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EFFECTS OF FERTILIZATION WITH UREA ON ROOT ROT DISEASES OF DOUGLAS-FIR AND WESTERN HEMLOCK CAUSED BY PORIA WEIRII MURR. AND FOMES ANNOSUS (FR.) KARST

by

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of the requirements for the degree of

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Approved by Chas. H. Driver III

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Date 11/21/72

Master's Thesis

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Abstract

EFFECTS OF FERTILIZATION WITH UREA ON ROOT ROT DISEASES OF DOUGLAS-FIR AND WESTERN HEMLOCK CAUSED BY *PORIA WEIRII* MURR. AND *FOMES ANNOSUS* (FR.) KARST

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The effect of urea fertilizer on root rot diseases of Douglas-fir and western hemlock caused by *Poria weirii* Murr. and *Fomes annosus* (Fr.) Karst was investigated. The basic approach of the study was to 1) compare the effects on artificially established host-pathogen associations of soil fertilized with urea and unfertilized soil, and 2) compare the decay resistance of wood from roots of urea fertilized and unfertilized Douglas-fir and western hemlock trees.

In the artificially established host-pathogen associations, the addition of urea fertilizer to the soil was found to reduce the number of infections in the Douglas-fir + *Poria weirii* association. The fertilizer had no effect on the Douglas-fir + *Fomes annosus* association. The effect of urea on the western hemlock + *Fomes annosus* association could not be entirely evaluated because the fertilizer proved to be very toxic to the western hemlock seedlings.

Rootwood from urea fertilized Douglas-fir trees was found to be more resistant to decay by *Poria weirii* and *Fomes annosus* than rootwood from unfertilized trees. The resistance of western hemlock rootwood to decay by *Fomes annosus* was not found to be affected by urea fertilization.

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Abstract (cont.)

Explanations are offered to account for the results obtained in the research.

TABLE OF CONTENTS

		page
	List of Figures	v
	List of Tables	vi
	Acknowledgments	vii
1.	INTRODUCTION	1
2.	LITERATURE REVIEW	3
	2.1 Effects of nitrogen fertilizer on the forest soil environment	3
	2.2 Effects of nitrogen fertilization on the physiological state of the root	5
	2.3 Review of studies concerning nitrogen fertilization and its effect on forest root diseases	8
3.	SEEDLING INOCULATION EXPERIMENTS	12
	3.1 Methods and materials	12
	3.1.1 Experimental design	12
	3.1.2 Description of nursery facilities and materials	12
	3.1.3 Inoculation techniques	14
	3.1.3.1 Description of inoculum	14
	3.1.3.2 Root contact method	15
	3.1.3.3 Root collar wound method	15
	3.1.4 Treatment of seedlings after inoculations	20
	3.1.5 Determination of disease induced mortality	20
	3.1.6 Determination of inoculum survival	22
	3.2 Results	23
	3.2.1 Survival of control seedlings	23
	3.2.2 Survival of unfertilized and fertilized seedlings with inoculum in contact with roots	23
	3.2.3 Survival of unfertilized and fertilized seedlings with inoculum attached at a root collar wound	27
	3.2.4 Survival of pathogens in unfertilized and fertilized soil	30

page

3.3 Discussion 30

3.3.1 Douglas-fir with Poria weirii associations 32

3.3.2 Douglas-fir with Fomes annosus associations 33

3.3.3 Western hemlock with Fomes annosus associations 34

4. ROOTWOOD DECAY EXPERIMENT 37

4.1 Methods and materials 37

4.1.1 Decay study 37

4.1.1.1 Experimental design 37

4.1.1.2 Description of field site 37

4.1.1.3 Collection and preparation of samples 39

4.1.1.4 Description of decay chambers 40

4.1.1.5 Inoculation technique 40

4.1.1.6 Methods for terminating experiment 45

4.1.2 Chemical analysis of rootwood 45

4.2 Results 47

4.2.1 Decay test 47

4.2.2 Chemical analysis of rootwood 49

4.3 Discussion 52

5. CONCLUSIONS 56

Literature Cited 57

Appendixes 63

I Description of stock cultures from which root rot pathogens used in the seedling experiment were obtained

II Description of fertilized and unfertilized trees sampled for rootwood in the decay experiment

III Procedure used to determine total nitrogen content of rootwood samples

IV Weight losses of decayed rootwood samples in decay test

V Results of nitrogen analysis of Douglas-fir rootwood samples from urea fertilized and unfertilized trees

page

Appendixes (cont.)

VII Results of starch analysis of rootwood from nitrogen fertilized and unfertilized Douglas-fir and western hemlock trees

LIST OF FIGURES

		page
1.	Outline of the experimental design for the seedling inoculation experiment	13
2.	Method of inoculating alder blocks with root rot pathogens	16
3.	Placement of alder inoculum block in the root contact inoculation method	17
4.	Root collar wound inoculation method showing seedling wound and bark strip removed from alder inoculum block	18
5.	Root collar wound inoculation method showing attachment of alder inoculum block with grafting rubber	19
6.	Potted seedlings with urea spread evenly over the soil surface	21
7.	Survival of Douglas-fir seedlings in the control group	24
8.	Survival of western hemlock seedlings in the control group	25
9.	Survival of seedlings with the inoculum in contact with the roots	26
10.	Survival of seedlings with the inoculum attached at a root collar wound	28
11.	Comparison of healthy Douglas-fir seedling with one exhibiting "root rot" symptoms. The seedling on the right is showing the advanced stage of root rot infection.	29
12.	Survival of root rot pathogens in urea fertilized and unfertilized soil	31
13.	Outline of rootwood decay experiment	38
14.	Decay chamber with hangar and sterile water	41
15.	Decay chamber with rootwood test specimen on hangar	42
16.	The first inoculation method used in the decay experiment. The inoculum plug is shown on the wood and the metal tube with plunger used to insert the inoculum is next to the decay chamber.	44
17.	The final inoculation method used in the decay experiment. The inoculum plug is shown on top of the malt agar block.	46

LIST OF TABLES

		page
1.	Average weight losses of decayed rootwood from fertilized and unfertilized trees and statistical comparisons of sample means.	48
2.	Results of total nitrogen analysis of sapwood from roots of nitrogen fertilized and unfertilized Douglas-fir trees and statistical comparison of sample means.	50
3.	Results of starch and sugar analysis of rootwood from nitrogen fertilized and unfertilized Douglas-fir and western hemlock trees.	51

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I. INTRODUCTION

With the advent of intensive forest management practices in the Pacific Northwest, forest fertilization is becoming a common method used to improve tree growth. Research has indicated that nitrogen deficiency is the most common limiting factor to growth in the Douglas-fir region (Gessel et al., 1965); therefore, nitrogen fertilizer is most widely used. It is estimated that 300,000 acres of Pacific Northwest forests have received an application of nitrogen fertilizer between the years 1966 and 1972 (Strand et al., 1972). Although fertilization can improve tree growth, it is necessary to know how it affects other aspects of the forest environment so that the total impact of nitrogen fertilization as a forestry practice can be evaluated.

Forest disease is one aspect of the forest environment which will undoubtedly be affected by nitrogen fertilization. Because of the increasing value of timber, minimizing losses from disease is an important part of forest management programs. Therefore, to understand fully the use of nitrogen fertilization as a cultural tool, it is necessary to know how it affects disease conditions.

In young growth stands of Douglas-fir and western hemlock, where today's emphasis of management is being directed, root rot diseases are responsible for annual losses of greater than 40 million cubic feet (Childs and Shea, 1967). The importance of dealing with root rot problems in intensively managed forests is easily seen when considering the following

statement by Childs and Shea (1967) from their report on annual losses from disease in Pacific Northwest forests: "Losses (from root rot) will increase considerably in the next several decades not only because of increase quantity of young growth but also because certain cultural practices tend to increase damage by root rots."

There is a lack of information on the effects of forest fertilization on root rot diseases in the Pacific Northwest. With the formation of the Regional Forest Nutrition Project, the impetus for basic research on that aspect of forest fertilization was provided. This thesis describes a study undertaken as a result of the Nutrition Project. The purpose of the study was to determine some of the effects of urea nitrogen fertilization on root rot diseases caused by Poria weirii Murr. and Fomes annosus (Fr.) Karst which are common in the Douglas-fir - western hemlock forests.

The basic approach of the study was to 1) compare the effects on artificially established host-pathogen associations of soil fertilized with urea and unfertilized soil and 2) compare the decay resistance of wood from roots of urea fertilized and unfertilized Douglas-fir and western hemlock trees. This approach should allow for some insight into how nitrogen fertilization affects root rot disease because the two environments which greatly influence the activity of the root rot pathogens, the soil and the internal tissues of the root, were investigated.

2. LITERATURE REVIEW

2.1 Effects of nitrogen fertilizer on the forest soil environment

Forest fertilization is an enrichment of soil with nutrients and thus will affect the various chemical and microbial characteristics of the soil. The activity of soil-borne pathogens is greatly influenced by the various factors of the soil environment (Garrett, 1970); therefore, a knowledge of the changes occurring after fertilization is important to understanding how the practice can affect root rot pathogens. In this section of the literature review, the results of research concerning the effects of nitrogen fertilization on the soil environment especially in the Douglas-fir region is presented.

In studies on nitrogen fertilization of black spruce stands, urea fertilizer has been found to affect the chemical and microbial characteristics of humus. Reddy and Knowles (1965) could find no consistent differences in the magnitude of the fungal population between fertilized (at 400 lbs urea N/acre) and unfertilized humus, but some qualitative differences were found between treatments. A similar study by Roberge and Knowles (1966) showed that urea (at 400 lbs N/acre) increased the bacterial and fungal populations and changed their composition. Roberge et al. (1970) also found that urea increased and changed the composition of the microbial population of humus. Urea fertilization also increased the pH and nitrogen content of the humus.

Mai and Fiedler (1970) studied the microbial populations of humus in urea fertilized and unfertilized stands of mature Norway spruce. In the

fertilized plots, there was a significant increase in the number of bacteria, a slight increase in the actinomycete population and a tendency toward a decrease in the size of the microfungi population.

Schalin (1967) tested the effect of four different types of nitrogen fertilizers on the microbial populations of a pine humus layer. He found the effect of urea was immediate. The pH rose and the bacterial density increased 20 to 30 times while the microfungi density decreased one third. In the ammonium sulphate plot, corresponding, but completely opposite, changes occurred almost as rapidly as in the case with urea. The other two nitrogen fertilizers, calcium ammonium nitrate and nitrate of lime, resulted in a gradual increase in bacterial and microfungi populations.

Fressenden et al. (1971) found urea but not ammonium nitrate to increase microorganism activity in the humus of a Jack pine stand.

In soils under Douglas-fir stands, Nelson (1970) applied sodium nitrate and ammonium chloride at concentrations equivalent to 150, 300, and 600 lbs of nitrogen per acre. Three months after fertilization, the $NH4^{+}$ and $NO3^{-}$ salts were not detected at the 6 to 8 inch depth. He also determined that over a one-year period, the populations of aerobic actinomycetes (at the 6 to 8 inch depth) in the fertilized plots were not significantly different from those in the control plots.

Shareeff (1955) and Gessel and Walker (1956) found that nitrogen fertilization increased the nitrogen content of the upper 6 inches of soil in low site Douglas-fir stands. Heilman (1961) also investigated nitrogen fertilized low site Douglas-fir stands. He determined that the nitrogen content in the upper 12 inches of soil was significantly increased by fertilization. Also urea fertilizer caused a significant lowering of pH in the surface

soil. Gallagher (1964) fertilized a young stand of Pacific silver fir with ammonium nitrate and urea and found that fertilization did not increase the nitrogen content of the upper 6 inches of soil.

The abundance of ammonium and nitrate in urea fertilized and unfertilized soil from two different sites (Douglas-fir stands) was determined by Bourgeois and Gessel (1972). In the upper 6 inches of soil in the site II area, the fertilized plot had the greatest production of nitrate and ammonium. On the site IV area, the soil in the fertilized plots showed little if any increase in nitrate and ammonium production over the control plots.

Crane (1972) has found that most of the urea fertilizer applied to a Douglas-fir stand stayed in the upper 4 cm of the soil. He determined that less than 1/2 percent of the nitrogen applied at levels of 112 and 224 kg of nitrogen per hectare moved through 15 cm of the soil. No nitrogen movement was measurable below the 100 cm depth. He also found a significant increase in the pH of the soil water following fertilization. Generally, the initial increase several days following treatment was 1 to 1.5 pH units. The effect was most intense at the 0 to 2 cm depth. At the 5 cm depth, there was an increase of only 1/2 pH unit or less.

2.2 Effects of nitrogen fertilization on the physiological state of the root

It has been clearly demonstrated that the physiological state of the root can affect the degree of activity of root rot pathogens (Miller and Kelman, 1966; Towers and Stambaugh, 1968). In this section of the review, information is presented which should serve as the basis for understanding how the activity of root rot pathogens will be affected in the roots of

fertilized trees. There is little known about this aspect of root physiology; most of the research has been conducted on fruit trees and conifer seedlings. Rarely have studies dealt with the larger roots of conifers.

The nitrogen nutrition of fruit trees has been actively investigated. Hill-Cottingham and Williams (1967) found total nitrogen in new and old roots of apple seedlings increased following a nitrogen application. Oland (1954) found nitrogen fertilizer increased total nitrogen, soluble nitrogen and protein nitrogen in roots of two-year apple seedlings. He also determined an increase in the amino nitrogen and amide nitrogen fraction of the total nitrogen. Similar findings were reported by Tromp (1970). His experiments with nitrogen fertilizer produced greater soluble nitrogen in the roots with arginine showing the greatest increase of the individual components. In roots of two-year-old peach seedlings, Taylor and May (1967) found nitrogen fertilization increased total nitrogen and soluble nitrogen. The increase in soluble nitrogen was mainly due to the increase in the amount of arginine. Taylor (1967) determined that nitrogen fertilization increased total nitrogen and the concentration of free amino acids in the roots (larger than 1/4-inch in diameter) of 25-year-old peach trees.

Pharis et al. (1964), in studies on loblolly pine seedlings, found that nitrogen fertilization increased the nitrogen content of the roots. They also showed that the amount of soluble nitrogen and the concentration of the individual amino acids was strongly influenced by the form of nitrogen used. Durzan and Steward (1967) also showed the same to be true for fertilized spruce and pine seedlings. Work by van den Driessche (1971) has shown that the nitrogen content of roots of Douglas-fir, western hemlock and spruce seedlings is increased by nitrogen fertilization. Other

investigations have also demonstrated that high levels of nitrogen nutrition increased the total and soluble nitrogen content of conifer seedling roots (Fowells and Krauss, 1959; Barnes, 1961; Nelson, 1964; Barnes and Bengtson, 1968; Meyer and Splittstoesser, 1971).

A few studies have found that nitrogen fertilization affects the nitrogen content of roots of mature conifers. Wheetman (1962) sampled the roots (1 cm in diameter) of fertilized and unfertilized 50-year-old black spruce trees. The trees had been fertilized with ammonium nitrate at 100 and 400 lbs of nitrogen per acre. He found that fertilization increased the nitrogen content of the roots. Goyer and Benjamin (1972) reported that nitrogen fertilization (ammonium nitrate at 250 lbs N/acre) of a young stand of Jack pine (25 to 30 years old) resulted in an increase in the nitrogen content of roots 2 cm in diameter.

The effect of nitrogen fertilization on the carbohydrates in tree roots is poorly understood. Lister et al. (1968) studied the translocation of 14C-photosynthate to the roots of white pine seedlings under various nutritional levels of nitrogen and phosphorus. They found that with increased nitrogen supply there was increased hydrolysis of sucrose to hexoses (sucrose is the main compound in which photosynthetically assimilated carbon was translocated from shoots to roots) and an increase in the recovery of 14C in the amino and organic acids. Meyer and Splitstoesser (1971) showed that nitrogen fertilization of Taxus media seedlings resulted in a slight decrease in the carbohydrate content of the roots.

The research conducted by Alcubilla et al. (1971) has provided some valuable information on the effects of nitrogen fertilization on root physiology (see discussion of Alcubilla's research in section 2.3). Their

studies showed that heavy nitrogen fertilization of a 35-year-old stand of Norway spruce increased the amino acid concentration (particularly of arginine) in the root cambium but did not affect the composition or the amount of polyphenolic compounds.

2.3 Review of studies concerning nitrogen fertilization and its effect on forest root diseases

Although it has been clearly demonstrated that forest fertilization affects forest disease conditions (Foster, 1968), there is very little known specifically about the effects of nitrogen fertilization on root rot diseases. The following is an attempt to describe in detail the studies which have dealt directly with nitrogen fertilization and forest root diseases.

Oksbjerg and West-Nielsen (1953) have published the results of an extensive survey made to determine the occurrence of root diseases in 40- to 70-year-old spruce plantations which had received some fertilizer applications during the early 1920's. The fertilizer treatments were 1) calcium carbonate, 2) calcium carbonate and phosphorus and 3) calcium carbonate and nitrogen. The survey showed that the attack of spruce by F. annosus was greatest in the fertilized areas of the plantations. Since the areas receiving treatment 1 had similar occurrences of attack as areas receiving treatments 2 and 3, the authors believed that limeing was largely responsible for the increased incidence of root disease in the fertilized areas. Although the effect of the phosphorus and nitrogen fertilizers on the occurrence of root disease in treatments 2 and 3 was uncertain, it was thought that their influence on the disease was minor.

Seibt (1964) made a study on the occurrence of red rot (mostly caused by F. annosus) in roots, stumps and stems of 30-year-old Scots pine, Japanese larch and Norway spruce on fertilized and unfertilized sites. The trees had been fertilized with a combination of nitrogen, potassium and sodium fertilizers. He found that fertilization clearly did not increase the amount of disease within the stand. However, within the tree he observed that the spread of the disease was favored by the greater and non-uniform increment associated with the fertilizer treatment.

Research by Nelson (1970) on a common Pacific Northwest root rot pathogen, P. weirii, has shown that nitrogen fertilization affects the survival of the pathogen in the soil. Nelson mixed sodium nitrate and ammonium chloride (at a rate equal to 600 lbs N/acre foot of soil) into a forest soil and then buried Douglas-fir wood cubes naturally infected with P. weirii in quart containers filled with the two fertilized soils and a control (unfertilized soil). After incubation at 15 degrees centigrade for six months, all the cubes were removed and isolations were made to test for survival of the pathogen. The results of his experiments were striking. P. weirii survived in less than 2 percent of the wood cubes buried in the fertilized soil, while survival was approximately 46 percent in the unfertilized soil. Although the factors responsible for the lower survival in the fertilized soil were unknown, Nelson felt that the additional supply of nitrogen stimulated development of microorganisms which invaded the wood cubes and replaced the pathogen.

The fungistatic nature of the cambial tissue in the roots of nitrogen fertilized spruce has been studied by Alcubilla et al. (1971). Root cambium tissue was sampled from fertilized (two applications of calcium ammonium

nitrate at 512 kg N/ha) 35-year-old Norway spruce trees and incorporated into a media to test its fungistatic nature on the growth of F. annosus. A similar test was conducted on root cambial tissue from unfertilized spruce trees. The results of the test showed that the root cambium from the fertilized trees was less inhibitory to the growth of F. annosus than the cambium from the unfertilized trees. A chemical analysis of the tissues revealed that nitrogen fertilization did not affect the composition or amount of polyhydroxyphenols in the root cambium (five phenolic compounds were found to inhibit F. annosus in vitro), but did increase greatly the amount of free amino acids. The investigators believed that the weaker fungal inhibition shown by the root cambium from the fertilized trees was due to the increase in amino acids which served as a food base for the growth of F. annosus.

The effect of fertilization on the occurrence of Armillaria root rot was investigated by Ono (1970) in a young plantation of Japanese larch and white pine. The trees were planted on a site with Armillaria mellea (Fr.) Kumm already established in the soil. After planting, a combination fertilizer (N 8%, P205 9%, K20 5%) was applied to two areas; one received the fertilizer at 100 grams per tree, the other at 200 grams per tree. Four years after establishing the plantation, a survey was conducted to record the incidence of root rot disease in the two fertilized areas and the control. No significant differences in occurrence of the disease between the fertilized and unfertilized parts of the plantation were found.

The decay susceptibility of rootwood from nitrogen fertilized and unfertilized Norway spruce and Scots pine was determined by Cowling et al. (1969). Trees of varying ages (25 to 140 years of age) were fertilized by an aerial application of granular urea at rates ranging from 130 to 258

kg N/ha. Approximately five years after fertilization, rootwood was collected from treated and untreated trees and the nitrogen content and decay susceptibility (by soil block test) determined. No significant difference in nitrogen content or decay susceptibility was found between fertilized and unfertilized tree rootwood.

One investigation not directly concerned with forest diseases but of interest to this review is the work by Cooley (1945). He inoculated the roots of apple trees with Xylaria mali Fromme., the organism which causes black root rot of apple trees, and applied various fertilizers to determine their effect on the root disease. The results of the experiment showed that none of the fertilizers including the nitrogen fertilizer (324 lbs sodium nitrate/acre) affected the disease. There was no appreciable difference in the percentage of successful infections or average size of root lesions between fertilized and unfertilized treatments.

Rowan (1971) made a study on the effects of soil fertilization, fumigation and temperature on black root rot of slash pine seedlings. Working with a soil heavily infested with Macrophomina phaseolina (Tassi) Goid and Fusarium oxysporum Schlecht. and maintained at 24 degrees centigrade and 35 degrees centigrade, he added nitrogen fertilizer (ammonium nitrate at 112.1 kg N/ha) and planted slash pine seeds. Nine months later, the severity of seedling root rot damage was recorded. In the soil maintained at 35 degrees, root rot damage and seedling mortality was most severe in the nitrogen fertilized treatment. However, at the 24 degree temperature, there was little difference in root rot damage or seedling mortality between the nitrogen fertilized and unfertilized soil.

3. SEEDLING INOCULATION EXPERIMENTS

3.1 Methods and materials

3.1.1 Experimental design

The experiment was designed to compare the effects on artificially established host-pathogen associations of soil fertilized with urea, and unfertilized soil. The methods used to create the associations will be referred to as the techniques of inoculation by root contact and by root collar wound. The host-pathogen associations were Douglas-fir (Pseudotsuga menziesii [Mirb.] Franco.) with P. weirii, Douglas-fir with F. annosus (Douglas-fir isolate), and western hemlock (Tsuga heterophylla [Raf.] Sarg.) with F. annosus (western hemlock isolate). The control group was designed to determine the effects of the urea fertilizer and the inoculation techniques on the growth of the seedlings. The experimental design is outlined in Figure 1.

3.1.2 Description of nursery facilities and materials

The Forest Land Management Center of the Washington State Department of Natural Resources provided the greenhouse facilities and nursery materials used in this experiment. Two-year-old Douglas-fir and western hemlock seedlings were lifted by hand from the Webster Nursery just prior to being inoculated (March-April, 1971). An attempt at using western hemlock seedlings obtained from cold storage was unsuccessful due to high mortality

Figure I Outline of the experimental design for the seedling inoculation experiment

I. Seedlings inoculated with the root contact method

Host pathogen association	Treatment and replications UF	F
DF+PW	20	20
DF+FA	20	20
WH+FA	20	20

II. Seedlings inoculated with the root collar wound method

Host-pathogen association	Treatment and replications UF	F
DF+PW	10	10
DF+FA	10	10
WH+FA	10	10

III. Control group

Seedlings	Treatment and replications: no alder block UF	no alder block F	root contact* UF	root contact* F	root collar wound* UF	root collar wound* F
Douglas fir	10	10	10	10	10	10
western hemlock	10	10	10	10	10	10

Key: DF.. Douglas fir
WH.. western hemlock
PW.. Poria weirii
FA.. Fomes annosus
UF.. unfertilized
F.. urea fertilized
*.. autoclaved alder block used in method

(90 percent) within the first two weeks after potting. Seedlings were carefully chosen on the basis of general vigorous appearance and to obtain a uniform size of the stem and root systems. Approximately 80 percent of the seedlings had some minor top damage as a result of the freeze in September of 1970.

The seedlings were potted in 6-inch diameter by 7-inch deep plastic pots with unsterilized soil obtained from the periphery of the nursery. The soil was classified as Tumwater fine sand, deficient in nitrogen and low in organic matter (U. S. Department of Agriculture, 1958).

The greenhouse was equipped with gas heaters, forced air coolers and an automatic watering system. Temperature was maintained at 60 ±5 degrees farenheit during spring, fall and winter months. The summer temperature was set at 70 degrees farenheit, but during the hottest of summer days, temperatures reached between 80 and 90 degrees for a few hours in the afternoon. Artificial lighting was not used during the experiment.

3.1.3 Inoculation techniques

3.1.3.1 Description of inoculum

Fresh red alder (Alnus rubra Bong.) stems approximately one-inch in diameter were cut into sections weighing between 25 and 30 grams (average length was about 2 inches). These were autoclaved in one-quart glass jars at 18 lbs psi. and 225 degrees centigrade for 25 minutes. This sterilization procedure was tested earlier for its effectiveness. Cultures of P. weirii and F. annosus were then grown in petri plates on 2 percent malt agar for two weeks at 20 degrees centigrade. A description of the stock

cultures of the fungi is included in the appendix. The alder blocks were then inoculated by placing aseptically in the jars three 1/2-inch strips of agar from the cultures (Figure 2). These inoculum cultures were then incubated at room temperatures for 11 weeks. At the end of the incubation period, the fungi had completely penetrated the alder wood and a hyphal mat covered the outside of the blocks.

3.1.3.2 Root contact method

The root contact method consisted of placing a single alder inoculum block under the root collar in contact with the roots. The seedling was then potted carefully to insure that the contact between the inoculum and the roots was not disturbed. Figure 3 shows the placement of the inoculum at the time of potting. Because of the mortality in the first group of western hemlocks, the inoculum was removed from these seedlings, washed free of soil and reused with the newly potted seedlings.

3.1.3.3 Root collar wound method

Following a technique developed by Kuhlman (1969), the seedling root collar was washed with tap water and surface sterilized with 65 percent ethyl alcohol. A wound was then made by aseptically removing a .3 x 2.5 cm bark and cambium section, exposing the xylem. A longitudinal strip of bark approximately .6 cm wide was aseptically removed from the alder inoculum block and this fresh surface of the inoculum was appressed to the seedling wound and secured with grafting rubber. The seedling was then potted carefully to avoid disturbing the inoculum attachment. Figures 4 and 5 illustrate this technique.

Figure 2 Method of inoculating alder blocks with root rot pathogens

Figure 3 Placement of alder inoculum block in the root contact inoculation method

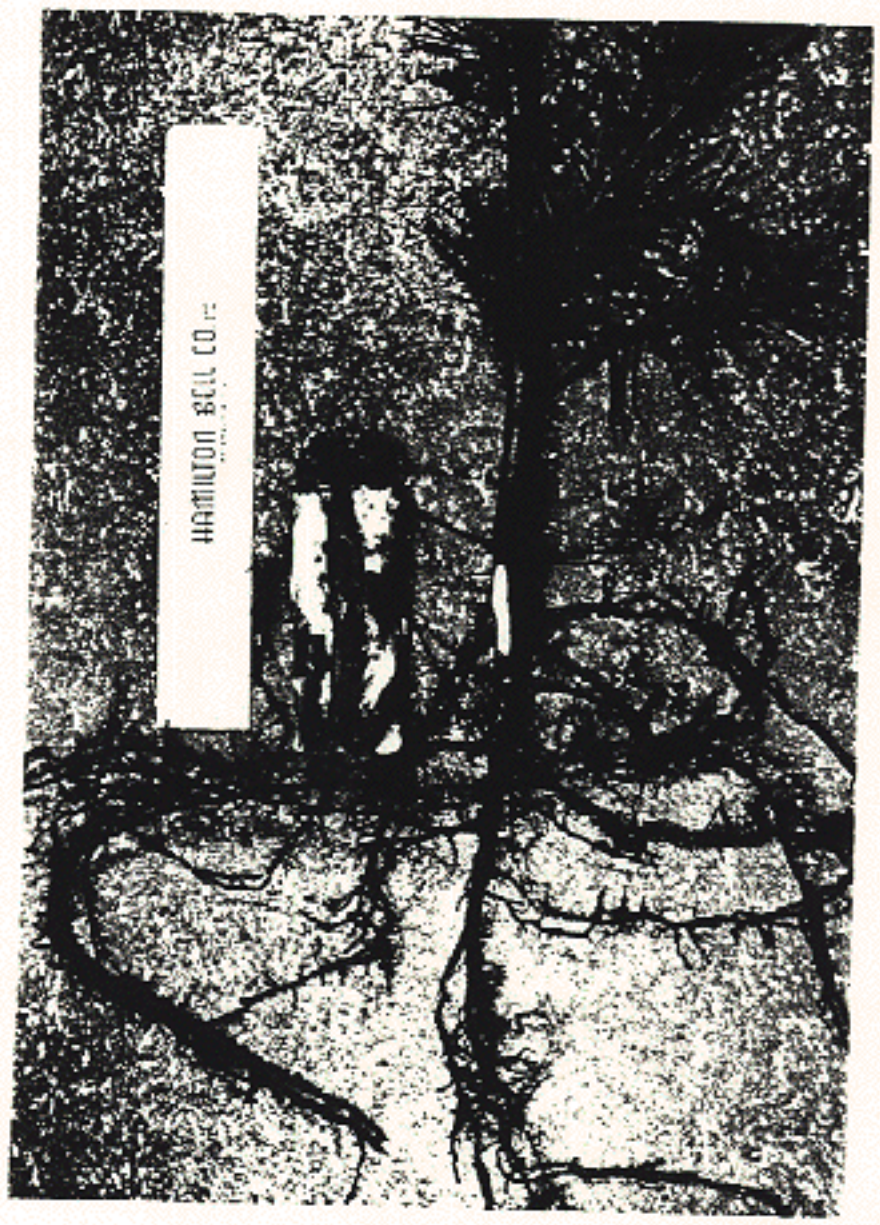


Figure 4 Root collar wound inoculation method showing seedling wound and bark strip removed from alder inoculum block

Figure 5 Root collar wound inoculation method showing attachment of alder inoculum block with grafting rubber

The mortality in the first group of western hemlock necessitated removing the inoculum from those seedlings and reusing it with the fresh seedlings. The inoculum was washed free of soil and a new bark strip was removed before attachment to the wound.

3.1.4 Treatment of seedlings after inoculations

Within 24 hours after the inoculations, the seedlings designated for the fertilizer treatment received the equivalent of 400 lbs of urea nitrogen per acre. The urea (1.8 grams) was spread evenly over the surface of the soil (Figure 6) and 60 milliliters of water was added to obtain rapid dissolution and percolation of urea into the soil.

The Douglas-fir seedlings were placed in the greenhouse immediately following the inoculations while the western hemlock seedlings were placed outside for five days before moving them inside. Because of the sensitive nature of western hemlock seedlings, it was thought that the gradual shift from nursery bed to greenhouse would give the seedlings time to adjust to the effects of handling and potting before entering the greenhouse environment.

3.1.5 Determination of disease induced mortality

Seedling mortality was determined by the presence of foliage and buds that turned brownish and brittle. When this criterion was met, the seedling was removed to the laboratory and an attempt made to isolate the pathogen from the area in contact with the inoculum.

The isolation procedure consisted of first carefully washing the roots and root collar area in tap water to remove all the soil. The plant

Figure 6 Potted seedlings with urea spread evenly over the soil surface

material was then surface sterilized in a .5-percent sodium hyperchlorite bath for one minute followed by a one-minute rinse in sterile, distilled water. Root isolations were made by cutting the entire root system into one-inch segments and placing them on media in petri plates. In isolations from the root collar wound area, the seedling tissue from one inch below the wound to one inch above was cut into segments, split longitudinally to expose the central xylem cylinder and placed on media in petri plates. A 2-percent malt agar media was used to isolate P. weirii and a selective media developed by Kuhlman and Hendrix (1962) was used to isolate both forms of F. annosus.

Identification of P. weirii cultures was based on the characteristic light brown mycelial mat formed on the malt agar (Nobles, 1948). Isolation of F. annosus was confirmed by the presence of the characteristic Oedocephalum stage of this fungus (Nobles, 1948).

3.1.6 Determination of inoculum survival

The alder inoculum blocks remained in the pots until January of 1972. Isolations were then attempted to determine if the inoculum was still viable. The inoculum blocks were washed with tap water then split aseptically to expose the inner portion. Chip cultures were made from the exposed wood surface. The isolation media and basis for identification of the pathogens were the same as previously noted for the seedling isolations.

3.2 Results

3.2.1 Survival of control seedlings

The survival of seedlings in the control group is shown in Figures 7 and 8.

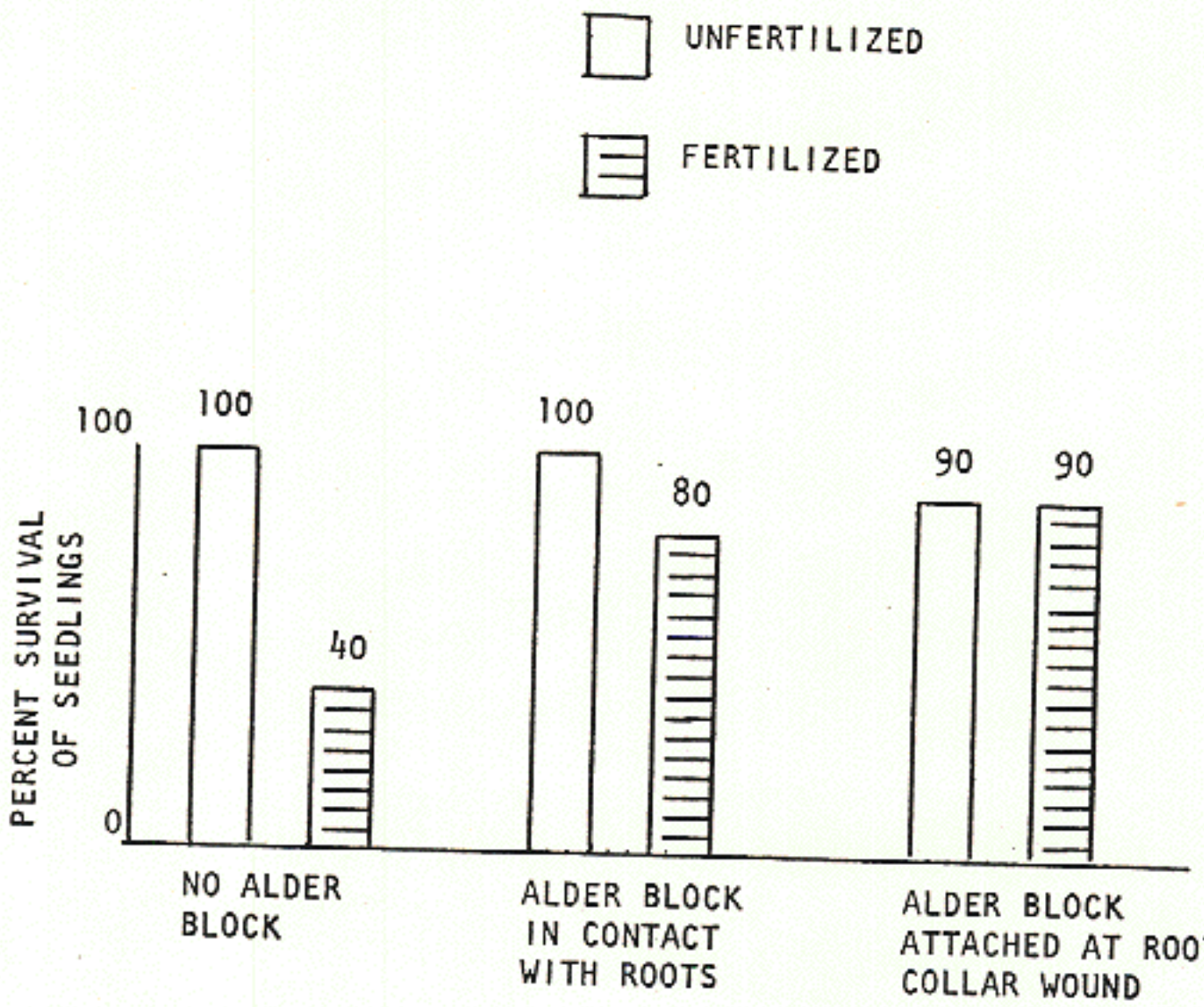
Considering the total number of Douglas-fir in each treatment, the unfertilized seedlings had a survival rate of 97 percent, compared to 73 percent in the fertilized treatment. The survival rates for seedlings receiving the two types of inoculations were high (between 80 and 100 percent) in both treatments.

In the western hemlock controls, the survival rate for the total number of seedlings in the unfertilized treatment was 83 percent, compared to 20 percent in the fertilized treatment. Survival of the seedlings receiving the inoculations was high (80 percent) in the unfertilized treatment, but low (0 to 20 percent) in the fertilized group.

The majority of seedlings that died in the fertilized treatments exhibited what will be referred to as "fertilizer kill" symptoms. This was basically a rapid browning and stiffening of all needles with a concurrent loss of some older needles. Most of the seedlings that exhibited these symptoms died within the first two months of the experiment.

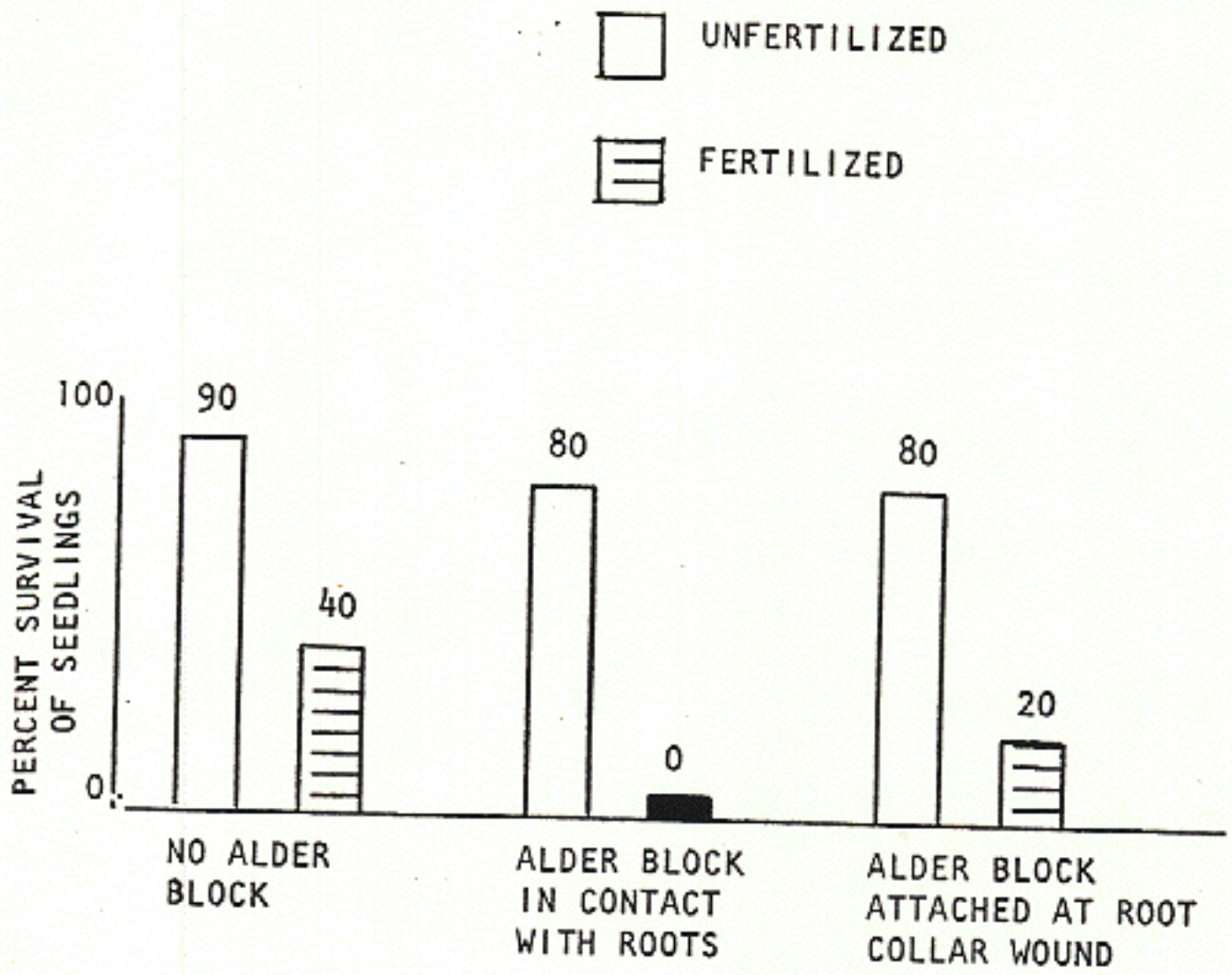
3.2.2 Survival of unfertilized and fertilized seedlings with inoculum in contact with roots

The survival of seedlings with the inoculum in contact with the roots is shown in Figure 9. The root rot pathogens were not isolated from the



SEEDLING AND AUTOCLAVED ALDER BLOCK ASSOCIATIONS

Figure 7 Survival of Douglas fir seedlings in the control treatments



SEEDLING AND AUTOCLAVED ALDER BLOCK ASSOCIATIONS

Figure 8 Survival of western hemlock seedlings in the control treatments

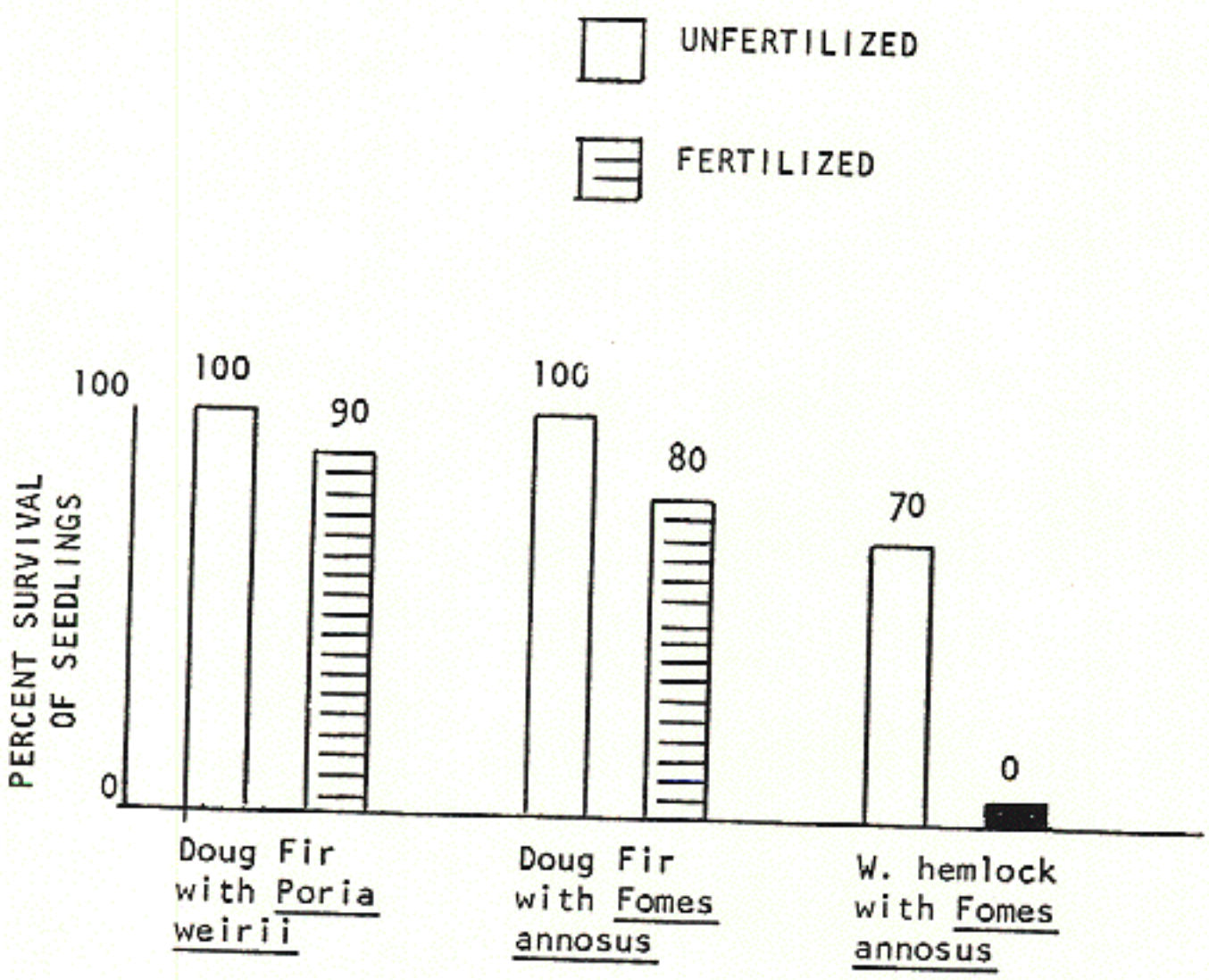


Figure 9 Survival of seedlings in fertilized and unfertilized soil with inoculum in contact with roots

roots of any dead Douglas-fir or western hemlock seedlings. The 100-percent mortality of western hemlock seedlings in the fertilized treatment occurred between the first and third month of the experiment. Most of those dead seedlings exhibited "fertilizer kill" symptoms.

3.2.3 Survival of unfertilized and fertilized seedlings with inoculum attached at root collar wound

Survival of seedlings with the inoculum attached at a root collar wound is shown in Figure 10.

In the Douglas-fir with *P. weirii* associations, there were six seedlings in the unfertilized and three seedlings in the fertilized treatment that died. Isolation of *P. weirii* from the dead seedlings was successful from only two in each treatment but all dead seedlings except one in the fertilized group exhibited what will be referred to as "root rot" symptoms. These symptoms were expressed over a two to three week period prior to seedling death and were very obvious.

The "root rot" symptoms started with a gradual wilting of the upper branches and needles, resulting in a droopy appearance as the lower foliage was affected (Figure 11). The wilting was followed by browning and drying of the foliage, eventually meeting the criterion for mortality used in this experiment. All the Douglas-fir and western hemlock seedlings that were positive for isolation of a pathogen displayed these symptoms.

The mortality in the Douglas-fir with *F. annosus* association was the same in each treatment. All dead seedlings exhibited "root rot" symptoms. Isolation of *F. annosus* was successful from the eight dead seedlings in the fertilized treatment, but only successful from six of eight in the unfertilized treatment.

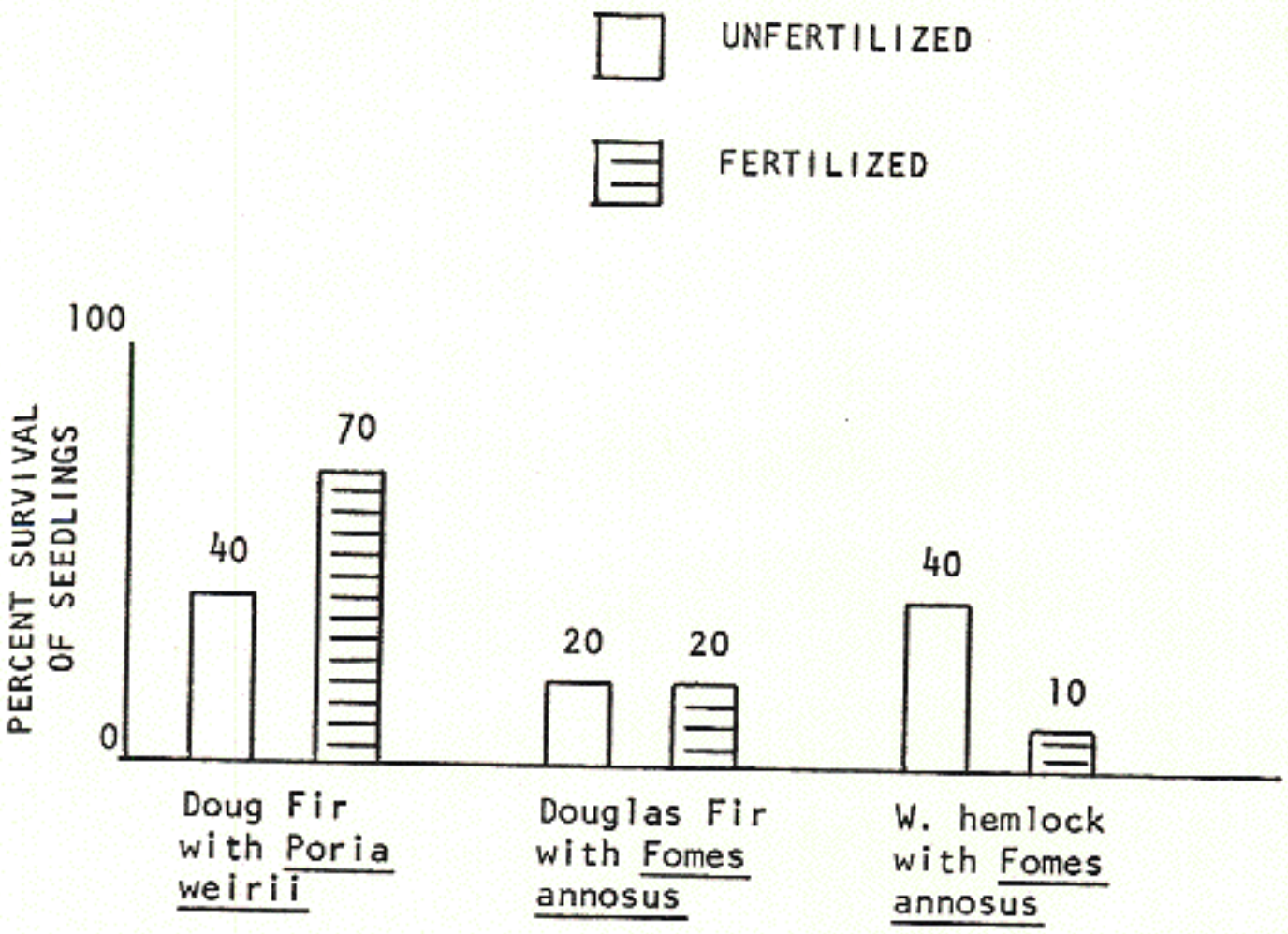


Figure 10 Survival of seedlings in fertilized and unfertilized soil with inoculum attached at a root collar wound

Figure 11 Comparison of healthy Douglas fir seedling with one exhibiting "root rot" symptoms. The seedling on the right is showing the advanced stage of root rot infection.

In the western hemlock with F. annosus associations, the unfertilized treatment resulted in six dead seedlings, three of which were positive for isolation of the pathogen. Nine seedlings died in the fertilized treatment and four were positive for isolation of the pathogen. Although "root rot" symptoms were shown on all unfertilized seedlings that died, the symptoms were not as obvious on the dying seedlings in the fertilized soil. There was a combination of "root rot" and "fertilizer kill" symptoms in the fertilized group of seedlings.

3.2.4 Survival of pathogens in unfertilized and fertilized soil

The results of isolating the pathogens from the alder inoculum blocks is shown in Figure 12. The survival of P. weirii was 4 percent in both the fertilized and unfertilized soil. The Douglas-fir isolate of F. annosus was recovered from more than 90 percent of the alder blocks in each soil treatment. The western hemlock isolate of F. annosus was recovered from only 6 percent of the alder blocks in the fertilized soil, as compared to 65 percent in the unfertilized soil.

3.3 Discussion

It is evident from the results in the control seedlings that the urea fertilizer was responsible for most of the western hemlock mortality in the fertilized treatments of this experiment. Even though some Douglas-fir were apparently killed by fertilizer in the controls, the importance of urea as a cause of mortality in the rest of the experiment was considered minor.

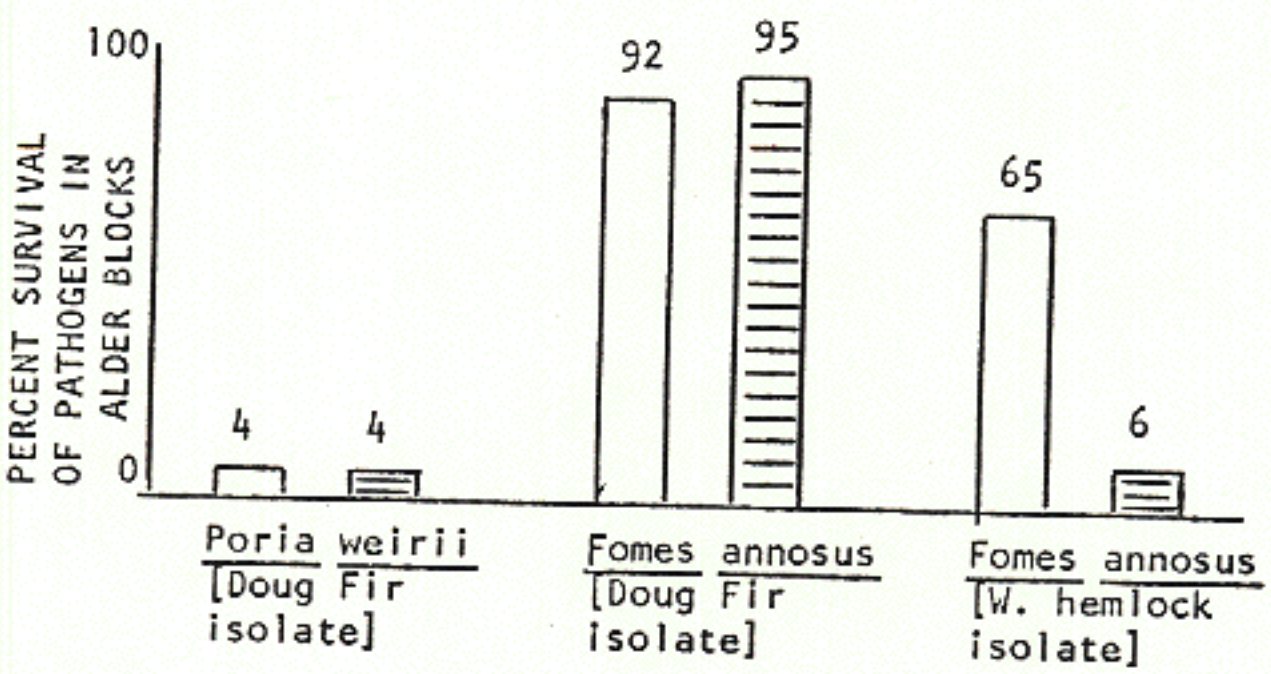


Figure 12 Survival of root rot pathogens in alder blocks burried in fertilized and unfertilized soil

3.3.1 Douglas-fir with *Poria weirii* associations

In the Douglas-fir with *P. weirii* associations, the results from the root contact inoculations indicate that urea fertilizer does not produce a favorable soil environment for infection or increase the susceptibility of the seedlings to infection. In fact, the evidence from the root collar wound inoculations suggest that urea creates either an adverse soil environment to infection or increases the disease resistance of the seedlings.

It is known that the microbial population plays an important role in the survival of *P. weirii* in the soil (Buckland *et al.*, 1954). The pathogen is a poor soil competitor compared to the many soil microorganisms (Nelson, 1967, 1968). Also there are actinomcetes, bacteria and other fungi which are antagonistic toward the pathogen (Li, 1969; Nelson, 1969).

It has been shown that the addition of nitrogen fertilizer can increase the microbial population in soil (Kaufman and Williams, 1964). If urea had a similar effect on the soil in this experiment, the increased population of microorganisms could explain the higher survival rate of Douglas-fir seedlings in the fertilized soil with the inoculum attached at a root collar wound. The greater microbial competition in the fertilized soil could have increased the chances for colonization of the inoculum-wound area by saprophytic soil microbes; this would account for the fewer infections by *P. weirii* in the fertilized soil.

Nelson (1970) has found that adding ammonium chloride and sodium nitrate to soil reduced the survival of *P. weirii* in buried Douglas-fir wood cubes. Although there was no experimental evidence to confirm which factors were rexponsible for the low survival, he felt the additional supply of nitrogen in the fertilized soil probably stimulated development

of soil microorganisms which invaded the wood cubes and replaced the pathogen.

Another possible but less likely explanation for the higher survival in the fertilized soil would be that the ammonium bicarbonate formed from the hydrolysis of urea had an inhibitory effect on the activity of P. weirii thereby reducing the chance for successful infection (see Court et al., 1964).

A beneficial effect of nitrogen fertilization on the resistance of Douglas-fir seedlings to infection by P. weirii could also explain the higher survival of the wound inoculated seedlings in the fertilized soil. This explanation would not appear to be very likely though, since there was no indication of increased resistance in the fertilized Douglas-fir seedlings inoculated with F. annosus at a root collar wound.

Survival of P. weirii in the alder inoculum blocks was very low in both treatments. If the fertilizer had an effect on the survival of the pathogen, it was masked by the overall low survival values. During the experiment, it was discovered that another investigator had found that P. weirii in buried wood cubes had low survival rates at greenhouse temperatures (E. Nelson, personal communication). Since the soil in the greenhouse was only 2 to 3 degrees lower than the air temperature, the high soil temperatures in the summer could be responsible for the low survival rates in both treatments.

3.3.2 Douglas-fir with Fomes annosus associations

The results from the Douglas-fir with F. annosus associations show the fertilizer did not change the soil environment enough to affect the activity

of the pathogen and did not affect the resistance of the seedlings to infection. The high mortality rate in the root collar wound inoculated seedlings indicates how virulent F. annosus is upon Douglas-fir. Although the virulence of F. annosus has been shown on a variety of conifer seedlings (Kuhlman, 1969, 1970; Koenings, 1970), this is the first demonstration of its virulence on Douglas-fir seedlings using an inoculation technique.

The survival of the Douglas-fir isolate of F. annosus was not affected by the addition of urea to the soil. After nine months in the soil, the pathogen was still viable in more than 90 percent of the alder blocks from both fertilized and unfertilized soil. The high survival of the fungus in the fertilized soil is probably indicative of the tolerance of the organism to varying environments (Cowling and Kelman, 1964; Koenings, 1969).

3.3.3 Western hemlock with Fomes annosus associations

In the western hemlock with F. annosus associations, 97 percent of the fertilized seedlings died. According to the results in the control group the urea fertilizer was responsible for this high mortality. Evidently western hemlock seedlings are very susceptible to fertilizer damage by urea when it is applied at the 400 lbs of nitrogen per acre rate. Because of the fertilizer damage, the effect of urea on the host-pathogen association can not be entirely evaluated.

In the root contact inoculations, the fertilized western hemlock seedlings died within three months after the start of the experiment. If adding urea to the soil resulted in an environment more conducive to seedling infection, the expression of this difference between soil treatments would

not have been seen because of the early death of the seedlings due to the fertilizer damange.

The fertilized western hemlock seedlings that died in the root collar wound inoculations expressed both "fertilizer kill" and "root rot" symptoms. Because there was greater mortality in the fertilized treatment and the dead seedlings exhibited "root rot" symptoms, it would appear that the fertilized soil was an environment more conducive to infection by F. annosus than was the unfertilized soil. Although a change in the soil environment could be responsible for the higher infection rate in the fertilized soil, a more plausible explanation is that the seedlings were predisposed to infection because of the fertilizer damage.

The survival of the western hemlock isolate of F. annosus in the alder blocks was 6 percent in the fertilized soil, compared to 65 percent in the unfertilized soil. Although there was a considerable difference in survival between treatments, the results may not be a true comparison of survival of the pathogen in fertilized and unfertilized soil. Another factor described in the following paragraphs may have influenced the survival of F. annosus more than the addition of urea.

In the fertilized treatments, all but one western hemlock was dead by the third month of the experiment. Due to this high mortality, 97 percent of the inoculum was buried for 6 months in pots containing no seedlings. In the unfertilized treatment, 60 percent of the inoculum blocks were still potted with living seedlings at the end of the experiment.

When a seedling died, the automatic watering tube was removed from the pot and those pots were watered manually each week to maintain a moist soil. A few times during the summer months, the soil in the fertilized treatments

became very dry during the time between weekly visits to the greenhouse. Out of a total of 30 pots in this treatment, there was only one with a living seedling; thus, the pots were not shaded and that probably contributed to the rapid drying of the soil between waterings.

The drying of the soil may have affected the survival of F. annosus by partially drying out the alder block or through a change in soil microorganisms capable of replacing F. annosus in the wood. Evidence for the relationship between the drying of the soil (associated with no seedling in pot) and the survival of the fungus is shown in the following data: 1) in the fertilized treatment, 3 percent of the pots had no seedlings in them and there was 6 percent survival of the pathogen, and 2) in the unfertilized treatment, 60 percent of the pots had seedlings in them and there was 65 percent survival of the pathogen.

4. ROOTWOOD DECAY EXPERIMENT

4.1 Methods and materials

4.1.1 Decay study

4.1.1.1 Experimental design

The rootwood decay experiment was designed to test the decay resistance of sapwood from roots of fertilized and unfertilized trees. The tree species and root rot pathogens tested in this experiment were in the same host-pathogen associations as in the seedling experiment (Douglas-fir with P. weirii; Douglas-fir with F. annosus; western hemlock with F. annosus). The decay test followed the same basic procedure as outlined in the ASTM "Standard method for accelerated laboratory test of natural decay resistance of wood" (1966). The experimental design is outlined in Figure 13.

To obtain a definitive test of the decay resistance of the two wood types, it was necessary in the decay chambers to exclude all sources of nutrients other than the wood samples themselves. Therefore, the normal soil block decay method was not used. Instead, a method similar to one used by Merrill and Cowling (1965) was followed.

4.1.1.2 Description of field site

Rootwood samples were collected from installation 14 of the Regional Forest Nutrition Research Project. The installation was located on the northeast side of highway 104, six miles northwest of the Hood Canal

Figure 13 Outline of rootwood decay experiment

I Rootwood test specimens

Rootwood and root rot pathogen	Rootwood type and replications UF	F
Douglas fir + Poria weirii	30	30
Douglas fir + Fomes annosus	30	30
western hemlock + Fomes annosus	30	30

II Reference blocks

Rootwood and root rot pathogen	Rootwood type and replications UF	F
Douglas fir + Poria weirii	5	5
Douglas fir + Fomes annosus	5	5
western hemlock + Fomes annosus	5	5

III Adjustment blocks

Wood type	Rootwood type and replications UF	F
Douglas fir	5	5
western hemlock	5	5

UF... from unfertilized trees

F... frem fertilized trees

bridge. The predominant tree was Douglas-fir with an average age of 59 years. The understory was western hemlock; the co-dominants and intermediate crown classes had an average age of 50 years. The stand exhibits a site index of 119 (site class II) based on Weyerhaeuser 50 year site class tables (King, 1966).

The installation consisted of six 2-chain x 1-chain plots; two fertilized at a rate of 400 lbs of urea nitrogen per acre, two fertilized at 200 lbs per acre and two control plots. The fertilized trees were sampled on plot 80 which received the 400 lbs application rate in the winter of 1969 to 1970. The unfertilized trees were sampled on control plot 82.

4.1.1.3 Collection and preparation of samples

Rootwood was collected in August of 1971. The roots sampled were at least five inches in diameter, within four feet of the root collar and free from any external evidence of abnormalities. A chain saw was used to cut a 1- to 2-foot section free and the ends of the root segments were checked for evidence of fungus infection or other undesirable properties. Root segments appearing to be normal were end coated with "Ortho" wound dressing and placed in plastic bags for transport to the laboratory.

A 1/2-inch disk was sawn off one end of each root segment and the heartwood-sapwood boundary for each root was determined (Barton and Gardner, 1963). The boundaries were distinct in Douglas-fir but no heartwood was detected in the western hemlock. The root segments were then cut into 3/8-inch serial disks and the sapwood specimens removed with a 1-inch plug cutter. Douglas-fir sapwood was taken from the area midway between the heartwood-sapwood boundary and the tissues formed since fertilization.

Western hemlock sapwood was taken from the area midway between the pith and the tissues formed since fertilization.

All rootwood blocks were placed on fiberglass screens in plastic trays and put into a conditioning room maintained at 70 degrees fahrenheit and 87 percent relative humidity. When the wood blocks reached equilibrium moisture content, they were weighed to the nearest mg and put in double polyethelene bags. The rootwood was then sterilized with 10^6 rads of gamma radiation. The irradiation source was a Gammacell 220 utilizing Co^{60} with a dosage rate of 2.4×10^5 rads per hour. This sterilization procedure was tested earlier for its effectiveness. Following irradiation, the rootwood blocks were stored at 35 degrees fahrenheit until placed in the decay chambers and inoculated.

4.1.1.4 Description of decay chambers

Eight-ounce French square bottles with plastic caps were employed as decay chambers. The wood samples were suspended over a free water surface by hangers made of fiberglass screen and nichrome wire (Figures 14 and 15). To obtain a uniform exchange of air in all bottles, a 1/2-inch hole was drilled in the plastic caps and each hole was plugged with cotton. To maintain high humidity around the wood, 100 milliliters of distilled water was added to each chamber. The decay chambers were steam sterilized before inserting the wood samples for inoculation.

4.1.1.5

Cultures of the wood rotting fungi were obtained from isolations made from pathogen killed seedlings in the seedling experiment. The fungi were

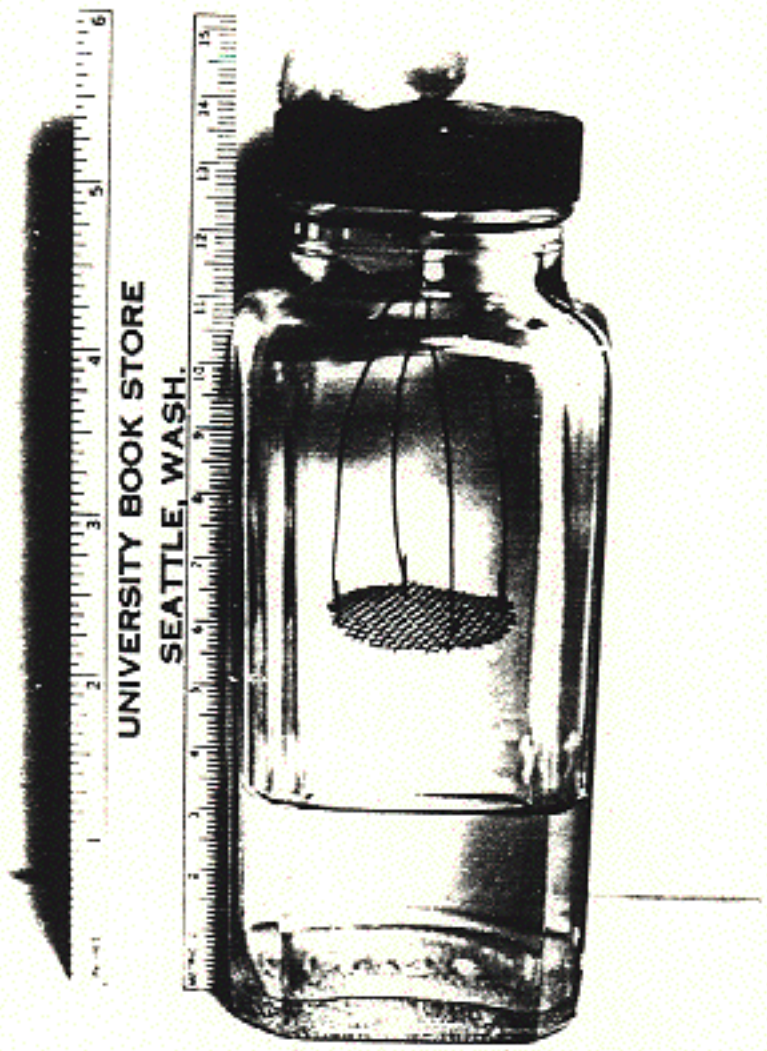


Figure 14 Decay chamber with hanger and sterile water

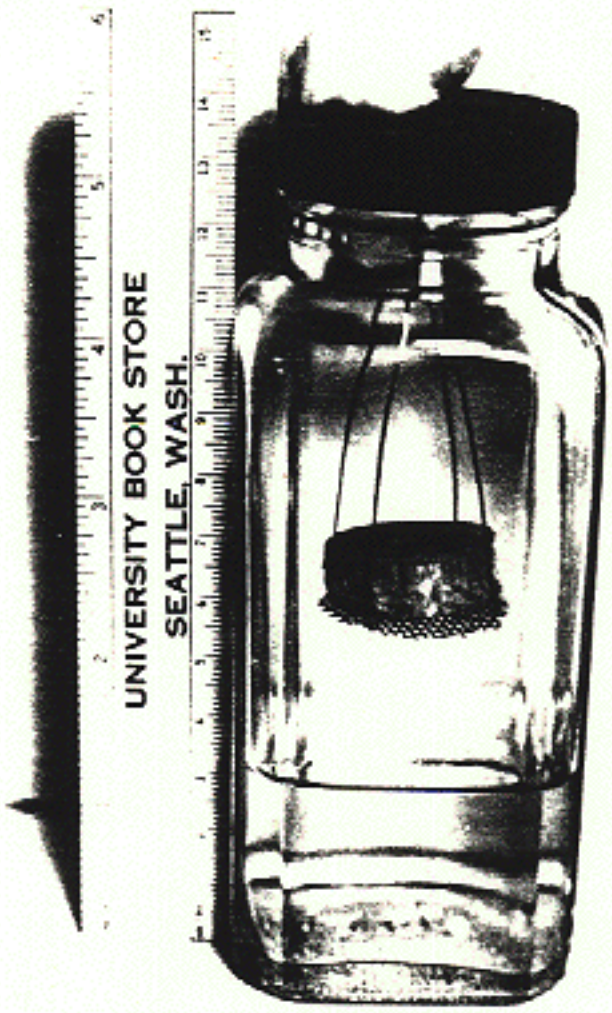


Figure 15 Decay chamber with rootwood test specimen on hanger

cultured on two-percent water agar for two weeks at 20 degrees centigrade. In a sterile inoculation chamber, the rootwood blocks were put in the decay chambers and inoculated by placing an agar plug with mycelium side down on top of the wood (Figure 16). A 9-cm diameter metal tube with plunger was used to make the inoculations. The decay chambers were incubated in the dark at 70 degrees fahrenheit and 87 percent relative humidity in a walk-in environmental room. Two weeks after the F. annosus inoculations, the environmental room failed to maintain the proper setting of temperature and relative humidity. The decay chambers were then moved to another environmental room which could maintain the proper settings. The P. weirii inoculations were made after the change in environmental rooms.

After incubation for two months, it was apparent that the F. annosus inoculations were not successful. The fungus had failed to grow out onto the wood from the inoculum plug and the agar was dried up. The P. weirii inoculations appeared to be succeeding because the fungus was slowly growing over the wood surface. It was thought that the problem with the first environmental room was responsible for the failure of the F. annosus inoculations, so all those decay chambers were reinoculated using the method previously described.

The second attempt at the F. annosus inoculations was also unsuccessful. The P. weirii inoculations after five months incubation had resulted in a hyphal mat between the fiberglass screen and the wood, but a check of the reference blocks showed no weight loss had occurred. It was concluded that the inoculation method did not establish enough fungus growth which could result in detectable decay. Therefore, all the decay chambers were reinoculated by a new technique.

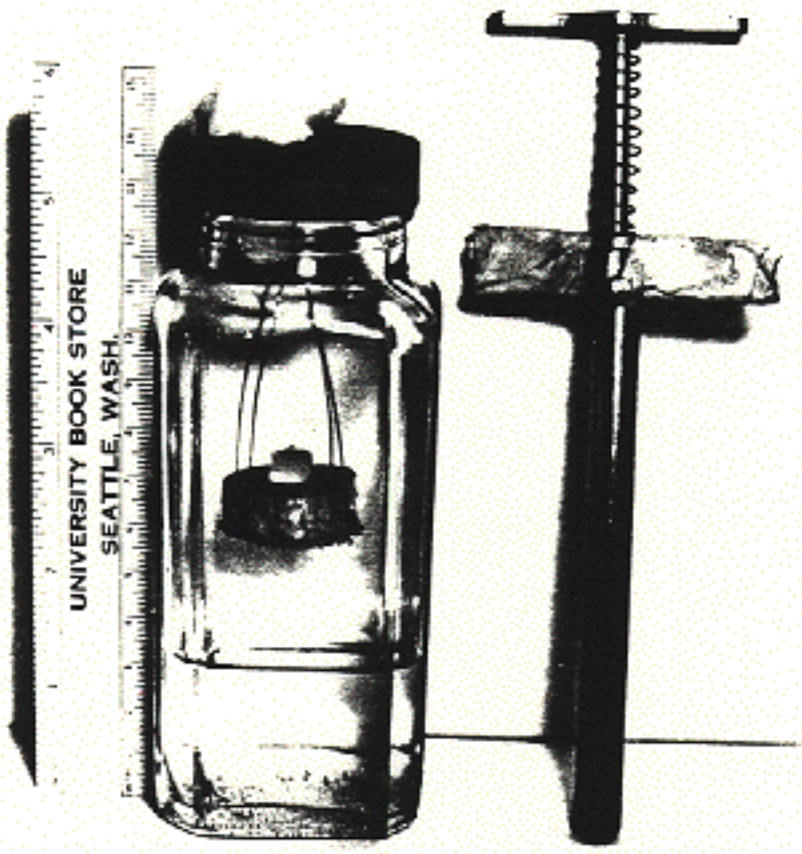


Figure 16 The first inoculation method used in the decay experiment. The inoculum plug is shown on the wood and the metal tube with plunger used to insert the inoculum is next to the decay chamber

The new inoculation method consisted of placing a 2 cm x 2 cm two-percent malt agar block on top of the wood and inoculating the agar with the wood rotting fungi (Figure 17). The F. annosus and P. weirii inoculum was cultured on two-percent malt agar for seven days at 20 degrees centigrade and the inoculum plug transferred to the decay chamber with the plug cutter.

4.1.1.6 Methods for terminating experiment

Two months after the final inoculations, a few reference blocks were checked to determine the progress of the experiment. The wood had a weight loss of less than five percent, indicating that decay was progressing very slowly. At the end of four months incubation, the rootwood blocks were removed from the decay chambers, placed on fiberglass screens in plastic trays and allowed to come to equilibrium moisture content in the conditioning room. Reminants of agar on the wood was carefully removed and the fungus mycelium left undisturbed. The rootwood blocks were then weighed to the nearest mg. Calculation of the percentage weight loss for each sample was made from the conditioning weights before and after exposure to the decay fungi.

4.1.2 Chemical analysis

Extra sapwood blocks were kept frozen until needed for analysis. They were then oven dried at 105 degrees centigrade for 24 hours and ground in a Wiley mill to pass a 40 mesh screen. The ground samples were stored in dessicators until removed for immediate analysis.

Figure 17 The final inoculation method used in the decay experiment. The inoculum plug is shown on top of the malt agar block.

The total nitrogen content of the rootwood was determined by a macro-Kjeldahl procedure. The basic technique used by Merrill and Cowling (1966a) was followed but the reagent volumes were increased four times to allow for analysis of a larger sample. Two 4-gram samples from each Douglas-fir root were analysed; results of the duplicate analysis were averaged to get the nitrogen content for each root. Western hemlock rootwood was not analysed for nitrogen.

Starch and sugar content was determined by glucose oxidase and anthrone procedures, respectively. The methods as described by Ebell (1969a, 1969b) were followed with only two changes in the procedures; oven dried, not freeze dried samples were used, and in the sugar analysis, ethanol was evaporated on a rotary evaporater, not on a steam bath. For each sample group (i.e., fertilized Douglas-fir, etc.), the dried rootwood was combined to make two composite samples representing the two crown classes of the group. From each of the composite samples two subsamples were analysed by the two procedures; results of the duplicate analysis were averaged to get the starch or sugar content of the sample.

4.2 Results

4.2.1 Decay test

The results of the decay test are shown in Table 1. The average percent weight loss for each rootwood group was low; no weight losses exceeded 12 percent. In the associations Douglas-fir with P. weirii and Douglas-fir with F. annosus, the rootwood from the unfertilized trees had a greater weight loss than rootwood from fertilized trees. There was only a small

Table 1 Average weight losses of decayed rootwood from fertilized and unfertilized trees and statistical comparisons of sample means

Association	Rootwood	Average percent decay weight loss	The t-test testing differences in sample means: value of t	degrees of freedom
Douglas fir with *Poria weirii*	F	7.86	4.779*	45
	UF	11.72		
Douglas fir with *Fomes annosus*	F	3.60	3.719*	51
	UF	6.51		
western hemlock with *Fomes annosus*	F	4.73	.882 NS	48
	UF	5.55		

F ..... from fertilized trees

UF..... from unfertilized trees

* ..... significant at 0.01 level

NS..... not significant

difference between the weight losses of the two rootwood types in the western hemlock with F. annosus association.

The results of a statistical t-test (Snedecor and Cochran, 1967) testing the differences between the mean weight losses of the two wood types in each association is included in Table 1. There is a highly significant difference (0.01 level) in the mean weight losses for the two wood types in the Douglas-fir with P. weirii and Douglas-fir with F. annosus associations. In the western hemlock with F. annosus association, no significant difference exists between the average weight losses of the two wood types.

4.2.2 Chemical analysis of rootwood

The results of the nitrogen analysis of Douglas-fir rootwood are shown in Table 2. The average nitrogen content of the rootwood from the fertilized plots is slightly lower than that for rootwood from the unfertilized plots. However, the difference between the two mean nitrogen contents is not significant at the 0.20 level in a statistical t-test.

Results of the starch and sugar analysis are given in Table 3. Douglas-fir rootwood from fertilized trees had 18 percent more sugar and 5 percent more starch than rootwood from unfertilized trees. Just the opposite was found for western hemlock rootwood. Rootwood from unfertilized western hemlock trees had 65 percent more sugar and 9 percent more starch than rootwood from fertilized trees. Douglas-fir rootwood from fertilized trees of the dominant crown class accounted for the increase in sugar content for that group; rootwood from fertilized and unfertilized trees of the co-dominant crown class had the same sugar content. In the starch analysis,

Table 2 Results of total nitrogen analysis of sapwood from roots of nitrogen fertilized and unfertilized Douglas fir trees and statistical comparison of sample means

Tree treatment	Tree No.	Crown* class	Percent nitrogen in rootwood	Average percent nitrogen in the rootwood from each treatment	The t-test testing difference in sample means: value of t	degrees of freedom
unfertilized	1	CD	.041			
	2	D	.043			
	3	CD	.040	.045		
	4	D	.047			
	5	D	.055			
fertilized	1	D	.050		.958 NS	20
	2	D	.043			
	3	CD	.039	.043		
	4	CD	.039			
	5	D	.046			

* ..... Dominant (D) and Co-dominant (CD)

NS..... not significant

Table 3 Results of starch and sugar analysis of rootwood from nitrogen fertilized and unfertilized Douglas fir and western hemlock trees

	Percent of sugar in rootwood tissue			Percentage of starch in rootwood tissue		
	composite sample analysis			composite sample analysis		
Rootwood	dominant crown class	co-dominant crown class	sample average	dominant crown class	co-dominant crown class	sample average
fertilized Douglas fir	.93	.51	.72	4.49	4.40	4.44
unfertilized Douglas fir	.72	.51	.61	4.28	4.19	4.23
	co-dominant crown class	intermediate crown class		co-dominant crown class	intermediate crown class	
fertilized western hemlock	.36	.22	.29	3.94	3.91	3.92
unfertilized western hemlock	.53	.44	.48	4.04	4.49	4.27

the roots from fertilized Douglas-fir trees of both crown classes were found to have similar increases in starch. Analysis of western hemlock rootwood showed that the difference in starch and sugar content between roots from fertilized and unfertilized trees was greatest in the intermediate crown class.

4.3 Discussion

Results of the decay test indicate that Douglas-fir root sapwood from nitrogen fertilized trees is more resistant to decay by P. weirii and F. annosus than is similar wood from unfertilized trees. The increased decay resistance of the rootwood from the fertilized trees was probably due to a greater amount of fungal inhibitory substances in the wood. The increase in the concentration of inhibitory compounds in the fertilized tree rootwood could be explained in two ways. The first is that the synthesis of inhibitory compounds was increased because of the greater photosynthetic capacity of the fertilized tree. Some evidence for the relationship of photosynthesis to synthesis of fungal inhibitory compounds has been found in the studies by Alcubilla et al. (1971). The second explanation is related to the greater starch and sugar content found in the fertilized tree rootwood. It has been found in pine sapwood that wounding (in this experiment, removing the sapwood from a living root and conditioning it to a lower moisture content can be considered to be wounding; see Jorgensen, 1961) results in the formation of fungal inhibiting substances (Jorgensen, 1961; Shain, 1967). The precursor to those substances is the carbohydrate stored in the xylem parenchyma cells (Rudloff and Jorgensen, 1963). It is conceivable that fungal inhibitory compounds were formed in the Douglas-fir sapwood blocks

after removal from the roots and that more of those compounds were formed in wood from the fertilized trees because of the greater starch and sugar content.

The relationship between fertilized Douglas-fir trees and rootwood decay resistance was not shown for fertilized western hemlock trees. Either nitrogen fertilization with urea does not affect the decay resistance of western hemlock root sapwood or the effect was minor and not detected by the methods used in this experiment. There was a definite chemical difference between the two western hemlock rootwood types as shown by the greater carbohydrate levels in the rootwood from unfertilized trees. Evidently the greater carbohydrate content is not associated with decay resistance properties as suggested in the case of Douglas-fir rootwood.

It was thought that the nitrogen analysis would show a higher nitrogen level in rootwood from fertilized Douglas-fir trees, since those trees would have greater amounts of organic nitrogen compounds being transported up the xylem. That was not the case, however, as the analysis revealed the nitrogen contents of the two rootwood types to be the same. This is in agreement with the results of Cowling's (1969) research, but in contrast to the findings of Wheetman (1962) and Goyer and Benjamin (1972). The low nitrogen values found in this experiment are in agreement with values of nitrogen in stem sapwood found by Merrill and Cowling (1966a).

The results of the starch and sugar analysis have some interesting implications concerning fertilized Douglas-fir and western hemlock trees and their relationship to the activity of root rot pathogens. In the roots from fertilized Douglas-fir trees, the higher carbohydrate content is probably a result of the greater photosynthetic capacity of the fertilized trees. Those trees could be considered to be more vigorous than the

unfertilized ones. In the western hemlock rootwood, the carbohydrate content was lowest in the roots from fertilized trees indicating that urea may have had an adverse effect on tree physiology. The results from the seedling experiment suggest that western hemlock is very susceptible to fertilizer damage when urea is applied at the 400 lbs of nitrogen per acre rate. The fertilized western hemlock trees sampled in this experiment had probably suffered some root damage from urea (see Court et al., 1964) and the low carbohydrate level was the result of a mobilization of carbohydrate reserves in the upper root which were transported to the damaged sites. If western hemlock trees do indeed suffer root damage from urea fertilizer, then it is possible that for a short time (at least two years is indicated by the sampling data) those trees are less vigorous physiologically than unfertilized trees.

The importance of tree vigor being brought out in connection with the carbohydrate analysis is its relationship to the activity of a root pathogen. It has been found in a number of investigations that when comparing the activity of F. annosus in trees of different physiological status, the activity of the pathogen is less in the most vigorous hosts (Rishbeth, 1951; Wallis, 1961; Miller and Kelman, 1966; and Shain, 1967). Thus, if fertilized Douglas-fir trees are more vigorous than their unfertilized counterparts, it is possible that the activity of P. weirii and F. annosus would be less in the root system of fertilized trees. Also, if the physiological vigor of western hemlock is reduced by urea fertilization, then for the period in which it is reduced, those trees may be more susceptible to damage by F. annosus than unfertilized trees.

Although the methods used in the decay study were successful in comparing the relative differences in decay resistance, an explanation of why

there were such low weight losses is in order. The enzymatic degradation of cellulose occurs when the moisture content of the wood is above the fiber saturation point by approximately 5 to 10 percent (Cowling, 1963). The fiber saturation point for Douglas-fir and western hemlock wood is 28 to 30 percent, respectively, of their own oven dry weights (Stamm, 1964). In the decay study, the moisture content of the wood blocks at the time of the final inoculation was approximately 19 percent. Because of this low moisture content (10 percent below fiber saturation point), it was necessary to add water to the wood so the decay fungi could become established and begin degradation. Adding the water was accomplished by tipping and rotating the decay chamber just long enough to cover the wood with the sterile water. The procedure was repeated five times during the four-month incubation period. Also, the wood was absorbing moisture from the decay chamber environment since the relative humidity was close to 100 percent. At the end of the experiment, the moisture content of the wood averaged 36 percent confirming the adequacy of the moisture-adding process.

Since the fungus began its activity on wood which was initially low in moisture, the decay progressed very slowly (reference blocks averaged less than 5 percent weight loss after two-months incubation), but gradually increased with increased moisture. Had the wood been inoculated when the moisture content was 36 percent instead of 19 percent, the decay weight losses would probably have been greater than those actually obtained. In the studies which have used the decay method employed in this experiment (Merrill and Cowling, 1966b; Levi and Cowling, 1968), the wood was infiltrated with water before the inoculations to insure an adequate moisture content for decay. Those studies obtained weight losses of 5 to 33 percent in a time of six to eight weeks.

5. CONCLUSIONS

Based on the results of the seedling-greenhouse and laboratory tests the following applied statements concerning these research results can be made:

1. Forest fertilization with urea will probably not increase Poria root rot problems in Douglas-fir stands.
2. Similarly, urea fertilization will probably not increase Annosus root rot problems in unthinned stands. However, there is evidence to indicate that the pathogen may become a serious problem in any stand under intensive management practices.
3. Roots of Douglas-fir trees may be more resistant to decay by Poria weirii and Fomes annosus after urea fertilization.
4. Young western hemlock stands may suffer some root damage from the fertilizer applications. If so, they could be predisposed to attack by root rot pathogens.
5. To determine the validity of the above applied statements, specific field tests will need to be conducted. Future research on the subject should concern itself with those field tests.

LITERATURE CITED

Alcubilla, Von M., M. P. Diaz-Palacio, K. Kreutzer, W. Laatsch, K. E. Rehfuess and G. Wenzel. 1971. Beziehungen zwischen dem Ernahrungszustand der Fichte (Picea abies Karst.), ihrem Kernfaulebefall und der Pilzhemmwirkung ihres Basts. (Relationships between nutrition and heart rot attack of Norway spruce (Picea abies Karst.) and the fungistatic effect of its inner bark. Eur. J. For. Pathology 1:100-114.

American society for testing and materials. 1966. Standard method for accelerated laboratory test of natural decay resistance of wood. (ASTM Designation: D2-17-63), part 16, p. 675-682. In Book of ASTM Standards, Philadelphia.

Barnes, R. L. 1961. Glutamine synthesis and translocation in pine. Plant Physiology 37:323-326.

Barnes, R. L. and G. W. Bengtson. 1968. Some aspects of nitrogen nutrition and metabolism in relation to fertilizer responses in southern pines, p. 58-63. In Forest fertilization, theory and practice. Tennessee Valley Authority National Fertilizer Development Center, Muscle Shoals, Ala. 306 p.

Barton, G. M. and J. A. F. Gardner. 1963. Color precursors in Douglas-fir. Forest Prod. J. 13:216-220.

Bourgeois, W. W. and S. P. Gessel. 1972. The abundance and distribution of ammonium and nitrate in two forest soils during a growing season. Soil Sci. Soc. Amer. Proc. (in press)

Buckland, D. C., A. C. Molnar and G. W. Wallis. 1954. Yellow laminated root rot of Douglas-fir. Can. J. Bot. 32:69-81.

Childs, T. W. and K. R. Shea. 1967. Annual losses from disease in Pacific Northwest forests. U.S. For. Ser. REsource Bull. Pac. Nthwest. For. Range Expt. Sta. No. PNW-20, 19 p.

Cowling, E. B. 1963. Structural features of cellulose that influence its susceptibility to enzymatic hydrolysis, p. 1-32. In Advances in Enzymatic Hydrolysis of Cellulose and Related Materials, E. T. Reese (Ed.). Pergamon Press, New York. 290 p.

Cowling, E. B. and A. Kelman. 1964. Influence of temperature on growth of Fomes annosus isolates. Phytopathology 54:373-378.

Cowling, E. B., B. Dillner and S. Rydholm. 1969. Comparative decay susceptibility of sapwood in nitrogen fertilized and nonfertilized stands of Norway spruce and Scots pine. Phytopathology 59:1022.

Cooley, J. S. 1945. The effect of manure and of commercial fertilizer on the susceptibility of young apple trees to black root rot. Phytopathology 35:207-209.

Court, M. N., R. C. Stephen and J. S. Waid. 1964. Toxicity as a cause of the inefficiency of urea as a fertilizer. I. Review. J. Soil Sci. 15: 42-48.

Crane, W. J. B. 1972. Urea nitrogen transformations, soil reactions and elemental movement via leaching and volatilization in a coniferous forest ecosystem following fertilization. Ph.D. Thesis, University of Washington, Seattle. 284 p.

Driessche, R. von den. 1971. Response of conifer seedlings to nitrate and ammonium sources of nitrogen. Plant and Soil 34:421-439.

Durzan, D. J. and F. C. Steward. 1967. The nitrogen metabolism of Picea glauca (Moench) Voss and Pinus banksiana Lamb. as influenced by mineral nutrition. Can. J. Bot. 45:695-710.

Ebell, L. F. 1969a. Specific total starch determinations in conifer tissue with glucose oxidase. Phytochemistry 8:25-36.

Ebell, L. F. 1969b. Variation in total soluble sugars of conifer tissue with method of analysis. Phytochemistry 8:227-233.

Foster, A. A. 1968. Damage to forests by fungi and insects as affected by fertilizers, p. 42-46. In Forest fertilization, theory and practice. Tennessee Valley Authority National Fertilizer Development Center, Muscle Shoals, Ala. 306 p.

Fowells, H. A. and R. W. Krauss. 1959. The inorganic nutrition of loblolly pine and Virginia pine with special reference to nitrogen and phosphorous. For. Sci. 5:95-112.

Fressenden, R. J., R. F. Calvert and K. A. Armson. 1971. Effect of some fertilizers and simazine on the activity of the microorganisms in Jack pine humus. For. Chron. 47:227-228.

Gallagher, L. U. 1964. A study on the effects of fertilization with nitrogen and potassium on the growth and nutrition of Abies amabilis with associated greenhouse trials. M. F. Thesis, University of Washington, Seattle. 112 p.

Garrett, S. D. 1970. Pathogenic root-infecting fungi. Cambridge University Press, London. 294 p.

Gessel, S. P. and R. B. Walker. 1956. Height growth response of Douglas-fir to nitrogen fertilization. Soil. Sci. Soc. Amer. Proc. 20:97-100.

Gessel, S. P., T. N. Stoate and K. J. Turnbull. 1965. The growth behavior of Douglas-fir with nitrogenous fertilizer. Res. Bull. 1. Coll. For. University of Washington, Seattle. 204 p.

Goyer, R. A. and D. M. Benjamin. 1972. Influence of soil fertility on infestation of Jack pine plantations by the pine root weevil. For. Sci. 18:139-147.

Heilman, P. E. 1961. Effects of nitrogen fertilization on the growth and nitrogen nutrition of low-site Douglas-fir stands. Ph.D. Thesis, University of Washington, Seattle. 213 p.

Hill-Cottingham, D. G. and R. R. Williams. 1967. Effect of time of application of fertilizer nitrogen on the growth, flower development and fruit set of maiden apple trees, var Lord Lambourne, and on the distribution of total nitrogen within the trees. J. Hort. Sci. 42:319-338.

Jorgensen, E. 1961. The formation of pinosylvin and its monomethyl ether in the sapwood of Pinus resinosa Ait. Can. J. Bot. 39:1765-1772.

Kaufman, D. D. and L. E. Williams. 1964. Effect of mineral fertilization and soil reaction on soil fungi. Phytopathology 54:134-139.

King, J. E. 1966. Site index curves for Douglas-fir in the Pacific Northwest. Weyerhaeuser Forestry Paper No. 8. 49 p.

Koenigs, J. W. 1969. Growth and survival of Fomes annosus at high concentrations of Borax. Phytopathology 59:1717-1721.

Koenigs, J. W. 1970. Inoculation of southern pine seedlings with Fomes annosus under aseptic conditions. For. Sci. 16:280-286.

Kuhlman, E. G. 1969. Inoculation of loblolly pine seedlings with Fomes annosus in the greenhouse. Can. J. Bot. 47:2079-2082.

Kuhlman, E. G. 1970. Seedling inoculations with Fomes annosus show variation in virulence and in host susceptibility. Phytopathology 60:1743-1746.

Kuhlman, E. G. and F. F. Hendrix. 1962. A selective medium for isolation of Fomes annosus. Phytopathology 52:1310-1312.

Levi, M. P. and E. B. Cowling. 1968. Role of nitrogen in wood deterioration. V. Changes in decay susceptibility of oak sapwood with season of cutting. Phytopathology 58:246-249.

Li, C. Y. 1969. Biological influence of red alder on Poria weirii and other root rot pathogens. Ph.D. Thesis, Oregon State University, Corvallis. 94 p. Diss. Abstr. 30 (Sec. B):1982.

Lister, G. R., V. Slankis, G. Krotkov and C. D. Nelson. 1968. The growth and physiology of Pinus strobus L. seedlings as affected by various nutritional levels of nitrogen and phosphorus. Ann. Bot. 32:33-43.

Mai, H. and H. J. Fiedler. 1970. Bodenmikrobiologische Untersuchungen an einem Stickstofformenversuch. (Soil microbiological studies in a trial of different forms of nitrogen.) Arch. Forstwes. 19:1049-1061.

Merrill, W. and E. B. Cowling. 1965. Effect of variation in nitrogen content of wood on rate of decay. Phytopathology 55:1067.

Merrill, W. and E. B. Cowling. 1966a. Role of nitrogen in wood deterioration: amounts and distribution of nitrogen in tree stems. Can. J. Bot. 44:1555-1580.

Merrill, W. and E. B. Cowling. 1966b. Role of nitrogen in wood deterioration. IV. Relationship of Natural variation in nitrogen content of wood to its susceptibility to decay. Phytopathology 56:1324-1325.

Meyer, M. M., Jr. and W. E. Splitstoesser. 1971. The utilization of carbohydrate and N reserves by Taxus during its spring growth period. Physiol. Plant. 24:306-314.

Miller, T. and A. Kelman. 1966. Growth of Fomes annosus in roots of suppressed and dominant loblolly pines. For. Sci. 12:225-333.

Nelson, C. D. 1964. The production and translocation of photosynthate-C^{14} in conifers, p. 243-257. In The Formation of Wood in Forest Trees, M. H. Zimmerman (Ed.). Academic Press, New York. 562 p.

Nelson, E. E. 1967. Factors affecting survival of Poria weirii in small buried cubes of Douglas-fir heartwood. For. Sci. 13:78-84.

Nelson, E. E. 1968. Survival of Poria weirii in conifer, alder, and mixed conifer-alder stands. U. S. For. Ser. Research Note Pac. Nthwest. For. Range Expt. Sta. No. PNW-83, 5 p.

Nelson, E. E. 1969. Occurrence of fungi antagonistic to Poria weirii in a Douglas-fir forest soil in western Oregon. For. Sci. 15:49-54.

Nelson, E. E. 1970. Effects of nitrogen fertilizer on survival of Poria weirii and populations of soil fungi and aeobic actinomycetes. Northwest Sci. 44:102-106.

Nobles, M. K. 1948. Studies in forest pathology. VI. Identification of cultures of wood-rotting fungi. Can. J. Res. C 26:281-431.

Oksbjerg, O. and G. West-Nielsen. 1953. Om rodfordaeverangreb. (Damage (to spruce) by Fomes annosus.) Hedeselkabets Tidsskrift 74:319-334.

Oland, K. 1954. Nitrogenous constituents of apple maidens grown under different nitrogen treatments. Physiol. Plant. 7:463-474.

Ono, K. 1970. Effect of soil conditions on the occurrence of Armillaria root rot of Japanese larch. Bull. For. Sta., Meguro (Japan) No. 229. p. 123-219.

Pharis, R. P., R. L. Barnes and A. W. Naylor. 1964. Effects of nitrogen level, calcium level and nitrogen source upon the growth and composition of Pinus taeda L. Physiol. Plant. 17:560-572.

Reddy, T. K. R. and R. Knowles. 1965. The fungal flora of a boreal forest raw humus. Woodl. Res. Index, Pulp Pap. Res. Inst. Can. No. 162, 12 p.

Rishbeth, J. 1951. Observations on the biology of Fomes annosus, with particular reference to East Anglian pine plantations. III. Natural and experimental infection of pines, and some factors affecting severity of disease. Ann. Bot. 15:221-246.

Roberge, M. R. and R. Knowles. 1966. Microbial population in a Black spruce humus fertilized with urea. Woodl. Res. Index, Pulp Pap. Res. Inst. Can. No. 180, 27 p.

Roberge, M. R., G. F. weetman and R. Knowles. 1970. An ecological and microbiological study of urea fertilization and thinning in a Black spruce stand. In Tree Growth and Forest Soils, C. T. Youngberg and C. B. Davey (Ed.). Oregon State University Press, Corvallis.

Rowan, S. J. 1971. Soil fertilization, fumigation and temperature affect severity of black root rot of slash pine. Phytopathology 61:184-187.

Rudloff, E. von and E. Jorgensen. 1963. The biosynthesis of pinosylvin in the sapwood of Pinus resinosa Ait. Phytochemistry 2:297-304.

Schalin, I. 1967. On the effect of nitrogen fertilization on the bacteria and microfungi in a humus layer. Silva Fennica 3:1-12.

Seibt, von G. 1964. Zur Frage des Einflusses von Dungung und Melioration auf die Faule von Wurzel-und Stammholz. (The effect of fertilization and site improvement on decay of root and stemwood.) Forstwiss Cbl. 83:101-118.

Shain, L. 1967. Resistance of sapwood in stems of loblolly pine to infection by Fomes annosus. Phytopathology 57:1034-1045.

Shareeff, A. 1955. Thinning and fertilizer studies on a poor site at Pack Forest. M. F. Thesis, University of Washington, Seattle, 69 p.

Snedecor, G. W. and W. G. Cochran. 1967. Statistical methods. Iowa State University Press, Ames. 593 p.

Stamm, A. J. 1964. Wood and cellulose science. The Ronald Press, New York. 549 p.

Strand, R. F., H. W. Anderson and R. T. Bergland. 1972. Forest fertilization in northwestern USA and western Canada. Forest Industries. (in press)

Taylor, B. K. 1967. The nitrogen nutrition of the peach tree. I. Seasonal changes in nitrogenous constituents in mature trees. Aust. J. Biol. Sci. 20:379-387.

Taylor, B. K. and L. H. May. 1967. The nitrogen nutrition of the peach tree. II. Storage and mobilization of nitrogen in young trees. Aust. J. Biol. Sci. 20:389-411.

Towers, B. and W. J. Stambaugh. 1968. The influence of induced soil moisture stress upon Fomes annosus root rot of loblolly pine. Phytopathology 58:269-272.

Tromp, J. 1970. Storage and mobilization of nitrogen compounds in apple trees with special reference to arginine, p. 143-158. In Physiology of Tree Crops, L. C. Luckwill and C. V. Cutting (Ed.). 382 p.

U. S. Department of Agriculture, Soil Conservation Service. 1958. Soil Survey, Thurston County, Washington. Series 1947, No. 6. U. S. Government Printing Office, Washington, D. C.

Wallis, G. W. 1961. Infection of Scots pine roots by Fomes annosus. Can. J. Bot. 39:109-121.

Weetman, G. F. 1962. Nitrogen relations in a Black spruce (Picea mariana Mill.) stand subject to various fertilizer and soil treatments. Woodl. REs. Index, Pulp Pap. Res. Inst. Can. No. 129, 112 p.

Appendix 1 Description of stock cultures from which root rot pathogens used in the seedling experiment were obtained

Pathogen	Date culture started	Description of host
Poria weirii	August, 1970	Heartwood of 40 year old Douglas fir killed by Poria root rot. Jackson county, Oregon.
Fomes annosus	June, 1970	Roots of 25 year old living western hemlock. Snoqualmie, Wash.
Fomes annosus	June, 1970	Roots of dead 10 year old Douglas fir. Wynoochee river area, Wash.

Appendix II Description of fertilized and unfertilized trees sampled for rootwood in the decay experiment

Tree type	Tree no.	Age	DBH	Crown class
fertilized Douglas fir	1	56	12.4	D
	2	52	18.5	D
	3	50	12.5	CD
	4	54	18.1	CD
	5	55	20.2	D
unfertilized Douglas fir	1	52	13.5	CD
	2	54	18.0	D
	3	51	13.7	CD
	4	56	13.0	D
	5	50	16.0	D
fertilized western hemlock	1	55	11.6	CD
	2	39	10.9	CD
	3	44	12.5	CD
	4	40	9.7	I
	5	43	8.4	I
unfertilized western hemlock	1	43	9.5	I
	2	45	8.2	I
	3	51	10.3	CD
	4	55	12.9	CD
	5	50	11.6	CD

DBH... diameter breast height (inches)
I... intermediate
D... dominant
CD... co-dominant

Appendix III Procedure used to determine total nitrogen content of rootwood samples

Specimens were cut into matchstick size pieces and dried to a constant weight at 105 degrees C. They were then ground in a Wiley mill to pass a screen with 0.42 mm openings (40 mesh) and stored in dessicators. Prior to nitrogen analysis the ground samples were checked to insure that they had reached a constant weight. The material was thoroughly mixed and the N content of duplicate subsamples of each specimen was determined by the following procedure.

Each 4 gram subsample was placed in a 800 ml digestion flask and 60 ml of a mixture containing 4 grams salicylic acid in 100 ml concentrated H_2SO_4 were added. The flasks were heated gently for 5 minutes and allowed to cool for 30 minutes. To each flask was added 4 grams $Na_2S_2O_3$ $5H_2O$. The flasks were warmed 5 minutes more and cooled 5 minutes. Sixteen grams of a catalyst mix containing 5 grams metallic selenium powder in 100 grams K_2SO_4 were then added to each flask. The contents of the flasks were boiled until clear or a pale-green color, and for an additional 30 minutes to insure complete digestion. The flasks were then cooled to 40 degrees C and to each was added 300 ml of distilled water. After the flasks had cooled to room temperature 100 ml of 40% (w/v) aqueous NaOH was added slowly to each. The flasks were immediately connected to the distillation apparatus and the contents of each was mixed by vigorous swirling. Flasks were heated to boiling and the distillate was collected in 40 ml of the boric acid indicator solution described below. Approximately 150 ml of distillate was collected and allowed to cool to room temperature. The ammonia present was titrated with .02 N H_2SO_4. The amount of N in each subsample was corrected for a reagent blank with each set of six digestions,

calculated as mg N, and expressed as a percentage of the dry weight of the original subsample. Results of duplicate analysis were averaged to give the N content of the specimen analyzed.

The indicator solution was prepared by dissolving 0.5 grams of bromcresol green and 0.1 gram methyl red in 100 ml of 95% ethanol and adjusting the pH to 4.5 with dilute NaOH or HCL. One milliliter of this solution was added to 100 ml of the 4% (w/v) aqueous H_3BO_3 to give a boric acid indicator solution.

Appendix IV Weight losses of decayed rootwood samples in decay test

Tree no.	Sample no.	Percent weight loss: Douglas fir decayed by Poria weirii UF	Douglas fir decayed by Poria weirii F	Douglas fir decayed by Fomes annosus UF	Douglas fir decayed by Fomes annosus F	western hemlock decayed by Fomes annosus UF	western hemlock decayed by Fomes annosus F
	1	9.76	5.16	3.88	2.01	D	10.10
	2	6.92	D	7.66	.60	3.95	4.34
	3	10.79	4.27	D	5.03	3.43	3.78
	4	9.49	5.06	5.02	.78	4.36	4.53
	5	14.42	4.17	4.25	1.58	3.17	4.24
	6	10.81	5.25	3.54	1.01	4.81	4.59
	1	10.25	D	3.43	4.68	4.99	4.21
	2	14.85	D	8.41	4.99	6.39	5.93
	3	10.75	5.52	4.37	2.67	6.04	3.94
	4	D	10.93	4.65	3.24	3.81	5.18
	5	15.60	D	D	2.06	2.49	15.44
	6	D	D	D	D	D	2.19
	1	10.07	D	D	4.32	3.55	5.59
	2	10.22	10.68	5.08	9.04	D	4.72
	3	11.43	6.95	4.91	4.83	4.28	5.97
	4	D	11.02	3.61	5.31	4.28	7.08
	5	8.68	13.01	4.57	4.28	3.63	5.25
	6	12.86	10.07	3.56	4.94	D	D
	1	13.75	D	5.37	4.01	6.97	1.88
	2	8.53	1.56	5.69	2.90	D	7.10
	3	9.98	10.50	6.69	7.79	7.00	1.84
	4	11.04	D	D	4.37	D	2.17
	5	11.26	D	4.37	4.29	5.81	2.27
	6	10.31	6.93	4.00	3.16	10.72	D
	1	14.44	9.63	6.40	3.29	6.06	D
	2	15.02	D	9.67	3.00	6.61	3.57
	3	14.37	6.24	17.20	D	6.61	4.42
	4	13.37	7.72	13.18	2.06	9.03	3.43
	5	16.16	10.46	10.47	2.44	6.13	D
	6	11.43	11.69	12.50	3.16	8.99	4.16

D... sample discarded because of contamination
UF... rootwood from unfertilized trees
F... rootwood from fertilized trees

Appendix V Results of nitrogen analysis of Douglas fir rootwood samples from urea fertilized and unfertilized trees

FERTILIZED TREE ROOTWOOD

Tree no.	Sample no.	Percent nitrogen
1	1	.0494
	2	.0501
2	1	.0431
	2	.0431
3	1	.0350
	2	.0441
	3	.0378
4	1	.0406
	2	.0371
5	1	.0441
	2	.0476

UNFERTILIZED TREE ROOTWOOD

1	1	.0406
	2	.0403
2	1	.0424
	2	.0427
3	1	.0406
	2	.0399
4	1	.0453
	2	.0504
	3	.0448
5	1	.0441
	2	.0476

Appendix VI Results of sugar analysis of rootwood from nitrogen fertilized and unfertilized Douglas fir and western hemlock trees

Rootwood sample	Crown class	Replication	Percentage of sugar in sample
fertilized Douglas fir	dominant	1	.92
		2	.93
	co-dominant	1	.44
		2	.57
unfertilized Douglas fir	dominant	1	.80
		2	.63
	co-dominant	1	.52
		2	.50
fertilized western hemlock	co-dominant	1	.38
		2	.33
	intermediate	1	.18
		2	.26
unfertilized western hemlock	codominant	1	.52
		2	.54
	intermediate	1	.47
		2	.40

Appendix VII Results of starch analysis of rootwood from nitrogen fertilized and unfertilized Douglas fir and western hemlock trees

Rootwood sample	Crown class	Replication	Percentage of starch in sample
fertilized Douglas fir	dominant	1	4.645
		2	4.325
	co-dominant	1	4.450
		2	4.325
unfertilized Douglas fir	dominant	1	4.290
		2	4.260
	co-dominant	1	4.445
		2	3.940
fertilized western hemlock	co-dominant	1	3.850
		2	4.025
	intermediate	1	3.995
		2	3.815
unfertilized western hemlock	co-dominant	1	3.795
		2	4.290
	intermediate	1	4.490
		2	4.490