included sapwood (Fig. 4), and the reduced thickness of included compared with normal sapwood i.e. the conversion to heartwood of the innermost sapwood present at the time of kino vein formation. The blocking effect of the vein, when still in sapwood, may cause an accumulation of carbohydrates immediately external to the barrier, resulting in the formation of relatively large quantities of certain extractives in this region during its conversion to heartwood (Fig. 4).

In the present study, the properties of included sapwood generally resemble more closely those of heartwood than those of sapwood (Table 1, Fig. 4). Indeed, for all quantitative wood properties examined, there is a significant difference between the values for true and for included sapwood. In this sense, 'included sapwood' is not an appropriate term (at least in the case of *E. macrorhyncha*), but the colour factor will ensure its continued use.

The pale coloured bands of tissue in the heartwood of species such as *Pseudotsuga menziesii* (Mirb.) Franco (Kennedy and Wilson 1956), *Thuja plicata* Donn ex D.Don (MacLean and Gardner 1958) and *E. marginata* Donn ex Sm. (Hillis 1956; Dadswell and Hillis 1962), forming target or moon ring patterns not obviously associated with stem wounding, contain less extractives than comparable heartwood. This form of 'included sapwood' may have much in common with that in *E. macrorhyncha* e.g. in agreement with the present findings, the content of phenols and natural decay resistance of the light coloured zones of *T. plicata* are exceptionally low in comparison with normal heartwood (Scheffer 1957; MacLean and Gardner 1958). The causes of formation of such zones, in the absence of obvious cambial injury, are speculative (McGinnes et al. 1969; Jane 1970).

Conclusions

In comparison with adjacent heartwood, included sapwood in *E. macrorhyncha* has a lower, and qualitatively altered content of extractives, apparently the cause of an appreciable reduction in resistance to decay. This decay susceptibility allows rapid deterioration of included sapwood in the standing trees. These findings probably extend to other species studied in this series [*E.*

Hart, J.H. and W.E. Hillis. 1974. Inhibition of wood-rotting fungi by stilbenes and other polyphenols in *Eucalyptus sideroxylon*. Phytopathology 64, 939-948.

Hillis, W.E. 1956. Leucoanthocyanins as the possible precursors of extractives in woody tissues. Aust. J. Biol. Sci. 9, 263-280.

Hillis, W.E. 1977. Secondary changes in wood. In: Loewus, F.A. and V.C. Runeckles (eds). Recent advances in phytochemistry. Vol. 11. Plenum Press, New York. pp.247-309.

Hillis, W.E. 1978. Wood quality and utilization. In: Hillis, W.E. and A.G. Brown (eds). Eucalypts for wood production. CSIRO Press, Australia. pp.259-289.

Hösli, J.P. and A. Osuský. 1978. The behaviour of beech red heart boundary zone plugged by tyloses under decomposition by *Coniophora puteana* (Schum.) Fr. and *Trametes versicolor* (L. ex Fr.) Pilat. Mater. Org. 13, 51-58.

Hulme, M.A. and J.K. Shields. 1970. Biological control of decay fungi in wood by competition for non-structural carbohydrates. Nature 227, 300-301.

Humphreys, F.R. and N. Humphreys. 1966. Starch levels in flooded gum sapwood. Aust. For. Res. 2, 35-40.

Humphreys, F.R. and J. Kelly. 1961. A method for the determination of starch in wood. Anal. Chim. Acta 24, 66-70.

Skene, D.S. 1965. The development of kino veins in *Eucalyptus obliqua* L'Herit. Aust. J. Bot. 13, 367-378.

Swain, T. and W.E. Hillis. 1959. The phenolic constituents of *Prunus domestica* I. The quantitative analysis of phenolic constituents. J. Sci. Food Agric. 10, 63-68.

Wardrop, A.B. and J. Cronshaw. 1962. Formation of phenolic substances in the ray parenchyma of angiosperms. Nature 193, 90-92.

Wilkes, J. 1985a. Host attributes affecting patterns of decay in a regrowth eucalypt forest I. Patterns of natural decay. Holzforschung 38, In press.

Wilkes, J. 1985b. Host attributes affecting patterns of decay in a regrowth eucalypt forest II. The resistance of heartwood to decay *in vitro*. Holzforschung 38, In press.

Wilkes, J. and W.A. Heather. 1983. Correlation of resistance to a pulsed current with several wood properties in living eucalypts. N.Z. J. For. Sci. 13, 139-145.

Table 1. Properties of sapwood, heartwood and included sapwood in *Eucalyptus macrorhyncha*

Radial position	Wood property		
	Unextracted basic density ($g\ cm^{-3}$)	Decay susceptibility[a] (% weight loss)	Starch content ($mg\ cm^{-3}$)
	b / c	b / c	b / c
Inner heartwood	0.62 / 0.62	5.0 / 4.8	1.1 / 1.0
Inner included sapwood	0.61 / 0.62	8.4 / 3.5*	1.0 / 1.0
Outer included sapwood	0.62 / 0.65*	18.6 / 2.9*	0.9 / 1.0
Heartwood bounding kino vein	0.68 / 0.68	2.4 / 2.8	1.1 / 0.9
Outer heartwood	0.67 / 0.67	3.1 / 3.8	1.0 / 1.1
Sapwood	0.54 / 0.54	26.7 / 31.2	8.0 / 9.0
LSD (P = 0.05) - within column comparisons	0.04 / 0.04	4.2 / 3.6	2.1 / 2.2

* Wood property significantly (P = 0.05) different between the two radii

a Blocks incubated for six weeks on cultures of *Trametes lilacino-gilva* in a soil-jar decay test

b,c Values for the kino vein (included sapwood) and control radii respectively

Each value the mean for five trees

Fig. 1. Stem cross-section showing sampling positions on two radii. **I.** Through a kino vein (K): 1 - inner heartwood (HW); 2 - inner included sapwood (SW); 3 - outer included SW; 4 - HW immediately external to kino vein; 5 - outer HW; 6 - central SW. **II.** Control samples (C) located in the same relative positions. [Not to scale].

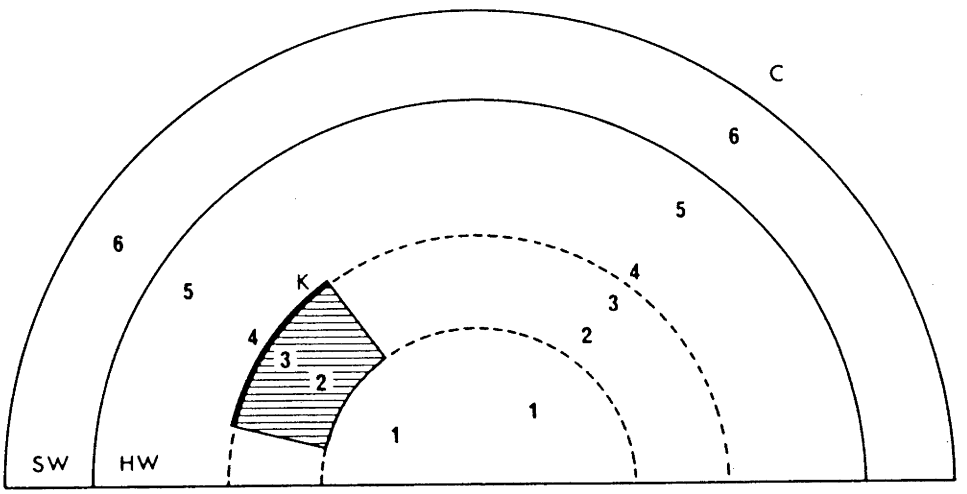

C
6
5
4
3
2
1
K
SW
HW

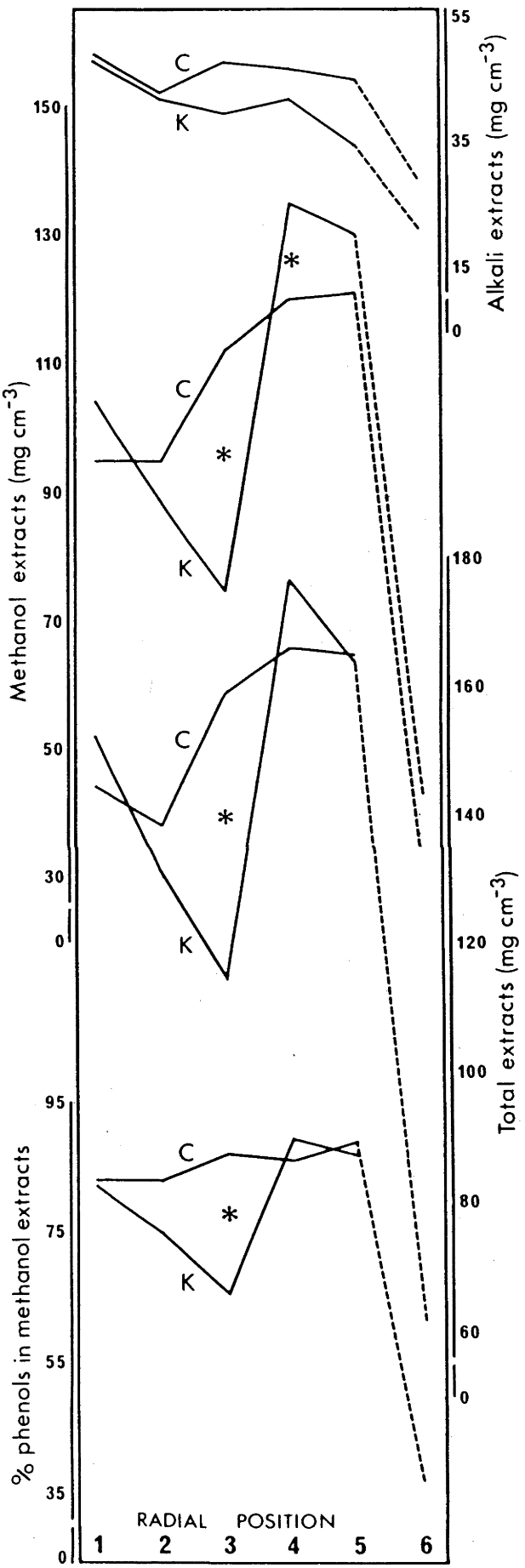
Fig. 2. Cross-section of an *E. macrorhyncha* stem containing several regions of included sapwood (I) internal to kino veins. An area of incipient decay (D) is present. Normal sapwood (S) to the top of this sample is abnormally narrow. [Stem diam ca. 30 cm].

Fig. 3. Included sapwood (I) in *E. macrorhyncha*. The formation of an extensive kino vein when the tree was young (ca. 4 cm diam) has resulted in the inclusion of a core of the light coloured tissue surrounding the pith.


S
I
D
I
I
I


I

Fig. 4. Radial variation in the extractives content of five stems of *E. macrorhyncha* through a kino vein (K) and on a control radius (C). Extraction with methanol preceded that with 0.1N sodium hydroxide. Position 1 - inner heartwood; 2,3 - included sapwood on radius K, or middle heartwood on radius C; 4,5 - outer heartwood; 6 - sapwood. Least significant difference (P = 0.05) for comparison of positions on each radius: alkali extracts C-8, K-8; methanol extracts C-22, K-20; total extracts C-19, K-22; percentage phenols C-12, K-15. * - radii differ significantly at the position.


Methanol extracts (mg cm^{-3})
150
130
110
90
70
50
30
0
Alkali extracts (mg cm^{-3})
55
35
15
0
Total extracts (mg cm^{-3})
180
160
140
120
100
80
60
0
% phenols in methanol extracts
95
75
55
35
0
C
K
*
RADIAL POSITION
1 2 3 4 5 6

CHAPTER 7. *GENERAL DISCUSSION*

Extensive discussion of a speculative nature was not possible in the manuscripts. In the following sections, selected topics, central to the theme of the paper series, are developed further. Again, space does not permit extensive elaboration in areas such as natural variation in wood properties.

RESUME OF SERIES

For the convenience of the reader of the 'General Discussion' sections which follow, the main findings/conclusions relating to the theme of the series are highlighted here and summarised in Table 1.

The series revealed three types of apparent (assumed hereafter to be real) influence of the host on the progression of decay within stems in the particular eucalypts studied:

1. The natural decay resistance of tissues (here regarded as synonymous with the 'resistance of tissues to decay *in vitro*'). Variations, between species, in the natural decay resistance of heartwood partially explain interspecific variation in the development of heartrot in living trees. Within individual trees, heartwood tissues of low natural resistance to decay e.g. included sapwood or inner heartwood, are also those most susceptible to deterioration *in vivo*.

2. The dynamic defence mechanisms of differentiated tissues. Although the heartwoods of the eucalypts are apparently unresponsive to injury and infection, the failure of microbes to invade extensive areas of living sapwood, either centrifugally from heartwood or verti-

cally and tangentially from sites of injury, appears to result from an active protective response by the host. The obstacle(s) to microbial invasion may be chemical (e.g. antibiotics) and/or physical (e.g. tyloses).

3. The dynamic defence mechanisms of cambial tissues. Barrier zones are effective in the medium-long term i.e. until outgrown by micro-organisms in heartwood. The causes of this effectiveness are speculative.

EXPANSION OF CONCEPTS

1. The Natural Decay Resistance of Tissues

For many years certain workers have assumed, but have not established directly, that the natural decay resistance of heartwood is important in determining the progression of heartrots in standing trees (Wagener and Davidson 1954; Rudman 1964; Da Costa 1973). More recently, the natural decay resistance factor has received little attention while the importance of protective responses of stem tissues, particularly in 'northern hardwoods' [e.g. beech (*Fagus*), birch (*Betula*) and maple (*Acer*)], has been emphasised (e.g. Shigo 1979a, 1983; Shortle 1979a). These species appear not to form true heartwood (Shigo 1965; Shortle 1979a). The present studies on *Eucalyptus* (Chs. 1, 2) demonstrate a probable importance of natural decay resistance in directing the development of rot within stems of this genus. Certainly, in the extreme case of included sapwood, the rapid progression of rot *in vivo* (Ch. 1) can reasonably be ascribed to the low natural decay resistance of this tissue (Ch. 6). Thus the argument is taken 'a full circle', and some substance is given to an assumed, but essentially untested, relationship.

Within stems of many tree species e.g. *E. citriodora* Hook., *Anadenanthera peregrina* (L.) Speg., *Piptadenia gonoacantha* (Mart.) Macbr. and *Platypodium elegans* Vog. (Reis 1973), and *Thuja plicata* Donn ex D.Don (Cartwright 1941; Scheffer 1957), the natural resistance to decay of heartwood tissues decreases with increasing height in the bole. The decrease in resistance of outer heartwood could partly reflect the sampling of more juvenile tissue at higher levels (where the cambium is necessarily younger than at lower positions). However, that for inner heartwood, where tissues are of approximately constant maturity (i.e. located a constant number of rings from the pith) indicates an additional effect associated with height *per se*. A common distribution of resistance to decay within the heartwood of a stem can thus be mapped diagramatically as in Fig. 1. Should such a distribution be representative of the eucalypts examined, heartrot could be expected to spread more rapidly at higher levels within stems, as reported for *Quercus nuttallii* Palmer (Toole 1964), and more rapidly up than down the boles.

The natural decay resistance of wood is largely determined by the quantity and quality of extractives present (Rudman 1964; Reis 1973; Da Costa 1975). However, information on factors controlling the type and quantity of heartwood extractives formed is fragmentary and inconclusive (Hillis 1977). An upper limit of extractive formation could be set by the quantity of carbohydrate stored at, or translocated centripetally to, the heartwood-sapwood boundary (Hillis et al. 1962; Hillis 1977). Hence it is often assumed that rapidly grown trees contain heartwood of low extractives content (and thus of low decay resistance) because a large proportion of photosynthates would be directed to the production of new cells (Hillis 1962). This assumption is not consistently supported by experimental evidence (e.g. see

Hemingway and Hillis 1970; Nelson 1975). Indeed, in the study eucalypts, the content of extractives is greater in the more rapidly grown stems (Wilkes 1984), suggesting that in this case, the abundance of photosynthates associated with rapid growth results in the production of greater quantities of both cell wall substance and heartwood extractives. The additional extractives deposited in such stems apparently lack appreciable fungitoxicity since the heartwoods of both fast and slow grown trees have a similar resistance to decay *in vitro* (Ch. 2). Thus it seems that environment also affects the quality of extractives formed.

Exceptions exist in the relationship between the resistance of heartwood tissues to decay *in vitro*, and their resistance to deterioration *in vivo* (Ch. 2). Thus, while in the field the inner heartwood of *E. dealbata* degrades relatively rapidly (Ch. 1), it is not unusually susceptible to decay (Ch. 2). The following are examples of the many factors which could contribute to a difference in the resistance of wood to decay between the *in vitro* and *in vivo* situations:

- Low oxygen and high carbon dioxide concentrations in stems could retard the activities of decay fungi (Jensen 1969; Highley and Kirk 1979; Worrall and Parmenter 1983). This effect would probably be most pronounced (i.e. gaseous exchange at a minimum) in dense tissues e.g. the outer heartwood of *E. sideroxylon* (Wilkes 1984), although such an influence would be tempered by variation in moisture content.
- It is likely that the quality and/or physical location of fungitoxic/fungistatic extractives in heartwood differs substantially between the near (ca. 90%; calc. from Table 2, Ch. 3) saturated condition of the living tree, and the moist decay test block e.g. *in situ* certain extractives may occur in solution, but this water solubility could be altered when blocks are oven dried prior to use in the soil-jar decay test.

- Nondecay (nonhymenomycetous) organisms often play a vital role in directing stem decay processes (Shigo 1966; Shortle and Cowling 1978; Wilkes and Heather 1983), and the progression of these microbes through a bole may not be influenced greatly by the natural resistance of tissues to decay. For example, following artificial inoculation, *Phialophora parasitica* Ajello et al. often spreads rapidly through both inner and outer heartwood tissues in living trees of the species studied here (Wilkes unpub. data).

2. The Dynamic Defence Mechanisms of Differentiated Tissues

Although devoid of living cells, the heartwood of healthy trees has been reported capable of protective responses (Shigo 1982, 1983). Thus injury to heartwood of *Q. rubra* L. resulted in more extensive discolouration i.e. more extensive microbial invasion, in ringbarked (dying), than in control (healthy), stems (Shigo and Shortle 1979). The nature of the response is unknown - certainly free carbohydrate reserves, which could act as precursors to antibiotics (Shortle 1979a; Hart 1981), are generally absent from heartwood (Hillis 1962). Possibly, in response to certain stimuli, residual enzymes within the heartwood (Kondo 1964; Shain and Mackay 1973) catalyse the conversion of one class of extractives to another. Phenolases, for example, can exist in plants in bound (Drawert and Gebbing 1967) or latent (Kenten 1957) forms, as could be appropriate in heartwood, and may function after tissue is injured or infected (Hyodo and Uritani 1965). The absence of living cells would preclude the development of (additional) tyloses (see Chattaway 1949), considered significant in the compartmentalisation of deterioration in the sapwood of some species (Shigo and Marx 1977; Ch. 4). Regardless, the reported responsiveness of oak contrasts with a nonreactiveness of the heartwood of the four *Eucalyptus* spp. (Ch. 3).

Differentiated sapwood of both eucalypts [Chs. 1, 4] and other genera [e.g. *Acer* (Sharon 1974; Shortle 1979b), *Picea* (Shain 1971; Hart et al. 1975), *Pinus* (Jorgensen 1961; Prior 1976) and *Quercus* (Hart and Johnson 1970; Wardell and Hart 1970)] responds dynamically to injury and/or infection. The antagonistic physiological responses of herbaceous plants and bole sapwood have much in common. In each case the disturbance in living cells results in a metabolic dysfunction, frequently including a shift in oxidative metabolism from glycolysis and tricarboxylic acid cycle pathways to acetate and shikimic acid pathways (Rohringer and Samborski 1967; Kosuge 1969; Shortle 1979a). The qualitatively altered and/or increased metabolism in sound tissues bordering the injured or infected region often results in an accumulation of phenols, which may assist in compartmentalising the deterioration (Craft and Audia 1962; Tomiyama 1963; Somers and Harrison 1967). Where such compounds, formed as part of a nonspecific defence mechanism of the host, are inhibitory to microbes, the materials can be classed as phytoalexins (Cruickshank 1963; Shain 1979). However, it is likely that the gross accumulation of certain phenols is unrelated to resistance to microbial invasion - possibly it is merely a result of infection, occurring after the success or failure of an attempted invasion has been determined by other factors (Cruickshank 1963; Hart 1981). These primary causes of resistance could be more subtle e.g. the degree to which host enzymes inhibit or inactivate toxins or enzymes released by microorganisms could be important (Kuć 1966). There is no reason to assume that a single mechanism controls the resistance of sapwood to microbial invasion (Kuć 1966).

Hart and Johnson (1970) have suggested that the ability of trees to produce antibiotic substances in response to injury of sapwood may be related to their capacity to form heartwood resistant to decay.

However, this hypothesis is not well supported by the present results e.g. in both *E. sideroxylon* and *E. macrorhyncha* [the latter containing less durable mature heartwood (Ch. 2)], injured sapwood has a similar resistance to decay by *T. lilacino-gilva in vitro* (Ch. 4). Possibly the sapwood-heartwood and sapwood-discoloured wood transition processes are different, which accords with the view (Hart and McNabb 1963; Shigo and Hillis 1973) that heartwood and discoloured sapwood are distinct tissues.

Shigo and Hillis (1973) suggest that the effectiveness of compartmentalisation responses is related positively to host vigour. Thus the spread of *Heterobasidion annosum* (Fr.) Bref. [*Fomes annosus* (Fr.) Cke.] in *Pinus* spp. is more rapid in suppressed than vigorous trees (Wallis 1961; Gibbs 1967). In *P. radiata* D.Don, following injury or infection of sapwood, polyphenols are produced more rapidly in trees of greater vigour (Shain and Hillis 1972). Similarly, stress from defoliation reduces the capacity of *Abies balsamea* L. to compartmentalise internal infections (Shortle and Ostrofsky 1983). No such effect of vigour was detected in the eucalypt sapwood wounding experiment (Ch. 4), possibly reflecting a lack of correlation between parenchyma cell vitality and tree vigour (growth rate) in the species studied.

The tyloses formed at the boundary of discolouration in the sapwood of the eucalypts (Ch. 4) may obstruct the growth of hypha and the movement of bacteria and fungal propagules in the longitudinal direction (Gerry 1914; Harrison and Clare 1970). Tylosis membranes rich in lignin, as detected in *E. dalrympleana* Maid. and *E. rubida* Deane & Maid. (Chattaway 1949), would probably be resistant to deterioration by nondecay organisms. Further, the polyphenols colouring many of the wound induced tyloses (Ch. 4) could have antimicrobial properties. The

tyloses could also have several less direct effects e.g. by restricting the access of air to clear tissues, the structures could retard the activities of aerobic microbes. Reports such as those by Toole (1965, 1967), showing a marked reduction in the progression of decay after wound closure, suggest tissue aeration to be important in regulating the activity of some microbes. Tyloses would also reduce both the desiccation of tissues in the vicinity of the wound, and the longitudinal movement of lethal agents such as microbial toxins and enzymes, autolytic materials produced in necrotic tissues, and plant hormones. A lack of tissue necrosis would restrict invasion by saprophytic organisms.

In studying the interaction between microorganisms and living sapwood, it is clearly desirable to examine the region at the forefront of the microbial invasion, where any host response would be based [i.e. the marginal zone (*sensu* Shortle 1979b); transition/reaction zones (*sensu* Shain 1979)]. Importantly, the properties of discoloured sapwood tend to reflect not only on host responsiveness, but also on the activities of mirobes in such tissues. Thus, in a poplar hybrid (*Populus deltoides* Marsh. X *P. trichocarpa* Hook.) and red maple (*Acer rubrum* L.), phenol levels are elevated in the marginal zone between clear and discoloured sapwood (as living cells react to microbial invasion), but decrease in discoloured (dead) tissues as the materials are utilised and/or altered by microorganisms (Shortle 1979b; Smith et al. 1981). Unfortunately the marginal zone is often narrow e.g. < 2 mm (Shortle 1979a), and in *Eucalyptus*, it is not visually distinct, creating obvious sampling difficulties.

The fate of discoloured sapwood on inclusion in heartwood is not known. It is unlikely that further extractives would be deposited in the tissue since these materials are formed *in situ* in living parenchyma cells (Hillis 1977), which are absent from discoloured wood (Hart and McNabb 1963; Shigo and Hillis 1973; Ch. 4). Consequently, the natural decay resistance of the injured tissue would probably be similar before and after inclusion in heartwood i.e. the resistance to decay should remain intermediate between that of sapwood and that of heartwood (compare Tables 3 Ch. 3, 2 Ch. 4). Presumably also, certain of the microbes present within the discoloured tissues in sapwood (Ch. 4) would persist in these in heartwood. Thus the injured tissue could be expected to degrade more rapidly than surrounding normal heartwood.

The injury with which discoloured sapwood is associated in *Eucalyptus*, is likely to cause the formation of both a kino vein, and subsequently, a region of included sapwood (Chs. 1, 5, 6; Fig. 2). Since included sapwood is commonly decaying in the standing eucalypts (Ch. 1), it is probable that the marginal zone bordering discoloured sapwood (Ch. 4; Fig. 2) does not remain effective as a limit to microbial invasion once incorporated in heartwood. Perhaps the effectiveness of the zone in sapwood is dependent on the continued presence of living cells within or proximal to the region.

In living trees containing both sapwood and heartwood, the latter is usually more susceptible to microbial degradation (Shain 1979; Ch. 1). Perhaps in eucalypts, where the width of sapwood is typically just 1.5 -2.5 cm (Hillis 1978), effective compartmentalisation of discolouration and decay in this region of the bole reflects evolutionary adaptation. Extensive deterioration in sapwood could have a serious adverse effect on the bole functions of support (where heartrot is

also extensive), food storage and 'sap flow'. *Abies alba* Mill. trees often die where wetwood is sufficiently widespread in sapwood to seriously reduce water supply to the crown (Bauch et al. 1979). Certainly, if the volume of sapwood within a tree is initially at a physiological optimum (Bamber 1976), loss of functional tissue would adversely affect tree health.

3. The Dynamic Defence Mechanisms of Cambial Tissues

As yet the effectiveness of barrier zones as limits to microbial invasion has not been conclusively related to any specific wood feature, although four tissue characteristics have received particular attention (Ch. 5): the variation in wood anatomy (e.g. Bauch et al. 1980); increased and/or altered cell wall lignification (e.g. Moore 1978); an abundance of extraneous materials frequently filling cells (e.g. Sharon, 1973); and the presence of living (responsive) cells when the zone is in sapwood (e.g. Tippett and Shigo 1981). However, barrier zones may be effective in the absence of each of these features (Ch. 5), and thus different features may be functionally important in different zones, or factors other than those listed could play a role. Such factors could be subtle e.g. host enzyme systems, capable of inhibiting fungal enzymes and toxins, may be produced in developing barrier zone tissues, and subsequently persist in heartwood. Alternatively, strongly antimicrobial compounds (possibly nonphenolic), could be present at low concentrations in barrier zones. If features such as these are important, studies attempting to relate the more obvious and gross properties of barrier zones (e.g. anatomy and total polyphenol content) to the effectiveness of the barriers, are misdirected.

Analyses of barrier zone tissue are made difficult as the areas are not always macroscopically obvious, and the narrowness of the region e.g. < 1 mm, necessitates the meticulous sampling of many and/or extensive surfaces to obtain suitable quantities of material for some tests. While these obstacles are not absolute, they can limit the replication logistically possible. Further, since we are ignorant of the causes of effectiveness of the barriers, it is not known exactly which tissues should be examined e.g. the spatial distributions of regions of anatomical and chemical response could differ.

Although the control of barrier zone formation has received little attention, it is probable that a localised hormonal imbalance occurs in the cambium after wounding. Smith (1980) has hypothesised that auxin concentrations may be reduced, resulting in the lack of cellular differentiation which is characteristic of many barrier zones, while increased concentrations of gibberellins and/or cytokinins could explain the accelerated cell division. Such an hypothesis is highly speculative and probably oversimplistic in that cambial growth is controlled by inhibitors and nonhormonal substances in addition to auxins, gibberellins and cytokinins, and interactions occur between the hormones (Leopold and Kriedeman 1975; Kramer and Kozlowski 1979). Further, under certain conditions, ethylene may stimulate phenol formation (Chalutz et al. 1969; Shain and Hillis 1973) and the radial expansion of growing cells (Burg 1968; Moore 1979), suggesting a possible role of this hormone in barrier zone formation. Indeed ethylene has been linked to the production of kino (veins) in *Eucalyptus* (Hillis 1975; Nelson and Hillis 1978a, b). Injured sapwood /cambia may produce ethylene (Cooper 1972; Shain and Hillis 1972), presumably accounting, at least in part, for the subsequent formation of a kino vein in many eucalypts. The similar extension of kino veins

above and below the drill wounds (Ch. 5) suggests comparable rates of diffusion of ethylene (and/or other hormones) in both the basipetal and acropetal directions, and the absence of substantial upward translocation of the hormone(s) in the transpiration stream (see Nelson and Hillis 1978b).

The physiological condition of the cambium at the time of wounding would presumably determine the degree of any hormone imbalance and/or govern the influence of this imbalance on cell growth. Consequently, such factors as cambial maturity (tree age), season of injury and site conditions could influence barrier zone formation e.g. water stress has been implicated in kino vein formation (Day 1959). Additionally, various stimuli of different intensity may give rise to varying cambial responses. Clearly, without a fuller understanding of the factors governing the development of barrier zones, caution is required in accepting that the zones (or particular regions of these) examined are representative of the species concerned - under different conditions the species may form zones with different properties. Thus, in studying *Acer rubrum*, Mulhern et al. (1979) detected an elevated content of fibres in barrier zones, yet the amount of fibres was normal in the comparable zones studied by Bauch et al. (1980).

Interspecific variation in the extent of barrier zones formed in response to a given stimulus is pronounced. In the eucalypts, the size of a barrier zone surface is loosely related to the severity of wounding; consequently microbes entering a stem via a small wound e.g. a larva tunnel, sometimes progress beyond the effective limits of the zone formed in response to that injury, and so move into tissues produced after wounding (Ch. 1). In contrast, the 'northern hardwoods' (Shigo 1965; Shigo and Larson 1969) and *Liquidamber styraciflua* L.

(Moore 1978) frequently form barrier zones which extend the full length of boles, and escape of discolouration and decay from tissues extant at wounding is apparently rare. These species differences are not easily rationalised when so little is known of the control of barrier zone formation.

In certain tree species, microorganisms present in sapwood are able to elicit the formation of barrier zones (Shigo 1979b; Tippett and Shigo 1981; Tippett et al. 1982). Thus barrier zones are extended and/or new zones are formed at the cambium as discolouration and decay extends within the tree. No evidence of this type of barrier development was detected in the eucalypts studied, perhaps because compartmentalisation of deterioration in the sapwood (Chs. 1, 4) ensures tissues internal to the cambium are healthy. Microbes invade wounded sapwood (Ch. 4), but in this case a barrier zone, produced in response to injury, is already present external to the discoloured tissue. Kino veins sometimes develop at the cambium of *E. bicostata* Maid. et al. when heartrot is exceptionally extensive and progresses centrifugally across sapwood (Wilkes pers. obs.), but it is not known if such formation is a direct response by the cambium to adjacent deterioration, or whether the cambium is injured by mechanical stresses in the structurally weakened stems.

While in the species investigated, included sapwood was a common feature of heartwood, this pseudo-sapwood is often not present internal to kino veins in the heartwood of some eucalypt species e.g. *E. maculata* Hook. (Wilkes pers. obs.). Perhaps the degree of cellular disorganisation in the parenchyma bridges of the veins in these species is relatively low; ray parenchyma or similar cells may traverse kino veins, allowing centripetal translocation of the precursors

of heartwood extractives. In *E. delegatensis* R.T. Bak. (a species in which visually obvious included sapwood is not common), parenchyma bridges are usually of a more normal xylem structure than in the eucalypts studied here e.g. rays and vessels may be present (Wilkes pers. obs).

Stem wounding may accelerate the centrifugal development of heartwood in *Acacia* spp. (Bamber 1976; Wilkes pers. obs.), but retard this development in *Quercus* spp. (Shigo 1972; McGinnes and Shigo 1975; Phelps and McGinnes 1977). Thus 2.4 m above ground level in a *Q. alba* L. stem wounded basally some 20 yrs previously, sapwood covered 20 annual increments directly above the wound, but only 14 increments on the opposite side of the tree (Phelps and McGinnes 1977). Such effects were not observed in this series of studies, but it seems likely that, as hypothesised for kino veins, the wound response barrier zones of *Acacia* and *Quercus* could alter normal patterns of movement of materials related to heartwood formation e.g. photosynthates and/or ethylene (Hillis 1977).

INTEGRATION OF CONCEPTS

The classical theory of heartrot may be summarised to three components (Shigo 1979a):

- Wounds initiate the deterioration processes.
- Decay fungi (Hymenomycetes) infect exposed heartwood.
- Decay of heartwood results.

Shigo (1979a) has proposed that this theory be revised to include further concepts:

- Many microorganisms, including nondecay species, are involved in the infection processes.

- Tissues in the vicinity of the injury react to wounding.
- Injured and infected tissues are compartmentalised, even in heartwood.

The inclusion of the latter factors represents an expansion, rather than replacement, of the original theory. Numerous studies support the relevance of the expanded concept to many tree species, including the 'northern hardwoods' (Shortle 1979a; Shigo 1983). However, the present studies and others on *E. microcorys* F. Muell. (Wilkes 1982b; Wilkes and Heather 1983) suggest that for the eucalypts, the expanded theory may require qualification - a reaction to compartmentalise injury and infection has not been detected in the tissues of eucalypt heartwood.

Despite the nonresponsiveness of eucalypt heartwood, an ability for passive compartmentalisation of decay is probably inherent in this tissue e.g. tyloses, which may resist the longitudinal progression of deterioration, are plentiful in the heartwood of most species (Dadswell 1972), including those studied in this series. Further, polyphenolic extractives, synthesised in parenchyma cells during the transformation of sapwood to heartwood, tend to remain concentrated in these cells (Hillis 1977), and if antibiotic, would resist the growth of heartrot organisms in the transverse (particularly the tangential) direction. Tyloses and extractives could be expected to have these effects *in vitro* also; thus the 'natural decay resistance' of, and effectiveness of 'compartmentalisation of decay' within, the eucalypt heartwood tissues, are concepts with much in common.

Previous studies on host resistance to stem decay have typically examined factors such as barrier and reaction zones in isolation, with little attention being given to the integration of the roles played by the various factors. This series demonstrates the usefulness of an

holistic approach. In effect, the three factors observed to influence the development of decay within stems of the eucalypts (the natural decay resistance of tissues, the responses of sapwood to injury and infection, and barrier zones) can be considered defence mechanisms of the host, and as such, each acts as a reserve ('back-up') for the others. Consider two examples:

- The breakage of a large, living branch. Microbes are likely to invade exposed heartwood, and to a very limited extent, the injured sapwood. The natural decay resistance of heartwood will influence the rate of development of heartrot, and in many instances, restrict deterioration to inner heartwood. Should this 'first line of defence' be relatively weak, the antagonistic response of sapwood should exclude decay from progressing centrifugally into this tissue, and the barrier zone, which formed when the limb fell, will eventually (i.e. once enveloped by heartwood) serve to confine the decay.
- A shallow injury to sapwood. Microbial invasion will initially be restricted by the responses of sapwood to injury and infection. The barrier zone formed after injury will limit the centrifugal development of deterioration. Following incorporation of the injured sapwood into heartwood, tissue decay resistance will substantially control the rate and direction of movement of the boundaries of any heartrot emanating from the region of injury.

In both cases, if heartrot organisms grow beyond the effective limits of the wound response barrier zone (probably in the axial direction), or if the zone is disrupted (e.g. by an insect tunnel), the activities of 'escaping' microbes will be influenced by the natural decay resistance of heartwood external to the barrier, and should the organisms reach the heartwood-sapwood boundary, the responsiveness of sapwood to microbial invasion will be important.

For simplicity, this discussion has assumed the existence of just three host attributes affecting the progression of decay in the eucalypts. Undoubtedly there are others, including the gaseous environment (e.g. concentrations of oxygen, carbon dioxide and volatile organic materials) within the stem (Wagener and Davidson 1954; Highley and Kirk 1979). Additionally, many nonhost factors will affect where, and how quickly, decay spreads in a bole e.g. the species of invading organisms and their physiological attributes, and ambient temperatures, would be of importance. As information becomes available on factors such as these, it can be used to augment current knowledge in developing a broad, yet integrative concept of stem decay in *Eucalyptus*.

GENERAL CONCLUSION

1. Achievements in Relation to Objectives

Principal findings are given in the 'Conclusions' and 'Summary' sections of each paper, in the general 'Abstract', and in this chapter (e.g. Table 1); thus they are not repeated. Rather achievements are here related, in the broadest terms, to the objectives of the group of studies (see 'Preface'):

- Primary objective: to ascertain the importance of three factors (the natural decay resistance of tissues, and protective responses of both differentiated tissues and of cambia) as determinants of the extent and patterns of decay within stems of four species of eucalypt in a regrowth forest. This objective was fulfilled in that an apparent importance of all three factors was demonstrated - in the sapwood, barrier zones and the protective responses of differentiated tissues to injury and/or infection both effectively compartmentalise microbial deterioration,

while in heartwood, barrier zones and the natural decay resistance of tissues influence the progression of heartrot.

- Secondary objective: to gain an initial understanding of the mechanisms of protective responses of the bole to injury and infection. Useful progress was made in this area. In the case of differentiated sapwood, it was shown that while the properties of injured (discoloured) and clear sapwood are generally similar, tyloses and phenols, produced in a marginal zone bordering injured and infected tissues, are likely agents of the effective compartmentalisation. The anatomy and chemistry of eucalypt barrier zones are partly understood, although the causes of effectiveness of the barriers remains speculative.

2. Future Research

The time and facilities available did not allow thorough examination of all aspects of each of the host attributes affecting the development of decay in the eucalypt trees. Thus the foregoing 'General Discussion' sections indicate several areas of possible fruitful research. Of particular usefulness would be study of:

- The factors, other than barrier zones, which complicate the potentially simple relationship between the natural decay resistance of heartwood tissues, and the rate of progression of heartrot. The gaseous environment within stems deserves further attention.
- The properties of the marginal region bordering infected sapwood which halt or hinder further microbial invasion. Tyloses and antibiotics are factors worthy of close consideration.
- The properties of barrier zones which confer effectiveness to the regions in sapwood, and in heartwood. The chemistry and responsiveness of the zones should be examined in detail.

Table 1. Summary of findings of six studies examining host influences on the extent and patterns of decay in four *Eucalyptus* spp.

Study	Examining	Findings/Conclusions
I	Extent and patterns of decay *in vivo*	- Main infection courts: basal injuries, branch stubs and insect bole wounds - Volumes of heartrot: *E. bancroftii, E. dealbata* > *E. macrorhyncha* > *E. sideroxylon* - Important medium-long term effect of barrier zones in heartwood - Within the confines of barrier zones, decay more extensive in inner than outer heartwood This pattern most obvious in *E. sideroxylon, E. dealbata* - Little deterioration of live sapwood. Included and killed sapwood very susceptible to decay
II	Resistance of heartwood to deay *in vitro*	- Variations in the volume of heartrot, both between species and radially within stems, can be partially explained by parallel variation in the natural decay susceptibility of tissues - No pronounced effect of rate of tree growth on the resistance of heartwood to decay *in vitro*
III	Responses of heartwood to injury and infection	- No evidence of responsiveness
IV	Responses of sapwood to injury	- Effective compartmentalisation of injured and infected tissues in sapwood may reflect the formation of tyloses and/or antibiotics
V	Barrier zones	- Anatomically and chemically the zones have features in common with, and distinct from, barrier zones of other hardwood species - Cause(s) of effectiveness not ascertained
VI	Included sapwood	- In comparison with surrounding heartwood, the included sapwood of *E. macrorhyncha* contains extractives in both reduced quantities, and a qualitatively altered form, explaining its susceptibility to decay *in vitro* and *in vivo*

Fig. 1. Schematic representation of a common pattern of variation in the natural decay resistance of heartwood within a tree bole. Numbers 1-4 indicate contours of increasing resistance to decay. For emphasis, vertical variation is exaggerated.

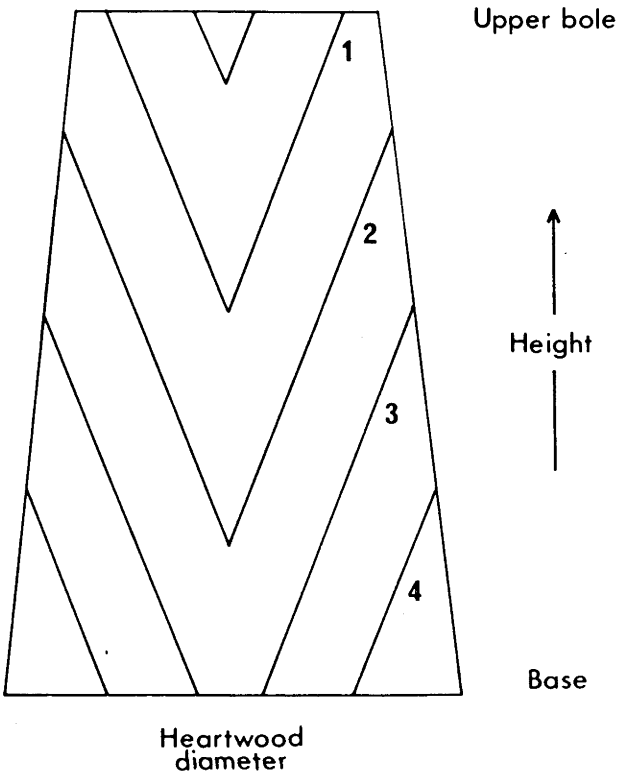
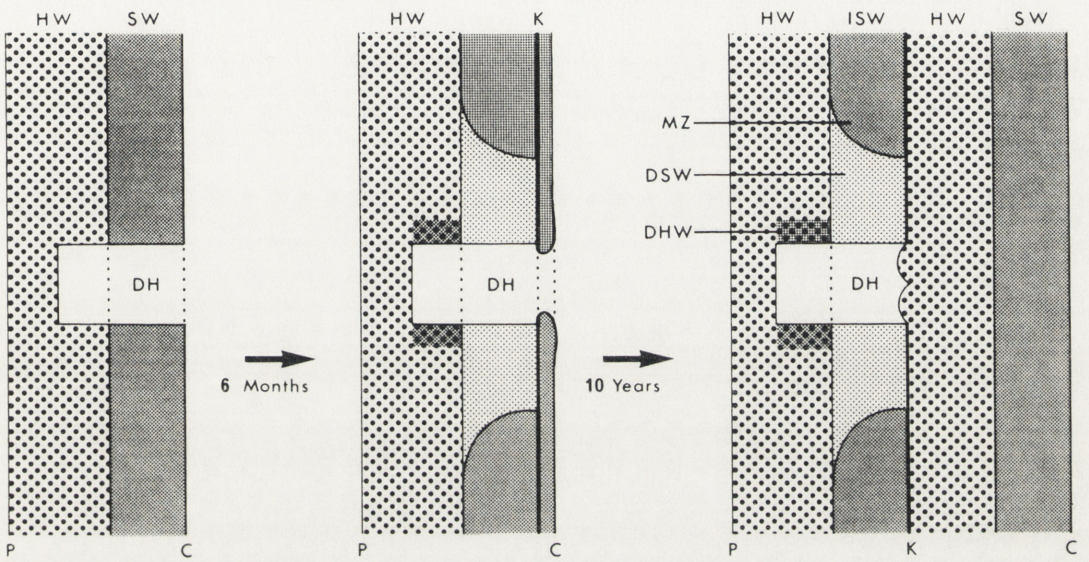

Upper bole
1
2
3
4
Height
Base
Heartwood
diameter

Fig. 2. Development of included sapwood after mechanical injury in certain species of *Eucalyptus*. HW - heartwood, DHW - discoloured heartwood, SW - sapwood, DSW - discoloured sapwood, ISW - included sapwood, P - pith, C - cambium, K - kino vein, DH - drill hole, MZ - marginal zone (not visible) bordering DSW. ISW and DSW (and perhaps the inner HW) would probably be decayed or decaying after 10 years. [Radial-longitudinal section, sapwood ca. 1.5 cm wide].


HW
SW
DH
P
C
6 Months
HW
K
DH
P
C
10 Years
HW
ISW
HW
SW
MZ
DSW
DHW
DH
P
K
C

REFERENCE LIST

Bamber, R.K. 1976. Heartwood, its function and formation. Wood Sci. Technol. 10, 1-8.

Bauch, J., P. Klein, A. Frühwald and H. Brill. 1979. Alterations of wood characteristics in *Abies alba* Mill. due to 'fir-dying' and considerations concerning its origin. Eur. J. For. Path. 9, 321-331.

Bauch, J., A.L. Shigo and M. Starck. 1980. Wound effects in the xylem of *Acer* and *Betula* species. Holzforschung 34, 153-160.

Brooker, M.I.H. and D.A. Kleinig. 1983. Field guide to eucalypts. Vol. 1. South-eastern Australia. Inkata Press, Melbourne. 288pp.

Burg, S.P. 1968. Ethylene, plant senescence and abscission. Plant Physiol. 43, 1503-1511.

Cartwright, K.St.G. 1941. The variability in resistance to decay of the heartwood of home-grown western red cedar (*Thuja plicata* D.Don.) and its relation to position in the log. Forestry 15, 65-75.

Chalutz, E., J.E. De Vay and E.C. Maxie. 1969. Ethylene-induced isocoumarin formation in carrot root tissue. Plant Physiol. 44, 235-241.

Chattaway, M.M. 1949. The development of tyloses and secretion of gum in heartwood formation. Aust. J. Sci. Res. (B) 2, 227-240.

Cooper, W.C. 1972. Trauma-induced ethylene production by citrus flowers, fruit, and wood. In: Carr, D.J. (ed). Plant growth substances 1970. Proc. 7th Int. Conf. Plant Growth Subs., Canberra. pp.543-548.

Craft, C.C. and W.V. Audia. 1962. Phenolic substances associated with wound-barrier formation in vegetables. Bot. Gaz. 123, 211-219.

Cruickshank, I.A.M. 1963. Phytoalexins. Annu. Rev. Phytopathol. 1, 351-374.

Da Costa, E.W.B. 1973. Natural durability and heart rots. 16th For. Prod. Res. Conf. (Aust.), Topic 1/15, 2pp.

Da Costa, E.W.B. 1975. Natural decay resistance of wood. In: Liese, W. (ed). Biological transformation of wood by microorganisms. Proc. 2nd Int. Cong. Plant Pathol., Minneapolis. pp.103-117.

Dadswell, H.E. 1972. The anatomy of eucalypt woods. CSIRO (Aust.), Div. Appl. Chem. Technol. Pap. No. 66, 28pp.

Davidson, J. 1974. Decayed wood in living trees of *Eucalyptus deglupta* Blume. Papua New Guinea Dept. Forests, Trop. For. Res. Note SR 18, 31pp.

Day, W.R. 1959. Observations on eucalypts in Cyprus I. The character of gum veins and anatomical indications for their origin. Emp. For. Rev. 38, 35-44.

Drawert, F. and H. Gebbing. 1967. Bound phenoloxidase and its release from plant cell structures. Naturwissenschaften 54, 226-227.

Edwards, D.W. 1973. Defects of fast-grown eucalypts in New South Wales, Australia. Proc. IUFRO Div. 5 Meet. 2, S. Africa. pp.256-270.

Gadgil, P.D. and A.D. Bawden. 1981. Infection of wounds in *Eucalyptus delegatensis*. N.Z. J. For. Sci. 11, 262-270.

Gerry, E. 1914. Tyloses: their occurrence and practical significance in some American woods. J. Agric. Res. 1, 445-469.

Gibbs, J.N. 1967. The role of host vigour in the susceptibility of pines to *Fomes annosus*. Ann. Bot. 31, 803-815.

Hall, N., R.D. Johnston and G.M. Chippendale. 1970. Forest trees of Australia. Aust. Govt. Pub. Serv., Canberra. 334pp.

Harrison, A.F. and B.G. Clare. 1970. Host reactions involved in the recovery of apricot trees from *Verticillium* wilt. Aust. J. Biol. Sci. 23, 1027-1032.

Hart, J.H. 1981. Role of phytostilbenes in decay and disease resistance. Annu. Rev. Phytopathol. 19, 437-458.

Hart, J.H. and K.C. Johnson. 1970. Production of decay-resistant sapwood in response to injury. Wood Sci. Technol. 4, 267-272.

Hart, J.H. and H.S. McNabb. 1963. Development of wound heartwood in Iowa hardwoods. (Abstr.). Phytopathology 53, 877.

Hart, J.H., J.F. Wardell and R.W. Hemingway. 1975. Formation of oleoresin and lignans in sapwood of white spruce in response to wounding. Phytopathology 65, 412-417.

Heather, W.A. 1962. The incidence and significance of disease in native forest crops. For. Timb. Bur. (Aust.), Leaflet No. 82, 10pp.

Hemingway, R.W. and W.E. Hillis. 1970. Heartwood formation in living stumps of Douglas-fir. Wood Sci. Technol. 4, 246-254.

Highley, T.L. and T.K. Kirk. 1979. Mechanisms of wood decay and unique features of heartrots. Phytopathology 69, 1151-1157.

Hillis, W.E. 1962. The distribution and formation of polyphenols within the tree. In: Hillis, W.E. (ed). Wood extractives and their significance to the pulp and paper industries. Academic Press, New York. pp.59-131.

Hillis, W.E. 1975. Ethylene and extraneous material formation in woody tissues. Phytochemistry 14, 2559-2562.

Hillis, W.E. 1977. Secondary changes in wood. In: Loewus, F.A. and V.C. Runeckles (eds). Recent advances in phytochemistry. Vol. 11. Plenum Press, New York. pp.247-309.

Hillis, W.E. 1978. Wood quality and utilization. In: Hillis, W.E. and A.G. Brown (eds). Eucalypts for wood production. CSIRO Press, Australia. pp.259-289.

Hillis, W.E., F.R. Humphreys, R.K. Bamber and A. Carle. 1962. Factors influencing the formation of phloem and heartwood polyphenols. Part II. The availability of stored and translocated carbohydrate. Holzforschung 16, 114-121.

Hyodo, H. and I. Uritani. 1965. σ-Diphenol oxidases in sweet potato infected by the black rot fungus. J. Biochem. (Japan) 57, 161-166.

Jensen, K.F. 1969. Oxygen and carbon dioxide concentrations in sound and decaying red oak trees. For. Sci. 15, 246-251.

Jorgensen, E. 1961. The formation of pinosylvin and its monomethyl ether in the sapwood of *Pinus resinosa* Ait. Can. J. Bot. 39, 1765-1772.

Kenten, R.H. 1957. Latent phenolase in extracts of broad-bean (*Vicia faba* L.) leaves I. Activation by acid and alkali. Biochem. J. 67, 300-307.

Kondo, T. 1964. On the wood enzyme. J. Jap. Wood Res. Soc. 10, 43-48.

Kosuge, T. 1969. The role of phenolics in host response to infection. Annu. Rev. Phytopathol. 7, 195-222.

Kramer, P.J. and T.T. Kozlowski. 1979. Physiology of woody plants. Academic Press, New York. 811pp.

Kuć, J. 1966. Resistance of plants to infectious agents. Annu. Rev. Microbiol. 20, 337-370.

Leopold, A.C. and P.E. Kriedemann. 1975. Plant growth and development. McGraw-Hill, New Delhi. 545pp.

McGinnes, E.A. and A.L. Shigo. 1975. A note on effects of wounds on heartwood formation in white oak (*Quercus alba* L.). Wood and Fiber 6, 327-331.

Moore, K.E. 1978. Barrier-zone formation in wounded stems of sweetgum. Can. J. For. Res. 8, 389-397.

Moore, T.C. 1979. Biochemistry and physiology of plant hormones. Springer-Verlag, New York. 274pp.

Mulhern, J., W. Shortle and A. Shigo. 1979. Barrier zones in red maple: an optical and scanning microscope examination. For. Sci. 25, 311-316.

Nelson, N.D. 1975. Extractives produced during heartwood formation in relation to amounts of parenchyma in *Juglans nigra* and *Quercus rubra*. Can. J. For. Res. 5, 291-301.

Nelson, N.D. and W.E. Hillis. 1978a. Genetic and biochemical aspects of kino vein formation in *Eucalyptus*. I Genetic variation in response to kino induction in *E. regnans*. Aust. For. Res. 8, 75-81.

Nelson, N.D. and W.E. Hillis. 1978b. Genetic and biochemical aspects of kino vein formation in *Eucalyptus*. II Hormonal influence on kino formation in *E. regnans*. Aust. For. Res. 8, 83-91.

Phelps, J.E. and E.A. McGinnes. 1977. Anatomical responses to basal injury in white and black oak. Wood Sci. 10, 15-21.

Prior, C. 1976. Resistance by Corsican pine to attack by *Heterobasidion annosum*. Ann. Bot. 40, 261-279.

Pryor, L.D. and L.A.S. Johnson. 1971. A classification of the eucalypts. Aust. Nat. Uni. Press, Canberra. 102pp.

Reis, M.S. 1973. Variation in decay resistance of four wood species from southeastern Brazil. Holzforschung 27, 103-111.

Rohringer, R. and D.J. Samborski. 1967. Aromatic compounds in the host-parasite interaction. Annu. Rev. Phytopathol. 5, 77-86.

Rudman, P. 1964. Durability in the genus *Eucalyptus*. Aust. For. 28, 242-257.

Scheffer, T.C. 1957. Decay resistance in western redcedar. J. For. 55, 434-442.

Shain, L. 1971. The response of sapwood of Norway spruce to infection by *Fomes annosus*. Phytopathology 61, 301-307.

Shain, L. 1979. Dynamic responses of differentiated sapwood to injury and infection. Phytopathology 69, 1143-1147.

Shain, L. and W.E. Hillis. 1972. Ethylene production in *Pinus radiata* in response to *Sirex-Amylostereum* attack. Phytopathology 62, 1407-1409.

Shain, L. and W.E. Hillis. 1973. Ethylene production in xylem of *Pinus radiata* in relation to heartwood formation. Can. J. Bot. 51, 1331-1335.

Shain, L. and J.F.G. Mackay. 1973. Phenol-oxidizing enzymes in the heartwood of *Pinus radiata*. For. Sci. 2, 153-155.

Sharon, E.M. 1973. Some histological features of *Acer saccharum* wood formed after wounding. Can. J. For. Res. 3, 83-89.

Sharon, E.M. 1974. An altered pattern of enzyme activity in tissues associated with wounds in *Acer saccharum*. Physiol. Plant Path. 4, 307-312.

Shigo, A.L. 1965. The pattern of decays and discolorations in northern hardwoods. Phytopathology 55, 648-652.

Shigo, A.L. 1966. Organism interactions in decay and discoloration in beech, birch, and maple. In: Becker, G. and W. Liese (eds). Holz und Organismen. Int. symp., Berlin-Dahlem. pp.309-324.

Shigo, A.L. 1972. Successions of microorganisms and patterns of discoloration and decay after wounding in red oak and white oak. Phytopathology 62, 256-259.

Shigo, A.L. 1979a. Tree decay - an expanded concept. U.S. Dept. Agric. Forest Service, Agric. Info. Bull. No. 419, 72pp.

Shigo, A.L. 1979b. Compartmentalization of decay associated with *Heterobasidion annosum* in roots of *Pinus resinosa*. Eur. J. For. Path. 9, 341-347.

Shigo, A.L. 1982. Trees resistant to spread of decay associated with wounds. In: Heybroek, H.M., B.R. Stephan and K. von Weissenberg (eds). Resistance to diseases and pests in forest trees. Proc. 3rd Int. Worksh. Genet. Host-Parasite Int. For., Wageningen. pp.103-109.

Shigo, A.L. 1983. Tree defects: a photo guide. U.S. Dept. Agric. Forest Service, Gen. Tech. Rep. NE-82, 167pp.

Shigo, A.L. and W.E. Hillis. 1973. Heartwood, discolored wood, and microorganisms in living trees. Annu. Rev. Phytopathol. 11, 197-222.

Shigo, A.L. and E.vH. Larson. 1969. A photo guide to patterns of discoloration and decay in living northern hardwood trees. U.S. Dept. Agric. Forest Service, Res. Pap. NE-127, 100pp.

Shigo, A.L. and H.G. Marx. 1977. Compartmentalization of decay in trees. U.S. Dept. Agric. Forest Service, Agric. Info. Bull. No. 405, 73pp.

Shigo, A.L. and W.C. Shortle. 1979. Compartmentalization of discolored wood in heartwood of red oak. Phytopathology 69, 710-711.

Shortle, W.C. 1979a. Mechanisms of compartmentalization of decay in living trees. Phytopathology 69, 1147-1151.

Shortle, W.C. 1979b. Compartmentalization of decay in red maple and hybrid poplar trees. Phytopathology 69, 410-413.

Shortle, W.C. and E.B. Cowling. 1978. Development of discoloration, decay, and microorganisms following wounding of sweetgum and yellow-poplar trees. Phytopathology 68, 609-616.

Shortle, W.C. and A. Ostrofsky. 1983. Decay susceptibility of wood in defoliated fir trees related to changing physical, chemical and biological properties. Eur. J. For. Path. 13, 1-11.

Smith, D.E. 1980. Abnormal wood formation following fall and spring injuries in black walnut. Wood Sci. 12, 243-251.

Smith, K.T., R.O. Blanchard and W.C. Shortle. 1981. Postulated mechanism of biological control of decay fungi in red maple wounds treated with *Trichoderma harzianum*. Phytopathology 71, 496-498.

Somers, T.C. and A.F. Harrison. 1967. Wood tannins - isolation and significance in host resistance to *Verticillium* wilt disease. Aust. J. Biol. Sci. 20, 475-479.

Specht, R.L. 1970. Vegetation. In: Leeper, G.W. (ed). The Australian environment. Melbourne Uni. Press, Melbourne. pp.44-67.

Tippett, J.T., A.L. Bogle and A.L. Shigo. 1982. Response of balsam fir and hemlock roots to injuries. Eur. J. For. Path. 12, 357-364.

Tippett, J.T. and A.L. Shigo. 1981. Barrier zone formation: a mechanism of tree defence against vascular pathogens. IAWA Bull. 2, 163-168.

Tomiyama, K. 1963. Physiology and biochemistry of disease resistance of plants. Annu. Rev. Phytopathol. 1, 295-324.

Toole, E.R. 1964. Progress of oak heart rot varies with height in tree. Plant Dis. Rep. 48, 585.

Toole, E.R. 1965. Inoculation of Bottom-land red oaks with *Poria ambigua, Polyporus fissilis,* and *Polyporus hispidus.* Plant Dis. Rep. 49, 81-83.

Toole, E.R. 1967. Rates of wood decay behind open and closed wounds. Plant Dis. Rep. 51, 600.

Wagener, W.W. and R.W. Davidson. 1954. Heart rots in living trees. Bot. Rev. 20, 61-134.

Wallis, G.W. 1961. Infection of Scots pine roots by *Fomes annosus.* Can. J. Bot. 39, 109-121.

Wardell, J.F. and J.H. Hart. 1970. Early responses of sapwood of *Quercus bicolor* to mechanical injury. Can. J. Bot. 48, 683-686.

Wilkes, J. 1981. Heartrot in tallowwood. Aust. Nat. Uni. Dept. Forestry, Grad. Dip. Sci. Res. Rep., 160pp.

Wilkes, J. 1982a. Stem decay in deciduous hardwoods - an overview. Aust. For. 45, 42-50.

Wilkes, J. 1982b. Pattern and process of heartrot in *Eucalyptus microcorys*. Aust. For. 45, 51-56.

Wilkes, J. 1984. The influence of rate of growth on the density and heartwood extractives content of eucalypt species. Wood Sci. Technol. 18, 113-120.

Wilkes, J. and W.A. Heather. 1983. Influence of wood extractives on fungi invading the stems of *Eucalyptus microcorys*. Holzforschung 37, 163-166.

Worrall, J.J. and J.R. Parmenter. 1983. Inhibition of wood-decay fungi by wetwood of white fir. Phytopathology 73, 1140-1145.

ADDENDUM - SUPPLEMENTARY INFORMATION

Chapters 1-2 (pp. 6, 7, 24).

As described in detail by Jacobs (1955) [ref. Ch. 2], in a eucalypt stem, the pressure of the expanding periphery against the stub of a small (< ca. 2-3 cm dia.) branch is usually sufficient to break that stub in the region of the stem cambium. After the stub has broken, but before it is excluded from the stem, kino is usually deposited over the fractured surfaces. Thus, when the stub is shed, a coating of kino prevents entry of microorganisms into the stem. Larger branches are often sufficiently strong to resist this breaking process, and until such time as the stub is overgrown, microorganisms have direct access to stem tissues.

Chapter 2 (pp. 17, 24).

Resistance to decay *in vitro* and resistance to heartrot *in vivo* are two quite separate concepts e.g. wood highly susceptible to decay *in vitro* may be highly resistant to decay *in situ* if the supply of oxygen within the stem is so low as to restrict microbial activity.

Chapters 2-6 (e.g. pp. 18, 47).

Blocks used in decay tests were elongated (e.g. 2x1x1 cm) rather than cubical, primarily to increase the ratio of surface area to volume. This allows slightly more rapid decay in the accelerated laboratory test. The dimensions of blocks used in other tests, e.g. in determining the presence of living cells, were usually those suggested in the relevant standard method to which reference is made.

Chapters 2-6 (e.g. pp. 32, 33, 46).

The possibility of contamination of wood (both *in vivo* and *in vitro*) with iron from steel based tools (drill bits, chisels, scalpels, etc.) has received little attention. Polyphenols within tissues of the standing trees apparently reacted little with the steel drill bits, since blue-black colouring was not evident around the holes.

Chapters 3-4 (e.g. pp. 10, 30, 45).

The concept of a response of dead tissue in heartwood is not universally accepted. Nevertheless, appreciable evidence suggests that in living trees of some species, microbial deterioration is actively localised in both heartwood and sapwood (Shigo 1983 [ref. Ch. 3]). The nature of the apparent response of heartwood has yet to be determined (p. 93). Various modes of response in sapwood are considered on p. 94.

Chapters 3-4.

Exposure of tissues of the stem to air as a result of mechanical injury may stimulate effects, such as polymerisation of extractives, not related directly to the injury *per se*. It may be possible to employ other types of injury e.g. bruising, in future studies.

Chapter 4 (p. 54)

In the concept of 'compartmentalisation of decay in trees' (Shigo and Marx 1977 [ref. Ch. 4]), 'Wall 1' refers to the physicochemical barriers, e.g. tyloses, which impede the spread of microorganisms in the axial direction.

Chapter 6 (p. 83).

Kino veins may also act as sinks for carbohydrates. These materials could accumulate adjacent to the vein, or be converted to phenols within the vein.

Chapter 6.

By the triphenyl tetrazolium chloride test, included sapwood was shown to be devoid of living cells.

Throughout. Terms are used as follows:

Microbe (e.g. pp. 9, 34, 36) - syn. microorganism.
Decay - degradation of wood structure by fungi.
Discolouration (e.g. pp. 43, 51) - abnormal (usually dark) colour in wood. The change in colour usually precedes decay. Discoloured wood often contains microorganisms.
Deterioration (e.g. pp. 43, 51) - includes discolouration and/or decay.

CORRIGENDA

Chapter 1. p. 7, para. 2, lines 3-4. 'precluded...ageing' to read 'precluded determination of the age of branches and stems'

Chapter 2. p. 19, line 15. 'by reference to' to read 'by deducting from them, the percentage weight loss values of'

Chapter 3. p. 30, lines 8-10. '(Wilkes 1985a)' to follow 'context', not 'forest'
p. 34, para 4, lines 2-3. 'also' to follow 'apparently', not 'tissues'

Chapter 4. p. 43, line 12. 'sapwood, and in' can read 'sapwood. Further in'

Chapter 5. p. 61, lines 5-6. 'undifferentiated parenchyma' - sensu ground tissue type
p. 61, line 9. 'lowly lignified' can read 'less lignified than adjacent tissues'
p. 66, line 13. 'lumens' should read 'lumina'
p. 66, para. 2, line 8. 'transgressed' syn. 'traversed'
p. 68, para. 2, lines 9-10. 'genera...Ulmus'can read 'timbers, including walnut (Juglans) (...), sweetgum (Liquidamber)(...) and elm (Ulmus)'
p. 68, para. 4, line 1. 'or' can read 'and/or'
p. 69, para. 3, lines 1-2. 'While...studied,' to read 'The anatomy of barrier zones has been commonly studied. However'
p. 70, para. 2, line 2. 'and distinct' can read 'and others distinct'
After p. 74 - caption to Fig. 7. 'filling' to read 'filled'

Chapter 6. p. 81, para. 3, lines 1-2. 'Paper...into' should read 'Using paper chromatography, the methanol extracts from both heartwood and included sapwood were separated into'
p. 82, line 5. 'further.' to be followed by 'In particular, the general increase in density, extractives content and natural decay resistance with distance from the pith (before a decline in sapwood) is well documented.'
p. 83, line 12. 'extraneous' syn. 'extractable'
p. 83, line 27. 'certain' can read 'methanol-soluble'

Chapter 7. p. 89, para. 3, lines 1-2. '(assumed hereafter to be real)' can be deleted
p. 89, para. 4, lines 1-2. '(here...in vitro')' can be deleted
p. 89, para. 5, line 1. 'The dynamic' to read 'The possible dynamic'