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Invited Review

Defense Mechanisms in the Sapwood of Living Trees against Microbial Infection

Toshihiro Yamada[1]

Laboratory of Forest Botany, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo 113–8657, Japan.

When pathogenic microorganisms invade living sapwood of woody plants, a series of defense responses occurs at the lesion margin. Putative active defense mechanisms include constitutive and induced inhibitory compounds, cell wall alterations, and occlusion of xylem elements. Active defenses play an important role in the sapwood, while constitutive and induced microenvironmental conditions in the wood might also constrain pathogen development. It is necessary to develop a unified understanding, in which these factors could act synergistically and provide effective defense barriers.

Key words: active defense, microenvironment, reaction zone, wood discoloration and decay

Trees may survive for many years despite suffering continuous harm by biotic and abiotic agents during their whole lives. Their longevity certainly depends on the mechanisms developed to protect themselves from wounding and microbial invasion. Infection in living sapwood from pathogenic agents, largely fungi, usually results in necrosis and deterioration of sapwood tissue, *i.e.*, wood discoloration and decay (Fig. 1). Resistance mechanisms limiting the development of fungi, and wood discoloration and decay in living trees have been discussed from several different viewpoints. Major models proposed are compartmentalization of decay in trees (CODIT), reaction zone formation, and an alternative view based on microenvironmental conditions in the tree.

Putative defense mechanisms of woody plant xylem against microbial invasion are described in this review chiefly using the terms of the reaction zone model. Defense responses have been investigated at the elemental, compound, cellular, tissue, and whole-plant levels. Histological considerations at tissue level, which are essential for understanding tree defense because of their size and longevity, are also discussed in combination with their biochemical and microenvironmental features.

Models for Defense Mechanisms in the Sapwood

1 Compartmentalization of decay in trees (CODIT) model

The CODIT model was proposed to describe and account for the pattern of wood discoloration and decay (Shigo and Marx, 1977; Shigo, 1984). According to this model, lesions are bounded by barriers termed walls 1–4, which enclose lesions within a defined compartment. The walls are essentially regarded as static barriers preventing the further spread of infection. Walls 1–3 are formed with wood extant at the time of wounding or infection. Wall 1 is a boundary to the axial spread of infection formed by plugging of conductive tissue (vessels and tracheids). Wall 2 is a boundary to the radial spread attributed to anatomical features such as terminal parenchyma or cell wall thickness of late wood. Wall 3 is a boundary to lateral spread composed of ray parenchyma. Wall 4 (barrier zone) differs from other walls in that it is formed by the cambium at the time of injuries and comprises traumatic parenchyma cells. It is the strongest and most durable of CODIT walls. Little evidence for the mechanisms of compartmentalization, however, was presented originally. Thereafter, anatomical and biochemical changes in the CODIT walls were established (Shigo, 1984).

2 Reaction zone model

The reaction zone model was developed by Shain (1967, 1971, 1979). A reaction zone is a zone of active host responses at a dynamic interface between living sapwood and wood colonized by the pathogen. The classical concept of the reaction zone is a necrotic tissue enriched with oleoresin and antifungal phenolics observed in *Pinus taeda* and *Picea abies* (Shain, 1967, 1971). It was conceived that the reaction zone retreated dynamically ahead of a continuously advancing lesion margin. The transition zone, where most parenchyma cells are living and metabolically active, is formed contiguous to the sound wood and encircles the reaction zone. Reaction zone formation is a nonspecific response in trees which are wounded or infected by various agents, and may be equated with CODIT walls 1–3 (Shigo, 1984). With the exception of the barrier zone (CODIT wall 4), most host-pathogen interfaces in living wood can be related to the reaction zone.

3 Model of microenvironmental restriction

A completely alternative mechanism was proposed for restriction of fungi in living wood (Boddy and Rayner, 1983; Rayner and Boddy, 1988; Boddy, 1992). According to this alternative model, the internal microenvironment of trees, such as high moisture content and concomitant low O_2 tension, itself precludes fungal colonization without active host defenses.

Formation of Reaction Zone Barriers

Chemical substances are the most important factor in defense mechanisms in the reaction zone model, but this barrier is not necessarily impenetrable to fungi permanently. Inhibitory compounds may be gradually detoxified by fungi (Loman, 1970; Popoff *et al.*, 1975; Prior, 1976), and gymnosperm hosts continue to respond (Shain, 1967). However, it is likely that a reaction zone located in outer sapwood is more effective and durable.

[1] Corresponding author (E-mail: yamari@fr.a.u-tokyo.ac.jp).

It has been suggested that reaction zones are not the dynamic structures initially envisaged, but normally formed static barriers to infection in woody angiosperms (Pearce, 1991; Boddy, 1992). When a reaction zone fails, a volume of contiguous wood is colonized with little or no expression of host responses, until a new reaction zone is established (Pearce, 1991). Relics of reaction zones formed at former lesion margins have been identified in the decayed wood of several woody angiosperms (Rayner and Boddy, 1988; Pearce, 1991; Boddy, 1992). These relics are distributed discontinuously in the decayed wood (Pearce, 1991). Discontinuous reaction zone relics, however, have not been detected in any conifers so far.

The transition zones of coniferous trees are nonconductive, dry tissue where metabolic activity is greatly elevated (Shain, 1971; Sharon, 1974; Yamada *et al.*, 1988). Ethylene production, which is implicated in triggering the synthesis of phenolic compounds, is attributable to host activity in the transition zone (Shain and Hillis, 1972, 1973). It has been reported that inhibitory substances accumulate not only in the reaction zone where parenchyma cells are not living, but in the transition zone in some cases (Table 1). Accumulation of phenolics in the transition zone was observed in *Picea abies* roots (Stenlid and Johansson, 1987) and *Cryptomeria japonica* (Yamada *et al.*, 1988). Whether parenchyma of phenol-

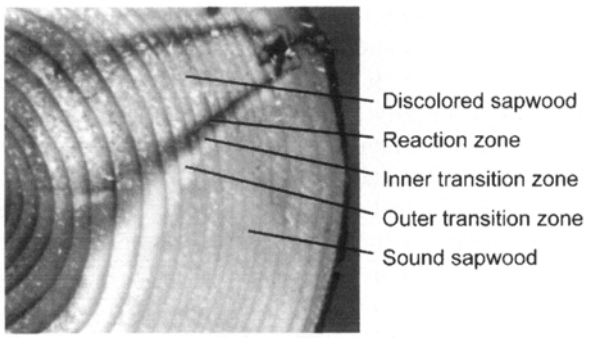


Fig. 1 Reaction zone barrier formed in *Cryptomeria japonica* sapwood infected with *Amylostereum laevigatum*.

enriched tissue is alive or dead may depend on tree species as well as the stage of reaction zone formation. The term reaction zone barrier will be used further to describe an active defense barrier, including reaction zones and transition zones, formed in differentiated sapwood (Fig. 1).

Negative hydrostatic pressure in functioning sapwood, combined with possible pressure relaxation in discolored and decayed wood, induces the formation of a nonconductive dry zone (transition zone) (Coutts, 1976). Exudation of deposits from parenchyma cells into tracheids and plugging of tracheids, combined with hydrostatic pressure, could also be associated with pit aspiration or gas emboli (Hessburg and Hansen, 1987). Coutts (1977) postulated that altered metabolism contributed to the extended dry zone formation. Although reaction zones and transition zones of gymnosperms are usually drier tissue, accumulation of water appears typical for reaction zones in several woody angiosperms (Pearce *et al.*, 1994, 1997b).

Chemical Aspects of the Defense Responses

1 Constitutive inhibitory compounds

Sound sapwood of many species usually contains small amount of preformed inhibitory compounds, although such compounds are more characteristic of heartwood than of sapwood. Examples for gymnosperms are lignans (Shain and Hillis, 1971), fatty acids, resin acids, and diterpene alcohols and aldehydes (Ekman, 1979) in *Picea abies*, and pinosylvins in *Pinus* spp. (Dumas and Hubbes, 1979; Dumas *et al.*, 1983). Examples for angiosperms are gallic acid and catechin, which occur in the sapwood of *Acer* spp. (Tatter and Rich, 1973), and ellagitannins in *Quercus* and *Castanea* spp. (Peng *et al.*, 1991).

Concentrations of inhibitory compounds found in sound sapwood usually are smaller those required for the activity (Shain and Hillis, 1971; Shortle *et al.*, 1971; Shaw, 1985; Stenlid and Johansson, 1987). Associations are suggested between constitutive compounds and disease resistance in some cases. For example, differences in resistance of *Pinus densiflora*

Table 1 Characteristics of reaction zone barrier in gymnosperms and woody angiosperms.

Tree species	Fungi	Portion	Parenchyma	Fungal invasion	Inhibitory substances	References
Gymnosperms						
Pinus taeda, *Picea abies*	*Heterobasidion annosum*	Transition zone	Alive	−	±	Shain (1967),
		Reaction zone	Dead	±	+	Shain and Hillis (1971) (Classical reaction zone concept)
Picea abies	*H. annosum*	Transition zone	Alive	−	+	Stenlid and Johansson (1987)
Cryptomeria japonica	*Guignardia cryptomeriae etc.*	Transition zone	Alive	−	+	Yamada *et al.* (1988)
		Reaction zone	Dead	+	± ~ +	
Angiosperms						
Acer saccharinum	*Ganoderma adspersum etc.*	Reaction zone (orange)	Alive	+	± ~ +	Pearce and Woodward (1986)
		Reaction zone (green)	Dead	−	+	

and *Pinus rigida* × *radiata* to *Heterobasidion annosum* may be attributed to constitutive compounds, especially to pinosylvins (pinosylvin and its related compounds) (Dumas and Hubbes, 1979; Dumas *et al.*, 1983).

2 Induced inhibitory compounds (phytoalexins)

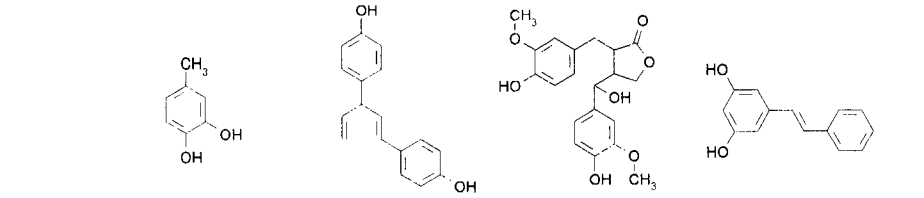
A number of classes of inhibitory compounds are induced in the sapwood against microbial infection (Fig. 2). Phytoalexins are low molecular weight antimicrobial compounds induced *de novo* against microbes after an infection (Paxton, 1981). Many of inhibitory compounds, such as lignans and pinosylvins, present at only very low levels in sound sapwood increase their quantity in response to microbial invasion at lesion margins (reaction zone barrier) (Shain, 1967, 1971; Popoff *et al.*, 1975; Wong and Preece, 1978c; Pearce, 1991; Yamada, 1992). These compounds exhibit features in common with phytoalexins and will be considered further in that context.

Inhibitory compounds in trees frequently are phenolic, and usually are also found in the heartwood of their respective species. A large number of other substances, such as terpenes, flavonoids, phenylpropanoids, resin, and fatty acids, are also involved in active defenses (Rowe, 1989), and accumulate in reaction zone barriers and barrier zones. It should be noted that antifungal activity in reaction zone barrier extracts can be related to only a few of the compounds accumulating at lesion margins (Yamada *et al.*, 1988; Yamada and Nakashima, 1997; Yamada, 1998b). Norlignans, lignans, stilbenes, and flavanones are the representative phenolic compounds. They occur in negligible amounts in sound sapwood and are produced in the process of heartwood formation.

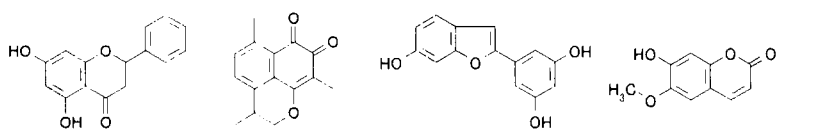
Norlignans are found in several coniferous families that lack resin canals in the xylem. Norlignans, such as hinokiresinol, agatharesinol, and sequirin-C, accumulate in the reaction zone barrier of *C. japonica* (Takahashi and Ogiyama, 1985a, b; Yamada *et al.*, 1988). Among norlignans produced in the reaction zone barrier, hinokiresinol and a few minor components have high inhibitory activity against fungi, but agatharesinol and sequirin-C have no inhibitory activity (Yamada *et al.*, 1988).

Lignans are distributed in several gymnosperms and woody angiosperms. Hydroxymatairesinol, matairesinol, liovil, conidendrin, and several other lignans were found in heartwood and reaction zones of *Picea abies* attacked by *H. annosum* (Shain and Hillis, 1971; Popoff *et al.*, 1975). Hydroxymatairesinol is significantly more inhibitory to *H. annosum* than is matairesinol or conidendrin (Shain and Hillis, 1971). It was thus concluded that hydroxymatairesinol in association with alkalinity in the reaction zone contributed to the resistance of the sapwood, while the general occurrence and antifungal activity of lignans, especially of hydroxymatairesinol, are somewhat controversial (Popoff *et al.*, 1975).

Stilbenes, such as pinosylvin and resveratrol, occur naturally in a number of herbaceous and woody plants. Details on the role of stilbenes as inhibitory compounds in woody plants have been reviewed (Hart and Shrimpton, 1979; Hart, 1981). Pinosylvins have been reported to accumulate in the reaction zone of many pine species (Shain, 1967; Prior, 1976), in response to fungal infection (Rishbeth, 1972; Shrimpton, 1973). Abiotic stresses, *e.g.*, desiccation, high humidity, ethylene, and UV, as well as biotic ones, are also triggering agents that induce pinosylvins production (Hart, 1981). Pinosylvins inhibited the growth of *Ceratocystis olivacea* (Rudman, 1965), *H. annosum* (Shain, 1967; Gibbs, 1972), and *Armillaria ostoyae* (Mwangi *et al.*, 1990). In addition, a



Class	Simple phenols	Norlignans	Lignans	Stilbenes
Compound	4-Methylcatechol	Hinokiresinol	Hydroxymatairesinol	Pinosylvin
Tree	*Picea abies*	*Cryptomeria japonica*	*Picea abies*	*Pinus* spp.
Reference	Popoff *et al.*, 1975	Yamada *et al.*, 1988	Shain, 1971	Shain, 1967



Class	Flavanones	Sesquiterpenes	Phenylbenzofurans	Phenylpropanoids
Compound	Pinocembrin	Mansonone E	Moracin M	Scopoletin
Tree	*Pinus* spp.	*Ulmus* spp.	*Morus alba*	*Prunus domestica*
Reference	Shain, 1967	Overeem and Elgersma, 1970	Takahashi and Shirata, 1982	Hillis and Swain, 1959

Fig. 2 Representative examples of induced antimicrobial compounds from reaction zone barriers.

negative correlation was shown between the extent of infection and pinosylvin concentration (Prior, 1976). The restriction of the fungal development in pine sapwood after infection appears to be due to the formation of stilbenes (Shain, 1967; Hillis and Inoue, 1968; Prior, 1976).

Flavanones are widely distributed in the plant kingdom. Several flavanones such as pinobanksin and pinocembrin are known to occur in pines. They also accumulate in wounded wood or reaction zones (Higuchi *et al.*, 1967; Shain, 1967; Shrimpton, 1973), and are inhibitory to fungi (Loman, 1970; Shain and Miller, 1982; Yamada and Ito, 1993).

Examples of xylem phytoalexins in woody angiosperms are the sesquiterpene mansonones in *Ulmus* spp. (Burden and Kemp, 1984), 7-hydroxycalamenene (Burden and Kemp, 1983) in *Tilia europea*, and stilbenes in *Morus alba* (Takasugi *et al.*, 1978; Takahashi and Shirata, 1982).

Secondary metabolites including phenolics identified in heartwood are also induced in sapwood, but commonly in different ratios (Shain, 1967; Shain and Hillis, 1971; Yamada *et al.*, 1988). An example is the ratio pinosylvin monomethylether: pinosylvin (Shain, 1967; Hillis and Inoue, 1968). Since pinosylvin is more toxic to *H. annosum* than pinosylvin monomethylether, a lower pinosylvin monomethylether: pinosylvin ratio in the reaction zone may be of some consequence for the defense mechanisms (Shain, 1967). Similar consideration is applicable to the ratio in *C. japonica*. Heartwood contains little hinokiresinol which is abundant in the reaction zone barrier (Takahashi and Ogiyama, 1985a, b, 1986). Further, outermost sapwood has a high capability to produce hinokiresinol (Takahashi and Ogiyama, 1985a). These facts indicate that younger tissue has a greater inhibitory activity against fungal invasion, and could explain the typical pattern of wood discoloration.

3 Resin

Conifer xylem, particularly that of pines, produces large amounts of oleoresin. Oleoresin is a hydrophobic mixture with volatile terpenes, resin acids, and fatty acids as major components (Mutton, 1962). Resin serves a mechanical or a chemical barrier protecting trees from wounds, insects, and pathogens. It is characteristic in many, but not all, conifers that the resin is located in a resin canal system. However, only some genera in the Pinaceae have well-developed resin canals. Some genera lack resin ducts at all, and some develop only traumatic resin canals. Even in trees that lack resin canals, terpenoids are the quantitatively important components of heartwood or the reaction zone barrier (Kondo *et al.*, 1959; Yamada and Nakashima, 1997).

Heavy resin impregnation after infection or wounding is a typical response in the xylem of pines, but not in other conifers. Fungal infection induces terpene accumulation in the xylem of pines (Shrimpton, 1973; Wong and Berryman, 1977; Cheniclet, 1987). Resin acids also increase (Croteau *et al.*, 1987), but at a lower rate than terpenes (Shrimpton, 1973).

Volatile terpenes from conifers are mostly fungitoxic (Rudman, 1962; Cobb *et al.*, 1968; Shrimpton and Whitney, 1968; Gibbs, 1972; Rishbeth, 1972; Flodin and Fries, 1978; Flodin, 1979; Schuck, 1982a), although the inhibitory effects vary widely with the fungi or substances tested (Bridges, 1987). Nonvolatile terpenes and resin acids also generally inhibit fungal growth (Rudman, 1965; Henricks *et al.*, 1979; Hartmann *et al.*, 1981).

Resin production is assumed to be an important defense mechanism in conifer xylem, particularly that of pines (Gibbs, 1968; Hart *et al.*, 1975). The significance of resin in resistance, however, is less clear. The principal effect of resin impregnation appears to be the mechanical inhibition of fungal growth (Verrall, 1938; Rishbeth, 1972; Hart *et al.*, 1975; Prior, 1976). Schuck (1982a) suggested that the physical barrier depends on resin acids, whereas the monoterpenes of the resin act as toxic chemical agents. However, little inhibition of pathogen growth, or even stimulation of growth by these compounds, has been reported (Prior, 1976; Flodin and Fries, 1978; Schuck, 1982a). Shain and Hillis (1972) found large quantities of phenolics and relatively less resin soaking in *Sirex* lesions of *Pinus radiata* trees. They suggested that phenolics, but not resin, participate in the resistance.

Two types of resin, primary and secondary resin, are distinguished on the basis of their origin and composition. Primary resin provides a rapid response to injury, since it is preformed. Secondary resin probably originates from living parenchyma cells of xylem ray and phloem, even in pines which have well-developed resin canal systems (Shigo, 1975; Lieutier and Berryman, 1988a, b). Secondary resin is secreted through pits into the lumina of contiguous tracheids (Birchem and Brown, 1979), and causes heavy resin soaking in pines (Shigo, 1975). Reid *et al.* (1967) pointed out the lack of secondary resinosis in trees successfully attacked by the beetle and blue stain fungus, and concluded that secondary resinosis was the more important factor in resistance to attack.

Phenolic substances are also produced by parenchyma cells, and are excreted into adjacent conductive cells (Shain, 1967, 1971; Gibbs, 1968). Furthermore, in several cases described above, "resin" probably includes both terpenoids and phenolics. Even in coniferous species that lack resin canals in the xylem (*e.g.*, *Cryptomeria* sp. and *Chamaecyparis* spp.), compartmentalization of infected tissue was demonstrated (Yamada and Okuda, 1987; Yamada *et al.*, 1988). Phenolics and terpenoids accumulated in the inner transition zone of *C. japonica* indicate that they originate from parenchyma.

Changes in the composition of terpenes (Schuck, 1982a, b; Paine and Stephen, 1987), resin acids (Shrimpton, 1973; Hart *et al.*, 1975), and the ratio resin acids : terpenes (Cheniclet, 1987) after infection or injury have been observed in *Pinus* spp. and *Picea abies* wood, although all components found in the defense responses were normal constituents of heartwood. These findings suggest that the accumulation of resins was not due simply to movement of resins from the preformed system.

Woody angiosperms also produce terpenoids in the wood. For example, mansonones accumulated in *Ulmus* spp. infected with Dutch elm disease fungus (Overeem and Elgersma, 1970; Elgersma and Overeem, 1971; Dumas *et al.*, 1983,

1986). In addition to terpenoids, chemically diverse resin-like materials (gums or gels) are produced by many woody angiosperms (Hillis, 1987).

4 Minerals and enzymes

Concentration of several minerals, such as K, Mg, Ca, Mn, and Zn, and the pH of reaction zones is elevated in some angiosperms [*e.g.*, *Acer saccharum* (Good *et al.*, 1955)] and gymnosperms [*e.g.*, *Picea abies* (Shain, 1971); *C. japonica* (Yamada *et al.*, 1987)]. While the mechanisms for mineral accumulation remain unclear, minerals associated with elevated pH probably contribute to sapwood defense in that growth of decay fungi is inhibited under alkaline conditions (Rennerfelt and Paris, 1953). Mn and Zn accumulated in the reaction zones may be originated from the oxidative enzymes of pathogens or hosts (Grime and Pearce, 1995).

Elevated peroxidase and polyphenol oxidase activity is a common feature of many plants' defense, including those in the sapwood of trees (Shain, 1971; Wong and Preece, 1978b; Yamada, 1987; Geiger *et al.*, 1989), and high levels of free radicals have been detected in the reaction zone barrier of *Acer pseudoplatanus* (Pearce *et al.*, 1997b). They might catalyze the oxidation of phenolics to more polymerized compounds, and contribute to the resistance either directly or indirectly.

Lytic enzymes, such as chitinase and glucanase, may be involved in defense mechanisms of xylem. There are no reports on the association of such enzymes with xylem responses of woody plants, although they are considered to be involved in the defense of bark, leaves, and roots of trees (Albrecht *et al.*, 1994; Hodge *et al.*, 1995; Clarke *et al.*, 1998).

Histological Aspects of the Defense Responses

1 Cell wall alterations and traumatic tissue formation

Cell wall alterations include suberization and lignification of cell walls of living xylem parenchyma cells. Suberin is not a normal component of sound sapwood, but the most signif-

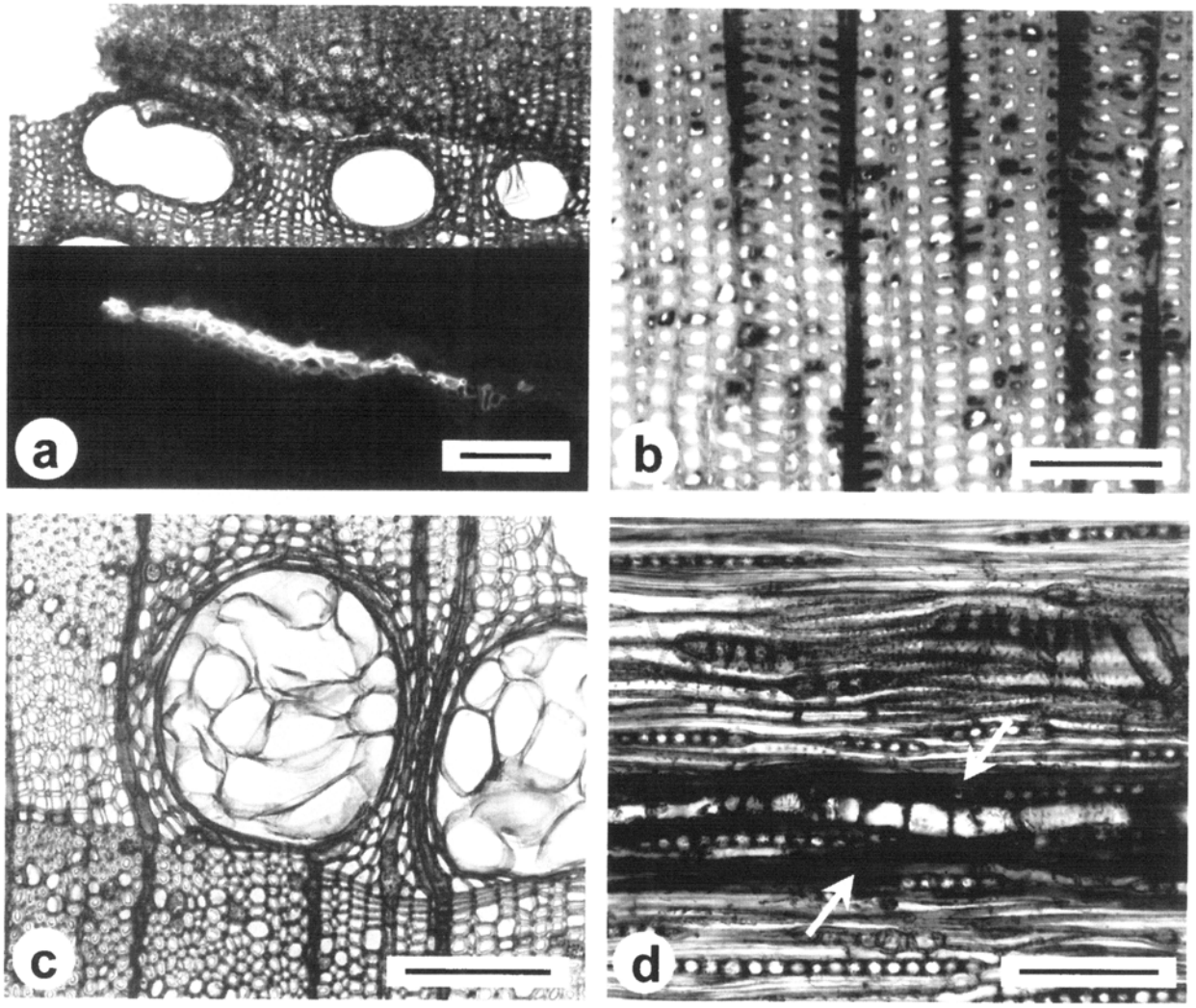


Fig. 3 Several putative defense barriers formed at the lesion margin in sapwood. (a) Suberization of barrier zone in *Quercus serrata*. Photograph under bright-field (upper) and ultraviolet illumination (lower). Suberin is fluorescent under ultraviolet illumination. Bar = 200 μm. (b) Deposition of resin-like material along the ray of *Cryptomeria japonica*. Bar = 200 μm. (c) Tyloses in the vessels of *Quercus mongolica* var. *grosseserrata*. Bar = 200 μm. (d) Deposition (arrows) in the xylem fibers of *Quercus serrata*. Bar = 200 μm.

icant cell wall polymer associated with xylem resistance.

Little is known on the ability of wood-inhabiting fungi to degrade suberin. Suberized tissue of *Quercus robur* are resistant to degradation by *Stereum gausapatum* (Pearce and Rutherford, 1981), although *Armillaria* and *Rosellinia* species were reported to degrade suberin slowly (Swift, 1965; Zimmermann and Seemüller, 1984; Ofong and Pearce, 1994).

Suberization of traumatic parenchyma cell walls can be involved in barrier zone (CODIT wall 4) (Fig. 3a; Pearce and Rutherford, 1981; Pearce and Holloway, 1984; Pearce and Woodward, 1986). Barrier zones are formed as a result of cambial response to injury, and are composed by the axial parenchyma cells, fibers, and gum canals or resin canals. Their function is to limit infection to the xylem present at injury, thus protecting the xylem formed after injury. Suberization is not a universal feature of the barrier zone, since some woody angiosperms and gymnosperms lack suberization (Tippett and Shigo, 1980, 1981; Tippett *et al.*, 1982; Pearce, 1990; Blanchette, 1992).

Suberization occurred also in tyloses and parenchyma cells at the reaction zone barrier (CODIT wall 3) of many tree species (Pearce and Rutherford, 1981; Biggs, 1987; Pearce, 1990). Discontinuous distribution of xylem ray and axial parenchyma in the wood formed before wounding suggests the limited efficiency of suberization in the reaction zone barrier, since the suberized tissues can be bypassed (Yamada *et al.*, 1988; Yamada, 1992). It is more likely in conifer xylem because of the limited extent of suberin formation after wounding. However, it should be also noted that suberized walls of tyloses and parenchyma cells can slow the invasion.

The extent of induced suberization in the reaction zone barrier differs among species. In some cases, for example *Fagus sylvatica* and *Quercus* spp., extensive suberization responses are observed in xylem parenchyma cells of all categories, vessel linings, and tyloses. In other species, the extent of suberization in reaction zones is much reduced or absent. Reaction zone suberization responses correlate closely with vessel occlusion by tyloses. Suberization is commonly absent in reaction zone barriers in which gummosis is the main mechanism for vessel occlusion, even in species which can form a suberized barrier zone (Pearce and Woodward, 1986; Pearce, 1990).

Induced lignification of parenchyma cell walls also appears to have a defensive function in normally non-lignified tissue in the wood (Vance *et al.*, 1980).

Traumatic resin canal formation in the wood laid down immediately after wounding or infection is a common response in several genera of conifers (Tippett and Shigo, 1980, 1981; Blanchette, 1982; Tippett *et al.*, 1982). These correspond in location to barrier zones. Comparable formation of traumatic gum canals has been reported following wounding in *Liquidambar styraciflua* (Moore, 1978). Kino veins form in *Eucalyptus* wood after wounding (Tippett, 1986) or after prolonged fungal activity in the bark tissues (Tippett *et al.*, 1983).

2 Occlusion of xylem elements

It is supposed that parenchyma can be a defense barrier by itself in the wood of certain angiosperms (Shigo and Marx, 1977). Because ray parenchyma of most gymnosperms, however, are single or consist of a few stranded cells that are short in height, fungal hyphae can easily bypass the ray parenchyma. Additive induced barriers are necessary to be developed for effective defense.

Compounds, plugging xylem elements, are commonly present in reaction zone barriers (Pearce and Woodward, 1986; Pearce, 1990; Blanchette, 1992; Yamada, 1992; Pearce *et al.*, 1994), where they might serve as chemical and mechanical barriers to further fungal penetration (Blanchette, 1992). They are unlikely to be impermeable barriers limiting the spread of cavitation and drying in gymnosperms sapwood (Yamada *et al.*, 1988; Yamada, 1992).

Wound-initiated tyloses, as well as tylosoids in resin canals, have been observed in tracheids of gymnosperms xylem with thin-walled epithelial cells such as pines. Tyloses, developed from ray parenchyma, protruded into the lumina of adjacent tracheids in *Pinus* spp. (Smith, 1967), and *Pseudotsuga menziesii* (Hessburg and Hansen, 1987) infected with *Ceratocystis wageneri*. Tyloses and tylosoids might play a partial role in confining the pathogen in these species.

Induced tracheid occlusion with resinous material has been observed in many coniferous species [Table 2; Fig. 3b; *Pinus* spp. (Shain, 1967; Lieutier and Berryman, 1988a, b), *Abies grandis* (Wong and Berryman, 1977), *Pseudotsuga menziesii* (Hessburg and Hansen, 1987), and *C. japonica* (Yamada *et al.*, 1988)], and may contribute to defense against fungi. Occlusion by resinous deposits in these species is considered a nonspecific response to fungal infection, because it also occurrs during heartwood formation (Nobuchi and Harada, 1983). Plugging is absent from the advancing margin of stain (Hessburg and Hansen, 1987). The barrier zone tracheids of *Pinus resinosa* also are often impregnated with resin (Tippett and Shigo, 1980).

Penetration of fungal hyphae colonizing discolored sapwood of *C. japonica* was prevented at the inner transition zone or reaction zone (Yamada *et al.*, 1988). The deposits containing inhibitory substances occluded tracheids and pits, through which fungal hyphae spread, and formed spatially continuous barriers at the lesion margin in *C. japonica*.

While tyloses often plug vessels of several woody angiosperms wood (Fig. 3c), occlusion of xylem elements with deposits is more common. Occlusion of fibers and tracheids with insoluble deposits has been observed in several species (Table 2; Fig. 3d; Phelps and McGinnes, 1977; Pearce and Woodward, 1986; Pearce *et al.*, 1994; Baum *et al.*, 2000; Schwarz and Baum, 2000). Occlusion of vessels with insoluble materials has been reported in *Acer* spp. and *Salix alba* var. *caerulea* (Table 2; Wong and Preece, 1978a; Pearce and Woodward, 1986; Pearce *et al.*, 1994).

Only a few chemical or serological analyses have been conducted on the deposits plugging xylem elements. The deposits in tracheid lumina of *Tsuga heterophylla* heartwood

Table 2 Examples of the occlusion of xylem elements at the reaction zone barrier.

Tree species	Xylem element	Occluding compound /tissue	References
Gymnosperms			
Pinus taeda, *Picea abies*	Tracheid	Resin/Resin-like compound	Shain (1967), Shain and Hillis (1971)
Cryptomeria japonica	Tracheid	Resin-like compounds	Yamada *et al.* (1988)
Angiosperms			
Acer pseudoplatanus	Vessel, fiber	Insoluble compound	Pearce *et al.* (1994)
Prunus pensylvanica	Vessel, fiber	Gel	Rioux *et al.* (1998)
Salix alba var. *caerulea*	Vessel	Insoluble compound	Wong and Preece (1978a)
Fagus sylvatica	Vessel Fiber	Tylosis Insoluble compound	Baum *et al.* (2000), Schwarze and Baum (2000)
Quercus velutina	Vessel Fiber	Tylosis Insoluble compound	Phelps and McGinnes (1977)
Ulmus hollandica	Vessel	Tylosis	Elgersma (1973), Newbanks *et al.* (1983)
Ulmus americana	Vessel Vessel, fiber	Alveolar reticulum Gel	Ouellette (1980), Ouellette and Rioux (1992)

contain the lignans matairesinol, hydroxymatairesinol, and conidendrin, often in quantities sufficient to plug tracheids (Krahmer *et al.*, 1970). The deposits from tracheid lumina of the inner transition zone of *C. japonica*, contain antifungal norlignans such as hinokiresinol (Yamada *et al.*, 1988). The deposits containing inhibitory substances block hyphal passages through tracheid lumina and pits, and form spatially continuous reaction zone barriers in the sapwood of *C. japonica*. Gels in *Prunus pensylvanica* wood, observed with monoclonal antibodies, have been demonstrated to contain pectins (Rioux *et al.*, 1998).

3 Genetic control of resistance

Genotype-dependent differences in the ability of trees to compartmentalize infections have been demonstrated (Shigo *et al.*, 1977; Garrett *et al.*, 1976, 1979; Lowerts and Kellison, 1981). Although the underlying mechanisms remain unknown in most cases, a correlation has been observed between wood structure and resistance to decay in hybrid poplar trees. Trees with fewer, smaller vessels might compartmentalize more strongly than trees with many, larger vessels (Eckstein *et al.*, 1979). Differences in vessel anatomy associated with susceptibility to Dutch elm disease have also been identified (Elgersma, 1970; McNabb *et al.*, 1970; Sinclair *et al.*, 1975).

Microenvironmental Factors in the Xylem

1 Water, O_2, and CO_2

It is suggested that the development of decay in living trees can be explained in terms of wood moisture status (Boddy and Rayner, 1983; Leben, 1985; Boddy, 1992). According to their theory, resistance of sapwood to fungal invasion can be attributed to the high moisture content and insufficient amount of O_2 assured by the waterproofing layer. Elevated CO_2 and reduced O_2 concentrations are commonly reported from living wood (Rayner and Boddy, 1988). They consider microenvironmental conditions in water-filled xylem to be inhospitable to fungi and conditions in an aerated tissue to be more favorable. Although low O_2 and high CO_2 concentrations are expected in the transition zone, many decay fungi can grow equally well in low O_2 and high CO_2 conditions (Gundersen, 1961; Jensen, 1967; Highley *et al.*, 1983; Scheffer, 1986). Furthermore, it is unlikely that a high moisture content directly prevents fungal development in most cases of gymnosperms, because conducting sapwood is separated from the pathogen by a nonconductive reaction zone or transition zone where the moisture content is rather low (Shain, 1971; Coutts, 1976; Yamada *et al.*, 1988). In addition, the highest O_2 concentrations in the wood have been found in the youngest sapwood (Jensen, 1969), which is the most resistant to fungal attack (Johansson and Stenlid, 1985). Accumulation of water in reaction zones of *Acer pseudoplatanus* has been demonstrated (Pearce *et al.*, 1994, 1997a), suggesting the significance of active enhancement on water-mediated tissue protection.

Resin-impregnated wood has been thought to act as a nontoxic waterproofing layer which prevents fungal penetration (Verrall, 1938; Rishbeth, 1972; Hart *et al.*, 1975). However, it appears impossible that resin impregnation *per se* constitutes the water sealant which divides water-filled and aerated tissue, because of the low moisture content of the transition zone between the resin-impregnated region and sound sapwood.

The characteristic patterns of discolored wood and hence those of the reaction zone barrier are explained as the result of increased penetration by the fungus with increasing distance from the cambium and greater penetration longitudinally

than transversely (Shain, 1967). Rayner and Boddy (1988) interpreted the positioning and relative strength of CODIT walls 1–3 according to purely anatomical considerations. Wong and Berryman (1977) also argued that long, tapered tracheids explained the greater vertical lesion expansion. The patterns of distribution of fungi colonizing from wounds and the corresponding position of reaction zones or barrier zones relate to the likely patterns of aeration and drying following injury (Rayner and Boddy, 1988).

Lesions commonly show a rapid expansion phase followed by reduced expansion and stabilization in fungus-inoculated trees (Wong and Berryman, 1977; Yamada, 1998b). Leben (1985) suggested that hydrostatic tension in the wood was a major determinant of the volume of the discolored wood column when a tree was infected by fungi via wounds. However, differences in the infected volume of sapwood between trees inoculated with virulent isolates and trees with avirulent isolates, and time course dynamics of lesion development (Yamada, 1998b) could not be explained in terms of hydrostatic tension. Also, the greater colonization of inner sapwood, explained as a result of less water and better aeration by Boddy and Rayner (1983), could be attributed to reduced parenchyma activity and hence a reduced response.

2 Nutrition

No correlation was found between the sugar content of sap from *Salix fragilis* and susceptibility to pathogens (Stanislawek *et al.*, 1987), although it was suggested that nutrients in the sap were a factor determining the rates of sapwood colonization (Beever, 1970). On the contrary, susceptibility to pathogens increases in stressed trees due to a reduction in starch reserves, which can be used for defensive responses (Wargo, 1972). Reciprocal conclusions may arise from neglecting to consider other factors such as defense responses.

Depletion of readily utilizable substances also may limit fungal spread. Shrimpton (1973) found a decrease in sugar levels that accompanied the increase in other extractives in the sapwood of *Pinus contorta* var. *latifolia* attacked by the bark beetle and associated fungi. The effective inhibition of fungal colonization could be due to lower sugar levels and the presence of growth inhibitors (Shrimpton, 1973; Worrall and Harrington, 1988). Wong and Berryman (1977) suggested that early fungal confinement in resistant *Abies grandis* was due to degenerative metabolism within the lesion.

Induction and Evaluation of Active Defense Responses

1 Induction of defense responses

The elicitation of inhibitory compounds in tree xylem remains largely unknown. Although elicitation has been reported to be a non-specific process following injury (Kemp and Burden, 1986; Duchesne *et al.*, 1992), a glycoprotein elicitor of the elm phytoalexin mansonone F has been isolated using callus culture (Yang *et al.*, 1989, 1994).

In stem lengths of *Acer pseudoplatanus*, inoculated with the aggressive pathogen or wounded but uninoculated, reaction zone responses were delayed in comparison with those in stems inoculated with the weakly aggressive fungus (Pearce *et al.*, 1994). Although the intensity of defense responses did not differ between living *C. japonica* trees inoculated with isolates of different virulence, concentrations of inhibitory compounds in the reaction zone barrier were higher in inoculated trees than in wounded trees (Yamada, 1998b). These results suggest that the defense response can be enhanced by the existence of pathogenic fungi, or by avirulent fungi.

2 Evaluation of active defense

Sapwood of nonliving logs is more susceptible to wood decay fungi than heartwood (Scheffer and Cowling, 1966), although sapwood of living trees is durable against decay. This implies that activity and quantity of constitutive antifungal compounds in sapwood are not enough to prevent attack by wood decay fungi.

No delay of fungal spread was observed in the sapwood of excised stems of *C. japonica* with high water content, when active defense responses were not induced (Yamada, 1998a). This fact suggests that constituents and high water content of sound sapwood do not inhibit fungal spread in the wood. Freshly excised stems responded to fungal invasion and interfered with fungal spread, indicating an important role of active defense responses in the inhibition of fungal spread in the sapwood.

The axial elongation of most xylem elements means that fungal colonization occurs most readily along the tree axis. In the absence of active defenses, fungal hyphae grow most rapidly along the axis in the sapwood of *C. japonica*. However, the axial-tangential ratio of fungal colonization (= wood discoloration in living tree) is higher when wood tissue is living (Yamada, 1998a). This suggests that active defense responses are also involved in the determination of the pattern of fungal colonization and wood discoloration, and that anatomical features might also influence the effectiveness of active defense.

The antimicrobial environment in the tree might work synergistically together with active responses. The effectiveness of the microenvironment, however, probably depends on the products of active defense, and the reaction zone barrier formed by the active defense can be regarded as an inhibitory field where these factors interact. Details of such xylem defense responses and trials for establishing a unified theory have been well documented by Pearce (1996). The dynamics of fungal invasion and active responses should be resolved to evaluate the models accounting for xylem defenses. It will help to develop a holistic understanding of the contribution of active defense responses and of microenvironmental factors.

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