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# Histology of canker of the Japanese cedar, *Cryptomeria japonica* D. DON, caused by *Cercospora sequoiae* ELLIS et EVERHART[1]

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Histological changes in xylem of the Japanese cedar, *Cryptomeria japonica*, infected with *Cercospora sequoiae* were investigated. Traumatic ray parenchyma were present in the vicinity of decayed parts. Hyphae were confined to the traumatic ray intercellular canal by swollen ray parenchyma. Suberin was deposited on the cell walls of traumatic ray parenchyma, and tracheids were plugged with a resin-like substance. Lignin and polyphenol were present in discolored sapwood in greater quantity than in non-discolored wood. Substances including polyphenol also occluded the tracheid lumina in the boundary between discolored and non-discolored sapwood. These changes in xylem imply resistance responses of the tree to invading fungi.
**Key words**: canker, *Cercospora sequoiae*, *Cryptomeria japonica*, resistance response

## Introduction

When seedlings of the Japanese cedar, *Cryptomeria japonica* D. DON, infected with *Cercospora sequoiae* ELLIS et EVERHART are planted in forests, longitudinal valleculae are formed as a symptom of canker on the surface of the trunk within about 1 m above the ground. Dieback marks often remain in the central part of the vallecula, and if valleculae progress in subsequent years, the stem twists and deforms further. Cross-sections of the diseased part shows that the xylem is discolored gray brown or dark brown, and in the aged part, it is decayed by secondary invasion of wood-decaying fungi. Thus, the timber quality is reduced because of decay and discoloration of the xylem, although diseased trees seldom die after planting (Zinno 1979).

In addition, it is known that this disease can spread from cankered to healthy cedars within the forest. The pathogen from the diseased part attacks branches or twigs of healthy trees and then invades the trunk and induces fresh canker 4~5 m above the ground (Chiba 1975).

There have been only a few reports on the anatomy of trees cankered by *C. sequoiae* (Ito *et al.* 1974) and none on trees damaged by natural infection. The present paper describes the histology of trees cankered by *C. sequoiae* and also of wood-decaying fungi, which invade secondarily, especially in relation to resistance factors.

## Materials and Methods

### 1. Plant materials

Three cankered trees were felled in a stand of *C. japonica* at Utano, Nara Prefecture, on 31 January and 6 July, 1994. This stand, which had been planted in 1985, was at 520 m a.s.l. and had an area of 1.3 ha. Seedlings infected with *C. sequoiae* had been planted without this being realized, and 48% of the trees had been damaged by this fungus (Nishiguchi *et al.* 1994).

Logs including infected parts of the trees felled on 31 January were immediately fixed with FAA (50% ethanol : formalin : acetic acid = 90 : 5 : 5 v/v) and those on 6 July were stored at −20°C until use.

### 2. Sectioning

Blocks containing both discolored and non-discolored sapwood were obtained from the fixed and stored logs. After slight moistening of the block surface, the block was sectioned to 20 to 30 $\mu$m thickness with a sledge microtome.

### 3. Histology

Sections fixed with FAA were used for histological observations, while sections stored at −20°C were used for histochemical tests. For histological observations using a light microscopy or scanning electron microscopy (SEM), sections were stained with toluidine blue O or ion-coated with gold, respectively.

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The phloroglucinol-HCl test for lignin, and the potassium bichromate and diazotized p-anisidine test for polyphenol were used for histochemical observation (Suzuki 1962). Both suberin and lipids were stained with Sudan black B (Suzuki 1962).

**4. Isolation of fungi**

Wood pieces (approximately 8 mm$^3$ in volume) were obtained from a log containing discolored and non-discolored sapwood, which had been stored at −20°C for 4 months. They were sterilized with 70% ethanol and 5-fold diluted antiformin, and then washed twice with sterilized water.

Sterilized pieces were placed on potato dextrose agar (PDA) in Petri dishes, and incubated at 25°C in darkness. Ten days after incubation, hyphae that had extended from the pieces were transplanted to PDA slants and then cultured under the same conditions.

## Results and Discussion

*C. sequoiae* was not isolated from the sapwood because this fungus is mainly present in the cambium of the Japanese cedar (Kiyohara and Tokushige 1967). However, the presence of other fungi, which seem to invade secondarily, was shown by isolation experiments. Six taxa of fungi, *Cryptosporiopsis* sp., *Phialophora* sp., *Acremonium* sp., *Aspergillus* sp., *Gliocladium* sp. and *Gliocephalis* sp., were detected from the wood pieces. *Cryptosporiopsis* sp. is known to induce dieback and canker, and some of the isolated fungi have already been found in discolored xylem of living Japanese cedar trees (Suzuki *et al.* 1984). The others are ordinarily present in xylem, soil and air (Udagawa *et al.* 1978).

Histological observation showed that in the sapwood, hyphae developed axially through tracheids and laterally through bordered pits and cross-field pitting (Fig. 1). The observed changes in tissue were considered to be resistance responses to hyphal spread. Traumatic ray parenchyma were present in the vicinity of decayed parts, especially in the early wood (Fig. 2). Hyphae were confined to the traumatic intercellular canal surrounded by swollen ray parenchyma (Fig. 3). Tracheids were plugged with a resin-like substance (Fig. 4), although there were no resin canals around resinous tracheids. Hyphal expansion was limited in the tracheids (Fig. 4B).

These histological changes will be discussed using the concept of compartmentalization of decay in trees (acronym CODIT) proposed by Shigo (1965, 1972, 1984) and Shigo and Hillis (1973). The CODIT model consists of two parts. Part 1 is represented by three walls: walls 1, 2 and 3 resist vertical, inward (radial) and lateral (tangential) spread, respectively. These walls are built into the tree prior to injury and infection. Part 2 starts when the tree forms wall 4 after infection. Wall 4 separates wood which is present at the time of injury or infection from new wood that forms subsequently (Shigo 1984).

In the present study, the thick cell walls of traumatic ray parenchyma in xylem hindered further hyphal expansion, suggesting that this traumatic ray parenchyma may correspond to wall 3. Occlusion of tracheids and pits by resin-like substances may include walls 1 and 3 because of limitation of vertical and lateral extension of hyphae. Resin production is assumed to be an important defense mechanism in the xylem of conifers, particularly pines (Gibbs 1968; Hart *et al.* 1975). The role of resin in resistance, however, is still not fully understood (Yamada 1992).

The reactions of discolored sapwood in the histochemical staining tests differed definitely from those of non-discolored sapwood (Table 1), tissues of discolored sapwood responding positively to lignin (Fig. 5), polyphenol (Fig. 6), suberin (Fig. 7) and lipids. Lipids could be distinguished from suberin by solution in acetone (Fig. 8). Suberization of cell walls is involved in walls 3 or 4. Suberin production has frequently been associated with antimicrobial defense reactions of plants (Biggs 1987). Suberin deposits may protect other cell wall components from attack by the enzymes of the pathogen (Pearce and Rutherford 1981). Histochemical tests and chemical analysis in xylem cells of *Quercus* spp. showed that suberin formed after wounding, and thus suberization may correspond to wall 4 (Biggs 1987; Pearce and Rutherford 1981). However, it is known that in other tree species, *e.g. Fraxinus americana* L., suberization of traumatic ray parenchyma may refer to wall 3 (Biggs 1987). Although it is not clear when suberin was produced in this study, suberization of cell walls is certainly related to the resistance in trees.

Substances including polyphenol also occluded the tracheid lumina at the boundary between discolored and non-discolored sapwood (Fig. 9). This tracheid occlusion by phenolic substances was similar in mechanical hindrance of hyphal extension (walls 1 and 3) to that by resin-like substances (Fig. 4). Phenolic compounds are critical to the formation of defense mechanisms in the xylem of conifers (Yamada 1992). It has been suggested that in sapwood of *C. japonica*, the deposits containing

Table 1. Staining and histochemical reactions of non-discolored and discolored sapwood of *C. japonica*

| Component stained | Stain or test | Response of | |
|---|---|---|---|
| | | Non-discolored sapwood | Discolored sapwood |
| Lignin | Phloroglucinol-HCl | Red | Red stain more intense than non-discolored wood |
| Polyphenol | Potassium bichromate | Unstained | Yellow-brown |
| | Diazoided p-anisidine | Unstained | Red-brown |
| Suberin (Lipids) | Sudan black B | Unstained | Blue-black |

inhibitory substances like polyphenol could block hyphal passage through tracheid lumina and pits, and form spatially continuous barriers (Yamada *et al.* 1988).

Occlusion of tracheids and pits by resin-like and phenolic substances observed in this study may also contribute to chemical resistance to fungi. There are many reports concerning the effects of terpene components of conifer resins on the growth of tree pathogens (Cobb *et al.* 1968; De Groot 1972; Ennos and Swales 1988; Shain 1971). The overall consensus is that the saturated atmosphere of monoterpenes, which are likely to be found in the vicinity of fresh wounds on conifers, reduce or prevent the growth of potentially pathogenic fungi (Kůc and Shain 1977; Shuck 1982). Defense responses of several coniferous genera, *Cryptomeria*, *Picea* and *Pinus* are related to phenols, some of which are considered phytoalexins (Yamada 1992). In *C. japonica*, hinokiresinol and a few minor components among norlignans have high inhibitory activity against fungi (Yamada *et al.* 1988). For the resin-like and phenolic substances observed in this study, however, further work is needed to analyze chemical resistance.

Thus, a series of observations in this study have suggested that changes in xylem of cankered trees of the Japanese cedar are likely to be resistance responses of the tree to invading fungi.

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(*These English titles are tentative translations by the author of this paper from the original Japanese.)

## スギ溝腐病罹病木の組織学的研究

吉田和広

スギ溝腐病による木部組織の変化を明らかにするため，罹病木の組織学的観察を行った。腐朽部の周辺には傷害柔組織が存在し，菌糸が閉じこめられている場合もあった。傷害柔組織の壁面にはスベリンが沈着していた。樹脂様物質は仮道管を閉塞していた。リグニンやポリフェノールは，非変色部より変色部に多く存在していた。変色部と非変色部の境界付近では，仮道管がポリフェノールによって閉塞されていた。今回観察された組織の変化は，菌の侵入に対するスギ木部の抵抗反応と考えられる。

キーワード：溝腐病，スギ，スギ赤枯病菌，抵抗反応

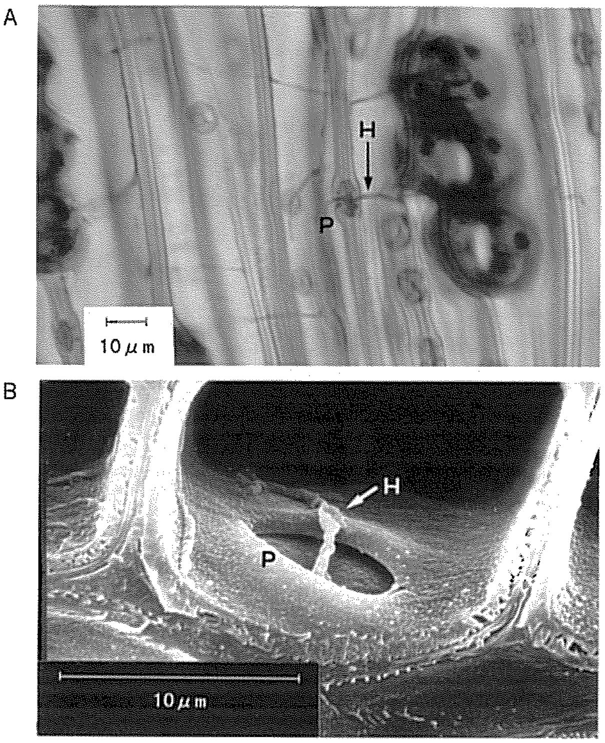


Fig. 1. Passage of hyphae through bordered pits. (A) observation by light microscopy (tangential section), (B) observation by SEM (cross-section). H: hyphae, P: bordered pit.

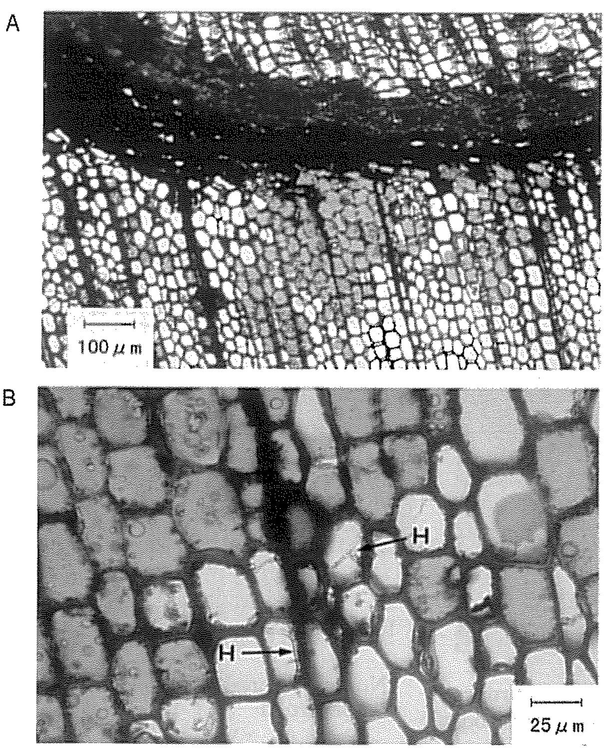


Fig. 4. Presence of resin-like substances in discolored sapwood of a cankered tree (cross-sections). (A) occlusion with resin-like substances in tracheid lumina, (B) hindrance of hyphal expansion. H: hyphae.

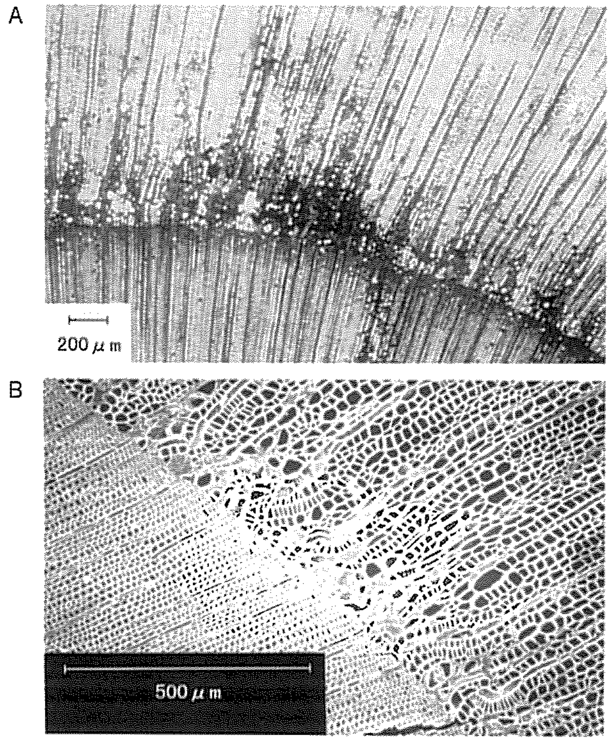


Fig. 2. Formation of traumatic ray parenchyma in xylem (cross-sections). (A) observation by light microscopy, (B) observation by SEM.

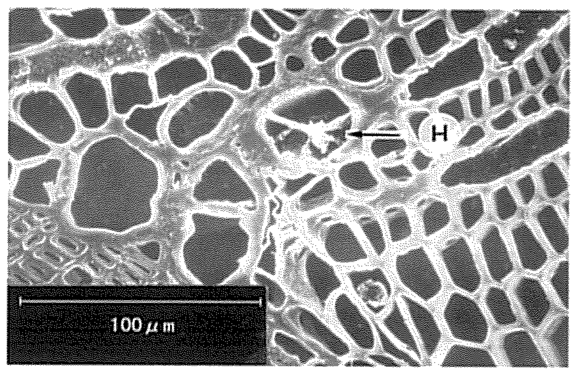


Fig. 3. Confinement of hyphae to the intercellular canal surrounded by swollen ray parenchyma (cross-section). H: hyphae.

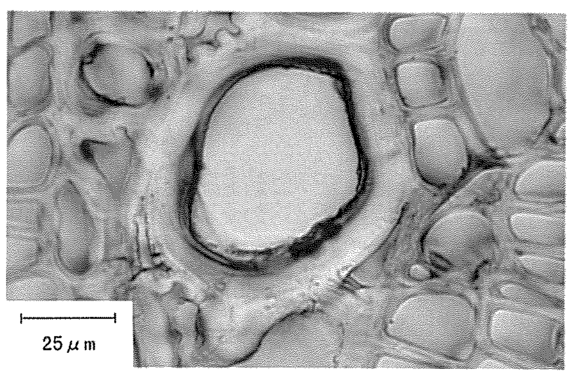


Fig. 7. Deposition of suberin on the cell wall in a cross-section stained with Sudan black B. The part stained blue-black shows the presence of suberin.

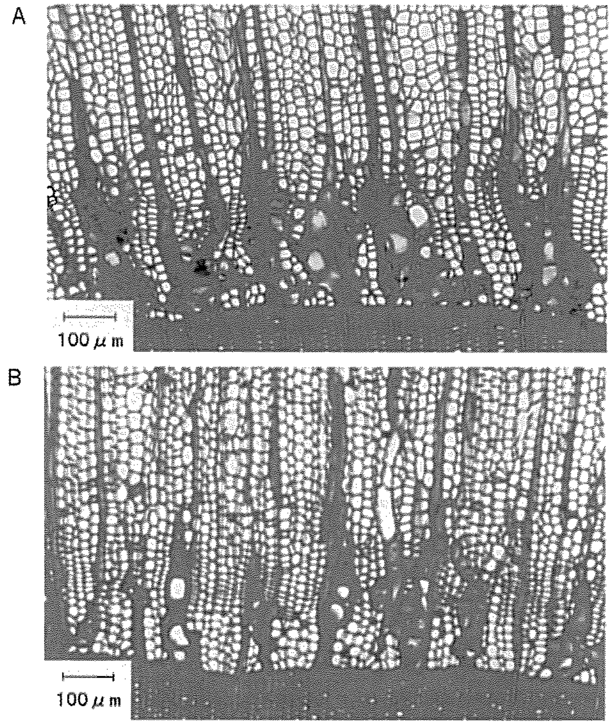


Fig. 5. Cross-sections stained with phloroglucinol-HCl. (A) non-discolored sapwood, (B) discolored sapwood.

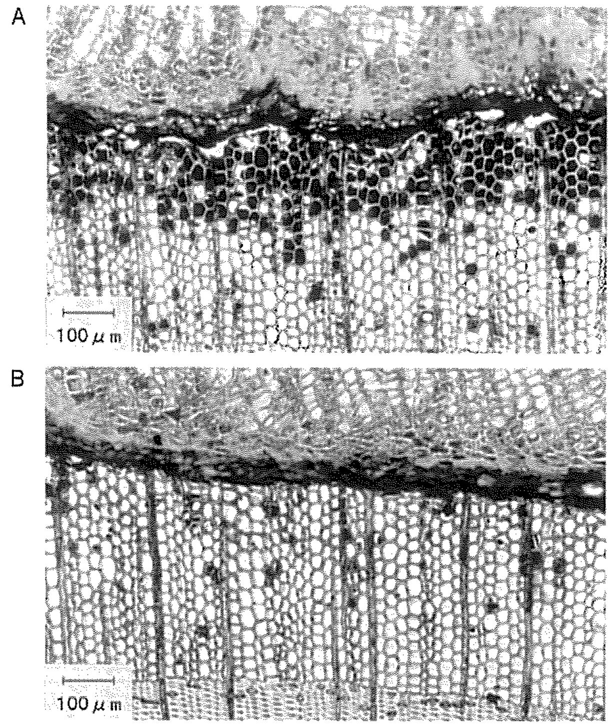


Fig. 8. Dissolution of lipids in cross sections stained with Sudan black B by acetone treatment. The part stained blue-black shows the presence of lipids. (A) before treatment, (B) after treatment.

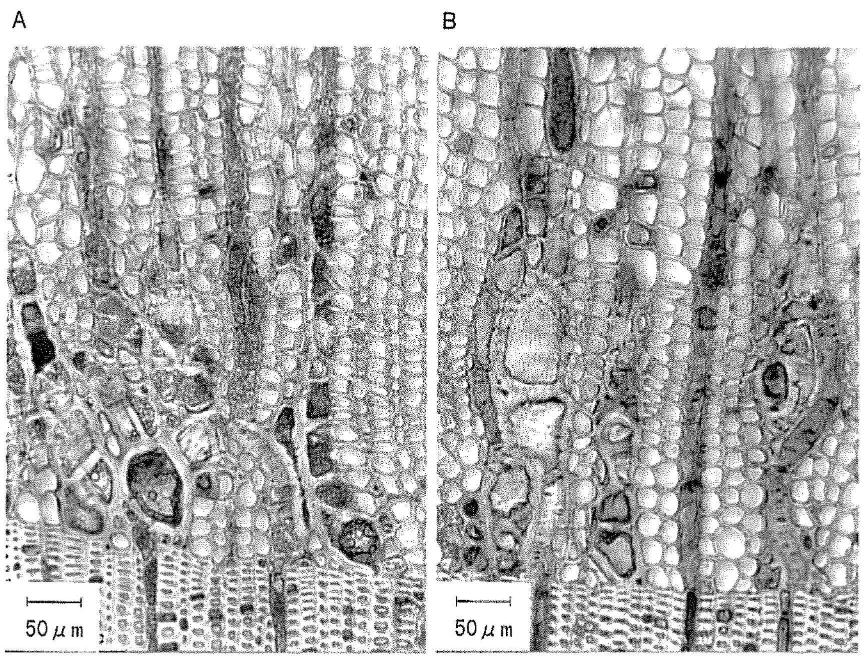


Fig. 6. Cross-sections stained with potassium bichromate. The part stained brown shows the presence of phenolic substances. (A) non-discolored sapwood, (B) discolored sapwood.

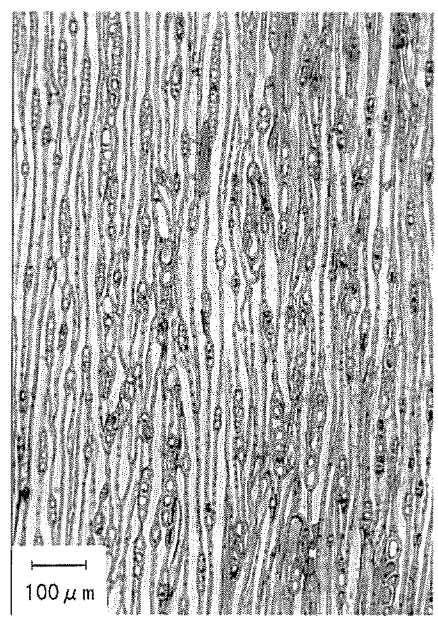


Fig. 9. Occlusion of tracheid lumina with phenolic substances in tangential section stained with diazoided panisidine. The part stained brown shows the presence of phenolic substances.