

Reducing *Phellinus weirii* inoculum by applying fumigants to living Douglas-fir

Walter G. Thies and Earl E. Nelson

Abstract: In 1982, Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) trees were placed in three disease classes based on signs and symptoms of laminated root rot caused by *Phellinus weirii* (Murr.) Gilb. Eight fumigation treatments and an untreated check were applied to five replicate trees within each disease class. The dose applied to each tree was based on the estimated biomass in the belowground portion of the bole, large roots, and first 2.4 m of the aboveground bole. The highest dosages tested were 1.5 g of methylisothiocyanate (MITC), 6.7 mL of chloropicrin (trichloronitromethane), and 6.7 mL of Vorlex (v/v 20% MITC, 80% chlorinated C₃ hydrocarbons)/kg biomass. In 1991 the roots of all surviving trees were excavated (roots of other trees were excavated at time of death), dissected, and sampled for viable *Phellinus weirii*. Twenty-four of the 30 trees treated with the two highest dosages of chloropicrin were killed, presumably by the fumigant. None of 45 trees treated with MITC and only 3 of 15 trees treated with Vorlex died, as did 3 of 15 untreated check trees. Volume of stained and decayed roots occupied by viable *Phellinus weirii* was reduced 78–90% by MITC or Vorlex compared with reductions of 51–65% by chloropicrin at the two lower, less phytotoxic doses, and 9% for untreated checks.

Résumé : En 1982, des Douglas taxifoliés (*Pseudotsuga menziesii* (Mirb.) Franco) furent regroupés en trois classes sur la base des signes et des symptômes de la carie de racines laminaire causée par *Phellinus weirii* (Murr.) Gilb. Huit traitements de fumigation et un témoin non traité furent répétés sur cinq arbres dans chaque classe. La dose administrée à chaque arbre était basée sur une estimation de la biomasse de la partie souterraine de la tige, des grosses racines et des 2,4 premiers mètres de tige au-dessus du sol. La plus forte dose testée atteignait 1,5 g de méthylisothiocyanate (MITC), 6,7 mL de chloropicrine (trichloronitrométhane) et 6,7 mL de Vorlex (v/v 20% de MITC, 80% d'hydrates de carbone C₃ chlorinés)/kg de biomasse. En 1991, les racines de tous les arbres encore vivants furent excavées (les racines des autres arbres avaient été excavées au moment où ils sont morts), disséquées et échantillonnées pour vérifier si *Phellinus weirii* était viable. Parmi les 30 arbres traités avec les deux plus fortes doses de chloropicrine, 24 furent tués, vraisemblablement par le fumigant. Aucun des 45 arbres traités avec le MITC et seulement 3 des 15 arbres traités avec le Vorlex sont morts ainsi que 3 des 15 arbres non traités. Le volume de racines colorées et cariées où *Phellinus weirii* étaient encore viable a été réduit de 78 à 90% par le MITC ou le Vorlex comparativement à 51 à 65% avec les deux plus faibles et moins phytotoxiques doses de chloropicrine et à 9% pour les arbres témoins non traités.

[Traduit par la Rédaction]

Introduction

Within the forests of the northwestern United States and British Columbia, Canada, laminated root rot, caused by the fungus *Phellinus weirii* (Murr.) Gilb., is the most widespread and important natural agent of disturbance that forest managers must deal with. The disease annually reduces wood production in western North America by about 4.4 million m³ (Nelson et al. 1981). Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) is the most important host economically, but nearly all conifers are susceptible. After diseased trees die, the pathogen lives saprophytically in the butts and large roots for as long as 50 years (Childs 1963; Hansen 1976, 1979). Disease begins in a young stand when roots of host trees become infected after contacting colonized stumps and roots from the preceding stand. The fungus then spreads between living trees across

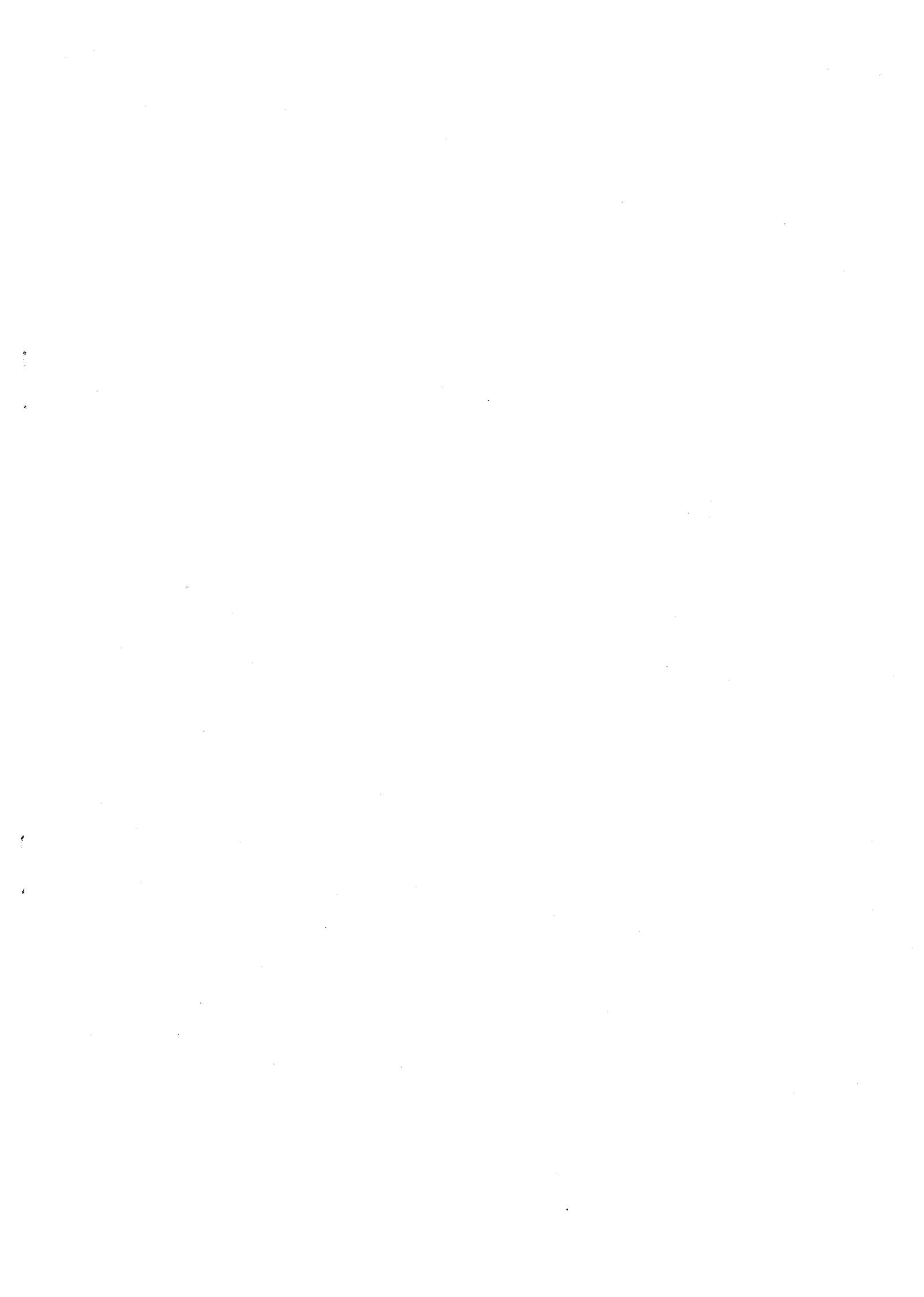
root contact (Wallis and Reynolds 1965). Biology, distribution, and impact of the disease, relative susceptibility of host species, and options for management were summarized by Thies and Sturrock (1995).

Strategies to manage the disease have focused on changing species composition or reducing inoculum before a susceptible stand is regenerated. Examination of excavated stumps colonized by *Phellinus weirii* showed that extensive stain or decay of the stump top indicates either advanced decay or a hollow at the root collar contiguous with advanced decay and stained wood within major roots. The presence of decay columns forming ducts to colonized portions of the root system suggested fumigation as a means of reducing inoculum. Application of fumigant to soil as well as directly to stumps to reduce or eliminate particular fungi was discussed previously (Filip and Roth 1977; Thies and Nelson 1982).

Fumigants were first applied in 1978 to stumps colonized by *Phellinus weirii*. Stumps were treated by drilling holes in their tops, pouring a dose of fumigant into the holes, and plugging the holes. One year later, *Phellinus weirii* had been eliminated from the stumps and most roots (Thies

Received August 9, 1995. Accepted December 22, 1995.

W.G. Thies and E.E. Nelson. USDA Forest Service, Pacific Northwest Research Station, Corvallis, OR 97331, U.S.A.



and Nelson 1982). A second study tested application techniques with doses based on estimated stump and root biomass. After 20 months, the fumigants eliminated the fungus from the stumps and reduced the volume of roots supporting *Phellinus weirii* to 22% of the prefumigation volume (Thies and Nelson 1987a). These studies demonstrated that fumigants could move through wood to eliminate *Phellinus weirii* inoculum and that an effective dose was related to the quantity of treated biomass. Additional evidence suggests that live Douglas-fir trees may tolerate injection of fumigants into the bole (Goodell et al. 1984; Morrell and Newbill 1990).

We began the study reported here in 1981 to determine if the fumigants chloropicrin (trichloronitromethane), methylisothiocyanate (MITC), or Vorlex¹ (v/v 20% MITC, 80% chlorinated C₃ hydrocarbons), injected into live Douglas-fir, could reduce the volume of roots occupied by *Phellinus weirii* without markedly reducing tree survival or vigor.

Methods

Study site

The study was initiated in 1981 in a 47-year-old, naturally regenerated Douglas-fir stand in the Oregon Coast Range near Apiary, Oregon (46°01'N, 123°04'W). Some site characteristics are the following: elevation, 420 m; slope, 0–35%; mean annual precipitation, 145 cm (U.S. Weather Bureau 1965); soil series, Bacon silt loam. Western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) was a minor constituent of the overstory. Ground cover was primarily salal (*Gaultheria shallon* Pursh), with some sword fern (*Polystichum munitum* (Kaulf.) Presl) and a lesser component of a diverse group of plants (Luoma and Thies 1994). The area has a site index for Douglas-fir of 52 m at 100 years (McArdle et al. 1961).

Selection of trees

Dominant and codominant Douglas-fir trees, with clearly visible crowns but lacking severe distress symptoms, were selected. Candidate trees were examined for *Phellinus weirii* by carefully exposing major lateral roots within 60 cm of the tree base, examining each root surface for characteristic ectotrophic mycelium, and at times using an increment borer to detect stained or decayed root wood (Thies and Sturrock 1995). Because their tolerance to fumigants might be affected by laminated root rot, we treated 45 trees in each of three disease classes: class I: infected, *Phellinus weirii* found on roots; class II: probably infected, has crown symptoms and an inoculum source is present within 5 m; and class III: probably uninfected, no symptoms and no identified inoculum source within 25 m. The anticipated frequencies of colonized roots for these classes were many, few, and none, respectively.

Trees in each disease class were separated (blocked) into five groups of nine based on similarities of diameter at breast height (DBH) and tree location. Study trees ranged in DBH

from 27.4 to 62.2 cm. Nine treatments were randomly assigned to trees within each group. Thus each of the nine treatments was applied to 15 trees, five trees in each of three disease classes, for a total of 135 trees. After trees had been designated for the blocked portion of the study, another five trees from disease class I were selected randomly to test the application of MITC in gelatin capsules. An additional 34 candidate trees from all disease classes with clearly visible crowns and terminals were included as photo trees to provide additional data on crown symptoms of nontreated trees.

Treatments

Chloropicrin and MITC were applied at several dosages, and Vorlex was applied at a single dosage (Table 1). Earlier we found that a dosage of 6.7 mL of either chloropicrin or Vorlex per kilogram of stump and root biomass eradicated *Phellinus weirii* (Thies and Nelson 1982). For this study, a standard dosage (1D) was 6.7 mL of chloropicrin, 6.7 mL of Vorlex, or 1.5 g of MITC per kg of treated biomass. The dosage for MITC was based on the concentration of MITC in Vorlex (232 g MITC per 1.0 L Vorlex). Thus, two trees of similar diameter receiving a treatment of MITC 0.5D or Vorlex 0.5D would receive equal amounts of MITC. We assumed that the dose tolerated by a tree and the dose needed to eradicate the pathogen would vary linearly with the tree's biomass in its large roots (those having a diameter equal to or greater than 10 mm), the belowground portion of the bole, and the first 2.4 m of the aboveground portion of the bole. The biomass to be treated was estimated from the DBH of the tree (Thies and Nelson 1987b). For ease of application, doses were rounded upward to the nearest quarter litre for the liquids chloropicrin or Vorlex. MITC was applied as a solid in small polyethylene sacks or capsules, each containing 58 g, the quantity of MITC in 250 mL of Vorlex.

In March 1982, seven of the nine treatments were applied: check; chloropicrin 1D, 0.5D, 0.25D; and MITC 1D, 0.5D, 0.25D. Additionally, encapsulated MITC at a dosage of 0.5D was applied to five trees. Application of a 2D dosage of both chloropicrin and MITC was planned for 1982 but postponed for one season pending evaluation of the toxicity of the lower dosages. By fall 1982, significant damage to trees treated with chloropicrin at dosages of 0.5D and 1D was obvious. Because of this adverse impact, 2D treatments were dropped in favor of two alternative treatments applied in April 1983: chloropicrin 0.125D and Vorlex 0.5D. Vorlex was added because it reduces the volume of roots supporting *Phellinus weirii* (Thies and Nelson 1987a).

Application of fumigant

Fumigants were applied to 3.2 cm diameter holes drilled down at a 45° angle below horizontal and extending past the center of the tree. For the lowest dosage the holes were equally spaced around the tree approximately 30 cm above the litter layer. For higher dosages the first hole was drilled 15 cm above the litter layer, and additional holes were drilled at 15-cm intervals around the base of the tree positioned to progress in a helical fashion 30 cm up the bole with each turn around the tree. Only enough holes were drilled to accommodate the dose of fumigant designated for a particular tree. A dose of chloropicrin or Vorlex was poured equally into all holes in a tree through a long-necked funnel to minimize contact with sapwood. MITC was applied as a solid in small polyethylene sacks. Each sack was broken as it was pushed into a hole. Capsules containing 58 g MITC were distributed evenly to the application holes and enough water added to cover the capsules. Zahora and Corden (1985) described capsule preparation. Each hole was tightly plugged with a 12.5 cm long by 3.3 cm diameter western

¹ This paper reports the results of research only. Mention of a pesticide does not constitute a recommendation for use by the U.S. Department of Agriculture, nor does it imply registration under the United States Federal Insecticide, Fungicide, and Rodenticide Act as amended. Also, mention of a commercial or proprietary product does not constitute recommendation or endorsement by the U.S. Department of Agriculture.

hemlock (*T. heterophylla*) dowel, the beveled end of which was coated with resorcinol glue to resist passage of the fumigant. The glue had hardened before plugs were used. Holes drilled in the tree to apply fumigant were part of the treatment. Check trees were neither drilled nor treated with fumigants.

Crown and bole observations

Trees were evaluated annually for survival or obvious changes in crown condition. Photo points allowing clear views of the upper crowns were permanently marked for 136 of the 140 study trees (tops of the remaining study trees were obscured) plus the 34 photo trees. The crown of each tree was photographed (color transparencies) on seven occasions during the study.

Crowns of all study trees were rated (August 1982, September 1983, September 1984, October 1985, and July 1991) on an 11-point scale of crown condition, from dead (0) to vigorous (10). Ratings were subjective but considered needle complement, needle length and color, number and distribution of dead needles and branches, length of the terminal internodes, and number and growth of new branches. Based on difficulty of assigning ratings, a new crown rating system was established and all study trees were rated by both systems in July 1991. The new crown rating is a total number of points (maximum of 12) based on four evaluations of crown vigor, each rated on a point scale of 0 to 3, as follows: branch stunting, based on the terminal growth of lateral branches in the upper crown: 0, no growth; 1, severe stunting; 2, slight stunting; 3, no stunting; crown color: 0, brown; 1, yellow; 2, slightly off color; 3, normal; crown density: 0, no needles; 1, less than 50%; 2, 50–80%; 3, 80% to normal; presence of live branches: 0, all dead; 1, more than 50% (but not all) dead; 2, 5% to 50% dead; 3, less than 5% dead.

In August 1991, 10 growing seasons after treatment, trees were felled and the crowns sampled. Terminal growth (for 1991) of 12 branchlets selected at random (four each from the upper, middle, and lower crown thirds) was recorded and a 10- to 15-needle sample was collected from the middle of each branchlet and combined (bulked) for each tree. A 100-needle subsample was taken for each tree (from the combined sample), dried to a constant weight in a 70°C oven, and weighed as a surrogate measure of needle length.

Stump and root observations

All study trees alive in August 1991 were marked on the north side, felled, and their stumps and roots removed from the soil and cleaned. Trees that died during the study were similarly processed, most within a year of dying. To reduce the risk of worker exposure to possible residual fumigant, each tree was severed 15 to 30 cm above the highest treatment hole.

A variety of heavy equipment, including a bulldozer, grapple skidder, and excavator, were used to remove the stump and roots with the least amount of breakage possible. Soil was then cleaned from stumps and all roots with hand tools. During cleaning, roots originating from the north and south sides of the stumps were examined and the presence of ectotrophic mycelium typical of *Phellinus weirii* was recorded when found. All roots were then marked at 30-cm intervals from the root collar to a root diameter of 5 cm. Starting at the outermost mark, roots were cut perpendicular to their axes at each marked location and the cut end examined. When stain or decay typical of that caused by *Phellinus weirii* was found, the root at that point was classified as infected, a 5 cm thick disk was removed, labeled, measured for diameter, placed in a sealed plastic bag, and kept up to 2 weeks in an unheated shed until processed. Root systems were diagrammed to record relative locations of collected disks, including their distance from the root collar.

To further reduce the risk of human exposure to fumigants, no roots were cut within 30 cm of the stump. Each major root was examined 30 cm from the root collar, its diameter measured, and the diameter recorded for those greater than 5 cm. A tree was classified as infected if stain or decay typical of that caused by *Phellinus weirii* was found in any of its roots.

Root disks were evaluated for viable *Phellinus weirii* by attempting to isolate the fungus from five chips taken from representative stained or decayed wood on freshly split faces of each disk. Each chip was placed in a culture tube containing 1.5% malt agar with 200 ppm streptomycin sulfate. Presence of *Phellinus weirii* was confirmed by distinctive morphological features of colonies that developed within 14 days (Mounce et al. 1940). Tubes without colonies were re-examined 14 days later.

Sampled disk pieces were placed in plastic bags that were loosely closed, incubated at ambient temperature (5 to 20°C) for 4–8 weeks, and then examined for the thick felt of mycelium with setal hyphae typical of *Phellinus weirii*. A disk was recorded containing living *Phellinus weirii* if either culturing of chips or incubation of disk wood yielded the fungus. Each disk collected represented a middle cross section of a 30 cm long root segment that showed symptoms of colonization by *Phellinus weirii*. The volume of each segment was calculated as a 30 cm long cylinder with a diameter equal to the disk. The total calculated volume of sections for any one tree was considered to be its colonized root volume. The root volume where *Phellinus weirii* was found to be no longer viable was calculated similarly. The treatment effect was evaluated on the basis of percent reduction of viable *Phellinus weirii* in the colonized portions of the root systems.

Inoculum contributed to the site by a diseased tree includes colonized wood of both roots and the belowground portion of the stump; however, in this study, stump volumes were not determined. Therefore, the volume of the belowground portion of the stump was not included in the calculation of either initial or residual inoculum and was not considered in the percent reduction of inoculum.

Data analysis

Data were subjected to analysis of variance (ANOVA) with a completely randomized design and a 2 × 7 factorial treatment structure to detect significant ($\alpha \leq 0.05$) differences between treated and untreated trees, with and without indications of infection by *Phellinus weirii*. Treatments were compared using least significant differences for DBH growth and surviving *Phellinus weirii* for both root volume and number of disks. Log transformation was used as needed to meet the assumptions of normality and equal variance of residuals. Logistic regression was used to assess the relation between the volume of the root and the likelihood that the fungus remained alive. We also made a linear regression of amount of roots on DBH. The Spearman rank correlation test was used to compare crown evaluation systems (Steel and Torrie 1980).

Results

Trees examined

In this study, 174 trees were identified and evaluated in fall 1981 and observed through fall 1991. A total of 146 trees were felled, and their stumps and roots were excavated and examined, including all 140 of the study trees plus 6 of the immediately adjacent photo trees. Of these 146 trees, 37 died and were excavated early; the 109 survivors were felled and examined in 1991. Based on evaluations of the excavated stumps and roots, 71 trees

Table 1. Reduction in roots colonized with *Phellinus weirii* and survival and growth of Douglas-fir trees treated with chloropicrin, methylisothiocyanate (MITC), or Vorlex in 1982 (initial) and evaluated in 1991 (live).

Treatment	Dose (D)	Trees (no.) ^a				Roots reduced (%) ^b	Infected		Uninfected		DBH increase (cm) ^d
		Initial 1982	Live 1991	Infected	Eradicated		Live 1991 (no.)	Branch length (cm) ^c	Live 1991 (no.)	Branch length (cm)	
Chloropicrin	1	15	2 ^e	9	7	87 _a	2	5.1	0	—	5.2
Chloropicrin	0.5	15	4 ^e	5	2	68 _a	1	—	3	5.0	2.9
Chloropicrin	0.25	15	10	9	4	65 _a	7	6.5 _b	3	6.1 _a	3.7
Chloropicrin	0.125 ^f	15	15	8	3	51 _a	8	6.5 _b	7	6.5 _a	5.5
MITC	1	15	15	6	3	83 _a	6	5.8 _a	9	5.8 _b	7.0
MITC	0.5	15	15	7	2	78 _a	7	5.8 _a	8	6.2 _a	5.4
MITC	0.25	15	15	8	5	90 _a	8	6.2 _b	7	6.1 _a	5.9
Vorlex	0.5 ^f	15	12	6	4	81 _a	5	6.7 _b	7	6.4 _a	4.6
Check	—	15	12	6	0	9 _b	3	5.1 _a	9	6.6 _a	7.0
MITC capsules	0.5 ^g	5	4	5	0	45	4	6.2	0	—	3.6

Note: Values in a column followed by the same letter are not significantly different.

^aNumber of study trees infected with *Phellinus weirii* per treatment and number of treated infected trees where *Phellinus weirii* was eradicated from the root system.

^bPercent reduced root volume, from all treated trees, containing stain or decay but without evidence of surviving *Phellinus weirii*.

^cBranchlet length based on 1991 branchlet elongation.

^dIncrease in DBH from 1982 until 1991 of surviving trees.

^eTreatments not included in statistical analysis of crown or DBH data because of the low number of survivors.

^fTreatment applied April 1983; all other treatments applied April 1982.

^gMITC capsules were applied to five infected trees only and are not included in the statistical analysis.

were classified as infected and 75 as uninfected. There were minor differences in DBH or height between the trees based on infection status (Table 2). Of the 120 treated trees in the replicated portion of the study, 58 were classified as infected when the stumps and roots were examined (Table 1).

Tree survival

Most (92 of 125) treated trees survived for 10 growing seasons after treatment (Table 1). Most mortality occurred within 2 years of treatment. Of the 33 treated trees that died, 27 (12 infected) died within 2 years of treatment. All nine of the uninfected check trees remained alive. Three of the six infected check trees died. Of the six photo trees that were excavated, two were infected and one died. The four uninfected trees survived to harvest.

All 45 MITC-treated trees survived through 10 growing seasons, as did 12 of 15 trees treated with Vorlex (Table 1). Two of the three Vorlex-treated trees that died were uninfected. Only 2 of the 15 trees treated with the highest dosage of chloropicrin (and only 4 of the 15 treated with half that dosage) remained alive after 10 growing seasons (Table 1). Most trees tolerated chloropicrin at the two lowest dosages: 10 of 15 trees treated with 0.25D and all 15 treated with 0.125D chloropicrin survived to harvest (Table 1).

Tree growth

In general, the crowns of surviving treated trees appeared healthier and more vigorous before harvest in 1991 than before treatment in 1982. The results of the two crown evaluation systems, compared by using the Spearman rank

Table 2. Diameters and heights of observed trees by disease class at initiation and completion of the study.

	n	DBH (cm)			Height (m)	
		Min.	Max.	Mean	n	Mean
1982						
Infected	71	27.4	67.3	45.2		
Uninfected	75	27.4	65.3	45.7		
1991						
Infected	52	30.0	68.8	50.1	49	34.55
Uninfected	55	31.2	70.4	51.6	52	34.67

correlation test, were found to be highly correlated ($R = 0.967$, $n = 93$). Inspection of the crown evaluation data and examination of the crown photos revealed that all fumigants caused an obvious decline in crown appearance for the first 2 years after treatment. Some crowns continued declining and some of these trees died; however, most crowns subsequently improved. For the 90 treated surviving trees, the percentage of crowns with the top rating (original system) rose from 15% 2 years after treatment to 37% by 1991. Only trees receiving MITC capsules failed to improve.

The two highest dosages of chloropicrin were dropped from statistical analysis of fumigant impact because many of the trees died. Examination of the remaining seven treatments by ANOVA showed that needle weights did not differ significantly among treatments ($p = 0.91$) between infected and uninfected trees ($p = 0.63$) or in the treatment by infection interaction ($p = 0.25$). Over the 10 growing

seasons of the study, DBH growth averaged 5.07 cm for infected trees and 5.72 cm for uninfected trees, but there was no detectable impact of infection ($p = 0.97$) or treatment by infection interaction ($p = 0.74$) on increase in DBH. As the ANOVA indicated a possible treatment effect ($p = 0.055$), treatment levels were compared with the check by using least significant differences. Among treatments, only chloropicrin 0.25D was significantly different (lower) from the check ($p = 0.014$) in DBH growth. Branch growth in the final season was influenced by treatment ($p = 0.017$), and there was a treatment – infection status (1991) interaction ($p = 0.04$). Branch growth of uninfected trees was significantly different (less) from the check trees for only one treatment, MITC 1D ($p = 0.008$). Branch growth of infected trees was significantly different (greater) in four of the treatments from the infected check trees: chloropicrin 0.25D ($p = 0.002$), chloropicrin 0.125D ($p = 0.002$), MITC 0.25D ($p = 0.017$), and Vorlex 0.5D ($p = 0.002$).

Since trees treated with MITC capsules were not selected at random from the pool of candidate trees, their data could not be included in the ANOVA. However, branch growth of infected trees treated with MITC capsules seemed to be the same as trees receiving MITC either in sacks or as Vorlex. Diameter growth of the four surviving trees (all infected) treated with MITC capsules was less than growth on the infected check trees (Table 1); however, the number of trees in the comparison was small, and we were unable to determine if the difference was the result of differences in treatment effects or random chance.

Inoculum reduction

The effectiveness of fumigant treatments to eradicate *Phellinus weirii* was judged by the reduction in volume of inoculum in roots. There was a highly significant effect of the fumigant treatments on the percentage of root volume with residual viable *Phellinus weirii* ($p = 0.004$) and the percentage of disks with residual *Phellinus weirii* ($p = 0.0045$). Comparison of fumigation treatments using least significant differences showed them to be different from the check, but none of the fumigant treatments was different from one another in residual *Phellinus weirii* for either volume (Table 1) or number of disks.

The fungus was eliminated from the roots of 30 of 58 treated, infected trees in the replicated portion of the study (Table 1). The standard dosage of chloropicrin (1D) eliminated *Phellinus weirii* from all roots of seven of the nine treated trees that had been infected. Of the 13 infected trees treated with chloropicrin that died during the study, *Phellinus weirii* was eradicated from seven. Of the 18 infected trees treated with chloropicrin that survived through the 10th growing season, the fungus was eradicated from nine. Fumigation with MITC gave similar results. All infected MITC-treated trees (21) survived, and the pathogen was eradicated from 10. Vorlex eradicated the pathogen from the one infected tree that died and from three of the five infected trees that survived to the end of the study. Chloropicrin and MITC killed *Phellinus weirii* in roots at comparable distances from the root crown. For chloropicrin, the pathogen was eliminated at a mean distance of 1 m (3 m maximum). For MITC, the mean distance was 1.3 m (2.7 m maximum).

MITC applied in capsules had less effect on residual root volume with *Phellinus weirii* (45% reduction) than the comparable dosage of MITC applied in sacks (78% reduction) or as Vorlex (81% reduction) (Table 1).

There was a positive relation between the log of the volume of the root piece and the probability that the fungus remained viable ($p = 0.044$); however, the amount of explained deviance was less than 0.01. This result was interpreted to mean that *Phellinus weirii* is more likely to have survived in large-diameter root pieces than in small-diameter pieces. This is of limited predictive or biological value because the amount of variation explained in the occurrence or nonoccurrence of *Phellinus weirii* based on root diameter is small.

Fumigant movement

Eight trees from six treatments (chloropicrin 1D, 0.25D, and 0.125D; MITC 1D, 0.5D, and 0.25D) had infected root wood separated from the stump by uninfected root wood. In these eight trees, *Phellinus weirii* was alive in only 1 of 33 disks collected from infected root wood not connected by a continuous decay column to the stump. The fungus was killed in all seven disks collected more than 2.1 m from the stump (one disk was 3.3 m from the stump).

The odor of chloropicrin or Vorlex was often detected and that of MITC was sometimes detected when treated trees were felled, indicating that some quantity of these chemicals remained near the application site. Distribution of the fumigants in the roots and boles at the time of harvest was previously reported (Morrell et al. 1994). Some stumps were inadvertently split open in 1991 while being removed. Frequently, fluid with the appearance and odor of either chloropicrin or Vorlex ran from the treatment holes. In some treatment holes, remnants of the gelatin capsules still containing MITC were visible. In one hole (two capsules), we estimated the residual MITC to be 50% of the original amount. No attempt was made to quantify the residual chemical in any stump. Based on earlier results (Thies and Nelson 1982, 1987a) and our observation of chemical still present near the site of application in split stumps, we accepted without further testing that stumps no longer contained viable inoculum.

Disease detection

Using logistic regression on data from the 104 study trees alive in 1991, we tested several variables to determine their suitability as predictors of infected trees. None of the tested variables proved to be a significant predictor of infection: old crown classification ($p = 0.37$), new crown classification ($p = 0.44$), branchlet length ($p = 0.56$), needle weight ($p = 0.95$), and DBH increase from 1982 to 1991 ($p = 0.27$). When we examined data from the 16 trees (12 check trees and 4 photo trees) not receiving a fumigant treatment, the branch stunting element of the new crown classification system was a strong predictor of infection ($p = 0.0005$). Of the 16 nontreated trees, all 4 infected trees had a branch length rating of 1. All uninfected trees had a branch length of either 2 (1 tree) or 3 (11 trees).

We excavated and examined 48 infected root systems from live trees in 1991. Roots emerging from nearest north and south were evaluated for ectotrophic mycelium as an

indicator of infection status. Examination of roots emerging from nearest north and south showed that 32 trees would have been declared infected; 7 trees had an infected root emerging either north or south while 25 trees had infected roots in both directions. Had examination been limited to north and south roots, 16 infected trees would have been missed. Often, when ectotrophic mycelium was not in evidence on the north or south root, it was present on the underside of the stump or major roots and appeared as a brown crustose mat composed largely of setal hyphae typical of *Phellinus weirii*. Location of the fungal mat would have precluded its being observed during any survey using less than total stump excavation.

Infected trees in each disease class were examined for the mean number of disks collected as an indicator of the relative volume of the root system that was colonized: class I, 14.3 disks; class II, 5.2 disks; class III, 5.3 disks.

We tested DBH as a predictor of root system size. As a measure of root system size we recorded the sum of cross-sectional area of all major roots on a stump taken 30 cm from the stump. We found a highly significant ($p = 0.0001$) correlation of cross-sectional area at breast height and total root cross section; however, the relation did not account for much of the variation in area ($R^2 = 60\%$) and thus would not be a good predictor. There were between 4 and 36 (mean of 15) roots greater than 5 cm in diameter on the 109 live trees excavated. Additionally, many diseased trees had many small adventitious roots that were not further quantified.

Discussion

In this study, survival and growth of treated trees compared with untreated trees and inoculum reduction in treated trees were the measures of treatment success. Although chloropicrin, MITC, and Vorlex are broad-spectrum biocides, this study demonstrated that Douglas-fir can survive injection with these fumigants at dosages found earlier to be sufficient to reduce or eradicate *Phellinus weirii* from colonized stumps and roots (Thies and Nelson 1987a). Of the 125 treated trees, 74% survived for 10 growing seasons after fumigant application. None of the nine nontreated, uninfected trees died during the same period. Of the 36 trees that died, 92% were treated. Of the treated trees that died, 85% died within 2 years of treatment. Of the 16 infected treated trees that died, 75% died within 2 years of treatment; on average we would have expected only 20% to have died. We attributed death of treated trees primarily to the fumigants, realizing that *Phellinus weirii*, when present, was likely a contributing factor.

MITC proved much less phytotoxic than chloropicrin and perhaps more effective at reducing residual *Phellinus weirii* inoculum. All 45 of the MITC-treated trees survived through 10 growing seasons, whereas only 31 of 60 chloropicrin-treated trees survived. Increased survival of chloropicrin-treated trees seems to be strongly related to decreased dosage (Table 1); however, reducing dosage of chloropicrin is a trade-off, increasing both the number of surviving trees and the amount of surviving inoculum.

Statistically, there was no difference among the fumigant treatments with regard to inoculum reduction, but that may

be an artifact of the analysis resulting in part from high variability and few survivors in some treatments. Although 10 trees survived the chloropicrin 0.25D treatment, the DBH growth of those 10 trees was significantly different (lower) from the check trees. Only the highest dosage of MITC appeared to have a slight, but statistically significant, adverse effect on branchlet growth of uninfected trees. On the positive side, four of the treatments resulted in improved crown vigor of infected trees as measured by increased branchlet growth: chloropicrin 0.25D and 0.125D, MITC 0.25D, and Vorlex 0.5D.

In this test, neither Vorlex nor MITC capsules seemed to be as satisfactory as any dosage of MITC in plastic sacks. Although only 3 of the 15 Vorlex-treated trees died, 2 were uninfected. Given that none of the uninfected nontreated trees died during the 10 growing seasons of the study and that MITC-treated trees all survived, we speculate that the carrier in Vorlex (chlorinated hydrocarbons) was largely responsible for killing the Vorlex-treated trees, either as a toxic compound itself or in association with the MITC.

The poor showing of the encapsulated MITC may have been related to our method of application. After capsules were placed in treatment holes, sufficient water was added to cover the capsules before plugging the holes. It has since been reported that little if any added water is necessary to allow the release of the MITC from the gelatin capsules. Due to the low solubility of MITC in water, excess water likely inhibits both release and the diffusion of MITC into the wood (Zahora and Corden 1985). This is consistent with our observations. A reduced release effectively lowers the dosage and may account for a greater than expected amount of residual inoculum. A mechanism was suggested (Zahora and Corden 1985) by which the excess water may lead to toxic breakdown products of MITC that could impact tree growth. Although speculative, this could account for the reduced DBH growth. Additionally, all five trees receiving the treatment were infected. Thus, while performance of trees receiving other treatments represented a mean growth of infected and uninfected trees, performance of trees receiving this treatment was judged only on trees with a reduced root capacity.

The reduction in inoculum volume is understated by the results given in Table 1. When a tree with laminated root rot dies or is cut, the belowground portion of the tree base (stump) and roots colonized by *Phellinus weirii* remain as inoculum. Compared with a root, the colonized stump is a massive piece of inoculum. Inoculum from untreated colonized stumps and roots loses viability from the distal portions (smallest roots) of the root system first and from the stumps last. Based on our earlier work with fumigation of stumps (Thies and Nelson 1982, 1987a) and the strong odor of fumigants detected around freshly cut trees or recently excavated stumps, it seems reasonable to conclude that *Phellinus weirii* had been eliminated from the stumps. By eliminating the stump inoculum, fumigation dramatically reduces the inoculum size and longevity on the site. We did not measure the volume of colonized stumps. The reduced *Phellinus weirii* inoculum volume (Table 1) represents the reduction in inoculum in roots over 15 cm away from the stump but does not reflect the reduction in stump inoculum.

There was significant variability in every aspect of the root systems that we attempted to characterize. We were unable to characterize either the number or size of roots based on tree DBH. We can use DBH to provide an estimate of belowground biomass (Thies and Cunningham 1996) and an indication of the total cross-sectional area of roots 30 cm from the stump, but root systems generally are too variable to predict from aboveground parameters. Further, neither size nor distance from the stump were absolute predictors of inoculum survival in fumigated trees. While a large-diameter piece is more likely to harbor *Phellinus weirii*, the amount of variation explained in the presence or absence of *Phellinus weirii* based on root diameter was very small and unlikely to be biologically important.

We expected that wood with stain and decay typical of *Phellinus weirii* would be more porous and would allow fumigants to diffuse from the root collar out through colonized roots more effectively than through sound roots. In this study as well as in our earlier work with stumps (Thies and Nelson 1987a), we found no relation between presence of decay and ability of the fumigants to contact and kill *Phellinus weirii*. Stained wood was not a prerequisite for the fumigants to diffuse through wood and eliminate *Phellinus weirii* from roots. Inspection of the data on distribution of residual inoculum did not suggest that fumigation was more effective when stained wood was contiguous with the stump or that one chemical diffused better than another.

Some observations from this study may be useful to those planning surveys for laminated root rot. Although our sample size was small, we interpreted the results to show that caution is required when the presence of *Phellinus weirii* ectotrophic mycelium is used to survey for laminated root rot. Had we checked only two roots on each stump (roots emerging closest to the north and the south sides of a stump), we would have missed 16 of 48 infected stumps for an error rate of 33%. Similar results were reported when a two-root sampling strategy was evaluated to survey for *Armillaria* sp. and *Heterobasidion annosum* (Baker et al. 1992).

Using the disease classes as a way to assure that each treatment was applied to some trees with numerous infected roots and some trees with few infected roots was partially successful. The class I trees yielded an average of about three times as many disks (and by inference, infected roots) as class II trees. It was unexpected, however, that class II and class III trees would yield about the same number of colonized disks.

Crown condition is a useful symptom in surveying for laminated root rot. Infected trees may exhibit reduced leader growth for 5 years or more before crown thinning or other symptoms appear. However, it is often difficult to see the leader. In this study, both our measurement of branchlet growth and our subjective crown evaluations of nontreated trees clearly separated infected and uninfected Douglas-fir. The correlation of reduced branchlet growth in the upper crown with laminated root rot is consistent with observations of the authors and other forest pathologists in the region and can be used as a reliable indicator.

Conclusions

(i) Douglas-fir trees can survive an appropriate dosage of chloropicrin, methylisothiocyanate or Vorlex injected into their boles. (ii) Fumigants injected into the bases of *Phellinus weirii*-infected Douglas-fir trees can diffuse into their root systems, dramatically reduce the amount of inoculum remaining on a site after harvest, and in the case of MITC, prolong the lives of the trees. (iii) Both the possibility of using branch growth and the shortcomings of using ectotrophic mycelium need to be considered when surveys are planned for laminated root rot.

Almost no literature relates to fumigation of live trees. As far as we are aware, ours is the first study to demonstrate a reduction in wood-decaying fungi in living trees by treatment with chemical fumigants. The methods described here are original and resulted in a major reduction of *Phellinus weirii* inoculum.

Acknowledgments

We thank K.C. VanNatta of Rainier, Oregon, for providing study trees, equipment, and personal services. We also thank NOR-AM Agricultural Products, Inc. (Naperville, Ill.) and Great Lakes Chemical Corporation (Fresno, Calif.) for supplying fumigants, advice, and financial assistance. A special thanks to the Siuslaw National Forest, Angel Job Corp Center, Yahats, Oreg., for help in cleaning root systems. We appreciate the advice of R.D. Graham, M.E. Corden, J.J. Morrell, and B.C. Goodell (Oregon State University, Corvallis, Oreg.). We thank Harlan Fay, Mike McWilliams, and Bob Merola for their hard work in the field and assistance in the laboratory, and Tom Sabin (Oregon State University, Corvallis, Oreg.) for statistical advice. This research was partially supported by the Coastal Oregon Productivity Enhancement (COPE) Program, U.S. Department of the Interior Bureau of Land Management, and USDA Forest Service, Pacific Northwest Research Station. Some of these results were presented as a poster at the 8th IUFRO Conference on Root and Butt Rot, Uppsala, Sweden, and Helsinki, Finland, August 9–16, 1993, and as a talk to the 41st Annual Western International Forest Disease Work Conference, Boise, Idaho, September 14–17, 1993, and will be published in the proceedings of these conferences.

References

- Baker, F.A., Shaw, C.G., III, Omdal, D.W., and Wargo, P.M. 1992. Evaluation of the root disease indicator used in the forest health monitoring program. *Phytopathology*, **82**: 1152.
- Childs, T.W. 1963. *Poria weirii* root rot. *Phytopathology*, **53**: 1124–1127.
- Filip, G.M., and Roth, L.F. 1977. Stump injections with soil fumigants to eradicate *Armillaria mellea* from young growth ponderosa pine killed by root rot. *Can. J. For. Res.* **7**: 226–231.
- Goodell, B.S., Helsing, G.S., and Graham, R.D. 1984. Responses of Douglas-fir trees to injection of chloropicrin. *Can. J. For. Res.* **14**: 623–627.

- Hansen, E.M. 1976. Twenty-year survival of *Phellinus (Poria) weirii* in Douglas-fir stumps. *Can. J. For. Res.* **6**: 123–129.
- Hansen, E.M. 1979. Survival of *Phellinus weirii* in Douglas-fir stumps after logging. *Can. J. For. Res.* **9**: 484–488.
- Luoma, D.L., and Thies, W.G. 1994. Effects of live tree fumigation on non-target vegetation. *Can. J. For. Res.* **24**: 2384–2389.
- McCardle, R.E., Meyer, W.H., and Bruce, D. 1961. The yield of Douglas-fir in the Pacific Northwest. USDA For. Serv. Tech. Bull. 201.
- Morrell, J.J., and Newbill, M.A. 1990. Movement of chloropicrin or methylisothiocyanate through the boles of Douglas-fir trees. *For. Sci.* **36**: 192–195.
- Morrell, J.J., Forsyth, P.G., and Thies, W.G. 1994. Distribution of methylisothiocyanate and chloropicrin in boles or roots of Douglas-fir trees. *Can. J. For. Res.* **24**: 2324–2329.
- Mounce, I., Bier, J.E., and Nobles, M.K. 1940. A root rot of Douglas-fir caused by *Poria weirii*. *Can. J. Bot.* **18**: 522–533.
- Nelson, E.E., Martin, N.E., and Williams, R.E. 1981. Laminated root rot of western conifers. USDA For. Serv. For. Insect & Dis. Leaflet. 159.
- Steel, G.D., and Torrie, J.D. 1980. Principles and procedures of statistics. 2nd ed. McGraw-Hill Co., New York.
- Thies, W.G., and Cunningham, P.G. 1996. Estimating large-root biomass from stump and breast-height diameters for Douglas-fir in western Oregon. *Can. J. For. Res.* **26**: 237–243.
- Thies, W.G., and Nelson, E.E. 1982. Control of *Phellinus weirii* in Douglas-fir stumps by the fumigants chloropicrin, allyl alcohol, Vapam, or Vorlex. *Can. J. For. Res.* **12**: 528–532.
- Thies, W.G., and Nelson, E.E. 1987a. Reduction of *Phellinus weirii* inoculum in Douglas-fir stumps by the fumigants chloropicrin, Vorlex, or methylisothiocyanate. *For. Sci.* **33**: 316–329.
- Thies, W.G., and Nelson, E.E. 1987b. Survival of Douglas-fir injected with the fumigants chloropicrin, methylisothiocyanate or Vorlex. *Northwest Sci.* **61**: 60–64.
- Thies, W.G., and Sturrock, R.N. 1995. Laminated root rot in western North America. USDA For. Serv. Gen. Tech. Rep. PNW-GTR-349.
- U.S. Weather Bureau. 1965. Climatic summary of the United States. Supplement for 1951 through 1960, Oregon. Climatographs of the United States. U.S. Weather Bureau, Washington, D.C. 86–31.
- Wallis, G.W., and Reynolds, G. 1965. The initiation and spread of *Poria weirii* root rot of Douglas-fir. *Can. J. Bot.* **43**: 1–9.
- Zahora, A.R., and Corden, M.E. 1985. Gelatin encapsulation of methylisothiocyanate for control of wood-decay fungi. *For. Prod. J.* **35**: 64–69.