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The formation of dry sapwood zones in conifers: A review

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The formation of dry sapwood zones in conifers: A review

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EXECUTIVE SUMMARY

Columns of white, dry wood are formed in the sapwood of many *Pinus radiata* stems infected with *Neonectria fuckeliana.* The columns of white wood, which may extend up to 2 m up the stem, are characteristically associated with infection centres at branch whorls. Radial, tangential and longitudinal extent of white wood is variable. *Neonectria fuckeliana* may sometimes be readily isolated from points throughout the white wood but in many instances is obtained only close to a pruned whorl, with the remainder of the white wood column apparently free of any colonising micro-organisms. Although this trait of white, dry wood has been previously observed in *P. radiata* the consistency with which it occurs in *N. fuckeliana*-infected stems, and the length of the columns, is considered unusual.

Pine-pathogen systems in which gas enters the sapwood in advance of colonising fungi have been recorded, albeit infrequently. Both the physical activity of hyphae forcing a passage through the pit membrane, and the activity of enzymes produced by the fungal pathogen and causing lysis of the pit membrane, thereby allowing air to enter the tracheids have been implicated in the initiation of dry-wood zones. Cavitation via air-seeding into surrounding tracheids may occur with a cascading effect that results in dry areas in the wood. In the northern hemisphere dry wood zones formed in conifers in response to inoculation with the pathogen *Heterobasidion annosum* extended from 0.8 m to 1 m above the region that the fungus had penetrated. Dry zones of lesser extent could also be induced in response to extracts of *H. annosum*-infected wood. The reason why dry wood zones are formed in some pathosystems and not others is not clear.

Dehydrated whitish wood has been reported in sapwood of *P. elliottii* alongside white zones with high levels of oleoresin deposit in the cells and tracheids but in the absence of fungal hyphae. The mechanism of the excessive resin production and of the dehydration was not determined. Similar resin-heavy wood zones have been found in *P. radiata* stems with Nectria flute cankers. The extent of this resin deposition and its association with dry wood is at present unknown.

Although sapwood drying has been reported to occur in pines solely in response to exposure to the air through wounding, particularly during the inactive period of growth, areas of dry wood are limited to small areas in proximity to the wound. Wounding itself is not therefore the primary cause of the formation of the dry wood observed in *P. radiata* stems.

This review sought to draw on the available information and establish likely causes for the formation of white, dry sapwood columns in *P. radiata* stems, particularly in the context of *N. fuckeliana* infection, and to enable us to interpret its role and importance in the development of Nectria flute cankers. A clear conclusion has not been reached but several lines of research are indicated.

Further Work

Further research examining the formation of white, dry wood zones in response to inoculation with both living *N. fuckeliana* and to fungal extracts is indicated. A comparison of the effect of extracts from wood infected with other stem-colonising fungi may demonstrate whether *N. fuckeliana* has a capability in the formation of dry wood zones that other common stem fungi do not possess.

As the phenomenon of air-seeding through the tracheids resulting in extensive zones of dry wood does not occur in response to all fungal colonisers, and has seldom been observed in relation to physical injury alone, the way in which *N. fuckeliana* infection influences cavitation will be further investigated. Field experiments will be complemented with biochemical studies and microscopy.
Microscopic studies will be undertaken to determine the extent and distribution of the zones with resin deposits and this wood will be compared with that from dry zones.

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Products investigated	Dry wood zones in xylem tissues
Wood species worked on	Pinus radiata
Other materials used	Neonectria fuckeliana
Location	

INTRODUCTION

Columns of white, dry wood are formed in the sapwood of many *Pinus radiata* stems infected with *Neonectria fuckeliana* in southern regions of New Zealand. The dry wood is characteristically associated with infection centres, or with branch stubs and the columns vary in how far they extend, both longitudinally and in the tangential and radial planes. Although this trait of white wood has been previously observed in *P. radiata* the consistency with which it occurred in *N. fuckeliana*-infected stems, and the length of the columns, is considered unusual. Transfer of white wood chips from over twenty *P. radiata* onto fungal growth media has given a range of isolation results. *Neonectria fuckeliana* was sometimes readily isolated from points throughout the white wood but this was not always so. In some instances the fungus was obtained only close to a pruned whorl and from stained sapwood that had the characteristic appearance of colonisation by a sapstain fungus while the surrounding white wood area appeared to be free of colonising organisms.

In addition to the dry white zones in the sapwood there may be areas of whitish wood that have high resin content (demonstrated by sectioning, staining and microscopic examination) and, when cut from the stem, are heavier than normal sapwood.

Although there is a large body of literature addressing the physiology of fungal invasion in tree stems, comparatively little literature directly discusses the topic of dry wood zones in conifer xylem tissues. What research has been carried out has largely taken place in the Northern Hemisphere and primarily on non-pine conifers. This review sought to draw on the available information and establish likely causes for the white dry wood in *P. radiata* stems in the context of observations in New Zealand, and to enable us to interpret its role and importance in the development of Nectria flute cankers. It has been necessary to include some background on the anatomy of conifer xylem tissues and on water movement to provide a framework for the discussion. Other aspects of fungal invasion of conifer stems that may be pertinent to the pathosystem have also been touched on briefly.

White, dry wood zones are illustrated in Figures 1-6.

Figs. 1-6: White wood zones in *P. radiata* stems with Nectria flute cankers

Fig, 1:

Fig. 2

Fig. 3

Fig. 4

Fig. 5

Fig. 6

WATER AND SAPWOOD

Pathological heartwood

Although the dry wood zones in *P. radiata* sapwood were initially referred to as 'pathological heartwood,' a phrase that has been commonly used for non-living areas of wood within what should be living sapwood, it became apparent that this term for the dry, white wood that was typically being found in *P. radiata* in southern regions of New Zealand was inappropriate.

In early studies of development of decay it was noted that a dark coloured tissue resembling heartwood was formed in response to wounding and the term 'pathological heartwood' to describe this tissue was coined by Hepting (1936). 'Normal' heartwood is the non-living wood that develops in the centre of the tree and which is not a result of injury. Subsequently with greater research into the responses of trees to wounding and infection (Jorgensen 1962; Shigo 1984; Shortle and Cowling 1978; Boddy and Rayner 1983; Blanchette 1992; Boddy 1992) it has become apparent that the term 'heartwood' in 'pathological heartwood' is inappropriate although it has continued to be widely used. The various types of tissue, some coloured and some not coloured, formed in response to injury and infection, are very different physically and chemically from 'heartwood', which is an age-modified tissue. The terms 'reaction zone', 'transition zone' or 'protection wood' are generally considered to be more appropriate to describe discoloured wood within the sapwood. Pending further anatomical studies the zones in *P. radiata* under discussion here will be referred to as 'dry wood zones' as they do not accurately match the criteria described above.

Xylem tissues and water movement in conifer stems

Conifer xylem is composed primarily of tracheids, single-celled structures serving the dual purposes of vascular conduction, and structural support. Water flows between the tracheids by means of numerous bordered pits. The Cohesion-Tension theory of how water moves in standing trees states that water is lifted by the force of evaporation at leaf surfaces. The cohesion of water molecules to each other and adhesion of water to tracheid walls results in water columns under tension, up to as much as -10 megapascals (Tyree 2003).

Water under tension is metastable and can be subject to cavitation. Injury to the xylem results in withdrawal of water under tension and its replacement by air in the broken tissue. However air is generally prevented from spreading continuously through the xylem of conifers by the valve-like action of the bordered pits, closure being brought about by the difference in pressure on either side of the pit membrane. Pits in conifer tracheids have a torus-margo membrane with the torus acting as a sealing mechanism while the margo permits passage of water (Fig 2). This achieves a compromise between hydraulic conductance and prevention of cavitation via air-seeding i.e. the passage of air from an embolised tracheid into an adjacent functioning tracheid (Hacke et al 2004, Tyree and Zimmerman 2002). If water pressure is equal on both sides, the torus remains in the middle and allows water conductance. If the water pressure drops, as may happen in injured tissue the torus is deflected to one side and pressed against the pit border to seal off the tracheid (Fig 7) (from Zimmerman 1983). However the torus-margo pit membranes can be deflected post-aspiration. When tracheids begin to cavitate in this manner, the pressure in surrounding tracheids becomes ever more negative, increasing the likelihood of further cavitations and cascading xylem collapse, particularly if a pathogen is present.

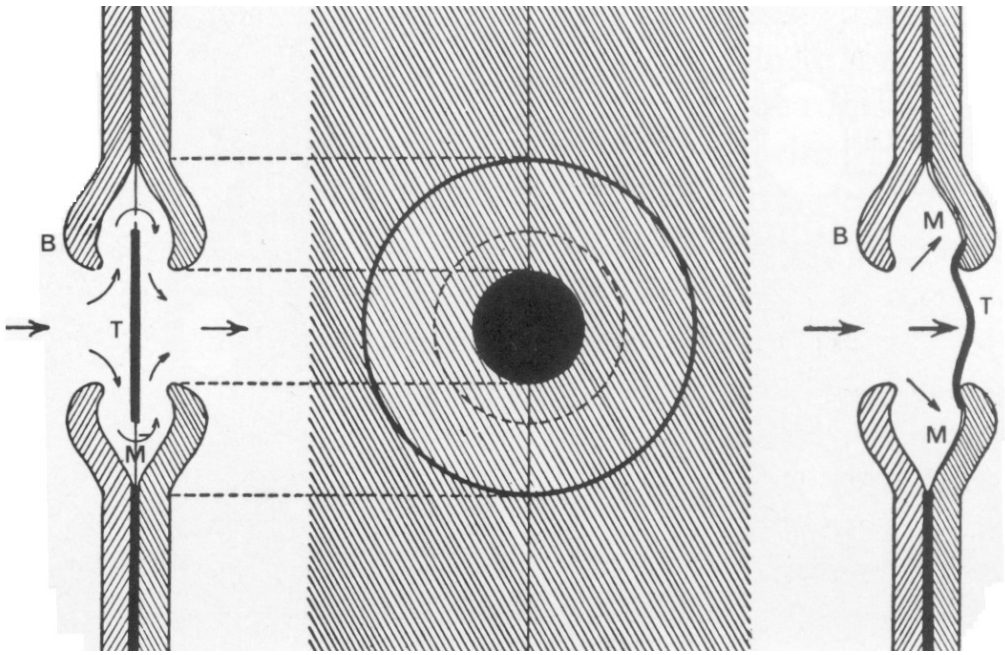

Fig 7. *Centre* – Surface view of the radial wall of a coniferous tracheid showing a bordered pit.
Left – The pit in sectionwith arrows indicating the path of water from one tracheid into the next.
Right – Section showing the valve-like action of the torus.
T torus; *M* pit membrane; *B* pit border (Zimmerman 1983)

Aeration of sapwood in Pinus spp.

Harris (1991) describes the typical reaction of *P. radiata* sapwood to the ingress of air. If a gas bubble develops within a tracheid, the water column is broken. Moreover under the high tensile stress that exists a small bubble could expand and block a large part of the conducting system. Generally the behaviour of the bordered pits prevents this happening (as described in the previous section). A gas bubble develops surface tension at the gas/water interface and when the gas bubble enters a pit chamber the effect of the surface tension is to pull the torus towards the pit membrane and seal it off, leaving a single aspirated pit. The water conducting system of the stem therefore suffers minimal damage.

Fungal infection of the sapwood of *Pinus* spp. may result in the replacement of free water in the xylem with gas (Nelson 1934; Caird 1935; Bramble & Holst 1940) with the subsequent formation of dry zones within the sapwood. Mathre (1964) and Basham (1970) found that gas entered the sapwood of *P. ponderosa* in advance of colonising blue stain fungi (*Ophiostoma* spp.) and believed that air replaced water which evaporated from the stem surface. Dry, stained wood forms in black stain root disease of pines and other conifers caused by the insect-vectored fungus *Leptographium wageneri*, an anamorphic *Ophiostoma* sp. (Harrington and Cobb 1983). *Leptographium wageneri* cannot penetrate roots without wounds (Smith 1969), is xylem-limited, cannot decay cell walls and therefore must use existing openings to move from tracheid to tracheid. A hypha forces the torus-margo membrane aside (Hessberg and Hansen 1987) allowing air-seeding into the adjacent tracheid. Hyphae are not present in many of the tracheids within a column of stain because cavitation through the tracheids cascades beyond the fungal growth. A similar effect was observed in conifers infected with the decay fungus *Heterobasidion annosum* (Gregory & Perry 1973).

Coutts & Dolezal (1966) examined dry zones that formed in *P. radiata* sapwood associated with infection by *Amylostereum,* the fungus transmitted by the wood wasp *Sirex noctilio*, and found that the drying extended longitudinally up to 0.2 m beyond the fungal mycelium. (Coutts, 1965) also found that prevention of evaporation through the bark or oviposition holes did not prevent the development of drying streaks in attacked stems. *Amylostereum areolatum*, both with and

without the addition of the toxic mucous that is also transmitted by *S. noctilio*, was subsequently inoculated into *P. radiata* stems by Kile and Turnbull (1972) who confirmed these results and also found that treatments at 3.5 and 6.0 m above the ground caused twice as much drying as the treatments at 1.0 m above ground. In the absence of the mucous the average amount of dry wood formed was reduced, the reduction being in lateral extension but not in the vertical length.

In following up these observations Coutts (1976) inoculated stems of several conifer species (*Abies grandis*, *Larix decidua*, *Picea abies*, *Pinus sylvestris* and *Tsuga heterophylla*) with *H. annosum* and found that after 4 months dry streaks of sapwood extended from 0.8 m to 1 m above the region that the fungus had penetrated. Dry zones extended further beyond the limit of fungal infection in the inner sapwood than in the outer sapwood, and further upwards than downwards. They extended further radially in towards the heart than transversely in the stem.

Coutts (1976) further examined the formation of dry zones in the wood of freshly cut pine logs in relation to fungal activity. Limited dry zones developed in response to exposure to air alone, but the extent of dry wood formed when holes drilled in the log were filled with extracts from wood infected with *H. annosum* were very much longer than when extracts from uninfected wood were applied. This was postulated by Coutts (1976) to be due to the presence of pectolytic enzymes in the *H. annosum* extract. Microscopic examination showed that although pit tori were completely lysed around the drill holes the bordered pits further away were aspirated but not lysed. No lysis, or extended dry wood zones occurred in logs to which extracts from uninfected wood were applied. Injection of these extracts of infected wood into autoclaved logs also caused the formation of dry zones in which the tori of bordered pits had been lysed. However the dry zone was smaller and did not extend as far as when the logs containing living cells were injected.

Dry zones were also induced in freshly cut logs of *P. nigra* and *P. sylvestris* by the injection of the poisons mercuric chloride, potassium cyanide or oxalic acid that killed the tissue (Coutts 1977) and these were observed to be similar to those extending beyond an area of fungal infection. Coutts (1977) therefore suggested that the effect was due to toxic substances diffusing from the infected region. In endeavouring to explain how gas could appear in the system so as to allow the release of water from the tracheids, and why the differences between logs with living and those with non-living cells occurred, Coutts (1977) proposed that the dry wood therefore may be formed at least partly as a result of water loss from damaged tracheids by hydrostatic tension. Fungi may play a role in causing lysis of bordered pit tori. However, as the dry zone extends far beyond the region of lysis of pit tori an active role of slowly dying ray parenchyma is indicated.

Jorgensen (1961) reported that drying of the sapwood of Pinus resinosa occurred in response to exposure to the air through wounding alone. This was particularly likely during the inactive period but areas of dry wood were limited to a few millimetres in close proximity to the wound.

Fergusson (2006) examined the reasons why the coastal varieties of *Pseudotsuga menziesii* (Douglas fir) experience higher incidence and severity of black stain root disease (caused by *Leptographium wagneri*) than inland varieties, although both the pathogen and vectors are widespread. He suggested that vulnerability to xylem cavitation may be a likely cause. Wood permeability is one factor, among others, that distinguish coastal and inland varieties of D fir. Inland D fir has greater resistance to xylem cavitation than the coastal variety (Kavanagh 1999) and the drying of the tracheids that is more characteristic of the coastal varieties may expose the tissues to fungal colonisation.

Resinous lightwood

White dry zones in the sapwood may be found in association with areas of whitish wood that have high resin content and, when cut from the stem, are heavier than normal sapwood. Miniutti (1977) discussed the formation of ‘lightwood’ in *P. elliottii* sapwood in response to application of the herbicide paraquat to trunk wounds. Two zones, both above the wound in a wedge-shape,

were described: a) an oleoresin soaked zone, and b) a paler zone of dehydrated wood without resin and with little, or no, free water in the lumen. Within the resinous zone deposits of oleoresin were found in resin ducts and in the lumens of all types of cells. Samples were sectioned and examined for fungal hyphae as enzymes secreted by sapwood-invading fungi were known to cause limited 'lightwood' formation in *P. elliottii*. Few hyphae were found and it was concluded that fungal presence is not necessary for the formation of these zones. The mechanism of the excessive resin production and of the dehydration was not determined.

Wound colonisation

Three 'models' or viewpoints have been proposed to attempt to explain the pattern of colonisation observed behind many wounds and the chemical and morphological changes between lesions and living sapwood. The models are based on:

- reaction zone formation (Shain 1967, 1971, 1979)
- compartmentalisation of decay in trees (referred to as 'Codit' by Shigo and Hillis 1973, Shigo 1984)
- responses brought on by changes in the microenvironment after wounding (Boddy and Rayner 1983, Boddy 1992)

The **reaction zone** separates the decayed and discoloured wood from healthy tissue. Reaction zones, in general resist digestion by most fungi, but the antifungal compounds present in the tissues may be eventually depleted; this results in a breach of the reaction zone. When this occurs, new reaction zones are formed, interior to the preceding one, by the same processes that formed the initial reaction zone (Shigo and Marx 1977; Pearce and Woodward 1986; Pearce 1996). The result is a succession of reaction zones formed and broken down that lead to the gradual decay of those woody tissues present at the time of wounding. Reaction zone formation is a non-specific response in trees which are wounded or infected by various agents.

The term **reaction zone barrier** is used to describe an active defence barrier formed in differentiated wood and includes the reaction zone and the **transition zone** (Yamada, 1992). The transition zone may also contributes to the confinement of colonising micro-organisms. It is a non-conducting, dry tissue where metabolic activity is greatly elevated (Shain 1971). The transition zone in conifers is thought to be the first stage in reaction zone formation, as a site of phenolic precursors for the production of polyphenols (Shain 1967, 1971, 1979). Ethylene production in the sapwood, which is implicated in triggering the synthesis of antifungal phenolic compounds, is also attributed to activity in the transition zone (Shain and Hillis 1973). In many hardwoods there is an accumulation of water in the reaction zone but in conifers the reverse is more common with reaction zones generally drier than adjacent sapwood (Shain 1971).

The role of the reaction zone, transition zone and transition zone barrier is complex and has been shown to differ between genera and even between species.

The **Codit model** explains the tree's response to infections as a two-step process. First, 'reaction zones' or chemical boundaries are formed at the time of injury in living parenchyma cells of the sapwood and by enzyme activity in the heartwood. Then the cambium responds by forming a 'barrier zone' to isolate new tissues from those present at the time of wounding. The Codit system describes these reactions as four 'walls' each based on the trees anatomical features (Shigo and Marx 1977; Shortle 1979). 'Wall 1' the weakest barrier, is formed when the vessels and tracheids are plugged above and below the wound. Spread inwards to the pith is limited by 'wall 2'. 'Wall 3' limits the spread perpendicular to the ray cells. 'Wall 4' the strongest wall, or the barrier zone, separates the xylem formed before wounding from xylem formed after wounding. The parenchyma cells of the barrier zone produce chemicals that result in a layer of new xylem more impervious than that produced by walls 1-3. 'Wall 4' prevents microbes from invading the tissues formed by the cambium after wounding (Shortle 1979).

Both the reaction zone and compartmentalisation models imply active host defences.

The **micro-environment** model suggests that changes in the internal micro-environment of trees as a result of wounding and associated xylem dysfunction, could determine lesion development without any need for active host responses. The high water content, and consequent low availability of oxygen, in functional wood will inhibit fungal growth. Boddy and Rayner (1983) suggest that resistance of sapwood to fungal invasion is due to the high moisture content and insufficient amount of oxygen provided by the waterproofing layer.

High moisture content and associated restriction of aeration are recognised factors limiting fungal activity in felled or fallen timber. Placing logs long-term under water sprays is an established method of protecting against invasion by decay fungi when environmental events such as forest blow-down mean that the wood cannot be processed in a suitable time frame. Some fungi, such as those causing vascular wilts (e.g. *Ophiostoma novo-ulmi* causing Dutch elm disease, can grow in functional sapwood. However although capable of some mycelial development spread within the xylem is probably largely through the abundant spores that are produced. This group of pathogens represents a specific, though minority, method of invasion and rapid pathogenesis (Gibbs 2001).

Extension of decay columns in sapwood of angiosperms may be very rapid and (Boddy 2001) suggests that it is unlikely to occur by mycelial spread from a single focus. A more likely scenario is that propagules (eg spores, mycelial fragments) may be already present in the sapwood of trunks and branches, but are prevented from developing when the xylem is functional due to the high water content and low nutrient availability. The latent presence of some decay fungi in sapwood has been demonstrated for a number of hardwood tree species (e.g. Chappela & Boddy 1988, Griffith & Boddy 1990, Fisher & Petrini 1990) by isolating from freshly felled wood or by allowing lengths to slowly dry. In the latter case pockets of stain or decay develop soon after drying begins. Often genetically identical propagules have been found well dispersed before visible development of symptoms (Boddy 2001). The way in which these propagules gain entry to the sapwood is unknown though a variety of pathways can be hypothesised.

If maintenance of water-saturated sapwood explains the limitation of fungal colonisation then sapwood that is newly formed outside the barrier zone after damage or fungal colonisation would be inaccessible to mycelial development. Rather than being a barrier to spread of infection the barrier zone could therefore be interpreted as a mechanism preventing movement of air or loss of water from the newly formed conducting tissues (Boddy and Raynor 1983). Fungal establishment at a wound is therefore likely related to the loss of water in wounded tissue. If fungal metabolites can then promote further loss of water from tracheids (Coutts 1976) the path is open to more extensive fungal colonisation. Invasion of sapwood in spring and summer when there is vigorous growth, and sap flow rates are high, are likely to be less effective than invasion occurring in the colder months (Boddy and Raynor 1983).

External stresses that affect various physiological functions can predispose trees to disease (Schoeneweiss 1981). The lowering of moisture content in the sapwood during periods of drought is likely to be one such function (Boddy and Raynor 1983). Indeed Chou and MacKenzie (1988) found that infection of the stem of *P. radiata* by *Diplodia pinea* (syn. *Sphaeropsis sapinea*) was highly facilitated by a predisposing period of drought.

These different models of colonisation of wood following wounding are in no way mutually incompatible: compartmentalisation and the formation of reaction zone barriers may have the dual functions of protecting against fungal attack directly, and helping to prevent aeration and maintaining the functional integrity of the healthy xylem adjacent to a wound or infection site (Pearce 2000). While it is uncertain which model most closely accounts for the mechanisms of tree defence, it is likely that elements of the three models may function together in protecting the tree when injured or infected. The exact strategy that the tree ‘employs’ will depend on the species and even the exact genotype within a species.

SILVICULTURAL PROCESSES THAT MAY INFLUENCE FUNGAL INVASION AND SAPWOOD DRYING

The branch protection zone and pruning

Pruning technique may affect the introduction of organisms into the tree. Flush cuts to remove branches result in larger, deeper wounds than wounds resulting from cuts close to but exterior to the branch collar. Flush cuts will also remove the branch protection zone (BPZ) - generally recognised as a double-cone-shaped resin-saturated zone that develops at the base of conifer branches. The xylem within the BPZ is occluded with decay resistant compounds (Green et al, 1981.) and the anatomy is such that the flow of water into the branch is somewhat reduced (Ewers and Zimmerman 1984). The importance of this branch protection zone in limiting stem infection after branch injury or removal has been described by Aufsess (1975), Shigo (1985) and Metzler (1997). Aufsess (1975) experimented with fungi and branches of *P. sylvestris* and *Picea abies* and found that when the protective zones were formed completely, fungi did not spread from the branch to the stem.

Pruning intensity

The number of wounds and the total size of the wounded area may affect the entry of pathogens. Pruning may also influence various physiological processes including the mobilisation of reserves, the synthesis or mobilisation of defence chemicals, water relationships within the tree and photosynthesis (through the reduction in leaf surface area). Langstrom and Hellqvist (1991) investigated the effect of different pruning regimes involving removal of 50 to 75% needle biomass on growth and sapwood area in 25 year old Scots pine (*P. sylvestris*). This intensity of pruning led to a decrease in conducting sapwood area, and an increase in non-conducting ‘heartwood’ area at breast height compared with unpruned controls. This finding is consistent with the understanding that the area of conducting xylem in a tree is directly related to foliage area and functional root area. Dry wood - normally a precursor to heartwood formation may form when roots and foliage can no longer maintain the full area of sapwood and when conditions favouring transpiration puts a strong pull or pressure on the outer sapwood. This pressure may result in breaks in the water column and drying out. The living cells in the sapwood require photosynthate to balance the loss of energy caused by cell respiration. However as a result of pruning and the reduced amount of photosynthesis that results from foliage removal, some of the living parenchyma or ray cells cannot be maintained and they slowly die. Slow death of ray cells appears to favour the formation of protection compounds in the cells while dry tissue without such compounds occurs when cell death is fast. The presence of dry wood can have a major influence on the progression of fungal invasion.

A dramatic illustration of the effect of pruning intensity on disease was described by Chou and MacKenzie in 1988. In a five-year-old *Pinus radiat*a stand, trees were pruned to remove 50%, 40% or 25% crown and the pruning stubs were inoculated with *Diplodia pinea* (syn. *Sphaeropsis sapinea*). In intensely pruned trees infection occurred in 65-68% of the trees, some of which also developed crown wilting and died, but in lightly pruned trees (25% crown removal) only 8% of the trees were infected and no mortality occurred.

Wound position

There is no real consensus in the literature on the effect of wound position on rates of infection. This is probably due the high degree of variation existing between different environments, host and fungal species. Many researchers have reported that root and stem wounds that contact the soil nearly always become infected regardless of size, and have faster decay rates than wounds higher in the stem; the observation applying to both hardwoods and softwoods (e.g. Hunt and Krueger, 1962; Isomaki and Kallio, 1974). However other researchers have reported limited colonisation of some basidiomycete species in the butt of *Pinus* spp. in comparison to that occurring further up the tree and attributed this finding to a lower resin content higher up the

stem. Kile and Turnbull 1972) inoculated *P. radiata* stems with *Amylostereum areolatum* at points 1, 3.5 and 6.0 m above the ground and found that on average, the treatments at 3.5 and 6.0 m caused twice as much drying as the treatments at 1.0 m. and drying extended twice as far above the treated zone as below it.In this context Chou and MacKenzie (1988) reported similar results with *D. pinea* infection of radiata pine. They found a gradient of resistance from the bottom of the tree (extremely resistant to infection) to the top (more susceptible). Changes in the moisture status of stem tissue with distance from the roots may also be a significant factor influencing colonisation of fungi high in the stem (Boddy, 1992).

Season

Vasiliauskas and Stenlid (1998) found that season of injury as well as position on the tree influenced the species composition of the fungi colonising wounds on *Picea abies* stems. An *Ophiostoma* sp. that was associated with butt wounds was more frequent in August (summer) wounds while the basidiomycetes *Stereum sanguinolentum* and *Cylindrobasidium evolvens* occurred more often in January (winter) wounds.

Shain (1967) found that both survival and distance of penetration by *Fomes annosus* in sapwood of inoculated loblolly pine (*P. taeda*) were related to season and therefore with the physiological status of the host. This was borne out in the following observations-

- The distance of penetration by *H. annosum* decreased most abruptly between April and July (Spring-Summer) the period during which the host was most active.
- The distance of penetration by *H. annosum* was greater when the host was dormant. This was especially true for dominant trees – for these trees there was little penetration during the growing season.

The seasonal difference were in agreement with expectations from earlier observations of wounds in stems of *Pinus sylvestris*, in that a lack of resin flow in trees wounded in winter permitted rapid desiccation of tissues and delayed production of pinosylvins in comparison with trees wounded during the growing season (Lyr, 1966). However, Shain's study (1967) observed no great difference in the amount of pinosylvins in reaction zone of trees harvested 3 months after inoculation in dormant or growing seasons.

DISCUSSION

The sapwood in conifers consists of tracheids through which water passes via bordered pits. These pits have a membrane that permits passage of water, but is also able to seal the junction in the event of exposure to air or gas. However this important function can be compromised. If air from an embolised tracheid moves into an adjacent functioning tracheid further cavitation via air-seeding into surrounding tracheids may occur with a cascading effect that results in dry areas in the wood. This has particularly been observed if a pathogen is present but is not a function of all pathosystems. Harris (1991) describes the structure of tracheids and bordered pits in the sapwood of *P. radiata* and states that the effect of gas in a tracheid is that the torus is pulled towards the pit membrane and seals it off, leaving a single aspirated pit. Air-seeding through the tracheids resulting in extensive dry zones has clearly not been a common feature of *P. radiata.* Although discussed in relation to the fungus vectored by *Sirex noctilio* the dry zones formed in this system are of limited extent (Kile & Turnbull 1972).

The formation of dry wood zones similar to those observed in association with *N. fuckeliana* in *P. radiata* in southern regions of New Zealand has been reported in the stems of *Pinus* spp., as well as other conifers in the Northern Hemisphere. Limited dry zones will develop in sapwood in response to exposure to air alone (Jorgensen 1962) but these are usually confined to small regions of a few millimetres diameter around the wound. Fungal infection, primarily by basidiomycetes but occasionally by ascomycetes, has been implicated as the initiating factor of longer dry wood zones in many instances and has been experimentally demonstrated. Dry sapwood has been measured at distances up to 1 m beyond the presence of the fungus.

Columns of dry wood can also be induced when extracts from fungal infected wood are inoculated into living stems although these are not as long as those formed in response to the actual pathogen. This phenomenon has been demonstrated by Coutts (1976), working with the pathogen *H. annosum* in pine, and in other conifers, Enzymes in the extracts have been proposed (Coutts 1976) as the cause of lysis of pit tori in tracheids around the inoculation point. Once this lysis has occurred, air-seeding through the pits may follow and lead to aspiration of tracheids further away. As the dry zone can extend far beyond the region of lysis of pit tori an active role of slowly dying ray parenchyma is indicated (Coutts 1977). Mycelium of *N. fuckeliana* colonises the stem tissues primarily through the ray cells (P. Crane, A. Hopkins, unpublished data) as also does *Diplodia pinea* (Chou 1987).

We can therefore postulate that, as has been reported for *H. annosum*, it is initial colonisation of sapwood by *N. fuckeliana* that triggers the dry zone formation. Fungal activity in the rays may have a function in inducing a biochemical response but this is untested.

Although we theorised that drying of sapwood could be initiated through exposure to air from pruning wounds, and that invasion of the stem by *N. fuckeliana* might be facilitated by the pre-formation of dry wood zones, this sequence of events is not supported by the literature. Dry wood formed when sapwood is exposed to the air is limited to regions of a few centimetres around the wound (Jorgensen 1962). Removal of areas of bark with concommitant exposure of the sapwood is a fairly common occurrence. It may be associated with silvicultural activities or damage caused by animals such as deer or possums. Yet development of extensive dry sapwood zones has not been reported in association with such injury. It therefore seems more likely that infection by *N. fuckeliana* occurs prior to the formation of dry wood zones in *P. radiata*. This possibility could be tested by performing experiments similar to those carried out by Coutts (1976) and using extracts from wood infected with *N. fuckeliana*.

Wood microscopists at Scion examined a piece of heavy whitish sapwood cut from a *P. radiata* stem exhibiting Nectria flute canker by sectioning, staining and microscopic examination. The sample was found to have oleoresin deposition in the cells similar to that described by Miniutti (1975) when he studied ‘lightwood’ in *P. elliottii* formed in response to paraquat treatment. A Scion wood biochemist described this phenomenon as unusual in *P. radiata* and worthy of

further investigation. The extent of this resin deposition and its association with dry wood is at present unknown.

The variables associated with silvicultural operations and the interaction of these variables with season has an effect on water levels within the sapwood. Research into the development of Nectria flute cankers in New Zealand has shown that fluting is more common in stubs after second-lift pruning operations i.e. higher up the stem, and that incidence is strongly correlated to stub size. Parallels with these findings can be found in some of the research reviewed. Kile and Turnbull (1972) and Chou and MacKenzie (1988) both found that invasion of *P. radiata* following inoculation with a fungal pathogen occurred at a higher frequency, and the changes in the sapwood were more extensive, with increasing height up the stem. It was suggested by Boddy (1992) that changes in the moisture status with distance up the stem (decreasing) was the significant factor influencing colonisation of fungi high in the stem. Decreasing resin content with height has also been suggested as a reason for this phenomenon (Chou & MacKenzie 1988) but this seems unlikely to be a factor with *N. fuckeliana* infection as in *in vitro* studies the fungus does not appear to be limited by the presence of resin (Hopkins unpublished data).

Overseas *N. fuckeliana* was more frequently found at 1.3 metres above the ground in *P. abies* trees than at 0.3 metres (Delatour, 1976). Vasiliauskas *et al*. (1996) also found *N. fuckeliana* infection to be positively correlated with increasing height of wounds above the ground and Metzler (1997) noted that *N. fuckeliana* invasion of green-pruned *P. abies* was more prolific at the higher tree levels. In trials carried out with *N. fuckeliana* and *P. radiata* in New Zealand higher levels of flute canker have consistently been recorded in second or third pruning lifts than in the first lift (Bulman 2009). Decreasing resin content and decreasing water pressure higher in the stem have been proposed as reasons for this happening. Mycelial growth of *N. fuckeliana* does not appear to be limited by pine resin (Hopkins unpublished data) and so the increased incidence of cankering at higher pruned whorls may be a function of stem moisture status as described by Boddy (1992).

Season of pruning also affects the formation of Nectria flute cankers with initiation more likely during winter than in summer (Bulman 2006). This is in contrast to Diplodia whorl canker development which seems confined to a short period in midsummer. There is however a correlation with a pre-infection period of dry weather that places the trees under drought stress.

Boddy (2001) suggested that the finding that fungi latently present in the sapwood of hardwoods play a key role as pioneers of wood breakdown and decay may not be the same for conifers. Initial studies by Power & Ramsfield (2007) and Power *et al.* (2008) indicated that propagules of *N. fuckeliana* were present in the sapwood of unpruned, as well as pruned, *P. radiata* that did not have any symptoms of Nectria flute canker. Subsequent studies (TD Ramsfield & MWP Power unpublished data) did not demonstrate that there was extensive colonisation of the sapwood by *N. fuckeliana*.

CONCLUSIONS

The exact cause of dry zone formation in *P. radiata* stems with Nectria flute cankers has not been established although some parallels with other pathosystems, both in *P. radiata* and in other conifers have been found. It is unlikely that the initial hypothesis proposed for the formation of dry wood in *P. radiata* (i.e. that drying of sapwood could be initiated through exposure to air either through pruning wounds or facilitated by pruning operations) is correct.

Further research will be required to determine the relationship between the formation of dry wood zones in *P. radiata* exhibiting flute cankers and the pathogen *N. fuckeliana*. There are some parallels between the response reported to inoculation of a number of conifer species with *H. annosum* and observations on the behaviour of *N. fuckeliana* in *P. radiata*. For example the distance of penetration by *H. annosum* was greater in the winter season when the host was dormant whereas the fungus was restricted in development in the stem in the growing season when sap and resin flow was at its peak. Results from Scion and forest industry trials in the

South Island indicate that flute canker formation is reduced when summer pruning is undertaken. Dry wood zones in *P. radiata* have also been shown to form after *Sirex* attack; these are related to both *Amylostereum areolatum*, the fungus vectored by *Sirex* and the toxic mucous that is deposited in the wood at the same time.

We can postulate that, as has been reported for *H. annosum*, it is initial colonisation of sapwood by *N. fuckeliana* that triggers the dry zone formation. Fungal activity in the rays may have a function in inducing a biochemical response but this is untested. The extent and frequency of wood containing deposits of oleoresin and its association with dry wood is at present unknown but should be considered further.

Several lines of future research have therefore been identified:

- Extracts from wood infected with *N. fuckeliana* and inorganic chemicals can be inoculated into stems in an attempt to induce dry zone formation in the absence of the living fungus. A comparison of the effect of extracts from a range of fungi may demonstrate whether *N. fuckeliana* has a capability in the formation of dry wood zones that other common stem fungi do not possess. Any extracts that show this capability can be analysed and the components identified.

- Microscopic studies will be undertaken to determine the extent and distribution of the zones with resin deposits and this wood will be compared with that from dry zones.

- The effect of season on establishment of *N. fuckeliana* within the sapwood and on the initiation of dry zones will be trialled.

- Further studies of sapwood colonisation by *N. fuckeliana,* both in the presence and in the absence of flute formation will be undertaken.

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