EFFECTS OF HEARTWOOD INHABITING FUNGI ON THUJAPLICIN CONTENT AND DECAY RESISTANCE OF WESTERN REDCEDAR (*THUJA PLICATA* DONN.)

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ABSTRACT

Western redcedar (*Thuja plicata* Donn.) outer heartwood blocks from a mature and an immature tree, inoculated with fungi commonly isolated from stained heartwood, had a thujaplicin content of less than 6% and a hot water solubility of about one-half that of controls after 10 weeks of exposure. Decay resistance of blocks from the mature tree was greatly reduced following exposure to the staining fungi. Naturally stained heartwood also had low thujaplicin content, but its decay resistance remained high in the same test.

Keywords: Thujaplicin, decay resistance, western redcedar

INTRODUCTION

Among the important timber species of western North America, western redcedar (*Thuja plicata* Donn.) is well known for the high decay resistance of its heartwood. This decay resistance is attributable to certain extractives, particularly the thujaplicins and the water-soluble phenolics (Barton and MacDonald 1971). Nevertheless, one finds extensive decay in mature and overmature western redcedar. In the province of British Columbia, for example, the volume of decay expressed as a percentage of total bole volume is greater for western redcedar (32%) than for any other major conifer (average 12%) (B.C. Forest Service 1957). The apparent contradiction implied by these observations led to the present study.

The heartwood color of mature western redcedar ranges from light straw at the periphery through pinks and reds to light and dark brown near the center. The various color zones typically have distinct but irregular boundaries, and can be visualized as a set of irregularly shaped nesting cones with sides lying roughly parallel to the cambium in longitudinal but not in transverse section. Eades and Alexander (1934) reported that the outer straw-colored heartwood is essentially sterile, while the darker colored wood is inhabited by a number of imperfect fungi. MacLean and Gardner (1956b) have shown that the thujaplicin content and hot water solubility of brown-stained heartwood is much lower than that of the outer, sterile, straw-colored heartwood. Van der Kamp (1975) has shown that the phenomenon can be regarded as a form of succession, with at least two stages preceding a decay-causing basidiomycete, and the decay fungus itself being replaced by a diverse microbial community of a complexity approaching that of soil. In that study the red-stained and outer brown-stained heartwood was inhabited by a single fungus tentatively identified as *Cylindrocephalum* sp. The inner brown-stained wood was inhabited by several fungi. The most common isolates from this zone were *Cylindrocephalum* and a second isolate tentatively identified as *Kirschteiniella thujina* (Peck) Pomerleau and Etheridge. Of all the isolates obtained from red- and brown-stained heartwood, only *Cylindrocephalum* and *K. thujina*

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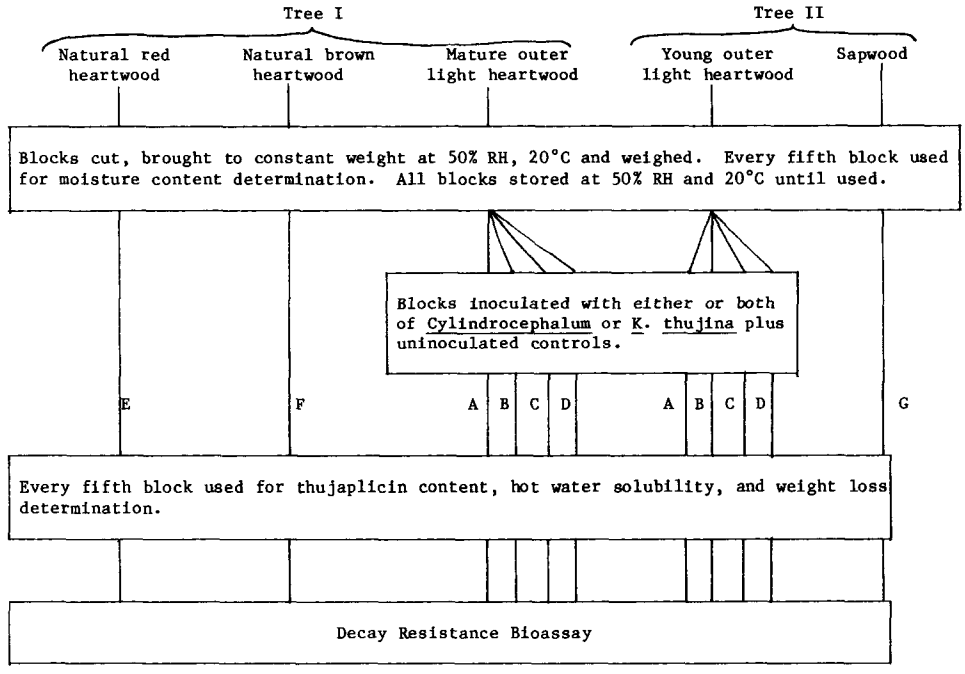

FIG. 1. Flow chart of methods showing the origin of test blocks and the sequence of measurements and treatments.

were able to invade sterile outer heartwood blocks with a high thujaplicin content (van der Kamp 1975).

This paper reports the effect of the two most common isolates from the red- and brown-stained heartwood on the thujaplicin content, hot water solubility, and decay resistance of western redcedar heartwood.

METHODS

A flow diagram showing the origin of test blocks and the order of treatments and measurements is given in Fig. 1.

Preparation of wood blocks

A 300+ year-old western redcedar (DBH = 84 cm) exhibiting typical heartwood discoloration and a central decay column (Tree I) and an 80-year-old redcedar (DBH = 40 cm), with no heartwood discoloration or decay (Tree II), both growing on the University Research Forest, Maple Ridge, B.C., were selected. A 40-cm bolt was cut from the base (1.0 to 1.4 m above ground) of each tree. The bolts were split into smaller, knot-free segments. These segments were sawn into 2- × 2-cm sticks with one true tangential face and lying wholly within one of the following zones: sapwood from the younger tree, straw-colored heartwood from near the heartwood-sapwood boundary of each tree, and red-stained and inner brown-stained wood from the older tree. The sticks were cut into 2- × 2- × 1-cm blocks, which were numbered sequentially. All the blocks were air-dried at a constant temperature (20 C) and relative humidity (50%) until equilibrium was

reached, and then weighed to the nearest 0.001 g. They were then stored under these conditions until they were used as described below. Oven-drying of test blocks was avoided to minimize volatilization or alteration of wood extractives. Every fifth block was used as a moisture content reference block. These blocks were oven-dried at 105 C for 24 hours in tared glass vials and weighed to the nearest 0.001 g. The moisture content of each treatment block was estimated as that of the adjacent reference block.

Origin of fungal isolates

The isolates of *Cylindrocephalum, K. thujina,* and *Poria albipellucida* Baxter used in this study were those obtained by van der Kamp (1975). *P. albipellucida* was selected for the decay resistance bioassay because it was the most common decay fungus isolated from the decay zone in western redcedar trees exhibiting the standard heartwood color pattern (van der Kamp 1975).

Isolation attempts on malt agar (3% malt extract, 2% agar) were made from freshly collected wood of all the wood zones used in this experiment to verify the results of van der Kamp (1975).

Inoculation of blocks with staining fungi

Cultures of *Cylindrocephalum* and *K. thujina* were grown on malt agar (3% malt extract, 2% bacto agar) in 25-mm deep petri plates. Sterile glass rods (3 mm) were placed on the surface of the culture medium. The outer heartwood blocks were divided into four groups of 25 blocks for each tree, by assigning each fourth block to a particular treatment. Blocks from Trees I and II assigned to the first treatment (Treatment AI and AII, respectively) were stored at 20 C and 50% relative humidity for 10 weeks. Each block was then surface sterilized by flaming, and placed in an empty sterile petri plate. Sterilization of test blocks by dry heat or autoclaving was avoided to minimize volatilization or alteration of wood extractives. Sterile distilled water was pipetted on the transverse surface to give a final moisture content of 80%. Blocks were left overnight and then placed on glass rods over cultures of *Cylindrocephalum* and incubated in the dark at 20 C for 10 weeks.

Treatment BI and BII blocks were treated similarly to Treatment A, except that they were placed on cultures of *K. thujina.*

Treatment CI and CII blocks were treated like those of Treatment A, except that they were placed on cultures of *Cylindrocephalum* immediately for 10 weeks and then immediately transferred to cultures of *K. thujina* for a further 10 weeks without intermediate sterilization.

Treatment D served as a control. These blocks were stored for 20 weeks as above and surface sterilized and wetted immediately before the decay resistance bioassay.

The red- (Treatment E) and brown-stained (Treatment F) heartwood and sapwood (Treatment G) blocks were stored at 20 C and 50% relative humidity until the start of the decay resistance bioassay (20 weeks).

At the end of 20 weeks, five blocks from each of the eleven treatments were selected randomly for thujaplicin and hot water solubility determinations. Blocks from Treatments DI, DII, E, F and G were surface sterilized and wetted. All 55 blocks were then weighed, air-dried at 20 C and 50% relative humidity to constant

weight, and weighed to the nearest 0.001 g. The moisture content of each block was estimated from the moisture content reference blocks described above. The weight loss (gain) of each block over the course of the 20-week period was then calculated. The thujaplicin content and hot water solubility were estimated using the method of MacLean and Gardner (1956a). The former involved hot water extraction of ground wood followed by n-hexane extraction of the water soluble fraction. Next the n-hexane solution was shaken with a ferric acetate solution and chloroform, and the organic portion was separated. Thujaplicin concentration was estimated by absorbance of the chelate in hexane-chloroform at 425 nm.

Decay resistance bioassay

Cultures of *P. albipellucida* were grown on malt agar (5% malt extract, 2% bacto agar) in 25-mm-deep petri plates (30 mL medium per plate) for 6 weeks. Sterile glass rods (3-mm diameter) were placed on the mycelial surface. One set of plates received one block of each of Treatments AI to DI per plate, a second set AII to DII, and the last set received one block of each of Treatments E–G. The average moisture content of blocks exposed to either or both of *Cylindrocephalum* and *K. thujina* (Treatments A, B, and C) was estimated from the blocks used for thujaplicin determination. Sterile distilled water was pipetted onto each block to yield an average moisture content of 80%. The blocks were then transferred to *P. albipellucida* without intermediate sterilization. Blocks from Treatments D–G were surface sterilized and wetted immediately before exposure to *P. albipellucida* as described above. All plates were incubated in the dark at 20 C for 12 weeks. At the end of this period, all blocks were examined and those showing evidence of contamination discarded. The remaining blocks were oven-dried to constant weight and weighed to the nearest 0.001 g. Weight loss since the start of the experiment was calculated. Analyses of variance of the results for thujaplicin content, hot water solubility, and weight loss was carried out. Duncan's multiple range test was used to compare treatments.

RESULTS AND DISCUSSION

Isolations from the various wood zones from both trees confirmed that the outer, straw-colored heartwood was sterile; *Cylindrocephalum* was the only fungus isolated from red-stained heartwood, while *Cylindrocephalum, K. thujina,* and three other fungi (unidentified) were isolated from the brown-stained wood.

Treatments A, B, and C all resulted in a marked decrease in thujaplicin content in both Tree I and Tree II (Table 1). The maximum thujaplicin content at the end of any of these treatments was less than 6% of the relevant controls. Thujaplicin concentrations of 0.02% or less are near the lower limits of detection of the method used, and represent trace amounts. Naturally stained red and brown heartwood also had low levels of thujaplicin. It may be concluded from these observations that both *Cylindrocephalum* and *K. thujina* can invade sterile heartwood and quickly reduce even high thujaplicin concentrations to trace amounts. In living trees, however, only *Cylindrocephalum* occurs near the outer edge of the stained heartwood, and since the thujaplicin content of red- and brown-stained wood is low, it appears that *K. thujina* does not degrade thujaplicin in living trees. Blocks exposed to either or both of *Cylindrocephalum* and *K. thujina* exhibited

TABLE 1. *Thujaplicin content, hot water solubility, and decay resistance of western redcedar heartwood inoculated with common heartwood inhabiting staining fungi from western redcedar.*

Source of test blocks	Initial treatment		Thujaplicin content (%)	Hot water solubility (%)	Weight loss (%)	Weight loss by *P. albipellucida* (%)
	Code	Staining fungi				
Outer heartwood of mature redcedar (Tree I)	AI	*Cylindrocephalum*	0.03c[1]	7.3bc	1.2a	8.3b
	BI	*K. thujina*	0.02c	6.2c	0.8a	10.6b
	CI	*Cylindrocephalum* + *K. thujina*	0.01c	6.7c	0.5a	8.6b
	DI	none	0.51a	15.0a	0.1a	2.5d
Outer heartwood of immature redcedar (Tree II)	AII	*Cylindrocephalum*	0.01c	3.4d	−0.5a	7.0bc
	BII	*K. thujina*	0.01c	3.8d	0.7a	10.3b
	CII	*Cylindrocephalum* + *K. thujina*	0.01c	3.7d	0.5a	11.1b
	DII	none	0.21b	6.2c	−0.1a	10.7b
Red-stained heartwood (Tree I)	E	none	0.05c	8.2bc	0.1a	3.0cd
Brown-stained heartwood (Tree I)	F	none	0.01c	11.9ab	−0.8a	1.5d
Sapwood (Tree II)	G	none	0.00c	0.9e	1.1a	22.5a

[1] Values followed by the same letter are not significantly different from each other at $P = 0.05$ according to Duncan's multiple range test.

a red-brown stain that was quite variable in intensity but generally lighter than that of naturally stained heartwood.

The hot water solubility was determined because this fraction contains the water-soluble phenolics (other than thujaplicins), which are also thought to be fungicidal (Roff and Atkinson 1954). Treatments A, B, and C all reduced the hot water solubility to about one-half of that of the controls. The hot water solubility of naturally stained red and brown heartwood was somewhat higher than that of the treated blocks from the same tree (Tree I). The reduction in hot water solubility presumably resulted from the condensation of some of the components of the fraction because the reduction in hot water solubility was in all cases much greater than the weight loss. It is not clear from the present study whether the fungicidal properties of the hot water soluble fraction were also affected by the two fungi.

None of the weight changes recorded at the end of the 20-week period were significantly different from each other or from zero. It appears, therefore, that the energy requirements of the two fungi are low. However, it may well be that a large part of the carbohydrate and nutrient requirement of these fungi was supplied by the malt agar medium and translocated to the sites of fungal activity in the blocks. In living trees these two fungi can inhabit heartwood for decades without affecting the structural integrity of the wood. Presumably they utilize some wood component for energy, but a marked decrease in specific gravity has not been recorded, and at any rate would be difficult to demonstrate because of the difficulty of selecting suitable controls.

The decay resistance bioassay showed that exposure of blocks from the mature tree to *Cylindrocephalum* and/or *K. thujina* not only decreased thujaplicin content and hot water solubility, but also resulted in a significant decrease in decay resistance. In blocks from the immature tree, however, the weight loss of the control

(Treatment DII) was not significantly different from that of Treatments AII, BII, and CII. Perhaps the thujaplicin content (0.21%) was too low to result in significant decay resistance under these test conditions. None of the treatments approached the weight loss incurred by sapwood. This indicates that even after virtually all detectable thujaplicin has been removed, and the water-soluble phenolic fraction has been considerably reduced, western redcedar heartwood still retains considerable residual decay resistance.

The performance of the naturally stained red and brown heartwood is of great interest since much of the commercial western redcedar currently produced is of this type. In spite of the fact that the thujaplicin content was very low in these zones, the decay resistance was not significantly different from that of the outer untreated heartwood of the mature tree. It may be that the decay resistance of such wood is attributable to a toxic thujaplicin derivative. It may also result from the antagonistic action of microorganisms present in the stained wood. However, *Cylindrocephalum* and *K. thujina* do not appear to contribute to such antagonism since blocks inoculated with either or both of these fungi exhibited a much lower decay resistance than natural red- and brown-stained heartwood. Since *Cylindrocephalum* was the only fungus isolated from red-stained heartwood, it is unlikely that antagonism is the cause of the high decay resistance of this zone.

CONCLUSION

It may be concluded from this study that the non-decay fungi tentatively identified as *Cylindrocephalum* sp. and *K. thujina,* which commonly inhabit the inner, sound but stained heartwood of western redcedar, have the ability to invade sterile heartwood containing high levels of thujaplicin. Once established, these fungi degrade or somehow alter the thujaplicins, and reduce the hot water solubility of the wood, resulting in considerable reductions in the natural decay resistance of test blocks with initial high levels of thujaplicin. Nevertheless, a similar loss of decay resistance of stained wood with low thujaplicin levels from a living tree could not be demonstrated. Some other mechanisms of decay resistance may be involved. Low levels of thujaplicin in stained western redcedar heartwood do not necessarily imply low decay resistance.

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