RADIAL DISTRIBUTION OF THUJAPLICINS AND THUJIC ACID IN OLD GROWTH AND SECOND GROWTH WESTERN REDCEDAR (THUJA PLICATA DONN)

by

JASON RAY NAULT

B.Sc., The University of Winnipeg, 1976

A THESIS SUBMITTED IN PARTIAL FULLFILLMENT OF THE REQUIREMENT FOR THE DEGREE OF MASTER OF SCIENCE

in

THE FACULTY OF GRADUATE STUDIES

(DEPARTMENT OF FORESTRY)

We accept this report as conforming to the required standard.

The University of British Columbia

April 13, 1986

In presenting this thesis in partial fulfilment of the requirements for an advanced degree at the University of British Columbia, I agree that the Library shall make it freely available for reference and study. I further agree that permission for extensive copying of this thesis for scholarly purposes may be granted by the head of my department or by his or her representatives. It is understood that copying or publication of this thesis for financial gain shall not be allowed without my written permission.

Department of FORESTRY

The University of British Columbia
1956 Main Mall
Vancouver, Canada
V6T 1Y3

Date APRIL 14, 1986

ABSTRACT

Radial cross sections of seven old growth cedars and ten second growth cedars taken at breast height were cut into increments averaging about 60 years growth for old growth trees and 10 years growth for second growth trees. These increments were extracted with ethanol:benzene (1:2). The extractives were analyzed for thujaplicin content by colorimetry of their ferric chelates, as well as by a new method utilizing capillary gas chromatography (GC) of their methylated derivatives. A statistical analysis of the two methods gave an r^2 value of 0.81 and a slope of 0.99. Thujic acid contents were also determined by the new GC method.

Distribution of thujaplicins and thujic acid generally increased from pith to outside heartwood, then decreased in the sapwood. Maximum thujaplicin contents were also related to the tree age.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

ACKNOWLEDGEMENT

The author wishes to express his gratitude to all those people who assisted with this study. In particular, assistance by Dr. J. W. Wilson, U.B.C. Faculty of Forestry, and Research Scientist Dr. E. P. Swan, Forintek Canada Corp. is appreciated. In addition, thanks are extended to Western Forest Products Ltd. (Port McNeill) for supplying the old growth material.

INTRODUCTION

Western redcedar (Thuja plicata Donn) has been long noted for the natural resistance of its heartwood to decay (11,26). This resistance has resulted in the use of western redcedar (WRC) in applications where this property is useful, especially where exposure to the elements is expected, for example in poles, shakes, shingles, siding and gutters.

This unique durability has been attributed to the presence of thujaplicins in the heartwood extractive components. Thujaplicins have been shown to be potent inhibitors of bacterial and fungal growth (24,25,26,27,28,30,33,37).

WRC is an important Canadian commercial wood. In the years 1977 to 1983 British Columbia's log production of WRC averaged 7.8 million M^3 per year (9), while exports of WRC shingles and shakes accounted for $68 million per year (6).

An increasing share of our timber production is coming from second growth forests, and silvacultural practices are resulting in faster growth of these stands. This has raised concerns over the quality of products being produced from this fast-grown wood. With WRC the concern is that the durability factor is maintained in second growth trees.

The purpose of this study is to analyze the radial distribution of thujaplicins and thujic acid to determine if they are related to tree age and how much they vary between old growth and second growth trees. As well, a new gas chromatographic method of analysis is compared to the previous colorimetric method.

LITERATURE REVIEW

Sowder (30) found that the hot and cold water soluble extractives of WRC heartwood and sapwood were toxic to fungi, while extracted wood meal was more susceptible to decay than unextracted. Rennerfelt (24) showed alpha, beta and gamma thujaplicin to be toxic to decay fungi in low concentrations, with beta being the most toxic, followed by gamma, then alpha. He also found them to be more effective in decay prevention than pinosylvin, the most potent fungicide in hard pine heartwood. The thujaplicins were of about the same effectiveness in decay inhibition as pentachlorophenol. Raa and Goksoyr (23) discovered that thujaplicins inhibited respiration in yeast in their study on yeast metabolic reactions. Rudman (27) studied toxicities of alpha, beta, and gamma thujaplicin, and beta thujaplicinol and demonstrated all to be potent inhibitors of fungal growth, although at low concentrations (<.1%) they were stimulants to growth for one species of fungus. He also compared their toxicity relative to other extractive chemicals (28) and found them to be the most toxic to all species of fungi tested. Trust and Coombs (33) showed beta thujaplicin to be a broad spectrum antibacterial agent. Coombs and Trust (8) found that antibacterial activity of beta thujaplicin was greatly reduced following exposure to light, and showed that photochemical decomposition had occurred.

Anderson and Gripenberg (1) elucidated the structure of beta thujaplicin, followed by Erdtman and Gripenberg (12), who isolated alpha and gamma thujaplicin and identified their

structures. The thujaplicins were discovered to be unusual seven member rings which are structural isomers. Nozoe et al (22) described the isolation of alpha thujaplicin from "Hiba-wood". Gardner et al (14) isolated and identified a related substance, 7-hydroxy-4- isopropyltropolone or beta thujaplicinol (which is beta thujaplicin with a hydroxyl group in the 7 position) from extractives of WRC. They reported that this substance occurred in concentrations of about one tenth that of thujaplicins, and that it had relatively low toxicity to fungi. Zavarin et al (38) isolated alpha thujaplicinol (7-hydroxy-6-isopropyltropolone) from Cupressus pygmaea Lemm. Roff and Whittaker (25) found beta thujaplicinol to be as toxic to brown rot fungi as gamma thujaplicin and sodium pentachlorophenate, but much less toxic to white rot fungi than either. Aulin-Erdtman (2) presented ultraviolet spectra for the thujaplicins, and molecular weight determinations for beta thujaplicin. Doering and Knox (10) and Cook et al (7) described methods for synthesizing various tropolones.

Gardner et al (14) studied the collection of tropolones including thujaplicins as copper chelates on a copper screen in a kiln drying WRC lumber. They also described the fractionation of this tropolone mixture. Gardner and Barton (13) studied the occurrence of thujaplicins in the steam volatile oil of WRC, and discussed separation of the thujaplicins from the oil.

Barton and Gardner (3) used acetone extraction of a large sample (2.8 kg) of WRC butt heartwood to determine the concentrations of beta and gamma thujaplicin and thujic acid

gravimetrically. They found that the wood contained 0.17 to 0.35% thujaplicins and 0.11 to 0.68% thujic acid. Maclean and Gardner (17) presented the first instrumental quantitative method for analyzing thujaplicins, involving colorimetry of the ferric chelates of these compounds. Johnson and Cserjesi (15) described a gas-liquid chromatographic method for analysis of tropolones and thujaplicin isomers. This method was used (16) to study the weathering effect on thujaplicin concentrations in WRC shakes. Zavarin and Anderson (35) and Zavarin et al (36,37) described the paper chromatography of tropolones, and determined optimal conditions for analysis, and discussed characterization of tropolones as dicyclohexylamine salts.

The decay resistance of WRC has been found to be widely variable. Buckland (5) studied and identified WRC decay organisms in British Columbia, and found that the extent of decay varied greatly between old and young trees and from stand to stand. Englerth and Scheffer (11) studied the decay resistance of four western species used as poles, and found WRC to be the most durable. They also showed that the decay resistance varied with radial position and height in the pole.

MacLean and Gardner (18) found the same variation in the concentration of thujaplicins in WRC heartwoods, ranging from 0 to 1.2%. MacLean and Gardner (19) also studied the concentration of thujaplicin in WRC "included sapwood" and found it to be the same as regular sapwood. Roff et al (26) studied WRC decay resistance relative to seasoning and log origin. They found that kiln drying did not significantly

change decay resistance, and that decay resistance varied with radial position. Scheffer (29) studied variations in WRC durability and found large tree to tree variations, and no relationships between durability and site or elevation. He also reported that decay resistance increased from pith to outer heartwood, then decreased in the sapwood. Swan and Jiang (31) and Swan et al (32) discussed the formation of heartwood extractives in WRC, (including thujaplicins and thujic acid) and reviewed their analysis by paper chromatography, thin layer chromatography, gas chromatography and colorimetry. They also proposed a sequence for formation of lignans through hydroxylation reactions in which thujaplicins play an inhibitory role. Their analysis of a 90 year old WRC (31) showed a maximum thujaplicin content of 0.34% occurring at 60 rings from the pith, but they did not analyze the complete radius. In their analysis of a 260 year old WRC (32) they found a maximum thujaplicin content of 0.42% at 210 rings from the pith, with concentrations increasing gradually from pith to this maximum, then decreasing rapidly in the sapwood.

Barton and MacDonald (4) presented a comprehensive review of WRC chemistry to 1971.

Nault (20) studied the radial distribution of thujaplicins in second growth WRC by a modification of the colorimetric method of Maclean and Gardner (17). He showed that the distribution of thujaplicin in second growth WRC followed the same pattern as in old growth trees, and that comparable maximum levels were reached in both.

Recently, Nault (21) developed a capillary gas-liquid chromatographic method for determining beta and gamma thujaplicin and thujic acid. This is the method used in the present study.

The major research period for the thujaplicins and related compounds was 1948-63. More recent work has been by Johnson and Cserjesi (15,16), Nault (20,21), Swan et al (31,32) Coombs and Trust (8) and Trust and Coombs (33).

MATERIALS AND METHODS

Breast height samples from six freshly felled old growth WRC were obtained from Western Forest Products at Port McNeill, B.C. These trees were cut from a stand about 11 kilometers west of Port McNeill, and ranged from 260 to 710 years of age (Appendix 1). Trees selected were free from obvious signs of butt rot.

A seventh old growth tree sample was obtained from UBC Forestry. It was cut from a freshly felled WRC grown at the UBC research forest in Haney, B.C. This tree was 420 years old (Appendix 1), and exhibited obvious signs of decay in the heartwood.

All old growth samples obtained were cut into strips across the diameter of the tree, about 10 cm wide and 5 cm deep. The sapwood was separated from these strips and the remainder was cut into sections consisting of enough growth rings to provide sufficient material for analyses (Figure 1, Appendix 2).

The samples were air-dried, then ground to pass a 50-mesh screen, with all material saved. The ground samples were extracted in a soxhlet with reagent grade ethanol:benzene (1:2), and the extractive content was determined by weight loss. The extractives from each sample were concentrated on a rotary evaporator, then made up to 25 ml.

Ethanol:benzene (1:2) extractives from a previous study by Nault (20) representing radial breast height samples from ten second growth WRC from two sites on Vancouver Island were also

included in the study (Appendix 4, Appendix 5). These samples and their extractives were prepared in a manner similar to that described. However, in these samples, heartwood and sapwood were not separated. The outermost sample in these radii thus contained heartwood and sapwood mixed in varying ratios.

Pure samples of beta and gamma thujaplicin and thujic acid were provided by Forintek Canada Corp. Diazomethane in ether was prepared from nitrosomethyl urea and potassium hydroxide (34).

Pure standards were prepared by dissolving the required amount of reagent in ethanol/benzene (1:2). The standards were methylated by addition of an equal volume of etheral diazomethane, then the solvent was evaporated at 70 °C. The methylated standards were then dissolved in ethanol/benzene (1:2) containing .00515 g/ml naphthalene as an internal standard. All extractive samples, pure standards, and derivatized samples were stored in a dark freezer to minimize degradation by heat or light.

All samples were analyzed on a Spectra-Physics SP7100 gas chromatograph with a dedicated data system and capillary capability. A 12M QC2/SE30 column with 0.02mm ID and 0.25 micrometer thick coating was used, with helium as the carrier gas. Oven temperature was held at 110 °C for 10 min., then raised to 200 C at 10 °C/min. Detection was accomplished with a flame ionization detector.

Peak area data were collected on the dedicated data system, and standard curves were prepared using 2 microliter

sample injections. Calibration curves were prepared for beta and gamma thujaplicin, and for thujic acid utilizing this technique. All extractive samples were analyzed using the same procedures as for the standards.

Because methylation of thujic acid yields methyl thujate, the reported concentrations are actually the sum of the concentrations of methyl thujate and thujic acid. Determination of methyl thujate could be performed easily by preparation of the ethyl derivatives, which would yield ethyl thujate from thujic acid leaving methyl thujate unchanged.

All samples were also analyzed colorimetrically for thujaplicin content by the method of MacLean and Gardner (17), as modified by Nault (20).

RESULTS

Seven old growth and ten second growth WRC trees were analyzed in radial increments from pith to bark (Appendices 3 and 6).

The ethanol:benzene (1:2) extractive content in all trees followed a general pattern of increasing from the pith to the most recently formed heartwood, then decreasing in the sapwood (Figures 2-4). All old growth trees attained much higher ethanol:benzene extractive contents than were exhibited in the second growth trees. Maxima ranged from 12.4% to 22.8% in the old growth trees, and from 6.2% to 9.8% in the second growth trees. Extractive contents in heartwoods seemed to be related to tree age (Figure 5).

Thujaplicin contents as determined by the colorimetric method (20) agreed roughly with those obtained by GLC (Figure 6,Appendix 7), yielding an r^2 value of 0.81 and a slope of 0.99 for GC vs colorimetric results. Colorimetric values tended to be slightly higher for second growth trees and slightly lower for old growth trees (Figures 7-23). To simplify reporting, all data in this paper are based on the GLC results, with thujaplicin content being the sum of beta and gamma isomer fractions.

Thujaplicin content in the second growth trees followed the pattern described by MacLean and Gardner (18), that is, very low content in the inner heartwood, with a rapid increase in the latest formed heartwood (Figures 7-16). However, the old growth trees did not demonstrate a similar pattern (Figures 17-22). The old growth trees showed appreciable thujaplicin

close to the pith. The maximum levels attained by old growth trees in this study were higher than any reported in the literature.

Thujaplicin contents also seemed to be directly related to tree age, with old growth trees having a maximum of 1.8% and second growth trees having a maximum of 0.7%. A plot of thujaplicins vs rings from pith shows this trend (Figures 24 and 25). Beta thujaplicin and gamma thujaplicin, when analyzed separately, also exhibited similar trends (Figures 26 to 29). No relationships were observed for relative amounts of beta and gamma thujaplicin and distance from pith. Thujaplicin contents seemed to be related also to extractive contents present in the samples (Figure 30).

Thujic acid contents (thujic acid plus methyl thujate) seemed to be related to extractive contents (Figure 31). No obvious relationship to thujaplicin content is seen (Figure 32), although thujic acid followed the same general pattern as thujaplicin, increasing from the pith through the outer heartwood, then decreasing in the sapwood (Figures 33-35). As well, no obvious relationship is seen between thujic acid and rings from pith (Figure 36).

The extractives from one second growth and one old growth tree analyzed with replication demonstrate the reproducibility of the GLC method (Table 1, Figures 37 and 38).

Figure 39 presents a summary of thujaplicin distribution in all 17 trees studied.

Table 2 presents a comparison of results from this study with results obtained by other researchers.

DISCUSSION

Ethanol:benzene extractive contents in the trees studied were generally in the ranges reported previously (Table 2), although some old growth trees exceeded any levels previously reported. Tree to tree variation was high, even in trees from the same stand (Figures 2,3,4). A surprising find is the general increase in extractive content with rings from pith (Figure 5).

This experiment is the first study of thujaplicins in WRC utilizing capillary GLC. The method is sensitive to low concentrations of thujaplicins, and requires no extensive sample preparation. The method gives repeatable results, as the two trees analyzed in triplicate demonstrate (Figures 37 and 38). This method is an improvement over the method of Johnson and Cserjesi (15) in that greater resolution of the components is possible due to the use of a capillary column. As well, the derivative preparation using diazomethane is easier and less time consuming than their method using silylation.

The data collected on the GC exhibited several major peaks which have not yet been identified. When these peaks are identified, and calibration curves prepared, more information on the overall pattern of extractive distribution will be available.

The small volumes of sample required for injection (2 microliters) and the high sensitivity of the method suggests that by decreasing sample size and extraction volumes, very small samples could be analyzed. This is a major advantage over

the standard colorimetric method, and could conceivably be used to analyze increment cores from standing trees.

Concerns have been expressed by the forest products industry on the quality of wood from our future forests, where intensive management will result in harvesting trees at much younger ages than has been the case for existing old-growth stands. This study suggests that level of thujaplicins in WRC is related to the age of the tree (Figures 24 and 25). In the case of second growth stands, harvesting at relatively young ages may give wood with lower concentrations of these compounds. This could conceivably yield wood that is less durable to decay and weathering (traditional characteristics for which WRC wood has been valued).

However, the high variability in thujaplicin concentrations in old growth and second growth trees suggests that there is more involved than simply the age of the tree. This variability is clearly illustrated by the differences between second growth Trees #1.1 (Figure 7) and #1.2 (Figure 8). Tree #1.1 has a maximum thujaplicin content of 0.323% in the last sample of pure heartwood, while Tree #1.2 has a maximum of only 0.108%, and reached that value at only 10-15 rings from pith. In the old growth trees studied, trees #5 (Figure 21) and #7 (Figure 23) represent extremes. Tree #5 attained a maximum of 1.77% thujaplicin, while tree #7 had a maximum content of only 0.28%. This agrees with earlier results presented by Buckland (5) and Scheffer (29), both of whom found great variability in WRC wood decay. Perhaps the level of

thujaplicin is a genetically controlled property, and trees could be selected for propagation with this property in mind. As stated, the new GC technique used with non-destructive increment cores could assist in such selection.

The maximum levels of thujaplicin reported here, as up to 1.8%, exceed any previously reported in the literature for WRC. The possibility exists that previous researchers had not protected their extractive samples from light, and that photochemical decomposition of the thujaplicins had occurred as described by Coombs and Trust (8). This would result in the reported values being lower than the actual values. Also, the colorimetric method (17) assumes a constant ratio of beta and gamma thujaplicin, with gamma making up 60% of the total thujaplicins. Any deviation from this ratio could change the absorbance maximum of the sample, and give erroneous results. Since the samples studied varied from 0% to 90% gamma thujaplicin (Appendices 3 and 6), this assumption is shown to be false. However, the magnitude of the error that this difference in ratios would cause is not presently known. As well, the pattern of fairly rapid increase of thujaplicin concentration in the heartwood of old growth trees (Figures 17 through 23) differs from the work of MacLean and Gardner (18) and Swan and Jiang (31), who found very low levels in the oldest heartwood of the trees they studied.

The one old growth WRC tree which did not have high levels of thujaplicin (Tree #7) was selected for sampling on the basis of evident decay. The fact that the tree showed signs of decay

and low thujaplicin content supports the theory that thujaplicins play a major role in WRC decay resistance. Whether the decay was caused by the absence of thujaplicins, or the thujaplicins had been inactivated by the decay organisms remains a question for further research. Also, it is noteworthy that this tree had roughly the same levels of thujic acid as did the other old growth trees (Figure 35).

CONCLUSION

The new GLC method is a sensitive and convenient method for analyzing thujaplicins and associated compounds in WRC extractives. The results obtained by this method are comparable to those obtained by colorimetric analysis.

Thujaplicin content seemed to be directly related to tree age, although tree to tree variability was found to be high. Further research to determine if this variabiltiy is a genetically controlled property could prove informative. Non-destructive increment cores would furnish enough material for analysis by the new GLC method.

Thujic acid was found to vary in generally the same pattern as thujaplicins.

LITERATURE CITED

1. Anderson, A. B. and J. Gripenberg. 1948. Antibiotic substances from the heartwood of Thuja plicata D. Don. IV. The constitution of thujaplicin. Acta. Chem. Scand. 2:644-650.

2. Aulin-Erdtman, G. 1950. Studies in the tropolone series. 1. Thujaplicins and nootkatin. Acta. Chem. Scand. 4:1031-1041.

3. Barton, G. M. and J.A.F. Gardner. 1954. The chemical nature of the acetone extractive of western red cedar. Pulp Paper Mag. Can. 55(10):132-137.

4. ---------- and B.F. MacDonald. 1971. The chemistry and utilization of western red cedar. Dept. Fisheries and Forestry, Canadian Forestry Service. Pub. No. 1023:13-19.

5. Buckland, D. C. 1946. Investigations of decay in western red cedar in British Columbia. Can. J. Res. C24:158-181.

6. Canadian Forestry Statistics. 1982. Statistics Canada. p33.

7. Cook, J. W. , Raphael, R. A. and A. I. Scott. 1951. Tropolones. Part II. The synthesis of alpha, beta, and gamma thujaplicins. J. Chem. Soc. (London). 1951:695-698.

8. Coombs, R. W. and T. J. Trust. 1973. The effect of light on the antibacterial activity of beta thujaplicin. Can. J. Microbiol. 19(10):1177-1180.

9. Council of Forest Industry Statistics. 1984. COFI.

10. Doering, W. and L.H. Knox. 1953. Synthesis of substituted tropolones. J. Amer. Chem. Soc. 75:297.

11. Englerth, G. H. and T. C. Scheffer. 1954. Tests of decay resistance of four western pole species. USDA Forest Service, Forest Products Laboratory, Madison. Pub. No. 2006. 8pp.

12. Erdtman, H. and J. Gripenberg. 1948. Antibiotic substances from the heartwood of Thuja plicata D. Don. 2. The constitution of gamma thujaplicin. Acta Chem. Scand. 2:625-643.

13. Gardner, J. A. F. and G. M. Barton. 1958. Occurrence of beta dolabrin (4-isopropenyl tropolone) in western red cedar. Can. J. Chem. 36:1612-1615.

14. Gardner, J. A. F., Barton, G. M. and H. MacLean. 1957. Occurrence of 2,7-dihydroxy-4-isopropyl-2,4,6-cycloheptatrien-1-one (7-hydroxy-4-isopropyl-tropolone) in western red cedar Thuja plicata D. Don. Canadian J. Chem. 35:1039-1048.

15. Johnson, E. L. and A.J. Cserjesi. 1975. Gas-liquid chromatography of some tropolone-TMS ethers. J. Chrom. 107:p388.

16. ----------. 1980. Weathering effect on thujaplicin concentration in western redcedar shakes. Forest Prod. J. 30(6):52-53.

17. MacLean, H. and J. A. F. Gardner. 1956. Analytical methods for thujaplicins. Anal. Chem. 28:509-512.

18. ----------. 1956. Distribution of fungicidal extractives (thujaplicins and water soluble phenols) in western red cedar heartwood. Forest Prod. J. 6(12):510-516.

19. ----------. 1958. Distribution of fungicidal extractives in target pattern heartwood of western red cedar. Forest Prod. J. 8(3):107-108.

20. Nault, J. R. 1985. Distribution of thujaplicins in fast-grown, second growth Thuja plicata Don. Unpublished research report, Dept. of Forestry, UBC.

21. ----------. 1986. A capillary gas chromatographic method for thujaplicins in extractives Thuja plicata Don. Unpublished research report, Dept. of Forestry, UBC.

22. Nozoe, T. Yasue, A. and K. Yamane. 1951. On the acidic constituents of Thujopsis dolabrata. Occurence of alpha thujaplicin. Proc. Japan Acad. 27(1):15-17.

23. Raa, J. and J. Goksoyr. 1965. Studies of the effects of the heartwood toxin beta-thujaplicin on the metabolism of yeast. Physiol. Plant. 18:159-176.

24. Rennerfelt, E. 1948. Thujaplicin, a fungicidal substance in the heartwood of Thuja plicata. Physiol. Plant. 1:245-254.

25. Roff, J. W. and E. I. Whittaker. 1959. Toxicity tests of a new tropolone, beta thujaplicinol (7-hydroxy-4-isopropyl tropolone) occuring in western red cedar. Can. J. Botany. 37:1132-1134.

26. Roff, J. W., Whittaker, E. I. and H. W. Eades. 1962. Decay resistance of western red cedar relative to kiln seasoning, colour and origin of the wood. Can. Dept. Forestry, Forest Products Research Branch, Tech. Note No.32:3-19.

27. Rudman, P. 1962. The causes of natural durability in timber. Part IX: The antifungal activity of heartwood extractives in a wood substrate. Holzforsch. 16(3):74-77.

28. ----------. 1963. The causes of natural durability in timber. Part XI: Some tests on the fungi toxicity of wood extractives and related compounds. Holzforsch. 17(2):54-57.

29. Scheffer, T. C. 1957. Decay resistance of western redcedar. J. Forestry. 55(6):434-442.

30. Sowder, A. M. 1929. Toxicity of water soluble extractives and relative durability of water-treated wood flour of western red cedar. Ind. Eng. Chem. 21:981-988.

31. Swan, E. P. and K. S. Jiang. 1970. Formation of heartwood extractives in western red cedar. TAPPI 53(5):844-846

32. ---------- and J. A. F. Gardner. 1969. The lignans of Thuja plicata and the sapwood-heartwood transformation. Phytochem. 8:345-351.

33. Trust, T. J. and R. W. Coombs. 1973. Antibacterial activity of beta thujaplicin. Can. J. Microbiol. 19(11):1341-1346.

34. Vogel, A. I. 1948. In Practical Organic Chemistry. Longmans, Green and Co., London. 1948:843-845.

35. Zavarin, E. and A. B. Anderson. 1956. Paper chromatography of the tropolones of Cupressacae. J. Org. Chem. 21:332-335.

36. ---------- and R. M. Smith. 1959. Paper chromatography of the tropolones of Cupressacae: J. Org. Chem. 24:1318-1321

37. ----------, Smith, R. M. and A. B. Anderson. 1959. Characterization of Cupressacae tropolones as dicyclohexylamine salts. J. Org. Chem. 24:1584-1585.

38. Zavarin, E., Smith, R. M. and A. B. Anderson. 1961. On the occurence of alpha thujaplicinol in the heartwood of Cupressus pygmaea (Lemm.) Sarg. J. Org. Chem. 26:173-176.

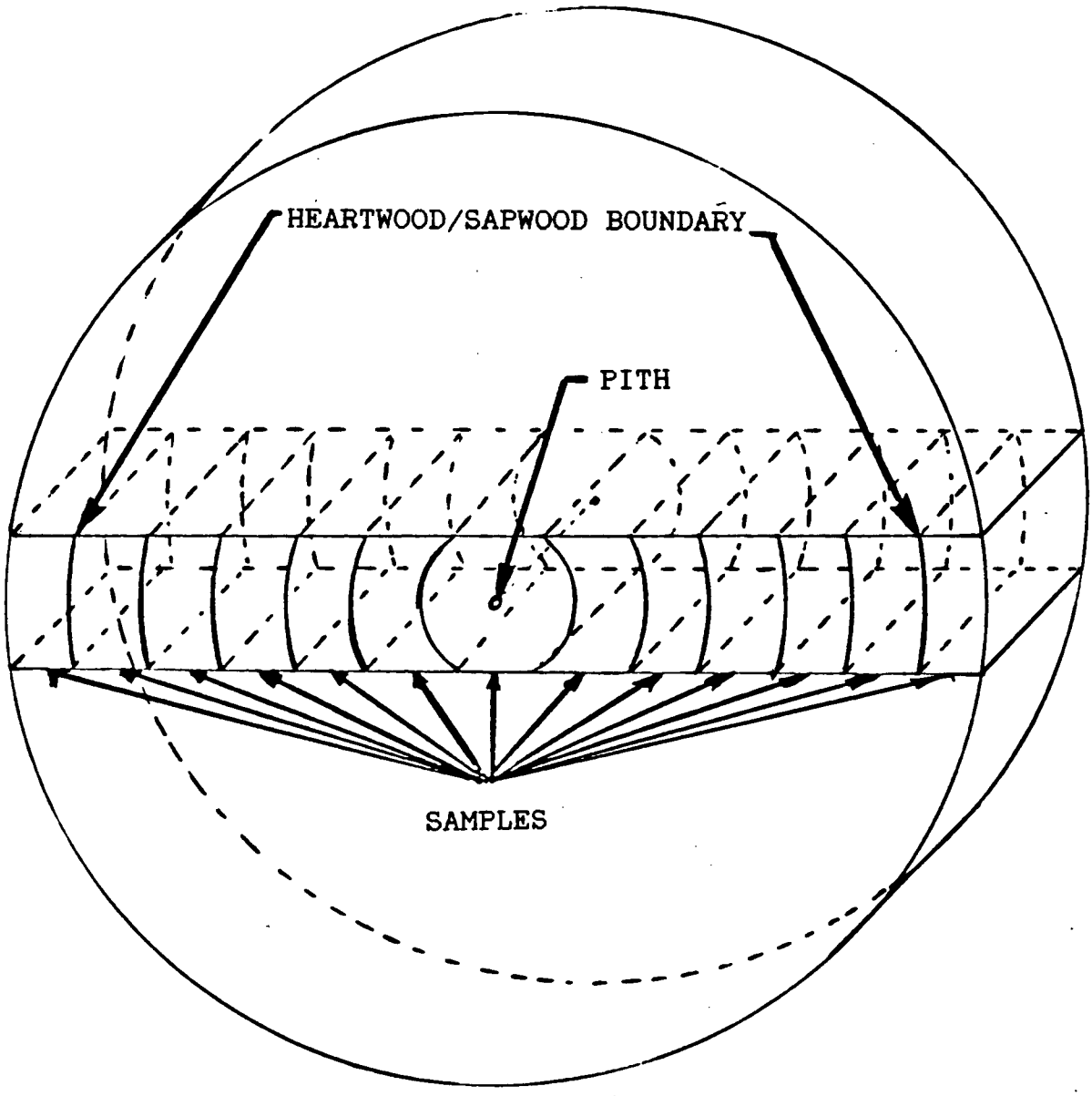
TABLE 1. GC SAMPLES ANALYZED WITH REPLICATION

SAMPLE NAME	AVERAGE # RINGS FROM PITH	THUJAPLICINS (% O.D.WOOD) TRIAL 1 BETA	TRIAL 1 GAMMA	TRIAL 2 BETA	TRIAL 2 GAMMA	TRIAL 3 BETA	TRIAL 3 GAMMA	AVERAGE BETA	AVERAGE GAMMA
SECOND	2.5	0	.03	0	.03	0	.03	0	.03
GROWTH	7.5	0	.02	0	.05	0	.04	0	.04
TREE	12.5	0	.05	0	.06	0	.07	0	.06
# 1.1	17.5	.07	.13	.04	.15	.08	.13	.06	.14
	25	.15	.27	.15	.25	.16	.28	.15	.27
	35	.19	.24	.21	.28	.25	.29	.22	.27
	45	.08	.11	.08	.13	.09	.21	.08	.15
OLD	40	.06	.07	.07	.08	.02	.08	.05	.08
GROWTH	125	.08	.24	.04	.27	.08	.36	.07	.29
TREE	210	.10	.50	.09	.44	.05	.48	.08	.47
# 3	310	.12	.34	.12	.33	.09	.31	.11	.33
	410	.14	.56	.14	.51	.11	.58	.13	.55
	475	.22	.38	.16	.39	.15	.38	.18	.38
	558	.30	.58	.21	.58	.18	.59	.23	.58
	610	.02	.04	.33	.04	.25	.03	.20	.04

TABLE 2. COMPARISON OF DATA WITH LITERATURE VALUES

SOURCE OF DATA	ETHANOL:BENZENE EXTRACTIVES	COMPONENT THUJAPLICINS (%)	THUJIC ACID (%)	ANALYTICAL METHOD
(3)	13.8	0.17-0.35	0.11-0.68	GRAVIMETRIC
(16)	----	0-0.546	----	GC
(18)	----	0-1.22	----	COLORIMETRY
(19)	----	0-0.27	----	COLORIMETRY
(31)	----	0-0.42	----	PAPER CHROM.
(32)	----	0.01-0.34	----	COLORIMETRY
NAULT	6.2-22.8	0.004-1.8	0.017-1.024	COLORIMETRY AND CAP. GC

FIGURE 1. SAMPLING PROCEDURE



BREAST HEIGHT RADIAL CROSS SECTION

FIGURE 2. RADIAL DISTRIBUTION OF ETHANOL:BENZENE EXTRACTIVES FOR SECOND GROWTH SITE #1

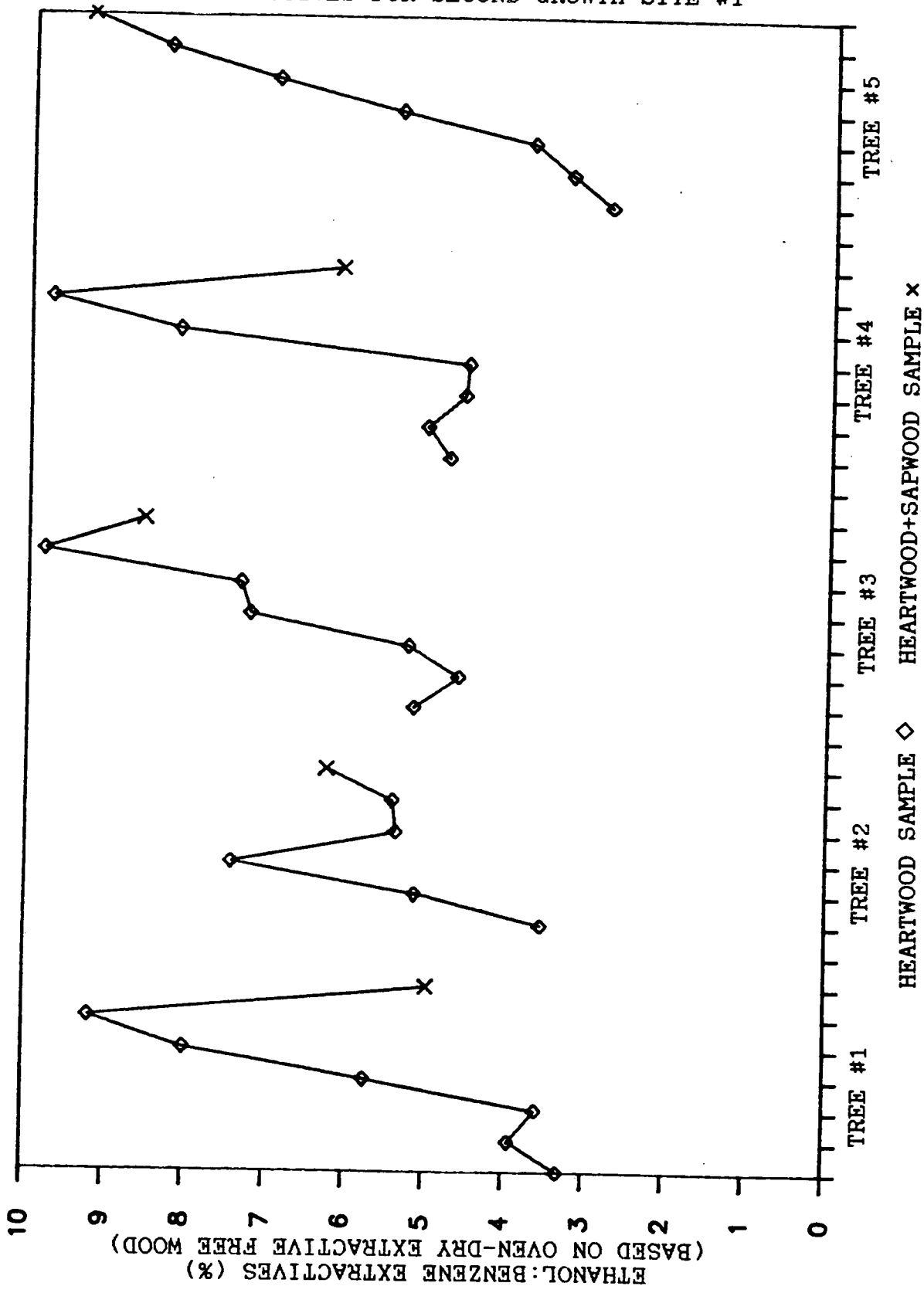


FIGURE 3. RADIAL DISTRIBUTION OF ETHANOL BENZENE EXTRACTIVES FOR SECOND GROWTH SITE #2

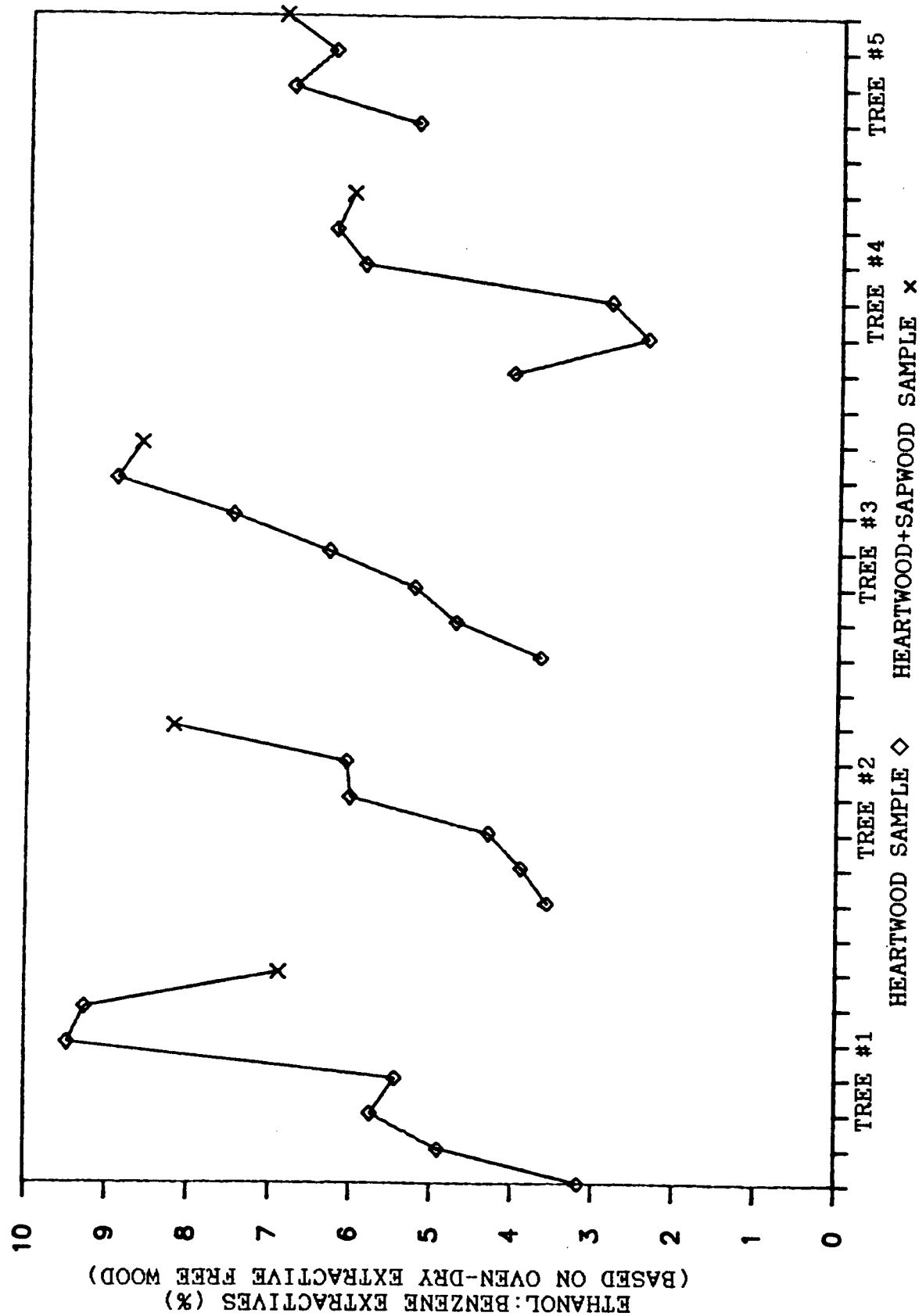


FIGURE 4. RADIAL DISTRIBUTION OF ETHANOL:BENZENE EXTRACTIVES FOR OLD GROWTH TREES

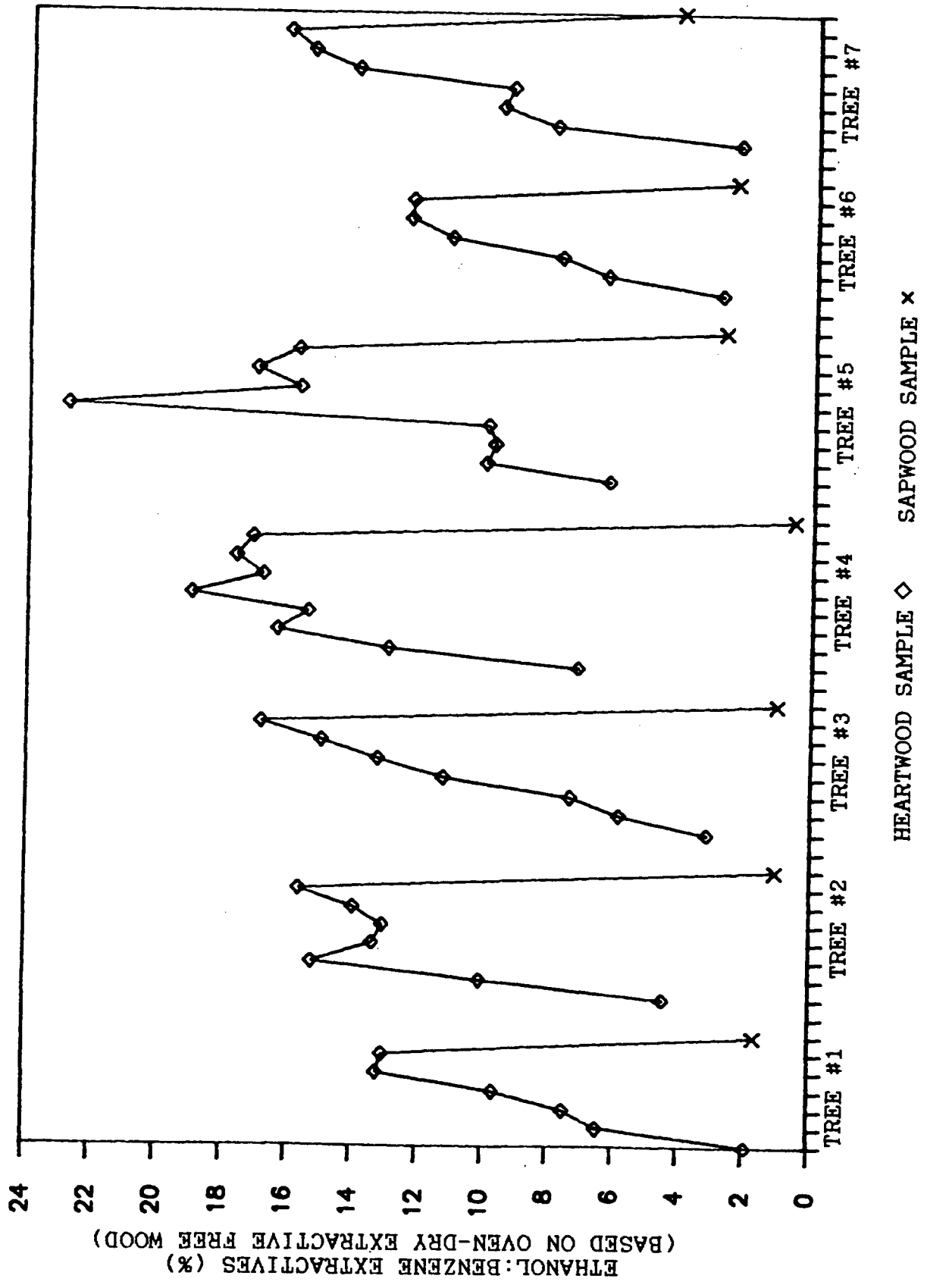


FIGURE 5. ETHANOL:BENZENE EXTRACTIVE CONTENT VS AVERAGE RINGS FROM PITH FOR ALL TREES

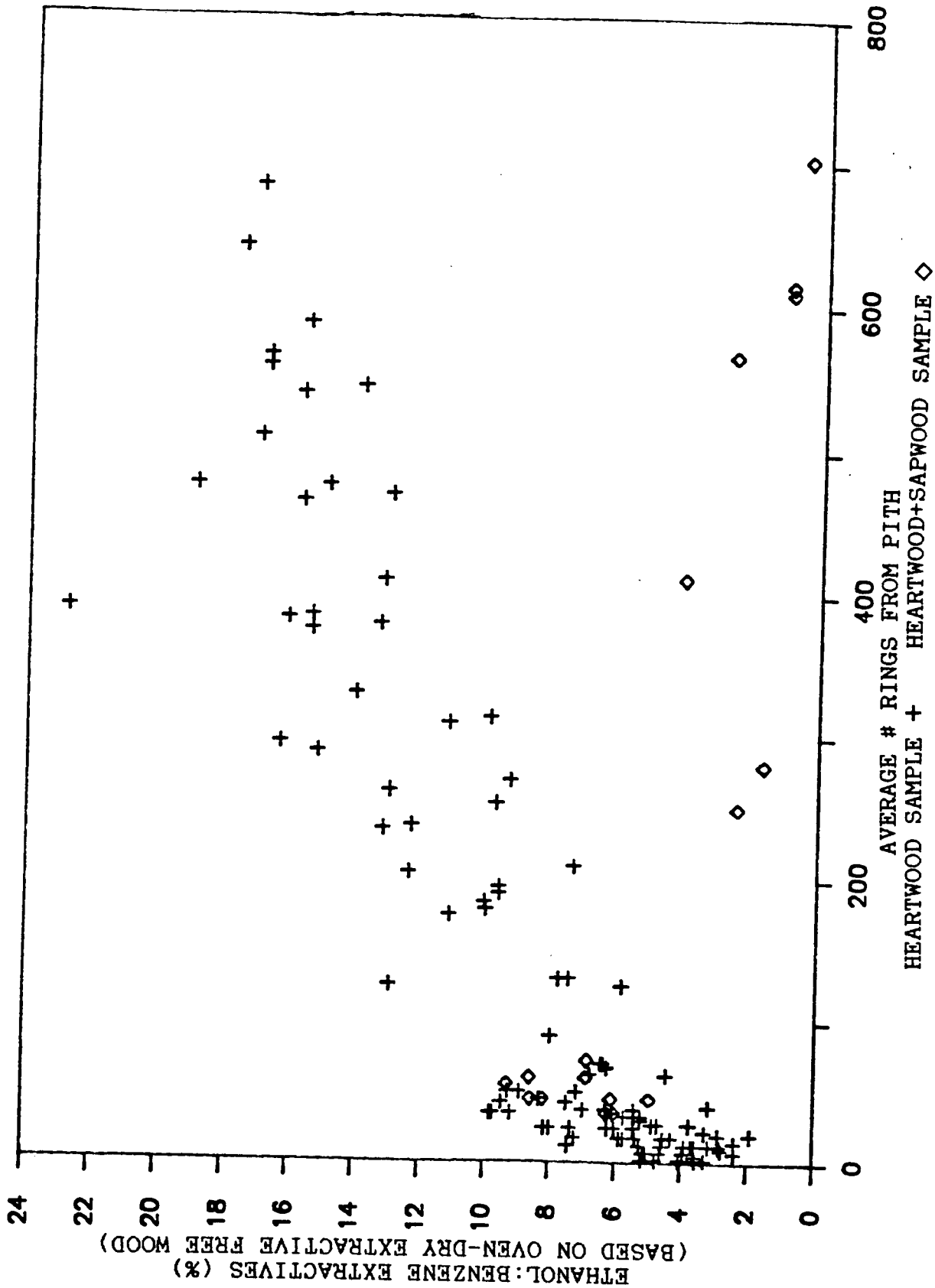


FIGURE 6. COMPARISON OF THUJAPLICIN RESULTS OBTAINED BY COLORIMETRY AND GLC

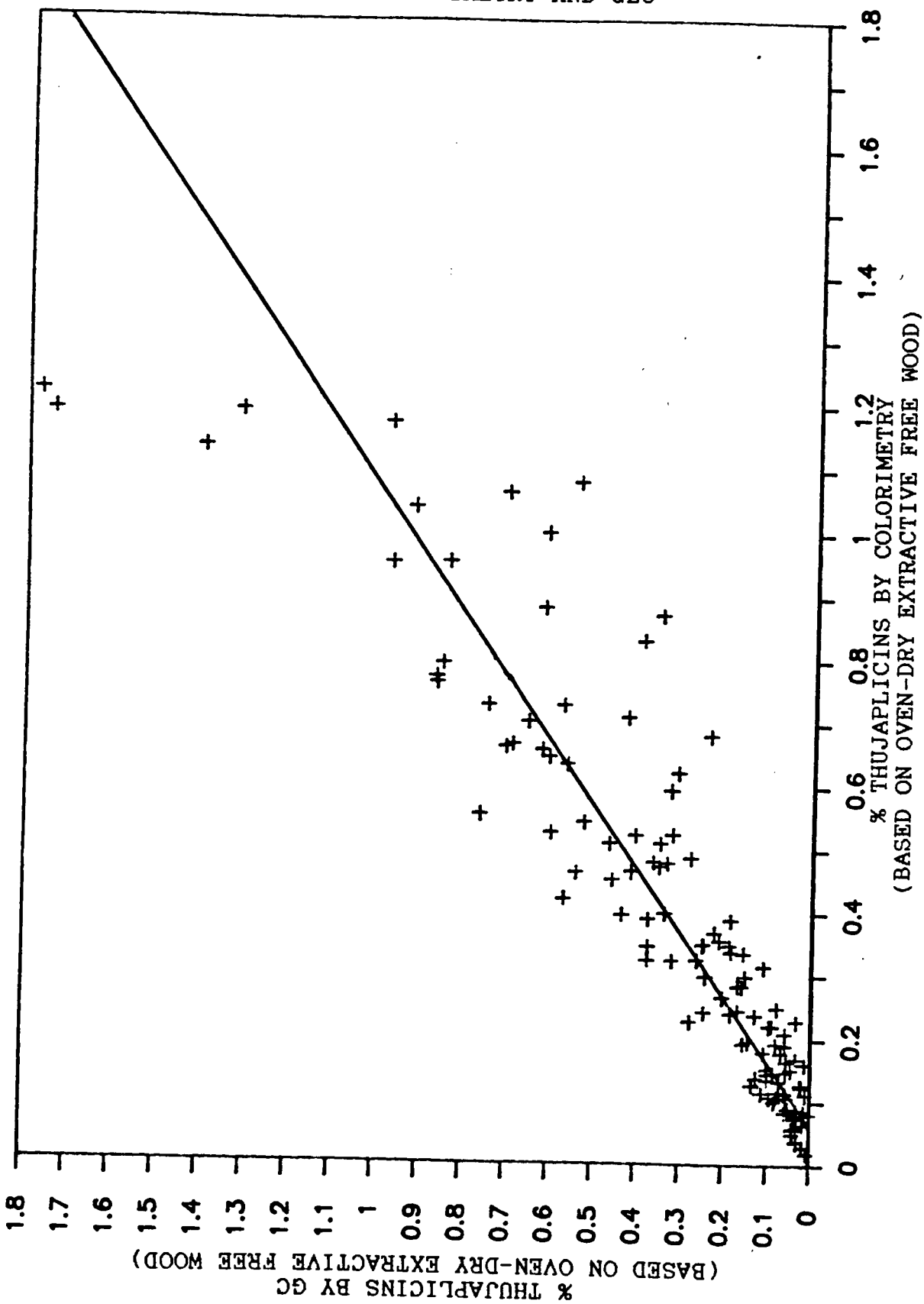


FIGURE 7. THUJAPLICIN CONTENT BY TWO METHODS VS AVERAGE RINGS FROM PITH FOR SECOND GROWTH TREE #1.1

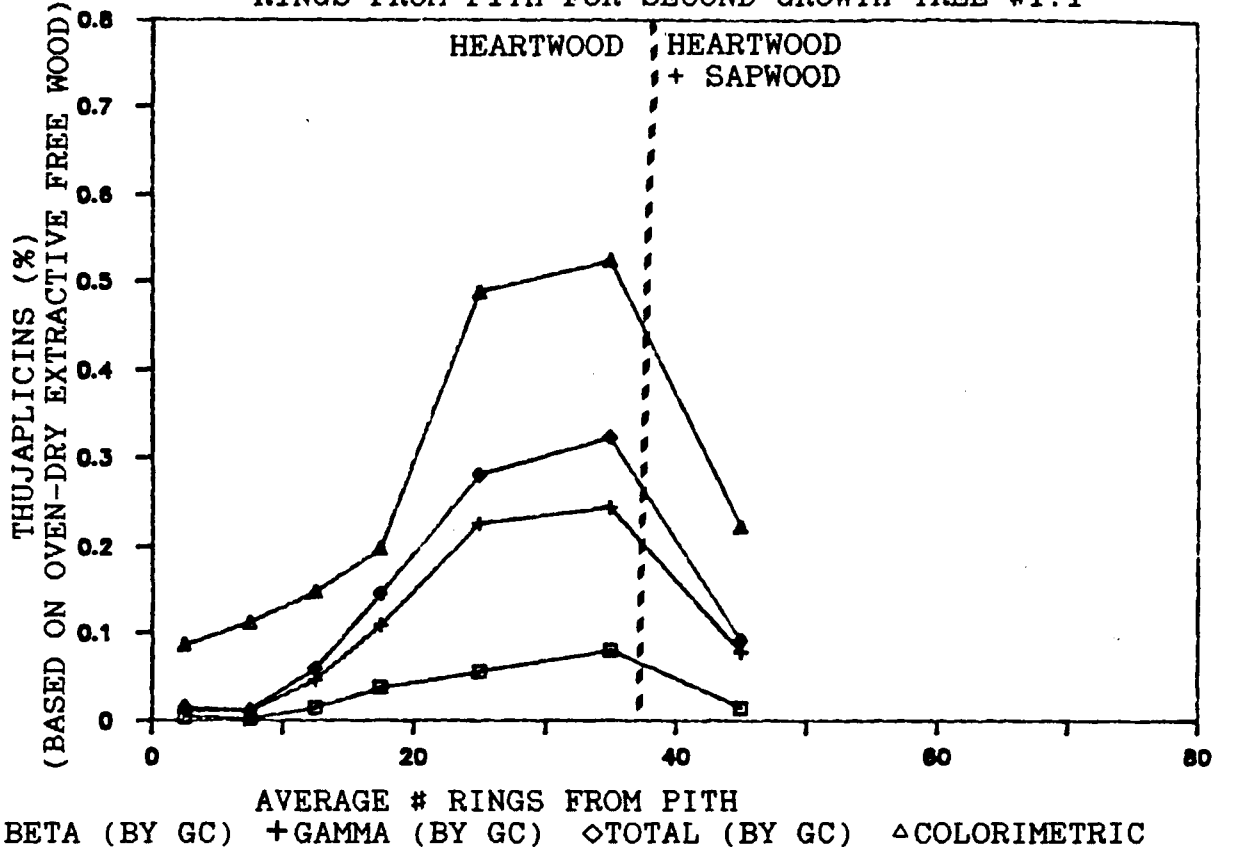


FIGURE 8. THUJAPLICIN CONTENT BY TWO METHODS VS AVERAGE RINGS FROM PITH FOR SECOND GROWTH TREE #1.2

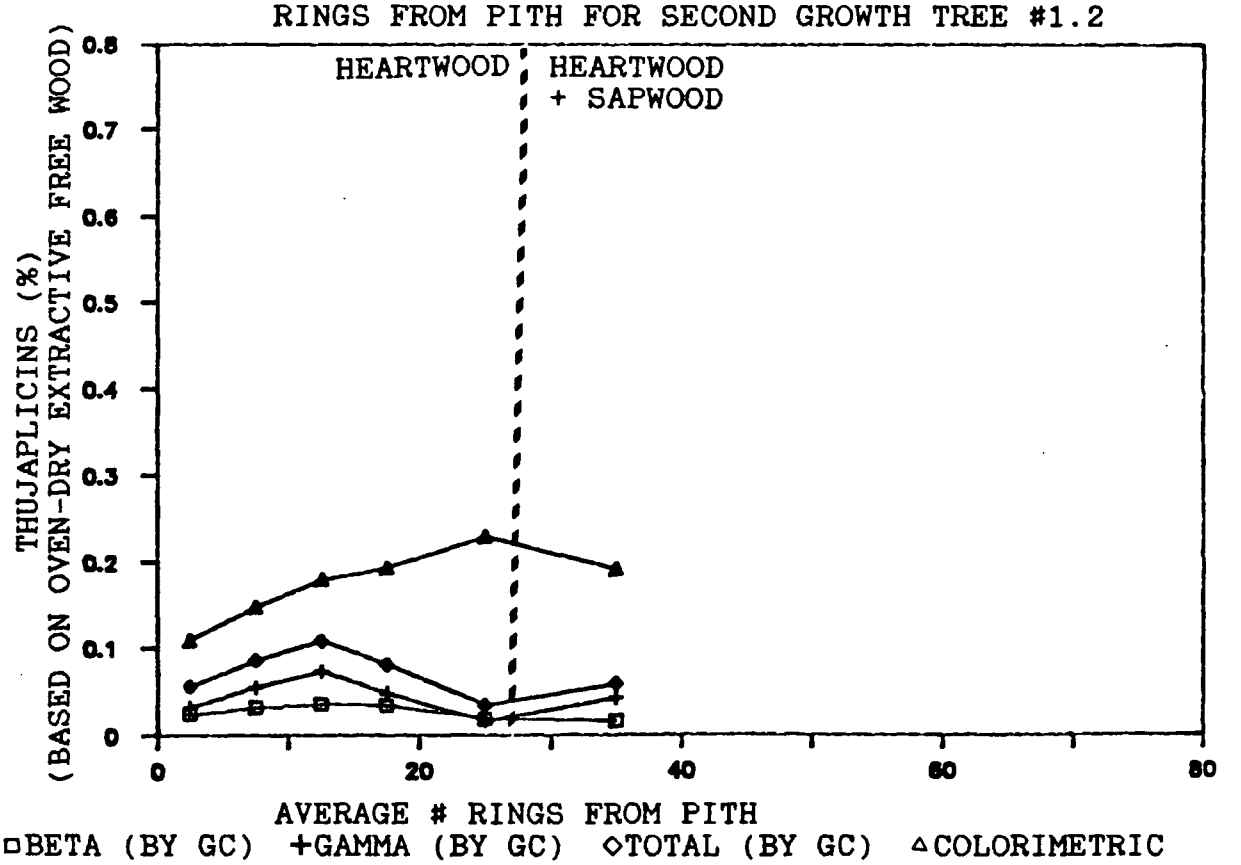


FIGURE 9. THUJAPLICIN CONTENT BY TWO METHODS VS AVERAGE RINGS FROM PITH FOR SECOND GROWTH TREE #1.3

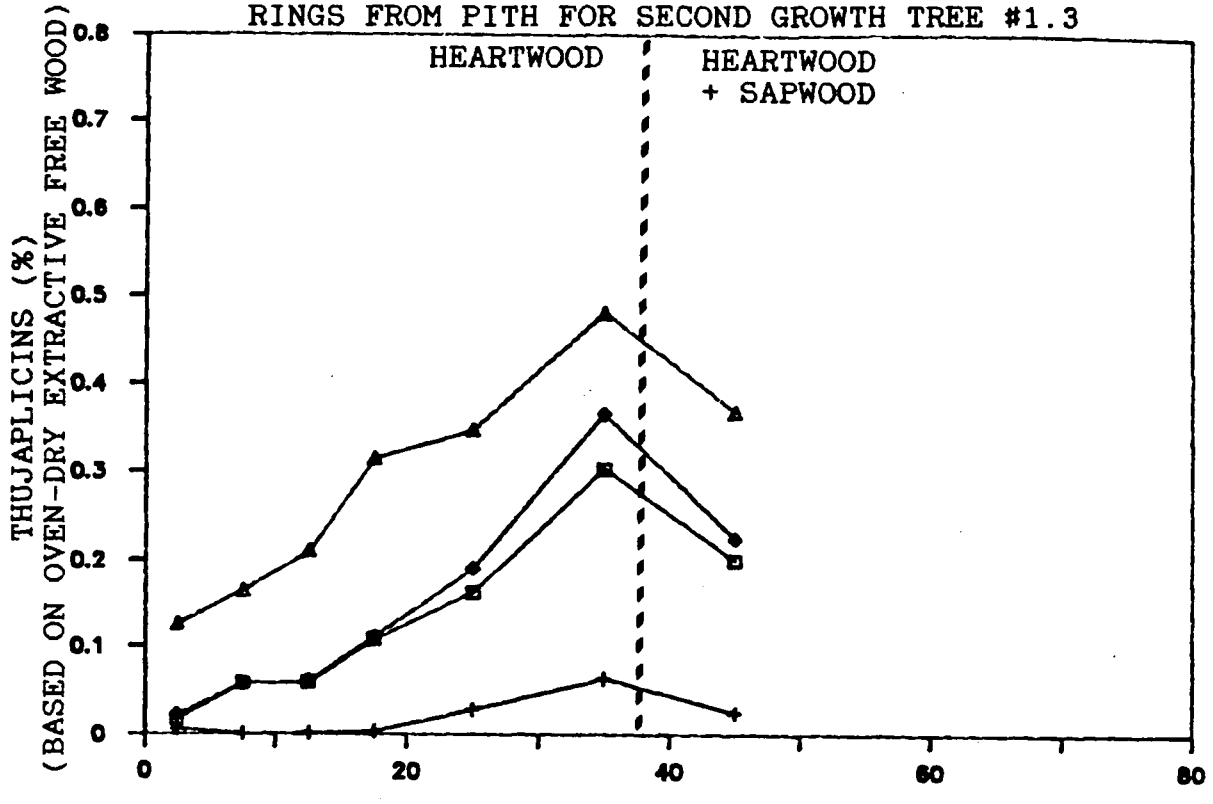


FIGURE 10. THUJAPLICIN CONTENT BY TWO METHODS VS AVERAGE RINGS FROM PITH FOR SECOND GROWTH TREE #1.4

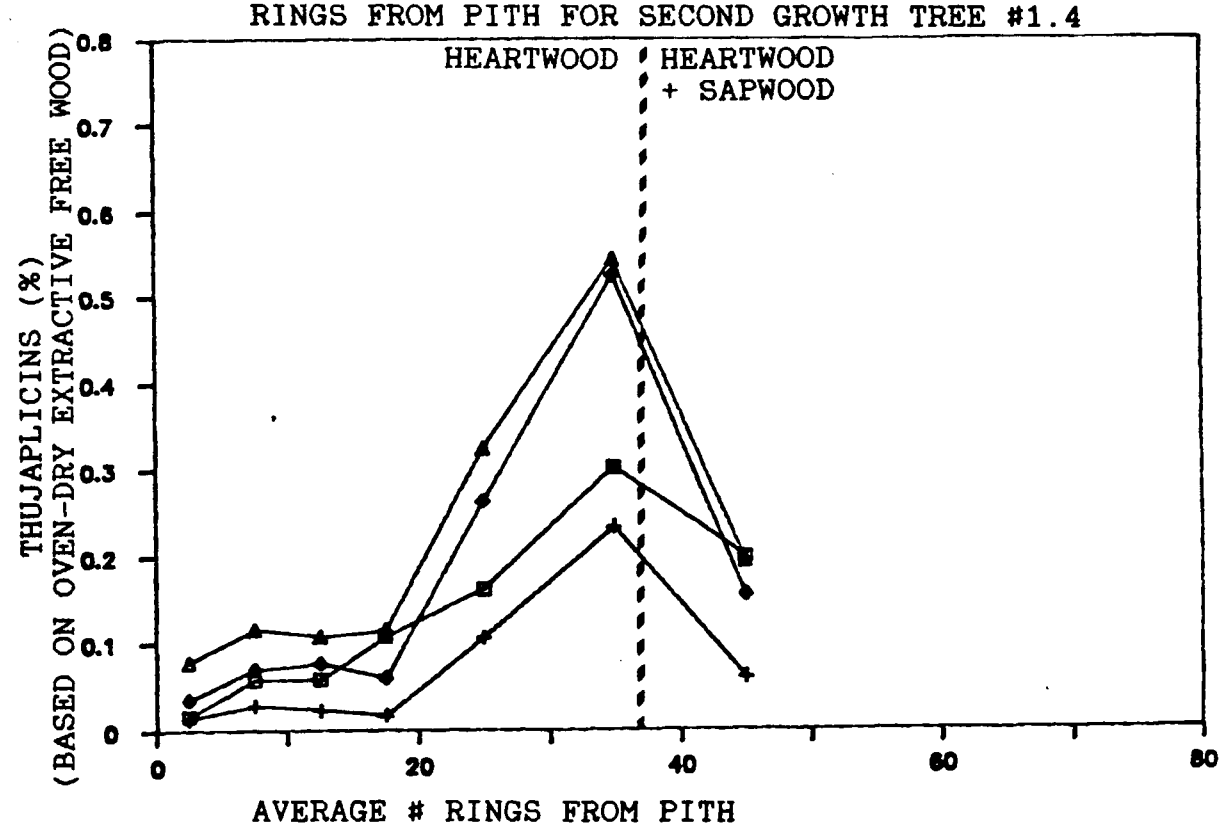


FIGURE 11. THUJAPLICIN CONTENT BY TWO METHODS VS AVERAGE RINGS FROM PITH FOR SECOND GROWTH TREE #1.5

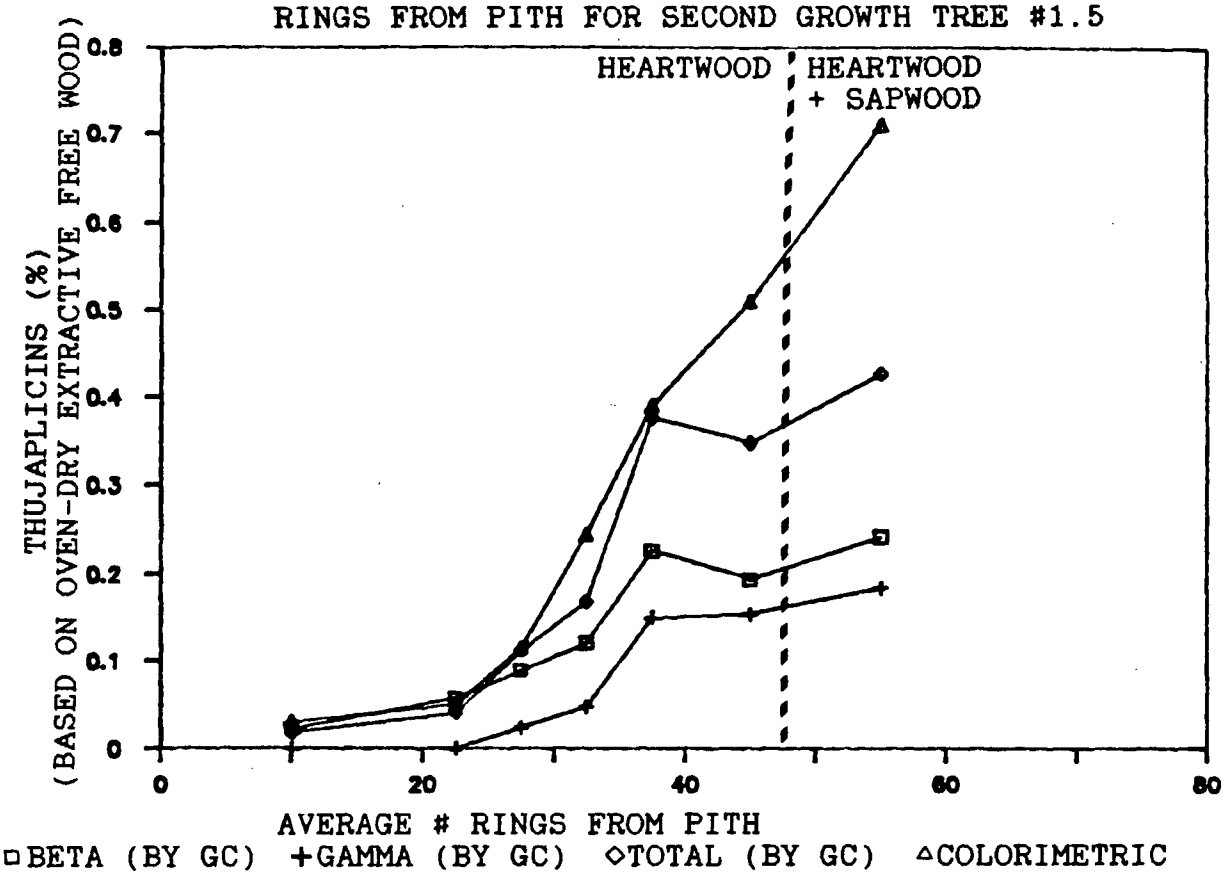


FIGURE 12. THUJAPLICIN CONTENT BY TWO METHODS VS AVERAGE RINGS FROM PITH FOR SECOND GROWTH TREE #2.1

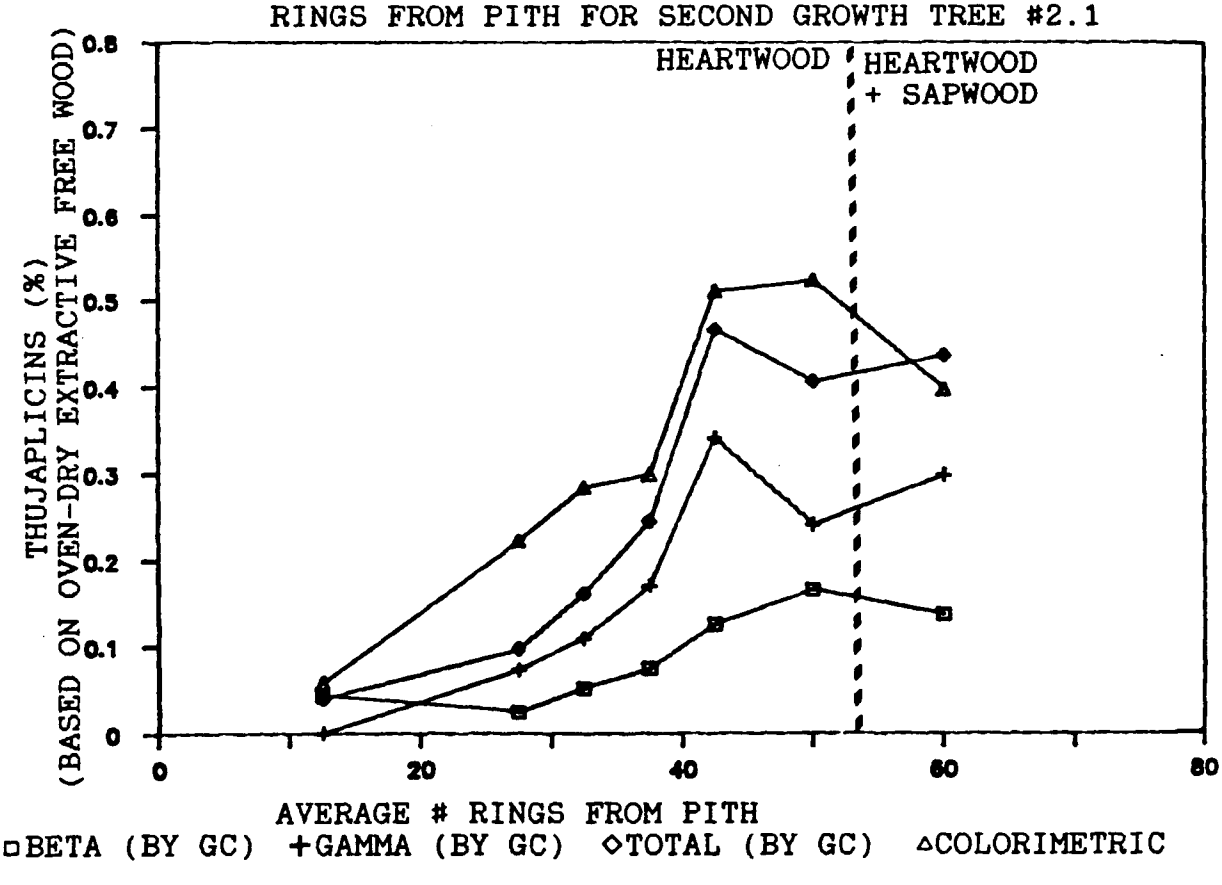


FIGURE 13. THUJAPLICIN CONTENT BY TWO METHODS VS AVERAGE RINGS FROM PITH FOR SECOND GROWTH TREE #2.2

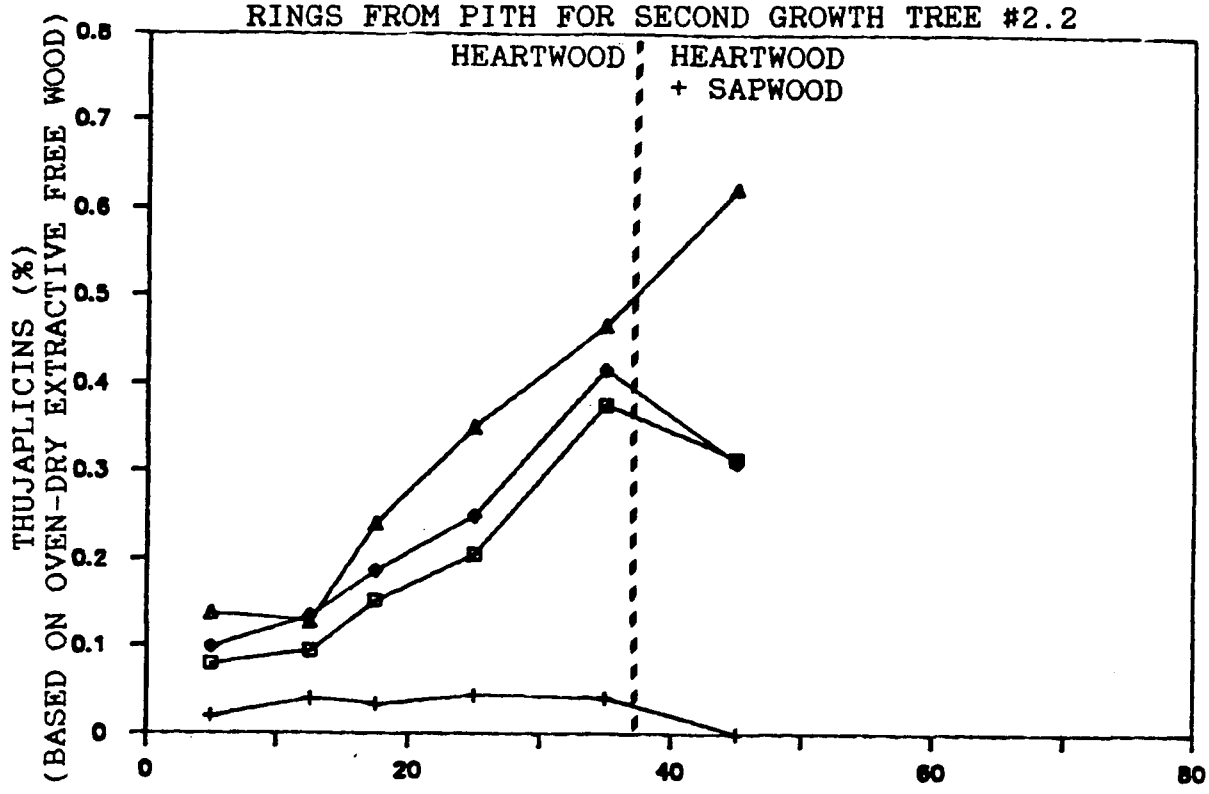


FIGURE 14. THUJAPLICIN CONTENT BY TWO METHODS VS AVERAGE RINGS FROM PITH FOR SECOND GROWTH TREE #2.3

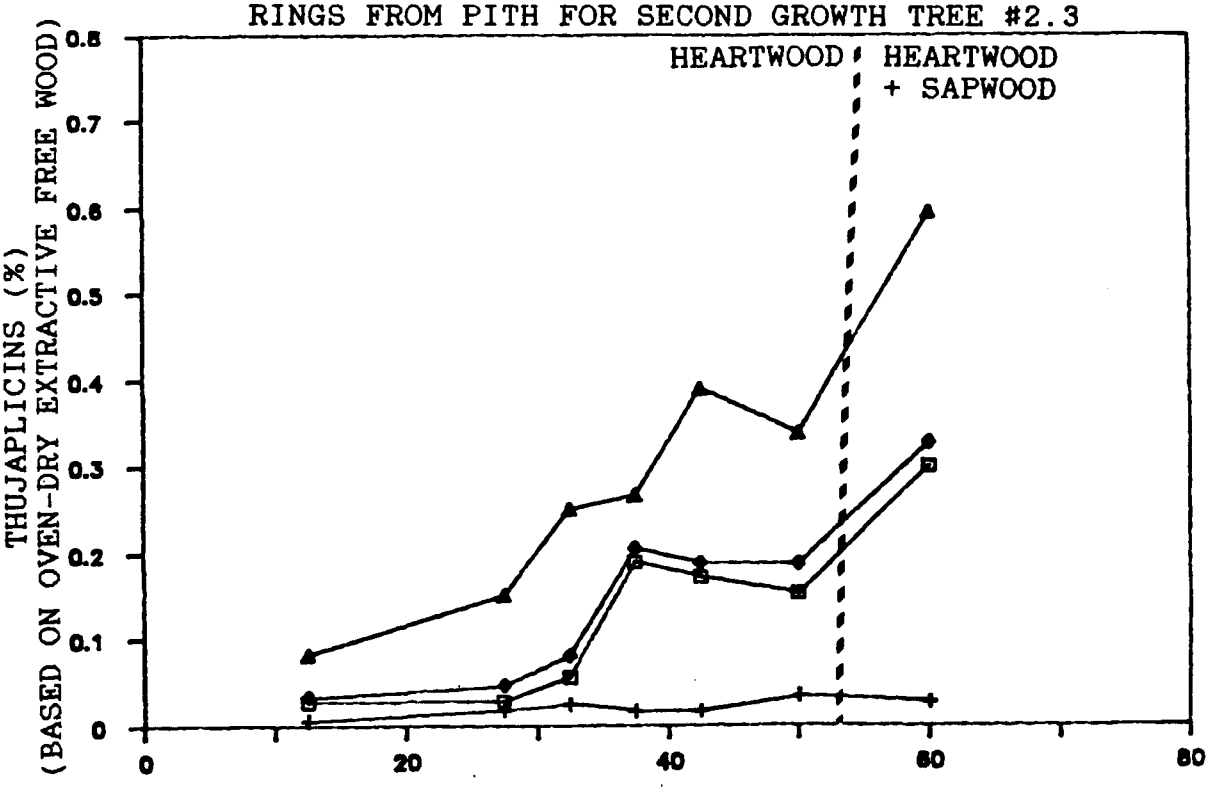
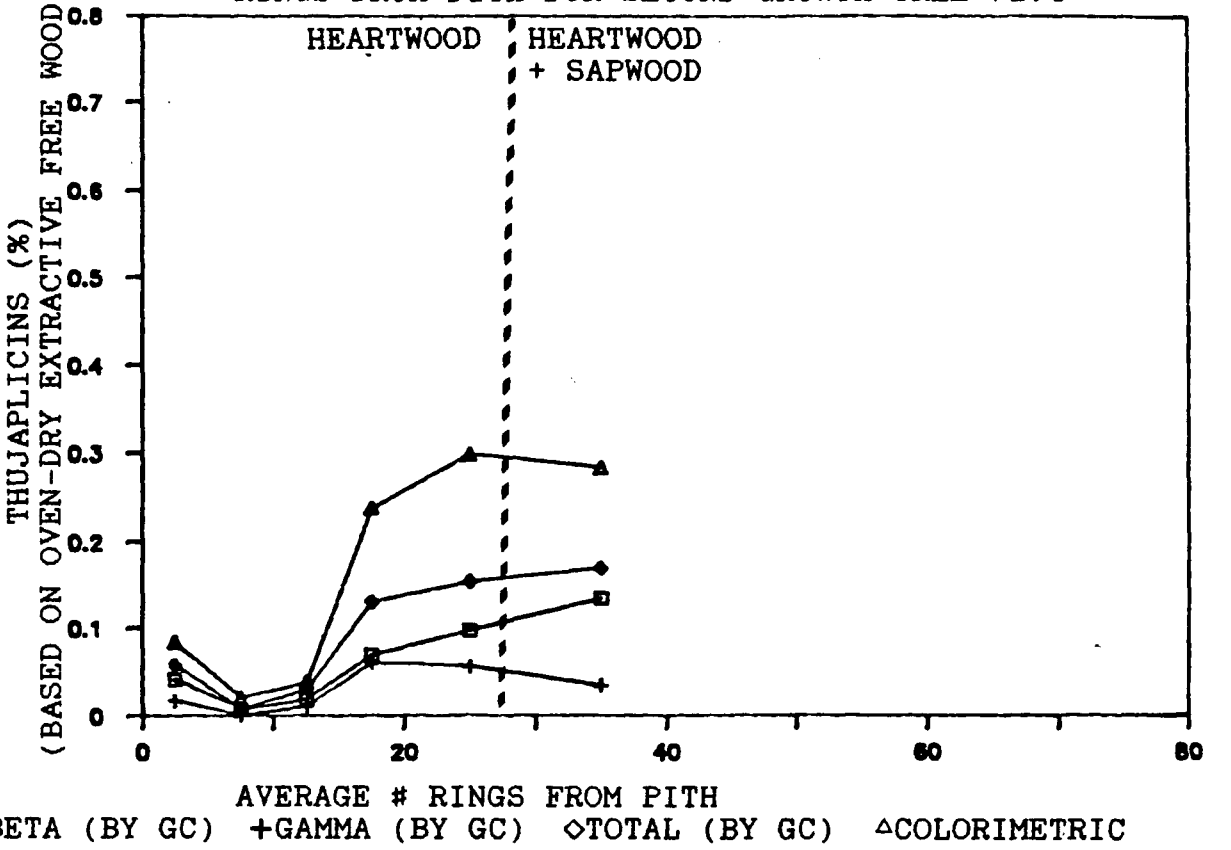
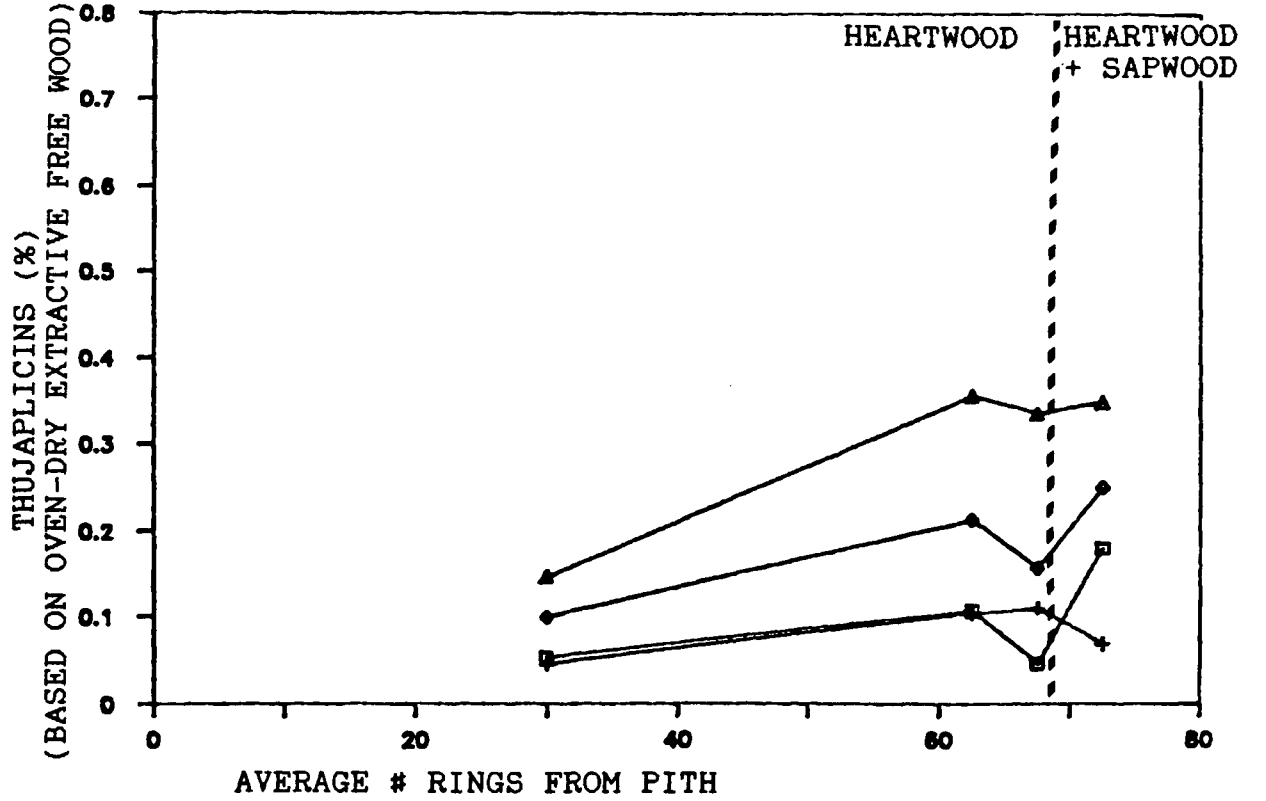


FIGURE 15. THUJAPLICIN CONTENT BY TWO METHODS VS AVERAGE RINGS FROM PITH FOR SECOND GROWTH TREE #2.4



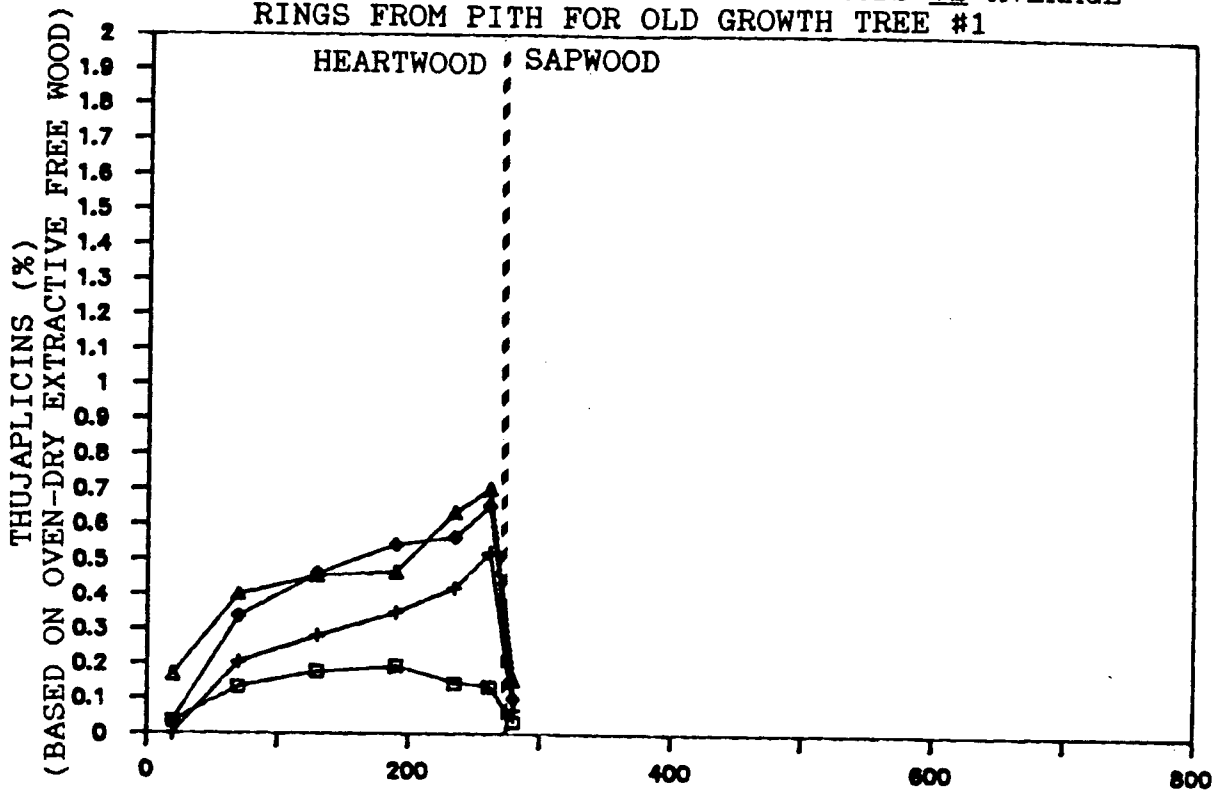
□BETA (BY GC) +GAMMA (BY GC) ◇TOTAL (BY GC) △COLORIMETRIC

FIGURE 16. THUJAPLICIN CONTENT BY TWO METHODS VS AVERAGE RINGS FROM PITH FOR SECOND GROWTH TREE #2.5



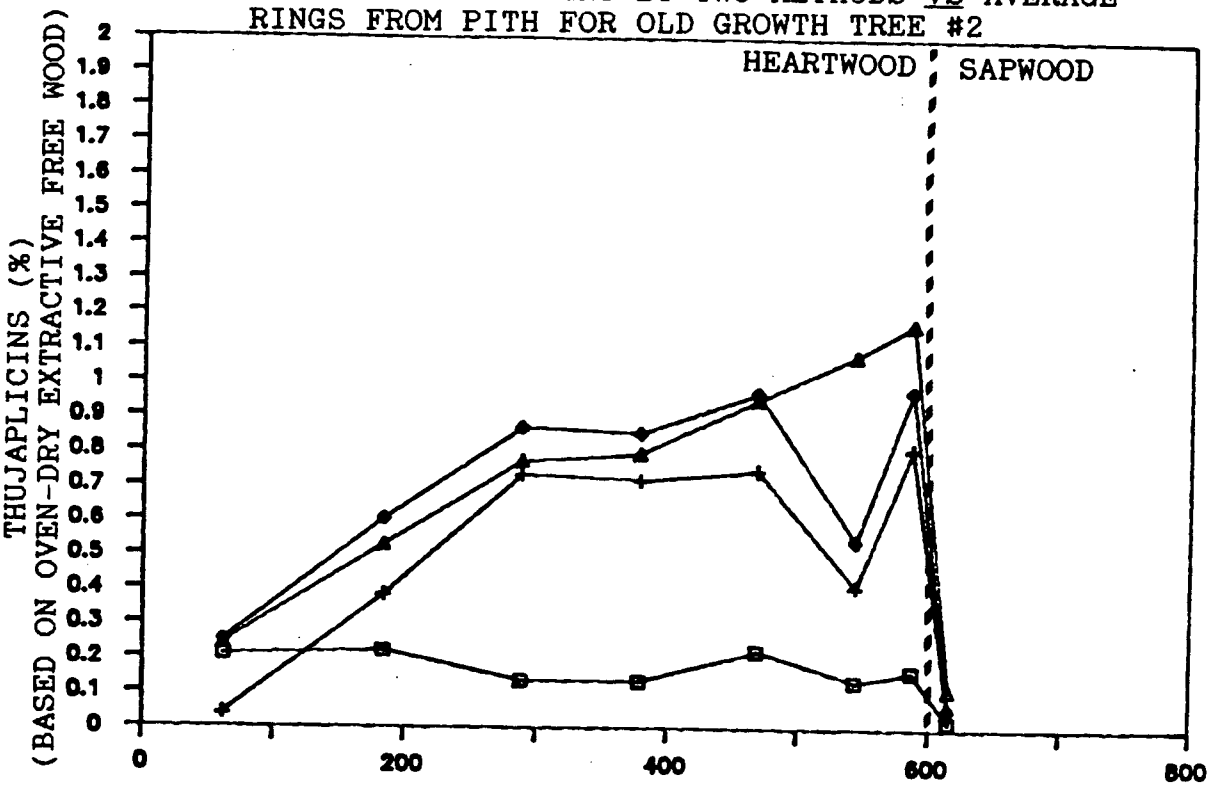
□BETA (BY GC) +GAMMA (BY GC) ◇TOTAL (BY GC) △COLORIMETRIC

FIGURE 17. THUJAPLICIN CONTENT BY TWO METHODS VS AVERAGE RINGS FROM PITH FOR OLD GROWTH TREE #1



□BETA (BY GC) +GAMMA (BY GC) ◇TOTAL (BY GC) △COLORIMETRIC

FIGURE 18. THUJAPLICIN CONTENT BY TWO METHODS VS AVERAGE RINGS FROM PITH FOR OLD GROWTH TREE #2



□BETA (BY GC) +GAMMA (BY GC) ◇TOTAL (BY GC) △COLORIMETRIC

FIGURE 19. THUJAPLICIN CONTENT BY TWO METHODS VS AVERAGE RINGS FROM PITH FOR OLD GROWTH TREE #3

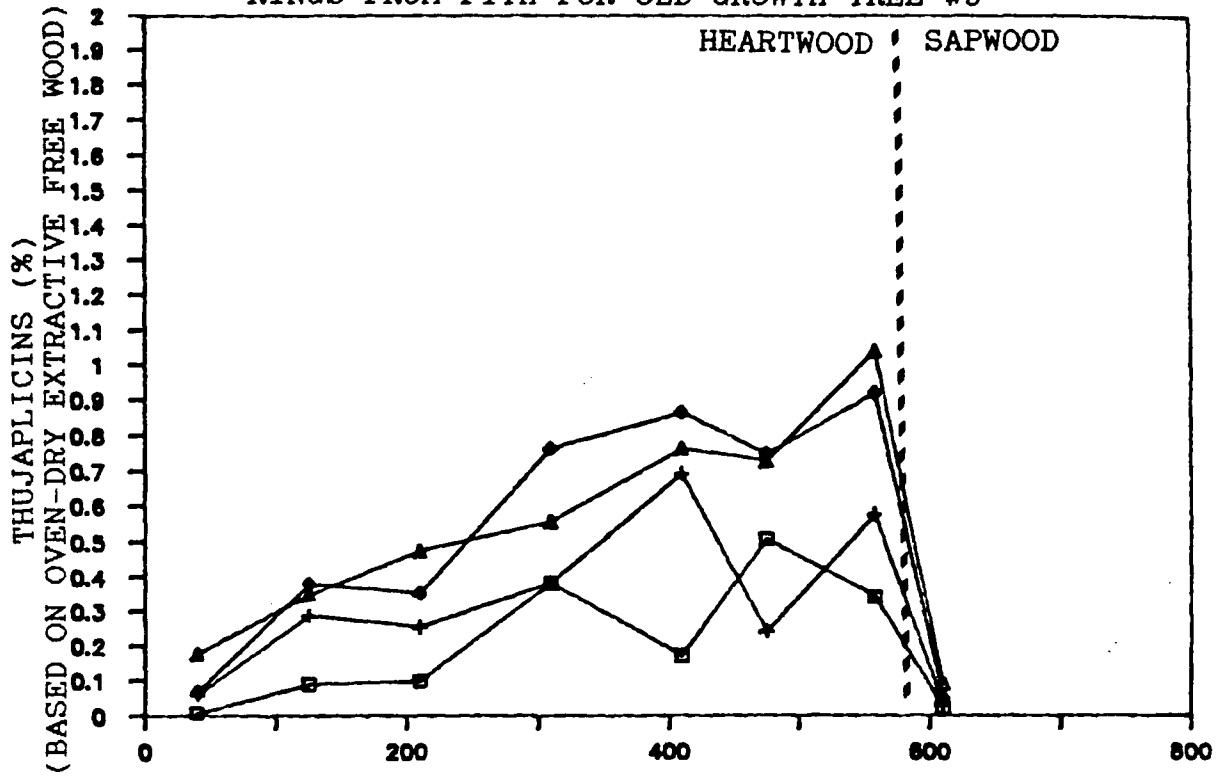


FIGURE 20. THUJAPLICIN CONTENT BY TWO METHODS VS AVERAGE RINGS FROM PITH FOR OLD GROWTH TREE #4

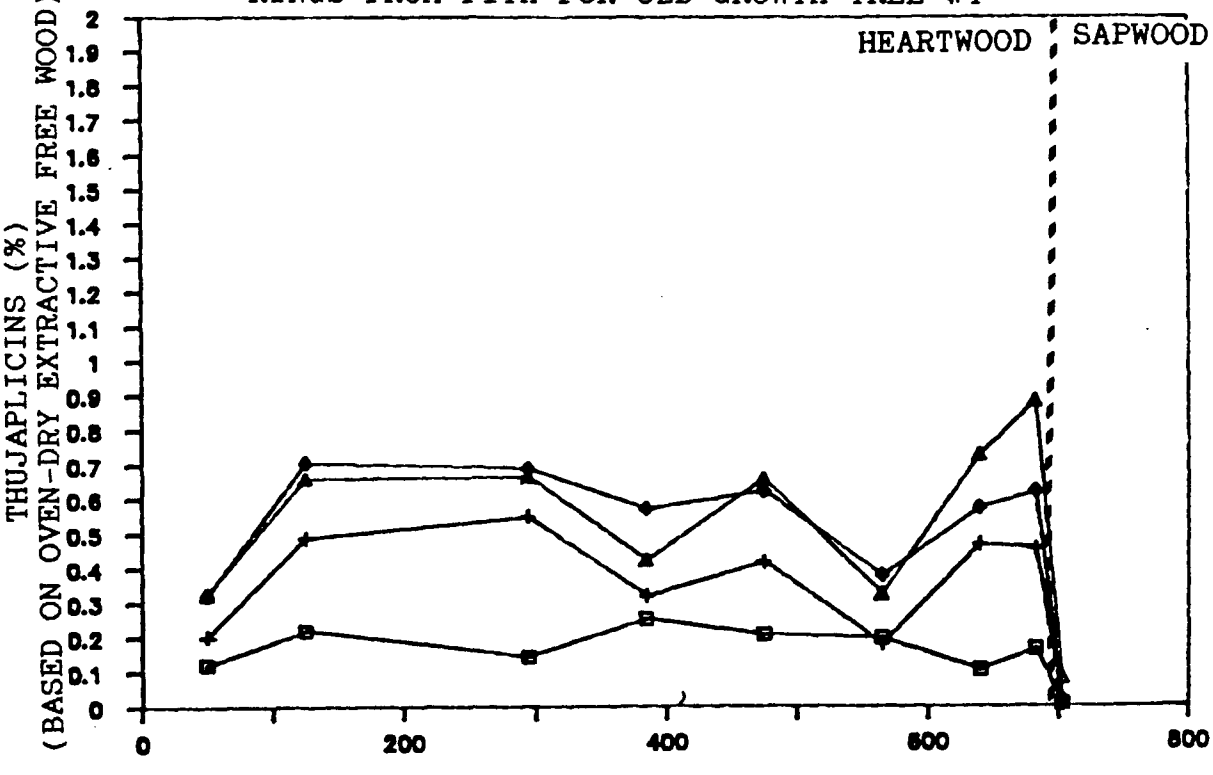
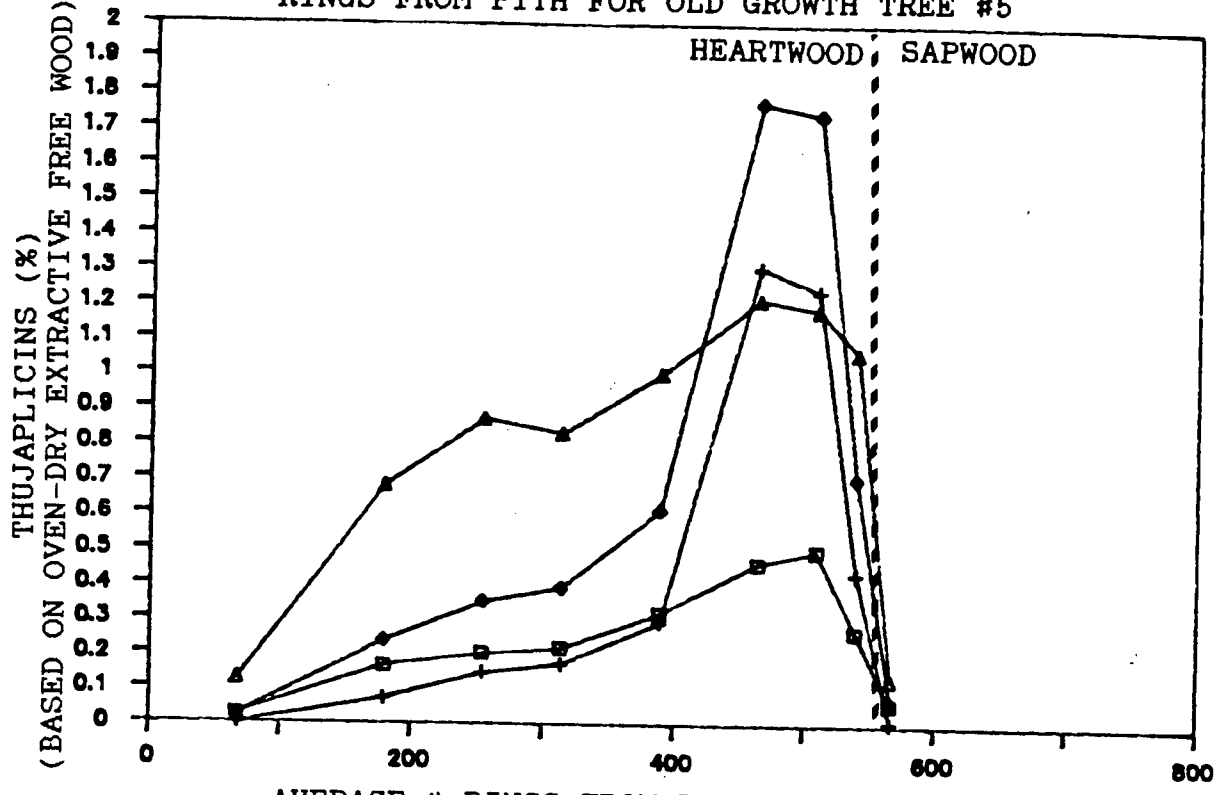
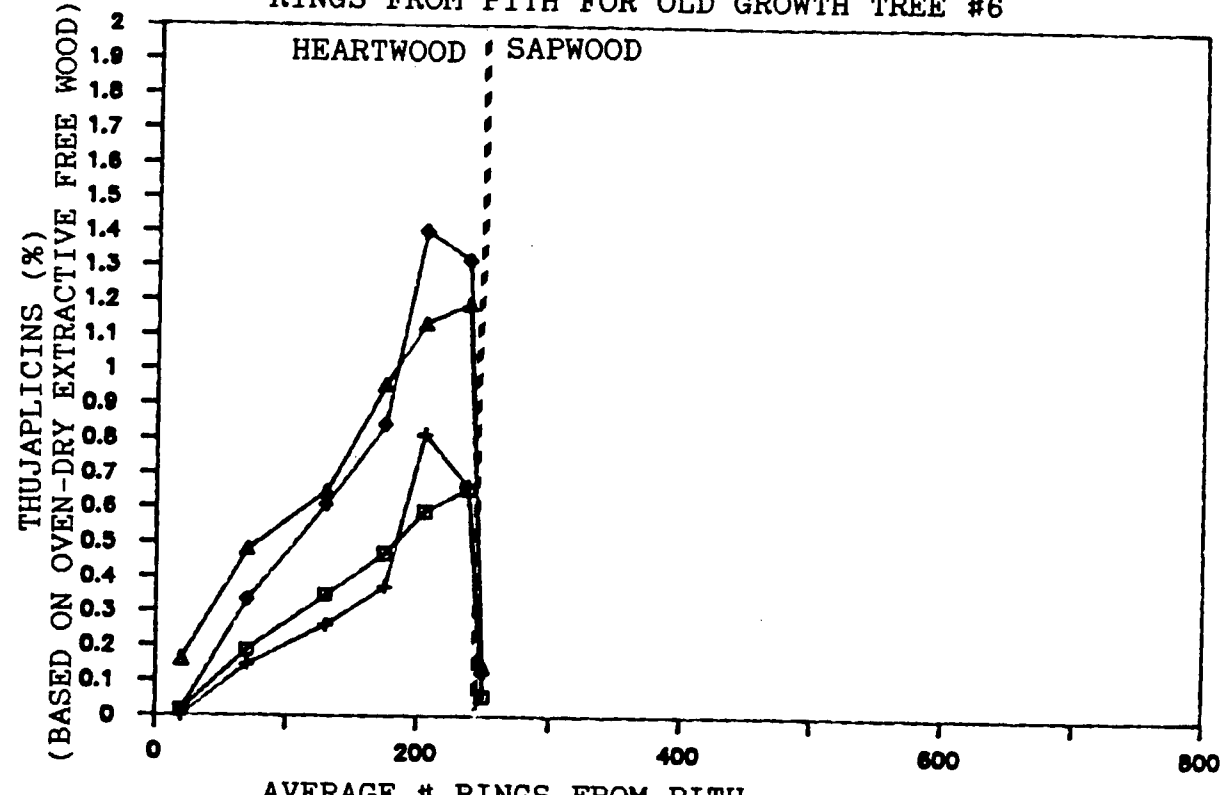


FIGURE 21. THUJAPLICIN CONTENT BY TWO METHODS VS AVERAGE RINGS FROM PITH FOR OLD GROWTH TREE #5



□BETA (BY GC) +GAMMA (BY GC) ◇TOTAL (BY GC) △COLORIMETRIC

FIGURE 22. THUJAPLICIN CONTENT BY TWO METHODS VS AVERAGE RINGS FROM PITH FOR OLD GROWTH TREE #6



□BETA (BY GC) +GAMMA (BY GC) ◇TOTAL (BY GC) △COLORIMETRIC

FIGURE 23. THUJAPLICIN CONTENT BY TWO METHODS VS AVERAGE RINGS FROM PITH FOR OLD GROWTH TREE #7

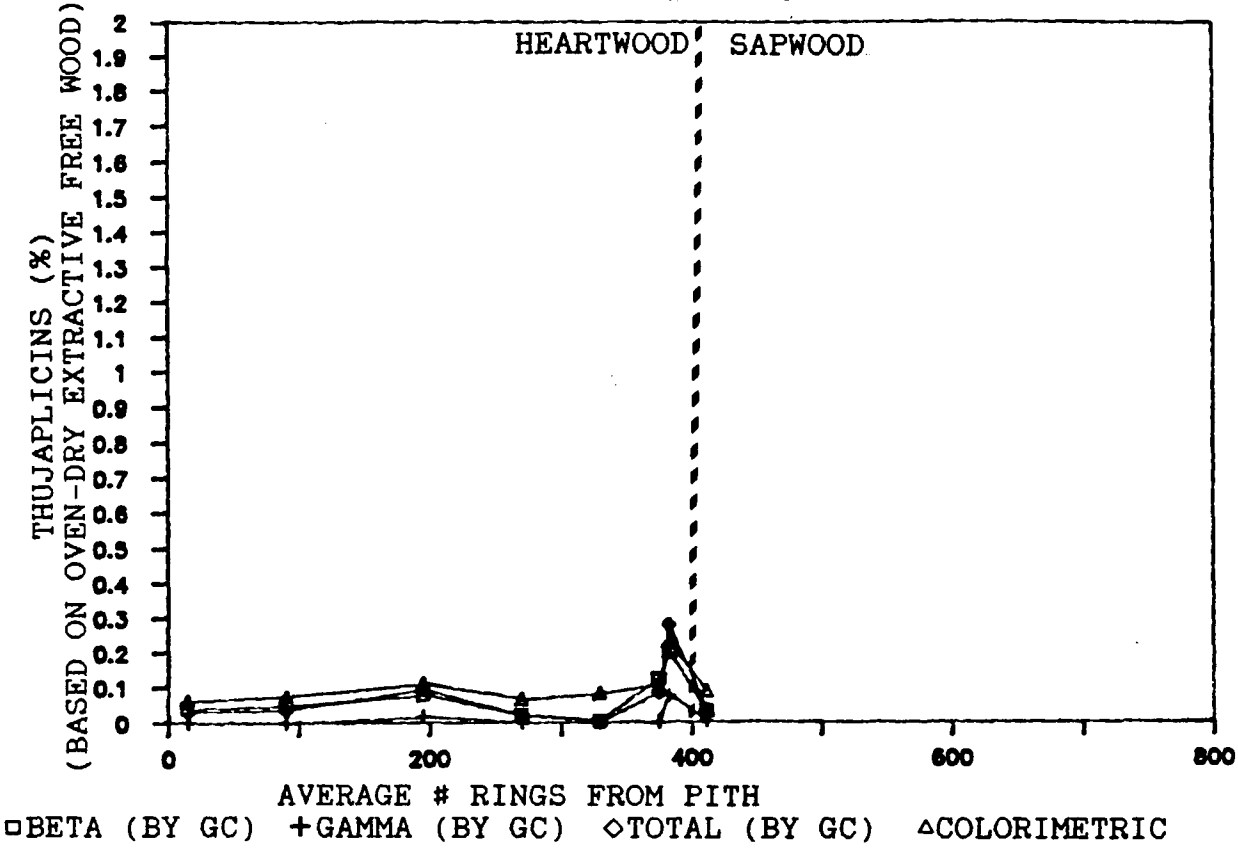


FIGURE 24. BETA+GAMMA THUJAPLICIN CONTENT VS AVERAGE RINGS FROM PITH FOR ALL TREES

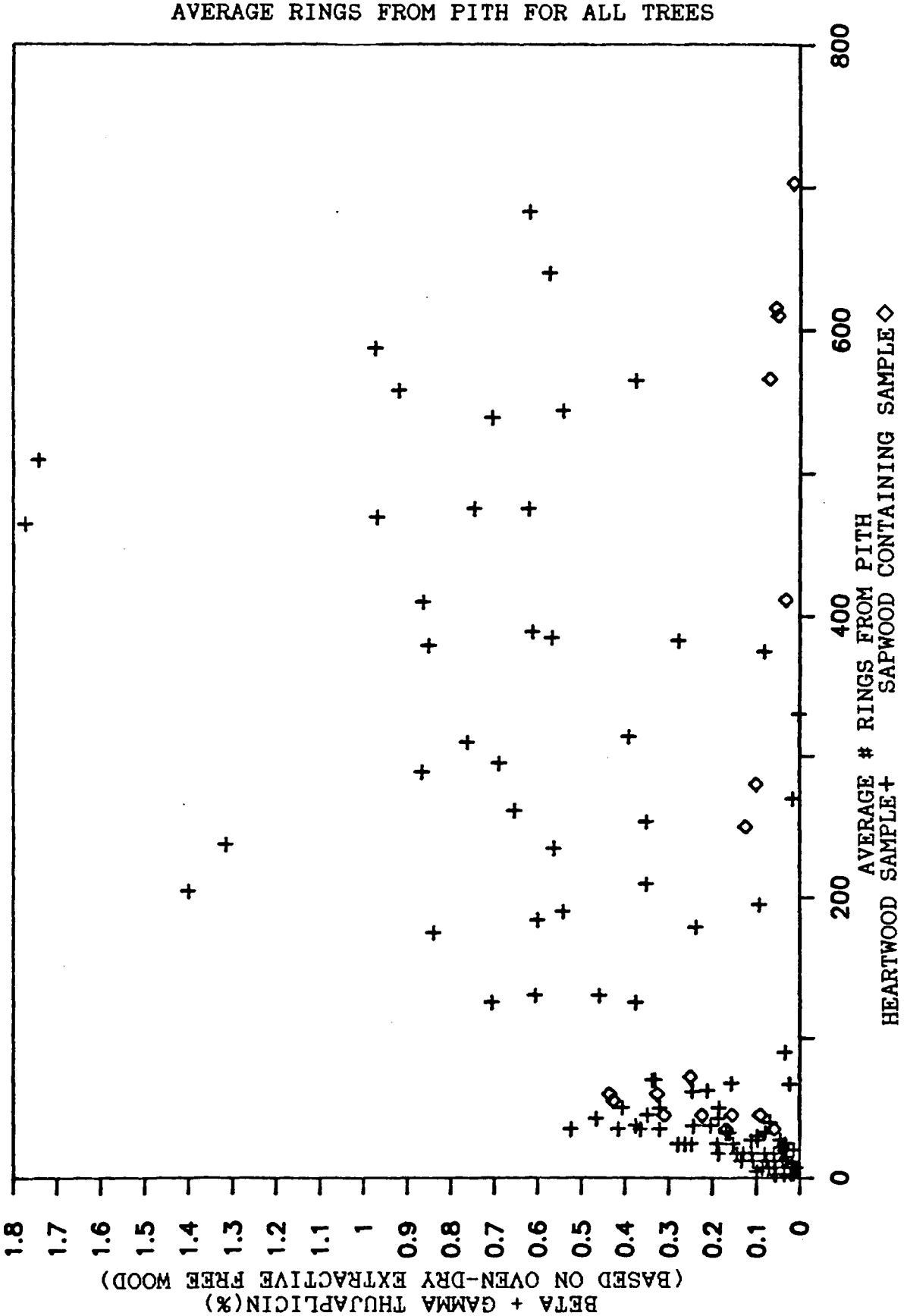


FIGURE 25. BETA+GAMMA THUJAPLICIN CONTENT VS LOG (AVERAGE RINGS FROM PITH) FOR ALL TREES

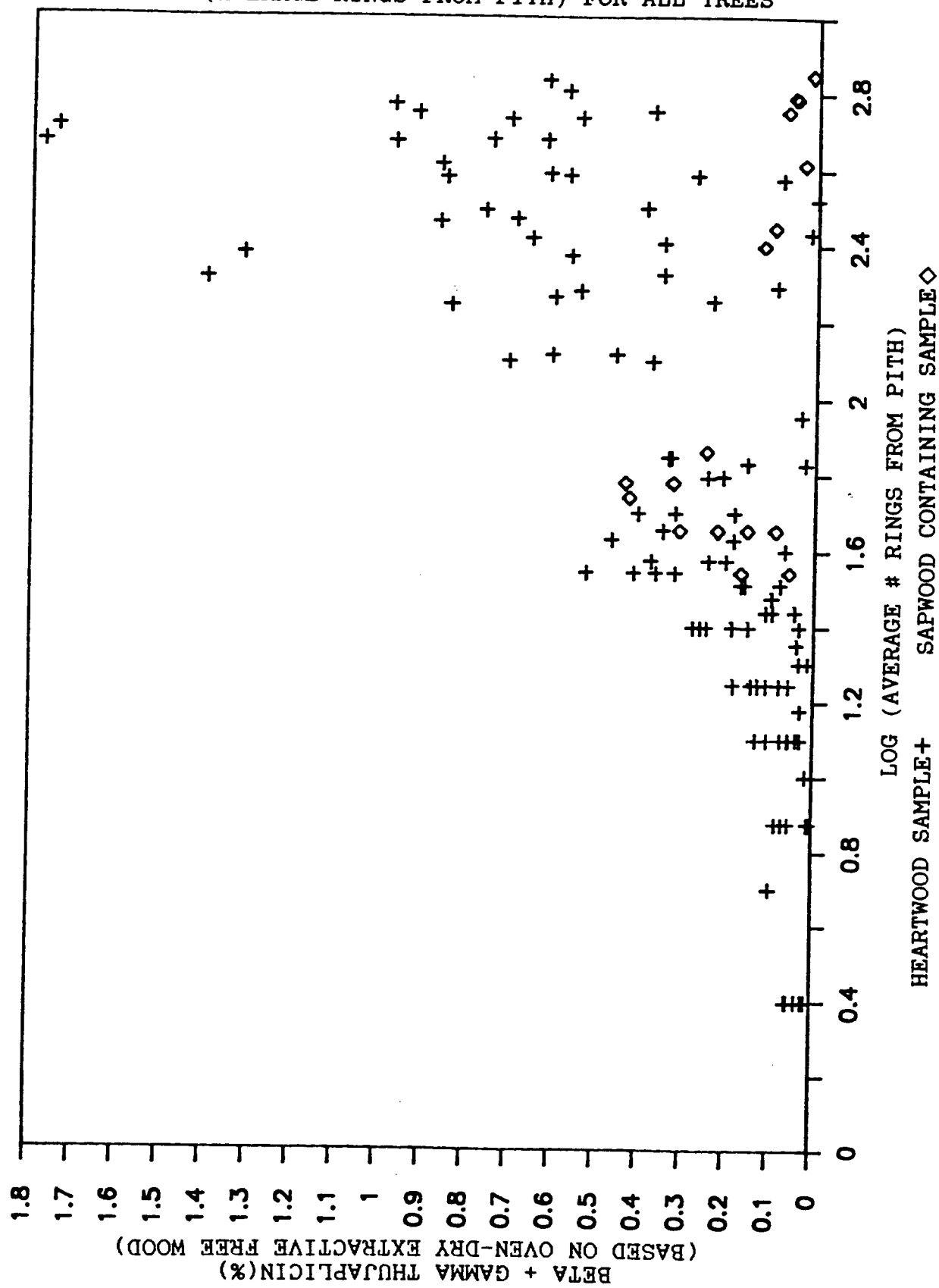


FIGURE 26. BETA THUJAPLICIN CONTENT VS AVERAGE RINGS FROM PITH FOR ALL TREES

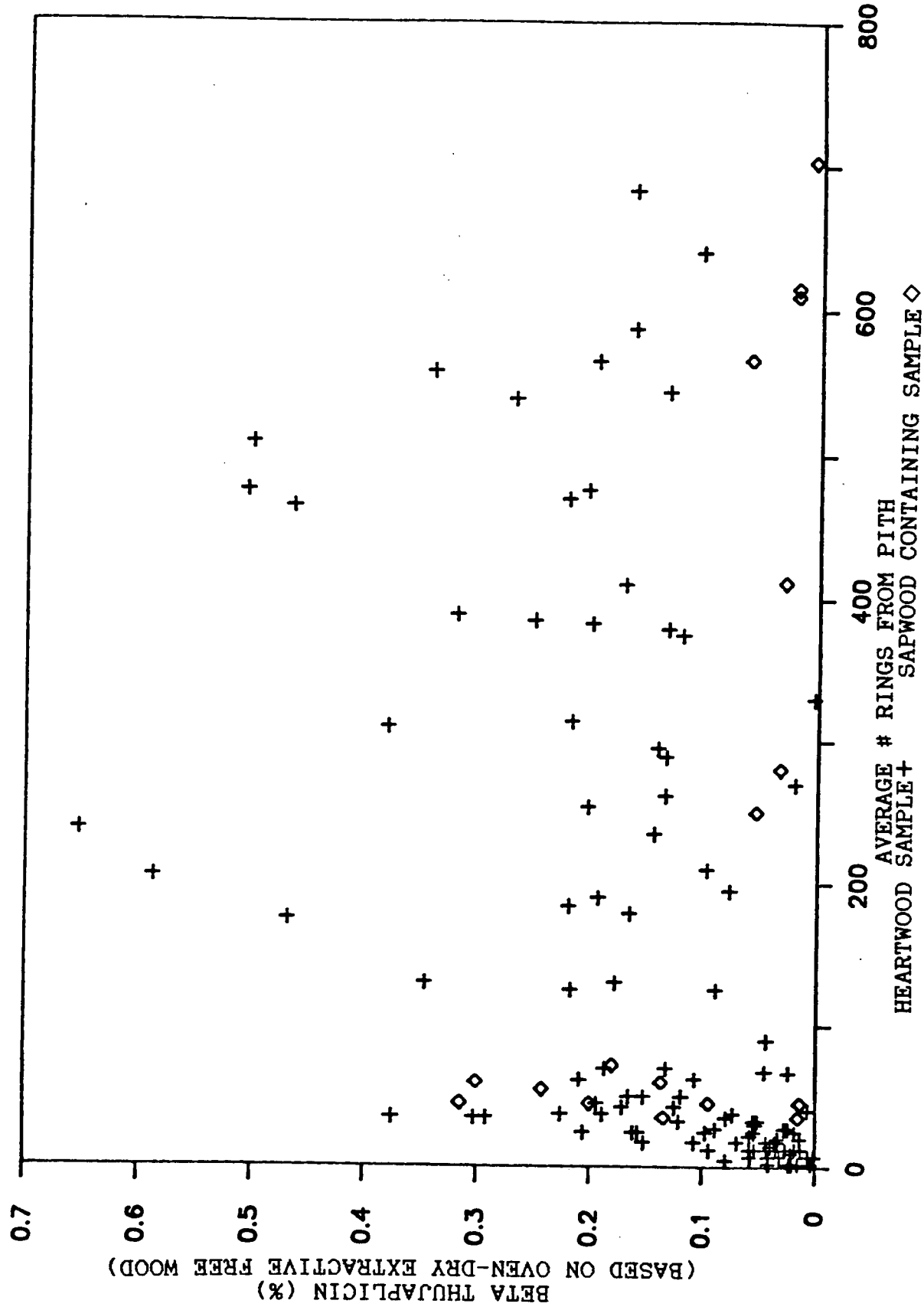


FIGURE 27. BETA THUJAPLICIN CONTENT VS LOG (AVERAGE RINGS FROM PITH) FOR ALL TREES

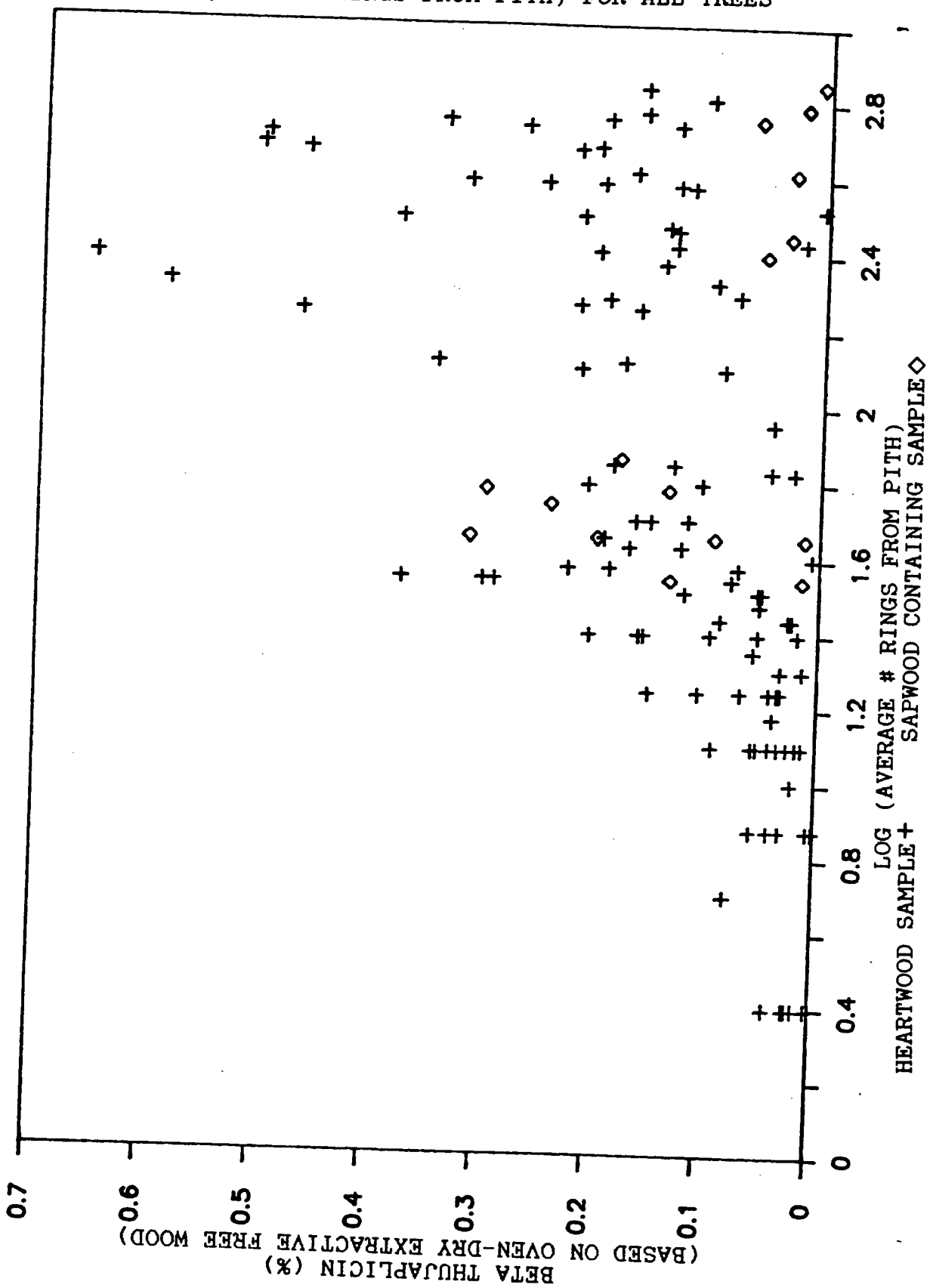


FIGURE 28. GAMMA THUJAPLICIN CONTENT VS AVERAGE RINGS FROM PITH FOR ALL TREES

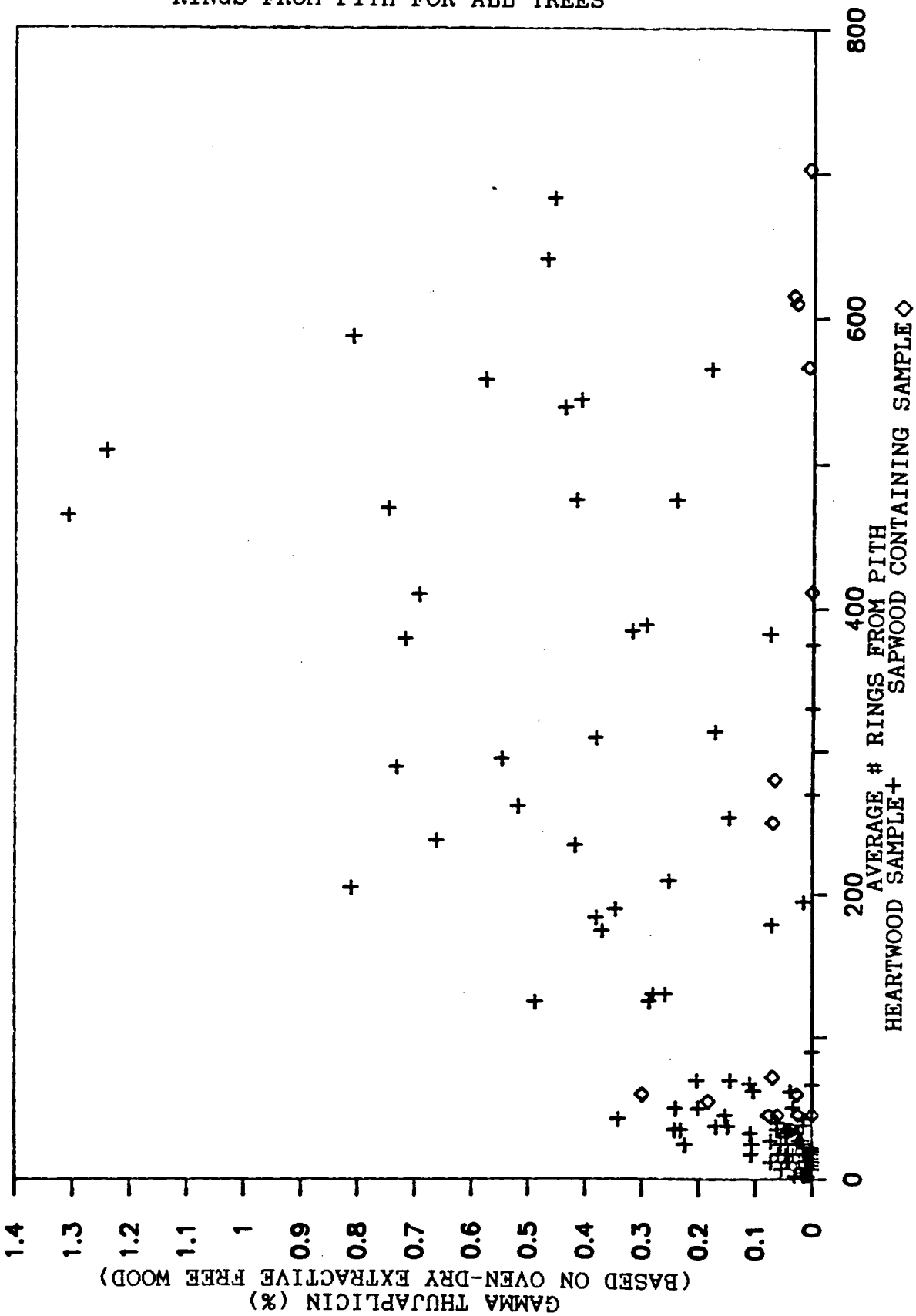


FIGURE 29. GAMMA THUJAPLICIN CONTENT VS LOG (AVERAGE RINGS FROM PITH) FOR ALL TREES

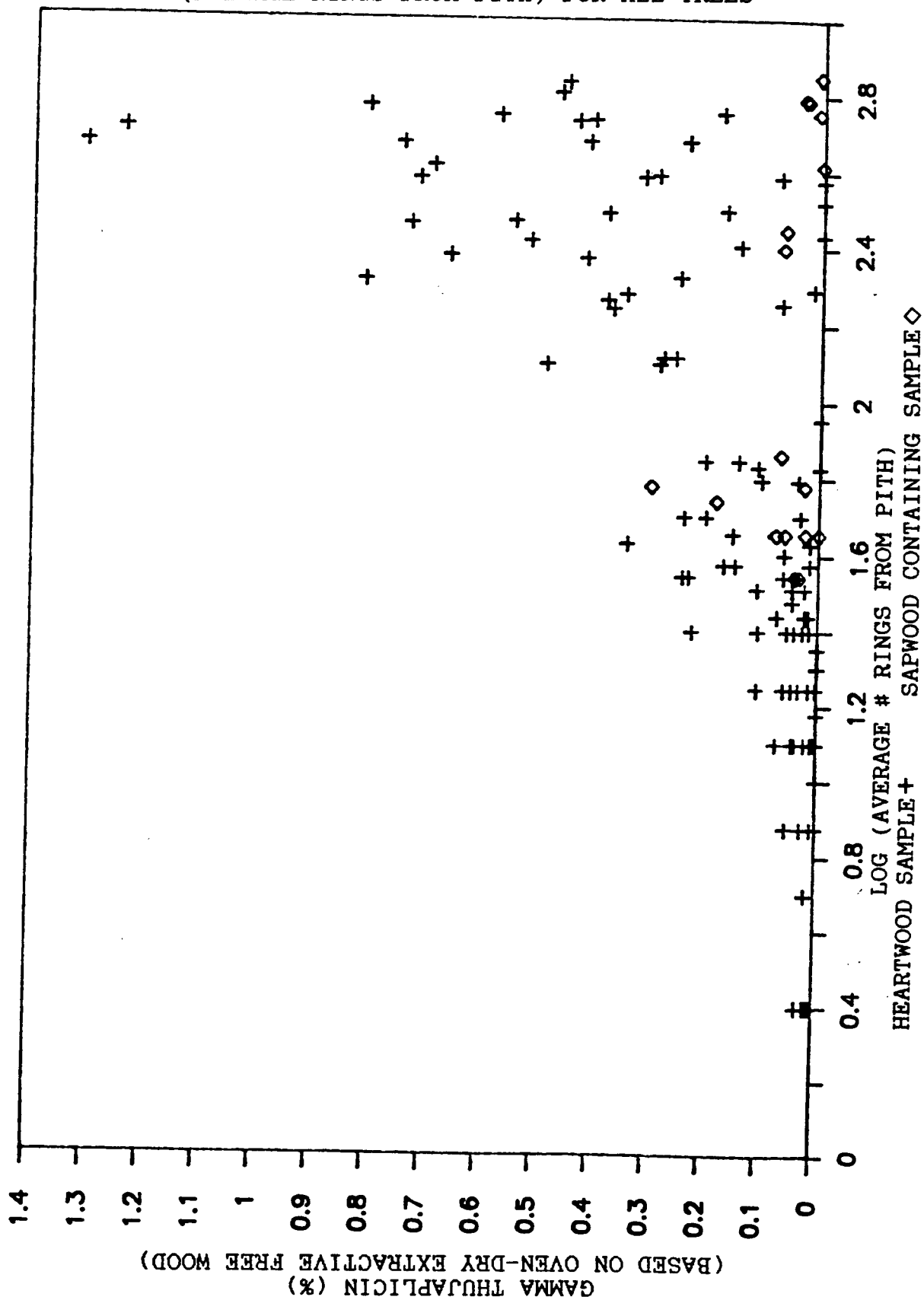


FIGURE 30. THUJAPLICIN CONTENT VS ETHANOL:BENZENE EXTRACTIVE CONTENT FOR ALL TREES

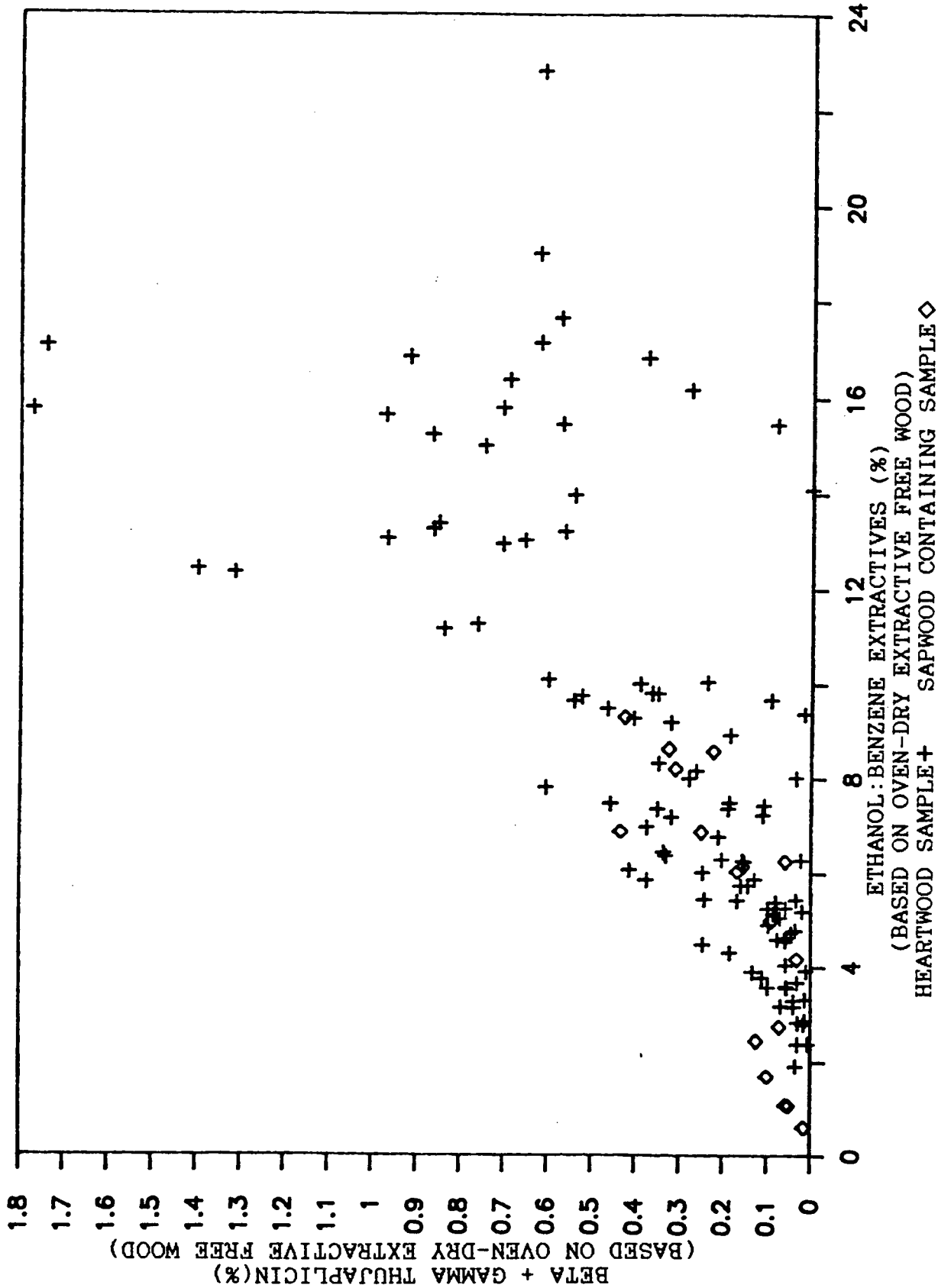


FIGURE 31. THUJIC ACID CONTENT VS ETHANOL:BENZENE EXTRACTIVE CONTENT FOR ALL TREES

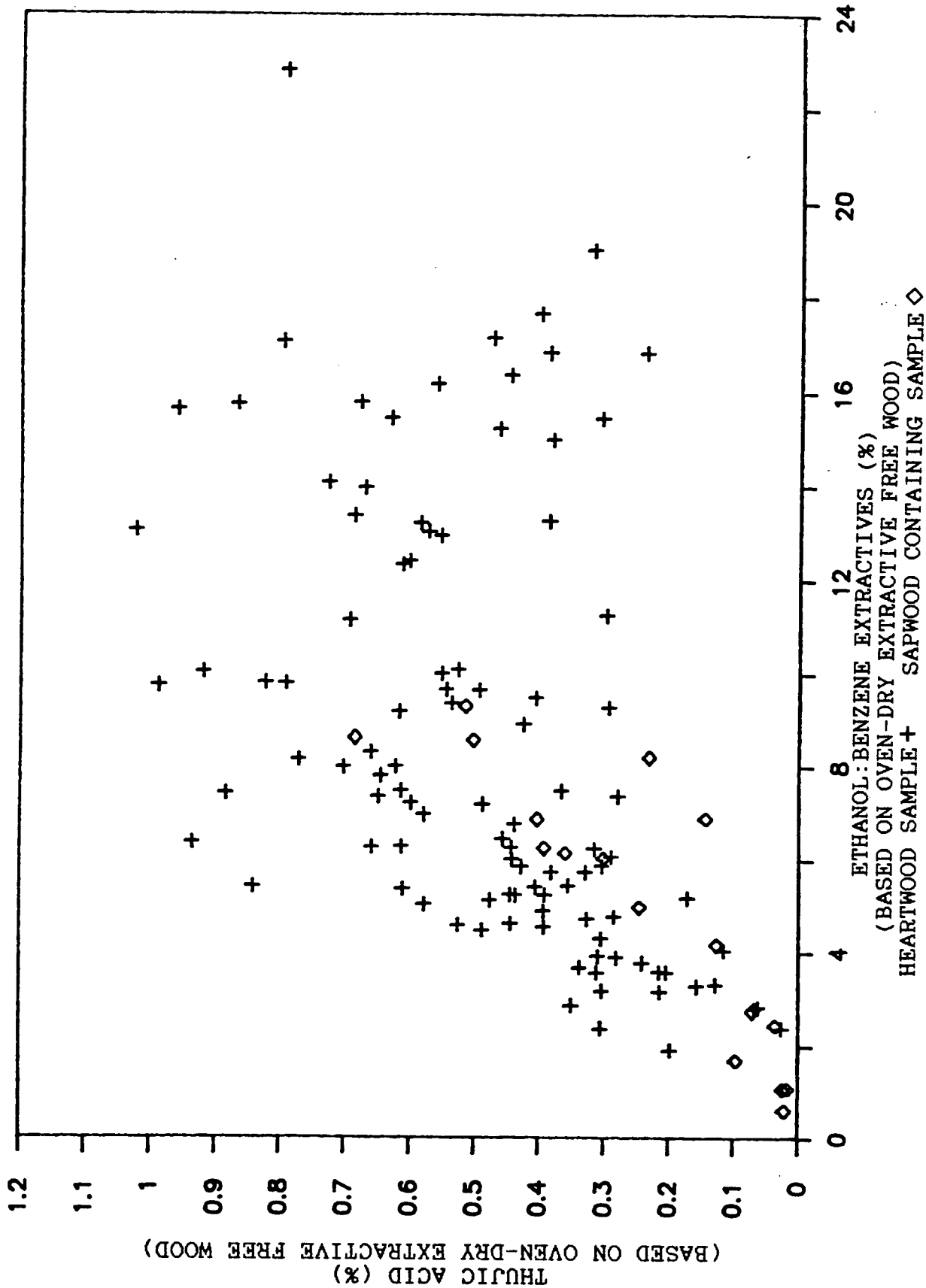


FIGURE 32. THUJIC ACID CONTENT VS THUJAPLICIN CONTENT FOR ALL TREES

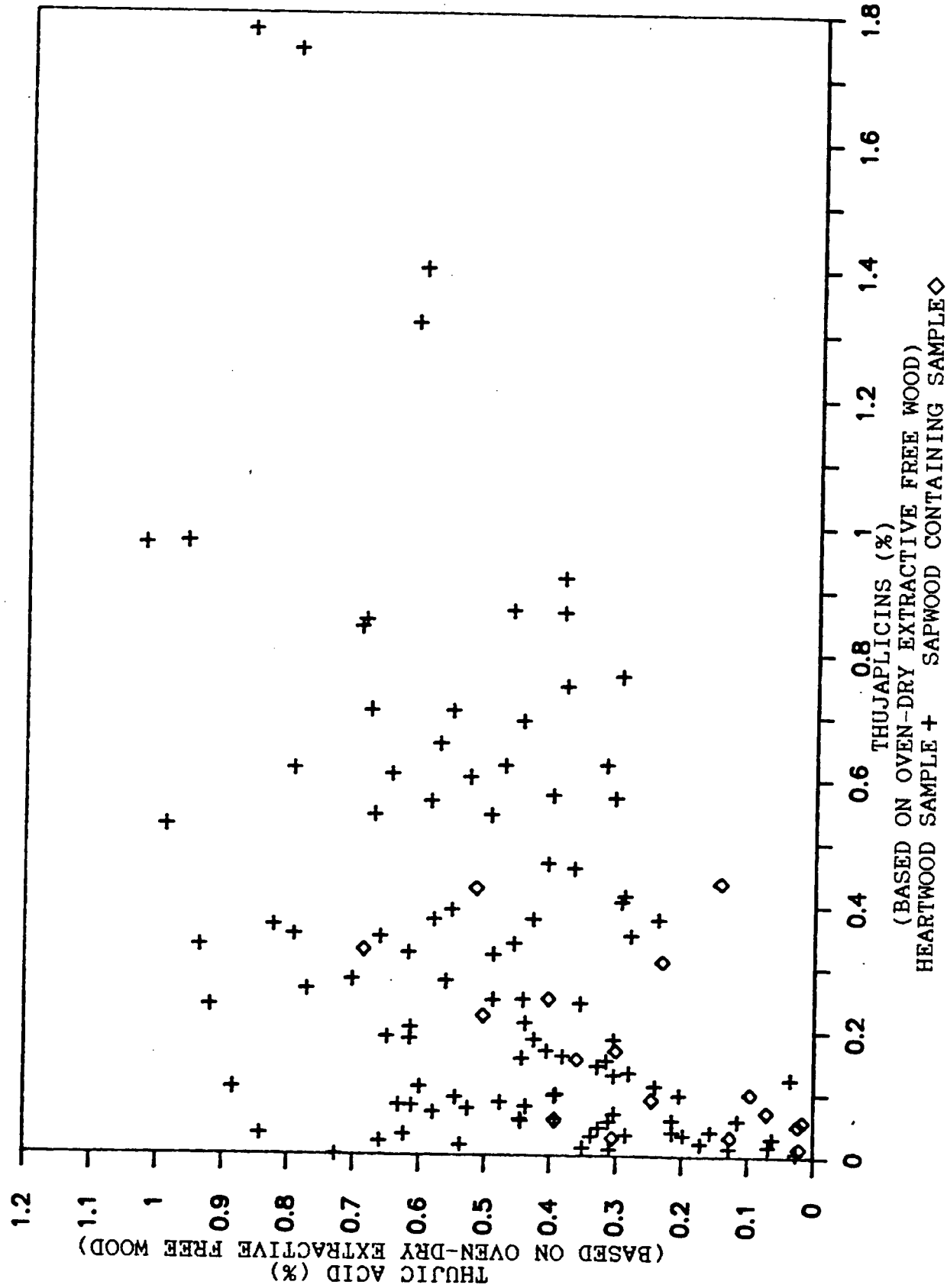


FIGURE 33. RADIAL DISTRIBUTION OF THUJIC ACID CONTENT FOR SECOND GROWTH SITE #1

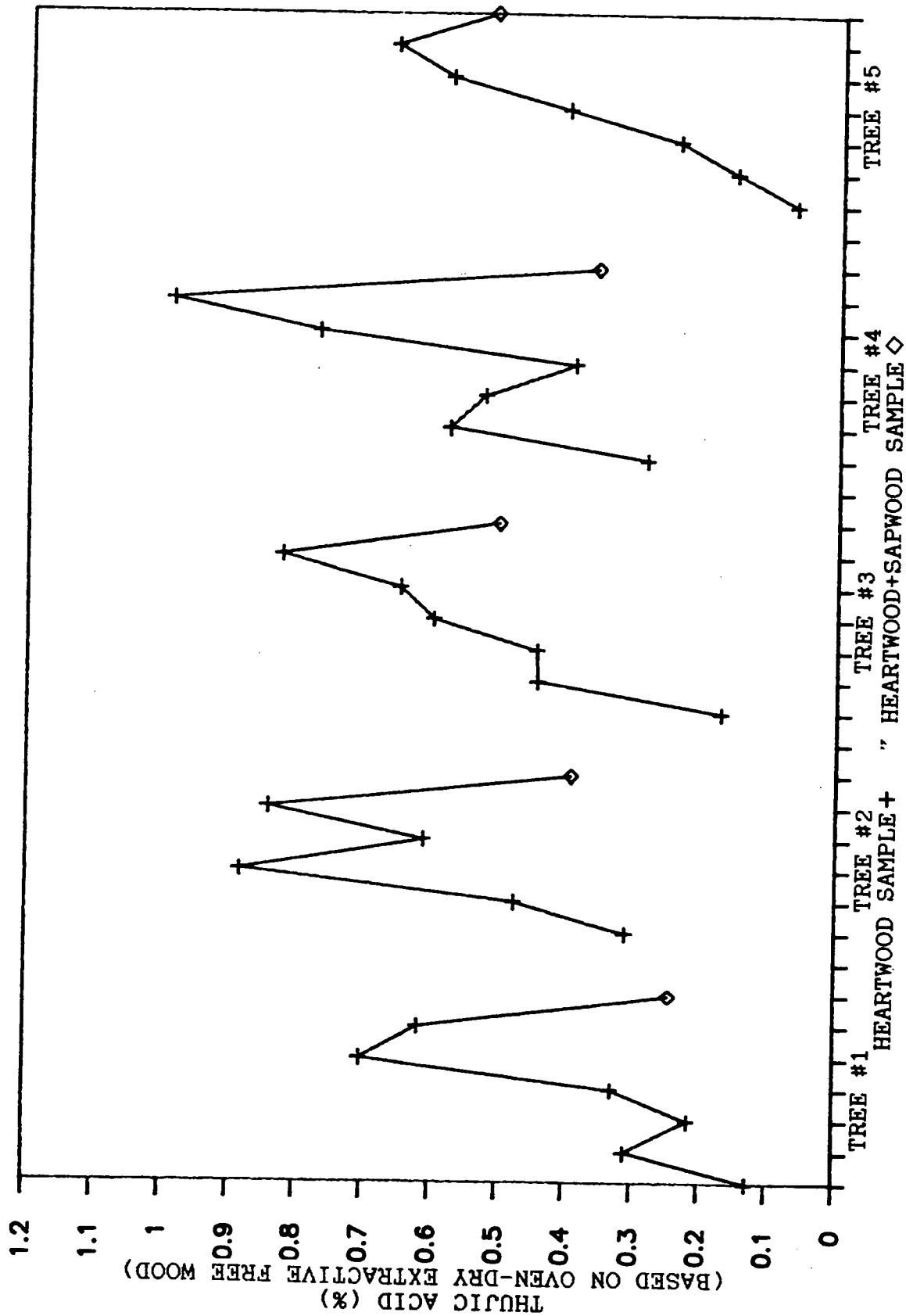


FIGURE 34. RADIAL DISTRIBUTION OF THUJIC ACID CONTENT FOR SECOND GROWTH SITE #2

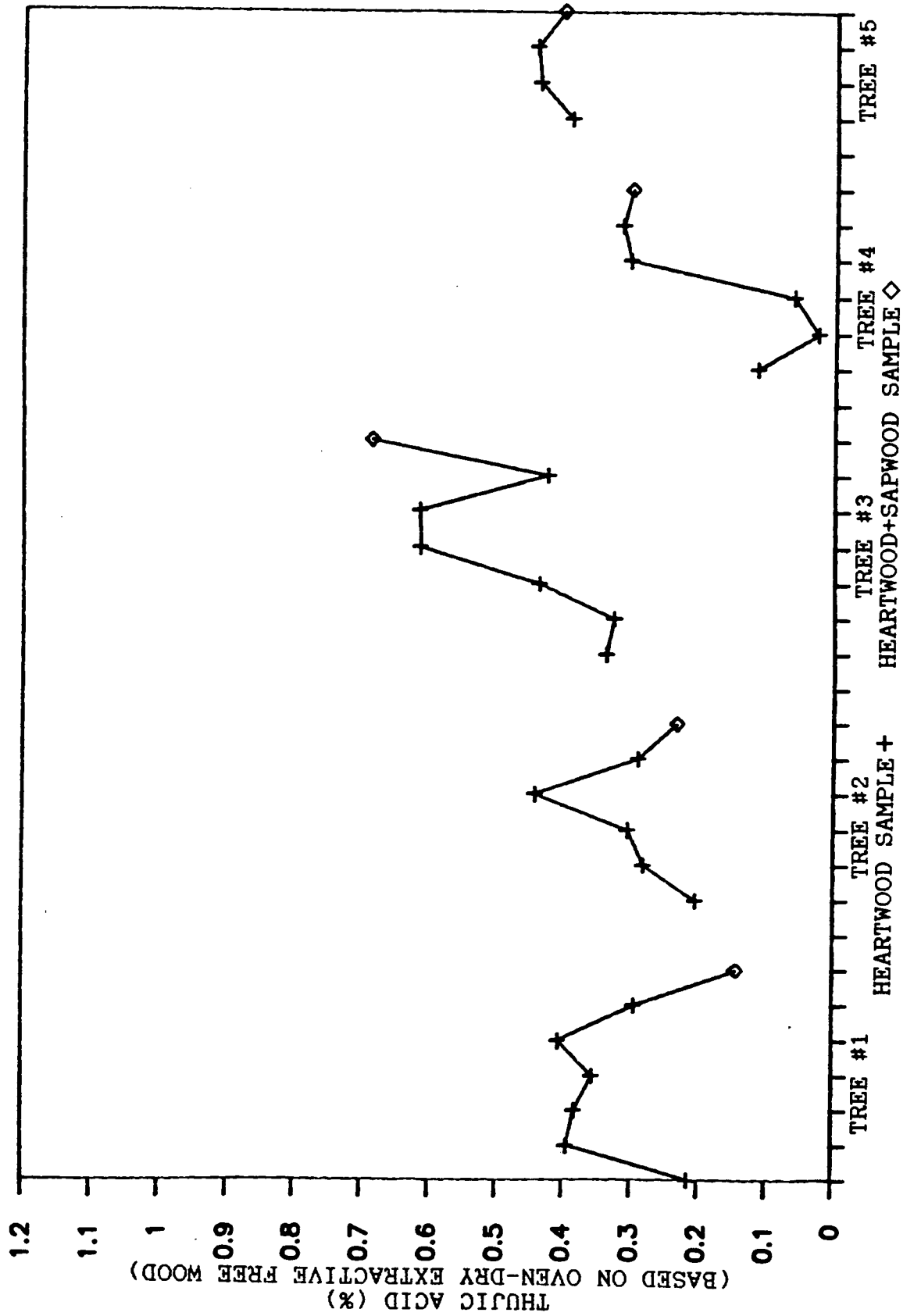


FIGURE 35. RADIAL DISTRIBUTION OF THUJIC ACID CONTENT FOR OLD GROWTH TREES

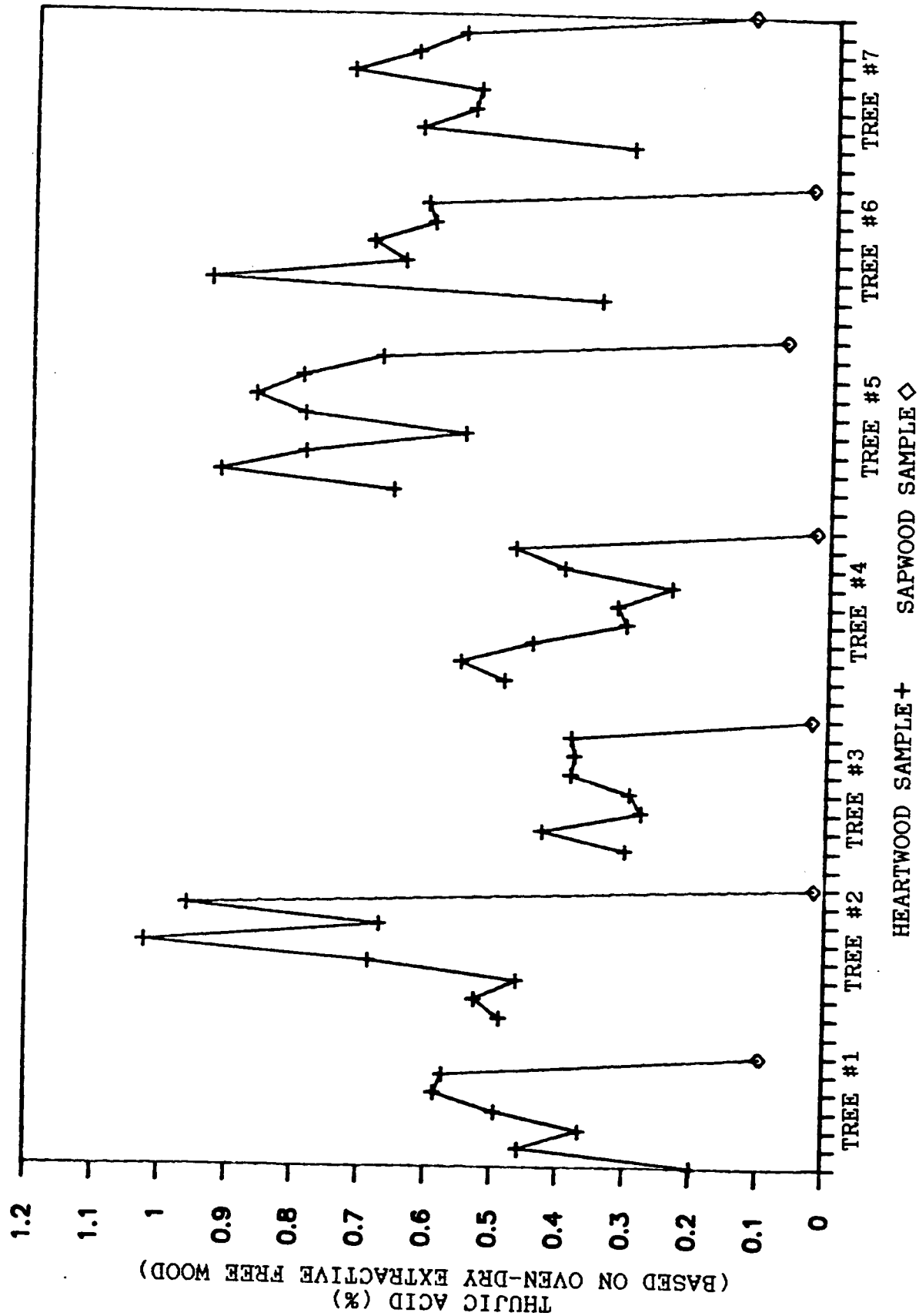
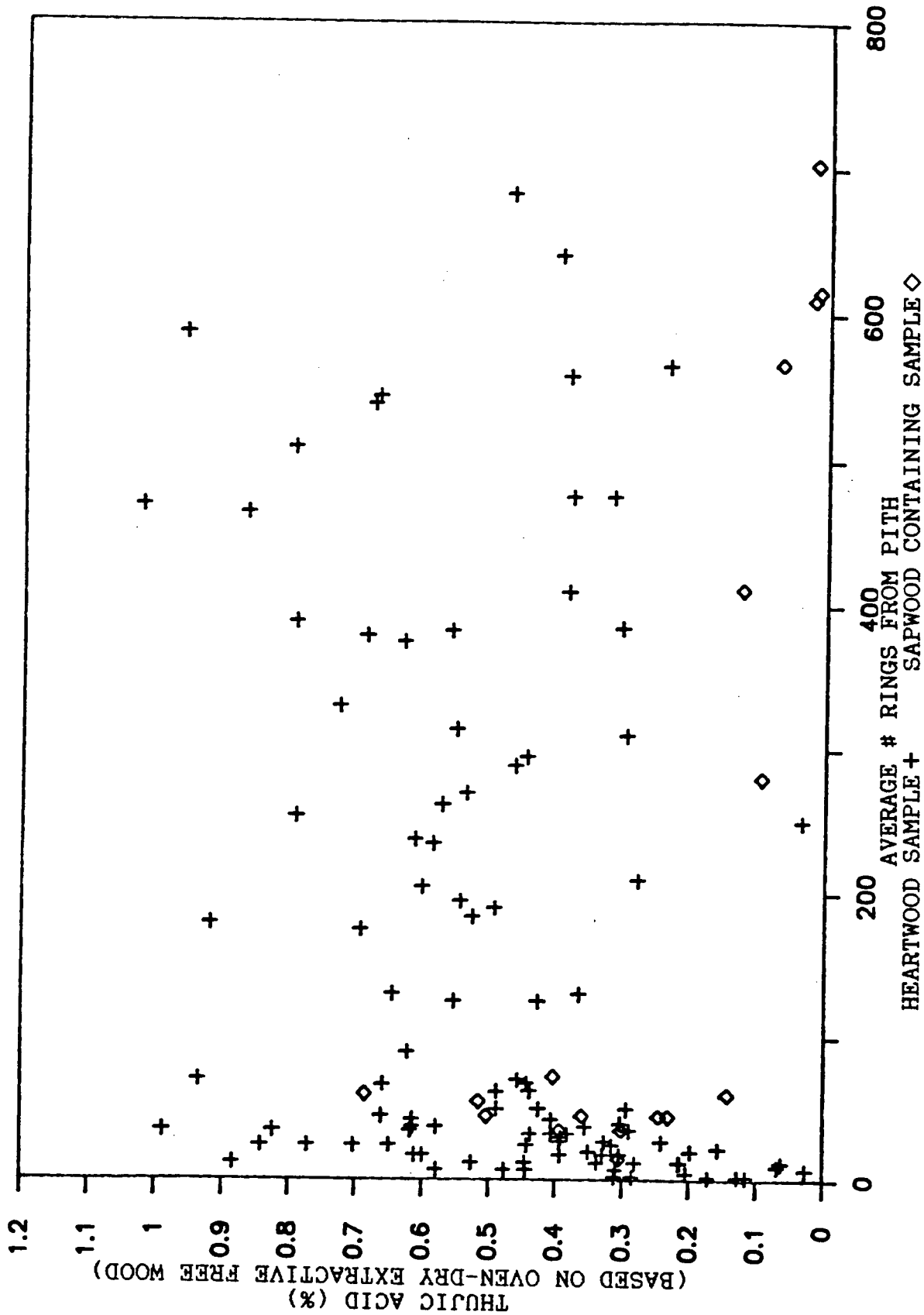


FIGURE 36. THUJIC ACID CONTENT VS AVERAGE RINGS FROM PITH FOR ALL TREES



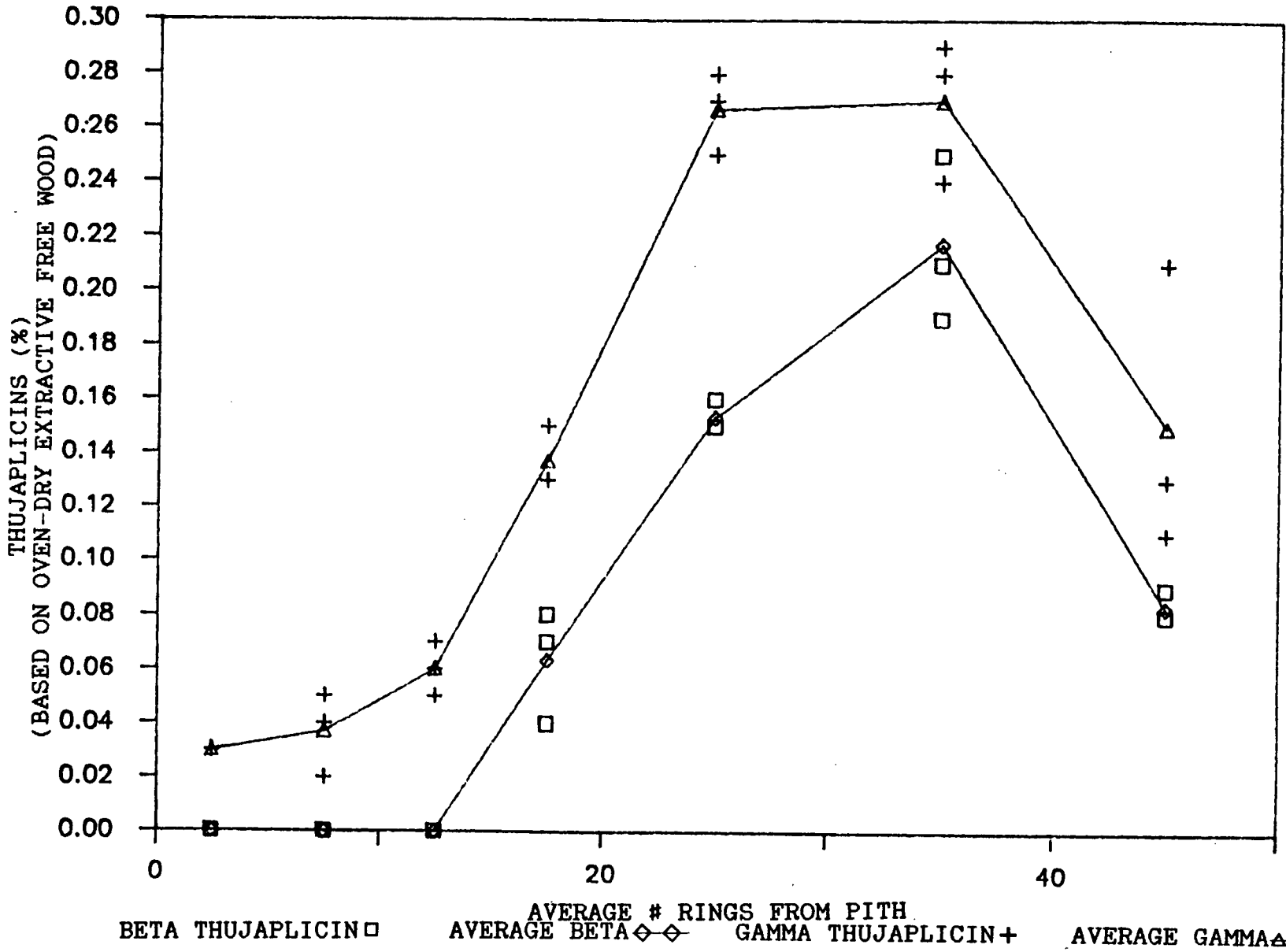


FIGURE 37. MULTIPLE ANALYSES OF THUJAPLICIN CONTENT IN SECOND GROWTH TREE #1.1

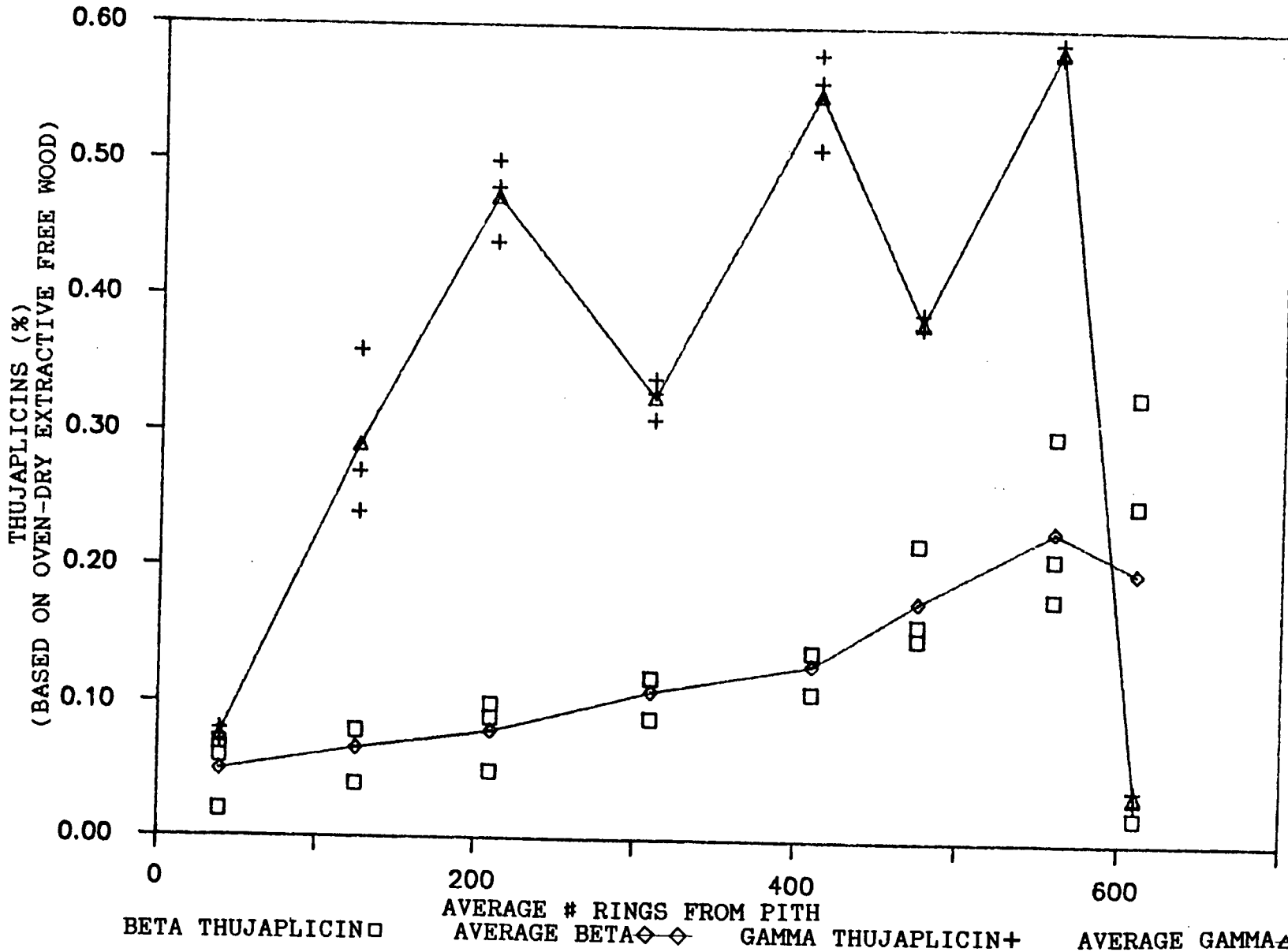
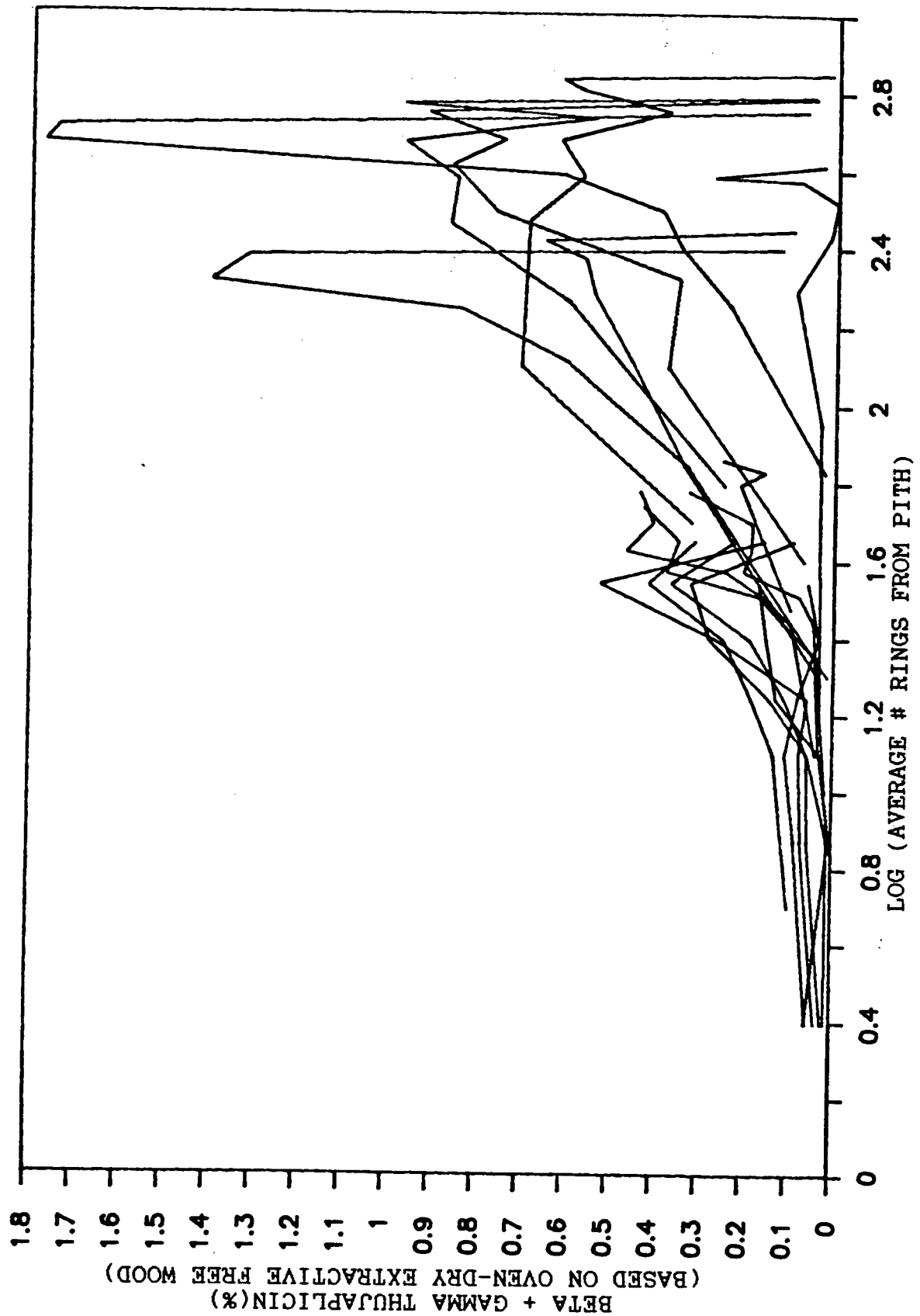


FIGURE 38. MULTIPLE ANALYSES OF THUJAPLICIN CONTENT IN OLD GROWTH TREE #3

FIGURE 39. BETA+GAMMA THUJAPLICIN CONTENT VS LOG (AVERAGE RINGS FROM PITH) FOR ALL TREES



APPENDIX 1. PHYSICAL DATA FOR OLD GROWTH TREES.

SAMPLE SITE	TREE #	DBH (cm)	# RINGS (AGE)	HEARTWOOD/SAPWOOD BOUNDARY (RINGS FROM PITH)
PORT McNEILL	1	100.8	287	273
VANCOUVER	2	115.6	622	604
ISLAND	3	134.0	622	597
B.C.	4	135.6	710	696
	5	130.4	579	554
	6	116.8	260	239
HANEY, B.C.	7	54.2	420	405

APPENDIX 2. OLD GROWTH SAMPLE DATA

TREE #	RINGS INCLUDED IN SAMPLE	AVERAGE RINGS FROM PITH	RADIUS TO LAST RING (cm)
1	1- 40	20	5.9
	41-100	70	17.0
	101-160	130	36.5
	161-220	190	41.9
	221-250	235	46.1
	251-273	262	48.6
	274-287	280	50.4
2	1-124	62	4.4
	125-244	184	16.6
	245-334	289	26.6
	335-424	379	36.6
	425-514	469	46.5
	515-574	544	53.1
	575-604	589	56.4
	604-622	613	58.6
3	1- 80	40	3.5
	81-170	125	17.5
	171-250	210	33.5
	251-370	310	45.8
	371-430	400	52.8
	431-520	475	58.3
	521-597	558	64.8
	598-622	610	67.3

OLD GROWTH SAMPLE DATA

TREE #	RINGS INCLUDED IN SAMPLE	AVERAGE RINGS FROM PITH	RADIUS TO LAST RING (cm)
4	1-100	50	6.7
	101-250	175	26.7
	251-340	295	35.2
	341-430	385	43.6
	431-520	475	50.4
	521-610	565	55.8
	611-670	640	62.5
	671-696	683	65.3
	697-710	703	67.7
5	1-134	67	4.9
	135-224	179	14.2
	225-284	254	26.5
	285-344	314	42.0
	345-434	389	54.5
	435-494	464	58.5
	495-524	509	60.5
	525-554	539	62.5
	555-579	566	63.8
6	1- 40	20	6.1
	41-100	70	17.6
	101-160	130	35.9
	161-190	175	47.4
	191-220	205	52.7
	221-239	230	56.3
	240-260	250	59.1
7	1- 30	15	4.8
	31-150	90	10.1
	151-240	195	15.1
	241-300	270	19.1
	301-360	330	23.1
	361-390	375	24.6
	391-405	397	25.9
	406-420	412	27.1

APPENDIX 3. EXPERIMENTAL DATA FROM OLD GROWTH TREES

(ALL RESULTS % OVEN DRY EXTRACTIVE FREE WOOD)

TREE #	AVE. # RINGS FROM PITH	EtOH: BENZ. EXTR.	BETA THUJA-PLICIN	GAMMA THUJA-PLICIN	BETA+ GAMMA THUJA-PLICIN	GAMMA /TOT.	COLOR. THUJA-PLICIN	THUJIC ACID + METHYL THUJATE
1	20	1.9	.034	.001	.034	.02	.170	.198
	70	6.5	.133	.204	.337	.60	.400	.457
	130	7.5	.179	.281	.460	.61	.454	.367
	190	9.6	.194	.349	.543	.64	.465	.494
	235	13.2	.145	.419	.564	.74	.635	.586
	262	13.0	.135	.520	.655	.79	.702	.573
	280	1.7	.033	.067	.100	.67	.153	.097
2	62	4.5	.209	.039	.248	.16	.243	.488
	184	10.1	.220	.382	.602	.63	.528	.527
	289	15.2	.135	.732	.867	.84	.772	.465
	379	13.4	.134	.718	.852	.84	.794	.688
	469	13.1	.223	.747	.970	.77	.952	1.024
	544	14.0	.134	.409	.543	.75	1.079	.671
	587	15.6	.164	.809	.974	.83	1.171	.960
	615	1.1	.021	.036	.057	.63	.114	.017
3	40	3.2	.007	.061	.068	.90	.178	.304
	125	5.9	.090	.288	.377	.76	.347	.429
	210	7.4	.098	.254	.352	.72	.474	.280
	310	11.2	.381	.382	.763	.50	.556	.298
	410	13.3	.172	.693	.865	.80	.763	.388
	475	15.0	.507	.240	.747	.32	.728	.382
	558	16.8	.342	.578	.919	.63	1.039	.387
	610	1.1	.021	.030	.051	.58	.091	.023
4	50	7.2	.119	.201	.320	.63	.324	.490
	125	13.0	.218	.488	.706	.69	.662	.554
	295	16.4	.142	.548	.690	.79	.666	.447
	385	15.4	.252	.318	.570	.56	.422	.306
	475	19.0	.205	.417	.622	.67	.658	.320
	565	16.8	.197	.181	.377	.48	.325	.238
	640	17.6	.106	.469	.575	.82	.728	.401
	683	17.1	.165	.456	.621	.73	.883	.475
	703	.6	.007	.008	.015	.51	.081	.022

EXPERIMENTAL DATA FOR OLD GROWTH TREES

(ALL RESULTS % OVEN DRY EXTRACTIVE FREE WOOD)

TREE #	AVE. # RINGS FROM PITH	EtOH: BENZ. EXTR.	BETA THUJA-PLICIN	GAMMA THUJA-PLICIN	BETA+ GAMMA THUJA-PLICIN	GAMMA /TOT.	COLOR. THUJA-PLICIN	THUJIC ACID + METHYL THUJATE
5	67	6.3	.024	.000	.024	.00	.127	.660
	179	10.0	.166	.072	.238	.30	.680	.920
	254	9.8	.204	.148	.352	.42	.872	.793
	314	10.0	.219	.173	.392	.44	.831	.553
	389	22.8	.321	.294	.615	.48	1.000	.794
	464	15.7	.466	1.308	1.774	.74	1.216	.868
	509	17.0	.502	1.240	1.742	.71	1.186	.797
	539	15.8	.270	.437	.707	.62	1.063	.679
	566	2.7	.062	.010	.072	.14	.134	.071
6	20	2.9	.014	.000	.014	.00	.161	.350
	70	6.4	.187	.146	.333	.44	.480	.936
	130	7.8	.347	.260	.607	.43	.647	.646
	175	11.2	.469	.372	.840	.44	.954	.694
	205	12.4	.587	.813	1.400	.58	1.131	.603
	238	12.4	.653	.663	1.315	.50	1.189	.613
	250	2.4	.055	.070	.125	.56	.139	.036
7	15	2.4	.040	.000	.040	.00	.061	.306
	90	8.0	.044	.000	.044	.00	.075	.623
	195	9.6	.078	.016	.093	.17	.109	.545
	270	9.4	.020	.000	.020	.00	.065	.537
	330	14.1	.004	.000	.004	.00	.082	.728
	375	15.4	.121	.000	.121	.00	.102	.632
	383	16.2	.201	.077	.278	.28	.228	.561
	412	4.2	.030	.002	.032	.06	.088	.127

APPENDIX 4. PHYSICAL DATA FOR SECOND GROWTH TREES

SAMPLE SITE	TREE #	DBH (cm)	# RINGS (AGE)	HEARTWOOD/SAPWOOD BOUNDARY (RINGS FROM PITH)
HORNE LAKE, VANCOUVER ISLAND, B.C.	1.1	46.8	50	44
	1.2	44.5	44	39
	1.3	37.8	52	46
	1.4	51.6	51	46
	1.5	44.9	63	58
SHAWNIGAN LAKE, VANCOUVER ISLAND, B.C.	2.1	64.6	68	60
	2.2	63.7	52	46
	2.3	56.0	71	64
	2.4	47.8	42	36
	2.5	52.7	77	72

APPENDIX 5. SECOND GROWTH SAMPLE DATA

TREE #	RINGS INCLUDED IN SAMPLE	AVERAGE RINGS FROM PITH	RADIUS TO LAST RING (cm)
1.1	1- 5	2.5	2.0
	6- 10	7.5	4.8
	11- 15	12.5	8.3
	16- 20	17.5	11.5
	21- 30	25	14.7
	31- 40	35	17.4
	41- 50	45	20.3
1.2	1- 5	2.5	3.3
	6- 10	7.5	5.6
	11- 15	12.5	7.2
	16- 20	17.5	9.0
	21- 30	25	12.3
	31- 44	35	16.2
1.3	1- 5	2.5	1.4
	6- 10	7.5	3.7
	11- 15	12.5	5.8
	16- 20	17.5	7.0
	21- 30	25	10.4
	31- 40	35	14.5
	41- 52	45	19.2
1.4	1- 5	2.5	1.5
	6- 10	7.5	3.1
	11- 15	12.5	5.2
	16- 20	17.5	8.4
	21- 30	25	15.9
	31- 40	35	20.1
	41- 51	45	23.5
1.5	1- 20	10	3.0
	21- 25	22.5	5.2
	26- 30	27.5	8.5
	31- 35	32.5	11.1
	36- 40	37.5	12.6
	41- 50	45	16.0
	51- 63	55	19.7

SECOND GROWTH SAMPLE DATA

TREE #	RINGS INCLUDED IN SAMPLE	AVERAGE RINGS FROM PITH	RADIUS TO LAST RING (cm)
2.1	1- 25	12.5	3.7
	26- 30	27.5	6.3
	31- 35	32.5	8.2
	36- 40	37.5	12.3
	41- 45	42.5	14.9
	46- 50	47.5	21.1
	51- 68	55	30.3
2.2	1- 10	5	1.9
	11- 15	12.5	5.0
	16- 20	17.5	8.0
	21- 30	25	13.1
	31- 40	35	20.2
	41- 52	45	30.1
2.3	1- 25	12.5	3.4
	26- 30	27.5	5.0
	31- 35	32.5	7.5
	36- 40	37.5	10.3
	41- 45	42.5	13.3
	46- 56	50	17.3
	57- 66	60	22.3
2.4	1- 5	2.5	1.3
	6- 10	7.5	3.5
	11- 15	12.5	6.5
	16- 20	17.5	8.8
	21- 30	25	14.2
	31- 42	35	19.2
2.5	1- 60	30	10.2
	61- 65	62.5	12.7
	66- 70	67.5	15.8
	71- 77	72.5	19.8

APPENDIX 6. EXPERIMENTAL DATA FOR SECOND GROWTH TREES

(ALL RESULTS % OVEN DRY EXTRACTIVE FREE WOOD)

TREE #	AVE. # RINGS FROM PITH	EtOH: BENZ. EXTR.	BETA THUJA-PLICIN	GAMMA THUJA-PLICIN	BETA+ GAMMA THUJA-PLICIN	GAMMA /TOT.	COLOR. THUJA-PLICIN	THUJIC ACID + METHYL THUJATE
1.1	2.5	3.3	.004	.010	.014	.72	.086	.128
	7.5	3.9	.002	.010	.011	.85	.112	.310
	12.5	3.6	.013	.044	.058	.77	.147	.216
	17.5	5.7	.036	.108	.145	.75	.197	.331
	25	8.0	.055	.225	.280	.80	.488	.703
	35	9.2	.080	.243	.323	.75	.525	.618
	45	5.0	.014	.077	.091	.85	.222	.246
1.2	2.5	3.6	.024	.032	.055	.57	.109	.312
	7.5	5.1	.032	.054	.086	.63	.148	.477
	12.5	7.4	.036	.073	.108	.67	.180	.884
	17.5	5.4	.034	.047	.081	.59	.193	.612
	25	5.4	.018	.016	.034	.46	.229	.843
	35	6.3	.016	.042	.058	.73	.191	.394
1.3	2.5	5.2	.016	.005	.021	.25	.125	.172
	7.5	4.6	.058	.000	.058	.00	.164	.445
	12.5	5.3	.059	.001	.060	.02	.210	.446
	17.5	7.2	.108	.003	.111	.03	.315	.600
	25	7.4	.162	.028	.190	.15	.348	.650
	35	9.8	.303	.063	.366	.17	.481	.824
	45	8.6	.200	.024	.224	.11	.368	.504
1.4	2.5	4.8	.021	.014	.035	.40	.078	.286
	7.5	5.0	.042	.028	.070	.40	.116	.579
	12.5	4.6	.054	.022	.077	.29	.108	.526
	17.5	4.6	.044	.016	.060	.27	.115	.393
	25	8.2	.157	.107	.264	.40	.325	.772
	35	9.7	.292	.233	.525	.44	.544	.989
	45	6.1	.095	.060	.156	.39	.194	.361
1.5	10	2.8	.022	.000	.022	.00	.030	.069
	22.5	3.3	.058	.000	.058	.00	.050	,157
	27.5	3.8	.089	.023	.113	.21	.115	.242
	32.5	5.4	.122	.047	.169	.28	.245	.407
	37.5	7.0	.226	.150	.376	.40	.390	.580
	45	8.3	.194	.154	.348	.44	.511	.661
	55	9.3	.242	.184	.426	.43	.710	.516

EXPERIMENTAL DATA FOR SECOND GROWTH TREES

(ALL RESULTS % OVEN DRY EXTRACTIVE FREE WOOD)

TREE #	AVE. # RINGS FROM PITH	EtOH: BENZ. EXTR.	BETA THUJA-PLICIN	GAMMA THUJA-PLICIN	BETA+ GAMMA THUJA-PLICIN	GAMMA /TOT.	COLOR. THUJA-PLICIN	THUJIC ACID + METHYL THUJATE
2.1	12.5	3.2	.043	.000	.043	.00	.058	.215
	27.5	4.9	.024	.073	.097	.75	.222	.395
	32.5	5.7	.052	.109	.161	.68	.284	.383
	37.5	5.4	.074	.170	.244	.70	.299	.357
	42.5	9.5	.125	.341	.467	.73	.511	.407
	50	9.3	.166	.240	.406	.59	.524	.295
	60	6.9	.137	.299	.436	.69	.397	.144
2.2	5	3.6	.079	.019	.099	.20	.137	.205
	12.5	3.9	.094	.040	.134	.30	.128	.282
	17.5	4.3	.152	.034	.186	.18	.240	.305
	25	6.0	.206	.044	.249	.18	.350	.444
	35	6.1	.376	.040	.415	.10	.466	.290
	45	8.2	.315	.000	.315	.00	.622	.232
2.3	12.5	3.7	.027	.006	.033	.18	.082	.338
	27.5	4.7	.028	.018	.046	.39	.151	.327
	32.5	5.2	.055	.024	.080	.30	.250	.438
	37.5	6.3	.189	.016	.205	.08	.266	.614
	42.5	7.5	.171	.016	.188	.09	.389	.616
	50	8.9	.152	.034	.186	.18	.338	.427
	60	8.6	.300	.027	.327	.08	.594	.686
2.4	2.5	4.0	.041	.017	.058	.29	.084	.116
	7.5	2.4	.007	.000	.007	.05	.020	.026
	12.5	2.8	.019	.011	.030	.37	.038	.064
	17.5	5.9	.070	.061	.130	.46	.238	.305
	25	6.2	.098	.056	.154	.36	.299	.317
	35	6.0	.135	.035	.169	.20	.284	.302
2.5	30	5.2	.054	.046	.099	.46	.146	.392
	62.5	6.8	.108	.105	.213	.49	.355	.440
	67.5	6.3	.046	.111	.157	.70	.336	.444
	72.5	6.9	.181	.070	.250	.28	.349	.404

APPENDIX 7. LINEAR REGRESSION STATISTICS FOR GC vs COLORIMETRIC RESULTS

SLOPE	INTERCEPT	CORRELATION COEFFICIENT	r^2
.994	-.062	.905	.819