

**INONOTUS TOMENTOSUS PLOT EXCAVATIONS
DATA ANALYSIS AND CONCLUSIONS**

by

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FOREWORD

This report is one of a series of internal reports distributed by Forest Protection Branch, B.C. Ministry of Forests, to record accomplishments and interim or provisional results for the information of staff, colleagues, and others.

In the Canada-British Columbia Forest Resources Development Agreement (FRDA) 1985 to 1990, over 100 pest control projects were directly funded by the province and administered by protection staff. To facilitate application of the results, and development of improved forest health management strategies, the contractors reports have been edited and distributed as internal reports. Accordingly, any opinions and recommendations expressed are those of the contractor, and not necessarily those of the ministry or the FRDA management committee.

For details or more information about the report or project, please contact forest health staff at Protection Branch or at the forest region offices.

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The guidelines for internal reports outlined in the Protection Manual were adhered to as closely as possible. All manuscripts were altered to comply with a common format, but entire reports were not rewritten to conform to a standardized outline.

Most Executive Summaries were the work of Graham Hawkins of MoF Inventory Branch (formerly with Pacific Coast Forest Biology). All word processing was accomplished by Catriona Kaufman of Top Kat Services. Will Gordon of R. White Woods inc. prepared all maps and figures.

R. White, editor
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EXECUTIVE SUMMARY

The project objective was to study the means and rates of infection and disease spread and to study the effects of *tomentosus* root rot on tree growth.

This project was the second part of a study initiated in 1986 at Beaver Forest Road, Prince George Forest Region, in which five permanent sample plots (PSP's) were established and some preliminary survey methods were investigated. All data had not been summarized during that project, thus the information and maps were collated and analyzed per plot. The site summary sheet included information on tree species, age, height, five-year increment, number and location of infected roots, infection rating up stem and infection rating in the roots. All trees were assigned a disease rating. Measurements taken for the last 15 years of growth were used to calculate any changes in basal area of infected trees over five-year periods as compared to control (healthy) trees by means of an analysis of variance.

The following findings were derived from a summary of the original survey observations and an analysis of the root system maps. Root collar weevils (*Hylobius warreni*) were found to be frequently associated with the presence of *tomentosus* root rot. The exact nature of this association was not established but it appeared that the role of the insect was secondary. Root collar weevils are probably not vectors of the disease, being attracted by stress due to infection instead. Regardless, some infection courts may have been initiated by weevil activity. Infection was seen to occur via the smaller (0.2-1.0cm) roots. Larger roots caused much less transmission. Decay generally moved from the smaller roots toward the root collar. Roots with stain only and no advanced decay were observed to be much less capable of causing infection. The fungus is more likely to be near the surface of the roots when roots are small or when advanced decay is exhibited. Trees which had stain without advanced decay in the root system had no decay in the stem even at the root collar. Observations on vegetative spread outlined in PM-PB-36 support these findings.

Despite an observed trend, the analysis of variance found that five-year basal area increment was not significantly affected by the pathogen. However, significant differences will be seen in basal area increment if the trend continues. Height increment of infected trees was found to be significantly less than in the control, mean growth over 15 years decreasing with increasing disease rating.

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1 INTRODUCTION

The purpose of this project was to study means and rates of infection and disease spread, and to study the effect of *Inonotus tomentosus* on tree growth by mapping healthy and diseased trees in disease centres. The plots are to be remeasured at 5-10 year intervals.

This study is the second part of a project established in 1986. The first part was a survey through a diseased stand using several means of disease detection, followed by spacing and examination of stump surfaces for disease detection. Results of part one are as an appendix to this report. Appendices are not included with this distributed report but are kept with the original document in the Forest Health Section, MoF Protection Branch, Victoria.

2 MATERIALS AND METHODS

2.1 Work by Richard Reich (1986)

Permanent sample plots with an eight-metre radius were established at Km 44 on the Beaver Forest Road, in a spruce stand approximately 60 years old. The plots were centred around disease centres, with three plots in the spaced area, and two plots in the unspaced area. All spacing slash was cleared from each plot and the trees in each plot carefully excavated by hand. Care was taken to avoid wounding the roots and after excavation, measurement and observation, the roots were covered with soil, duff and moss.

Measurements of dbh, height, percent live crown and age were taken for all trees in the plots, and trees were located by distance and bearing from plot centre (Appendix 3). To measure the root systems, a string grid was laid over the roots, and the roots mapped to scale on graph paper in the field. Roots were sampled using an increment corer from the root collar distally at 25cm intervals. Each sample was rated 0 = healthy, 1 = stain or 2 = decay, and the ratings were noted directly onto the field maps. Samples were also taken at the root collar, at 0.3m up the stem and at dbh (except for the spaced trees which could only be sampled at root collar), and rated by the same system. Radial five-year growth increments were measured for the last 15 years of growth using an increment corer (infection rating and growth increment data in Appendix 4). Resin crusts on the roots and signs of *Hylobius warreni* activity were also noted on the maps.

Field maps were reduced 65% on a photocopier, then traced over on drafting paper. The maps were colored by infection class.

2.2 Subsequent Work (1988)

The large maps were very awkward to handle; therefore, they were further reduced to a scale of 1:45 (Appendix 1). The original excavator's notes were collected and typed (Appendix 2), then used in conjunction with the maps to analyze sources of infection, movement of the fungus in the root systems and the association of *Hylobius warreni* with *Inonotus tomentosus*.

Summaries of tree data and infection ratings were made once the data had been entered into a spreadsheet. All trees were given a disease rating from 0-5 based on the following criteria:

- 0 = healthy;
- 1 = one root infected < 25cm advanced decay;
- 2 = one to two roots infected > 25cm advanced decay in at least one root, or one to three roots with stain only;

- 3 = $\geq$ two roots infected with $<$ half with advanced decay, or $>$ three roots with stain only;
- 4 = more than half of all roots infected; half with advanced decay;
- 5 = all roots infected; most with advanced decay.

The summaries and disease ratings are in Appendix 5.

The measurements of five-year increment taken for the last 15 years of growth were used to calculate the change in basal area over five-year periods. Blocking first by diseased and healthy trees, and then by disease class, the change in basal area was tested for significance between treatment blocks by Analyses of Variance (ANOVA). Appendix 6 contains the raw data and Appendix 7 the ANOVA calculations.

3 RESULTS

The maps reduced from the original field maps are in Appendix 1. Notes made by the original excavator on Plots 2, 3, and 4 are in Appendix 2. Notes for Plots 1 and 5 either were not made or have been lost. Using the maps and notes together, the following summary of observations has been made with respect to source of infection, movement of the fungus in the roots and the association with *Hylobius warreni*, a root collar weevil.

Plot 1

- Tree 132 infection came in several roots from the tips, stem decay has progressed only to the stain stage (infection rating 1) at the root collar.
- On most of the other trees, stain is distal to decay on the root, suggesting the fungus is growing from the root collar outward.
- Several trees have resin crusts but do not show signs of infection, suggesting that the crust is due to the activity of the weevil, and that this activity did not lead to infection.
- Windthrown, decayed trees, even part way up stem, appear to be able to cause infection. *Hylobius* activity also seems to concentrate here.

Plot 2

- A very typical pattern was shown several times here, where infection occurred through one or two roots, then moved around the stem and out another root.
- In this plot and in several others, stain was observed originating from areas of bark damage. Most of the samples were not plated onto petri dishes; therefore, it is impossible to say whether or not the observed stain was due to *I. tomentosus*. However, the regularity of stain occurrence in the root disease centres, and the similarity to stain observed associated with decay suggest that the stain under the wounds was that of *I. tomentosus*.
- Descent of the fungus from the root surface to the heartwood following infection occurred in a short distance.
- Small decayed stumps and their residual roots were prime sources of inoculum.
- Infection occurred in very small roots (0.2cm diameter), and root contacts with larger roots (2-5cm) as inoculum sources were less likely to cause infection.

- The association of *H. warreni* with *I. tomentosus* appeared to be secondary; the weevil was attracted to diseased roots. There may be more of a primary role for the weevil in the creation of bark wounds, but conclusive evidence for this was lacking.
- Stain in roots preceded decay by several metres in living trees.
- In heavily diseased areas, multiple points of infection on one tree or on one root were quite common.
- Infection (Tree 135) may have been picked up from a 4mm diameter root. It may be that these smaller roots are more readily colonized by the fungus, such that the fungus is on the root surface and available as an inoculum source. Total colonization of larger roots takes more time and is often restricted by resin-soaked outerwood layers, although overall fungal biomass is greater.
- Several trees with stain or decay in the roots were not in contact with any observed sources of infection; some of these trees' roots did have bark wounds and exhibited signs of *H. warreni* activity.
- Tree 148 was infected through contact with a very small (2cm diameter at root collar) dead stump. There were no signs of weevil activity.

Plot 3

- In the earlier stages of decay the fungus was sometimes confined to one branch in a forked root.
- Two small roots from Trees 184 and 182 crossed the roots of an old windfall where infection was thought to occur. The root ends were girdled by *H. warreni* and were dead. In the same area, stain in a root of Tree 176 appeared to originate at an *H. warreni* larvae site. The evidence suggests, however, that the weevil came in after the roots had become infected from the old windfall.
- In Tree 176, the fungus again appeared to have come in one root, then proceeded away from the tree in another root.
- Contacts with diseased roots that have only stain and no advanced decay were not sufficient sources of inoculum to cause infection.
- Tree 187 is a good example of multiple infections occurring within one root, where a colonized root serves as an inoculum source for several other roots of the same tree.

- Again, the smaller roots (less than approximately 4cm diameter) seemed to cause more infections than larger roots.

Plot 4

- Small residual stump roots were a prime source of inoculum.
- *Hylobius* galleries were especially prevalent where the root contacted an old rotten log and there was a heavy duff layer.
- Some of the observed stain may be more a reaction to weevil activity than to the fungus.
- Based on estimated percentages of root contacts causing infection, large roots of trees were less susceptible to infection than smaller roots (0.5-4cm).
- *I. tomentosus* spreads radially in roots (both stain and decay) as well as longitudinally but spreads much slower radially in living trees.
- Trees which had stain without advanced decay in the root systems had no decay in the stem, even at the root collar.
- The edge of the disease centre seemed to occupy the eastern portion of this plot.

Plot 5

- Diseased trees in this plot had very little advanced decay.
- In most cases, the infection occurred at the root ends (2-4cm) and moved towards the butt.
- There was little *H. warreni* activity associated with stain, which further indicates that *H. warreni* is secondary to *I. tomentosus*.

Tree size between plots did not vary much; trees in Plots 4 and 5 were slightly smaller but were also younger. The percentage of trees infected by *I. tomentosus* and the mean disease rating were different between the plots (Table 1). Plots 4 and 5 had the fewest infected trees and the least number of roots with advanced decay. These two plots were probably located on the edge of a disease centre in an area less heavily infested with *I. tomentosus*.

Butt cull in *I. tomentosus*-infected logs can cause significant losses. Table 2 shows the percentage of trees in each infection rating (0-2 scale) at each sampling location. Figure 1 gives the mean infection rating at each sampling location. Two observations are made: the overall disease rating of the tree usually is greater than 3.0 before decay is found above the root collar, and spread in the stem appears to be slower than in the roots because little stain is seen in the stem. The samples show either healthy wood, or wood with advanced decay.

Analyses of Variance of the changes in basal area blocked by disease classes showed no significant differences in basal area increment. The tests were run on the 0 - 5 year increment data, and on the 0 - 15 year increment data blocked by disease class. None of the ANOVA's showed significant differences (Table 3) in the mean change in basal area, although Figure 2 shows a trend of decreasing basal area increment with increasing disease ratings.

The number of root contacts leading to infection could not be calculated because the excavator did not consistently note which overlapping roots, as drawn on the map, actually contacted.

4 CONCLUSIONS

The primary sources of infection of trees in the stand are from roots of old stumps and windfalls, some of which may be as old as 60 years, dating back to a fire (evidenced by charred wood in the duff layer) which probably initiated the current stand. Some of the sources of inoculum which were directly related to new infections were quite small (less than 1cm diameter).

Furthermore, the most common observations of infection were made on small roots, less than four or five centimetres in diameter. Small pieces of root can be more readily colonized by *I. tomentosus* than larger pieces; therefore, the fungus is more likely to be at the surface of the root where it can more readily contact a healthy root. The resin-soaking response to wounding or invasion by fungi is of much greater volume in larger roots and, therefore, may serve to confine the fungus more to the interior of the root, as seen by "hollow shells" of wood and bark surrounding thoroughly decayed material. Roots with stain only and no advanced decay are less capable of causing infection.

The role of *Hylobius warreni* in these plots appears to be secondary. There are many examples of *Hylobius* activity in areas where no disease was detected. Weevil activity in diseased roots was sometimes at a distance from the area of colonization suggesting that the weevils did not transmit the fungus. Most of the evidence supports the hypothesis that the weevils are attracted to diseased roots, especially roots showing dark stain, and therefore arrive after infection. A few observations indicate, however, that the weevils may play a role in creating infection courts for the fungus by feeding on the rots and creating openings in the bark. Stain was observed originating from several weevil gallery sites, and from wounds created by other means. Although isolations were made from these samples, identification of the stain is supported by its location, pattern, color, and the overall abundance of *I. tomentosus*.

Mean tree growth over 15 years decreased with increasing disease rating, although the difference in mean change in basal area between classes was not statistically significant. There was a large amount of variation within disease classes which may account for the lack of statistical significance. In addition, the disease classes are subjective classifications, not linearly related, which also may have affected variance. Should the trend observed in this data continue, statistically significant differences in basal area increment will be observed in a few years. Tree height growth is usually more severely affected by root disease than is radial growth. Estimates of height growth loss due to certain levels of disease may be more defined between classes, and of greater magnitude than radial growth loss.

TABLE 1 MEAN TREE MEASUREMENTS AND DISEASE RATINGS

Plot	dbh (cm)	Ht (m)	Age	% Infected	Disease Rating
1	20.5	21.4	62.8	83	2.76
2	21.6	20.3	62.6	90	3.10
3	19.9	19.2	65.1	96	2.73
4	19.5	16.9	59.8	77	1.88
5	16.8	19.5	59.7	71	1.63

TABLE 2 STEM COLONIZATION BY INONOTUS TOMENTOSUS
Percent of Trees in Each Infection Rating
(0 = healthy, 1 = stain, 2 = decay) by Location

Plot	Location								
	Root Collar			0.6m			dbh		
	0	1	2	0	1	2	0	1	2
1	45	10	45	50	0	50	58	0	42
2	45	3	52	52	3	45	86	7	7
3	52	8	40	64	0	36	64	0	36
4	89	4	7	89	7	4	100	0	0
5	73	19	8	85	11	4	100	0	0

TABLE 3 ANALYSES OF VARIANCE ON BASAL AREA INCREMENT BY DISEASE CLASS

Ho: mean class 0, 1 is equal to the mean of class 2, 3, 4, and 5 for the 0-5 year increment data

Source	SS	DF	MS	F
Treatment	404.15	1	404.15	1.25 NS
Error	38013.99	118	322.15	
Total	8418.14	119		

Ho: mean class 0, 1 is equal to the mean of class 2, 3, 4, and 5 for the 0-15 year increment data

Source	SS	DF	MS	F
Treatment	1777.53	1	1777.53	0.68 NS
Error	307345.88	117	2626.89	
Total	309123.41	118		

Ho: all of the means in class 0, 1, 2, 3, 4 and 5 are equal

Source	SS	DF	MS	F
Treatment	6041.56	5	1208.31	0.126 NS
Error	186848.8	113	9618.13	
Total	1092890.4	118		

FIGURE 1 **AVERAGE TREE DISEASE RATING PER PLOT AND AVERAGE INFECTION**
RATING UP THE TREE STEMS BY SAMPLE LOCATION

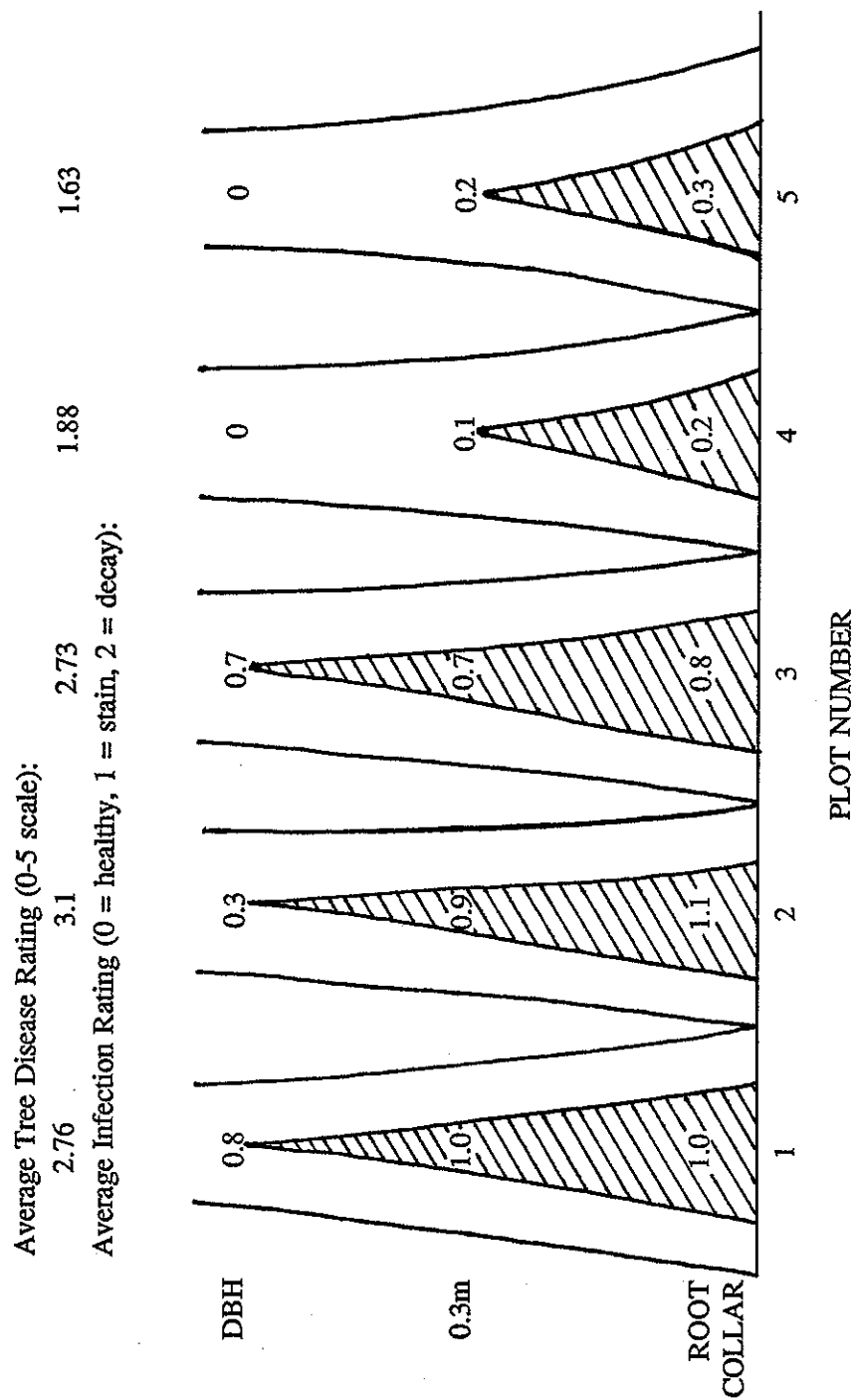


FIGURE 2 **MEAN CHANGE IN BASAL AREA OVER 15 YEARS BY INFECTION CLASS**

