

**EFFECT OF SEASON ON THE EXTENSION OF WOOD DISCOLORATION
COLUMN AFTER MECHANICAL WOUNDING**

(Master's Thesis)

物理的傷害に対する材変色の季節による相違

Course of Natural Environmental Studies
Graduate School of Frontier Science
Laboratory of Biosphere Function
The University of Tokyo

Sule ZUHAIR

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The study entitled **Effects of season on the extension of wood discoloration column after mechanical wounding** was completed and submitted in partial fulfillment of the requirements for the degree of Master of Science in Environmental Studies of The Graduate School of Frontier Sciences, The University of Tokyo. The Study was conducted under the supervision of Dr. Kenji FUKUDA, Professor of Forest Ecology.

ACCEPTED AND APPROVED BY
Professor. Dr. Kenji FUKUDA

Laboratory of Evaluation of Natural Environment
Institute of Environmental Studies
Graduate School of Frontier Sciences
The University of Tokyo.

Effects of season on the extension of wood discoloration column after mechanical wounding

Mar.2007, Department of Natural Environmental studies. Evaluation of Natural
Environment
47-56864 Sule Zuhair

Supervisor: Professor; Kenji Fukuda

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Introduction.

During their development, trees commonly experience mechanical injuries and damages, these wounds provide an infection courts for many serious forest diseases and wood discoloration and decay. There are various opinions about the effects of season and wound closure on the extension of discoloration column and subsequent decay. For this reason the effects of season on the extension of wood discoloration were studied macroscopically and microscopically in four different species.

Materials and Methods

Ninety-six trees belonging to 4 different species, Shirakashi evergreen oak (*Quercus myrsinaefolia*), Konara deciduous oak (*Quercus serrata*), Hinoki cypress (*Chamaecyparis obtusa*) and Metasequoia (*Metasequoia glyptostroboides*) were used. With drill borer, 5 holes were bored into the lower trunk at every 25 cm, height from 1 to 2 meters above the ground in spiral line. Wounding was done during the months of January, April, July and October of 2005. The trees were felled at 2 weeks, 2 months and 6 months after wounding. Wood discoloration columns were measured on the axial and tangential direction. Wound closure index after six months were also determined. The presence of accessory substances on the reaction and barrier zone were determined histochemically.

Results and Discussion.

Trees examined macroscopically showed discoloration in woody tissues, which extended from the drill wounds in the axial direction more rapidly than in the tangential direction. In conifers the longest discoloration length was at the inner most xylem next to heartwood, while in oaks it was the middle part of the hole. Wound made in winter or autumn had reduced amount of discoloration compared to those made during the growing seasons. Wound closure index differed between species, with Konara and Metasequoia trees wounds completely closed after spring wounding, primarily because of the stem growth rates. There is no correlation between wound closure and wood discoloration column length.

The reaction of the barrier -and reaction zones in the histochemical staining tests differed definitely from those of non-discolored sapwood. Lignin, suberin and phenol substances were observed in various concentrations in these tissues. Shirakashi had the highest concentration of accessory substances and tyloses in the reaction zone, in which, vessels, ray parenchyma and fiber cells were totally or partially filled by accessory substances

Traumatic resin canals were observed in the xylem of *Metasekoia*, this is the first report on the formation of traumatic resin canals from sample other than fossilize wood. The epithelial cells of the traumatic resin canals were stained positive for the accessory substances.

(Figs 1-2).

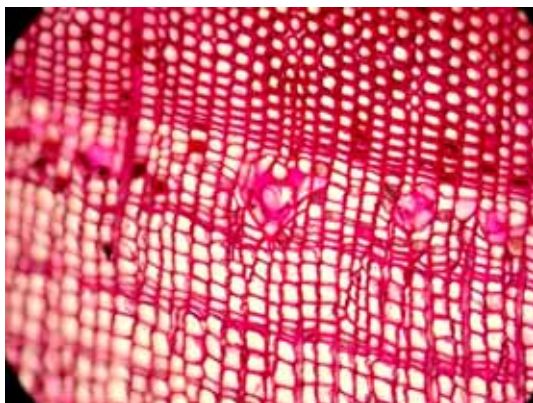


Fig.1 lignification of the traumatic resin canal lining in *Metasekoia* xylem. Schiff's reaction, six months after spring wounding.

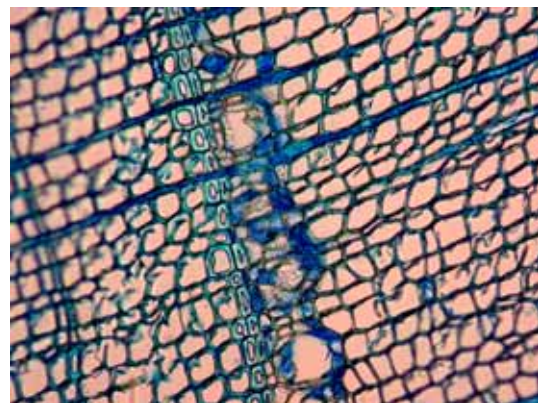


Fig.2 Suberin deposition in the traumatic resin canals lining in *Metasekoia* xylem. Nile blue, six months after summer wounding.

物理的傷害に対する材変色の季節による相違

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47-56864 Sule Zuhair

指導教員 教授 福田健二

キーワード: 変色部, 季節, 傷害樹脂道, 巻き込み率

はじめに

一般的に、樹木は成長の過程において物理的な傷害・損傷を受ける。これらの傷害は、多くの森林病害や材変色・材腐朽を引き起こす菌類の侵入門戸となる。変色部の拡大とその後の腐朽は、季節や巻き込みにより影響を受けるとされているが、詳細は明らかにされていない。本研究では、材変色部の拡大における、季節の影響を解明するために、4 種の樹木を対象として、肉眼・顕微鏡スケールの実験を行った。

材料と方法

常緑広葉樹のシラカシ(*Quercus myrsinaefolia*)、落葉広葉樹のコナラ(*Quercus serrata*)、常緑針葉樹のヒノキ(*Chamaecyparis obtusa*)、落葉針葉樹木のメタセコイア(*Metasequoia glyptostroboides*)の 4 種について各 24 個体を選び供試木とした。2005 年 1 月・4 月・7 月・10 月の各月にドリルを用い、各供試木 6 本の樹高 1 ~ 2 m の高さに、らせん状に 25cm おきで 5 つの穴を開けた。付傷から、2 週間後、2 ヶ月間後、6 ヶ月間後に 2 本ずつ伐採し解剖した。材変色の広がりを軸方向と接線方向について計測した(図 1.)。6 ヶ月後の試料を用いて巻き込み率(Wound closure index)を算出した。反応帯、防御帯から材片を採取し、組織化学的観察を行った。

結果と考察

肉眼スケールでは、いずれの種でも、ドリル損傷部から広がった変色部は、接線方向よりも軸方向でより大きくなったが、広葉樹では中間の深さで、針葉樹では最も内側で変色長が最大となった。冬期・秋期の傷による変色部は、春期・夏期の傷に比べても小さかった。巻き込み率は供試木の種によって異なり、成長の良かったコナラとメタセコイアでは春以降の傷害で完全に巻き込みを生じた。巻き込み率と材変色の大きさには、相関関係は認められなかった。

組織化学的な染色試験を行った結果では、反応帯、防御帯は無変色域とはまったく異なる反応を示した。反応帯・防御帯の組織細胞では、リグニン・スベリン・フェノール類などの化学物質が、様々な濃度で存在した。シラカシは二次物質とチロースが最も多く見られ、樹脂道の導管・放射柔組織・木繊維が全般的にこれらにより閉塞していた。本研究ではメタセコイアで、傷害樹脂道の形成が初めて確認された。しかし、ヒノキや広葉樹では生じなかった。樹脂道のエピセリウム細胞と内腔には二次化学物質が観察された(図 2,3.)。



図 1. 冬期付傷のコナラを 6 ヶ月後に割材し、観察した例。 A.軸方向に割材。 B.接線方向に割材、巻き込みが認められる。

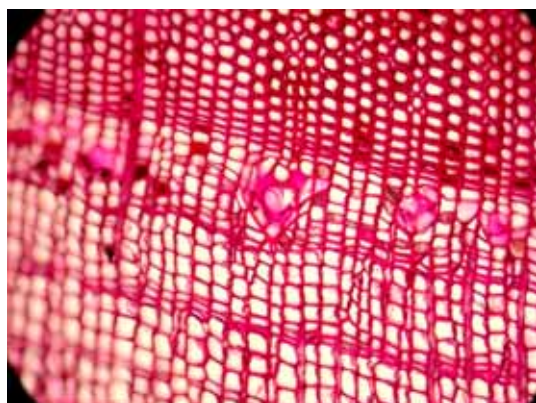


図 2. メタセコイア木部、付害樹脂道内部でのリグニン沈着反応。春期付傷、6 ヶ月経過での観察。Shiff 試薬で染色

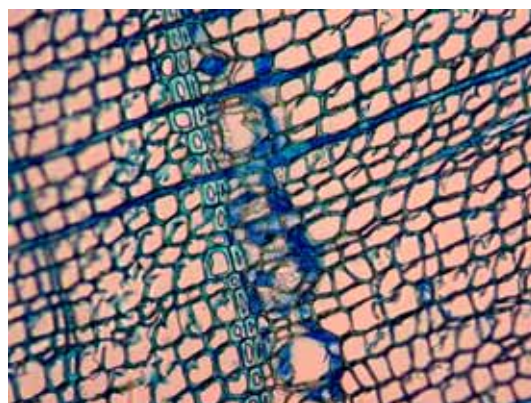


図 3. メタセコイア木部、障害樹脂道内部でのスベリン沈着反応。夏期付傷、6 ヶ月経過での観察。ナイル・ブルー染色

1. Introduction.

During their development, trees commonly experience damages. In natural forest the damage is often in the form of branch or stem breakage or bark abrasions or gouges, typically caused by falling debris, limbfalls, animal activity, or severe storms (Aide 1987). In managed or exploited forests, it's also associated with felling, skidding, and tending operations. In urban environment it's mainly by construction works, strong storms and by boreholes made in trees to examine them to ensure public safety (Mattheck and breloer 1994), these mechanical damages results in wounds that lead to discoloration and may result in a colonization of the wood by decay fungi.

Wood discoloration and decay are the largest sources of lost economic value of tree's wood as it affects the natural beauty of wood, which is the major reason for its widespread use. Discoloration and the subsequence decay are processes initiated by wounding and involve the injured tree, microorganisms, and the environment.

The basic structure of living trees is among the factors that dictate the extension of wood discoloration columns in the extant of wood after wounding and subsequent invasion by microorganisms.

There are many anatomical differences of wood between gymnosperm (conifer) and angiosperm (hard wood), which has effects on how they response to wounding and the restriction of the resulting discoloration and the subsequent decay.

Gymnosperm wood is relatively homogeneous in structure and consists primarily of trachieds and ray parenchyma cells. In some genera axial and epithelial parenchyma are found (Esau 1977). Trachieds are closed cells that overlap adjacent cells. Bordered pits are found throughout the trachied but are usually numerous on the radial plane and the end of cells that overlap other trachieds. Resin ducts, axial and radial parenchyma, are found in the wood of some genera, such as *Pinus* and *Larix*.

Dicotyledonous wood consists largely of vessels, fibers and parenchyma cells. Large variation in number and distribution of cell types exists among genera of cotyledons. Diffuse and ring porous species exhibit major anatomical differences. In diffuse porous wood, such as *Betula*, the vessels are relatively uniform in size and distributed throughout a growth ring, whereas ring porous wood, such as *Quercus*, has extremely large vessels in the early wood. The anatomy of wood with an axial alignment of its principal structural elements (vessels, trachieds and fibers), and the discontinuous distribution of living cells, is reflected in the host-pathogen interactions observed in sapwood. These features impose constraints on both the extension of discoloration and spread of the pathogens.

1.2 Models for the host-pathogen interaction in the wood of living trees

Four principal models have been proposed to describe the development and restriction of discoloration and decay in living trees.

The heart rot concept

The foundation for tree decay studies was laid over a century ago by Robert Hartig and gave rise to the classical heartrot concept (Pearce 1991). In which decay was perceived as predominantly saprotrophic process, confined within the non-living heartwood. Wounds or dead organs exposing the heartwood allowed the entry of fungal propagules. No killing of living tissues was envisaged and the fungus could only leave the living trees through interfaces between dead wood and the external environment.

Compartmentalization of decay in trees (CODIT)

Any wound or break that exposes wood causes a defensive reaction in the tree and provides an opportunity for infection by microorganisms. Trees resist the spread of injury and infection through the process of compartmentalization. The details of compartmentalization vary among tree species. In all wounded trees, effective compartmentalization leads to relatively little loss of function and small amounts of discolored and decaying wood. This CODIT model was proposed and developed by Shigo and co-workers (Shigo and Marx, 1977) to describe and account for the pattern of discoloration and decay developing in living wood behind tree wounds. According to the CODIT model, lesions in living wood are bounded and, by interference, restricted by barriers laid down in the wood. These have been termed walls 1-4, and enclose any lesion or incipient lesion within a defined compartment. They were envisaged as essentially static barriers preventing the further spread of infection. Walls 1-3 are formed within wood extant at the time of wounding. Wall 1 is formed transversely across the xylem, and acts as a boundary or barrier to the axial spread of infection. It was envisaged as resulting from vessel plugging responses in the conductive tissues and regarded as the weakest of the compartmentalization walls. Wall 2, resisting the radial spread of pathogen inwards from the wound, has been attributed mainly to anatomical features of the wood, and wall 3, resisting the lateral spread of infection, has been attributed to the activities of ray parenchyma cells. Wall 2 and 3 were perceived as being stronger than wall 1, but ultimately capable of being defeated by pathogen, following which they could be reinstated to contain a lesion of increased volume (Shortle, 1979; Blanchette, 1992). Wall 4 barrier differs from wall 1 and 3 in that it is formed in the plane of the cambium at the time of wounding as a response to damage (Pearce, 1987). It is the strongest and most durable of compartmentalization walls, comprises of cells laid down *de novo*, which can form a structurally homogeneous barrier, rather than being formed after differentiation in a tissue containing diverse cell types as

occurs in wall 1-3 (Shigo 1984). Functionally it's the most important barrier, protecting the youngest wood and the cambial tissues, both of which are vital to the continuing growth and survival of the trees.

Reaction model

(Shain 1979) developed the concept of the reaction zone as a region of active host response at the dynamic interface between living sapwood and wood colonized by pathogen. Reaction zone were initially described as a dynamic structure (Shain 1967), however evidence has accumulated from several different angiosperm pathosystems to suggest that reaction zones are not the dynamic structures initially envisaged, but normally form static boundaries to infection (Pearce 1991). When reaction zone boundaries fail, a volume of wood is colonized with little or no expression of characteristic reaction zone responses, until ultimately a new reaction zone boundary is established. Distinctive alteration at both tissues and cellular levels regarded as representing reaction zone formation, induced cell wall alterations (suberization), antimicrobial compounds, polyphenolic deposits and cell necrosis have been associated with reaction zone.

Environmental restriction of fungal colonization

As alternative to the CODIT and reaction zone models in which the growth of wood decay fungi is restricted by active host defenses, (Boddy and Rayner 1983) proposed and subsequently developed a model that does not require the operation of active host defenses. They suggested that high water content and concomitant low oxygen tension in functional sapwood is itself sufficient to preclude fungal development. According to this model, the limits of decay would occur at the junction between microenvironmental conditions conducive to fungal colonization in wood aerated as a result of drying and dysfunction. The cell wall alteration and polyphenolic deposits characteristic of reaction zone model would then have a wound repair function, their often hydrophobic nature serving to limit the spread of drying and cavitation and to maintain the hydraulic integrity of the adjacent functional xylem, rather than providing an inhibitory barrier.

All models mentioned are believed to create the barriers that resist invasion by decay fungi. However, these physical and morphological barriers do not make the tree immune to microbial colonization. As long as the wound remains open, its surface can be invaded by microorganisms that occur on the bark and in the air.

These different models are in no way mutually incompatible, and elements of all may function in concert to protect the living trees. Compartmentalization and reaction zone barriers may have the dual functions of protecting against fungal attack directly and helping to prevent aeration and maintain the functional integrity of healthy xylem adjacent to a wound or infection site.

1.3 Significance and importance of the research.

Research on wound responses of trees is required in order to understand the processes that favor or impede the development of discoloration, decay and fungal infection in wood. Many important and serious diseases of trees are caused by pathogens that initiate infection at wounds caused by many factors, like insects, animals, and human during management and silvicultural operations, these wounds become the starting points that may lead to discoloration and decay caused by invading microorganisms. Therefore, it's possible that more precise information about wound responses and the influence of the seasons could lead to better control and management measures based on better understanding of the chronology of the wounds responses.

A clear understanding of trees responses is of critical importance to the development of trees with enhanced resistance to pathogens or to the elaboration of silvicultural practices, which optimize the trees natural means of disease resistance.

The incidence and extend of discoloration and decay in trees is highly variable and depends on many factors among them the season of injury. There are various opinions and discrepancy about the effect of season on the extension of discoloration column and subsequent decay. For this reason the effects of season and exposure time on the extension of wood discoloration columns were studied.

1.4 Research Objectives

The objectives of the study are to determine the response of four different species, Shirakashi *Quercus myrsinaefolia*, Konara, *Quercus serrata* Metasequia *Metasequoia glyptostroboides* and Hinok *Chamaecyparis obtuse*, to wounds made at four different seasons.

The main focus was set to the extension of discoloration column, wound closure and deposition of accessory substances at the barrier zone and reaction zone.

2. Back ground.

The first visible reaction to wounding is the discoloration of the xylem that surrounds the wound. This response is relatively rapid and most of the changes occur within a few weeks of the injury (Shigo 1986).

Above and below the wound, vessels or tracheids that would normally conduct sap become plugged with pigmented substances that tend to make wood toxic to microorganism and more or less impervious to water (Blanchette 1992). Tyloses, which are the hypertrophied parenchyma cells that balloon out into the lumen of vessels plugging them, are induced through injury. After formation, tyloses can undergo secondary thickening, and if abundant may completely occlude vessels. Although tyloses may slow the colonization process, many decay fungi are capable of growing through them (Blanchette 1992).

The processes of compartmentalization create the barriers that resist invasion by decay fungi. However, these physical and morphological barriers do not make the tree immune to microbial colonization. As long as the wound remains open, microorganisms that occur on the bark and in the air can invade its surface, leading to greater discoloration and decay.

The reaction of the tree to wounding cannot be separated from its reaction to microorganisms because trees possess no mechanisms to prevent microbial invasions (Shigo and Marx 1977). Bacteria and yeast are the primary colonists followed by organisms, chiefly ascomycetous fungi that grow into the reaction zone and detoxify inhibitory chemicals or use them as nutrients. These organisms may be resisted, but not halted, and their advance causes a continual expansion of the reaction zone away from the wound. The extent and amount of wood discoloration and the subsequent decay in trees is highly variable and depends on internal as well as external factors.

The size of the wound and the depth of penetration into xylem, have great influence in the discoloration. Deep wounds have more discoloration than surface wounds (Shigo and Sharon 1968). For the discoloration to occur, the xylem must interact with air. In studies where the xylem was injured without breaking the bark, no xylem discoloration occurred (Sucoff et al. 1967). The presence of microorganism in the xylem also is not necessary for discoloration processes (Sharon 1974). However, the presence of bacteria and fungi may intensify the formation of cell occlusions, resulting into greater amount of discoloration (Blanchette and Sharon 1975, Blanchette 1982).

The tree species in which wounds are created is an important variable influencing host-pathogen interactions. When wounded xylem from different tree species was examined and compared, the extent of discoloration was found to differ among the tree species examined (Shigo and Sharon 1968, Shigo 1986). In study on the Patterns of Storm

Injury and Tree Response reported that the least effective compartmentalization and the greatest amounts of wood discoloration are in paper and yellow birch, with intermediate amounts found in red maple, and the least amounts of wood discoloration in sugar maple and white ash (Smith et al. 2001). The anatomical structure of a tree species and the functional behavior of the xylem cell after wounding influenced the amount of discoloration (Bauch et al. 1980). There exist two groups of different compartmentalizing trees (Dujesiefken et al 1991). Weak compartmentalizing genera are e.g. *Aesculus*, *Betula*, *Malus*, *Populus*, *Prunus*, and *Salix*. In comparison effective compartmentalizing genera are e.g. *Carpinus*, *Fagus*, *Quercus*, and *Tilia*.

The season in which wounds occur may also influence the degree of discoloration, although some discrepancies in the literature exist. Several authors (Leben 1985; Shain and Miller 1988; Mireku and Wilkes 1989) reported that the most extensive discoloration occurs with autumn or winter wounds, intermediate discoloration with summer wounds, and the least with spring wounds. (Armstrong et al. 1981) reported greater volumes of discolored wood were associated with fall wounds than with spring wounds. However, (Mohammed et al 2000) in a study on *Eucalyptus nitens*, found that after 12 months there was a slightly higher number of decay lesions in the sapwood associated with wounds pruned in spring and summer than in wounds pruned in autumn and winter. In addition, decay columns were longest following pruning in summer and autumn. (White and Kile 1993) found that in wounded *Eucalyptus regnans*, spring and summer wounds had significantly greater defect volume than autumn wounds during the first 6 months. However this seasonal effect was not apparent after 12 and 24 months. Therefore it may be expected that differences in defense responses are also likely to be most distinguishable at the early stages following wounding. (Mireku and Wilkes 1989) found that extent of infected tissue resulting from spring and summer stem wounds in *Eucalyptus maculata* was less than for autumn and winter wounds. In summer, higher phenol production was detected and wounds were sealed with kino. (Basham 1978) reported greater discoloration to *Acer saccharum* (Marsh) from spring wounds compared with summer or autumn wounds, but also noted that after two years spring wounds had less decay than those made in the autumn.

Moisture loss from the tree is also an important consideration that directly influences bark die back and withdrawal of water from the xylem. Loss of moisture and replacement with air in xylem can affect discoloration processes and subsequent invasion by fungi (Boddy and Rayner 1983, Rayner 1986). The extent to which air will enter the xylem can vary at different time of the year depending upon whether water columns are under positive or negative hydrostatic pressure when the xylem is wounded (Zimmermann 1983, Rayner 1986). These differences appear to be important considerations when the season of wounding is being examined.

Location of initial injury on the stem affects the frequency of infection and amount of wood discoloration and subsequent decay. Generally, wounds below diameter breast height are more likely to be infected by fungi than those occurring higher. Root and stem wounds that contacted the soil nearly always become infected, and have faster discoloration and decay rates than wounds higher in stem (Aho et al 1983). This may be due to microclimatic effects that favor fungal colonization.

The above and below extension of the wood discoloration columns differ along the axis of the tree. (Mireku and Wilkes 1989) reported that the mean vertical extension of discoloration from the drill holes was significantly greater above than below the wounds. (Armstrong et al. 1981) reported that, fall and spring upper wounds were associated with larger volumes of discolored wood than their lower counterparts.

When trees are wounded or infected with pathogens a number of induced defenses takes place, which can be divided into cell wall alteration and induced chemical defenses. In the primary tissues of both herbaceous and woody plants, cell wall appositions at sites of damage or attempted penetration are commonly observed. These may incorporate a number of components not normally present in the unmodified walls, including lignin and other phenolic compounds and suberin, together with normal cell-wall materials (Aist, 1983). Such papillae have been found in both angiosperm (Edwards and Ayres, 1981) and gymnosperm (Bonello et al., 1991) trees.

Lignins are complex, heterogeneous, cell wall bound polymers synthesised by almost all land plants ([Kubitzki, 1987](#)). At present, lignins are the second most abundant biopolymers on Earth. In extant plant species, lignins are found in the cell walls of specific tissue types, principally the wood fiber and vessel elements of xylem, but also sclerenchyma, phloem fibers and periderm ([Esau, 1977](#)). Lignins provide mechanical support for the plant body, and enable water and minerals to be transported through the xylem under negative pressure without collapse of the tissue ([Jones et al., 2001](#)). In addition, lignins may also be generated in response to pathogen challenge and wounding ([Vance et al., 1980](#); [Lange et al., 1995](#)). The synthesis of lignins in response to pathogens and wounding plays a dual role. Lignins waterproof plant cells ([Reina et al., 2001](#)), and thereby decrease the potential for dehydration. In addition, due to the complex nature of lignin polymers, lignins are difficult to degrade. Consequently, lignins provide a significant barrier to pathogen ingress ([Hammond-Kosack and Jones, 1996](#)). Induced lignification might not be expected to be effective in defense in the highly lignified xylem.

Suberin is one of the main cell wall polymer that is deposited together with suberin associated waxes at distinct locations during plant growth that is species-specific. Its typical function is to seal off the entire plant or one of its tissues against loss of water and solutes, but also to contribute to the strength of the cell wall. Suberin is constitutively present in the

secondary growth periderm of aerial tissues and in several underground tissues, e.g. epidermis, hypodermis, peridermis and the Casparian strips of the root endodermis. It may be deposited in bundle sheaths, the chalazea and abscission zone during seed development, and in secretory organs as well as fibers ([Kolattukudy 1981](#)). Where its resistance to microbial degradation and hydrophobic properties are important in maintaining the integrity of this plant environment interface. It is not a normal component of healthy sapwood, although it is present in some heartwoods (Pearce and Holloway, 1984), and is associated with the resin duct system in *Pinus* spp. (Biggs 1987; Pearce, 1990). Xylem suberization responses at the margins of decay lesions were first recognized in the compartmentalization wall 4 barrier formed in the vicinity of pruning wounds in oak (*Quercus robur*) where sapwood was attacked by *Stereum gausapatum* (Pearce and Rutherford 1981; Pearce and Holloway 1984). Similar suberized multicellular compartmentalization wall 4 barriers have been reported from various other woody angiosperms (Pearce and Woodward 1986; Pearce, 1990). Although most of the species examined exhibited a xylem suberization response, two (*Fraxinus excelsior* and *Sophora japonica*) showed no suberization of the traumatic axial parenchyma adjacent to decayed wood (Pearce, 1990). In conifers in which wall 4 barrier zones have been examined, a traumatic tissue of parenchyma cells and resin ducts can be formed, but suberization of this tissue does not appear to have been reported (Pearce 1990; Blanchette, 1992). Suberization is not, therefore, a universal feature of the compartmentalization wall 4. In addition to forming a decay-resistant zone around the site of a major wound, protecting the youngest xylem, cambium and bark from attack by micro-organisms entering via the wound, the suberized tissue could also act as an effective waterproof seal, reducing drying and the entry of air around the lesion, and helping to maintain an inimical microenvironment for fungal development in new wood adjacent to the wound (Boddy, 1992).

Differentiated sapwood could exhibit suberization responses as a result of mechanical wounding (Schmitt and Liese. 1991, 1993), or as a consequence of fungal colonization (Pearce. 1990). The extent of this induced suberization differed between species. In some, for example *Fagus sylvatica* and *Quercus* spp., an extensive response was seen, in which xylem parenchyma cells of all categories, vessel linings and tyloses stained for suberin. In other species the extent of suberization in reaction zones was much reduced or absent, in general, strong reaction zone suberization responses correlated closely with vessel occlusion by tyloses. In conifers, where the proportion of living cells in the xylem is normally low, suberization responses were generally poorly developed (Pearce, 1990). It is interesting to note, however, that in a reaction zone in *Picea abies* suberin was associated only with ray parenchyma cell end wall pits (Pearce, 1987). In a circumferential reaction zone this is precisely where suberin might have the greatest effectiveness, both in

maintaining the hydraulic integrity of the functional sapwood and in blocking the major route of radial fungal spread (Pearce and Woodward, 1986).

Tyloses, which develop from xylem parenchyma cells and project through pits to occlude the lumens of vessels (Koran and Cote, 1965), are commonly suberized (Pearce and Holloway, 1984; Pearce, 1990; Rioux et al., 1995). Such suberized tyloses are resistant to degradation by decay fungi, persisting recognizably after the remainder of the wood has been completely degraded (Pearce, 1989). Tyloses might block the axial spread of pathogens along the vessels, which otherwise offer little anatomical restriction to microbial spread (Rioux et al., 1995). Because of their hydrophobic properties, largely conferred by suberin, they might also be important in maintaining xylem function by restricting the extent of cavitation and air access (Rayner and Boddy, 1988).

In contrast to the wall 4 barrier zone, which often comprises a continuous sheet of suberized parenchyma, reaction zones are discontinuously suberized, reflecting the distribution of parenchyma cells capable of expressing this response. Although tracheids in conifers, and xylem fibres in woody angiosperms, have been observed with suberized linings, these were near the cambium and appeared to have been incompletely differentiated at the time of wounding (Biggs, 1987; Pearce, 1990). Induced suberization alone cannot, therefore, render reaction zones resistant to penetration, since the suberized tissues can be bypassed. However, the principal axial and radial routes for fungal spread in wood - tyloses and ray parenchyma cells, respectively - can be blocked by degradation-resistant suberized walls, thereby slowing invasion along these key anatomical pathways. Although xylem suberization has been interpreted as a response to wounding and tissue drying (Biggs, 1987; Schmitt and Liese, 1991, 1993) the experimental systems used did not preclude the possibility of fungal contamination and hence a role for microbial infection in the induction of xylem suberization.

Increased levels of phenolic compounds have been reported from lesion margins in living sapwood in many tree species (Shain, 1967, 1971; Pearce, 1987; Yamada, 1992), often the compounds induced at sites of host-pathogen interaction are absent from, or present at only very low levels in healthy sapwood (e.g, *Aesculus hippocastanum*, *Fagus sylvatica* (Pearce, 1991), *Pinus taeda* (Shain 1967). These chemical changes often accompany cell wall alterations and the deposition of insoluble polymeric materials in CBL (column boundary layer) reaction zones and compartmentalization wall 4 barriers (Pearce and Woodward, 1986; Pearce 1991).

3. Materials and Methods.

3.1 Research site.

Experimental Station at Tanashi, the University Forests, the University of Tokyo, is established in 1929 under the name of Tama nursery until it was entrusted to the University Forests in 1982. The experimental forest is located in Nishitokyo city, Tokyo metropolitan area (35° 45' N, 139° 32' E). The total area is about 9.1 hectares, which is located on a flat area that is about 60m above sea level. The annual mean temperature is 13.7 C and annual mean rainfall is 1,400 mm.

The soil type Andosol covering the forest site, with the upper black soil layer of about 50cm deep and a red loam below it.

The main species are *Quercus serrata*, *Quercus acutissima*, and *Pinus densifolia*. In the demonstration forest, there are about 350 species of which 80 are coniferous trees and 270 broad leaved

3.2 Plant materials.

In the experimental station, 96 apparently healthy trees belonging to the four below mentioned species were selected for the wounding experiment. (Table 1).

Metasequoia glyptostroboides.

Order Coniferales. Family Taxodiaceae (Redwood family).

Japanese name: Metasekoia

Known as the Dawn Redwood and also has been called "a living fossil" because it was first discovered in Japan in 1939 as a fossil and then, found growing in the wild in China. The species is over 50 million years old, native to the [Sichuan-Hubei](#) region of [China](#). It is the only living [species](#) in the [genus](#) *Metasequoia*. It is a fast growing tree to 40-45 m tall and 2 m trunk diameter.

It is a deciduous conifer, with soft needle-like leaves that look like evergreens, which bright -green in the spring and brilliant orange/red in the fall. The needles are shed in the cold season of winter. Metasekoia are a very popular large ornamental and tree, one of the few deciduous conifers in the world. It is feathery pyramidal in form with a straight, fluted trunk. The bark is red-brown, fissured and exfoliating in long strips. It is a beautiful and stately tree, well suited for large areas. It makes a very effective, fast growing screen, perfect as a long driveway alley.

Chamaecyparis obtusa.

Order Pinales, Family Cupressaceae (Cypress family).

Japanese name: Hinoki

Native to central [Japan](#), commonly known as Hinoki Cypress or Hinoki. It is a slow growing [tree](#), which grows to 35 m tall with a trunk up to 1 m in diameter. It is planted for its very high quality timber in Japan, where it is used as a material for building [palaces](#), [temples](#), shrines, traditional [noh](#) theatres, [table tennis](#) blades and baths. The wood is lemon-scented and light-colored with a rich, straight grain, and is highly rot resistant.

It is also a popular ornamental tree in [parks](#) and [gardens](#), both in Japan and elsewhere in [temperate](#) climates including Western [Europe](#) and parts of [North America](#). A large number of [cultivars](#) have been selected for garden planting, including dwarf forms, forms with yellow leaves, and forms with congested foliage. Wood is, light and soft, sapwood pale yellow to cream color, distinct from the orange/red to pinkish heartwood.

Quercus serrata.

Order Fagales, Family fagaceae (Beech family).

Japanese name: Konara.

Konara is the most common tree in temperate zone of Japan distributed mainly in Hokkaido, Honshu, Shikoku and Kyushu. The wood is used for fuel and beds of mushroom.

Its a tall deciduous tree with dark gray bark, leaves are alternative with pretty long petioles, serrate with callous tips. The wood is very hard, strong, red-brown in color. Wood is ring-porous , with extremely large vessels in the early wood.

Quercus myrsinaefolia

Order Fagales, Family fagaceae (Beech family).

Japanese name: Shirakashi.

Shirakashi is a tall evergreen tree with grayish black or greenish dark bark. Leaves are alternate, lanceolate, acuminate at the apex, deep green and lustrous above. This tree is scattered in the temperate zone of Japan in Honshu, Shikoku and Kuyshu. The wood is used for building, utensils, ships and machineries.

Table 1. Characteristics of the four species

Scientific name	Japanese name	Average DBH cm	Average Height m	Description
<i>Metasequoia glyptostroboides</i>	Metasekoia	6.1	7.4	Conifer
<i>Chamaecyparis obtusa</i>	Hinoki	10.4	10.2	Conifer
<i>Quercus serrata</i>	Konara	5.0	4.0	Hardwood
<i>Quercus myrsinaefolia</i>	Shirakashi	7.8	10.9	Hardwood

3.3 Wounding experiment

To characterize species response to mechanical wounding, an experiment was set to stimulate mechanical damages to trees. An electric drill was used to create wounds on the trees. Wounding were done in winter (January), spring (April), summer (July) and autumn (October) of 2005, each season 6 trees from each species were wounded. Five wounds were made through the bark deep into the xylem at 100, 125, 150, 175 and 200 cm above the ground, with the first wound stand 1 meter above the ground level where the last wound stand 2 meters from the ground level, except for Konara where the wounds were between 20 cm to 120 cm.

Wounds were position one above other in spiral line, with the lower most wound face north direction and the second wound was east, 90 degree left and so on.

Wound depths were made to the half of DBH of the respected trees, while the width of the wound were 1/6 to 1/7 of the DBH. Wounds were left open and untreated.

Three exposure durations were adapted, 2 weeks, 2 months and 6 months. At the end of each duration, destructive sampling method was used, the trees were felled and the portion of the trunk that contained the wounds was then taken to the warehouse for further dissection.

3.4 Measurement of wood discoloration columns.

At the completion of each experimental exposure duration, the trunk portion that contains the wounds where further sectioned to a 5 cm disks starting from the drill hole using electric saw, the disks were then split axially through the center of the wound. The extent of the wood discoloration columns in the axial and tangential direction were recorded as any changed in the color of the normal sapwood, measurements were taken at the start, middle and end of the wound length (Figs 1-2).

The extension of the discoloration columns above and below the drill holes were measured at it's the longest extension portion of the wood discoloration column

Photos of the wood discolored columns were taken as permanent records. The disks were them stored at -30 degree.



Fig.1 Axial extension of the discoloration column. Shirakashi, six months summer. After summer wounding.



Fig.2.tangential extension of the discoloration column. Shirakashi, six months after summer wounding.

3.5 Wound closure index

Activity of the cambium around wounds exceeds that of non- wounded parts of the tree. This activity produces more wood that elsewhere at the same level on the stem. Callus-bark plus sapwood produced at the edge of the wound expands faster tangentially than radially, which allows the wound to close while tree expands faster in girth.

After six months of each wounding season, the rate of wound closure was measured as a closure index.

Wound closure index $= (\text{hole diameter} - \text{extant opening diameter}) / \text{hole diameter} \times 100$

3.6 Anatomical and Histochemical experiment

This experiment is concerned with the spatial distribution of the accessory substances that were produced in the discolored, reaction zone and barrier zone after wounding.

Six months after each wounding season, small blocks of 2x0.5x1cm, at the interface of the discolored and normal wood, were taken from the frozen disks, blocks also contained parts of the barrier zone.

After 30 minutes of thawing at 5 degree, a 20 -24 μm transverse sections were obtained by

using sliding microtome.

The presence and spatial localization of the starch, lignin, suberin and phenols, which are characteristic substances, produced as the results of wounding and fungal infection.

A Nikon light microscope (ECLIPSE E600) were used, and the photos showing the presence and the location of these substances were analyzed, and then saved as a permanent records. These accessory substances were detected using the following methods. (Jensen 1962).

Starch.

Iodine solution in potassium iodine IKI: Microtome sections were placed in the solution for several minutes and mounted on the slide using water. Starch is stained black to dark brown color.

Lignin.

Three different stains were used.

Weisner Reaction phloroglucinol-Hcl (Jensen 1962): Large drop of phloroglucinol solution was place on the microtome section, after few minutes a red color developed, but quickly faded.

Muäle Reaction (Jensen 1962): Microtome sections were immerse in 1% aqueous potassium permanganate for 5 minutes, rinsed thoroughly with water, and then treated with 1% aqueous hydrochloric acid until color beige, then added concentrated ammonium hydroxide to developed a magenta color in angiosperm and brown color in gymnosperm. The color also faded quickly.

Schiff's Reaction (Jensen 1962): Microtome sections were immersed in Schiff's solution for about 15 minutes to 4 hours, lignin stained red.

Suberin.

Two types of stained were used.

Nile Blue (Jensen 1962): Microtome sections were dipped in the Nile blue solution at 37 degree for about 30 seconds and the transferred to 1% acetic acid for 30 seconds, washed with distilled water and mounted on glycerol. Suberin containing tissues stained blue.

Sudan black (Jensen 1962): Microtome sections were placed in 50% methanol for several minutes, then immersed in 1% Sudan black solution for 5 to 20 minutes and differentiated in 50 % methanol for about one minutes, then mounted in glycerol. Suberin containing tissues are stained dark blue to black.

Phenols

Three different stains were used.

Nitrosophenol Reaction (Suzuki 1957): Water was suck from the microtome sections, then one drop of concentrated acetic acid were added, 20% sodium nitrate were then added for

several minutes, then the sections were dried of the solution, 10% sodium hydroxide were then added. Phenols are stained red.

Diazo Blue Reaction (Burstone 1955): Microtome sections were placed in 0.2 % fresh solution diazo blue for 5 minutes, then briefly rinsed in 0.1% HCL, then transferred to a buffer solution of. Phenols are stained brown.

Fast Red Reaction (Lillie 1965): Microtome sections were place on a fresh 0.12 % solution of fast red for several minutes then mounted on slide. Phenols are stained oze color.

4. RESULTS

4.1 Description of the wood discoloration columns.

Injuries that extend into the tree's sapwood disrupt the water conduction system, expose the injured xylem to the air and microorganism, that lead to drying of the tissues that surround the wound. The first macroscopically visible change that occurs in the xylem after the wounding was the formation of discolored wood. The four tree species examined had similar wound reactions, wood discoloration columns extended above and below the drill holes. At first the color changes lighter than in sound wood, but darken with time. In Konara and Shirakashi, the wood discoloration columns were brown in color and clearly zoned and encircled with a dark brown reaction zone in Shirakashi while it is greenish in Konara. This reaction zones separate the discolored wood from the normal sapwood. The reaction zone was only 1-2 millimeters in width, and was clearer in Shirakashi than in Konara. In Metasekoia, the wood discoloration column was encircled by a pink color reaction zone, which was broad and diffused and about 2-3 mm width, while in Hinoki, the light brown wood discoloration column was encircled by a darker reaction zone of 2-3 millimeters in wide. The colors of the reaction zone in all species examined became darker with increase time after wounding.

4.2 Pattern of the wood discoloration columns

The Pattern of wood discoloration columns that extended from the drill wound in the axial direction was generally consistent within each species. There were two distinctive shapes, in which the four species can be grouped into.

In Shirakashi and Konara, the longest extension of the wood discoloration column was at the middle part of the wound. While, in Hinoki and Metasekoia the longest extension of the discoloration column was at the inner part of the trunk near to the heartwood. (Figs .3-6).



Fig.3 wood discoloration column shape. Shirakashi six months after summer wounding.



Fig.4 Wood discoloration column shape. Hinoki six months after summer wounding.



Fig.5 Wood discoloration column shape. Metasekoia, six months after autumn wounding.



Fig.6 Wood discoloration column shape. Konara, six months after winter wounding

4.3 Axial extension of the wood discoloration columns

The maximum length of the wood discoloration columns was measured from the axially split disks. Wood discoloration columns extension on the axial direction was more clear and rapid compared to the tangential directions, and it ranged from few millimeters to more than 40 centimeters.

In Konara, two weeks after wounding, there were no great differences between the lengths of the discoloration columns between the different seasons. However, spring discoloration column was slightly longer than the summer discoloration column while, autumn discoloration column length was the shorter among all. Two months after wounding there were great differences between the different seasons. Spring wood discoloration columns were the longer with a length of nearly 50 centimeters. While, winter wood discoloration columns length was the shorter with less than 4 millimeters, autumn wood discoloration columns length was longer than summer wood discoloration. Six months after wounding, winter wood discoloration column length was shorter with less than 25 millimeter length, while the wood discoloration columns on the other seasons ranged between 25-30 centimeters, with autumn wound discoloration length is slightly longer than spring and summer discoloration (Fig 7).

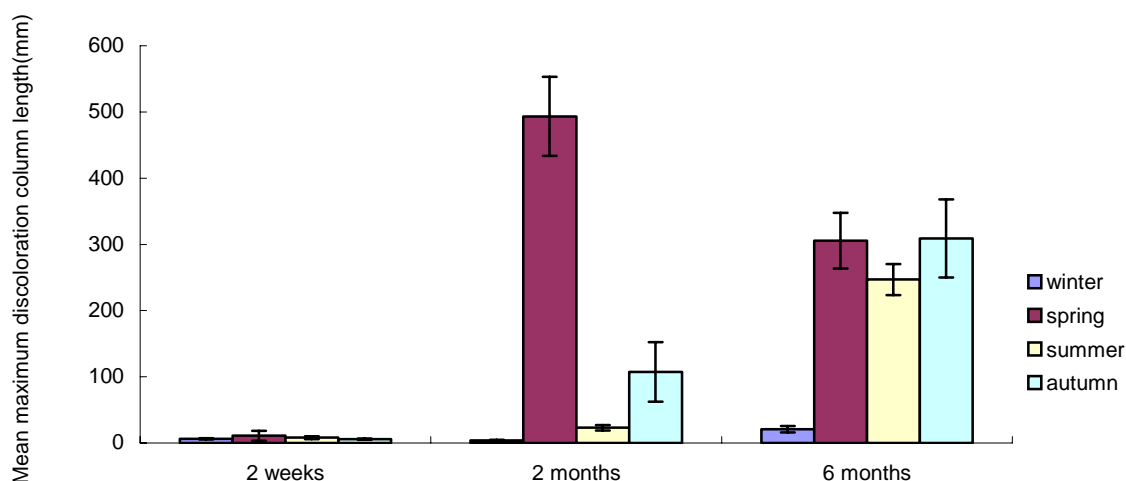


Fig.7 Mean maximum discoloration column length In Konara

In Shirakashi, two weeks after wounding, summer wood discoloration column length was about 23 millimeters long and was greatly longer than the winter and spring wood discoloration columns. While, autumn wound discoloration column length was greatly shorter with less than 1 millimeter in length. Two months after wounding, winter wood discoloration column was greatly shorter than spring and summer wood discoloration columns. Six months after wounding, summer and spring wound discoloration lengths were 85 and 78 millimeters respectively and were longer than winter and autumn wood discoloration columns length, which were 35 and 54 millimeters in length respectively (Fig 8).

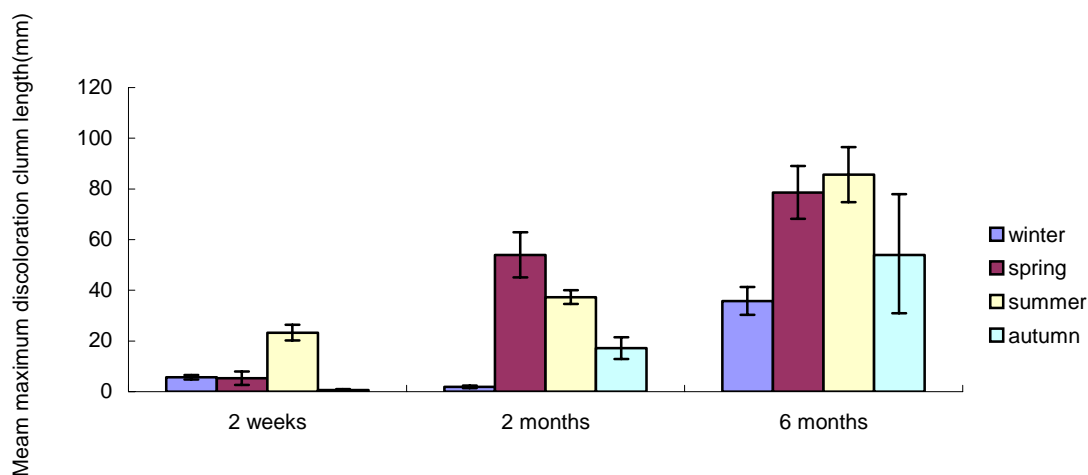


Fig.8 Mean maximum discoloration column length In Shirakashi.

In *Metasekoia*, two weeks after wounding, summer wound discoloration column length was longer, with a length of 50 millimeters followed by spring wound, while winter discoloration length was the shorter with a length of less than 8 millimeters. Two months after wounding, there were no great differences between the different seasons. However, spring and summer discoloration columns lengths were slightly longer than winter and autumn wood discoloration columns, with spring wound discoloration columns slightly longer than the summer discoloration columns. Six months after wounding, winter discoloration column length was longer than the other seasons with length of 90 millimeters (Fig 9).

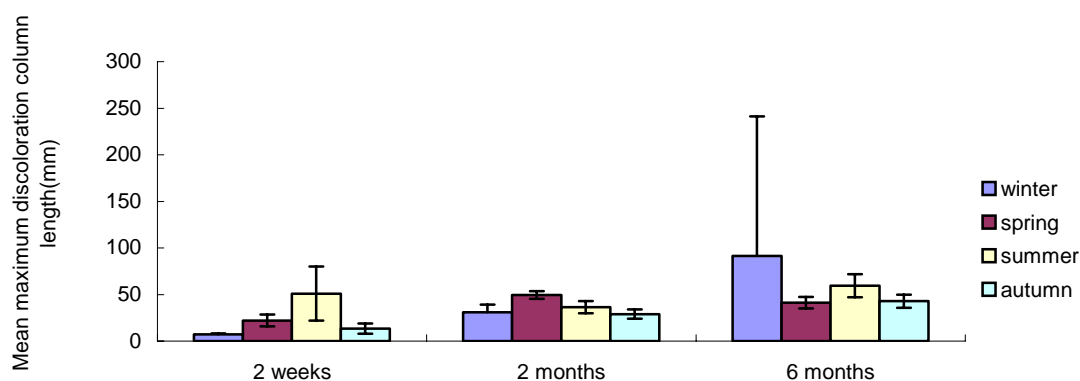


Fig.9 Mean maximum discoloration column length in *Metasekoia*

In *Hinoki*, two weeks after wounding, summer wound discoloration columns length was greatly longer with a length of 77 millimeters, while autumn discoloration length was the shorter with a length of 6 millimeters. Two months after wounding, summer discoloration columns length was longer with a length of 100 millimeters, followed by spring and autumn respectively, while winter discoloration column length was only 4 millimeters in length. Summer discoloration length was slightly longer than autumn wound discoloration length. Six months after wounding, spring wound discoloration column length was greatly longer than winter discoloration column length. Autumn discoloration column length was slightly longer than summer discoloration columns but both were longer than winter discoloration columns length (Fig 10).

For the all tree species examined, the wood discoloration columns length in the axial direction is highly related to the duration of exposure after wounding, six months after wounding in all seasons has significantly longer discoloration compared to that of two months and two weeks.

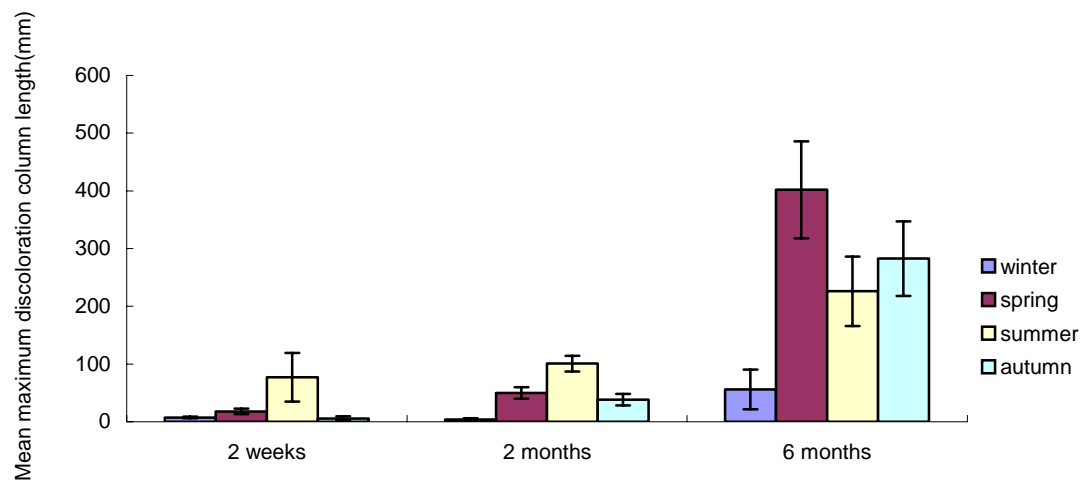
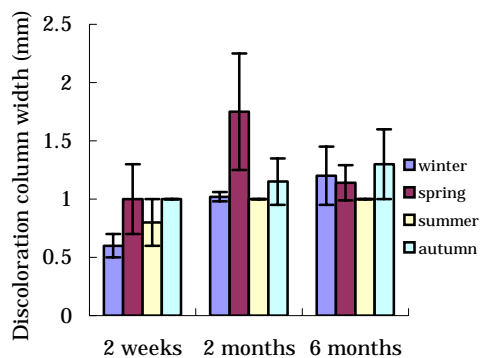


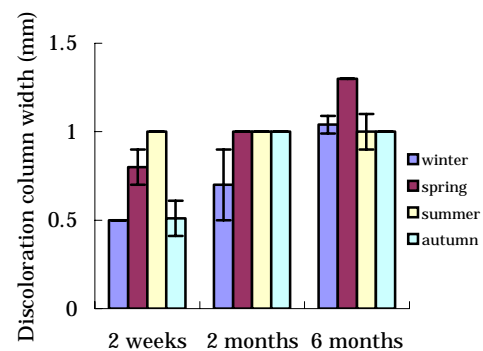
Fig.10 Mean maximum discoloration column length in Hinoki.

4.3 Tangential extension of the wood discoloration columns

In all trees examined, wood discoloration columns extension on the tangential direction was between 1-2 millimeters, with no much difference between species, seasons and exposure durations (Figs 11).



Konara



Shirakashi

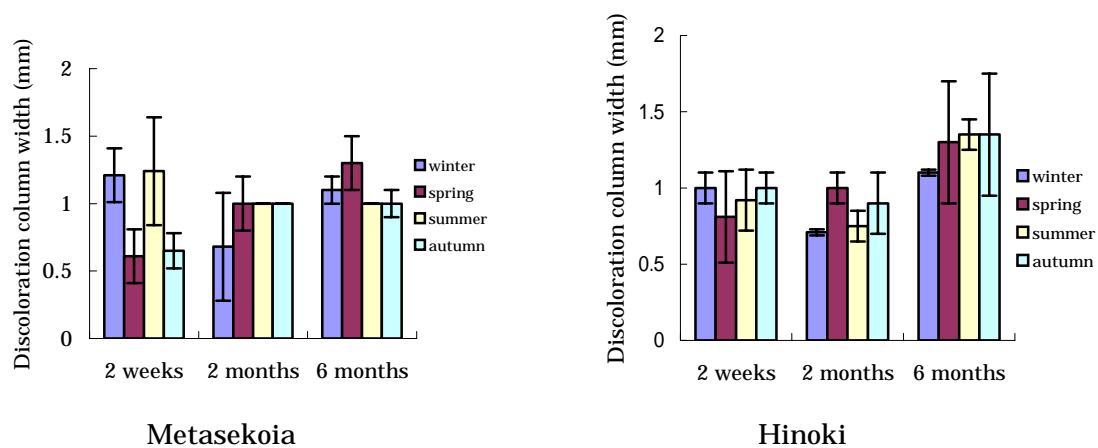


Fig.11 Wood discoloration column extension on the tangential direction.

4.4 Species response rates two months after wounding

Wood discoloration column length was longer in spring for *Konara Shirakashi* and *Metasequoia*, while the longest discoloration column lengths correspond to summer wounding in *Hinoki*, with *Konara* showing the longest discoloration. The shorter discoloration column length was in winter wounding in all tree species examined. Summer wounding discoloration column was longer than autumn wounding discoloration for all species except in *Konara*.

Generally *Metasekoia* and *Shirakashi* species showed the strongest wound response compare to *Hinoki* and *Konara* respectively (Fig 12).

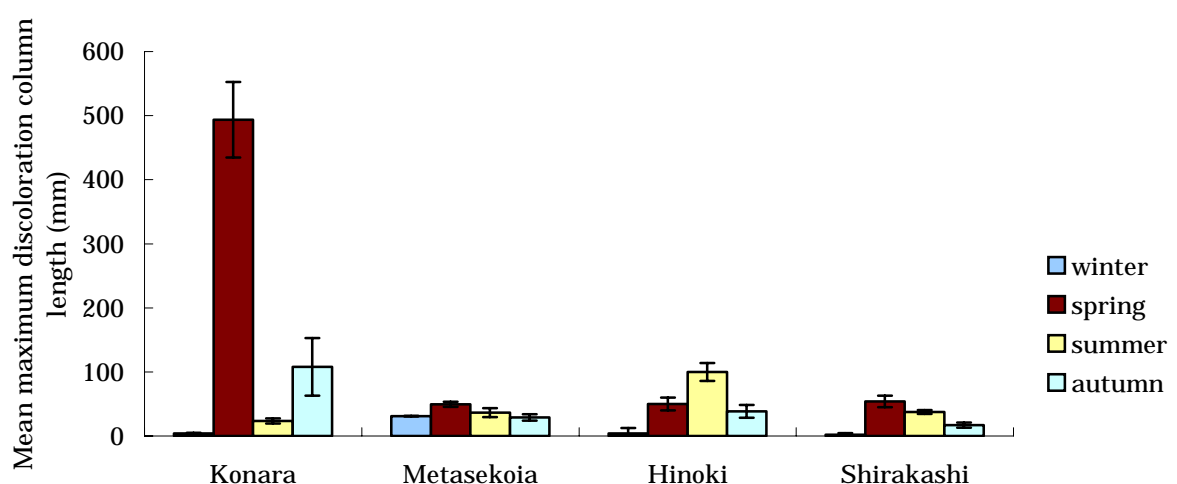


Fig.12 Mean discoloration length after two months

4.5 Species response rate six months after wounding

Among all tree species examined, Metasekoia, and Shirakashi had shorter discoloration length when compared to Konara and Hinoki.

In Hinoki and Konara, the longest discoloration length corresponded to spring wounding in six months after wounding, followed by autumn wounding while winter wounding has the least discoloration length with Konara has the shorter discoloration length among tree species examined. Six months after wounding, Hinoki and Konara had the longer discoloration columns, than Shirakashi, and Metasekoia. For Konara, and Hinoki, autumn discoloration column length was longer than summer discoloration column while in Shirakashi, and Metasequoia, summer discoloration column length was longer than spring and autumn discoloration columns length respectively.

Winter discoloration column of Metasekoia, was the longest among tree species examined and also the longest between seasons for the Metasequoia.

Generally Shirakashi and Metasekoia had the strongest wound response among species examined when comparing the length of the discoloration columns (Fig 13).

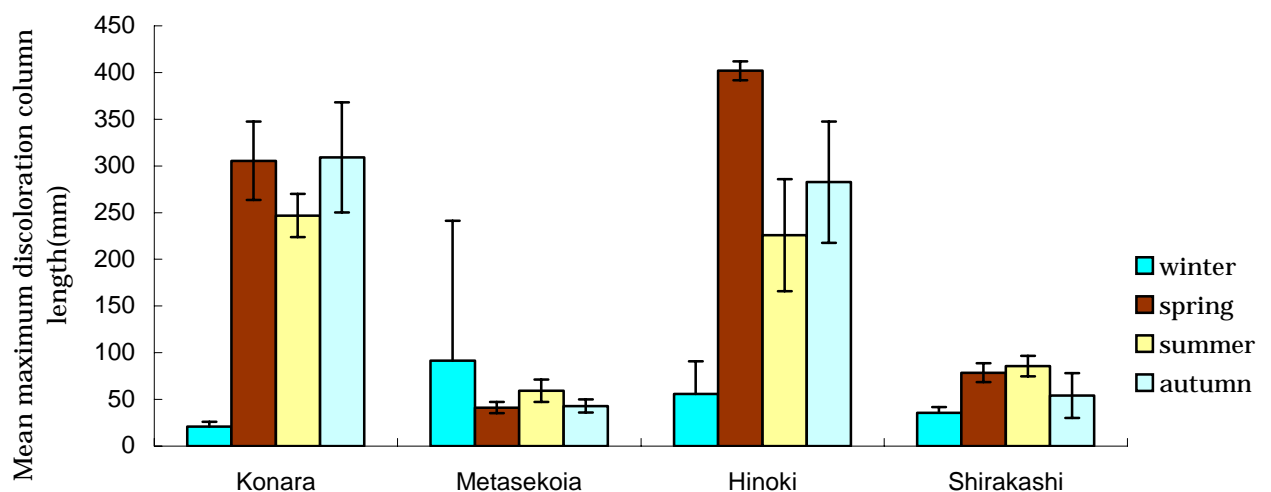


Fig.13. Mean discoloration length after six months

4.7 Effects of wounds position

Two weeks after wounding, generally there were no clear differences between the length of the discoloration column between the top (5th) and the bottom (1st) wounds for all species in all season (Figs 14-15).

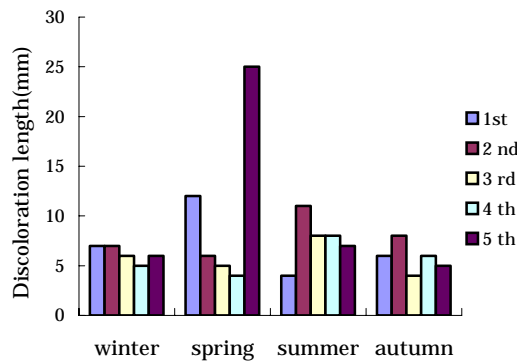


Fig. 14 Konara discoloration column length of wounds 1st to 5th. Two weeks after wounding

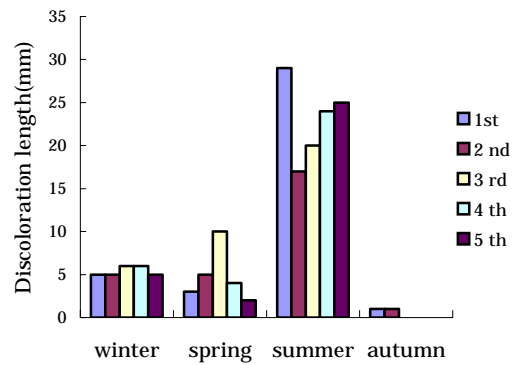


Fig. 15 Hinoki discoloration column length of wounds 1st to 5th. Two weeks after wounding

Two months after wounding and during the active growing seasons of spring and summer, there were some differences between the length of the discoloration column from the bottom (1st) wound and top (5th) wound, with (1st) wound discoloration column had longer discoloration. During the dormant season of winter and autumn generally the top (5th) wounds had slightly longer discoloration column than lower wound (Figs 16-19).

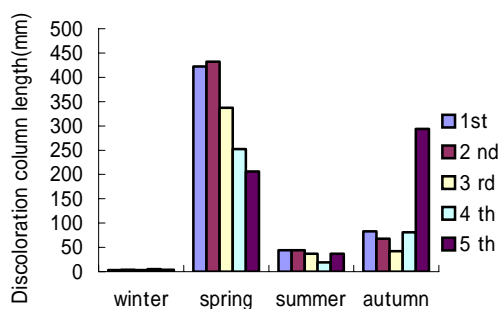


Fig. 16 Konara discoloration column length of wounds 1st to 5th. Two months after wounding.

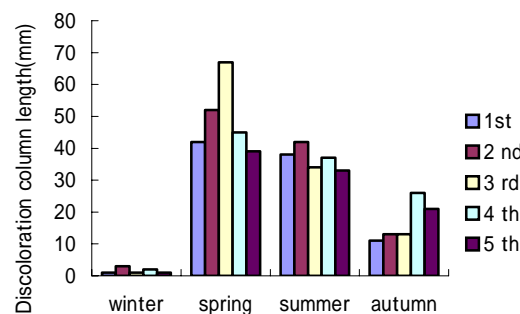


Fig. 17 Shirakashi discoloration column length of wounds 1st to 5th. Two months after wounding.

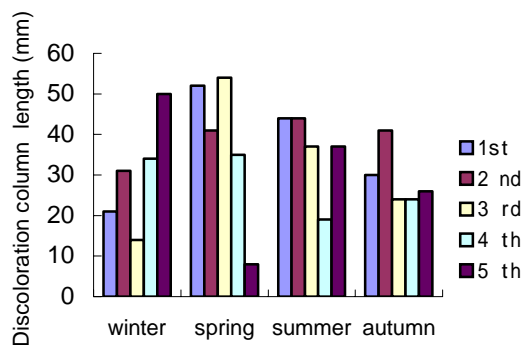


Fig. 18 Metasekoia discoloration column Length of wounds 1st to 5th. Two months after wounding.

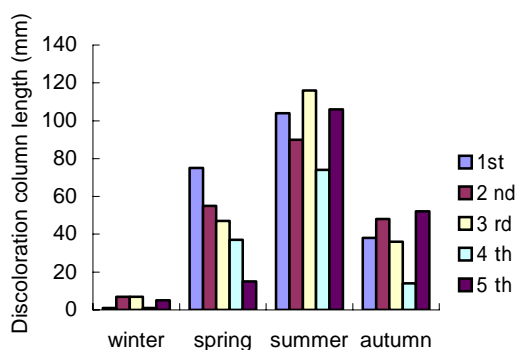


Fig. 19 Hinoki discoloration column length of wounds 1st to 5th. Two months after wounding.

Six months after wounding, there was no fixed trend. For Hinoki, generally the top (5th) wound discoloration column was slightly longer when compared to the bottom (1st) wound, which become clear in summer and autumn wounding. For Metasekoia, generally there were no clear different in discoloration column length between top (5th) and bottom (1st) wounds. For Shirakashi, the top (5th) wound showed longer discoloration column length in winter and spring however the trend was opposite in summer and autumn wounding. For Hinoki, the bottom (1st) wound discoloration columns were longer than the top (5th) wound during winter, spring and autumn wounding while it's the opposite during the summer wounding (Figs20-23).

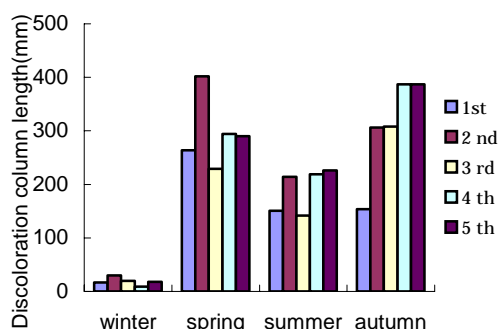


Fig. 20 Konara discoloration column length of wounds 1st to 5th. Six months after wounding.

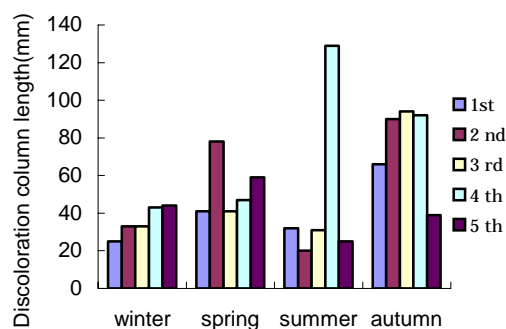


Fig. 21 Shirakashi discoloration column length of wounds 1st to 5th months after wounding.

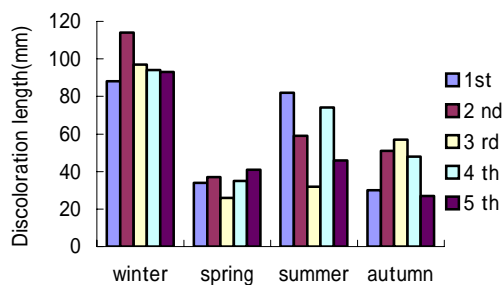


Fig.22 Metasekoia discoloration column length of wounds 1st to 5th. Six months after wounding.

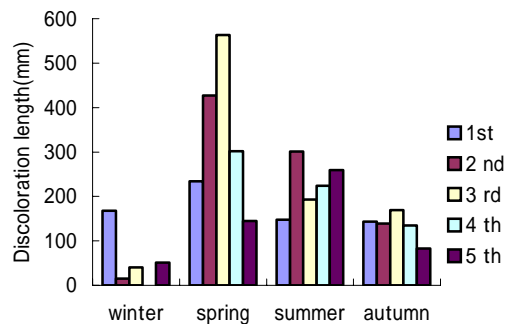
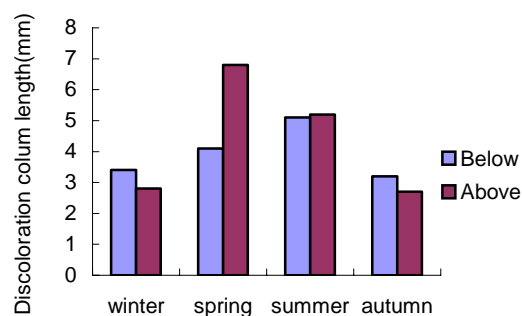


Fig. 23 Hinoki discoloration column length of wounds 1st to 5th. Six months after wounding.

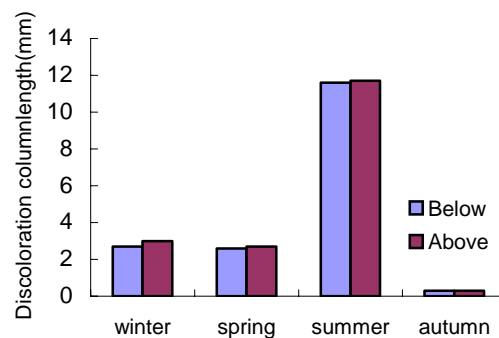
4.8 Discoloration extension below and above the wound

When the trees were wounded, wood discoloration extended from the wound hole in both direction above and below the wounds.

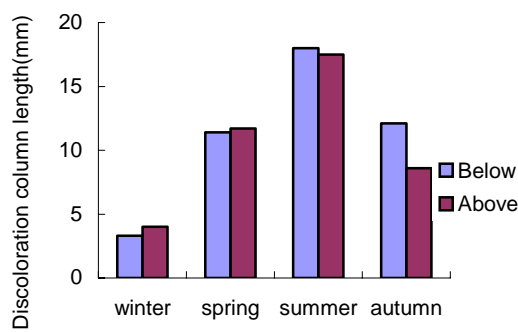
Two weeks after wounding there were no clear differences between the length of the discoloration column above and below the wound. However, in spring wounding, konara showed a longer discoloration column extension on the above direction (Figs 24).



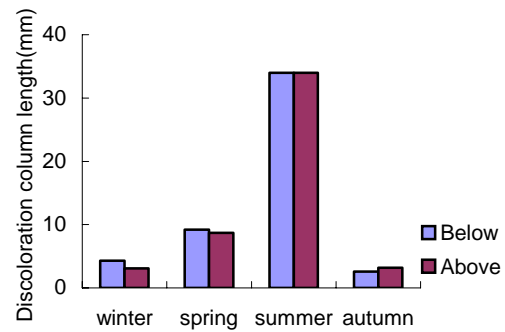
Konara



Shirakashi



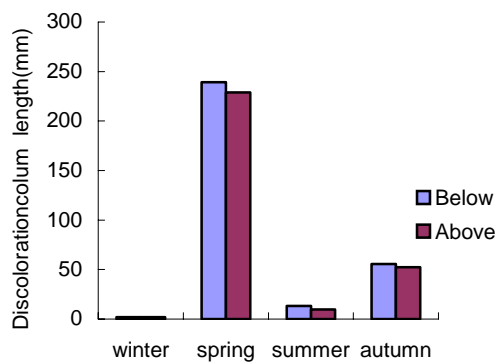
Metasekoia



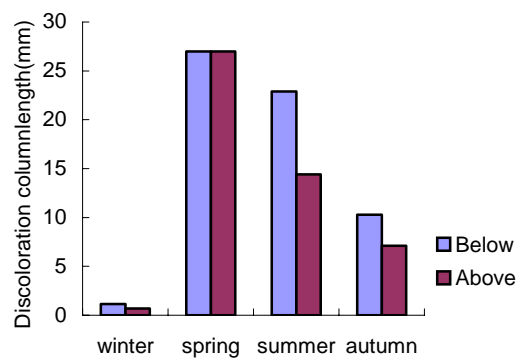
Hinoki

Fig.24 Discoloration column extension above and below the wound 2 weeks after wounding

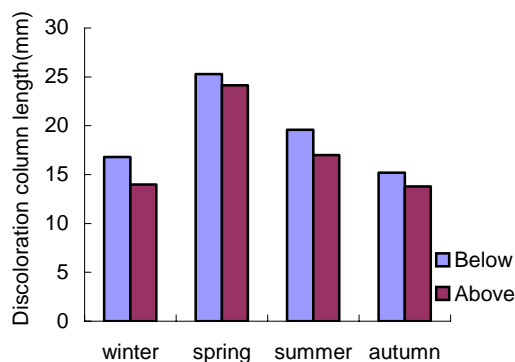
Two months after wounding again there were no clear differences between the length of the discoloration column above and below the wound in all tree species. However, the wood discoloration columns extension in the direction below the wound were slightly longer than the above direction in all species except Hinoki (Figs 25).



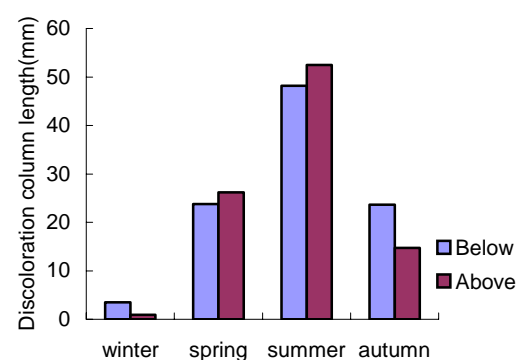
Konara



Shirakashi



Metasekoia



Hinoki

Fig.25 Discoloration column extension above and below the wound 2 months after wounding

Six months after wounding again there were no differences between the discoloration column extension above and below the wound for all seasons. However, in spring there were slight differences between the below and above extension of the discoloration column in Shirakashi and Metasequoia, where the below extension of the discoloration column was longer than the above extension (Figs 26).

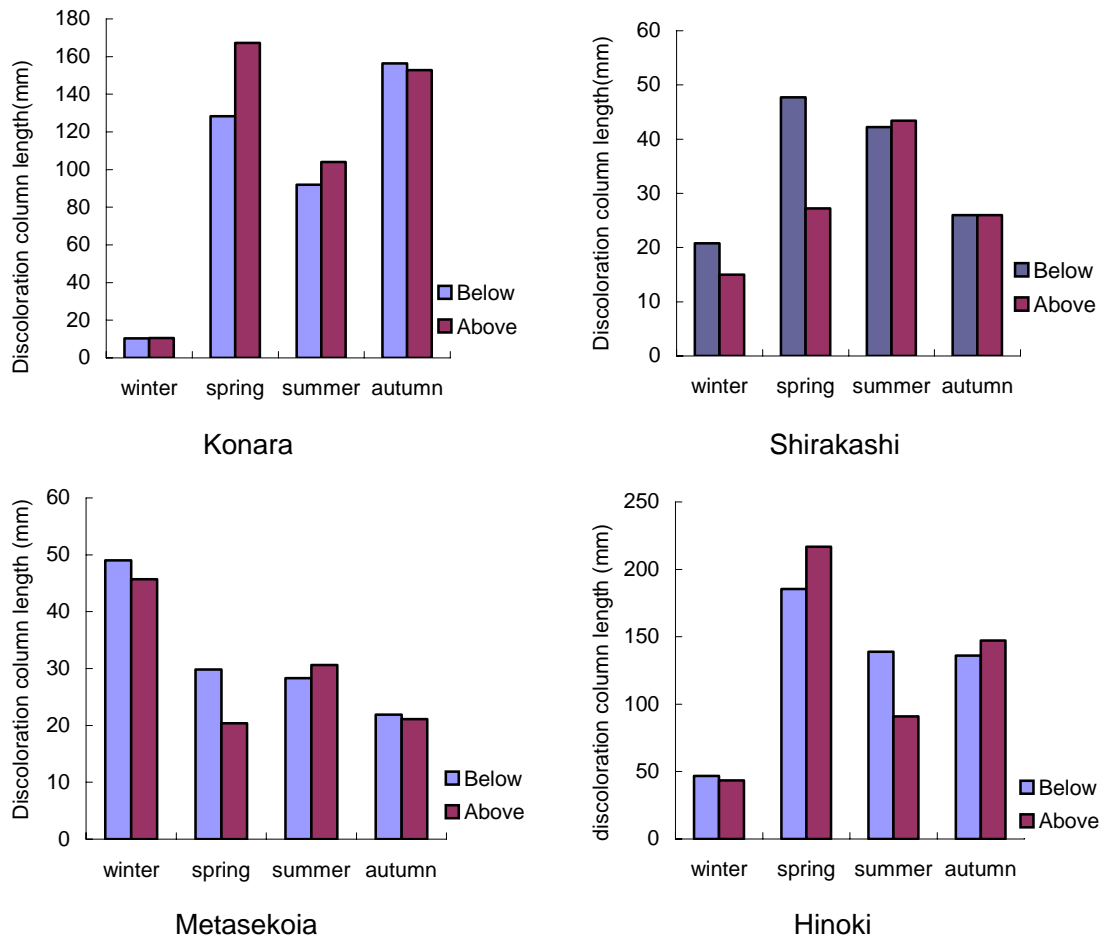


Fig.26. Discoloration column extension above and below the wound 6 months after wounding

4.9 Wound closure index six months after wounding.

In Konara, wound closure index was maximum for spring wounds as the wounds were completely closed while its minimum for the autumn wounds. Winter and summer wound closure indexes were high where the wounds were nearly close with winter wound closure index was slightly higher. Metasekoia, wound closure index showed the same trends. In shirakashi, wound closure index was slightly higher in spring wounds compared to

summer wounds while its was minimum for the autumn wounds. While in Hinoki, wound closure index were slightly higher in winter wounds compared to spring and summer wounds respectively.

Generally wound closure was maximum for spring wounds, with konara and Metasequoia, wounds closing faster than Shirakashi, and Hinoki. For winter and summer wounds, konara and Metasequoia were again closing faster than Shirakashi and Hinoki. Wound closure index were minimum for autumn wounds in all tree species examined. (Fig 27).

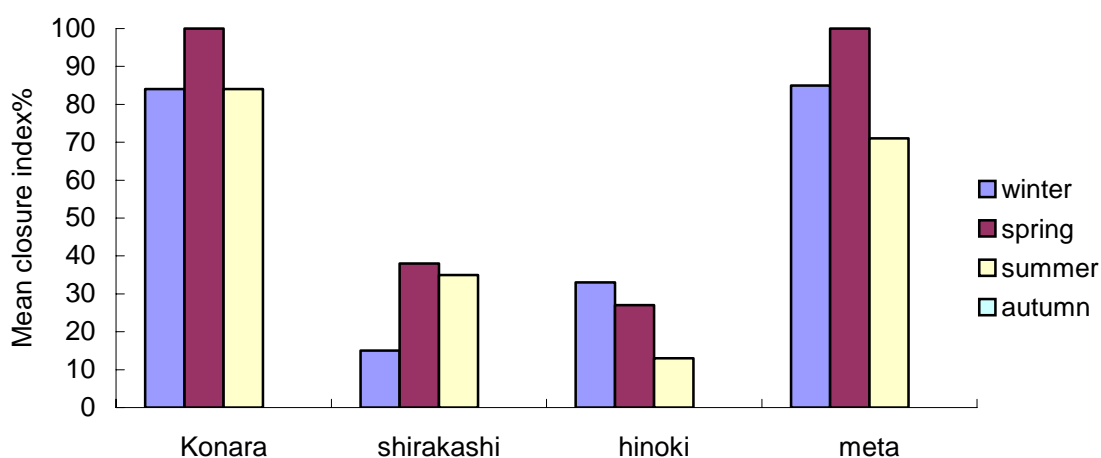


Fig.27 Mean closure index after 6 months

4.10 Anatomy and Histochemistry of the wood discoloration.

Anatomical response of the xylary cells to wounding and subsequent invasion by fungi are profound changes that represent a dynamic sequence of events facilitating tree defense. These morphological transformations are only a part of multifaceted alteration that occurs within the xylem of living trees. An anatomical response in xylem is clearly function for protection. It's debatable whether this defense system is produced as an active mechanism for resistance (Shigo and Marx 1977, Shigo 1984), a nonspecific response designed to maintain sapwood function (Boddy and Rayner 1983, Rayner 1986), or combination of the two. The reaction of the barrier zone and reaction zone in the histochemical staining tests differed from those of the non discolored sap wood, tissues of the discolored wood respond positively to lignin, phenol and suberin.

There were many alterations in the reaction zone and barrier zone in all tree species examined. Reaction zone macroscopically appeared as dark band adjacent to sound wood, which extended only a few mm wide, while microscopically it's appeared as a region where

the tissues and cell were occluded with pigmented substances. The barrier zone appeared microscopically as an area of cell with abnormal morphology that separate the wood made after wounding from those made before wounding.

In Shirakashi, investigations of the anatomical characteristics of xylem formed after wounding have shown that the callus tissues and barrier zone that separate the wood formed before and that formed after wounding to consist of abnormal cells, with irregular shape and they have more number of axial parenchyma cells, broader axial parenchyma cells and fewer vessels compare to normal sapwood.

Starch granules were observed at different concentration between the normal sapwood, reaction zone and barrier zone, starch granules were much more in the barrier zone compared to normal sapwood and reaction zone respectively.

The tissues of the callus and barrier zone responded positively to lignin by stained intense red compare to normal sapwood. The spatial localization of lignin was clearer when using Phloroglucinol HCL stain. Lignin deposits were found in the axial parenchyma and to little extend in the ray parenchyma (Fig 28).

Suberin deposits were detected in the callus and barrier zone tissues that were formed after wounding specially in the area closer to the wounded tissues. Axial parenchyma and ray parenchyma stained positive for suberin (Fig 29).

Reaction zone in Shirakashi, was seen as dark brown band at the interface between healthy and discolored wood. In this reaction zone, deposition of lignin, suberin and phenols were observed in a form of discontinuous batches. Ray parenchyma, apotracheal parenchyma and paratracheal parenchyma that were associated with the vessels elements were obstructed by either a complete or partially occlusion of cells lumina by these deposits. Fiber elements were also stained positively for the accessory substances. Extensive response by tyloses were found blocking lumina of vessels through out the reaction zone (Fig 30). Starch granules were predominantly found in ray parenchyma, apotracheal parenchyma and paratracheal parenchyma cells of the normal sapwood, their concentration were greatly reduced in these cells of the reaction zone. There were strong lignification in the reaction zone elements, in which the paratracheal parenchyma around the vessels, apotracheal parenchyma cells, vessels lining and fiber cells, all stained positive. In addition, cell wall, intercellular spaces and cell lumina of different elements in the reaction zone showed strong lignification. Tyloses that were abundant in the reaction zone and were also showed strong lignification (Fig 31). In sections stained for suberin deposits, Nile blue stain produced clearer and positive response for suberin when compared to Sudan black, all elements in the reaction zone showed suberization, ray and axial parenchyma cells were strongly occluded with suberin, in which, their cell wall and lumina strongly stained for suberin. Vessels lining and vessels tyloses also showed suberization (Fig 32). Phenolic

substances deposition were mainly observed in the ray parenchyma in which, the cell lumina were occluded with these substances. There were little phenolic substances deposition in the lumina of paratracheal parenchyma and apotracheal parenchyma cells. However, no phenolic substances were observed in the vessels lining and tyloses in the reaction zone (Fig 33).



Fig. 28 Presence of lignin in callus and barrier zone of Shirakashi (cross section). PG-HCL, six months after summer wounding.

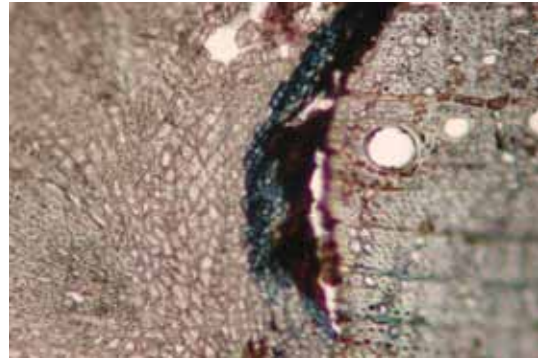


Fig. 29 Suberization of the callus tissues in reaction zone of Shirakashi (cross section). Sudan black, six months after summer wounding.

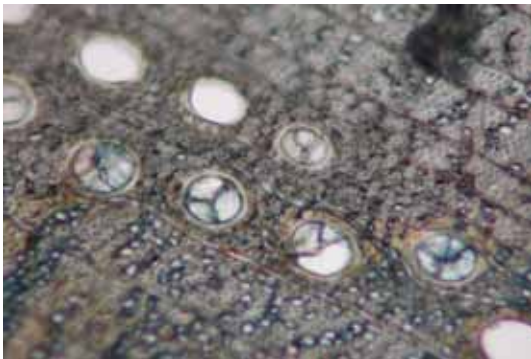


Fig.30 Suberization of tyloses in reaction Zone in shirakashi (cross section) Sudan black, six months after spring wounding.

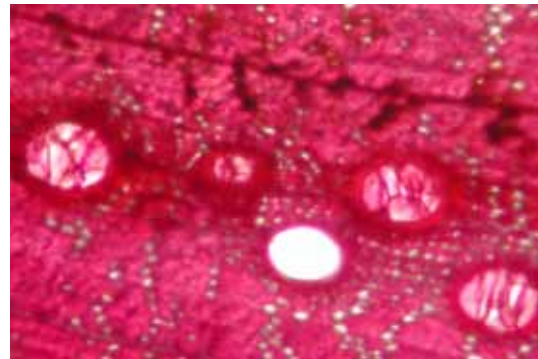


Fig.31 Strong lignification of xylary cells and tyloses in reaction zone of Shirakash (cross section). PG-HCL, six months after spring wounding.

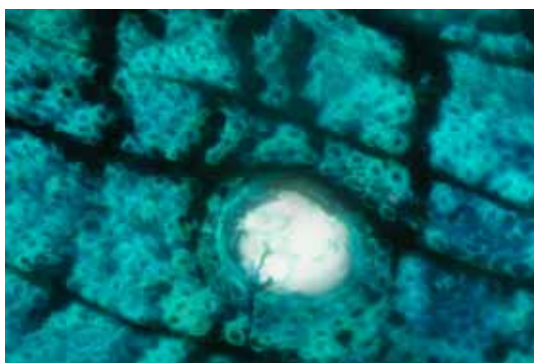


Fig.32 Deposition of suberin on the ray and axial parenchyma in reaction zone Konara (cross section). Nile blue, six months after spring wounding.

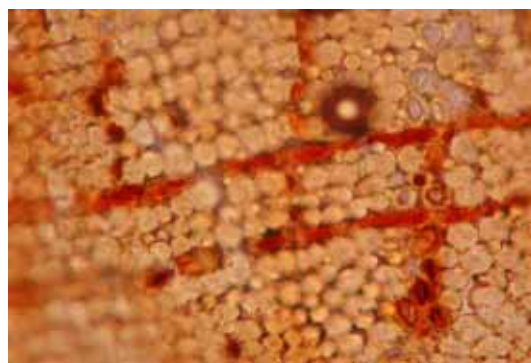


Fig.33 Deposition of phenols on the ray and axial parenchyma in reaction zone. Konara (cross section). Nitrosophenol, six months after spring wounding.

The barrier zone in Konara was clearer and broader than that of Shirakashi. Microscopically, different cell elements had irregular shapes, ray parenchyma cells increased in number and size, there is also increase in the number of axial parenchyma cell and the vessels were smaller and fewer compared to normal wood. Starch granules were less observed in the discolored wood compared to the barrier zone as more starch was used for the production of accessory substances. Within the sections treated with Nile blue and Sudan black, Nile blue treated sections resulted into more clearer suberin spatial localization. Ray parenchyma and axial parenchyma cells in the barrier zone were occluded with suberin (Fig 34). Lignification was clear in the apotracheal parenchyma and paratracheal parenchyma cells, in which cell wall and lumina stained positive for lignin. Ray parenchyma cell also stained positive for lignin (Fig 35). Phenolic substances occluded the apotracheal parenchyma and paratracheal parenchyma cell's lumina as well as the cell walls. Vessels lining stained positive for the phenolic substances (Fig 36).

The reaction zone in Konara, appeared as brown regions, typically 1 to 2 millimeters wide at the interface between healthy sapwood and the discolored wood and was not easy to distinguished from the discolored wood. Cells occlusion with accessory deposits were less observed in the reaction zone of Konara, reaction by tyloses was less observed and it's mainly in the larger vessels, moreover, lignification and suberization of the tyloses were less when compared with Shirakashi.

Within sections treated with Nile blue and Sudan black, ray parenchyma, apotracheal parenchyma and small vessels stained positive for suberin (Fig 37). Lignification was also observed in the same elements (Fig 38). Within sections stained for the localization of phenolic deposits, ray parenchyma, axial parenchyma and to a little extend tyloses in the smaller vessels, showed positive response (Fig 39).

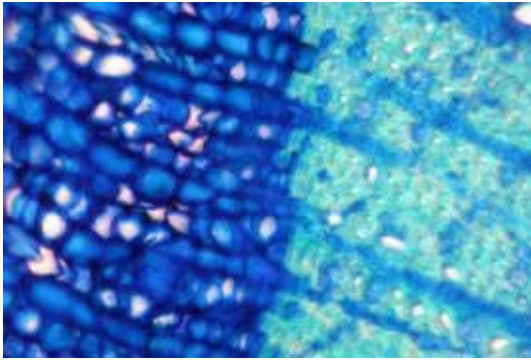


Fig.34 suberization of cell wall and lumina of traumatic parenchyma cell in Konara (cross section). Nile blue, six months autumn wounding.

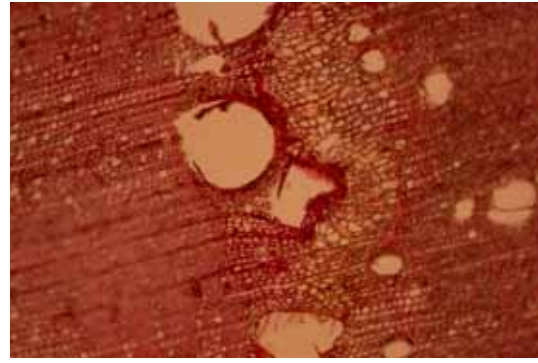


Fig.35 lignification of ray parenchyma, axial parenchyma and vessels cell walls (cross section). Schiff's, six months winter wounding.

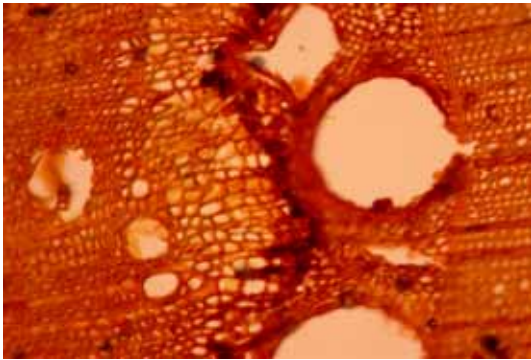


Fig.36 Phenol deposition and occlusion of traumatic Parenchyma cells and vessel in barrier zone of Konara (cross section) Fast red, six months spring wounding.

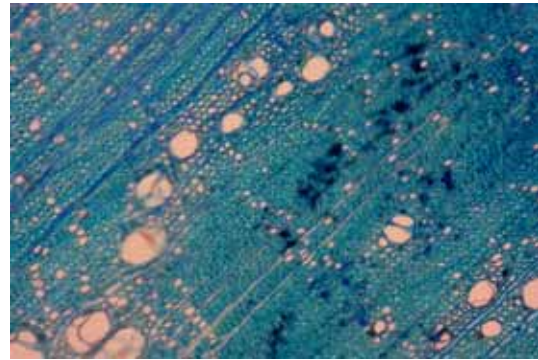


Fig.37 Presence of suberin deposition and suberized tyloses in reaction zone of Konara (cross section). Nile blue, six months summer wounding.



Fig.38 lignification of ray parenchyma and axial parenchyma of Konara (cross section). Schiff's. Six months after autumn wounding.

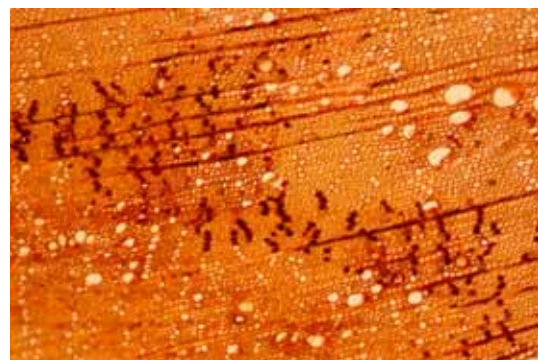


Fig.39 Phenols deposits in ray parenchyma and axial elements of Konara (cross section). Fast red, six months after spring wounding.

In Hinoki, the barrier zone was also produced as a result of mechanical wounding, its most prevalent in the area separating wood formed before from those formed after wounding. The cells formed after wounding contained distorted trachieds, abundant axial parenchyma cells that accumulate accessory substances and it had expanded and larger ray parenchyma cells.

Resin canals were not observed in the normal xylem of Hinoki, neither normal nor traumatic resin canals. Starch granules were clearly abundant in the ray parenchyma of the barrier zone of Hinoki, compared to the normal wood and reaction zone. The cells walls of all elements in the barrier zone responded uniformly positive to lignin and to some extent the cell lumina of the trachieds were filled with the lignin (Fig 40). Within sections stained for suberin, there was strong suberization of the ray parenchyma cells in which cell wall as well as cells lumina stained positively dark blue. Suberin deposits occluded the trachied's lumina, suberin deposits appeared as fine grained oil droplets (Fig 41). All cells types in the barrier zone of Hinoki, *Chamaecyparis obtusa* were strongly occluded with phenolic deposits, cell walls and lumina of parenchyma and trachied cells were occluded with phenols (Fig 42).

The anatomical features of sound wood of Hinoki, and the alterations within the reaction zones were mainly characterized by occlusion of cells elements with different types of deposits as the result of wounding and subsequent fungal invasion. Reaction zone in Hinoki appeared macroscopically as dark 2-3 millimeters wide band adjacent to normal sapwood. In these zones occlusion of ray parenchyma was clearly observed in the cell wall as well as the cells Lumina. Some of the trachied elements of the reaction zones were also occluded with the deposits. The ray parenchyma's lumina, cell wall and intercellular spaces are heavily occluded with phenols (Fig 43). Within sections treated with Nile blue and Sudan black for detection of suberin, a positive response was found mainly in the ray parenchyma's lumina and cell wall of all elements in the reaction zone, some of the trachied's lumina in the reaction zone (Fig 44). The same elements stained also positively for lignin (Fig 45). Starch granules completely or partially disappeared from the ray parenchyma in the reaction zones.

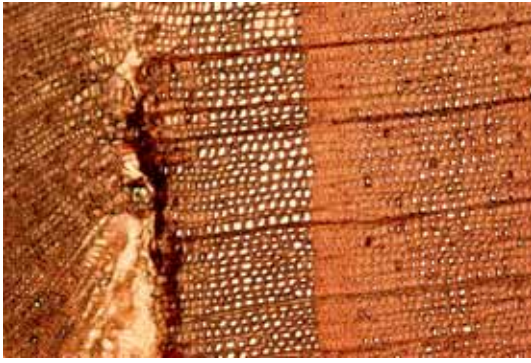


Fig.40 Lignification of traumatic paren chyma elements and trachieds in callus and barrier zone. Hinoki (cross section) Maule, six months after summer wounding.

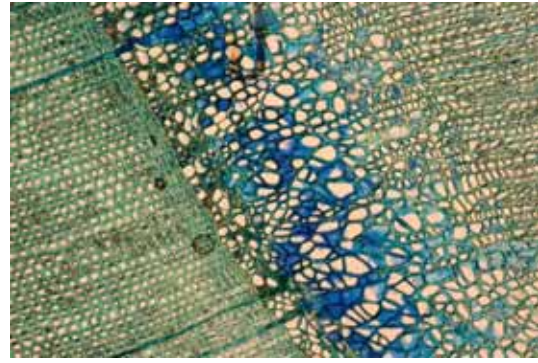


Fig.41 Suberin deposition in the traumatic parenchyma and trachieds in barrier zone Hinoki (cross section). Nile blue, six months after summer wounding.

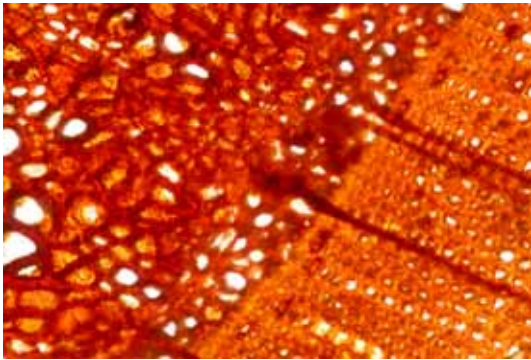


Fig.42 phenol deposition in cell wall and lumina of the traumatic elements in barrier zone. Hinoki (cross section) Fast red, six months spring wounding.

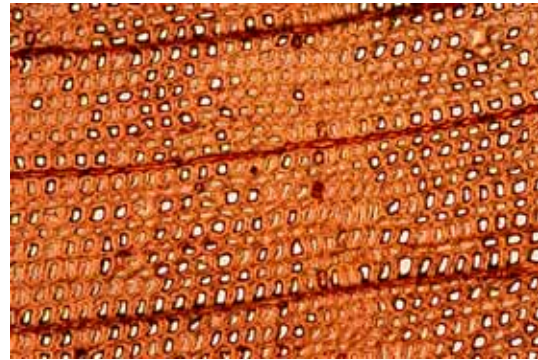


Fig.43 Lignin deposition in ray parenchyma and trachieds in reaction zone. Hinoki (cross section). Maule, six months summer wounding.

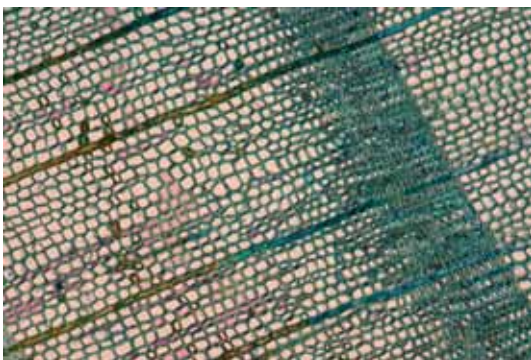


Fig.44 suberin deposition in ray parenchyma in reaction zone of Hinoki (cross section). Nile blue, six months after spring wounding.

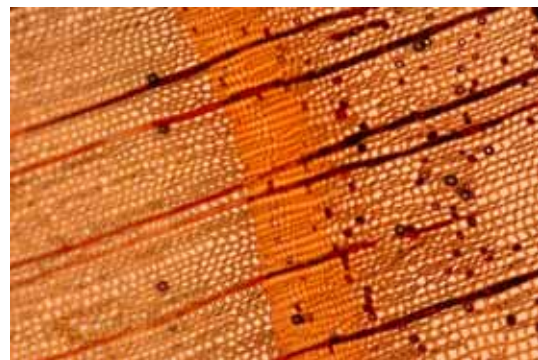


Fig.45 Phenols deposition in ray parenchyma and occlusion of trachieds in barrier zone. Hinoki (cross section). Diazo blue, six months after spring wounding.

In *Metasekoia*, investigation of the anatomical characteristics of the xylem formed after wounding have shown that the barrier zone consist of a row of tangential traumatic resin canals, which was not observed in the normal sapwood of *Metasekoia*. Abnormally distorted trachieds and more ray parenchyma with distorted and larger parenchyma cells were also observed in the barrier zones of *Metasekoia*. Lignification of the barrier zone as the result of wounding was very significant on the cell walls of the tracheids, intercellular spaces, ray parenchyma and resin canals and resin canal linings. In a few cases tracheal occlusion with lignin was also observed (Fig 46).

Suberization of the callus and barrier zone tissues of *Metasekoia*, were mainly concentrated in the ray parenchyma, where the cell walls, lumina and intercellular spaces showed suberization. Traumatic resin canals epithelial lining and ray parenchyma were suberized . (Fig 47). Within sections stained for phenolic deposits, resin canals and their epithelial lining cells, strongly stained for phenols. Ray parenchyma cells also showed strong phenolic deposits in their cell wall, cell lumina and intercellular spaces. Some of trachieds were occluded with phenolic deposits (Fig 48).

The reaction zones in *Metasekoia* appeared as a reddish pink band that encircle the discolored wood separate it from the normal sap wood, its extended 2-3 millimeters wide. The basic alterations as the result of wounding was the occlusion of the ray parenchyma with deposits that filled intercellular spaces, cell walls and cell lumina. Occasional occlusions of trachieds were also observed in the reaction zone (Figs 49-51).

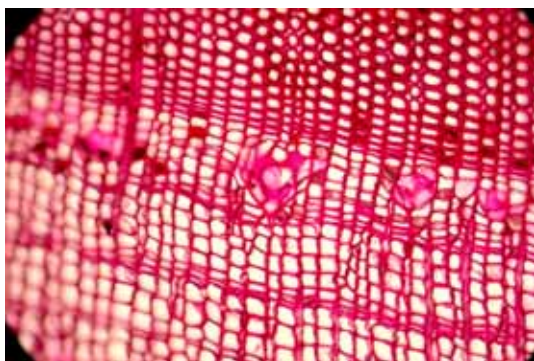


Fig.46 .Lignification of traumatic resin canal lining and occlusion of barrier zone trachieds of *Metasekoia* (cross section). Schiff's, six months after spring wounding.

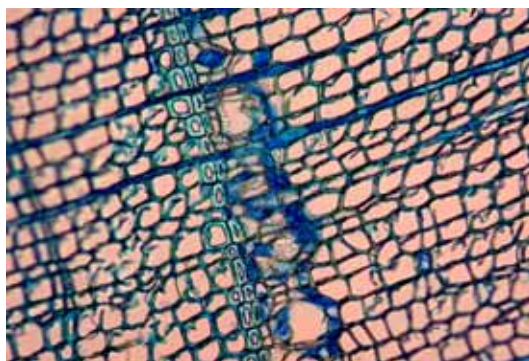


Fig.47 Suberin deposition in the traumatic resin canals lining and ray parenchyma in barrier zone of *Metasekoia* (cross section). Nile blue, six months after summer wounding.

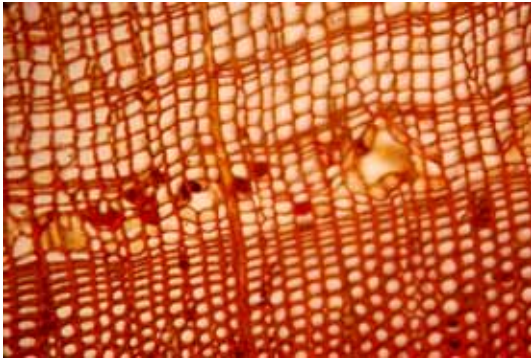


Fig. 48 Phenol depositions in traumatic trachieds and parenchyma cell in barrier zone of Metasekoia (cross section). Fast red, six months after spring wounding.

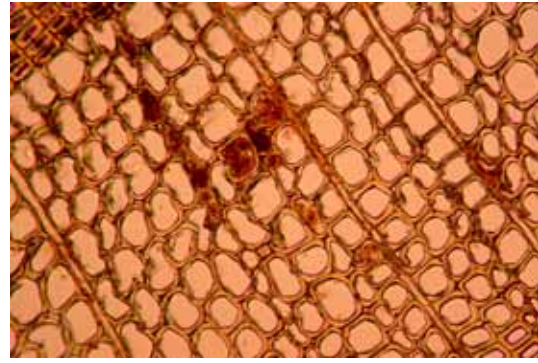


Fig. 49 Trachieds occlusion and ray parenchyma lignification in reaction zone of Metasekoia (cross section). Maule, six months after summer wounding.

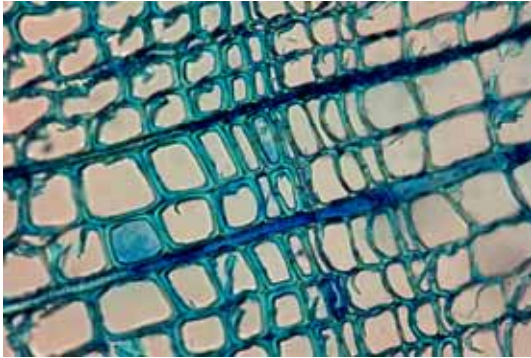


Fig.50 Suberization of ray parenchyma in reaction zone of Metasekoia (cross section). Nile blue, six months after winter wounding.

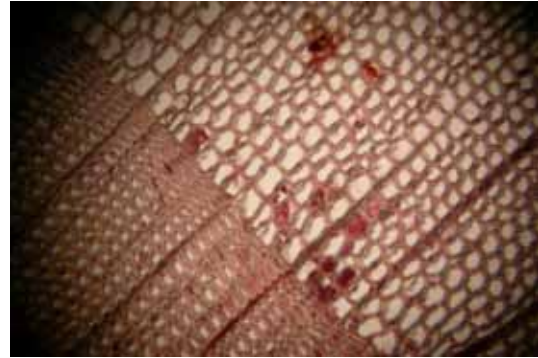


Fig.51 Occlusion of trachieds by phenols and deposition of phenols in ray parenchyma in reaction zone of Metasekoia (cross section). Diazo blue, six months after spring wounding.

5. Discussion.

5.1 Description of the discoloration columns.

The first visible response to mechanical wounding was the discoloration of the xylem that surrounded the wounds. At first the color turn into a paler color than the normal sapwood then, the color darken with time. as starch is removed and cell lumina fill with phenols, suberin, lignin and pigmented substances which is a results of degeneration of nuclei and cytoplasm that leads to cell death (Blanchette and Sharon 1975). As long as the wounds remain open, air entry and drying of the tissues results into more accessory and pigmented substances. This lead to darker color of the discoloration.

Air embolism interrupt the water flow through the vessels and tracheids. Fungi can colonize the wood through the wound holes thus; the trees compartmentalized this area and separate it to from sound wood (pearce 1991).

The intensity of compartmentalization and barrier zone formation in Shirakashi, Konara, Metasequoia and Hinoki varied greatly. All four species contained a large amount of accessory substances in the reaction zone and area correspond to wall 1 according to CODIT model. This area at the interface between normal and discolored wood is the place where these accessory substances formed. The limited area of discoloration and the height quantity of accessory substances in the reaction zone and wall 1 (CODIT) suggest that Shirakashi have the stronger wound response of the trees examined. Metasequoia also formed reaction zone after being wounded, but its redish color and not much distinct as Shirakashi, However it show strong wound response explained by the quantity of accessory substances. Konara showed weak wounds response.

5.2 Pattern of the wood discoloration columns.

Above and below the wounds, the discoloration columns extended in a particular pattern which was dictated by the wood anatomy according to CODIT and reaction zones models which described wood discoloration and its restriction in trees. The vertical extension of the discoloration is restricted when the vessels and tracheids are plugged with the accessory substances, but the restriction in this direction is the weakest compared to lateral and tangential restrictions, thus the discolored columns shape is often elongate and narrow (Shigo and Marx 1977).

In this study, the discoloration shape of Shirakashi and Konara, could be directly explained by the above models the pattern and shape of the discoloration columns. In Metasequoia and Hinoki, the discoloration columns was also elongate and narrow, but the longest extension is the area near to heartwood, this indicated that in conifers, inner sapwood just

before heartwood formation are the most sensitive and susceptible to discoloration. In these areas cells are dying or deprived from living cells so it lacks the active defense mechanism.

5.3 Effects of wounds position

Location of the initial wounds on the stem affects the frequency of fungal infection and hence the amount of discoloration and decay. Wounds near ground and below diameter at breast height are more subject to infection (Isomaki and Kallio 1974). In this study, there was no significant differences between the wounds positions. This can be attributed mainly to the shorter distance between the wounds, which was only 25 cm so the possibility of differences in the fungal infection or microclimate is very small.

5.4 Wound closure.

Wounds close as the result of callus formation by vascular cambium. Activity of the cambium around wounds exceeds that of non-wounded parts of the tree. This activity produces more wood than elsewhere at the same level on the stem this allows wounds to close as the tree expand in growth.

In this study, wound closure was maximum during the active growing seasons and minimum in the dormant seasons. Once the wounds are closed and the wound is no longer aerated, the activity of wood destroying fungi is greatly suppressed and likely terminated. The rate of wound closure differs between species, Konara and Metasekoia had the highest closure rates followed by Hinoki and Shirakashi. This result agreed in principle a previous research (Martin and Sydnor 1987) that showed the amount and rate of callus production and hence the closure of wounds following wounding varies according to tree species. The results also showed that the timing of wounding could delay closure. Wounding in spring results into faster wound closure but also results into longer discoloration. Wounding in autumn results into zero closure rates but also results into shorter discoloration. In this particular study there is no indication that wound closure is directly correlated with lesser amount of wood discoloration, this results agree with Shain and Miller (1988).

The rate of wounds closure depends primarily on the growth rate of the stem. The influences of growth respectively supply of nutrients are also shown by Herms and Mattson (1992). Trees with broad tree rings close faster than slow growing trees (Dujesiefken et al 1989). In this study Konara and Metasekoia grew faster than Hinoki and Shirakashi.

5.5 Seasonal effects on the wood discoloration columns.

In general, wounds made during the growing seasons of spring and summer had longer discoloration columns when compared to those formed after wounding in dormant seasons of winter and autumn. This results agreed to many reports on the effects of season in which injuries occur and resulting discoloration and decay, it refer to sap season i.e., spring through early summer when sap in trees is actively flowing (Zeglen 1997; White and Kile 1993) found that in wounded *Eucalyptus regnans*, spring and summer wounds had significantly greater defect volume than autumn wounds during the first 6 months. However, this seasonal effect was not apparent after 12 and 24 months. However, some results in this study could not be explained by this regime. Winter wounding resulted in the longest discoloration after 6 months in *Metasekoia*, and summer wounding discoloration showed shorter discoloration than autumn, 2 months after wounding. 6 months after wounding, autumn discoloration was greater than summer discoloration in *Hinoki*.

On the other hand, some researchers (Leben 1985; Shain and Miller 1988; Mireku and Wilkes 1989) reported that the most extensive discoloration occurs with autumn or winter wounds, intermediate discoloration with summer wounds, and the least with spring wounds.

A number of factors may contribute to seasonal differences in discoloration, decay and defense response. Differences in rainfall and temperature may influence the hydraulic status of xylem rendering it more or less compromised by wounding. Distribution of resources may change with varied environmental factors and climate (Wainhouse et al. 1998). Factors affecting fungal infection could include seasonal differences in sporulation and dissemination for various fungi (Gadgil and Bawden 1981) as well as factors affecting subsequent growth rate.

The conflicting reports on the seasonal effects on wound discoloration and subsequent decay may be partly due to the different in the wounds type between small experimental wound to large pruning wounds. The differences of the length of wound exposure time through seasons may have also influence on the resulting discoloration and decay.

5.6 Anatomy and histochemistry of the discoloration columns.

In this study, when the trees were wounded, the cells generated after wounding in the area, that separate wood formed after wounding from the wood formed before wounding, consists of abnormal elements. This is a universal phenomenon and has been shown in many reports (Blanchette and Biggs 1992).

Formation of traumatic resin canals in the wood laid down immediately after wounding is a common response to wounding and infection and have been reported from barrier zones of many *Pinaceae*, but it's not a universal phenomena. Several authors have observed the presence of traumatic resin canals or cysts in extant *Metasequoia* (Liang et al. 1948; Gerry 1950; Greguss 1955; Schönfeld 1955). Basinger (1981) and Schönfeld (1955) reported the presence of traumatic resin canals in fossil wood identified as *Metasequoia*. However it seems to be reported only in *Metasequoia* type fossil wood. In the study a row of tangential traumatic resin canals was shown in the barrier zone of *Metasequoia*, these traumatic resin canals shown deposition of the accessory substances. No tylosoid (occlusion of resin canal by epithelial cells) were observed in the extant samples. This is the first report on the formation of traumatic resin canals in the xylem of *Metasequoia* from non-fossilized samples.

No traumatic resin canals were reported from the xylem tissues of Hinoki in this study, which agreed with previous reports (eg Yamanaka 1984).

Tyloses, which develop from xylem parenchyma cells and project through pits to occlude the lumen of vessels in *Konara*, and *Shirakashi* were suberized and lignified. Tyloses might block the axial spread of fungi along the vessel as well as reducing aeration and loss of water (Rioux et al., 1995). High tyloses formation and strong suberization and lignification may explain the strong response of *Shirakashi*, compare to *Konara*, which in addition characterized by larger vessels.

In this study a relationship was observed between the discoloration column length and the intensity of the anatomical and histochemical reaction of the xylem tissues, the concentration of the accessory substances in the reaction – and barrier zones is greater during the growing seasons compared to the dormant seasons. During the active growing season, trees are able to respond strongly to wounding by producing the accessory substances as part of their active defense mechanism, which may lead to more discoloration in the short term.

6. Conclusion

In forest management, wounding of trees cannot be completely avoided. Although many discrepancies are found in the literature and our understanding of the interaction between trees and seasonal factors does not yet allow us to prescribe the optimal strategy and time to prevent and reduce discoloration and decay on trees.

A better understanding of the nature and dynamics of defensive responses in different species and temporal analysis of the tree's responses through a unified methodology and longer duration, may identify favorable times for tree management operation, or unfavorable times that are more likely to allow the ingress of infection. Seasonal differences in susceptibility to fungal attack may contribute, in part at least, to alteration in the efficacy of defense responses in different seasons. Understanding of defense responses in trees may facilitate the development and accelerated methods for screening trees for better compartmentalization.

In this study, the experiments and observations are based on apparently healthy trees only. Particularly extensive discolorations can be caused by drilling into already discolored or decayed area inside the trunk. This breaks through the marginal zone of the damage in the stem and renews the danger of fungi reaching the healthy wood, causing more discoloration (Shigo 1986).

7. References.

- Aho, P.E, Fiddler G. and Filip GE 1983.** How to reduce injury to residual trees during stand management activities. U.S. Department of Agriculture Forest Service, Portland, Oreg. General Technical Report PNW-156. 17pp
- Aide, T.M. 1987.** Limbfalls: a major cause of sapling mortality for tropical forest plants. *Biotropica*, 284-285.
- Aist JR, 1976.** Papillae and related wound plugs of plant cells. *Annual Review of Phytopathology* 14; 145-163.
- Aist JR. 1983.** Structural responses as resistance mechanisms. In: Bailey JA Deverall BJ, eds. *The Dynamics of Host Defence*. NewYork: Academic Press, 33-70.
- Bauch J, Shigo AL, Starck M 1980.** Wound effects in the xylem of Acer and Betula species. In: Blanchette RA, Biggs AR, eds. *Defense Mechanisms of woody plants against Fungi*. Berlin: Springer-Verlag.
- Armstrong JE, Shigo AL, Funk DT, McGinnes EA. 1981.** Amicroscopis and microscopic study of compartmentalization and wound closure after mechanical wounding of black walnut tree. *Wood and fiber science*. Vol. 13, No 4, pp. 275-291.
- Basham JT 1978.** Early sugar maple stem discoloration and microorganism invasion in simulated wounds of felling and fire scars. *Phytopathology* 68:1693-1699.
- Biggs AR. 1987.** Occurrence and location of suberin in wound reaction zones in xylem of 17 tree species. *Phytopathology* 77:718-725.
- Biggs AR. 1992.** Anatomical and physiological responses of bark tissues to mechanical injury. In: Blanchette RA, Biggs AR, eds. *Defence Mechanisms of Woody Plants Against Fungi*. Berlin: Springer-Verlag, 13-40.
- Blanchette RA. 1982.** Decay and canker formation by *Phellinus pini* in white and balsam fir. *Canadian Journal of Forest Research* 12: 538-544.
- Blanchette RA. 1992.** Anatomical responses of xylem to injury and invasion by fungi. In:
- Blanchette RA, Biggs AR ed. 1992.** *Defense Mechanisms of Woody Plants Against Fungi*. Berlin: Springer-Verlag.
- Blanchette RA, Sharon EM 1975.** Agrobacterium tumefaciens, a promoter of wound healing in Betula alleghaniensis. *Can J For Res* 5:722-730.
- Boddy L. 1992.** Microenvironmental aspects of xylem defenses to wood decay fungi. In: Blanchette RA, Biggs AR. eds. *Defense Mechanisms of Woody Plants Against Fungi*. Berlin: Springer-Verlag, 96-132.
- Boddy L, Rayner ADM. 1983.** Origins of decay in living deciduous trees: the role of moisture content and re-appraisal of the expanded concept of tree decay. *New Phytologist* 94:623-641.

- Dujesiefken D, Kronenschnitt D. 1991** in der Baumpflege, *Neue Landschaft*, pp. 27–31: In The Hamburg Tree Pruning System – A framework for pruning of individual trees. *Urban Forestry and Urban Greening, Volume 1, Issue 2, 2002, Pages 75-82.*
- Dujesiefken D, Ebenriffer S, Liese W 1989.** Wood reaction in the wound of birch, beech and basswood. *Holz als Roh-und- Werkstoff* 47:495-500.
- Dujesiefken D, Rhasea A, Eckstein D 1999.** Tree wound reaction of differently treated boreholes. *Journal of Arboriculture* 25:113-123.
- Dujesiefken D, Liese W, Shortle W, Minocha R 2005.** Response of beech and oaks to wounds made at different time of year. *European Journal of Forest Research*. Online springer-Verlag.
- Esau K. 1977.** *Anatomy of seed plants*. 2nd edn. New York: Wiley.
- Farjon, A. 2005.** *Monograph of Cupressaceae and Sciadopitys*. Royal Botanic Gardens, Kew. [ISBN 1-84246-068-4](https://doi.org/10.1017/9781842460684).
- Flodin K. 1979.** Effects of monoterpenes on *Fomes annosus* (Fr.) Cooke and its phenol oxidase activity, *European Journal of Forest Pathology* 9: 1-6.
- Forrest GI. 1982.** Preliminary work on the relation between resistance to *Fomes annosus* and the monoterpene composition of Sitka spruce resin. In: Heybroek HM, Stephan BR, von Weissenberg K, eds. *Resistance to Diseases and Pests in Forest Trees*. Wageningen: PUDOC, 194-197.
- Franich RA, Gadgil PD, Shain L. 1983.** Fungistatic effects of *Pinus radiata* needle epicuticular fatty and resin acids on *Dothistroma pini*. *Physiological Plant Pathology* 23: 183-195.
- Franich RA, Gaskin RE, Wells LG, Zabkiewicz JA. 1982.** Effect of *Pinus radiata* needle monoterpenes on spore germination and mycelial growth of *Dothistroma pini* *in vitro* in relation to mature tree resistance. *Physiological Plant Pathology* 21: 55-63.
- Gadgil PD, Badwen AD. 1981.** Infection of wounds in *Eucalyptus delegatensis*. *NZ J. For. Sci* 11:262-270.
- Geiger J-P, Rio B, Nicole M, Nandris D. 1986.** Biodegradation of *Hevea brasiliensis* wood by *Rigidoporus lignosus* and *Phellinus noxius*. *European Journal of Forest Pathology* 16:147-159,
- Grime GW, Pearce RB 1995.** External beam analysis of living sycamore xylem infected by pathogenic fungi. *Nuclear Instruments and Methods in Physics Research B*. 104: 299-305.
- Hermes DA, Mattson WJ. 1992.** The dilemma of plants to grow or defend. *The Quarterly Review of Biology* 67:283-335.
- Hillis, W.E., 1987.** Heartwood and Tree Exudates. Springer, Berlin, 268 pp. In Pearce RB. Antimicrobial defences in the wood of living trees. *New phytologist* 132:203-233.
- Homans AL, Fuchs A. 1970.** Direct bioautography on thin-layer chromatograms as a method for detecting fungitoxic substances. *Journal of Chromatography* 51: 327-329.
- Isomälik A, Kallio T. 1974.** Consequences of injury caused by timber harvesting machines and growth and decay of spruce. *Acta Forestalia Fennica* 136.25 pp.

- Jensen W A. 1962.** Botanical Histochemistry. W.H. Freeman and Company.USA.
- Jones L, Ennos AR, Turner SR . 2001.** Cloning and characterization of irregular xylem4 (irx4): a severely lignin-deficient mutant of Arabidopsis. *Plant Journal* 26: 205– 216.
- Kolattukudy PE. 1984.** Biochemistry and function of cutin and suberin. *Canadian Journal of Botany* 62: 2918-2933.
- Koran Z, Cote WA. 1965.** The ultrastructure of tyloses. In: Cote WA, ed. *The Cellular Ultrastructure of Woody Plants*. New York-. Syracuse University Press, 319-333.
- Kubitzki K. 1987.** Phenylpropanoid metabolism in relation to land plant origin and diversification. *Journal of Plant Physiology* 131: 17- 24.
- Lange BM, Lapierre C, Sandermann H Jr . 1995.** Elicitor-Induced Spruce Stress Lignin (Structural Similarity to Early Developmental Lignins). *Plant Physiology* 108: 1277– 1287
- Leben C. 1985** Wound occlusion and discoloration column in red maple. *New phytol* 99:485-490.
- Mattheck, C, and Breloer, H. 1994:** The body language of Trees. A Handbook for failure analysis. Research for Amenity trees No.4, Department of Environment, HMSO, London, UK.240 pp.
- Mireku E and Wilkes J. 1989:** Seasonal variation in the ability of the sapwood of *Eucalyptus maculata* to compartmentalize discolouration and decay. *For Ecol. Manage.* 28, 131–140.
- Mohammed, C.; Barry, K.; Battaglia, M.; Beadle, C.; Eyles, A.; Mollon, A.; Pinkard, E., 2000.** Pruning-associated stem defects in plantation *E. nitens* and *E. globulus* grown for sawlog and veneer in Tasmania, Australia. In: Proceedings of the Future of Eucalypts for Wood Products, 19–24 March, Launceston, Australia. Launceston, Australia: International Union of Forestry Research Organisations, pp. 357–364.
- Martin JM, Sydnor TD. 1987.** Differences in wound closure rate in 12 species. *Hort Science* 22: 442-444.
- Nicole MR, Geiger JP, Nandris D. 1992.** Defence of angiosperm roots against fungal invasion, in: Blanchette- RA, Biggs .AR, eds. *Defense Mechanisms of Woody Plants Against Fungi*. Berlin: Springer-Verlag, 181-206.
- Pearce RB. 1987.** Antimicrobial defences in secondary tissues of woody plants. In : Pegg GF, Ayres PG, eds. *Fungal Infection of Plants*. Cambridge; Cambridge University Press, 219-238.
- Pearce RB. 1989.** Cell wall alterations and antimicrobial defence in perennial plants. In: Lewis NG, Paice .MG, eds. *Plant Cell Wall Polymers: Biogenesis and Biodegradation*. American Chemical Society Symposium Series No 399 Washington DC: American Chemical Society, 346-360,
- Pearce RB. 1990.** Occurrence of decay-associated xylem suberization in a range of woody species. *European Journal of forest Pathology* 20: 275-28.
- Pearce RB. 1991.** Reaction zone relics and the dynamics of fungal spread in the xylem of woody angiosperms. *Physiological and Molecular Plant Pathology*. 39: 41-55.

- Pearce RB. 2000.** Decay development and its restriction in trees. *Journal of Arboriculture* 26: 1-11.
- Pearce RB, Holloway PJ. 1984.** Suberin in the sapwood of oak (*Quercus robur* L.): its composition from a compartmentalization barrier and its occurrence in tyloses in undecayed wood. *Physiological Plant Pathology* 24: 71-81,
- Pearce RB, Rutherford J. 1981.** A wound-associated suberized barrier to the spread of decay in the sapwood of oak (*Quercus robur* L.) *Physiological Plant Pathology* 19:359-369
- Pearce RB, Woodward S. 1986.** Compartmentalization and reaction zone barriers in *Acer saccharinum* L, *Physiological and Molecular Plant Pathology* 29; 197-216,
- Prior C. 1976.** Resistance by Corsican pine to attack by *Heterobasidion annosum*. *Annals of Botany* 40; 261-279,
- Rayner ADM. 1986.** Water and the origins of decay in trees. In : Ayres PG, Boddy L. eds. *Water, Fungi and Plants*. Cambridge; Cambridge University Press, 321-341,
- Rayner ADM, Boddy L. 1988.** *Fungal decomposition of wood*. Chichester: John Wiley.
- Reina JJ, Dominguez E, Heredia A . 2001.** Water sorption-desorption in conifer cuticles: the role of lignin. *Physiologia Plantarum* 112: 372– 378.
- Rioux D, Chamberland H, Simard M, Ouellette GB. 1995.** Suberized tyloses in trees: an ultrastructural and cytochemical study, *Planta* 196; 125-140,
- Schmitt U, Liese W. 1991.** Suberin in wound reaction parenchyma of birch xylem (*Betula pendula* Rotb): an electron microscope study, *Holzforschung* 45: 313-315.
- Schmitt U, Liese W. 1993.** Response of xylem parenchyma by suberization in some hardwoods after mechanical injury. *Trees* 8: 23-30.
- Shain L. 1967.** Resistance of sapwood in stems 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, Miller JB 1988.** Ethylene production by excised sapwood of clonal eastern cottonwood and compartmentalization and closure of seasonal wounds. *Phytopathology* 78: 1261-1265.
- Sharon EM. 1974.** An altered pattern of enzyme activity in tissues associated with wounds in *Acer saccharum*. *Physiological Plant Pathology* 4: 307-312.
- Shibata N, Harada H, Saiki H. 1981.** Development and structure of traumatic tylosis in *Quercus serrata* thub L Development of traumatic tylosis in various boring sesean. *Mokuzai Gakkaishi*. 27: 618-625.
- Shigo AL. 1979.** Tree decay-an expanded concept, *United States Department of. Agriculture Forest Service, Agriculture information Bulletin No 419*.

- Shigo AL. 1984.** Compartmentalization: a conceptual framework for understanding how trees grow and defend themselves. *Annual Review of Phytopathology* 22: 189-214.
- Shigo AL. 1986.** *A new tree biology*. Durham. New Hampshire: Shigo and Trees, Associates.
- Shigo AL, Marx HG. 1977.** Compartmentalization of decay in trees. *United States Department of Agriculture Forest Service. Agriculture Information Bulletin No 405*.
- Shigo AI, Sharon EM 1970.** Discoloration and decay in hardwoods following inoculation with hymenomycetes. *Phytopathology* 58:1493-1498.
- Shigo AL, Shortle WC, Garrett PW. 1977.** Genetic control suggested in compartmentalization of discolored wood associated with tree wounds. *Forest Science* 23: 179-182.
- Shortle WC. 1979.** Mechanisms of compartmentalization of decay in living trees. *Phytopathology* 69: 1147-1151.
- Shortle WC, Cowling EB. 1978.** Interaction of live sapwood and fungi commonly found in discolored and decayed wood. *Phytopathology* 68: 617-62.1,
- Smith K T, Shortle WC, Dudzik KR.** USDA Forest Service Northeastern Research Station Publication number: NA-TP-02-01.
- Sucoff E, Ratsch H, Hook DD. 1967.** Early development of wound initiated discoloration in *Populus tremuloides* Michx. *Can J Bot* 45:649-656.
- Vance CP, Kirk TK, Sherwood RT. 1980.** Lignification as a Mechanism of disease resistance. *Annual Review of Phytopathology* 18: 259-288.
- Visscher GE, Jagels R. 2003.** Separation of *Metasequoia* and *Glyptostrobus* (*Cupressaceae*) based on wood anatomy. *IAWA Journal* 24: 439-450.
- Wardell JF, Hart JH 1970.** Early response of sapwood of *Quercus bicolor* to mechanical injury. *Can J Bot* 48:683-686.
- Wainhouse D, Ashburner R, Ward E. 1998.** The effect of variation in light and nitrogen on growth and defense in young sitka spruce. *Funct. Ecol.* 12: 561-572.
- White DA, Kile GA. 1993.** Discoloration and decay from Artificial wounds in 20-year-old *Eucalyptus regnans*. *European Journal of Forest Pathology* 23: 4.31-440.
- Yamada T. 1992.** Biochemistry of gymnosperm xylem response.s to fungal invasion. In: Blanchette RA, Biggs AR, eds. *Defense Mechanisms of Woody Plants Against Fungi*. Berlin: Springer-Verlag, 147-164.
- Yamanaka K. 1984.** Normal and traumatic resin canal in the secondary phloem of conifers. *Mokuzai Gakkaishi.* 30: 347-353.
- Zeglen Stefan. 1997.** Tree wounding and partial cut harvesting. A literature review of British Columbia. Pest management report
- Zimmermann MH. 1983.** Xylem structure and the ascent of sap. Springer, Berlin Heidelberg. New York, 143 pp.

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