

TROPOLONE CONTENT OF INCREMENT CORES AS AN INDICATOR OF DECAY RESISTANCE IN WESTERN REDCEDAR

*Jeffrey D. DeBell*¹

Former Graduate Research Assistant

Jeffrey J. Morrell

Associate Professor

and

Barbara L. Gartner

Assistant Professor

Department of Forest Products
Forest Research Laboratory
Oregon State University
Corvallis, OR 97331-7402

(Received January 1997)

ABSTRACT

The high decay resistance of western redcedar (*Thuja plicata* Donn) is due to the presence of toxic extractives, called tropolones, in the heartwood. Therefore, tropolone content may be used as an indicator of decay resistance. With increment core-sized samples of western redcedar heartwood, we used gas chromatography to measure tropolone content and soil block tests to assess decay resistance. Results showed that decay resistance was extremely variable at low tropolone levels, but was uniformly high at tropolone levels of 0.25% or greater. Analyzing tropolone content of western redcedar increment cores is a useful way to assess decay resistance of standing trees.

Keywords: *Thuja plicata*, tropolones, thujaplicins, decay resistance.

INTRODUCTION

Western redcedar (*Thuja plicata* Donn) is a valuable commercial species in the northwestern United States and Canada. One of its most important characteristics is high decay resistance, which is due to the presence of toxic extractives in the heartwood. While a number of heartwood extractives have been shown to be toxic to fungi, the tropolones are the most important; they are comparable to pentachlorophenol in toxicity (Barton and MacDonald 1971; Rennerfelt 1948; Rudman 1962, 1963). Five tropolones have been identified in west-

ern redcedar. Of these, β -thujaplicin, γ -thujaplicin, and β -thujaplicinol are the most important in terms of quantity, together making up 98% of the total tropolone content (Barton and MacDonald 1971; Frazier 1987).

Since tropolones are primarily responsible for the decay resistance of western redcedar, it should be possible to use tropolone content of the wood as an indicator of decay resistance. Tropolone content can be assessed in days; the standard method of assessing decay resistance is the soil block test, which takes 12–16 weeks. Nault (1988) noted that measurement of tropolones using gas chromatography can be accomplished with very small samples, making it possible to use increment cores to assess tropolone content of standing trees.

¹ Present address: Natural Resource Scientist, Washington Dept. of Natural Resources, P.O. Box 47018, Olympia, WA 98504-7018.

This would be particularly useful for studying tropolone content in situations where destructive sampling is undesirable.

Techniques have been developed previously for measuring thujaplicin content using gas chromatography (Johnson and Cserjesi 1975, 1980; Nault 1987, 1988). The first step is extraction in a soxhlet apparatus; thus, the number of samples that can be processed at one time is limited by the number of soxhlet setups available. For some studies, it would be desirable to use an extraction method that allows more efficient processing of large numbers of samples.

We modified the technique of Johnson and Cserjesi (1980) to allow efficient handling of large numbers of increment cores or similar sized samples. We then used the technique on small samples collected from various positions within the heartwood of western redcedar trees. This paper is limited to an examination of the technique, including its repeatability and the relationship between tropolone content and decay resistance; variation of tropolone content and decay resistance as a function of position in the stem will be discussed in another paper.

METHODS

Collection of wood samples

Material for this study came from western redcedar trees growing near Clatskanie, Oregon, on the Oregon State University College of Forestry's Blodgett Tract. Eleven trees were felled (Table 1), and disks were cut every 2 m from breast height (1.37 m) to the top of the tree. The disks were taken to the lab, where they were air-dried. A drill equipped with a 9.5-mm plug cutter was used to remove two plugs, parallel to the stem axis and oriented side by side tangentially, from the outermost heartwood of each disk. A second pair of plugs was removed approximately 1.5 cm from the pith. One of the plugs from each pair was used for tropolone analysis, while the other was used in the decay tests. We selected the plug size because it is close to the diameter of

TABLE 1. *Selected characteristics of sample trees.*

Tree	Total height (m)	BH age	Crown Percent	DBH (cm)
1	36.2	91	36	52.6
2	39.3	*	66	87.0
3	34.6	*	44	67.6
4	35.2	*	43	68.8
5	33.8	95	44	47.8
6	30.8	93	39	33.4
7	34.8	*	47	57.3
8	32.4	92	46	50.2
9	28.7	*	47	43.2
10	36.0	94	47	62.6
11	30.8	*	44	67.2

* Breast-height disk was decayed in center, so accurate ring count was not possible.

the 8-mm increment cores we often use in wood quality studies.

Tropolone analysis

We modified the extraction technique of Johnson and Cserjesi (1980) by replacing soxhlet extraction with cold extraction in centrifuge tubes. The benefit of this modification was the ability to process larger numbers of samples quickly. The disadvantage was that complete extraction was not possible. Thus the method is suitable for studying relative differences in tropolone content between wood samples rather than absolute tropolone content of a sample. Based on a few comparisons we made, cold extraction is capable of extracting 90% or more of the tropolones extracted by the soxhlet method.

Each redcedar plug was cut into pieces and ground in a small Wiley mill to pass through a 30-mesh screen. Approximately 0.5 g (± 0.01 g) of wood meal from each sample was weighed and placed in a 50-ml plastic centrifuge tube, along with 9 ml of acetone and 1 ml of an internal standard solution (3,4,5-trimethoxyphenol in acetone, with 0.35 mg/ml). The tube was capped and allowed to sit overnight (16 h) at room temperature (23–25°C). The sample was then centrifuged for 5 min, and the extract was transferred by Pasteur pipet to a clean 50-ml plastic centrifuge tube.

The samples were evaporated to 1 ml by

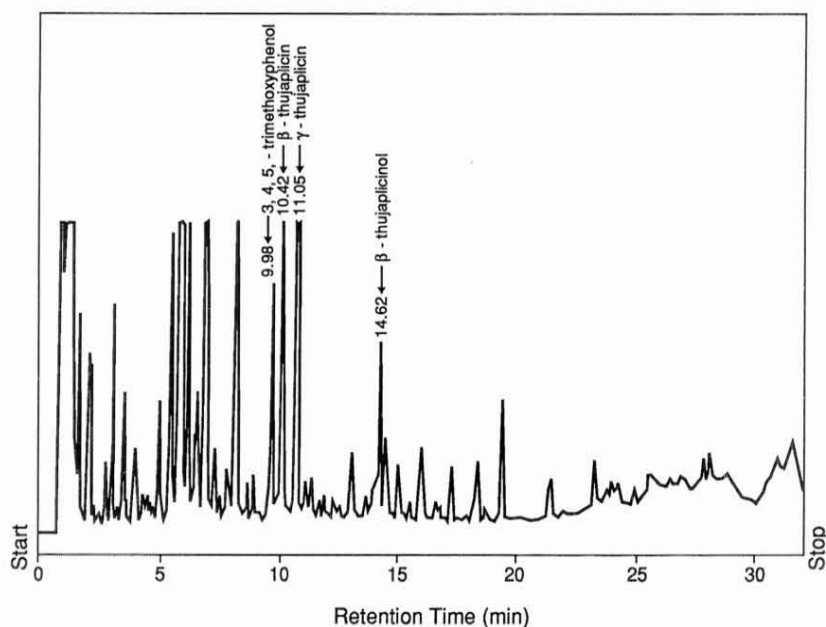


FIG. 1. Typical chromatogram showing retention times for 3,4,5-trimethoxyphenol, β -thujaplicin, γ -thujaplicin, and β -thujaplicinol.

blowing air through a small hose into the tube. The sample was then transferred to a small glass vial, and evaporated to dryness using air as described above. When the sample was dry, 0.2 ml of B.S.A. (N,O-bis (trimethylsilyl) acetamide) was added, and the sample was placed in a warming tray at 70°C for 10 min. A needle and syringe were then used to transfer the solution to an autosampler vial, which was capped and placed in the autosampler tray to await injection into the gas chromatograph. One-microliter injections were made.

A Hewlett Packard HP-5890 gas chromatograph equipped with a flame ionization detector and an autosampler was used for analyses. The column was a Supelco SPB-5 (30 m \times 0.75 mm). Hydrogen was the carrier gas, with a flow rate of 15 ml/min. The initial oven temperature of 125°C was held for 4 min, then raised at 5°C/min to 200°C. Injector and detector temperatures were 250°C. Retention times for the internal standard and tropolones were as follows (Fig. 1): 3,4,5-trimethoxyphenol: 9.9 min; β -thujaplicin: 10.4 min;

γ -thujaplicin: 11.0 min; β -thujaplicinol: 14.6 min.

Tropolone content was determined by comparison of tropolone peak areas with that of the internal standard. Pure samples of γ -thujaplicin and β -thujaplicinol were provided by Forintek Canada Corporation. β -thujaplicin was purchased under the name Hinokitil from a commercial supplier (TCI America). Tropolone content was expressed as a percentage of air-dried wood weight.

Small-scale analyses

In addition to analyzing tropolone content in the samples described above, we ran two small scale tests to check the precision of the method, and to assess the variation in tropolone level around the outer heartwood of a western redcedar tree. The precision of the method was checked by separately analyzing 5 subsamples of a single bulk sample of ground cedar heartwood. The variation in tropolone content around an individual stem was

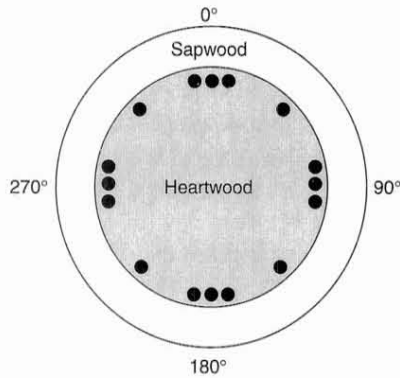


FIG. 2. Positions of samples removed from a western redcedar disk to assess variation in tropolone content around the circumference of the stem.

assessed by analyzing 16 plugs from a single disk, as shown in Fig. 2.

Soil block tests

Soil block tests were performed using procedures described by Scheffer et al. (1987). Briefly, 113-ml glass bottles were half-filled with moist forest loam, and a single $15 \times 15 \times 3$ -mm-thick western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) feeder strip was placed on the soil surface. Water was added to raise the moisture content to 100% (weight basis); then the jars were loosely capped prior to autoclaving for 45 min at 121°C . After cooling, the feeder strips were inoculated with 3-mm-diameter disks of agar cut from the actively growing edge of cultures of *Postia placenta* (Fr.) M. Larson & Lombard, a fungus which causes brown rot of many coniferous species. The bottles were incubated at 28°C until the feeder strips were thoroughly colonized by the test fungus. The cedar heartwood plugs were oven-dried (54°C), weighed, and sealed in plastic bags and subjected to 2.5 Mrads of ionizing radiation from a cobalt 60 source. The plugs were placed on the feeder strips (1 plug/bottle) and the jars were then incubated at 28°C for 16 weeks. The plugs were removed, scraped clean of adhering mycelium prior to oven-drying (54°C), and weighed. Loss in oven-dried weight was used as the measure of decay resistance (samples with the least

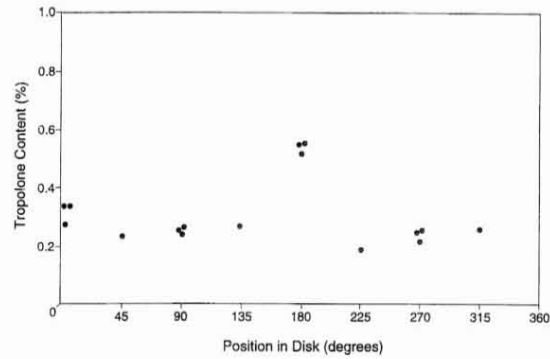


FIG. 3. Variation in heartwood tropolone content around the circumference of a western redcedar stem.

weight loss were considered the most decay-resistant).

RESULTS AND DISCUSSION

Small-scale analyses

The coefficient of variation for tropolone content of the five subsamples from a single batch of cedar meal was 4.5%. This low level of variation indicates that the modified method gives consistent results; we consider this level of precision to be acceptable for wood quality studies.

Tropolone content around the outer heartwood was fairly uniform around most of the disk, averaging between 0.2 and 0.3% (Fig. 3). But samples from the 180 degree position contained nearly double the tropolone content of samples from the rest of the disk. There was no obvious reason for the higher level at this position. From these data, it appears that a single increment core should be a useful indicator of tropolone content at a given location in a tree. With a single core, the tropolone content of some trees could be significantly over- or underestimated if an increment core includes an atypical zone like the one we encountered at the 180 degree position (Fig. 3). If only a few trees are sampled, average tropolone content at the sampling height could also be over- or underestimated. In this case, one might want to get several samples per tree to improve the reliability of the estimate for each tree. However, if the sample size (number of

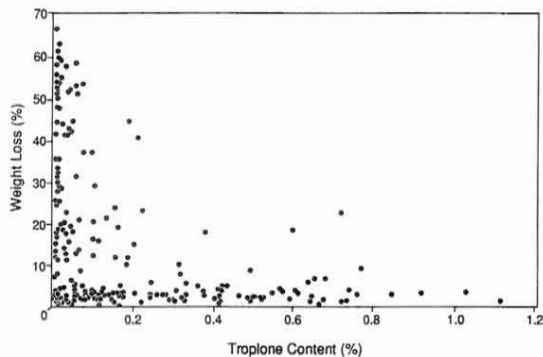


FIG. 4. Relationship between tropolone content and weight loss in soil block tests for paired samples of western redcedar heartwood.

trees) is large enough, the effect of hitting an atypical zone in a few trees should be unimportant to the overall results, and the extra work of collecting multiple cores from each tree may not be justified.

The consistency in tropolone content at positions where clusters of three samples were taken increased our confidence in the precision of the method (Fig. 3). The data also suggested that if multiple increment cores are taken from a single tree, the cores should be separated by at least 90 degrees, since taking two cores near the same point may do little to improve the estimate of the average tropolone content at that height in the tree.

Relationship of tropolone measurements to soil block tests

Tropolone content of the wood samples ranged from 0 to 1.2% (weight basis), while weight loss ranged from 0 to 70% (Fig. 4). Weight loss was highly variable for samples with tropolone content <0.10%, but averaged 21% loss. Weight loss was less variable for samples with tropolone contents between 0.10% and 0.24%, averaging 9% loss. Samples with tropolone content >0.25% had consistently low weight loss; average weight loss was 4%. Only 3 out of the 59 samples with high tropolone content had more than 10% weight loss.

We are unsure of the reasons for the high

variability in weight loss in samples with low tropolone levels. One possibility is that other substances in the heartwood prevented significant decay in some samples. However, there were no obvious patterns in the chromatograms to suggest any major differences in other extractives between low tropolone samples. Differences in tropolone distribution could influence the results, since the extract and decay samples were separate but adjacent plugs; however, the results from repeated sampling of a single disk (Fig. 3) suggest that these differences should be minimal. The variability of the soil block test may be a factor, since individual decay tests can sometimes vary widely.

These results indicate that tropolone analysis of increment cores is a useful way to assess decay resistance at a given position in standing trees. The modified analysis method, using cold extraction, gave consistent results and could be used with large numbers of samples efficiently. This technique should be particularly useful for wood quality studies, where many trees must be sampled.

ACKNOWLEDGMENTS

We are indebted to Bob Daniels of Forintek Canada Corporation for supplying tropolone samples and discussing extraction techniques when we began this project. We also thank Associate Professors Chris Biermann, Joe Karchesy, and Murray Laver of Oregon State University's Department of Forest Products for providing us with laboratory equipment and valuable technical assistance. This research was funded by a USDA Special Grant to Oregon State University for Wood Utilization Research.

REFERENCES

- BARTON, G. M., AND B. F. MACDONALD. 1971. The chemistry and utilization of western red cedar. Canadian For. Serv. Publ. No. 1023. 31 pp.
- FRAZIER, C. E. 1987. The essential oil of western red cedar, (*Thuja plicata*). M.S. thesis, University of Washington, Seattle, WA. 112 pp.
- JOHNSON, E. L., AND A. J. CSERJESI. 1975. Gas-liquid

- chromatography of some tropolone-TMS ethers. *J. Chromatog.* 107(2):388.
- , AND ———. 1980. Weathering effect on thujaplicin concentration in western redcedar shakes. *Forest Prod. J.* 30(6):52–53.
- NAULT, J. 1987. A capillary gas chromatographic method for thujaplicins in western redcedar extractives. *Wood Sci. Technol.* 21(4):311–316.
- . 1988. Radial distribution of thujaplicins in old growth and second growth western red cedar (*Thuja plicata* Donn). *Wood Sci. Technol.* 22(1):73–80.
- RENNERFELT, E. 1948. Investigations of thujaplicin, a fungicidal substance in the heartwood of *Thuja plicata* D. Don. *Physiologia Plantarum* 1(3):245–254.
- RUDMAN, P. 1962. The causes of natural durability in timber. IX. The antifungal activity of heartwood extractives in a wood substrate. *Holzforschung* 16(3):74–77.
- . 1963. The causes of natural durability in timber. Part XI. Some tests on the fungi toxicity of wood extractives and related compounds. *Holzforschung* 17(2):54–57.
- SCHEFFER, T. C., J. J. MORRELL, AND M. A. NEWBILL. 1987. Shellrot control in western redcedar: potential replacements for pentachlorophenol spray. *Forest Prod. J.* 37(7/8):51–54.